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# FORMULATION AND *IN-VITRO* EVALUATION OF ORAL HYDROGEL CONTAINING MICONAZOLE NITRATE

**Umesh T. Jadhao*, Bharat W. Tekade, Rupesh Patil, Amol. P. Patil., Vijay R. Patil**

**TVES's Honourable Loksevak Madhukarrao Chaudhary College of Pharmacy, Faizpur**

*Corresponding Author's E mail: Umj81@rediffmail.com

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**ABSTRACT**

The aim of the present work was to formulate and evaluate formulation of Miconazole nitrate as oral hydrogel to improve the efficacy & bioavailability of Miconazole nitrate. Miconazole Nitrate gel was prepared with Gellan gum and Carbopol 934P as gelling agents with polyethylene glycol as a penetration enhancer. The formulations were examined for pH, spreadability, consistency, viscosity, homogeneity, drug content and stability. In vitro drug release was evaluated using Franz diffusion cell. The viscosity of all formulation follows a pseudo-plastic flow behaviour. The pH of formulation Lies in between 6.3 to 7.2. The formulation C2 and G2 showed good released pattern. The formulation shown good resistance of bacterial growth as compared to the other formulation antifungal activity was more potent on fungal growth by the formulation G2 and C3.

**Keywords:** Miconazole nitrate, Gellan gum, antifungal, Rheology, *in- vitro*; Diffusion.

## INTRODUCTION

Hydrogels, cross-linked 3D networks of hydrophilic polymer chains, are capable of holding large amounts of water due to their hydrophilic structure.[1] Thus, the hydrogel networks can extensively swell in water media. Since water is the greatest component of the human body, a hydrogel, which can absorb large quantities of water, is considered to have great potential when applied for biomedical purposes. Recently, wide investigation has been going on into the feasibility of applying hydrogels in fields including tissue engineering, drug delivery, self-healing materials, biosensors, and hemostasis bandages. Compared with other types of biomaterials, hydrogels have the advantages of increased biocompatibility, tunable biodegradability, properly mechanical strength, porous structure, and so on. However, due to the low mechanical strength and fragile nature of the hydrogels, the feasibility of applying hydrogels is still limited. Thus, novel hydrogels with strong and more stable properties are still needed and remain an important direction for research.

As expected, naturally formed hydrogels are gradually replaced by synthetic hydrogels to achieve longer service life, high capacity of water absorption, and high gel strength. Fortunately, with various developed synthetic strategies, hydrogels with defined network structures, desirable chemical compositions, and mechanical strength can be designed. Hydrogels can be prepared from completely artificial components and show remarkable stability even under severe conditions such as high temperature or a very acidic or basic environment. Additionally, by modifying the polymer chains with stimuli-responsive functional groups, the hydrogel properties can be switched by stimuli including heat, light, magnetic fields, chemical agents, and pH.

Miconazole interacts with 14-α demethylase, a cytochrome P-450 enzyme necessary to convert lanosterol to ergosterol. As ergosterol is an essential component of the fungal cell membrane, inhibition of its synthesis results in increased cellular permeability causing leakage of cellular contents. Miconazole may also inhibit endogenous respiration, interact with membrane phospholipids, inhibit the transformation of yeasts to mycelial forms, inhibit purine uptake, and impair triglyceride and/or phospholipid biosynthesis.[2,3]

## MATERIAL AND METHOD

### Material

Miconazole nitrate was obtained as kind gift sample from Mahrshee Laboratories Pvt. Ltd.Mumbai., Carbopol 934 was purchased from Vishal-Chem, Mumbai, Gellan gum was purchased from Research-Lab Fine Chem Industries, Mumbai. All other materials used of analytical grades.

