Development and Evaluation of Vaginal gel Containing Ketoconazole in Combination with Probiotic Bacteria

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ABSTRACT:
Ketoconazole an imidazole derivative has been mainly used for treatment of vaginal candidiasis. The identity of Ketoconazole was confirmed by UV spectrophotometric and IR analysis. The identity, purity, viability and stability of Lactobacilli species was confirmed by various macroscopic, microscopic and chemical tests. The gel formulations were characterized by their physical and performance tests. Polymers from both the classes (semi synthetic and synthetic) were selected on the basis of their viscosity, spreadability. The selected formulations were further characterized for suitability using appearance, viscosity, spreadability, pH and drug content, bioadhesive strength and in-vitro drug release. Only those formulations which indicated the prolonged release of Ketoconazole from the preformed gels over period of 7 hrs were short listed for determining antifungal efficacy of formulation, animal study and stability study. The antifungal efficacy of Ketoconazole was comparable with marketed vaginal gel formulations. The selected formulations were not affected much when stored at 5^0C and 25^0C over 45 days. At 40^0C, slight decrease in viscosity and drug content was observed. The topical vaginal gel formulation did not produce any dermal toxicity in the rabbits.

1. INTRODUCTION:

The design and development of vaginal drug delivery systems (VDDS) also requires a comprehensive understanding of the various types of drugs being delivered and their specific requirements. Different drugs, such as antifungal agents, hormones, antibiotics, and anti-inflammatory drugs, may have unique pharmacokinetic profiles and require tailored formulations to maximize their effectiveness. For example, hormones used in vaginal rings for contraception or hormone replacement therapy need to be released at a controlled rate to maintain therapeutic plasma concentrations over time. Similarly, antimicrobial agents may require rapid absorption and sustained release to ensure effective treatment of vaginal infections without causing resistance. Additionally, the pH of the vaginal environment can significantly impact the stability and release of drugs, with certain drugs being more effective in a mildly acidic environment. Formulating drug delivery systems that can maintain the drug's stability and ensure optimal release rates based on the specific needs of each therapeutic application is vital for maximizing both efficacy and safety. Integrating modern drug delivery technologies, such as smart polymers that respond to pH changes

or temperature-sensitive formulations, can further improve the performance of VDDS, allowing for more personalized, effective treatment options. By addressing the diverse needs of different drug classes and ensuring compatibility with the vaginal environment, researchers can significantly advance the field of vaginal drug delivery. [1-5]

2. MATERIALS AND METHODS [16-17]

1. MATERIALS :

Chemicals - The chemicals used in the present study include a variety of reagents and materials sourced from reputable suppliers. Ampicillin discs were obtained from Dynamicro, Thane, while bile salt (Sodium Taurocholate) was supplied by High Media Labs, Pune. Carbomer 1342 and Carbomer 974, both essential for the formulation process, were provided by Noveon, Mumbai. Additionally, HPMC K 15 M, a polymer used for controlled release, was sourced from Colorcon Asia Pvt. Ltd., Goa, and ketoconazole was obtained from Yanshu Chemicals, Mumbai. MRS broth (369), which is commonly used for microbial growth, was also supplied by High Media Labs, Pune. For the formulation of various drug delivery systems, PEG 400 from Qualigens Fine Chemicals, Mumbai, and Polycarbophil from both Noveon, Mumbai, and Emcure Pharmaceuticals, Pune, were utilized. These high-quality chemicals were crucial to the success of the experimental procedures, ensuring the reliability and consistency of the results.

Biologicals: The study utilized several Lactobacilli strains, which were sourced from established microbiological collections. Lactobacillus plantarum (NCIM No. 2083) was obtained from the National Collection of Industrial Microorganisms (NCIM), Pune. Additionally, Lactobacillus casei (NCIM No. 2125) and Lactobacillus acidophilus (NCIM No. 2285) were also sourced from NCIM, Pune. Another strain, Lactobacillus rhamnosus (LGG, MTCC No. 1408B), was provided by the Microbial Type Culture Collection Bank (MTCC) in Chandigarh. These Lactobacilli strains were selected for their relevance to the study's focus on microbial interactions and their potential roles in maintaining health.

Cell lines: The study also utilized two important human cell lines for various experimental purposes. The *Caco-2* cell line, a human intestinal epithelial cell line, was sourced from the National Centre for Cell Science (NCCS) in Pune. This cell line is commonly used to model the intestinal barrier and study drug absorption. Additionally, the *A-431* cell line, a human skin carcinoma squamous cell line, was used for research involving skin-related assays and treatments. These cell lines were essential for evaluating the cellular responses and interactions relevant to the experimental objectives.

2. METHODS

i. Composition of de Man, Rogosa and Sharpe (MRS) broth:

The composition of MRS (De Man, Rogosa, and Sharpe) broth is as follows: for 1000 mL of the broth, 20.00 g of dextrose, 10.00 g of beef extract, 10.00 g of protease peptone, and 5.00 g of sodium acetate are included. Additionally, 5.00 g of yeast extract, 2.00 g of ammonium citrate, and 2.00 g of dipotassium phosphate are added. Polysorbate-80 is included at 1.00 g, along with 0.10 g of magnesium sulfate and 0.05 g of manganese sulfate. The final pH of the broth, when prepared and measured at 25°C, is 6.5 ± 0.2.

ii. Preparation of MRS broth medium: [16]

MRS broth was prepared by accurately weighing the required quantity of MRS powder, dissolving it in a minimum volume of distilled water, and adjusting the final volume to 100 mL. The broth was sterilized by autoclaving at 121°C for 15 minutes under moist heat and pressure, then cooled and stored for future use.

iii. Subculturing of pure cultures of *Lactobacillus* strains: [16]

To subculture lyophilized cultures and obtain the vegetative form of Lactobacilli, MRS broth and MRS agar are prepared and sterilized. The cultures are then inoculated onto petriplates using the four-quadrant streaking method and incubated at 25-30°C for 24 hours to allow colony formation.

i. Inoculation of *Lactobacilli* in MRS broth: [17]

A loopful of the four bacterial strains subcultured previously were inoculated aseptically in separate four sets of 10 X 50 ml sterile MRS broth quantities contained in conical flasks. These flasks were tightly closed with cotton plugs and were shaken on laboratory shaker for 48 hrs (25-30^0C ^{0} C)

ii. Determination of viable count of lyophilized powders of *Lactobacilli*: -

Procedure for serial dilution technique: [17]

The procedure begins by transferring 5 mg of lyophilized Lactobacilli cultures into test tubes with sterile distilled water, followed by serial dilutions and plating onto MRS agar. After incubation at 37°C for 24 hours, colony counts are recorded to determine the concentration of Lactobacilli in the original suspension based on the dilution factor.

iii. Determination of acid tolerance of lyophilized powders of *Lactobacilli* strains:

To determine the viable bacterial count (CFU) of Lactobacilli cultures, 5 mg of lyophilized powder from each culture was transferred into MRS broth with pH adjustments, then incubated at 37°C for 0, 2, and 4 hours. The viable bacterial count was determined using the Standard Plate Count (SPC) method, with the test conducted in duplicate for accuracy.

Determination of bile tolerance of lyophilized powders of *Lactobacilli* strains:

To determine the viable bacterial count (CFU) of Lactobacilli cultures, 5 mg of lyophilized powder was transferred into MRS broth with varying bile concentrations (0.3%, 1%, and a control). The cultures were incubated at 37°C for 0, 3, 6, 24, and 48 hours, and viable counts were determined using the Standard Plate Count (SPC) method, with duplicate testing for accuracy.

iv. Determination of compatibility of *Lactobacilli* cultures with Ketoconazole:

To assess the compatibility of Lactobacilli cultures with Ketoconazole, bacterial suspensions were inoculated onto MRS agar plates, and wells were filled with Ketoconazole and Ampicillin solutions. After 48 hours of incubation at 37°C, the zones of inhibition were measured to evaluate the effects of Ketoconazole and Ampicillin on the bacterial cultures..

v. Determination of adhesion of *Lactobacilli* to cell lines:

Materials:

The bacterial strains used were Lactobacillus plantarum (NCIM No. 2083), Lactobacillus casei (NCIM No. 2125), Lactobacillus acidophilus (NCIM No. 2285), and Lactobacillus rhamnosus (MTCC No. 1408B). The study utilized MRS broth, Eagle's MEM, PBS pH 7.2, formaldehyde

(3.7%), crystal violet stain, Caco-2 and A-431 cell lines, and equipment such as a 12-well tissue culture plate, CO2 incubator, and microscope.

Composition of Eagle's 1959 medium (MEM) for cell lines:

The ingredients used included traces of L-glutamine, sodium bicarbonate for pH adjustment, 5% fetal calf serum, and antibiotics consisting of 100 UI/ml penicillin, 100 UI/ml streptomycin, and 200 mg/ml neomycin. These components were used in the preparation of the culture medium.

Methods:

The cell lines were sliced, washed with PBS, and incubated in MEM for 24 hours to allow growth before testing. Lactobacilli cultures were prepared by inoculating strains into MRS broth, incubating, and transferring to fresh medium, and then tested for adhesion by inoculating them onto the cell lines, fixing with formaldehyde, and performing Gram staining for microscopic examination.

3. Characterization of Ketoconazole:

A. Determination of physical constants

The melting point of Ketoconazole was determined using the glass capillary method with a programmable melting point apparatus, with the last solid particle liquefying indicating the melting point. The saturation solubility of Ketoconazole was determined in methanol, methanol: distilled water (40:60), and vaginal simulated fluid by shaking excess drug in each solvent for 48 hours, followed by UV spectrophotometric analysis.

Spectral analysis of Ketoconazole:

B. UV – Visible spectrophotometry:

The λmax of Ketoconazole was determined by dissolving it in methanol and scanning the solutions in the range of 400 nm to 200 nm, with the same procedure followed for methanol: distilled water and vaginal simulated fluid. A calibration curve was prepared by dissolving Ketoconazole in methanol and creating dilutions, with UV absorbance recorded at specific λmax values of 243.5 nm, 232 nm, and 225 nm for different solvent systems.

C. Infrared spectrophotometry:

The infrared absorption spectrum of Ketoconazole was determined using the potassium bromide dispersion technique, with 1-2 mg of the drug sample. The spectrum was recorded using an FTIR spectrophotometer.The potential interactions of Ketoconazole with different polymers used in gel formulations were studied using IR spectroscopy, analyzing spectra between 600-4000 cm-1 for any changes. UV interference was assessed by scanning 0.5% w/v methanolic solutions of the polymers between 200-400 nm to check for any overlap with Ketoconazole's absorption profile.

