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Research Article

Novel Self-microemulsifying Drug Delivery System for Enhanced Diabetes Management: Evaluating the Combined Effects of Glimepiride and *Boswellia serrata* Extract

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ABSTRACT

This study aimed to create and assess a novel self-microemulsifying drug delivery system (SMEDDS) for treating diabetes mellitus that contains glimepiride and *Boswellia serrata* extract. In the creation of SMEDDS formulations, Transcutol-P was employed as the base oil, while the surfactants Tween 80 and propylene glycol/polyethylene glycol 400 served as the co-surfactants. To improve the formulation, pseudoternary phase diagrams were created. While the SMEDDS of *B. serrata* extract showed a mean droplet size of 27.63 nm, 98.3% transmittance, a zeta potential of -0.11 mV, and a polydispersity index of 0.287, the glimepiride-optimized SMEDDS showed a mean droplet size of 14.8 nm, 98.5% transmittance, a zeta potential of -0.10 mV. *In-vivo* evaluation on diabetes-induced rats demonstrated significant reductions in SGOT and SGPT levels with the glimepiride and *B. serrata* extract SMEDDS compared to diabetic control rats and the marketed glimepiride formulation. The formulation showed promising results in controlling serum total protein, triglyceride, and cholesterol levels. The glimepiride and *B. serrata* extract SMEDDS also exhibited antioxidant activity, reducing malondialdehyde (MDA) absorbance. Histopathological assessment of kidney and pancreas tissues revealed the protective effects of the concomitant administration of glimepiride and *B. serrata* extract SMEDDS formulation against diabetes-induced damage. Overall, the developed SMEDDS formulation kit showed superior *in-vitro* and *in-vivo* performance, suggesting its potential as an effective therapeutic option for managing diabetes mellitus.

Introduction

Diabetes, characterized by hyperglycemia, glycosuria, negative nitrogen balance, hyperlipidemia, and ketonemia,[1] poses a significant global burden on healthcare systems and society. The prevalence of diabetes has been steadily increasing, with an estimated 425 million people affected in 2017 and projected to rise to 629 million by 2045.[2] Current drug therapies for diabetes includes biguanide, meglitinide, α-Glucosidase inhibitor, thiazolidine, sulphonyl-urease (SU), and newer drugs like exenatide and sitagliptin.[3] glimepiride, a second-generation sulfonyl-urease, offers advantages over other sulphonyl-urease drugs, such as reduced side effects and lower risk of hypoglycemia and weight gain.[4] However, these conventional therapies may not adequately address the long-term complications associated with diabetes, which are often related to altered antioxidant status and peroxide levels.[5] This study explores the potential advantages of combining glimepiride with a natural phytochemical, *Boswellia serrata* extract from the plant *B. serrata*, using a self-micro emulsifying drug delivery system (SMEDDS) in order to investigate a novel approach to managing diabetes that takes into account the underlying vascular complications.[6] *B. serrata* extract and glimepiride both fall into distinct Biopharmaceutics Classification System (BCS) indicating poor water solubility and dissolution that may impact medication bioavailability.[7,8] To address this, we have

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chosen the self-microemulsifying drug delivery system (SMEDDS) approach, which offers improved solubility, ease of manufacturing, and scalability. SMEDDS enables the efficient transportation of active molecules through the gastrointestinal tract, promoting drug partitioning from oil to the aqueous phase, thereby enhancing drug absorption. It provides constant plasma time profiles and exhibits greater stability on the shelf life compared to conventional drug delivery systems.[9] Here are some potential pharmaceutical advantages for this novel combination. Boswellic acid has been shown to produce a synergistic effect with glimepiride, leading to improved glucose control in diabetes. This combination may help in better-regulating blood glucose levels by enhancing the action of glimepiride in stimulating insulin release from pancreatic β cells. Having diabetes mellitus increases the risk of long-term effects such as diabetic nephropathy, retinopathy, and neuropathy. Boswellic acids, which are included in the extract of *B. serrata* and have anti-inflammatory and antioxidant properties, may offer a defense against these issues. By combining it with glimepiride, the formulation could potentially provide a comprehensive novel approach to managing diabetes and reducing the risk of these complications.

Materials and Methods

Resources

Glimepiride was obtained as gift sample from Zydus life sciences (Ahmedabad, India) and Micro Labs Limited (Mumbai, India). Gurjar Phytochem Pvt. Ltd. in Indore sold the *B. serrata* extract that was purchased. A free sample of Aeroperl 300 was received from Evonic Pharma in Mumbai. Syloid XDP 3150 sample from Grace GmbH & Co. KG, Germany, Trancutol P by Gattefossé. Propylene glycol and PEG 400 were supplied by Merck life science Pvt. Ltd., Vikhroli, Mumbai, India, Tween- 80 supplied by Alpha Chemika Mumbai, Avicel® PH102 (Microcrystalline cellulose) is supplied by FMC Biopolymer. The uric acid Kit is procured from Beacon Diagnostic Pvt. Ltd, Navsari, India. Blood urea nitrogen kit, Creatinine Kit, and Total protein kit is purchased from Span Diagnostic Pvt. Ltd, Surat, India. Histopathological studies carried out in Gujarat Veterinary Research and Diagnostic Center

Methods

Infrared spectroscopy using the Fourier Transform

A fourier transform infrared spectrophotometer (FTIR) (Perkin Elmer - spectrum Bx, USA) was used to get the infrared spectrum of glimepiride and a *B. serrata* extract. To create the 10% combination a sample was diluted with KBr, mashed in a mortar, and pestle with KBr before being compacted into pellets. The spectra of these pellets was captured with a resolution of 2 cm^{-1} spanning a frequency range of 4000 to 400 cm^{-1}. For measurement, the KBr background spectrum was employed as a control.[10]

Simultaneous estimation of the drug- using RP- HPLC method

A straightforward, quick, and repeatable reverse-phase high-performance liquid chromatographic (RP-HPLC) approach was created for the simultaneous quantification of *B. serrata* extract and glimepiride in both bulk and pharmaceutical dosage forms by HPLC. On a Waters Exterra C18 analytical column, gradient elution with an acetonitrile-water mobile phase was used to produce chromatographic separation. The detection wavelengths for glimepiride and *B. serrata* extract were 228 and 260 nm. The elution times were 9.3 and 12.5 minutes for glimepiride and *B. serrata* extract, respectively. The analytical method was successfully developed and found to be useful for the routine estimation of glimepiride and *B. serrata* extract in the optimized formulation.[11-13]