### Methods

### Preparation of Miconazole oral hydrogel

Weight quantity of carbopol and gellan gum were measured and allowed to swell in a beaker for 24hrs. Accurately weight amount of drug solubilized in PEG was then added to soaked polymer solution with stirring; then the solution was neutralized by using Triethanolamine, Glycerin as a moistening agent and ascorbic acid with methyl paraben as preservative to then formulation.[4]

**Table No. 1: Formulation chart of Carbopol and Gellan Gum Batches.**

| Ingredient | C1 | C2 | C3 | G1 | G2 | G3 | GC |
|---|---|---|---|---|---|---|---|
| Miconazole Nitrate(mg) | 300 | 300 | 300 | 300 | 300 | 300 | 300 |
| Carbopol (mg) | 1000 | 1000 | 1000 | -- | -- | -- | 500 |
| Gellan gum (mg) | -- | -- | -- | 1000 | 1000 | 1000 | 500 |
| Ascorbic Acid (mg) | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| Glcerine (mg) | 0.5 | 1 | 1.5 | -- | -- | -- | 1 |
| Polyethylene glycol (ml) | -- | -- | -- | 0.5 | 1 | 1.5 | 0.5 |
| Methyl paraben (mg) | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 |
| Triethanolamine(ml) | q.s. | q.s. | q.s. | -- | -- | -- | q.s. |
| Water(ml) | q.s. | q.s. | q.s. | q.s. | q.s. | q.s. | q.s. |

## EVALUATION OF TOPICAL PREPARATION:

The factors to be evaluated for semisolid at various stages of its development are described below. These must be within prescribed specification, and all must remain so over the stated lifetime for the product.

### Organoleptic properties:

Colour, odour, and appearance may get changed on storage. The change in properties indicates decomposition, so it is necessary to check the organoleptic properties of gel formulation.[5]

### pH:

The pH of gel is measured, using a pH meter, which was calibrated before each use with standard buffer solution. The electrode was inserting into the sample prior to taking the reading at room temperature. The determinations were carried out in triplicate and the average of three reading is recorded.[6]

### Spredability:

The spreadability of the gel was determined using the following technique; spreadability was measured on the basis of 'Slip' and 'Drag' characteristics of gels. A ground glass slide was fixed on this block. An excess of gel (about 2 gm) under study was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. A 1 Kg weight was placed on the top of the two slides for 5 minutes to expel air and to provide

a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 80 gms. With the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5 cm be noted. A shorter interval indicates better Spreadability

calculation of spreadability (S) is as follows;

$$S = M \times L / T$$

Where:

S, is the spreadabliy of gel formations

M, is the weight (g) tied on the upper plate,

L, is the length (cm) of the glass plates, and

T, is the time taken for plate to slide the entire length.

**Drug content (%):**

Accurately weight amount of gel was mixed with methanol and allowed to stand for 24 hrs and filter the solution and making the appropriate concentration is analysed spectrophotometrically to determine percentage drug content.[7]

**Viscosity:**

Time dependent rheological behavior of semisolids may also signal physical or chemical change. The tools for rheological assessments are cone and plate research viscometer, which, in principle, precisely quantify viscosity, or simply utilitarian rheometers, that include extrusion rheometers, penetrometers and Brookfeild viscometers with spindle helically through a semisolid. Increase or decreases in viscosity by any of these measuring tools indicate changes in the structural elements of the formulation. Substantial irreversible rheological changes are a sign of poor physical stability. [8,9]

**Rheological properties:**

The Rheological property is determined by Brook-field viscometer. Oral hydrogel system containing polymers in various ratio and combinations such as gellan gum and carbopol was prepared and evaluated viscosity in order to identify the composition suitable for hydrogel systems. Many experiments were conducted by varying the concentration of these polymers in order to identify the optimum concentration required for the gel. The hydrogel system containing gellan gum and carbopol are improving the rheology.

Rheological properties of the delivery system may be achieved by the addition of viscosity enhancing polymers such as gellan gum and pH. It also helped the gels for its adhesion. All the formulations were behaving as shear thinning systems. [10]

**Gelling capacity:**

The gelling capacity was determined by placing a drop of the system in a vial containing 2 ml of simulated salivary fluid (pH 6.8) freshly prepared and equilibrated at 37ºC and visually assessing the gel formation and nothing the time taken for the gelation formed and the time taken for the gel formed dissolve. Weight and rate of formation of gel with respect to time. Average of three reading; - No gelation; + Gel after few minutes, dissolved rapidly; ++ Gelation immediately, remains for few hours; +++ Gelation immediately, remains for extended period. [11]

