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Research Article

ASSESSMENT OF ANTI-DERMATOPHYTIC ACTIVITY OF ZINCODERM GM CREAM IN EXPERIMENTAL TINEA PEDIS IN WISTAR RATS

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ABSTRACT

Zincoderm GM cream is a fixed dose combination of clobetasol, gentamicin, miconazole and zinc. Studies have been done for efficacy of each component like clobetasol, gentamicin and miconazole but as fixed dose combination including Zinc has not been reported yet. Hence a study was planned to assess the efficacy of Zincoderm GM cream in experimental Tinea pedis in animal models. In the experiment a total of 24 Wistar rats (male, pathogen free, 6-8 weeks old) were used. The rats were divided into 4 groups of 6 rats each. 25-30 mg of test drugs (Zincoderm GM cream with or without Zinc) was applied on infected paws from 10th day of post-infection. Fungal infection was assessed by clinical response scoring system and fungal culture examination. Clinical response ($p<0.003$) as well as fungal scores ($p<0.001$) was significantly lower in Tinea pedis induced rats treated with Zincoderm GM cream with Zinc when compared to Tinea pedis induced control (untreated) rats. There was no significant difference in clinical response scores ($P<0.0264$) between Zincoderm GM with zinc and Zincoderm GM without zinc treatment groups. However, there was significantly lower fungal score($P<0.019$) in Tinea pedis induced rats treated with Zincoderm GM cream with Zinc in comparison with Tinea pedis induced rats treated with Zincoderm GM cream without Zinc.

Keywords: Tinea pedis, *Trichophyton rubrum*, Zincoderm GM, Zinc.

INTRODUCTION

The skin is an external organ that covers the entire body surface. It is responsible for the communication between an organism and the environment and is constantly subjected to exogenous stimuli[1]. The main function of the skin is to protect the organism from environmental insults. Fulfilling its role, the skin is able to activate a defence mechanism aimed at pathogen elimination and tissue repair. Most skin infections can be treated outpatient although physicians should be on alert for signs and symptoms of more severe infections. Therefore, clinical assessment of the severity of the infection, diagnosis, and knowledge of pathogen-specific antibiotic resistance is important. Tinea cruris, corporis, and pedis, named for the body sites involved, are superficial fungal infections (dermatophytosis) caused by three genera of dermatophytes: Trichophyton, Microsporum, and Epidermophyton.

These dermatophytes are a homogenous group of fungi that live on the keratin of the stratum corneum, nails, and hair. The estimated lifetime risk of acquiring tinea infections is between 10 and 20 % [1-5]. Dryness of the skin's outer layer discourages colonization by microorganisms, and shedding of epidermal cells keeps many microbes from establishing residence. With inhibition or failure of the skin's protective mechanisms, cutaneous infection may occur with subsequent pruritus, redness, and scaling. Since dermatophytes require keratin for growth, they are restricted to hair, nails, and superficial skin; therefore, most can be treated with topical antifungal medications. Tinea pedis (athlete's foot) is one of the most common superficial fungal infections of the skin and is most often caused by the dermatophytes *Trichophyton rubrum*, *Trichophyton mentagrophytes*, and *Epidermophyton floccosum*[6-8]. Affected skin is usually pruritic with scaling plaques on the soles extending to the lateral aspect of the feet and interdigital spaces. Tinea cruris is a dermatophyte infection of the groin (jock itch) also caused by T. rubrum, T. mentagrophytes, and E. floccosum[8-10]. This condition affects the skin of the medial and upper parts of the thighs, usually bilaterally, with severe pruritis. In general, the selection of topical antifungal agent will be dependent on the probable microorganism causing the infection. Patients who have compromised epidermis, poor hygiene, live in crowded conditions, have co-morbidities, and have close contact with people having skin and soft tissue infections are at high risk of acquiring a skin and soft tissue infection themselves. Many topical antifungal preparations are available as prescription medications and over-the-counter (OTC) products.

Zincoderm GM cream is a fixed dose combination of Clobetasol, Gentamicin, Miconazole and Zinc. Studies have been done for efficacy of each component like Clobetasol, Gentamicin and Miconazole but as fixed dose combination including Zinc has not been reported yet. Hence a study was planned to assess the efficacy of Zincoderm GM cream in experimental Tinea pedis in animal models.

MATERIALS AND METHODS

Animals

24 adult male Wistar rats (150–200 g) were housed in polypropylene cages, maintained under standard conditions with temperature (22–240 C), 12-h light/12-h dark cycle and relative air humidity 40–60%. Rats had continuous access to norm caloric standard rat pellet diet (Hindustan Lever Ltd., Mumbai, India) and to tap water. The animals were acclimatized to the laboratory conditions for one week before the start of the experiment. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC/KMC/89/2013) and experiments were conducted according to the ethical norms approved by Ministry of Social Justices and Empowerment (Government of India), Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines.