Screening of excipients - Oil

The screening of oils for glimepiride and *B. serrata* extract formulation was conducted separately using Transcutol-P, Accogel, Acconon CC-6, castor oil, olive oil, and coconut oil. Saturated glimepiride and *B. serrata* extract solutions were prepared in 1-mL of each oil solvent in separate Eppendorf tubes. The solutions were then subjected to 2 hours of ultrasonication and rotated at 37°C for 72 hours on an orbital shaker to reach equilibrium. Then, each eppendorf tube was centrifuged at 6000 rpm for 20 minutes using a centrifuge. After additional dilution with phosphate buffer solution, the resultant supernatant was filtered through a 0.45 m filter disc, and the concentration was measured.[14] Based on the solubility of both drug substances, transcutol P (a highly purified form of diethylene glycol monoethyl ether) was selected as the oil for the optimized formulation.

Ratio of co-surfactant to surfactant chosen

The selection of the surfactant to co-surfactant ratio is of prime importance for the successful design of a microemulsion-based drug delivery system.[15] The efficacy of the optimized ratio for mircoemulsification ability was evaluated using the turbidimetric method (Table 1). The highest solubility of glimepiride was observed in propylene glycol + Tween 80 (1:2) ratio (0.397 ± 0.098 mg/mL). For *B. serrata* extract, it was in the ratio of polyethylene glycol (PEG) 400 + Tween 80 (3:1). In both SMEDDS formulations of glimepiride and *B. serrata* extract, Tween 80 was selected as the surfactant. In contrast, propylene glycol was chosen as the co-surfactant for glimepiride, and PEG 400 for *B. serrata* extract SMEDDS.

Ternary phase diagram construction

The phase diagrams for the pseudo-ternary systems were built using the water titration method. By combining transcutol P, Tween 80, and propylene glycol for glimepiride and transcutol P, Tween 80, and PEG 400 for *B. serrata* extract with distilled water in a variety of ratios such as 1:1, 1:2, 1:3, 2:1, and 3:1 the pseudo-ternary phase diagrams

Table 1: Composition of glimepiride SMEDDS batch 1 to 17 (oil/Surfactant: Co-Surfactant)

Batch Code	*Composition of SMEDDS formulation of glimepiride*			*Composition of SMEDDS formulation of B. serrata extract*		
	mL of oil Transcutol-P	*mL of surfactant Tween-80*	*mL of co-surfactant propylene glycol*	*mL of oil Transcutol-P*	*mL of surfactant Tween-80*	*mL of co-surfactant PEG400*
1	0.4	0.35	0.15	0.4	0.9	1.1
2	0.33	0.4	0.16	0.5	0.9	1
3	0.33	0.36	0.2	0.46	1.03	0.9
4	0.4	0.4	0.1	0.6	0.9	0.9
5	0.36	0.36	0.16	0.4	1.1	0.9
6	0.36	0.33	0.2	0.46	0.96	0.96
7	0.35	0.4	0.15	0.5	0.9	1
8	0.4	0.3	0.2	0.4	0.1	1
9	0.38	0.33	0.18	0.53	0.96	0.9
10	0.4	0.4	0.1	0.43	0.93	1.03
11	0.4	0.35	0.15	0.4	0.1	1
12	0.4	0.35	0.15	0.43	1.03	0.93
13	0.35	0.4	0.15	0.43	0.9	0.96
14	0.4	0.3	0.2	0.53	0.9	1.1
15	0.35	0.35	0.2	0.4	1.0	0.9
16	0.38	0.38	0.13	0.4	1.1	0.9
17	0.30	0.4	0.2	0.4	1	1

of O/W microemulsions were created. Emulsions were produced by changing the oil mass ratio in S_{mix} from 9:1 to 1:9. At room temperature, distilled water was added drop by drop to each pre-concentrate mixture while stirring the liquid in between drops. The solution became hazy and turbid at the end of the titration process, and the quantity of water used was noted to determine the needed microemulsion. The pseudo-ternary phase diagram was used to pinpoint the microemulsion zone and the boundaries between phases. Pseudo-ternary phase diagrams were created using the ProSim simulation programme.[16]

Preparation of SMEDDS formulation of glimepiride

In 4 mg of glimepiride were accurately weighed and dissolved in the necessary quantity of oil. A magnetic stirrer was used to gently agitate the liquid until it became transparent. The formulation was then left to equilibrate for 24 hours at 37°C.[17] The suggested ratio from the pseudo ternary phase diagram was used to calculate the proper ratio of Tween 80 and propylene glycol. The optimized SMEDDS mixture was then adsorbed on Syloid XDP 3150 and Aeroperl 300, forming sprinkle granules. Additionally, microcrystalline cellulose was used as a diluent.

Preparation of SMEDDS formulations of B. serrata extract

Accurately weighed, 220 mg of *B. serrata* extract (equivalent to 200 mg of 3-O-Acetyl-11-keto-β-Boswellic Acid i.e., AKBA) was dissolved in the required amount of oil. A magnetic stirrer was used to combine the ingredients until they were homogenous gently. The formulation was then allowed to equilibrate for 24 hours at 37°C. The ratio suggested by the pseudo-ternary phase diagram was used to calculate the desired concentration of Tween 80, propylene glycol, and PEG 400. The optimized SMEDDS mixture was then adsorbed on Syloid XDP 3150 and Aeroperl 300 to form sprinkle granules. Additionally, microcrystalline cellulose was used as a diluent.

Formulation optimization

Through the use of the Design-Expert programme, the formulation was optimised using a D-Optimal mixture design.[18] Concentrations of oil [X1], surfactant [X2], and co-surfactant [X3] were taken into consideration as independent variables. Percentage Transmittance [Y1], Mean Droplet size [Y2], and Polydispersity Index [Y3] were the responses that were measured. In order to guarantee unbiased experimentation, randomized experiments were carried out. Multiple linear regression and analysis of variance (ANOVA) were used to determine the importance of the variables.

