Topical Delivery of Fenugreek Seed Extract Loaded Solid Lipid Nanoparticles Based Hydrogels for Alopecia

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Original Research Article	

ABSTRACT

Background: Alopecia, a chronic dermatological inflammatory condition affecting the hair follicles. Conventional treatments are associated with the risk of serious side effects. The stratum corneum limits the percutaneous absorption of drugs. Hence, the development of novel herbal formulations for topical delivery has been the target, with the enhancement of their therapeutic efficacy and safety of use.
Aims: To formulate and characterize Fenugreek seed extract loaded solid lipid nanoparticles carrier for the management of Alopecia to reduce the systemic side effects.
Methodology: Fenugreek seed extract loaded solid lipid nanoparticles (SLN) were prepared by melt emulsification accompanied by probe sonication. The formulation was prepared using GMS, Tween 80, and Soya lecithin as Lipid, Surfactant, and Co-Surfactant. The SLN was incorporated into carbapol 934 dispersion to convert it into a gel. The SLN formulation was evaluated for size, Polydispersity Index, Zeta Potential, Entrapment efficiency, Transmission Electron Microscopy. After that, the SLN gel was examined for Spreadability, Extrudabilty, Viscosity, *In vitro* drug release, *Ex vivo* permeation, and Skin deposition studies.
Results: The formulated Fenugreek seed extract loaded showed a particle size of 223.36 nm with a narrow PDI of 0.313. Entrapment efficiency revealed that 74.56±0.12% of the drug was

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entrapped. Transmission electron microscopy images confirmed the spherical nature of the SLN. The extended-release pattern of the formulated SLN for 24h was observed in the *in vitro* release studies and followed Higuchi model(R^2=0.9964). *Ex vivo* permeability showed a 72.05±0.15% deposition of Fenugreek seed extract loaded SLN. The formulation was stable for three months without significant changes.

Conclusion: Fenugreek seed extract loaded NLC demonstrated enhanced permeation, improved skin retention, and extended release compared to conventional gel. The developed formulation would be further used for *in vivo* studies and by seeing above results it can be an alternative for Alopecia in the future.

1. INTRODUCTION

Alopecia, a chronic dermatological inflammatory condition affecting the hair follicles which arises from the combination of genetic and environmental influences [1]. In many cases, even drug-induced alopecia can be encountered. The very thought of becoming bald can lead to emotional stress and traumatic experience for the individual [2]. Non-scarring (non cicatricial) and scarring (cicatricial) alopecias are the two forms of hair loss. Conventional treatments for non-cicatricial alopecia include topical minoxidil and oral type II 5-α-reductase inhibitors like finasteride; pulsed high doses of oral or intravenous steroids, photochemotherapy, and topical immunotherapy. For cicatricial alopecia, conventional treatments include topical and oral antibiotics, oral peroxisome proliferator-activated receptor-γ agonist like pioglitazone hydrochloride [3]. However, these treatment approaches are associated with the risk of serious side effects such as skin irritation, bruising, itching, allergic contact dermatitis, blisters at the site of application, and also suppression of immunity due to prolonged treatment.

Topical products are available for the treatment of alopecia but, due to the low permeability through the keratin layer, only a fraction of the applied dose reaches the site for action, after penetrating the pores and hair follicles [4]., Hair growth using such products does not meet consumer expectations, which may lead to a lack of treatment adherence. The stratum corneum forms a significant barrier to limit the percutaneous absorption of drugs [5].

Thus there is a need to develop better formulations to improve topical delivery of the drug through the skin. The current trend moves towards herbalism and the use of natural products. Indian herbs are the richest source to be used in this aspect [6].

Hence, the development of novel herbal formulations for topical delivery has been the target, with the enhancement of their therapeutic efficacy and safety of use. Few herbs are known to have the properties of stimulating hair growth; one among them is *Trigonella foenum graecum*, which is popularly known as fenugreek [7]. It is an aromatic herb, belonging to the family Leguminosae (Fabaceae). It mainly contains flavonoids, quercetin, saponins, tigogenin, trigonelline, protein, fats, carbohydrates, galactomannan. It has the property to initiate and complete hair growth; it also has the best hair lengthening properties [7, 8]. Marketed formulations of fenugreek seed extract are available such as fenugreek hair oils, shampoos, creams. But the efficacy of such formulations is questionable. The permeation efficiency of the topical formulations can be improved by using nanoparticulate carriers, especially those with superior lipophilicity that will be able to permeate the stratum corneum [9]. Hence an attempt was made in the current study to incorporate fenugreek seed extract into a nanocarrier such as a solid lipid nanoparticle to increase its solubility and permeability, thereby improving the bioavailability and therapeutic activity.

