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Original Article

FORMULATION, CHARACTERIZATION AND EVALUATION OF DAIDZEIN-LOADED SOLID-LIPID NANOPARTICLES GEL FOR ANTI-INFLAMMATORY POTENTIAL

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ABSTRACT

Objective: Daidzein is a natural isoflavone present in various leguminous plants. It has broad therapeutic effects, including cardioprotective, anticancer, antidiabetic, anti-inflammatory, and anti-oxidative effects. Objective of present study was to enhance the solubility and bioavailability of daidzein through the development of Solid-Lipid Nanoparticle (SLN) gel formulation for anti-inflammatory effect.

Methods: Daidzein-loaded SLNs were prepared using ultrasonication solvent emulsification method and optimized SLNs were converted into gel using Carbopol 934. Prepared Daidzein Loaded Solid Lipid Nanoparticles (DAID–SLNs) gel was characterized for drug release, pH, viscosity and spreadability. DAID–SLNs gel was further evaluated for *in vitro* anti-inflammatory effect using albumin denaturation inhibition, anti-proteinase assay and anti-lipoxygenase assay.

Results: Optimized SLNs were successfully converted into a homogeneous, semisolid gel with good spreadability. The percentage drug release was 61.2±3.01 in 180 min, while nanoparticle formulation DAID-SLNs showed 70.9±3.18 percentage release in 180 min. Optimized formulation showed small particle size (193.62±5.89 nm), polydispersity Index (0.21±0.42) and increased Zeta potential with negatively charged (-33.17±1.24 mV), which showed good physical stability of the formulation. *In vitro* anti-inflammatory effect in protein denaturation inhibition assay of DAID–SLNs loaded gel showed with 91.42±3.18% was comparable to Standard Diclofenac sodium showed 95.24±3.85%. Proteinase inhibitory effect at concentration of 100µg/ml was showed 92.46±3.45% that was comparable to the standard Diclofenac sodium (98.28±3.72 %).

Conclusion: Daidzein-loaded solid lipid nanoparticles gel was found effective for treatment of inflammation through inhibition of protein denaturation and essential enzymes proteinase as well as lipoxygenase.

Keywords: Daidzein, Anti-inflammatory, Solid lipid nanoparticles, Isoflavone, Gel, Diclofenac sodium

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INTRODUCTION

The most effective way to administer medication to address problems connected to the skin is through topical application. With dermal and transdermal drug delivery, topical drug delivery gives both local and systemic effects, avoids first-pass metabolism, enhances patient compliance, and allows for self-administration. The main challenge in topical delivery is to overcome the skin protective barriers [1]. To overcome the barrier properties of the skin and to enhance the delivery of drugs through topical route, various novel delivery systems such as liposomes, niosomes, microemulsions and polymeric nanoparticles have been introduced. Amongst these particulate systems, Solid Lipid Nanoparticles (SLNs) have gained significant interest in both pharmaceutical applications and cosmetics.

SLN comprises range of biocompatible lipids such as triglycerides, alkanoic acid, cetyl palmitic, and various synthetic or natural lipophilic compounds. As a result, SLN have shown great promise as pharmacological carriers and are appropriate for the oral and topical bioavailability of particular biocompatible drugs. They are cheaper and easier to scale up than other polymeric nanoparticles. Lipid nanoparticles are non-toxic, easy to manufacture, and biocompatible; they can be used to deliver both small molecule drugs and advanced biologics than the liposomes. Small particle size range of SLN is one of the major advantages over microsphere and liposomes [2].

For topical application, solid lipid nanoparticles offer a promising method of drug delivery. Better penetration through the stratum cornea and effective medication deposition in the dermal matrix were the goals of their formulation. They were also assessed for rheological behavior, pharmacokinetic and pharmacodynamic studies, and *in vitro* drug release characteristics. They demonstrated good physical stability, high entrapment efficiency, and controlled drug release, making them a viable carrier for topical drug delivery [3].

