Polish Pharmaceutical Society

Gugulipid is an ethyl acetate extract of guggul resin, obtained from Commiphora wightii (Fam.: Burseraceae) and is official in Indian Pharmacopoeia (1). The active constituent of gugulipid is guggulsterone (4,17(20)-pregnadiene-3,16-dione) (2), which is present in a concentration of 4.0–6.0%. The guggulsterone is present in gugulipid in the form of stereoisomers E-guggulsterone and Z-guggulsterone.

Pharmacokinetic studies conducted in rats after intravenous and oral administration indicated that the absolute bioavailability of the guggulsterone after oral administration was 42.9%. Further, it was observed that in vivo Z-guggulsterone isomerizes to give E-guggulsterone (3).

Gugulipid is a potent hypolipidemic agent. Apart from its hypolipidemic activity, a large number of therapeutic activities like antimicrobial, anthelmintic, anti-inflammatory, antiarthritic and antioxidant have been reported (4–6). A wide range of therapeutic activities, low bioavailability and aqueous insolubility of gugulipid prompted us to explore an approach, which in addition to removing its undesired pharmacological action also improves its therapeutic concentration at the site of inflammation.

Topical application of anti-inflammatory agents at the site of inflammation can overcome their systemic side effects and improve their therapeutic activity. Proniosomal gels have earlier been reported to enhance the topical delivery of levonorgestrel and estradiol (7, 8). Proniosomal gels are the formulations, which on in situ hydration with water from the skin are converted into niosomes.

Proniosomal gels overcome the disadvantage of vesicular instability associated with niosomes. The drug entrapped in the niosomal vesicles penetrates the skin at a faster rate than the free drug (9–11). Thus, taking view of the topical delivery potential of the proniosomal gel, a guggulipid loaded proniosomal gel based formulation was developed in the present study. The formulated proniosomal based gel formulation was characterized for particle size entrapment efficiency, in vitro drug release and in vivo anti-inflammatory activity using carrageenan-induced rat hind-paw method (12, 13).

EXPERIMENTAL

Materials

Soya lecithin and dialysis membrane were purchased from Hi-Media Laboratories Ltd. (Mumbai, India). Span 40 was procured from SD Fine Chemicals (Mumbai, India). Cholesterol, potassium dihydrogen phosphate and sodium hydroxide were obtained from CDH Chemicals Pvt. Ltd. (Delhi, India). Standard gugulipid was generously gifted by Ayush Herbs (U.P., India). Propanol was purchased from Spectrochem Pvt. Ltd. (Mumbai, India).

Preparation of proniosomal gel

Pronosome gel was prepared by the method reported earlier (11). Surfactant : alcohol 1:1 (total surfactant 100 mg) and gugulipid (100 and 200 mg) were taken in a clean, dry, wide mouth small glass tube. Surfactant ratio used was Soya PC : Span 40 : cholesterol (4.5:4.5:1). All ingredients were mixed properly and open end of the tube was covered to
prevent any loss of solvent from it and warmed on water bath at 60–70°C for about 5 min until the surfactants were dissolved completely. The aqueous phase (phosphate buffer pH 7.4) was added and warmed on water bath and allowed to cool to room temperature to give proniosomal gel.

**Characterization of proniosomal gel**

**Particle size**

Evaluation of particle size was done for niosomes prepared from proniosomes by hydration with agitation. The proniosomes gel (100 mg) was hydrated in a small glass test tube using 10 mL of normal saline solution with manual shaking for 5 min. The dispersion was observed under optical microscope. Particle size of 300 niosomes was measured using calibrated stage and ocular micrometer.

**Entrapment efficiency**

Total amount of drug present in proniosomal gel was determined by adding 10 mg of gel to 10 mL of methanol, sonicated and filtered through 0.2-µm syringe filter. The filtrate was analyzed by HPLC.

**HPLC analysis**

Assay of gugulipid in samples was carried out by injecting 20 µL of the solution, spiked with testosterone as an internal standard into a chromatographic system equipped with 600 pump controller (Waters, Milford USA), 2487 dual λ absorbance detector (Waters), and 7725i Rheodyne injector. The resolution of gugulipid was achieved using acetonitrile:water (65:35, v/v) at a flow rate of 1 mL/min, as mobile phase in an isocratic run through Spherisorb (Waters) C18, 5 µ (250×4.6 mm i.d.) column. The eluent was monitored for gugulipid at 242 nm.

**In vitro drug release**

The release of gugulipid from proniosome formulation was determined by using modified Franz diffusion cell. The semi-permeable membrane was placed between donor and receptor compartment of diffusion cell. The area available for diffusion was 0.95 cm². The receptor compartment was filled with 11 mL of freshly prepared phosphate buffer (pH 7.4). One gram of gel was placed on the membrane and the opening of donor compartment was sealed with a glass cover slip, while receptor fluid was maintained at 35°C with constant stirring using a Teflon coated magnetic stir bead. Two-milliliter sample was withdrawn from receptor compartment at various time intervals up to 8 h and was analyzed by HPLC.

