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**Research Article**

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# Antifungal Activity and Skin Irritation Study of Formulated Flutrimazole Gel

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**Abstract:** Fungal infections of the skin are a widespread dermatological concern, necessitating effective topical treatments with enhanced drug retention and minimal side effects. Flutrimazole, a broad-spectrum imidazole-class antifungal agent, is widely used for treating fungal infections; however, conventional formulations exhibit limitations such as poor skin retention and potential irritation. This study aimed to formulate and evaluate a Flutrimazole gel with a microsponge-based delivery system to improve drug penetration, sustain antifungal activity, and reduce skin irritation. The antifungal activity of the formulated Flutrimazole gel was assessed in vitro using the cup plate method against Candida albicans. Comparative analysis was conducted against free Flutrimazole gel and marketed formulations. Additionally, the Draize skin irritation test was performed on White New Zealand rabbits to evaluate the dermatological safety of the formulations. Statistical analysis, including ANOVA and Dunnett's Multiple Comparison Test, was used to determine significance levels.

**Keywords:** Flutrimazole, microsponge, antifungal activity, skin irritation, topical gel, dermatological safety.

## Introduction

Fungal infections of the skin are a prevalent dermatological concern, affecting millions of individuals worldwide. These infections, caused primarily by dermatophytes, yeasts, and molds, often lead to conditions such as athlete's foot, ringworm, and candidiasis [1-3]. The treatment of such infections requires effective antifungal agents with high skin penetration, prolonged activity, and minimal adverse effects. Flutrimazole, a broad-spectrum imidazole-class antifungal agent, has shown potent efficacy against various fungal strains by inhibiting ergosterol biosynthesis, a critical component of fungal cell membranes [4-6].

Despite its effectiveness, conventional formulations of Flutrimazole, such as creams and lotions, have limitations, including poor skin retention, rapid degradation, and potential irritation. To overcome these challenges, the development of a Flutrimazole gel formulation offers a promising alternative, providing better drug penetration, controlled release, and enhanced patient compliance. Gel-based formulations are advantageous due to their non-greasy nature, ease of application, and ability to form a uniform drug reservoir on the skin, ensuring sustained antifungal activity [7].

A crucial aspect of evaluating a topical formulation is assessing its antifungal efficacy and potential irritation to the skin [8-10]. The antifungal activity of the formulated Flutrimazole gel must be rigorously tested against common fungal pathogens to establish its therapeutic effectiveness. Simultaneously, a skin irritation study is essential to ensure the formulation is dermatologically safe, causing minimal adverse reactions upon prolonged use [11-12]. These studies are critical in optimizing the formulation to provide a balance between efficacy and safety.

This study aims to evaluate a Flutrimazole gel formulation earlier prepared [13] by analyzing its antifungal properties and skin irritation potential. The research involves in vitro antifungal assessments and in vivo dermatological safety evaluations to establish a patient-friendly, effective antifungal treatment for topical applications.

## Material And methods

**Table 1:** Microsponge formulations using Eudragit RS100 [13]

| Constituents | Flutrimazole Microsponges |
|---|---|
| **Inner phase** | |
| Flutrimazole | 2.5 |
| Eudragit RS 100 (g) | 0.19 |
| Methanol (mL) | 3 |
| **Outer phase** | |
| Distilled water (mL) | 200 |
| PVA 72000 (mg) | 50 |

### Antifungal Activity of Flutrimazole Gels

The antifungal activity of Flutrimazole from the optimum formula (microspongic-gels) as well as the free Flutrimazole and marketed formulations of the same were determined using Candida albicans as a representative fungus, adopting the cup plate method. The mean inhibition zone was calculated for each plate, and this value was taken as an indicator for the antifungal activity.

### Microbiological assay of Flutrimazole using cup plate method

A single well-isolated colony of Candida albicans of at least 1 mm in diameter was picked from the culture plate (Sabouraud dextrose agar) using a disposable plastic loop (10 μL) and suspended into a tube containing 10 mL of Sabouraud dextrose broth. The resulting suspension was mechanically shaken for 30 seconds, and then incubated at 35°C for 24 hrs. One mL of the inoculum was mixed with the melted Mueller-Hinton agar, then poured into a sterile petri dish, and allowed to solidify. Wells were made by using sterile cork-borers. Six concentrations of Flutrimazole were made by dissolving the desired amount of Flutrimazole in a sterile Dimethyl sulphoxide (DMSO). Each concentration was placed in each well, and the plates were incubated aerobically at 37°C for 24 hrs. After incubation, the inhibition zone diameter around each well was measured using a ruler, and a graph of inhibition zone versus drug concentration was plotted [14-17].

