Original Research Article (Experimental)

Pharmacodynamic evaluation of self micro-emulsifying formulation of standardized extract of *Lagerstroemia speciosa* for antidiabetic activity

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**A B S T R A C T**

Background: *Lagerstroemia speciosa* (SEL) leaves are a popular folk medicine for diabetes treatment due to presence of corosolic acid. It has low water solubility resulting poor absorption after oral administration. Self micro-emulsified drug delivery system is the way by which we can improve the oral absorption of drug.

Objective: The objective of this study was to develop the self micro-emulsifying formulation of standardized extract of SEL leaves and evaluate its pharmacodynamic performance for antidiabetic activity.

Materials and methods: The SME formulation was prepared by using sefsof-218 as oil, cremophor-EL as surfactant and transcutol-P as co-surfactant. The ratio of surfactant and co-surfactant was determined by pseudoternary phase diagram. SME formulations were characterized for dilution at different pH, self emulsification, optical clarity, globule size and thermodynamic stability. Pharmacodynamic evaluation of formulations was assessed in Wistar rats by using parameters viz. blood glucose level and serum lipid profile.

Results: SEL loaded SME formulation was successfully developed by using sefsof-218, cremophor-EL and transcutol-P with a droplet size 23.53 nm. Pharmacodynamic results showed a higher reduction in blood glucose by SME formulation than SEL without SMES respectively at 50 mg/kg dose while reduction produced at dose of 100 mg/kg was found significant and better on 15th day of study. The percentage reduction produced by SME formulation on serum lipid profile was also significant and was more prominent than SEL.

Conclusion: This study confirms that the formulation elevates the pharmacodynamic performance of SEL approximately two fold.

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1. Introduction

Diabetes mellitus is a disorder of endocrine system in which there is lack of insulin release or the biological cells are not responding against insulin resulting in higher level of glucose in blood [1]. The World Health Organization data said that, in year 2000, the diabetic patients were 2.8% of the total world’s population and it may increase to 4.4% by 2030 becoming 7th leading cause of death [2,3]. It was estimated, in 2014, that about 8.5% adults aged 18+ years were diabetic globally [4]. The number of diabetic patients in India alone was 15 million in 1995 and the proposed increment in 2025 is 57 million [5]. The major cause for its prevalence in modern culture is due to decreased physical activity, obesity, stress, and changes in food consumption [6]. Diabetes is of two types, viz. type 1 and type 2. Type 1 diabetes (10% of total world population) often produces more serious consequences than type 2 diabetes (90% of total world population) and will be treated only by insulin injection while type 2 with oral hypoglycemic agents (OHAs) [7]. Several different types of OHAs are available in the market but there is a need for use of herbal products with antidiabetic potential because the cells may show resistance against OHAs [8]. For this, several phytomolecules have been isolated from plants and studied for antidiabetic activity. These phytomolecules have good clinical efficacy *in vitro* but may have no or less *in-vivo* actions. It may be due to its poor solubility/
dispersion in gastrointestinal tract (GIT) or not having ability to cross the biological membrane resulting in poor bioavailability [9].

Lagerstroemia speciosa leaves are a popular folk medicine for diabetes treatment [10]. A number of studies have shown that the corosolic acid, 2α-hydroxy ursolic acid (Fig. 1), present in L. speciosa promotes the absorption and utilization of glucose in the cells by induction of GLUT4 and has low water solubility, which leads to poor oral bioavailability, high inter and intra subject variability and lack of dose proportionality [11–13]. So it is necessary to draw attention on formulation approaches to enhance the bioavailability of poorly water-soluble active components, and furthermore obtain some more successful therapeutic effects.

Self microemulsified formulation is an approach for enhancing the absorption of poorly soluble phytomolecules due to their lipidic nature and small particle size [14]. Self microemulsified formulation is a mixture of water insoluble extract/phytomolecule, oil/lipid, surfactant and co-surfactant. After oral administration, they are diluted in aqueous media of GIT and form oil-in-water microemulsion/nanomulsion having particle size less than or equal to 100–500 nm. The energy required, for dispersion, will be provided by digestive motility. The formed microemulsion presents the phytomolecule in a dissolved form which is a premier requirement for poorly water soluble phytomolecule for absorption. Along with this, the specific lipid excipients of self microemulsified formulation promotes lymphatic transport of phytomolecules resulting in increase in bioavailability through first pass metabolism reduction. Another reason of increase in intracellular concentration of phytomolecules is due to reduction in strength of P-glycoprotein efflux system by used lipid and surfactant [15–20]. In this study, the self microemulsifying formulation of standardized extract of L. speciosa (SEL) leaf was prepared and characterized for dilution at different pH, self emulsification, optical clarity, globule size and thermodynamic stability. The optimized formulation was evaluated for its pharmacodynamic performance for antidiabetic activity in experimental animals.

