Impact of Composition and Morphology of Ketoconazole-Loaded Solid Lipid Nanoparticles on Intestinal Permeation and GastroPlus-Based Prediction Studies

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ABSTRACT: Ketoconazole (KTZ) is a potential oral antifungal agent to control systemic and local infections. This study addresses the impact of composition (tween 80 and compritol as CATO) and morphology on permeation (stomach, jejunum, and ileum) profiles of KTZ-loaded solid lipid nanoparticles (SLNs) in rats followed by in vivo pharmacokinetic prediction and simulation using GastroPlus. The selected formulations were characterized for size, size distribution, zeta potential, entrapment efficiency, total drug content, morphology, in vitro drug release, ex vivo permeation and drug deposition, penetration potential, and GastroPlus-based in vivo prediction in rats. The results showed that there was considerable impact of pH, composition (CATO and tween 80), size, total drug content, and entrapment efficiency on in vitro drug release and permeation across the stomach, jejunum, and ileum. Ex vivo findings suggested pH, composition, size, and permeability coefficient-dependent permeation of SLNs across the stomach, jejunum, and ileum. Confocal laser scanning microscopy (CLSM) confirmed a relatively high degree of penetration of the optimized formulation "K-SLN4" (66.1% across the stomach, 51.5% across the jejunum, and 47.9% across the ileum) as compared to KSUS (corresponding values of 21.7%, 18.2%, and 17.4%). Finally, GastroPlus predicted in vivo dissolution/absorption as 0.012 μg/mL of K-SLN4 as compared to KSUS (the drug suspension with 0.0058 μg/mL) and a total regional absorption of 80.0% by K-SLN4 as compared to 60.1% of KSUS. There was only an impact of dose on C_max (maximum plasma concentration) and area under the curve (AUC) in rats. Thus, the present strategy could be a promising alternative to parenteral and topical delivery systems for long-term therapy against systemic and local mycoses with high patient compliance.

1. INTRODUCTION

The oral route is the most common and preferred for its benefits (ease of administration, high patient compliance, high doses, and cost effectiveness) over others to control fatal and chronic diseases (systemic fungal infections and bacterial diseases). Various systemic fungal diseases (mycoses) have emerged as life-threatening infections due to clinical drug resistance, emerging new strains, and the involvement of various immunocompromised patients. Continued progressive developments in drug delivery of available established drugs (ketoconazole, terbinafine, amphotericin B, itraconazole, griseofulvin, and fluconazole) has resulted in promising findings to treat these infections. However, frequent clinical application of these molecules has been restricted due to associated serious limitations. Itraconazole and terbinafine (idiiosyncratic liver and skin reaction) are not recommended in the U.K. for children. Fluconazole is unapproved in the USA by the US-FDA to control onychomycosis due to its high doses and unclear duration of treatment. Griseofulvin was disappointing due to its low cure rate (30–40%) of toenail infection. Notably, ketoconazole (KTZ) is used orally to control systemic mucocutaneous fungal infections with a cure rate of 15–30% and 50–70%, in toenail and fingernail infections, respectively. However, oral delivery of KTZ is associated with various clinical limitations such as (a) nausea and vomiting, (b) abdominal discomfort, (c) US-FDA warnings that using oral Nizoral tablets could cause potential fatal liver injury, risky drug interactions, unusual production of corticosteroid hormones, jaundice, and adrenal gland issues (US FDA 2013 Report; https://www.fda.gov/drugs/drug-safety-and-availability/fda-drug-safety

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Table 1. Summary of Compositions and Characterized Parameters of Reformulated Formulations Used in This Study$^a$

| code      | CATO | tween 80 | PEG600 | PL90 | KTZ  |
|-----------|------|----------|--------|------|------|
| K-SLN1    | 3.5  | 1.4      | 1.0    | 0.06 | 0.6  |
| K-SLN2    | 2    | 0.7      | 0.9    | 0.06 | 0.6  |
| K-SLN4    | 2    | 1.4      | 1.5    | 0.06 | 0.6  |
| K-SLN7    | 3.5  | 0.7      | 0.9    | 0.02 | 0.6  |
| K-SLN13   | 1.5  | 1.1      | 1.2    | 0.02 | 0.6  |
| KSUS      | 0.1% Na-CMC as suspending agent in water |

$^a$CATO: Compritol 888 ATO. % EE: Percent entrapment efficiency. % TDC: Percent total drug content. KTZ: Ketoconazole. PDI: Polydispersity index. FE = Fold error. PL90 = Phospholipon 90G.

Ketoconazole is a chemically imidazole-based antifungal drug with limited water solubility (0.04 mg/mL), dermal irritation (free for topical/transdermal delivery or commercials), and safety concerns in vivo. In various studies, either limited permeation and subsequent simulation (reducing clinical studies). The drug is also potentially effective against various systemic fungal infections such as candidiasis and blastomycosis. Commercially available topical products (~2%) strength) have been challenged for therapeutic effectiveness due to limited permeation across the skin and short residence time at the applied site.

KTZ is a chemically imidazole-based antifungal drug with limited water solubility (0.04 mg/mL), dermal irritation (free for topical/transdermal delivery or commercials), and safety concerns in vivo. In various studies, either limited permeation and subsequent simulation (reducing clinical studies). The drug is also potentially effective against various systemic fungal infections such as candidiasis and blastomycosis. Commercially available topical products (~2%) strength) have been challenged for therapeutic effectiveness due to limited permeation across the skin and short residence time at the applied site.

In our recent investigations, we reported improved permeation, efficacy, cellular uptake (L929 and J774A.1), and safety concerns in vivo. Furthermore, we explored comprehensive mechanistic perspective of SLN-loaded ketoconazole for enhanced ex vivo penetration across rat skin, dermatokinetics, and long-term stability studies (photostability and chemical stability for 12 months). The optimized KTZ-loaded SLN formulation was characterized for particle size, zeta potential, solid-state properties, in vitro/ex vivo (rat skin and human dermatome skin), and in vivo pharmacokinetics in the rat model. The long-term stability study at different temperatures and photostability ensured the success of the product over a period of one year. In continuation of previous research, it was required to assess the intestinal permeation profile of the presently investigated KTZ-loaded SLNs in a rat model followed by GastroPlus-based simulation/prediction studies. The selected formulations (in terms of varied size and composition) were used in the current study to investigate the effect of various factors (morphology and composition) on permeation profile and subsequent simulation—prediction for in vivo performance in an animal model using the GastroPlus program. Therefore, we address the impact of the solid lipid (compritol 888 ATO), surfactant (tween 80), dosing volume, and particle size on the intestinal permeation profile followed by GastroPlus-based predictions in rat. In the simulation and prediction section, various physicochemical properties of the drug, formulation-related parameters, and physiological input parameters were studied using experimental, reported, and by-default values.

