Original
ISSN 2413-0516

Utility of Piperine and Liquisolid Tablets to Enhance the Pharmacological Profile of Tacrolimus

Ahmed S. Ali[1*], I.H. Oumar[1], O.A.A. Ahmed[2], K.M. El-Say[2], M. Alsieni[1], H.M. Alkreathy[1]

[1]Department of Clinical Pharmacology, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia.
[2]Department of Pharmaceutics, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia.
*Correspondence to: Ahmed S. Ali (E-mail: asali@kau.edu.sa; profahmedali@gmail.com)
(Submitted: 14 October 2024 – Revised version received: 29 October 2024 – Accepted: 17 November 2024 – Published online: 26 December 2024)

Abstract

Objective: To evaluate the impact of a self-nanoemulsifying drug delivery system (SNEDDS) incorporated into liquisolid tablets (LSTs) and the natural bioenhancer piperine (Pip) on the bioavailability, immunosuppressive efficacy, and toxicity of tacrolimus (Tac).

Methods: An optimized Tac-SNEDDS was developed and formulated into four LST compositions using various carriers and coatings (Neusilin®-Avicel®/FujiSil®-fumed silica). The optimal Tac-LST formulation was selected based on drug content and tested in Wistar rats. Bioavailability was assessed using the limited area under the curve (AUC_{0-6}), while immunosuppressive efficacy was evaluated through IL-2 levels. Renal histology and serum cystatin C levels were analyzed to assess potential nephrotoxicity.

Results: Among the four Tac-LST formulations, LST-4 (Avicel®/FujiSil®) exhibited the highest drug content and was chosen for in vivo studies. Co-administration of Pip, LSTs, and their combination significantly improved Tac bioavailability without compromising immunosuppressive activity. Pip co-administration demonstrated superior IL-2 inhibition compared to Tac alone or Tac-LSTs. Additionally, Pip reduced Tac-induced increases in serum cystatin C levels and mitigated renal histological damage, indicating a nephroprotective effect. The combination of Pip and Tac-LSTs provided the most stable absorption profile with minimal AUC fluctuations, suggesting a more predictable pharmacokinetic profile.

Conclusion: Pip and LST formulations significantly enhance Tac bioavailability while maintaining its immunosuppressive efficacy. Moreover, Pip exhibits a protective effect against Tac-induced nephrotoxicity. The combination of Pip and Tac-LSTs offers a stable pharmacokinetic profile, potentially improving therapeutic outcomes by maintaining consistent drug levels.

Keywords: Tacrolimus, bioenhancer, calcineurin inhibitors, immunosuppressant, self-nano emulsifying drug delivery system, liquisolid tablets

Introduction

Tacrolimus (Tac) is a calcineurin inhibitor widely used to manage organ transplant patients and several autoimmune disorders. It has a narrow therapeutic range, low bioavailability, and a long list of adverse drug reactions.[1] Various bio-enhancers, including curcumin, gingerols, and quercetin, improved the bioavailability of specific immunosuppressants.[2] However, due to its good safety and favorable therapeutic activity, piperine (Pip) is one of the most comprehensively studied bio-enhancers.[3] Several approaches were utilized to overcome the poor bioavailability of Tac, e.g., self-micro emulsifying drug delivery system (SMEDDS),[4] pro-liposomes,[5] and nanosomes.[6]

Self-nano emulsifying drug delivery systems (SNEDDS) is a lipid-based formulation that spontaneously develops oil in water (o/w) emulsion at micro or nanoscale in aqueous media upon gentle agitation. It offers a high solubilization capacity, blocks P-GP drug efflux, and facilitates GIT absorption. Therefore, these formulations have been developed to improve the bioavailability of many drugs or phytochemicals.[7,8] Moreover, they were developed into solid dosage forms with improved stability and manufacturability.[9]

Several solidification methods change the liquid SNEDDS into a solid state, including spray drying, adsorption into a solid carrier, nano-particle technology, and melt extrusion. Adsorption into the solid carrier is one of the most economical and simplest techniques for developing stable, free-flowing solid SEDDS powder. It can then be formulated as tablets, pellets, granules, solid dispersions, nanoparticles, or microspheres.[10] Thus, liquisolid (LS) formulation is one of the newly established and promising techniques to improve the dissolution, bioavailability, and sustain the release of drugs. However, its possible pharmaceutical applications are still being expanded.

A compelling strategy to boost medication bioavailability and potentially mitigate side effects is the combined use of a natural bioenhancer and SNEDDS in a single formulation.[11] Thus, the present study aimed to assess the impact of Pip and liquisolid tablets (LSTs) formulation co-administration on Tac bioavailability, immunosuppressant activity, and nephrotoxicity. Developing immunosuppressant LSTs and integrating them with a natural bioenhancer in one dosage form is considered an innovative approach.

Methodology

Tacrolimus (Tac) was from Beijing Mesochem Technology Co., Ltd. (Beijing, China). Piperine (Pip) was procured from Fluorochrome Ltd. (Sheffield, UK), while Lauroglycol FCC and polyoxyethylene glycol 35 castor oil (Cremophor® EL) were from Gattefosse (Saint-Priest, France). Microcrystalline cellulose (Avicel® PH 101), fumed silica (0.007 µm), magnesium stearate, methanol, Polyethylene Glycol (PEG) 200, Plasdone®, Polyvinylpyrrolidone (PVP), sodium carboxymethylcellulose (NaCMC), and tocopherol polyethylene glycol succinate (TPGS) were from (Sigma-Aldrich -St. Louis, USA), Magnesium trisilicate was from JRS PHARMA GmbH & Co. KG (Rosenberg, Germany) and Premium LV (Methocel® LV) from Dow Chemical Company (Midland, MI, USA).