Characterization of SMEDDS Formulations

Visual observation

The formulation was visually observed, and parameters such as transparency, translucency, phase separation, and clarity were examined. Formulations exhibiting excellent clarity with no phase separation were chosen, as clarity

is a fundamental requirement for designing SMEDDS formulations. [18]

Evaluation of droplet size

Using photon correlation spectroscopy, which examines light scattering fluctuations brought on by the Brownian motion of particles, the droplet size of batches B1–B17 was identified. A Zetasizer, which can measure particle sizes between 10 nm and 5000 nm, was used for the measurements carried out at 25°C at a 90° angle.

Polydispersity index

The polydispersity index (PDI) of batches B1-B17 was determined using the Malvern Zeta sizer. The SMEDDS formulations were diluted with water before measuring the polydispersity index. A lower PDI value indicates a more significant dispersion of globules in the emulsion. [19]

Percent transmittance

The percent transmittance is a measure of the formulation's transparency. A formulation is considered transparent if the percent transmittance is greater than 99%. A UV spectrophotometer was used to calculate the system's percent transmittance at a certain wavelength.

Characterization of the Granules

The granules underwent evaluation for various parameters, including appearance, bulk density, tap density, compressibility, angle of repose, and Hausner's ratio.

In-vitro dissolution study

Drug release studies were conducted in triplicate at a controlled temperature of 37 ± 0.5°C, utilizing USP apparatus 2 (paddle) operating at 75 rpm. The dissolution study was performed using phosphate buffer pH 7.8 for glimepiride and phosphate buffer pH 7.4 for *B. serrata* extract SMEDDS as dissolution media.

Robustness to dilution for SMEDDS

By using distilled water to dilute the SMEDDS 20, 50, and 100 times, the resilience to dilution was assessed. Following a 12 hour period of storage, the diluted SMEDDS samples were examined closely for any indications of phase separation or drug precipitation.

Self-emulsification time

In this test, 20 mL of distilled water were added to 0.5 g of powder from each SMEDDS formulation, and the temperature was kept at 37 +/- 0.5°C. Using a magnetic stirrer moving at a constant speed of 100 rpm, the ingredients were gently blended. By visually witnessing the disappearance of the SMEDDS and the subsequent development of the microemulsion, the emulsification time was tracked.

Results and Discussion

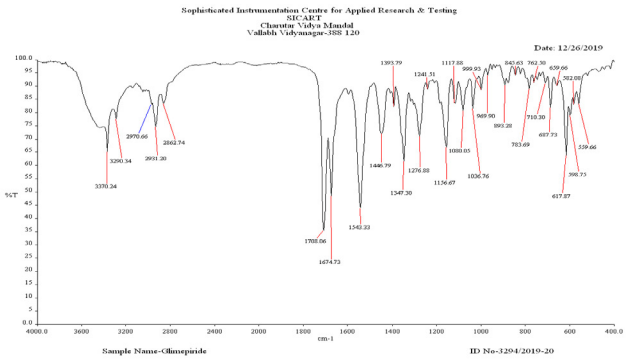

Fig. 1: FTIR Spectra of Glimepiride API

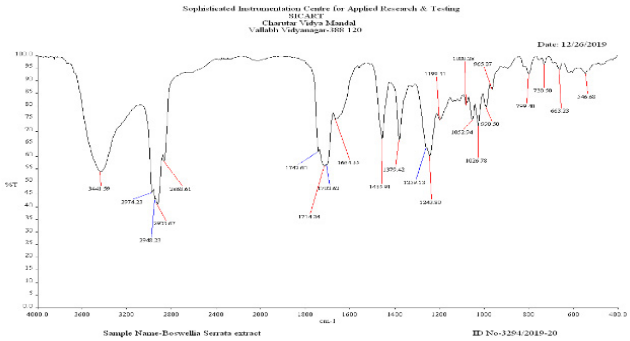

Fig. 2: FTIR Spectra of *B. serrata* extract API

Fourier Transform Infrared Spectroscopy Study

The FTIR spectra of glimepiride exhibited characteristic peaks, including N-H at 3368.27 cm^{-1}, –C=O bond at 1704.10 cm^{-1}, and S=O bond at 1343.32 cm^{-1}. Additionally, as shown in Fig. 1, the Glimepiride formulation had substantial C-O stretching at 1050.98 cm^{-1} and C-H stretching at 2873.44 cm^{-1}.

The FTIR spectra of *B. serrata* extract revealed peaks at C=O stretching (1731.19 cm^{-1}), C-H stretching (2816.22 cm^{-1}), and strong C-O stretching (1032.44 cm^{-1}). As shown in Fig. 2, the *B. serrata* extract formulation displayed substantial C-O stretching at 1050.98 cm^{-1} and C-H stretching at 2869.16 cm^{-1}.

The FTIR spectra of both formulations were found to be comparable with the individual drug peaks, and the additional peaks observed in the formulations can be attributed to the presence of excipients, as shown in Fig. 3.

HPLC Method Development for Simultaneous Estimation of the Formulation

The standard solutions were prepared based on the solubility of glimepiride and *B. serrata* extract using different combinations of Water: Methanol, Water: Acetonitrile (ACN), and Methanol: CAN. The diluent was chosen to be a 60:40 blend of acetonitrile and methanol. For the development of a rapid analytical method for both glimepiride and *B. serrata* extract, 200 ppm solutions were prepared in the diluent and injected into HPLC using 100% gradient trails. HPLC grade water and acetonitrile