D. Stability of Ketoconazole in simulated vaginal fluid (pH 4.2):

Interactions between Ketoconazole, individual polymers, and their mixtures were analyzed using IR spectroscopy (600-4000 cm-1) to detect any changes. UV interference was assessed by

scanning 0.5% w/v methanolic polymer solutions between 200-400 nm to ensure no overlap with Ketoconazole's absorption profile, ensuring accurate future analyses.

E. Minimum inhibitory concentration (MIC)-

The minimum inhibitory concentration (MIC) of Ketoconazole was determined by preparing varying concentrations of the drug in Sabouraud glucose agar plates, inoculating them with Candida albicans, and incubating at 37°C for 24 hours. Growth was observed, and CFU counts were recorded to assess the drug's effectiveness, with negative and positive control plates included for comparison.

4. Preparation of gel formulations:

The formulation involves using Carbopol 1342, Carbopol 974, polycarbophil, and lyophilized powder of lactobacillus strains as key materials. Ethanol (99.9%) serves as a solvent, while polyethylene glycol 400 and propylene glycol are included for their moisturizing and stabilizing properties. Triethanolamine is used to adjust the pH of the mixture. The preparation process utilizes an overhead stirrer to ensure uniform mixing of the components. The resulting mixture is intended for use in pharmaceutical or cosmetic applications, with a focus on probiotic delivery.

Formulations were developed in three parts

a. Plain gel bases
b. Gels containing Ketoconazole
c. Gels containing Ketoconazole and probiotic bacteria.

i. The preparation of topical vaginal gels was carried out as follows

Solubilization of Ketoconazole:

Ketoconazole, being poorly soluble in water (0.017 mg/ml), required a suitable solvent system for incorporation into a clear gel dosage form. Various cosolvent systems were tested, combining ethanol (99.9%) with different concentrations of PEG 400 and propylene glycol (PG) to enhance solubilization. The optimized cosolvent ratio was selected to develop effective gel formulations containing dissolved Ketoconazole.

ii. Preparation and evaluation of plain gel bases

The preparation involved two phases: in Phase I, Carbomer 1342, 974, and Polycarbophil were soaked in distilled water for 2-3 hours. In Phase II, ethanol (99.9%), PEG 400, and propylene glycol were mixed in appropriate proportions. Phase II was then added slowly to Phase I while stirring with an overhead stirrer. The gelling agents' dispersion was neutralized dropwise with triethanolamine (TEA) under continuous stirring. The final formulation was adjusted to the desired weight with distilled water, and the pH was maintained between 4.0 and 4.5.

iii. Preparation and evaluation of medicated gels containing Ketoconazole

In the preparation process, Carbomer 1342, 974, and Polycarbophil were soaked in distilled water for 2-3 hours (Phase I). Ketoconazole, ethanol (99.9%), PEG 400, and propylene glycol were

mixed in appropriate proportions in Phase II. Phase II was then slowly added to Phase I while stirring with an overhead stirrer. The dispersion of gelling agents was neutralized dropwise with triethanolamine (TEA) under continuous stirring. The final formulation was adjusted to the desired weight using distilled water, and the pH was maintained between 4.0 and 4.5.

iv. Preparation and evaluation of medicated gels containing Ketoconazole and lyophilized powders of individual *Lactobacilli* strains:

For this, the plain gel bases were prepared and then lyophilized probiotic cultures of individual strain and solubilized Ketoconazole were incorporated into the gel bases so as to get a homogeneous dispersion. The incorporation process was conducted with care to ensure a homogeneous dispersion throughout the gel matrix. The lyophilized probiotic cultures were gently mixed into the gel base, allowing them to be evenly distributed without damaging the sensitive microbial cells. Similarly, the solubilized Ketoconazole was introduced, ensuring that the drug was fully integrated into the gel formulation.

v. Preparation and evaluation of medicated gels containing Ketoconazole and combination of lyophilized powders of *Lactobacilli* strains:

Plain gel bases were prepared, and then mixed culture powder of Lactobacilli strains and solubilized Ketoconazole were incorporated to achieve a homogeneous dispersion. The solubilization ensured the drug was fully dissolved and evenly distributed, enhancing bioavailability. The mixed culture powder and Ketoconazole were gently blended into the gel base using stirring or vortexing, avoiding air bubbles to maintain the gel's consistency.

vi. Preparation of plain gels using Carbomers viz Carbopol 1342, Carbopol 974 and Polycarbophil

In Phase I, Carbopol 1342, Carbopol 974, Polycarbophil, and distilled water are soaked for 2-3 hours, while Phase II combines ethanol, PEG 400, and propylene glycol. The Phase II mixture is added to Phase I, neutralized with triethanolamine, and stored, with gel bases evaluated for appearance, pH, viscosity, and spreadability.

vii. Preparation of gels containing Ketoconazole

a. Preparation of Ketoconazole gel with Carbopol 1342, Carbopol 974 and Polycarbophil

In Phase I, Carbopol 1342, Carbopol 974, Polycarbophil, and distilled water are soaked, while in Phase II, Ketoconazole, ethanol, PEG 400, and propylene glycol are mixed. The Phase II mixture is added to Phase I, neutralized with triethanolamine, and stored, with the gels evaluated for appearance, pH, viscosity, spreadability, average % Ketoconazole content, and in-vitro drug release.

b. Preparation of Ketoconazole gel with Carbopol 1342, Carbopol 974, Polycarbophil and HPMC K 15M

In Phase I, Carbopol 1342, Carbopol 974, Polycarbophil, HPMC K 15M, and distilled water are soaked. In Phase II, Ketoconazole, PEG 400, ethanol, and propylene glycol are mixed. The Phase II mixture is then added to Phase I while stirring, and the medicated gel is neutralized using triethanolamine (TEA). The final gel is stored as per Scheme No. 3.

Formulation of medicated Ketoconazole gel with Carbopol 1342, Carbopol 974, Polycarbophil and HPMC K 15M

The formulation includes Ketoconazole, Carbopol 1342/974, Polycarbophil, HPMC K 15M, ethanol, PEG 400, propylene glycol, triethanolamine, and distilled water, mixed in specified ratios. The gels are evaluated for appearance, pH, viscosity, spreadability, average % Ketoconazole content, and in-vitro drug release.

c. Preparation of Ketoconazole gel with Carbopol 974 and Polycarbophil

Phase I of the gel formulation involves the preparation of the base gel using Carbopol 974, Polycarbophil, and distilled water. These ingredients are blended together to form a gel matrix. In Phase II, the active ingredient, Ketoconazole, is incorporated into the gel along with other excipients such as ethanol, PEG 400, and propylene glycol. The gel is then neutralized using triethanolamine (TEA), which adjusts the pH to enhance the gel's consistency and stability. Finally, the gel is stored according to Scheme No. 4, ensuring proper conditions to maintain its efficacy and stability over time.

Formulation of medicated Ketoconazole gel with Carbopol 974 and Polycarbophil

The formulation consists of Ketoconazole, Carbopol 974, Polycarbophil, ethanol, PEG 400, propylene glycol, triethanolamine, and distilled water, mixed in specified ratios. The gels are evaluated for appearance, pH, viscosity, spreadability, average % Ketoconazole content, and in-vitro drug release.

d. Preparation of Ketoconazole gel with Carbopol 974, Polycarbophil and HPMC K 15M

The formulation involves preparing a gel base with Carbopol 974, Polycarbophil, HPMC K 15M, and distilled water in Phase I, followed by the addition of Ketoconazole, ethanol, PEG 400, and propylene glycol in Phase II. The gels are evaluated for appearance, pH, viscosity, spreadability, average % Ketoconazole content, and in-vitro drug release.

viii. Preparation and evaluation of medicated probiotic gels containing Ketoconazole with individual *Lactobacillus* strains:

a. Preparation of medicated probiotic gel of *L. plantarum* containing Ketoconazole with Carbopol 974, Polycarbophil and HPMC K 15M

The preparation of the medicated probiotic gel involves mixing Carbopol 974, Polycarbophil, HPMC K 15M, and distilled water in Phase I to form the gel base. In Phase II, Ketoconazole, L. plantarum, PEG 400, and propylene glycol are added, followed by neutralization with triethanolamine (TEA) to adjust the pH and enhance gel stability, before storing the final formulation under controlled conditions.

b. Preparation of medicated probiotic gel of *L. casei* containing Ketoconazole with Carbopol 974, Polycarbophil, HPMC K 15M

The formulation of the medicated probiotic gel starts with Phase I, where Carbopol 974, Polycarbophil, HPMC K 15M, and distilled water are combined to create the gel base. In Phase II, Ketoconazole, L. casei, PEG 400, and propylene glycol are added, followed by pH adjustment

with triethanolamine (TEA), ensuring proper gel consistency and stability before storing the final formulation under controlled conditions.

c. Preparation of medicated probiotic gel of *L. acidophilus* containing Ketoconazole with Carbopol 974, Polycarbophil and HPMC K 15M

The formulation of the medicated probiotic gel begins with Phase I, where Carbopol 974, Polycarbophil, HPMC K 15M, and distilled water are mixed to form a stable gel base. In Phase II, Ketoconazole, L. acidophilus, PEG 400, and propylene glycol are added, and the pH is adjusted using triethanolamine (TEA), followed by storage under controlled conditions to maintain stability and therapeutic efficacy.

d. Preparation of medicated probiotic gel of *L. rhamnosus (LGG)* containing Ketoconazole with Carbopol 974, Polycarbophil and HPMC K 15M

The preparation of the medicated probiotic gel starts with Phase I, where Carbopol 974, Polycarbophil, HPMC K 15M, and distilled water are combined to create the gel base. In Phase II, Ketoconazole, L. rhamnosus (LGG), PEG 400, and propylene glycol are added, followed by pH adjustment using triethanolamine (TEA), with the final gel stored under controlled conditions for stability. The formulations are then evaluated for appearance, pH, viscosity, spreadability, Ketoconazole content, in-vitro drug release, viable Lactobacillus count, and stability under different environmental conditions.

***ix.* Preparation and evaluation of medicated probiotic gel containing Ketoconazole and four strains of *Lactobacilli* (*L. plantarum, L. casei, L. acidophilus and L.rhamnosus (LGG)* with Carbopol 974, Polycarbophil and HPMCK15 M.**

The preparation of the medicated probiotic gel begins with Phase I, where Carbopol 974, Polycarbophil, HPMC K 15M, and distilled water form the gel base. In Phase II, Ketoconazole and a combination of Lactobacillus strains are added, along with ethanol, PEG 400, and propylene glycol, and the gel is neutralized with triethanolamine (TEA). The gel is then stored according to Scheme No. 10, ensuring stability and optimal performance. The formulation was evaluated for appearance, pH, viscosity, spreadability, Ketoconazole content, drug release, viable Lactobacillus count, and stability under various environmental conditions.