2. MATERIALS AND METHODS

2.1 Materials

The seeds of *Trigonella foenum greacum* were obtained from the local market. Soya lecithin, Tween 80, and carbapol 934 were purchased from LOBA Chemical Pvt. Ltd., India. Ultra-pure deionized water was used throughout the experiments. All other solvents and chemicals used were of analytical grades or higher.

2.2 Preparation of Ethanolic Extract of Fenugreek

The seeds were, pulverized, sieved through 40 mesh to obtain a coarse powder. A hundred grams of powdered seeds were extracted with ethanol as a solvent by hot extraction method using soxhlet apparatus. The resulting extract was cooled and filtered. The filtrate was evaporated in a vacuum to give a residue [10].

2.3 Preparation of Fenugreek Seed Extract Loaded Solid Lipid Nanoparticles

Solid lipid nanoparticles (SLNs) of fenugreek seed extract were prepared from the melt emulsification-probe sonication method. Previously, the SLN was optimized in terms of particle size, polydispersity index, and entrapment efficiency (data not shown). The procedure for the optimized batch is briefly discussed here. Weighed quantity of Glyceryl monostearate was taken to prepare the lipid phase. Tween 80 (surfactant) and Soya lecithin (cosurfactant) were dissolved in Milli-Q water to make the aqueous phase. Both of these phases were kept at 70°C. Extract (2.5%) was introduced to the molten lipid mixture when stirring constantly, and then the aqueous phase was added drop by drop to the lipid phase under steady stirring at 2000 rpm for 30 minutes until a homogeneous emulsion was created. The resulting primary emulsion was ultrasonicated for 15 minutes at 75% amplitude under hot conditions. After cooling the solution to room temperature, the SLN dispersion was obtained [11].

2.4 Characterisation of Fenugreek Seed Extract Loaded Solid Lipid Nanoparticles

2.4.1 Determination of particle size, polydispersity index, and zeta potential

The particle size, polydispersity index, and zeta potential of the SLN were determined by Dynamic light scattering, using Zetasizer Nano-Series (Nano-ZS 90, Malvern Instruments, U.K)., at 25°C, with a scattering angle of 90°. Samples were diluted with distilled water before analysis for better accuracy. Estimations were performed in triplicates, and the average was considered [12].

2.4.2 Entrapment efficiency

Entrapment efficiency was calculated by centrifugation of the known amount of SLN dispersion at 10000 RPM for 15 minutes. The free drug present in supernatant was analyzed at 265 nm, using UV visible spectrophotometer after suitable dilution with phosphate buffer saline of ph 7.4. All estimations were performed in triplicate [13].

2.4.3 Transmission electron microscopy (TEM)

The surface morphology of the SLN was determined by a transmission electron microscope. A drop of diluted SLN dispersion was placed on a carbon-coated copper grid, after staining for 1 min. Afterwards the sample was tapped with filter paper to remove excess dispersion and air-dried, for examination [14].

2.5 Incorporation of the SLN Dispersion into the Hydrogel

Carbopol 934 was chosen as a gelling agent because of its excellent adhesive properties, elegant appearance, and ease of removal from the skin. It was dispersed in distilled water with continuous stirring to obtain three different hydrogels concentrations: 1%, 1.5%, and 2%. Glycerol was added to this dispersion, optimized SLN was then added to the aqueous dispersion, and was neutralized by dropwise addition of triethanolamine until the gel was formed [15].

2.6 Characterisation of Fenugreek Seed Extract Loaded SLN Based Hydrogel

2.6.1 Visual appearance, spreadability, extrudability, and measurement of pH

All developed gels were tested for clarity, colour, and homogeneity by visual inspection after the gels have been set in the container. The spreadability of the gel formulations was determined by measuring the spreading diameter of 1 g of gel between two glass slides after 1 min, the standard weight was applied to the upper plate.

The gel formulations were filled in standard capped collapsible aluminum tubes and sealed by crimping to the end. Weights of the tubes were recorded. The tubes were placed between two glass slides and were clamped. 500 gm was

placed over the slides and then the cap was removed. The amount of the extruded gel was collected and weighed. The pH of the gel was determined by using a pen pH meter [16,17].