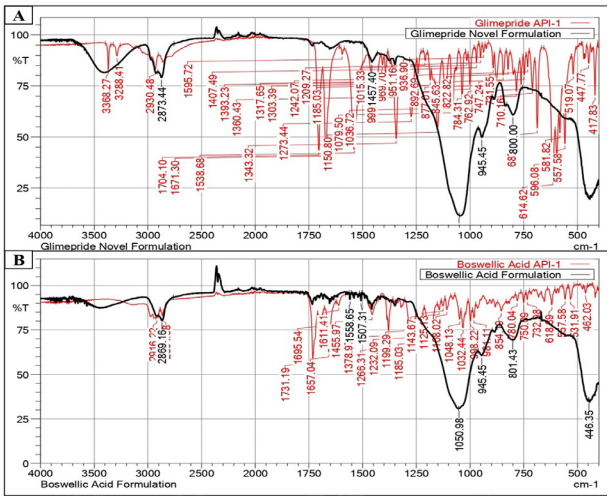

Fig. 3: FTIR Spectra of Glimepiride and *B. serrata* extract SMEDDS Formulations

were used with different concentrations (50:50, 70:30, 20:80) at a 1.0 and 1.2 mL/minute flow rate. Detection was performed using the PDA detector with a wavelength range of 200 to 400 nm. After the completion of the run, chromatograms were evaluated at each wavelength with an interval of 1-nm from 200 to 400 nm to select the wavelength of maximum absorbance (λ_{max}) for both glimepiride and *B. serrata* extract. The examination of glimepiride and a *B. serrata* extract used wavelengths of 228 and 260 nm.

Screening of Excipients

Screening of oil

The hydrophobic nature of oil contributes to improved drug loading in SMEDDS and enhances the bioavailability of poorly water-soluble drugs. The selection of the appropriate oil is crucial in this type of formulation. Medium-chain triglycerides are commonly used in SMEDDS due to their resistance to oxidation and high solvent capacity compared to long-chain triglycerides found in vegetable oils. Transcutol-P has demonstrated good solubility for both glimepiride and *B. serrata* extract. The solubility data obtained using various oils for glimepiride and *B. serrata* extract is presented in (Table 2) (Fig. 5 and 6).

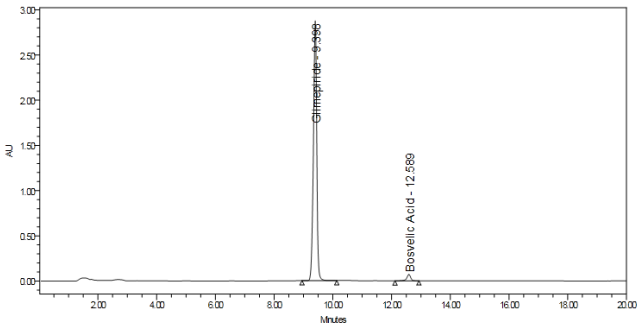

Fig. 4: HPLC wavelength detection of glimepiride and extract of *B. serrata*

Screening of surfactant, co-surfactant, carrier, and coating materials

For the glimepiride SMEDDS formulation, Tween 80 and propylene glycol were chosen as surfactant and co-surfactant, respectively. Similarly, for the *B. serrata* extract SMEDDS, Tween 80 and PEG 400 were used as surfactant and co-surfactant. As for the carriers and coating materials, Aeroperl 300 and Syloid XDP 3150 were selected. The combination of Syloid XDP 3150 as a carrier with Aeroperl 300 as a coating material provided favorable flow properties, with a flow angle of 29° and the highest Phi value of 0.78. This combination demonstrated the highest sorption capacity, making it ideal for formulation.

Pseudo ternary phase diagram construction

The first three S_{mix} ratio combinations for glimepiride SMEDDS entailed raising the co-surfactant concentration while holding the surfactant concentration constant. These ratios were 1:1, 1:2, and 1:3. While maintaining the same proportion of the co-surfactant, the other two ratios [2:1,

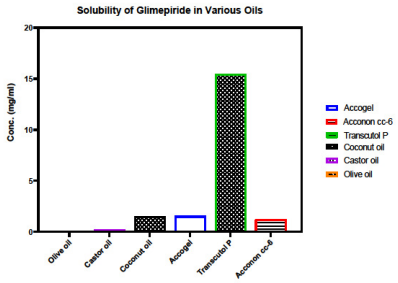

Fig. 5: Solubility of Glimepiride in Various Oils

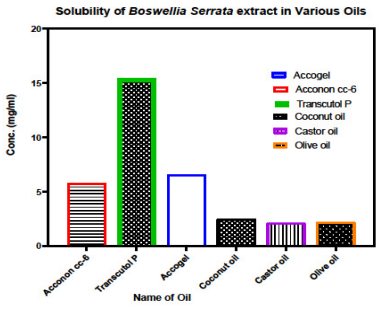

Fig. 6: Solubility of *B. serrata* extract in Various Oils.

Table 2: Screening of oils

Name of oil	*Glimepiride Conc. (mg/mL)*	*B. serrata extract Conc. (mg/mL)*
Transcutol -P	15.45 ± 0.23	15.42 ± 0.27
Accogel	1.55 ± 0.19	6.55 ± 0.29
Acconon cc-6	1.24 ± 0.42	5.78 ± 0.18
Castor oil	0.22 ± 0.06	2.14 ± 0.19
Olive oil	0.11 ± 0.08	2.19 ± 0.09
Coconut oil	1.48 ± 0.07	2.47 ± 0.07

3:1] necessitated raising the surfactant concentration. Fig. 7 shows that among these combinations, the [S_{mix} 1:1] ratio demonstrated a higher microemulsion region (shown by the shaded color zone) in comparison to the others.
Fig. 8 shows that when *B. serrata* extract SMEDDS with S_{mix} [3:1] was used, the microemulsion region (the darker colour zone) grew as the surfactant concentration was raised. This expansion was more significant compared to the microemulsion regions in combinations of S_{mix} [1:1], [1:2], and [1:3]. On the other hand, the microemulsion area of S_{mix} [1:1], [1:2], and [1:3] decreased when compared to S_{mix} [3:1]. As a result, the [1:1], [1:2], and [1:3] S_{mix} ratios were dropped from the investigation.

