Formulation and Evaluation of Ketoconazole Microemulgel with Mixture of Penetration Enhancers

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ABSTRACT

The present research work was to develop ketoconazole loaded microemulgel formulation. The main objective was to enhance the penetration capability of ketoconazole by incorporating penetration boosters and to administer the drug in a sustained release fashion. Currently available dermal creams refuse to provide intended action as needed. Screening of oils, surfactants, and co-surfactants was done by the construction of pseudo ternary phase diagrams with 2% ketoconazole. Compatibility studies like FT-IR, DSC were performed to determine incompatibilities. The microemulsion was characterized for droplet size, zeta potential, viscosity , thermodynamic stability. Moreover for enhancement of patient compliance the optimized microemulsion was modified into microemulsion based gel. *Ex-vivo* studies were carried out for microemulgel using Franz diffusion cell by help of porcine skin membrane. The antifungal activity of microemulgel was evaluated using cup plate method incorporating *Candida albicans (MTCC Code: 3018)*.The optimized microemulsion had a composition of 20% Oleic acid: coconut oil (2:1), 34.06% Tween 80: Propylene glycol (2:1), and 43.94% water and was later incorporated into polymeric gel base. The microemulgel exhibited 10hr sustained release profile when compared to the Kz cream®. *In-vivo* investigation i.e; skin irritation test on albino mice was done by grouping into standard[Kz cream®],control[placebo], test[microemulgel] and it was identified that no irritation caused by microemulgel as well as standard Kz cream. The control showed signs of irritation as it does not possess active moiety**.** The optimized microemulsion showed 99.02%

drug loading and 98.07% transmittance.The thermodynamic stability, sustained drug release with greater penetration and enhanced activity due to the presence of oleic acid in microemulgel warrant its application as an excellent formulation for treating opportunistic fungal infections.

Keywords: Microemulgel, oleic acid, Albino mice,Ketoconazole,Stratum Corneum,Sustained release

INTRODUCTION

Topical formulations are utilised for localised effects at the point of application due to medication penetration into the underlying layers of skin or mucous membranes. It allows the use of medications with a short biological half-life and a narrow therapeutic window to extend the duration of activity.[1,2] The natural barrier for topical distribution is skin[3,4] which makes drug delivery problematic. Taking this into account, microemulsions are formed that have low skin irritation a high drug loading capacity, and may minimise the diffusion barrier of the *Stratum corneum* and boosting drug absorption[5,6].

Oleic acid, (9Z)-Octadec-9-enoic acid classified under monounsaturated omega-9 fatty acid acts as penetration booster by disruption of skin's barrier functionality and there by enhancing drug partitioning into *Stratum Corneum* and it boosts the delivery of both lipophilic and hydrophilic drugs.Due to the penetration enhancer property oleic acid possess wide range of applications in pharmaceutical formulations like excipient and in the solution phase synthesis of the nanoparticles[7].

Microemulsions are thermodynamically stable isotropically transparent dispersions of two immiscible liquids such as oil and water stabilised by an interfacial layer of surfactant molecules with a size range of 10-200nm and very low interfacial tension.[8] It consists mostly of oil, surfactant (SA) and water with varying amounts of cosurfactant (Co-SA). This combination is clear and steady.[9] The current study focuses on ketoconazole microemulsion based gel with penetration enhancers for the treatment of topical infections. Ketoconazole,2,2,2-trideuterio-1-[4-[4-[[(2*R*,4*S*)-2-(2,4-dichlorophenyl)-2-(imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]ethanone an imidazole antifungal drug, preferentially inhibits ergosterol production in fungal cell wall as a result occurrence of flaws in fungal cell wall and there by acting as a fungistatic agent.[10]

MATERIALS AND METHODS

Materials

Ketoconazole (KTZ) drug was a gift sample from Chalapathi Institute of Pharmaceutical Sciences, Guntur , India. Coconut oil (oil phase) was purchased from a local vendor in Guntur (India). Oleic acid (oil phase) and Tween 80 (surfactant) from Loba Chemie Pvt.Ltd and propylene glycol (co-surfactant) from Qualigens. Double distilled water was incorporated all over the formulation procedure and all other chemicals were of analytical grade.