2.6.2 Determination of viscosity and percentage drug content

The viscosity of the prepared gels was measured using Brookfield viscometer using Helipath spindle no. 95, at 50 rpm. Experiments were carried out in triplicate for each sample Viscosities were determined at different rpm to understand the rheological properties of the formulation.

Gel equivalent to 5mg of trigonelline was taken and dissolved in 100 ml of PBS 7.4 in a volumetric flask. Gel solution was subjected to shaking on a mechanical shaker to obtain complete solubility. The solution was estimated spectrophotometrically at 265 nm using methanol as solvent [12].

2.6.3 In vitro drug release studies

I*n vitro* release studies were performed using modified Franz diffusion cells. Dialysis membrane having pore size 2.4 nm and molecular weight cut off of 12000–14000 (HIMEDIA) was used. The membrane was soaked in phosphate buffer saline pH 7.4 overnight. SLN formulation was filled in the donor compartment and the recipient compartment was filled with phosphate buffer saline of pH 7.4. The content of the cell was stirred with the help of a magnetic stirrer at 37°C. At the fixed time intervals pre-determined aliquots of the sample were withdrawn from the receiver compartment through the side tube. Fresh phosphate buffer saline of pH 7.4 was used to replace the samples to maintain sink condition. Samples were analyzed by UV spectrophotometer [18,19,20].

The amount of trigonelline in the withdrawn samples was assessed by UV spectroscopy at 265 nm. All the measurements were carried out in triplicate.

2.6.4 Kinetic analysis of the release data

To understand the release kinetics of the formulations, the in vitro release data was fitted into different kinetic models such as zero order, first order, Higuchi diffusion, and Korsmeyer-Peppas. The model with the highest correlation coefficient (r^2) was chosen as the best fit.

2.6.5 Ex vivo permeation studies

Ex vivo release studies were performed using modified Franz diffusion cells. The excised rat skin was sliced into a suitable size and clamped between the donor and receptor compartments of the Franz diffusion cell with the stratum corneum facing the donor compartment. The skin was soaked overnight in phosphate buffer saline of pH 7.4. SLN loaded gel formulation was filled in the donor compartment and the recipient compartment was filled with phosphate buffer saline of pH 7.4. The experiments were carried out in the same way as in *in-vitro* release studies(section 2.4.3) except for the change that rat skin was used as the membrane instead of the dialysis membrane. Fresh phosphate buffer saline of pH 7.4 was used to replace the withdrawn medium to maintain sink condition. Samples were analyzed by UV spectrophotometer [19, 20].

The cumulative amount of drug that permeated through the skin per unit area over time was plotted. The steady-state flux (Jss) was calculated using the slope of the linear portion of the plotted curve, and the permeability coefficient (Kp) was calculated using the equation below;

.Kp=Jss/Co

J_{ss} is the drug flux at steady-state, and Co is the initial drug concentration in the donor cell.

2.6.6 Drug deposition studies

This study was performed after the completion of the permeation study. For determination of drug deposited in the skin, Franz diffusion cell was dismantled after a period of 24 h and the skin (from diffusion study) was carefully removed from the cell. The formulation applied on the skin surface was swabbed with phosphate buffer saline pH 7.4. The procedure was repeated twice to ensure no traces of formulation are left on the skin surface. The skin was then cut into small pieces and kept in a buffer to extract the drug present in the skin for 48 h. Then, It was analyzed spectrophotometrically after suitable dilution and filtration. The standard calibration curve equation was used to determine the amount of drug deposited in the skin [21]

2.7 Stability Studies

Fenugreek seed extract loaded SLN was subjected to stability studies for 90 days at three different

temperature conditions following ICH guidelines. Sampling was done at day 0, day 30, day 60, and day 90, respectively, and evaluated for parameters like pH, Drug content, Viscosity spreadability, and phase separation [22, 23].

3. RESULTS AND DISCUSSIONS

The melt emulsification-probe sonication process was used to prepare fenugreek seed extract loaded SLN gels. Table 1 shows the composition of the prepared SLN. The prepared SLN gels were further characterized for in vitro investigations.