2. Material and methods

2.1. Materials

Sample of Sefsol-218 was kindly gifted by Nikko Chemicals (Tokyo, Japan). Diethylene glycol monoethyl ether (Transcutol-P) was kindly provided by Gattefosse Corp. (France). Polyoxyxyl-35 castor oil (Cremophor-EL) and co-surfactant (Transcutol-P) in a glass vial. Pseudoternary phase diagram was constructed to determine the concentration of SME formulation components. Surfactant and co-surfactant (Smix) in each composition were mixed in different volume ratio (1:0, 2:1, 3:1, 1:1, 1:2, 1:3) in increasing concentration of surfactant with respect to co-surfactant and increasing concentration of co-surfactant with respect to surfactant for detailed study of the phase diagrams. For each phase diagram, oil and specific Smix ratio was mixed thoroughly in different volume ratio from 1:9 to 9:1 in different glass vials. The prepared mixture was vortexed and then titrated with water drop-wise. After each addition, mixtures were observed visually for phase separation or clarity.

2.2. Plant material

The leaves of L. speciosa were freshly collected from the roadside of Lucknow, Uttar Pradesh. The leaves were identified and authenticated taxonomically by Dr. A.K.S. Rawat, Scientist, Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute, Lucknow, India. The herbarium, (NBR/CIF/256/2011), was preserved at the department for future reference.

2.3. Preparation of extract

The matured leaves were collected, washed with distilled water to remove dirt and soil, and shade dried up-to 20–25 days. Routine pharmacognostic studies including organoleptic tests, macroscopic and microscopic observations were carried out to confirm the identity of the materials [21]. The dried materials were powdered by grinder and passed through a 10-mesh sieve. The coarsely powdered leaves were defatted by immersing the powder into petroleum ether up to 12 h by regular shaking. Extraction was done by hot continuous Soxhlet apparatus using 50% alcohol at 60 °C for 6 h. After extraction the excess solvent was removed by using a rotary evaporator (Buchi, USA) and then freeze-dried (Freezone™ 4.5, Labconco, USA) at high vacuum (133 × 10⁻³ mbar) and at temperature −40 ± 2 °C [22,23]; A net yield of 12.8 gm per 100 gm was obtained. The extract was also standardized (SEL) with Ultra Fast Liquid Chromatography (UFLC) for quantity of corosolic acid present in it [24].

2.4. Pre-formulation study for self microemulsifying system (SMES)

The selection of oil, used in SMES was based on solubility of herbal drug. The solubility provided by natural oils (such as castor oil, corn oil, olive oil, soybean oil and peanut oil) usually enhances the solubility of herbal drugs. However, their ability to solubilize herbal drugs cannot catch up with some medical liquid glycerin series (such as Labrafortm PG, Maisintm 35–1, Labrafac™, Lipophile WL 1349 and capryloltm 90), which can greatly improve the solubility of herbal drugs [15]. Hence, for this study a medical liquid glycerin, Sefsol-218, was used as oil. Pseudoternary phase diagram was constructed to determine the concentration of SME formulation components. Surfactant and co-surfactant (Smix) in each composition were mixed in different volume ratio (1:0, 2:1, 3:1, 1:1, 1:2, 1:3) in increasing concentration of surfactant with respect to co-surfactant and increasing concentration of co-surfactant with respect to surfactant for detailed study of the phase diagrams. For each phase diagram, oil and specific Smix ratio was mixed thoroughly in different volume ratio from 1:9 to 9:1 in different glass vials. The prepared mixture was vortexed and then titrated with water drop-wise. After each addition, mixtures were observed visually for phase separation or clarity.

2.5. Formulation study for self microemulsifying system

SMES (1 ml) were prepared by using oil (Sefsol-218), surfactant (Cremophor-EL) and co-surfactant (Transcutol-P) in a glass vial.

