RESEARCH ARTICLE

Development and Characterization of Ethanolic Extract of *Cleome gynandra L. Loaded* Solid Lipid Nanoparticles for the Management of Diabetes Mellitus

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ABSTRACT

The bioactive components of the botanical remedy *Cleome gynandra* L., which is famous for its antidiabetic effects, were extracted using ethanol. To improve its stability, bioavailability, and therapeutic potential, the extract was subsequently encapsulated within solid lipid nanoparticles (SLNs) using a high-pressure homogenization method with varying concentrations of lipids. Zeta potential, entrapment efficiency, kinetics of drug release, and particle size were among the numerous characteristics of the SLNs that were thoroughly examined. One important measure for determining whether bioactive compounds were successfully encapsulated within the SLNs is the entrapment efficiency. An impressive entrapment efficiency of more than 82.13% was shown by the optimized formulation F4. The optimized formulation F4 exhibited a mean particle size ranging from 243.5 to 261.7 nm, according to particle size analysis. This size range is ideal for improving cellular uptake and bioavailability. A negative charge was observed in the zeta potential measurements, which means that the SLNs are stable and do not aggregate. A PDI of 0.416 provided additional evidence of the nanoparticles' stability and uniformity. A sustained release of about 62.57% over 7 hours was shown in the *in-vitro* drug release profile data, indicating a long-lasting therapeutic effect. Improved therapeutic efficacy of antidiabetic treatment and more stable blood glucose levels are both benefits of the sustained release profile. The *in-vitro* results of formulation F4 suggest that SLNs have the good stability, bioavailability, and therapeutic potential of *C. gynandra* L.'s ethanolic extract when used as a delivery system.

Keywords: *Cleome gynandra L.*, Solid lipid nanoparticles, Ethanolic extract, Antidiabetic activity, Diabetes mellitus.

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INTRODUCTION

Diabetes mellitus is characterized by hyperglycemia and glucose intolerance, stemming from either a relative deficiency in insulin secretion or impaired insulin action that inhibits glucose uptake. It is a chronic medical condition that, although manageable, persists throughout one's lifetime. Chronic hyperglycemia associated with diabetes leads to long-term damage and dysfunction in various organs, notably the kidneys, eyes, heart, blood vessels, and nerves. While type 1 and 2 diabetes differ in their pathological processes, they both share hyperglycemia as a common hallmark. In type 2 diabetes, hyperglycemia arises from insulin secretion impairment, with or without concomitant insulin action impairment. The World Health Organization has declared a global diabetes epidemic affecting the worldwide population.

The African spider plant, also known as *Cleome gynandra*, is highly valued in many African and Asian regions for its traditional medicinal properties. "It is a member of the Cleome genus, which is part of the Cleomaceae family."[1] This family, formerly known as Capparaceae, includes a wide variety of flowering plants that are mainly found in tropical and subtropical regions around the world. In addition to its medicinal properties, Cleome is also grown for its edible greens. *C. gynandra* is a particularly popular variety for this purpose. These leafy greens offer a wide range of nutritional benefits, making them a valuable source of nourishment worldwide.[2,3]

MATERIALS AND METHODS

The Botanical Survey of India, Coimbatore, confirmed in May 2019 that "fresh aerial parts of *C. gynandra* L." "Specimens

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were collected from Kalapatti, Coimbatore, Tamil Nadu, India" "(BSI/SRC/5/23/2019/Tech)".

Plant Material Extraction

An initial 48-hour extraction with petroleum ether was performed on one kilogram of finely ground *C. gynandra* L. powder using a soxhlet apparatus.[4,5] The subsequent 48-hour extraction with ethanol was then carried out. The extracts were dried at room temperature until they became a thick solid after being concentrated in different amounts. The raw extracts were weighed after collection and stored at 40°C for further analysis.