Daidzein (DAID) (7-hydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one) is a natural isoflavone present as a glucoside in leguminous plants, especially in soybean plant. DAID exhibits a variety of beneficial effects on human health; therefore, it is a compound of great clinical and pharmacological interest. Its broad therapeutic activities include cardioprotective, anticancer (prostate, ovary, colon, lung, breast, bladder) [4], anti-allergic, antidiabetic, anti-inflammatory, and anti-oxidative effects [5]. DAID is a significant candidate for an experimental lead molecule in therapeutic development because of these activities and minimal toxicity. However, because of its limited solubility, DAID has a lower pharmacological benefit rate and a higher dose. Low oral bioavailability of DAID appears to be the main cause for the unfavorable physicochemical characteristics, which include low solubility, a low partition coefficient and possibly a strong metabolism in the liver and intestines [6].

Thus, it implies that daidzein's poor solubility takes precedence over first-pass metabolism, and as a result, its low solubility is responsible for its bioavailability. So, its poor lipophilicity and hydrophilicity brings a major challenge in pharmaceutical applications for this molecule. The production of solid lipid nanoparticles as topical gel was a highly suitable strategy to improve their solubility and bioavailability. We hypothesize that daidzein-loaded SLN gel will exhibit enhanced anti-inflammatory effects compared to conventional formulations. The objective of present study was to prepare daidzein-loaded solid lipid nanoparticle gel formulation to treat inflammation using different *in vitro* assays.

MATERIALS AND METHODS

Materials

Daidzein drug was purchased from Yucca Enterprises,Mumbai. Glycerol Monostearate, Tween 80, Lecithin, polyethylene glycol 400, Carbopol 934, were procured from Loba Chemie Pvt. Ltd., Mumbai,

India. The orthophosphoric acid (HPLC grade 88%), acetonitrile (HPLC grade, 99.9%) and methanol (HPLC grade, 99.9%), chloroform, ethyl alcohol were obtained from Merck Specialties Pvt Ltd., Mumbai, India. Furthermore, all the other chemicals, reagents, solvents were used in the present study of analytical grade.

Preparation of daidzein-loaded solid-lipid nanoparticles (SLNs)

Daidzein-loaded SLNs were prepared using ultrasonication solvent emulsification method as describe previously by Wang *et al.* [7] with slight modification. Based on the solubility study of Daidzein, Glycerol Monostearate as the lipid phase and methanol used as a solvent, Tween 80 as a surfactant were optimized with an objective to get an average size below 200 nm (data not shown). The desired amount of Daidzein (50 mg) was dissolved in Methanol at 70 °C and added to molten lipids mixture of Glycerol Monostearate and Lecithin as lipid phase (1:1). Lipids were melted above to their melting point. Aqueous solution of surfactant (2% Tween 80) was heated up to same temperature as lipid phase and blended drop wise with the lipid phase under continue stirring. The resulting dispersion was sonicated using Ultra-Turrax® (IKA India Private Limited) for 10 min at 20000 rpm to get primary emulsion. This primary emulsion was subjected to process for high pressure homogenizer (1000 bars) up to 10 cycles to obtained finally Daidzein loaded SLNs by allowing the hot nanoemulsions to cool to room temperature. The dispersion was filtered through 0.45 μm filter in order to remove any impurity. Blank solid liquid nanoparticle was prepared in similar way without adding the daidzein.

Characterization of prepared daidzein loaded solid-lipid nanoparticles

Because of the dynamic nature of the delivery system, the size, complexity, and nature of the particles, physical and chemical characterization is necessary following the development of SLNs. The parameters needed to evaluate SLNs include particle size, zeta potential, polydispersity index, drug release, Entrapment Efficiency (% EE) and surface morphology [8].

Determination of particle size, polydispersity index (PDI), and zeta potential

The average particle size, Polydispersity (PDI), and zeta potential of SLNs were determined by a dynamic light scattering analyzer (Zetasizer Nano ZS90; Malvern Instruments, Malvern, UK) at room temperature, 230 V and 50 HZ. All SLNs samples were diluted 10 times with distilled water to obtain the optimal intensity before measured at room temperature. Each measurement was performed in triplicate manner [9, 10].

Drug entrapment efficacy

In order to know the drug entrapment efficiency, the amount of daidzein incorporated in SLNs was determined using HPLC (1220 infinity LC, Agilent Technologies, United Kingdom). Briefly, 10 mg of SLNs was added to 20 ml of methanol. After lipids were dissolved when heated to 65 °C, the solution was cooled to room temperature and centrifuged at 20,000 rpm for 10 min to remove Lipid. The supernatant (10 ml) was directly injected into HPLC with C18 column, the mobile phase: acetonitrile-water (65:35), detection wavelength: 249 nm, the flow rate: 1 ml/min. The drug entrapment efficiency was defined as the percentage of daidzein recovered from SLNs compared with the initial drug amount. The drug loading was calculated as the amount entrapped daidzein compared with the total amount of SLNs [11].