One gram each of free Flutrimazole gel and gel containing microspongic Flutrimazole and Flutrimazole marketed formulations were placed in each well with a control (blank gel). Mean inhibition zone of Flutrimazole released from 5 plates for each formula was calculated. Statistical analysis using ANOVA test followed by Dunnett's Multiple Comparison Test at level of significance of 0.05 was carried out to determine the degree of significance between the test and the reference standard.

### Safety Considerations (Draize Skin Irritation Testing)

The irritation potential of the gels containing free drug and drugs entrapped in microsponges were evaluated in comparison to marketed gel by carrying out the Draize patch test on rabbits. Animal care and handling throughout the experimental procedure was performed in accordance to the CPCSEA guidelines. The experimental protocol was approved by the Institutional Animal Ethical Committee (N. 1709/RcBi/S/13/CPCSEA/246). White New Zealand rabbits weighing 2.5-3 kg were obtained and acclimatized before the beginning of the study.

### Primary Dermal Irritation Test

### Rabbit screening procedure

A group of at least 6 White rabbits were screened for the study.

All rabbits selected for the study were in good health (rabbit exhibiting snuffles, hair loss, loose stools, or apparent weight loss was rejected and replaced).

18 hrs. prior to application of the test substance, each rabbit was prepared by clipping the hair from the back and sides using a small animal clipper.

Six animals with skin sites that were free from hyperemia or abrasion, due to shaving were selected for each group.

**Study procedure**

Four areas of skin, two on each side of the rabbit's back, were utilized for sites of application.

Each animal serves as its own control.

Besides the test substance (marketed/in-house gels containing free drug and gels containing drug entrapped in microsponges), a positive control substance (a known skin irritant, formalin) and a negative control (untreated patch) were applied to the skin.

The four intact (flee of abrasion) sites of administration were assigned a code number as Site 1 (Positive control), Site 2 (Flutrimazole microspongic gel), Site 3 (Marketed product) and Site 4 (Negative control) have 6 animals in each group.

The pattern of administration makes certain that the test substance and controls were applied to each position at least once. Each test or control substance was held in place with a 1 X 1 sq. in. 12-ply surgical gauze patch. The gauze patch was applied to the appropriate skin site and secured with 1 in. wide strips of surgical tape at the four edges, leaving the center of the gauze patch non-occluded. 0.5 g of gel was weighed and placed on the gauze patch. The test substance patch was placed on the appropriate skin site and secured. The patch was subsequently moistened with 0.5 mL of physiological saline. The negative control site was covered with an untreated 12 -ply surgical gauze patch (1 X1 sq. in). The positive control substance and vehicle control substance were applied to a gauze patch in the same manner. The entire trunk of the animal was covered with an impervious material for a 24 hrs. period of exposure, secured by wrapping several long strips of athletic adhesive tape around the trunk of the animal. The impervious material aids in maintaining the position of the patches and retards evaporation of volatile test substances. An Elizabethan collar was fitted and fastened around the neck of each test animal. The collar remains in place for 24 hrs. exposure period. The collars were utilized to prevent removal of wrappings and patches by the animals, while allowing the animal's food and water ad libitum. The wrapping was removed at the end of the 24 hrs. exposure period. The test substance skin site was wiped to remove any test substance still remaining. Immediately after removal of the patches, each 1 X 1 sq. in. test or control site was outlined with an indelible marker by dotting each of the four corners [18-20]. This procedure delineates the site for identification.

**Observations**

Observations were made of the test and control skin sites 1 hr. after removal of the patches (25 hrs. post-initiation of application). Erythema and edema were evaluated and scored on the basis of the designated values presented in Table 2.