1. Codes for medicated gels containing Ketoconazole with Carbopol 1342, Carbopol 974, Polycarbophil and HPMC K15 M

The medicated gel formulations of Ketoconazole were developed using various combinations of Carbopol 1342, Carbopol 974, Polycarbophil, and HPMC K15M in different proportions. These formulations, with subcodes for different Lactobacillus strains such as *L. plantarum*, *L. casei*, *L. acidophilus*, and *L. rhamnosus*, were evaluated for their effectiveness in delivering both the drug and probiotics, with 10 mg of lyophilized Lactobacilli in each formulation**.**

5. Characterization of gels: [20]

The physical characteristics of the gel formulations were evaluated through appearance, pH, spreadability, viscosity, and bioadhesive strength. The pH was measured using a calibrated pH meter, spreadability was assessed with a glass slide apparatus, viscosity was determined using a

Brookfield viscometer, and bioadhesive strength was tested with a modified balance assembly and excised cow vaginal mucosa.

a. Preparation of vaginal mucosa:

In our study, cow vaginal mucosa (Bos Taurus) was used as a model for mucoadhesion testing due to its similarity to human vaginal mucosa. The gel samples were applied to the mucosa, and the force required to detach them from the mucosal membrane was measured by applying a constant weight and recording the detachment force.

b. In-vitro drug release:

For the release studies of vaginal gels, a modified USP dissolution apparatus II was used, with the gel placed in a mesh box submerged in simulated vaginal fluid (SVF) at 37±0.5°C. The fluid was analyzed for Ketoconazole content and Lactobacilli viability, with aliquots taken every 30 minutes and MRS agar used for colony counting.

c. Antifungal efficacy:

The antifungal efficacy of the medicated Ketoconazole gel was evaluated using both solid and liquid media. In the solid media test, C. albicans was cultured on Sabouraud glucose agar (SGA), and the zones of inhibition of the drug, placebo gel, and KTZ gel were compared to the marketed Candid V gel. In the liquid media test, KTZ gel was dissolved in DMSO, mixed with Sabouraud glucose broth (SGB), and inoculated with C. albicans. The presence of turbidity after 48 hours at 37°C was used to assess antifungal activity, compared to Candid V gel.

d. Test for antifungal efficacy (*in-vivo*) of KTZ gel containing mixture of Probiotic *Lactobacilli* species against induced vaginosis in mice:

The antifungal efficacy of the KTZ gel was tested in Swiss albino female mice, aged 6-8 weeks, weighing 25-30 grams. The mice were administered the drug intravaginally twice daily for 9 days, with Candida albicans (10^7-10^8 inoculum) used as the fungal strain. The study included five groups: infected (Group I), positive control (CTZ gel, Group II), placebo control (Group III), no infection (Group IV), and the test formulation (Group V), with a total of 24 animals. Estradiol valerate was used to induce pseudoestrus, and vaginal scrapings were taken on days 3, 6, and 9 to assess fungal growth

e. Test for determination of acute dermal toxicity of vaginal gels containing 1%w/w of Ketoconazole and mixture of lactobacilli strains for application into vagina for antifungal activity:

The acute dermal toxicity of vaginal gels containing 1% Ketoconazole and Lactobacilli strains was tested on female New Zealand white rabbits. Gels were applied to shaved areas on the rabbits' backs (0.5 g per area) on intact and abraded skin, and toxicity was observed at 1, 4, 12, 24, 48, and 72 hours. The animals were divided into three groups: control, medicated probiotic gel, and marketed formulation, with three animals per group. Toxic reactions, including erythema and edema, were recorded and compared between groups.

xi) Stability studies of gel formulations:

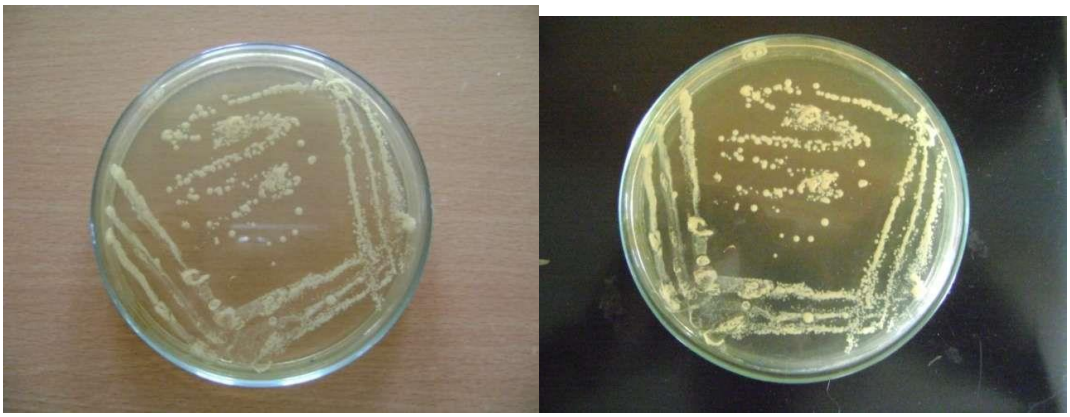
The shelf life of the prepared gels was evaluated following ICH guidelines over 45 days under refrigerated (5°C), ambient (25°C/75% RH), and accelerated (40°C/75% RH) conditions. Samples

were tested at 0, 15, 30, and 45 days for appearance, pH, viscosity, spreadability, drug content, in vitro drug release, and Lactobacilli viability to assess the gel's stability, drug efficacy, and probiotic content.

6. RESULTS AND DISCUSSION

1. Characteristics of probiotic cultures:

Subcultured *Lactobacillus* strains:



a. L. plantarum **b. *L. Casei***

c. *L. acidophilus* **d. *L. rhamnosus (LGG)***

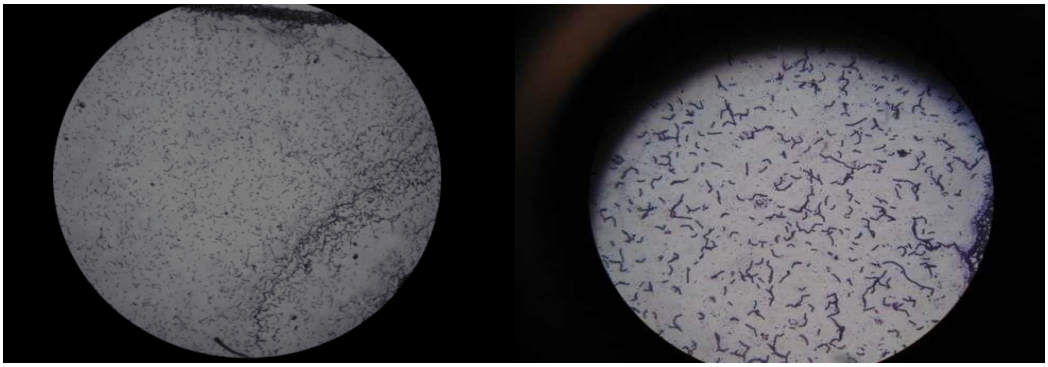
Fig 01: Subcultured *Lactobacillus* strains.

3. Characteristics of *Lactobacilli* strains

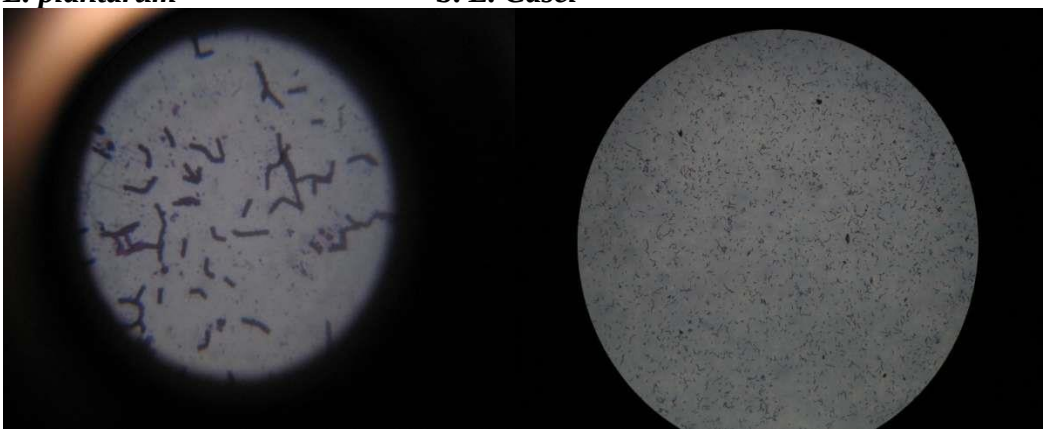
A. Morphological characteristics of *Lactobacilli* strains

Fig 02: Morphological characteristics of *Lactobacilli* strain

A. Microscopic characteristics of *Lactobacilli* strains:



a. ***L. plantarum*** **b.** ***L. Casei***



c. L. acidophilus **d.** ***L. rhamnosus (LGG)***

Fig 03: Microscopic characteristics of *Lactobacilli* strains

Colony characteristics of *Lactobacillus* strains observed

Macroscopically and microscopically

C- i. viable cell count of lyophilized powders (5mg) of *Lactobacilli*

Table 01 : Viable cell count of *Lactobacilli*

Characteristics of colony	***Lactobacillus plantarum***	***Lactobacillus casei***	***Lactobacillus acidophilus***	***Lactobacillus rhamnosus***
Size	2.8-3 mm	3 mm	2.7-3 mm	2.6-3 mm
Shape	Round	Round	Round	Round/Circular
Colour	Light yellow	White to gray	White to gray	White to gray
Margin	Entire	Entire	Entire	Entire
Elevation	Convex	Raised	Flat	Convex
Consistency	Mucoid/ smooth	Mucoid/ smooth	Mucoid/ smooth	Mucoid/ smooth
Opacity	Opaque	Opaque	Opaque	Opaque
Gram Staining	Gram +ve, short rods	Gram +ve, short rods	Gram +ve, short rods	Gram +ve, short rods
Motility	Motile	Motile	Motile	Motile

iv. Compatibility of *Lactobacilli* cultures with Ketoconazole:

Table No 2. Zone of inhibition for *L. plantarum* cells incubated with Ketoconazole and standard Ampicillin respectively.

Sr. No.	**Plate No.**	**Ketoconazole concentration (µg)**	**Ampicillin concentration (µg / disc)**	**Zone of inhibition for Ketoconazole (mm)**	**Zone of inhibition for Ampicillin (mm)**
1	1	10	10	9.1 - 10.2	10.1-10.5
2		100	10	9.8 - 11.1	10.2-10.6
3	2	10	10	9.0 - 10.3	9.9-10.3
4		10 0	10	10.4 - 11	10.4-10.7

***Note:** Zone of inhibition diameter <11mm considered to be resistant.

Table No. 3. Zone of inhibition for *L. casei* cells incubated with Ketoconazole and standard Ampicillin respectively.

