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Research Article

Fabrication of Ketoconazole Nanoformulation Using Extra Virgin Olive Oil: A Comparative Pharmacokinetic and Stability Study

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ABSTRACT

Ketoconazole belongs to the class II drugs of the biopharmaceutical classification system and has low solubility issues. In the market, the available drug ketoconazole is 200-400 mg and is associated with low solubility. To cope with these issues nanoemulsion is formulated. During the fabrication of the nanoemulsion, olive oil was used as a lipid, tween 80 as a surfactant, and PEG 600 as a co-surfactant. By changing these ratios of surfactants and co-surfactants, formulations with different particle sizes and polydispersity index were obtained. The optimized lipid-based nanoparticles obtained have a particle size of 50.29 nm, polydispersity index of 0.377, and Zeta potential -17.2 mv. They have an entrapment efficiency of 78% and a drug loading capacity of 3.97%. Further characterization through the FTIR study showed that there is no interaction between drug excipients. The scanning electron microscopy showed white patches, which also confirmed nanoparticles formation. The XRD analysis showed that the drug changed from crystalline to amorphous form. The in vitro study was conducted, and the nanoformulation showed better bioavailability than the marketed one. A statistical model was applied to the in vitro study. The stability study was conducted at different temperatures, and the formulation was found stable at refrigerated, room, and hot temperatures. The in vivo antifungal study proved that nanoformulation has a better recovery rate as compared to the marketed one. The similarity index was 69.3, and the peaks of nanoformulation were above the marketed one in terms of release kinetics. It is concluded from the study that nanoformulation was better in terms of recovery, release, and cost than the marketed drug.

Keywords: ketoconazole, olive oil, bioavailability, *in-vitro*, i*n-vivo*, stability

INTRODUCTION

The oral route for drug delivery is considered optimal for achieving therapeutic and prophylactic effects, especially against chronic and various other diseases [1]. Oral drug delivery routes have maximum patient compliance compared to others, but the major hurdle with oral drug delivery is their poor water solubility, which poses a great challenge in pharmaceutical drug research for researchers and manufacturers [2]. Bioavailability is a major Pharmacokinetic property. The US Food and Drug Administration defined bioavailability as the rate and extent to which the active moiety or active ingredient is absorbed from a drug product and becomes available at the site of action [3]. As a result of reduced bioavailability,

the drug dose is high, and only a small amount is present at the action site. The high dose of drugs not only causes wastage of drugs but also has various side effects and causes an economic burden to patients [4]. A few examples of drugs that are used in high doses due to poor bioavailability are given below. The absolute bioavailability of commercially available danazol is (6.2%) which is very low. The poor bioavailability of danazol is related to its first-pass metabolism and poor aqueous bioavailability. Because of its poor bioavailability, danazol formulations are advised at high doses, i.e., 300-400 mg twice daily for endometriosis treatment. Side effects like weight gain, virilism, and a decrease in bone density can be noted in such high doses [5]. Paracetamol (acetaminophen) is another common example. Paracetamol is known to have extensive first-pass metabolism, which is responsible for its partial bioavailability, leading to high dose concentration, the result of which is severe kidney and liver damage [6].

Various efforts have been made to cope with these problems, such as molecular optimization. Nanomedicines have recently gained a reputation for enhancing the bioavailability of poor water-soluble drugs [7]. Improvement in nanoparticle formulation of the same shape, composition, and size has brought innovation to the field of nanopharmaceutical sciences. For targeting drugs at the secondary and tertiary levels, the drug assimilation into the nanocarrier system gives a new prototype in drug delivery. Drug delivery systems based on lipids have attracted the interest of scientists for the past few years. In the pharmaceutical nanotechnology field, lipid-based nanoparticles (LBN) are gaining importance due to many prospective uses in pharmaceutical drug delivery systems [7][8]. For enhancement of bioavailability and targeted drug delivery of biopharmaceutical classification system class II drugs (high permeability and low solubility), lipid nanoparticles are being extensively used[3]. Lipid-based nanoparticles offer distinct opportunities for the growth of novel ways to cure disorders because of their unique size-dependent properties [4, 5]. Lipid-based nanoparticles in oral dosage form reduce hepatic first-pass metabolism by potentially enhancing lymphatic transport and thus increasing oral bioavailability [3, 6].