Fourier transform infrared spectroscopy

FT-IR (Cary 630, Agilent Technologies, United Kingdom) analysis was used to investigate the drug, lipid, and surfactant compatibility and records the distinctive peaks of the infrared spectroscopy of Daidzein, Tween 80, and Glycerol monosterate. The sample was first mixed with 1% KBr and then pressed. It was then scanned with a wavenumber range of 400–4000 cm^{-1}wavenumber [12].

Surface morphology

The morphological characters of Daidzein powder and the lyophilized SLNs formulations were examined using Transmission electron microscopy (Jeol/JEM 2100, USA). A drop of diluted nanosuspension (with distilled water) was applied to a carbon-coated copper grid. The negative stain was used with 2% phosphotungstic acid and kept for 30 second. The grid was analyzed using the Transmission Electron Microscope with an accelerating voltage of 60–80 kV [5].

Stability study

The developed DAID-SLNs were stored at different temperature up to three months as per International Conference on Harmonization (ICH) guidelines. Sufficient quantity of optimized formulations were kept at 40 °C±2 °C/75%±5% RH in humidity chamber, at room temperature 25 °C±2 °C/60%±5% RH and under refrigeration (5 °C±2 °C). The formulation sample was removed after 1, 30, 60, and 90 days. The samples were taken out at prearranged intervals and examined for changes in particle size and PDI over time [13].

Preparation of daidzein-loaded SLN (DAID-SLNs) gel

Carbopol 934 was used as the gelling agent for converting the DAID-SLNs dispersion into a gel carrier system. Distilled water was added to 1% w/w Carbopol 934, and the mixture was swirled for 10 min at 1500 rpm. After adding the appropriate amount of freshly made DAID-SLNs dispersion and mixing for ten minutes, drops of triethanolamine were added to adjust the pH to 5.5. In order to release trapped air, the prepared gels were left to stand for an additional night and proceed further for characterization [3].

Characterization of DAID-SLNs gel

DAID–SLNs loaded gel was characterized for drug release, pH, viscosity and spreadability. Every test was performed in triplicate manner.

***In vitro* release study**

The dialysis bag diffusion method was used to conduct the *in vitro* release investigation. Using a thermal shaker bath system (Thermo Shaker Incubator BSTH-103, Biolab Scientific Ltd. Canada), the HPLC method was used to measure the rate at which daidzein was released from the nanoparticles, which was maintained at 37 °C and shaken horizontally at 300 rpm. A dialysis membrane bag with a molecular cut-off of 3500 Da containing 1 mg of pure daidzein or freeze-dried nanoparticles gel was used [5]. All sample groups were in sink condition with this amount of drug and each group of samples was immersed in 30 ml of phosphate buffer solution (pH 7.4). After a particular time, interval (0, 1, 2, 4, 6, 12, 24, 36, 48, 60, and 72 h), 1 ml of the dissolution medium was withdrawn and the nanoparticles were resuspended in medium which was replaced with equal volume of fresh dissolution medium at the same temperature. The released daidzein in the dissolution media was determined by HPLC. Measurements were performed in triplicate for each batch.

pH of gel

Accurately weigh the amount of gel (1g) and dissolved in 100 ml of purified water and stored for two hour. The three samples were checked for pH using digital pH meter (Labindia Analytical Instruments Pvt Ltd.).

Homogeneity and viscosity

After the gels were placed in the container, a visual inspection was used to verify that all of the generated gels were homogenous. A Brookfield digital viscometer was utilized to gauge the viscosity of gel compositions that were created at 10 rpm, spindle number six was turned. The reading, near to 100% torque was noted.