Sr. No.	**Plate No.**	**Ketoconazole Concentration (µg)**	**Ampicillin concentration (µg / disc)**	**Zone of inhibition for Ketoconazole (mm)**	**Zone of inhibition for Ampicillin (mm)**
1	1	10	10	11.0 - 9.8	10.1-10.5
2		100	10	9.4 – 10.6	10.2-10.6
3	2	10	10	8.9-10.2	9.9-10.3
4		100	10	8.3 – 10.1	10.4-10.7

***Note:** Zone of inhibition diameter <11mm considered to be resistant.

Table No. 4. Zone of inhibition for *L. acidophilus* cells incubated with Ketoconazole and standard Ampicillin respectively.

Sr. No.	Plate No.	Ketoconazole concentration (µg)	Ampicillin concentration (µg / disc)	Zone of inhibition for Ketoconazole (mm)	Zone of inhibition for Ampicillin (mm)
1	1	10	10	9.7 - 10.2	10.1-10.5
2		100	10	10.0 – 10.7	10.2-10.6
3	2	10	10	10.1 - 10.44	9.9-10.3
4		10 0	10	10.2 – 11.1	10.4-10.7

*** Note:** Zone of inhibition diameter <11mm considered to be resistant.

Table No. 5. Zone of inhibition for *L. rhamnosus* cells incubated with Ketoconazole and standard Ampicillin respectively.

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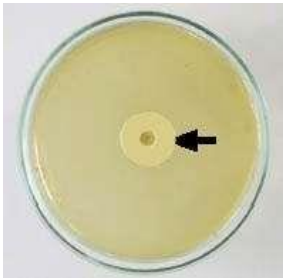
Sr. No.	Plate No.	Ketoconazole concentration (µg)	Ampicillin concentration (µg / disc)	Zone of inhibition for Ketoconazole (mm)	Zone of inhibition for Ampicillin (mm)
1	1	10	10	10.2 - 10.4	10.1-10.5
2		100	10	10.1 – 10.9	10.2-10.6
3	2	10	10	9.7 - 10.4	9.9-10.3
4		10 0	10	10.4 – 11.1	10.4-10.7

Note: Zone of inhibition diameter <11mm considered to be resistant.

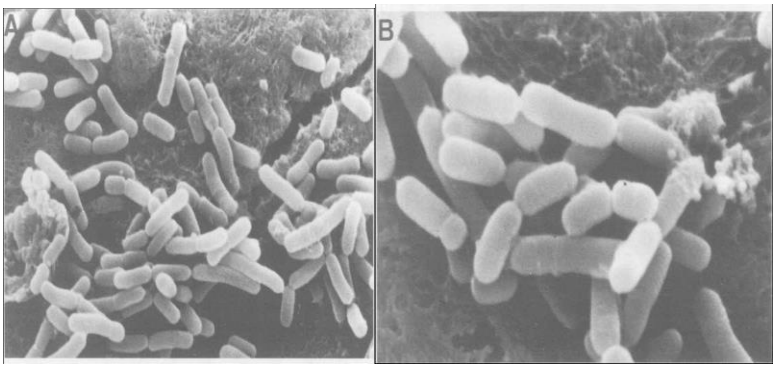
Table No. 6. Standard zone size interpretation data for Ampicillin



Sensitivity of *Lactobacilli*	Diameter of zone of inhibition (mm)
Resistant	≤ 11
Moderate	12 – 13
Sensitive	≥ 14

*** Note:** Values reported on the label of Ampicillin discs manufactured by **Dynmicro, Thane.**

v. Adhesion of *Lactobacilli* to cell lines:



a. A-431 **b. Caco-2**

Fig. No. 4. Adhesion of *Lactobacilli* to cell lines a.A-431, b. Caco-2.

8.2. Characteristics of Ketoconazole:

A. Physical constants:

i.Melting point : 148ºC-152ºC

ii.Saturation solubility : Practically insoluble in water.

B. Spectroscopic characteristics:

a. UV – Visible spectrophotometry :

i.λmax value :

Table no. 7. λmax value of Ketoconazole in various solvents.

Sr. No.	Solvent system	λ max
	Methanol	243.5 nm
	Distilled water : Methanol (60:40)	232 nm
	Vaginal simulated fluid (pH 4.5)	225.5 nm

ii) Calibration curves of Ketoconazole:

Table No. 8. Particulars of calibration curves of Ketoconazole in different solvents.

Sr. No.	Solvent system	Equation of line (y = mx + C)	Coefficient of regression (r^2)
1.	Methanol	y = 0.044 + 0.0	0.998
2.	Distilled water : Methanol	y = 0.033 + 0.0	0.997
3.	Vaginal simulated fluid (pH 4.5)	y = 0.036 + 0.0	0.999

D. Stability of Ketoconazole (10µg/ml) in vaginal simulated fluid (VSF) pH 4.2

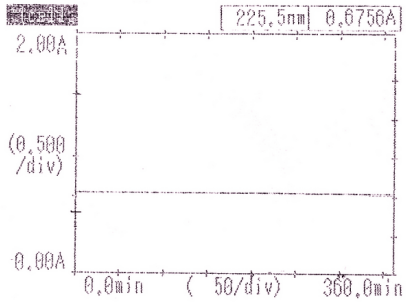


Fig. No. 5. UV spectrum of Ketoconazole indicating constant absorbance at 225.5nm.

C. MIC of Ketoconazole against *C. albicans* on SGA:

Table No. 9. MIC value of Ketoconazole

Conc of Ketoconazole (µg/ml)	Growth status		
	Unbuffered SGA	Buffered SGA	
		pH3-3.5	pH4.5-5
80.00	-	-	-
40.00	-	-	-
10.00	-	-	-

Conc of Ketoconazole (µg/ml)	Growth status		
	Unbuffered SGA	Buffered SGA	
		pH3-3.5	pH4.5-5
30.00	-	-	-
20.00	-	-	-
10.00	-	-	-

Conc of Ketoconazole (µg/ml)	Growth status		
	Unbuffered SGA	Buffered SGA	
		pH3-3.5	pH4.5-5
5.00	+	+	+
10.00	-	-	-
15.00	-	-	-

Conc of Ketoconazole (µg/ml)	Growth status		
	Unbuffered SGA	Buffered SGA	
		pH3-3.5	pH4.5-5
7.00	+	+	+
10.00	-	-	-
12.00	-	-	-

Conc of Ketoconazole (µg/ml)	Growth status		
	Unbuffered SGA	Buffered SGA	
		pH3-3.5	pH4.5-5
7.00	+	+	+
8.00	+	+	-
9.00	+	+	-

(Unbuffered medium with unadjusted pH 5.3-5.5)

- Absence of bacterial growth
+ Presence of bacterial growth

*** Note:** + ve control and – ve control maintained in duplicate for buffered SGA and Unbuffered SGA.

- ve control: Plate with medium containing drug (No *C.albicans* added)
+ ve control: Plate with medium containing *C.albicans* (No drug added)

8.3. Formulation of gels:

Table No. 10. Ratios of cosolvents for solubilization of Ketoconazole

	Cosolvent Ratio			Homogeneity of Solutions
	Ethanol	**PEG400**	**PG**	
Ketoconazole (1 gm)	20	10	15.52	Non Homogenous
			15.52	Homogenous
			15.52	Homogenous
			15.52	Homogenous

8.4. Characteristics of gels
Physical characteristics
i) Appearance
Appearance of formulations containing Ketoconazole with Carbopol 1342, Carbopol 974, Polycarbophil and HPMC K15 M
All formulations (A1a, A1b, A1c, A2a, A2b, A2c, A3a, A3b, A3c, A4a, A4b, A4c) appeared transparent, with most displaying a colorless and smooth texture. The overall appearance of each formulation remained clear and uniform throughout.

Appearance of formulations of Ketoconazole containing combination of Carbopol 1342, Carbopol 974 and Polycarbophil with HPMC K 15M

All formulations (C1a, C1b, C1c, C2a, C2b, C2c, C3a, C3b, C3c, Da, Db, Dc, Ea, Eb, Ec) were transparent in appearance. Each formulation displayed a consistent, clear, and uniform look.

Appearance of formulations of Ketoconazole containing *Lactobacill* strains with combination of Carbopol 974, Polycarbophil and HPMC K 15M

All formulations (La, Lb, Lc, Ld, G) were transparent in appearance. Each formulation exhibited a clear and uniform look.

ii) pH measurements

pH of formulations containing Ketoconazole with Carbopol 1342, Carbopol 974, Polycarbophil and HPMC K15 M

The pH values of the formulations ranged from 4.3 to 6.4, with A1a, A1b, and A1c having pH values between 4.3 and 4.4. Formulations A4a, A4b, and A4c had higher pH values, ranging from 6.2 to 6.4.

Appearance of formulations of Ketoconazole containing combination of Carbopol 1342, Carbopol 974 and Polycarbophil with HPMC K 15M

The pH values of the formulations ranged from 4.2 to 4.6, with C1a, C1b, and C1c having pH values around 4.3 to 4.4. The pH values of Da, Db, and Dc were slightly higher, ranging from 4.5 to 4.6, while Ea, Eb, and Ec remained between 4.3 and 4.4.

Appearance of formulations of Ketoconazole containing *Lactobacillus* strains with combination of Carbopol 974,Polycarbophil and HPMC K 15M

The pH values of the formulations ranged from 4.4 to 4.5, with La, Lc, and G having a pH of 4.4, while Lb and Ld were slightly higher at 4.5. All formulations maintained similar pH values within this narrow range.

iii) Drug content.

Drug content of formulations containing Ketoconazole with Carbopol 1342, Carbopol 974, Polycarbophil and HPMC K15 M

The average drug content of the formulations ranged from 98% to 103%, with A1a, A1b, A1c showing values between 98% and 99%, while A2a, A2b, A2c, and others ranged from 99% to 103%. Formulations A4a, A4b, and A4c had drug content values around 99% to 101%, indicating consistent formulation quality.

Drug content of formulations of Ketoconazole containing combination of Carbopol 1342, Carbopol 974 and Polycarbophil with HPMC K 15M

The average drug content of the formulations ranged from 98% to 101%, with formulations C1a, C1b, C1c, C2a, C2b, and others showing values near 98% to 101%. Formulations Da, Db, Dc, Ea, Eb, and Ec consistently showed drug content percentages around 98% to 101%, reflecting stable drug incorporation.

