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ORIGINAL ARTICLE

Formulation Development and Evaluation of Mustard Oil Based Organogel for Effective Topical Delivery of Clotrimazole

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ABSTRACT
The purpose of the study was to develop mustard oil-based organogels for topical administration in order to ensure effective transport of Clotrimazole deeper into the skin layers. The hot-melt procedure was used to develop four formulations containing Sorbitan Monostearate, Clotrimazole, and Mustard Oil. Macro evaluations, pH, spreadability, viscosity, and in-vitro diffusion investigations are performed on formulated formulations. An improved formulation's antifungal efficacy was tested in vitro. Because of the findings of the investigated parameters, the created mustard oil-based organogels are stable and effective. According to in-vitro diffusion research, drug release increases when surfactant concentration rises due to viscosity. Furthermore, in an in-vitro antifungal study, the enhanced formulation inhibited more fungal strains than the control and commercially available product.
Keywords: *Clotrimazole, Mustard oil, Organogel, Antifungal, Diffusion Study.*

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INTRODUCTION

The USP defines gel as a formulation in a base that is water soluble and can be thought of as a greaseless ointment. Depending on the type of liquid component, gels are broadly divided into two categories: organogels and hydrogels. Hydrogel is the name given to a gel when a polar solvent is utilised as the gel's solvent. Organogel is a term used to describe a gel that contains a non-polar solvent. Organogel is defined as a cross-linked, soft matrix that contains a significant proportion of organic or lipophilic solvent. The current work uses mustard oil as the oil phase, while sorbitan monostearate serves as the organogelator to create the organogel [1-5]. Globally, the prevalence of fungal infection has increased; twenty million people survive with significant morbidity and around 300 million people are affected by superficial fungal illness. Particularly in industrialized and developing nations, fungus infection decreased the patient's immune system and its most susceptible disease. Numerous topical creams and ointments with antifungal marketing formulations are available. The benefits of topical treatment for a fungus infection include concentrating on the infection site, lowering the possibility of systemic side effects, improving treatment effectiveness, and high patient compliance. Different types of topical antifungal chemical moieties are commercially accessible in conventional dose forms as cream, gels, lotions, and sprays; however, these have a number of drawbacks [6-10]. Clotrimazole, an imidazole antifungal drug is commonly used to treat candida infections of the vaginal, oral, and cutaneous tracts. Clotrimazole is often taken orally or intravenously. Lotrimin is the brand name for the drug Clotrimazole. It is used to treat ringworm, oral thrush, diaper rash, and vaginal yeast infections, among other things [11-12].

MATERIAL AND METHODS

Clotrimazole was purchased from Glenmark Pharmaceuticals, Sinnar, Nashik. Mustard oil is purchased from local market. KBHSS Trust Institute of Pharmacy, Malegaon provides Span 60. The only additional chemicals and reagents utilized were analytical grade.

Formulation of Mustard oil based Organogel

The hot-melt approach was used to generate mustard oil organogels. Table 1 shows the composition of four formulations generated by maintaining a constant concentration of clotrimazole. Formulations were developed by varying the percentage concentrations of mustard oil and organogelators. The required amount of sorbitan monostearate (Span 60) was added to the beaker, which was then heated at 60°C on a thermostatically controlled magnetic stirrer at 400 rpm. Clotrimazole was added to the heated SMS after being dissolved in mustard oil and constantly stirred until a homogeneous dispersion was obtained. After 24 hours at room temperature (RT), the formulations were subjected to macroscopic and other evaluations.

Table 1: Formulation Table

Formulation code	Drug (%)	Span 60 (%)	Mustard oil Up to (%)
F1	1	05	100
F2	1	10	100
F3	1	15	100
F4	1	20	100

Evaluation of Mustard oil based Organogel

pH measurement

The pH of different gel formulations was calculated using a digital pH meter. A 1% aqueous solution of the gel formulation was prepared, stored for two hours, and then analyzed to determine the pH. The pH level of each formulation was measured three times, and the average value and standard deviation were calculated.

Viscosity measurement

A Brookfield digital viscometer was used to measure the viscosity of the formed gel compositions. The gel sample was loaded to spindle number 7, which was then rotated at speeds ranging from 10 to 100 rpm for 15 minutes at 25^{o} C, with the reading recorded in three copies. The viscosity was measured in centipoise units.