Magnesium aluminometasilicate (Neusilin® US2) and silicon dioxide (FujiSil®) were from Fuji Chemical Industries Co., Ltd. (Burlington, NJ, USA), while Talc powder was from Whittaker Clark & Daniels-South (Plainfield, NJ, USA). Formic acid, acetonitrile of high-performance liquid chromatography (HPLC) grade, and all other analytical grade chemicals were procured from Merck Inc. (Darmstadt, Germany).

Rat Tacrolimus - FK506 Enzyme-Linked Immunosorbent Assay Kit (Cat No. MBS3808320), Rat Interleukin-2 ELISA kit (Cat No. MBS2505890), Rat Cys C ELISA kit (Cat No. MBS042119) were from MyBioSource Inc. (San Diego, USA). The study was conducted in two phases: Formulation and Pharmacological studies, as shown in Figure 1.

Formulation Study

HPLC Analysis for Tac in the Formulation

Tac solution was prepared with methanol to achieve a concentration range of 5 µg/ml to 50 µg/ml. Standard Tac solutions were measured at a λ_{max} 215 nm using methanol as a blank. The linear regression equation from the calibration curve was utilized to determine sample concentrations. HPLC Agilent 1200 system was used with a DAD detector controlled by Mass Hunter software. The separation was performed on an HPLC column (Agilent Eclipse XDB-C18, 150 mm x 4.6 mm, 5 µm, Agilent Technologies, Palo Alto, USA). The mobile phase comprised 0.1% formic acid and acetonitrile (60:40, v/v), and the flow rate was 0.8 ml/min. The total run time was 7.5 min, and the injection volume was 5 µl. Linearity of the assay method was verified within the concentration range of 5–50 µg/ml with a regression coefficient (R^2) = 0.9992 for Tac.

Formulation of Tac-Loaded SNEDDS

Extreme vertices mixture design of the special cubic model was utilized to study and statistically optimize the effects of three SNEDDS components in thirteen runs (10 mg of Tac in 1 g of SNEDDS formula) in a randomized order. The three components system was designed using the fraction of oil phase, Louroglycol FCC (X_1), the fraction of surfactants' mixture, chromophore EL/TPGS (X_2), and the fraction of co-surfactant; PEG 200 (X_3). Each component was used in several ratios with a total concentration of 1 to develop Tac-SNEDDS. The required amount of Tac powder was dissolved into the oil, surfactant, and co-surfactant mixture in a glass vial, vortex, then heated at 40–50°C in a water bath until a transparent/homogenous mixture was obtained. Tac-SNEDDS formulations were stored at room temperature until used.

Characterization and Optimization of Tac-SNEDDS Formulations

Tac-SNEDDS formulations were evaluated visually for the spontaneous emulsification tendency and the appearance of the final emulsion. Dynamic light scattering (DLS) (NanoZS90, Malvern Instrument Worcestershire, UK) determined globule size and Zeta potential. Samples were diluted 10-fold with double distilled water. Measurements were performed using a standard laser 4 m WHeANe, 633 nm, at room temperature 25°C, and fixed angle (90°).

The impact of formulation variables (X_1, X_2, and X_3) on the globule size (Y_1) and Zeta potential (Y_2) was statistically correlated with a regression equation using Statgraphics® Centurion XV version 15.2.05 software (StatPoint Technologies Inc, Warrenton, VA, USA). The optimized formulation, which possesses the smallest globules and the highest Zeta potential, was utilized to prepare Tac-LSTs. A series of SNEDDS were prepared with varying amounts of Tac (20, 30, 60, 80, and 100 mg) using the optimized composition, and then globule size and zeta potential were determined.

Preparation and Evaluation of Tac-LSTs

Tac-LSTs were prepared with two carriers and two coating materials to achieve the most suitable additives with the highest drug content and acceptable tablet characteristics. The optimized Tac-SNEDDS was developed into LSTs after the incorporation of several excipients. First, the calculated weights (W) of SNEDDS (containing 100 mg of Tac/1 g of SNEDDS were incorporated into the calculated quantities of the carrier material (Neusilin®) (Q1) and mixed manually. Then, the resulting wet mixture was combined with the specified amount of coating (FujiSil®) (q_1) or (Fumed silica) (q_2), using a standard mixing process to form a simple admixture. Binder (Methocel®), disintegrant/thickener (plasdone®, polyvinylpyrrolidone (PVP) or Mg-trisilicate, and finally lubricants/anti-adherents (Talc powder and Mg stearate) were added. The same procedures were repeated to prepare liquisolid powdered systems using Avicel® as the carrier material. These formulations were subjected to pre- and post-compression analysis. The flow properties and compressibility of the liquisolid powdered systems were determined by calculating the angle of repose, Carr's index (CI), and Hausner's ratio (HR). Weight variation, thickness, hardness, friability, and disintegration Tests of Tac-LSTs were evaluated based on United States Pharmacopeia USP 29 guidelines. The content of Tac in three randomly selected tablets of each patch was analyzed using HPLC-DAD (as described before).

