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Enhancing oral bioavailability of escitalopram by self-nanoemulsifying drug delivery systems (SNEDDS): An *in-vivo, in-vitro* and *in-silico* study

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Abstract: This study aimed to develop SNEDDS to address the issue of low water solubility of escitalopram (ETP), which consequently leads to inadequate oral bioavailability. The formulation consisted of Tween 80, geranium oil, and polyethylene glycol 400. An evaluation was conducted to determine the surface charge and particle size of ETP-SNEDDS. The stability and compatibility of excipients were evaluated by TGA, DSC, and FTIR. Studies were carried out to examine the dissolution, digestion, permeability, in *in-vitro, in-vivo,* and *ex-vivo* settings. ETP-SNEDDS bioavailability was assessed in a group of six albino rats under normal conditions. The synthesized SNEDDS exhibited thermodynamic stability, characterized by a 145nm droplet size with a polydispersity index of 0.120 and a minute emulsification duration. Developed SNEDDS containing ETP in FSSIF had a dissolution rate of 96%. Based on the permeation results, the SNEDDS demonstrated a 4.2-fold and 3.1-fold increase in drug penetration relative to standard powder-ETP drug and a reference tablet, correspondingly. Statistically significant improvements ($p<0.05$) were reported in *in-vitro* digestion, dissolution and *ex-vivo* permeability. SNEDDS exhibited a 5.34-fold increase in C_{max} and a 4.71-fold rise in AUC compared to the reference. Based on findings, the formulated SNEDDS have a valuable method for enhancing ETP oral bioavailability.

Keywords: Escitalopram; self-nano-emulsifying drug delivery system (SNEDDS); solubility boost; in-vivo ex-vivo and *in-vitro* studies; bioavailability; permeability.

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INTRODUCTION

Giving medications orally is the easiest and safest way to give medication since it allows patients to manage their conditions more effectively and is convenient for them to administer (Shahba *et al*., 2022). Nevertheless, patients may find it challenging to ingest medicinal molecules orally if the compounds have low bioavailability due to restricted water solubility (Mahmood *et al*., 2023a). Regretfully, only over 70% of newly developed bioactive compounds and pharmaceuticals have poor water solubility (Buya *et al*., 2020a). Exploring the SNEDDS preparation for oral route is now underway amongst drug delivery techniques. According to the literature, SNEDDS enhances an oral medication's bioavailability, particularly when competed to dispersion in solid forms (Nasr *et al*., 2016). The SNEDDS has been used to increase the bioavailability of several drugs because it is very effective at producing pharmaceuticals that are poorly soluble in water (Ashfaq *et al*., 2022). Research has been done on a variety of techniques for developing SNEDDS formulations that boost a medication's bioavailability. There isn't any research being done on SNEDDS formulations for ETP administration orally at this time (Abushal *et al*., 2022, Teaima *et al*., 2022).

ETP is a selective serotonin reuptake inhibitor (SSRI) utilized for treating depression. ETP is white to light yellowish and slightly soluble in water and has a molecular weight of 324.4 g/mol (Devunuri *et al*., 2016). ETP operates on the serotonin transporter and has an oral bioavailability of 80%. Over a period of 24 hours, people who consumed a daily dosage of 10mg of ETP experienced a maximum concentration (C_{max}) of 21 ng/mL and an area under the curve (AUC) of 360 ng× h/mL. They have a 55% protein binding and are processed in the liver. An overdose of ETP can cause symptoms like dizziness, nausea, vomiting, seizures and abnormal heartbeats (Hayes *et al*., 2010). ETP is insoluble in heptane, barely soluble in ethyl acetate and hardly miscible in water and ethanol. In an isotonic saline solution, it dissolves (Akay *et al*., 2021, Woo *et al*., 2024).

According to recent findings, the bioavailability of ETP has increased due to the development of nanotechnology. Due to its strong lipophilicity, ETP has limited solubility in aqueous conditions and minimal gastrointestinal absorption (Mutingwende *et al*., 2021). Polymeric nanoparticles, liposomes and nanoemulsions can carry

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complicated pharmaceuticals. These methods reduce drug toxicity and allow for a regulated release, all while enhancing therapeutic efficacy, stability and solubility (Yadav *et al*., 2022). When gently stirred in a water-based media, SNEDDS spontaneously emulsifies like digestive juices. SNEDDS are isotropic oil, surfactant, co-surfactant, and drug or bioactive mixes (Jain *et al*., 2017). Particles in SNEDDS-produced oil-in-water nanoemulsions range in size from 20 nm to 200 nm after dilution. By increasing enterocyte barrier lipid mobility and inhibiting efflux pumps, SNEDDS increase oral bioavailability and transcellular permeability (Rathore *et al*., 2023, Cao *et al*., 2022).

We added ETP to SNEDDS, which were assessed by globule size and PDI, to take advantage of their prospective benefits. Albino rats were used to assess and enhance the ETP oral bioavailability from loaded SNEDDS and the systems' stability under simulated gastrointestinal circumstances.

MATERIALS AND METHODS

Materials

ETP was sampled and given by Saffron Pharmaceuticals Pvt. Ltd. in Faisalabad, Pakistan. Dimethyl sulfoxide (DMSO), triton X-100, potassium dihydrogen phosphate, methanol, Glacial acetic acid, acetonitrile, tween 80, 60, 40, 20, and 4-bromophenylboronic were purchased from Dae-Jung Chemicals, Korea. Cinnamon, tea tree, olive, geranium, sesame, castor, coconut, sunflower, and ginger oils were obtained from Sigma-Aldrich Gmbh, Darmstadt, Germany. Merck, Darmstadt, Germany, supplied PEG 600, 400 and 200.

ETP Solubility Study

ETP solubility was assessed using Bravo-Alfaro *et al*.'s method with some modifications(Bravo-Alfaro *et al*., 2022). This research sought to discover ETP's oil phase of dissolution. In Eppendorf tubes with 1 mL of oils (cinnamon, tea tree, olive, geranium, sesame, castor, coconut, sunflower, and ginger oils), surfactants (tween 20, 40, 60 and 80), and co-surfactants, different quantities of ETP were applied. After 10 minutes of vortex mixing, the mixtures were immersed in ultrasonic bath at 37°C for five minutes.

The mixture was placed in the orbital shaker and subjected to agitation for a duration of 72 hours, maintaining constant temperature conditions throughout the experiments. After that, the samples were centrifuged for 20 minutes at 13,000 rpm to extract the medication that had not dissolved. After dilution in methanol for ETP extraction, each oil supernatant, co-surfactant and surfactant was filtered using a 0.45 µm membrane filter. A UV-visible spectrophotometer (Perkin Elmer, New York, USA) was used to measure the amount of ETP at 235 nm, which was extracted from the different oils, surfactants, and co-surfactants and methanol was dissolved into it.

Pseudo ternary phase diagram

For the ternary phase diagram, oils, surfactants, and co-surfactants were used. These surfactants, essential oils, and co-surfactants were selected according to ETP's solubility (Natesh *et al*., 2024). A range of self-emulsifying series was made using surfactants (0 to 20%), surfactants (25 to 65%), and essential oils (25 to 65%). The self-emulsifying area was determined by formulating several combinations of co-surfactants, essential oils, and surfactants. Since an ETP was not available, a schematic was generated using the CHEMIX® software.

Preparation of the SNEDDS of ETP

By identifying self-emulsifying zones, we were able to select the appropriate ratios of various oils for the formulation of SNEDDS. Tween 80 and PEG 400 zones were also selected by this method. The chemicals geranium oil, PEG 400 and tween 80 were added to the 10 mg of ETP in the sequence specified in table 1. The next step was to use a sonicator to create a crystal-clear solution. Subsequently, the SNEDDS formulation was preserved for use in future studies.