MATERIALS AND METHODS

Materials

Ketoconazole was provided by Atco Laboratories Pvt. Ltd Karachi, Pakistan, tween 80, and Polyethylene glycol-600, manufactured by VMR International Belgium, were purchased from Scientific traders. Olive oil was obtained from Buraq Scientific traders, Pakistan. Dialysis bags were purchased from Spectrum Lab.

Methods

Thirteen different formulations were fabricated using the microemulsion method using different concentrations of surfactant tween 80, cosurfactant Polyethylene glycol-600, and changing stirring time and speed given in table 1.

While preparing the blank formulation organic phase, olive oil was warmed, and PEG was melted at 75 °C, above its melting point. For the preparation of the aqueous phase, tween 80 was dissolved in water by stirring. The organic phase was then incorporated into the aqueous phase, stirring was started, and cold water was added dropwise to obtain microemulsion. The microemulsion was then subjected to centrifugation at 10,000 rpm for 15 minutes. The supernatant was filtered, and the precipitate was redispersed for particle sizing, zeta potential, and PDI (Polydispersity Index).

Fabrication of ketoconazole-loaded lipid-based nanoparticles

The best-optimized formulations (Table 2) were selected from the above blank formulations, loaded with ketoconazole drug, and checked for particle size, polydispersity index, and zeta potential. The rest of the protocols were the same as for the unloaded one.

Lyophilization

The optimized formulation was lyophilized through a freeze dryer (HumanLab Korea). 10% D-Glucose solution (7 mL) was added as a cryoprotectant for better dispersion and aggregation prevention. The formulation, which was optimized, was kept for one night at -20 °C and then shifted to freeze drier at -75 °C. The particles were freeze-dried for two days (48 h) [9], which were further subjected to characterization like X-ray diffraction (XRD), scanning electron microscopy (SEM), and Fourier transform infrared spectroscopy (FTIR).

Entrapment efficiency and drug loading capacity determination

The optimized microemulsion formulation centrifugation was done at 10,000 rpm for 15 minutes; the supernatant was separated and passed through UV light to check for unentrapped drugs. The UV Shimadzu 1800 was used for analysis. The following equation (1) was used for the calculation of drug entrapment, and equation (2) was used for drug loading capacity.

Equation (1) $$EE\% = \frac{\text{Total drug amount-unloaded drug}}{\text{Total drug amount}} \times 100$$

Equation (2) $$DLC = \frac{\text{Total qty of drug}}{\text{Total qty of drug + qty of excipients}} \times 100$$

CHARACTERIZATION

Zeta sizing

Malvern zeta sizer (UK) was used to determine particle size, Polydispersity Index, and zeta potential [10]. Prior to determining these parameters, the precipitates were redispersed with 200 µL Deionized water with the help of a vortex mixer. All the three parameters were checked, and the average of 03 readings was considered.

X-ray diffraction analysis (XRD)

XRD studies were carried out to know any differences in the crystalline structure and physical nature of pure drug and nanoformulation. XR diffractometer (JDX-3532 Jeol Japan) was used for structure differences [11].

Scanning Electron Microscopy

To determine surface morphology, the scanning electron microscope (JSM-5910 Jeol Japan) was used. The magnifying range used was from 100 to 10,000X at changing voltage [12].

Table 1: Fabrication of blank Solid lipid nanoparticles

Formulation	Olive Oil	PEG	Tween 80	Stirring Time	RPM
UME-1	1 g	0.4 g	1.5 g	15 minutes	800 rpm
UME-2	1 g	0.4 g	1.5 g	15 minutes	800 rpm
UME-3	1 g	0.4 g	1.5 g	15 minutes	800 rpm
UME-4	1 g	0.4 g	1.5 g	15 minutes	800 rpm
UME-5	1 g	0.4 g	1.5 g	15 minutes	800 rpm
UME-6	1 g	0.2 g	1 g	15 minutes	800 rpm
UME-7	1 g	0.2 g	1 g	10 minutes	800 rpm
UME-8	1 g	0.4 g	1.5 g	15 minutes	800 rpm
UME-9	1 g	0.4 g	1.5 g	15 minutes	900 rpm
UME-10	1 g	0.4 g	1.5 g	30 minutes	600 rpm
UME-11	1 g	0.4 g	1.5 g	10 minutes	1200 rpm
UME-12	1 g	0.4 g	1.5 g	07 minutes	1200 rpm
UME-13	1 g	0.4 g	1.5 g	10 minutes	1000 rpm
UME-14	1 g	0.4 g	1.5 g	15 minutes	1000 rpm

Table 2: Fabrication of Ketoconazole-loaded lipid-based nanoparticles

Formulation	Olive Oil	PEG 600	Tween 80	KZ	Stirring Time	RPM
RKZME1	1 g	0.4 g	1.5 g	0.12 g	15 minutes	800 rpm
RKZME11	1 g	0.4 g	1.5 g	0.12 g	10 minutes	1200 rpm
RKZME13	1 g	0.4 g	1.5 g	0.12 g	10 minutes	1000 rpm

Fourier Transform Infrared Spectroscopy (FTIR)

Instrument Model Spectrum Two, Serial Number 103385 was used for drug excipients interactions. The default scan range used was 4000 -450 cm^{-1} [13, 14].

