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Research Article

SKIN PERMEATION STUDY OF NANOMIEMGEL FOR ANTIFUNGAL ACTIVITY OF EXTRACT *SAMADERA INDICA*

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ABSTRACT

The transdermal route of drugs has received increased attention in recent years due to numerous advantages over the oral and injectable routes, such as avoidance of the hepatic metabolism, protection of drugs from the gastrointestinal tract, sustained drug delivery, and good patient compliance. The assessment of *ex vivo* permeation during the pharmaceutical development process helps in understanding the product quality and performance of a transdermal delivery system. Generally, excised human skin relevant to the application site or animal skin is recommended for ex vivo permeation studies. However, the limited availability of the human skin and ethical issues surrounding the use of animal skin rendered these models less attractive in the permeation study. The aim of this study to determine the skin permeation of Nanomiemgel for using antifungal plant extract by using rat abdominal skin was conducted through Franz diffusion cell. The *ex vivo* skin permeation studies were performed using Franz diffusion cell with rat skin as permeation membrane. Significant increase in permeability parameters was observed in nanomiemgel formulations ($P < 0.05$). This research discussed the alternative skin models that have been used for skin permeation. *Ex vivo* permeation studies of rat were performed for each animal and trail, five parameters are to be collected. After removal of patch on completion of a week, each of the areas was examined for any sign of erythema or edema.

Keywords: Nanomiemgel, rat abdominal skin, Franz diffusion cell.

1. INTRODUCTION

The fungal infections are a dynamic and complex bio-logical process which requires orchestration of different cellular processes to help damaged skin restores its normal function and structure. These include, for example, non-invasive treatment, gastroin-testinal tract protection, and avoiding the first pass metabolism of the liver. The ideal physicochemical characteristics of a drug chosen for cutaneous adminis-tration are very low that reaches systemic circulation and drug absorption by vessels diffusion across the viable epidermis layers into the dermisdrug partitioning from the stratum corneum into viable epidermis layers.

1.1. Modifying Factors of Skin Penetration

The interactions between the drug, skin, and vehicle determine: (1) The drug release, (2) the penetration through the SC, (3) the penetration through the viable skin layers. In ancient times herbal remedies contains hundreds of phytopharmaceuticals of plants and it has been increasing day by day among physicians and patients. Various dietary products and supplements are also derived from the natural origin and gaining more interest in the industry and the global market for phytopharmaceuticals [1, 2]. *Samadera indica* is a medicinal plant obtained from Western Ghats of India including Kerala, Ernakulum and Karnataka forest region in India. *Samadera indica* is also known as Neipa bark tree, *Quassia indica*, karinjotta, karingota etc. It is bitter in taste and is a small tree up to 11 m in height with stout branches and pale yellow bark. It is widely distributed in evergreen forests and along backwaters of south India. *Samadera indica* leaves are used for the treatment of number of diseases [2]. It is traditionally used in vitiated conditions of kapha and pita, leprosy, scabies, pruritis, skin diseases, for treating burns, inflammations, bacterial infections, gonorrhoea, asthma etc. [1, 3, 4]. A typical extraction process may contain following steps [4]: Collection and authentication of plant material & drying, Size reduction, Extraction, Filtration, Concentration, Drying & reconstitution [5, 6]. Quality of an extract is influenced by several factors such as, plant parts used as starting material, solvent used for extraction, extraction procedure and plant

material, solvent ratio etc. From laboratory scale to pilot scale, all the parameters are optimized and controlled during extraction. Extraction techniques separate the soluble plant metabolites through selective use of solvents. It is the oldest method of preparation of plant extract. It is a main constituent source of bioactive compound which are used for medicine and other applications. Plant extract contains many bioactive compounds like as flavonoids, steroids, tannins, alkaloids, fixed oils, volatile oils etc. All of these bioactive compounds are obtained from specific parts of plants like as flowers, leaves, fruits, barks, seeds etc [6-8]. Now a day's herbal formulations are mostly used which is converted into novel herbal formulations and extraction method is one of the important method for obtaining bioactive compounds. There are number of methods available for extraction- maceration, percolation, infusion, decoction, soxhlet extraction etc. [9-11]. It should be noted that choice of appropriate solvent is of essential importance along with application of a compatible extraction method. For selection of solvents 'like dissolves like' principle is applicable. Thus polar solvents will extract out polar substances and non-polar material will be extracted out by non-polar solvents. Succecive Solvent extraction method is the most popular method of extraction. [7, 10-12].

2. MATERIAL AND METHOD

2.1. Plant Material

The plant is generally found in South, Ernakulum district Kerala during the month of February. For the study plant was obtained from DKC Agro tech Pvt. Ltd., New Delhi, India.