Drug content of formulations of Ketoconazole containing *Lactobacillus* strains with combination of Carbopol 974,Polycarbophil and HPMC K 15M

The average drug content for formulations La, Lb, Lc, Ld, and G ranged from 99% to 102%, with La and Ld showing the highest values of 101% and 102%, respectively. The results indicate consistent drug incorporation across all formulations, with minimal variation.

iv) Spreadability

Spreadability time of formulations containing Ketoconazole with Carbopol 1342, Carbopol 974, Polycarbophil and HPMC K15

The spreading times of formulations A1a to A4c ranged from 37.48 ± 0.31 sec (A3a) to 60.11 ± 0.36 sec (A4c), indicating a variation in spreadability. A3 formulations exhibited the fastest spreading times, while A4 formulations showed slower spreadability.

Spreadability time of formulations of Ketoconazole containing combination of Carbopol 1342, Carbopol 974 and Polycarbophil with HPMC K 15M

The spreading times of formulations C1a to Ec varied from 41.74 ± 0.33 sec (C3a) to 56.99 ± 0.56 sec (C1c), with C3 formulations exhibiting the fastest spreading. The Da to Ec formulations displayed moderate spreading times, generally ranging between 45.03 ± 0.91 sec and 49.73 ± 0.53 sec.

Spreadability time of formulations of Ketoconazole containing *Lactobacillus* strains with combination of Carbopol 974, Polycarbophil and HPMC K 15M

The spreading times of formulations La to G ranged from 43.89 ± 0.36 sec (La) to 46.34 ± 0.73 sec (G), with La showing the fastest spreading. The other formulations (Lb, Lc, Ld) had similar spreading times, ranging from 45.67 ± 0.33 sec to 46.01 ± 0.31 sec.

v) Rheology of vaginal gels
Viscosity values of formulations containing Ketoconazole with Carbopol 1342, Carbopol 974, Polycarbophil and HPMC K15 M

The viscosities of the formulations ranged from 3,360 cp (A3a) to 20,590 cp (A1c), with A1b and A1c exhibiting higher viscosities. The viscosities of other formulations like A2a, A2b, A2c, A4a, A4b, and A4c varied between 6,760 cp and 17,430 cp.

Viscosity values of formulations of Ketoconazole containing combination of Carbopol 1342, Carbopol 974 and Polycarbophil with HPMC K 15M

The viscosities of the formulations ranged from 4,620 cp (C3a) to 23,110 cp (C1c), with C1a, C1b, and C1c showing the highest viscosities. The other formulations, including C2a, C2b, C2c, Da, Db, Dc, Ea, Eb, and Ec, had viscosities ranging from 7,370 cp to 12,310 cp.

Viscosity values of formulations of Ketoconazole containing *Lactobacillus* strains with combination of Carbopol 974, Polycarbophil and HPMC K 15M

The viscosities of the formulations ranged from 6,540 cp (Lc) to 7,860 cp (G), with La, Lb, and Ld showing viscosities of 7,320 cp, 7,500 cp, and 7,780 cp, respectively. These values indicate a relatively consistent viscosity across the formulations.

vi) Bioadhesive strength

Bioadhesive strength of formulations containing Ketoconazole with Carbopol 1342, Carbopol 974, Polycarbophil and HPMC K15 M

The bioadhesive strength of the formulations ranged from 2.7 g (A1a) to 4.7 g (A3c), with A1b and A1c showing strengths of 2.9 g and 3.3 g, respectively. Formulations A2a to A3c exhibited progressively higher strengths, peaking at 4.7 g.

Bioadhesive strength of formulations of Ketoconazole containing combination of Carbopol 1342, Carbopol 974 and Polycarbophil with HPMC K 15M

The bioadhesive strength of the formulations ranged from 3.9 g (C1a) to 6.5 g (Eb), with C1b and C1c showing strengths of 4.3 g and 4.6 g, respectively. Formulations C2a to Ea exhibited progressively higher strengths, peaking at 6.5 g for Eb.

Bioadhesive strength of formulations of Ketoconazole containing *Lactobacillus* strains with combination of Carbopol 974, Polycarbophil and HPMC K 15M

The bioadhesive strength of the formulations La, Lb, Lc, and Ld ranged from 6.1 g to 6.2 g, while formulation G showed a slightly lower strength of 6.0 g. These formulations exhibited similar bioadhesive properties.

vii) Release of Ketoconazole from gels (*in-vitro*)

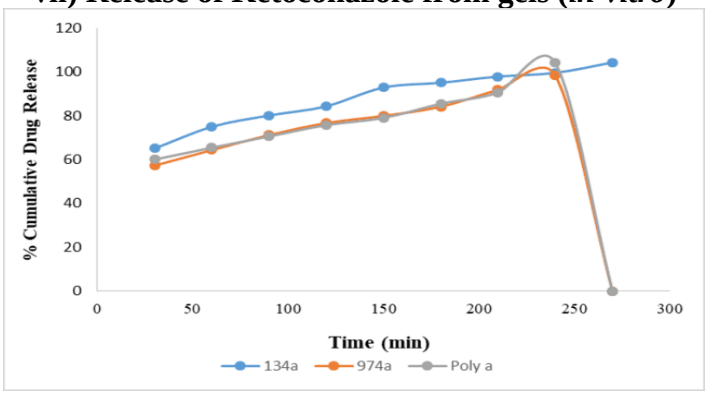


Fig No. 6. Release profile of KTZ from gels prepared with individual polymers (0.5% w/w)

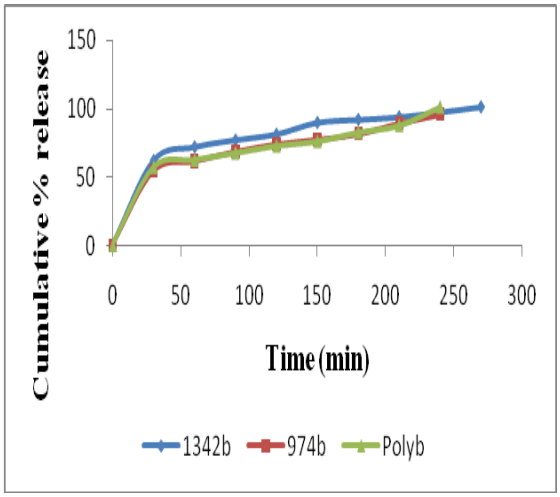


Fig No. 7. Release profile of KTZ from gels prepared with individual polymers (1%w/w)

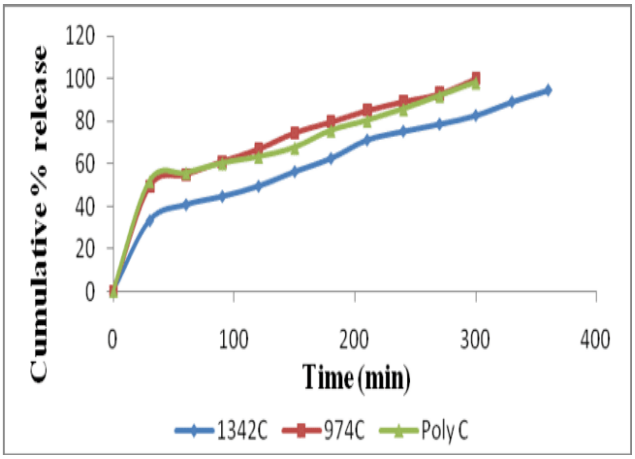


Fig No. 8. Average cumulative % release of Ketoconazole from gels prepared with individual polymers (1.5 %w/w)

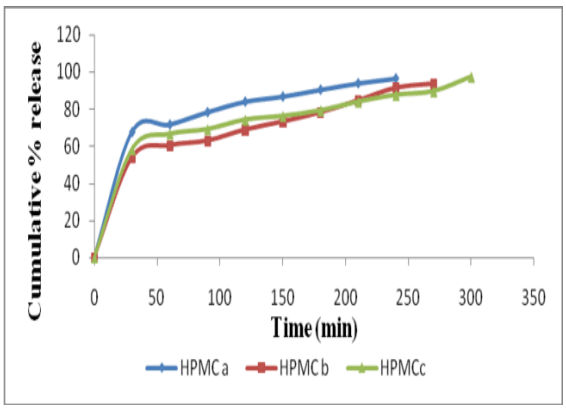


Fig No. 9. Average cumulative % release of Ketoconazole from gels prepared with HPMC K15 M (2%, 3%, 4%w/w)

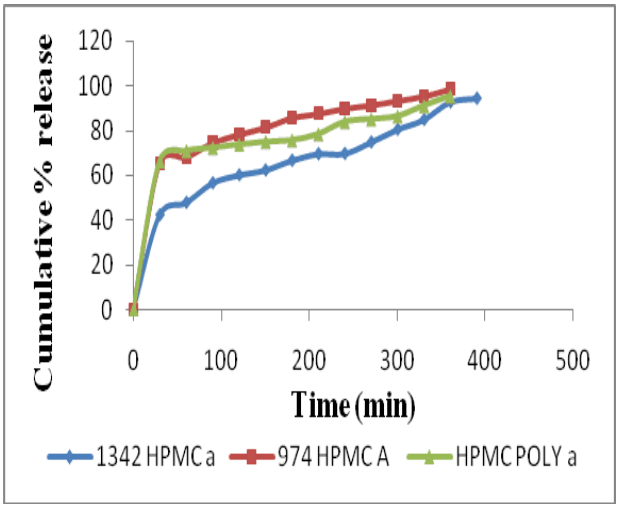


Fig No. 10. Average cumulative % release of Ketoconazole from gels prepared combination of polymers (1:0.5)

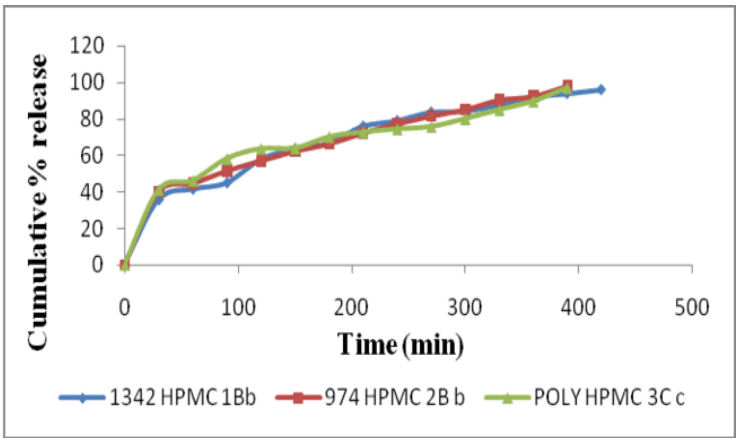


Fig No. 11. Average cumulative % release of Ketoconazole from gels prepare combination of polymers (1:1)