Simulated gastrointestinal fluids preparation

The fasted-state simulated gastric fluid (FSSGF) and fasted-state simulated intestinal fluid (FSSIF) were prepared as per instructions given by *Biorelevant.com Ltd*. To put it simply, 34.22 mM NaCl and 25 mM HCl were mixed to make a 1.6 pH solution in DI water for the FSSGF. The pH was checked and added 6 M hydrochloric acid to get it down to 1.6 when needed. At a concentration of 0.0596 mg/mL, the FSSGF powder was added to the buffer per the supplier's instructions. A solution of sodium chloride (68.62 mM), maleic acid (19.12 mM), and sodium hydroxide pellets (34.8 mM) was prepared for FSSIF in dilute hydrochloric acid (pH 6.5). Hydrochloric acid (6M) or sodium chloride (10M) was used to adjust pH 6.5.Calcium chloride dihydrate increased buffer calcium to 1.4 mM. FSSIF powder was dissolved in 1.79 mg/mL buffer and left for 1hr before usage.

Characterization of ETP-SNEDDS

Determination of Emulsification Time

Mix 500 mL of 0.1 HCl with 1 gram of each formulation, stir gently at 100 rpm and keep at 37±0.5°C. The duration of time required to attain a transparent dispersion was used to compute the process of time for emulsification (Singh *et al*., 2011).

Determination of zeta potential and droplet size

Ten milliliters of media were used to dissolve the ETP-SNEDDS formulations after pre-warming the FSSGF and FSSIF to 37°C. The liquid was mixed for 1 hour at 100 rpm prior to droplet size measurements. Next, we used 37°C

dynamic light scattering to find the size and polydispersity index (PDI) of the droplets. We analyzed droplet sizes and PDI using parametric paired t-tests. ETP-SNEDDS' zeta potential was measured using a Malvern Zeta Sizer Nano Series ZS90.

Fourier transforms infrared spectroscopy (FTIR)

The following ingredients were used in the formulations: ETP, tween 80, PEG 400, geranium oil and FTIR spectroscopy: FTIR by Bruker Alpha, Germany was used to analyze the spectra, with a wavelength range of 400-4000 cm-1 and 10 scan rates.

Differential scanning calorimetry (DSC)

Using a DSC-60 calorimeter from Shimadzu, Germany, 5±0.5 mg of ETP, oil, tween 80, PEG 400, blank, and ETP-loaded SEP5 were heated at 50-400°C at 5°C/min while nitrogen flowed at 40mL/min.

Thermogravimetric analysis (TGA)

For TGA, crucibles made of aluminum were filled with ETP, PEG 400, tween 80, geranium oil, blank and SEP5 ETP-loaded samples. Under an arc of nitrogen gas flowing at 40mL/min, the temperature ranged from 50 to 400°C and it was heated at 10°C/min. It was thought that the proportion of weight loss function was regulated by heat.

Scanning electron microscopy

The Nova Nano FE-SEM 450 (FEI) scanning electron microscope was used to investigate the samples for their morphological characteristics of SNEDDS. The entire sample was covered with gold before being subjected to SEM examination.

Dissolution studies

This USP Apparatus II research assessed drug release in vitro using a dialysis bag method (Buya *et al*., 2020b). After filling the dialysis bag with 10 mg of ready-to-use ETP SNEDDS, it was sealed. After that, the sealed bag was transported to USP apparatus II with 900mL FSSIF (Miao *et al*., 2024). At 37°C, the solution pH was 6.8 and the paddle spun at 100 rpm. Consistently adding 5mL of buffer following 5mL of sample volume helped keep the sink state. A comparison was made between the ETP release of the prepared formulas and the SNEDDS that were created. The drug content was measured with a 235-nm UV spectrophotometry. Quantification controls included ETP-unloaded formulations to decrease oil absorption. ETP release kinetics and mechanism were examined by fitting the ETP release data to established mathematical models.

Permeation studies

Spanish researchers employed a Franz diffusion cell to assess the ex-vivo SNIDDS permeability, powder drug, and standard tablet (Karavasili *et al*., 2020). A 1 cm^2 diffusional cross-sectional area was studied. The receiver chamber of volume 2.5 ml was used. A new goat intestinal membrane for permeation experiments was used. The permeation media (FSSIF) mimic natural settings. The donor and receptor compartments were pressed and fastened with a new goat intestinal membrane since the epithelial surface faces the donor area. Place reference tablet, ETP powder and SNEDDS in the donor compartment after diluting with 1 mL medium.

For the 12-hour test at 37°C, the media were regularly mixed to ensure uniformity. At 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, and 12 hours, 0.25mL samples were obtained. All the samples were filtered using a 0.45 µm before analysis by HPLC at 238 nm. Permeation profiles were created by graphing medicine penetration through the membrane over time. The portion of the curves in a stable state was utilized to calculate the flux (J) values.

$$0J = dQ/Adt(ug/cm^2min) \quad (1)$$

Here, A is the area of the membrane, Q is the amount of drug that permeates, and t is time exposed.

The equation for pressure is given by:

$$P=J/C_0\ (min/cm) \quad (2)$$

In this case, J stands for the flux value and C_0 for the initial medicine concentration in the donor compartment.

In vitro dispersion study

ETP5-SNEDDS (1.5g) formulations received 50mL of pre-warmed FSSGF or FSSIF at 37°C and with stirring at 100 rpm. Replaced 700µL of dispersion medium with fresh media at intervals of 2, 5, 10, 20, 30, 45, 60, 90 and 120 minutes (Kok *et al*., 2022). Before HPLC analysis, the material was filtered with a 0.45 µm syringe filter and was diluted 1:1 with dimethyl sulfoxide and methanol. To assess extraction, ETP5-SNEDDS were dispersed in FSSIF and FSSGF at 0.50mg/mL ETP in a physiologically suitable medium. ETP was dissolved in FSSIF and FSSGF at 2mg/mL in the same formulations with w/w 2.5% of ETP. Equation 3 was applied to assess extraction efficiency after all formulations were subjected to the same conditions and treatment. To adjust the concentrations according to the extraction efficiency found in the dispersion study, we utilized Equation 3. The developed SNEDDS's initial dispersion rate was determined using Equation 4, which considers concentration-time curve slope between 0 and 2 minutes (Reyna-Lázaro *et al*., 2024).

$$\text{Adjusted concentration} = \frac{\text{Obtained concentration}}{\text{Extraction efficiency}} \quad (3)$$

$$\text{Dispertion rate} = \frac{dc}{dt} \approx \frac{\text{Concentration at 2min - Concentration at 0min}}{\text{2min - 0min}} \quad (4)$$

AUC values were computed using Equation 5, a linear trapezoidal technique, where C is the concentration, t is the time and n is the time point number.

$$AUC_{0-t} = \Sigma_{i-1}^{n-1} \frac{c_{i+1} - c_i}{2}(t_{i+1} - t_i) \quad (5)$$

In vitro digestion study

Kok et al. had previously published these same methods in 2022(Kok *et al*., 2022), which were utilized in this

investigation of the digestive system. The digestion process began with the addition of 40mL of SNEDDS-containing dispersion media dried vial with 667 mg pancreatic lipase. The digesting study used a 100-rpm medium swirling at 37°C. Removed 800µL of medium at 5, 15, 30, 60, 90 and 120 minutes. Diluted in 1:1 dimethyl sulfoxide to methanol, they were filtered using a 0.45 µm PVDF syringe filter. At regular intervals, digestion vessels were replenished with a new digestion medium, specifically FSSIF with pancreatic lipase. To evaluate each sample, HPLC was utilized. To calculate the AUC values, equation 5 was used.

ETP SNEDDS antidepressant effect

Albino male mice that weigh 25 to 30 g were examined for antidepressant effects and behavior. Before conducting any studies, the animals were in a light-dark cycle with 12 hours between each cycle. They were also provided with complimentary access to tap water and regular meals for three days. International animal testing and biodiversity rules were followed for each experiment. The mice were divided into three groups. International animal experimentation and biodiversity rights standards were followed during the research (Saibabu and Malyadri, 2021). One mg/kg was given for each drug. The vector for control mice was a 0.5% aqueous carboxymethyl cellulose (CMC) solution. Oral drug delivery systems with SNEDDS-loaded test drugs like ETP were used to provide the medicine. As a reference, ETP in 0.5% CMC was utilized(Yankelevitch-Yahav *et al*., 2015, Saibabu and Malyadri, 2021).