Spreadability study

Spreadability was measured with a device that had 11 pulleys at the bottom and a wooden block on top. The "slip" and "drag" principles were employed to gauge the gel's spreadability. A 2 g gel was applied on the ground slide. Entrapped air between the slides was eliminated and another glass slide was added on top of the ground slide. For 5 min, a weight of 1 kg was placed on the slide. The excess gel was removed from the slide's corner. When an 80g weight was applied to the pulley, the top slide's time to travel 7.5 cm was recorded. A short interval indicates better spreadability [14]. Spreadability was then calculated using the following formula:

$$S = \frac{M \times L}{T}$$

Where, S: Spreadability; M: Weight in the pan (tied to the upper slide); L: Is the length moved by the glass slide and; T: The time taken to separate the slide completely from each other

***In vitro* anti-inflammatory activity of DAID-SLNs gel**

Albumin denaturation inhibition assay

The anti-inflammatory activity of prepared DAID-SLNs Gel was studied with slight modification in the reported method [15]. The protocol was followed for inhibiting albumin denaturation. Any anti-inflammatory drugs help in prevention of the protein or albumin denaturation, which acts as antigens and prompts autoimmune diseases and; hence, the present study was undertaken. The reaction mixture contains fresh prepared 0.2 ml of egg albumin, 2.8 ml of phosphate-buffered saline (pH 6.4) and DAID-SLNs Gel (0.6 ml) with different concentrations in DMSO. The concentrations of DAID-SLNs Gel in the total reaction solution ranged from 20-100 µg/ml. The sample mixture was incubated at 37 °C for 20 min before being heated to 51 °C for another 20 min to induce denaturation of the egg albumin. Diclofenac sodium salt and double-distilled water were used as positive and negative controls, respectively. After cooling the mixture, the absorbance was measured at 660 nm. The controls group contains similar ingredients except the drug formulation including 0.2 ml of fresh egg albumin, 0.6 ml of DMSO, and 2.8 ml of phosphate-buffered saline. The percentage of protein denaturation inhibition, which indicates the anti-inflammatory activity of the DAID-SLNs Gel, was calculated by the following equation:

$$\text{Percent Inhibition} = \frac{\text{As} - \text{Ac}}{\text{Ac}} \times 100$$

Where, As = absorbance of sample, Ac = absorbance of control

Anti-proteinase assay

Proteases inhibition assay is directly related to anti-inflammatory activity because protease enzymes can hydrolyze peptide bonds and degrading other proteins. They also can cause inflammation by controlling the expression and activity of pro-inflammatory cytokines, chemokines, and other immune components. Proteinase inhibitory effect of DAID-SLNs Gel was determined by some modification in the reported method of Gunathilake *et al.*, [16]. In brief, the reaction mixture consists of trypsin (0.06 mg), 1 ml of 20 mmol Tris-HCl buffer (pH 7.4), and 1 ml DAID-SLNs Gel solution (in DMSO). The mixture was incubated (37 °C for 5 min), and then 1 ml of casein (0.8% w/v) was added, and the mixture was further incubated for an additional 20 min. Two milliliters of 70% perchloric acid were added to stop the reaction at the end of the incubation period. After the mixture was centrifuged, the percentage of proteases inhibition was calculated by measuring the absorbance of the supernatant at 210 nm against a blank solution.

Anti-lipoxygenase assay

The assay for lipoxygenase inhibition was employed as a screening method to evaluate the anti-inflammatory properties of different test drug substance. Lipoxygenase inhibition activity of the prepared DAID-SLNs Gel was assayed as per reported method of Wu [17], with slight modification. In brief, the lipoxygenase enzyme (10 µl) was incubated with the mixture of gel sample in 0.2 M borate buffer (pH 8.7) up to 5 min for its inhibition determination. Then, the addition of linoleic acid initiated the process. A difference in absorbance was observed between the test group and the control, which contain only buffer at 234 nm at room temperature.

Statistical analysis

All the results obtained from this study are expressed as mean±SEM. Probability values of $P<0.05$ were considered to be statistically significant. The variation between mean values determined via one-way Analysis Of Variance (ANOVA) and multiple comparisons using Tukey's test. Graphpad instat software was used for statistical analysis of the results.

RESULTS AND DISCUSSION

Preparation and characterization of daidzein-loaded solid lipid nanoparticles (DAID-SLNs)

An essential stage in the development of solid lipid nanoparticles is the appropriate selection of the solid lipid phase. The solubility and stability of the components served as the primary selection criteria. The most crucial factor in ensuring that the drug stays in its dissolved state in the SLN formulation is its solubility in lipids. Out of the various lipids employed, the maximum solubility of Daidzein was observed in methanol. Tween 80 was used as surfactant. After screening different concentration of solid lipid mixture and surfactants, Daidzein loaded solid lipid nanoparticles were prepared by the ultrasonication solvent emulsification method.

