RESEARCH ARTICLE

Antifungal and Antioxidant Therapy for the Treatment of Fungal Infection with Microemulsion Gel Containing Curcumin and Vitamin C

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Abstract

Objective: Fungal infection on the skin is one of the common dermatological diseases especially for the people living in hot and humid climatic conditions. Conventional topical formulations suffer from its own limitations such as lack of site-specific delivery, drug bioavailability, and patient compliance. Curcumin oil was selected as bioactive molecules which target fungal infection which also act as an oil phase for microemulsion preparation. Another strategy was implemented in this research study was to protect the skin from excessive exposure from free radicals by supporting the skin endogenous antioxidant system by adding ascorbic acid (Vitamin C) as an adjunct. **Materials and Methods:** Micro emulsion gel was formulated with curcumin oil as an active ingredient as well as an oil phase along with ascorbic acid as an adjunct (antioxidant), tween 20, and isopropanol as surfactant and cosurfactant, respectively. Carbopol 940 was chosen as a polymer for the preparation of gel. Pseudoternary phase diagram of microemulsion with various surfactant/cosurfactant ratios (1:1–4:1) were constructed to study the phase behavior. Minimum inhibitory concentration study and Fourier transform infrared (FTIR) study was conducted to check antifungal activity and possible drug-excipient interactions, respectively. The developed formulations were characterized for physicochemical parameters such as globule size, zeta potential, viscosity, pH, etc. The optimized microemulsion formulations were further loaded into the carbopol gel base and evaluated. **Results:** Surfactant/cosurfactant weight ratio 3:1 was found to be largest in the percentage area of micro emulsion region. FTIR study did not show any potential drug-excipient interaction and curcumin oil showed considerably greater antifungal activity as compared to clotrimazole. The physical appearances of all the formulations were found to be yellowish, transparent, clear, glossy, and consistent. The pH, viscosity, droplet size of all the formulations was found to be in the acceptable range. From the results of *in vitro* drug release, approximately 90% of drug released was noted at the end of 10 h and *ex vivo* release study showed possible permeation mechanism of drug. *In vitro* antioxidant activity showed that microemulsion gel containing ascorbic acid possess significant free radical scavenging activity in a concentration-dependent manner. *In vivo* study showed that microemulsion gel containing curcumin oil and ascorbic acid has significant antifungal and antioxidant effect. **Conclusions:** Curcumin oil was successfully incorporated into topical microemulsion gel prepared with Vitamin C used as an adjunct and showed good spreadability, viscosity, drug release, in vitro antioxidant activity, and in vivo antifungal activity.

Key words: Curcumin oil, Carbopol 940, microemulsion, Vitamin C

INTRODUCTION

Fungal infection is the most common dermatological disorder as it is a complex and unstable disease where conventional treatment is marginally effective. Various study revealed that conventional formulations with antifungal agents are less penetrative and therefore they showed a less significant result, in contrast to advanced novel formulations such as

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microemulsions, nanoemulsions, transfersomes, ethosomes. These novel formulations play an important role in optimizing and enhancing the topical delivery of the drug by modulating physicochemical and biopharmaceutical property of the drug candidate. Microemulsion has shown promising results as a drug delivery system due to its enhanced drug solubilization, target specificity, ease of formulation, sustained drug release, prolonged shelf life, and slow degradation properties.[1-3]

A high degree of an oxidative stress from both endogenous and exogenous sources always exhibits on the skin before the fungal attack and even after treatment with antifungal agents. Propitious techniques should be developed to enhance the skin protection and provide support to endogenous skin antioxidant system. The disease is mainly caused by keratinophilic fungi called dermatophytes and cause disease of the skin, hairs, and nails. Physicians have a wide choice for the treatment from the solid dosage form to semisolid and to liquid formulations. However, no marketed formulations are available till date which can provide dual effect, i.e., sustained antifungal with antioxidant effect together.[4,5]

Microemulsions have opened new vistas for the treatment of fungal infection in the field of preventive care medication as compared to conventional formulations. Curcumin oil is a herbal drug with the larger number of bioactive molecules are present and provide broad spectrum antifungal activity. Vitamin C helps to facilitate recovery from fungal infection and reduces symptoms of inflammation associated with fungal and yeast infections.[6-9]

With all the above aspects keeping in mind, the present work was developed to investigate the potential of microemulsion-based topical drug delivery system containing curcumin oil as active moiety and Vitamin C as an adjunct for the treatment of fungal infection with antifungal and antioxidant activity together.

