Journal of Ayurveda and Integrative Medicine 9 (2018) 38–44

Contents lists available at ScienceDirect

Journal of Ayurveda and Integrative Medicine

journal homepage: http://elsevier.com/locate/jaim

Original Research Article (Experimental)

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

Vipin Kumar Agarwal [a], Gupta Amresh [b, *], Phool Chandra [c]

[a] Department of Pharmaceutics, Invertis Institute of Pharmacy, Invertis University, NH 24 Lucknow Bareilly Highway, Bareilly, Uttar Pradesh, India
[b] Department of Pharmacognosy, Faculty of Pharmacy, Uttar Pradesh University of Medical Sciences, Saifai, Etawah, Uttar Pradesh, India
[c] Department of Physiology & Pharmacology, School of Pharmaceutical Sciences, IFTM University, Moradabad, Uttar Pradesh, India

ARTICLE INFO

Article history:
Received 23 December 2016
Received in revised form
31 January 2017
Accepted 11 February 2017
Available online 8 December 2017

Keywords:
Self micro-emulsifying formulation
Lagerstroemia speciosa
antidiabetic activity

ABSTRACT

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 sefsol-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 sefsol-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.

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/

* Corresponding author
E-mail: amreshgupta@gmail.com
Peer review under responsibility of Transdisciplinary University, Bangalore.

http://dx.doi.org/10.1016/j.jaim.2017.02.007

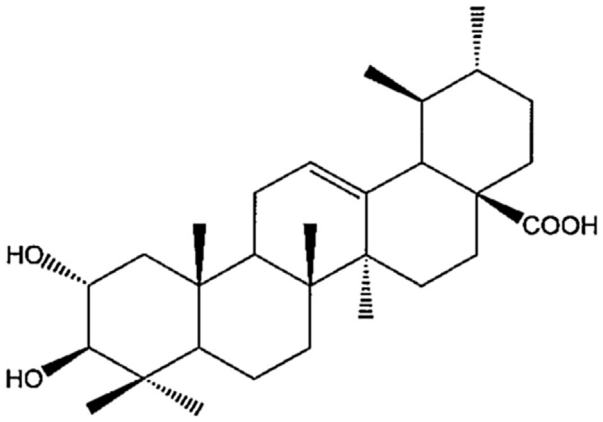

Fig. 1. Structure of corosolic acid.

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 poor 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/nanoemulsion 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 phytomolecule 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). Polyoxyl-35 castor oil (Cremophor-EL) was obtained as gift sample from BASF Co. (Germany). Streptozotocin (STZ) was purchased from Himedia Laboratories Pvt. Ltd (Mumbai, India). Glimepiride was obtained as a gift sample from USV Pvt. Ltd. (Baddi, India). All other chemicals and reagents used were of analytical grade.

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, (NBRI/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 upto 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 Labrafac™ PG, Maisine™ 35-1, Labrafac™, Lipophile WL 1349 and capryol™ 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 of different formulation.

Composition	Formulation			
	F1	F2	F3	F4
SEL (in mg)	10	10	10	10
Sefsol-218 (in % v/v)	33	25	20	16
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 poly-dispersity 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

STZ induced diabetic rats were divided into seven groups (Groups II–VIII).

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

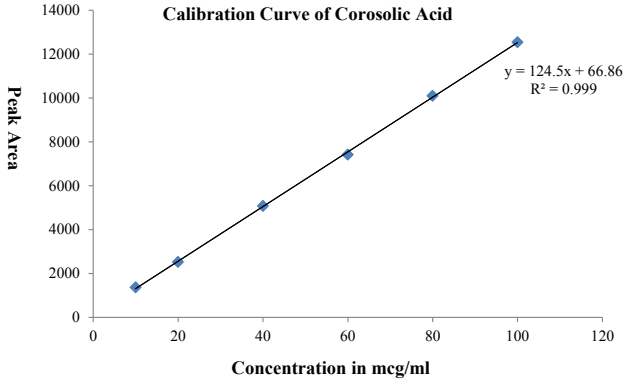

Fig. 2. Calibration curve of corosolic acid for its quantification.

Table 2
Characterization of SMES.

