DEVELOPMENT OF MUCOADHESIVE GEL OF FLUCONAZOLE FOR VAGINAL CANDIDIASIS

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ABSTRACT
Fluconazole is a triazole antifungal, widely prescribed for the treatment of vaginal Candidiasis. Topical application of FZ localizes the drug in tissue and reduces the side effects as compared to systemic administration. In contrast to the lower retention of conventional formulations in vagina, mucoadhesive formulations would prolong the retention time resulting in effective topical therapy. The aim of the present study was to develop a mucoadhesive vaginal gel of Fluconazole for treatment of Candidiasis. Various mucoadhesive polymers including, Carbopol 974, Carbopol 980, Polycarbophil, Hydroxy propyl methyl cellulose K 100 M, Hydroxy propyl cellulose, Hydroxy ethyl cellulose (Natrosol 250) and Xanthan gum were evaluated for their potential as gel matrices in vaginal drug delivery. The gel formulations were prepared at different concentrations of polymers and evaluated for appearance, pH, spreadability, extrudability, viscosity, effect of dilution on viscosity, ex vivo mucoadhesion and in vitro drug release. The optimized formulations were evaluated for in vitro antifungal activity, in vivo vaginal irritation and stability. The gel formulations prepared using polycarbophil (2 %w/w) and hydroxyl ethyl cellulose (6 % w/w) were optimized since these formulations were found to be clear, easily spreadable and extrudable, viscous with shear thinning property, resistant to dilution by acidic pH of vagina, and revealed excellent mucoadhesive behaviour. The developed formulations exhibited sustained drug release pattern with diffusion as the mechanism of drug release. The formulations indicated in vitro antifungal activity against Candida albicans and did not show any sign of vaginal irritation, when tested in mice vagina. Thus, the study demonstrated the potential of developed mucoadhesive gel of Fluconazole in treatment of vaginal Candidiasis.

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INTRODUCTION

Vaginitis is a very common gynaecological disease affecting women of reproductive age [1]. It has been reported that 70 to 75 % women are affected by vulvovaginal Candidiasis which is the second most prevalent infection [2-4]. The effective treatment includes immediate topical treatment with azole antifungal for relief from symptoms of vaginal inflammation. Recurrent infections may need prolonged topical treatment with antifungal agents along with systemic therapy. Topical treatment with antifungal agents is intended to provide rapid drug release, increased tissue levels and lower systemic exposure [2].

Fluconazole (FZ) is a triazole antifungal agent with broad spectrum of activity against many fungal species including Candida albicans, mainly responsible for Vaginal Candidiasis [5]. FZ interfere with ergosterol synthesis which is a main component maintaining the fluidity and integrity of fungal cell membrane. It shows lower mucosal toxicity and excellent safety profile, hence remained as one of the most prescribed drug for treatment of vaginal Candidiasis [6]. Topical application of FZ has been reported to reduce the side effects as compared to its oral administration [7].

The topical vaginal drug delivery systems include a large variety of pharmaceutical dosage forms such as semi-solids, tablets, capsules, pessaries, liquid preparations, vaginal films, vaginal rings, foams, and tampons. Most preferred conventional dosage forms, for treatment of infection include semi-solid preparations like creams, ointments, and gels. These semisolid preparations may suffer from the limitation of messiness, difficulty in spreading and leakage from the vaginal cavity due to self cleansing action of vagina [8].

These limitations can be overcome by formulating a mucoadhesive gel with good gel strength and resistant to dilutions. Mucoadhesive polymers are able to swell rapidly when placed in aqueous environment. The polymer chains interpenetrate across the mucus layer of vaginal mucosa which results in adhesion, thus the formulation is retained at the biological surface for a longer time and the drug is released in a controlled manner close to the absorptive membrane, with a consequent enhancement of bioavailability [9, 10]. To date, the most commonly studied mucoadhesive polymers for mucosal applications are synthetic polyacrylates like carbopol (C 974 and C980), polycarbophil (PC) [11], cellulose polymers like hydroxy ethyl cellulose (HEC) [12, 13], hydroxy propyl cellulose (HPC) and hydroxy propyl methyl cellulose (HPMC) [14]. The mucoadhesive gels of these polymers have been studied for vaginal lubrication, for delivery of anti-infectives [15] and as vehicle for microemulsions [16].