Table 1

| Composition | F1 | F2 | F3 | F4 |
|-------------|----|----|----|----|
| SEL (in mg) | 10 | 10 | 10 | 10 |
| Sefsol-218 (in % v/v) | 33.5 | 37.5 | 40 | 42 |
| Cremophor-EL (in % v/v) | 33.5 | 37.5 | 40 | 42 |
| Transcutol-P (in % v/v) | 33.5 | 37.5 | 40 | 42 |
Then the standardized extract of L. speciosa leaves (SEL) (10 mg) was mixed by gentle stirring. The mixture was vortexed and heated at 40 °C on water bath for 15 min. The prepared formulation was stored in tightly closed container at ambient conditions until further use. The composition of different formulations is summarized in Table 1.

2.6. Characterization of self microemulsifying (SME) formulations

2.6.1. Thermodynamic stability study

The objective of thermodynamic stability study was to evaluate the phase separation and effect of temperature variation on SME formulations. Different formulations were diluted with deionized water (1:20) and centrifuged at 3,000 rpm for 30 min, and observed visually for phase separation. Formulations that did not show any sign of phase separation after centrifugation were subjected to freeze–thaw cycles between (−20 °C and +25 °C) with storage at each temperature for not less than 48 h. Formulation that passed the test was used for further study [25].

2.6.2. Self-microemulsification efficiency and precipitation assessment

Self-emulsifying properties of the formulations were observed by visual assessment. Different SME formulations were categorized on the basis of emulsification time, clarity and precipitation. The assessment was performed by drop-wise addition of 0.2 ml of each SME formulation into 200 ml of purified water at room temperature and the mixture was gently stirred by magnetic stirrer at 100 rpm. The dispersibility of formulations were visually assessed using the following grading system: A, denoting a rapidly forming micro-emulsion within 1 min (clear or slightly bluish); B, rapidly forming microemulsion (slightly less clear had a bluish white appearance); C, microemulsion formed within 2 min (bright white emulsion); D, microemulsion formed in longer than 2 min (a dull, grayish white emulsion). The sign of precipitation of resultant microemulsion was noted after 24 h [26,27].

2.6.3. Spectroscopic characterization of optical clarity

The optical clarity of the aqueous dispersions of the SME formulations were measured spectroscopically by UV–visible spectrophotometer (UV-3200, Lab India) using distilled water as blank. Briefly, the sample was diluted in ratio of 1:20 with distilled water. The absorbance of each solution was measured at 638 nm [28].

2.6.4. Robustness to dilution

All the SME formulations were diluted to 10 and 100 times with distilled water, 0.1 N hydrochloric acid and 6.8 pH phosphate buffers, to evaluate the effect of volume and pH of dispersion medium. The diluted micro-emulsions were stored for 12 h and observed for any signs of phase separation or drug precipitation [29].

2.6.5. Globule size

SME formulations were diluted to 100 time with distilled water and diluted sample was subjected for globule size and polydispersity index (PDI) using Zetasizer (Model: Zetasizer NanoZS90, Malvern Ltd, UK) based on the 90° scattering angle using dynamic light scattering. Polydispersity index (PDI) provides pattern of size distribution and uniformity of size [30].

2.7. In-vivo pharmacodynamic study

The experimental protocol was approved by the Institutional Animal Ethics Committee. The guideline provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India (CPCSEA-837/ac/2004), on the use and care of experimental animals, was followed during the study. Wistar albino male rats (150 ± 20 g) were selected for pharmacodynamic study of L. speciosa leaves extract loaded SME formulation. Before conducting the experiment, animals were kept in departmental animal house for a period of 15 days at 25 ± 2 °C, with 12 h light and 12 h dark cycles. They were provided with standard pellet diet. The experiment was conducted in overnight fasting animals and only water was allowed ad libitum [31].

Diabetes was induced in overnight fasted male albino Wistar rats by a single intraperitoneal injection of 60 mg/kg body wt. STZ, dissolved in ice cold citrate buffer (0.1 M, pH 4.5) [32]. After STZ injection, 5% glucose was provided to the rats to prevent mortality associated with hyperinsulinemia. After 48 h of STZ injection, animals having more than 250 mg/dL but less than 350 mg/dL fasting blood glucose levels were selected for this experiment.

2.7.1. Experimental design

Total eight groups of animals (one group = 6 animals) were selected for study and treated as follows:

Group I: Normal Control

Group II: Diabetic Control

Group III: STZ + SEL (50 mg/kg, p.o.)