Spreadability

Method: Parallel plate method

Spreadability was assessed using a glass slide and a wooden block device. The "Slip" and "Drag" properties of gels were employed to determine spreadability in this method. After the glass slide was bonded to the block, a ground glass slide with 1 gram of gel sample was placed on it. The gel was then sandwiched between this glass slide and a second glass slide with a hook and a specified ground slide dimension. A 1 kg weight was placed on top of two slides. Extra gel has been scraped off the edges. The top plate was then given a 20gm pull. A piece of string connected to the top slide was used to measure and estimate spreadability.

$$S = WL/T$$

Where S= Spreadability, W= weight tide to upper slide, L= length of glass and, T = time taken to separate the slide completely from each other.

Consistency

The consistency of the gel was assessed by dropping a cone attached to a holding rod from a fixed distance of 10 cm and falling in the center of a glass cup filled with gel. The cone's penetration was measured from the gel's surface to the cone's tip inside the gel. After ten, the distance traveled by the cone was calculated.

Homogeneity

All gel compositions were visually examined after allowing the gel to solidify in an appropriate container. The presence of gel was noted.

Drug content

To completely dissolve the medication, 100 mg of gel sample was dissolved in 100 ml of phosphate buffer 6.8 and methanol solution. The gel solution was then prepared and stirred on a mechanical shaker for two hours. As a control, a 6.8 Phosphate Buffer and Methanol mixture was used. The drug content of the formulation was evaluated in triplicate to obtain the average value and standard deviation.

***In vitro* Drug Diffusion study**

A modified Franz diffusion cell with a receptor compartment capacity of 10 ml was used to calculate it. An egg membrane was connected between the diffusion cell's donor and receptor sections. The entire apparatus was supported by a magnetic stirrer. The combination of phosphate buffer and methanol was placed within the receptor compartment of the diffusion cell. A 0.1gm sample of gel was put across the membrane, and magnetic beads were used to mix the receptor compartment solution continuously at 50 rpm. A constant 37°–10°C was maintained. At 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4.5 hours, 1 ml of the sample was taken out and diluted with 10 ml of a blank solution before being tested by UV spectrophotometer at 263 nm. A three-fold diffusion investigation was carried out.

Skin irritation test

Any issue with cutaneous irritation can be treated with it. On human volunteers, a skin irritancy test for the gel was conducted. The three human participants served as the study's subjects. A two square inch region of the hand near the wrist received a topically administered 1gm gel, and any lesions, irritation, or redness was checked for.

Antifungal activity of Clotrimazole

The cup-plate technique was used to assess the antifungal activity of the optimised organogel formulation using sabouraud dextrose agar medium. Selected organisms are applied to solidified media, and then the formulation is added to a cup with a 1 cm diameter that has been drilled into the medium. The potency of Clotrimazole, a pure medication, and commercial Clotrimazole against the chosen fungus strains was evaluated (candida albicans). Following about three days of incubation at a regulated temperature of 250°C, the zone of organism growth inhibition was examined.

RESULT AND DISCUSSION

Organoleptic properties

Organoleptic characteristics, such as colour, appearance, odour, melting point, and pH, were examined for the drug sample and Span 60. The outcome depicted in Table 2.

Table 2: Organoleptic properties

Sr. No.	Properties	Active drug	Span 60
1	Color	White to pale yellow	White to tan waxy solid
2	Appearance	Crystalline	Pellets large crystal
3	Odor	Odorless	Slight odor
4	Melting point	149 ◦ c – 151 ºC	149 ◦ c – 151 ºC
5	pH	4.3 -5.1	-

Solubility measurement

The solubility of Clotrimazole was determined by using different solvent

Table 3: Solubility

Sr. No.	Solvent	Types of solubility
1	Alcohol	Soluble
2	Mixture of Phosphate buffer 6.8 and methanol	Soluble

Construction of Calibration Curve

10 mg drug was taken and transferred to a 100 ml volumetric flask. The active ingredient in clotrimazole was diluted to the proper concentration using a solution of phosphate buffer (pH 6.8) and methanol (6:4). The varied concentrations of Clotrimazole (5, 10, 15, 20, 25, and 30 g/ml) were prepared, and the absorbance was taken at chosen absorbance maxima (263 nm). This concentration absorbance data was used to construct a Beer Lambert graph [13-16].

