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Topical therapy of 1-2, 4, 5 Trimethoxy phenyl 1' methoxypropionaldehyde in experimental *Tinea pedis* in Wistar rats

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Abstract

Dermatophytic infections are cutaneous, restricted to nonliving cornified layers because of the inability of the fungi to penetrate deeper into immunocompetent hosts. The current therapeutics includes azoles, allylamines, and polyenes. The unfavourable side effects and the resistance exhibited by the fungus demand new drugs. *Acorus calamus* is a traditional Indian medicinal plant used for curing epilepsy, insomnia etc. In the present study, *in vivo* efficacy of 1-2, 4, 5 trimethoxy phenyl-1' methoxy-propionaldehyde (TMPMP) was evaluated using Wistar rats. Dermatophytic infection was created in the Wistar rats using *T. rubrum* in the plantar region of rats. The infection was monitored using physical, microscopic, and microbiological observations. Therapy was topical application of TMPMP on infection site for 7 days. The curing of infections was again monitored by physical, microscopic, and microbiological observations.

Keywords: *Tinea pedis*; dermatophytes; *Acorus calamus*; Wistar rats.

Introduction

Microbes cause wide range of diseases in human and fungal infections are uncommon in normal healthy persons. However, in immunocompromised host, a variety of normally mild or nonpathogenic fungi can cause potentially fatal infections (Peters and Gilles 1995). Dermatophytic infections are cutaneous, restricted to nonliving cornified layers because of the inability of the fungi to penetrate the deeper in immunocompetent hosts (Dei Cas and Vernes 1986). The etiologic agents of the dermatophytosis are classified in three anamorphic (asexual or imperfect) genera, *Epidermophyton*, *Microsporum*, and *Trichophyton*, belonging to class Hyphomycetes of Deuteromycota (Fungi Imperfecti). Dermatophytes are types of fungi that cause skin, hair and nail infections. Infections caused by these fungi are also known by the names "tinea" and "ringworm". Traditionally, infections caused by dermatophytes have been named on the basis of anatomic locations (i) *Tinea barbae* (ringworm of the beard and moustache); (ii) *Tinea capitis* (scalp, eyebrows, and eyelashes); (iii) *Tinea corporis* (glabrous skin); (iv) *Tinea cruris* (groin); (v) *Tinea favosa* (favus); (vi) *Tinea imbricata* (ringworm caused by *T. concentricum*); (vii) *Tinea manuum* (hand); (viii) *Tinea pedis* (feet); and (ix) *Tinea unguium* (nails) (Weitzmann and Summerbell 1995).

Fungal infections are normally treated with polyenes, azoles and candins, however, cross-resistance is exhibited by various classes of fungi towards these drugs. Resistance to antimicrobial agents has important implications for morbidity, mortality, and health care. These developments and the associated increase in fungal infections intensified the search for new, safer, and more efficacious agents to combat serious fungal infections (Ghannoum and Rice 1999). The alarming side effects and increase in the incidence of fungal disease and the resistance of fungus towards the present day drug demand a new drug discovery.

Despite the existing synthetic drugs, natural products have always been a potent antimicrobial source. Plant derived antimicrobial compounds have already known to possess high antifungal efficacy (Hammer *et al.*, 1998, 2002; Docherty *et al.*, 2001; Fukai *et al.*, 2003; Atai *et al.*, 2009). In our preliminary studies, we found methanolic extracts and unidentified active fractions have antidermatophytic as well as anticandidal properties (Subha and Gnanamani 2008, 2009). In the past, terpenoids have been reported to have antidermatophytic efficacies (Inouye *et al.*, 2006). The present study deals with the *in vivo* efficacy of a novel terpenoid, TMPMP (1-2, 4, 5 trimethoxy phenyl 1' methoxy propionaldehyde) extracted from *A. calamus*.

Materials and Methods

Test organism

Dermatophytic strain *T. rubrum* GH0510 was obtained from Govt. General Hospital, Chennai. The cultures were grown on a slant of Sabouraud's dextrose agar (Himedia, India) at 37°C for 21 days and a conidial suspension of *T. rubrum* GH0510 was prepared in sterile saline containing 0.05% (W/V) Tween 80. The conidial suspension was adjusted to a density of 1×10^8 conidias /ml using haemocytometer. This conidial suspension was used as inoculum.

Procurement of animals

The whole experimental plan was approved by CLRI ethical committee with wide Ref No: 466/01/a/CPCSEA/2001/IAEC/06/001/07. Six-week-old specific-pathogen-free male and female albino rats (Wistar strain) weighing approximately 180 g were used. The rats were housed in 480mm × 270mm × 200mm Apec cages on corncob granules. Photoperiods were adjusted to 12 hr of light and 12 hr of darkness daily, and the environmental temperature was constantly maintained at $21 \pm 1^\circ\text{C}$. Rats were given *ad libitum* access to food and water.