2.0. MATERIALS AND METHODS

2.1. Materials. Ketoconazole (KTZ >99.5% pure, AR grade) was procured from a local pharmaceutical company (Velite Pharmaceuticals, Ludhiana, Punjab, India). Phospholipon 90G (P 90G) and Compritol 888 ATO (CATO) were obtained as gift samples from Lipoid (Germany) and Gattefosse (France), respectively. Tween 80 (as surfactant) was procured from CDH, Mumbai (India). Both dyes probe fluorescein sodium and rhodamine 123 were purchased from Sigma-Aldrich (Mumbai, India). Millipore water was used as aqueous solvent wherever required in the study. Fluorescence dye (fluorescein sodium) was purchased from Sigma-Aldrich (Mumbai, India). All chemicals were of analytical grade. All other solvents and reagents used were of AR grade. Distilled water was used as aqueous media for buffers and related preparations.

2.2. Methods. 2.2.1. Analytical Method. The content of the drugs was quantified using the validated HPLC method.
(reverse-phase high-performance liquid chromatography) as per a previously reported method. The isocratic HPLC (Agilent, 1200 series, California, United States of America) was operated using a reverse-phase C18 column (150 mm × 4.6 mm, 5 μm) coupled with a photodiode array (PDA) detector operating at ambient temperature (25 ± 1 °C) and an absorption wavelength of 230 nm. The working calibration standard curve (5.0–50.0 μg/mL) was prepared in a mobile phase (regression coefficient of 0.999). The mobile phase contained acetonitrile (ACN) and phosphate buffer (pH 4.8) in 48:52 v/v ratio, respectively. The mobile phase was filtered (0.45 μm membrane filter), sonicated (bath sonicator to avoid entrapped air and bubbles), and transferred to the HPLC assembly slot. The isocratic column was operated at the flow rate of 1.0 mL/min over a period of 10 min after the sample injection (10 μL). The study was carried out in triplicate for the mean and standard deviation.

2.2.2. Formulation Compositions. In our recent report, we prepared KTZ-loaded solid lipid nanoparticles (KTZ-SLNs) using CATO, tween 80, PEG600 (polyethylene glycol 600), and phospholipon 90G (PG90) as the solid lipid, surfactant, cosurfactant, and stabilizer, respectively. Several batches of the drug-loaded formulations were prepared, optimized, and characterized for particle size (nm), entrapment efficiency (% EE), and total drug content (%) using a suitable standard method. These formulations were intended for topical application to control nail and cutaneous fungal infections. In all these formulations, a constant amount (0.1% w/v) of fluorescent dye (fluorescein sodium) was added for permeation study. A detailed description of composition and three evaluation parameters with four formulations are presented in Table 1, as reported previously.

In brief, precisely weighed amounts of KTZ (2.0 g), solid CATO (2 and 3.5 g), and PEG600 (0.01 g) were melted at 70 °C to get a homogeneous organic phase. Similarly, tween 80 (0.7 and 1.4 g) and PG90 (0.03 g) were solubilized in distilled water at the same temperature separately. Both phases were kept at the same temperature and under constant stirring. The organic phase was slowly emulsified into the aqueous phase under constant stirring using a stirrer to obtain a primary microemulsion. The obtained microemulsion was further subjected to high-pressure homogenization to get small solid lipid nanoparticles. In all formulations, the content of PG90 and PEG600 was constant. For comparison, the KTZ suspension was prepared using NaCMC (sodium carboxymethyl cellulose) as a suspending agent (0.1% w/v). The final strength of all formulations was 2% w/v.

2.2.3. Formulation Characterized. The prepared formulations (KTZ-SLN1, KTZ-SLN2, KTZ-SLN4, and KTZ-SLN7) were evaluated for particle size, zeta potential (mV), and polydispersity index using the Malvern zetasizer (Malvern zetasizer, United States of America). To assess particle size and polydispersity index (PDI), the sample was diluted (100 times) with Millipore water before analysis. This was required to avoid error in size analysis. However, the sample was processed without dilution for zeta potential measurement. Transmission electron microscopy (an advanced and sophisticated instrument) was used for morphological assessment (particle size) for the formulations. Each individual sample was placed on the copper grid using a double adhesive tape. Then, the sample was negatively stained using 0.1% phosphotungstic acid (negative staining agent) followed by complete drying before scanning under TEM. Notably, the size obtained from TEM (electron beam as a source of light and location-based size measurement) was slightly varied due to instrumental error, sample preparation, and the principle of size measurement (Malvern working on the principle of diffraction of light scattering, DLS). Therefore, this error was resolved by calculating a “fold error” value for each sample using eq 1. This difference is generated due to preferential adsorption of relatively smaller particles by the grid surface of the TEM sample holder as compared to larger particles (as an instrumental error).

\[
\text{FE (fold error)} = 10^{\log_{10}(\text{size by zeta size})/\text{size by TEM}}
\]

where the value of “n” indicated the number of experimental points repeated. The values of “FE” less than 2 can be considered as acceptable.

2.2.4. In Vitro Drug Release Studies at Varied pH. The four optimized and selected formulations such as ketoconazole-loaded SLN1, SLN2, SLN3, and SLN4 were selected for in vitro drug release profiles in three different media. These media were acid media using 0.1 N HCl (pH 1.2), phosphate buffer at pH 4.5, and phosphate buffer at pH 7.4 (prepared as per IP 1996). All of the studies were carried out at the same experimental conditions. The opted volume of the release media was 500 mL, previously simulated at 37 ± 1 °C and a constant stirring rate (using a magnetic Teflon-coated bead). The sample (1 mL) was transferred to a dialysis membrane (12–14K Dalton cutoff for molecular weight) (Sigma-Aldrich, Mumbai, India) bag, tied with one end, and suspended in a glass beaker containing the respective release medium. Sampling (1 mL) was carried out at different time points (0.5, 1, 2, 4, 8, 10, 12, 16, 20, and 24 h) followed by replenishment with an equal volume of fresh medium. In order to maintain a sink condition, 5% DMSO (dimethyl sulfoxide) was added into the release medium.