Optimization By D-optimal Mixture Design

A three-component system consisting of the oil phase (A), surfactant (B), and co-surfactant (C) was used in experimental mixing research. These three components have a combined concentration of 100%. The drug content in the SMEDDS was kept constant. Based on the phase diagram results, the range for each component was chosen. The dependent variables that were measured were the polydispersity index (Y3), mean droplet size (nm), and %transmittance. We choose the cubic models with the lowest press value. Table 3 provides a summary of the D optimum design matrix produced by design expert software.
For batches B1 to B17, a statistical model was created that included interactive and polynomial terms to evaluate the responses of % transmittance, mean droplet size, and PDI. To get conclusions, it was looked into how the fitted equations, both whole and reduced, related to the replies Y1, Y2, and Y3. In the case of glimepiride SMEDDS, all batches exhibited % transmittance greater than 91.3, with batch 5 outperforming others at 98.5% transmittance. Batch 5 also showed a PDI of 0.207, and its droplet size ranged from 14 to 72 nm (Fig. 9), with the smallest droplet size observed in this batch. The

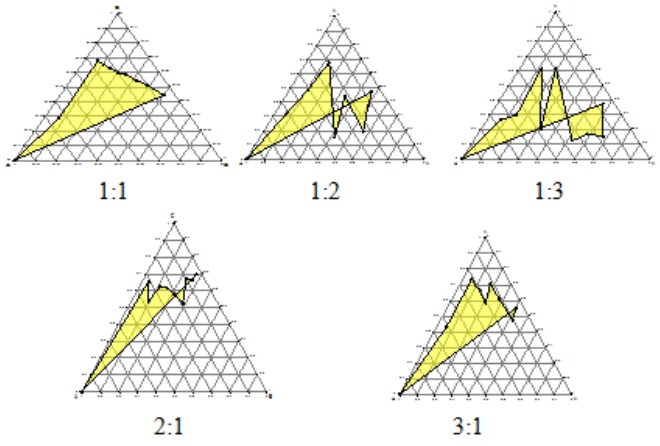

Fig. 7: Pseudo-ternary phase diagram of glimepiride SMEDDS, illustrating the impact of various surfactant, co-surfactant, and oil ratios.

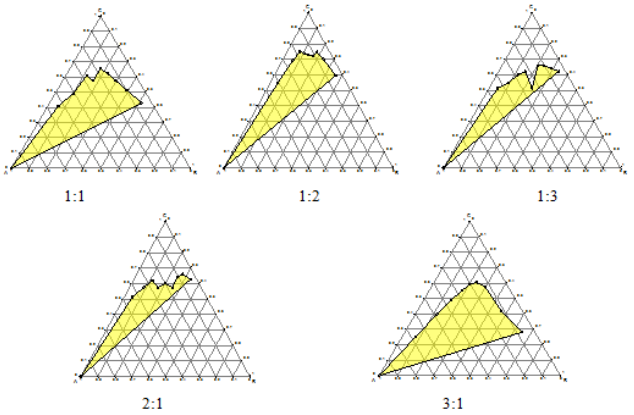

Fig. 8: *B. serrata* Extracts SMEDDS pseudo-ternary phase diagram illustrating the impact of different surfactant, co-surfactant, and oil ratios.

polydispersity index for glimepiride batches ranged from 0.151 to 0.292. All batches for *B. serrata* extract SMEDDS (Table 5) demonstrated % transmittance higher than 96%, indicating clarity and transparency. Batch 5 showed the highest % Transmittance at 98.3%. The mean droplet size for *B. serrata* extract batches ranged from 25 to 29 nm, with batch 5 having a droplet size of 27.63 nm (Fig. 10). The Polydispersity index for these batches ranged from 0.206 to 0.287, with batch 5 having a PDI of 0.287.
Polynomial equations for Glimepiride SMEDDS are as follows:

Y_1= 91.80A + 96.57B + 98.37C + 13.65AB + 11.42AC -1.31BC

Y_2= 19.24A +36.55B +61.27C +51.14AB +82.79AC +58.14BC -1069.46ABC +290.21AB (A-B) -506.98AC (A-C) +611.63BC (B-C)

Y3 = 0.2913A + 0.1509B + 0.1666C -0.1207AB -0.0053AC -0.0266BC +0.1339AB (A-B) + 0.1583ABC - 0.7682AC (A-C) +0.5091BC (B-C).

Table 3: Independent and dependent variable to apply D-optimal design

Independent variable	*Glimepiride Actual value*		*B. serrata extract actual value*	
	Low level (-1)	*High level (+1)*	*Low level (-1)*	*High level (+1)*
A = Amount of oil	0.3	0.4	0.4	0.5
B = Amount of surfactant	0.3	0.4	0.9	1.1
C= Amount of co-surfactant	0.1	0.2	0.9	1.1
Dependent variable				
Y1 = %Transmittance	95-100 %		95-100 %	
Y2 = Mean droplet size	10-100 nm		10-100 nm	
Y3 = Poly dispersity index	0.1-0.5		0.1-0.5	

Table 4: Design matrix with responses for glimepiride SMEDDS

Batch code	*Amount of oil (mL) = A*	*Amount of surfactant (mL) = B*	*Amount of co-surfactant (mL) = C*	*Transmittance (%) = Y_1*	*Mean droplet size(nm) = Y_2*	*Poly dispersity Index = Y_3*
1	0.4	0.35	0.15	97.3	71.23	0.168
2	0.33	0.4	0.16	97.2	18.56	0.197
3	0.33	0.36	0.2	97.1	71.05	0.229
4	0.4	0.4	0.1	98.4	60.86	0.197
5	0.36	0.36	0.16	98.5	14.8	0.207
6	0.36	0.33	0.2	97.3	29.36	0.167
7	0.35	0.4	0.15	97.2	58.64	0.245
8	0.4	0.3	0.2	96.8	35.65	0.151
9	0.38	0.33	0.18	96.7	56.23	0.183
10	0.4	0.4	0.1	98.4	60.86	0.197
11	0.4	0.35	0.15	97.3	58.23	0.168
12	0.4	0.35	0.15	97.3	58.23	0.168
13	0.35	0.4	0.15	98.2	58.64	0.245
14	0.4	0.3	0.2	96.8	35.65	0.151
15	0.35	0.35	0.2	98.0	20.56	0.185
16	0.38	0.38	0.13	98.2	47.56	0.209
17	0.3	0.4	0.2	91.3	16.95	0.292

