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ORIGINAL ARTICLE

Olanzapine Liquisolid Tablets Using Kolliphor EL with Improved Flowability and Bioavailability: *In vitro* and *In vivo* Characterization

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ABSTRACT

Objectives: Liquisolid tablets are an innovative approach to enhance the dissolution rate and, thereby, the bioavailability of therapeutic agents with poor aqueous solubility.

Materials and Methods: The objective of the current research was to compare the bioavailability of the optimized formulation of the olanzapine (OLZ) liquisolid tablet with that of the marketed tablet (MT) by conducting pharmacokinetic and behavioral assessment studies. Ten formulations were designed using Kolliphor EL as a non-volatile solvent, and the respective tablets were prepared by the direct compression method.

Results: Pre-compression studies of powders of all the formulations showed good/excellent flow properties and compressibility. The drug release profiles of liquisolid tablets were determined and compared with those of MT. Based on the *in vitro* results, K250 was considered as an optimized formulation and selected for further *in vivo* studies. $AUC_{0-\infty}$ value of K250 formulation was found to be 357.2 ± 35.5 ng.h.mL^{-1}, which was higher than that of the MT (258.4 ± 29.9 ng.h.mL^{-1}). The reduction in locomotor activity was enhanced remarkably in K250 compared with MTs at $p < 0.05$. The time periods taken to fall in the rotarod test were approximately equal in the experimental groups, which indicated the absence of extrapyramidal side effects. There was a remarkable decrease in the number of boxes covered in the open field test.

Conclusion: Kolliphor EL was found to be a potential non-volatile solvent that can be used to produce liquisolid tablets of OLZ with improved flow, compressibility, dissolution, and bioavailability.

Keywords: Liquisolid tablets, olanzapine, Kolliphor EL, pharmacokinetic study, behavioral assessments

INTRODUCTION

The oral route continues to be the major route of drug administration, as it is advantages outweigh it is a major limitation of low and varied bioavailability. Bioavailability depends on several factors, the most important being the drug's solubility and permeability. Drugs are categorized into four classes based on their solubility in aqueous media and permeability through biological membranes.[1] The low bioavailability of drugs of classes II and IV of the biopharmaceutical classification system (BCS) can be improved by solubility enhancement techniques. Chemical synthetic techniques and high-throughput analysis were used for drug targeting and reducing side effects, but this approach resulted in the generation of drug molecules with high lipophilicity.[2] Approximately 40% of currently marketed drugs and 70% of new drug molecules are poorly water-soluble.[3] Poor water solubility is a crucial challenge for a formulation scientist. A number of pharmaceutical methods have been advanced to enhance the drug's aqueous solubility, including micronization,[4] solid dispersion,[5] cyclodextrin complexation,[6] and liquisolid system.[7] Liquisolid method is a new method developed for improving the rate of dissolution and bioavailability of drugs having poor aqueous solubility.[8] It is the transformation of a liquid drug or a solid drug solubilized in a liquid vehicle into a dry powder that is non-tacky, free-flowing, and highly compressible.[9]

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Olanzapine (OLZ) is a second-generation antipsychotic medication used in the treatment of bipolar disorder and schizophrenia. It is a member of class II of the BCS, which comprises drugs having low aqueous solubility and high permeability. It is a yellow crystalline powder that is practically not soluble in aqueous media.[10] The oral bioavailability of OLZ is 60%. It can be administered orally as a conventional tablet and an orally disintegrating tablet available in 2.5, 5, 7.5, 10, 15, and 20 mg dosages. OLZ can also be administered parenterally available in 5 $mg.mL^{-1}$ dosage. Plasma protein binding of OLZ is high (93%) and it undergoes extensive pre-systemic metabolism. The drug's poor water solubility and presystemic metabolism are responsible for its low oral bioavailability. The dissolution rate of OLZ is enhanced by solid dispersions,[11] inclusion complexes,[12] nanosuspensions,[13] liquisolid tablets,[14,15] and self-nanoemulsifying drug delivery systems.[16] Natarajan et al.[13] conducted pharmacokinetic (PK) studies of OLZ nanosuspensions using albino Wistar rats.

Shah and Patel[14] formulated liquisolid compacts of OLZ using PEG 400 as a non-volatile solvent, neusilin as a carrier material, and Aerosil 200 as a coating material. The effect of formulation parameters such as drug: non-volatile solvent ratio and carrier: coating ratio on the angle of repose and percentage drug release was studied by 3^2 full factorial designs. The prepared tablets were evaluated by *in vitro* quality control tests. Ramadevi et al.[15] increased the dissolution rate of OLZ using the liquisolid technique employing tween 80 and propylene glycol as non-volatile solvents. Although the dissolution rate of the drug was increased, the flow properties of the developed pre-compression powder remained poor. Kolliphor EL is a non-ionic solubilizer used for solubility enhancement. The present work aimed at the formulation of liquisolid tablets employing Kolliphor EL as a non-volatile solvent with a view to improving bioavailability by conducting *in vivo* studies. The bioavailability of the optimized formulation was compared with that of the marketed tablet (MT) using PK and behavioral assessment methods. To date, no work has been reported on the PK and behavioral assessment studies of OLZ liquisolid tablets.