Forced swimming test

The forced swimming test (FST) described by *Butterweck et al.,* was employed to assess the anti-depressant effect, with minor modifications (Butterweck *et al*., 2001). Oral administration of the reference and test formulations was performed. After the doses were given, the mice swam for an hour. Every animal was placed within a transparent glass beaker that was 20 cm in height. The beaker was filled halfway with tap water at a temperature of 23±2°C. Every mouse was used only once in an experiment and the water was replaced between each usage. To assess the total period of inactivity, the last 2-minutes of each six-minute study were chrono-metered. All experiments were videotaped. Except for the movements needed to maintain their forelimbs in water, animals were thought to be passive while they tried to run (Saibabu and Malyadri, 2021, García-Durán *et al*., 2022).

Tail suspension test (TST)

The reference and experimental formulations were administered orally. One hour following administration of the drug, each mouse was affixed to a position 50 cm above the floor and 1 cm away from its tail using tape. Each trial was recorded, and the final six minutes of every ten-minute session were analyzed for periods of inactivity. Mice were considered immobile when they remained still for one minute after the tape was applied (Saibabu and Malyadri, 2021, Nakagawasai *et al*., 2022).

Stability studies

Hermetically sealed bottles were used to store SEP5 for a period of ninety days to check the stability studies. Conditions for storage comprised 75±5% humidity and 45°C±2.0°C temperature. At 0, 30, 60 and 90 days, particle size, self-emulsification time, zeta potential, color were measured.

Cytotoxicity studies

SNEDDS was tested for cytotoxicity in Caco-2 cells using the MTT assay. The Caco-2 cell line was acquired from the ATCC. The Caco-2 cells were cultured in 96-well plates using Eagles Minimum Essential Medium (MEM) supplemented with fetal bovine serum (FBS) of 20% concentration. A DMEM solution containing 0.5% DMSO was used to dilute the medication, excipients, and SEP5 before delivery. Cells were treated with 200 µg/mL of SEP5 formulation, along with the same quantity of excipients utilized in the formulation, as well as blank preparations. Subsequently, they were incubated in DMEM medium devoid of fetal bovine serum (FBS). Samples were collected at a certain time after exposure, and PBS was employed to cleanse the specimen. The medium without FBS was subsequently exposed to an MTT solution and incubated for one hour. Afterward, the clear solution on top was removed and dissolved in DMSO. The absorbance of the produced solution was quantified at a recorded wavelength of 570 nm. The following formula is utilized to calculate the ratio of viable cells.

$$\text{Cellviability}\,(\%) = \frac{\text{Absorbance of treated sample dispersion}}{\text{Absorbance following treatment with DMEM}} \times 100 \quad (6)$$

RBC lysis test

ETP-loaded SNEDDS acute *in-vitro* toxicity was assessed by RBC lysis. A solution containing anticoagulant was introduced to a sample of healthy human blood. The mixture was then subjected to centrifugation for a duration of 15 minutes at a temperature of 4°C and a speed of 10,000 revolutions per minute. As a result, the plasma and buffy coat were separated and removed from the sample. The pellet containing red blood cells (RBCs) was washed and exposed to an isotonic solution to achieve a hematocrit of 50%. A minute quantity of the red blood cell mixture was administered with a concentration of 4 mg/mL for each component, for a duration of one hour at a temperature of 37°C, for research purposes. The samples were centrifuged at a speed of 15000 revolutions per minute for a one-hour duration. The liquid was then tested for haemoglobin concentration using a UV spectrophotometer at 576 nm (Zhang *et al*., 2015).

Quantification of ETP by HPLC

An HPLC technique was developed for quantifying ETP. Mobile phase selection, flow rate, detection wavelength,

and pH were optimized during the study. The isocratic mobile phase is formed using a 40:60 volumetric ratio of acetonitrile and methanol. The detection was performed at wavelength of 235nm and the chromatographic conditions was set at 25°C. ETP was isocratically eluted from a C18 column at 1.0 mL/min. The technique was deemed acceptable according to the requirements set by the International Council for Harmonization (ICH). The precision of the method was evaluated for both interday and intraday measurements, and correlation coefficients (R2) were generated to assess the linearity of the results for ETP. ETP recovery, limit of detection (LOD) limit of quantification (LOQ) estimation, and relative standard deviation (RSD) calculations were performed alongside all the HPLC operations to validate the parameters. The HPLC system includes LC-10AT VPand Shimadzu-LC-10AT pumps, a manually operated 20µL sample loop, and an SPD 10A VP UV-visible detector.

Pharmacokinetics of escitalopram

The University's animal house provided albino rats aged between 35 and 55 days. All animals were given a one-week interval to acclimate before starting the trial. The animals had unrestricted water and food during the research, showing they were well-nourished. ETP-SNEDDS (SEP5) and ETP suspended in 5% CMC were tested for pharmacokinetics. Five albino rats per group received the ETP solution or one of the two ETP-SNEDDS (SEP5) formulations orally.

Each rat received the corresponding formulations orally at 10 mg/kg ETP. Serial blood samples were obtained from the tail vein of rats at 0, 0.5, 1, 2, 4, 6, 8 and 12 hours after injection using heparin-treated tubes. A technique called extraction was used to separate ETP from plasma. To sum up, 200 µL of acetonitrile and 20 µL of plasma were mixed. After 30 seconds of forceful mixing, the materials were centrifuged at 10,000 rpm for 20 minutes. The supernatant that was left over from each sample was carefully put into a clean tube. To concentrate the samples, evaporate the solvent under nitrogen at 37°C and then add 100 µL of acetonitrile to make them whole again. The materials that had been reconstituted were then sonicated for 5 minutes and HPLC was used to examine them.

In vivo, plasma concentrations were corrected for extraction efficiency. The individual data was used for non-compartmental pharmacokinetic analysis. The regression slope of the log-linear terminal elimination phase was used to derive the terminal elimination rate constant (kel) and half-life ($t_{1/2}$). Each animal's C_{max} and T_{max} were measured. C_{max} was stated as the mean value, while T_{max} was recorded as the median value. The linear trapezoidal rule was used to find the AUC_{0-t}, which covered the range from 0 to the final observed plasma quantity. The values for $AUC_{0-\infty}$ (area under the curve from time zero to infinity) and MRT (mean residence time) were also determined. The pharmacokinetic parameters were analyzed using statistical methods in SPSS version 17 (SPSS Inc., Chicago, IL, USA). The test and control groups were compared using the student's t-test.

In silico study

The 5HT2A serotonin receptor is a viable target for treating migraine, depression, and anxiety (Al-Massarani *et al*., 2023, Panda *et al*.). Depression is linked to decreased post-junctional 5HT1A signaling caused by forebrain 5HT2A receptor activation (Zięba *et al*., 2021). The amino acids TRP336 PHE332 and ILE163 are necessary for biological function and activation of receptor (Rathore *et al*., 2022). The 5HT2A activates potassium channels to increase the action of other 5HT1A serotonin receptors which regulate neuronal firing, and alleviate depression (Lv and Liu, 2017). The 5HT2A receptor-blocking drug risperidone is used to treat unipolar depression (Mahmoud *et al*., 2007, Kemp *et al*., 2009). When combined with other antidepressants like SSRIs and MAOIs, the 5HT2A receptor antagonist has a synergistic impact on depression that is resistant to normal therapy (Marek *et al*., 2003, Lin *et al*., 2014).

This study examines ETP's antidepressant effects by antagonizing 5HT2A. The protein data bank (PDB ID 6A93) was searched for 5HT2A receptor complexed with risperidone for *in silico* molecular docking. Before further processing, the protein was run using Maestro 12.8 of the Schrodinger Suite's protein-preparation wizard function (http://www.schrodinger.com). Following the addition of polar hydrogen, the repair of chains that were missing with amino acids and the assignment of protein charges, the OPLS-2005 force field was employed to decrease the system's energy (Harder *et al*., 2016). Lastly, the working directory was updated with the improved 3D structure. Maestro was used to import the SDF file containing the three-dimensional structure of the ligand ETP that was obtained from PubChem. A stable ligand conformation was achieved by employing the Ligprep module with the OPLS-2005 force field. Around the active binding sites, a grid box was made. Glide suite was used to dock the ligand under study with the target protein to evaluate its theoretical binding affinity (Friesner *et al*., 2004, Friesner *et al*., 2006).