Methods

Preformulation studies

Solubility studies

Ketoconazole solubility was tested using a variety of oils, surfactants and cosurfactants. solubility investigations were carried out by using an addition of excessive dose of the drug to 2mL of the vehicle contained in a 5mL glass vial. The drug was dissolved by heating the mixture in a water bath at 50°C with vortexing, then mixing for 2hr in an orbital shaker at 25^0 ± 1°C (Remi) . Heat was used to create the kinetic energy needed to disrupt the intermolecular interactions that hold the solute molecules together. The supernatant of an equilibrated combination centrifuged at 5000rpm for 15min was examined using a UV-Vis spectrophotometer at 292.4nm.[11]

Construction of standard plot of ketoconazole

Accurately weighed quantity of drug (10mg) was solubilised in appropriate quantity of methanol and diluted to 10mL with saline phosphate buffer of pH 5.5 and ethanol in the ratio of 1:1 to obtain 1000µg/ml solution and from above solution 1mL was pipetted out and made up to 10mL with buffer to get 100µg/ml solution. Serial dilutions were made from above solution and analysed spectrophotometrically at a λ_{max} of 292.4nm.

Compatibility studies

FTIR study

The drug's infrared spectrum was analyzed using the potassium bromide (KBr) dispersion technique on an FTIR Spectrometer (Bruker alpha-T). The drug was triturated in a 1:100 ratio with KBr to ensure uniform dispersion. The mixture was made into a pellet for FTIR spectrophotometer spectrum recording (wavelength range: 400 to 4000cm^{-1}). FTIR spectra were captured using OPUS software on a BRUKER alpha model infrared spectrophotometer.[12]

Differential scanning calorimetry (DSC) studies

The DSC thermogram for pure drug and formulation was generated with TG-instrument DSC-Q20 and evaluated using TA explorer to determine the probable interactions of excipients with the drug. Samples were weighed (2-5mg) sealed in aluminium pans, then heated to 300^0C. The heating rate was around 10^0C/min**.**[13]

Method: Phase titration

Construction of Pseudo ternary phase diagrams and preparation of Microemulsion[14,15]

The pseudoternary phase diagrams were developed by titrating a homogenous combination of oil, surfactants and cosurfactant with water at room temperature. The oil mix (Oleic acid and coconut oil)(1:1, 2:1) and S_{mix} (Tween 80 and Propylene glycol) (1:1, 2:1) were dispersed into separate vials in weight ratios varying from 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 to 0:10. Each vial holding the liquid mix was titrated with water, vortexed and set aside for 48hr to examine the change in transperancy.The vials that showed a crucial change in transparency after 48hr were removed from the research design because they failed to create the micro emulsion. The % weight composition of each vial was utilized to develop the pseudoternary phase diagram using Chemix 10.0 software.

S_{mix} ratio with maximum microemulsion area was chosen from the pseudoternary phase diagrams. Depending on the positions inside the shaded area of the pseudoternary phase, different quantities of oil mix and S_{mix} were blended. At room temperature ketoconazole was dissolved in a combination of oil and S_{mix} with magnetic stirring to get oil phase and this oil phase was added dropwise to the double distilled water under magnetic stirring to obtain a clear transparent, low viscous microemulsion. Then the mixture was allowed to stabilise and

reach equilibrium for 15min. All ketoconazole containing microemulsions were then kept at room temperature and monitored. Following a 24hr interval for phase separation.

Characterization of microemulsion

The optimised microemulsion was evaluated for droplet size, zeta potential (nanoparticle analyzer SZ-100, Horiba scientific), pH (LABINDIA), transmittance, percent drug loading, viscosity (Brookfield viscometer), thermodynamic stability against centrifugation at 5000 rpm for 15min.