Table 1. Composition of the fenugreek seed extract loaded SLN

Components	% in formulation (w/w)
Fenugreek seed extract	2.5
Glyceryl Monosterate (GMS)	2.33
Tween 80	2
Soya Lecithin	0.87
Deionized water	92.3

3.1 Characterisation of Fenugreek Seed Extract Loaded SLN

3.1.1 Determination of particle size, polydispersity index, and zeta potential

The particles were in the nano range, and the narrow PDI indicates that the particles had a uniform size distribution. The zeta potential is important in determining the stability of SLNs since it indicates particle attraction or repulsion. The negative charge on SLN was due to fatty acids released from the hydrolysis of GMS. Table 2 shows the particle size and polydispersity index of the prepared SLN.

3.1.2 Entrapment efficiency

The entrapment efficiency of the optimized SLN was 74.56 ± 0.12%, which suggests the minimum loss of drug in the formulation. This shows that for increased entrapment efficiency, the drug should be encapsulated in the lipid matrix of SLN, moreover extract solubility and affinity were higher in lipid, also due to the matrix nature of SLN a substantial amount of drug to be entrapped successfully [24].

3.1.3 Transmission electron microscopy (TEM)

TEM images of the Fenugreek seed extract loaded SLN showed uniform size distribution of the colloidal particles in nanometric range and are spherical. As seen from Fig. 1 there are no signs of aggregation or adherence.

## 3.2 Characterisation of Apremilast Loaded NLC Gel

In distilled water, gel bases containing varying concentrations of carbopol 934 (1, 1.5, and 2%) were produced, to which Fenugreek seed extract loaded SLN was added. The physicochemical features of the produced SLN gels, such as spreadability, extrudability, consistency, and pH drug content, were investigated. Table 3 displays the results.
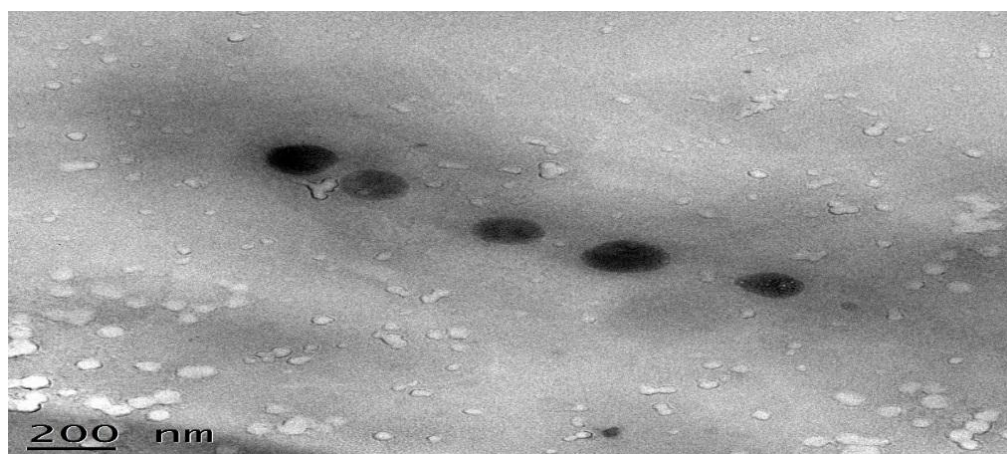
Fig. 1. TEM image of the optimized Fenugreek seed extract loaded SLN

Table 2. Particle size, PDI, and Zeta potential of the prepared Fenugreek seed extract loaded SLN

Formulation	Particle size	Polydispersity Index	Zeta Potential
Fenugreek seed Extract Loaded SLN	223.36 ± 0.45 nm	0.313 ± 0.023	-16.64 ±1.32mV
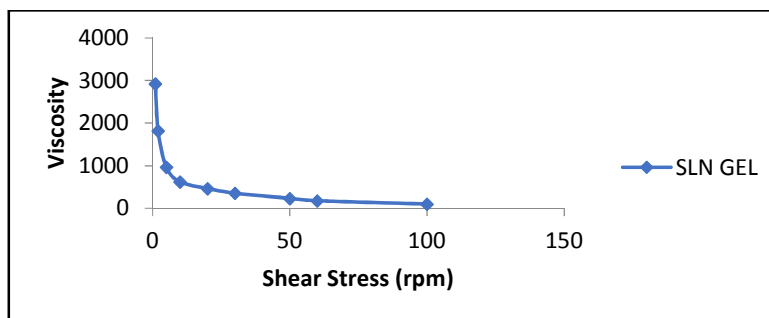
Fig. 2. Viscosity of the SLN gel under different shear rates

All of the gels that were made were visually pleasing and firm. The gel was homogeneous and free of coarse particles. Based on its good flowability, excellent spreadability, maximum drug content and extrudability, and near-neutral pH, carbopol 934 gel prepared at a concentration of 2% w/w was the most suitable for incorporation into the formulation and further use.