2.2.5. Ex Vivo Permeation Study Using a Rat Model. The developed formulations (K-SLN1, K-SLN2, K-SLN4, K-SLN7, and K-SLN13) were subjected to ex vivo permeation behavior across the stomach, and an intestinal segment was excised from an ethically sacrificed rat. Male Sprague–Dawley rats of weight about 180–200 g were issued from the Department of Institutional Ethical Committee (Panjab University, regd. No. 45/GO/ReBiS/99/CPCSEA) and approved for the study as per ARRIVE guidelines. They were housed (air-conditioned room) with free access to water and food and acclimatized for 5 days. They were in a fasting condition for 12 h before the experiment. For comparison, the drug-loaded suspension was used as a control group. The excised stomach and intestine were freed from the inner food content by rapid flow of distilled water using a syringe. The identifies sections of the stomach and intestine were cut from the gastrointestinal tract of the rat. To avoid variation in results, an equal length (2.0 cm) of each GIT segment was used in the study for the sample. One end of the excised segment was tied with thread and loaded with the sample (1 mL containing 20 mg). Next, both ends were tightly tied and closed using the same thread to avoid any leakage in the release medium. The final pH was adjusted with freshly prepared buffer solution (0.1 N HCl, acetate buffer for pH 4.5, and phosphate buffer for 7.4). The tissue (stomach, duodenum, and intestine) loaded with the respective sample was suspended in the modified USP dissolution apparatus II using a sinker. The release medium (900 mL) was phosphate buffer solution (pH 7.4 containing 5% dimethyl sulfoxide) set at a temperature of 37 ± 1 °C and a paddle rotation of 100 rpm. The tissue was properly aerated using an aerator. The study was conducted for 4 h due to the viability of the used tissue. Therefore, sampling (1 mL) was carried out at 0.5, 1, 2, 4, and 6 h. The equal volume of fresh medium was transferred to the same release medium to...
maintain sink sink conditions. The permeated drug was quantified using the HPLC method at 230 nm. All ex vivo permeation parameters (cumulative drug permeation rate, flux, apparent permeability coefficient, and enhancement ratio) were calculated.

2.2.6. Drug Deposition Study. After completion of the ex vivo permeation study, the sample-loaded tissues were removed from the dissolution medium, and the remaining content was removed from the intestine or stomach. The remaining content (nonpermeated) of the drug was assayed. The tissue was sliced into small pieces and placed in a beaker containing a methanol−chloroform mixture (2:1, v/v). The mixture was rotated using a magnetic bead over 12 h for extraction of the deposited drug.

Finally, the mixture of tissue was homogenized using a homogenizer (T18 digital Ultra Turrax, IKA, Staufen Germany/Deutschland) for 5 min at 1000 rpm. Then, the mixture was filtered and assayed using the HPLC method. The study was performed for each sample (stomach, duodenum, and intestine) and treated individually.

2.2.7. Penetration Study Using Confocal Laser Scanning Microscopy (CLSM). In order to conform to a mechanistic understanding of the intestinal penetration of the drug-load formulation (rhodamine-123 probed K-SLN4) as compared to the respective suspension (containing 0.05% rhodamine-123), it was essential to visualize under CLSM after completion of ex vivo permeation. Rhodamine-123 aqueous solution was used as a control for the penetration study. The sample was subjected to microtome-based tissue preparation for visualization. The degree of penetration in terms of fluorescence intensity was correlated with the permeation profile. The scanning of the sample was performed at excitation and emission wavelengths of 540 and 600 nm, respectively.

2.2.8. GastroPlus-Based Simulation and Prediction Studies. In general, the GastroPlus program (version 9.7, Simulation Plus, Inc., Lancaster, USA) is a mechanical-based simulation and prediction software to simulate and predict pharmacokinetics (PK) and pharmacodynamics (PD) parameters after administration of any drug or formulation. The physiological-based absorption model is widely applied as a commercial software tool. The program provides various routes of drug administration. In this study, we opted for an oral route of administration. There are three basic tabs for input parameters which can be categorized as (a) compound tab (physicochemical properties of ketoconazole) for the entry of drug-related required information (experimental and theoretical), (b) formulation tab (experimental values such as solubility, particle size, and permeation coefficients), and (c) physiological tab for prediction and simulation conditions. The program simulates an advanced compartmental absorption and transit (ACAT) model based on oral absorption of the pure KSUS and the optimized K-SLN4 containing nine different compartments of the GIT. These nine compartments are termed as the “regional absorption compartment model” linked in series wherein the first is the stomach and the subsequent compartments are the duodenum, jejunum (three), ileum (three), and ascending colon. Moreover, we conducted the prediction study by opting for a rat model and simulation time of 24 h (considering sustained release K-SLN4). For input tabs, we used experimental, theoretical (literature based), and by-default values for each simulation and prediction run.

Plasma Concentration Time Profile Prediction of Pure Drug (KSUS) and K-SLN4. GastroPlus was applied to carry out virtual trials of KSUS and K-SLN4 to assess whether particle size, composition, physiological condition (intestine, stomach, and duodenum), pH reference solubility, and apparent permeability could influence oral absorption and pharmacokinetic parameters in rats. The analysis was evaluated under rat fasting conditions to avoid food−drug interactions. This would be the basis for predicting the effect in humans in a future study. Using input values from Table 3 for pure drug-related information (experimental, literature, and default values), the program predicted a plasma drug concentration time profile at a 20 mg/mL dose for 24 h in rats weighing 0.3 kg.

Prediction of in Vivo Dissolution Rate and Absorption. Having a poor water solubility of ketoconazole in KSUS, it was mandatory to predict in vivo drug dissolution and subsequent absorption in a rat model at the explored dose, dosing volume, and simulation time, as given in Table 3. Similarly, K-SLN4 was the optimized formulation considering the entrapped drug in soluble form and facilitated drug release in the physiological medium (PBS). Therefore, the simulation and prediction were run for both formulations, and a comparison was established in terms of in vivo dissolution/absorption predicted patterns and values under similar experimental conditions.

The software program simulates and predicts the in vivo dissolution based on the diffusion layer model of the Noyes−Whitney equation.

\[
\frac{dM}{dt} = D(C_s - C_l)(1 + 2s)\mu/dr
\]

where \(\rho\), \(r\), \(h\), and \(s\) are the drug density, particle radius, diffusion layer thickness, and shape factor (for spherical shape factor, \(s = 1\), respectively. Moreover, \(C_s\) and \(C_l\) represent the drug solubility in the diffusion layer and the drug concentration in the lumen, respectively. Notably, a drug exhibiting greater in vivo dissolution rate (significant in vivo solubility) as compared to pure drug dissolution (in vitro) in the aqueous system may be overpredicted due to the slower diffusion rate of micelle (bile salt) based drug diffusion than free drug.

Parameter Sensitivity Analysis (PSA) Study. In the PSA study, we investigated the dependence of various selected factors (particle size, shape, oral hold time, particle density, and oral dose) on pharmacokinetic parameters (AUC, \(C_{max}\) and \(T_{max}\)) of KSUS and K-SLN4. It was expected that K-SLN4 may be executed to have relatively improved these pharmacokinetic parameters as compared to KSUS.