Ethical Approval

All animal protocols were approved by the Pharmacy Ethical Committee, Faculty of Pharmacy at Bahauddin Zakariya University in Multan, Punjab (Pakistan) with reference No.254/PEC/2023.

STATISTICAL ANALYSIS

One-way Analysis of Variances (ANOVA) was used for the evaluation of the in-vitro drug release data. The confidence interval was set to 95% for analysis.

RESULTS

ETP solubility in SNEDDS excipients was tested to develop a stable zone for self-emulsification. fig. 1(A-C) depicts the process of ETP dissolution in different excipients. A phase diagram is used to calculate the self-emulsification zone and adjust excipient quantities for the best formulation without ETP (fig. 1D). The oils utilized in this investigation were olive, geranium, cinnamon, castor, and ginger. These oils were chosen as oil phases because they dissolved the medication well. A multiple-phase diagram was created using PEG 400 and tween 80 to discover the best self-emulsification area. It took less than sixty seconds for the three enhanced formulations (SEP5-SEP7) to emulsify, which demonstrates their ability to rapidly and fully disperse in diluted aqueous solutions with a minimum of agitation. The droplet diameters of lead ETP-SNEDDS formulations (SEP5-SEP7) dispersed in FSSGF and FSSIF were assessed. The droplet sizes of SEP5, SEP6, and SEP7 formulations in FSSGF were 145±35, 161±10, and 148±18 nm, respectively. Droplet sizes for SEP5, SEP6, and SEP7 formulas in FSSIF were 147±41, 165±37, and 144±26 nm, respectively, as shown in table 2.

Using Fourier transform infrared spectroscopy (FTIR), both quantitative and qualitative studies of both organic and inorganic compounds were carried out as shown in fig. 2. Using infrared absorption spectra, this method identifies different types of chemical bonds in every molecule. It is a precise technique for determining the kinds of covalent bonds, functional groups, and chemical groups that are present in SNEDDS. In the pharmaceutical research, DSC, a thermal diagnostic technique, is often utilized to identify drug-excipient incompatibility related to the formulation (fig. 3). The thermal stability and degradation pattern of the excipients along with final formulations are assessed by TGA (fig. 4). Surface morphology was shown in fig. 5. The drug release investigation was conducted in a 900 mL solution at 37°C and a pH of 6.8. The discharge of ETP from the SNEDDS can be observed in the pattern depicted in fig. 6. The permeation studies assessed ETP release in vitro and predicted absorption in vivo. SEP5's ex vivo intestinal permeability was compared to ETP powder (control) and a non-everted sac commercial tablet. The 12-hour mean ETP plasma concentrations are shown in fig. 7.

FSSGF and FSSIF dispersion tests were performed on SEP5, SEP6 and SEP7 formulations. After being applied to either medium, all formulations were seen to disperse within 5 minutes. After FSSIF dispersion experiments, we tested each dispersed ETP-SNEDDS formulation for in vitro digestion by porcine pancreatin. Drug precipitation occurred in all formulations as shown in table 5. Table 6 presents a concise overview of the results obtained from the FST and TST. The mice immobility demonstrates depressed behavior similar to that of humans. The primary objectives of antidepressants are to ease symptoms and minimize the duration of onset before achieving efficacy. A favorable mouse reaction involves locomotor activity under stress with minimal inactivity and sensitivity to stimuli.

The developed SNEDDS was stable and our goal in doing these experiments was to determine how well the ETP, Tween 80, PEG 400, geranium oil, and the SEP5 formulation affect Caco-2 cell cultures. An in vitro experiment was conducted to assess the potential harm of the SNEDDS formulation on RBCs. The bioavailability investigation chose the SEP5 ETP-loaded formulation because of its small droplet size. The pharmacokinetic profiles shown in fig. 8 illustrate the effects of administering SEP5 ETP-loaded SNEDDS and orally suspending ETP in rats. The pharmacokinetic parameters have been calculated and are documented in table 7. Fig. 9 shows the binding interactions between the crystalline ligand and ETP within 2d and 3d dimensions of distinct amino acids.

DISCUSSION

Solubility study

The solubility of ETP in coconut oil was found to be limited, with a concentration of 3.58mg/mL. Geranium oil (15.67mg/mL) and olive oil (5.18mg/mL) were the two oils that exhibited the highest solubility of ETP. Since they dissolve ETP, cinnamon, geranium, olive, ginger and castor oils were used for supplementary study. Escitalopram dissolved best in 20.33mg/mL Tween 80 and 8.16mg/mL Tween 20 (Ames-Sibin *et al*., 2024). Given the proven ability of tween 80 to improve the absorption of medication, it was employed as a surfactant in this study. ETP has demonstrated effective solubilization properties for PEG 400 at a concentration of 16.4mg/mL and PEG 200 at a concentration of

12.6mg/mL. The stated excipients will be employed in future studies due to their statistical relevance.

Phase diagram using pseudo ternary method

Turbid emulsions were created using 40–50% surfactants. Increasing the co-surfactant concentration in SNEDDS increased its ability to spontaneously form an emulsion within self-emulsification. The minimal Smix ratio for self-emulsion was 45%–75%. The S_{mix} ratio above 60% increased the self-emulsifying ability of SNEDDS. A phase diagram demonstrated that the size of the droplets decreased from 280 to 80 nanometers when the concentration of the surfactant increased from 30 to 60 percent. Conversely, the larger average droplet size was caused by the increase in surfactant level to 70%. A total of nine different formulations were subjected to testing.

Table 1: SNEDDS formulations of ETP.

Formulation	SEP1	SEP2	SEP3	SEP4	SEP5	SEP6	SEP7	SEP8	SEP9
*ETP	10	10	10	10	10	10	10	10	10
*Oil	25	30	35	40	45	50	55	60	65
*Tween80	65	60	55	50	45	40	35	30	25
*PEG400	10	10	10	10	10	10	10	10	10

*ETP in mg, Oil, Tween80 and PEG400 in percentage (%)

Table 2: ETP-loaded SNEDDS surface charge, particle size in FSSGF, FSSIF and PDI.

Formulation	SEP1	SEP2	SEP3	SEP4	SEP5	SEP6	SEP7	SEP8	SEP9
*Particle Size FSSGF	167±23	176±29	183±38	169±36	169±36	145±15	161±10	148±18	166±28
*Particle size FSSIF	171±24	174±31	187±34	170±31	147±41	165±37	144±26	172±32	190±29
Zeta Potential	-15	-14	-15	-14	-26	-21	-18	-15	-16
PDI	0.234	0.401	0.367	0.398	0.120	0.289	0.235	0.278	0.309

*Particle sizes in nm.