**Extrudability:**

it is useful empirical test to the measure the force required to extrude the materials from a tube. Since the packing of have a gained a considerable importance in delivery of desired quantity of gel from collapsible tube, therefore measurement of extrudability becomes an important criteria for oral gel. Evaluating gel formulation for extrudability was based upon the quantity in percentage of gel and gel extruded from lacquered aluminum collapsible tube on application of weight in grams required to extrude at least 0.5 cm ribbon of gel in 10 seconds. More quantity extruded better was extrudability. The measurement of extrudability of each formulation was in triplicate and the average values are presented. The extrudability was than calculated by using the following formula:

**Extrudability = Applied weight to extrude gel from tube (in gm) / Area (in $cm^2$)**

***In-vitro* permeation study:**

The cellophane membrane was mounted between the compartments of the Frantz diffusion cell with stratum corneum facing the donor compartment. Reservoir compartment was filled with 10 ml artificial saliva of pH 7.2. Franz diffusion cell with cellophane mounted between compartments. The study was carried out at 37° ± 1°C and speed was adjusted untill the vortex touches the membrane and it carried out for 20mins with the 1 ml of sample was withdrawn from reservoir compartment at 2 min interval and absorbance was measured spectrophotometrically at 230 nm. Each time the reservoir compartment was replenished with the diffusion medium to maintain constant volume. [12]

### *In-vitro* antifungal activity

Agar cup-plate method was adopted for this study. Different concentrations of Miconazole Nitrate in DMSO (1%, 0.5%, 0.1%, 0.05% and 0.01%) were use to study in-vitro antifungal activity of Miconazole Nitrate against Candida albicans. Also the solvent use; DMSO was tested as positive growth control. A single isolate of each fungus was picked from the agar slab culture to prepare spores suspension in sterile water and was adjusted to the 1 x 106 spores/ml. one ml of spores suspension was mixed with saboureaud agar(15-20 ml ) in sterile petri dish (9cm in diameter ) and the agar plate were allow to solidify after solidification a signal well was Made in each agar plate using a porer of size 5 mm and filled with 50µl of the specified concentration of miconazole solution using DMSO as control solution. The plate was incubated at 25± 1 º C for 3 days (for candida isolates) and (pseudomonas auregenosa) and then they were examined for the inhibition zone diameter which is and indicator for antifungal activity.

The selected gel formulation that showed the best release was subjected to this study. The formulation was tested for its in-vitro release was miconazole nitrate to the agar and its antifungal activity against the send to fungi using the same method. Plane gel formulation (without drug) was also tested as a positive growth control result. The same methodology as mention previously was repeated with changing the diameter of the wells to 1cm and filling them with an accurately weighed 0.5gm each formula (either medicated or plain). The mean value of the inhibition zone diameter from 3 plates was calculated. [13]

### Accelerated stability study of gel formulation

It is responsibility of manufacturers to see that the medicine reaches the consumer in an active form. Stability of medicinal product may be define as the capability of particular formulation in a specific container to remain with its physical, chemical, microbial, therapeutic and toxicological specification, i.e. stability of drug is ability to resist deterioration. 90% of labelled potency is generally recognized as the minimum acceptable potency level. So stability study was carried out for three months at 40º C/ 75 % RH. [14]

## RESULT AND DISCUSSION

### Evaluation and characterization of Gel

### Appearance:

The gel formulations were observed for their visual appearance, odour, colour, texture and feel upon application such as grittiness, uniformity, and stickiness of formulation. Result shown in table No. 2

**Table No. 2: physical Evaluation of Gel formulations**

| Properties Formulation | Colour | Odour | Consistency | Appearance | Grittiness | Stikiness |
|---|---|---|---|---|---|---|
| C1 | White | Characteristic | Good | Smooth | No | None |
| C2 | White | Characteristic | Good | Smooth | No | None |
| C3 | White | Characteristic | Good | Smooth | No | None |
| G1 | White | Characteristic | Good | Smooth | No | None |
| G2 | White | Characteristic | Good | Smooth | No | None |
| G3 | White | characteristic | Good | Smooth | No | None |
| GC | White | characteristic | Good | Smooth | No | None |

The values of spreadability indicate that all gels are easily spreadable by applying just a small amount of shear. Result of homogeneity all formulation of Miconazole Nitrate oral hydrogel posses the homogeneous in nature. pH measurement is very important because the pH of the formulation should not deviate from oral pH The pH of prepared Miconazole gel was measured using a pH meter. Result of Gelling capacity are shown in the Table no. 3 grades were allotted (+ + +Excellent, + + Good, + fair) All the gel formulation were also evaluated for spreadability test it was found in the range 53 to 71 mm. the highest and good spreadability of gel C2 gel formulation is 71 mm. and lowest Spreadability value is G3 formulation is 54 mm.