Pharmacological Study

Preparation of Oral Suspensions

Oral suspensions of Pip, Tac raw, or its formulations were prepared daily using 0.5% w/v sodium carboxymethylcellulose (NaCMC) solution as the vehicle. Tac-LSTs were powdered and sieved through a 200 µm sieve. Tac dose was (1 mg/kg/day).[12] Tac-LST powder was suspended in 15 ml of the vehicle (0.25 mg Tac/ml). Tac raw dispersion (5 mg Pip/ml vehicle) was prepared, and the dose of Pip was 20 mg/kg/day.[13]

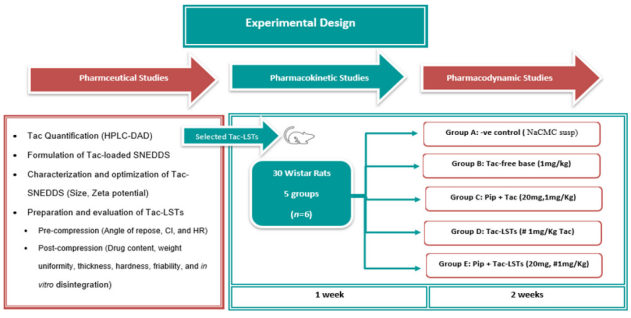

Fig. 1 **Study design to assess the impact of Pip on the bioavailability of Tac.**

Experimental Animals

Thirty healthy male Wistar rats (160 g ± 30 g) were used. The study followed the recommendations of the Research Ethics Committee of the College of Pharmacy (King Abdulaziz University, Reference No: PH-1443-03). Rats were allowed to be fed on a standard pellet diet, drinking water ad libitum, and fasting for 2 h before administering suspensions by gavages. The animals were randomly allocated into five groups ($n = 6$). Administration was repeated for 14 days for efficacy and nephrotoxicity studies. The following protocol was adopted: a) Negative Control: vehicle (NaCMC aqueous solution), b) Positive control: Tac raw (1 mg/Kg), c) Pip & Tac raw: Pip (20 mg/Kg/day) 1 h before Tac raw (1 mg/Kg), d) Tac-LSTs: Tac-LSTs suspension equivalent to (1 mg Tac/Kg), and Pip &Tac-LSTs: Pip (20 mg/Kg/day) suspension 1 h before Tac- LSTs suspension (1 mg/Kg).

Plasma Samples and Analysis of Tac

For the relative bioavailability study of Tac samples were collected after repeated administration over 6 days, assuming steady state had been reached. Approximately 500 µl of blood was drawn from the retro-orbital plexus of anesthetized animals at the following post-oral administration time points: 0.5, 1, 2, 4, and 6 hours. Blood was collected into EDTA tubes and stored at 4°C for analysis within 7 days. Tac levels in the blood samples were determined using a sandwich enzyme-linked immunosorbent assay (ELISA) technique, specifically with the Rat Tac - ELISA Kit from MyBioSource (Cat No. MBS3808320). The assay sensitivity limit was 0.1 ng/m.

Bioavailability of Tac

Limited Area Under the Tac-blood concentration *vs.* time curve (AUC_{0-6}) was estimated for each group as a relative parameter for bioavailability. This was attained by using the linear trapezoidal method from time zero to the time for the final concentration obtained (6 h). The AUC was determined using Pksolver[14] and graphed using GraphPad Prism program version 9.2.0 (Graph Pad® Inc., USA).

Efficacy and Nephrotoxicity

This *in vivo* study has two domains: efficacy (measuring IL-2 level) and nephrotoxicity (Cyratin C and histology). These studies were conducted after 14 days of repeated oral administration. Interleukin-2 ELISA kit was used to determine IL-2 concentrations in the blood samples. A Rat Cys C ELISA kit was used to determine serum Cys-C levels. Both were based on the sandwich-ELISA technique. On day 15, rats were anesthetized using IV ketamine (70 mg/kg), sacrificed by decapitation, and dissected in aseptic conditions. The kidney tissues were removed and preserved in 10% buffer formalin. Samples of kidney tissues were processed for paraffin embedding. Several sections were cut (5 µm) thickness, stained with Hematoxylin and eosin (H&E), and examined using a light microscope. The morphometric study was performed on samples from three animals per group using an image analyzer (Image J analyzer version 1.43, National Institutes of Health, USA). The renal corpuscles' diameters (µm) were measured (magnification ×40).

Statistical Analysis

Statistical Package for the Social Science Software (version 26; SPSS Inc., Chicago, Illinois, USA) was used. values were represented as means ± standard deviation (mean ± SD). A comparison between the means of the variables was performed using a one-way analysis of variance (ANOVA). Post hoc analysis was the Mann-Whitney test or the least significant difference as appropriate. *P*-values < 0.05 were considered significant. GraphPad Prism program version 9.3.0 (Graph Pad® Inc., USA) was used to create graphs.

RESULTS

Formulation Study

Table 1 shows the composition, globule size, and zeta potential of thirteen Tac-SNEDDS formulations (F-1 to F-13). Table 2 presents the composition of optimized formulations. These formulations were successfully prepared using Lauriglycol FCC (the oily phase), Cremophor EL/TPGS (the surfactants' mixture), and PEG 200 (the co-surfactant). These SNEDDS formulations likely provide optimal solubility.[15] In addition, TPGS and Cremophor EL act as inhibitors of CYP 450 and P-gp.[16] These effects could enhance drug bioavailability and reduce the intra-inter individual variability of Tac.