Observation of particle size, polydispersity index (PDI), and zeta potential

Particle sizes of prepared DAID-SLNs were found between 193.62±5.89 to 307.19±8.32 nm (table 1). Zeta potential is a surface characterization technique that aids in figuring out the surface charge and potential stability of nanoparticulates system. Large values of the zeta potential, either positive or negative, are typically needed for the stability of SLNs because they prevent particle aggregation by electrostatically repelling particles that have the same charge. Despite the slight differences in zeta values-21mV to-33 mV were observed in all DAID-SLNs formulations. SLNF3 formulation showed less particle size (193.62±5.89 nm), polydispersity Index (0.21±0.42) and increased Zeta potential with negatively charged (-33.17±1.24 mV) particles, which prevents particle aggregation or coalescence and ensures good physical stability of the formulation through electrostatic repulsion.

Drug entrapment efficacy

Various factors were investigated to attain optimal drug encapsulation efficiency, such as the nature and quantity of lipids and surfactants. The corresponding percentage of entrapment efficiency of DAID-SLNs was found satisfactory high which is ranging from 59.03±1.82 to 75.44±1.21% (table 1). Out of all formulations, SLNF3 formulation showed highest entrapment efficiency 75.44±2.21%.

Table 1: Observation of different formulations with their droplet size, polydispersity index, zeta potential and entrapment efficiency

Formulations	Mean particle size (nm)	Polydispersity Index (PDI)	Zeta potential (mV)	Entrapment Efficiency (%)
SLNF1	307.19±8.32	0.39±0.24	-21.52±1.76	62.28±2.08
SLNF2	292.28±7.79	0.38±0.12	-22.92±1.66	59.03±1.82
SLNF3	193.62±5.89	0.21±0.42	-33.17±1.24	75.44±2.21
SLNF4	282.74±6.77	0.45±0.32	-31.71±1.40	64.52±2.22

Data represent as mean±SD, n =3

Fourier transform infrared spectroscopy (FTIR)

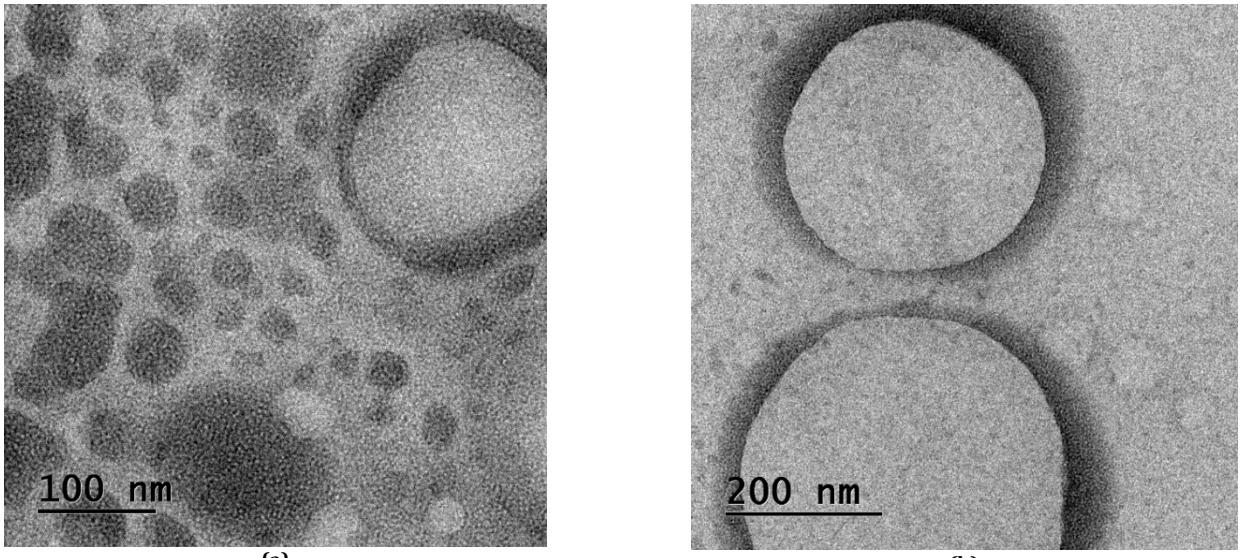
Observation of FT-IR spectra of Daidzein and prepared formulations represent to the information of different functional group by using infrared scattered light. The observations record the characteristic peaks of the different functional groups of Daidzein and Glycerol monosterate. In the FTIR spectrum of Daidzein, several characteristics peaks such as at about 3222 cm^{-1} (represent to-OH group) stretching vibration), 2836 cm^{-1} (assigned to-CH stretching vibrations), 1632 cm^{-1}(due to-C=O stretching vibrations) and 1596 cm^{-1}(assigned to-C=C vibration). It showed that there was no significant interaction between Daidzein and the excipients. FTIR spectral data were also found similar major characteristic peaks in the reported literature [18].