MATERIALS AND METHODS

Materials

Curcumin oil was a kind gift sample from JSS Pharmacy College, Mysore, India. Vitamin C (ascorbic acid) was purchased from Merck, Germany. Tween 20, Carbopol 940, and propylene glycol were purchased from LobaChemie Pvt. Ltd, Mumbai, India. Sabouraud dextrose agar (SDA) was purchased from HiMedia Pvt. Ltd, Mumbai, India. Other chemicals and reagents used were of analytical grade.

Animals

Albino Wistar rats weighing 150–200 g of either sex were used for the preclinical skin irritancy and antifungal test experiments. The housing of the rats was done in polypropylene cages and maintained at a temperature of 25°C. The experimental protocol, and study were approved by Institutional Animal Ethical Committee, NGSM Institute of Pharmaceutical Sciences, Nitte University, Mangaluru (Approval no.: NGSMIPS/IAE/MAY-2016/32).

Methods

Preformulation studies

Drug-excipients compatibility study

Fourier-transform infrared (FTIR) spectroscopy was carried out to detect any possible chemical interaction between drug/s and excipients. The IR spectra of physical mixtures ofcurcumin, Vitamin C, tween 20, isopropyl alcohol, and Carbopol 940 were carried out. The wave number of the characteristic peak of physical mixture was compared with the pure sample and interpreted.[10]

Susceptibility test

The determination of the antifungal activity of curcumin was done by disc-diffusion method using SDA as the culture medium. In this method, SDA medium was inoculated with the test organism by pour plate method and incubated for 3–4 days. The test organism was seeded in the medium and its sensitivity to the drug was determined from the inhibition of its growth. In this study, the antifungal disc was preferred to apply by sterile forceps. After the overnight incubation, sensitivity was determined by zone of inhibition of the growth around the disc. Growth was inhibited around the disc containing antifungal. The diameter of the zone of inhibition was influenced by the variety of factor such as disc concentration, diffusibility of the drug, thickness, and composition of the medium, the presence of inhibitory or stimulatory substance, pH, and time of incubation. The different dilution of the curcumin oil was made as 8% w/v, 10% w/v, 15% w/v, and 20% w/v in ethanol. 25 µg of each sample was pipetted into the each disc of the agar plates seeded with test organism, and the standard drug was used as control. Then, the plates were incubated at 28°C for 10 days. The clear zone formed surrounding each disc if observed and taken as the minimal inhibitory concentration of the curcumin oil.[10,11]

Formulation of microemulsion

Construction of pseudo-ternary phase diagram

To analyze the microemulsion region, pseudoternary phase diagrams were constructed by using water titration method at ambient temperature 25°C using software CHEMIX SCHOOL. Phase diagrams were prepared with the ratio of 1:1, 2:1, 3:1, and 4:1 of tween 20 and iso-propranolol act as surfactant and co-surfactant, respectively. By considering input from ratios, microemulsions were prepared by lowering the oil concentration from 90% to 10% to determine the maximum water intake by microemulsion by which a clear transparent isotropic microemulsion was obtained. The maximum uptake of water was mainly depends on oil phase, surfactant, and surfactant optimized. Based on the

pseudoternary phase diagram, one suitable ratio was optimized and accordingly microemulsion was formulated.[12,13]

Preparation of microemulsion formulation (o/w type)

The microemulsion was prepared by mixing tween 20 (surfactant), isopropanol (co-surfactant), and oil together and then diluted dropwise with water, under the moderate magnetic stirring for 5 min at room temperature. During the process, mixture was being monitored visually for sign of turbidity or phase separation. Thereafter, Vitamin C was incorporated under the moderate magnetic stirring for 30 min. Microemulsion was kept covered for 24 h before evaluation.[4]

Characterization of microemulsion[4,10,14]

Visual inspection

The formulations were visually inspected for homogeneity, optical clarity, and fluidity.