The zeta potential values showed good colloidal dispersion stability, ranging from –11.2 to –20.1 mV. Since a rise in surface charge inhibits droplet aggregation, its high value for smaller droplets will confirm the formulation's electrical stability. Since repulsion predominates over attraction, the system will spread and deflocculate, preventing it from breaking.

Table 1. **Composition, globule size (Y_1), and Zeta potential (Y_2) of Tac-SNEDDS formulations as suggested by the mixture design**

Run	Oil (X_1)	Surfactant (X_2)	Cosurfactant (X_3)	Y_1 (nm)	Y_2 (mV)
1	0.100	0.5000	0.4000	123.7	–18.6
2	0.100	0.3000	0.6000	148.5	–14.7
3	0.200	0.4000	0.4000	265.5	–20.1
4	0.200	0.3000	0.5000	233.3	–17.4
5	0.125	0.4375	0.4375	149.5	–13
6	0.125	0.3375	0.5375	163.1	–18.6
7	0.175	0.3875	0.4375	139.5	–17.6
8	0.175	0.3375	0.4875	126.9	–18.3
9	0.100	0.4000	0.5000	114.1	–11.2
10	0.150	0.4500	0.4000	132.8	–15
11	0.150	0.3000	0.5500	128.9	–17.7
12	0.200	0.3500	0.4500	240.7	–16.7
13	0.150	0.3750	0.4750	115.8	–17.4

Table 2. **Composition of the optimized Tac-SNEDDS formulation**

Component	High	Low	Optimum	Response
Oil (X_1)	0.2	0.1	0.1	**Y_1 = 127.899 nm**
Surfactant (X_2)	0.5	0.3	0.36	
Cosurfactant (X_3)	0.6	0.4	0.54	**Y_2 = –24.0 mV**

Globule size and Zeta potential were assessed as indicators for stability, and both were utilized for fitting the special cubic model (Figure 2A and 2B). The globule sizes were 114.1 nm to 265.5 nm in F-9 and F-3, respectively. There was a good relationship between the components and the response (Y_1) (P = 0.003). F-9, which had the lowest oil concentration, showed the smallest particle size. There is a significant relationship between the components and Zeta potential (Y_2) (P = 0.0092). Zeta potential values were 11.4 mV and 24.7 mV in F-4 and F-2, respectively.

Optimization of Tac-SNEDDS

The mixture experimental design was utilized to optimize the prepared Tac-SNEDDS. Figure 2C shows the combined factors' effect that maximizes the desirability function over the indicated region. As shown in Figure 2C, increasing X_3 levels (co-surfactant) and decreasing X_1 and X_2 (oil and surfactant mix, respectively) maximized the formulation's desirability.

Preparation and Evaluation of Tac-LSTs

The composition of Tac-LSTs formulations is presented in Table 3. Table 4 shows the pre- and post-compression characteristics of LST formulations. Pre-compression (angle of repose, CI, and HR) and post-compression (thickness, hardness, friability, and disintegration time) evaluation of the four LST formulations indicated the physical acceptability of the formulations and ability to resist the mechanical stress conditions during the handling. The flow properties of the four formulations were all acceptable. For example, the angle of repose varied from 41.3° to 37.5°.

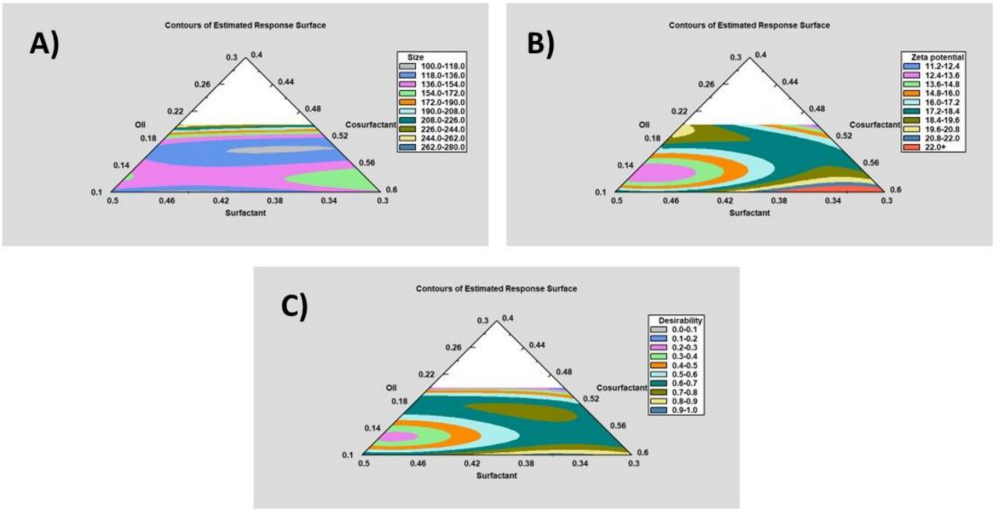

Fig. 2 **Two-dimensional contour plots for the effect of independent variables on the globule size (A), Zeta potential (B), and the desirability functions (C) of both responses.**

