Clinical Trials of New Permethrin Preparation Efficacy on Sarcoptic Mite Infestation in Sheep and Rabbits

Bassem A. Fayed, Mohamed K. EL-Bayoumy, Mohamed A. El-Nabarawi and Randa Tag A. El Rehem

Quality Control Department, Atos Pharma, SEKEM, Egypt
Parasitology and Animal Diseases Department, National Research Center, Giza, Egypt
Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Cairo University, Egypt

Abstract: The present study was conducted to evaluate the efficacy of topically applied Permethrin proniosome (PP) Emulgel 5%, (PP) Powder 5% and compare it with commercially available Permethrin lotion 5% for treatment of naturally infested sarcoptic mange in sheep and rabbits. A total of 10 rabbits and 2 sheep were enrolled in this study, diagnosis of naturally infested sarcoptic mange in animals was made by identifying skin scrapings and all cases were severely infested with relevant significant clinical signs of scabies. Evaluation of clinical signs suggested that faster complete clinical cure of lesions of infested animals used topically applied (PP) Emulgel was obvious than animals treated with 5% permethrin lotion while (PP) topical powder was not effective as scabicide. In conclusion, the use of proniosome dispersed in Emulgel base which act as penetration enhancer could be used together propelarly as good drug delivery system with Permethrin as a new era for treatment of Sarcoptes scabiei in sheep and rabbits.

Key words: Emulgel • Proniosome • Mite • Sarcoptic Mange

INTRODUCTION

Mange is a contagious skin disease, characterized by crusty, pruritic dermatitis and hair loss, it caused by a variety of parasitic mites burrowing in or living on the skin [1]. Sarcoptid mites are all obligate, burrowing skin parasites of mammals, with over 100 described species [2, 3]. According to Bornstein et al. [4] it is among the most common, widespread and serious types of mange extant, more than 100 known species of infested hosts occur worldwide in at least 10 mammalian orders and 26 families. Domestic hosts include camels, cattle, dogs, sheep, goats, horses, swine, llamas and alpacas. Humans are host to the readily transmitted Sarcoptes scabiei and human scabies occurs most frequently in elderly nursing homes and children’s day-care centers. Some other mange mites may cause transient disease in humans, but infestations seldom persist [1].

Different therapies for scabies consist of topical antiscabiotics such as benzyl benzoate, crotamiton, lindane and permethrin [5]. The most common treatments in human and veterinary settings are topical 5% permethrin and/or systemic treatment with a macrocyclic lactone, such as ivermectin [6]. These acaricides have relatively little activity against arthropod eggs, hence multiple treatments are generally required to achieve cure [7]. Permethrin is a pyrethroid insecticide. It’s mode of action is reacting with the voltage-gated sodium channels causing paralysis of the insect [8], despite the varied methodological quality of trials, a recent meta-analysis suggested that topical permethrin is the most effective [9]. Five percent permethrin is recommended by the Centers for Disease Control and Prevention (CDC) as first-line topical therapy for scabies [10].

Powders for cutaneous application are preparations consisting of solid, loose, dry particles of varying degrees of fineness. They contain one or more active substances, with or without excipients and, if necessary, coloring matter authorized by the competent authority [11].

Number of medicated product is applied to the skin or mucous membrane that either enhances or restores a fundamental function of skin or pharmacologically alters
an action in the underlined tissues. Such products are referred as topical or dermatological products. Many widely used topical agents like ointments, creams lotions have many disadvantages, they are sticky in nature causing uneasiness when applied, have lesser spreading coefficient so applied by rubbing and they also exhibit the problem of stability. Due to all these factors within the major group of semisolid preparations, the use of transparent gels has expanded both in cosmetics and in pharmaceutical preparations. In spite of many advantages of gels a major limitation is in the delivery of hydrophobic drugs. So to overcome this limitation an emulsion based approach is being used so that even a hydrophobic therapeutic moiety can be successfully incorporated and delivered through gels [12]. The presence of a gelling agent in the water phase converts a classical emulsion into an emulgel. These emulgel are having major advantages on novel vesicular systems as well as on conventional systems in various aspects. Various permeation enhancers can potentiate the effect, so emulgel can be used as better topical drug delivery systems over present systems. [13]. Emulgel for dermatological use have several favorable properties such as being thixotropic, greaseless, easily spreadable, easily removable, emollient, non-staining, water soluble, Longer shelf life, bio-friendly, Pleasing appearance [14].

