Zotepine loaded lipid nanoparticles for oral delivery: development, characterization, and in vivo pharmacokinetic studies

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Abstract

Background: The purpose of this work was to prepare and evaluate the zotepine (ZT) loaded solid lipid nanoparticles (SLNs) that might improve the oral bioavailability. ZT is an anti-psychotic drug used for the treatment of schizophrenia. Currently, it is available as parenteral and oral dosage form. But, ZT has a poor oral bioavailability of about 7–13% due to limited aqueous solubility and first-pass effect. ZT-SLNs were developed using homogenization method and characterized for optimal system based on physicochemical characteristics and in vitro release. The optimized ZT-SLNs were evaluated for permeation through rat intestine using evvert sac method. The crystalline nature of the ZT-SLNs was studied using DSC and XRD analysis. Surface morphology studies were conducted using SEM. Physical stability of the optimized ZT-SLN was evaluated at refrigerator and room temperature over 2 months. Further, pharmacokinetic (PK) studies of ZT-SLN were conducted in male Wistar rats, in comparison with ZT coarse suspension (ZT-CS), in vivo.

Results: Among all the developed ZT-SLN formulations, optimized formulation (F1) showed Z-avg, PDI, and ZP of 104.3 ± 1.6 nm, 0.17 ± 0.01, and −30.5 ± 2.5 mV, respectively. In vitro release and permeation studies exhibited 82.9 ± 1.6% of drug release and 19.6 ± 2.1% of percentage drug permeation over 48 h and 120 min, respectively. DSC and XRD studies revealed the conversion of ZT to amorphous form. SEM studies showed spherical shape with improved PDI of ZT-SLN formulation. PK studies showed a significant (p < 0.05) improvement in AUC of about 1.3-fold, in comparison with ZT-CS in Wistar rats.

Conclusion: Therefore, the results concluded that SLNs could be considered as a new alternative delivery system for the enhancement of oral bioavailability of ZT.

Keywords: Zotepine, Solid lipid nanoparticles, Crystallinity, In vitro release, Ex vivo permeation, Oral bioavailability

Background

The amount of the drug reaching to the systemic circulation mainly depends on the rate of absorption, which in turn depends on the drug solubility [1]. Oral delivery of the drugs is the most conventional and widely used technique for the administration of majority of active moieties. However, several compounds are unsuccessful and failed owing to their low absorption and low bioavailability upon oral administration [2]. The drugs with poor oral bioavailability are unable to reach the minimum effective concentration to exhibit therapeutic action. Some of the reasons for poor bioavailability include poor solubility, inappropriate partition coefficient as it influences the permeation of drug through lipid membrane, first-pass metabolism, P-glycoprotein (P-gp) mediated efflux, and degradation of drug in the gastrointestinal tract (GIT) due to pH of the stomach or enzymatic degradation or by chemical [3].

To overcome the solubility and oral bioavailability problems of biopharmaceutical classification system (BCS) classes II and IV drugs, the research in the present scenario is focused on bioavailability improvement, by
using various solubility enhancement techniques like liquisolid compacts [4, 5], micronization using nanosuspensions [6], solid dispersions using complexation [7, 8], and salt formation [9]. Majority of the drugs undergo first-pass effect after oral administration; thereby, the therapeutic concentration required in the systemic circulation is lowered. However, by using the lipid-based delivery systems, both the solubility and first-pass effect problems are resolved, and subsequently oral absorption was improved [3].

Lipid-based drug delivery systems are one of the approaches, which favors the lymphatic transport of oral administered drugs by escaping the first-pass effect [10, 11]. Colloidal lipid nanocarriers such as solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) are the promising approaches in the lipid-based delivery systems. SLNs are mainly developed with the solid lipid carrier, used for incorporation of drug, and lipid dispersion is stabilized by the use of biocompatible surfactants [12]. SLNs offer remarkable advantages such as low toxicity, high bioavailability (pharmacokinetic and pharmacodynamic) of drugs, versatility of incorporation of both hydrophilic and lipophilic drugs, and feasibility of large-scale production. SLN formulations have controlled drug release properties and provide enhanced chemical stability of drug molecules [13]. SLNs also used for drug targeting to various tissues such as the liver [14] and ocular [15] for treatment of various diseases.