Table 3. **Formulations composition of Tac-LSTs**

Run	Tac-SNEDDS	LF	Carrier material	Coating material	Disintegrant	Methocel	Plasdone (6 %)	Talc powder (0.5 %)	Mg Stearate (0.5 %)
LST-1	50	0.5	100 Neusilin®	5 silica	50 (PVP)	30	14.1	1.175	1.175
LST-2	50	0.5	100 Neusilin®	5 FujiSil®	50 (PVP)	30	14.1	1.75	1.175
LST-3	50	0.25	200 Avicel®	20 silica	20 (Mg trisilicate)	40	19.8	1.65	1.65
LST-4	50	0.25	200 Avicel®	20 FujiSil®	20 (Mg trisilicate)	40	19.8	1.65	1.65

Table 4. **Pre- and post-compression evaluation of Tac-LSTs**

	Pre-compression				Post-compression					
Run	Carr's Index	HR	Ang. of repose	Type of flow	Weight (mg)	Drug (%)	Thickness (mm)	Hardness (N)	Friability (%)	Disintegration time (min)
LST-1	23.07	1.29	41.34	Passable	251.45	19.8	4.16	0.487	0.14	5.57
LST-2	18.17	1.22	40.17	Fair	251.45	40.4	4.18	0.488	0.12	7
LST-3	12.5	1.1	39.8	Good-Fair	353.10	36	4.83	0.487	0.39	2.11
LST-4	20	1.25	37.5	Fair	353.10	45.6	5.1	0.488	1.03	3.3

The four formulations were successfully compressed. However, Neusilin®, in general, provides higher liquid loading capacity than Avicel®, so LST-1 and LST-2 have lower tablet weight and thickness. Drug content was higher in LSTs containing FujiSil® (LST-2 and LST-4). LST-3 and LST-4 showed lower disintegration time as Avicel® has higher aqueous diffusion and can act as a disintegrant, while the others containing Neusilin® were harder due to the silicate (they also have higher R values (carrier/coating ratio).[17,18]

Pharmacological Study

The data from Table 5 demonstrates a significant enhancement in Tac bioavailability when administered with Pip, Tac-LSTs, or a combination of both. Notably, Pip alone increased Tac bioavailability by approximately 300%, while Tac-LSTs alone enhanced it by about 250%. When Pip and Tac-LSTs were administered together, the bioavailability was also close to 300%, indicating that Pip plays a crucial role in enhancing Tac absorption. Interestingly, the combination of Pip and Tac-LSTs resulted in the least fluctuation in the AUC (Area Under the Curve), suggesting more stable absorption and a more predictable pharmacokinetic profile compared to the individual treatments. This stability may offer a therapeutic advantage by ensuring more consistent drug levels.

IL-2 levels were measured as an indicator of Tac-immunosuppressive activity.[19] As shown in Table 5 IL-2 levels were significantly decreased in Pip-treated groups (Pip + Tac and Pip-LSTs groups) versus the positive control group (Tac-raw) ($P < 0.05$). A slight reduction in mean IL-2 level was observed in the case of Tac-LSTs ($P = 0.31$). The effect of Tac administration (14 days) on the Cys C is indicated in Table 5 Tac-induced renal injury as evidenced by the high level of serum Cys C in the control group ($P = 0.001$). Cys C levels were significantly ($P = 0.001$) decreased in Pip-treated groups (either Pip + Tac or Pip-LSTs) versus positive control (Tac-raw).

The histology of a Section of the renal cortex is shown in Figure 3. Major changes in the architecture were seen in sections of tac-raw as well as Tac-LSTs e.g decreased glomerular capillary tuft and a widening of Bowman's space. Extravasation of RBCs peritubular infiltration of inflammatory cells, and a thick-walled dilated congested BV with endothelial cell

Table 5. **Tac Area Under the Curve (AUC_{0-6}) following repeated oral dosing and Mean IL-2 Blood Levels, Cys C, Renal corpuscle diameter in the study groups**

Study groups	AUC_{0-6} (ng.h/ml)	IL-2 (pg/ml)	Cys C (mg/dl)	Renal corpuscle diameter (µm)
Control group	--	--	--	263.6 ± 19.96
Tac-raw	5.662 ± 0.7	8.4 ± 0.79	5.23 ± 0.31	178.8 ± 11.81
Pip + Tac	18.12* ± 4.6	3.9* ± 0.80	1.12* ± 0.15	263.5 ± 4.608@
Tac-LSTs	13.80* ± 4.3	7.2# ± 1.04	4.42# ± 0.66	165.6 ± 8.017
Pip + Tac-LSTs	16.78* ± 2.4	5.33* ± 0.32	1.46*,# ± 0.37	259.4 ± 5.857@

Data were expressed as mean ± SD. *Significant vs. Tac-free group; #: significance vs. Pip + Tac group; @: significance vs. control group. Tac dose (1 mg P.O/kg/day), Pip dose (20 mg/kg) ($n = 6$). * $P < 0.05$ compared with oral Tac-raw. Values are expressed as the means ± S.D. Tac = Tacrolimus, LSTs = Liquisolid tablets, Pip = Piperine.