Parameters		Formulation			
		F1	F2	F3	F4
Centrifugation test		Passed	Passed	Passed	Passed
Freez–thaw test		Passed	Passed	Passed	Passed
Dispersibility grade		B	A	A	A
Emulsification time (s)		48	36	28	32
Precipitation		Yes	No	No	No
UV Absorbance at 638 nm		0.06	0.033	0.01	0.056
Globule size (nm)		94.18	39.76	23.53	26.63
PDI		0.234	0.226	0.138	0.188
Dilution study in distilled water	10 ml	Unstable	Stable	Stable	Stable
	100 ml	Unstable	Stable	Stable	Stable
Dilution study in pH 1.2	10 ml	Unstable	Stable	Stable	Stable
	100 ml	Unstable	Stable	Stable	Sable
Dilution study in pH 6.8	10 ml	Unstable	Stable	Stable	Stable
	100 ml	Unstable	Stable	Stable	Stable

(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 co-surfactant 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 3
Blood glucose level (mg/dL) in STZ induced diabetic rats.

Group	0 Days	Day 1	Day 5	Day 10	Day 15
I	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 ± 5.60***	252.0 ± 4.51***	208.4 ± 6.67***	175.7 ± 3.92***
VI	299.3 ± 4.16	254.2 ± 8.64***	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 ± 6.01*	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 ± 9.54***	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 SME formulation i.e. from 286.5 ± 5.54 to 90.5 ± 4.19 mg/dL.

3.5. Serum lipid profile

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 (31.18%, 34.13%) 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.

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% while its formulation reduces 40.23% whereas the reduction produced by SEL at 100 mg/kg was 29.48% compared 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 uninhibited actions of lipolytic hormones on the fat depots, 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].

In the study SC, ST, LDL, VLDL was found to be significantly ($p < 0.001$) higher in STZ control group and in placebo group as compared to normal control group. Treatment with SME formulation of SEL significantly ($p < 0.001$) brought their levels towards normal level while SEL without formulation brought it at significant value $p < 0.05$ only. The concentrations of VLDL and LDL were also reduced and approached toward normal value as compared with diabetic control. Total HDL was found to be significantly decreased in group II ($p < 0.001$) as compared with group I and its value was found to increase after treatment with SME formulation compared to SEL, in diabetic animals at $p < 0.01$ significant value.

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 SME formulation consisting of Sefsol-218 (20% v/v), Cremophor-EL (40% v/v) and Transcutol-P (40% v/v) as compared to standardized extract without formulation. Therefore, SME formulation shows a promising approach for enhancement of the *in vivo* performance of standardized *L. speciosa* leaf extract.