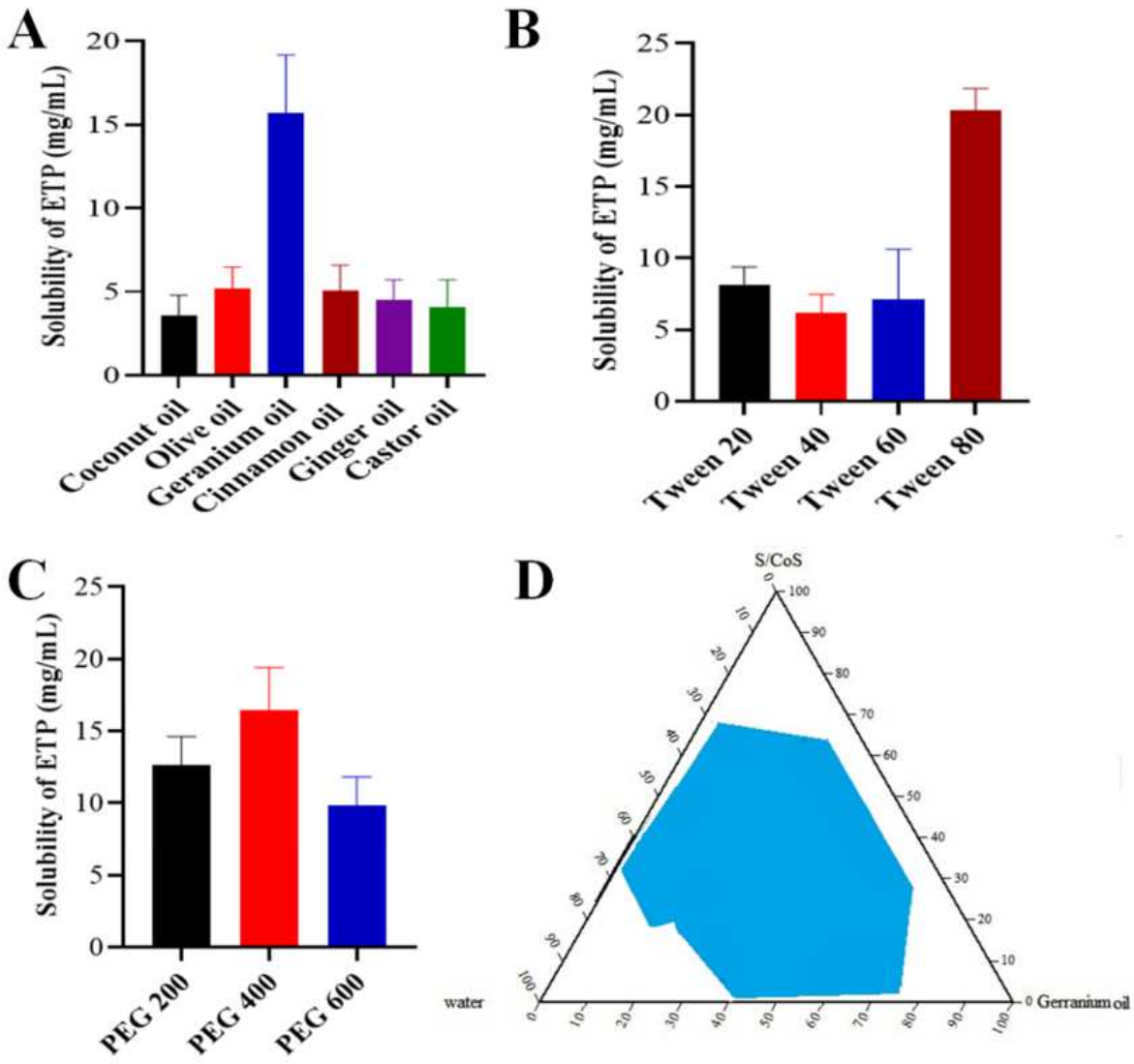

Fig. 1: The solubility of ETP in different oils (A), surfactants (B), co-surfactants (C), and a pseudo ternary phase diagram illustrating the usage of geranium oil in the formulation of SNEDDS (D).

Table 3: Kinetic values of ETP release from ETP-SNEDDS formulations.

Formulation	SEP1	SEP2	SEP3	SEP4	SEP5	SEP6	SEP7	SEP8	SEP9
Hixon-Crowell (R^2)	0.876	0.993	0.990	0.786	0.956	0.786	0.567	0.675	0.876
Higuchi(R^2)	0.921	0.932	0.959	0.966	0.976	0.924	0.913	0.924	0.979
Korsmeyer(R^2)	0.990	0.991	0.992	0.993	0.994	0.994	0.995	0.990	0.991
Peppas(n)	0.29	0.40	0.45	0.19	0.14	0.36	0.43	0.28	0.41
Zero Order(R^2)	0.991	0.992	0.999	0.996	0.999	0.994	0.993	0.991	0.996
First Order(R^2)	0.891	0.792	0.899	0.696	0.796	0.894	0.793	0.691	0.599

Table 4: Flux and permeability coefficient values for the SNEDDS and reference tablet containing ETP in goat gut membrane.

Parameters	Medium			
	FSSGF		FSSIF	
	Flux	Permeability coefficient	Flux	Permeability coefficient
Units	$\mu g\ cm^{-2}\ min^{-1}$	$x\ 10^{-2} cm\ min^{-1}$	$\mu g\ cm^{-2}\ min^{-1}$	$x\ 10^{-2} cm\ min^{-1}$
SNEDDS-SEP5	115±31	0.439±0.09	234±41	0.709±0.07
Tablet (reference)	12±0.76	0.108±0.003	4.56±0.23	0.035±0.003

Table 5: Assessment of *in vitro* dispersion and digestion characteristics of SNEDDS in FSSGF and FSSIF.

Formulations		units	SEP5	SEP6	SEP7
FSSGF	Initial dispersion rate	$mg.mL^{-1}min^{-1}$	1.20±0.07	1.24±0.05	1.26±0.06
	Dispersion AUC	$min.mg/mL$	279±10	259±11	273±12
FSSIF	Initial dispersion rate	$mg.mL^{-1}min^{-1}$	1.11±0.05	1.34±0.2	1.39±0.03
	Dispersion AUC	$min.mg/mL$	291±11	269±13	284±12
	Digestion AUC	$min.mg/mL$	190±5	189±7	183±6

Table 6: Results of ETP as antidepressant in albino mice.

Material	Forced swimming test			Tail suspension test		
	Control	Pure Drug (ETP)	ETP-loaded SNEDDS (SEP5)	Control	Pure Drug (ETP)	ETP-loaded SNEDDS (SEP5)
Dose (mg/kg)	1	1	1	1	1	1
Immobility duration (seconds)	185±3.18	163±4.69	131±4.34	135±4.04	112±3.55	89±2.43

Table 7: The average pharmacokinetic characteristics for ETP in albino rats afterward they were given a 10mg oral dosage of the test formulation (SEP5) and the reference formulation (ETP suspension).

Parameters	$t_{1/2}$ (*hours*)	MRT (*hours*)	$AUC_{0\text{-}12h}$ (*ng.h/mL*)	$AUC_{0\text{-}\infty}$ (*ng.h/mL*)	C_{max} (*ng/mL*)	T_{max} (*hours*)
ETP	6.3±1.6	6.4±1.2	645±278	1876±245	135±50	0.5±0.2
SEP5	12.5±2.1	23.3±2.5	1435±498	2850±654	867±191	6±1.3

Analysis of self-nanoemulsifying drug delivery systems (SNEDDS)

Duration of emulsification

There is a correlation between the time of emulsification and the ability of formulations to self-emulsify without the need of heat or mechanical energy. Water penetration into the droplet surface's complex colloidal structure connected with the emulsification rate. The observed result can be attributed to a decrease in the amount of oil and an increase in the amount of co-surfactant, resulting in a decrease in viscosity in the system containing Tween 80.

Analysis of droplet size

Formulations were developed with 10% w/w ETP, ranging from SEP5 to SEP7. The average size of the droplets in both FSSGF and FSSIF formulations of ETP-SNEDDS was the same. The zeta potential investigation used SNEDDS formulations SEP5, SEP6 and SEP7. Formulation stability is affected by the zeta potential, which stops particles from forming. The zeta potentials for SEP5 to SEP7 are -26, -21 and -18, respectively, as shown in table 2. The most stable chemical was SEP5. Whether a formulation is negatively charged depends on its free fatty acid content. A stable system was achieved with a Zeta potential of ±30 mV.

Fourier transforms infrared spectroscopy (FTIR)

The FTIR analysis of ETP revealed peaks at 3005, 1520, 1210, and 1023 cm^{-1}, which correspond to the stretching vibrations of –NH, -CH, -C-O, and –OH, respectively, as indicated in fig. 2 (Sonia *et al*., 2023). The geranium oil exhibited characteristic peaks at 2982, 1650 and 1192cm^{-1},

which confirmed the existence of several functional groups (Nur, 2021). The Tween 80 displayed distinct peaks at 2867, 1654, and 1234 cm^{-1}, which can be ascribed to the vibrations of -CH, stretching of -OH, and other existing functional groups. The SEP5 exhibited coinciding peaks with tween 80, geranium oil, and ETP, suggesting a harmonious interaction between ETP and the co-surfactants and surfactants.

DSC (Differential scanning calorimetry)

A 143°C endothermic peak indicated the drug's melting point. fig. 3 also shows a 152°C exothermic peak (Hoffmann *et al*., 2023). The temperature at which geranium oil exhibited endothermic and exothermic reactions were recorded at 99°C and 289°C respectively (Mahmood *et al*., 2023b).The exothermic peak seen at 289°C in geranium oil was a result of the evaporation of the essential oil. A peak indicating endothermic behavior was detected in Tween 80 at a temperature of 79 °C. Exothermic and endothermic peaks in the improved SEP5 formulation indicated drug stability. The presence of an amorphous molecule was indicated by the disappearance of ETP peaks in SEP5-ETP formulation. It was clear from peak shifting and disappearance of peaks in the SEP5 thermogram that the medication had been correctly placed into the SNEDDS. No water is lost up to this high temperature since the formulation is thermally stable.