**Table 2:** Evaluation of skin reactions

| Skin Reaction | Value |
|---|---|
| **Erythema and eschar formation** | |
| No erythema | 0 |
| Very slight erythema (barely perceptible) | 1 |
| Well-defined erythema | 2 |
| Moderate to severe erythema | 3 |
| Severe erythema (beet redness) to slight eschar formation(injuries in depth) | 4 |
| Necrosis (death of tissue) | +N |
| Eschar (sloughing or scab formation) | +E |
| **Edema formation** | |
| No edema | 0 |
| Very slight edema (barely perceptible) | 1 |
| Slight edema (edges of area well defined by definite raising) | 2 |

| | |
|---|---|
| Moderate edema (raised approximately 1 mm) | 3 |
| Severe edema (raised more than 1 mm and extending beyond the area of exposure) | 4 |
| Total possible score of primary irritation | 8 |

Observations were made again at 48 and 72 hrs. after application and scores were recorded. If necrosis was present or the dermal reaction needs description, the reaction should be described. Necrosis should receive the maximum score for erythema and eschar formation (4) with a (+N) to designate necrosis. When a test substance produces dermal irritation that persists for 72 hrs. post- application, daily observations of test and control sites were continued on all animals until all irritation caused by the test substance resolves or until Day 14 post-application.

**Evaluation of Results**

A subtotal irritation value for erythema or eschar formation was determined for each rabbit by adding the values observed at 25, 48, and 72 hrs. of post application. A subtotal irritation value for edema formation was determined for each rabbit by adding the values observed at 25, 48, and 72 hrs. of post application. A total irritation score was calculated for each rabbit by adding the subtotal irritation value for erythema or eschar formation to the subtotal irritation value for edema formation. The primary dermal irritation index (PDII) was calculated for the test substance or control substance by dividing the sum of total irritation scores by the number of observations, 18 (3 days X 6 animals =18 observations).

**Table 3:** The categorization of dermal irritation; modification of the original classification described by Draize (1944)

| Score (PDII) | Interpretation |
|---|---|
| 0.0 | Non irritant |
| >0.0 and ≤ 0.5 | Negligible irritant |
| >0.5 and ≤2.0 | Mild irritant |
| >2.0 and ≤5.0 | Moderate irritant |
| >5.0 and ≤ 8.0 | Severe irritant |

**Results and Discussion**

**Antifungal Activity of Flutrimazole Gels**

Standard calibration curve of Flutrimazole using cup plate method

**Table 4:** Zone of inhibitions for standard Flutrimazole for calibration curve

| Concentration of Flutrimazole (µg/mL) | Zone of inhibition in mm | | | | | Mean ± SD |
|---|---|---|---|---|---|---|
| 500 | 9.2 | 10.3 | 9.5 | 9.7 | 9.4 | 9.2±0.42 |
| 750 | 12.6 | 13.4 | 12.6 | 12.5 | 13.6 | 12.5±0.52 |
| 1000 | 15.5 | 15.6 | 15.7 | 15.9 | 15.5 | 15.5±0.17 |
| 1250 | 18.3 | 17.9 | 17.8 | 18.4 | 17.8 | 17.8±0.29 |
| 1500 | 20.6 | 20.5 | 20.5 | 20.6 | 20.6 | 20.5±0.05 |
| 1750 | 23.7 | 23.4 | 23.8 | 22.8 | 23.9 | 22.8±0.44 |
| 2000 | 26.5 | 25.9 | 26.9 | 26.7 | 25.8 | 25.8±0.49 |