Group IV: STZ + SEL (100 mg/kg, p.o.)

Group V: STZ + SME formulation of SEL (equivalent to 50 mg/kg, p.o.)

Group VI: STZ + SME formulation of SEL (equivalent to 100 mg/kg, p.o.)

Group VII: STZ + Blank SME formulation (Placebo)

Group VIII: STZ + Glimepiride (0.5 mg/kg, p.o.)

2.7.2. Measurement of blood glucose levels

Blood sample for determination of glucose level was collected from tail-vein of normal and STZ induced diabetic rats on day zero (before the treatment), day 1, day 5, day 10 and on day 15. Glucose in blood was estimated by using standard kits from company, Accu-Chek Active, TM Roche Group, Germany.

2.7.3. Estimation of serum lipid profile

For estimation of serum lipid profile, all animals were sacrificed on 15th day of study at fasting state; whole blood was collected in centrifugation tube and serum was separated by centrifuging it at 5000 rotation/min for 15 min. The various parameters for estimation of serum lipid profiles were serum cholesterol (SC), triglyceride...
(ST) and high density lipoprotein (HDL) by using kit (Span diagnostic kit, Surat, Gujrat, India). Friedewald’s formula was used for estimation of very low density lipoprotein (VLDL) and low density lipoprotein (LDL) cholesterol [33].

2.8. Statistical analysis

The results were analyzed using one-way analysis of variance followed by Dunnett’s test using GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, California, USA). The data were expressed as mean ± SEM. The value of p < 0.05 was considered statistically significant.

3. Results

3.1. Standardization of extract

The SEL was standardized for corosolic acid and found that about 1% corosolic acid was present in it. The calibration curve of corosolic acid for quantitative analysis is shown in Fig. 2.

3.2. Pre-formulation study of SMES

The primary criterion for selection of the surfactant and cosurfactant was that they should be pharmaceutically acceptable for oral administration and should fall under generally recognized as safe (GRAS) category. Amongst all the surfactants, nonionic surfactants are less toxic and have lower CMCs than their ionic counterparts. Another important criterion for selection of the surfactants is that the required HLB value to form o/w nanoemulsion should be greater than 10 [26]. So Cremophor-EL having HLB value 14, was selected as surfactant. The presence of co-surfactants decreases the bending stress of interface and allows the interfacial film sufficient flexibility to take up different curvatures required to form nanoemulsion over a wide range of composition [34,35]. Thus, the co-surfactant selected for the study was Transcutol-P which again is a nonionic surfactant.

Pseudoternary phase diagram was constructed to determine the highest microemulsion region provided by Smix at different volume ratios (1:0, 2:1, 3:1, 1:1, 1:2, 1:3) of surfactant and co-surfactant. Based on diagram it was observed that the Smix in ratio 1:1 showed the maximum microemulsion area; hence it was selected as the optimal ratio for development of formulation.

3.3. Characterization of SME formulation

The result of different characterization parameters of SME formulations are listed in Table 2. Centrifugation and freeze-thaw tests were conducted to study thermodynamic stability and all formulations were found to have passed these tests. The self emulsification test result showed that the emulsification time was varying between 48 and 28 s with clarity of formulation between bluish to transparent. The UV absorbance at 638 nm varied between 0.06 and 0.01. The formulation F1 was found to be unstable in dilution test at different media while rest formulations were stable. The globule size and PDI of prepared SME formulations decreased as the concentration of surfactant was increased and the minimum size was observed for F3 formulation i.e. 23.53 nm and 0.138 respectively.

Table 2

Characterization of SMES.