2.2. Chemicals

The chemicals used in this study were obtained from laboratory of Dr. APJ Abdul Kalam University, Indore, [M. P.], India.

2.3. Plant Profile

Common name: *Samadera indica*
Synonyms: Karinjotta, Quassia Indica, Lokhandi, Niepa bark tree
Family: Simaroubaceae
Medicinal use: Fever, skin disease, rheumatic, febrifuge, erysipelas, anti inflammatory etc.

Fig. 1: ***Samadera indica*** **plant**

2.4. Preparation of plant extracts

Leaves were collected, separated out and washed to remove impurities. Leaves were dried in the presence of sunlight, then leaves were blended in the mechanical grinder and converted into fine powder by passing through sieve no 40 and stored in an airtight container for further use. 50gm of fine powder in round bottom flask was soaked in 250ml of methanol for 24 hours with intermittent shaking. The extraction process was done by hot extraction method using soxhlet apparatus. This process was continued until the solvent became clear and collected the sample in container. After the extraction, the extract was kept in heating mantle for evaporating the solvent until the extract obtained in crude form.

2.5. Determination of Extraction Yield (% Yield)

The yield (%W/W) from the dried extracts was calculated as

% Yield = (W_1 X 100)/W_2

Where W_1 is the weight of extract after evaporation of solvent and W_2 is the weight of the plant powder.

2.6. Formulation of Nanomiemgel (NMG)

2.6.1. Formulation of nanoemulsion

Nanoemulsion (NEM) was prepared by first dissolving Extract (800 mg) in 8 mL of olive oil and miglyol (1:1), followed by the addition of 6 mL of "Polysorbate 80 and Transcutol" mixture (1:1). The oil and surfactant mixture-containing drug were sonicated for about 15 minutes to get clear oil & surfactant mixture. To the mixture, 11 mL of deionized water was added while homogenizing to get a primary emulsion. The obtained o/w emulsion was homogenized further for 5 minutes at 3000 rpm to obtain a microemulsion.

2.6.2. Formulation of nanomicelle

Nanomicelle was prepared with Vitamin E TPGS using the solvent evaporation method where the organic solvent was removed through evaporation. The Vitamin E TPGS (7.55 gm), Extract (800 mg) were added to 2 mL of acetone. When a clear solution was obtained, 25 mL of distilled water was added. Then the organic solvent was removed gradually through evaporation. Change of solvent quality and hence, selectivity, from organic to aqueous was gradual; the polymer and the extract were able to aggregate into micelles rather than precipitating from the solution into the bulk. The solvent of choice was acetone, due to its high water miscibility and low vapour pressure, which simplified the solvent removal.

2.6.3. Preparation of nanomiemgel

50 mL of purified distilled water and propylene glycol (1:1) were put into a beaker and heated up to 70°C. EDTA (0.5%) and pluronic F-127 (0.5%) were added into the warmed purified water with continuous stirring after which the mixture was cooled down to 50°C. The mixture was then added to carbopol (1g) under continuous low rpm stirring to form a uniform gel that was free from lumps and bubbles. The pH of gel was then neutralized with triethanolamine (TEA). After cooling the gel phase to 40°C, the NEM and NMI formulations were incorporated into the carbopol gel and mixed uniformly to obtain the NMG. The NEM and NMI were dispersed into the carbopol gel to achieve the final concentration of extract at 2% respectively, whereas the final concentration of carbopol was maintained at 2%. This NMG was used for the drug release, skin permeation and other *in vivo* studies.

2.7. Evaluation of Nanomiemgel

2.7.1. Ex-vivo skin permeation studies

The *ex-vivo* skin permeation of NMG through depilated rat abdominal skin was conducted using a modified Franz diffusion cell. The study was conducted in accordance with the Helsinki declaration and animal care and facilities in Principles and Methods of Toxicology.

2.7.2. Preparation of skin

The abdominal skin of Long-Evans rat was used. Hairs on the abdominal area were shaved after sacrificing by prolonged chloroform inhalation. Abdominal skin was excised and the subcutaneous tissue was surgically removed. Dermis side was wiped with isopropyl alcohol (IPA) to remove residual adhering fat. The skin was then washed with distilled water. The prepared full thickness skin was treated with 2 M sodium bromide solution in water for 6 h. The epidermis was separated by using a cotton swab moistened with distilled water. Then, epidermis sheet was cleaned by washing with distilled water. The skin so prepared was wrapped in aluminum foil and stored in a freezer at-20°C till further use.