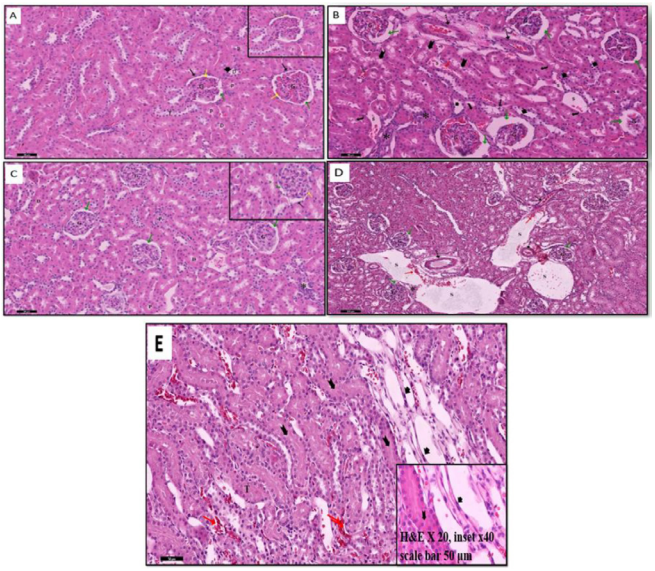

Fig. 3 **Sections of the renal cortex of the study groups using H&E stain (X20, Image inserts X40). (A) Control; normal renal glomeruli (G) and tubules (B) Tac-raw; decreased glomerular capillary tuft and a widening of Bowman's space. Extravasation of RBCs (peritubular infiltration of inflammatory cells (*), and a thick-walled dilated congested BV with endothelial cell proliferation in the wide interstitial spaces (s). (C) Pip + Tac; glomerulus is nearly as a control group with few inflammatory cell infiltrations. (D) Tac-LSTs; severe distorted architecture with marked glomerular sclerosis and obliteration of the Bowman's space. Extravasation of the RBCs and inflammatory cell infiltration. Marked widening of the interstitial spaces between tubules, and a thick-walled dilated congested BV. (E) Pip + Tac-LSTs; the glomerulus is near as a control group with some proliferation with narrow Bowman's space.**

proliferation in the wide interstitial spaces. Most of these histological changes were attenuated in sections of Pip + tac or Pip + Tac-LSTs.

Morphometric analysis is presented in Table 5 (renal mean corpuscle diameter). Reduction in mean corpuscle was considered as a marker for renal toxicity induced by Tac. Either Tac-raw or Tac-LSTs lead to significantly lower renal mean corpuscle diameter ($P < 0.05$). In contrast, either Pip + tac or Pip + tac-LSts showed mean values comparable to the control. These results confirmed the ability of Pip to attenuate Tac-induced nephrotoxicity.

Discussion

The optimal SNEDDS composition, through the achievement of small particle size, provides an adequate decrease in the system's free energy and stabilizes the nanoemulsion.[20] Moreover, the large surface area could allow more rapid absorption and decrease drug exposure to the metabolizing enzymes.[11] In general, the average globule size is preferred to be lower than 200 nm to achieve the optimal SNEDDS characteristics.[20]

The present study successfully utilized the optimized formula to develop four Tac-LSTs. The preparation involved using different carriers and coating materials. To attain a liquisolid system with acceptable flowable and compressible properties, the appropriate quantities of carrier and coating material were determined based on the R-value. PVP was a disintegrant in LSTs-1 and LSTs-2, but Mg-trisilicate was used in the other formulations. MethoCel® was added as a binder, plasdone®, PVP, or Mg-trisilicate as a super disintegrant or thickener, talc powder, and Mg stearate lubricants or anti-adherents. The level of each component was determined based on the preliminary study results of the holding capacity of excipients (Lf), the flow properties, and the compressibility behavior of the liquisolid powder blends. These formulations ensure the achievement of a free-flowing powder suitable for compression into tablets with good quality attributes. As the R-value is associated with the flowability, compressibility, and disintegration of the liquisolid system, an optimum value of R is recommended to be 20.[18] The formulations containing Neusilin® provided tablets with optimal characteristics. LST-4 was selected for pharmacological studies as it showed the highest drug content and proper disintegration time.

The limited AUC_{0-6} provides a reliable measure of the bioavailability of tacrolimus, as it captures the early phase of drug absorption and reflects the extent of drug exposure during the initial critical period, offering valuable insights into the formulation's performance.[21] PK evaluation involved observation of the impact of utilizing LSTs and Pip alone or in combination on Tac limited AUC_{0-6}. Regarding Tac-LSTs, the results suggested that optimized Tac-LTS-4 significantly increased Tac's oral bioavailability. This is likely due to the improved Tac dissolution rate (Class II compound).[22] Moreover, chromophore EL, TPGS, and PEG 200 have been reported as P-gp inhibitors, which contribute to Tac's lower bioavailability.[16] Similarly, Tac-limited AUC_{0-6} was significantly increased after administration of either Pip + Tac or Pip + Tac-LSTs. The enhancement of Tac bioavailability was greater than that after tac-LSTs alone. These observations document the favorable effect of Pip on Tac bioavailability. Similar effects of Pip were reported with other drugs, such as gemifloxacin[23] and acyclovir (Dudhatra, Mody,.[24] *In vitro* and *in vivo* studies indicated that Pip mediates CYP450 and P-gp inhibition, which explains the increase in Tac bioavailability.[24]

