

# Experimentation on *Hibiscus rosa* Flower Mucilage as a Potential Nasal Mucoadhesive its Characterization as an *In-situ* Gel for some Anxiolytics

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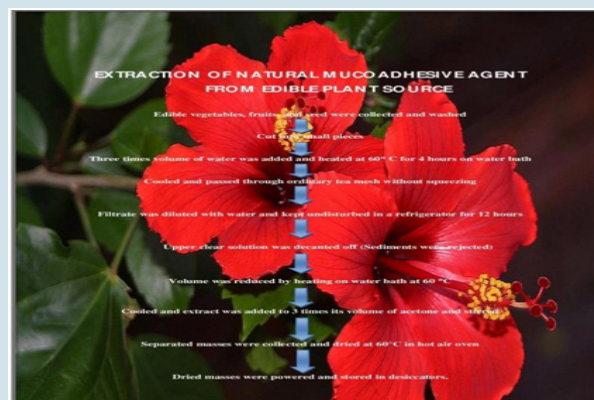
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## ABSTRACT

**Objective:** The objective of the present study was to prepare mucoadhesive *in situ* nasal gels with mucilage isolated from hibiscus flower (*Hibiscus rosa sinensis*, family: malveceae) containing alprazolam. Methods-Nasal gels of alprazolam were prepared using three different concentration (0.5%, 1% and 1.5% w/v) of H Rosa mucilage (HRM) and synthetic polymers (Hydroxy propyl methyl cellulose and carbopol 934). *In situ* gels were prepared with mixture of pluronic F127 and mucoadhesive agent. **Result:** Evaluation of HRM showed that it was as safe as the synthetic polymer for nasal administration. Evaluation of the prepared gel was carried out, including determination of viscosity, texture profile analysis and mucoadhesive strength. *In vitro* drug permeation study was conducted with the gels prepared with and without permeation enhancer (0.5% w/v sodium taurocholate) using excised goat nasal mucosa. For the determination of any further histological changes, before and after *in vitro* permeation study of the gels, with and without the permeation enhancer was also conducted. *In vivo* experiment conducted in rabbits further confirms that *in situ* nasal gel prepared with HRM provided better bioavailability of alprazolam than the gels prepared from synthetic mucoadhesive polymer. **Conclusion:** It was observed that the nasal gel containing 0.5% HRM and 0.5% sodium taurocholate exhibited appropriate rheological, mechanical and mucoadhesive properties and showed better drug release profile. More over this formulation produced no damage to the nasal mucosa used for the permeation study, and absolute bioavailability was also higher compare to gels prepared from synthetic polymers.

**Key word:** Alprazolam, Mucoadhesive agent, Nasal gel, *Hibiscus rosa*.

## PICTORIAL ABSTRACT



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## INTRODUCTION

Alprazolam is a freely water soluble benzodiazepine. It is a short acting anxiolytic of the benzodiazepine class of psychoactive drugs. Alprazolam, like other benzodiazepines, binds to specific sites on the GABA<sub>A</sub> gamma-amino-butyric acid receptor. Alprazolam is commonly used and FDA approved for the treatment of panic disorder and anxiety disorders such as generalized anxiety disorder (GAD) or social anxiety disorder (SAD). Alprazolam is available for oral administration in compressed tablet (CT) and extended-release capsule (XR) formulations. Alprazolam possesses anxiolytic, sedative, hypnotic, skeletal muscle relaxant, anticonvulsant and amnesic properties. Oral administration of alprazolam is preferable, but it undergoes extensive first pass metabolism in the liver, owing to which its oral bioavailability ranges from 15% to 27% in children and from 31% to 72% in adults. In that case, administration of alprazolam via transmucosal route is advantageous. Sublingual and buccal administration of alprazolam has been studied, but nasal route is most important among them in terms of drug absorption and availability of the drug for systemic effect.<sup>1,2</sup>

Administration of alprazolam via the nasal route is a comparative new method of delivering the drugs. For that purpose, various nasal alprazolam formulations were developed like solutions and sprays. However, these systems suffer from a number of disadvantages. Solu-

tions are cleared off rapidly from nasal cavity and do not allow prolonged residence of the drug in the cavity. Parenteral solution of alprazolam for intranasal administration caused irritation that is produced by its acidic pH and the relatively large volume that has to be administered.

So the idea of administration of alprazolam via nasal gels was conceived, but conventional gels pose difficulty in administering accurate dose of the drug owing to their high viscosity. This problem could, however, be overcome by formulating *in situ* mucoadhesive gels which are liquid at room temperature but change to a firm gel at the temperature of the nasal cavity. Pluronic F127 is widely used as a thermosensitive gelling agent since it is non-toxic, non-irritating, poses good release characteristics and biocompatible with other chemicals.<sup>2,3</sup>

In the present investigation, mucilage isolated from Hibiscus flowers (*Hibiscus rosa sinensis*) was used as a mucoadhesive agent along with Pluronic F127 to prepare mucoadhesive *in situ* nasal gels containing alprazolam and to characterise them accordingly, in terms of texture profile analysis, viscosity, mucoadhesive strength, *in vitro* release profile, *in vivo* absorption kinetics and histological studies. Mucilages isolated from *Hibiscus rosa* used in formulations is compared with synthetic polymers hydroxypropyl methyl cellulose (HPMC) and Carbopol 934 (C-934). This mucilage is biocompatible and biodegradable since it is edible in nature.<sup>4,21</sup>

## MATERIALS AND METHODS

Alprazolam was obtained as a gift from Sun Pharmaceutical Industries Ltd., Gujarat, India. *Hibiscus rosa* flowers were collected from local flower market. Pluronic F127 was purchased from Sigma Aldrich Pvt. Ltd., India. Ruthenium red, HPMC, Carbopol 934 and Sodium taurocholate were purchased from Loba Chemie Pvt. Ltd., Mumbai, India. All other reagents and chemicals used were of analytical grade.