Zotepine (ZT) is an atypical anti-psychotic drug, which belongs to BCS class II category. The mechanism of action of ZT is mediated through combined dopamine and serotonin antagonisms [16]. It has a poor oral bioavailability of about 7–13%, due to poor aqueous solubility (0.046 μg/L), highly lipophilic (log p 5.6), and also hepatic first-pass metabolism. The drug levels were also decreased in the presence of CYP enzyme [17, 18]. In oral route, 30% of the drug metabolizes into nor-zotepine (active metabolite), and remaining 70% of drug transforms to inactive metabolites such as 3-hydroxyzotepine, 2-hydroxyzotepine, and zotepine-S-oxide [16]. Previously, Pailla et al. [19] developed intranasal delivery of ZT for enhanced brain targeting, using nanosuspension formulation. The enhanced solubility and in vitro dissolution rate of ZT was observed and reported with self-emulsifying drug delivery systems [20]. But, till now there are no lipid nanocarrier systems were reported for enhanced oral delivery of ZT. Hence, in this attempt, ZT loaded SLNs were developed that might improve the oral bioavailability.

The objective of the present investigation was aimed to develop and characterize ZT loaded SLNs for enhanced oral delivery. Accordingly, ZT-SLNs were prepared using film hydration method and evaluated for an optimized formulation based on the physical and chemical parameters, in vitro release studies. Further, optimized formulation also characterized for solid state characterization for crystallinity, SEM for surface morphology, and ex vivo permeation studies. In vivo performance of optimized ZT-SLN was observed by pharmacokinetic (PK) studies in male Wistar rats, comparison with ZT-coarse suspension (ZT-CS) as control formulation.

Methods
Zotepine was a kind gifted sample from Symed labs, Hyderabad, India. Dynasan®118 and Dynasan®114 were purchased from Sigma-Aldrich, Hyderabad, India. Compritol® 888 ATO was gift sample from Neuheit Pharma Technologies Private Ltd., Hyderabad. Soylecithin was a gift sample from Lipoïd, Ludwigshafen, Germany. Captex®355 and Captex®200 were gift samples from Abitec Corporation. All other chemicals were of analytical grade, and solvents were of HPLC grade.

Animals
Male Wister rats (210 ± 30 g) used for the study were procured from Teena Biolabs Pvt Ltd., Hyderabad, India. The studies were conducted with prior approval of Institutional Animal Ethical Committee, Kakatiya University, India (IAEC/01/UCPSC/KU/2018).

Solubility studies
ZT solubility in various solid lipids was determined by using shaking method. Solid lipids were heated to above 5 °C of their melting points. An excess amount of ZT was added and was continuously stirred on gyrator shaker at 180 rpm for 48 h. The supernatant was collected and filtered through 0.45-μ membrane filter after centrifugation [21]. Necessary dilutions were made to the filtrate with methanol and estimated the drug concentration using UV-Visible spectrophotometer at λ_{max} of 261 nm (SL-159, Elico, Hyderabad, India). Simultaneously, solubility of ZT in release media such as distilled water, 0.1 N HCl (pH 1.2), phosphate buffer of pH 6.8 and pH 7.4.

Preparation of zotepine loaded solid lipid nanoparticles
Zotepine loaded solid lipid nanoparticles (ZT-SLN) were prepared using homogenization-probe sonication method, based on film hydration method [22]. Required amounts of ZT, solid lipid, and soy lecithin were dissolved in 10 mL mixture of chloroform and methanol (1:1) in a round bottom flask. Organic solvents were removed using rota evaporator (Heidolph, Germany). Drug embedded lipid layer was molten by heating at 5 °C above the melting point of the solid lipid. Aqueous phase was prepared by dissolving Poloxamer-188 in double distilled water and heated to the same
temperature of oil phase. Hot aqueous phase was added to oil phase. Homogenization was carried out at 12,000 rpm for 5 min to form pre-emulsion. The obtained emulsion was ultra-sonicated using Probe sonicator (Bandelin, Germany) for 20 min. ZT-SLNs were obtained upon cooling to room temperature. The compositions of the SLNs are given in Table 1.

Preparation of ZT coarse suspension (ZT-CS)
About 100 mg of sodium carboxy methyl cellulose (suspending agent) was taken in a mortar and trititated. Then, 10 mg of ZT was added to it and together triturated. To it, 10 mL of double distilled water was added and again triturated for about 5 min to obtain ZT-CS (1 mg/mL). This was used as control formulation.