Table 5: Design matrix with response for *B. serrata* extract SMEDDS

Batch no.	*Amount of oil (mL) = A*	*Amount of surfactant (mL) = B*	*Amount of co-surfactant (mL) = C*	*Transmittance (%) = Y_1*	*Mean droplet size(nm) = Y_2*	*Poly dispersity Index = Y_3*
1	0.4	0.9	1.1	97.1	25.25	0.206
2	0.5	0.9	1	97.3	27.18	0.272
3	0.46	1.03	0.9	97.5	27.27	0.213
4	0.6	0.9	0.9	96.3	27.13	0.237
5	0.4	1.1	0.9	98.3	27.63	0.287
6	0.46	0.96	0.96	97.1	27.36	0.268
7	0.5	0.9	1.0	98.2	26.89	0.286
8	0.4	1.0	1.0	96.9	25.01	0.256
9	0.53	0.96	0.9	97.1	28.3	0.245
10	0.43	0.93	1.03	98.2	26.89	0.286
11	0.4	1.0	1.0	97.8	27.15	0.294
12	0.43	1.03	0.93	97.3	28.74	0.273
13	0.53	0.9	0.96	96.0	25.25	0.206
14	0.4	0.9	1.1	96.8	27.8	0.249
15	0.5	1.0	0.9	96.3	27.13	0.237
16	0.4	1.1	0.9	98.2	26.89	0.286
17	0.4	1.0	1.0	96.0	25.25	0.206

Reduced model

Y_1= 91.80A + 96.57B + 98.37C + 13.65AB + 11.42AC

Y_2= 19.24A +36.55B +61.27C -1069.46ABC +290.21AB (A-B) -506.98AC (A-C) +611.63BC (B-C)

Polynomial equations for *B. serrata* extract SMEDDS are as follows

Y_1= 97.48A +96.30B +95.99C +0.4151AB +1.17AC +8.12BC +11.92ABC -4.38AB (A-B) +2.75AC (A-C) +1.90BC (B-C)

Y_2= 27.25A +27.11B +25.27C -1.98AB +4.87AC +2.98BC +20.02ABC -16.53AB (A-B) +11.25AC (A-C) -27.38BC (B-C)

Y2 = 0.2135A + 0.2373B + 0.2059C + 0.1425AB + 0.2315AC + 0.2569BC - 0.0814ABC -0.0497AB (A-B) +0.1519AC (A-C) +0.2656BC (B-C).

Reduced model

Y_1= 8.12BC -4.38AB(A-B)

Y_2= 4.87AC -16.53AB(A-B) -27.38BC(B-C)

Y_2= 0.2136A +0.2373B +0.2059C +0.1425AB +0.2315AC +0.2569BC +0.2656BC(B-C)

Y3 = 0.2913A + 0.1509B + 0.1666C -0.1207AB + 0.1339 AB (A-B) -0.7682AC(A-C) + 0.5091BC(B-C).

Counter plots and response surface plots

Figs. 11 and 12, respectively display three-dimensional response surface plots and two-dimensional contour plots. These illustrations were used to illustrate the interactions between the dependent and independent variables. The contour figure demonstrates various microemulsion components' effects on the percent transmittance, polydispersity index and mean droplet size.

Overlay plot

Following the creation of the polynomial equations linking the dependent and independent variables, the SMEDDS formulations for the solutions Y1, Y2, and Y3 were optimised. The optimal values of the variables were determined using graphical and numerical investigations with Design-Expert software, as shown in Figs 13 and 14.

In-vitro dissolution study

Dissolution testing was conducted on the optimized SMEDDS formulation for both drug substances and compared with marketed formulations, as depicted in (Table 6 and 7). The results revealed a substantial enhancement in the dissolution rate for both Glimepiride and B. serrata extract SMEDDS (Fig. 15 and 16). Glimepiride exhibited 99.0% drug release within 20 minutes, while B. serrata extract demonstrated 107.1% drug release within 120 minutes.

Optimized Formulation Evaluation on Antidiabetic Rats [20]

The optimized formulation of glimepiride and *B. serrata* extract SMEDDS were treated with diabetes induced rats. The impact glimepiride SMEEDS alone and in combination with *B. serrata* extract SMEDDS, a commercially available glimepiride formulation, on blood glucose levels was examined for up to 120 minutes. Serum levels of glutamate oxaloacetate transaminase (SGOT), glutamate pyruvate transaminase (SGPT), fasting blood glucose, urea nitrogen, uric acid, serum triglyceride, and serum total proteins, as well as cholesterol, were assessed in

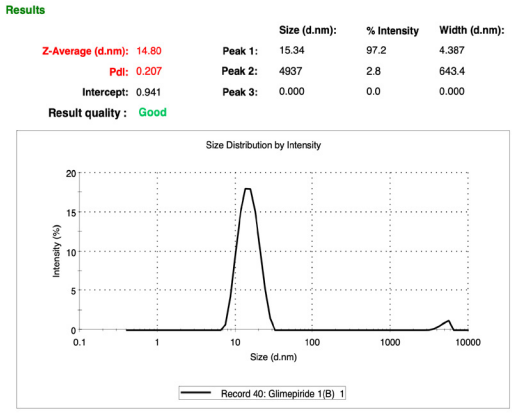

Fig. 9: Glimepiride SMEDDS formulation size distribution report

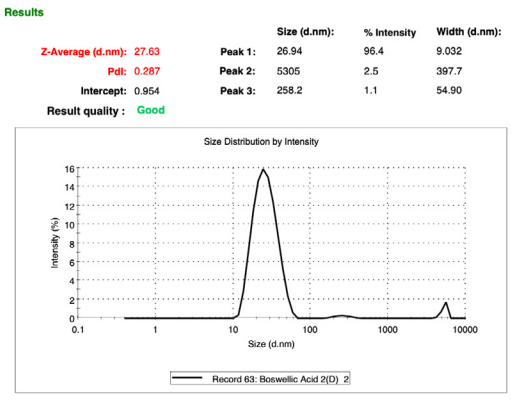

Fig. 10: *B. serrata* extract SMEDDS formulation size distribution report

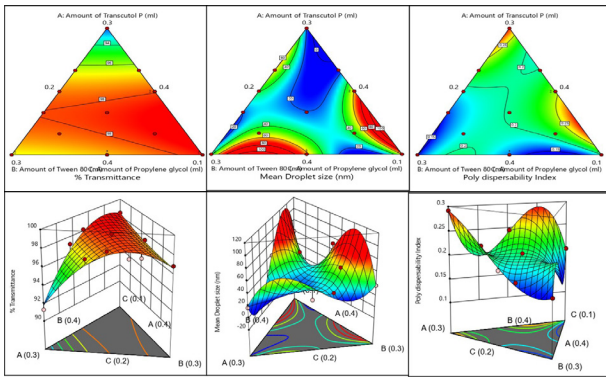

Fig. 11: Shows counter plots and response surface plots of the Glimepiride SMEDDS's mean droplet size, polydispersity index, and transmittance percentage