Droplet size determination

The Dynamic light scattering particle size analyser (nanoparticle analyzer SZ-100, Horiba scientific) was used to determine the droplet size of microemulsion. Dilution was performed by taking 1mL of microemulsion and diluting it to 10mL with water before obtaining measurements .[16]

pH

The pH electrode of pH meter was calibrated with acetate buffer of pH 5.5 and a 1% aqueous formulation solution before being submerged until the variability in readings ended and a consistent reading was achieved .[17]

Viscosity

Approximately 1g of microemulsion was poured on the plate of the Brookfield viscometer and allowed to settle for 5-10min before the spindle(S62) was placed on the plate containing micro emulsion and rotated at 5rpm. The temperature was kept at 25℃ and the appropriate dial reading was recorded in cps.[18]

Zeta potential

Zeta potential is a process of scattering electrophoretic light and it was measured using HORIBA SZ-100 analyzer by keeping the sample in cuvette having cathode and anode with the help of syringe.[19]

Thermodynamic stability

Microemulsion stability was tested by centrifuging it for 15min at 5000rpm. Any changes such as phase separation and clarity were visually observed. As the centrifugation technique produced the intended outcomes no freeze-thaw cycles or heating and cooling cycles were performed.[20]

Drug content

The drug content of the microemulsion corresponding to 200mg was determined by double beam UV-Visible spectrophotometer after dilution with methanol at 292.4 nm against methanol as blank. The total drug content was estimated by using the formula.[21]

$$\% \text{ Drug content} = \frac{\text{Actual drug content}}{Theoreticaldrugcontent} \times 100$$

Formulation of microemulsion loaded microemulgel

Gelling agents were applied to these systems to improve the microemulsion viscosity and thereby increasing its retention period. Gelling agents like HPMC K4M and sodium alginate were soaked overnight to obtain consistent microemulgels. The optimum ketoconazole loaded microemulsion was introduced into the microemulgel base.

Characterization of microemulsion loaded microemulgel

The pH (LABINDIA), viscosity (Brookfield viscometer) and drug content of the optimized microemulgel were measured.

Drug content

The drug content was determined by dissolving 1g of microemulgel in 100mL of pH 5.5 phosphate buffer: ethanol (1:1) buffer. 1mL of this was taken and diluted up to 10mL with phosphate buffer. Because ketoconazole was insoluble in water it was dissolved in phosphate buffer, which had the maximum solubility. This solution was sonicated for 30min and then filtered. There was no drug precipitate seen since it disintegrated entirely following sonication. the absorbance was measured spectrophotometrically at λ_{max} of 292.4nm.[22]

***Ex vivo* skin penetration studies**

The experiments were carried out on excised porcine skin utilising Franz diffusion cell construction.[23] Phosphate buffered saline (PBS) pH 5.5 and ethanol (1:1) was employed as receptor medium and cell contents were kept at 37.0°C. For lipophilic drugs such as ketoconazole, solubility would be a rate limiting step via skin absorption in receptor fluid potentially affecting overall flow. As a result, methanol was used as solubilizer for ketoconazole without affecting skin integrity. 1mL aliquot was removed from the receptor chamber at appropriate time intervals and replaced with new buffer. Each experiment was carried in triplicates. The residual formulation was collected from the skin and weighed at end of *ex vivo* drug release testing. The pig skin was made to small pieces and put in 10mL of phosphate buffer pH 5.5 and methanol (1:1) in water bath for 30min followed by 15min sonication and centrifugation at 5000rpm. After diluting 1mL of sample with phosphate

buffer pH 5.5: methanol (1:1). The quantity retained was measured spectrophotometrically at 292.4 nm.

Antifungal activity

By employing the agar plate diffusion method the microemulsion loaded gel[24] and placebo formulation was evaluated for activity on fungal strain *Candida albicans* which was cultured on dextrose agar medium. A suspension of *C.albicans* of 100µl was inoculated in three agar plates now plates were bored and filled with 0.3g of formulation and control, after filling the plates were incubated at 37^0C for a period of 24hr and measured the zone of inhibition. Experiment performed in triplicates.

***In-vivo* study**

All the experimentation procedures were reviewed and approved by IAEC(Approval number: 16/IAEC/CLPT/2021-22). The institution of reviewers committee was Acharya Nagarjuna University,Guntur.

Accelerated Stability studies[25]

The optimized gel formulation was taken in collapsible aluminium tubes and stored at 5 ± 3^0C, 25 ± 2^0C /60%±5%RH and 40 ± 2^0C/75% ±5%RH for 3months. Samples were analyzed for appearance, drug content, release profiles.

RESULTS AND DISCUSSION

Solubility studies

From the solubility studies, it was evident that ketoconazole was highly soluble in Coconut oil and nigella oil mixture among the different oils and then oleic acid+coconut oil. Drug showed maximum solubilization in tween 80+propylene glycol among three combinations followed by tween 20+PEG400.

