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Formulation and Evaluation of Standardised *Withania somnifera* Leaf Extract Loaded Transdermal Gel

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Several technological advances have been made in the recent years to overcome skin barrier properties and to enhance drug penetration. In this regard, transdermal drug delivery system provides a suitable solution of the problems associated with conventional routes of drug administration. In the present study, *Withania somnifera* leaf extract loaded proniosomal gel was prepared and evaluated for better delivery and improved anti-inflammatory property. The formulated gel was characterized for vesicle physical analysis, entrapment efficiency and *in vitro* release study. The method used for preparation of proniosomal gel resulted in higher drug entrapment value of 87.2%. Scanning electron microscopy analysis showed that the surface of the particles was smooth. The formulations showed prolonged *in vitro* drug release of 60.8% over a period of 24 h and significant anti-inflammatory response. From this study, it is concluded that proniosomes are very stable and promising delivery system for *Withania somnifera* leaf extract.

Key words: Proniosomes, *Withania somnifera*, transdermal gel

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INTRODUCTION

Withania somnifera (L.) Dunal (Family-Solanaceae) is an erect evergreen shrub distributed almost all parts of India. Commonly known as Aswagandha, it is very popular for its widespread use in Ayurvedic medicines. In folklore medicines, Aswagandha root is used in several health disorders like adenopathy, arthritis, asthma, hypertension, inflammation and as immunomodulator while leaves of this plant are recommended for topical applications only (Pawar *et al*., 2011).

The major chemical constituents present in *Withania Somnifera* are withanolides (0.001-0.5% w/w). Withaferin-A, the main bio active compound of *Withania* leaves is absent in roots, stems and seeds (Singh *et al*., 2011). Withaferin-A exhibits many therapeutic activities such as antitumor, anti-inflammatory, radiosensiting, immunosuppressive and antibacterial (Mishra *et al*., 2000) but water solubility of this bio active compound is very low, hence the bioavailability and in turn efficacy is also becomes very low (Gupta *et al*., 1996). A wide range of potential effects and low water solubility explored it a model compound to carry out the present research work with an aim to enhance its bioavailability and hence therapeutic efficacy.

Several studies conducted in past have suggested that transdermal delivery of drugs through the skin is a suitable and convenient route of administration. In this approach, the drug penetration through skin is enhanced by decreasing the main skin barrier i.e., stratum corneum. Out of the various transdermal delivery approaches, the liposomes, niosomes and proniosomes have been proved as potential methods to enhance permeation of drugs through skin (Scheuplein *et al*., 1969).

Proniosomes are the transdermal delivery system in which water-soluble carrier particles are coated with surfactant and hydrated to form dry formulations. While coming in contact with hot aqueous media, the niosomal dispersion takes place in a short span (Hu and Rhodes, 1999). Previous experimental attempts and theoretical analysis has supported the mechanisms involved in the ability of niosomes to affect drug transfer across skin. (Barry, 2001). Both phospholipids and non-ionic surfactants in proniosomes can act as penetration enhancers, since it was found that some phospholipids are able to fluidize the stratum corneum lipid bilayers and diffuse through them (Kirjavainen *et al*., 1996).

Proniosomes delivery system also increases the physical stability to the preparation thus makes it convenient in transportation and storage. Studies have indicated that proniosomal preparations are less likely to aggregate or fuse which are considered as one of the main problems associated with other transdermal delivery systems (Frfkjaer *et al*., 1984). Proniosomes not only offer a promising means of drug delivery, but also enhance the recovery rate of the skin barrier (Hatziantoniou *et al*., 2000). Due to these properties, the proniosomes are seen as future industrial products (Hu and Rhodes, 1999).

Thus, taking view of the topical delivery potential of the proniosomal gel, *Withania sominifera* leaf extract loaded proniosomal gel based formulation was developed. The developed formulation was then characterized for particle size, entrapment efficiency, *in vitro* drug release and *in vivo* anti-inflammatory activity using carrageenan induced rat hind-paw method.

MATERIALS AND METHODS

Chemicals: Withaferin-A was procured from Alta-Vista Phytochemicals, Hyderabad. Acetonitrile HPLC Grade was procured from Spectrochem Pvt. Ltd., Potassium dihydrogen phosphate from CDH Chemicals Pvt. Ltd., Lecithin and disodium hydrogen phosphate from Hi-Media Laboratories Ltd., Cholesterol, Ethanol and Span 60 from Loba Chemie Pvt. Ltd. and Glycerol was obtained from Merck Chemicals. Pure Carrageenan was procured from Sigma Aldrich.

Plant materials: Fresh leaves of *Withania somnifera* were collected from the Botanical Garden, Ghronda (Karnal district), Haryana, India. The sample material was properly identified by Dr. H.B. Singh, taxonomist at National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. Voucher specimen (No. MDU/Phcog/VS/115) has been retained at the Department of Pharmacognosy, Maharshi Dayanand University, Rohtak, Haryana for future reference. The leaves were then air-dried at room temperature (30-40°C), powdered and kept in polythene bags until used for the next step.