**Table No.3: Spreadability, pH, Homogeneity, Extrudability, Drug content of Gel formulations.**

| Batch | Spreadability (mm) | Homogeneity | pH | Drug content | Extrudability (g) | Gelling Capcity |
|---|---|---|---|---|---|---|
| C1 | 68 | Homogeneous | 6.9 | 99.92±0.23 | 165 | ++ |
| C2 | 71 | Homogeneous | 6.9 | 99.91±0.62 | 149 | ++ |
| C3 | 70 | Homogeneous | 6.9 | 98.99±0.51 | 150 | +++ |
| G1 | 60 | Homogeneous | 6.9 | 99.89 ±0.12 | 129 | ++ |
| G2 | 63 | Homogeneous | 7.1 | 99.90±0.27 | 158 | ++ |
| G3 | 54 | Homogeneous | 7.1 | 99.91±0.18 | 162 | +++ |
| GC | 67 | Homogeneous | 7.2 | 99.93±0.48 | 160 | ++ |

Drug content was determined by titrimetric method which was found within the limits. The viscosity of formulated gel was determined. The viscosity determination was carried out by Brook-field viscometer.

**Table No. 4: Viscosity of gel (cps)**

| RPM | C1 | C2 | C3 | G1 | G2 | G3 | GC |
|---|---|---|---|---|---|---|---|
| 1 | 7498 | 24220 | 19121 | 7499 | 23450 | 18121 | 10987 |
| 2 | 3842 | 11151 | 12641 | 3937 | 11051 | 13647 | 3897 |
| 5 | 2630 | 7438 | 4258 | 2598 | 7338 | 4357 | 2678 |
| 10 | 1507 | 3637 | 2354 | 1577 | 3530 | 2458 | 1687 |
| 20 | 1057 | 1186 | 1856 | 1134 | 1167 | 1899 | 1184 |
| 30 | 653 | 906 | 1120 | 657 | 950 | 1124 | 797 |
| 60 | 409 | 419 | 468 | 450 | 461 | 470 | 580 |

The viscosity of all formulation follows a pseudoplastic flow behavior. The material flows as soon as a shear stress is applied; the slope of the curve gradually decreases with increasing rate of shear. The viscosity was derived from the slope which is found to decrease as the shear rate is increased.