Considering the unique anatomy and physiology of vagina, for effective treatment of infection, an ideal mucoadhesive gel should easily spread in the vaginal cavity and cover the entire infected mucosa. At the same time, it should retain its viscosity in presence of vaginal fluids and resist the dilution due to stress applied by squeezing action of elastic vaginal walls which otherwise can cause leakage through vaginal cavity [8]. Similarly, the formulation should be stable and robust in the vaginal acidic pH (4.2-5.5) range, preventing faster erosion of gel matrix. Thus, it is required to evaluate the reported mucoadhesive polymers for their potential as gel matrices for vaginal drug delivery.

The objective of the present study was development of mucoadhesive vaginal gel for FZ for treatment of Vaginal Candidiasis. For this work, we selected Carbopol 974 (C974), Carbopol 980 (C 980), Polycarbophil (PC), hydroxypropyl methyl cellulose K 100 M (HPMC), hydroxyethyl cellulose (HEC), hydroxypropyl cellulose (HPC) and Xanthan gum (XG) as gelling polymers. Various mucoadhesive gel formulations of FZ were prepared using these polymers and comparative evaluation of gel formulations was done for various in vitro characteristics, viz. appearance, pH, spreadability, extrudability, viscosity, ex vivo mucoadhesion and in vitro drug release. The best suitable formulations were optimized on the basis of these in vitro characteristics and the optimized formulations were evaluated for in vitro antifungal activity and in vivo vaginal irritation in mice.

MATERIALS

Fluconazole was kindly supplied by Zim laboratories, Nagpur. Carbopol 974 (C974), Carbopol 980 (C980), Polycarbophil (PC) were received from Lubrizol, Mumbai. Klucel HF (Hydroxy propyl cellulose, HPC) and hydroxyethyl cellulose (Natrosol 250, HEC) by Aqualon USA was supplied by Signet chemical corporation, Mumbai. Hydroxypropyl methyl cellulose K 100 M (HPMC) was received from Colorcon Asia, Mumbai. Xanthan gum (XG) was purchased from Himedia, Mumbai, India.

METHODS

Formulation of mucoadhesive gel of FZ

Mucoadhesive vaginal gel formulations of FZ were prepared using gelling agents at different concentrations as shown in table 1. The cold dispersion method [17] was used for the preparation of gel formulations with gelling agents, C974 P, C980, PC, HPC and xanthan gum. In this method, to the previously prepared solution of benzalkonium chloride, glycerine and water, a gelling agent was dispersed by means of a mechanical stirrer (Remi, RQ/226, India). The stirring was continued for 1 hour at slow speed (50 rpm) to obtain the viscous gel. Weighed quantity of FZ was added to propylene glycol and dissolved using ultrasonication for 15 min. The drug solution was added dropwise with stirring to the previously formed viscous gel and stirring was continued further for 15 min to ensure proper uniform mixing of drug within gel. In case of Carbopol and PC gels, gel consistency was obtained after addition of triethanolamine to adjust the pH in the range of 6.0 to 6.5.

For the gel formulations of HPMC and HEC, hot dispersion method was used. The previously mixed solution of glycerine, water and benzalkonium chloride was heated to 80 °C. Weighed amount of gelling agent was slowly added to this solution maintained at 80 °C. The dispersion was continuously stirred by mechanical stirrer and slowly cooled to room temperature to obtain gel formulation. Weighed amount of FZ was dissolved in propylene glycol and this drug solution was added to the previously formed gel and stirred for 15 min to ensure the uniform distribution of drug within the gel. Table 1 indicates the general formula for preparation of FZ gel using various gelling agents.
Evaluation of Fluconazole gel

Appearance

All the gel formulations were examined for clarity and general appearance. Formulations were graded for clarity as, turbid (-), slightly turbid (+), clear and transparent (++).

pH

pH of the gel formulations was measured using pH meter (Toshniwal, CL54, India) previously calibrated before each use with standard buffer solutions.

Drug content

FZ gel (1 g) was dissolved in 100 ml of citrate buffer pH 4.0. The resultant dispersion was ultrasonicated and shaken for 1 hour and then filtered. The absorbance of the filtrate was measured at 260 nm by UV spectrophotometer (Shimadzu, UV-1700, Japan) using citrate buffer pH 4.0 as blank. The percent drug content of the gel formulation was calculated using calibration curve of FZ in citrate buffer pH 4.0.