MATERIALS AND METHODS

Materials

The drug OLZ and non-volatile solvent Kolliphor EL were obtained from Dr. Reddy's Laboratories, Hyderabad, India, and Baden Aniline and Soda Factory (BASF), Mumbai, India, respectively, as gift samples. Starch and Avicel PH 102 were purchased from Yarrow Chem Products, Mumbai; Aerosil 200 was procured from Oxford Laboratory, Mumbai; hydrochloric acid from Emplura, Mumbai; high-performance liquid chromatography (HPLC) grade methanol and acetonitrile from Rankem, Haryana; and ketamine from a local hospital. All other chemicals used were of analytical grade. The MT Oleanz 10 was obtained from a local pharmacy (Sun Pharma, Gujarat, India).

Determination of the solubility of OLZ in Kolliphor EL and distilled water

The drug's solubility was estimated in Kolliphor EL and distilled water. An excessive quantity of drug (30 mg) was put into each conical flask containing 10 mL of solvent. The flasks were shaken for 24 hours at 25 ± 2 °C on a rotary shaker (Sisco).[17] The drug suspensions were centrifuged, and the clear supernatant liquids were collected. The liquids were appropriately diluted using 0.1 N HCl, and the OLZ content in the supernatants was determined using an ultraviolet-visible (UV-vis) spectrophotometer (Elico, SL159) at 259 nm.[18]

Preparation of liquisolid tablets and directly compressible DCTs

The direct compression technique was used for preparing liquisolid tablets. Kolliphor EL was used as the liquid vehicle, and the composition of ten formulations is shown in Table 1. The required amounts of Avicel PH 102, used as a carrier material, and Aerosil 200, used as a coating material, were

Table 1. Formulation of OLZ liquisolid tablets

Formulations	Wt. of OLZ (mg)	Wt. of Kolliphor EL (mg)	Wt. of avicel PH 102 (mg)	Wt. of aerosil 200 (mg)	Wt. of starch (mg)	Total wt. (mg)	Carrier: coating ratio (R)	Liquid load factor (L_f)
K110	10	10	55.56	5.56	4.06	85.18	10	0.360
K120	10	10	63.49	3.17	4.33	90.99	20	0.315
K130	10	10	66.67	2.22	4.44	93.33	30	0.300
K140	10	10	68.26	1.71	4.50	94.47	40	0.293
K150	10	10	69.44	1.39	4.54	95.37	50	0.288
K210	10	20	83.33	8.33	6.08	127.74	10	0.360
K220	10	20	95.24	4.76	6.50	136.50	20	0.315
K230	10	20	100.00	3.33	6.67	140.00	30	0.300
K240	10	20	102.39	2.56	6.75	141.70	40	0.293
K250	10	20	104.17	2.08	6.81	143.06	50	0.288

Wt.: Weight, OLZ: Olanzapine

obtained from their flowable liquid retention potential (denoted as ϕ) values. Avicel PH 102 and Aerosil 200 have ϕ values of 0.27 and 0.9, respectively, for Kolliphor EL.[19] The drug was mixed thoroughly using a glass rod in a preheated non-volatile solvent (Kolliphor EL) in a beaker until a uniform solution was obtained. The carrier and coating materials were added to the drug solution and transferred to a mortar. The mixing of liquid-powder contents was carried out according to the three-stage standard mixing process described by Spireas.[20] In the first stage, the mixture was blended using a spatula for 1 min to disperse the liquid containing the drug in the carrier and coat the mixture evenly. As the second step, the drug in the liquid/carrier and coating mixture was layered in the mortar for 5 m to permit drug permeation into powder particles. The last step involves scraping off the powder from the mortar and mixing it with starch (5% *w/w*) for 30 s. A rotary tablet machine (Shakti, India) was used to compress tablets from the uniform powder mixture. The number of tablets prepared in each batch was 50.

Micromeritics of the precompression powders

The micromeritic properties of the precompression powder mixtures were evaluated by estimating the Hausner ratio, Carr's index, and angle of repose.[21] Bulk density and tapped density values were measured using a bulk density apparatus (Excel Enterprises), and the Hausner ratio and Carr's index were determined using these values. The experiments were conducted in triplicate, and the mean and standard deviation were calculated.

Characterization of the liquisolid tablets

Hardness, friability, drug content, and disintegration time were determined for the prepared liquisolid tablets. The drug release of liquisolid tablets was evaluated in distilled water (900 mL) using a USP type II apparatus at 37 ± 0.5 °C and operated at 50 rpm. The amount of OLZ released was estimated using a UV-vis spectrophotometer at 259 nm. The dissolution profiles of liquisolid tablets were compared with that of the MT Oleanz 10 (Sun Pharma, Gujarat, India), containing 10 mg of the drug. Fourier transform infrared (FTIR) spectroscopy (Bruker, Alpha-T) was used to determine drug-excipient compatibility. The solid state characterization of OLZ and optimized liquisolid tablets was carried out by differential scanning calorimetry (DSC) (Hitachi, STA-7300), X-ray diffractometer (PANalytical, X'Pert PRO), and scanning electron microscope (SEM) (Jeol Asia PTE Ltd, JSM-6610LV). The hardness, drug content, disintegration time, and dissolution studies were determined in triplicate, and the mean and standard deviation were calculated.

In vivo bioavailability studies

These studies were conducted to quantify optimized liquisolid tablets after oral administration and to compare their bioavailability with that of MT. The studies were conducted according to the Committee for the Purpose of Control and Supervision of Experiments on Animals recommendations and after acquiring owing approval from the Institutional Animal Ethics Committee of Raghu College of Pharmacy, with number RCP/1549/PO/Re/5/11/21/06 dated 11/10/21.