### Extraction of mucilage from *Hibiscus rosa* flowers

*Hibiscus rosa* flowers were washed with double distilled water to remove any adherent material. About three times its volume of water was added and heated at  $60 \pm 1^\circ\text{C}$  on a water bath for about 4 h until the slurry was prepared. The viscous solution was then filtered, and the filtrate was diluted with three times its volume of water and kept undisturbed overnight in a refrigerator so that most of the undissolved portion settled down. In the following day, the clear supernatant portion was decanted and concentrated at  $60 \pm 1^\circ\text{C}$  in a rotary vacuum evaporator. The concentrate was cooled to room temperature and precipitated in about three times its volume of acetone. The precipitate was washed repeatedly with acetone and dried at  $60 \pm 1^\circ\text{C}$ . The dried material was ground by a mechanical grinder and passed through # 80 mesh sieve and kept in a desiccator till further use.<sup>5</sup>

### Characterisation of mucilage

#### Chemical identification of *hibiscus rosa* mucilage

*Hibiscus Rosa* mucilage (HRM) was tested for the presence of carbohydrates and reducing sugars with Molisch reagent and Fehling's solution, respectively. The mucilage was treated with  $\alpha$ -naphthol and concentrated sulphuric acid which gave purple colour. For detecting reducing sugars, the mucilage is treated with Fehling's solution A and B. After heating, brick-red precipitate was obtained. The mucilage was further treated with ferric chloride solution for detection of tannins, but no precipitate was observed. Further presence of mucilage was tested by treating the mucilage with 0.1% w/v ruthenium red solution. Presence of protein was detected by Biuret reagent. No test for determination of ficin was done.

#### Determination of $p_H$

pH of 1% (w/v) aqueous solution of HRM was measured using a Toshniwal pH meter (Toshniwal Inst. Mfg. Pvt. Ltd., Ajmer, India).

#### Swelling capacity

Swelling capacity of HRM was determined by keeping 1 g of the mucilage in 20 ml water in a measuring cylinder for 24 h. Swollen volume was recorded and determined as follows:

$$\text{Swollen Volume} = (V_2 - V_1)$$

where  $V_1$  and  $V_2$  are initial volume of the material prior to hydration and volume of the hydrated material, respectively.

#### Determination of zeta potential

Zeta potential of the mucilage was measured with the help of a zeta-sizer (Malvern Instruments Limited, UK). One millilitre of the mucilage solution was taken in a zeta potential cell and placed inside the instrument.<sup>6</sup>

#### Determination of viscosity

Viscosity of 1% aqueous solution of HRM was studied using Toki Sangyo Viscometer TV-10 (Toki Sangyo Co. Ltd., Japan) at five different speeds of 10, 20, 30, 60 and 100 rpm, respectively using spindle M4 and cord no. 23 at  $34 \pm 1^\circ\text{C}$  (Figure 1).

### Evaluation of mucoadhesive strength

Mucoadhesive strength of 1% aqueous solution of HRM was studied with QTS-25 Texture Analyser (Brookfield Engineering Labs., USA). Freshly excised goat nasal membrane was attached to the upper probe of the instrument, and drop of HRM solution was kept below that. The upper probe was then lowered at a speed of 10 mm/min. to touch the surface of the solution. A force of 0.1 N was applied for 5, 10, 15, 20 and 30 min, respectively, to ensure intimate contact between the membrane and the gel. The surface area of exposed mucous membrane was  $1.13 \text{ cm}^2$ .

The above studies were also conducted for HPMC and Carbopol 934, and results were compared.<sup>7,8</sup>

### Drug polymer interaction study

#### Fourier Transform Infrared Radiation measurement (FT-IR)

Infrared spectra of the pure drug and pure drug with several mucoadhesives were recorded on a FT-IR spectrophotometer (Model FT-IR 8400S, Shimadzu, Japan). The disc method was employed to study the possible interactions between the pure drug Alprazolam and the selected mucoadhesive, HBR. KBr (IR grade) discs in a proportion of 1:100: sample:KBr, were prepared from the samples and eventually analyzed over arrange of  $400\text{-}4000 \text{ cm}^{-1}$ . Transmittance (T) spectra were recorded and displayed accordingly in (Figures 2). (pure Alprazolam), Figure 3. (Alprazolam+HRM).<sup>9,10</sup>

### Preparation of mucoadhesive nasal gel of alprazolam

*In situ* gels are prepared by cold method. A 16% w/v Pluronic F127 was prepared in a mixture of propylene glycol/ water (45:55), and then the mucoadhesive agents (HRM, HPMC and Carbopol 934) were added to it in three different concentrations of 0.5%, 1.0% and 1.5% w/v, respectively. Alprazolam was then added to the dispersion, which was kept at  $4^\circ\text{C}$  overnight for complete swelling to form a homogenous gel. Gels containing enhancer (0.5% w/v sodium taurocholate) were also prepared to study the effect of enhancer on drug release pattern. Formulation codes of the gels are provided in Table 1.<sup>8,11,12,19,24</sup>

### Physicochemical characterization of the nasal gels

#### Determination of viscosity

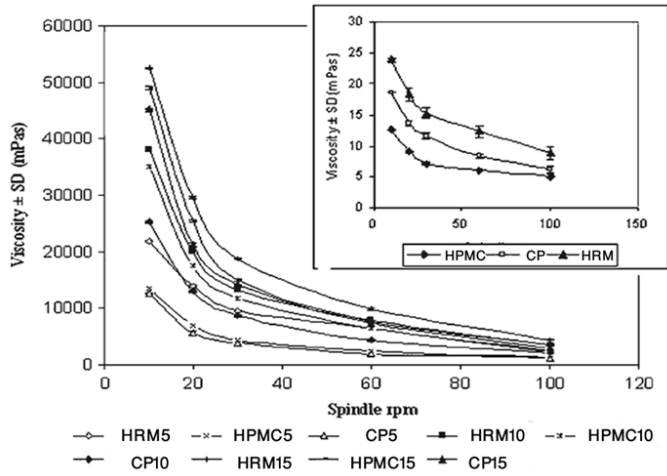
Viscosities of different formulations were measured with the same viscometer at five different speeds of 10, 20, 30, 60 and 100 rpm, respectively, using spindle M4 and cord no. 23 at  $34 \pm 1^\circ\text{C}$ . The corresponding viscosities were plotted against spindle rpm as depicted in Figure 1.

#### Gelation temperature measurements

Gelation temperature was determined by pouring 5 ml formulation in a 20-ml beaker containing magnetic bar and placing it upon a hot plate. A thermometer was immersed in the gel, which was heated at a rate of  $2^\circ\text{C}/\text{min}$  with constant stirring at 20 rpm. When the bar stopped moving due to gelation, the temperature was recorded as the gelation temperature as depicted in Table 2.