Spreadability [18]

The spreadability of the gel was determined using a fabricated spreadability apparatus. The apparatus consisted of two glass slides (7.5 × 2.5 cm), one of which was fixed onto the wooden board and the other was movable, tied to a thread which passed over a pulley, carrying weight. About 0.5 gm of gel sample was placed between the two glass slides. 100 gm weight was allowed to rest on the upper slide for 1 to 2 minutes so as to allow expulsion of the entrapped air between the slides and hence provide a uniform film of the gel. The weight was removed and the top slide was subjected to a pull of 20 gm. The time (sec) taken by the top slide to travel a previously marked distance (6.5 cm) was noted. Spreadability was then calculated using the following formula:

\[ S = \frac{M \times L}{T} \]

Where, \( S \) = is the spreadability, \( M \) = is the weight tied to the upper slide, \( L \) = is the length moved by the glass slide and \( T \) = represents the time taken to separate the slide completely from each other. The measurements were taken in triplicate and the average values were reported. The method gave an idea of relative spreadability of gel products with different polymers.

Extrudability

Extrudability of the gels was evaluated on the basis of the gel extruded from a vertically assembled gel syringe type applicator, on application of weight. The fixed weight (300 g) was applied on the piston of applicator and the time required to extrude gel to a distance of 0.5 cm was noted. The measurement of time required for extrusion of each formulation was done in triplicate and the average values were noted.

Viscosity [19]

The viscosity of the gel formulations was determined using programmable Brookfield Viscometer (Brookfield Viscometer, RVDV pro II, USA) at a temperature of 25.0 ± 0.5 °C with t bar spindle and helipath adjustment. The viscosity of gel samples was noted at different speed of rotations of spindles changed from 0.5 RPM to 100 RPM.

Viscosity after dilution [20]

Generally, 2-5 g of gel formulation is applied by vaginal route and the volume of ambient fluid present in the vagina (vaginal fluid transudate and mucus) is approximately 0.5–0.75 mL [23]. To simulate the dilution of formulation that might occur after application, 0.25 mL of citrate phosphate buffer per g of gel was layered on formulations and the formulations were equilibrated at 37 °C for 30 min. The viscosity of formulations was measured using Brookfield Viscometer with t bar spindle at 20 RPM.

In-vitro drug release [21]

The drug release studies from gel formulations were performed using dialysis bag method. The gel formulations (1 to 1.5 g) were placed in previously soaked dialysis tube (mol.wt. cutoff-11000 Da) and both the ends of tube were tied. This tube was further tied to the basket rod of USP dissolution apparatus Type I (Veego, DT 60, India). The assembly was inserted to dissolution medium containing 100 ml of 5.2 pH citrate buffer medium at 37 ± 0.5 °C. The dissolution was conducted with stirring speed of 100 rpm for 8 h. The samples (5 ml) were withdrawn at an interval of 1 hr, filtered through Whatman filter paper and analyzed for the content of FZ by UV- spectrophotometer at 260 nm. An equivalent volume of 5ml of fresh dissolution medium was added to compensate for the loss due to sampling.

Drug release kinetics

The cumulative drug release data obtained from dissolution studies were evaluated by using PCP Disso software (PCP Disso, V 3, India). The data was fitted to various dissolution models including zero order, first order, Higuchi matrix, Korsemayer Peppas to find out the mechanism of drug release.
Mucoadhesion [22]

The mucoadhesive strength of gel formulations was measured using female goat vaginal mucosa on modified balance assembly. The vaginal mucosa of newly sacrificed adult goat was collected freshly from local slaughter house. It was carefully separated from underlying tissues, washed 2-3 times with water and was cleaned with solution of Gentamycin injection (0.5% w/v). The mucosa was stored at 2-8 °C in saline solution till further use. The modified balance consisted of a lever, on which was mounted a stainless steel pan on one arm and other arm was fitted to a glass vial with stainless steel wire. At the base of this vial a double sided adhesive tape was placed on which the sample of gel was affixed. Another glass vial was fixed to wooden board in inverted position using double sided adhesive tape. The excised vaginal mucosa was fitted on its base. The distance between two vials was adjusted in such a way that the gel sample remained adhered to mucosal membrane. Sufficient pressure was applied on both of the vials for 10 seconds to allow proper adhesion of gel to mucosa. A constant weight was added to the pan connected to the other arm of lever, which pulled the gel away from the mucosa. The weight required for detaching the two vials and hence the gel sample from the mucosal membrane was noted. The force of adhesion was calculated using the following equation.

\[
\text{Force of adhesion (dyne/cm}^2\text{)} = \frac{\text{Biodehesive strength(g)} \times \text{Gravitational Force (cm)} \text{ }} {\text{Area of the mucosal surface exposed (cm}^2\text{)}}
\]

In vitro Antifungal activity

The antifungal activity of the optimized gel formulations was determined by agar well diffusion method. The sabouraud’s dextrose agar plates were prepared, sterilized and swabbed with previously standarized (10^6 CFU/ml) culture of Candida albicans. The wells (10 mm) were made in agar with the help of bores. The gel formulations (FG9 and FG15) were added to the well with the help of syringe. Placebo gels were added as control and marketed gel was taken as control. The plates were incubated at 37 °C for 48 h. the diameter of zone of inhibition (mm) was measured and the readings were taken in triplicate in different directions.