Niosomes are vesicles composed of non-ionic surfactants, such as polyoxyethylene alkyl ethers and may be prepared as single or multilamellar vesicles. Surfactants of this type are known to enhance skin permeation and this is likely to play a role in any modification of permeation using these vehicles [13]. Although niosomes as drug carriers have shown advantages such as being cheap and chemically stable, they are associated with problems related to physical stability such as fusion, aggregation, sedimentation and leakage on storage. The proniosome approach minimizes these problems as it is a dry and free flowing product which allows for ease of transfer, distribution, measuring and storage, thus making it a versatile delivery system [15]. Proniosomes are dry formulations of water-soluble carrier particles that are coated with surfactant and hydrated by agitation in hot water for a short period of time [16, 17], yet in our study the carrier used was aerosil 200, a water insoluble carrier. Proniosomes are successful formulation for transdermal delivery systems [18-25], nebulisable delivery [26], Oral use [17] and topical preparations [27].

The goal of the current study is to prepare permethrin proniosomes (PP) and disperse it in topical powder and emulgel bases to be evaluated through in-vivo trials as a treatment against S. scabiei and comparing its action with Ectomethrin 5 % lotion (Permethrin 5 % lotion).

**MATERIAL AND METHODS**

**Humane Care and Use of Animals:** All animals were housed at National Research Center (NRC) (Giza-Egypt) to be operated in accordance with applicable local laws, regulations, policies and guidelines of NRC.

**Formula of Preparations**

- Permethrin proniosomes (PP) was prepared, using solvent evaporation technique, according to the formula presented in Table 1.
- Permethrin 5% powder was prepared according to formulae presented in Tables 2 and 3 to make a final 5% powder for topical application.
- Permethrin 5% Emulgel was prepared according to formulae presented in Tables 4 and 5 to make a final 5% Emulgel for topical application.

**Detecting the Agent:** According to Deger and Ural et al. [28] prior to enrollment in the study, rabbits and sheep had to have existing S. scabiei infestation, as assessed by the determination of live mites (larva, nymph and adult) within skin scrapings of at least 3-4 sites. Scrapings were performed at those sites adjunctive to healthy tissues, believed to be most likely to yield mites and to those of visible suspected lesions. Hair was clipped thoroughly and then the lesion was scraped until capillary bleeding

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### Table 1: (PP) formula

<table>
<thead>
<tr>
<th>Serial no.</th>
<th>Ingredient</th>
<th>Quantity</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Permethrin</td>
<td>5.00 gm</td>
<td>Insecticide</td>
</tr>
<tr>
<td>2</td>
<td>Cyclohexane</td>
<td>QS*</td>
<td>Solvent</td>
</tr>
<tr>
<td>3</td>
<td>Cholesterol</td>
<td>QS</td>
<td>Basic proniosomal component</td>
</tr>
<tr>
<td>4</td>
<td>Brij 97</td>
<td>QS</td>
<td>Non ionic surfactant</td>
</tr>
<tr>
<td>5</td>
<td>Aerosil 200</td>
<td>QS</td>
<td>Carrier</td>
</tr>
</tbody>
</table>

*Quantity sufficient

### Table 2: Powder vehicle

<table>
<thead>
<tr>
<th>Serial no.</th>
<th>Ingredient</th>
<th>Quantity</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Palmitic acid</td>
<td>QS</td>
<td>Absorption enhancer</td>
</tr>
<tr>
<td>2</td>
<td>Talc</td>
<td>QS</td>
<td>Carrier</td>
</tr>
<tr>
<td>3</td>
<td>Ethyl alcohol</td>
<td>Q S</td>
<td>Solvent</td>
</tr>
</tbody>
</table>

### Table 3: 5% permethrin topical powder

<table>
<thead>
<tr>
<th>Serial no.</th>
<th>Ingredient</th>
<th>Quantity</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PP</td>
<td>9.38 gm</td>
<td>Insecticide</td>
</tr>
<tr>
<td>2</td>
<td>Powder Vehicle</td>
<td>40.62 gm</td>
<td>Vehicle</td>
</tr>
</tbody>
</table>
Table 4: Emulgel vehicle

