ABSTRACT

Objective: The objective of this study was to develop, characterize, and conduct pharmacokinetic and pharmacodynamic studies on ursolic acid solid self-microemulsifying drug delivery system (UA-S-SMEDDS) for the treatment of diabetic complications.

Methods: Liquid self microemulsifying drug delivery system (L-SMEDDS) were made with Capryol 90 as an oil, Cremophor EL as a surfactant, and polyethylene glycol (PEG) 400 as a co-surfactant. The surfactant and co-surfactant (Smix) ratios were calculated using a pseudo ternary phase diagram. At different pH levels and with water, the globule size, polydispersity index (PDI), zeta potential (ZP), and dilution were all assessed. S-SMEDDS has developed adsorption to a solid carrier by utilizing L-SMEDDS formulation. The powder properties, liquid retention potential, globule size, PDI, ZP, assay, and pharmacokinetic studies were all evaluated. The pharmacodynamic investigations of the S-SMEDDS formulation in streptozotocin (STZ) induced Wistar rats were evaluated using malondialdehyde (MDA) and glutathione (GSH) determination in tissues and section studies.

Results: S-SMEDDS formulation was successfully developed with a droplet size of 163.4±1.475 nm, PDI of 0.251±0.042, a ZP of -21.3±1.02, an assay of 96.21±0.75%. The release studies showed 26.28% (0.1N HCl) and 83.57% (6.8 phosphate buffer) were released in 15 min. When comparing the pharmacokinetics of a UA-loaded SMEDDS to the coarse solid, the S-SMEDDS (F2A) showed a 4.12 fold improvement in UA oral bioavailability. The pharmacodynamic results showed that S-SMEDDS was a higher recovery rate.

Conclusion: The developed solid SMEDDS (F2A) formulation proved effective in treating diabetic complications in STZ induced Wistar rats by inhibiting the aldose reductase enzyme.

Keywords: Bioavailability, Diabetic complications, Pharmacokinetic, Pharmacodynamic, SMEDDS, and Ursolic acid
each vehicle [13]. The components with the highest UA solubility were chosen for further investigation.

### Construction of pseudo-ternary phase diagrams

Based on solubility tests, oils (Capryol 90, Capmul MCM), surfactant (Cremophor EL), and co-surfactant (PEG 400) were chosen for the study. The water titration approach was used to construct pseudo ternary phase diagrams to determine the microemulsion area. In this experiment, surfactant and co-surfactant (Smix) were combined in 1:1 and 2:1 ratios. With a total weight of 1g, oil and a specific Smix mixture were prepared in 1:9 to 9:1 ratios. The mixtures were vortexed and titrated with water while gently agitated, and any turbidity was observed. Tripod software (version 4.1.2) was used to produce the pseudo ternary phase diagrams [14].

### Preparation of L-SMEDDS and S-SMEDDS

SMEDDS was prepared in a vial by dissolving UA (10 mg), Capryol 90 (oil), surfactant (Cremophor EL), and co-surfactant (PEG 400). After that, the mixture was cycled mixed for 20 min to produce a translucent uniform mixture. The formulations oil, surfactant, and co-surfactant ratios were altered [15]. The various compositions are listed in table 2. The adsorbed (aerosil 200, flogel pH 101, and mannitol) were combined with L-SMEDDS and triturated using a mortar and pestle until a free-flowing powder portion was formed. The S-SMEDDS that were produced were kept in desiccators until they could be tested further.

### Characterization of L-SMEDDS and S-SMEDDS

#### Measurement of size, PDI and ZP

Photon correlation spectroscopy (Nano ZS90, Malvern, UK) was used to assess the particle size, ZP, and PDI of developed SMEDDS. The measurements were made at a 90° angle at a temperature of 25 °C. About 100 µl of L-SMEDDS were collected and diluted with milli-Q water (5 ml, 1:50) for size, ZP, and ZP analysis [16]. In the case of S-SMEDDS, 10 ml of the sample was diluted with double distilled water, and then approximately 100 µl of the sample was taken and diluted (5 ml, 1:50), and the size, PDI, and ZP were determined.