Vaginal irritation study [23]

Vaginal irritation study of the optimized formulation was performed using mice as animal model. The study was performed on six mice per group. The study was conducted with prior approval from institutional animal ethical committee as per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India, guidelines. The animals were provided with food and water ad libitum. The test formulation was applied intravaginally to first group of mice and placebo formulation was applied to second group of mice. The gel was administered once daily for 10 days. Animals were humanely killed on day 11, and parts of the cervico-vagina, mid-vagina, and uro-vagina of each animal were fixed in 10% neutral-buffered formalin. Fixed vaginal tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Stained sections were examined by light microscopy. Each of the 3 regions of vagina was scored for epithelial ulceration, leukocyte infiltration, edema, and vascular congestion. The findings were recorded in terms of the numerical scores for each animal on following grades, no irritation (0), minimal (1), mild (2), moderate (3), severe (4).

Stability studies

In order to assess the physical stability of gel formulations, the optimized gel formulations were placed in wide mouth stoppered glass bottles and kept in the stability chamber (Remi, CHM 12 S, India) at 30±2 °C and 65%±5% RH (Conditions of long term stability study for zone IV). After 90 days, formulations were evaluated for appearance, drug content, viscosity at 20 rpm and drug release.

RESULT AND DISCUSSION

Mucodhesive polymers like carbopol (974 and 980), polycarbophil, HPMC, HPC, HEC and XG were selected to prepare various gel formulations of FZ. The polymers are reported in the literature for drug delivery to mucosa. Gel was selected as dosage form considering its higher mucoadhesion, reduced irritation and possibility of application for effective treatment of Candidiasis. The evaluation of gel was done and results are reported in table 2.

Appearance, clarity and pH

All the prepared gel formulations were found to be clear and transparent except for XG gel which was found to be translucent. pH of all the formulations was found to be in the range of 5.6 to 6.5 (table 2) which is acceptable for administration to vaginal cavity (pH range 4.0 to 5.5). For Carbopol and PC formulations, pH of the formulations was adjusted to 6.5 during gel preparation since gel formation of carbopol depends on ionization of its acrylic acid backbone. Carbopol is anionic polymer with pKa 6.0 which undergoes ionization at pH more than 6.0. After addition of basic solution (triethanolamine), complete dissociation and swelling of polymer chain occur which leads to mutual repulsion of these chains resulting into gel formation [24].

Drug content

The FZ content of gel formulations was found to be in the range of 93.8±1.4 to 99.1±2.3.
Spreadability

Good spreadability of the semisolid formulation is required for the better efficacy of the product. The gels are expected to spread evenly on the infected mucosal surface when applied. Spreadability of formulation depends on contact angle formed by the semisolid formulation to that of mucosa after application. The spreadability of the gel depends on its viscosity and yield stress. The greater the viscosity the longer will be the time taken for spreading. Spreadability of prepared gel formulations was found to be affected by the type as well as percentage of the gelling polymers used (table 2). Based on the type of gelling polymer, the spreadability of gel formulations was found to be in the following order:

\[ \text{XG} > \text{PC} > \text{C 974} > \text{HPMC} > \text{C 980} > \text{HPC} > \text{HEC} \].

The spreadability of carbopol, polycarbophil and xanthan gum gels was found to be more as compared to gel prepared with cellulose derivatives. This might be because of high yield stress value of poly (acrylic acid) than cellulose derivates. High stress values indicate higher viscosity at low shear and lower viscosity at high shear. Spreadability of gel prepared with 2% of Carbopol 980 (F6) was found to be less due to drastic increase in the viscosity of polymer. Spreadability was also found to be decreased with increasing concentration of polymer. Increase in polymer concentration increased the viscosity of gel which led to decrease in the spreadability.