Characterization of ZT-SLNs
Measurement of particle size, polydispersity index (PDI), and zeta potential (ZP)
The mean sizes as Z-avg (nm), polydispersity index (PDI), and zeta potential (ZP) of ZT-SLNs were measured by photon correlation spectroscopy (PCS) using a Malvern Zetasizer (Nano ZS90, UK). About 100 μL of prepared SLN formulation was diluted to 5 mL with double distilled water to get optimum kilo counts per second (Kcps) of 50–200 for measurements [23]. All measurements were in triplicate at 25 °C.

Drug Content and entrapment efficiency (EE)
About 0.1 mL of ZT-SLN formulation was taken and diluted with chloroform to methanol (1:1), and drug content of the diluted samples was estimated by HPLC method. The entrapment efficiency (EE, %) in ZT-SLNs was calculated by estimating the concentration of the free drug in the aqueous phase of an undiluted formulation, using an ultrafiltration method centrisort tubes (Sartorius, Germany). The aqueous medium was separated by ultra-filtration using centrisort tubes, which consisting a filter membrane (M.Wt.20,000 Da) at the base of the sample recovery chamber. Centrifugation was carried at 4000 rpm for 30 min [24]. The SLN along with the encapsulated drug remained in outer chamber, and aqueous phase moved into sample recovery chamber through filter membrane. The amount of zotepine in aqueous phase was estimated by HPLC method. The EE was calculated by using the Eq. 1:

\[
\frac{0}{\text{D}} \cdot \frac{D_f}{\text{D}} \times 100
\]

where \(D_f\) is the total drug content and \(D_i\) is the free drug present in the aqueous phase.

In vitro drug release studies
The in vitro drug release study was performed using dialysis method. Dialysis membrane (Hi-Media, Hyderabad, India) having average pore size 2.4 nm and molecular weight cut-off between12,000–14,000 Da were used for the release studies [23, 25]. Dialysis membrane was soaked overnight in double distilled water prior to the study. Release studies were carried out for first 2 h in 0.1 N HCl, followed by pH 6.8 phosphate buffer for 46 h by open tube method. Dialysis membrane was tied to open tube which acts as a donor compartment. Temperature of receptor compartment was maintained at 37 ± 0.5 °C. Aliquot of 2 mL sample was withdrawn at regular time points of 0, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 36, and 48 h from receiver compartment and replenished with same volume of fresh buffer. The collected samples were suitably diluted and analyzed by UV-Visible spectrophotometer.

Ex vivo studies by normal sac method
Ex vivo studies were performed for optimized ZT-SLN formulation by using evert sac method [26]. The studies were conducted with prior approval of Institutional Animal Ethical Committee (IAEC/01/UCPSC /KU/2018). Evert sac studies were carried out in order to investigate the permeation behavior of the drug solution and optimized formulation (1 mL) across the small intestine. In this study, male Wistar rats of 210 ± 30 g (n = 3) were taken and subjected to overnight fasting. The rats were

| Table 1 Composition of zotepine loaded solid lipid nanoparticles | F1 | F2 | F3 | F4 | F5 | F6 | F7 |
|---|---|---|---|---|---|---|---|
| Ingredients (% w/v) | Zotepine (mg) | Dynasan®118 | Dynasan®114 | Compritol ATO 888 | Soylecithin | Poloxamer 188 | Na CMC |
| Water (mL) | QS 10 | QS 10 | QS 10 | QS 10 | QS 10 | QS 10 | QS 10 |
| Na CMC, sodium carboxy methyl cellulose; F7, coarse suspension of ZT |
sacrificed by cervical dislocation technique, and jejunum of 4 cm² were isolated, flushed with saline solution, and transferred into oxygenated Krebs’s ringer solution. The one end of the sac was tied with thread and filled with the optimized ZT-SLN or ZT-CS as control (1 mL) formulation, and the other end was tied. The segment was immersed in 100 mL in Krebs’s ringer solution, and the medium was oxygenated using aerator. At regular time intervals (0, 15, 30, 45, 60, 90, and 120 min), samples were withdrawn from beaker and analyzed for drug content [27].