Compartmental Regional Absorption Study. Data obtained from in vitro dissolution and ex vivo permeation studies were used for simulation and prediction of ketoconazole permeability across the rat stomach and intestine (duodenum and jejunum). Limited intestinal permeability studies have been performed to investigate in vivo perfusion for poorly soluble drugs. In this study, in vitro dissolution data (experimental values) and ex vivo permeation (experimental data) were simulated to establish a correlation between these two. Moreover, the software was used to predict in vivo permeability of the pure drug suspension and formulation in a rat model. The model was used to model passive absorption for individual compartments (regional compartmental absorption). Each compartment was expected to have a different extent of absorption due to varied absorption mechanisms working differently in each compartment and other factors (trans- and paracellular transport, varied surface area, different dimensions, population of villi/microvilli, and degree of ionization at regional pH). The software program provides a log D model to scale regional permeability, and subsequently effective permeability declines as the ionized fraction of the drug.
increases. The software-optimized log D model reproduced the fraction of drug absorbed for the investigated drug in rats.

2.2.9. Hemolysis Study. An in vitro hemolysis study was conducted using rat RBCs (4% suspension of red blood cells). The study was designed for time- and concentration-dependent hemolysis of K-SLN4 and KSUS. Triton X100 and PBS served as the positive and negative controls, respectively. Two concentrations (0.12% and 0.24%) of K-SLN4 and KSUS were formulated under similar experimental conditions. In brief, 1.5 mL of RBC suspension and 0.5 mL of the test sample were transferred to a sterile blood collection tube containing heparin (as anticoagulant). The final volume was adjusted to 4 mL using PBS. Each tube was tightly closed and incubated for 1 and 12 h, separately. After completion of incubation time, the tube was removed and centrifuged, and the supernatant was used to estimate released hemoglobin using a UV−vis spectrophotometer. The experiment was conducted in triplicate to get the mean and SD values (n = 3).

2.2.10. Statistical Analysis. Release kinetics was expressed as mean and SD. The result was compared by a Student’s t test. A p < 0.05 (two-tailed) value was considered significant. All of the studies were performed in triplicate to obtain the mean and SD.

3.0. RESULTS AND DISCUSSION

3.1. Formulations and Evaluated Parameters. As described before, this study is an extension of our previous investigation wherein we prepared several batches of ketoconazole-loaded solid lipid nanoparticles, optimized using Design Expert (experimental design tool) and evaluated for particle size, PDI, % EE, % TDC, and morphology (Table 1).9,10 The selected five (K-SLN1, K-SLN2, K-SLN4, K-SLN7, and K-SLN13) formulations were reformulated and evaluated for particle size, PDI, ZP, and morphological assessment using the TEM technique. The results are presented in Table 1. The particle size ranged between 299 and 831 nm, whereas the values of PDI ranged from 0.22 to 0.83 as shown in Table 1. It is apparent that the content of surfactant “tween 80” had a significant impact on the particle size. As observed in K-SLN1 and K-SLN7, the particle size was substantially increased from 393 to 831 nm due to half of the concentration of tween 80 used later. Moreover, the inhomogeneous nature (higher PDI values) of the particle was observed in both formulations which may be attributed to the high concentration of lipid, resulting in insufficient emulsiﬁcation at the explored content of surfactant tween 80. A similar pattern was observed in K-SKN2 and K-SLN4. Comparing K-SLN2 with K-SLN13, the particle size was further decreased due to a minimum content (1.5%) of the solid lipid CATO and relatively higher value of tween 80 (1.1%) as compared to K-SLN2. The particle size values of K-SLN1, K-SLN2, K-SLN4, K-SLN7, and K-SLN13 were found to be 393, 775, 299, 831, and 635 nm, respectively. Comparing K-SLN1 and K-SLN7, it is apparent that surfactant concentration played a significant role in reducing SLN size. The largest particle observed in K-SLN7 could be attributed to the relatively low content of tween 80 and insufficient emulsiﬁcation of 3.5% oil in the formulation. Likewise, a similar relation can be established by comparing K-SLN2 and K-SLN4 wherein the particle size was quite reduced to 299 nm (K-SLN4) on increasing tween 80 content and decreasing relative lipid content (2% compared to K-SLN1 and K-SLN7). Moreover, a similar trend was observed with PDI values, suggesting a homogeneous particle was obtained by increasing the content of tween 80 over lipid concentration. That is why K-SLN4 executed the lowest value of

![Figure 1](https://doi.org/10.1021/acsomega.2c01272)
particle size and PDI values for the expected maximum permeation and detrimental effect against pathogenic strains.

The size obtained from DLS and TEM exhibited a slight difference (TEM result biased) due to preferential adsorption of smaller particles by the perforated carbon grid (through small pores) as compared to larger particles. This results in a high propensity to diffuse smaller particles across the streamline and settle at the carbon film.\textsuperscript{20,21} The biased results of TEM are in accordance with a previous report with obvious reason (drying attributed to aggregation in particles and medium interaction in DLS).\textsuperscript{22} This has been reported as an instrumental error which can be expressed as fold error and calculated using eq 2. The results have been presented in Table 1 which are below 2, suggesting the acceptable error.

The values of negative ZP were found to be in the range of 25–34.2 mV, which may be attributed to fatty acid content (mixture of different esters of behenic acid with glycerol) of the lipid (solid lipid) in the composition.\textsuperscript{23} The lipid is a unique, well-established excipient and multifunctional excipient in drug delivery and nanopharmaceuticals. CATO is a well-documented solid lipid for nanoproducts due to its biocompatibility and high drug loading. Here, we observed (a) high % EE, (b) optimal zeta potential, (c) high drug content, (d) stabilized particle size at explored manufacturing temperature, and (e) the least drug

Figure 2. \textit{In vitro} drug release pattern of ketoconazole at varied pH (A–C) over period of 24 h. Data represent mean ± SD, \(n = 3\).

