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SHORT COMMUNICATION

Efficacy of CIM 1166, a combination of compounds derived from *Mentha* spp. in alleviating experimental vulvovaginal candidiasis in mice

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Abstract *Candida albicans* is yeast that is most often associated with serious fungal infections and can cause fungal diseases in immuno-compromised patients especially patients suffering from AIDS, cancer and cases of organ transplant. Amongst women, candidal vaginitis is predominantly caused by strains of *Candida albicans* and also remains to be a common problem in immuno-competent or healthy women. A study was undertaken to assess the efficacy of a compound CIM 1166 obtained from plant source which was found to possess promising antimicrobial property under in vitro conditions especially against *C. albicans*. Taking the lead further, a small animal model utilizing aged Swiss albino females that had parturated at least three times were taken up for model development. Infection (7×10^6 cfu/ml) was instilled into the vagina in a volume of 20 µl for 3 days. Vaginal washings were aseptically collected on day 4th to confirm the establishment of infection following which the treatment was started which continued for the next 5 days through vaginal route. Vaginal washings were collected on 6th day and the colony forming units were enumerated on chloramphenicol incorporated SDA plates. The results indicated that there was a significant decrease in the colony forming units in vaginal washings (8.0×10^2 cfu/ml) of the treated animals as compared to blank control group (6.0×10^4 cfu/ml). The positive control group administered with clotrimazole also showed a recovery from infection with a fungal load of 8.78×10^2 cfu/ml. The study proves the efficacy of CIM 1166 in curing vaginal candidiasis in mice, which can be taken up for formulation development and further studies.

Keywords Vulvovaginal candidiasis · Mice · Menthol and Menthyl acetate · *Candida albicans*

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Introduction

Invasive fungal infections, particularly in immunocompromised patients have continued to increase in incidence during past few decades and are presently the cause of significant morbidity and mortality. Immune deficiencies as a consequence of HIV infection, aggressive chemotherapy for cancer treatment, growing use of organ transplants besides iatrogenic conditions have been the major etiological causes for the invasive fungal infections especially by *Candida albicans* and *Aspergillus* spp; It is widely accepted that immunocompromised hosts, particularly those with impaired phagocytic cell function (mainly neutrophils and or monocytes/macrophages) are at high risk of infection caused by *Candida* and other filamentous fungi.

Results of epidemiological surveys indicate that *Candida* organisms are present as commensals in oral cavities of approximately 40% of healthy subjects (Challacombe 1994)and that *C. albicans* specifically is carried as

commensal organism in the mouth of approximately one third of the population (Odds 1988). The most common yeasts isolated from vagina are *C. albicans* (85–90%) followed by other spp. of the same genera (Horowithz 1991). Vaginitis is a common gynaecological disorder responsible for 10 million office visits to physicians each year (Haefner 1999) and *C. albicans* is the second most common cause of vaginal infection, bacterial vaginosis being the most common.(March 2000). *C. albicans* may be isolated from genital tracts of approximately 10–55% of asymptomatic healthy women in their child bearing years (MacNeill and Carey (2001). Healthy women develop recurrent symptomatic candidal vaginitis when estrogen levels are high especially during pregnancy (Sobel 1992) though not lethal and rarely progressing to systematic disease, produce significant morbidity, affects a large no. of patients and may respond poorly to conventional therapy.

Research in the past decade have led to many natural compounds being isolated from plant sources showing potential biological activity and essential oils amongst them have been of particular interest, hypothesizing their use in pharmaceutical sector.

Since natural products have been proven to be an excellent source of novel chemical entities, we came across a combination of two essential oil components (Menthol and Menthyl acetate) derived from Mentha spp. that was exhibiting promising antifungal activity under in vitro conditions (Khanuja et al. 2003). The MIC of Menthyl Acetate and Menthol was reported to be 1/400 and 500 µg/ml against *C. albicans*, respectively, in the above study. Further, it was observed that a combination of both, in the ration of 1:1 proved to be synergistic, leading to an MIC of 1/1600 against the same organism. The in vitro results needed to be translated under in vivo condition and so, to prove its efficacy, mice that had parturated more than three times were selected and immuno-suppressed using cyclophosphamide. A pseudo estrous condition was simulated by injecting estradial valereate, to enable the pathogen to establish itself on to the uterine wall. The pathogen was then instilled and allowed to establish itself which was confirmed by spread plating the vaginal washings. Upon establishment of the vaginal candidiasis, the treatment commenced for a period of 5 days. Animals were divided into test groups keeping one group as blank and one for clotrimazole as positive control. Clotrimazole is a synthetic imidazole derivative primarily used locally in the treatement of vaginal and skin infections due to yeasts and dermatophytes. Clotrimazole has been used successfully in patients who had failed to respond to other antifungal agents such as nystatin and amphotericin B but a limiting factor in clotrimazole therapy is the high incidence of gastro-intestinal disturbances and neurological reactions (Sawyer et al. 1975).