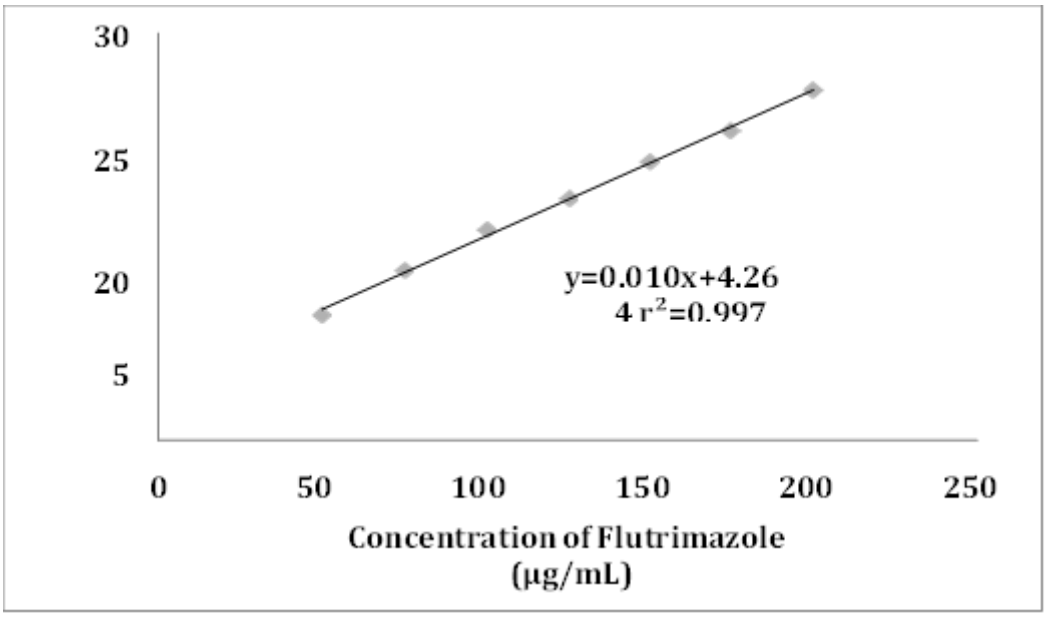


***Figure 1:*** *Standard curve for antifungal assay of Flutrimazole gels with coefficient of correlation 0.997*

**Table 5:** Anti-fungal activity of microspongic gels in comparison to reference standard using Candida albicans (n=5), P value (<0.05)

| Formulation | Zone diameters (mm) | | | | | Mean zone diameter ±SD (mm) |
|---|---|---|---|---|---|---|
| Flutrimazole | 18 | 19 | 21 | 18 | 22 | 18±1.82 |
| Marketed Formulation | | | | | | |
| Free Flutrimazole gel | 17 | 21 | 22 | 19 | 18 | 17±2.07 |
| F7gel | 22 | 23 | 26 | 24 | 25 | 22±1.58 |

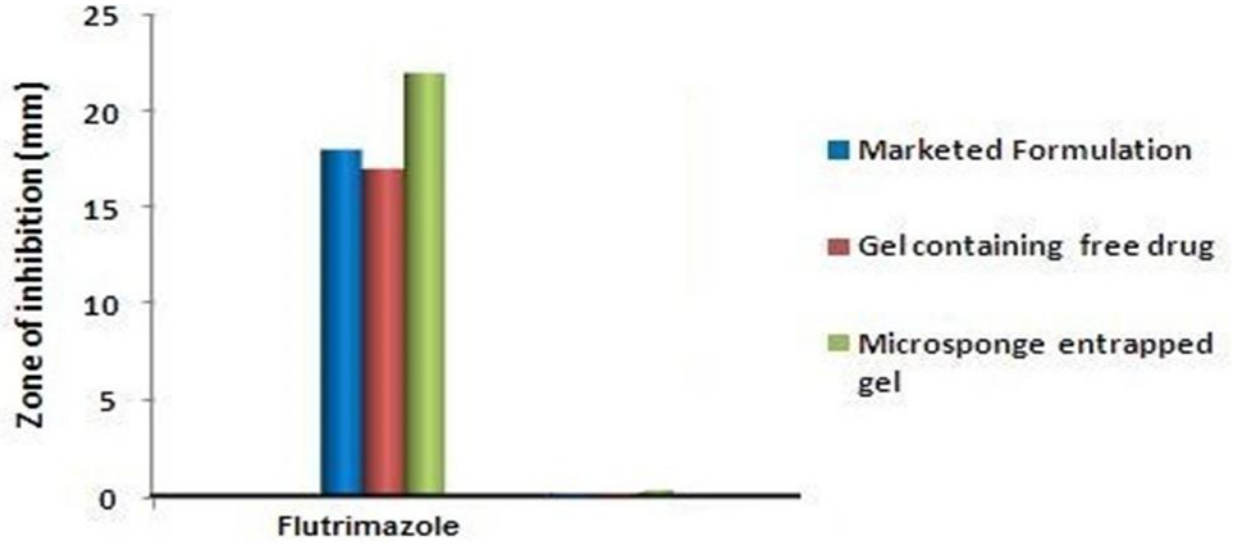


*Figure 2: Comparative Zone of inhibition of Flutrimazole Marketed formulation, Gel containing free drug and Microsponge entrapped gel*