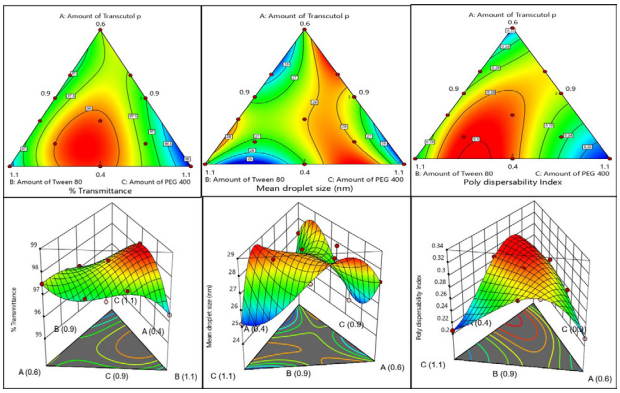

Fig. 12: Counter plots and response surface plots of mean droplet size, polydispersity index, and transmittance of *B. serrata* extract SMEDDS

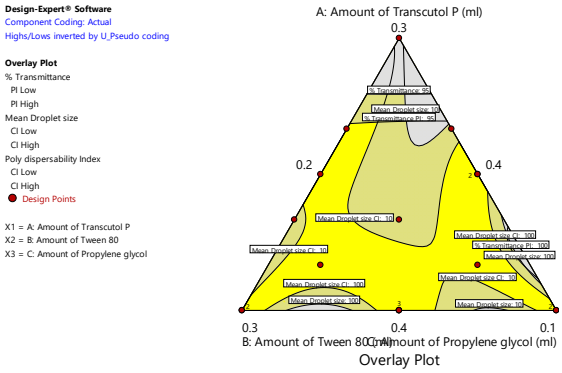

Fig. 13: Overlay plot of glimepiride SMEDDS

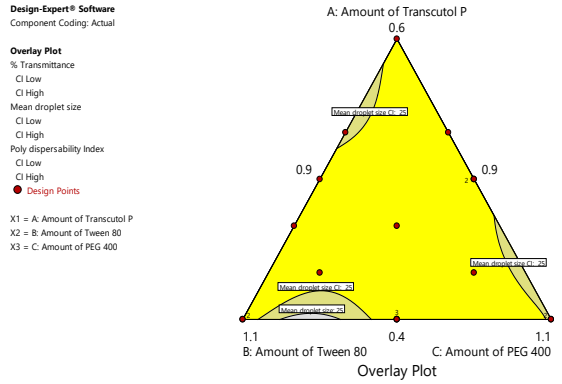

Fig. 14: Overlay plot of *B. serrata* extract SMEDDS

control and streptozotocin-induced diabetic rats. At end of study, the activity of superoxide dismutase (SOD), and malondialdehyde (MDA) levels were studied in kidney homogenate. Histopathological evaluation performed on kidneys and pancreas. Observed results are summarized below

Effect on serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT)

The findings of this research reveal notable differences in SGOT and SGPT levels among the various experimental groups.

The observed reduction in both SGOT and SGPT levels in the experimental groups further reinforces the potential antidiabetogenic effect of these drugs. Given that diabetes is associated with increased gluconeogenesis and ketogenesis, which can be linked to elevated SGOT and SGPT levels, the significant decrease in these enzymes after treatment suggests a positive impact on diabetes management. Particularly, the glimepiride and *B. serrata* extract SMEDDS combination and the alone glimepiride SMEDDS formulation show promise as potential treatments for diabetes, as they effectively mitigate the rise in SGPT levels (Table 8). Further research in this direction is essential to fully comprehend the therapeutic capabilities of these drug formulations in diabetes management.

Table 6: Comparative dissolution study of optimized formulation with marketed drug products

Time Points	*Diapride Tablets 4 Mg [Marketed Formulation] - % Drug Release*	*Glimepiride SMEDDS % Drug Release*
0	0	0.0
5	30.1	41.3
10	76.4	88.2
15	89.8	95.2
20	95.1	99.0

Table 7: Comparative dissolution study of optimized formulation with marketed drug products

Time Points	*Himalaya Shallaki Tablets [Marketed Formulation] - % Drug Release*	*B. Serrata Extract Smedds Sachet - % Drug Release*
0	0.0	0.0
5	0.0	4.9
10	1.6	14.1
15	2.4	19.4
20	6.2	28.9
30	12.0	43.7
45	20.9	59.1
60	27.5	74.7
90	34.5	91.3
120	40.3	107.1
180	58.3	107.8

Tests for oral glucose tolerance in diabetic rats induced by STZ

After an overnight fast, six groups (n=6) of STZ-induced diabetic rats were divided for this study. The aim of the study was to compare the effects of different medications on fasting blood glucose levels for up to 120 minutes, including the commercially available glimepiride formulation, *B. serrata* extract SMEDDS, glimepiride SMEDDS alone, and their combinations. At time '0' (the initial fasting blood sample), blood samples from the rats' retro-orbital plexus were taken. Next, a 50% glucose load was administered at a dose of 2 mL/kg. Blood sugar levels were tested 15, 30, 60, 90, and 120 minutes after the treatment (Table 9).