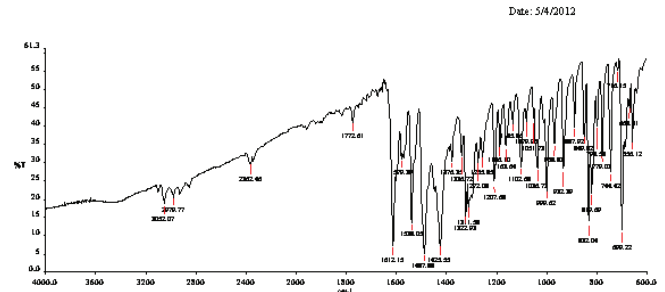
#### Texture Profile Analysis

Texture profile analyses of the prepared gels were evaluated using QTS-25 Texture Analyser to determine the mechanical parameters like hardness, cohesiveness and adhesiveness. An analytical probe of diameter 1.2 cm was depressed twice into each sample to a defined depth (15 mm), at a defined rate (30 mm/min), with a defined recovery period (15 s), between the end of the first compression and the beginning of the second. A trigger force of 4 g was applied. At least six replicate analyses



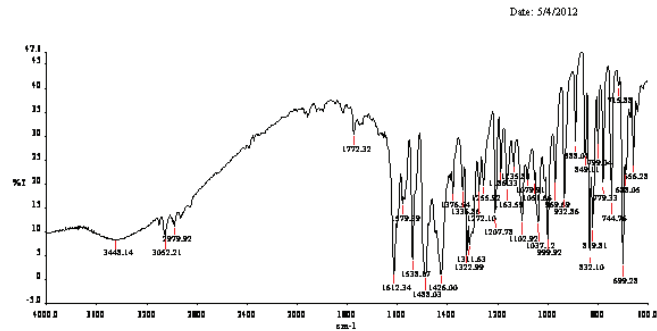
**Figure 1:** Comparative result of viscosities of 1% aqueous solutions of mucoadhesive agents and of *in situ* gels measured at 34 ± 1°C

Data represent mean values ± SD (n=6).



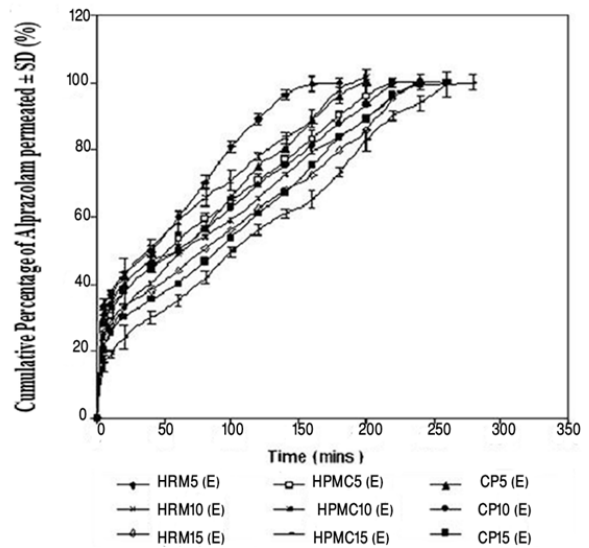
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 Alprazolam  
 REF 4000 17.27 2000 39.64 600  
 3052.07 18.30 2979.77 20.45 2362.46 29.58 1772.61 40.94 1612.15 7.08  
 1579.39 30.68 1538.05 13.24 1487.88 4.77 1425.55 7.23 1376.55 30.17  
 1336.72 31.78 1322.93 15.52 1311.58 17.76 1272.08 29.99 1255.85 31.53  
 1207.68 23.65 1186.10 34.33 1163.64 33.86 1135.96 40.64 1102.68 28.44  
 1079.95 41.40 1051.73 41.09 1036.75 28.14 999.62 22.54 969.83 34.77  
 932.39 27.74 887.92 39.26 849.02 37.21 832.04 12.92 819.69 21.28  
 798.58 39.69 779.03 32.01 744.42 26.80 716.15 55.08 699.22 11.14

**Figure 2:** FTIR spectra of the pure drug Alprazolam



Amitabha Roy S1 04052012.pk  
 AMITAV-1.SP 851 4000.00 600.00 0.48 47.63 4.00 %T 4 2.00  
 H.Rosa + Alp  
 REF 4000 9.59 2000 35.22 600  
 3448.14 8.28 3052.21 8.47 2979.92 10.93 1772.32 30.12 1612.34 1.00  
 1579.39 15.68 1528.17 4.03 1498.03 0.48 1426.00 0.97 1376.54 18.05  
 1336.86 18.61 1322.99 5.31 1311.65 7.97 1272.10 18.54 1255.92 19.53  
 1207.78 12.75 1186.33 22.65 1163.59 18.97 1135.88 23.74 1102.92 12.11  
 1079.91 21.01 1051.66 20.06 1037.12 11.77 999.92 8.46 969.69 20.42  
 932.86 16.80 888.01 28.19 849.11 25.84 832.10 4.87 819.81 10.44  
 790.04 28.42 770.22 10.56 744.76 15.07 714.88 40.12 699.28 2.08

**Figure 3:** FTIR spectra of the pure drug Alprazolam along with the mucoadhesive HRM



**Figure 4:** Cumulative percentage permeation of Alprazolam

**Table 1: Formulation Codes of Nasal Gels**

Formulation code	HRM 5	HRM 10	HRM 15	HPMC 5	HPMC 10	HPMC 15	CP 5	CP 10	CP 15
Alprazolam (% w/v)	5	5	5	5	5	5	5	5	5
Pluronic F127 (% w/v)	16	16	16	16	16	16	16	16	16
FCM (% w/v)	0.5	1.0	1.5	-	-	-	-	-	-
HPMC (% w/v)	-	-	-	0.5	1.0	1.5	-	-	-
Carbopol 934 (% w/v)	-	-	-	-	-	-	0.5	1.0	1.5

HRM-*Hibiscus rosa mucilage*, HPMC-hydroxypropyl methyl cellulose, CP-Carbopol 934.