Thermogravimetric Analysis (TGA)

Two times, ETP degraded, as indicated by TGA measurements. The initial substantial decrease in mass, observed from the melting point of ETP up to 201°C, is attributed to the decomposition of the oxalate. The subsequent major decrease in mass, observed up to 310°C (Hoffmann *et al*., 2023), is caused by the deterioration of ETP, as depicted in fig. 4. The medication TGA thermogram exhibited a loss of just 11% at a temperature of 201°C and a loss of 50% at a temperature of 310°C. At a temperature of 293°C, the TGA analysis of geranium oil indicated a little 12% decrease in weight. However, at a higher temperature of 347°C, a significant loss of 80% was seen (Mahmood *et al*., 2023b). The thermal gravimetric analysis (TGA) of Tween 80 revealed a weight loss of just 11% at a temperature of 253°C, whereas a significant weight loss of 80% was seen at a higher temperature of 352°C. The TGA analysis of the formulation revealed a weight loss of 13% at 250°C and a weight loss of 80% at 327°C. Nevertheless, its stability increased after loading into the SNEDDS. After 249°C, there was a sudden and obvious decrease in weight. There was a 78% reduction in weight at 327°C. After that, the formulation is stable up to 500°C.

Scanning electron microscopy (SEM)

Significant intermolecular interactions were indicated by the particles' non-uniform dispersion and aggregation, which is typical of stabilized SNEDDS formulations. The SEM picture in fig. 5A indicates that SEP5 was roughly 156 nm in size.

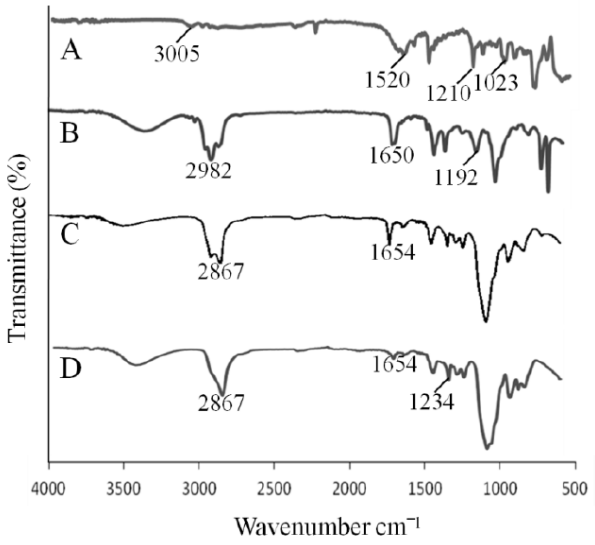

Fig. 2: The FTIR spectra of ETP (A), geranium oil (B), tween 80 (C) and SEP5 ETP loaded SNEDDS formulation (D).

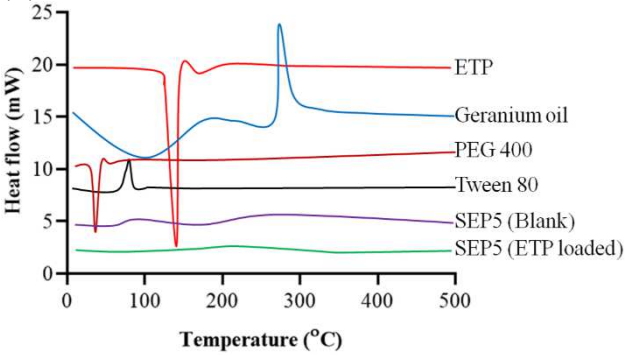

Fig. 3: DSC curves for ETP, tween 80, PEG 400, geranium oil, SEP5 blank and SEP5-ETP loaded SNEDDS formulation.

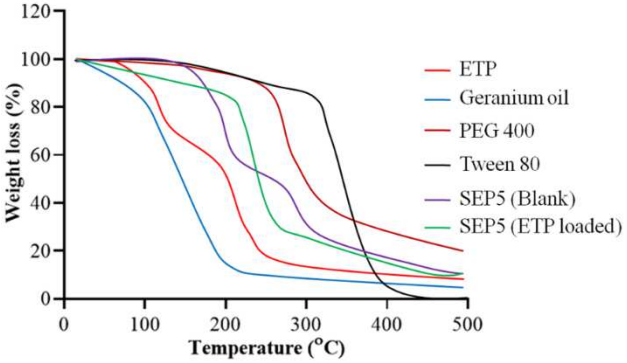

Fig. 4: TGA curves of SEP5 blank, SEP5 ETP loaded SNEDDS formulation, ETP, geranium oil, Tween 80 and PEG 400.

ETP Release and Kinetics

After 12 hours, the SEP5 formulation delivered the highest possible amount of the drug (96%). System oil and Smix

mixture can allow this. Due to greater oil content, bigger droplets and a smaller medium-exposed surface area, SEP1 gave up 66% less medicine. Stats demonstrate that SNEDDS formulations release ETP faster than the present pharmaceutical recipe. The ETP was discharged from SEP5 at a rate of 96% after a duration of 12 hours, which was notably greater than the 86% observed in the commercial formulation (Chaudhuri *et al*., 2022). In vitro results showed enhanced ETP release from SNEDDS. Table 3 shows ETP release kinetics. The ETP-SNEDDS formulation has zero-order kinetics due to a non-Fickian diffusion-based mechanism (Parveen *et al*., 2023).

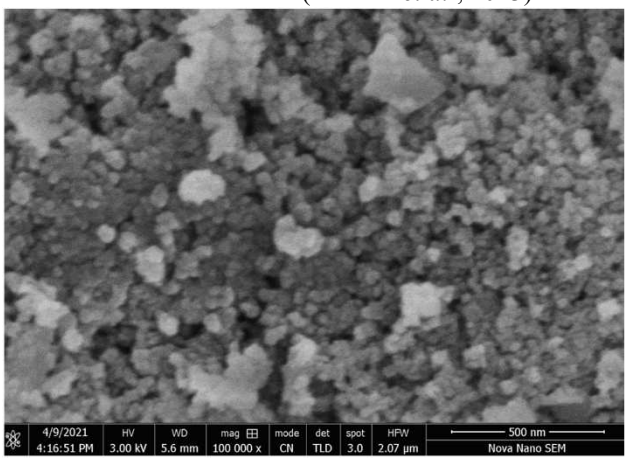

Fig. 5: SEM image of SNEDDS of SEP5 formulation.

Permeability investigations

The improved SEP5 formulation demonstrated superior intestinal permeability compared to both ETP powder and traditional tablets. At the 12-hour, the optimized SEP5 SNEDDS demonstrated an intestinal permeability of 56.56%, while the marketed tablet had a permeability of 19.87% and the ETP powder had a permeability of 15.56%, as shown in fig. 6. SNEDDS may improve intestinal ETP absorption due to several factors. The fast intestinal sac permeability and diffusion of ETP allow for significant penetration. Nano-sized emulsion droplets in the gut increase ETP absorption. Nanoemulsions increase medication penetration into the gut by increasing surface area. SEG3's excellent drug solubility and quick self-emulsification may have increased intestinal ETP absorption. By breaking cell membrane lipids, Tween 80 and PEG 400 improve permeability (Chaudhuri *et al*., 2022).

Table 4 presents the flux and permeability coefficients for both SNEDDS and reference tablets. The study revealed that ETP exhibited greater permeability and dissolution in FSSIF as compared to the reference. The study revealed a statistically significant difference in permeability between SNEDDS and tablets, as determined by FSSIF. The SNEDDS formulation incorporates Tween 80 as a surfactant, hence enhancing the drug's intestinal permeability. The drug permeated better through SNEDDS than conventional and ETP powders. Due to oil droplet absorption, SNEDDS increases ETP permeability. These methods include passive diffusion, pinocytosis, endocytosis, and nano-sized droplets that increase interfacial surface area. BOS solubility increases, increasing its permeability (Ansari *et al*., 2021).