Table 4: Calibration Curve

Sr. No.	Concentration in µg/ml	Absorption in nm						
		I	II	III	IV	V	VI	Mean
1	0	0	0	0	0	0	0	0
2	5	0.074	0.081	0.074	0.085	0.080	0.076	0.078
3	10	0.168	0.164	0.172	0.170	0.174	0.174	0.162
4	15	0.240	0.240	0.244	0.242	0.246	0.245	0.242
5	20	0.336	0.335	0.340	0.341	0.337	0.334	0.338
6	25	0.424	0.421	0.429	0.430	0.420	0.422	0.425
7	30	0.510	0.501	0.510	0.505	0.508	0.503	0.505

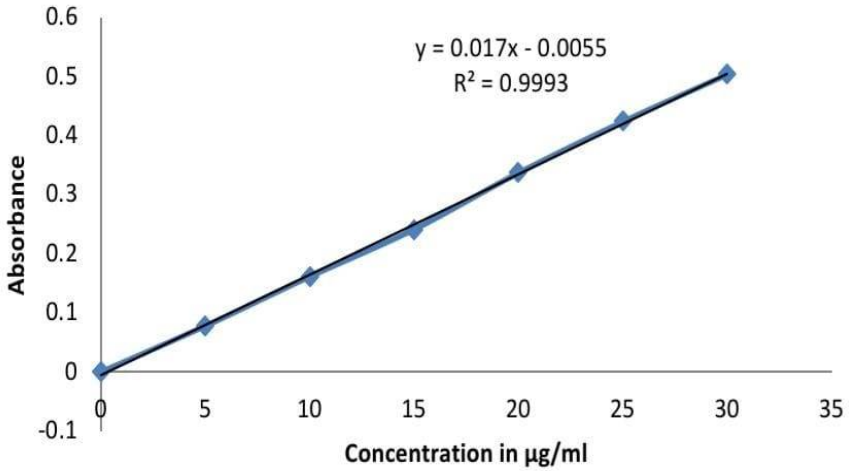

Figure 2: Calibration Curve of Clotrimazole

pH measurement

The pH readings are within the typical pH range, which is compatible with the average pH range of skin, and all gel formulations had pH values ranging from 6.42 to 6.71, which were within the normal pH range.

Viscosity measurement

The viscosity of all four formulations was determined to range from 45000 CPS to 15 CPS at various RPM and 25°C. With an increased RPM, the viscosity of formulation was decreased. The compositions therefore possess both viscous and elastic characteristics. The formulated organogel appears to exhibit viscoelasticity according to the Maxwell model. When subjected to increased shear, the organogel becomes less viscous and behaves more like a solid [17-23].

Table 5: Viscosity measurement

Formulation Code	RPM	10	12	20	30	50	60	100
F1	Viscosity In Centipoise	-	-	-	-	-	20	15
F2		-	-	-	-	60	45	20
F3		600	315	250	200	120	90	60
F4		45000	31000	29000	23000	14000	10050	1710

Spreadability measurement

Spreadability was determined using the parallel plate technique. Spreadability in the parallel plate approach ranges from 18.81 to 28.12 gm/cm.

Consistency measurement

Consistency values for the prepared gel ranges from 08 to 11 mm, and found to be consistent.

Homogeneity

All topical gel formulations showed excellent homogeneity and a lack of lumps.

Skin irritation test

The skin sensitivity test is crucial before using a gel formulation. Each gel underwent a skin irritancy test on three participants, which was then visually analysed. No formulation caused skin irritation or redness [24-28].

Drug content measurement

Drug content provides the formulation's consistent drug distribution. All of the topical gel formulations in the table were assessed for their percentage drug content. The outcome showed that all topical gels had almost identical medication contents and low standard deviation values.

