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Comparison of Therapeutic effects of the *Myrtus communis* Nano-essence and Topical 1% terbinafine cream in Guinea pigs infected by *microsporum canis*

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Abstract

The use of traditional medicine for centuries to treat various illnesses, is known in many societies.Nanoparticle drug carriers are very important because it carries a variety of drugs to the different parts of the body. They can effectively deliver the drug to a target site and thus increase the therapeutic benefit, while minimizing side effects. In this study, Myrtus communis nanoessence was used to treat dermatophytosis caused by Microsporum canis (M.canis) in an experiment on guinea pigs. In vivo and in vitro methods were used to investigate the antifungal properties of the nanoessence. Minimum inhibitory concentration of nanoessence was 3.2-6.5 µg/ml. The treatment was started 5 days after infection as 12 hours regimen for 40 days after the infection. Both treatment groups, terbinafine and nanoessence, were completely cured over the 40-day period. Results show that the nanoessence under study is an effective drug to treat Microsporum canis caused by dermatophytosis.

Keywords: Nano-essence, myrtus communis, Terbinafine, Microsporum canis, guinea- pig.

Introduction

Dermatophytosis caused by a species of microsporum, trichophyton, or epidermophyton. Dermatophytosis attracts keratinized tissues, hair, and *stratum corneum*[1]. *Microsporum canis* (*M.canis*) is the most common cause of dermatophytosis in animals and human being[2-4]. Over 90% of feline dermatophytosis cases worldwide are caused by *M.canis*[5,6]. Every confirmed case of dermatophytosis should receive topical therapy. Creams and lotions used to treat focal lesions are typically applied every 12 hours to cover a 6-cm margin of clinically normal skin[2].

Terbinafine is an antifungal agent that is taken by mouth or applied to the skin[7].

Therapeutic nanoparticle (NP) technologies have the potential to revolutionize the drug development process, transforming the landscape of the pharmaceutical industry. Nanoparticles could also improve the bioavailability of water-insoluble drugs, enhance absorption, protect therapeutic agents from physiological barriers, and help obtain sustained-release characteristics in nano-sized drugs[8-12].

Chitosan (CS),is the main agent in the production of nano-essence,and its ability to enhance absorption by increasing cellular permeability[13-16].

True Myrtle or *Myrtus communis* L. is one of the famous and ancient medicinal herbs of Iran. The popular Persian name for the plant is *Moord.* The leaves of this "sacred" medicinal plant have been used in Iranian Traditional Medicine for antifungal, antibacterial, anticandidal, antiseptic, anti-inflammatory, mucolytic, carminative, astringent treatments[17,18]. Although the chemical composition of this herb varies according to the geography in which the plant grows, all of the species share the main components including α-Pinene, 1,8-Cineole, Linalool and Limonene[19]. The purpose of this study is to use the *Myrtus communis* nanoessence to treat *M.canis* caused by dermatophytosis under experimental conditions.

Material and Methods

Animals: In this study, 24 male guinea pigs with the same weight (ranging from 350 to 450 grams) were obtained from Pasture institute (Tehran, Iran). All of the animals were kept in separate polycarbonate cages under controlled condition (12 hours light period, relative humidity 50±3%, and temperature 25±1°C). The animals were put in an optimized condition and fed with basic diet for 1 week.

Drugs: *Myrtus communis* essence was purchased from Barij Essence Pharmaceutical Company (Kashan, Iran) and nanoencapsulation was done in Zist Shimi Azma Roshd Company (Tehran, Iran). Five cc of *Myrtus communis* essence is sufficient to produce 1 litre of nanoessence. To confirm the reliability of the product, Fourier Transform Infrared Spectrometer and Screening Electron Microscopy were used (figure 1 and figure 2). The terbinafine hdydrocoloride topical cream used in this study was purchased from Tehran Chemi Pharmaceutical Company (Tehran, Iran).