Pharmacokinetic study

Male rabbits weighing 1.5-2.2 kg were randomly assigned to the two treatments (n= 6). The rabbits were abstained from food for 12 hours before starting the experiment and fed 4 hours after dosing. Rabbits were given water as much as desired throughout the study.

Marketed and liquisolid tablets were powdered and dispersed in water. Two milliliters of suspension containing OLZ equivalent to the rabbit dose (1.0 mg) were administered orally. The rabbit equivalent dose was calculated based on the average weight of rabbits (2 kg).[22,23] One milliliter of blood was taken through the marginal vein of the rabbit ear at specific hour intervals of 0, 1, 2, 3, 4, 6, 8, 12, and 24 and was transferred into Eppendorf tubes containing ethylene diamine tetraacetic acid to avoid clotting of the blood sample. The blood samples were subjected to centrifugation using a cooling centrifuge (CM-12 plus, Remi) at 4000 rpm for 10 min to obtain plasma. The samples containing plasma were kept at -20 °C in a deep freezer (Subzero, ULT80) until further evaluation. The proteins in plasma were separated from the drug by a protein precipitation technique using acetonitrile as a protein precipitating agent. To 100 µL of plasma, 1 mL of acetonitrile was added and centrifuged for 15 min at 4000 rpm. OLZ present in the clear supernatant was analyzed by an HPLC (Shimadzu, Prominence) method with a UV detector, which was developed and validated earlier. The HPLC conditions were as follows: column, Hypersil-BDS C18; mobile phase, a mixture of 50 mM phosphate buffer (pH 5.5), acetonitrile, and methanol (50:30:20 *v/v/v*); flow rate-1.2 $mL.min^{-1}$, run time, 10 min; wavelength, 214 nm.

The PK parameters were calculated using PK Solver 2 software. The peak height concentration, which is denoted by the symbol Cmax, and the time of peak height concentration, which is denoted by the symbol tmax, was verified through the drug plasma level-time profile. K_E, the elimination rate constant was obtained by multiplying the slope of the linear elimination phase with 2.303. $t_{1/2}$, the biological half-life was obtained using $0.693/K_E$. The trapezoidal method was used to calculate the area beneath the plasma level-time profile from zero to the last time point, which is denoted as the area under the curve (AUC_{0-24}). The AUC from the last time point to infinity ($AUC_{24-\infty}$) was obtained by dividing the last measured concentration by K_E. The sum of both areas gives the total area beneath the plasma level-time profile from zero to infinity ($AUC_{0-\infty}$). The PK parameters of the two groups were compared for any significant differences using the *t*-test with a probability value < 0.05.

Behavioral assessments

Schizophrenia is a complex psychiatric disorder. Symptoms associated with the disorder are classified into positive symptoms, negative symptoms, and disorganized symptoms.[24] The potency of the drugs to exhibit pharmacological activity can be evaluated using animal models. Various animal models include studies on rats, mice, and monkeys. Behavioral abnormalities are symptoms of psychosis and can be studied using different animal models.[25] In this study, spontaneous

motor activity, rotarod test, and open field test were used to evaluate the pharmacological response of the drug. Male Swiss albino mice were used for spontaneous motor activity and the rotarod test, [25] and male Wistar rats were used for the open-field test.[24] The open field test was performed on rats because it is easy to observe their movements. It is difficult to conduct the test on mice because of their fast movements and small size.

Spontaneous motor activity

Male Swiss albino mice, with an average weight of 25 g, were selected and separated into three groups based on randomization, with six animals in each group. Mice were given access to water and food as much as desired. Locomotor activity was determined by using a digital phototachometer (Indosati, CAT2002E). Normal mice exhibit typical locomotor activity when placed in a phototachometer. The neurotransmitter dopamine regulates a wide array of physiological functions, including locomotor activity, in the central nervous system. The pharmacological blockade of dopamine transmission inhibits locomotor activity.[26] Marketed and liquisolid tablets were powdered and dispersed in water. A volume of suspension (0.1 mL) containing OLZ equivalent to the mice dose (0.05 mg) was administered orally. The mice equivalent dose was calculated based on the average weight of mice (25 g). The third group of mice received distilled water and were considered as controls. The animal to be tested was individually placed in the phototachometer for 10 min, and the score on the digital phototachometer was recorded. The procedure was repeated every hour for 4 hours. The percentage decrease in locomotor activity after the administration of different formulations was calculated based on the locomotion exhibited by the animals in terms of the score given by the digital phototachometer.

Rotarod test

Male Swiss albino mice were placed into three groups, with 6 mice in each group. The rotarod test was conducted according to the method outlined by Dunhan and Miya[27] in 1957. The rotarod apparatus (Indosati, two compartments) is electronic equipment that contains a rotating rod, speed knobs, and a lever. The lever functions to stop the timer, when the mouse drops down from the rod. The mice to be tested were kept on a rotating rod[28] and their latency or time taken to fall was recorded. Drugs that alter neuromuscular coordination decrease the time taken by the animals to stay on the rod.[29]

Open field test

Male Wistar rats with weights between 200 and 250 g were selected for the study. The rats were marked and assigned to four different groups by randomization with six rats in each group. The rats were given water and food as much as required. When ketamine is injected at subanesthetic doses, it induces stereotypic behavior in animals.[24,30] The intensity of behavioral patterns increases, and this occurs because of disturbances in the brain. These disturbances are similar to those experienced by patients suffering from schizophrenia. The effect of the formulations on reversing the stereotypic behavior induced by ketamine was determined. The effect can be used to interpret the efficiency of formulations in controlling the symptoms of schizophrenia. An open-field apparatus (Indosati) was used to observe behavioral changes in the animals. The open field area was equally divided into squares. Marketed and liquisolid tablets were powdered and suspended in water. A suspension of 0.5 mL containing OLZ equivalent to the rat dose (0.25 mg) was administered orally. The rat equivalent dose was calculated based on the average weight of rats (250 g). Ketamine at a dosage of 30 $mg.kg^{-1}$ was injected 30 min after administration of the formulations. The third group received only ketamine and the fourth group received normal saline. The rats were positioned in the open field apparatus and the number of squares crossed was measured.