Table-1: Solubility profile of drug

Ingredients	**Solubility(mg/ml)±SD**
Coconut oil+nigella oil (oil mix)	27.8±2.34mg/ml
Oleic acid+Coconut oil (oil mix)	24.9±1.87mg/ml
Tween 80+propylene glycol (S_{mix})	28.9±1.76mg/ml
Tween 20+PEG400 (S_{mix})	19.8±2.5mg/ml

All values are mean of 3 trials±SD(n=3)

Table-2 : Construction of calibration curve of ketoconazole: λ_{max}: 292.4nm

S.NO	Concentration(µg/ml)	Absorbance±SD
1	0	0
2	10	0.125±0.07
3	20	0.216±0.09
4	30	0.341±0.03
5	40	0.445±0.11
6	50	0.553±0.04
7	60	0.642±0.08

All values are mean of 3 trials±SD(n=3)

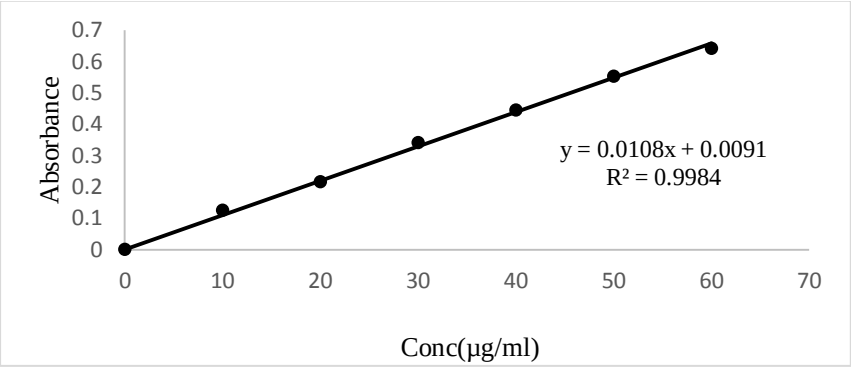


Figure 1: Calibration curve of ketoconazole in pH 5.5: methanol (1:1) ratio.

Compatibility studies

FT-IR Study

The infrared spectrum of ketoconazole pure drug in figure-2(a) shows strong absorption bands at 1643.57cm^{-1}(C=O carbonyl stretching) and 1024.52cm^{-1}(C–O aliphatic ether stretching), 1242.78cm^{-1}(C–O cyclic ether stretching). For microemulgel in figure-2(b) the absorption bands were observed at 1641.17cm^{-1} and at 1084.23cm^{-1} respectively indicating no interactions.

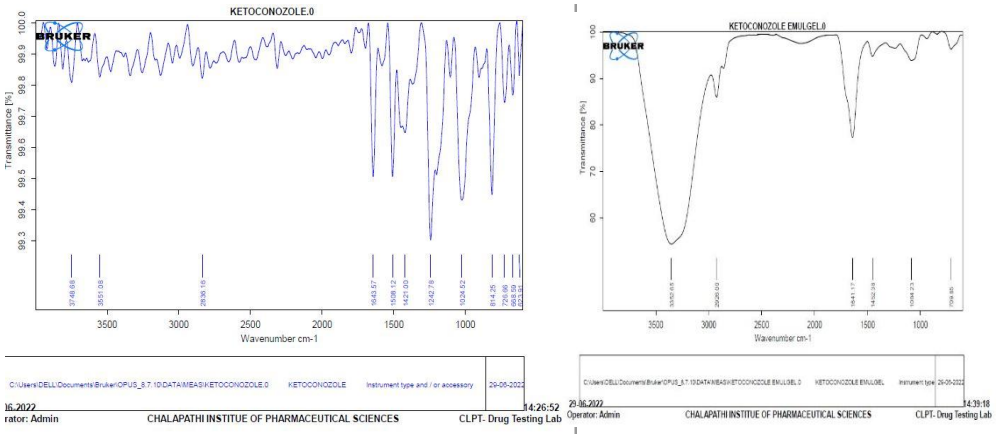


Figure-2: (a) FT-IR Spectrum of pure drug (b) FT-IR Spectrum of microemulgel

DSC Studies

In DSC study, a sharp endothermic peak was appeared at 151.91^{0}C denoting the melting point of ketoconazole drug and the peak in microemulgel was similar to that of pure drug denoting no possible interactions.