Sources of funding

None

Conflict of interest

None

References

[1] Verma N, Amresh G, Sahu PK, Mishra N, Singh AP, Rao ChV. Antihyperglycemic activity, antihyperlipedemic activity, haematological effects and histopathological analysis of *Sapindus mukorossi* Gaerten fruits in streptozotocin induced diabetic rats. Asian Pac J Trop Med 2012;5(7):518–22.
[2] Sabitha V, Ramachandran S, Naveen KR, Panneerselvam K. Antidiabetic and antihyperlipidemic potential of *Abelmoschus esculentus* (L.) Moench. in streptozotocin-induced diabetic rats. J Pharm Bioallied Sci 2011;3(3):397–402.
[3] Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med 2006;3(11):2011–30.
[4] Global report on diabetes. Geneva: World Health Organization; 2016. p. 6. Available from: http://apps.who.int/iris/bitstream/10665/204871/1/9789241565257_eng.pdf.
[5] Sikarwar MS, Patil MB. Antidiabetic activity of *Crateva nurvala* stem bark extracts in alloxan-induced diabetic rats. J Pharm Bioallied Sci 2010;2(1):18–21.
[6] Shastri K. Comments on Charaka Samhita. Varanasi: Chanukah bharati; 1980. p. 22.
[7] Modak M, Dixit P, Londhe J, Ghaskadbi S, Devasagayam TPA. Indian herbs and herbal drugs used for the treatment of diabetes. J Clin Biochem Nutr 2007;40(3):163–73.
[8] Mohan V. Evaluation of Diabecon (D-400) as an antidiabetic agent - a double-blind placebo - controlled trial in NIDDM patients with secondary failure to oral drugs. Indian J Clin Pract 1998;8(9):18–25.
[9] Kesarwani K, Gupta R. Bioavailability enhancers of herbal origin: an overview. Asian Pac J Trop Biomed 2013;3(4):253–66.
[10] Judy WV, Hari SP, Stogsdill WW, Judy JS, Naquib YMA, Passwater R. Antidiabetic activity of a standardized extract (Glucosol) from *Lagerstroemia speciosa* leaves in Type II diabetics: a dose-dependence study. J Ethnopharmacol 2003;87(1):115–7.
[11] Park C, Lee JS. Banaba: the natural remedy as antidiabetic drug. Biomed Res 2011;22(2):125–9.
[12] Udell RG, Hari SP. Corosolic acid formulation and its application for weight loss management and blood sugar balance. US Application Publication; 2004; April, 15. US 2004/0072901.
[13] Fukushima M, Matsuyama F, Ueda N, Egawa K, Takemoto J, Kajimoto Y, et al. Effect of corosolic acid on postchallenge plasma glucose levels. Diabetes Res Clin Pract 2006;73(2):174–7.
[14] Gursoy RN, Benita S. Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs. Biomed Pharmacother 2004;58(3):173–82.
[15] Zhang L, Zhang L, Zhang M, Pang Y, Li Z, Zhao A, et al. Self-emulsifying drug delivery system and the applications in herbal drugs. Drug Deliv 2015;22(4):475–86.
[16] Humberstone AJ, Charman WN. Lipid-based vehicles for the oral delivery of poorly water soluble drugs. Adv Drug Deliv Rev 1997;25(1):103–28.
[17] O'Driscoll CM. Lipid-based formulations for intestinal lymphatic delivery. Eur J Pharm Sci 2002;15(5):405–15.
[18] Swenson ES, Curatolo WJ. (C) Means to enhance penetration: (2) Intestinal permeability enhancement for proteins, peptides and other polar drugs: mechanisms and potential toxicity. Adv Drug Deliv Rev 1992;8(1):39–92.
[19] Haus DJ, Mehta SC, Radebaugh GW. Targeting lymphatic transport and modified systemic distribution of Cl-976, a lipophilic lipid-regulator drug, via a formulation approach. Int J Pharm 1994;108(2):85–93.
[20] Porter CJH, Charman WN. *In vitro* assessment of oral lipid based formulations. Adv Drug Deliv Rev 2001;50(Suppl):S127–47.
[21] Amresh G, Rao ChV, Mehrotra S, Shirwaikar A. Standardization and ethnopharmacological evaluation of antidiarrhoeal herbal formulation. Manipal: Manipal Academy of Higher Education; 2003. p. 24–40 (Dissertation).
[22] Rao ChV, Amresh, Kartik R, Irfan A, Rawat AKS, Pushpangadan P. Protective effect of *Aegle marmelos* fruit in gastrointestinal dysfunction in rats. Pharm Biol 2003;41(8):558–63.
[23] Amresh G, Kant R, Rao ChV, Singh PN. Chemomodulatory influence of *Cissampelos pareira* (L.) Hirsuta on gastric cancer and antioxidant system in experimental animal. Acta Pharm Sci 2007;49(1):71–83.
[24] Vijaykumar K, Murthy PB, Kannababu S, Syamasundar B, Subbaraju GV. Quantitative determination of corosolic acid in *Lagerstroemia speciosa* leaves, extracts and dosage forms. Int J Appl Sci Eng 2006;4(2):103–14.
[25] Singh AK, Chaurasiya A, Awasthi A, Mishra G, Asati D, Khar RK, et al. Oral bioavailability enhancement of exemestane from self-microemulsifying drug delivery system (SMEDDS). AAPS Pharm Sci Tech 2009;10(3):906–16.
[26] Kommuru TR, Gurley B, Khan MA, Reddy IK. Self-emulsifying drug delivery systems (SEDDS) of coenzyme Q10: formulation development and bioavailability assessment. Int J Pharm 2001;212(2):233–46.
[27] Patel AR, Vavia PR. Preparation and *in-vivo* evaluation of SMEDDS (self-microemulsifying drug delivery system) containing fenofibrate. AAPS J 2007;9(3):344–52.
[28] Fahmy UA, Ahmed O, Hosny KM. Development and evaluation of avanafil self-nanoemulsifying drug delivery system with rapid onset of action and enhanced bioavailability. AAPS Pharm Sci Tech 2015;16(1):53–8.
[29] Dixit AR, Rajput SJ, Patel SG. Preparation and bioavailability assessment of SMEDDS containing Valsartan. AAPS Pharm Sci Tech 2010;11(1):314–21.
[30] Kadu PJ, Kushare SS, Thacker DD, Gattani SG. Enhancement of oral bioavailability of atorvastatin calcium by self-emulsifying drug delivery systems (SEDDS). Pharm Dev Technol 2011;16(1):65–74.
[31] Ved A, Gupta A, Rawat AKS. Antioxidant and hepatoprotective potential of phenol-rich fraction of *Juniperus communis* Linn. leaves. Pharmacogn Mag 2017;13(49):108–13.
[32] Verma N, Amresh G, Sahu PK, Mishra N, Rao ChV, Singh AP. Pharmacological evaluation of hyperin for antihyperglycemic activity and effect on lipid profile in diabetic rats. Indian J Exp Biol 2013;51(1):65–72.
[33] Naresh KR, Sundaram R, Shanthi P, Sachdanandam P. Protective role of 20-OH ecdysone on lipid profile and tissue fatty acid changes in streptozotocin induced diabetic rats. Eur J Pharmacol 2013;698(1–3):489–98.
[34] Eccleston J. Microemulsions. In: Swarbrick J, Boylan JC, editors. Encyclopedia of pharmaceutical technology, vol. 9. New York: Marcel Dekker; 1994. p. 375–421.
[35] Kawakami K, Yoshikawa T, Moroto Y, Kanaoka E, Takahashi K, Nishihara Y, et al. Microemulsion formulation for enhanced absorption of poorly soluble drugs I. Prescription design. J Control Release 2002;81(1–2):65–74.