#### Liquid retention potential (Φ) and flow properties of powder blend

The maximum quantity of L-SMEDDS formulation that may be adsorbed onto a solid carrier without affecting flow quality is described by the liquid retention potential of the carrier at a 33° angle of a slide. The angle of repose, Carr’s compressibility index, and Hausner’s ratio [17] were used to evaluate the flow parameters.

### Drug-excipient compatibility studies by DSC

Differential scanning calorimetry (DSC) was used to determine the drug-excipient compatibility and crystalline behavior of the drug and excipients. Perkin Elmer (DSC 4000, USA) obtained DSC thermograms of pure drug, and optimized S-SMEDDS. The instrument was calibrated with indium. Dry nitrogen was utilized as the effluent gas to heat all of the samples (10 mg) in aluminum pans. The thermograms were taken at temperatures ranging from 20 to 200°C, with a heating rate of 10°C/min [18].

### Field emission scanning electron microscopy (FESEM)

FESEM (JEOL, Japan, and 0.1kV to 30kV) was used to evaluate the morphology of pure drug, aerosil 200, and the formulation of UA solid-SMEDDS. The stubs of aluminium were used to keep the measured samples. A thin coating of gold-palladium (5-10 nm) was sputter deposited on the surface of the samples at various magnifications as a reflective layer [19].

### In vitro dissolution studies

In vitro dissolution studies were performed on both pure UA and S-SMEDDS. The formulation, which contained 10 mg of UA, was packaged in a capsule with a size of "0". The experiment was carried out in a dissolution bath with 500 ml of dissolution medium (pH 6.8 phosphate buffer and 0.1N HCl) at 37±0.5 °C at a rotating speed of 100 rpm using USP type II dissolution (Electrolab). To maintain sink condition, 1 ml aliquots were withdrawn from the dissolution medium and replaced with fresh medium at 15, 30, 45, 60, 75, 90, 105, and 120 min. The aliquots were then filtered through a 0.45 µm membrane and the samples were analyzed with HPLC [20].

### Bioavailability studies

Male Wistar rats were used in the bioavailability study, and were fasting at the time. The study was approved by the Institutional Animal Ethical Committee (IAEC) (IAEC/10/UCPSC/KU/2020) at UCPSC, Kakatiya University, and Warangal, India. In the experiment, Wistar rats weighing 200±30 g were employed (n=6 rats per group). In Wistar rats, the oral bioavailability of the optimized formulation of S-SMEDDS and UA coarse suspension was assessed at a dose of 10 mg/kg body weight. At regular specified intervals (0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, and 30 h following the injection), 0.5 ml of blood was collected from the retro-orbital plexus into eppendorf tubes and centrifuged at 3,000 rpm for 30 min to separate serum. Until the HPLC analysis was done, it was held at 20°C.

### Extraction procedure and HPLC analysis

As an internal standard, 100 µl glycyrrettinic acid (GA (IS), 2g/ml) was added to 100 µl serum and mixed for 2 min with a cyclomixer. As a precipitating and extracting solvent, 300 µl methanol was utilized. The resulting mixture was vortexed and centrifuged at 3,000 rpm for 30 min. The supernatant was collected, and a 20 µl sample aliquot was put into the HPLC apparatus. UA and IS had retention durations of 5.7 and 8.2 min, respectively. The serum components were unaffected by the retention times [21].

### Estimation of pharmacokinetic parameters and statistical significance

Non-compartmental estimations were used to compute pharmacokinetic (PK) parameters such as Cmax, Tmax, AUC, C Last, and MRT using the Kinetica software (version 5.0). The data were presented as a mean±SD. For statistical comparison of data, Graph pad prism software (version 6.4) was used to do a one-way ANOVA.

### Induction of diabetes

To induce diabetes, a freshly prepared STZ (55 mg/kg, i.p.) was mixed in 0.1 mol ice-cold citrate buffer (pH 4.5) and administered to fasted Wistar rats (200±30 gm). After 2 h of STZ therapy, blood samples were taken through the retro-orbital puncture, and serum glucose levels were measured using a glucometer (Onetouch, Crina-GNO). When Wistar rats blood glucose levels reached>250 mg/dl, they were labelled diabetic and used in additional investigations [22].