Statistical analysis

The *t*-test was used to compare the PK parameters. One-way ANOVA was used to observe the remarkable differences between groups in behavioral assessment studies at $p < 0.05$. Dunnett's test was performed to compare the optimized formulation with the control group in the spontaneous motor activity test and with the ketamine group in the open field test at $p < 0.05$. Statistical analysis was performed using Prism 5.0 software.

RESULTS AND DISCUSSION

Solubility analysis of OLZ in Kolliphor EL and distilled water

The determination of the solubility of OLZ in liquid vehicles is the first step in the design of liquisolid systems. A higher quantity of solubilized drug in a liquid vehicle indicates a higher solubility of the drug, thereby improving its dissolution rate. OLZ is a BCS class II drug that is not freely soluble in water. The solubility of OLZ in distilled water was 0.044 $mg.mL^{-1}$ which agrees with the value given in literature.[31] The drug presented higher solubility in Kolliphor EL (3.63 $mg.mL^{-1}$).

Micromeritics of the precompression powders

Uniform and reproducible powder flow from the hopper to the die cavity is highly essential to obtain tablets of constant weight and drug content. The nature of powder flow can be determined using the Hausner ratio, Carr's index, and angle of repose. Good (K210-K250) to excellent (K110-K150) flow properties were observed when liquisolid powders were prepared using Kolliphor EL as the liquid vehicle (Table 2). Liquisolid formulations containing higher drug concentrations (K110-K150) exhibited good flow and compactibility compared with liquisolid formulations containing lower drug concentrations (K210-K250). The results were analogous to those reported by earlier workers.[32] The quantity of Avicel PH 102 increased with an increase in the R value, and thereby the flow properties of liquisolid powders improved with an increase in the R value. These findings can be attributed to the good flow properties of Avicel PH 102.[33]

Characterization of the liquisolid tablets

The values of the post compression parameters of the liquisolid tablets are presented in Table 3 and were found to be within the limits. The findings of the dissolution study of the developed tablets and MT are presented in Figures 1 and 2. The percentage released in 60 min was found to be 44.87 for MTs

Table 2. Flow parameters of precompression blends (mean ± SD, n= 3)

Formulations	Carr's index (%)	Hausner ratio	Angle of repose (degrees)
K110	18.81 ± 0.36	1.23 ± 0.04	21.98 ± 0.44
K120	16.67 ± 0.21	1.20 ± 0.02	21.47 ± 0.63
K130	15.27 ± 0.78	1.18 ± 0.03	19.65 ± 0.21
K140	14.32 ± 0.55	1.16 ± 0.03	18.97 ± 0.28
K150	11.78 ± 0.47	1.13 ± 0.01	17.69 ± 0.57
K210	28.57 ± 0.19	1.40 ± 0.02	24.74 ± 0.17
K220	23.08 ± 0.34	1.30 ± 0.01	23.47 ± 0.26
K230	22.25 ± 0.26	1.29 ± 0.05	23.04 ± 0.31
K240	21.24 ± 0.22	1.27 ± 0.04	22.73 ± 0.38
K250	16.72 ± 0.57	1.20 ± 0.02	22.04 ± 0.54

SD: Standard deviation, n= Number of trials

Table 3. Post compression parameters of liquisolid tablets (mean ± SD, n= 3)

Formulations	Hardness (kg.cm^{-2})	Friability (%)	Drug content (%)	Disintegration time (seconds)
K110	2.98 ± 0.37	0.980	97.26 ± 1.62	125.62 ± 2.40
K120	3.21 ± 0.45	0.914	97.31 ± 2.08	123.75 ± 3.28
K130	3.54 ± 0.28	0.790	99.26 ± 2.74	120.39 ± 2.60
K140	3.78 ± 0.67	0.826	99.86 ± 3.54	119.41 ± 3.34
K150	3.89 ± 0.31	0.880	101.65 ± 3.62	118.47 ± 2.35
K210	3.33 ± 0.34	0.538	98.75 ± 2.46	114.26 ± 1.59
K220	3.81 ± 0.26	0.612	99.29 ± 1.98	113.64 ± 1.56
K230	3.92 ± 0.69	0.674	101.28 ± 2.59	112.4 8 ± 3.42
K240	4.12 ± 0.62	0.706	101.67 ± 3.62	112.23 ± 3.45
K250	4.33 ± 0.56	0.791	102.54 ± 2.76	109.68 ± 5.12