Surface morphology

Study of surface morphology of prepared DAID-SLNs was done by, Transmission Electron Microscopy (TEM). Photomicrograph of prepared DAID-SLNs formulations was found to be spherical in shape with a smooth surface. There is no aggregation of nano particles was appeared and uniformly dispersed in the media (fig. 1). This indicates that the employed method of preparation ultrasonication solvent emulsification method was successfully achieved SLNs systems with uniformly distributed particles and with size range of nanometers.

Stability study

The stability study showed no significance change in particle size and PDI during 90 days. The particle size and PDI remains stable. It was concluded that optimized formulation (SLNF3) was stable at under refrigeration, room temperature and under accelerated temperature and humidity with respect to particle size and PDI during 90 days (table 2). These results indicated that optimized formulation is stable at three different temperature conditions as there were no significant changes in physical parameters and can be recommended for topical application in drug delivery system.



(a) (b)

Fig. 1: Photomicrograph of transmission electron microscopy (TEM) of prepared DAID-SLNs: (a) Scale bar is 100 nm; (b) Scale bar is 200 nm

Table 2: Stability study of optimized formulation (SLNF3) batch for particle size (nm), and PDI at different time period and temperatures

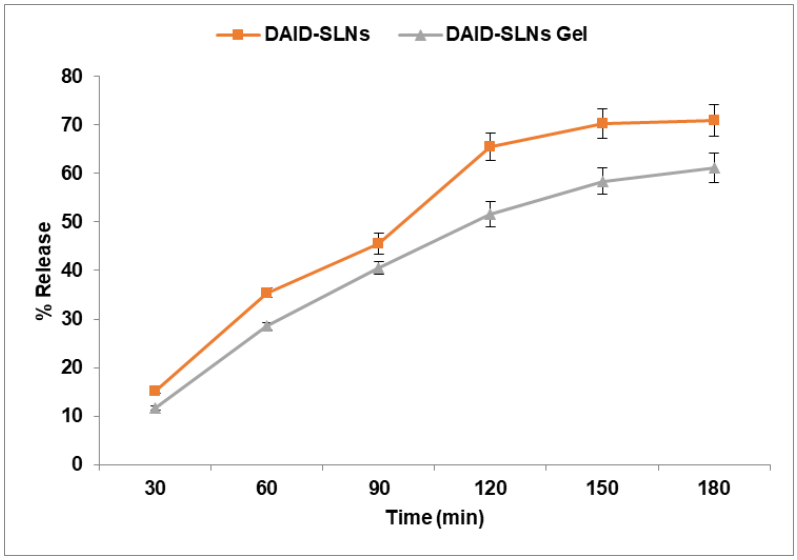
Temperature	Time (days)	Particle size (nm)	PDI
5 °C	T1	192.62±5.89	0.20±0.040
	T30	193.45±4.58	0.21±0.042
	T60	193.50±6.34	0.22±0.041
	T90	191.63±4.28	0.21±0.042
25 °C	T1	193.50±5.12	0.20±0.043
	T30	193.62±6.22	0.21±0.041
	T60	192.34±5.87	0.23±0.040
	T90	191.61±5.43	0.22±0.039
40 °C	T1	190.62±6.22	0.20±0.043
	T30	193.55±5.75	0.21±0.040
	T60	191.52±5.48	0.21±0.042
	T90	189.65±4.92	0.22±0.041