### Experimental design

Experimentally induced diabetic Wistar rats were divided into 4 groups (n=6). Group I was a control group (Saline solution treatment, p. o.). Group II (Diabetic induced group), received only STZ (i.p.) and no further treatment was given. Group III was treated with UA coarse suspension (10 mg/kg/day, p. o.). Group IV Wistar rats were treated with ursoic acid of S-SMEDDS (10 mg/kg/day, p. o) respectively for 28 d.

### Biochemical analysis

Wistar rats were anesthetized with isoflurane, and blood was taken from the retro-orbital plexus. After centrifuging the blood for 10 min at 4000 g, the serum was collected and stored at-20°C for further research. Using the Erba diagnostic kit, standard techniques were used to estimate blood glucose levels (Transasia Biomedicals Ltd, HP, and India).

### Estimation of MDA and GSH levels in kidney, retina, and sciatic nerve

The rats were sacrificed by cervical decapitation at the end of the 28th-day study, and their kidneys, eyes, and sciatic nerve were collected. Before being centrifuged at 2000 rpm for 10 min, the kidney slab was homogenized in phosphate buffer (pH 7.4). The eye was removed, the retina was separated, and the supernatant was
used for estimation tests after centrifugation for 10 min at 3,500 rpm. The sciatic nerve was removed, cleaned in ice-cold saline, and subjected to biochemical testing. In the tissue homogenates, the levels of malondialdehyde (Ohkawa et al.) and glutathione (Ellman’s technique) were measured [23].

**Pharmacodynamic studies**

STZ-induced Wistar rats kidney, retina, and sciatic nerve tissue sections were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned using a microtome at a thickness of 5 m, and stained with hematoxylin and eosin (H and E) for light microscopy section evaluation. On the acquired tissue slices, Wistar rats sections changes were examined at 400X magnification.

**Preparation of UA coarse suspension**

The UA coarse suspension was prepared using the mortar and the trituration process. Sodium CMC (50 mg) was triturated in 10 ml distilled water for 10 min. This combination was triturated after 10 mg of UA was introduced. Finally, to get the volume up to 10 ml, distilled water was employed.

**Stability studies**

Changes in particle size, ZP, PDI, and the S-SMEDDS assay were used to assess stability. Optimized S-SMEDDS formulation was stored at room temperature (25 °C/60% RH) for 90 d [24]. The physical stability of the sample was tested on the first, 30th, 60th, and 90th days (n=3).

**RESULTS AND DISCUSSION**

**Solubility studies**

The solubility of UA in different oils, surfactants, and co-surfactants was investigated using HPLC analysis. Solubility tests for UA were carried out to find the vehicles for optimal drug loading. The vehicles in a SMEDDS formulation have a high solubilization capacity, ensuring that the drug is completely dissolved, with the results reported in table 1. The most effective oil for dissolving UA was Capryol 90 (11.75±0.26 mg/g), followed by Capmul MCM (8.12±0.31 mg/g). Furthermore, the surfactant Cremophor EL (14.37±0.71 mg/g) and co-surfactant PEG 400 (16.82±0.54 mg/g) had the highest solubility [25].

| Components | Vehicles | Solubility of UA (mg/g) |
|------------|----------|------------------------|
| **Oils**   |          |                        |
| Pecol      |          | 2.58±0.14              |
| Captex 355 |          | 3.74±0.17              |
| Capmul MCM |          | 8.12±0.31              |
| Capryol 90 |          | 11.75±0.26             |
| Labrafil M 1944CS | | 4.18±0.12              |
| **Surfactants** |   |                        |
| Tween 80   |          | 6.89±0.35              |
| Labrasol   |          | 9.26±0.68              |
| Cremophor EL |       | 14.37±0.71             |
| **Co-surfactants** | |                        |
| Propylene glycol | | 5.46±0.38             |
| PEG 200    |          | 11.58±0.41             |
| PEG 400    |          | 16.82±0.54             |

(mean±SD; n=3)