SD: Standard deviation, n= Number of trials

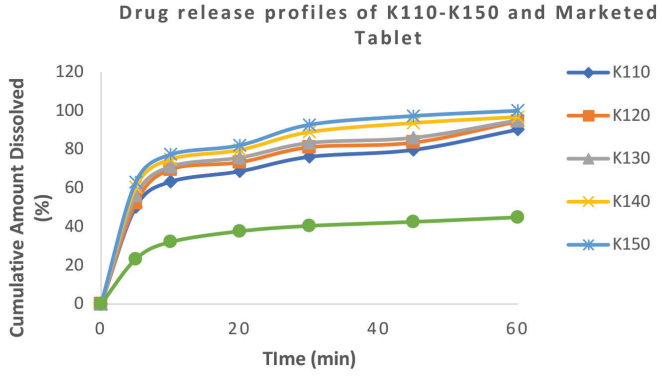

Figure 1. Drug release profiles of K110-K150 and marketed tablets

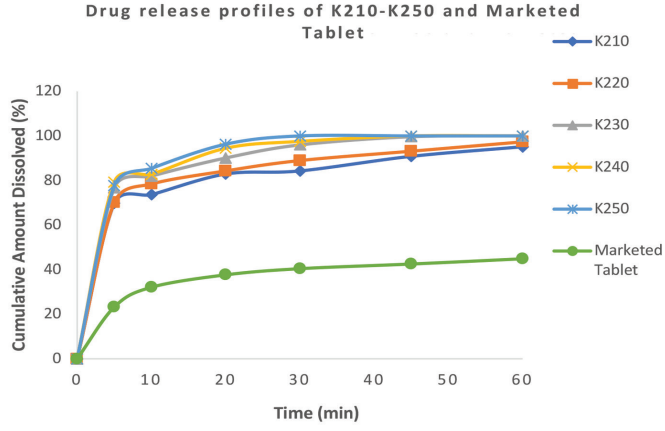

Figure 2. Drug release profiles of K210-K250 and marketed tablets

and 90.21-100 for liquisolid tablets. The results clearly indicate that the dissolution rate of liquisolid tablets is higher than that of MTs. This is best described by the Noyes-Whitney equation given in Equation 1.

$$D_R = \frac{D}{h} S(C_s - C) \quad (1)$$

Equation 1 where, D_R = rate of dissolution of the dissolved drug substances, D= diffusion coefficient of the dissolved drug substances, S= surface area of drug substances opening to the dissolution vehicle, h= diffusion layer thickness, C_S = maximum drug solubility in diffusion layer and C= drug's concentration in the dissolution medium.

The dissolution medium is the same in all studies; hence, there will be no change in the values of D and h. S and (C_s - C) were the variables that influenced the dissolution rate. In the tablet prepared without any liquid vehicle, the surface area of the drug open to the dissolution vehicle is delimited because of the drug's low aqueous solubility. In contrast, the drug substances in the liquisolid tablets were dispersed in a non-volatile solvent, which enormously enhanced the surface area of the drug molecules. The drug's saturation solubility (C_s) is increased as the drug molecules are available in a state of molecular dispersion in liquisolid tablets. The quantity of non-volatile solvent used in the formulation of liquisolid tablets is negligible, and it may not be sufficient to enhance the total saturation solubility of drug molecules in the dissolution vehicle. However, at the junction between liquisolid particles and dissolution vehicles, the quantity of non-volatile solvent that diffuses with the drug substances is sufficient to augment the solubility of drug molecules. The non-volatile solvent acts as a cosolvent in the diffusion layer. In addition, an increase in R value from 10:1 to 50:1 increased the dissolution of liquisolid tablets. The presence of a high amount of Avicel PH 102 in liquisolid tablets with higher R values is responsible for improved imbibing, disintegration, and disaggregation.[34] Liquisolid tablets, K210-K250 comprising 67.77% of Kolliphor EL, exhibited larger drug release in contrast to formulations containing lower Kolliphor EL concentration (50% in K110-150). The calculated difference factors (f1) and similarity factors (f2) show a large difference in the dissolution profiles of K250 and MT. f1 and f2 for K250 and MT were 153.38 and 12.40, respectively. Formulation K250, described with Kolliphor EL as a liquid vehicle containing 67.77% of non-volatile solvent with a carrier: coating ratio of 50, was the best formula selected considering the outcomes obtained from all the studies.

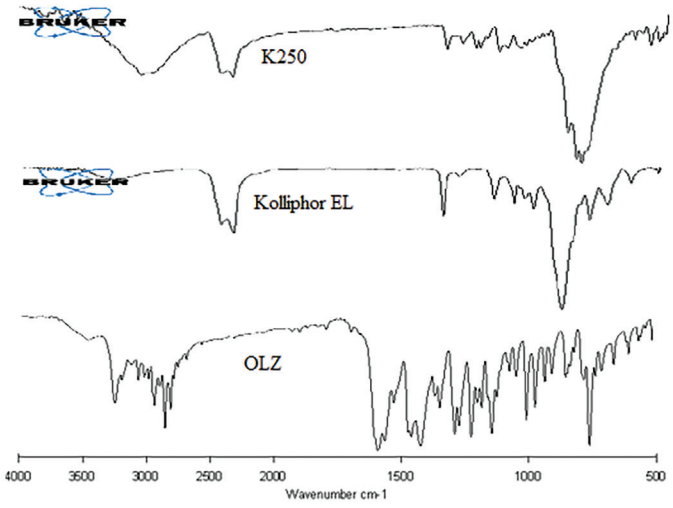

Figure 3. FTIR spectra of OLZ, Kolliphor EL, and K250
FTIR: Fourier transform infrared, OLZ: Olanzapine