Drugs

Zincoderm GM cream with Zinc, Zincoderm GM cream without Zinc and cream base were obtained as kind gift from Apex Laboratories Private Limited, Chennai (India).

Test micro-organism

The cultures of dermatophytic strain *Trichophyton rubrum* was grown on a slant of Sabouraud's dextrose agar at 37°C for 21 days and a conidial suspension of *T. rubrum* was prepared in sterile saline containing 0.05% (W/V) Tween 80 [3]. The conidial suspension was adjusted to a density of $1x10^8$ conidias /ml using haemocytometer. This conidial suspension was used as inoculum[11-12].

Inoculation of test micro-organism (*Trichophyton rubrum*) in rats

The site of inoculation was the planta of both the hind paws of Wistar rats. A paper disk (diameter, 11mm; AA disk; Whatmann) was wetted with 50 µl, 10^6 cells of the conidial suspension, and applied onto the planta and fixed with cotton and an adhesive elastic tape[3]. To avoid cross-contamination and to improve growth conditions for the bacteria, an occlusive dressing (Tegaderm, 6x7 cm; 3 m) was applied immediately after the application of the conidial suspension. The rats were bandaged with Peha-haft for stabilization and protection clipped with VisiStat. The disk was removed on day 7 post infection. The infection was monitored physically.

Experimental procedure

In the experiment a total of 24 Wistar rats (male, pathogen free, 6-8 weeks old) were used. The rats were divided into 4 groups of 6 rats each. 25-30 mg of test drug was applied on infected paws from 10th day of post-infection. The treatment was carried for a period of 7 days with one time per day application of the drugs as follow-

Group I: Normal control rats- 25-30 mg of cream base was applied on both hind paws.
Group II: Tinea pedis toxic control rats- 25-30 mg of cream base was applied at the affected area.
Group III: Tinea pedis positive control rats- 25-30 mg of Zincoderm GM cream without Zinc was applied at the affected area.
Group IV: Tinea pedis test drug treatment rats- 25-30 mg of Zincoderm GM cream with Zinc was applied at the affected area.

Assessment of infection

Clinical scoring system

At the end of the treatment (on day 18 of post-infection), clinical response was estimated by assigning a score between 0 and 4 to each infection site. The scoring was done as Table 1.

Fungal culture examination (Fungal Score)

On the last day of the experiment (Day 18), all animals were killed and the skin block from each hind foot was taken. Each piece of skin block from each hind foot was placed on a plate of Sabouraud-glucose agar containing cycloheximide 500 mg/L, chloramphenicol 50 mg/L and sisomicin 50 mg/L and plates were then incubated at 37°C for 21 days (Figure 1). Skin pieces that yielded fungal growth (*Trichophyton rubrum*) were considered culture-positive. The fungal score was assessed with scores ranging from 0 (Culture negative for *Trichophyton rubrum*) to 1 (Culture positive for *Trichophyton rubrum*).

Table 1: The Scoring System Used for Estimating the Severity of Infection

Score	Symptoms
0	Normal skin
1	Flaking skin with mild inflammation
2	Slight lesions with moderate inflammation
3	Moderate lesions with severe inflammation
4	Severe inflammation plus crust formation