Drug in vitro release study

The in vitro drug release study was conducted using the dialysis membrane. The dialysis bag was soaked for 24 hours in deionized water. The optimized formulation, microemulsion (2.5 mL) was transferred to the dialysis membrane. Both ends were tied with a thread and placed in simulated gastric fluid pH (1.2) 250 mL volume. The other bag filled with the same dispersion was placed in simulated intestinal fluid pH (6.8) 250 mL volume. After an interval of 1-12 hours, a 2 mL sample was withdrawn for UV study, and the same volume was replaced to maintain sink condition. The same procedure was repeated for marketed drug and reference drug (Pure ketoconazole).

Table 3. Changing stirring time, speed, and its effect on particle size and PDI

Formulations	PDI	Particle Size (nm)	Stirring time (min)	Stirring Speed (rpm)
RKZME1	0.377	50.29	15	800
RKZME2	0.931	64.96	15	800
RKZME3	0.672	56.17	15	800
RKZME4	0.85	56.05	15	800
RKZME5	0.946	72.62	15	800
RKZME6	1	49.4	15	800
RKZME7	1	65.7	10	800
RKZME8	1	57.64	15	800
RKZME9	0.9	40	15	900
RKZME10	1	57	30	600
RKZME11	0.4	39	10	1200
RKZME12	1	48	07	1200
RKZME13	0.08	22	10	1000
RME14	0.5	18	15	800

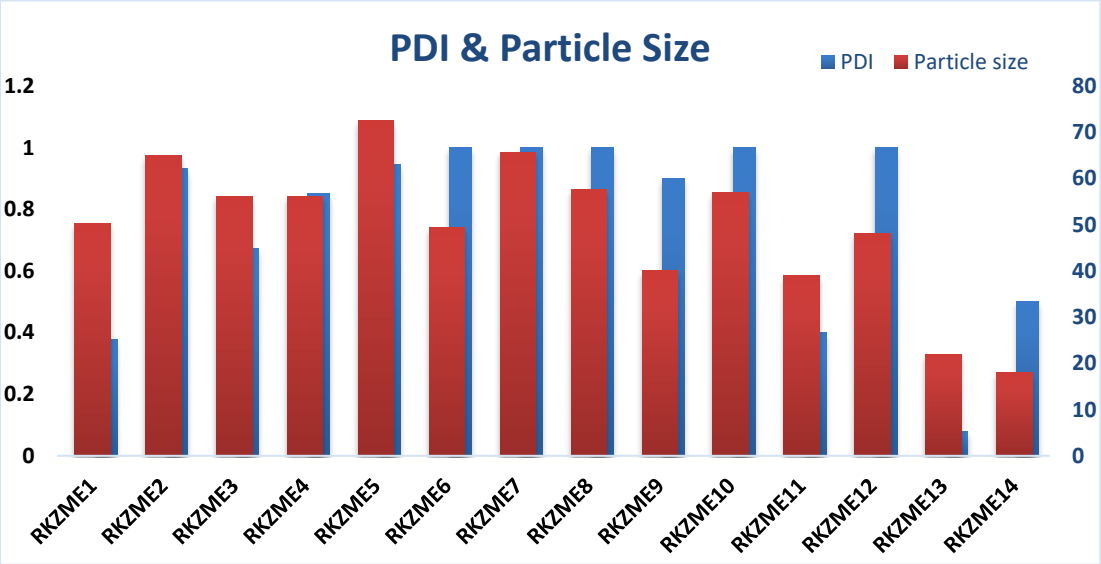

Figure 1: PDI and Particle Size

Drug in vivo activity

Healthy rabbits were used to conduct in vivo antifungal activity. The ethical committee (Department of Pharmacy, University of Malakand) accepted all rabbits for experimental purposes. All experimental animals were kept for 24 hours to adapt to the new environment. The experimental animals found to be in any discomfort were skipped from the study.