Compared to other time points during the pharmacodynamic examination, the mean blood glucose levels and the %change in glucose level at 60 minutes were significantly greater for all groups. The data demonstrated that the combination of Glimepiride and *B. serrata* extract SMEDDS in pre-treated groups exhibited the most significant control over serum glucose levels concerning their initial values, with only a 26% increase. In comparison, the marketed glimepiride formulation showed a 76% increase, the control group exhibited a 38% increase, and the diabetic group showed a substantial 85%

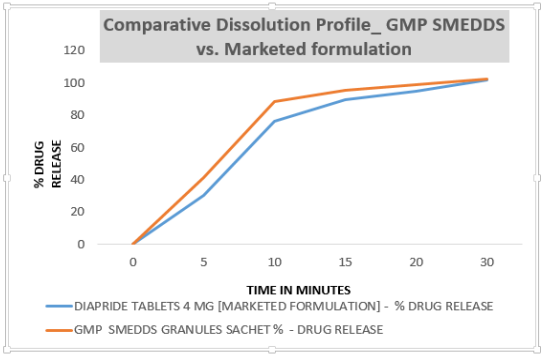

Fig. 15: Comparative dissolution profile of glimepiride SMEDDS and glimepiride tablets marketed formulation

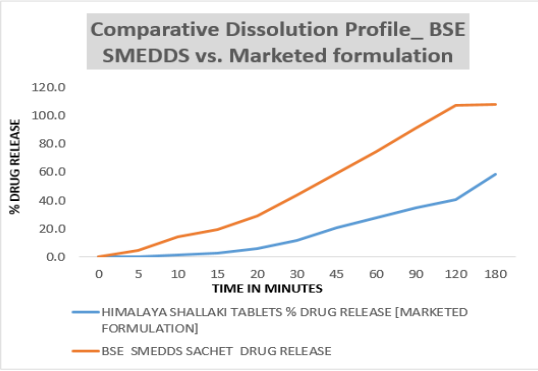

Fig. 16: Comparative dissolution profile of *B. serrata* extract (BSE) SMEDDS and *B. serrata* extract tablets marketed formulation

increase. The enhancement in the hypoglycemic action, particularly in diabetic rats, was more pronounced when glimepiride SMEDDS and *B. serrata* extract SMEDDS were co-administered. This combined treatment demonstrated an increased ability of glimepiride SMEDDS to lower glucose in diabetic rats as compared to when each drug was administered alone or compared to the control group. These results indicate a synergistic effect on glucose reduction when glimepiride SMEDDS is used in combination with *B. serrata* extract SMEDDS (Fig. 17).

Histopathology of Kidney and Pancreas

In the histopathology study of the kidney, various groups were examined. The research paper investigates the histopathological effects of a novel combination of glimepiride and *B. serrata* extract SMEDDS on kidney and pancreas tissues in diabetic rats. The study focused on evaluating the protective effects of this formulation against diabetes-induced damage.

Reviewing the histopathology data reveals that the novel formulation combining glimepiride and *B. serrata* extract SMEDDS demonstrates protective effects against diabetes-induced damage to both kidney and pancreas tissues. The pancreas of rats treated with this formulation showed a preserved architecture, while the presence of mitotic suggests potential regenerative processes (Fig. 18 and 19).

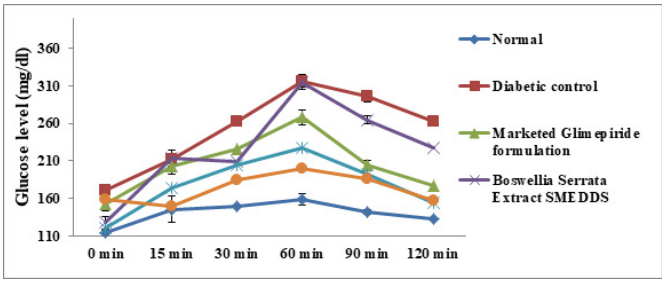

Fig. 17: Effect of various formulations on blood sugar levels (mg/dl) at various times

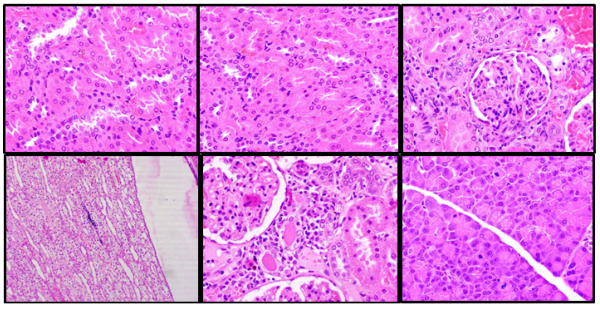

Fig. 18: Histopathology of Kidney tissues of rats treated with, (a): Normal control group, (b): Diabetic control, (c): Glimepiride SMEDDS, (d): *B. serrata* extract SMEDDS group, (e): Marketed glimepiride formulation group, (f): Glimepiride and *B. serrata* extract SMEDDS group.

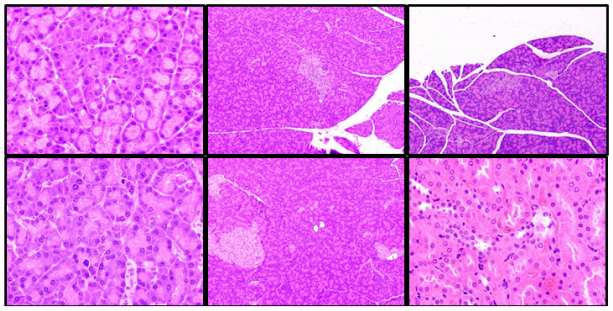

Fig. 19: Histopathology of pancreas tissues of rats treated with, (a): Normal control group, (b): Diabetic control, (c): Glimepiride SMEDDS, (d): *B. serrata* extract SMEDDS group, (e): Marketed glimepiride formulation group, (f): Glimepiride and *B. serrata* extract SMEDDS group.

Table 8: Effect of formulation on SGOT and SGPT level

After 28th Day of treatment	Group treated with various formulations and observed results					
	Normal	Diabetic control	Marketed glimepiride formulation	*B. serrata* extract SMEDDS	Glimepiride SMEDDS	Glimepiride + *B. serrata* extract SMEDDS
SGOT (mg/dl)	21.91	45.22	32.10	33.14	29.69	26.71
SGPT (mg/dl)	23.35	46.63	35.43	31.88	27.39	26.65