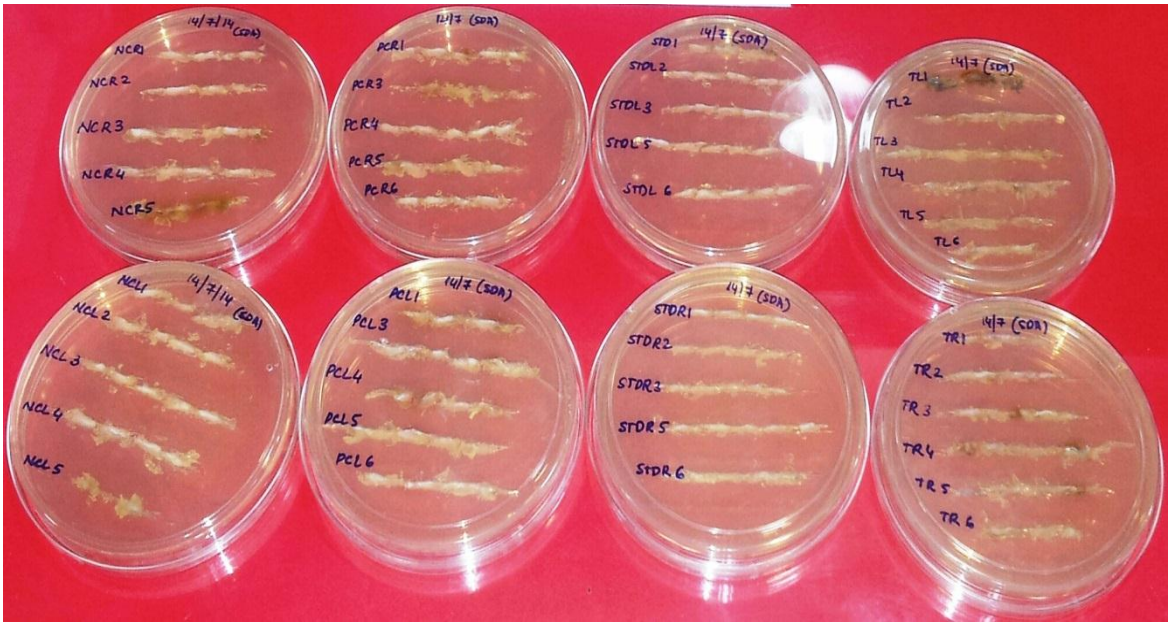

Fig. 1: Incubation of tissue samples for *Trichophyton rubrum* culture examination

Data analysis

Using SPSS 20.0, data were expressed as mean ± standard error of mean and analyzed by one way analysis of variance (ANOVA) followed by post hoc Tukey test. P value less than 0.05 was considered as statistically significant.

RESULTS

There was significant increase for fungal growth (fungal culture positive) as well as clinical response score ($p<0.001$) in the Tinea pedis control (untreated) rats when compared to normal healthy control rats. There was no significant difference for clinical response scores ($p=0.139$) and fungal scores ($p=0.084$) in Tinea pedis induced rats treated with Zincoderm GM without Zinc in comparison with the Tinea pedis control (untreated) rats. Clinical response ($p<0.003$) as well as fungal scores ($p<0.001$) was significantly lower in Tinea pedis induced rats treated with Zincoderm GM cream with Zinc when compared to Tinea pedis induced control (untreated) rats (Table 2 and 3). There was no significant difference in clinical response scores ($P<0.0264$) between Zincoderm GM with zinc and Zincoderm GM without zinc. However, , there was significantly lower fungal score($P<0.019$) in Tinea pedis induced rats treated with Zincoderm GM cream with Zinc in comparison with Tinea pedis induced rats treated with Zincoderm GM cream without Zinc.

Table 2: Effect of Zincoderm GM cream with/without Zinc on Clinical response score

Groups	Dose	Mean ±SEM
I	Normal control group- 25-30 mg cream base	0.00±0.00
II	Tinea pedis + 25-30 mg cream base	1.00±0.00^{***a}
III	Tinea pedis + 25-30 mg 25-30 mg Zincoderm GM cream without Zinc	0.66±0.14
IV	Tinea pedis + 25-30 mg cream Zincoderm GM cream with Zinc treated	0.25±0.13$^{***b, **c}$

[c]ompared to normal control rats, [c]ompared to Tinea pedis toxic control rats, * **p<0.001, **p<0.01

Table 3: Effect of Zincoderm GM cream with/without Zinc on Fungal score (*Trichophyton rubrum*; Absent = 0, Present = 1)

Groups	Dose	Mean ±SEM
I	Normal control group- 25-30 mg cream base	0.00±0.00
II	Tinea pedis + 25-30 mg cream base	3.83±0.16^{a***}
III	Tinea pedis + 25-30 mg 25-30 mg Zincoderm GM cream without Zinc	2.83±0.40
IV	Tinea pedis + 25-30 mg cream Zincoderm GM cream with Zinc treated	2.00±0.44^{b**}

[a]compared to normal control rats, [b]compared to Tinea pedis toxic control rats, [c]compared Tinea pedis + Zincoderm GM cream without Zinc treated rats ***p<0.001, **p<0.01

DISCUSSION

The present study revealed that Zincoderm GM cream with Zinc (Apex Laboratories Private Limited, Chennai, India) is the potential drug that offered a novel therapeutic benefit mainly against *Trichophyton rubrum* inoculated Tinea pedis infection in Wistar rats. The significant decrease in fungal culture positive samples for Zincoderm GM with Zinc treated group in comparison with Zincoderm GM without Zinc may be attributed to the inhibitory effects of Zinc on *Trichophyton rubrum* which has been studied by few researchers [7-9]. Further, clinical evaluation has to be performed to precisely define the therapeutic role

of Zincoderm GM with Zinc in *Trichophyton rubrum* induced tinea pedis infection conditions of human subjects.

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