The FTIR spectra of OLZ, Kolliphor EL, and the optimized liquisolid formulation with Kolliphor EL are shown in Figure 3 and the values are shown in Table 4. The distinct peaks of OLZ are observed at 2933 cm^{-1} (CH stretching), 1600-1500 cm^{-1} (double bonds attached partially to CH and NH bending deformation), 1500-1300 cm^{-1} (deformation of methyl, methylene and CH groups), 1300-1100 cm^{-1} (CC and CN stretching), 1009 cm^{-1} (deformation of piperazinyl group attached to methyl group) and 745 cm^{-1} (out of plane deformation of CH bonds belonging to the same group).[35] Any drug degradation or drug interaction with additives results in changes in ischemical structure, which is reflected by the changes in the FTIR spectra. The FT-IR spectra of liquisolid tablets exhibited the same distinct drug absorption peaks, indicating the absence of drug-liquid vehicle interaction.[36]

The DSC thermograms of OLZ and K250 are displayed in Figure 4, and their values are given in Table 5. A sharp endothermic peak at 194.25 °C was observed in the OLZ thermogram, which is related to the melting point of the drug.[37] The thermogram of the liquisolid system showed a shift of the endothermic peak to a lower temperature, indicating partial amorphization of the drug.[38]

Table 4. FT-IR peak values of OLZ and K250

Wavenumber (cm^{-1})						
	CH stretching	NH bending	CH_3, CH_2, CH deformation	CC and CN deformation	Piperazinyl deformation	CH out of plane deformation
Literature values	2933	1600-1500	1500-1300	1300-1100	1009	745
OLZ	2926.62	1587.79	1468.23	1268.89	1004.83	746.91
K250	2925.15	1580.63	1463.66	1276.48	1007.35	743.28

FT-IR: Fourier transform infrared, OLZ: Olanzapine

Polymorphism of a drug is an important factor that affects its rate of dissolution, and, eventually, its bioavailability. Hence, it is essential to observe any changes in the drug's polymorphism after formulation as liquisolid tablets. Sharp and distinct peaks at 20.47˚, 21.64˚, and 24.56˚ were observed in the X-ray diffraction (XRD) pattern of the pure drug (Figure 5, Table 5), indicating its high crystalline character.[39] The XRD pattern of the optimized liquisolid formulation (Figure 5) showed the disappearance or a decrease in the intensity of the drug's characteristic peaks, which indicates that the crystallinity of the drug is reduced. This effect was also observed in the reports of earlier workers.[40,41]

Drug crystals of irregular shape were observed in the SEM photomicrograph of OLZ (Figure 6)[12]. The inability to differentiate crystals of OLZ in the photomicrographs of K250 (Figure 6) indicates a solid-state transition in the drug.[42,43] The results of DSC, XRD, and SEM indicate a reduction in the crystallinity of the drug, which is another mechanism that explains the enhancement of dissolution in liquisolid tablets.

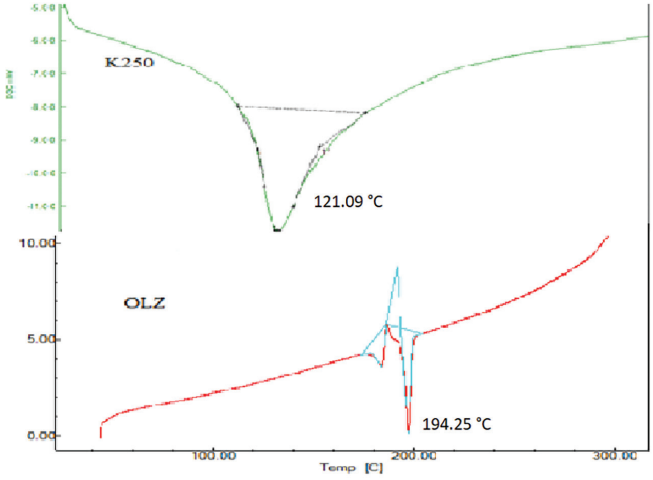

Figure 4. DSC of OLZ and K250
OLZ: Olanzapine, DSC: Differential scanning calorimetry

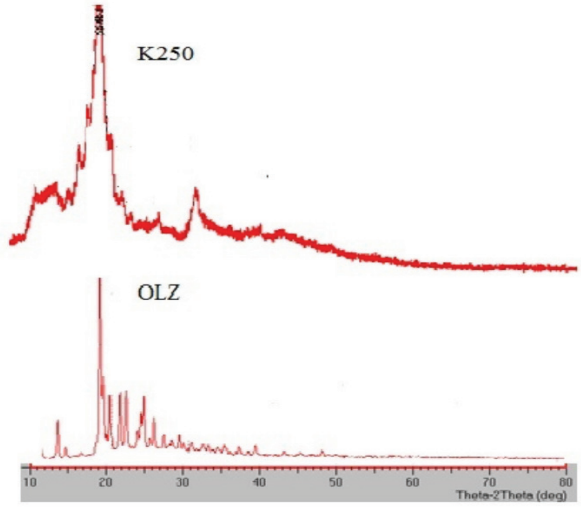

Figure 5. XRD of OLZ and K250
OLZ: Olanzapine, XRD: X-ray diffraction

In vivo studies

Pharmacokinetic study

The plasma drug concentration *versus* time graphs of the marketed formulation and optimized liquisolid formulation are presented in Figure 7, and the PK parameters obtained are given in Table 6. The peak plasma concentration obtained was higher for the optimized liquisolid tablets than for the MTs. However, the time taken to attain C_{max} was 3 hours and it was similar in both treatments. The $AUC_{0-\infty}$ which denotes the quantity of drug absorbed completely from zero to infinite time, was higher for liquisolid tablets (357.2 ± 35.5 ng.h.mL^{-1}) compared to MTs (258.4 ± 29.9 ng.h.mL^{-1}) and a significant difference was observed at $p < 0.05$ among the two groups. The higher