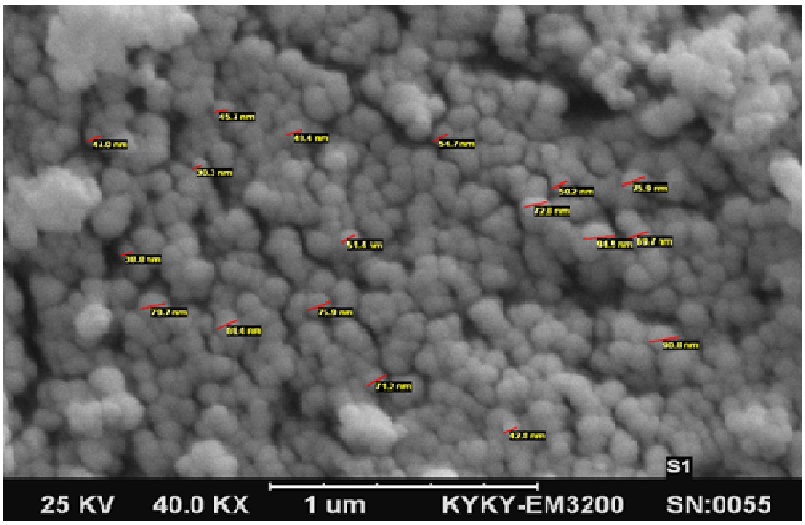

Figure-1
Loaded screening electron microscopy (SE M)

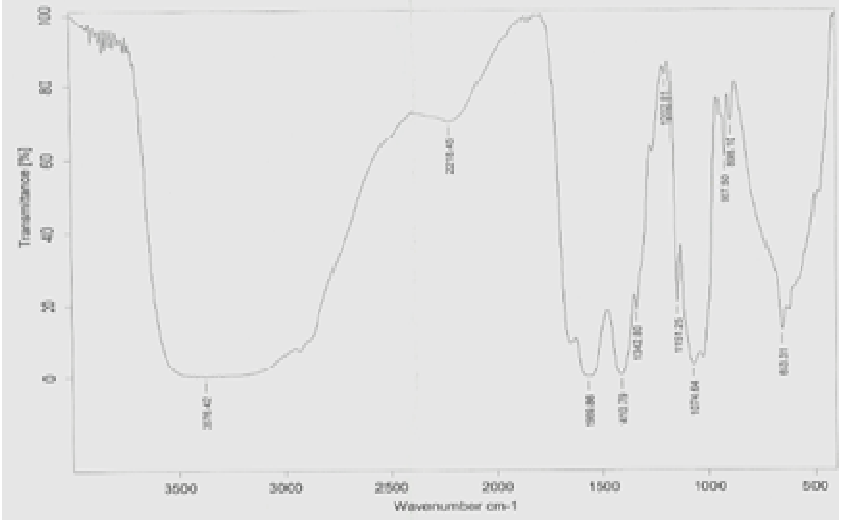

Figure-2
Loaded Fourier Transform Infrareded Spectrometer (FTIRs)

Test organism: *M.canis* standard isolate (PTCC 5069) and 4 field isolates were used to measure the minimum inhibitory concentration (MIC), while infection was caused by the standard isolate.

MIC determination: Clinical and Laboratory Standards Institute (CLSI) broth microdilution M38-A protocol was used to determine MIC *in vitro*. Through the use of RPMI1640 medium, a 0.5-5x10^4 cells/ml suspension was obtaine[20-22].

Animal infection: An area of 2 cm x 2 cm on the back of each animal was clipped and gently scraped with the edge of a sterile scalpel[23,24]. Such gentle skin traumatization makes the animal more susceptible to infection. As a result, the animals were inoculated with *M.canis*, while suspension adjusted to a 0.5 McFarland turbidity standard (1-5x10^6 CFU/ml). The entire area was occluded with Vaseline® in order to keep the area closed just for 24 hours. Suspension was prepared from *M. canis* colonies. The colonies were covered with sterile saline and were gently scraped with the tip of a Pasteur pipette[16]. Experimental animals were divided to 4 groups randomly: positive control group, negative control group, nanoessence group, and 1% terbinafine hydrochloride treatment group. All of the animals, except those of the negative control group, were diagnosed to have been infected on day 5[25,26].

Treatment: Treatment was started on day 5 after infection, when clinical features of infection were most evident. Based on

previous research, we started topical treatment every 12 hours on the 5th day with both nano-essence and terbinafine cream. During the 40-day treatment, the nano-essence was sprayed by a sprinkler on and around the infected area. Following the same pattern, terbinafine cream was applied on the infected area. In both positive and negative control groups, saline was used as the placebo during treatment period. Changes in lesion scaling, erythema, ulceration or alopecia were examined and recorded every 5 days.

Efficacy evaluation: Therapeutic effects of various treatments were evaluated by clinical lesion scoring and fungal culture. Changes in lesion scores were divided into 6 grades which are as follows: 0 – No signs of infection; hair fully re-grew. 1– Skin was calm; half-length long hair; no scaling. 2 – Hair re-grew on entire lesion surface; little scaling. 3 – No redness; little scaling; hair started to re-grow; few bald patches. 4– Slightly erythematous skin; loss of hair; evident scaling. 5 – Extensive skin damage; redness; crusting, ulceration, loss of hair[27].