<table>
<thead>
<tr>
<th>Serial no.</th>
<th>Ingredient</th>
<th>Quantity</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>LAS** (Polyethyleneglycol-8 Caprylic/Capric Glycerides)</td>
<td>Q S</td>
<td>Surfactant</td>
</tr>
<tr>
<td>2.</td>
<td>Plurol Isostearique</td>
<td>Q S</td>
<td>Surfactant</td>
</tr>
<tr>
<td>3.</td>
<td>Oleic acid</td>
<td>Q S</td>
<td>Absorption enhancer</td>
</tr>
<tr>
<td>4.</td>
<td>Isopropyl myristate</td>
<td>Q S</td>
<td>Absorption enhancer</td>
</tr>
<tr>
<td>5.</td>
<td>Methyl paraben sodium</td>
<td>Q S</td>
<td>preservative</td>
</tr>
<tr>
<td>6.</td>
<td>Propyl paraben sodium</td>
<td>Q S</td>
<td>preservative</td>
</tr>
<tr>
<td>7.</td>
<td>Carbomer 934</td>
<td>QS</td>
<td>Gelling agent</td>
</tr>
<tr>
<td>8.</td>
<td>Water</td>
<td>QS</td>
<td>Solvent</td>
</tr>
</tbody>
</table>

**Labrasol

Table 5: 5% permethrin topical Emulgel

<table>
<thead>
<tr>
<th>Serial no.</th>
<th>Ingredient</th>
<th>Quantity</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>PP</td>
<td>9.38 gm</td>
<td>Insecticide</td>
</tr>
<tr>
<td>2.</td>
<td>Emulgel</td>
<td>40.62 gm</td>
<td>Vehicle</td>
</tr>
</tbody>
</table>

was evident. The obtained scraping material was mounted in paraffin liquid and microscopically examined for live mites and when necessary the samples were cleared with potassium hydroxide.

**Administration of Topical Treatment:** A study size of 10 Rabbits and 2 local breed “Baladi” sheep naturally infested with *S. scabiei* were enrolled in the study, none of them had been treated with any ecto-parasiticidal or steroidal anti-inflammatory drugs in the 30 days before the study. Treatment for 14 days regimen was selected for this study to identify an effect at a high dose regimen that could subsequently be optimized.

To facilitate examination and identification of each infested leg of tested rabbit, legs position was distributed as following diagram:

![Diagram showing leg positions](image)

Rabbits were restrained manually, after cleaning rabbit's legs with tap water and soap then washing with tap water, it dried with clean towel for each leg. Permethrin proniosome Emulgel 5%, Permethrin lotion 5% and Permethrin Powder 5% were applied topically on the R, R, and L leg respectively, Ll leg was left as control and no treatment was applied.

Sheep ears, nose and left leg were cleaned and dried with same precautions; Permethrin Emulgel 5%, Permethrin lotion 5% and Permethrin Powder 5% were applied topically on the left ear (LE), nose (N) and front left leg (FL) respectively. Right ear (RE) was kept infected without treatment as control.

After application, rabbits and sheep were returned to the cage and drugs were allowed to air dry on the animals’ skin and hair coat. Animals were observed daily for any adverse effects, including signs of toxicity, mortality and (for the efficacy study) clinical signs of acariasis, including pruritus, alopecia and ulcerative dermatitis for the duration of the study as well as skin scraping of cured sites were examined microscopically for mite detection [29].

**RESULTS**

On the 1st day Skin scrapings of all animals indicated the presence of *S. scabiei* mange mites. The total number of each life stage found in a scraping used as an indication of the severity of the infestation (i.e., low, moderate or high), severe lesions were detected on all rabbits and sheep.

On day 15, lesions received topical permethrin emulgel (R, leg of rabbits and LE of sheep) showed complete clinical remission of clinical signs. In lesions treated with topical permethrin lotion (R, leg of rabbits and N of sheep) 7 (70%) of rabbits showed complete remission of clinical signs, 3 rabbits and 2 sheep showed low signs of infestation (not completely recovered).

Lesions treated with topical Permethrin powder (L, leg for rabbits and FL of sheep) and those enrolled in the control group (L, leg for rabbits and RE of sheep) appeared to show no recovery or any degree of self-cure respectively.