Extrudability

The gel is required to be extrudable from the container during application. Extrudability of gel formulation is related to shear thinning ability of the gelling polymer. Extrudability was measured in terms of time taken for a formulation to be extruded from applicator under the applied weight. C 974, PC and Xanthan gum were found to be easily extrudable as compared to cellulose polymers. HPC, HPMC, HEC and C 980 (at higher concentration, 2% w/w) gels were found to be less extrudable. This might be related to lower yield stress value leading to less shear thinning behaviour making the gel difficult to extrude. At higher concentrations of gelling agents, the extrudability of gels was found to be decreased.

Viscosity & effect of dilution on viscosity

**Fig. 1: Effect of RPM on viscosity of carbopol gel (FG3 to FG9).**

**Fig. 2: Effect of RPM on viscosity of gel (FG11 to FG16).**
Fig. 3: Viscosity of gel formulations at 20 RPM, n=3, Mean±SD.

Fig. 4: Effect of dilution on viscosity of gel formulations, n=3, Mean±SD. (*represents percent reduction in viscosity).

The viscosity of gels is expected to affect their retention & spreading at the vaginal site of application. Higher viscosities would minimize the problem of seepage of product, through vagina (which can cause discomfort to the patient). The viscous gels will also offer slower dissolution & diffusion of actives and hence, prolonged duration of action. All prepared gels exhibited shear thinning behaviour indicating decrease in viscosity with increasing shear rate (fig 1 and 2). This is because, as shear stress is increased, disarranged/random molecules of gelling material are aligned in direction of flow thus, decreasing internal resistance of material & decreasing the viscosity. Viscosity of gels was found to be affected by both type & concentration of polymers. The viscosity of gels (at 20 rpm) prepared with different polymers at their higher concentrations (fig 3) was found to be in the order, 

**HPMC > C 980 > HPC > HEC > C 974 > PC > XG.**

Among all the gel formulations, HPMC gels exhibited highest viscosity (fig 3) (124000 cp at 20 rpm). On the other hand, lowest viscosity was observed with Xanthan gum (32250 cp at 20 rpm). Among the carbopol gels, C 980 gels were found to possess higher viscosity as compared to PC and C 974. This might be the reason for lower extrudability and spreadability of C 980 gels. Viscosity is greatly influenced by degree of crosslinking and physical entanglement of polymeric chains. In presence of water, polymer chains are decoiled, hydrated and form hydrogen bonds with water. In addition, increase in concentration of polymer results in increased interaction or crosslinking between polymer chains. Intermolecular associations such as hydrogen bonding and electrostatic and hydrophobic interactions play a crucial role in the crosslinking of polymeric chain [20]. These polymer-polymer and polymer-water interactions impart viscosity to the gel formulation. There was corresponding increase in viscosity values with increasing concentration of each polymer. Higher concentration of polymers leads to high degree of crosslinking, thus increasing the viscosity of polymer.
After application to vaginal cavity, gel formulation is intended to retain its structure and release the drug in a sustained manner. When the formulation is applied to vaginal cavity, there are chances of formulation getting diluted with vaginal fluid. Thus, it is required to develop a formulation which is insensitive to dilution and be capable of releasing the drug in sustained manner for prolonged period of time. Generally, 2 to 5 g of formulation is applied to vaginal cavity which might get diluted with 0.25 to 0.5 ml of vaginal secretions. Hence, 0.25 ml of citrate buffer (pH 5.2) per g of formulation was added and viscosity of diluted formulation (at 20 rpm) was determined (fig 4). It was observed that there was significant dilution of carbopol and PC gel at lower concentrations (1 %) which lead to significant decrease in the viscosity. Percentage reduction in viscosity for 1 % C 974, C 980 and PC (FG 1, FG 4, FG 7) was 76 %, 52.6% and 51.7% respectively. With increase in concentration of polymer, percentage reduction in viscosity was lowered (fig 4). For 2 % C 974, C 980 and PC (FG3, FG 6 and FG 9), reduction in viscosity was 41.4 %, 40.8 % and 28.8% respectively. The decrease in the viscosity of carbopol gels might be because of presence of unfavourable pH conditions for ionization of Carbopol. Thus, it was inferred that for preparation of robust vaginal gel, higher concentration (2 %) of carbopol and PC was needed. The reduction in the viscosity of HPMC, HPC, HEC and XG (FG 11, FG 13, FG 15 and FG 16) was 18.3, 20.8, 11.5, and 22.4 % respectively. Higher hydrophilicity of HPMC and HEC polymers when compared to HPC and XG, led to increased swelling of these polymer in presence of additional fluid. This might be the reason for lower reduction of their viscosity when compared to HPC and XG. HEC gel (6 % w/w) was found to be less sensitive to the dilution effect (11.5 % reduction in viscosity after dilution) and shear effect (viscosity 32750 cp at 100 rpm).