In the present study, IL-2 was utilized as a biomarker for the Tac immunosuppressant effect.[25] The results indicated Pip improves Tac's bioavailability without compromising its immunosuppressive activity. Several studies suggested serum cystatin C levels as a sensitive parameter of renal function.[26] The present study confirms the reported nephroprotective effect of Pip against drug-induced nephrotoxicity.[27,28] Tac-induced nephrotoxicity is mediated by the activation of the major vasoconstrictor systems, including the renin-angiotensin system, endothelin system, and NADPH oxidase, which induce oxidative stress and sympathetic nerve activity.[29] It reduces prostacyclin/NO synthesis and NO-mediated vasodilation.[30] Pip has been studied for its anti-oxidant and nephroprotective activity.[29] Pip possesses anti-oxidant and anti-inflammatory effects that could overcome the major causes of Tac-induced nephrotoxicity.[31]

The absence of a nephroprotective effect observed after oral administration of Tac-LSTs could be explained by the fact that the LST formulation mediates an increase in Tac-bioavailability without providing adequate antioxidant action. Histological findings were consistent with the results of cystatin C level, which confirm the superiority of Pip in terms of attenuation of drug-induced nephrotoxicity.

Conclusion

The study demonstrates that both piperine (Pip) and (LSTs), individually or combined, significantly improved the bioavailability of (Tac) without compromising its immunosuppressive efficacy. Pip + Tac showed superior inhibition of IL-2 compared to comparable dose of Tac and Tac-LSTs. Additionally, Pip mitigated Tac-induced increases in serum cystatin C (Cys C) and reduced renal histological damage, suggesting a nephroprotective effect. Notably, the combination of Pip and Tac-LSTs yielded the most stable absorption, with minimal fluctuations in the Area Under the Curve (AUC), indicating a more predictable pharmacokinetic profile and potentially offering therapeutic advantages by maintaining consistent drug levels.

Conflict of Interest

All authors declare that they have no conflict of interest.

Authors' Contributions

A.S.A and H.M.A designed the study and supervised the project. I.H.O performed the analysis and wrote the draft manuscript. O.A. and, K M E supervised the pharmaceutical analysis. M.I contributed to discussion, revised and edit all versions. All authors approved the final manuscript.

Ethics Approval

The study was approved by the Research Ethics Committee of the College of Pharmacy (King Abdulaziz University, Reference No "PH-1443-03").

Informed Consent

Not applicable.

Availability of Data and Materials

The combined datasets and materials were available upon reasonable request.

Funding

This project was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, under grant no. G-73-140-1441. The authors, therefore, acknowledge with thanks DSR for technical and financial support.

Acknowledgment

The authors acknowledge Deanship of Scientific Research (DSR) - King Abdulaziz University, Jeddah, for supporting this project grant no. G-73-140-1441.

We would also like to acknowledge Prof. Soad S. Ali, Professor of Histology at Assiut University, Egypt, for her valuable guidance and comments on the histology studies.