Figure 3. \textit{Ex vivo} permeation of ketoconazole-loaded SLNs across (A) rat jejunum, (B) duodenum, and (C) rat stomach (pH 1–2) (data represent mean ± SD, \(n = 3\)).
Table 2. Summary of Permeation Flux Parameters of the Selected Formulations Using the Rat Stomach, Duodenum, and Jejunum

| parameters                                      | K-SLN4      | K-SLN13     | K-SLN2      | KSUS       | K-SLN1     | K-SLN7     |
|------------------------------------------------|-------------|-------------|-------------|------------|------------|------------|
| permeation flux (μg/cm²/h)                     | 347.12      | 154.48      | 147.25      | 74.25      | 231.18     | 99.57      |
| enhancement ratio (ER)                         | 4.67        | 2.08        | 1.99        | 3.11       | 1.34       | 1.04       |
| $P_{app}$ (cm/s, x10⁻⁵)                        | 2.44        | 1.08        | 1.04        | 0.523      | 1.62       | 0.701      |
| permeation flux (μg/cm²/h)                     | 239.30      | 97.81       | 43.27       | 35.17      | 214.5      | 39.56      |
| enhancement ratio (ER)                         | 9.39        | 2.78        | 1.23        | 6.1        | 1.12       |            |
| $P_{app}$ (cm/s, x10⁻⁵)                        | 17.5        | 5.19        | 2.29        | 1.86       | 11.4       | 2.09       |
| permeation flux (μg/cm²/h)                     | 224.66      | 42.13       | 40.58       | 30.47      | 192.08     | 31.33      |
| enhancement ratio (ER)                         | 7.37        | 1.38        | 1.33        | 3.39       | 1.02       |            |
| $P_{app}$ (cm/s, x10⁻⁵)                        | 6.63        | 1.24        | 1.19        | 0.89       | 5.66       | 0.924      |

Apparent permeability coefficient ($P_{app}$) in μm.

SLN4 could be considered as the most optimized formulation possessing minimum size (299 ± 11 nm), relatively high homogeneous nanosuspension (PDI ≈ 0.22), optimal zeta potential (~28 mV), high % EE (84%), and high % TDC (96%). These studies were replicated for mean and standard deviation.

A comprehensive study was conducted to confirm improved permeation across rat skin and potentiated efficacy against the explored fungal strains in a previous report. In this study, we selected few formulations among them and addressed the impact of composition (CATO and tween 80), particle size (nm), drug content (%), and % EE on the intestinal permeation profile using a rat model. Moreover, a confocal laser scanning electron microscopy (CLSM) study was used to investigate degree of penetration. Finally, the GastroPlus program was used to predict the in vivo performance of the optimized formulation as compared to drug suspension in rats and humans. The input data for GastroPlus prediction were used from experimental studies, the literature, and by-default values in the program.

3.2. In Vitro Drug Release Studies at Varied pH. The result of the in vitro drug release profile has been illustrated in Figure 2A–C. It is clear that the selected K-SLN1, K-SLN13, K-SLN2, K-SLN7, and K-SLN4 formulations exhibited improved drug release at the explored pH-based mediums as compared to leaching. The presence of negative charge on the particle surface would provide sufficient repulsive force to prevent aggregation in the long term.

The results of % EE (percent drug entrapment) and % drug content (% TDC) have already been reported in previous publications for KTZ-SLN1, KTZ-SLN2, KTZ-SLN4, and KTZ-SLN7. K-SLN4 exhibited maximum % EE and % TDC which may be due to the smaller size and relatively high content of tween 80 to lipid CATO. Figure 1A–C illustrated a chemical structure of the drug and brief information on the K-SLN4 product (morphology using transmission electron microscopy and size distribution intensity graph). Notably, K-

Figure 4. Impact of tween 80 on (A) permeation flux (μg/cm²/h) across the rat jejunum, duodenum, and stomach and (B) apparent permeability coefficient ($P_{app}$) × 10⁻⁵ cm/s. Data represent mean ± SD, n = 3.

Figure 5. Percent drug deposition (stomach, duodenum, and jejunum) of ketoconazole-loaded formulations after permeation study (DD = drug deposition) (data represent mean ± SD, n = 3).
the suspension (KSUS).24 The drug release was extended over 24 h at all pH values. However, KTZ release from the suspension was significantly increased with a decrease in pH value. The drug release from KSUS was found to be 18.6 ± 0.9%, 31.2 ± 1.7%, and 33.4 ± 1.6% at pH 7.4, 4.5, and 0.1 N HCl (1−2), respectively, at the end of 24 h (Figure 2). This may be correlated to the weak dibasic nature of ketoconazole (two pK\textsubscript{a} values of 2.91 and 6.5), and one can anticipate the maximum dissolution at a lower pH range (pH 2−3) as compared to pH > 6.0.24 The drug released at pH 7.4 was approximately 18%, which may be due to drug precipitation (exceeding pH 5.5), and the result is in close agreement with the previous reported value wherein only 10% KTZ was dissolved at pH 6.0 over a period of 60 min.25 Comparing all formulations against control KSUS, these formulations followed the same release pattern due to the obvious reasons shown in Figure 2A−C. Maximum drug release was obtained at pH 1.2 and 4.5 as compared to pH 7.4 by all the developed formulations due to pH-dependent free drug (unentrapped) solubilization and available as three different chemical species (two protonated and one nonprotonated species).26 Below pH 4.5, free KTZ may be available as two protonated species (imidazole and piperazine N atom protonated), whereas at pH > 6.0, there is only one protonated (piperazine ring) and one unprotonated species.24 Moreover, formulations of K-SLN2 (having 35% as % EE) and K-SLN13 (45.6% as % EE) revealed rapid burst release at 60 min which

**Table 3. Summary of Input Parameters in GastroPlus Software Tabs**

| input parameters         | values                  | reference |
|--------------------------|-------------------------|-----------|
| molecular formula        | C\textsubscript{26}H\textsubscript{35}Cl\textsubscript{2}N\textsubscript{4}O\textsubscript{4} | 10        |
| molecular weight (g/mol) | 531.44                  |           |
| log P                    | 3.74                    | ADMET predictor module |
| aqueous solubility (mg/mL)| 0.24                    | 11        |
| pK\textsubscript{a} values| 2.94 (imidazole amine) and 6.51 (piperazine imine) | 24, 29    |
| dose (mg)                | 20                      | by default |
| dosing volume (mL)       | 1                       | by default |
| body weight (kg)         | 0.25−0.3                | set conditions |
| particle density (g/mL)  | 1.2                     | by default |
| mean precipitation time (s)| 900                    | by default |
| permeability coefficient (cm/s) | 3.7 × 10\textsuperscript{-4} | by default |
| pH for reference solubility | 4.4                    | 43        |
| simulation time (h)      | 24                      |           |

**Figure 6.** Ex vivo penetration study of the optimized formulation (K-SLN4) across the stomach, duodenum, and jejunum using CLSM technique and comparison: (A−C) treated with rhodamine-123-probed K-SLN4, (D,E) treated with rhodamine-123-probed KSUS, and (G−I) treated with 0.05% w/v rhodamine-123 aqueous solution as a control (scale bar = 25 μm).