**Table 1: Solubility results of UA in various vehicles**

**Table 2: Formulation composition of UA L-SMEDDS**

| Components | Quantity (g) |
|------------|--------------|
| UA (mg)    | 10 10 10 10 10 10 10 10 |
| Capryol    | 10 15% 20 25% |
| 90         | (60 mg) (12 mg) |
| Capmul MCM | - - - - - - - - - - |
| 10%        | (60 mg) (12 mg) |
| 15%        | (60 mg) (12 mg) |
| 20%        | (60 mg) (12 mg) |
| 25%        | (60 mg) (12 mg) |
| Cremophor EL | 40 37.5 35 32.5 |
| 40%        | (24 225) (21 205) |
| 37.5%      | (24 225) (21 205) |
| 35%        | (24 225) (21 205) |
| 32.5%      | (24 225) (21 205) |
| PEG 400    | 40 37.5 35 32.5 |
| %          | (24 225) (21 205) |
| %          | (24 225) (21 205) |
| %          | (24 225) (21 205) |
| %          | (24 225) (21 205) |

Pseudo-ternary phase diagrams were used to identify the emulsion development in fig. 1. The microemulsion area was developed by Smix at various 1:1 and 2:1 ratios. Oil and surfactant mixes with ratios

![Pseudo-ternary phase diagrams](image-url)
The size, PDI, and ZP of all developed formulations were measured, and the findings are listed in table 3. When compared to Capmul MCM formulations, Capryol 90 formulations had better size, PDI, and ZP characteristics. The formulations [F1-F4] had PDI values ranging from 0.192±0.049 to 0.294±0.065 and ZP values ranging from -18.3±1.05 to 23.7±1.84 mV, with sizes ranging from 148.6±3.97 to 193.5±4.75 nm. The ZP values for the formulations (F5-F8) ranged from -15.4±0.95 to -22.6±1.42 mV, with sizes ranging from 237.1±7.84 to 301.7±7.92 nm, PDI values ranging from 0.267±0.075 to 0.326±0.069, and sizes ranging from 237.1±7.84 to 301.7±7.92 nm. The rise in oil content and decrease in surfactant resulted in increased globule size, which was reported as a common occurrence. The size of the globules decreased as the surfactant amount was increased. The smallest size, PDI, and ZP were found in the F2 formulation, which contains Capryol 90 at a 15% concentration. F2 was chosen for further study and, as a result, adsorbs on carriers.

Table 3: Characterization parameters of developed L-SMEDDS

| Formulations | Size (nm)±SD | PDI±SD | ZP±SD (mV) |
|--------------|-------------|--------|------------|
| F1           | 156.2±5.32  | 0.205±0.051 | -20.1±1.32 |
| F2           | 161.6±3.97  | 0.192±0.034 | -2.34±1.48 |
| F3           | 188.2±6.91  | 0.253±0.049 | -18.3±1.05 |
| F4           | 193.5±4.75  | 0.294±0.065 | -19.7±1.26 |
| F5           | 237.1±7.84  | 0.267±0.075 | -20.9±1.31 |
| F6           | 256.4±8.29  | 0.302±0.062 | -15.4±0.95 |
| F7           | 289.3±5.36  | 0.281±0.058 | -22.6±1.42 |
| F8           | 301.7±7.92  | 0.326±0.069 | -18.5±1.06 |

(mean±SD; n=3)

Adsorption of L-SMEDDS onto a carrier

L-SMEDDS that had been optimized were used to develop S-SMEDDS. Aerosil® 200 (F2A), Flocel®101 (F2F), and mannitol (F2M) were the carriers used, with Aerosil® 200 (F2A) providing the greatest results when compared to the others. The liquid retention potentials of all carriers were determined and evaluated as follows: the data in the table reveal that aerosil® 200 (F2A) had better flow characteristics than the other carriers in the formulations. The findings of powder characteristics were 29.36±0.618, 13.82±0.829, and 10.7±0.038, respectively, as shown in table 4. The results were based on S-SMEDDS flow characteristics: F2A was then examined for globule size, PDI, and ZP, and assay, and the results were 162.4±1.475 nm, 0.25±0.042-21.3±1.02 mV, and assay 96.21±0.75%, respectively, results are given in table 4. There were no significant differences in globule size, PDI, or ZP among the formulations. Aerosil® 200 had better flow characteristics than the other carriers in the formulations. Aerosil® 200 had better flow characteristics than the other carriers in the formulations. Aerosil® 200 had better flow characteristics than the other carriers in the formulations. Aerosil® 200 had better flow characteristics than the other carriers in the formulations.