Lyophilization of ZT-SLNs
The optimized ZT-SLN formulation was subjected to lyophilization. The SLN formulation was prepared, using 10% w/w of trehalose as cryoprotectant. Prepared SLN formulation was kept in −80 °C freezer for overnight and subjected to lyophilization (Lyodel, Chennai, India) with applied vacuum [28]. Lyophilized formulation was subjected to particle size analysis, drug content, EE, and in vitro release before and after lyophilization.

Solid-state characterization
Differential scanning calorimetry
Differential scanning calorimetry (DSC) of sample was performed through using Perkin Elmer DSC 4000 model to find out the presence of any interaction between drug and the excipients and also to check the alterations in crystallinity of the drug. About 8 mg of the pure drug, physical mixture and optimized lyophilized ZT-SLN formulation samples were taken in aluminum pans, using dry nitrogen as purging gas. The heating rate was 10 °C/min, and the obtained thermograms were observed for any type of interaction [29]. Empty pan was used as reference for the study.

X-ray diffraction studies
Crystalline nature of the nanoparticle formulation was analyzed through powder X-ray diffractometer (XRD-6000, Shimadzu, Japan) [25]. Powder XRD studies were performed on the samples by exposing them to nickel filtered CuKα radiation (40 kV, 30 mA) and scanned from 2 to 70°, 2θ at a step size of 0.045° and step time of 0.5 s. Samples used for PXRD analysis were pure ZT, pure lipid, and lyophilized ZT loaded solid lipid nanoparticles [30].

Surface morphology by scanning electron microscopy (SEM)
The morphology of pure ZT and freeze-dried ZT-SLN formulation was investigated by scanning electron microscope (SEM S-3700, Hitachi, Japan). The samples were fixed on a brass stub and were coated by thin layer of gold to make electrically conductive [25]. SEM images were recorded at applied fixed voltage.

Bioavailability study
Study design and sampling schedule
Healthy male Wistar rats (weighing 210 ± 30 g) were used for the PK study. The animals were fasted overnight and had free access to water. The studies were conducted with prior approval of Institutional Animal Ethical Committee (IAEC/01/UCPSC /KU/2018). The animals were divided into two groups (n = 6) and were orally administered with ZT-CS (F7) and optimized ZT-SLN (F1) formulation at a dose of 2.2 mg/kg body weight with the help of rat oral feeding tube. At appropriate predetermined time intervals after oral administration, blood samples were collected (0, 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 48, 72, and 96 h) by retro-orbital venous plexus puncture. The blood samples were allowed to clot and centrifuged for 15 min at 3000 rpm. The serum was separated and transferred into clean micro centrifuge tubes and stored at −20 °C until analysis.

HPLC method
HPLC analysis was conducted with C18 column (5 μm; 250 mm × 4.6 mm). Mobile phase consisting of 45:55 (v/v) ratio of acetonitrile and phosphate buffer adjusted to pH 4.7 at a flow rate of 1 mL/min. Wavelength of 261 nm was used for detection of peaks. The retention times of ZT and ramipril (used as internal standard; IS) were found to be 7.8 min and 4.5 min, respectively.

Extraction procedure from rat serum sample
To 100 μL of serum, 100 μL of IS (ramipril, 2 μg/mL), and 300 μL of methanol were added. After vortex mixing
for 5 min at room temperature, the samples were centrifuged at 5000 rpm for 15 min. After centrifugation, the whole organic layer was separated and transferred into another eppendorf tube. Finally, a volume of 20 μL was injected into HPLC system.

**Estimation of pharmacokinetic parameters and statistical significance**

The Kinetica 2000 software (version 5.0, USA) was used for the estimation of PK parameters like $C_{\text{max}}$, $T_{\text{max}}$, AUC_total, $t_{1/2}$, and MRT, and the values were expressed in mean ± SD. One-way ANOVA was performed using the Graph pad prism software (version 5.03) for statistical comparison of data at $p$ value of 0.05.

**Physical stability studies**

An optimized ZT-SLN formulation was stored at room temperature and refrigerated temperature for 2 months. Z-avg, PDI, ZP, assay, and EE of the samples were observed at predetermined time intervals.