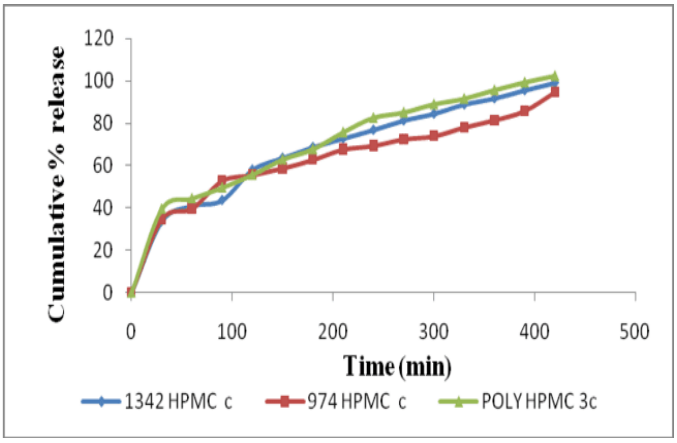


Fig No. 12. Average cumulative % release of Ketoconazole from gels prepared combination of polymers (1:1.5)

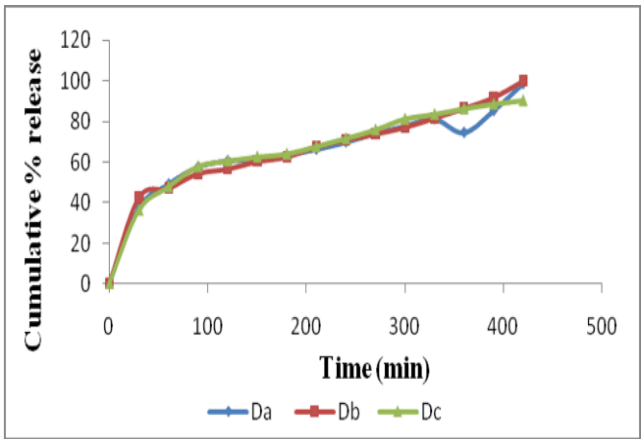


Fig. No. 13. Average cumulative % release of Ketoconazole from gels prepared combination of polymers (formulations Da, Db, Dc)

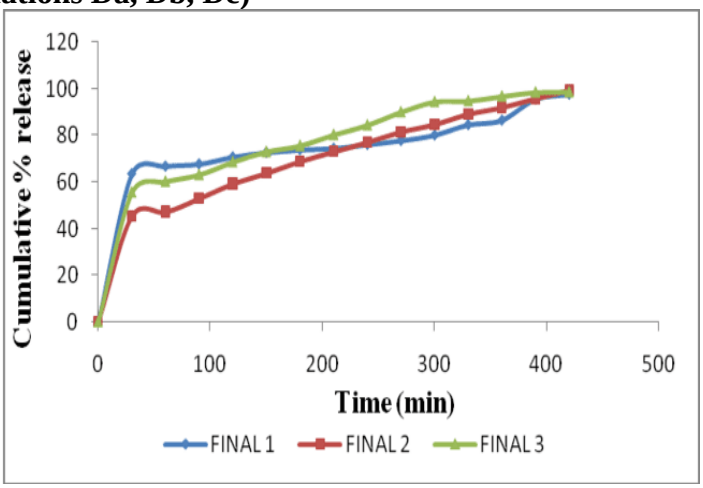


Fig No. 14. Average cumulative % release of Ketoconazole from gels prepared combination of polymers (formulations Ea, Eb, Ec)

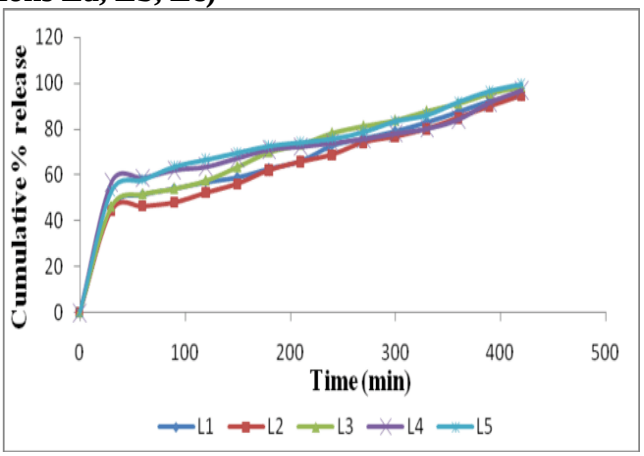


Fig No. 15. Average cumulative % release of Ketoconazole from gels containing *Lactobacilli* (formulations La, Lb, Lc, Ld, G)

viii) Viable Cell Count:

C- i. viable cell count of lyophilized powders of *Lactobacilli* (Formulation Code La or Lb)

Table No. 11. Viable cell count of *Lactobacillus plantarum.*

Plate No.	Dilution (a)	No. of colonies (b)	Corresponding no. of microorganisms (a×b)	Average cell count (approx.)
6	10^6	dense growth	-	109-1010
8	10^8	250	25 x 10^{10}	
9	10^9	39	39 x 10^9	
10	1010	5	5 x 10^{10}	

Table No. 12. Viable cell count of *L. casei*.

Plate No.	Dilution (a)	No. of colonies (b)	Corresponding no. of microorganisms (a×b)	Average cell count (approx.)
6	10^6	**dense growth**	-	10^9-10^{10}
8	10^8	240	24 x 10^9	
9	10^9	42	42 x 10^9	
10	1010	3	3 x 10^{10}	

Table No. 13. Viable cell count of *L. acidophillus*.

Plate No.	Dilution (a)	No. of colonies (b)	Corresponding no. of microorganisms (a×b)	Average cell count (approx.)
6	10^6	dense growth	-	10^9-10^{10}
8	10^8	290	29 x 10^9	
9	10^9	65	65 x 10^9	
10	1010	9	9 x 10^9	

Table No. 14. Viable cell count of *L. rhamnosus*.

Plate No.	Dilution (a)	No. of colonies (b)	Corresponding no. of microorganisms (a×b)	Average cell count (approx.)
6	10^6	dense growth	-	
8	10^8	160	16 x 10^9	
9	10^9	35	35 x 10^9	
10	1010	7	7x 10^{10}	10^9-10^{10}

ix) Antifungal efficacy of selected gels of Ketoconazole

1) **Antifungal efficacy of gel of Ketoconazole against *C. albicans* on SGA**

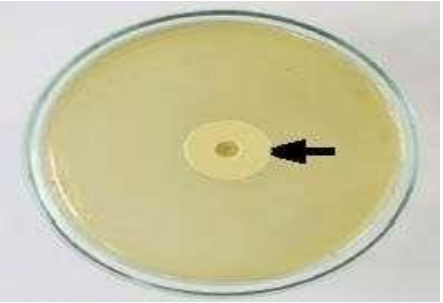


Fig No. 16. Zone of inhibition against *C. albicans*

Table No. 15. Zone of inhibition against *C. albicans*

Sr. No.	Test material	Diameter of zone (mm)
1	Placebo gel	-
2	Ketoconazole alone (10µg/ml)	33± 0.12 mm
3	Medicated gel(1%w/w)	32.3 ± 0.6 mm
4	Candid-V gel(2%w/w)	33.1 ± 0.6 mm

2) Antifungal efficacy of gel of Ketoconazole against *C. albicans* using SGB

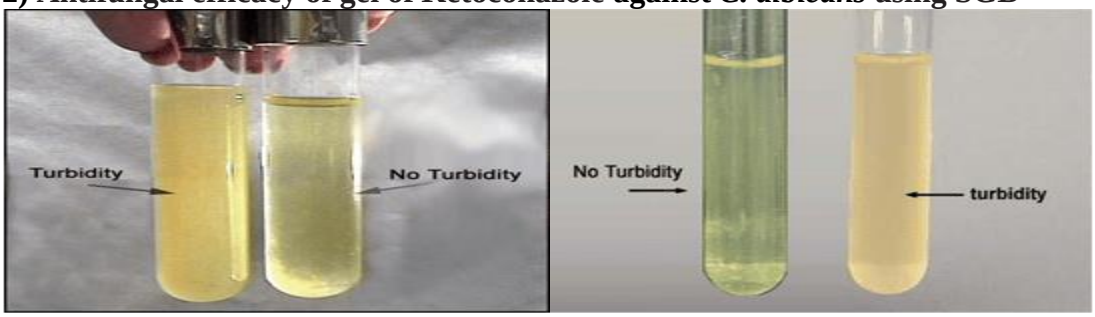


Fig. No. 17. Turbidity of SGB indicating antifungal efficacy of gels of Ketoconazole against *C. albicans*

ix) Antifungal efficacy (in-vivo) of KTZ gel containing mixture of probiotic Lactobacilli species against induced vaginosis in mice

Observations –

i) Initial

Initial	**No. of colony forming units (cfu)**				
Groups	**Group I (P)**	**Group II (KTZ)**	**Group III (CTZ)**	**Group IV (CA)**	**Group V (NO)**
	C. albicans				
1.	358	222	412	176	34
2.	184	158	327	231	47
3.	210	324	367	311	54
4.	150	210	296		
5.	136	186	228		
6.	412	138	196		
	Lactobacilli				
1.	36	64	68	20	130
2.	48	71	53	33	92
3.	52	34	26	28	122
4.	28	21	31		
5.	32	18	23		
6.	76	23	18		

ii) 3rd day of treatment

3 rd day	**No. of colony forming units (cfu)**				
Groups	**Group I (P)**	**Group II (KTZ)**	**Group III (CTZ)**	**Group IV (CA)**	**Group V (NO)**
	C. albicans				
1.	352	202	250	252	36
2.	180	148	312	294	51

3.	198	207	218	384	60
4.	161	214	224		
5.	142	152	189		
6.	430	140	164		
	Lactobacilli				
1.	30	78	63	17	110
2.	43	86	49	28	68
3.	46	54	28	23	124
4.	22	32	26		
5.	30	27	19		
6.	72	34	16		

iii) 6th day of treatment

6 th day	**No. of colony forming units (cfu)**				
Groups	**Group I (P)**	**Group II (KTZ)**	**Group III (CTZ)**	**Group IV (CA)**	**Group V (NO)**
	C. albicans				
1.	362	128	141	280	32
2.	190	102	122	310	53
3.	212	144	130	420	52
4.	142	118	192		
5.	162	104	98		
6.	490	78	62		
	Lactobacilli				
1.	28	93	58	14	124
2.	36	102	40	22	88
3.	40	78	22	19	134
4.	18	52	30		
5.	26	61	26		
6.	66	69	14		

iv) 9th day of treatment

Table No. 16. Comparative efficacy of drug Ketoconazole

9 th day	**No. of colony forming units (cfu)**				
Groups	**Group I (P)**	**Group II (KTZ)**	**Group III (CTZ)**	**Group IV (CA)**	**Group V (NO)**
	C. albicans				
1.	371	43	45	350	30
2.	210	52	58	420	52
3.	242	34	30	520	49