Table 4: Adsorption of L-SMEDDS on solid carriers

| Formulations | Solid carrier  | Weight of L-SMEDDS (mg) | Weight of carrier (mg) | Total weight of S-SMEDDS (mg) | Liquid retention potential (φ) |
|--------------|----------------|-------------------------|------------------------|------------------------------|-------------------------------|
| F2A          | Aerosil® 200   | 550                     | 350                    | 900                          | 2.368                         |
| F2F          | Flocel® 101    | 550                     | 500                    | 1050                         | 1.529                         |
| F3M          | Mannitol       | 550                     | 900                    | 1450                         | 0.418                         |

(mean±SD; n=3)

In vitro dissolution studies of S-SMEDDS (F2A)

In vitro dissolution studies for pure drug powder and S-SMEDDS (F2A) loaded into "0" size capsules were done using the USP type II apparatus. In the pH 6.8 phosphate buffer as dissolution media, the S-SMEDDS (F2A) released 83.57% of the UA after 15 min. In the case of 0.1N HCl, 26.28% of the drug was released are shown in fig. 2 and fig. 3. Because UA is a weak acid, it is less ionized in this medium, this type of drug release is expected. On the other hand, the developed S-SMEDDS revealed a promising strategy for increasing UA solubility (BCS class IV). In 0.1N HCl, the pure drug and optimized formulation of S-SMEDDS showed 9.15% and 62.23%, respectively. On the other hand, phosphate buffers exhibit 32.77% and 96.15% [28].
Solid-state characterization

Differential scanning calorimetry (DSC)

DSC was employed for the pure drug (UA) and optimized S-SMEDDS (F2A) formulation. UA showed a sharp endothermic peak at 280.24 °C. The absence of a DSC thermogram of the F2A peak shown in fig. 4 could be due to the bulkiness of other components present rather than a loss of crystallinity of the drug.

Surface morphology by FESEM

FESEM images of UA, aerosil® 200, and the S-SMEDDS (F2A) formulation are shown in fig. 5. Pure UA crystals had an irregular shape. In developed formulations, SMEDDS was adsorbed on the solid carrier's surface. There was no sign of crystallization or precipitation, implying that the drug had been solubilized or micronized [29].

Pharmacokinetic study

The plasma profiles of UA were studied after oral administration of UA coarse suspension and the developed S-SMEDDS (F2A) formulation. The pharmacokinetic parameters are reported in table 7, and the plasma concentrations versus time graphs are displayed in fig. 6. The S-SMEDDS (F2A) formulation had statistically significantly higher PK values in terms of Cmax, AUC, t1/2, and MRT when compared to UA coarse suspension. The AUC values for UA coarse suspension and S-SMEDDS (F2A) were respectively 6.897±0.089 (g/ml. h) and 28.415±1.385 (g/ml. h), respectively. S-SMEDDS (F2A) showed a higher relative bioavailability of 4.12-fold that of UA coarse suspension.
Fig. 4: DSC thermograms of UA pure drug (A); optimized S-SMEDDS (F2A)

Fig. 5: FESEM studies of a) Pure drug; b) Aerosil 200; c) S-SMEDDS (FA2)

Table 7: Consolidated table showing the pharmacokinetic parameters of UA in rats: UA coarse suspension and optimized formulation of S-SMEDDS (F2A)

| P/K parameter | UA coarse suspension | S-SMEDDS (F2A) |
|---------------|----------------------|----------------|
| C<sub>max</sub> (µg/ml) | 1.33±0.021 | 6.24±0.79** |
| T<sub>max</sub> (h) | 2 | 2 |
| AUC<sub>tot</sub> (µg/ml.h) | 6.89±0.089 | 28.41±1.385*** |
| t<sub>1/2</sub> (h) | 3.92±0.166 | 8.75±0.132** |
| MRT (h) | 5.41±0.134 | 9.22±0.226** |

(mean±SD; n=6) ***p<0.001 and **p<0.01, Statistically significant at p<0.01 when compared with coarse suspension