| Parameters                                      | Formulation |
|-------------------------------------------------|-------------|
| Centrifugation test                             | F1          |
| Passed                                           | Passed      |
| Passed                                           | Passed      |
| Passed                                           | Passed      |
| Freez–thaw test                                 |             |
| Passed                                           | Passed      |
| Passed                                           | Passed      |
| Passed                                           | Passed      |
| Dispersibility grade                            | B           |
| A                                               |
| Emulsification time (s)                         | 48          |
| 36                                              |
| 28                                              |
| 32                                              |
| Precipitation                                   | Yes         |
| No                                              |
| No                                              |
| No                                              |
| UV Absorbance at 638 nm                         | 94.18       |
| 39.76                                           |
| 23.53                                           |
| 26.63                                           |
| Globule size (nm)                               | 0.234       |
| 0.226                                           |
| 0.138                                           |
| 0.188                                           |
| Dilution study in pH 6.8                         | 10 ml       |
| Unstable                                        |
| Stable                                          |
| Stable                                          |
| Stable                                          |
| Dilution study in pH 1.2                         | 100 ml      |
| Unstable                                        |
| Stable                                          |
| Stable                                          |
| Stable                                          |
| Dilution study in distilled water               |             |
| 10 ml                                           |
| Unstable                                        |
| Stable                                          |
| Stable                                          |
| Stable                                          |
| Dilution study in pH 6.8                         | 100 ml      |
| Unstable                                        |
| Stable                                          |
| Stable                                          |
| Stable                                          |

Table 3

Blood glucose level (mg/dL) in STZ induced diabetic rats.

| Group   | 0 Days   | Day 1   | Day 5   | Day 10  | Day 15  |
|---------|----------|---------|---------|---------|---------|
|         | 82.6 ± 1.76 | 84.1 ± 2.01 | 83.5 ± 1.23 | 84.8 ± 1.47 | 85.3 ± 2.03 |
| II      | 292.8 ± 2.01* | 295.5 ± 2.00* | 301.7 ± 1.84* | 313.2 ± 2.04* | 322.1 ± 3.84* |
| III     | 293.5 ± 4.29 | 287.5 ± 3.71| 269.3 ± 3.78* | 253.2 ± 4.21* | 239.5 ± 2.76* |
| IV      | 283.8 ± 9.91 | 269.5 ± 3.80* | 242.3 ± 4.76* | 220.8 ± 3.44* | 200.1 ± 4.85* |
| V       | 294 ± 3.62   | 268.5 ± 5.90* | 252.0 ± 4.51*** | 208.4 ± 6.87*** | 175.7 ± 3.92*** |
| VI      | 299.3 ± 4.16 | 254.2 ± 6.64* | 210.0 ± 4.68*** | 180.3 ± 12.51*** | 121.9 ± 3.57*** |
| VII     | 291.5 ± 3.38 | 298.1 ± 1.94 | 303.2 ± 4.06 | 310.9 ± 2.94 | 320 ± 2.89 |
| VIII    | 286.5 ± 5.54 | 135.5 ± 5.92* | 118.8 ± 10.74*** | 110.1 ± 5.24*** | 90.5 ± 4.19*** |

Values are mean ± Standard error of mean (SEM); n = 6 in each group. Results of test and standard group were compared with diabetic control. *p < 0.05, ***p < 0.001.

Table 4

Effect of L. speciosa extract and drug formulation on serum lipid profile on diabetic rats.

| Group   | Serum cholesterol (mg/dL) | Triglyceride (mg/dL) | VLDL (mg/dL) | HDL (mg/dL) | LDL (mg/dL) |
|---------|---------------------------|----------------------|--------------|-------------|-------------|
| I       | 118.5 ± 2.28              | 92.83 ± 1.80         | 18.57 ± 0.36 | 81.88 ± 5.76 | 18.06 ± 5.48 |
| II      | 197.70 ± 8.99             | 213.92 ± 10.41       | 42.78 ± 2.08 | 42.33 ± 4.22 | 112.58 ± 10.47 |
| III     | 178.88 ± 8.99             | 187.17 ± 9.80        | 37.43 ± 1.96 | 52.70 ± 3.04 | 88.75 ± 9.22 |
| IV      | 165.17 ± 4.47             | 174.67 ± 6.04        | 34.93 ± 1.20 | 53.83 ± 3.40 | 76.40 ± 7.03 |
| V       | 136.05 ± 4.50***          | 144.50 ± 3.58        | 28.90 ± 0.72 | 62.25 ± 2.14** | 44.90 ± 4.25 |
| VI      | 130.21 ± 1.91             | 136.67 ± 6.01        | 27.33 ± 1.05 | 65.50 ± 5.58* | 37.38 ± 5.52 |
| VII     | 196.42 ± 5.60             | 212.50 ± 5.34        | 42.50 ± 1.07 | 42.67 ± 2.54 | 111.26 ± 3.46 |
| VIII    | 124.47 ± 4.00***          | 105.17 ± 5.54        | 21.03 ± 0.78 | 80.83 ± 2.65*** | 22.60 ± 4.80 |