Extraction and standardizaion: The air-dried and coarsely powdered leaves (200 g) were exhaustively extracted with methanol in a soxhlet apparatus for around 24 h till the sample became colourless. The extract was then concentrated and finally evaporated to dryness in a rotary evaporator.

Qualitative and quantitative analysis of withaferin-A in *Withania somnifera* leaves extract was carried out using HPLC-DAD chromatographic system (Hitachi Elite

Lachrom) equipped with quaternary gradient pump (L2130) and autosampler (L2200). The HPLC conditions listed below were used for both purity control and quantitative determination using UV detection at a x wavelength of 210 nm. The injection volume was 10 μL and the column temperature was maintained at 27°C. A number of trials for chromatographic separation was performed as per method described by Mulgund *et al.* (2009). Using a Reliasil reversed phase C18, 5 μm, 250×4.6 mm column with a mobile phase consisting of acetonitrile/water (50:50), at varying pH, with a flow rate of 1.0 mL min^{-1}.

Stock solution of 100 μg mL^{-1} was prepared and further diluted to obtain final concentration of 20, 40, 60 and 80 μg mL^{-1}.

Preparation of proniosomal gel: Proniosomal gel was prepared by coacervation phase separation method according to method reported by Vora *et al.* (1998). Precisely, hundred milligrams of surfactant mixture, surfactants: alcohol (1:1) and drug (1% w/w) were weighed in a clean and dry, wide mouth small glass tube. Surfactant ratio used was soya lecithin: span 60: cholesterol (9:9:2). After mixing all the ingredients, the open end of the glass tube was covered with a lid to prevent loss of solvent and then warmed on a water bath at 60-70°C for about 5 min, until the surfactants were dissolved completely. The aqueous phase (0.1% glycerol solution) was then added and warmed on a water bath till clear solution was formed. The mixture was allowed to cool to room temperature until the dispersion was converted to proniosomal gel. The final ratio of surfactant: alcohol: aqueous phase was 5:5:4 by weight (Fang *et al.*, 2001). The gel obtained was preserved in dark until characterization.

Characterization of proniosomal gel

Particle size analysis: The average size of the prepared proniosomes was performed by laser diffraction particle size analysis (LD) using Microtrac S3500, Laser Diffraction Particle size Analyzer. For analysis small amount of formulation was suspended in aqueous dispersing phase and stirring for 5 min on a votex mixer at room temperature.

Scanning electron microscopy (SEM): The surface morphology (roundness, smoothness and formation of aggregates) of the prepared proniosomes formulation was determined by SEM (JSM-6510 scanning electron microscope at 15 KV). A very small amount of the gel was mounted on an aluminum stub with double sided adhesive carbon tape. The formulation was then sputter coated with gold using a vacuum evaporator and examined with the scanning electron microscope.

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.

The percentage of entrapped drug was calculated by the following equation:

$$\text{Entrapment (\%)} = \frac{\text{Total amount of unentrapped drug}}{\text{Total amount of drug}} \times 100$$

***In vitro* release study:** The release of withaferin-A from proniosomal formulation was determined using Franz diffusion cell. The semi-permeable membrane (cellophane) was placed between donor and receptor compartment of diffusion cell. Before the permeation study, the skin was hydrated in phosphate buffer (pH 5.8) at room temperature overnight. The area available for diffusion was 2.97 cm^2. The receptor compartment was filled with 16.4 mL of freshly prepared 25% ethanolic phosphate buffer (pH 7.4). 500 mg of gel was placed on the membrane and the opening of donor compartment was sealed with paraffin paper while receptor fluid was maintained at 35±0.5°C with constant stirring using magnetic beads. The samples (1 mL) from the receptor compartment were withdrawn at predetermined time intervals and immediately replaced by an equal volume of fresh buffer solution. The samples withdrawn from the receptor compartment were then analyzed by HPLC.

Anti-inflammatory activity: Anti-inflammatory activity of *Withania somnifera* extract loaded proniosomal gel formulation was evaluated using carrageenan-induced rat hind paw edema method (Gupta and Gaud, 2006; Goyal *et al.*, 2011). The experimental protocol was duly got approved by Institutional Animal Ethics Committee (IAEC) with reference no. Pharma Sc./91-101. Healthy Wistar rats of either sex weighing between 150-200 g were obtained from the animal house of DIPSAR, New Delhi. The animals were divided into three groups each containing 6 rats. Localized inflammation was induced by sub-plantar injection of 0.1 mL carrageenan suspension (1% w/v in distilled water) into right hind paw, 1 h before drug administration where maximum oedema was reached. The paw size was determined before and after administration of the sample drug at different time

intervals using vernier caliper. First group received only vehicle (control group), second group was treated with the *Withania somnifera* extract loaded transdermal formulation and third group was treated with marketed diclofenac gel (Voveran® Emulgel) (standard group). Readings were taken every hour for 5 h duration and percentage inhibition of inflammation was calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{C-T}{C} \times 100$$

Where:

C = Control paw edema
T = Test paw edema

Statistical analysis: Statistical analysis was done by means of Student's t-test (one-tailed); $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Characterization of Proniosomal gel formulation: Proniosomes prepared were found to had an average size of 2-11 μm (Fig. 1) with an good entrapment of 87.2%. This high entrapment efficiency may be attributed to the high lipophilic nature of the drug. Scanning Electron Microscopy (SEM) study of the formulated proniosomal gel showed that the proniosomes prepared by coacervation phase separation method were small in size, unilamellar and spherical in shape with a smooth surface (Fig. 2).