**In vitro drug release**

![Fig. 5: In vitro FZ release from gel formulations.](image)

*Fig. 5: In vitro FZ release from gel formulations.

*n=3, Mean±SD

For the drug release study from gel matrices, the formulations with higher polymer concentrations were selected, as these were found to be resistant to dilution effect. Fig 5 indicates the drug release profile from selected gel formulations. The drug release from the gel matrices were found to be in the following order;

C 974 > PC > HEC > HPMC > C 980 > HPC

The formulations FG3, FG6, FG9, FG11, FG13 and FG15 were found to release 82.5 ± 1.5, 73.8±1.4, 76.3±0.6, 75.4±1.4, 61.1±1.2 and 77.4±0.6 % of FZ in 8 h of dissolution. The drug release, thus, was found to be dependent on viscosity and erosion of gel matrix in acidic pH (5.2) during dissolution study. The drug release from carbopol matrices was attributed to unfavourable pH conditions for ionization of carbopol, resulting into faster erosion and drug release. HPC formulation was found to be showing sustained drug release due to higher viscosity of matrix.

**Drug release kinetics**

The mechanism of drug release from gel matrix is complex and is based on diffusion of drug through hydrated portion of the gel matrix and erosion of the outer fully hydrated polymer on the surface of the matrix. Due to permeation of excess water into core of gel matrix, there is increase in hydration of gel matrix which provides a diffusion barrier to drug release. As gel matrix becomes fully hydrated, the polymer chains become completely relaxed and can no longer maintain the integrity of the gel leading to disentanglement and erosion from the surface of the gel matrix.
For formulations FG3, FG9 and FG 15, n value in Korsemayer-Peppas model closely approximated 0.45 to 0.5 indicating Fickian diffusion (table 3). The drug release from these gel formulations was mostly governed by diffusion of FZ through gel matrix. For gel formulations, F6, F11 and F13, n value approximated from 0.51 to 0.69 which indicated non-Fickian/anomalous behaviour. Thus, drug release in these formulations was mostly governed by both drug diffusion through gel matrix and disentanglement or erosion of polymer chains.

**Mucoadhesion**

Mucoadhesion involves association of reactive functional groups of polymers with those of mucin lining of the tissue. The adhesive force was measured to indicate the strength of the adhesive bond between the film and the biological tissue, which is considered a function of both the interaction energy between the adhesive and the mucosa and the viscoelastic properties of the formulation. The mucoadhesive strength was found to be increased in following order of polymers used,

\[
\text{PC} > \text{HEC} > \text{C 980} > \text{HPMC} > \text{HPC} > \text{C 974}.
\]

As compared to other polymers, HEC & PC showed better mucoadhesion (fig 6). The force required to detach 2 % PC and 6 % HEC from goat vaginal mucosa was 387 and 419.25 dynes/cm² respectively. High mucoadhesion of PC and HEC was attributed to formation of hydrogen bond between carboxylic acid group of polymer to that of mucin. PC is a homopolymer of acrylic acid crosslinked with divinyl glycol [23]. In addition, PC also permits high degree of entanglement within mucus layer. Mucoadhesion was found to be increased with increase in concentration of polymer (table 2). This is reported to be due to increased entanglement of polymer chains within mucin.

![Fig. 6: Mucoadhesive strength of gel formulations.](image)

\*n=3, Mean±SD

**In vitro antifungal activity**

The *in vitro* antifungal activity of optimized gel formulations (F9 and F15) was compared with marketed gel of FZ (table 4). The zones of inhibition of gel formulations were found to be slightly higher than the marketed gel formulation. The placebo did not show any antifungal activity. Thus, the developed gel was found to possess the required antifungal activity.