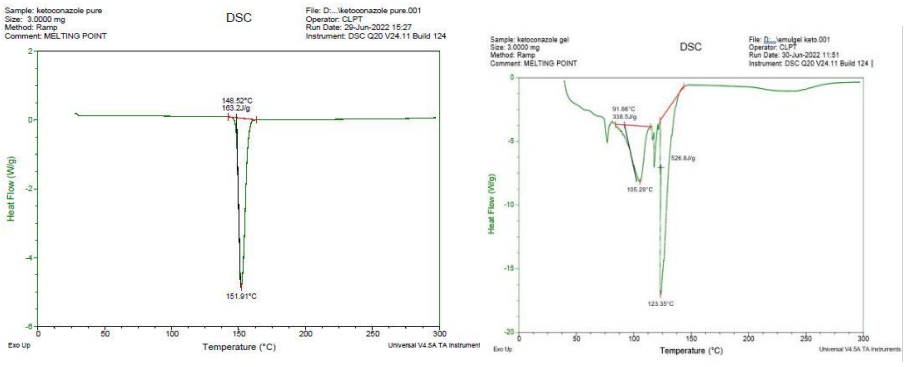


Figure-3: (a) DSC of pure drug (b) DSC of microemulgel

Table-3: Formulation batches of Ketoconazole Microemulsions.

Formulation	**Drug(%w/w)**	**Oil mix (%w/w) (2:1)**	**S_{mix} (%w/w) (2:1)**	**Double distilled water (mL)**
P1	2%	10	38.12	49.88
P2	2%	15	40.04	42.96
P3	2%	5	39.60	53.40
P4	2%	28	40.23	29.77
P5	2%	20	34.06	43.94
P6	2%	11	35.98	51.02

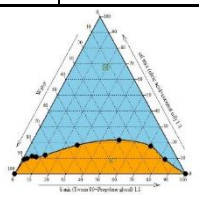
For microemulsion, the oil mix and S_{mix} of 2:1 ratio was chosen because the microemulsion occurrence region in 1:1 ratio is comparatively low and they were degraded after a period of 48hr because of equal proportions of oil mix and S_{mix}.

Construction of pseudoternary phase diagram and preparation of KTZ microemulsion

The oil mix (oleic acid+coconut oil) and Smix (Tween 80+propylene glycol) concentrations of 1:1 and 2:1 were used to create the pseudoternary phase diagrams (Figure 4A,B), which show the microemulsion area(for 1:1 it was in yellow colour and for 2:1 it was in red). There was no phase inversion from w/o to o/w for the microemulsion at equilibrium. For further investigation, the ratio with stable and distinct solutions was chosen. Because an increase in

concentration may enhance the irritation and toxicity to skin and nails, the ratios with high surfactant levels were not further investigated.[26]

Formulation	**pH±SD**	**Viscosity(cps)±SD**	**Mean droplet size(nm)±SD**	**PDI**	**Zeta potential(mV)±SD**	**Drug content (%)±SD**
P1	6.23±0.06	47.23±0.31	73.82±2.45	0.204	-28.51±1.11	97.06±1.02
P2	6.46±0.55	36.8±0.42	61.87±0.92	0.194	-33.41±0.19	98.23±0.14
P3	6.07±0.31	54.08±0.32	57.24±1.82	0.185	-40.26±0.14	97.02±0.74
P4	6.34±0.41	77.21±0.18	78.72±2.41	0.209	-35.17±1.50	98.94±0.24
P5	5.01±0.03	34.5±0.74	54.27±0.99	0.180	-45.41±2.01	99.18±0.94
P6	5.97±0.04	57.91±0.28	77.34±0.17	0.196	-31.98±0.98	98.03±0.95