**Table 2: Values of gelation Temperature and Mechanical properties of the Gels**

Formulation code	Gelation temperature (°C)	Hardness (g)	Adhesiveness (g)	Cohesiveness	Mucoadhesive strength (g)
HRM 5	27.20 ± 0.33	141.67 ± 2.08	-2,560.96 ± 12.71	1.07 ± 0.05	67.56 ± 0.34
HRM 5 (E)	26.50 ± 0.21	-	-	-	-
HRM 10	24.60 ± 0.24	245.67 ± 2.62	-3,628.05 ± 22.87	0.96 ± 0.02	75.25 ± 0.55
HRM 10 (E)	24.20 ± 0.31	-	-	-	-
HRM 15	22.80 ± 0.25	468.11 ± 2.60	-6,644.66 ± 20.29	0.92 ± 0.07	83.75 ± 0.65
HRM 15 (E)	22.40 ± 0.11	-	-	-	-
CP 5	28.50 ± 0.02	135.50 ± 2.91	-2,269.82 ± 17.67	1.08 ± 0.08	44.27 ± 0.33
CP 5 (E)	28.00 ± 0.04	-	-	-	-
CP 10	25.50 ± 0.03	204.00 ± 2.24	-3,628.09 ± 12.26	1.03 ± 0.02	62.08 ± 0.98
CP 10 (E)	25.00 ± 0.05	-	-	-	-
CP 15	23.30 ± 0.04	335.00 ± 1.85	-5,508.83 ± 9.90	0.93 ± 0.02	73.26 ± 0.53
CP 15 (E)	22.80 ± 0.06	-	-	-	-
HPMC 5	29.50 ± 0.05	118.00 ± 0.82	-2,158.04 ± 17.67	1.04 ± 0.14	10.23 ± 0.95
HPMC 5 (E)	29.00 ± 0.03	-	-	-	-
HPMC 10	27.80 ± 0.11	175.67 ± 3.62	-3,084.31 ± 25.92	1.03 ± 0.03	30.67 ± 1.15
HPMC 10 (E)	27.20 ± 0.05	-	-	-	-
HPMC 15	25.20 ± 0.15	295.00 ± 2.85	-4,438.49 ± 30.26	0.96 ± 0.04	43.67 ± 1.13
HPMC 15 (E)	24.60 ± 0.04	-	-	-	-

Data shows mean ± SD (n=6), E- With enhancer.

**Table 3: Interday and Intraday Accuracy and Precision Data for Quantitation of Alprazolam in Rabbit plasma**

Amount of drug added (ng/ml)	Concentration in plasma (ng/ml)		Accuracy		Precision (% CV)	
	Intraday	Interday	Intraday	Interday	Intraday	Interday
10	9.91 ± 0.24	9.55 ± 0.38	99.10	95.50	2.42	3.97
200	199.05 ± 2.15	195.99 ± 4.51	99.53	98.46	1.08	2.30
600	599.30 ± 2.65	596.08 ± 6.58	99.88	99.34	0.44	1.10
1,000	998.05 ± 3.95	994.09 ± 5.01	99.80	99.41	0.39	0.50

Data represent mean ± SD (n=6). CV-Coefficient of variation.

**Table 4: Comparative Pharmacokinetic Parameters of Alprazolam Following Administration of Intravenous and Nasal *In situ* Gels in Rabbits (dose=2 mg/kg)**

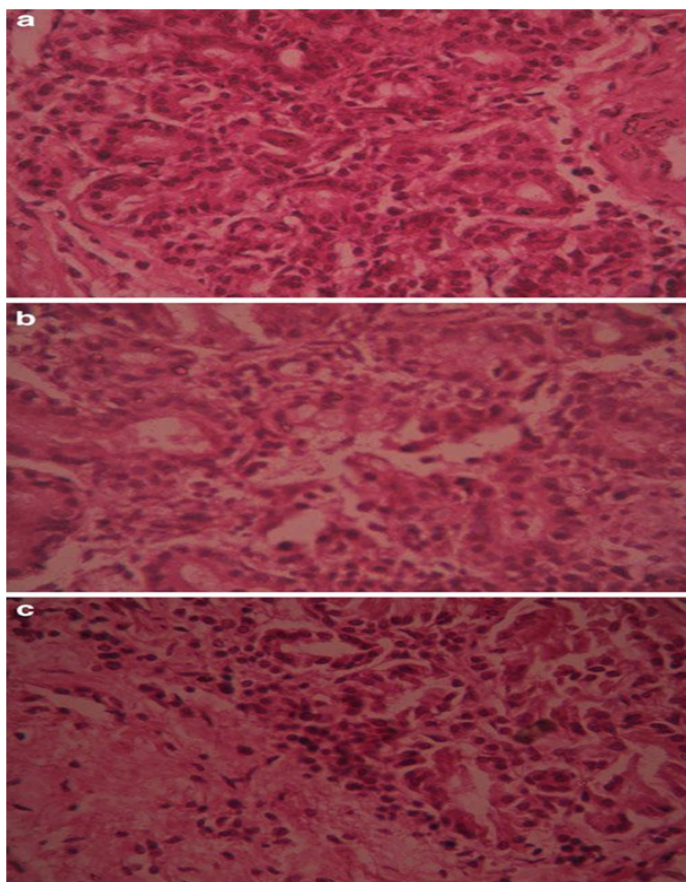
Parameter	Intravenous solution	HRM gel	HPMC gel	Carbopol gel
C <sub>max</sub> (ng/ml)	73.64 ± 5.23	199.59 ± 9.28	151.47 ± 7.22	164.07 ± 6.85
t <sub>max</sub> (min)	0.00	20.00 ± 5.11	60.00 ± 5.68	45.00 ± 3.66
AUC <sub>(0-t)</sub> (min(ng/ml))	32,671.50 ± 90.56	25,311.30 ± 115.05	18,034.30 ± 101.45	22,074.10 ± 96.72
AUC <sub>(t-∞)</sub> (min(ng/ml))	865.87 ± 37.25	6,907.46 ± 55.41	1,191.83 ± 45.26	3,684.71 ± 60.58
AUC <sub>total</sub> (min(ng/ml))	33,537.40 ± 101.25	32,218.80 ± 86.25	19,226.10 ± 97.25	25,758.90 ± 95.46
t <sub>1/2</sub> (min)	58.78 ± 6.32	133.52 ± 8.07	68.44 ± 5.28	99.68 ± 4.89
MRT (min)	71.61 ± 5.05	196.20 ± 5.35	130.16 ± 6.58	162.50 ± 7.25
CL (×10 <sup>-5</sup> ) (mg/kg/(ng/ml min))	5.79 ± 6.95	6.02 ± 7.58	10.93 ± 8.15	7.53 ± 5.66

Data represent mean ± SD (n=3).