Drug administration by oral route

The rabbits were divided into three groups before treatment with ketoconazole drug (120 mg). Group I (06) rabbits received the market-available drug. Group II received microemulsion with two different concentrations, and group III (control group) rabbits received just food and water.

Data collection

After inducing fungal infection (aspergillus Niger), drug administration by oral route was started, and observation was done after a fixed period, and the infected and the recovered area were measured in terms of centimeters reduction in the infected area.

RESULTS

Particle size analysis

After optimizing the blank formulation, the loaded formulations (RKZME1, RKZME11, and RKZME13) showed reduced particle size, with sizes of 50.29 nm, 39 nm, and 22 nm, PDI 0.377, 0.4, and 0.08, respectively, and zeta potentials of -17.2 mv, -14.8 mv, and -20. 9 mv (given in Figure 1 and Table 3).

Scanning electron microscopy

Scanning electron microscopy was done for both loaded and unloaded formulations, and the changes in surface morphology are given in Figures 3 and 4. The unloaded formulation showed non-uniformity on the surface. In contrast, for the loaded formulation, there are small beads like white globules, which show that the lipid is properly incorporated with the drug. The round globules in the form of white beads also confirm nanoparticle production in the loaded formulation. The scanning electron microscopy for both formulations was performed at different resolutions.

Fourier transform Infrared Spectroscopy

Fourier transform infrared spectroscopy is an easy way to discover drug-excipient interactions [15]. The FTIR spectra of both loaded and unloaded drugs showed various characteristic peaks. The loaded formulation NFKZ in Figure 6 showed OH stretch at 3300 cm^{-1}, which is strong and broad. At 2900 cm-1, it has a medium stretch, which is characteristic of the CH bond. Around 1700 cm^{-1}, it has C=O (carbonyl stretch). In the fingerprint regions, it has an aromatic stretch at 1300 cm-1, which is characteristic of the ketoconazole ring. All the peaks of the unloaded formulation were present in the loaded formulation at the same wave number, which showed that no such drug excipient interaction was present.

X-ray diffraction

The XRD pattern showed that the original ketoconazole drug shows sharp and high peaks, while in nanoformulation (KZME-1), these peaks are reduced from high intensity to short and small peaks (Figure 7 and Figure 8). This shows that the drug is changed from crystalline to amorphous form in nanoemulsion. which further increases the solubility and bioavailability of nano formulation because of having greater free energy than crystalline form. Thus nanosizing was also confirmed by XRD pattern which is also reported in the literature [16].