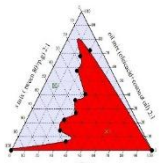


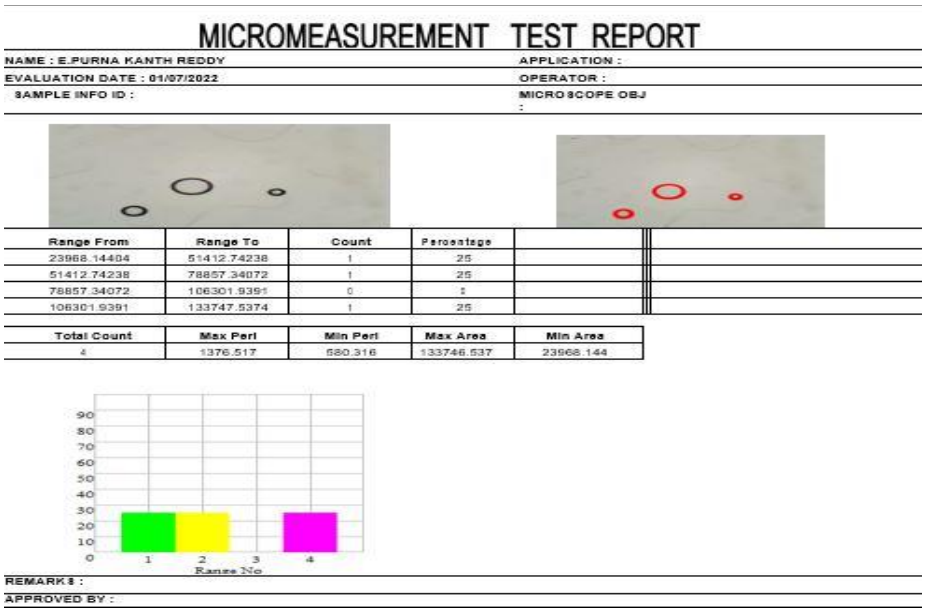
Figure-4(a): Ternary plot of oil mix(1:1) +S_{mix} (1:1) 4(b): Ternary plot of oil mix(2:1)+S_{mix}(2:1)

Table 4 : Measurement of Viscosity, pH, Mean droplet size,PDI,zeta potential and drug content

All values are mean of 3 trials±SD(n=3). It was evident that by increase in S_{mix} concentration the mean droplet size and PDI decreased and zeta potential is increased. Hence the formulation P5 was chosen as optimized formulation for further studies based on its zeta potential,PDI,drug content and its mean droplet size.

Microscopic observation of microemulsion:

The microemulsion was observed under the electronic imaging microscopy and the magnification of the lens is 40X.



MICROMEASUREMENT TEST REPORT

NAME : E.PURNA KANTH REDDY
APPLICATION :
EVALUATION DATE : 01/07/2022
OPERATOR :
SAMPLE INFO ID :
MICROSCOPE OBJ :

Range From	Range To	Count	Percentage
23968.14404	51412.74238	1	25
51412.74238	78857.34072	1	25
78857.34072	106301.9391	0	0
106301.9391	133747.5374	1	25

Total Count	Max Perl	Min Perl	Max Area	Min Area
4	1376.517	580.316	133746.537	23968.144

REMARKS :
APPROVED BY :

Figure-5: Measurement of particle size of microemulsion

Formulation of microemulgel

The optimized formulation of microemulsion was incorporated into carbopol gel base which was previously prepared then an immediate colour change to pink was observed due to interactions of oil and drug. So, carbopol was not preferred.[27] So, HPMCK_4M and sodium alginate in 1:1 proportion yielded required consistency hence it was chosen for formation of microemulgel and the resultant microemulgel was evaluated for drug release, drug content and antifungal activity.

Characterization of microemulsion loaded microemulgel

The pH, viscosity, and drug content of microemulgel were found to be 6.12± 0.15, 7510 cps, and 96.25%.