Types of emulsion

The type of emulsion was determined by:

Dilution test

Small amount of formulation was placed on clean glass slide. A drop of water added to the microemulsion and mixed with the help of glass rod, and their transparency was assessed visually.

Dye solubility test

Water soluble dye amaranth was mixed with water and was placed on a slide to which drop of prepared microemulsion was added and mixed again and was observed under the microscope.

Measurement of droplet size

Droplet size of the microemulsion was measured using particle size analyzer using BIOVIS software.

Zeta potential

Zeta potential must be negative or neutral, which indicate that the droplet of microemulsions have no charge so that the system is stable. Zeta potential was measured using Malvern Nano Zeta Sizer (Malvern Instruments Ltd., UK). Zeta potential is essentially useful for assessing flocculation since the electric charge on particles influence the rate of flocculation. Based on the results, the best formulation was selected and incorporated into a gel formulation.

Percentage transmission

Transparency of microemulsion formulation was determined by measuring the percentage transmittance at 650 nm with water taken as blank through UV spectrophotometer.

Preparation of microemulsion based gel

The microemulsion-based gel was prepared by dispersing 0.5% w/w, 1.00% w/w, and 1.5% w/w of Carbopol 940 in distilled water taken in a separate 50 ml beaker. This dispersion was then kept in dark room about 24 h to allow the Carbopol 940 to swell completely after the complete swelling the suitable concentration of gel was optimized, and optimized microemulsion was incorporated into the gel base at 300 rpm for 15 min at room temperature.

Characterization of microemulsion-based gel

pH

The microemulsion pH was determined using pH meter. The pH meter was calibrated using pH buffer solution and pH of each microemulsion prepared was determined in triplicate.

Viscosity measurements

The viscosity of each microemulsion containing the different percentage of water content was determined using digital Brookfield viscometer LVDV II (Elscolab Netherlands B.V Tolboomwerg, The Netherlands). The viscosity of the microemulsion was measured at different rpm.

Spreadability

The special apparatus has been designed to study the spreadability of the formulations. Spreadability is expressed in terms of time in seconds taken by two slides to slip off from the formulation, placed between, under the application of certain load. Lesser the time is taken for the separation of the two, better the spreadability.

Method

Two glass of standard dimension was selected. The formulation whose spreadability has to be determined is placed over one of the slides. The other slide is placed over the formulation was sandwiched between the two slides across the length of 5 cm along the slide. 100 g weight was placed up on the upper slide so that the formulation between the two glass slides is pressed uniformly to form a thin layer. The weight was removed and the excess of the formulation adhering to the slide was scrapped off. One of the slides is fixed in which formulation is placed. The second movable slide is placed over it with one end tied to a string to which load could be applied with the help of a simple pulley and a pan.

A 30 g weight was put on the pan and the time taken for the upper slide to travel the distance of 5.0 cm and separate away from the lower slide under the direction of the weight was noted.

The spreadability was then calculated from following formula:

$$\text{Spreadability}=(m\times l)/t$$

where m is weight tied to the upper slide (30 g), l is length of the glass slide (5 cm), t is time taken in seconds.

Drug content

The drug content of microemulsion formulation estimated by dissolving 1ml of the formulation in 10 ml of ethanol. After the suitable dilutions with ethanol, absorbance was analyzed using Shimadzu Double beam UV-visible spectrophotometer(UV-1700 Shimadzu Corporation, Tokyo, Japan) at wavelength of 425 nm.

Release study and kinetic mechanism

In vitro drug release study was carried out using beaker method. The receptor compartment was filled with phosphate buffer pH 5.8. The temperature of dissolution medium was maintained at 37°C ± 0.5°C. Samples were collected at suitable interval and diluted. Absorbance was read at 425 for curcumin using UV- visible spectrophotometer.