**Anti-inflammatory activity**

The anti-inflammatory activity of gugulipid-loaded proniosomal gel formulations was evaluated by the carrageenan-induced rat hind paw edema method (13). The experimental protocol was designed and approval of Institutional Animal Ethics Committee (IAEC) (Reg. No. 0436) was obtained. Healthy Wistar rats of either sex weighing between 150–200 g were obtained from the disease-free small animal house of CCSHAU, Hisar. The animals were housed in institutional animal house under standard conditions with free access to food and water. Anti-inflammatory activity of the gugulipid-loaded proniosomal gel was compared to the marketed gel of diclofenac (Voveran® Emulgel). Fifteen albino Wistar rats were divided into three groups of five animals each as follows:

- **Group 1 (Control group):** animals were treated with plain proniosomal gel.
- **Group 2 (Standard group):** animals were treated with diclofenac gel B.P.
- **Group 3 (PNG2):** animals were treated with gugulipid loaded proniosomal formulation.

Inflammation was induced by sub-plantar carrageenan injection and after 1 hour, formulations were applied topically on the inflamed paw of rats by gently rubbing with index finger and the volume of the paw was measured (12, 13). The thickness of paw was measured at 1 h time intervals till 5 h after carrageenan injection. A digital vernier caliper (Aerospace, China) was used for measuring paw thickness of rats. The percentage inhibition of inflammation was calculated by the following formula:

\[
\text{Percentage inhibition} = \left[ \frac{(C-T)}{C} \right] \times 100
\]

where C = control paw edema, T = test paw edema.

**Statistical analysis**

Statistical analysis was done by means of one-way ANOVA followed by Tukey’s post-hoc test; p value < 0.05 was considered statistically significant.

**RESULTS AND DISCUSSION**

Proniosomal gel formulation was prepared employing Span-40 as surfactant, as earlier studies.

Table 1. Particle size and entrapment efficiency of proniosomal gel formulations.

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Size (µm)</th>
<th>% Entrapment</th>
</tr>
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<tbody>
<tr>
<td>PNG1</td>
<td>30.1 ± 0.4</td>
<td>89.3 ± 2.4</td>
</tr>
<tr>
<td>PNG2</td>
<td>38.2 ± 1.2</td>
<td>87.6 ± 3.6</td>
</tr>
</tbody>
</table>
Preparation and evaluation of anti-inflammatory activity of gugulipid-loaded proniosomal gel

reported that Span-40 provides vesicles of larger size, with higher entrapment efficiency and least rate of leakage of the drug (8, 9). Table 1 shows the results of particle size analysis and entrapment efficiency of the niosomal dispersion obtained by hydration of gugulipid-loaded proniosomal gels. The niosomes had an average size of 30–38 µm, with a fairly good entrapment of 87–89% of gugulipid. Figure 1 shows the results of in-vitro release of proniosomal gels, studied across the semi-permeable membrane through the modified Franz diffusion cell. It can be observed that gugulipids were released at faster rate initially followed by a slow and sustained release for 8 h duration of study.

The rapid initial release rate of the drug may be due to the diffusion of unentrapped drug. Further, a higher release of drug obtained from formulation containing higher amount of gugulipid.

Table 2 shows the results of paw edema and percentage inhibition of carrageenan-induced paw edema in rats treated with gugulipid proniosomal gels and commercial formulation of diclofenac (Voveran® Emulgel). The control group consisted of rats treated with plain proniosomal gel base. The results revealed a significantly higher (p < 0.05) inhibition of carrageenan-induced paw edema in rats treated with gugulipid-loaded proniosomal gel as compared with the control animals. However, an extremely significant (p < 0.001) inhibition of carrageenan induced paw edema was observed in animals treated with commercial reference product (Voveran® Emulgel) gel in comparison with the control during the entire 5 h duration of the study. Further, the results of % inhibition of paw edema produced by reference products were statistically higher as compared to the gugulipid-loaded proniosomal gel. The study shows that the gugulipid-loaded proniosomal gel possesses fair anti-inflammatory activity but it is not as good anti-inflammatory as the commercial product of diclofenac. Thus, proniosomal gel of gugulipid is a potential herbal anti-inflammatory formulation.

CONCLUSION

The proniosomal gel on hydration with water produced niosomal dispersions of average size 30 µm. The entrapment efficiency of gugulipid in proniosomal gel conducted across semi-permeable membrane revealed the initial faster release followed by slow sustained release of the drug for 8 h

Table 2. Paw thickness and percentage inhibition in different groups at various time intervals.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Paw volume (mm)* (Percentage inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 h</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>5.84 ± 0.13</td>
</tr>
<tr>
<td>2</td>
<td>Standard</td>
<td>4.96 ± 0.05 (15.0%)</td>
</tr>
<tr>
<td>3</td>
<td>PNG2</td>
<td>5.12 ± 0.06 (12.35%)</td>
</tr>
<tr>
<td>One way ANOVA followed by Tukey’s test</td>
<td>F</td>
<td>28.661</td>
</tr>
<tr>
<td>p</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

*Values are the mean ± SEM (n = 5). Significantly different from control at: ‘p < 0.001,’ ‘p < 0.01,’ ‘p < 0.05’
duration of study. Evaluation of proniosomal gel for topical anti-inflammatory activity in carrageenan-induced rat hind paw edema model, demonstrated that the proniosomal gel possess fairly good anti-inflammatory activity but not as good as commercial product. However, future studies with inclusion of transcutaneous permeation enhancers in the proniosomal gel formulation may provide a gugulipid loaded proniosomal gel with anti-inflammatory activity comparable to commercial formulations of NSAIDs. Thus, in conclusion, proniosomal formulation of gugulipids holds an immense potential for development of topical herbal anti-inflammatory formulation comparable to topical NSAIDs.

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REFERENCES


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