The drug-release study of investigated formulations showed slow release of the drug at almost constant rate and gave sustained release for 5 h duration of study. This may be due to the fact that most of the drug got entrapped within the vesicles which releases the drug at a slow rate (Sudhamani *et al*., 2010). The lesser rate may also be attributed to presence of less amount of marker compound in the extract which was used as active in the proniosomal formulation. Thus, a higher release can be obtained from formulation containing higher amount of marker compound rich extract.

Assessment of anti-inflammatory effect: In the anti-inflammatory activity test using carrageenan as phlogistic compound, pronisomal and standard diclofenac gel (voveran) exhibited anti-inflammatory activity up to 5 h and peak activity was observed between 3-5 h for both formulations (Table 1). Pronisomal gel exhibited almost comparable anti-inflammatory activity compared to standard gel ($p<0.05$). Pronisomal gel application resulted in 7.8% inhibition at the end of first hr after application of the gel which was increased further to 32.16% after 5th h.

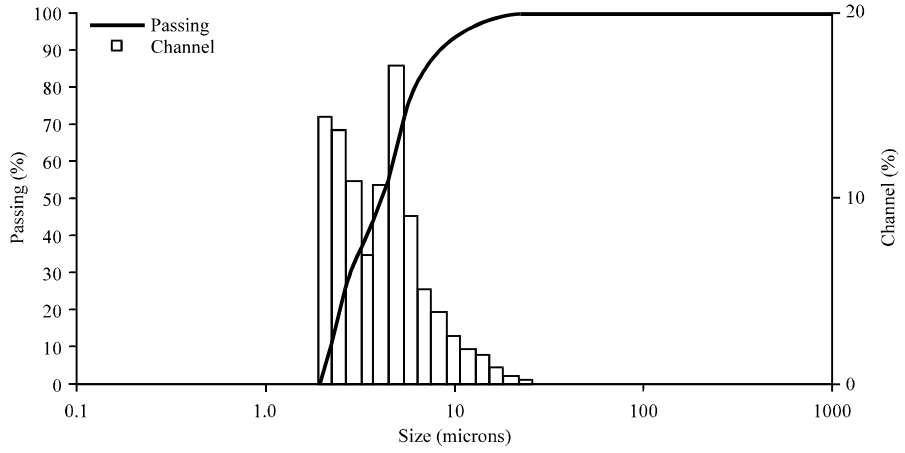

Fig. 1: Bar graph showing particle size range

Table 1: Data showing paw edema volume and % inhibition

	Paw Edema (mm) (% inhibition)				
Compound	1 h	2 h	3 h	4 h	5 h
Control group	3.97±0.19	5.50±0.27	6.20±0.28	6.51±0.21	6.28±0.26
Proniosomal gel	3.66±0.332* (7.8%)	5.23±0.250 (4.9%)	4.73±0.18* (23.7%)	4.4±0.282* (32.4%)	4.26±0.302* (32.16%)
Voveran gel	3.52±0.377 (11.3%)	5.15±0.372 (6.36%)	4.66±0.38* (24.8%)	4.38±0.278* (32.7%)	4.11±0.348* (34.5%)

Values are the Mean±SEM (n = 5), *Significantly different from control at $p<0.05$

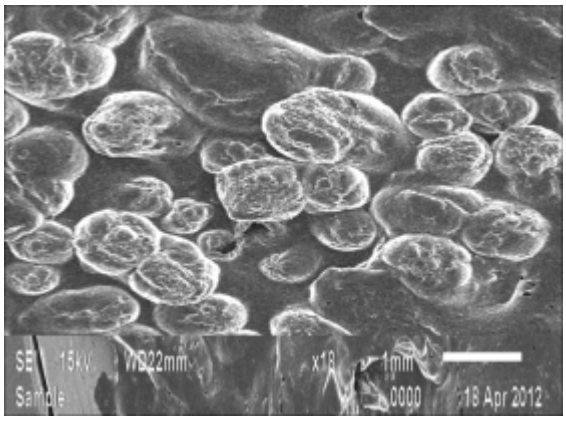

Fig. 2: SEM image of the proniosomal gel

Inhibition produced by the application of standard gel was 11.3 and 34.5% after 1st and 5th h, respectively. The results confirm the fact that a significant amount of drug was delivered from the gel through rat skin to induce the anti-inflammatory effect.

CONCLUSION

Present study has shown that entrapment of *Withania somnifera* leaves extract using nonionic surfactants into the proniosomal gel provided significant transdermal flux and high entrapment efficiency. Pharmacological activity was quite comparable with standard anti-inflammatory gel. Thus concluded that the developed formulation offers a suitable approach for transdermal delivery for Withaferin-A.

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