Materials and methods

Experimental animals

Apparently healthy, post parturient Swiss albino female mice (30 ± 3 gm) that had parturated at least three times were selected. Thirty such mice in groups of ten were taken for the study and the animals were maintained in the in vivo testing facility under 25 ± 3°C and 70% humidity with 12 h of light and dark cycle and were fed with *adlibbed* diet and water. The study was approved by the Institutional Animal Ethics Committee

Vaginal candidiasis

All the post parturient female mice were injected with a single dose of cyclophosphamide (200 mg/kg bd.wt. i.p.) on day-3. Estradial valereate, to produce pseudooestrous condition (0.5 mg/mice s.c.) was administered from day-3 to day +4 (8 days). *C. albicans* (20 µl) from a suspension of 0.5 Mc Farland's (7×10^6 cfu/ml) was instilled into the vagina on day 0 and day 1. 24 h later, vaginal washings (20 µl) were collected from all the mice to know the establishment of infection and plated on to chloramphenicol treated SDA plates after serial two fold dilution. Upon confirmation of infection, the treatment commenced for a period of 5 days b.i.d. through intravaginal instillation of 20 µl of the compound diluted in 0.7% of sterile Carboxy Methyl Cellulose (CMC) (Fig. 1). All the animals of blank control were treated with only 0.7% sterile CMC. Finally on 6th day fungal load was quantified in a similar manner

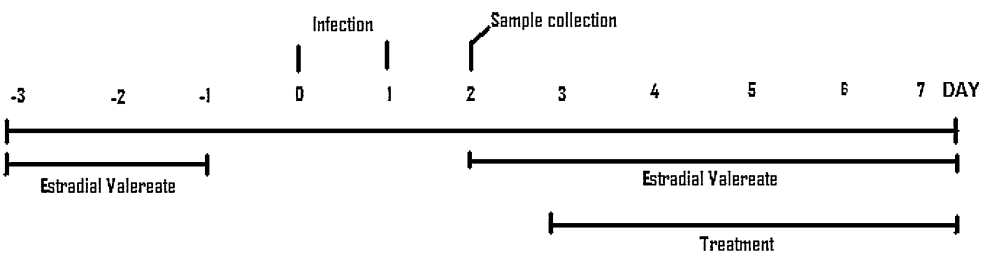

Fig. 1 Schematic representation of the experimental design

as described initially. Results were analysed statistically for their significance through students *t*-test using Graph Pad Prism software.

Results and discussion

In recent years there has been a remarkable increase in the interest of therapeutic use of natural medicines in the form of essential oils, standardized extracts or other botanicals for the therapeutic use. We have examined specifically the therapeutic activity of a synergistic combination of two compounds from Mentha spp. In an experimental model of vaginal candidiasis. It was observed that 20 µl of a suspension containing 1.4×10^5 *C. albicans* were sufficient to establish an infection when instilled for 2 days into the vagina of pseudoestrous female mice (Fig. 2). Previous studies have indicated resistance of some mouse strains to vaginal candidiasis infection (Calderon et al. 2003) but our experiments show the succeptibility of the swiss albino mouse strain to *C. albicans* infection intravaginally. The dose of infection was found to be optimum to achieve the colonization of *C. albicans* after 2 days which was approximately 3.7×10^4 cells per 20 µl of washing. Upon treatment with our compound for 5 days, the fungal load in the vaginal washings were lowered significantly. It was further observed that the progression of colonization by *C. albicans* in the control group rose almost by two folds from 3.7×10^4 to 6.0×10^4 cfu/20 µl (Fig. 3). This observation further strengthens the fact that CIM 1166 and clotrimazole inhibit the colonization and replication of *C. albicans* thus decreasing the probability of recurrence of the infection. CMC was used as a vehicle throughout our studies because it could be used to formulate all the drugs, further, CMC lacks intrinsic antifungal activity. The compound can be taken up for synergistic studies along with clotrimazole with an assumption of lowering the MIC of clotrimazole and selection pressure on *C. albicans* to develop resistance amongst the azoles.

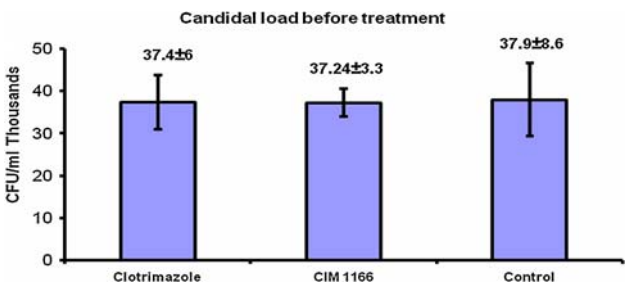

Fig. 2 Vaginal candidal load before start of treatment

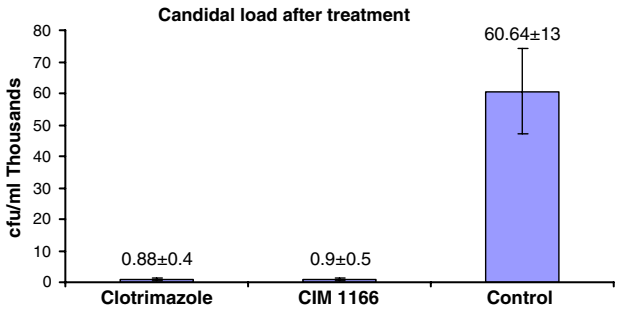

Fig. 3 Vaginal candidal load after treatment

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