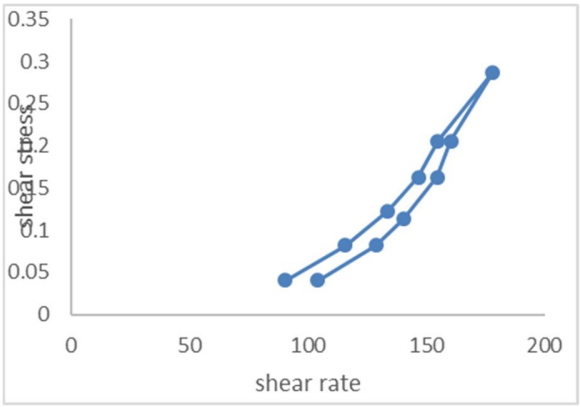


**Figure No. 1: Rheogram of C1**

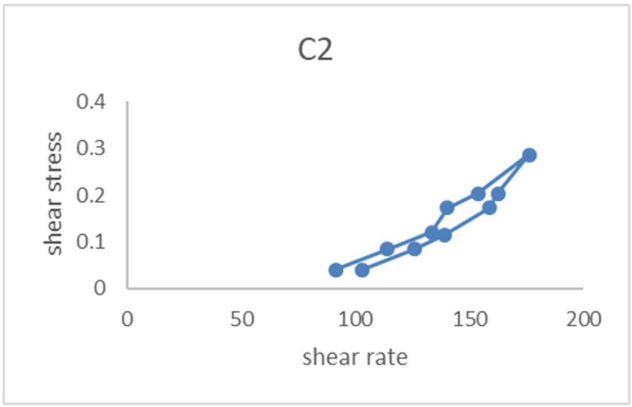


**Figure No. 2: Rheogram of C2**

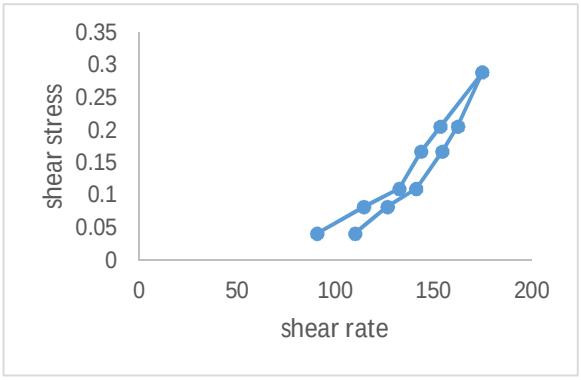


**Figure No. 3 : Rheogram of C3**

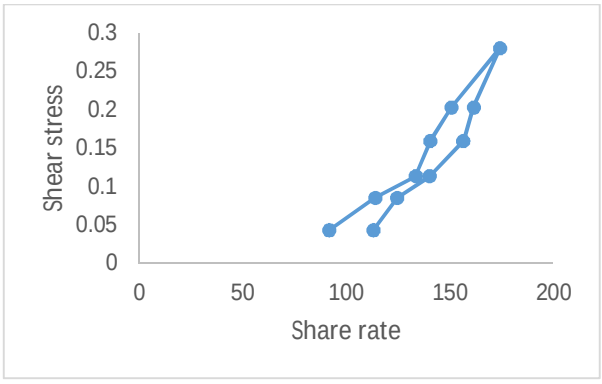


**Figure No. 4: Rheogram of G1**

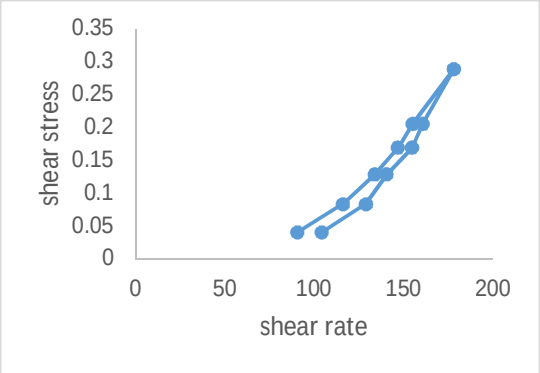


**Figure No. 5: Rheogram of G2**

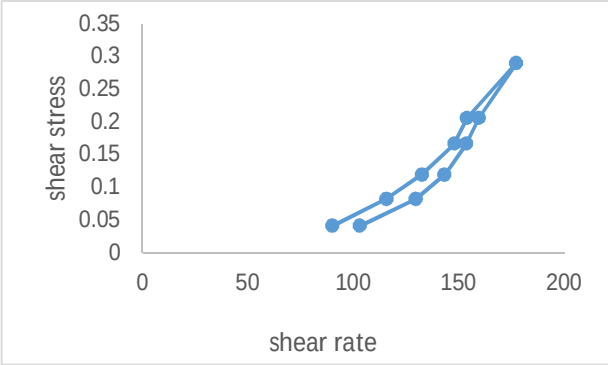


**Figure No.6: Rheogram of G3**

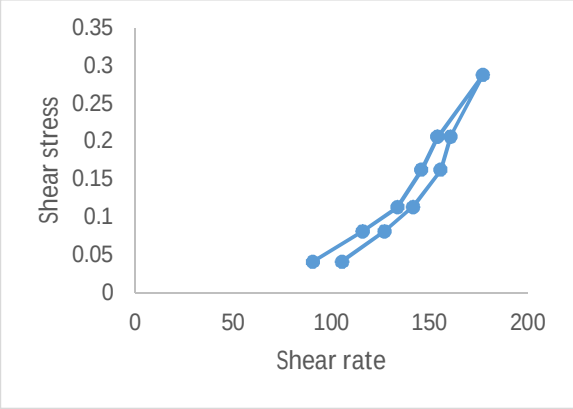


**Figure No. 7: Rheogram of GC**

## In-Vitro diffusion study

The In-vitro diffusion study was taken by using Franz diffusion cell which shows Cumulative % drug release of Miconazole nitrate gel formulation was C1-91.84±0.62, C2- 94.99±0.16, C3-92.84±0.37, G1-93.62±0.27, G2-95.23±0.39, G3-92.84±0.40 And GC- 92.23±0.12 The formulation C2 and G2 showed good released pattern.

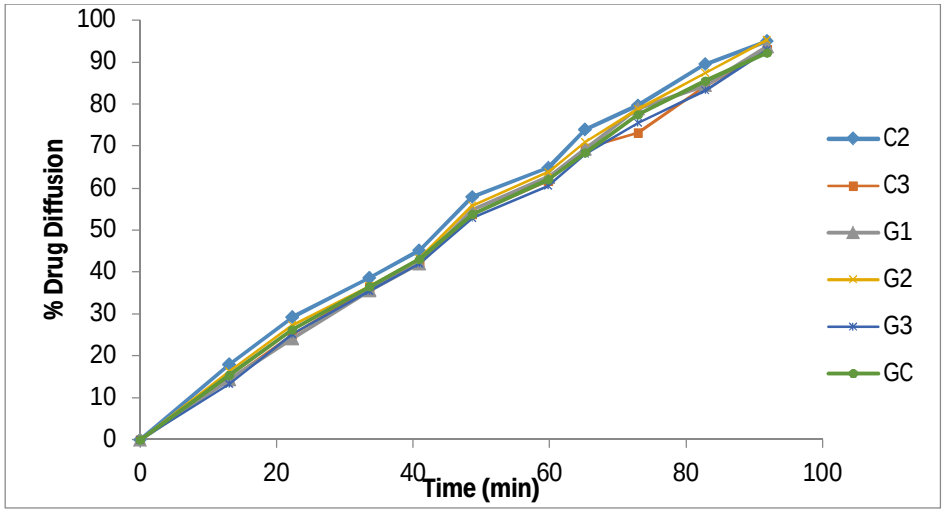


**Figure No. 8: In-vitro release of Gel.**

### In-Vitro Antifungal Activity

Agar cup-plate method was adopted for this study. Different concentration of Miconazole Nitrate in DMSO (1%, 0.5%, 0.1%, and 0.01%) were used to study the in vitro antifungal activity of Miconazole against candida albicans. Also the solvent used; DMSO was tested as positive growth control. The formulation shown good resistance of bacterial growth as compared to the other formulation antifungal activity was more potent on fungal growth by the formulation G2 and C3.