To determine the release mechanism that provides the best description to the pattern of drug release, the *in vitro* release data were fitted to first order, zero order, Korsmeyer–Peppas and Higuchi model. The data were processed for regression analysis using MS-EXCEL statistical function.[15-17]

In vitro antioxidant activity using 1, 1-diphenyl, 2- picrylhydrazyl (DPPH)

The scavenging activity of DPPH radical was investigated according to the method described by Ramadan *et al.,* a methanol solution of DPPH (2.95 ml) was added to 50 µl sample (microemulsion gel was dissolved in methanol at different concentrations (10,000 - 25 µg/ml) in a disposable cuvette. Ascorbic acid was used as a standard at 0.1 M concentration which is equal to 17613 µg/ml.[18] The absorbance of the standard and samples was measured at 517 nm spectrophotometrically at regular interval of 15 s for 5 min. The inhibition percent for each sample was calculated using the following formula:

$$\text{\% inhibition (reactive reaction rate)} = \frac{\text{Abs.(DPPH solution)} - \text{Abs. (Sample)}}{\text{Abs. (DPPH soultion)}} \times 100$$

Permeation study

Albino Wistar rat skin was obtained and kept frozen until use. Before the start of the experiment, it was washed by water thoroughly, and hairs were removed. Further, it was placed in the hot water for 15 min to remove the fat and was mounted on Franz diffusion cells. Phosphate buffer pH 5.8 was filled in the evening before the experiment and allows it to equilibrate overnight in water bath maintaining skin surface temperature at 37°C. 10 mg of the formulation was spread in the membrane which is fixed to the donor chamber. The glass cylinder was attached to the shaft and suspended in 50 ml of dissolution medium so that the membrane is just touched to the receptor medium surface temperature was maintained at 37°C and stirred at 50 rpm using magnetic stirrer. A volume of 3 ml were collected at a suitable interval and absorbance was read at 425 nm for curcumin using UV-visible spectrophotometer.

Skin irritation and antifungal study

The skin irritation and antifungal study were performed on 12 healthy Albino Wistar rats weighing between 150 and 200 g. The study was approved by the institutional animal ethical committee proposal no. NGSMIPS/IAE/MAY-2016/32. The selected 12 rats were divided into three groups (Group I, group II Group II) and caged separately according to the group. The study animals were provided with normal food, water, and environmental conditions. On the day of procedure, individual rats were weighed again and hair was depilated from the lower back portion while the animal was under light anesthesia.[19] To induce fungal activity in rats, *Trichophyton mentagrophytes* was used as an infectious agent. The organism was isolated from the patient at the K.S Hegde medical academy, center for preventive medicine, Deralakatte, Mangaluru, India. SDA was used to maintain micromycetes which contain 40 g of glucose, 10 g of agar and 10 g of peptone in 1 L of distilled water. The culture was stored at 4°C and subcultured once a month.[20]

The suitable numbers of animals were divided into the three groups formed based on the number of optimized formulation. Group I (control group) animals from this group were not exposed to any formulation and were considered as a reference standard for other two groups. Group II (reference group) animals were treated with the formulation of marketed fluconozole gel. Group III (test group) animals from this group were applied with microemulsion gel (optimized formulation).

On the back of each animal, the areas of 4 cm^2 were cleaned and depilated. The infectious inoculum was prepared from a 7-day-old culture of *T. mentagrophytes*. The inoculum was applied on the animal's back immediately after the depilation and left for 3 days. The establishment of active infection was confirmed on the 4th day by visual examination of animals as well as by isolating the pathogens from skin scales cultured from infected loci on SDA plates, containing 100 units/ml of penicillin and streptomycin.[21]

Group I left untreated as a control group, Group II treated with marketed cream as a reference group, and Group III was treated with optimized microemulsion formulation. At first reaction in the animals were noted after 24 h, 48 h, and 72 h, respectively, for 7 days of depilation. The rats were observed for sensitivity, skin irritation, and any reaction by visual observation, if any and were graded as non-irritant, irritant, and highly irritant.

The total scores given for the test was calculated using following equation:

$$\text{Average irritation} = \frac{\left(\text{Erythema reaction scores} + \text{Edema reaction scores}\right)}{\text{Time interval (h)}}$$

Animals were treated once a day and the infected areas were scored visually for inflammation and scaling as well as for the presence of the pathogens by cultivating skin scales from infected loci in SDA plates each day.

Assessment of thermodynamic stability of microemulsion

The stability study of the microemulsion-based gel was carried out. All the prepared microemulsion-based gels were placed in the airtight glass container in a clean and dry place at room temperature for more than 2 months. Afterward formulations were withdrawn and checked for pH, viscosity, visual appearance, particle size, and drug content.