Ex vivo skin penetration studies

A comparison of conventionally marketed Kz cream and microemulgel loaded with ketoconazole microemulsion was conducted.Figure 6 shows a study of the *ex vivo* release of a microemulgel against the marketed formulation(Kz cream®). During the beginning hour, the drug release from microemulgel and Kz cream® were found to be 4.73± 0.03% and 11.65 ± 0.34, respectively. This demonstrated that the release of drug from Kz cream® was three times that of microemulgel formulation. At the last of tenth hour, 19.82±0.22% release was found out from microemulgel as well as 48.47±0.06% was released from conventional Kz cream®. For treatment of infections caused by fungus a formulation with sustained release is recommended as it decreases the frequency of application when in comparison to the conventional cream. The skin retention study states that there were no significant variations among the two formulations.The percent of microemulgel and Kz cream® remained on the porcine skin membrane was be 23.56% ± 0.63 and 21.08% ± 0.36 respectively

Table-5: *Ex vivo* drug release study

S.no	**Time(hr)**	**%Drug release from microemulgel±SD**	**%Drug release from Kz cream®±SD**
1	0	0	0
2	1	4.73±0.03	11.65±0.34
3	2	4.81±0.10	12.14±0.46
4	3	4.91±1.17	14.98±0.11
5	4	10.05±0.12	17.90±0.09
6	5	11.12±0.01	19.78±0.55
7	6	12.13±0.07	20.89±0.77
8	7	14.23±0.09	28.89±0.01
9	8	15.67±0.23	38.97±0.36
10	9	18.98±0.13	44.98±0.41
11	10	19.82±0.22	48.47±0.06

All values are mean of 3 trials±SD(n=3)

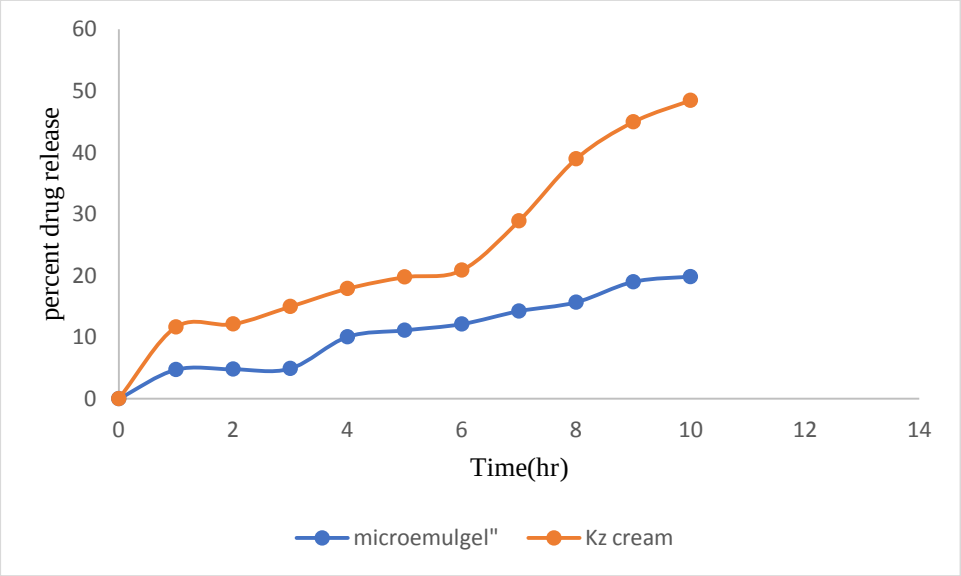


Figure-6: *Ex vivo* comparative release study between microemulgel and Kz cream®

***In-vivo* study: Grouping of animals and IAEC approval**

15 albino mice were grouped 24hr prior to experimentation into three namely standard control and test each group comprising of five mice. All the animals were habituated to 12hr dark cycle and 12hr light cycle. The study protocols were reviewed and approved by the IAEC(Approval number:16/IAEC/CLPT/2021-22).All the experimentation was carried out in animal house of Chalapathi institute of pharmaceutical sciences.

Procedure: Skin irritancy test

Arrange the mice in aseptic condition by cleaning of experimental area with 70% v/v alcohol. The albino mice of 200g weight were taken and the hair of the mice were scraped off upto 1-2cm^2 on previous day of experiment. After that application of Kz cream(Standard) to group-1 and application of oleic acid containing placebo to group-2 and formulated microemulgel(test) to group-3. Allow the mice to withstand for more than 24hr then observe for any irritation or redness on the skin.

Study outcome

The experimentation revealed that there was no irritation or redness observed after a period of more than 24hr for both test and standard groups followed by signs of irritancy was observed for control group. Indicating that the formulation is safe for application.