**Vaginal irritation study**

The frequent use of vaginal formulations can induce mucosal irritation & damage of epithelium. Therefore, vaginal irritation studies for newly developed vaginal formulations are required. The vaginal irritation studies of optimized gel formulations were conducted on mice for 10 consecutive days. Table 5 shows the numerical score assigned to gel formulations on the basis of histopathological examination. None of the treatment group revealed any sign of irritation viz. epithelial erythema or edema (mean score 0 out of 4). The histopathological evaluation also suggested no erythema, ulceration or leukotriene infiltration (fig 7). These findings suggested the safety & non-toxicity of developed vaginal gel formulations.
Stability study

After 90 days of storage at 30±2 °C and 65±5% RH, the optimized gel formulations, FG9 and FG15 were evaluated for appearance, pH, viscosity at 20 rpm, percent drug content and drug release. All the formulations were found to be stable and there was no significant change in appearance, viscosity, pH, drug content and drug release of the gel formulations. Thus, formulations were found to be stable for the entire period of stability assessment.

Table 1: Composition of FZ gel formulations with different gelling agents.

<table>
<thead>
<tr>
<th>Formulation Code*</th>
<th>C 974P (% w/w)</th>
<th>C 980 (% w/w)</th>
<th>PC (% w/w)</th>
<th>HPMC K100M (% w/w)</th>
<th>HPC (% w/w)</th>
<th>HEC (% w/w)</th>
<th>Xanthan gum (% w/w)</th>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>4.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FG14</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FG15</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FG16</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.0</td>
<td>-</td>
</tr>
</tbody>
</table>

*Each formulation contained 1% w/w of FZ, 10% w/w of propylene glycol, 5% w/w of glycerine, 0.01% w/w of benzalkonium chloride as preservative and water to make up the weight.
Table 2: Evaluation of prepared gel formulations.

<table>
<thead>
<tr>
<th>Code</th>
<th>Appearance and Clarity*</th>
<th>pH</th>
<th>Spreadability (g.cm/s)**</th>
<th>Time for extrusion (sec)**</th>
<th>Drug content (%)**</th>
<th>Mucoadhesion (dynes/cm²)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>FG1</td>
<td>++</td>
<td>6.4</td>
<td>4.4±0.26</td>
<td>18.3±3.5</td>
<td>97.4±2.3</td>
<td>122.5±23.2</td>
</tr>
<tr>
<td>FG2</td>
<td>++</td>
<td>6.3</td>
<td>2.9±0.08</td>
<td>39.3±4.0</td>
<td>93.8±1.4</td>
<td>187.0±23.2</td>
</tr>
<tr>
<td>FG3</td>
<td>++</td>
<td>6.5</td>
<td>1.7±0.09</td>
<td>100.7±5.1</td>
<td>96.8±1.7</td>
<td>251.5±23.2</td>
</tr>
<tr>
<td>FG4</td>
<td>++</td>
<td>6.2</td>
<td>3.8±0.15</td>
<td>22.3±2.5</td>
<td>98.4±1.8</td>
<td>174.1±23.2</td>
</tr>
<tr>
<td>FG5</td>
<td>++</td>
<td>6.3</td>
<td>1.7±0.08</td>
<td>55.7±8.1</td>
<td>98.3±1.0</td>
<td>307.4±16.2</td>
</tr>
<tr>
<td>FG6</td>
<td>++</td>
<td>6.4</td>
<td>1.5±0.1</td>
<td>114.0±5.3</td>
<td>96.0±2.6</td>
<td>337.5±16.2</td>
</tr>
<tr>
<td>FG7</td>
<td>++</td>
<td>6.4</td>
<td>6.2±0.38</td>
<td>10.3±1.5</td>
<td>97.3±1.3</td>
<td>277.3±11.1</td>
</tr>
<tr>
<td>FG8</td>
<td>++</td>
<td>6.3</td>
<td>3.9±0.16</td>
<td>27.7±2.5</td>
<td>95.7±1.3</td>
<td>337.5±16.2</td>
</tr>
<tr>
<td>FG9</td>
<td>++</td>
<td>6.2</td>
<td>2.4±1</td>
<td>92.0±5.3</td>
<td>97.9±0.9</td>
<td>384.8±41.9</td>
</tr>
<tr>
<td>FG10</td>
<td>++</td>
<td>5.8</td>
<td>3.4±0.19</td>
<td>69.7±5.0</td>
<td>94.8±2.2</td>
<td>219.3±11.1</td>
</tr>
<tr>
<td>FG11</td>
<td>++</td>
<td>5.6</td>
<td>1.6±0.13</td>
<td>140.7±3.8</td>
<td>97.4±1.4</td>
<td>290.2±19.3</td>
</tr>
<tr>
<td>FG12</td>
<td>++</td>
<td>5.8</td>
<td>2.7±0.1</td>
<td>72.3±7.5</td>
<td>97.7±0.8</td>
<td>215.0±18.6</td>
</tr>
<tr>
<td>FG13</td>
<td>++</td>
<td>5.9</td>
<td>1.0±0.02</td>
<td>129.0±4.0</td>
<td>96.8±0.6</td>
<td>253.7±26.0</td>
</tr>
<tr>
<td>FG14</td>
<td>++</td>
<td>6.0</td>
<td>2.0±0.1</td>
<td>132.0±5.3</td>
<td>96.3±2.6</td>
<td>273.0±16.2</td>
</tr>
<tr>
<td>FG15</td>
<td>++</td>
<td>5.8</td>
<td>0.8±0.03</td>
<td>171.3±11.0</td>
<td>99.1±2.3</td>
<td>380.5±23.2</td>
</tr>
<tr>
<td>FG16</td>
<td>+</td>
<td>5.7</td>
<td>3.9±0.37</td>
<td>46.0±8.5</td>
<td>97.8±1.3</td>
<td>333.2±22.6</td>
</tr>
</tbody>
</table>