RESULTS AND DISCUSSION

Preformulation studies

Drug-excipients compatibility study

FTIR study was carried out to point out any possible interaction between the curcumin oil, Vitamin C, surfactant, cosurfactant, and polymer used. The IR spectra of oil (drug), Vitamin C, individual polymer, and surfactants as well as physical mixtures of the drug with each of the excipients were obtained. The IR spectrum of pure drug, i.e., curcumin showed characteristic peaks at 3511.10/cm, 1507.01/cm, 1279.54/cm, and 1206.25/cm because of C=C, =O and –OH groups, respectively. The IR spectrum of ascorbic acid showed characteristics at 1674/cm to 1322/cm. The IR spectrum of carbopol 934 showed characteristics peak carbonyl group (C=O) band from 1702.40/cm to 1645.90/cm. These peaks were retained in the IR spectra of the final formulation. This indicates that the drug and the gelling agents are compatible with each other. The findings of FTIR studies revealed the absence of any potential drug-excipient interaction and ruled out any possible physicochemical incompatibility between the active and inactive moiety.

Susceptibility test

Antifungal activity of curcumin oil was determined against *Candida albicans* using well diffusion method where SDA was used as culture media and clotrimazole was used as reference drug. Curcumin oil showed greater zones of inhibition than clotrimazole. Zone of inhibition of curcumin oil was found to be 23 mm at a concentration of 20% of curcumin oil whereas zone of inhibition of clotrimazole was found to be 21 mm at the same concentration. From the result it was evident that that curcumin oil showed considerably greater antifungal activity as compared to clotrimazole and could inhibit the dermatophytes.

Preparation of microemulsion

Construction of phase diagram

Pseudoternary phase diagrams are frequently used to obtain concentration range of the oil, surfactant, and cosurfactant for clear microemulsion formation. Figure 1 exhibits the phase diagrams composed of water, surfactant, cosurfactant, and oil. It was observed that percentage area of microemulsion region in the majority of phase diagrams was largest at surfactant/cosurfactant weight ratio of 3:1 compared to 1:1, 2:1 and 4:1 ratios. This can be explained on the basis of the enhancement in micelle formation with the increase in surfactant/cosurfactant ratio, which consequently increases the solubilizing capacity of the microemulsion.

Formulation of microemulsion

Ternary phase diagram characterizing the phase behavior of surfactant blend with oil and water. It generally highlights the formation of the micro emulsion. From the result of ternary phase diagram, suitable ratio of micro emulsion was selected and micro emulsions were prepared by mixing tween 20, iso-propyl alcohol, and oil together and then, diluted with water drop-wise, under moderate magnetic stirring at ambient temperature. The micro emulsion was prepared according to the formula was given in Table 1. The formulations were then stored in glass containers and closed with a screw cap.

Evaluation of prepared microemulsion

Visual examination

All the formulations were visually examined for their color, homogeneity, appearance, and consistency. The physical appearances of all the formulations were found to be yellowish, transparent, clear, glossy, and consistent. Phase separation was not observed in none of the formulations. However, formulation F6 was found to be not clear and transparent in contrast to other formulations.

Type of emulsion

Several tests are available for distinguishing between o/w and w/o type emulsions which include tests of miscibility,

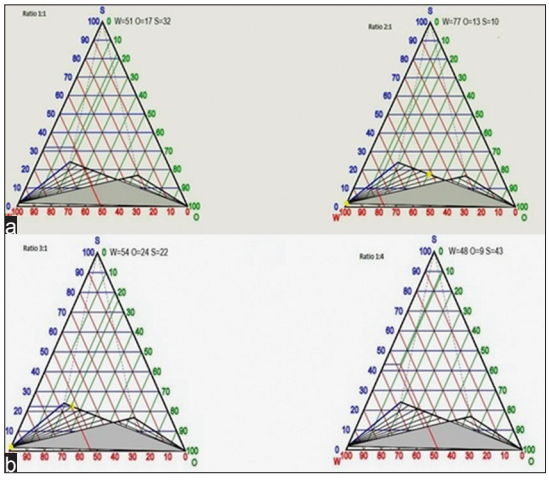

Figure 1: (a) Phase diagram of 1:1 and 2:1 ratio, (b) phase diagram of 3:1 and 1:4 ratio

dye test, electrical conductivity measurements, etc. We have considered here dye and dilution test to check the nature of the emulsion. From the dye test result, it was found that dispersed globules were appeared red due to oil soluble dye and water was observed as the continuous phase or colorless. However, from the dilution test, water was observed as continuous phase which ensures that formulation F3, F4, and F5 were o/w type of microemulsion.