**Results**

**Solubility studies**

Solubility of ZT was studied in various solid lipids. Dynasan®118, Dynasan®114, and Compritol® ATO 888 were selected as solid lipids for SLN formulations development. Order of solubility of ZT in solid lipids was Dynasan®118 > Dynasan®114 > Compritol® ATO888 > Dynasan®112 > Precirol®ATO5. The solubility of ZT was decreased with increased pH of dissolution media and
indicates pH dependent solubility. The order of solubility in release media was as follows: 0.1 N HCl > pH 6.8 phosphate buffer ≫ water > pH 7.4 phosphate buffer.

Characterization of ZT-SLN
Measurement of particle size, PDI, and ZP
Prepared ZT-SLNs were characterized for particle size, PDI, and ZP, using Zetasizer and reported in Table 2. Particle size, PDI, and ZP of the ZT-SLNs formulations (F1–F6) were 104.3 ± 2.4 to 340.4 ± 3.0 nm, 0.17 ± 0.01 to 0.61 ± 0.05, and −10.66 ± 0.6 to −30.5 ± 2.5 mV.

Drug content and entrapment efficiency
All the formulations were analyzed for EE and total drug content by HPLC (Table 2). EE of all the formulations was ranged from 91.1 ± 1.5 to 98.4 ± 2.8%. Among all the developed formulations, F1 formulation is having the high EE. Drug contents of all the formulations were in the range of 8.7 ± 0.4 to 9.8 ± 0.3 mg.

In vitro release studies
All the prepared SLNs were subjected to in vitro release studies, using dialysis method. In this study, 0.1 N HCl pH 1.2 followed by pH 6.8 phosphate buffer for the release studies. In vitro release of ZT from SLN formulations was observed to 69 ± 2.1 to 82.9 ± 3.6% range, over 48 h (Fig. 1). However, formulations prepared with 1% and 2% w/v lipid concentrations were not significantly influence the drug release behavior from the SLN formulations. Formulations prepared with Dynasan®118, Dynasan®114, and Compritol ATO888 showed 82.9 ± 3.6 and 74.4 ± 2.1% (from F1 and F2), 77.5 ± 1.1 and 75.1 ± 2.9% (from F3 and F4), and 72 ± 1.9 and 69 ± 2.1% (from F5 and F6), respectively. The drug release from the ZT-CS (F7) formulation showed 51.9 ± 2.7% in 120 min (data not showed). In all the prepared formulations, ZT-SLN of F1 formulation prepared with Dynasan®118 was having less particle size and PDI, stable ZP, with high EE, acceptable drug content and also showed maximum drug release of 82.9 ± 3.6% in 48 h, comparison with other formulations. Therefore, F1 formulation was

| Table 3 Physicochemical characteristics of optimized ZT-SLN before and after lyophilization (mean ± SD, n = 3) |
| Condition | Size (nm) | PDI | ZP (mV) | Assay (mg) | EE (%) |
|-----------|----------|-----|---------|------------|--------|
| Pre-lyo   | 109.4 ± 1.7 | 0.18 ± 0.03 | −30.1 ± 1.9 | 9.9 ± 0.2 | 98.0 ± 2.1 |
| Post-lyo  | 272.8 ± 4.1 | 0.42 ± 0.09 | −29.5 ± 2.1 | 9.8 ± 0.1 | 97.9 ± 2.8 |

Fig. 3 DSC thermograms of pure ZT (a), pure Dynasan-118 (b), physical mixture of ZT + lipid (1:1) (c), and lyophilized optimized ZT-SLN (F1) formulation (d)
considered as an optimized formulation and selected for further studies.

**Ex vivo permeation studies**
Ex vivo permeation studies were performed for optimized ZT-SLN formulation (F1) in comparison with ZT-CS (F7) through rat intestine using everted sac perfusion method. The duration of the study was 120 min and represented in Fig. 2. From the results, the percentage of ZT permeation from both F7 and F1 formulations was $19.6 \pm 2.1\%$ and $29.6 \pm 2.6\%$, respectively. Statistically significance difference ($p < 0.05$) was observed from the F1 and F7 formulations.

**Lyophilization of ZT-SLN**
Optimized ZT-SLN formulation was subject to lyophilization, using 10% w/w of trehalose as cryoprotectant. The cryoprotectant and its concentration were selected based on the previously reported methods [24]. The lyophilized formulation was characterized for before and after lyophilization and presented in Table 3.