Values are mean ± Standard error of mean (SEM); n = 6 in each group. Results of test and standard group were compared with diabetic control. *p < 0.05, **p < 0.01, ***p < 0.001.
3.4. Blood glucose level

The results of fasting blood glucose levels of various groups of rats are summarized in Table 3. It was found that the blood sugar level was continuously increasing from day 0 to day 15 in group II (diabetic control) i.e. from 292.8 ± 2.01 to 322.1 ± 3.84 mg/dL compared to group I animals (Normal Control). On comparison with group II, SEL without formulation, at two different doses (50 mg/kg and 100 mg/kg, p.o.), shows antihyperglycemic effect i.e. from 293.5 ± 4.29 to 239.5 ± 2.76 mg/dL (p < 0.001), and 283.8 ± 9.91 to 200.1 ± 4.85 mg/dL (p < 0.001), for group III and IV while the reduction in blood glucose level produced by SME formulation was found 294.5 ± 3.62 to 175.7 ± 3.92 mg/dL, and 299.3 ± 4.16 to 121.9 ± 3.57 mg/dL for group V and VI respectively on 15th day of study. The reduction produced by SME formulation was significantly higher (p < 0.001), than SEL without SME formulation. The reduction of blood glucose level was in time and dose dependent manner i.e. higher the dose higher was the reduction.

The result of comparison with placebo group showed reducing effect produced by the standardized extract (SEL). However, the reduction in blood glucose by Glimepiride (group VIII) was as good as effect produced by the standardized extract (SEL). However, the manner i.e. higher the dose higher was the reduction.

The results of serum lipid profile of various groups of rats are summarized in Table 4. After 15-day treatment, comparison of group III, IV, V and VI against group II showed the lipid-lowering effect of SEL and its formulation. After treatment with SEL without SME formulation at doses of 50 mg/kg (group III) and 100 mg/kg (group IV) p.o., the significant reductions in SC was 9.51%, 16.4%, while 12.5%, 18.34% reduction was found in ST. Also, there was increase in HDL (24.4%, 27.16%) in SEL without formulation treated diabetic rats. On comparison of SME formulation with control rats, the significant (p < 0.001) reductions in SC was 9.51%, 16.4%, and ST (32.4%, 36.11%) were found at doses equivalent to 50 mg/kg (group V) and 100 mg/kg (group VI) respectively. Also, there was a significant (p < 0.01) increase in (47.0%, 54.7%) HDL in SME formulation treated diabetic rats. The reduction in serum lipid profile produced by formulation of SEL was significantly higher (p < 0.01–0.001) than SEL without SME formulation on comparison between both of them. The serum lipid profile of standardized extract with formulation is comparable with standard drug Glimepiride.

3.5. Serum lipid profile

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4. Discussion

This study was conducted to evaluate the in-vivo antidiabetic performance of SME formulation of SEL. The SME formulations were formed when a particular concentration of oil and Smix are combined. The concentration of surfactant and co-surfactant (Smix) was determined by pseudoternary phase diagram. The combination of Sefsol 218, Cremophor-EL and Transcutol-P as oil, surfactant and co-surfactant was capable for producing microemulsion region. When Smix ratio was 1:0 the system was turbid and unable to flow while the flowability was increased by using Smix 1:1. This behavior may be due to greater penetration of the oil phase in hydrophobic region of surfactant monomer resulting in decrease in interfacial tension [36]. On further increasing the co-surfactant concentration in Smix 1:2 and 1:3, the total microemulsion area was found to be decreased as compared to 1:1 ratio. On the contrary, when the surfactant concentration was increased keeping co-surfactant concentration constant, Smix ratio 2:1 and 3:1, the microemulsion region was again found to be decreased due to increased surfactant character. So with the help of phase diagram study it was found that the Smix ratio 1:1 showed maximum microemulsion region; hence this ratio was selected for formulation development. By keeping in mind that large amount of surfactant may cause gastric irritation the formulation should be developed with minimum quantity of surfactant. The formula for different developed formulations is given in Table 1. After preparation, the formulations were characterized for, thermodynamic stability, self-microemulsification efficiency and precipitation assessment, spectroscopic characterization of optical clarity, robustness to dilution, globule size and polydispersity index.