3.3 Rheological Properties of Apremilast Loaded-SLN Gel Formulation

Because the gels are intended to be applied topically, understanding the rheological flow pattern is critical. The viscosities of optimized Fenugreek seed extract loaded SLN gel and Plain Fenugreek gels were investigated at various shear rates (rpm). The viscosity of the SLN gel reduced as the shear rate increased from 0.5 to 100 rpm, indicating that the formulation was shear-thinning. [25]. The pseudoplastic flow behavior of the SLN gel was evident from the rheogram obtained in Fig. 2.

3.4 In-vitro Drug Release Studies

For drug release studies, phosphate buffer saline pH 7.4 was selected as the receptor media. The cumulative percentage of drug release from the drug-loaded gel and SLN loaded gel was studied over 24 hours [26]. The ideal topical formulation should show controlled release for a more extended period to avoid frequent application for better patient compliance.

From the *in vitro* release study, fenugreek gel showed the faster release of the drug, i.e.100% in 12h, whereas Fenugreek seed extract loaded SLN gel demonstrated a sustained release profile (90.2% for 24h).

This result was probably due to the release-retarding effect of the polymeric matrix of gelling agents.

As seen from Fig. 3, Fenugreek seed extract loaded SLN gel demonstrated an initial burst effect, which was due to surface-absorbed actives on SLNs, followed by the sustained release for an extended period, due to the actives slowly diffusing through the lipid core. Further prolongation in drug release was observed, which can be attributed to the probable retention of the drug inside the microchannel structures of the carbopol gel system. It was observed that *in vitro* release of fenugreek gels followed zero-order release kinetics (r^2 =0.9874) whereas SLN gel (r2=0.984) followed Higuchi kinetics which explains that the drug incorporated into the lipid matrix was released in a sustained manner via the. matrix diffusion-controlled mechanism.

3.5 Ex vivo Permeation Studies

The *ex vivo* permeation profiles Fig. 4 revealed that 210 µg/cm^2 trigonelline permeated through the rat skin from the SLN gel compared to 35 µg/cm^2 trigonelline permeated from the aqueous solution of extract.
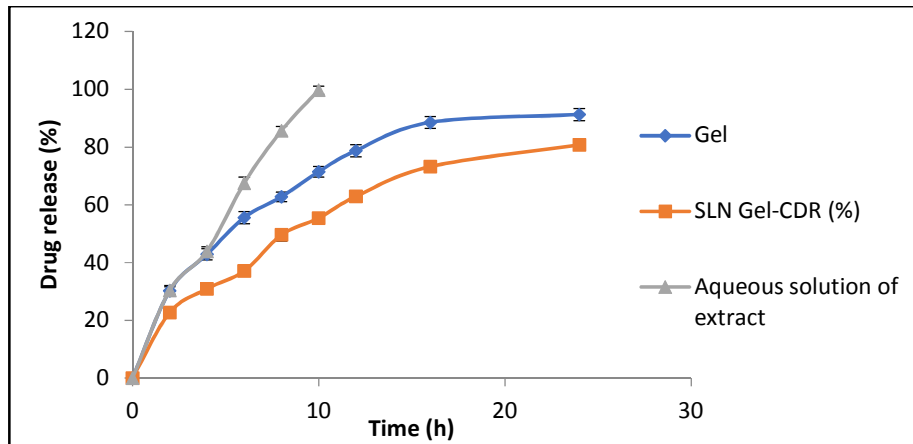
Fig. 3. *In vitro* **drug release of trigonelline from Aqueous solution of extract, Fenugreek seed extract Gel and SLN gel through cellophane membrane using Phosphate buffer saline of pH 7.4 as the diffusion medium**

The nanoparticulate gel shows higher localization of trigonelline in the skin as compared to conventional gel (Fig. 4). Thus, the drug-localizing effect in the skin seems possible with novel colloidal particulate drug carriers such as SLN. This colloidal carrier, being submicron in size, enhances the drug penetration into the skin, and, because of its lipoidal nature, the penetrated drug concentrates in the skin and remains localized for a longer period, thus enabling drug targeting to the skin.