**Figure 7.** GastroPlus-based simulated curves of the plasma drug concentration time profile of KSUS and K-SLN4 in a rat model.

the suspension (KSUS). The drug release was extended over 24 h at all pH values. However, KTZ release from the suspension was significantly increased with a decrease in pH value. The drug release from KSUS was found to be 18.6 ± 0.9%, 31.2 ± 1.7%, and 33.4 ± 1.6% at pH 7.4, 4.5, and 0.1 N HCl (1−2), respectively, at the end of 24 h (Figure 2). This may be correlated to the weak dibasic nature of ketoconazole (two pK\textsubscript{a} values of 2.91 and 6.5), and one can anticipate the maximum dissolution at a lower pH range (pH 2−3) as compared to pH > 6.0. The drug released at pH 7.4 was approximately 18%, which may be due to drug precipitation (exceeding pH 5.5), and the result is in close agreement with the previous reported value wherein only 10% KTZ was dissolved at pH 6.0 over a period of 60 min. Comparing all formulations against control KSUS, these formulations followed the same release pattern due to the obvious reasons shown in Figure 2A−C. Maximum drug release was obtained at pH 1.2 and 4.5 as compared to pH 7.4 by all the developed formulations due to pH-dependent free drug (unentrapped) solubilization and available as three different chemical species (two protonated and one nonprotonated species). Below pH 4.5, free KTZ may be available as two protonated species (imidazole and piperazine N atom protonated), whereas at pH > 6.0, there is only one protonated (piperazine ring) and one unprotonated species. Moreover, formulations of K-SLN2 (having 35% as % EE) and K-SLN13 (45.6% as % EE) revealed rapid burst release at 60 min which

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stomach > duodenum > jejunum due to pH-dependent ketoconazole solubilization/dissolution as discussed in the in vitro drug release section. KTZ is highly soluble and stable at low pH (<3.0). The drug dissolution rate decreases with an increase in pH value (facilitates drug precipitation at higher pH).29,30 At the high pH (7.4) region (jejunum), K-SLN7, K-SLN2, and K-SLN13 showed low cumulative permeation as compared to K-SLN1 and K-SLN4 at the end of 6 h. This may be due to larger particle size, less drug solubility, and low % EE in K-SLN2 and K-SLN13. Despite the high % EE of K-SLN7, it elicited low cumulative permeation due to higher particle size (831 nm) and low tween 80 content (0.7%) as compared to K-SLN1 and K-SLN4. K-SLN1 and K-SLN7 have comparable % EE values as shown in Table 1. However, K-SLN1 showed relatively higher permeation across jejunum as compared to K-SLN7 (376.03 μg/cm²), which can be rationalized based on the lower value of particle size (393 nm) and higher tween 80 concentration (1.4%) responsible for enhanced permeation. Thus, particle size and surfactant concentration played a major role in improving cumulative drug permeation of KTZ across jejunum at pH 7.4. This pattern of permeation was also observed in the stomach and duodenum because the absorption of KTZ is dependent upon gastric acidity.29 Notably, K-SLN13 and K-SLN2 demonstrated maximum cumulative permeation across the stomach at 60 min which may be due to high drug solubilization at low pH < 3.0 and that they are readily solubilized and unentrapped for permeation as compared to K-SLN4.25 In this project, we attempted to address the impact of composition, particle size, and % EE on ex vivo permeation parameters and correlated the predicted values using the GastroPlus in silico program.

The values of permeation flux of K-SLN4 across the stomach, duodenum, and jejunum were found to be 4.8-, 9.4-, and 7.4-fold higher than KSUS, respectively. This suggested that the loaded KTZ was preferentially permeated across duodenum as compared to the stomach and jejunum, which may be due to suitable pH (4.5) for permeation.29 A similar pattern of permeation was observed with K-SLN1, which may be due to smaller particle size, high % EE, and maximum surfactant concentration as compared to K-SLN7, K-SLN2, and K-SLN13.30 Moreover, enhancement ratios of K-SLN1 were observed as 3.1, 6.1, and 6.3 in the stomach, duodenum, and jejunum, respectively. Thus, K-SLN4 elicited maximum permeation flux and enhancement ratio as compared to other formulations (Table 2). Furthermore, the apparent permeability coefficient values of K-SLN4 and KSUS were found to be maximum in the stomach, duodenum and jejunum, respectively. Maximum P_app values may be attributed to facilitated permeation of the SLN of the lowest size ferrying KTZ (Table 1 and Table 2).30

Figure 4A,B illustrates the impact of tween 80 content on permeation flux and P_app parameters of all developed formulations across the stomach, duodenum, and jejunum. Both K-SLN1 and K-SLN4 shared the same concentration of tween 80 and exhibited maximum flux across the stomach, duodenum, and jejunum (Figure 4A). The improved permeation flux of K-SLN4 and K-SLN1 (Table 2) may be due to small particle size (299 nm vs 393 nm), high % EE (84% vs 60.7%), and high concentration (1.4%) of tween 80 among them.30 The permeation flux values of K-SLN2 were profoundly decreased across the stomach, duodenum, and jejunum as compared to K-SLN4 due to reduced content of tween 80 (0.7% in K-SLN2), high particle size (775 nm), low % EE (35%), and low
permeation flux (Figure 4A, Table 1, and Table 2). Further reduction in tween 80 content (1.1%) and the content of CATO (K-SLN13) resulted in reduced size (635 nm) and increased % EE (45%). K-SLN7 exhibited the least permeation flux due to larger particle size generated through a relatively low content of tween 80 (0.7%) as compared to K-SLN1 at the same CATO content. Permeation parameters of K-SLN4 compared to KSUS across the stomach, duodenum, and jejunum were attributed to the combined effect of low particle size, tween 80 based modulation for permeation at the enterocyte surface (a potential P-gp efflux inhibitor), optimum CATO (2%), maximized content of tween 80 (1.4%), and adjusted pH of the lumen (Figure 4B). Moreover, the impregnated lipophilic KTZ (log p ~ 4.35) inside the solid lipid matrix core of SLN was mediated for attenuated permeation across the cellular barrier via facilitated internalization. The lipophilic nature of KTZ, modulating the intestinal mucus barrier by nanoparticles, pH close to the drug pKa, and high % EE worked together to improve its permeation across the lipophilic mucosal membrane after tween 80 (nonionic, HLB 15, CMC: 0.015 mmol/L).