Antifungal activity

Ketoconazole microemulgel shown the highest zone of inhibition against *C albicans*(39mm) when compared to placebo(35mm) .The placebo formulation also shown appreciable antifungal activity due to the presence of oleic acid which seems to possess antifungal activity and as well as a penetration enhancer.

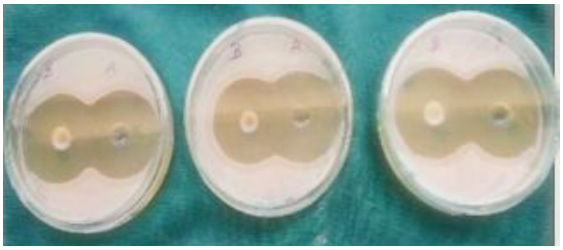


Figure-7: Zone of Inhibition of placebo and microemulgels

Accelerated stability studies

The stability studies were performed on microemulsion loaded microemulgel according to ICH-Q1A guidelines. The formulation was checked for appearance, drug content, and *in vitro* diffusion.

The stability studies were performed from 0 to 2 months mainly there exists 3 key parameters.

Refrigeration at 5 ± 3^0C

1. Appearance remained same from 0 to 2months i.e; smooth white gel.
2. Drug content was 96.34% at 0months and 95.10% at 1month and at 2months the drug content was 94.11%.
3. Drug release by *in-vitro* diffusion below 10hr was 90.01% at 0months and 89.76% at 1 month and 88.98% at 2months.

Room temperature 25^0C ± 2°C/ 60% ± 5%RH

1. Appearance: Smooth, white gel Smooth, white gel Smooth, white gel(0-2months)
2. Drug content: 96.31%(0months) 94.40%(1month) 93.52%(2months)
3. Drug release by *in- vitro* diffusion study within 10h:90.06%(0months) 88.85%(1month) 87.36% (2months).

Accelerated temperature 40^0C ± 2°C/ 75% ± 5%RH

1. Appearance: Smooth, white gel Smooth, white gel Smooth, white gel(0-2months)
2. Drug content:96.33%(0months) 94.75% (1month) 93.69% (2months)

3. Drug release by *in-vitro* diffusion study within 10h:90.08%(0months) 88.15% (1 month) 86.21%(2months).

CONCLUSION

The optimized formulation comprising 2% ketoconazole, 20% oil mix(Oleic acid: coconut oil::2:1), 34.06% S_{mix} (Tween 80:PG::2:1) and 43.94 % water was selected on the basis of drug loading, percent transmittance, thermodynamic stability and *ex- vivo* drug release. The chosen micro emulsion showed 99.02% drug loading and 98.07% transmittance. The microemulsion when loaded into different grades of Carbopol gel colour change occurs indicating chemical interaction. The microemulsion was loaded into a gel base comprising of 1% HPMC K4M and 2% sodium alginate in the ratio of 1:1. The microemulgel containing 1% HPMC K4M and 2% sodium alginate in the ratio of 1:1 was white in appearance, and smooth in consistency. It did not exhibit any colour change. The *ex- vivo* study was done on porcine skin due to unavailability of human cadaver nail plate. The microemulgel showed a slow release of 19.82% whereas Kz cream® showed a release of 48.47% at the end of 10th hr. The microemulgel was stable for a period of two months. *In-vivo* study revealed no irritation on animal skin. Thus, ketoconazole and oleic acid microemulgel formulation could show promising and beneficial results in treating opportunistic fungal infections.

Acknowledgements

The work is supported by Chalapathi institute of pharmaceutical sciences, Lam, Guntur.

Conflicts of interest

We declare that there are no conflicts of interest.

Abbreviations

FT-IR-Fourier transform infrared,DSC-Differential scanning calorimetry,MTCC-Microbial type culture collection,KTZ-Ketoconazole,UV-Ultraviolet,nm-nanometer,µg-microgram,λ_{max}-absorption maxima,KBr-potassium bromide,mg-milligram,hr-hours, rpm-revolutions per minute, ml-milliliter,g-grams, cps-centipoise,HPMC-Hydroxy propyl methyl cellulose,PBS-phosphate saline buffer,PDI-polydispersity index,RH-Relative humidity,IAEC-Institutional animal ethics committee,mm-millimeter,ICH-International council for harmonisation,PG-Propylene glycol.

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