Formulation of *C. gynandra* L. Extract-Loaded Solid Lipid Nanoparticle (Table 1)

Hybridization with ultrasonication and heat allowed for the preparation of solid lipid nanoparticles that were loaded with extract from *C. gynandra* L. After dissolving the solid lipid and stearic acid in a 1:1 mixture of methanol and chloroform, the ethanolic extract of *C. gynandra* L. (EECG) was added using the heat homogenization technique. To get rid of any organic solvents that were left, the solution was allowed to evaporate completely.[6,7] The remaining residue had a melting point that was about 5 to 10°C greater than the lipids. At the same time, 100 mL of a 2% aqueous surfactant solution and the lipid phase were heated. When the lipid phase and the surfactant solution reached the same temperature, the heated surfactant solution was added to it. Achieving homogeneity was accomplished using a high-speed homogenizer that ran for three hours at 15,000 rpm. The temperature was meticulously maintained 5 to 10°C higher than the lipid's melting point to prevent recrystallization. After the coarse emulsion had cooled to room temperature, it was homogenized and then ultrasonicated using a probe sonicator for 30 minutes.[8]

Characterization of the Prepared SLN

Entrapment efficiency

At the detected wavelength, the spectrophotometric determination of the entrapped extract percentage was made. Centrifugation of the water solution at 15,000 rpm for 15 minutes yielded the amount of unbound drug in the supernatant. After subtracting the amount of unbound drug from the initial drug amount, the amount of encapsulated drug was determined.

Table 1: Formulation of *C. gynandra* L. extract-loaded solid lipid nanoparticle

Formulation code	*Lipid (g)*	*Drug (extract) g*	*Surfactant (2% of Tween 80)*
F1	2 (Stearic acid)	1	2
F2	4 (Stearic acid)	1	2
F3	6 (Stearic acid)	1	2
F4	8 (Stearic acid	1	2
B1	2 (Beeswax)	1	2
B2	4 (Beeswax)	1	2
B3	6 (Beeswax)	1	2
B4	8 (Beeswax)	1	2
C	-	-	2

Particle size and zeta potential analysis

A Malvern Zetasizer and photon correlation spectroscopy were utilized to ascertain the particle size, with the dynamic light scattering methodology serving as the basis. Additionally, the zeta potential of the core particles was determined by applying an electric field and the electrophoretic mobility principle. The optimal formulation was selected from the array and subjected to further characterization based on the results of the particle size analysis. Formulations F1–F4 and B1–B5 were analyzed using the Malvern Zetasizer.[9]

Scanning electron microscopy

Electron microscopy "(Zeiss, Jena, Germany) was used to examine the surface morphology of the solid lipid nanoparticle (SLN) that was prepared." The specimen was attached to adhesive tape after being freeze-dried. Next, the tape was placed on the scanning electron microscope (SEM) and viewed at a low voltage of 20 kV.[10]

X-Ray diffractometry

This analysis helps determine the crystal lattice, phase, and planes of solid lipid nanoparticles.

In-vitro drug release[11]

A dissolution medium of phosphate buffer with a pH of 7.4 was used in the 24-hour in-vitro drug release experiment. The mixture was transferred to a "small cylinder coated with 12000 Da cellophane membranes and left to incubate at 37°C with continuous stirring using a magnetic stirrer in 50 mL of pH 7.4 aqueous buffer. Every time a new batch of the medium was added, two milliliters of the sample was taken. A UV spectrophotometer was used to measure the absorbance of the extracted samples to determine the amount of drug that was released.

Drug release kinetic studies

A thorough evaluation of the drug's release is necessary to clarify the product, assure consistency in pharmacological and biological effects from batch to batch, and establish reliability in the formulation. A variety of models were employed for the dissolution data analysis, including the Higuchi, Korsmeyer-Peppas, zero-order, and first-order rate equations. Each rate order had its own correlation coefficient (R2).

RESULTS AND DISCUSSION

Calibration Curve

Partitions of "1, 2, 3, 4, and 5 µg/mL of standard solution were carefully added" to a series of 5 mL calibrated flasks while the rest of the volume was filled with methanol. At 272 nm, the absorbance of the resulting solution was compared to that of a blank. The calibration curve was made using a plot of absorbance against concentration.