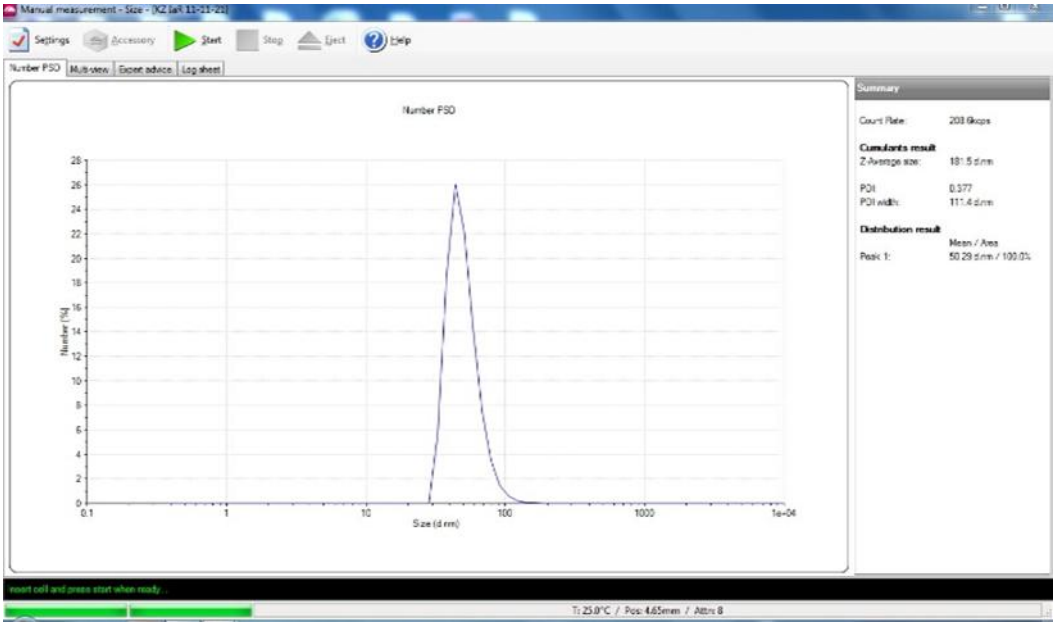

Figure 2: Average particle size of the optimized formulation

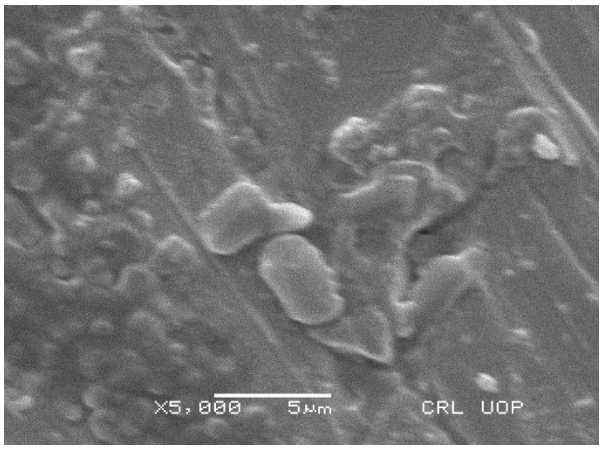

Figure 3: Unloaded Formulation

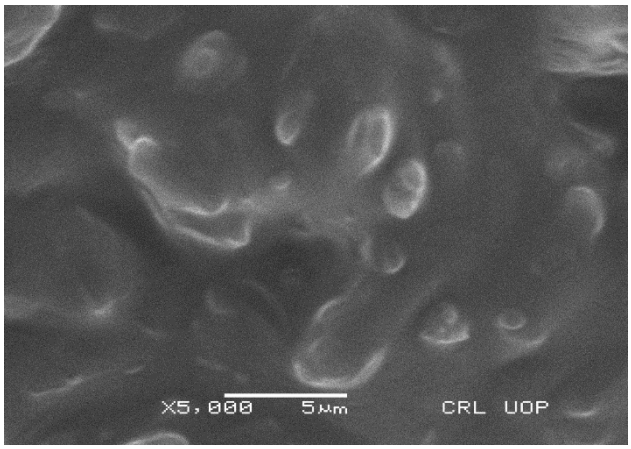

Figure 4: Loaded formulation

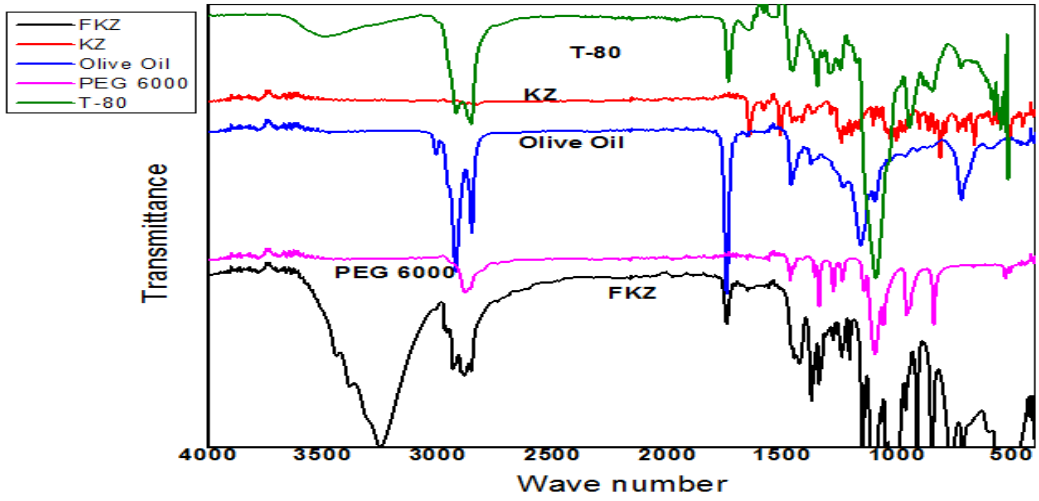

Figure 5: Excipients FTIR

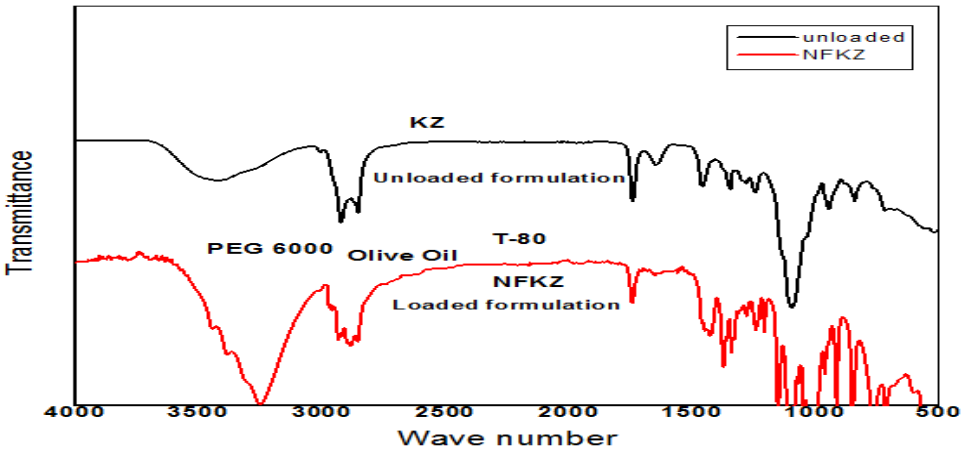

Figure 6: Unloaded and loaded formulation

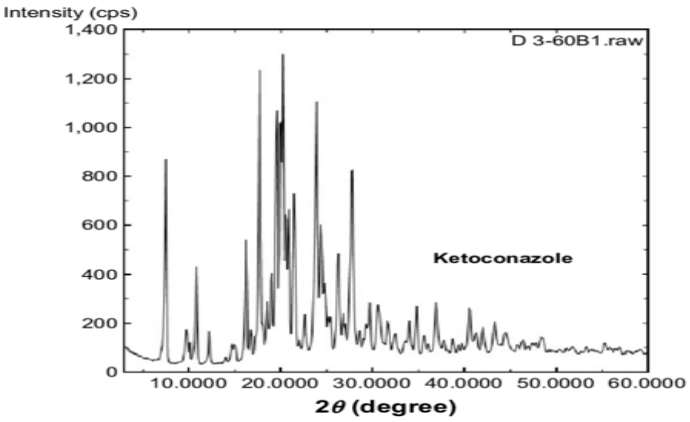

Figure 7: XRD Pattern of Ketoconazole drug

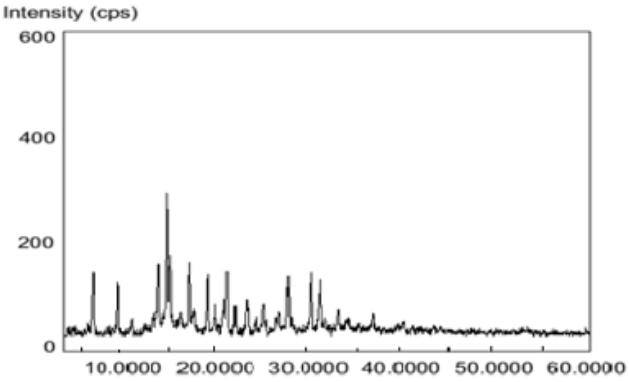

Figure 8: XRD Pattern of Nano formulation

Entrapment Efficiency and DLC

Table 4 and bar charts showed that decreasing the drug amount from 120 mg to 100, 80, 60, and 50 mg, respectively, decreased the drug efficiency from 78% to 47.20%, while increasing the quantity of the drug from 120 to 140 mg increased the drug entrapment efficiency. The optimized formulations with lipid, surfactant, and co-surfactant showed drug entrapment efficiency of 78% and drug loading capacity of 3.97%, with desirable particle size and PDI.

Table 4: Entrapment Efficiency and Drug Loading capacity

Formulations	Entrapment Efficiency	Drug loading capacity
KZME1	78%	3.97%
KZME2	73.60%	3.30%

KZME3	67%	2.68%
KZME4	56%	2.02%
KZME5	47.20%	1.69%
KZME6	78%	3.97%
KZME7	78%	3.97%
KZME8	81.14%	3.97%
KZME9	78%	3.97%
KZME10	78%	3.97%
KZME11	78%	3.97%
KZME12	73.60%	3.30%
KZME13	78%	3.97%
KZME14	78%	3.97%