*Appearance and clarity: (-) Turbid, (+) slightly turbid, (++) clear and transparent, **n=3, Mean±SD

Table 3: Release kinetics of FZ gel.

<table>
<thead>
<tr>
<th>Code</th>
<th>Zero order R²</th>
<th>First order R²</th>
<th>Matrix R²</th>
<th>Korsmeyer Peppas R²</th>
<th>Hixson Crowell R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>FG3</td>
<td>0.7344</td>
<td>0.9273</td>
<td>0.9768</td>
<td>32.0</td>
<td>0.9765</td>
</tr>
<tr>
<td>FG6</td>
<td>0.8637</td>
<td>0.9603</td>
<td>0.9790</td>
<td>28.21</td>
<td>0.9780</td>
</tr>
<tr>
<td>FG9</td>
<td>0.7494</td>
<td>0.9319</td>
<td>0.9826</td>
<td>29.74</td>
<td>0.9729</td>
</tr>
<tr>
<td>FG11</td>
<td>0.8084</td>
<td>0.9532</td>
<td>0.9894</td>
<td>29.01</td>
<td>0.9827</td>
</tr>
<tr>
<td>FG13</td>
<td>0.8841</td>
<td>0.9648</td>
<td>0.9964</td>
<td>21.82</td>
<td>0.9950</td>
</tr>
<tr>
<td>FG15</td>
<td>0.7549</td>
<td>0.9478</td>
<td>0.9890</td>
<td>29.36</td>
<td>0.9863</td>
</tr>
</tbody>
</table>

Table 4: In vitro antifungal activity of selected gel formulations.

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Diameter of zone of inhibition (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent control</td>
<td>No zone</td>
</tr>
<tr>
<td>FG9</td>
<td>2.3±0.1</td>
</tr>
<tr>
<td>FG16</td>
<td>2.2±0.2</td>
</tr>
<tr>
<td>FZ marketed gel</td>
<td>2.0±0.1</td>
</tr>
</tbody>
</table>

**Average of three readings± SD.

Table 5: Numerical score for vaginal irritation study of gel formulations.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Score for epithelial ulceration/erythema</th>
<th>Score for edema</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Placebo gel)</td>
<td>0±0</td>
<td>0±0</td>
</tr>
<tr>
<td>II (FG9)</td>
<td>0±0</td>
<td>0±0</td>
</tr>
<tr>
<td>III (FG15)</td>
<td>0±0</td>
<td>0±0</td>
</tr>
</tbody>
</table>

**n = 6, Mean±SD.
CONCLUSION
In this study, various mucoadhesive gel formulations of FZ were prepared and evaluated for their suitability for vaginal drug delivery. Out of those mucoadhesive polymers, Polycarbophil and hydroxyl ethyl cellulose were found to be suitable gel matrices for vaginal route. The developed gels of PC (2% w/w) and HEC (6% w/w) were clear, easily extrudable, spreadable, viscous showing sustained drug release. The formulations exhibited shear thinning behaviour and found to be resistant to dilution effect. The developed gels indicated excellent mucoadhesion on goat vaginal mucosa and in vitro antifungal activity against Candida albicans. The formulations did not show any irritation in mice vagina. Thus, the developed mucoadhesive gel matrices hold potential for vaginal drug delivery and could be used for effective topical treatment of Candidiasis. In future, these gel matrices could be used as potential drug delivery vehicles to vagina.

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Conflict of interest
Authors declare no conflict of interest.

REFERENCES