**Solid state characterization**

**DSC**
DSC studies were used to determine the compatibility status of the solid lipids and other excipients used in the SLN formulation, crystalline nature of the drug in the nanoformulations and was based on the fact that different lipids possessed different melting points and enthalpies. DSC thermograms of pure ZT, pure Dynasan®118, physical mixture of ZT, and Dynasan®118 in 1:1 ratio and lyophilized ZT-SLN formulation are shown in Fig. 3. The DSC thermogram of pure ZT showed a sharp endothermic peak at $93.76 ^\circ C$ (Fig. 3a), and it is corresponding to reported melting point. Pure Dynasan®118 showed a sharp endothermic peak at $76.06 ^\circ C$ (Fig. 3b). Physical mixture of ZT with Dynasan®118 showed drug peaks at $94.82 ^\circ C$, however with less enthalpy compared with pure ZT enthalpy. But, melting endotherm of drug was well preserved with slight changes in terms of shifting in the temperature of the melt (Fig. 3c). The absence of endotherm peak of drug in lyophilized ZT-SLN formulation (Fig. 3d) was observed.

**XRD**
X-ray diffractometer (XRD) studies of pure ZT, pure lipid, and lyophilized ZT-SLN (F1) were showed in Fig. 4. From the results, powder XRD patterns of ZT showed sharp peaks at 2$\theta$ scattered angles of $10.3, 11.4, 13.5, 17.1, 18.3, 19.8, 20.3, 23.2,$ and $24.1^\circ$; these were indicating the crystalline nature of drug. These characteristic peaks of ZT peaks were absent in the lyophilized ZT-SLN sample. This specified that the ZT was not in crystalline form and converted to amorphous nature, after lyophilization of optimized ZT-SLN (F1).

**SEM**
The shape of the ZT before and after loading onto SLN formulation was observed using SEM method. The
results are showed in Fig. 5. The results of the SEM studies also correlated with particle size of after reconstituted lyophilized sample.

**Stability study of optimized ZT-SLN**

The stability of the optimized ZT-SLN was monitored by storage at room temperature (25 °C) and refrigerated temp (4 °C) for 60 days. The formulations were analyzed on 1st day, 30th day, and 60th day, respectively, for size, PDI, ZP, EE, and drug content. The results indicated that no significant variations were noticed in all the measured parameters and found to be stable up to 2 months (Table 4).

**Pharmacokinetic (PK) study**

Oral bioavailability study for ZT-CS (F7) and optimized ZT-SLN (F1) formulations were performed in male Wistar rats. The collected serum samples were analyzed for drug content, using HPLC method. Ramipril is used as IS. Retention times of both ZT and IS were 7.8 and 4.5 min, respectively. The linearity of the ZT was observed in the concentration range of 0.25–12 μg/mL. The limit of detection and limit of quantification of the ZT were 0.1 and 0.2 μg/mL, respectively. About 98.5% of extraction efficiency was observed from recovery studies.

The PK parameters were calculated using the Kinetica 2000 software. Mean serum concentration vs time profiles of F7 and F1 formulation after oral administration of single dose are shown in Fig. 6. PK parameters like AUC_total, C_{max}, T_{max}, MRT, and t_{1/2} of the formulations were calculated and shown in Table 5. A significant difference observed in C_{max} and MRT of the F1 formulation indicates the prolonged release of the ZT. AUC is the major determining factor of the bioavailability of the formulations. From the results, AUC_total values of F7 and F1 were found to be 32.3 ± 1.1 and 43.3 ± 2.2 μg/mLh, respectively, and were statistically significant (p < 0.05). From the results, about 1.3-fold improvement in the bioavailability observed from F1 formulation in comparison with F7 formulation.

**Discussion**

ZT-SLNs formulations were prepared using three different lipids such as Dynasan*118, Dynasan*114, and Compritol *ATO 888, each at 1 and 2% w/v, respectively. Homogenization followed by probe sonication method used for the preparation of ZT- SLNs. The homogenization time and probe sonication time used at 12,000 rpm for 5 min and 20 min at 40% amplitude, respectively. The conditions were optimized based on the earlier reported methods.