**Table 5: Values of pH, Swollen volumes, Zeta Potentials and Mucoadhesive Strengths of Mucoadhesive Agents**

Mucoadhesive agents	pH	Swollen volume (cm <sup>3</sup> /g) ± SD (n=6)	Zeta Potential (mV) ± SD (n=6)	Mucoadhesive strength (g) ± SD (n=6)				
				5 min	10 min	15 min	20 min	30 min
HRM	7.02	8.04 ± 0.33	-20.54 ± 0.05	5.50 ± 0.39	5.86 ± 0.36	6.46 ± 0.44	6.89 ± 0.52	7.52 ± 0.61
HPMC	6.73	7.85 ± 0.95	0.00 ± 0.00	3.63 ± 0.15	4.15 ± 0.09	4.44 ± 0.06	4.76 ± 0.03	5.25 ± 0.04
CP	3.27	15.75 ± 0.65	-23.64 ± 0.03	4.24 ± 0.08	4.72 ± 0.05	5.20 ± 0.06	5.36 ± 0.08	5.74 ± 0.09

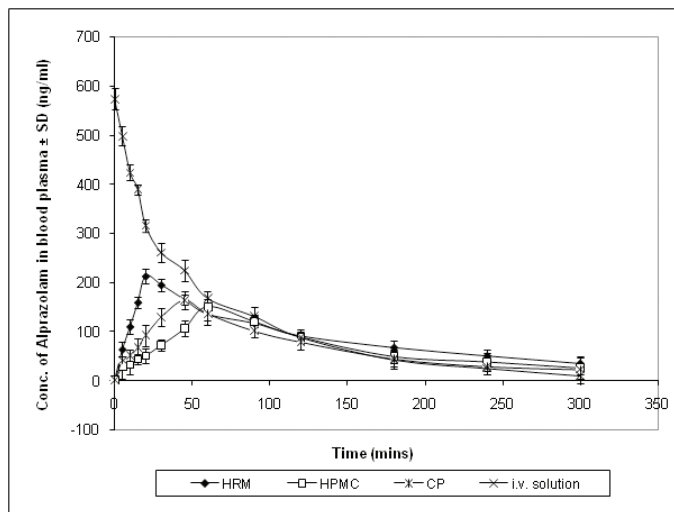
HRM-*Hibiscus rosa* mucilage, HPMC-hydroxypropylmethyl cellulose, CP-Carbopol 934, SD-standard deviation.



**Figure 5: Histological photomicrograph of eosin-haematoxylin stained nasal mucosa (40×0.55 μ PHP magnification) a Control-mucosa without application of nasal gel. b Nasal mucosa after application of HRM nasal gel. c Nasal mucosa after application of HRM nasal gel containing 0.5% sodium taurocholate**

of each sample were performed at 34 ± 1°C. Data collection and calculation were done by Texture pro software, version 2.1.<sup>14</sup> Datas are depicted in Table 2.

The analysis was done using a 5-kg load cell instrument. Validation of the method was done by calibrating the instrument for distance and load cell. While calibrating the instrument in terms of distance, the force required to travel a particular distance (15 mm) was measured. For calibrating the instrument for load cell, distance travelled by the probe to attain a particular force (5 kg) was determined. The other parameters like probe speed of 30 mm/min before and after the test and trigger force of 4 g were kept constant. In both the cases, net work done was calculated from area under the curve. Intraday and interday variability



**Figure 6: Plasma concentration-time profiles of alprazolam after administration of intravenous solution and the *in situ* nasal gels in rabbits**

Data represent mean ± SD (n=3).

were determined, and for both, the methods, accuracy and precision were determined by performing the experiment on six successive days and six times in a day.<sup>14</sup>

### Evaluation of mucoadhesive strength

Mucoadhesive strength of each formulation was determined by measuring force required to detach nasal mucous membrane from the formulation using the same texture analyser. Freshly excised goat nasal membrane was attached to the upper probe of the instrument, and fixed amount of gel was kept below that. The upper probe was then lowered at a speed of 10 mm/min to touch the surface of the gel. A force of 0.1 N was applied for 5 min to ensure intimate contact between the membrane and the gel.<sup>8,14,15,20</sup> The surface area of exposed mucous membrane was 1.13 cm<sup>2</sup>.

### *In vitro* permeation study

*In vitro* permeation study was conducted using a Franz diffusion cell containing 100 ml of phosphate buffer (pH 6, 0.1 M) using an excised goat nasal mucosa. The goat nose was obtained from local slaughterhouse within 15 min after the goat was sacrificed. After removing the skin, the nose was stored on ice cold phosphate buffer (pH 7.4, 0.05 M). The septum was fully exposed, and nasal mucosa was carefully removed using forceps and surgical scissors. The mucosal tissues were immediately immersed in Ringer's solution. The freshly excised nasal mucosa was mounted on the diffusion cell, and 100 μl of gel containing 5 mg alprazolam was placed on it. Throughout the study, the buffer solution in the chamber was maintained at 34 ± 1°C by connecting the Franz diffusion cell with water bath. At predetermined time intervals, 1 ml of the samples was withdrawn at a time and replenished with an equal

amount of phosphate buffer. The samples were diluted appropriately and filtered. Absorbances of the samples were measured spectrophotometrically at 218 nm using Jasco V-550 UV/Vis Spectrophotometer (Tokyo, Japan), taking phosphate buffer (pH 6) as the blank.<sup>13,15,23</sup>

The amount of drug permeated was calculated from the calibration curve (linearity range=2.4 to 120 µg/ml;  $r^2=0.9996$ ). The mean cumulative percentage of drug permeated with enhancer was plotted against time (Figure 4). Permeation area was 2.54 cm<sup>2</sup>.

### Histological evaluation of nasal mucosa

Histological study of excised nasal mucosa was conducted by comparing a fresh mucosa with the mucosae used for 5 h in the *in vitro* permeation study to detect if any significant histological change has occurred during the experiment. After permeation study, nasal mucosa was cleared off the gel, sectioned with a rotary microtome (Model 1090 A, The Western Electric and Scientific Works, India) and fixed in 10% formalin solution. The sectioned tissue was then stained with hematoxylin and eosin. Another normal mucosa was taken as a control and treated similarly. Tissue sections were observed under Olympus CKX41 optical microscope (Olympus Optical Co. Ltd., Tokyo, Japan).<sup>16,22</sup> The photographs were taken with an Olympus-SC 35 camera (Figure 5).