Measurement of droplet size

The mean droplet size of the prepared microemulsions was found to be in the range between 0.5 and 5 μ which is the stable size of the microemulsion. The smaller the droplet size better will be the microemulsion because it prevents from clumping, phase separation, and flocculation.

Zeta potential

The zeta potential was found to be −32.7, −22.2, and −18 mV, respectively, for F-2, F-3 and F-4, respectively and hence proved that prepared microemulsion is a stable because the negative zeta potential indicates that globules of microemulsion had no charge. If there is no charge on globules, no flocculation of globules will occur, and hence microemulsion was found to be stable.

Percentage transmittance

Percentage transmittance was carried out to prove that microemulsion forms clear and transparent systems. The percentage transmittance was carried out as per the method describe in methodology. The percentage transmittance was found to be 98.34 ± 0.23, 98.12 ± 0.17, and 97.96 ± 0.21, respectively, for F-2, F-3, and F-4, respectively, and hence proved that prepared microemulsion is optically clear which is a prerequisite for microemulsions.

Formulation of microemulsion base gel

Carbopol 940, were used to prepare the blank gel. Different concentrations of Carbopol 940 (0.5%, 1%, 1.5%, and 2%) were taken to prepare gel. After optimizing suitable formula for gelation, microemulsions were incorporated into the gel.

Evaluation of microemulsion based gel

pH

pH evaluation is a very important parameter for topical formulations as it may cause irritation to the skin if varied from normal skin pH condition. All the formulations were evaluated for pH and the results are given in Table 2. The pH of all the formulations was found to be in the range of 6.13–6.47 which is nearer to the skin pH.

Drug content

The estimated drug content of the formulated microemulsion was found to be in the range between 90% and 92%. The drug content determination also showed that the drug was uniformly distributed throughout. Results of drug content are shown in Table 2.

Viscosity

It is an important rheological parameter which is related to the mechanical and physical properties such as spreadability, consistency, and hardness of the preparation which in turn are related to ease of product removal from containers, ease of application on the skin surface and product feel on the application site. The viscosity was determined using Brookfield viscometer. For viscosity determination of curcumin oil microemulsion gel, 150 g of the formulation was filled in a 250 ml beaker, and the viscosity was measured using Spindle number LV4. The viscosity of optimized formulations were found to be in the ranged between 148 cps and 160 cps.

Table 1: Preparation of microemulsion

Formulation	% w/w					
	S_{mix} (ratio)	Oil	Surfactant	Co-surfactant	Vitamin C	Water
F1	3:1	2.5	4.66	2.3	0.40	0.5
F2	3:1	2.0	4.66	2.3	0.40	1.0
F3	3:1	1.0	4.66	2.3	0.40	2.0
F4	3:1	1.5	4.66	2.3	0.40	2.5
F5	3:1	0.8	4.66	2.3	0.40	3.0
F6	3:1	0.6	4.66	2.3	0.40	4.0

Table 2: Results of physical characterization

Formulation code	pH (cps)	Viscosity (cm)	Spreadability curcumin	% Drug content
C1	6.28	160±01	2.6	92.4±0.14
C2	6.47	154±01	2.9	93.86±0.22
C3	6.13	148±01	3.4	90.12±0.29

Data are represented as mean±SD (n=3). SD: Standard deviation

Spreadability

Spreadability of all the formulations were measured according to the method mentioned in methodology. The data of spreadability weregiven in Table 2. The spreadability of formulations C1-C3 which was found to be in the ranged from 6.78 to 5.73 cm. From the result of viscosity and spreadability parameters, C1 and C2 were selected as best formulation for further studies.