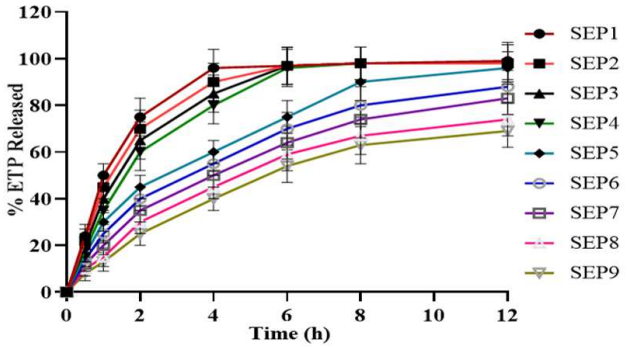

Fig. 6: ETP release profile from ETP-SNEDDS in FSSIF medium (n=6).

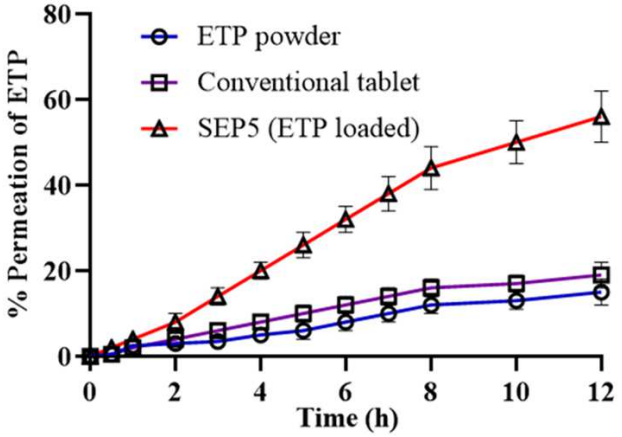

Fig. 7: Permeation profile of ETP from powder, reference tablet, and experimental SEP5 ETP-loaded formulation in FSSIF medium (n=6).

Study of dispersion in vitro

Table 5 shows that the formulations SEP5, SEP6, and SEP7 exhibited initial dispersion rates of 1.20±0.07, 1.24±0.05, and 1.26±0.06 $mg.mL^{-1}$ min^{-1}in FSSGF. Consequently, there was no noticeable variation in the rates at which the three lead formulations dispersed in FSSGF. The initial dispersion rates of the SEP5, SEP6, and SEP7 formulations in FSSIF were 1.11±0.05, 1.34±0.2, and 1.39±0.03 $mg.mL^{-1}$ min^{-1}. When tested in FSSIF, the SEP5 formulation had a much slower dispersion rate than the SEP6 and SEP7 formulations. To find differences in ETP solubilization, the ETP-SNEDDS were compared by AUC, but no significant difference was identified. The presence of drug precipitation was not detected in any of the dispersion investigations.

In vitro digestion analysis

Table 5 shows that SNEDDS preserved 80% of the original ETP solubilized concentration during digesting testing.

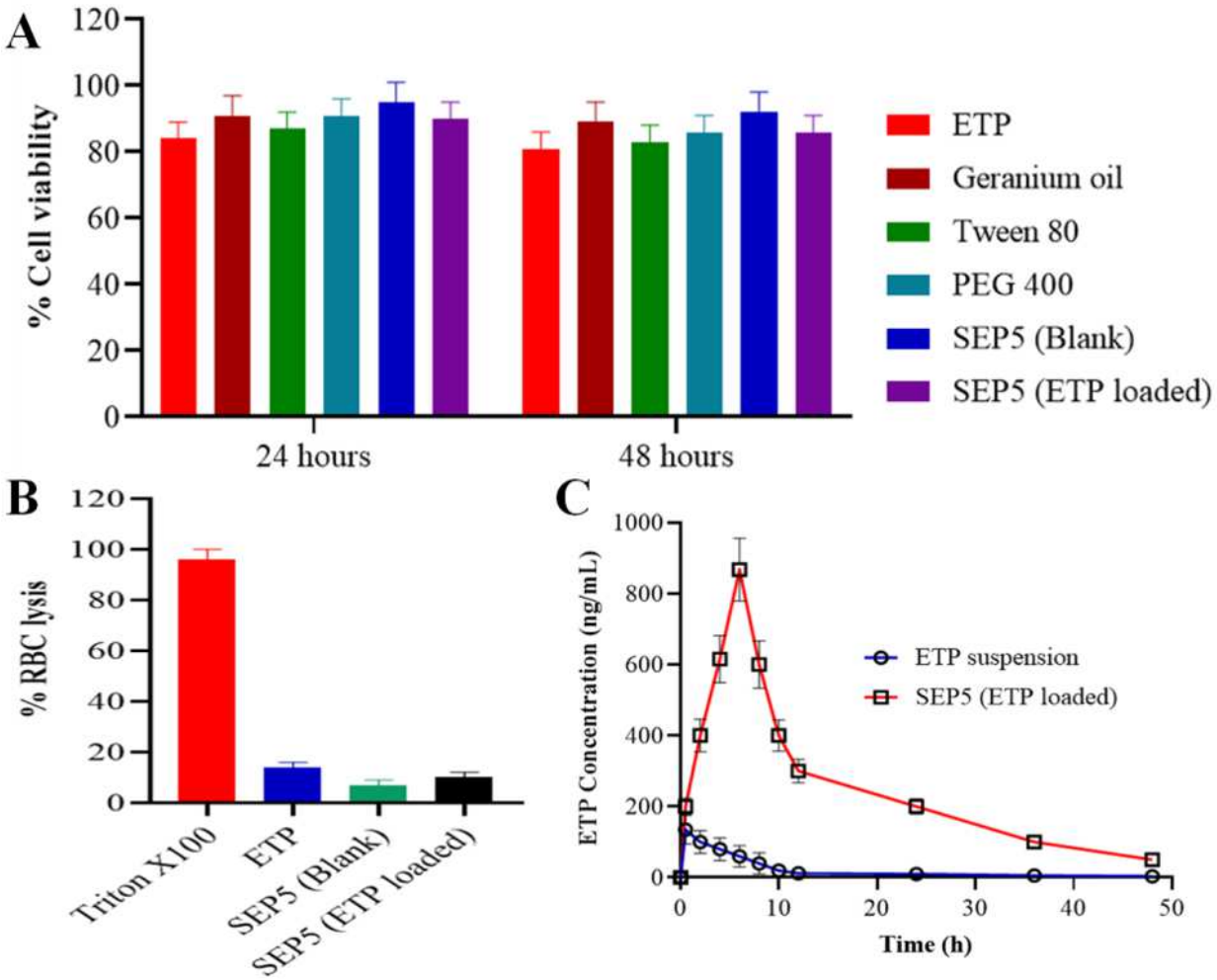

Fig. 8: Cell viability of ETP-loaded SNEDDS (SEP5) for48 hours (A). Percentage of lysis of red blood cells exposed to SEP5 blank, ETP-loaded SNEDDS and triton X100(B). Relationship between time and ETP concentration from ETP suspension and SNEDDS (C).

SEP5, SEP6 and SEP7 formulations have similar ETP solubilization levels, according to this test. The ETP formulation slurry was dispersed and digested in a lab to test its solubility. The formulations could not disperse in the biorelevant medium, hence ETP did not solubilize in the media (Karavasili *et al*., 2020).

The antidepressant effects of self-nanoemulsifying drug delivery systems (SNEDDS)

The ETP-loaded SNEDDS group had much less mouse immobility than the control and pure drug groups. Shorter durations of inactivity in mice show they respond to stressful situations faster than people, explaining why the medicine works faster than pure drug. The pure drug suspension and ETP-loaded SNEDDS were taken orally. The rapid uptake of ETP from the SNEDDS resulted a quick efficiency (Mahdi *et al*., 2022).

Study on stability

While there was no change in color, the time needed for self-emulsification significantly changed ($p<0.05$). SEP5 particle size was 148 nm at 1 month, 151 at 2 months, and 155 at 3 months. After 30, 60, and 90 days, the zeta potential was -26, -25 and -24 mV (Lim *et al*., 2023).