Fig. 6: Pharmacokinetic profile of UA in rat serum following oral administration of UA coarse suspension and optimized S-SMEDDS (F2A) formulation (mean±SD, n=6)
The S-SMEDDS UA formulation's greater bioavailability could be attributable to many reasons (F2A). In the GIT, the SMEDDS converts to a microemulsion, which keeps the drug distributed and improves absorption. When the SMEDDS enters the GIT, it produces a fine oil-in-water microemulsion with droplet sizes of less than 100 nm. Because of the small droplet size, the drug interfacial surface area comes into contact with the cell membrane, potentially increasing drug release and absorption. SMEDDS high surfactant content may increase permeability by disrupting the cell membrane. SMEDDS contains a surfactant that lowers interfacial surface tension, allowing medicines to penetrate epithelial cells. Song et al. developed ursolic acid nanocrystals to increase the drug's aqueous dispersibility and dissolving rate. Ursolic acid nanocrystals had superior water dispersibility and a faster dissolving rate than the free drug, they discovered. According to Yang et al., oleanolic acid (an isomer of ursolic acid) improved oral bioavailability for the SMEDDS by 5.07 times.

Biochemical analysis

Effect of S-SMEDDS (F2A) on blood glucose level

STZ induced Wistar rats had a considerable increase in blood glucose levels of around 68.90% (91.48±4.85 to 294.18±6.24 mg/dl) as compared to the control group fig. 7. The blood glucose levels of induced Wistar rats treated with ursolic acid coarse suspension and optimized S-SMEDDS (F2A) were lowered to 46.86% (294.51±6.15 to 156.49±5.18 mg/dl) and 60.01% (289.84±6.73 to 115.83±6.24 mg/dl), respectively. When comparing S-SMEDDS (F2A) to ursolic acid coarse suspension, the reduction in blood glucose level was greater with the formulation of S-SMEDDS (F2A). These findings show that optimized S-SMEDDS (F2A) have a pronounced hypoglycemic effect in diabetic rats.

Effect of S-SMEDDS (F2A) on MDA and GSH levels in kidney, retina, and sciatic nerve

The levels of the intracellular oxidant enzyme reduced glutathione and lipid peroxidation (LPO) were measured in the kidney, retina, and sciatic nerve tissue homogenate to assess oxidative stress, and results are represented in fig. 8.

STZ-induced Wistar rats had a considerable rise in kidney MDA levels, which increased by 34.42% (21.37±1.65 to 32.59±2.16 nmol/mg) as compared to the normal group. Ursolic acid coarse suspension and optimized S-SMEDDS (F2A) formulations were administered to STZ-induced rats, which lowered MDA levels by 37.92% (32.59±2.16 to 20.23±1.89 nmol/mg) and 51.73% (32.59±2.16 to 15.73±1.24 nmol/mg), respectively. STZ-induced Wistar rats had a significant drop in kidney GSH levels 37.14% (67.38±2.86 to 43.13±2.13 µmol/gm) as compared to the control group. GSH levels increased by 22.10% (49.13±2.13 to 58.7±2.52 µmol/gm) and 55.24% (49.13±2.13 to 75.41±2.45 µmol/gm) in STZ-induced Wistar rats treated with coarse suspension and S-SMEDDS (F2A), respectively.

The STZ-induced Wistar rats showed significant up-regulation of MDA levels 43.38% (35.81±2.18 to 51.25±2.53 nmol/mg) as compared to the normal group. Treatment of STZ-induced rats with formulations decreased elevated MDA levels 59.62% (63.25±2.53 to 44.51±2.35 nmol/mg) and 38.17% (63.25±2.53 to 38.47±1.96 nmol/mg), respectively. STZ-induced Wistar rats showed 76.44% (5.69±0.28 to 1.34±0.75 µmol/mg) lower levels of GSH in retina tissue homogenate than the control group. After treatment with ursolic acid coarse suspension and optimized S-SMEDDS (F2A) formulation, GSH levels in STZ-induced Wistar rats increased by 21.96% (5.69±0.28 to 6.94±0.75 µmol/mg) and 60.45% (5.69±0.28 to 9.13±0.69 µmol/mg).

In Wistar rats, STZ induced a 57.66% (7.81±0.72 to 18.45±1.39 nmol/mg) increase in MDA levels as compared to the control group. MDA levels were lowered by 44.22% (18.45±1.39 to 10.29±1.12 nmol/mg) and 53.17% (18.45±1.39 to 8.66±0.60 nmol/mg) in STZ-induced Wistar rats treated with UA coarse suspension and S-SMEDDS (F2A), respectively. In comparison to the control group, STZ-induced Wistar rats had a considerable fall in sciatic nerve glutathione levels of 83.06% (6.27±3.27 to 3.96±2.24 µmol/mg). After treatment with UA coarse suspension and S-SMEDDS (F2A), GSH levels increased by 36.48% (33.96±2.24 to 46.35±2.85 nmol/mg) and 68.69% (33.96±2.24 to 57.29±3.19 nmol/mg), respectively [30].