In vitro drug release study

Based on the drug release comparison studies, it was observed that the drug release from the microemulsion formulation C2 was found to be significantly higher when compared with C1 and C3 formulation. In addition, it was revealed that there was 92.481% drug release form the formulation C2 at the end of 10 h. The order of drug release decreases as follows: C2>C1>C3. It was suggested that the spontaneous formation of a microemulsion with a small droplet size, which permitted a faster rate of drug release. Thus, this greater permeability of dissolved curcumin oil from the microemulsion formulation could lead to better targeting through the skin and considered as an optimized formulation for further studies. The difference in release behavior which was found can be attributed to the oil content. Results are given in the Figure 2a**.**

The various kinetic models were applied to *in vitro* release data for prediction of the drug release kinetic mechanism. The release constants were calculated from the slope of appropriate plots, and the regression coefficient (R^2) was determined. It was found that the *in vitro* drug release of microemulsion was best explained by first-order kinetics as the plots show highest linearity. The regression coefficient (R^2) was found to be 0.9863, indicating that the drug release was nearly independent of concentration, followed by Higuchi's.

Permeation study

Results of the *ex vivo* permeation studies revealed that there was 86.34% drug release form the formulation C2 at the end of 10 h across the skin. The skin permeation profile showed the same pattern as it was shown by *in vitro* release profile across the cellophane membrane. The results also revealed that formulation C2 showed significantly higher permeation for curcumin. It indicated that the permeability of the curcumin was more enhanced by the polymers. Results are given in the Figure 2b.

In vitro antioxidant activity

Showing reaction reactive rate (inhibition %) of different concentrations of microemulsion gel at different time intervals as compared with ascorbic acid (standard). Antioxidant activity of microemulsion gel (C-2) was evaluated *in vitro* as free radical scavenger activity. The reactive reaction rates (inhibition %) with a mean value ± standard error of microemulsion gel were found to be 95.11 ± 1.71%, 54.16 ± 2.87%, and 19.88 ± 0.52%, at concentrations of 10000, 1000, and 25 µg/ml of methanol solution of the plant extract, respectively as shown in Table 3. The results of the study showed that all the microemulsion gel containing ascorbic acid possess significant free radical scavenging activity in a concentration-dependent manner.

Skin irritation study and an antifungal study

Skin irritation test were conducted on healthy Albino Wister rats. Group I (control group**)** was used as a control, Group II (Reference group) was used as placebo group where the animals were treated with the formulation of marketed fluconazole gel and Group III (test group) were applied with microemulsion gel formulation containing curcumin and ascorbic acid. The optimized formulations C-2 were applied onto the rats. After inspecting visually the prepared microemulsion was found to be compatible with rat skin and showed no irritation. The rats were observed after 0 h, 24 h, and 78 h and the readings were noted. There were no sign of any erythema or edema formation in the entire period of 72 h after the application of marketed and in-house formulation. From the results, it can be concluded that curcumin microemulsion was found to be irritation free [Table 4].

Beginning of the infection which was inoculated with *T. mentagrophytes* in rats was observed on the 5th day of the experiment as it is depicted in Figure 3 as well as the treatment was also initiated on the same day. After the 10th day of the experiment, treatment with marketed and microemulsion formulations were evaluated. Group I was left untreated and kept for observation till the end of the treatment. Group III treated with microemulsion gel was found to be free from dryness, rough, and a scaly skin condition, as well as the culture, was found to be negative. Group II which was treated with fluconazole gel were cured after the 16th day of treatment and it was found that macroscopic lesions were not completely disappeared. Apart from this, skin was found to be dry, rough, and scaly in Group II animals as it is depicted in Figure 4.

From these results, it can be concluded that microemulsion gel containing curcumin oil and ascorbic acid has significant antifungal and antioxidant effect *in vivo*. This study model represents possible alternative therapy for the treatment of fungal infection by dermatomycetes. Moreover, ascorbic acid may provide added benefit to the patients by providing extra protection from free radicals by which skin maintains its tone even after the dermatocyte attack and also after the treatment which is quite apparent in the Group III animals [Figure 4].

Stability studies

The final optimized formulations were subjected to stability studies for a period of 2 months as given in methodology section. The formulations were evaluated for physical appearance, pH, spreadability, viscosity, drug content, and *in vitro* drug release. According to the results, there were no significant changes in the parameters mentioned above.