Table 2: Entrapment efficiency of SLN

S. No	*Formulation code*	*%Entrapment efficiency*
1	B1	52.13
2	B2	49.98
3	B3	35.26
4	B4	28.42
5	F1	78.52
6	F2	65.85
7	F3	46.34
8	F4	82.13

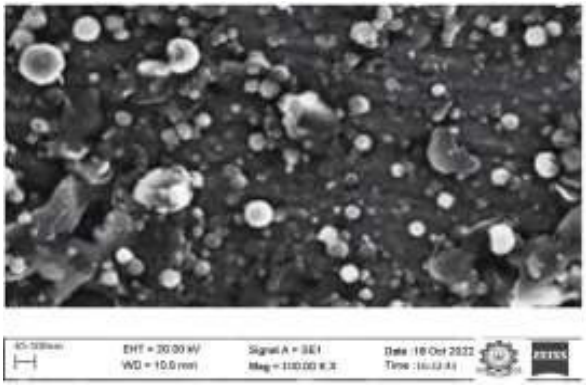

Figure 1: Scanning electron microscopy

Entrapment Efficiency (Table 2)

The entrapment efficiency of the nanosuspension ranges from 52.13% to 82.13%. Formulation F4 exhibited the highest entrapment efficiency (82.13%) among all formulations.

Scanning Electron Microscopy

Most of the formed particles were round, and their sizes were rather evenly distributed in the results. The SLNs surface morphology seemed uniform and smooth. Particles ranged in size from 80 to 110 nm. The absence of lumps or aggregations in the SLNs was also noted, as shown in Figure 1.

Particle Size and Zeta Potential Analysis (Table 3)

The formulations that were developed had particle sizes ranging from 243.5 to 591.4 nm. The average particle size for F4 was 243.5 nm, with a PDI of 0.416 and a zeta potential of 0.0147 mV.

Table 3: Particle and zeta potential of SLN

S. No.	*Formulation code*	*Average particle size (nm)*	*Zeta potential (mV)*	*Polydispersity index (PdI)*
1	B1	591.4	0.00118	0.706
4	B2	1062	-0.00306	1.000
7	B3		0.0424	
10	B4	597.9	-0.0926	0.764
13	F1	1998	0.0329	1
16	F2	437.1	4.954	0.628
19	F3	326.	0.0531	0.581
22	F4	261.7	0.130	0.498

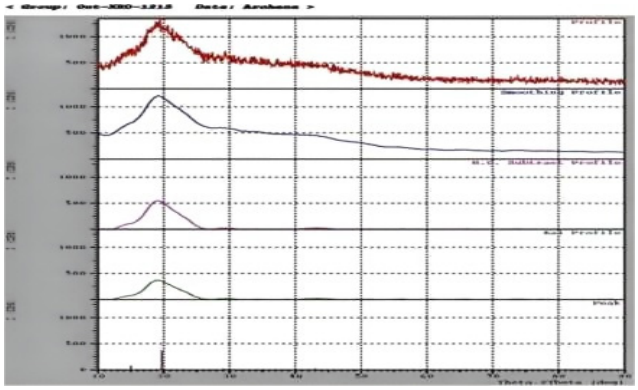

Figure 2: X-ray diffraction spectrum

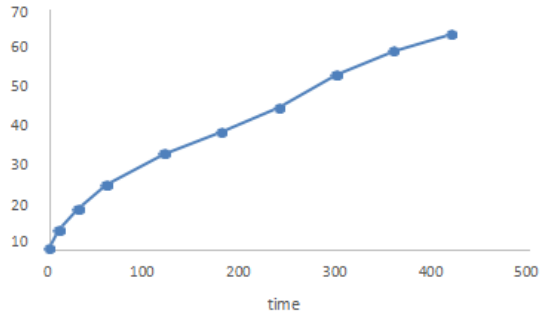

Figure 3: *In-vitro* release graph of F4 formulation of *C. gynandra L* extract loaded SLN

Table 4: *In-vitro* release for the F4 formulation of *C. gynandra L* extract loaded SLNs