Table 5 and Figure 2 manifested that the antifungal activity of tested microspongic gels was larger than that of the gels containing free drug and marketed formulation. The ANOVA and Dunnett's Multiple Comparision Test showed

that there was a significant difference in the microspongic gel zone of inhibition in comparison to the gels containing free drug and marketed formulation at P<0.

**Safety Considerations (Draize Skin Irritation Test)**

Figure 3 shows photographs of primary skin irritation studies where Site 1 indicates positive control; Site 2 indicates test drug entrapped in the microsponges; Site 3 indicates marketed product and Site 4 indicates negative control.

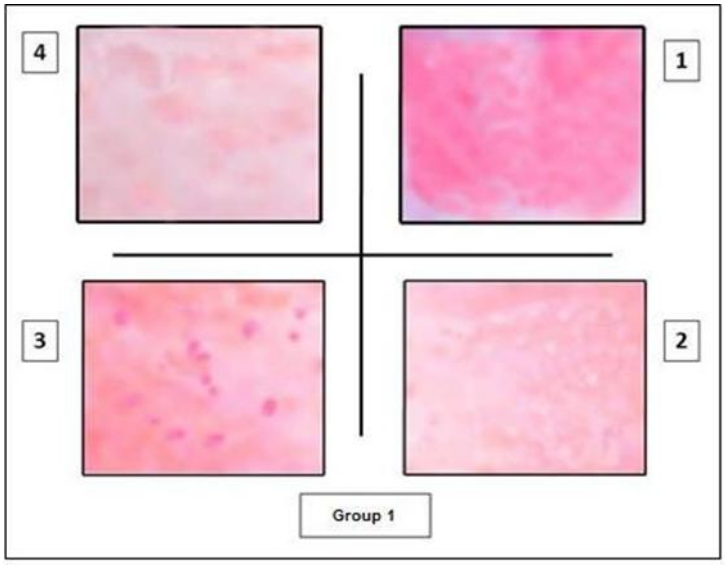


*Figure 3: Photographs of skin irritation studies carried out on rabbits*

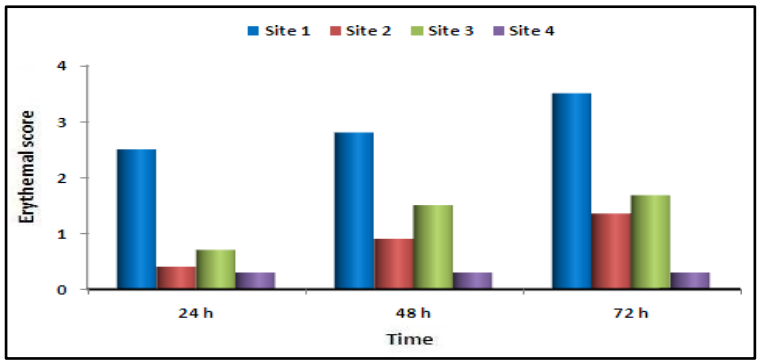


*Figure 4: Erythema scores observed for Flutrimazole formulations, recorded at 24, 48 and 72 hrs.*

PDIIs of all the marketed gels and gels containing entrapped drug are given in Figure 4. It was observed that the marketed gel shows more irritation than all the gels containing drug entrapped in microsponge drug delivery system.

**Conclusion**

Gels containing microsponges encapsulating flutrimazole showed retention of anti-fungal properties and were compared to gels containing free drug and commercial samples. Antifungal assays indicated that microsponges loaded gels maintained or improved efficacy relative to free drug gels and commercial formulations, along with reduced skin irritation.

These findings highlight the potential of microsponge delivery systems for the topical administration of Flutrimazole, offering controlled release, reduced irritation, and potentially enhanced therapeutic outcomes.

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