Since the phase separation may occur due to variation in storage temperature, therefore, the formulations were subjected to thermodynamic stability study. All formulations passed the test; this shows that the formulations were physically stable. This means that the concentration of Smix is appropriate for maintaining the oil in solubilized state.

The self emulsification assessment was conducted without any external energy source. The SME formulation should disperse completely and quickly in GIT under mild agitation provided by peristaltic activity. It has been reported that self emulsification mechanism involves the erosion of a fine cloud of small droplets from the monolayer around emulsion droplets, rather than progressive reduction in droplet size. The ease of emulsification was suggested to be related to the ease of water penetration into the colloidal or gel phases formed on the surface of the droplet [37].

The result showed that the formulation F3 provides minimum emulsification time with no sign of precipitation after 24 h.

The spectroscopic absorbance of the aqueous dispersions of the F3 formulation was found minimum among all formulations. The compositions with the lower absorbance values showed the smallest droplet size because aqueous dispersions with small absorbance values are optically clear, and oil droplets are thought to be in a state of finer dispersion [38].

The effects of GI pH and volume on SME formulation were determined by dilution test. It was observed that the formulation F1 was found to be unstable while all others were stable. This may be due to presence of higher quantity of oil which could not be emulsifying properly by the provided Smix.

Globule size is a decisive factor in self-emulsification performance of formulation. It determines the rate and extent of drug release as well as drug absorption. The small size of microemulsion globules may lead to more rapid absorption, thereby improving the bioavailability [15]. From Table 2, it can be seen that the formulation F3 has smallest droplet size i.e. 23.53 nm.

PDI is the ratio of standard deviation to mean droplet size, which signifies uniformity of droplet size within the formulation. The higher the value of PDI, the lower is the uniformity of droplet size [39]. Results revealed that the formulation F3 has lowest PDI i.e. 0.138 means, the globules were uniformly distributed in formulation.

The result of different formulations in Table 1 has showed that the formulation F3 possesses all characteristics of SMES; hence it was selected for pharmacodynamic evaluation.

The antidiabetic activity of SEL loaded SME formulation and the SEL were evaluated by blood glucose level and serum lipid profile. The primary mechanism in diabetes mellitus involves the overproduction and decreased utilization of glucose by the tissues [40]. STZ is a slightly cytotoxic agent of pancreatic β-cells and selectively destroys the pancreatic insulin secreting β-cells, leaving less active cells and resulting in a diabetic state [41–43]. Hence STZ, at a dose of 60 mg/kg (i.p.), produced significant (p < 0.001) rise in fasting blood glucose levels in STZ treated groups on comparison with normal rats. After treatment with SEL in group III and IV and its SME formulation in group V and VI, produced significant (p < 0.001) lowering of blood glucose levels on comparison with diabetic
control on day 15. The percentage reduction in blood glucose produced by SEL at 50 mg/kg was 18.37% whereas its formulation reduces 40.23% whereas the reduction produced by SEL at 100 mg/kg was 29.48% with its formulation reduces 59.30% at 15th day of study. The reduction in blood glucose level was approximately two folds higher with formulation as compared with extract without formulation. The self microemulsified formulation, after oral administration, produces microemulsion and higher pharmacodynamic response of the formulation may be due to any reason as presentation of extract in solubilized form, reduced particle size enhances interfacial area for absorption, improve dissolution in the presence of Cremophor-EL, and Transcutol-P. SMES also plays an important role in improvement of permeability through intestinal membrane due to presence of Cremophor-EL, and Transcutol-P as these components have the ability to interfere with the lipid bi-layer of the epithelial cell membrane and inhibit the activity of P-glycoprotein efflux system [14,44–46].

In diabetic patients, hyperglycemia is associated with dyslipidemia due to the inhibited actions of lipolytic hormones on the fat deposits, mainly due to the action of insulin [32]. The dyslipidemia is characterized by increase in SC, ST, LDL, VLDL, and fall in HDL [47,48].

5. Conclusion

SMES is successfully attracting attention to improve the bioavailability of lipid soluble drugs. The result of study reveals higher pharmacodynamic activity with an optimized SMES formulation consisting of Selosil-218 (20% v/v), Cremophor-EL (40% v/v) and Transcutol-P (40% v/v) as compared to standardized extract without formulation. Therefore, SMES formulation shows a promising approach for enhancement of the in vivo performance of standardized L. speciosa leaf extract.

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Conflict of interest

None

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