From the results, as the concentration of lipids increased from 1% w/v to 2% w/v, increased in particle size and PDI, decreased in ZP were observed for F1–F4 formulations. But, F5 and F6 formulations showed revere trend. This could be due to the nature of lipid, i.e., Compritol *ATO 888. This might be due to the changes in the orientation of the drug and presence of the void spaces of lipid and drug. PDI of the formulations (F1–F6) was ranged from 0.17 to 0.61. The PDI value of up to 0.3 was considered to be homogeneous dispersion and was evidenced from the earlier reports [15]. The formulation ZP of the ZT-SLNs was crucial factor the stability assessment. In this case, the combination of surfactants imparts the electrostatic repulsion and steric

| Time (day) | At room temperature (25°C) | At refrigerated temperature (4°C) |
|------------|-----------------------------|----------------------------------|
|            | Size (nm) | PDI | ZP | EE | Assay | Size (nm) | PDI | ZP | EE | Assay |
| 1          | 104.3 ± 2.4 | 0.17 ± 0.01 | 30.5 ± 2.5 | 98 ± 0.3 | 98.4 ± 2.8 | 105.1 ± 2.0 | 0.17 ± 0.01 | 30.8 ± 2.2 | 9.9 ± 0.2 | 98.1 ± 2.3 |
| 30         | 110.3 ± 3.1 | 0.18 ± 0.03 | 29.4 ± 1.9 | 98 ± 0.5 | 97.4 ± 3.0 | 107.5 ± 1.8 | 0.17 ± 0.06 | 30.4 ± 1.9 | 9.9 ± 0.2 | 98.4 ± 2.8 |
| 60         | 114.7 ± 2.7 | 0.19 ± 0.04 | 28.1 ± 3.1 | 97 ± 0.4 | 97.1 ± 1.9 | 108.3 ± 0.9 | 0.18 ± 0.03 | 30.1 ± 1.4 | 9.8 ± 0.6 | 97.9 ± 1.7 |
stabilization as well. The presence of Poloxamer-188 in the development of SLNs could be favored for the maintenance of this ZP [31]. In general, ZP with ± 30 mV could be considered as stable dispersion system. Formulations prepared with Dynasan®118 and Dynasen-114 showed above −25 mV and indicates stability. But, formulations F3 and F6 had below −16 mV of ZP.

The crystal lattice of the lipid matrix might be the reason for difference in the drug release from ZT-SLNs. Triglyceride formulations (Dynasan-118 and Dynasen-114) showed slightly better release than mixed glyceride (Compritol ATO888) formulations. All the formulations showed burst release in initial 2 h and followed by slow prolonged release of the drug. This probably due to the unentrapped drug and the drug which present in the outer layer of the lipid showed burst effect. The drug embedded inside the lipid matrix slowly diffuse the drug as time progress.

From the ex vivo results, a statistically significance difference (p < 0.05) was observed in percentage of permeation of ZT from SLN formulation compared with control formulation through rat intestine. The prolonged release of the ZT from the ZT-SLN formulations was observed from the in vitro release and ex vivo permeation studies.

The crystalline nature, surface morphology, and conversion of lipid nanoparticles into solid dosage forms by incorporating in capsules or compression to tablets were easy by the conversion of dispersion to solid form of the nanoparticles. This is generally achieved with either spray drying or freeze drying techniques. From the results of reconstituted lyophilized powder, about two to three-fold increase in the particle size and PDI of the formulation was observed after lyophilization. This could be due to the aggregation of the particles during the process of freeze-drying. But, there were no significant changes noticed in the other parameters. There were no significant changes assay, and EE in the reconstituted lyophilized SLN formulation indicates that there were no structural changes in the nanoparticle formulation.

From the DSC studies, in physical mixture, the intensity of drug peak was reduced compared with pure drug. It is known that the quantity of material used, especially in drug-excipient mixtures, could influence the peak shape and enthalpy. Thus, these minor changes in the melting endotherm of drug could be due to the mixing of drug and excipient, which lowered the purity of each component in the mixture, and this might not necessarily indicate potential incompatibility. Drug endothermic peak was absent in the lyophilized SLN formulation. The absence of endotherm peak of drug in lyophilized ZT-SLN formulation unravels the conversion of native crystalline state of the drug to amorphous state. XRD studies also confirm the conversion of crystalline nature of ZT to amorphous form. This reduction in crystallinity or conversion to amorphous state of ZT from ZT-SLN was noticed in DSC analysis as well [32]. Physical stability of the formulation at refrigerated and room temperature was observed to be stable over 60 days.