Table 5. DSC and XRD peak values of OLZ and K250

	DSC peak values (˚C)	XRD peak values (˚2θ)		
OLZ	194.25	20.47	21.64	24.56
K250	121.09	19.31	Absent	23.98

OLZ: Olanzapine, XRD: X-ray diffraction, DSC: Differential scanning calorimetry

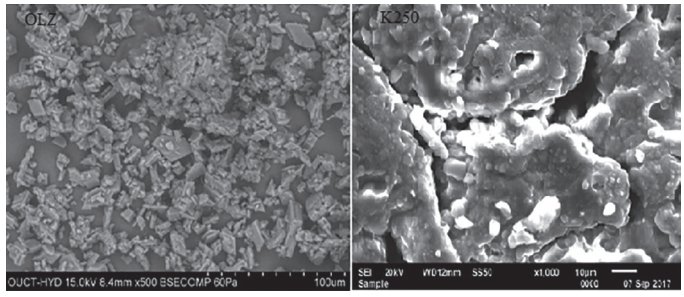

Figure 6. SEM images of OLZ and K250
OLZ: Olanzapine, SEM: Scanning electron microscope

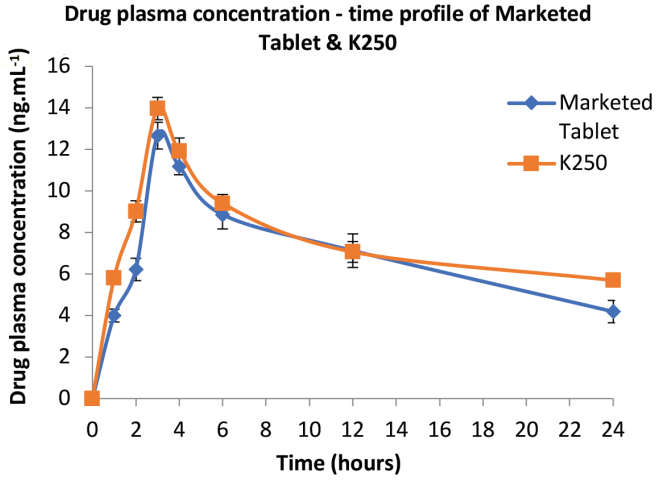

Figure 7. Drug plasma concentration-time profile of marketed tablets and K250, n= 6

Table 6. Pharmacokinetic parameters of marketed tablet and K250 (mean ± SD, n= 6)

Formulations	Pharmacokinetic parameters					
	C_{max}	t_{max}	$t_{1/2}$	AUC_{0-t}	$AUC_{0-\infty}$	K_E
	($ng.mL^{-1}$)	(h)	(h)	($ng.h.mL^{-1}$)	($ng.h.mL^{-1}$)	(h^{-1})
Marketed tablet	12.663 ± 0.643	3.000 ± 0.000	15.238 ± 2.598	165.032 ± 7.218	258.482 ± 29.926	0.046 ± 0.007
K250	13.966 ± 0.538	3.000 ± 0.000	21.276 ± 4.141	182.705 ± 5.979	357.276 ± 35.598	0.034 ± 0.006

SD: Standard deviation, AUC: Area under the curve, n= Number of trials

$AUC_{0-\infty}$ and C_{max} values obtained for the liquisolid tablet relative to the MT could be attributed to the improved dissolution rate of OLZ from the liquisolid tablets, leading to higher absorption. The non-volatile solvent Kolliphor EL used in the formulation of liquisolid tablets inhibits P-glycoprotein.[44] P-glycoprotein is present in the cell membrane and is responsible for the efflux transportation of drugs and toxins. P-glycoprotein reduces the absorption of many drugs through the intestine, thereby decreasing the drug plasma concentration. Thus, the inhibitory effect of Kolliphor EL on P-glycoprotein is an added benefit for enhancing the bioavailability of the drug in the formulation.

Behavioral assessments

The results of locomotor activity are presented in Table 7. The liquisolid tablet formulation showed a higher reduction in locomotor activity compared with the MT. A remarkable difference at $p < 0.05$ was observed in the phototachometer scores using One-Way ANOVA among the three groups. Dunnett's test was performed to determine where the significant difference lay, *i.e.*, whichever two among the three groups were significantly different. It was found that the effect of the liquisolid tablet was significantly different from that of the control.

The results of the rotarod test are given in Figure 8 and Table 8. The test was conducted to check for extrapyramidal side effects of the optimized formulation because of increased bioavailability. The latency to fall after 3 hours after administration of distilled water for control was 236.83 s; for the MT it was 223 s; and for K250 it was 220.35 s. There was no remarkable statistical difference among the three groups for the time taken to fall by the mice. The results indicate the absence of side effects in the optimized formulation.