Table 3: *In vitro* antioxidant activity

Tested material (inhibition %)	Concentration	Reaction reactive rate (µg/ml)
Ascorbic acid (standard)	17613	99.21±0.64
	10000	95.11±1.71
	5000	71.75±3.65
Microemulsion gel (C-2)	1000	54.16±2.87
	100	31.72±0.38
	50	23.40±0.26
	25	19.88±0.52

Data are represented as mean±SD (n=3). SD: Standard deviation

Table 4: Scores of skin irritation tests

Rat Group	Rat number	Erythema and scar formation edema time (h)								Primary skin irritation (scores)
		0	24	48	72	0	24	48	72	
Group I	1	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0
Group II	4	0	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0
Group III	7	0	0	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0	0	0
	9	0	0	0	0	0	0	0	0	0

Data are represented as mean±SD (n=3). SD: Standard deviation

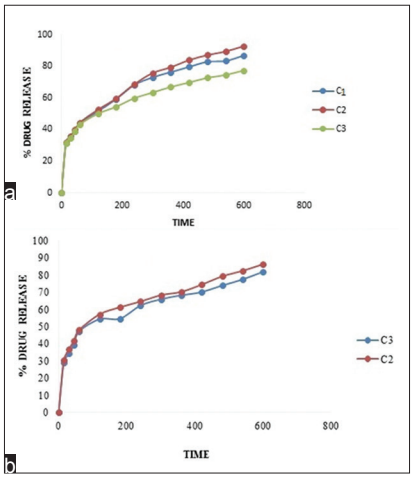

Figure 2: (a) *In vitro* drug release study of curcumin. (b) *Ex vivo* drug release of curcumin

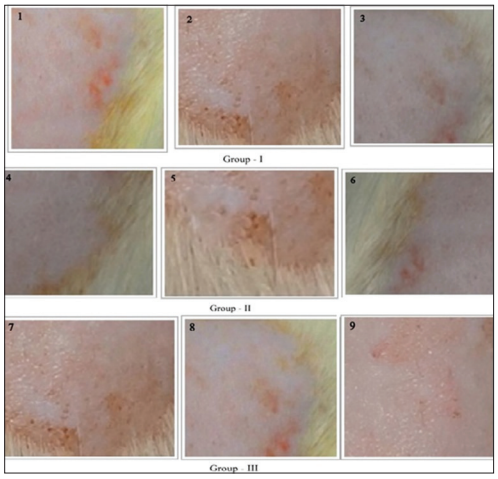

Figure 3: Rat skin condition on 5th day after incubation

CONCLUSION

This study demonstrated that the curcumin oil was successfully incorporated into topical microemulsiongel prepared with Vitamin C used as an adjunct and showed good spreadability, viscosity, drug release, *in vitro* antioxidant activity and *in vivo* antifungal activity. Curcumin oil microemulsion gel showed promising results against the marketed product and could be possible alternatives as herbal medication for the treatment of dermatological infections. However, it is preliminary investigation further intense preclinical study is required to establish more standard.

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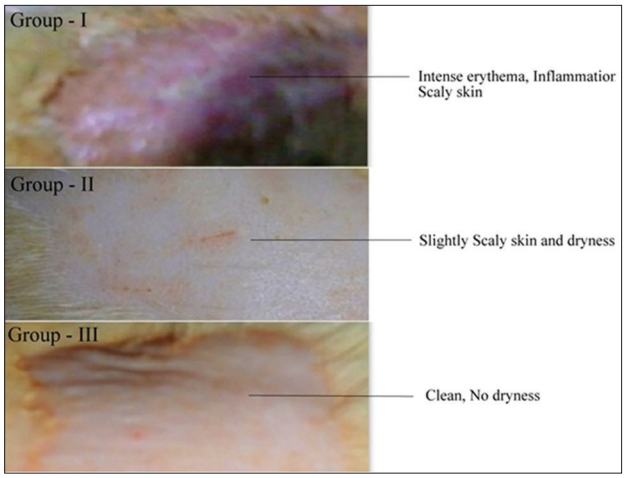

Figure 4: Skin condition after treatment

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