4.	184	70	76		
5.	158	41	45		
6.	550	18	22		
	Lactobacilli				
1.	20	144	50	4	134
2.	24	134	32	12	78
3.	29	98	20	7	152
4.	9	91	21		
5.	18	112	19		
6.	40	124	13		

x) Acute dermal toxicity of gels of Ketoconazole

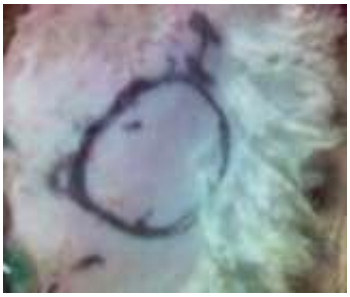
Table no 8.53 gives these scores for Gr I, II, and III respectively

Gr I: Control

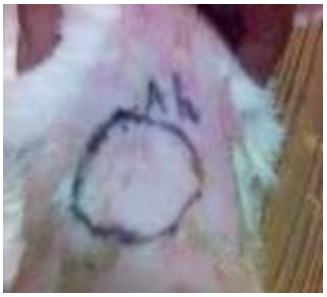
Table no. 17. Numerical score for irritancy potential

Sr. No.	Animal Code	1 hrs		4 hrs		12 hrs		24 hrs		48 hrs		72 hrs	
		I	A	I	A	I	A	I	A	I	A	I	A
1	H	0	0	0	0	0	0	0	0	0	0	0	0
2	B	0	0	0	0	0	0	0	0	0	0	0	0
3	T	0	0	0	0	0	0	0	0	0	0	0	0

* I: Intact, A: Abraded



Intact skin



Abraded skin

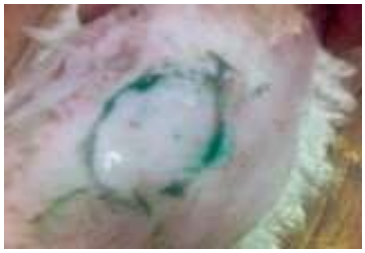
Gr II: medicated gel containing KTZ.

Numerical score for irritancy potential.

Sr. No.	Animal Code	1 hrs		4 hrs		12 hrs		24 hrs		48 hrs		72 hrs	
		I	A	I	A	I	A	I	A	I	A	I	A
1	H	0	0	0	0	0	0	0	0	0	0	0	0
2	HB	0	0	0	0	0	0	0	0	0	0	0	0
3	HBT	0	0	0	0	0	0	0	0	0	0	0	0

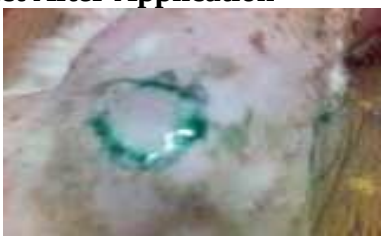
* I: Intact, A: Abraded

Intact before Application



Intact After Application

Abrasion before Application



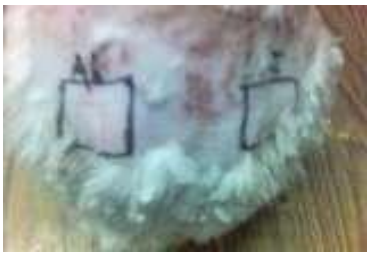
Abrasion after Application

Gr. III medicated gels containing KTZ and mixture of *Lactobacilli* species.

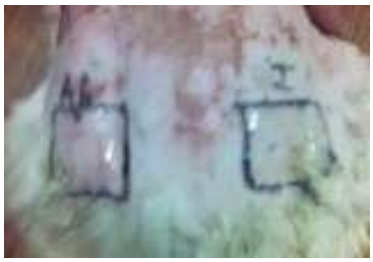
Numerical score for irritancy potential

Sr. No.	Animal code	1 hrs		4 hrs		12 hrs		24 hrs		48 hrs		72 hrs	
		I	A	I	A	I	A	I	A	I	A	I	A
1	W	0	0	0	0	0	0	0	0	0	0	0	0
2	BT	0	0	0	0	0	0	0	0	0	0	0	0
3	HT	0	0	0	0	0	0	0	0	0	0	0	0

* I: Intact, A: Abraded

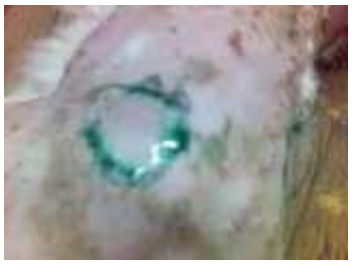


Intact before Application



Intact After Application

Abrasion before Application



Abrasion after Application

Fig. 18. Acute dermal toxicity study

Stability studies

i) Appearance:

All the formulations were found to be clear, transparent in appearance.

ii) pH

The pH values of formulations La, Lb, Lc, Ld, and G remained relatively stable during the stability study, with slight variations observed across different storage conditions. For instance, formulation La exhibited a pH range of 4.5 to 4.9, while formulation G showed a decrease in pH from 4.6 to 4.3 at 40°C/75% RH over 45 days.

i) Drug contents

The stability study indicated minimal changes in the drug content of formulations La, Lb, Lc, Ld, and G over time. At 40°C/75% RH, the drug content for formulation La decreased from 101.01% to 95.32%, while formulation Lb maintained a more stable drug content, with a decline from 101.95% to 94.33%.

iv) Viscosity (cps)

The viscosity study of formulations La, Lb, Lc, Ld, and G showed slight decreases over the storage period. At 40°C/75% RH, formulation La dropped from 6723 cps to 6430 cps, while formulation Lb decreased from 6380 cps to 6022 cps, demonstrating some viscosity loss during the stability study.

v) Viability count

The viscosity study of medicated gel formulations La, Lb, Lc, Ld, and G revealed a slight decrease in viscosity over 45 days, with formulation La reducing from 6723 cps to 6430 cps at 40°C/75% RH. Formulations Lb and Lc also experienced reductions, indicating stability changes during the storage period.

vi) Dissolution studies

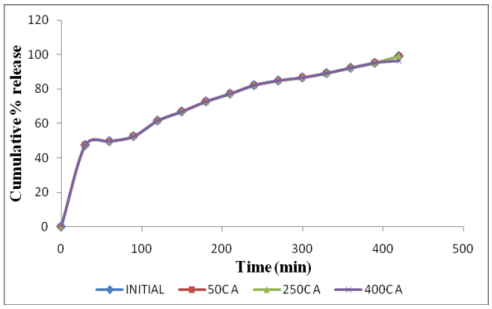


Fig No. 19. Average cumulative % release of Ketoconazole from medicated probiotic gel containing *L. plantarum* after 15 days.

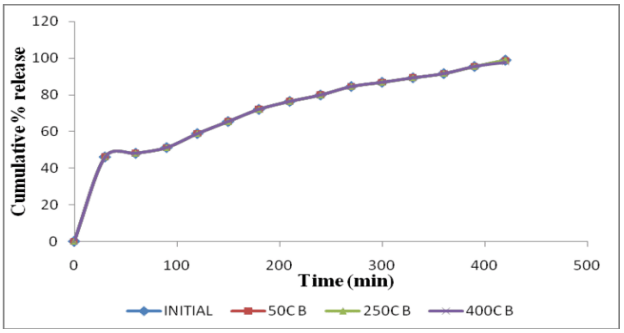


Fig No. 20. Average cumulative % release of Ketoconazole from medicated Probiotic gel containing *L. casei* after 15 days.

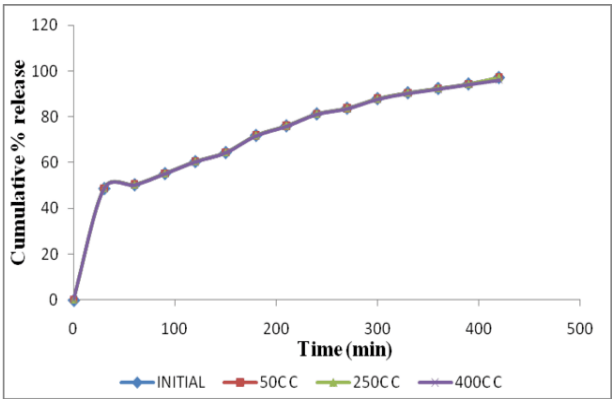


Fig No. 21. Average cumulative % release of Ketoconazole from medicated probiotic gel Containing *L. acidophilus* after 15 days

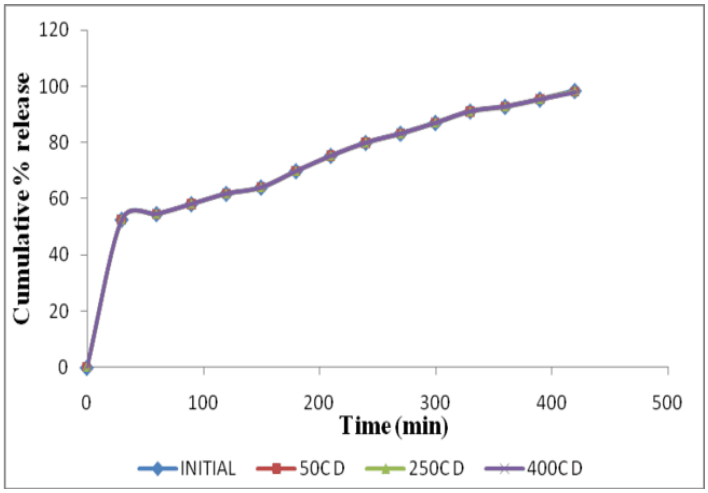


Fig No. 22. Average cumulative % release of Ketoconazole from medicated probiotic gel containing *L. rhamnosus* after 15 days

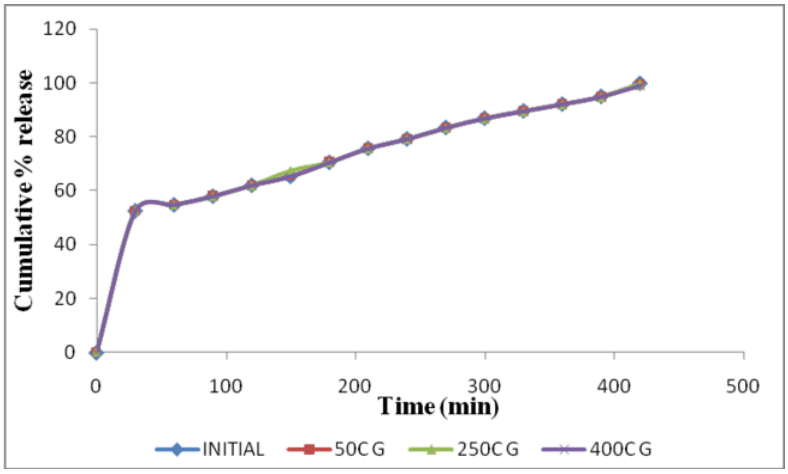


Fig No. 23. Average cumulative % release of Ketoconazole from medicated probiotic gel containing combination of *Lactobacilli* after 15 days