This enhanced permeation showed by SLN can be attributed to the composition of the SLN. SLN consists of a mixture of lipids and surfactants, and the latter enhances the lipid bilayers disruption and denaturation of keratin in the stratum corneum, thus allowing drug-loaded SLNs to permeate passively across the skin via the transfollicular route. Since lipids in the SLN binds to sebum the drug release is still sustained.

The higher dynamic viscosity of the SLN loaded gel and slow diffusion from the carbopol matrix of the SLN Gel seemed to retard the drug release further, similar to that obtained in the *in vitro* release study [27].

The lower flux and permeation coefficient of trigonelline in the aqueous dispersion is due to poor solubilization and partition of trigonelline in the skin layers. The low permeation of trigonelline from the normal gel might be due to the low solubilization of the gel in the hydrophilic matrix and insufficient partitioning of the drug between the matrix and skin layers. It was observed that there was a difference in the order

of drug release in the *in vitro* and *ex vivo* studies. This is mainly due to different characteristics of the cellophane membrane and the animal skin.

3.6 Skin Deposition Studies

As seen in Fig. 5, the highest drug deposition among different formulations was found in SLN gel (72.05±0.15%). This is due to nanosized particles that enhance the solubilization and the sustained release of trigonelline from the polymeric gel matrix. The nanosize as well as the influence of surfactant are the major factors for contributing to better penetration. So surfactant swells the stratum corneum and the intact vesicle can penetrate into and through the intact skin.

In dermatological treatment, improving the efficacy demands high drug levels in the skin. With nanoparticle dispersion, a greater quantity of drugs remained localized in the skin, with lesser amounts penetrating the receptor compartment as compared with the conventional formulation. Thus, the drug-localizing effect in the skin seems possible with novel colloidal particulate drug carriers such as SLN. This colloidal carrier, being submicron in size, enhances the drug penetration into the skin, and because of its lipoidal nature, the penetrated drug concentrates in the skin and remains localized for a longer period, thus enabling drug targeting to the skin.

Furthermore, the high affinity of SLN lipids for lipophilic skin layers and sebum led to trigonelline skin accumulation after topical

application of SLN gel. The increase in the deposition of SLN gel can also be attributed to the improved adhesiveness of the hydrogel with the follicle. Thus SLN based gels are suitable for topical administration in treating diseases like alopecia, where the local effect is desired [28].

3.7 Stability Studies

The stability of the SLN Gel was examined in terms of viscosity, spreadability, and visual appearance (Phase separation). The results obtained from the studies in Table 4 indicated no significant changes in Spreadability and Viscosity after subjecting the tested formulations to stability studies at 4°C & 25°C as per ICH guidelines. Whereas at 45°C, there were slight changes concerning spreadability and viscosity. These results indicate that the prepared SLN can be best stored at refrigerated or room temperature conditions.