Animal model

Infection was induced by a slight modification of the method by Fujita and Matsuyama (1987). In brief, the site of inoculation was the planta of the hind paw of wistar rats. A paper disk (diameter, 11mm; AA disk; Whatman) was wetted with 50µl. 10^6 cells of the conidial suspension, and applied onto the planta and fixed with an adhesive elastic tape (Johnson and Johnson, India). The disk was removed on day 7 post infection. The infection was monitored physically and microscopically. All the infected rats were grouped into 4 groups with 6 animals each, a healthy control was maintained. Animal groups were as follows; Group-1 (control) Healthy; Group-2 TMPMP (1-2, 4, 5 trimethoxy phenyl 1' methoxy propionaldehyde) treated; Group-3 Fluconazole (Standard drug) treated; and Group-4 Diseased (untreated). Topical application of the drug was carried out as follows, a stock solution of drug was prepared at concentration of 100 µg/ml, 0.1 ml of test compound was applied on infected paws from tenth day of post-infection. The treatment was

carried for a period of 7 days with one time per day application of the drugs.

Assessment of infection

Physical observation on the hair loss, swelling, microscopic evaluations of the tissue scrapping were observed. For microbiological studies, infected skin tissue scraping was excised and cut into bits. The blocks implanted on Sabouraud's dextrose agar, plates were incubated at 37°C for 21 days. Fungal burden was monitored.

Results and Discussion

In humans, *Tinea* is a representative superficial dermatomycosis consisting of various clinical forms depending on the site of infections, depth on invasion and the degree of inflammatory responses during infection, among them *Tinea pedis* is the most prevalent clinical form (Padhye and Weitzman 1998). The *in vivo* study has conventionally been carried out using a hairy skin tinea model by using *T. rubrum*.

In last two decades, a large number of antifungal agents have been successfully developed for topical treatment of superficial dermatomycosis; still the number of cases of relapsing of the infection was common. However, the reason for the mechanism of relapse remains unknown. Dermatophytic lesion or the surrounding tissue may serve as reservoirs. Hence, the relapsing could be avoided by complete eradication of the fungus from the infection site. Thus, the need for new fungicidal antifungal drugs with fewer side effects arises.

In the present study, a novel antifungal terpenoids isolated from *A. calamus* was used. Our previous reports suggest that crude extracts and active fractions possessed antifungal activity. In this animal model, the infections were restricted to the plantar region. The infection was monitored using microscopic, morphological, and microbiologic evaluations. Figure 1 shows the micrograph of infected skin scrapping stained with lactophenol cotton blue showing the dense hyphal mat of *T. rubrum* with macro conidias. Infection was characterised by loss of hair in plantar region, and swelling, reddening of the feet (Figure 2). Microbiological evaluations show that all the animals were infected (Table 1).

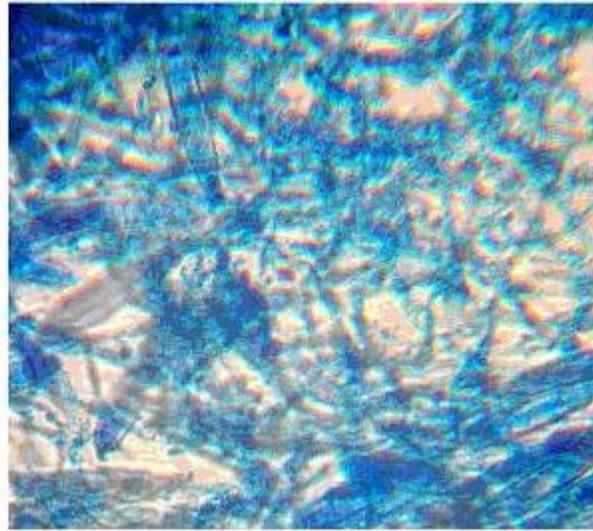


Figure 1: Skin Scrapping showing the dense hyphal mat with macro conidias.



HEALTHY

INFECTED Day 10

INFECTED Day 20

Figure 2: Showing the healthy and infected plantar of Wistar rats.

Groups 2 and 3 represented the drug treated animals. In both TMPMP and Fluconazole treated groups, reduction in swelling followed by initiation of hair growth was

observed. In the animal group treated with TMPMP, initiation of hair growth was observed from third day onwards; on tenth day complete hair growth was observed.



Figure 3: Shows the various stages of curing of *Tinea pedis* on treatment with TMPMP (1-2, 4, 5 trimethoxy phenyl 1' methoxy propionaldehyde).

Microscopic evaluations display the absence of fungal hyphae in the plantar region. The microbiological evaluation shows absence of growth of hyphae. After seven days of topical application of TMPMP, the rats were observed for reoccurrence of infection. There was no reoccurrence of tinea pedis for a period of 8 months. Similar to our findings, Arika *et al.* (1990) reported *in vivo* efficacy of a novel benzyl amine–butenafine in *Tinea pedis* animal model, the authors reported enhanced efficacy in comparison with that of the standard drugs. Uchida *et al.* (2003) reported new imidazoles antimycotic agent NND-502 was effective in controlling tinea pedis in guinea pigs up to 20 weeks of post infection and relapsing of the infections were not reported.

Conclusion

In the present study, *in vivo* efficacy of 1-2, 4, 5 trimethoxy phenyl 1' methoxy propionaldehyde (TMPMP) was evaluated using tinea pedis model in Wistar rats. *T. rubrum* was used to create infections in plantar regions of the paw. Infected animals groups were treated with TMPMP, and fluconazole. Infections and curing was monitored using physical, microscopic, and

microbiological methods. In TMPMP treated animals groups, the infections were cured within seven days and no reoccurrence was observed.

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