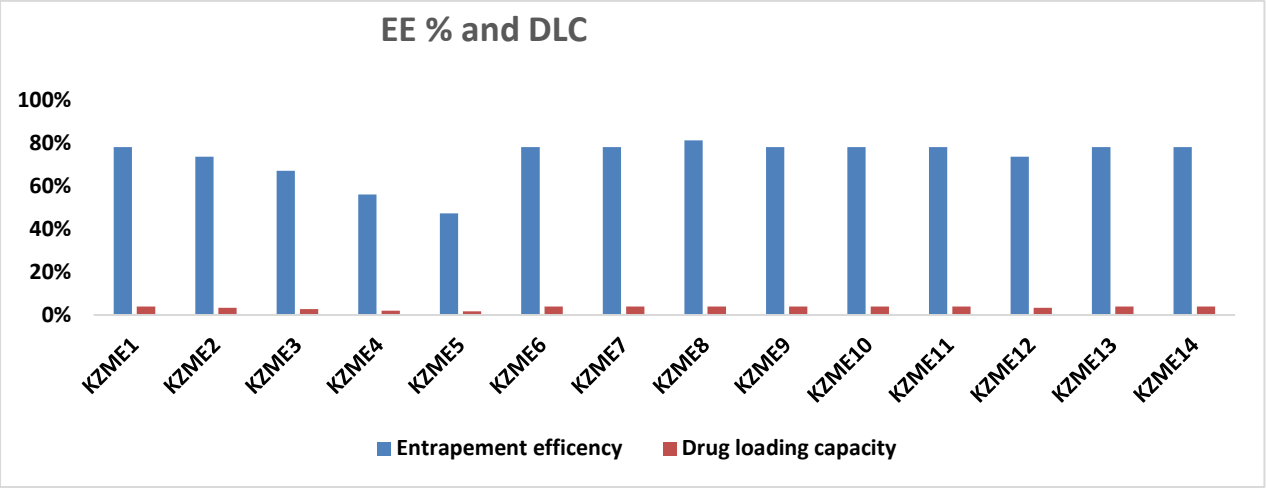

Figure 9: Entrapment Efficiency and Drug Loading Capacity

In Vitro Study

By choosing the appropriate formulation and excipients, in vitro drug release of a drug can be altered [17].

In this study, an in vitro study was designed for both market-available drug and lab-prepared formulations and conducted on dialysis bags in simulated intestinal (pH 7.4) and GI fluids (pH 6.8). It was observed that the lab-prepared formulation release rate was doubled every hour up to 08 hours, and then a slight decline was observed in the release rate of the drug after the 9^{th}, 10^{th}, 11^{th}, and 12^{th} hour. Another observation that was noted in this study is that lab-prepared formulation was released more (66%) than marketed drugs (Fig 10). This nanoformulation also has better solubility in the stomach as compared to the marketed drug, which may be attributed to the addition of olive oil [18] .The nanoformulation drug was released 64% in simulated intestinal fluids.

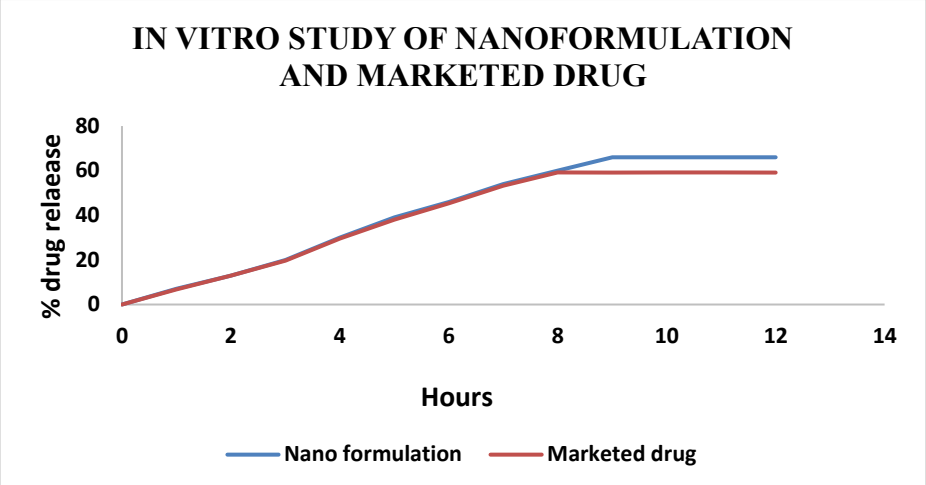

Figure 10: In vitro study of nanoformulation and marketed drug.