The above results indicate that optimized S-SMEDDS (F2A) are more effective in treating diabetes complications such as nephropathy, retinopathy, and neuropathy had considerably reduced MDA levels and increased in STZ-induced Wistar. In the presence of oils (Caproyl 90), reduced particle size enhances the interfacial area for absorption and improves bioavailability.

Pharmacodynamic studies

In STZ-induced Wistar rats, pharmacodynamic studies of renal, retinal, and sciatic nerve sections were seen; the results are displayed in fig. 9.

There were no anomalies in the control group, which had normal tubules and glomeruli. Many localized infiltrations of inflammatory cells were found in the interstitial zone between tubules in STZ-induced Wistar rats. STZ-induced Wistar rats given with ursolic acid coarse suspension
develop significant tubular inflammation and inflammatory cell infiltration, whereas treatment with the optimized S-SMEDDS (F2A) formulation restores most tubule function and reverses the STZ-induced renal section damage.

The retinal cone and rod layers in the control group had normal morphology. The retinal basement layer of STZ-induced Wistar rats displayed degenerative changes. Induced Wistar rats fed with a coarse suspension of ursolic acid showed mild retinal degeneration in the basement layer, while an optimized formulation of S-SMEDDS (F2A) showed significant improvement in retinal tissue damage and recovered to normal morphology.

A control group with normal sciatic nerve morphology was included. Wistar rats that had been given STZ had severe demyelination or degeneration. Induced Wistar rats given ursolic acid coarse suspension showed considerable demyelination or degeneration, but the S-SMEDDS (F2A) formulation showed no degeneration or demyelination [31].

Stability studies

Storage of the developed S-SMEDDS at room temperature (25 °C) for three months was used to assess its stability. All of the examined parameters showed virtually minimal change in the results, and they were shown to be stable for up to three months; results are summarized in table 8.

Fig. 8: Effect of coarse suspension and S-SMEDDS (F2C) on MDA and GSH levels in STZ induced Wistar rat tissues of kidney, retina, and sciatic nerve. Data are displayed as mean±SEM; n=3. Statistical analysis by ANOVA followed by Dunnett’s multiple comparisons, *p<0.05; **p<0.01
CONCLUSION

The present study illustrated the potential utility of S-SMEDDS for ursolic acid delivery via the oral route. The SMEDDS of UA have been successfully developed in both liquid and solid forms. L-SMEDDS (F2) is an optimized formulation including Capryol 90 (15%), Cremophor EL (37.5%), and PEG 400 (37.5%). Using the adsorption technique and Aerosil® 200 as an optimized adsorbent carrier, the formulation (F2) was used to prepare S-SMEDDS (F2A). The in vitro release studies showed 83.57% of the UA was released after 15 min, release studies conducted in acidic (62.23±0.869%) and alkaline (96.15±0.11%) conditions for 2 h. FESEM was used to study the surface morphology. The DSC studies showed that the drug could not exhibit crystallinity in S-SMEDDS formulations due to the bulkiness of excipients. When compared to coarse suspension, S-SMEDDS had a 4.12 fold higher bioavailability in Wistar rats, according to a pharmacokinetic study. The formulation S-SMEDDS (F2A) was studied on Wistar rat sections of kidney, retina, and sciatic nerve tissue, indicating the protective effect of ursolic acid on the amelioration of diabetic complications.

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AUTHORS CONTRIBUTIONS

The authors contributed to the study’s conception and design. Formulation development, characterization, and analysis were performed by Golla Chandra Mouli. The corresponding author is the one individual who takes primary responsibility for planning the research work, making modifications as appropriate as the work progresses, communicating with the journal during the manuscript submission, peer review, and publication process by Ciddi Veeresham (Supervisor). The authors read and approved the final manuscript.
CONFLICT OF INTERESTS

The authors report no conflicts of interest. The authors are alone responsible for the content and writing of this paper.

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