Time in Hours	*%CDR*
0.25	5.428
0.5	11.55231
1	18.65356
2	27.6331
3	34.08273
4	41.23054
5	50.81923
6	57.6895
7	62.578

Table 5: Release kinetics of F4 formulation of *C. gynandra* L. extract loaded SLNs

Release kinetics	*Zero order* r^2	*First order*^{2}	*Higuchi* r^2	*Koresmeyer* r^2
SLN	0.9917	0.8829	0.994	0.8921

X-ray Diffractometry

The X-ray diffraction patterns of the SLN loaded with *C. gynandra* L. extract" did not exhibit any distinct peaks, indicating that the formulations were amorphous. The

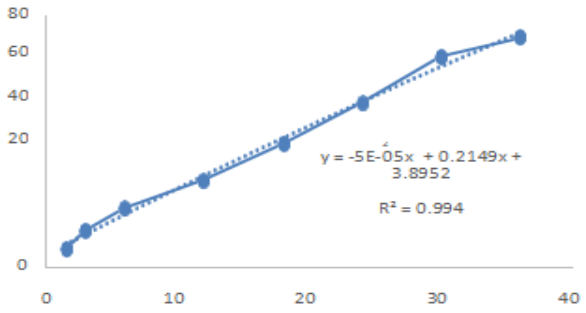

Figure 4: Drug release graph of F4 formulation of *C. gynandra L* extract loaded SLNs

diffractograms indicated a lack of clearly defined distinct characteristics in terms of crystallinity. The extract's structure was indicated by the broad diffractogram, which also verified its dispersion throughout the SLN. The measurement was conducted with a step time of 0.24 s and a scanning rate of 0.02° 20 s^{-1}, spanning an angular range of 4 to 50°. Examining the diffraction patterns in counts per step using Topas software (Bruker, AXS) allowed for the determination of the crystallinity (polymorphism) and orientation of polymers. Crystalline materials exhibit unique X-ray diffraction patterns, showcasing clearly defined edges and faces that effectively diffract X-rays. Additionally, they possess precise melting points. However, amorphous solids exhibit irregular or curved surfaces and lack distinct peaks in their spectra, as shown in Figure 2.

In-vitro Drug Release (Table 4)

The formulation (F4) demonstrated a drug release of 62.57% within 7 hours, indicating a rapid and effective release of the drug using the dissolution apparatus. With such a high percentage of drug release in a relatively short period, it appears that the formulation possesses favorable attributes for drug delivery, as displayed in Figure 3.

Kinetic Modeling

Analyzed the percentage cumulative drug release (Figure 4) data using various mathematical models to investigate the drug release pattern and mechanism. Here are the figures displaying the Higuchi matrix, zero order, first order, and Koresmeyer Peppas plots. Here are the regression coefficient (r2) values for each formulation, as shown in the table below. The formulations displayed a remarkably strong correlation in the Higuchi plot. The formulation adheres to Higuchi release kinetics, as indicated by the results of the release kinetics analysis (Table 5). Based on an impressive r2 value of 0.994, Higuchi's formula reveals that the drug is released from the matrix through a time-dependent square root process. The diffusion of the non-Fickian Super Case II type drives this process.

CONCLUSION

This research successfully developed and evaluated SLNs containing *C. gynandra* L. extract as a promising new formulation for the treatment of diabetes. Formulation F4 showed an impressive entrapment efficiency, with a peak rate of 82.13%, indicating the successful encapsulation of the *C. gynandra* L. extract within the SLNs. The optimized formulation (F4) showed an average particle size of 243.5 nm, which falls within the ideal range for enhanced drug delivery. Based on the X-ray diffraction (XRD) analysis, it was found that the SLNs possess amorphous characteristics. This suggests that the SLNs have improved drug stability and dissolution properties. The drug release study showed that F4 had a sustained release pattern, with over 62% of the drug being released within a 7-hour period. Through kinetic modeling, it was discovered that the drug release followed the Higuchi model, indicating a mechanism controlled by diffusion. These findings suggest that the use of *C. gynandra* L. extracted-loaded SLNs could be a promising approach for managing diabetes.

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