[36] Shafiq S, Shakeel F, Talegaonkar S, Ahmad FJ, Khar RK, Ali M. Development and bioavailability assessment of ramipril nanoemulsion formulation. Eur J Pharm Biopharm 2007;66(2):227–43.
[37] Yadav PK, Yadav E, Verma A, Amin S. *In-vitro* characterization and pharmacodynamic evaluation of furosemide loaded self nano emulsifying drug delivery systems (SNEDDS). J Pharm Invest 2014;44(6):443–53.
[38] Basalious EB, Shawky N, Badr-Eldin SM. SNEDDS containing bioenhancers for improvement of dissolution and oral absorption of lacidipine. I: development and optimization. Int J Pharm 2010;391:203–11.
[39] Date AA, Desai N, Dixit R, Nagarsenker M. Self-nanoemulsifying drug delivery systems: formulation insights, applications and advances. Nanomed 2010;5(10):1595–616.
[40] Latner A. Clinical bio chemistry. Philadelphia: Saunders; 1958. p. 48.
[41] Elsner M, Guldbakke B, Tiedge M, Munday R, Lenzen S. Relative importance of transport and alkylation for pancreatic beta-cell toxicity of streptozotocin. Diabetalogia 2000;43(12):1528–33.
[42] Verma N, Amresh G, Sahu PK, Rao ChV, Singh AP. Antihyperglycemic and antihyperlipidemic activity of ethyl acetate fraction of *Rhododendron arboreum* Smith flowers in streptozotocin induced diabetic rats and its role in regulating carbohydrate metabolism. Asian Pac J Trop Biomed 2012;2(9):696–701.
[43] Verma N, Amresh G, Sahu PK, Rao ChV, Singh AP. Antihyperglycemic activity of *Woodfordia fruticosa* (Kurz) flowers extracts in glucose metabolism and lipid peroxidation in streptozotocin-induced diabetic rats. Indian J Exp Biol 2012;50(5):351–8.
[44] Porter CJH, Pouton CW, Cuine JF, Charman WN. Enhancing intestinal drug solubilisation using lipid-based delivery systems. Adv Drug Deliv Rev 2008;60(6):673–91.
[45] Tang J, Sun J, He ZG. Self-emulsifying drug delivery systems: strategy for improving oral delivery of poorly soluble drugs. Curr Drug Ther 2007;2(1):85–93.
[46] Singh B, Bandopadhyay S, Kapil R, Singh R, Katare OP. Self-emulsifying drug delivery systems (SEDDS): formulation development, characterization, and applications. Crit Rev Ther Drug Carr Syst 2009;26(5):427–521.
[47] Zeashan H, Amresh G, Singh S, Rao Ch V. Protective effect of *Amaranthus spinosus* against D-galactosamine/lipopolysaccharide induced hepatic failure. Pharm Biol 2010;48(10):1157–63.
[48] Tiwary BK, Dutta S, Dey P, Hossain M, Kumar A, Bihani S, et al. Radical scavenging activities of *Lagerstroemia speciosa* (L.) Pers. Petal extracts and its hepato-protection in CCl_4-intoxicated mice. BMC Complement Altern Med 2017 Jan 18;17(1):17–55.