Data represent as mean±SD, n =3

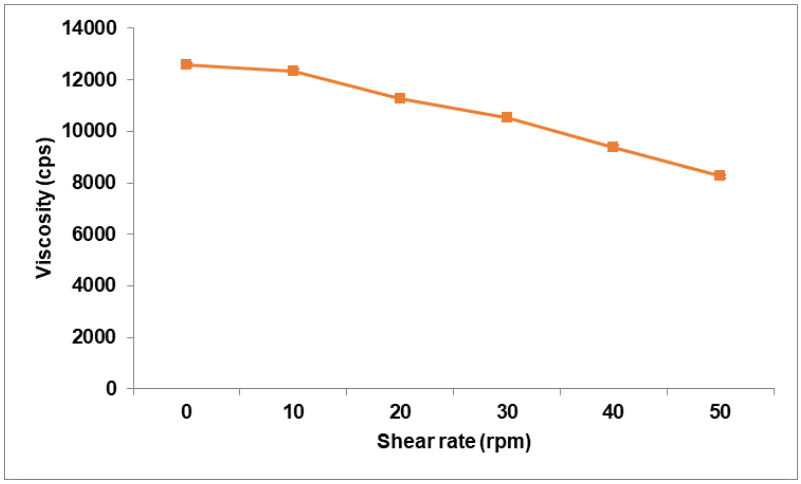
Characterization of formulated daidzein-loaded (DAID-SLNs) gel

The prepared formulation DAID-SLNs gel was smooth homogenous with semisolid consistency. The actual measured pH of DAID-SLNs gel was found as 6.8±0.05 that is found appropriate for topical application and it is physiologically compatible with the pH of the skin. Non-irritant and non-allergic property is the primary requirement of suitable topical formulation, pH of formulation should be compatible to the skin. The percentage drug release 61.2±3.01 in 180 min, while nanoparticle formulation DAID-SLNs showed 70.9±3.18percentage release in 180 min. Spreadability is another important parameter of gel that can affect the therapeutic efficacy of topical formulation. The appropriate spreadability of any topical formulation is support to the easy application on the skin. Spreadability of the prepared gel was found to be 6.24±0.073 to 7.45±0.058 g. cm/s that is good spreadability of gel and suitable for topical administration.

An essential component in the setup for transdermal delivery of medications is viscosity. The topical preparation's viscosity directly affects the drug's release, spreadability, stability, and simplicity of application. The polymers and excipients used in the formulations of topical applications also influence their viscosity [19]. The viscosity of DAID-SLNs Gel was measured at different shear rates, and the results indicated that the connection between shear rate and gel viscosity was inverse (fig. 2a and 2b). It was evident that the viscosity of the gel reduced with an increase in shear rate and vice versa. The DAID-SLNs Gel's decreased viscosity in response to an increase in shear rate suggests that pseudo-plastic flow was present in the gel.



(a)



(b)

Fig. 2: (a) Percent drug release from DAID-SLNs and DAID-SLNs gel (b) Observation of viscosity of prepared DAID-SLNs gel

***In vitro* anti-inflammatory effect**

Protein denaturation inhibition assay

The results of protein denaturation inhibition assay were confirmed maximum inhibition with DAID–SLNs loaded gel at 100µg/ml with 91.42±3.18%, was comparable to Standard Diclofenac sodium showed 95.24±3.85% at 100µg/ml (table 3). Control group does not show any inhibition.

Table 3: Anti-inflammatory effect of daidzein loaded solid lipid nanoparticles (DAID-SLNs) gel in albumin denaturation inhibition assay

Treatment groups	% Albumin denaturation inhibitory effect at various concentrations (µg/ml)				
	20	40	60	80	100
DAID–SLNs loaded gel	42.37±1.53	51.20±2.61	67.26±2.85	82.31±3.64	91.42±3.18
Standard (Diclofenac sodium)	39.85±1.53	50.27±2.75	72.34±3.41	85.34±3.61	95.24±3.85

Tabulated value represented as mean±SD (n = 6). DAID–SLNs: Daidzein-loaded solid lipid nanoparticles

Table 4: Anti-inflammatory effect of daidzein loaded solid lipid nanoparticles (DAID-SLNs) gel in Anti-proteinase assay

Groups	% Proteinase inhibitory effect at various concentrations (µg/ml)				
	20	40	60	80	100
DAID–SLNs loaded gel	24.61±0.87	34.26±1.62	58.37±2.45	74.24±3.18	92.46±3.45
Standard (Diclofenac sodium)	31.85±1.27	45.18±1.08	64.42±2.17	77.62±3.62	98.28±3.72

Tabulated value represented as mean±SD (n = 6). DAID–SLNs: Daidzein-loaded solid lipid nanoparticles

Proteinase inhibitory efficacy

DAID–SLNs loaded gel also tested for proteinase inhibitory efficacy and found to have a strong inhibitory effect (table 4). The higher concentration 100µg/ml was showed 92.46±3.45% inhibition that was comparable to the standard Diclofenac sodium (98.28±3.72 %).