In vivo antifungal study

During the in vitro antifungal study, the animals were divided into 03 groups: Group I (G-I) was a controlled group, Group II (G-II) was given a market-available drug, and Group III (G-III) was given a lab-prepared formulation. Firstly, the animal skin was trimmed off for about a 5 cm area. It was cleaned with alcohol swabs. Then it was abraded with the help of clean sandpaper, and fungal inoculum (2 mL suspension) was inoculated to the abraded skin with the help of cotton soaked in inoculum and was attached to the abraded skin for 3 to 5 days with the help of nitro tape. After 3 to 5 days, the fungal infection was induced and confirmed by the in vivo method. The drug was administered to the rabbits by oral route, and observation was done after each 05 days and noted. It was confirmed that the group treated with lab-prepared formulation has the potential to treat quickly in a shorter duration of time than the marketed group.

Stability study

Stability studies were performed at three different temperature conditions, i.e., room temperature, refrigerated, and hot temperature (45 °C), for three months. The formulation was observed both physically and by UV study. The same UV absorbance was observed after the second and 10th day, at the end of the first, second, and third month, and the formulations were found stable.

Similarity index

Similarity factors f2 were calculated for marketed and lab-prepared nanoformulations using the following equation (equation 3). f2 was found to be 69.3, which was greater for nanoformulations than for market-available drugs, as given in Figure 9.

$$50\,log\left[\frac{100}{\sqrt{1+\frac{\sum_{t=1}^{t=n}[R(t)-T(t)]^2}{n}}}\right] \quad \text{.....equation 3}$$

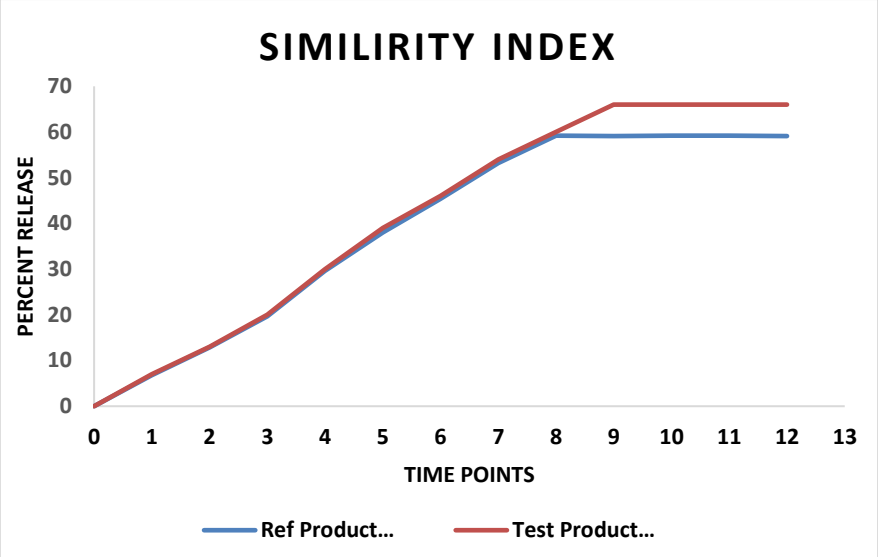

Figure 11: Similarity Index

DISCUSSION

The aim of the present study was to fabricate lipid-based nanoparticles for ketoconazole, which showed better solubility with decreased dose and good stability. The optimized formulation was obtained as a result of fabrication using the microemulsion technique and by incorporating surfactants like tween 80 [19, 20] and cosurfactants like PEG 600 and olive oil as a lipid. It had a particle size of 50.29 nm, polydispersity index of 0.377, and zeta potential of -17. 2mv. It has an entrapment efficiency of 78% and a drug loading capacity of 3.97%.

FTIR showed no drug excipients interaction. The scanning electron microscopy confirmed nanoparticle formation with white patches showed a decrease in peaks in the case of loaded formulation, which is a shift from crystalline to amorphous form.

An in vitro study was conducted, and the nanoformulation showed better bioavailability as compared to the marketed one [21, 22]. The stability study was conducted at different temperatures, and the formulation was found stable at refrigerated, room, and hot temperatures. There was also no change in UV absorption spectra. The in vivo antifungal study proved that nanoformulation has a better recovery rate than the marketed one. The similarity index was 69.3, and the peaks of nanoformulation were above those of the marketed one in terms of release kinetics. It is concluded from the study that the nanoformulation was better in terms of recovery, release, stability, and cost as compared to marketed drugs.

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