The reversal of ketamine-induced stereotypic behavior was determined by the number of boxes covered by rats in the open field test apparatus (Figure 9, Table 9). The number of boxes covered was 44 in the control group, 82.5 in the ketamine-administered group, 66.75 in the MT group, and 61.17 in the K250 group. The liquisolid tablet showed a remarkable decrease in the number of boxes covered. A remarkable statistical difference was noticed in the number of boxes covered among the four groups at $p < 0.05$ using One-Way ANOVA. Dunnett's test showed that the effect produced by the liquisolid tablet formulation was significantly different from the group that received only ketamine.

Table 7. Results of spontaneous motor activity of different groups (mean ± SD, n= 6)

Time (hours)	Photoactometer score		
	Control	Marketed tablet	K250
1	591.83 ± 15.82	240.50 ± 6.70	224.17 ± 8.07
2	538.00 ± 14.51	183.00 ± 2.47	159.67 ± 4.86
3	551.83 ± 15.69	159.67 ± 4.86	100.17 ± 5.81
4	540.33 ± 10.43	121.00 ± 3.76	193.50 ± 3.35

SD: Standard deviation, n= Number of trials

CONCLUSION

The dissolution rate and bioavailability of the optimized formulation were assessed by conducting relevant *in vitro* and *in vivo* experiments. The incorporation of Kolliphor EL in the formulation of the liquisolid tablets has resulted in a remarkable improvement in the dissolution rate of OLZ. Precompression powders with improved flow properties were obtained. Formulation K250 prepared with an OLZ:Kolliphor EL ratio of 1:2 and an Avicel PH 102: Aersosil 200 ratio of 50:1 showed the

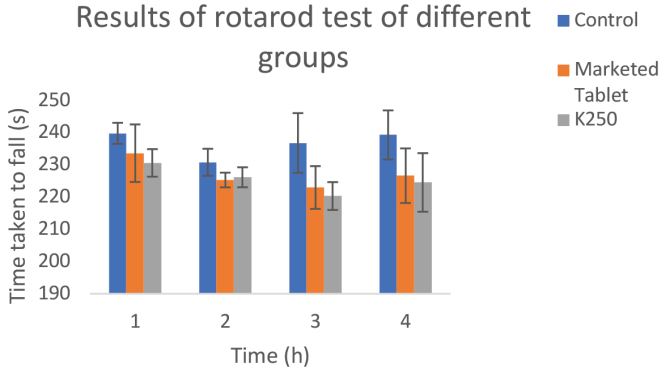

Figure 8. Results of the rotarod test of different groups, n= 6

Table 8. Results of rota rod test of different groups (mean ± SD, n= 6)

Time (hours)	Time taken to fall (seconds)		
	Control	Marketed tablet	K250
1	239.83 ± 3.26	233.67 ± 8.93	230.65 ± 4.28
2	230.83 ± 4.20	225.33 ± 2.28	226.19 ± 3.10
3	236.83 ± 9.28	223.00 ± 6.65	220.35 ± 4.33
4	239.33 ± 7.62	226.67 ± 8.48	224.58 ± 9.10

SD: Standard deviation, n= Number of trials

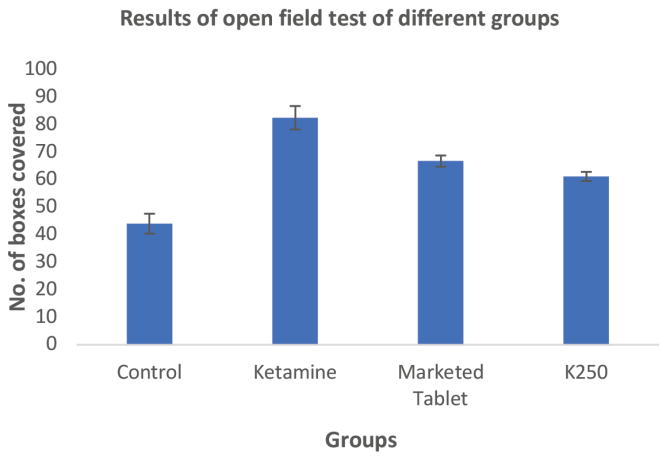

Figure 9. Results of the open field test of different groups, n= 6

Table 9. Results of open field test of different groups (mean ± SD, n= 6)

Groups	No. of boxes covered
Control	44.00 ± 3.63
Ketamine	82.50 ± 4.26
Marketed tablet	66.75 ± 2.05
K250	61.17 ± 1.67

SD: Standard deviation, n= Number of trials

highest dissolution rate and is thus the optimized formulation. A reduction in drug crystallinity is another reason for the additional improvement in the dissolution rate, and it is distinctly observed in the results of solid-state characterization. The PK and behavioral assessment study results clearly indicated the enhancement in bioavailability of the optimized formulation. Kolliphor EL is a potential solubility enhancer for OLZ liquisolid tablets.

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Ethics

Ethics Committee Approval: The studies were conducted according to the Committee for the Purpose of Control and Supervision of Experiments on Animals recommendations and after acquiring due approval from the Institutional Animal Ethics Committee of Raghu College of Pharmacy with number RCP/1549/PO/Re/5/11/21/06 dated 11/10/21.

Informed Consent: Not required.

Authorship Contributions

Surgical and Medical Practices: R.D.K., Concept: R.D.K., C.S.R.G., Design: R.D. K., Data Collection or Processing: R.D.K., Analysis or Interpretation: R.D.K., Literature Search: R.D.K., Writing: R.D.K., C.S.R.G.

Conflict of Interest: No conflict of interest was declared by the authors.

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