Table 6: Evaluation parameter

Formulation code	pH	Spreadability (gm/cm)	Homogeneity	Skin irritation test	Drug content measurement	Consistency
F1	6.71± 0.015	28.13 ± 2.18	Homogenous	Nil	95.20 ± 0.32	11.00 ± 01.0
F2	6.54± 0.011	24.32 ± 1.93	Homogenous	Nil	95.11 ± 0.61	11.66 ±0.57
F3	6.50 ± 0.020	21.14 ± 2.32	Homogenous	Nil	95.01 ± 0.32	09.33± 0.57
F4	6.42 ± 0.015	18.81 ± 0.93	Homogenous	Nil	96.01 ± 0.34	08.00 ±0.10

Drug diffusion study

To evaluate drug diffusion over the egg membrane, drug diffusion research was conducted. Franz diffusion cell is used to examine the diffusion of prepared Organogel. For all four formulations, the CDR percentage is indicated at various time intervals. After 4 hours and 30 minutes, the % CDR of F4 formulation was revealed to be 97.846%, the highest of all four formulations.

Table 7: Diffusion Study

Time	Percent CDR			
	F1	F2	F3	F4
0	0	0	0	0
30	13.42± 0.669	16.42 ±0.76	14.66± 0.712	13.65± 0.015
60	30.711± .045	32.07 ± 0.48	30.69 ±0.83	38.96 ± 1.249
90	40.37 ± 0.47	40.54 ± 2.32	40.58 ± 1.23	45.89 ± 2.205
120	52.04 ± 0.47	55.32 ± 1.01	56.43 ± 1.24	55.719 ±1.103
150	59.40 ± 0.28	61.70 ±1.75	62.10± 0.313	63.7 ±.0221
180	66.21 ±0.19	70.85 ± 0.70	69.93 ± 93	72.533 ± 1.269
210	71.71 ± 0.20	77.92 ± 1.41	74.81 ± 0.81	79.610 ± 0.93
240	81.73 ± 0.78	86.26 ± 0.33	84.12 ± 12	89.318 ± 0.90
270	88.96± 0.67	94.74 ± 0.70	93.11 ± 1.14	97.84 ± 0.96

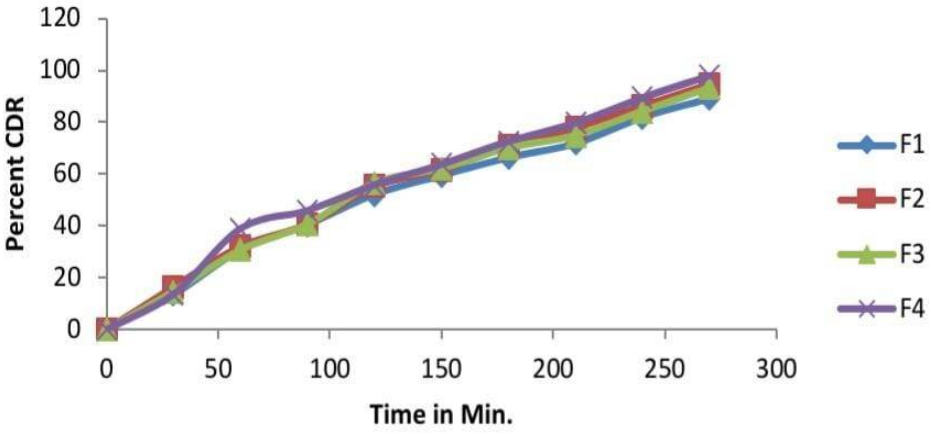

Figure 2: Drug Diffusion Study

Antifungal Study

Clotrimazole's antifungal activity was shown to be stronger than that of commercial gel formulations against various fungal strains. The order of inhibition was revealed to be pure drug, control, commercialized, and enhanced F4 formulation. F4 organogel was shown to be more effective against *Candida albicans* and *A. Niger* [29-31].

Table 8: Antifungal activity

Fungal strain	Pure drug	Control	Mkd gel	F4
C. albicans	09± 1.41	11± 1.36	15± 1.54	19± 1.54
A. Niger	12± 1.36	13 ±2.45	17± 2.33	19± 2.4

CONCLUSION

The antifungal topical Organogel F4 comprising Clotrimazole and Mustard oil revealed greater antifungal activity as compared to its prior formulation. At Mustard oil 80% and Surfactant 20% concentrations, F4 formulation shows maximum drug release. Prepared Organogel was found to be effective for topical application due to its good spreadability, neutral pH, normal viscosity, lack of signs and symptoms of redness or itching when applied to human skin, and lack of skin irritation.

CONFLICT OF INTEREST

There are no conflict of interest and disclosures regarding the work.

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