### Accelerated Stability Testing

The formulation was kept for stability studies there was no significance change observed in physical parameter i.e. (appearance colour change and grittiness) at 40º C±2 / 75%± RH. There was negligible difference in the drug content observed after stability study suggested that all the formulations are stable under the given conditions for 60 days.

**Table No. 5: Result of accelerated stability testing**

| **Days** | **0** | **15** | **30** | **45** | **60** |
|---|---|---|---|---|---|
| **Colour** | No Change | No Change | No Change | No Change | No Change |
| **Drug Content** | 99.93 ± 0.48 | 99.80 ± 0.51 | 99.23 ± 0.16 | 98.93 ± 0.16 | 98.13 ± 0.13 |
| **%Drug Release** | 92.23 ± 0.12 | 92.13 ± 0.72 | 91.80 ± 0.52 | 91.63 ± 0.62 | 91.23 ± 0.42 |
| **Spreadability (mm)** | 65 | 66 | 64 | 63 | 62 |

## CONCLUSION

As per the results of the present study showed that the permeation rate of formulation C2 and G2 was enhanced without any significant change in the pH, Viscosity, Spreadability and drug content and Gelling capacity. The formulations G2 and C3 have good significant level during antifungal study. The C1 and GC formulation are show the good drug content Miconazole nitrate oral hydrogel. It was more worthwhile to evaluate the formulation at the clinical level as a further study.

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