Table 3. Characterization of the optimized Fenugreek seed extract loaded SLN gel

Homogeneity	+++
Texture of gel	Free from grittiness
Viscosity at 50 rpm (cp)	245.3 ± 6.34
pH	6.5±1.3
Spreadability Diameter (cm)	11.6 ± 1.2
Extrudability	Excellent(>90%)
Drug content (%w/w)	90.52 %
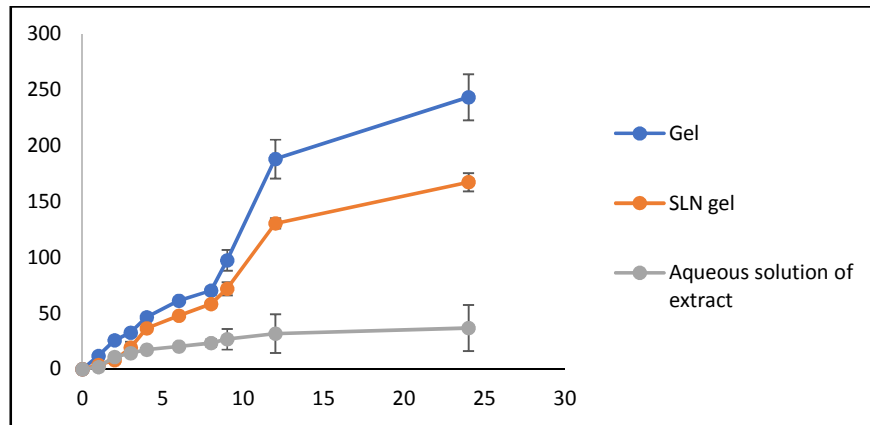
Fig. 4. *Ex vivo* Permeability studies of trigonelline from different formulations through rat skin using Phosphate buffer saline 7.4 as the diffusion medium
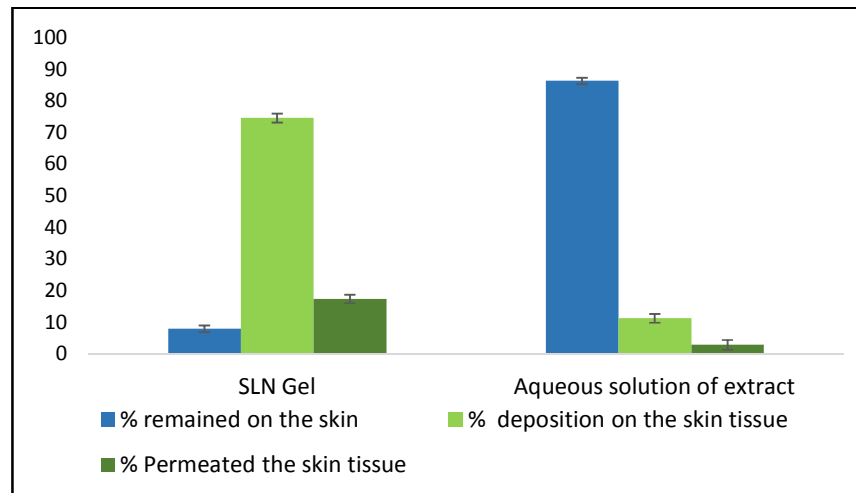
Fig. 5. *Ex vivo* skin deposition studies of trigonelline from SLN gel and aqueous extract

Table 4. Stability studies of the optimized SLN gel under different temperature conditions for 90 days

S.No	Time	Storage Condition	pH	Drug content (%)	Viscosity at 50 rpm (cps)	Spreadability(cm)	Phase separation
1	Day 0	4±2°C	6.5±0.50	90.52±0.23%	245.3 ± 6.3	11.6 ± 1.2	No
		25±2°C	6.5±0.45	90.45±0.31%	246.5 ± 5.1	11.4 ± 0.26	
		45±2°C	6.5±0.42	90.36±0.63%	251.7 ± 3.5	11.3 ± 0.35	
2	Day 30	4±2°C	6.6±0.55	90.25±0.36%	255.3 ± 6.2	11 ± 0.21	No
		25±2°C	6.5±0.10	90.35±0.89%	234.8 ± 6.4	11.4 ± 0.33	
		45±2°C	6.6±1.02	90.24±0.55%	227.5 ± 7.3	9.7 ± 0.15	
3	Day 60	4±2°C	6.5±0.31	90.15±0.89%	215.3 ± 6.7	11.1 ± 0.74	No
		25±2°C	6.5±0.60	90.28±0.55%	262.4 ± 7.1	10.5 ± 0.76	
		45±2°C	6.5±0.76	90.27±0.69%	251.3 ± 7.4	9.4 ± 0.36	
4	Day 90	4±2°C	6.5±0.64	90.42±0.17%	241.3 ± 6.5	10.1 ± 0.22	No
		25±2°C	6.5±0.25	90.14±0.25%	254.3 ± 6.2	10.7 ± 0.39	
		45±2°C	6.5±0.87	90.33±0.34%	205.2 ± 5.9	9.4 ± 0.25	

4. CONCLUSION

The present research work could be surmised as successful production of SLNs using GMS by melt- emulsification probe sonication technique. Further, developed SLNs were meaningfully utilized for the topical delivery of fenugreek seed extract. Greater skin deposition and slow drug release were observed with the developed SLN. SLN topical gel containing fenugreek seed extract demonstrated superior permeability and skin retention compared to conventional gels. Production of fenugreek seed extract loaded SLNs and its formulation as a topical gel could be a new, cost-effective, and commercially viable alternative to conventional products with minimal side effects. This is expected to improve the therapeutic efficacy of constituents by the exploitation of nanotechnology applications in drug development. Fenugreek seed extract SLNs demonstrated similar, if not superior activity compared to the crude drug extract. This opens up avenues to explore SLNs as carriers for the effective delivery of phytopharmaceuticals.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

ACKNOWLEDGEMENT

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/71828