### *In vivo* drug absorption study

*In vivo* studies were conducted on 12 New Zealand albino male rabbits weighing between 1.5 and 2 kg. Based on texture profile analysis and drug release pattern of the formulations, HRM5, HPMC5 and CP5 containing 0.5% sodium taurocholate were selected for *in vivo* study. The animals were kept in individual metal cages and maintained at 25°C for 10 days prior to the experiment. They were provided with standard diet and water ad libitum. The approval of the Institutional Animal Ethics Committee was obtained before starting the study, and it was conducted according to the institutional guidelines. The rabbits were kept in fasting condition for 24 h before the experiment commenced. The rabbits were grouped into four (group I, II, III and IV), each group containing three rabbits. Group I was administered intravenous bolus injection of alprazolam. Groups II, III and IV were administered nasal gels of HRM, HPMC and Carbopol 934. Single dose of alprazolam (2 mg/kg body weight of rabbit) was administered intravenously to compare the pharmacokinetic parameters. No anaesthesia was used for the intravenous study. Alprazolam was injected through cannulated marginal ear vein. Two millilitres of blood samples each time were collected before intravenous injection and then at 5-, 10-, 15-, 20-, 30-, 45-, 60-, 90-, 120-, 180-, 240- and 300-min intervals in eppendorfs containing heparin sodium (100 U/ml). In case of nasal gels, the dose of alprazolam that was administered was also 2 mg/kg body weight of rabbit. Before application of gel, each rabbit was lightly anaesthetised by intramuscular injection of a mixture of xylazine (3 mg/kg) and ketamine (35 mg/kg). Following induction of anaesthesia, a catheter was fixed into the central artery for blood sample collection. About 2 ml blood sample was collected prior to the application of gel and then at 5-, 10-, 15-, 20-, 30-, 45-, 60-, 90-, 120-, 180-, 240- and 300-min intervals in eppendorfs as above. After every 20 min, each rabbit was administered one-third of the initial dose of xylazine and ketamine intramuscularly to maintain a light plane of anaesthesia. The blood samples were kept on ice and centrifuged at 3,000 rpm for 10 min immediately after collection to separate the plasma and stored at -20°C until the time of analysis. Immediately after each blood sample collection, the catheter was flushed with 0.2 ml of a 10% (v/v) heparin/normal saline solution to prevent blood clotting inside the catheter.

All animal experiments are performed as per the standard norms and guidelines of the Animal Ethics Committee of Himalayan Pharmacy

Institute, Sikkim Central University, Tadong, Sikkim as recognised by the Committee for the Purpose of Control and Supervision on Experiments on Animals, India.<sup>17,18</sup>

### High performance liquid chromatographic (HPLC) analysis

Reverse phase HPLC was used to quantitate alprazolam in plasma samples. Alprazolam was extracted with 3 ml of cyclohexane/diethyl ether (3:7) after the addition of 10 µl of 2% sodium hydroxide. The organic phase was removed and evaporated to dryness under nitrogen, and the residue was reconstituted in 200 µl of the mobile phase (10 mM phosphate buffer (pH 6.0)/acetonitrile, 80:20). Of the mixture, 100 µl was injected for chromatographic analysis. The mobile phase consisted of phosphate buffer/acetonitrile (80:20) v/v. The mobile phase was delivered into the HPLC apparatus at a flow rate of 1 ml/min (isocratic pump, Model LC-10AS, Jasco, Japan). The detection wavelength was 218 nm (ultraviolet variable wave-length detector, Model SPD-10A), and a C18 column was used. All assays were performed at ambient temperature.<sup>2,18</sup> Calibration curve of alprazolam prepared in rabbit plasma was found to be linear over the concentration range of 10–1,000 ng/ml ( $r^2=0.9999$ ). The experiment was repeated six times a day and for six consecutive days. Interday and intraday accuracy and precision values are displayed in Table 3.

### Pharmacokinetic analysis

Pharmacokinetic parameters like peak plasma concentration ( $C_{max}$ ), time to reach peak plasma concentration ( $t_{max}$ ), area under the (concentration–time) curve (AUC), mean residence time (MRT), elimination half-life ( $t_{1/2}$ ) and total body clearance (CL) were calculated following non-compartment model by Kinetic 4.4, PK/PD Analysis, Thermo-electron Corporation. All the parameters were calculated for i.v. bolus injection of alprazolam and *in situ* nasal gels.

Fraction of dose absorbed (F) was calculated by the equation:

$$F = \frac{\text{Dose(i.v.)} \times \text{AUC(nasal)}}{\text{Dose(nasal)} \times \text{AUC(i.v.)}}$$

where dose<sub>(i.v.)</sub> = dose of alprazolam given as i.v. solution, dose<sub>(nasal)</sub> = dose of alprazolam in nasal gels, AUC<sub>(i.v.)</sub> = AUC after i.v. administration of alprazolam and AUC<sub>(nasal)</sub> = AUC after nasal administration of alprazolam. Results are shown in Table 4.

### Statistics

Data were analysed by one-way ANOVA followed by Tukey's HSD test using Vassar stat software (USA).  $p < 0.01$  has been considered to be significant statistically.<sup>10</sup>

## RESULTS

Chemical identification of mucoadhesive agent isolated from *H. rosa* revealed that it consisted of carbohydrates, reducing sugar and protein but no tannin. Treatment of the material with ruthenium red confirmed that it was mucilage and not gum.

(Table 5) depicts the values of pH and swollen volumes of the mucoadhesive agents. pH of HRM was found to be 7.04 which is within the pH range of the nasal cavity (5.5–7.5) and also comparable with the synthetic polymers like HPMC and Carbopol 934 that are widely used in formulation of nasal gels.

Swollen volume of HRM ( $8.04 \pm 0.80$  cm<sup>3</sup>/g) was found to be slightly greater than that of HPMC ( $7.89 \pm 0.95$  cm<sup>3</sup>/g) but significantly less than that of Carbopol 934 ( $15.57 \pm 0.65$  cm<sup>3</sup>/g).

Values of zeta potential also are presented in Table 5. Zeta potential of HRM, HPMC and Carbopol are reported to be  $-20.54 \pm 0.05$ ,  $0.00 \pm 0.00$  and  $-23.64 \pm 0.03$  mV, respectively.