References

1. Brunet, M. and M. Pastor-Anglada (2022). "Insights into the Pharmacogenetics of Tacrolimus Pharmacokinetics and Pharmacodynamics." Pharmaceutics 14(9): 1755.
2. Peterson, B., M. Weyers, J. H. Steenekamp, J. D. Steyn, C. Gouws and J. H. Hamman (2019). "Drug bioavailability enhancing agents of natural origin (bioenhancers) that modulate drug membrane permeation and pre-systemic metabolism." Pharmaceutics 11(1): 33.
3. Haq, I. U., M. Imran, M. Nadeem, T. Tufail, T. A. Gondal and M. S. Mubarak (2021). "Piperine: A review of its biological effects." Phytotherapy Research 35(2): 680–700.
4. Wang, Y., J. Sun, T. Zhang, H. Liu, F. He and Z. He (2011). "Enhanced oral bioavailability of tacrolimus in rats by self-microemulsifying drug delivery systems." Drug Development and Industrial Pharmacy 37(10): 1225–1230.
5. Nekkanti, V., J. Rueda, Z. Wang and G.V. Betageri (2016). "Design, characterization, and in vivo pharmacokinetics of tacrolimus proliposomes." AAPS PharmSciTech 17(5): 1019–1029.
6. Bobbala, S.K.R. and P.R. Veerareddy (2012). "Formulation, evaluation, and pharmacokinetics of isradipine proliposomes for oral delivery." Journal of Liposome Research 22(4): 285–294.
7. Rehman, F.U., K.U. Shah, S.U. Shah, I.U. Khan, G.M. Khan and A. Khan (2017). "From nanoemulsions to self-nanoemulsions, with recent advances in self-nanoemulsifying drug delivery systems (SNEDDS)." Expert Opin Drug Deliv 14(11): 1325–1340.
8. Mohanty, S., S. Sahoo, S. Patra and B.M. Sahoo (2022). "Self-micro emulsifying drug delivery systems: State-of-art a technology to enhance the solubility of poorly water-soluble drug." Journal of Medical Pharmaceutical and Allied Sciences, 11(6): 5368–5374.
9. Seo, E.B., L.H. du Plessis and J.M. Viljoen (2022). "Solidification of Self-Emulsifying Drug Delivery Systems as a Novel Approach to the Management of Uncomplicated Malaria." Pharmaceuticals 15(2): 120.
10. Patel, J., Němcová, L., Maguire, P., Graham, W. G., & Mariotti, D. (2013). Synthesis of surfactant-free electrostatically stabilized gold nanoparticles by plasma-induced liquid chemistry. Nanotechnology, 24(24), 245604.
11. Thakur, P.S., N. Singh, A.T. Sangamwar and A.K. Bansal (2017). "Investigation of Need of Natural Bioenhancer for a Metabolism Susceptible Drug—Raloxifene, in a Designed Self-Emulsifying Drug Delivery System." AAPS PharmSciTech 18(7): 2529–2540.
12. Diehl, R., F. Ferrara, C. Müller, A.Y. Dreyer, D.D. McLeod, S. Fricke and J. Boltze (2017). "Immunosuppression for in vivo research: State-of-the-art protocols and experimental approaches." Cellular & Molecular Immunology 14(2): 146–179.
13. Kumar-Sarangi, M., Chandra-Joshi, B., & Ritchie, B. (2018). Natural bioenhancers in drug delivery: An overview. Puerto Rico health sciences journal, 37(1), 12–18.
14. Zhang, Y., M. Huo, J. Zhou and S. Xie (2010). "PKSolver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel." Computer Methods and Programs in Biomedicine 99(3): 306–314.
15. Dheer, D., P.N. Gupta and R. Shankar (2018). "Tacrolimus: An updated review on delivering strategies for multifarious diseases." European Journal of Pharmaceutical Sciences 114: 217–227.
16. Alqahtani, M.S., M. Kazi, M.A. Alsenaidy and M.Z. Ahmad (2021). "Advances in oral drug delivery." Frontiers in Pharmacology 12: 618411.
17. Garud, A.A. and R.R. Shah (2017). "Formulation and optimization of liquisolid tablets of olmesartan medoxomil using 3 (2) factorial design." International Journal of Pharmaceutical Sciences and Research 8(11): 4682–4693.
18. Lu, M., H. Xing, J. Jiang, X. Chen, T. Yang, D. Wang and P. Ding (2017). "Liquisolid technique and its applications in pharmaceutics." Asian Journal of Pharmaceutical Sciences 12(2): 115–123.
19. Meçule, A., F. Tinti, A. Bachetoni, L. Poli, M. D'Alessandro, C. Alessandri, I. Umbro, I. Nofroni, P. Berloco and A. Mitterhofer (2011). Interleukin-2 profiles shortly after tacrolimus conversion from a twice-daily to once-daily regimen. Transplantation proceedings, Elsevier 43(4):1017–9.
20. Aboul-Einien, M. (2012). "Design and in-Vitro Evaluation of Olanzapine-Loaded Self Nanoemulsifying Drug Delivery System." Int. J. Ind. Pharm. Life Sci 2: 12–32.
21. Almeida-Paulo, G.N., R. Lubomirov, N.L. Alonso-Sanchez, L. Espinosa-Román, C. Fernández Camblor, C. Díaz, G. Muñoz Bartola and A.J. Carcas-Sansuán (2014). "Limited sampling strategies for tacrolimus exposure (AUC 0-24) prediction after Prograf® and Advagraf® administration in children and adolescents with liver or kidney transplants." Transplant International 27(9): 939–948.
22. El-Say, K.M., O.A. Ahmed, B.M. Aljaeid and A.S. Zidan (2017). "Matrix-type transdermal films to enhance simvastatin ex vivo skin permeability." Pharmaceutical Development and Technology 22(4): 492–499.
23. Gorgani, L., Mohammadi, M., Najafpour, G. D., & Nikzad, M. (2017). Piperine—the bioactive compound of black pepper: from isolation to medicinal formulations. Comprehensive reviews in food science and food safety, 16(1), 124–140.
24. Dudhatra, G.B., S.K. Mody, M.M. Awale, H.B. Patel, C.M. Modi, A. Kumar, D.R. Kamani and B.N. Chauhan (2012). "A comprehensive review on pharmacotherapeutics of herbal bioenhancers." The Scientific World Journal 2012: 637953.
25. Lim, T.Y. and M. Heneghan (2016). "Biomarkers of immunosuppression." Clinical Liver Disease 8(2): 34.
26. Pan, Y., B. Hu, M. Li, L. Fan, Y. Ni, J. Zhou and X. Shi (2014). "A meta-analysis on diagnostic value of serum cystatin C and creatinine for the evaluation of glomerular filtration function in renal transplant patients." African Health Sciences 14(4): 1025–1035.
27. Kakalij, R.M., B.D. Kumar and P.V. Diwan (2016). "Comparative evaluation of nephroprotective potential of resveratrol and piperine on nephrotic BALB/c mice." Indian Journal of Pharmacology 48(4): 382.
28. Sudjarwo, S.A., K. Eraiko and G.W. Sudjarwo (2017). "Protective effects of piperine on lead acetate induced-nephrotoxicity in rats." Iranian Journal of Basic Medical Sciences 20(11): 1227.
29. Luo, K., S.W. Lim, J. Jin, L. Jin, H.W. Gil, D.S. Im, H.S. Hwang and C.W. Yang (2019). "Cilastatin protects against tacrolimus-induced nephrotoxicity via anti-oxidative and anti-apoptotic properties." BMC Nephrology 20(1): 1–11.
30. Hošková, L., I. Málek, L. Kopkan and J. Kautzner (2017). "Pathophysiological mechanisms of calcineurin inhibitor-induced nephrotoxicity and arterial hypertension." Physiological Research 66(2): 167.
31. Lee, M.-K. (2020). "Liposomes for enhanced bioavailability of water-insoluble drugs: In vivo evidence and recent approaches." Pharmaceutics 12(3): 264.