Based on clinical examination, the topical treatment of Permethrin 5% Emulgel regimen was well tolerated in all animals (Rabbits and Sheep). There were total reductions in the severity of the clinical signs for the Permethrin 5%
Fig. 1: Left: *S. scabiei* heavy infestation on four legs at day "0". Right: 15 days after treatment

Fig. 2: Left: *S. scabiei* heavy infestation on four legs at day "0". Right: 15 days after treatment

Fig. 3: Left: *S. scabiei* Heavy infestation at day "0". Right: 15 days after treatment

Fig. 4: *S. scabiei* infestation in sheep at day "0"

Emulgel treated organs compared to those of permethrin 5% lotion treated organs. In contrast, permethrin 5% powder showed no efficacy for therapy compared with control organs.

Cured legions gave no any adult, larva, nymph of sarcoptic mites on microscopic examination of both rabbits and sheep skin scrapings.

There was no adverse drug experience nor adverse treatment related mortality during the study in any of the animals. Only one rabbit developed a small area of alopecia on the treated leg with Permethrin 5% Emulgel at the site of application of the drug, which resolved within 3 days.

**DISCUSSION**

Topical treatment against *S. scabiei* includes permethrin, lindane, benzyl benzoate, bioallethrin, crotamiton and precipitated sulfur, topical scabicides have neurotoxic effects on mites and larvae [29]. Permethrin cream (5%) was introduced in 1989 for the treatment of scabies and seems to be a good substitute for previous medications, it is considered to be the drug of choice in many countries [30, 31]. Permethrin is a photostable synthetic pyrethroid with potent insecticidal activity; it is cosmetically elegant and easy to use, without any unpleasant odor. It acts by disrupting the current in sodium channels that are ubiquitous and therefore acts at all stages of the life cycle of the mite. Neither lindane nor ivermectin has this effect [32].

Permethrin products are EPA (Environmental protection agency) registered for use in lactating and beef cattle as well as small ruminants and swine; the meat withdrawal time is 5 days in swine and 0 days in approved species [33]. The 5% permethrin preparation has an extremely low rate of absorption, making the toxicity potential nonexistent. It is probably the most reliable topical scabicide [34]. Permethrin is rapidly broken down by skin esterases to an inactive metabolite and therefore has very low mammalian toxicity [32], so in our study we applied it on different lesions on the same animal as there is no systemic effect.

This study was planned to evaluate the efficacy and safety of topical Permethrin 5% Emulgel, permethrin topical powder and compare it with commercial Permethrin 5% lotion. The total reductions
in the severity of the clinical signs for the Permethrin 5 % Emulgel treated skin sites compared to those of permethrin 5% lotion treated sites may be due to, emulgels have emerged as one of the most interesting topical delivery system as it has dual release control system i.e. gel and emulsion. The major objective behind this formulation is the effective penetration of hydrophobic drug (permethrin) to scabies mites through the skin. In fact presence of a gelling agent in water phase converts a classical emulsion in to emulgel. The emulgel for dermatological use has several favorable properties such as being thixotropic, greaseless, easily spreadable, easily removable, emollient, non-staining, water-soluble, longer shelf life, bio-friendly, transparent and pleasing appearance [14]. Various penetration enhancers can potentiate the effect, so this can be used as better topical drug delivery systems over present conventional systems available in market as presented by Singla [12].

The Interaction Between Skin and Proniosomes May Be an Important contribution to the improvement of transdermal drug delivery. One of the possible mechanisms for niosomal permeability enhancement is structural modification of stratum corneum. Both phospholipids and nonionic surfactants used in proniosomes act as penetration enhancers, leading to increase the permeation of many drugs [35].

Although proniosomes may act as penetration enhancer, yet topical powder containing proniosomes did not give the same efficacy as that of the emulgel. This result may be attributed to less adherence of topical powder to skin than emulgel.

In conclusion, as emulgel have better spreadibility, adhesion, viscosity and extrusion and proniosomes act as penetration enhancer they could be used together as a good drug delivery system for treatment of S. scabiei in different animals. In this study Permethrin 5 % proniosomes Emulgel was more effective as scabicide than 5% permethrin lotion while (PP) topical powder is not effective. As the meat withdrawal time is 0 days in meat producing species except swine (5 days), it is economically recommended to use topical Permethrin 5 % proniosomes Emulgel instead of other systemic treatment.

ACKNOWLEDGMENT

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REFERENCES