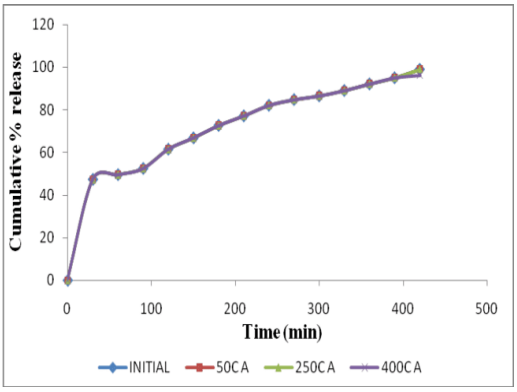


Fig No. 24. Average cumulative % release of Ketoconazole from medicated Probiotic gel containing *L. plantarum* after 30 days

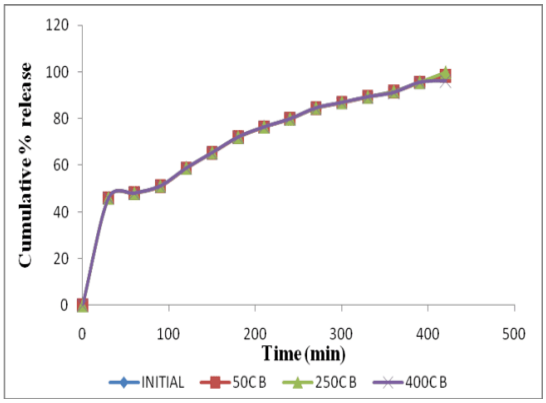


Fig No. 25. Average cumulative % release of Ketoconazole from medicated probiotic gel containing *L. casei* after 30 days

Table No. 26. Average cumulative % release of Ketoconazole from medicated Probiotic

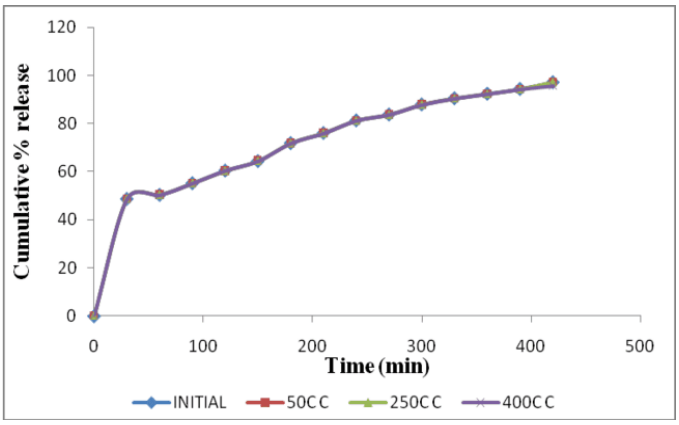


Fig No. 27. Average cumulative % release of Ketoconazole from medicated Probiotic gel containing *L. acidophilus* after 30days

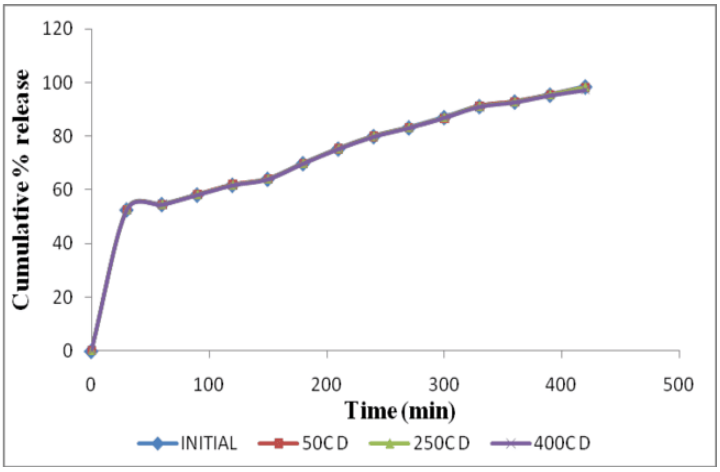


Fig No. 28. Average cumulative % release of Ketoconazole from medicated probiotic gel containing *L. rhamnosus* after 30 days

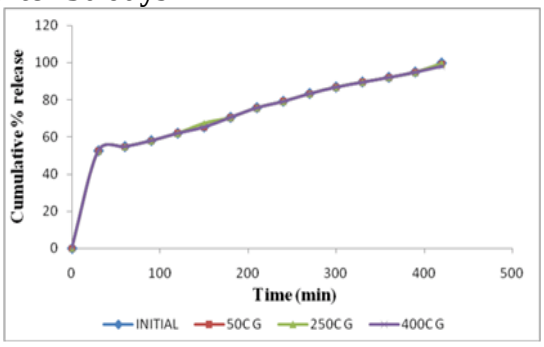


Fig No. 29. Average cumulative % release of Ketoconazole from medicated probiotic gel containing combination of *Lactobacilli* after 30 days

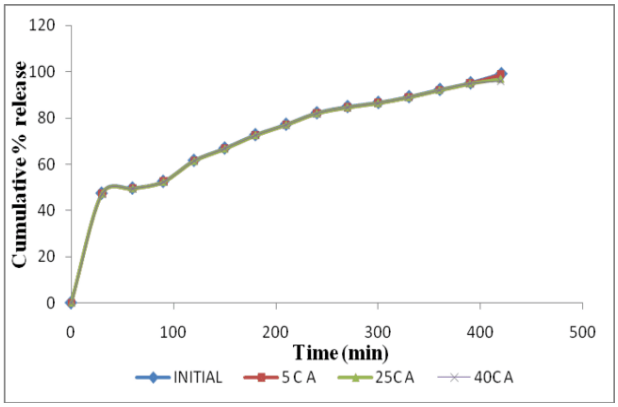


Fig No. 30. Average cumulative % release of Ketoconazole from medicated Probiotic gel containing *L. plantarum* after 45days

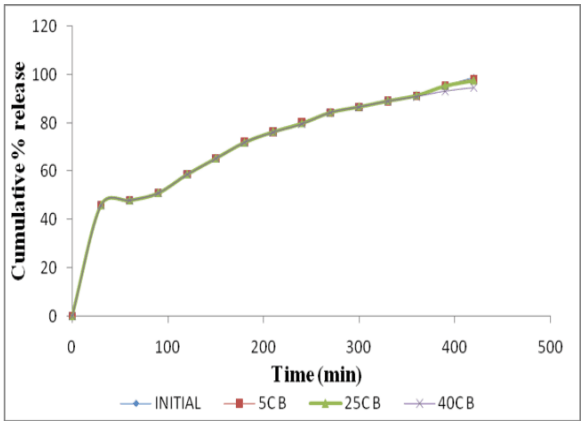


Fig No. 31. Average cumulative % release of Ketoconazole from medicated probiotic gel containing *L. casei* after 45days

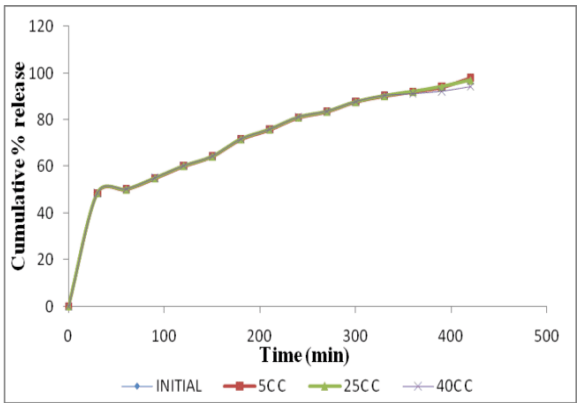


Fig No. 32. Average cumulative % release of Ketoconazole from medicated Probiotic gel containing *L. acidophilus* after 45days

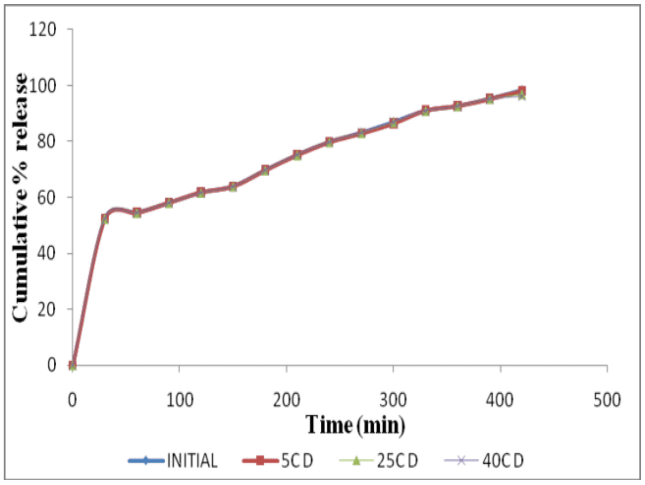


Fig No. 33. Average cumulative % release of Ketoconazole from medicated Probiotic gel containing *L. rhamnosus* after 45days

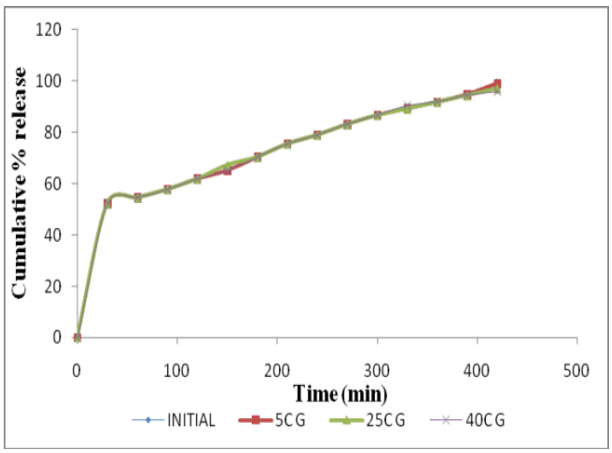


Fig No. 34. Average cumulative % release of Ketoconazole from medicated probiotic gel containing combination of *Lactobacilli* after 45

CONCLUSION

In the present work, a novel combination of Ketoconazole and the four *Lactobacillus* species (*L. plantarum, L casei, L acidophilus and L. rhamnosus*/LGG*)* was employed for the formulation of vaginal gels, which is meant for local application in the vagina. The experimental work included characterization of the bacterial cultures for suitability in the gel formulation and characterization of drug. Ketoconazole for its availability in the vaginal pH. The lyophilised powders of these cultures were incorporated in suitable proportions along with the drug Ketoconazole in suitable gel base formulae.

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