Surface morphology study was conducted for pure drug and lyophilized ZT-SLN formulation. SEM picture revealed that pure drugs of ZT particles are possessed different shaped (cubic and spherical) but highly aggregated (Fig. 5a). Optimized formulations of ZT-SLN (F1) particles are nearly spherical shaped and also aggregated [33].

The PK studies in Wistar male rats confirmed the enhanced oral bioavailability of the ZT from ZT-SLN compared with coarse suspension formulation, after a single oral dose administration. From the AUC<sub>total</sub> about 1.3-fold improvement in the bioavailability observed from F1 formulation in comparison with F7 formulation. Similar type of results for improved oral delivery with

| Parameter          | ZT-CS (F7) | ZT-SLN (F1) |
|--------------------|------------|-------------|
| C<sub>max</sub> (μg/mL) | 1.8 ± 0.2  | 2.4 ± 0.6*  |
| T<sub>max</sub> (h) | 4          | 4           |
| AUC<sub>total</sub> (μg/mL.h) | 32.3 ± 1.1 | 43.3 ± 2.2* |
| t<sub>1/2</sub> (h) | 16.0 ± 1.0 | 18.3 ± 2.3  |
| MRT (h)            | 19.7 ± 2.6 | 25.6 ± 1.5* |

*Statistically significant at p < 0.05 in comparison with ZT-CS
SLN system was reported earlier [34–37]. Recently, ZT loaded NLCs also showed enhanced oral bioavailability in Wistar rats [38]. ZT-NLCs showed 1.6-fold enhancement in the oral bioavailability compared with coarse suspension. The NLCs are second generation of SLNs and exhibit better entrapment than SLNs. The expulsion of the drug is also avoided through NLCs. This could be the reason for changes (but non-significant) in the oral bioavailability in SLNs and NLCs. The improved oral bioavailability of SLNs of ZT could be due to the contribution of individual and/or combined effects of nanosized particles of the SLNs. The effective surface would influence the adhesion to GI tract. Consequently, there is increased contact time of the SLN particles. In addition, the soylecitin and poloxamer could alter the permeability characters of the GI membrane. The fatty acid chains present in the lipids of SLNs improve the uptake by lymphatic transport. This lymphatic transport minimizes the first-pass effect of the drug [39, 40].

Conclusion
ZT loaded solid lipid nanoparticles were successfully developed for the improved oral delivery. The ZT-SLN formulation was prepared with homogenization followed by sonication method. The optimized formulation was selected. DSC and XRD studies revealed the conversion to amorphous form of ZT in SLN formulation. In vitro and ex vivo permeation studies confirm the sustained release of the ZT from SLN formulation comparison with ZT coarse suspension as control formulation. Optimized ZT-SLN formulation was stable over 2 months at refrigerated and room temperature. Pharmacokinetic study in male Wistar rats revealed that there was 1.3-fold improvement in oral bioavailability from SLN formulation when compared to coarse suspension. The overall results indicated that the lipid-based delivery systems considerably improved the bioavailability of ZT.

Abbreviations
BCS: Biopharmaceutical classification system; CS: Coarse suspension; DSC: Differential scanning calorimetry; EE: Entrapment efficiency; GIT: Gastrointestinal tract; IS: Internal standard; NLCs: Nanostructured lipid carriers; PDI: Poly dispersity index; PK: Pharmacokinetics; SEM: Scanning electron microscope; SLNs: Solid lipid nanoparticles; XRD: X-ray diffractometer; ZP: Zeta potential; ZT: Zotepine

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Authors’ contributions
We declare that this work was done by the authors named in this article: ND conceived and designed and drafted the manuscript study. TC and DS carried out the laboratory work, collected and analyzed the data, and drafted the manuscript. NB supervised the work and drafted the manuscript. All authors have read and approved the final manuscript.

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Availability of data and materials
All necessary data generated or analyzed during this study are included in this published article. Any additional data could be available from the corresponding author upon request.

Ethics approval and consent to participate
The studies were conducted with prior approval of Institutional Animal Ethical Committee (IAEC/01/UCPSC/AU/2018).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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