Anti-lipoxygenase efficacy

Anti-lipoxygenase efficacy of DAID–SLNs gel was revealed maximum 96.31±3.94% at 100µg/ml and it also showed comparable to the standard Diclofenac sodium (99.53±3.85%). These assays were showed percent inhibition in a dose-dependent manner (table 5).

Table 5: Anti-inflammatory effect of daidzein-loaded solid lipid nanoparticles (DAID-SLNs) gel in Anti-lipoxygenase assay

Groups	% Lipoxygenase inhibitory effect at various concentrations (µg/ml)				
	20	40	60	80	100
DAID–SLNs loaded gel	41.82±1.05	57.61±2.16	69.27±2.88	82.32±3.42	96.31±3.94
Standard (Diclofenac sodium)	40.13±1.06	61.78±2.21	75.48±3.42	88.18±3.77	99.53±3.85

Tabulated value represented as mean±SD, (n = 6). DAID–SLNs: Daidzein loaded solid lipid nanoparticles

Denaturation of proteins is one of the major causes of inflammation (20). As part of the investigation on the mechanism of the anti-inflammatory activity, ability of the DAID–SLNs gel to inhibit protein denaturation was studied. In the literature, anti-inflammatory effects of isoflavone daidzein, has been reported partially mediated through inactivation of MAP K and/or NF-κB signaling pathways in macrophages [21].

Reactions involving inflammation linked to arthritis have been linked to proteinases. Numerous serine proteinases are included in the lysosomal granules of neutrophils. During inflammatory processes, leukocyte proteinases are crucial in the development of tissue damage [22]. Proteinase inhibitors offered a notable degree of protection. Numerous flavonoids have been demonstrated in recent studies to have a major role in the antioxidant and anti-inflammatory properties [23, 24].

The essential enzymes for the synthesis of leukotrienes are lipoxygenases. Leukotrienes have a significant impact on a number of inflammatory illnesses, including cancer, asthma, arthritis, and allergy disorders. Arachidonic acid metabolism is a key component of the anti-inflammatory mechanism, which may entail a number of different processes [25, 26]. In this mechanism, when neutrophils are stimulated appropriately, arachidonic acid is broken down from the membrane phospholipids and can be transformed via the lipoxygenase and cyclooxygenase routes into prostaglandins and leukotrienes, respectively [16]. This process may be inhibited by the daidzein which act as potent anti-inflammatory agent. Moreover, the anti-inflammatory property of the Daidzein Loaded Solid Lipid Nanoparticles (DAID-SLNs) gel was comparable to the standard diclofenac sodium. By using solvent emulsification method, researchers produced DAI-loaded nanostructured lipid carriers for transdermal application [27]. They found that the permeation rate was 3.78 times higher than that of pure DAI solution. This report was supportive for the present research work for improvement of topical permeation of daidzein through development of DAID-SLNs gel. The major limitation of this study is the cost of daidzein and purity. Long-term stability study and *in vivo* study can be plan for further study for different inflammatory diseases.

CONCLUSION

Results of various characterization parameters confirmed that prepared DAID-SLNs gel was found suitable for the topical delivery of daidzein. Daidzein-loaded solid lipid nanoparticles gel was found effective for treatment of inflammation tested by *in vitro* assay i. e. albumin denaturation inhibition, anti-proteinase and anti-lipoxygenase assay. The significant anti-inflammatory effects correlated with improve solubility and bioavailability of daidzein in the selected solvent and lipids. The possible anti-inflammatory mechanism of daidzein is inhibition of protein denaturation and essential enzymes proteinase as well as lipoxygenase. Findings of the present study were also recommended that the prepared daidzein-loaded solid lipid nanoparticles gel may applicable in the treatment of various inflammatory disorders.

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AUTHORS CONTRIBUTIONS

Manuscript drafting, revision and formatting were done by Ms. Amrita Chourasia. Design of the manuscript, language editing and data was verified by Dr. Santram Lodhi.

CONFLICT OF INTERESTS

Declared none

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