Viscosity plot of 1% aqueous solutions of HRM, HPMC and Carbopol 934 at five different speeds of 10, 20, 30, 60 and 100 rpm (Figure 1) shows that viscosities of 1% aqueous solutions of HPMC, Carbopol 934 and HRM were in the range of 5–12, 6–18 and 8–28 mPa, respectively.

Table 5 depicts the mucoadhesive strengths of 1% solutions of HRM, HPMC and Carbopol 934. HRM was found to exhibit greater mucoadhesive strength compared to the synthetic polymers.

Regarding formulation development, various concentrations of Pluronic in a mixture of propylene glycol/water (45:55) were evaluated from 15% to 18% w/v, prior to selecting the optimum concentration of Pluronic necessary for the present study. Solutions containing 15%, 16%, 17% and 18% w/v of Pluronic had gelation temperatures of 38.5°C, 29.8°C, 24.5°C and 23.8°C, respectively. Only 16% w/v Pluronic F127 in a mixture of propylene glycol/water (45:55) was selected for further investigation. Addition of mucoadhesive agents lowered the gelation temperature. The temperature range of the nasal cavity is within 32–35°C.

Gelation temperatures of the formulations, prepared with or without sodium taurocholate, are displayed in Table 2. The concentration of mucoadhesive agent at which gelation of formulation occurs at the lower range of nasal temperature is found to be optimum. From the Table 2, it is evident that gelation temperatures of gel containing HRM and synthetic polymers were comparable in all possible concentrations. It is observed that addition of sodium taurocholate lowered the gelation temperature in the case of all the formulations. Viscosities of different formulations at different spindle rpm at  $34 \pm 1^\circ\text{C}$  are shown in Figure 1. All the formulations exhibited pseudoplastic behaviour. It was observed that all the HRM gels showed higher viscosity than the gels prepared from synthetic polymers.

Regarding validation of the texture profile analysis method, in terms of distance, interday and intraday accuracy were 97.50% and 98.25%, respectively, while interday and intraday precision were 2.17% and 1.15%, respectively. Regarding validation of the instrument in terms of load cell, interday and intraday accuracy were 96.5% and 98.99%, respectively, whereas interday and intraday precision were 3.15% and 2.74%, respectively.

Texture profile analyses of the gels were studied at  $34 \pm 1^\circ\text{C}$ . The results of texture profile analysis were displayed in Table 4. Minimum hardness was shown by HPMC5 gel, and maximum was shown by HRM15 gel. Adhesiveness of the formulations showed that with the increase in concentration of HRM from 0.5% to 1.5%, adhesiveness increased by 2.6 times (from  $-2,560.96 \pm 12.71$  to  $-6,644.66 \pm 20.29$  g). For HPMC gels, with the increase in concentration from 0.5% to 1.5%, it increased by 2.05 times (from  $-2,158.04 \pm 17.67$  to  $-4,438.49 \pm 30.26$  g). For Carbopol gels, it was enhanced by 2.1 times (from  $-2,269.82 \pm 17.67$  to  $-5,508.83 \pm 9.90$  g). Cohesiveness was found to decrease as the concentration of the mucoadhesive agents increased from 0.5% to 1.5% w/v. Maximum cohesiveness was observed in HPMC 5 gel and minimum in HRM 15 gel. However, the formulations containing sodium taurocholate did not show any change in the values of hardness, adhesiveness and cohesiveness.

Mucoadhesive strengths of the gels were also measured in texture analyzer. Results are displayed in Table 2 which shows that mucoadhesive strength of HRM gels was higher than those prepared with synthetic mucoadhesive polymers and it increased with corresponding increase in concentration of mucoadhesive agent used.

*In vitro* release profiles of alprazolam from various gels were studied in phosphate buffer (pH 6.0). (Figure 4). depicts *in vitro* release profiles of alprazolam from various formulations. Results reveal that drug release

from HRM gels was faster than the synthetic ones. In (Figure 4), it is shown that drug release from gels containing 0.5% sodium taurocholate as enhancer was faster in comparison to gels without enhancer.

Safety of a nasal formulation can be assessed by a comparative histological study of nasal mucosae after the *in vivo* study and normal mucosae (control). The histological micrographs as shown in Figure 5 revealed that no significant alteration of nasal epithelium and/or necrosis occurred. This implies that HRM is as safe as HPMC and Carbopol 934 to be used as a mucoadhesive agent for nasal administration and the concentration of sodium taurocholate used is also safe.

The accuracy and precision values of the HPLC method displayed in Table 4 shows that inter and intraday coefficients of variation were found to be less than 4%, and accuracy varied from 95.50% to 99.88%. The limit of quantification was 10 ng/ml.

Plasma concentration–time profiles of alprazolam after administration of *i.v.* solution and the nasal gels are shown in Figure 6. Pharmacokinetic parameters were displayed in Table 4.  $C_{\text{max}}$  values of *i.v.* injection, HRM, HPMC and CP gels were  $573.64 \pm 5.23$ ,  $199.59 \pm 9.28$ ,  $151.47 \pm 7.22$  and  $164.07 \pm 6.85$  ng/ml, respectively.  $t_{\text{max}}$  values of *i.v.* injection, HRM, HPMC and CP gels were 0.00, 20.00  $\pm$  5.11, 60.00  $\pm$  5.68 and 45.00  $\pm$  3.66 min, respectively.  $t_{1/2}$  values of *i.v.* injection, HRM, HPMC and CP gels were 58.78  $\pm$  6.32, 133.52  $\pm$  8.07, 68.44  $\pm$  5.28 and 99.68  $\pm$  4.89 min, respectively.

## DISCUSSION

Chemical identification of mucoadhesive agent isolated from *H. rosa* flowers shows that it consists of reducing sugars, protein and mucilage, but tannin is absent.

The pH of the mucilage isolated from *H. rosa* was found to be consistent with that of the nasal mucosa as shown in Table 5. Hence, HRM can be effectively used in the formulation of nasal mucoadhesive gels.

Swellability is an indispensable property of mucoadhesive polymers. There is a critical degree of swelling that is necessary for optimum bioadhesion. However, excessive swelling converts the gel network to a slippery surface that causes a decrease in bioadhesion. From Table 5, it is found that swollen volume of Carbopol 934 was greater than HPMC, but Carbopol has very high percentage of carboxyl groups which form hydrogen bonding with sugar residues in oligosaccharide chains in the mucus membrane leading to formation of a strong network between the polymer and mucus membrane. As a result, Carbopol 934 is found to provide better mucoadhesive property than HPMC. However, it is observed that HRM exhibited moderate swellability compared to HPMC and Carbopol suggesting better mucoadhesion.