2.7.3. Assembling of the Franz diffusion cell

The skin membranes were first hydrated for 30 min in the buffer solution (pH 7.4) at room temperature to remove extraneous debris and leachable enzymes. They were then placed between the donor and receptor compartments of the cells, with the dermal side in direct contact with the receptor medium. Approx. 150 ml of the phosphate buffer (pH 7.4) was placed in the receptor compartment. Its temperature was maintained at 37±0.5°C using a thermostatic water bath. This whole assembly was kept on a magnetic stirrer and solution in the receiver compartment was continuously stirred during the whole experiment using magnetic bead. The samples were withdrawn (200 μ ml each time) at different time intervals and an equal amount of phosphate buffer (pH 7.4) was replaced each time. Absorbances of the samples were read spectrophotometrically at 280 nm taking phosphate buffer solution

(pH 7.4) as blank. The amount of drug permeated per square centimetre at each time interval was calculated and plotted against time. A similar set was run simultaneously using the patch (without drug) at the donor compartment as a skin patch control system to avoid the influence of inherent extracts from the skin or leaching of any material from the patch without drug on the absorbance at 284 nm.

2.8. Evaluation of skin irritation potential of polymeric matrices

The primary skin irritation studies were carried out using modified Draize test [13]. The hair of rats was removed by shaving from the dorsal area on both sides 24 h before test. One side of the back of each rat *i.e.* untreated skin area served as the control for the test. Medicated patch was secured on experimental side using adhesive tape and the non-medicated patch was adhered on the control side of 6 rats. These patches were covered with occlusive covering to approximate the condition of use. The medicated patches were changed after 48 hands the fresh patches were secured at the same site. However, the patches on the control side were not changed. The patches were secured on the back for seven days. After removal of patch on completion of a week, each of the areas was examined for any sign of erythema or edema.

Table 1: Observation of skin permeation

Formulation	Visual observation	
	Erythema	Edema
Normal	0.00±0.00	0.00±0.00
Adhesive tape (USP)	1.18±0.18	1.37±0.12
NMG	1.28±0.27	1.47±0.14
Blank	1.32±0.11	1.07±0.36
Formalin (0.8% v/v)	2.59±0.07	2.19±0.27

Values are expressed as Mean±SEM, n=6 Significant when compared to Formalin (p<0.05)*

3. RESULTS AND DISCUSSION

The *ex-vivo* skin permeation of NMG through depilated rat abdominal skin was conducted using a modified Franz diffusion cell. The study was conducted in accordance with the Helsinki declaration and animal care and facilities in Principles and Methods of Toxicology. Prepared skin NMG by using Isopropyl alcohol and water. and then, epidermis sheet was cleaned by washing with distilled water. The skin so prepared was wrapped in aluminum foil and stored in a freezer at -20°C till further use. After that France diffusion cell assembly was set and performed the operation firstly with drug and second time without drug. Absorbance of the samples was read spectro-photometrically at 280 nm taking phosphate buffer solution (pH 7.4) as blank. The primary skin irritation studies were carried out using modified Draize test. The hair of rats was removed by shaving from the dorsal area on both sides 24 h before test. Medicated patch was secured one experimental side using adhesive tape and the non-medicated patch was adhered on the control side of 6 rats. The patches on the control side were not changed. After removal of patchon completion of a week, each of the areas was examined for any sign of erythema or edema.

During the permeation study, it is essential to maintain the sink condition in the receptor compartment of the Franz diffusion cell. An ideal sink condition would have a zero-drug concentration in the receptor medium. However, it is not practically possible to achieve a perfect sink condition with static diffusion cells. The experimental setup conditions of Franz cells have a great influence on the results while carrying in vitro permeation studies. The variation in permeability data is influenced by experimental parameters like temperature, sampling frequency, stirring condition, and membrane chemistry.

4. CONCLUSION

For fungal infection healing purposes, several studies have shown the effectiveness of NMG in restoring endothelial function and accelerating tissue repair of a skin lesion in both animal and human studies. To completely utilize the potential of this administration route, it is necessary to optimize the drug release and skin permeation study. This research work drug administration into a skin layer and permeation through the skin is a complex challenge and develop combination therapy in the form of nanomiemgel for the treatment of skin disease. The success of topical and transdermal therapy is used for the evaluation of the preparations, which facilitate the optimization of the skin permeation of the plant extract so that it can reach the therapeutic site. In present methodology, it was concluded that the Nanomicelles of methanolic extract of *samadera indica* plant was successfully prepared. It was safe and non-toxic. The selected plant extract shows the presence of flavonoids, triterpenes, alkaloids which was responsible for antifungal activity. Current research in drug discovery from medicinal plants involves a multi-

disciplinary approach combining botanical, phyto-chemical, biological and molecular techniques.

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