Figure 9. Parameter sensitivity assessment (PSA) using the GastroPlus-based prediction program. Impact of various factors on (A) Cmax, (B) AUC, and (C) Tmax values in the rat model based on input parameters in the program.
polyoxyethylene as a higher ratio of the hydrophilic part) mediated interaction (reduced rheological properties) with the hydrophilic mucosal content.\(^{32,33}\) Nanoscale SLNs may protect KTZ from chemical, physical, and photolytic degradation within the matrix core.\(^{34}\)

KTZ has an apparent permeability coefficient (\(P_{\text{app}}\)) value in the range of 11.3–1.5 \(\times\) 10\(^{-5}\) cm/s across caco-2 as reported previously.\(^{35}\) The values of the \(P_{\text{app}}\) of K-SLN4 across the rat stomach, duodenum, and jejunum were in the range of 2.44 \(\times\) 10\(^{-5}\) to 0.7 \(\times\) 10\(^{-3}\) cm/s, 17.5 \(\times\) 10\(^{-5}\) to 2.09 \(\times\) 10\(^{-3}\) cm/s, and 6.63 \(\times\) 10\(^{-5}\) to 0.92 \(\times\) 10\(^{-3}\) cm/s, respectively, at the explored concentrations, whereas these values of KSUS were found to be 0.523 \(\times\) 10\(^{-5}\) cm/s, 1.86 \(\times\) 10\(^{-5}\) cm/s, and 0.89 \(\times\) 10\(^{-5}\) cm/s, respectively (Table 2). Theoretically, the coefficient varies according to the physicochemical properties of the drug, such as particle size, lipophilicity, functionalization, and molecular weight.\(^{36}\) Thus, the drug was primarily permeated across the duodenum region as compared to the acidic stomach and jejunum area. These improvements may be correlated to the protective effect of lipid nanoparticles, increased \(P_{\text{app}}\) values, and chemical stability of ketoconazole at a higher pH (\(>4.0\)).\(^{37}\)

### 3.4. Ex Vivo Permeation and % Drug Deposition

The result of % DD is illustrated in Figure 5 wherein K-SLN4 (29, 15.5, and 14.5%) and K-SLN1 (26, 13.5, and 9.2%) exhibited maximum % DD deposition across the stomach, duodenum, and jejunum as compared to others, respectively. % DD values of KSUS across the stomach, duodenum, and jejunum were observed as 4, 1.75, and 1.4% due to the poor aqueous solubility and lipophilic nature of the drug in aqueous suspension.\(^{28}\) Considering the impact of size, K-SLN4 and K-SLN1 executed enhanced permeation across the explored segments of the GIT which may be correlated with relatively low particle size, high tween 80 content, and high % EE of formulations as compared to others. K-SLN7 elicited the lowest values of % DD (17.0, 7.0, and 6.0% in respective regions) among the selected formulations, and this outcome may probably be related to the large particle size that is incapable of passing across the mucosal membrane of the stomach, duodenum, and jejunum. Thus, the higher the % DD, the more permeation flux across the stomach, duodenum, and jejunum that can be achieved. The most critical barriers lining the inner construction of GIT are the enterocyte cellular layer and the first hydrophilic mucous layers. Therefore, smaller nanoparticle size, surfactant-based sufficient emulsifica-

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**Figure 10.** Regional compartmental absorption: (A) pure KETO suspension (KSUS) and (B) K-SLN4.

**Figure 11.** *In vitro* hemolysis study at varied time points and concentrations (data are expressed as mean and SD, \(n = 3\)).
tion in hydrophilic mucous, and augmented internalization with enterocyte lining are responsible for improved permeation across the stomach, duodenum, and jejunum. Conclusively, composition, particle size, and % EE are major factors to attain enhanced permeation of KTZ to control systemic fungal infection using oral administration. Notably, other factors also play a major role for improved permeation and drug stability for improved in vivo performance. Lipophilic KTZ (log \( P = 4.3 \)) is associated with low molecular weight (531 g/mol), zero hydrogen bond donor groups, and six hydrogen bond acceptor groups, which are favorable properties for improved intestinal permeation through the passive lipoidal diffusion mechanism. Moreover, the lipid matrix internalizes substantially with the biological membrane, resulting in increased permeation through transcellular pathways. Tween 80 being a substrate of the P-gp modulation of permeation pathways (paracellular, transcellular, and carrier mediated). CATO has been reported to have benefited oral absorption in K-SLN4 while passing through the diverse absorption in K-SLN4 while passing through the diverse

3.5. Ex Vivo Penetration Study Using the CLSM Technique. The result of CLSM-based scanning of a treated stomach, duodenum, and jejunum using the optimized rhodamine-123-probed K-SLN4, rhodamine-123-probed KSUS, and dye solution has been portrayed in Figure 6A–I. The dye-solution-treated group served as a control for comparison purposes in terms of fluorescence intensity. The observed intensity values of the K-SLN4-treated stomach, duodenum, and jejunum were found to be 66.1%, 51.5%, and 47.9%, respectively, whereas these were observed as 21.7%, 18.2%, and 17.4% for KSUS, respectively. The intensity values of dye-solution-treated samples were found to be in the range of 11.8–13.5%, which may be due to the inability of permeation of aqueous dye solution across the lipophilic biological membrane. The result suggested that K-SLN4 exhibited relatively high fluorescence intensity in the treated stomach, duodenum, and jejunum as compared to both KSUS and control dye solution. This may be attributed to CATO and tween-80-based modulation of permeation pathways (paracellular, transcellular, intercellular, and carrier mediated). CATO has been reported to have benefits over other solid lipids (stearic acid, tristearin, and monostearin) for improved lymphatic uptake (transcellular route) after oral administration of SLNs with low particle size (~100 nm). Lipophilic biological membrane interacts with lipid nanoparticles through lipid–lipid interaction (hydrophobic) and then results in enhanced permeation from the apical side to the basolateral side (A→B) for systemic availability (as shown in Figure 6). Solid lipid nanoparticles may protect the drug carrying inside the matrix and allowed it to permeate across the biological membrane without being exposed to lumen, which results in intestinal and hepatic abnormalities (free-drug-mediated toxicity). In general, nanoparticles preferentially access the lymphatic system of the intestines and improve systemic transport, avoiding hepatic circulation. This strategy may reduce the possible hepatic toxicity and enterohepatic recycling of ketoconazole on oral administration.

3.6. GastroPlus-Based Simulation and Prediction Studies. 3.6.1. Plasma Concentration Time Profile Prediction in a Rat Model. The software predicted the plasma concentration time profile of KTZ using in vitro and ex vivo data obtained for KSUS and K-SLN4. Using input parameters (Table 3), the program was run to predict plasma drug concentration in rats and compared between both formulations.

The result has been illustrated in Figure 7 wherein KSUS showed relatively lower plasma concentration (0.0058 \( \mu g/mL \)) as compared to K-SLN4 (~0.012 \( \mu g/mL \)). Higher plasma concentrations in K-SLN4 may be attributed to maximized permeation across the physiological membrane and stabilized particles in systemic circulation. KTZ has been reported to be highly degraded under in vivo conditions. Moreover, the biphasic pharmacokinetics (PK) behavior caused two half-lives (as ~2.0 h and ~8 h) of disposition and predicted that reported in humans. The pharmacokinetic profiles were reported to be dependent upon the dose of oral administration where PK parameters (\( C_{max} \) and AUC) were linearly increased from 50 to 200 mg, and then no linearity was observed at 400 mg or more. This dose dependency was attained due to saturation-based drug absorption from the intestinal membrane of KTZ.