The negative values of zeta potential displayed in Table 3 indicate that in colloidal condition incidences of repulsive forces seem to prevail between particles of mucoadhesive agents in aqueous solution, which helps the mucilage preparation to settle down quickly.

Viscosity of HRM was found to be higher than that of HPMC and Carbopol 934 at the same concentration as is depicted in Figure 1. Hence, HRM can be considered to be a better gel forming agent than synthetic polymers.

Determination of mucoadhesive strength showed that HRM has better bioadhesive strength than synthetic polymers. From Table 2, it is observed that the greater the contact time, the higher is the force of adhesion and, hence, the greater the mucoadhesive strength. It can be concluded that the isolated mucilage with higher viscosity and moderate swellability has better mucoadhesive property compared to synthetic polymers. This may be due to presence of certain functional groups in the mucilage that were able to establish a more intimate contact with mucin of the mucosa.

During preformulation study, FTIR (Fourier Transform Infrared) spectroscopy patterns of the pure drug Alprazolam alone and in combination with mucoadhesives (HRM) were observed. IR spectra are given in Figure 2 and Figure 3. Major frequencies of functional groups of pure drug remained unchanged in presence of mucoadhesives. Hence chances of less possible major interaction between the drug and the polymers can be concluded.

The aim of the present study was to prepare nasal *in situ* gels within a temperature range of 25-34°C so that the preparation is liquid at room temperature for easy administration and providing accurate dose of the drug but forms a firm gel at the temperature of the nasal cavity. The lowering of gelation temperature with addition of mucoadhesive agents attributed to the fact that the mucoadhesive agents formed hydrogen bonds with Pluronic to promote dehydration leading to increased entanglement of adjacent molecules and thereby enhancing micellar association.

Mucoadhesive agents contributed to increased viscosity of the formulations which is evident from (Figure 1). Viscosity of the gels was enhanced with increase in concentration of the mucoadhesive agents.

The validation confirms that the texture analyzer is suitable for use and produces results that are accurate and precise.

Texture profile analysis was used to evaluate the mechanical properties of the gels, like hardness, adhesiveness and cohesiveness. Hardness is the maximum force during first compression cycle. It has been found to increase with the increase in the amount of mucoadhesive agent. Adhesiveness is considered as an important parameter since it determines proper gel contact and retention at mucosal surface thereby leading to enhanced bioavailability of the drug. Results show that adhesiveness was augmented significantly with increase in amount of the mucoadhesive agent. Cohesiveness is the ratio of area of the curve during second compression to that during first compression of the gel with the probe. It was observed to be reduced with increase in amount of mucoadhesive agents. With increase in amount of dispersed solids, that is the mucoadhesive agents, semisolid nature of the gels increased, which caused the gel to become less coherent.

Results show that 5-min contact time was enough to achieve optimum mucoadhesive strength. However, increase in contact time did not affect mucoadhesive strength. It is expected that presence of numerous carboxyl and hydroxyl groups of carbohydrates present in HRM contributed to enhanced mucoadhesive strength of the HRM gels than those prepared from synthetic polymers. This shows that HRM has better mucoadhesive properties than HPMC and Carbopol 934.

Drug release pattern from *in situ* gels showed that with the increase in concentration of mucoadhesive agents, drug release was retarded, but Carbopol gels were exceptional. Since Carbopol itself has a penetration-enhancing effect, hence, drug release was enhanced with increase in amount of Carbopol. However, drug release from gels containing enhancer was relatively faster than the gels containing no enhancer.

Histopathological study of the nasal mucosae suggests that HRM nasal gel prepared with enhancer was safe for nasal administration after 5 h permeation study when compared with the normal mucosa. Bile salts like sodium taurocholate enhance paracellular transport of drugs by opening the tight junctions of the nasal mucosa owing to bile salt-induced calcium complexation. However, this change is temporary and reversible. Since no necrosis or removal of epithelial layer was observed, hence, it can be inferred that no major histological change has occurred.

Higher plasma concentration of alprazolam is observed in case of HRM gels in comparison to HPMC and Carbopol 934 gels (Figure 6), and accordingly, absolute bioavailabilities of alprazolam from HRM, HPMC and Carbopol gels were reported to be  $96.07 \pm 3.45\%$ ,  $57.33 \pm 5.85\%$  and  $76.81 \pm 5.09\%$ , respectively.

Mucilages obtained from *H. Rosa* flowers were collected from plants of different gardens. They, however, exhibited similar basic physical and chemical characteristics, and it is observed that there were certain variations in results of viscosity and mucoadhesive strength, but the deviations were within 10%. However, when gels were prepared from such mucilages, the concentration of HRM was very small (0.5–1.5% w/v) compared to that of the Pluronic F127 (16% w/v), owing to which the results of viscosity, texture analysis and mucoadhesive strengths showed minimal deviations.

## CONCLUSION

The mucilage extracted from *H. Rosa* flowers was found to possess better mucoadhesive properties than the synthetic polymers HPMC and Carbopol 934 that are widely used in preparation of nasal gels. The *in situ* nasal gels prepared from HRM showed better rheological, mechanical and mucoadhesive properties than the gels prepared from synthetic polymers. Histopathological study confirmed that this natural mucilage had no adverse impact on the structural integrity of the nasal mucosa. Further, *in vivo* study proves that bioavailability of alprazolam from HRM gels was far better than those prepared from HPMC and Carbopol 934 gels. Results obtained from the present study show that nasal gel prepared from 0.5% w/v HRM containing 0.5% sodium taurocholate as enhancer gave reproducible results. This type of nasal *in situ* gel of alprazolam prepared from natural mucilage extracted from *H. Rosa* flowers will undoubtedly provide a cheaper dosage form of alprazolam and will thus add a new dimension in this regard.

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## CONFLICT OF INTEREST

The author declare no conflict of interest.

## ABBREVIATIONS USED

**GAD:** Generalized anxiety disorder; **SAD:** Social anxiety disorder; **CT:** Compressed tablet; **XR:** Extended release, **HPMC:** Hydroxypropyl methyl cellulose, **C-934:** Carbopol 934, **HRM:** *Hibiscus rosa* mucilage, **FCM:** Ficus carica mucilage, **HPLC:** High performance liquid chromatography, **AUC:** Area under curve **MRT:** Mean residence time.



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