Study on cytotoxicity

There was no effect on cell survival at the doses examined when SEP5 was present in the culture media, according to the data. Fig. 8A shows the cell viability plot, which clearly shows that most of the cells were still alive after incubating with the SEP5 formulation for 48 hours. Even when examined under a microscope, the cell lines maintained their original appearance.

RBC lysis test

All red blood cells were lysed by Triton X-100 as a positive control. Fig. 8B shows that SNEDDS containing ETP produced less red blood cell (RBC) lysis than empty SNEDDS and pure ETP powder.

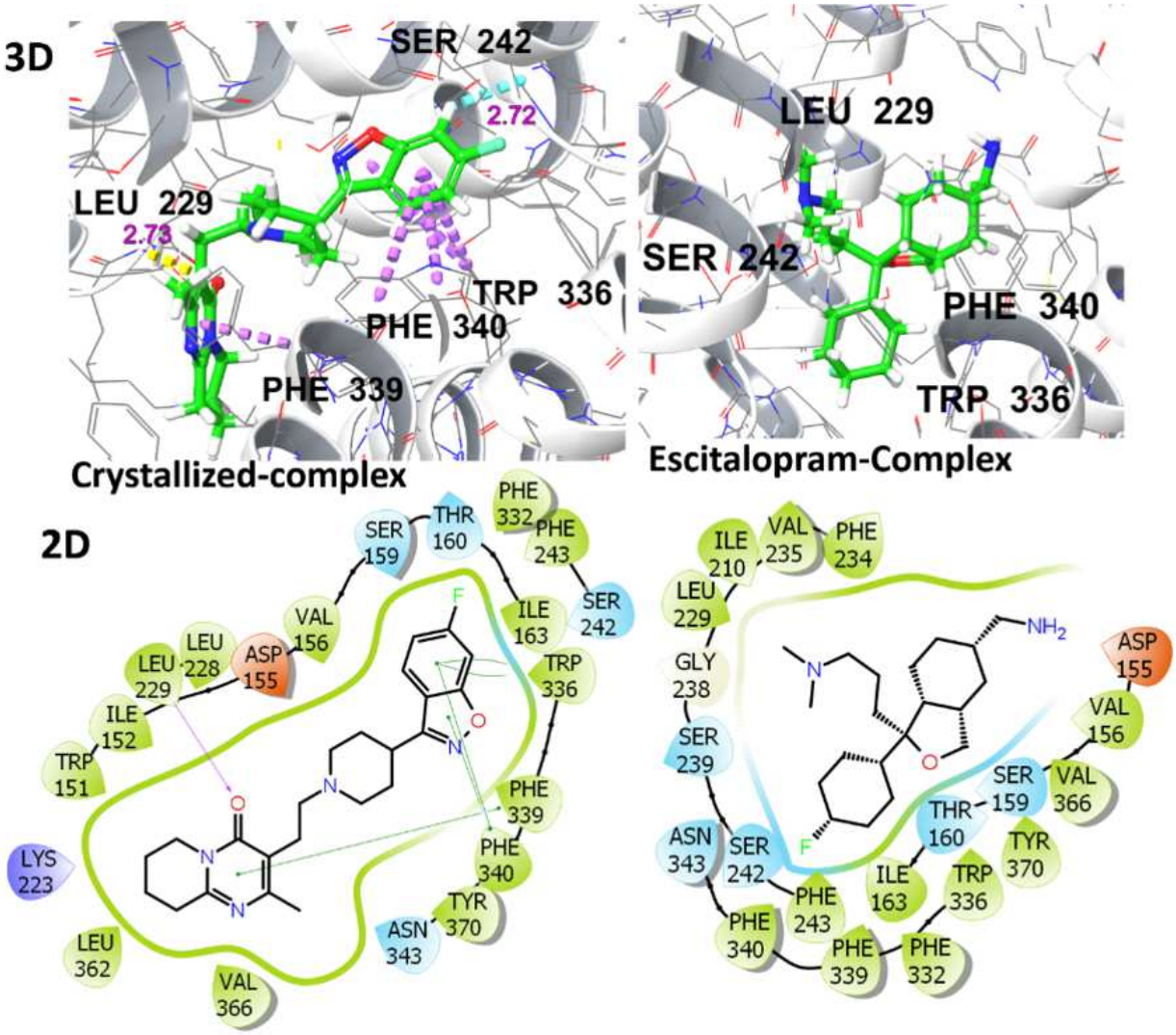

Fig. 9: Analysis of the binding interactions between a co-crystal ligand and ETP with specific amino acid residues in both 2D and 3D dimensions.

Development and validation of an HPLC technique for quantifying ETP

The detection time for ETP was determined to be 4.53 minutes and the linearity graph shows a regression value of 0.9992, indicating a strong correlation. LOD and LOQ for ETP were found to be 3.91µg/mL and 6.87µg/mL, respectively, according to studies. ETP recovery of 97.91% proved the method's accuracy. ETP intra-day and inter-day measurements were below 2% accurate.

Pharmacokinetic Analysis

The C_{max} values did not significantly differ across the groups. Nevertheless, as fig. 8C illustrates, the group receiving ETP suspension had the lowest C_{max} (135±50 ng/mL), while the group receiving ETP in the SEP5 formulation had the greatest C_{max} (867±191 ng/mL). Compared to SEP5, the T_{max} value for the administration of ETP suspension (0.5 hours) was lower. In contrast, the group that got the SEP5 formulation took a longer period (6 hours) to achieve the maximum concentration (C_{max})(Rao, 2007). Plasma concentrations were tested 12 or 24 hours after treatment. The ETP half-life (t1/2) values for SEP5 and ETP suspension groups were 12.5±2.1 hours and 6.3±1.6 hours, respectively. SEP5 ETP AUC results for the treatment group were compared to ETP suspension formulation AUC values. The SEP5 formulation has an AUC value of 1435±498 ng.h/mL from 0-12 hours. The AUC value for endogenous thrombin potential (ETP) was 645±278 ng.h/mL after injection of the ETP suspension formulation. The AUC0-12h value of the treatment group was significantly higher ($p<0.05$) than that of ETP suspension administration. After delivering the SEP5 formulation, the ETP total results increased 2.3-fold compared to the suspension. Animals with estimated elimination rate constants (kel) had their AUC values extrapolated to infinity. The AUC0-∞ values for SEP5 were 2850±654 and 1876±245 ng.h/mL after ETP suspension injection. Significant differences in AUC0-∞ values were

seen between SEP5 and ETP suspension (Søgaard *et al*., 2005).

Insights into molecular docking

The co-crystallized ligand H-bonded with LEU229 and π-π interacted with important amino acid TRP336, subsequent in a significant binding affinity of -7.4 Kcal/mol. Escitalopram binds strongly to LEU229, SER242, TRP346, and PHE340 amino acid residues due to their hydrophobic interactions, with-5.86 Kcal/mol G-score. The amino acid residues that are polar like SER159, THR160, ASP155, SER239, SER242, and ASN343 encircled ETP's amino and fluoro (F) groups, making a stable protein-ligand combination in the binding pocket.

CONCLUSION

To summarize, our findings suggest that complex formulations can enhance the absorption of lipophilic drugs such as ETP when taken orally. In comparison to ETP suspension, administering ETP-SNEDDS resulted in faster ETP absorption. ETP-SNEDDS preparations enhanced ETP bioavailability from 0 to 12 hours post-delivery compared to ETP solution, but they also improved ETP availability above the reference preparation. Further research is needed to understand the various absorption routes for encapsulated drugs administered in SNEDDS formulations, as the absorption process remains poorly understood. This requires more investigation, and improving SNEDDS dose may increase ETP and other hydrophobic drug dispersion. ETP and 5HT2A are linked in the computational analysis, suggesting it may cure depression. A 3D representation of drug-receptor interaction can help build more polar Escitalopram analogues that can hydrogen bind with critical amino acid residues like TRP336. To bridge this information gap and effectively imitate the absorption process in living organisms, innovative in vitro experiments must be carefully selected, particularly when examining lipid-based drug delivery methods. Considering all aspects, these limitations underscore the difficulties and complexities of establishing effective oral delivery systems for lipophilic drugs.

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