3.6.2. In Vivo Dissolution and Absorption Prediction. The software predicted in vivo drug dissolution and oral absorption in rats using the ACAT model. The model takes into consideration factors that have a great impact on the drug absorption and bioavailability such as the physicochemical attributes of the investigated drug (data fed in the compound tab), formulation attributes (nanoefect, size, dose, shape, and density), and physiological tab. The dibasic KTZ is highly lipophilic and chemically stable for expected rapid absorption from intestinal lumen. The result of in vivo absorption and dissolution predicted profiles has been portrayed in Figure 8A,B. The predicted pattern of in vivo kinetics of the drug absorption and dissolution supported the in vitro findings and is considered a good way to predict in vivo performance of K-SLN4 and KSUS in rats. The drug was highly soluble at low pH, and therefore, it was predicted to be rapidly dissolved within 10 min (Figure 8A); however, the drug absorption was extended over 24 h due to the matrix-based SLN carrier from the varied region of intestinal lumen (green curve). K-SLN4 was predicted to be slowly dissolved with extended absorption over 24 h, which is prudent to correlate that the entrapped drug in the SLN matrix was slowly released from the inner matrix. Moreover, the unentrapped drug was less available for dissolution and absorption in K-SLN4 while passing through the diverse environments (in terms of pH and physiological conditions) of lumen.

3.6.3. Parameter Sensitivity Analysis (PSA) Study. In general, drug dissolution and subsequent absorption depend upon several factors related to the drug properties, formulation, and physiological factors (gastric pH, fast and fed conditions, hypochlorhydria, food, and other disease conditions). We attempted to predict the impact of various factors on PK parameters using K-SLN4. Pure KSUS is a chemically weak base and soluble at low acidic pH, which exhibited no difference in PK parameters (data not presented) in running the program. However, it was mandatory to investigate the impact of K-SLN4 characteristics (size, shape, oral hold time, density, and dose) on PK parameters as K-SLN4 was not completely dissolved in gastric lumen and revealed extended drug release over time as evidenced with the in vitro drug release profile. Particle size matters only when the dissolution is limited in the gastric region due to hypochlorhydria for KTZ. The result is illustrated in Figure 9A–C. It is clear from the result that two prime PK parameters such as \( C_{max} \) and AUC are substantially affected by the oral dose. Both parameters exponentially increased with an increase in dose. However, there was no impact of these factors on \( T_{max} \) as shown in Figure 9C. This result is in good agreement with the published findings wherein the half-life of KTZ was about 4 h and the dose dependency was observed in terms of PK.
parameters. GastroPlus predicted no impact of oral hold time, particle density, and particle morphology of K-SLN4 on the investigated PK parameters.

3.6.4. Regional Compartmental Absorption Model. The present study addressed the safe delivery of KTZ-loaded SLNs for oral delivery to improve intestinal absorption and the impact of pharmaceutical properties (particle size, composition, and % EE) on permeation parameters in the rat model. Based on ex vivo permeation, DD, and the in vitro drug release study (12 h), the GastroPlus program predicted regional absorption of KTZ from K-SLN4 through nine different segments and compared it against KSUS. The predicted values are illustrated in Figure 10A,B. It is apparently obvious that duodenum and jejunum are the two major sites of KTZ absorption which constituted 55.4% (duodenum, jejunum 1, and jejunum 2) and 69.1% (duodenum, jejunum 1, and jejunum 2) absorption for KSUS and K-SLN4, respectively. Moreover, overall % absorption was significantly higher in K-SLN4 (80%) as compared to KSUS (60.1%) (Figure 10A,B). Notably, the applied model is the best fit model and supportive to the ex vivo permeation outcomes wherein duodenum and jejunal elicited maximum permeation parameters. However, the model predicted contradictory results for the stomach which may be due to perfusion-limited kinetics of KTZ in humans (as default data in the input tab). In the literature, KTZ is reported to be highly lipophilic and maximally absorbed from these major sites and cases. However, any free drug causes several intestinal side effects and hepatic disease on oral delivery. Therefore, the current approach can be promising for safe delivery and attenuated systemic availability by protecting the drug from exposure to intestinal content.

3.7. Hemolysis Study. The finally optimized formulation was K-SLN4 in the study. Predictive software revealed 80% of the total drug absorption. Therefore, two concentrations were decided based on predictive values (one as half of the maximum absorption and the second as the maximum absorption). Thus, two concentrations of K-SLN4 (0.12% and 0.24%) and KSUS were used. Moreover, K-SLN4 is a slow and extended release product. Therefore, it was mandatory to understand the time-dependent hemolysis profile. The results have been portrayed in Figure 11, wherein all formulations executed hemolysis of <15% except positive control (100%). Negative control showed hemolysis at approximately 8.5 and 11.9% at 1 and 12 h, respectively. Hemolysis caused by the positive control was considered as 100% at 12 h. This was a preliminary toxicity study of the product for oral administration. Thus, the developed product was safe and biocompatible as evidenced with the report.

4. CONCLUSION
The present investigation addressed the impact of SLN composition and formulation attributes on permeation across the stomach, duodenum, and jejunum of rats followed by a prediction study. Based on in vitro and ex vivo results, it was observed that KTZ was rapidly released in an acidic stomach which may be due to protonation of imidazole (after in vivo dissolution at pH 4.5) and piperazine rings (free drug). Duodenum exhibited maximum P_app due to being unprotonated. Moreover, jejunum showed relatively high permeation parameters (P_app and ER) as compared to the stomach being soluble in the unprotonated form (>80%). Moreover, a relatively high content of tween 80 over CAO improved permeation parameters. The in silico software program predicted that the dose had a significant impact on PK parameters, whereas particle shape, size, hold time, and density showed an insignificant impact on the in vivo performance of K-SLN4. Furthermore, regional absorption results showed the highest absorption (~80%) of K-SLN4 compared to KSUS (60%). The approach is promising for enhanced oral absorption from the distal region of the GIT for reduced intestinal side effects and more systemic access.

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Notes
The authors declare no competing financial interest.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of the Department of Institutional Ethical Committee (Panjab University, regd. No. 45/GO/ReBiBt/S/99/CPCSEA) and approved for the study as per ARRIVE guidelines. The protocol was followed as per ARRIVE guidelines.

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