Microscopic examination and fungal culture of plucked hairs and scraped scales were observed on days 30, 37 and 44 respectively.

Data analysis: Kruskal-Wallis Test was used to analyse lesion scores in SPSS.

Results and Discussion

MIC: MIC ranges of *Myrtus communis* nano-essence were 3.2-6.5 µg/ml. For this study MIC was considered as 4.8µg/ml.

Lesion scoring: All treatment groups except the negative control group at the start of treatment were found to be infected. Clinical lesion score averages on day 5 (start of the treatment) for all of the groups, except the negative control group, was between 3.8 and 4.8. The average of the clinical lesion scores in the nano-essence treatment group was lower than that of the Terbinafine treatment group when treatment had started (figure 4), but the average of lesion scores on days 10 and 15 in the nano-essence treatment group was higher than that of the Terbinafine treatment group. On day 20, the average of clinical lesion scores in the nano-essence treatment group was lower than that of the Terbinafine treatment group. This decreasing trend continued until day 40 of treatment. Nano-essence and positive control groups showed significant statistical difference on days 5, 10, 15, 20, 25, 30, 35 and 40 ($p<0.05$). A similar statistical difference exists between the Terbinafine group and positive control group. A comparison of the Terbinafine group and the nano-essence treatment groups on various dates revealed a significant difference on days 10, 15, 20, 25, 30 and 35 ($p<0.05$).

Culture results: Three consecutive culture results for all animals was negative on days30, 37 and 44, in treatment groups and negative control group.

Discussion: Dermatophyte infections can be treated with local and systemic treatment. From standpoint of clinical practice, topical drugs are cornerstone of treatment.

Various herbal extracts have been tested for their antifungal properties. In the present study, the *Myrtus communis* nano-essence was used to treat *M.canis* caused by dermatophytosis in guinea pigs. Microdilution broth using CLSI M38-A protocol was used for MIC determination. This technique is widely used in mycology laboratory. MIC was determined at the range of 3.2-6.5 µg/ml. Chitosan is used in production of many nano drugs, because it exhibits very attractive characteristics for drug delivery and has proved very effective when formulated in a nanoparticulate form.

All experimental animals in this study were successfully infected and treatment was started on day 5 after the infection, when clinical features of infection were most visible. The treatment period continued for 40 days after the infection. All of the animals under study except those of the positive control group clinically improved by day 40.

Clinical lesion score averages on day 5 (start of the treatment) for all of the groups, except the negative control group, between 3.8 and 4.8.

The average of clinical lesion scores in the nano-essence treatment group was 3.8 (63.3%), whereas the average of the Terbinafine group was 4.1 (68.3%) when treatment had started (figure 4). The average of lesion scores on days 10 and 15 in the nano-essence treatment group was 4 (66.6%) and 3.5 (58.3%) respectively, whereas the average of the Terbinafine treatment group on these two days was 3.6 (60%) and 3.3 (55%) respectively. On days 20, 25, 30, 35 and 40 after the start of treatment, the average of clinical lesion scores in the nano-essence treatment group was 2.6 (43.3%), 2.1(35%), 1 (16.6%), 0.3 (5%), 0 (0%) respectively, whereas the average of the Terbinafine treatment group on the same days was 3.1 (51.6%), 2.1 (35%), 1.5 (25%), 1.3 (21.6%), 0.6 (10%) respectively. This declining trend in the average of clinical scores from day 30 after the infection was followed by 3 consecutive negative cultures in 100% of the animals in both nano-essence and Terbinafine groups, as opposed to 100% positive culture observed on days 30 and 37, 83.3% on day 44 in the positive control group.

According to the results obtained on days 10 and 15 clinical lesions in nano-essence is more than Terbinafine However, the clinical scores in nano-essence from day 15 to 40 were less than terbinafine. nano-essence groups differences in clinical scores at days 15 to 20 is 15%, if the difference is only 4% in the terbinafine group. The findings reveal that the nano-essence treatment group showed improved clinical symptoms faster than the Terbinafine treatment group over a shorter treatment period. Generalization of results in animals and human patients needs further clinical trials.

Figure-3
Time manner gross finding in different groups infected with M.canis. PC, positive control; NC, negative control; Nano, nanoessence; Terbi, Terbinafin

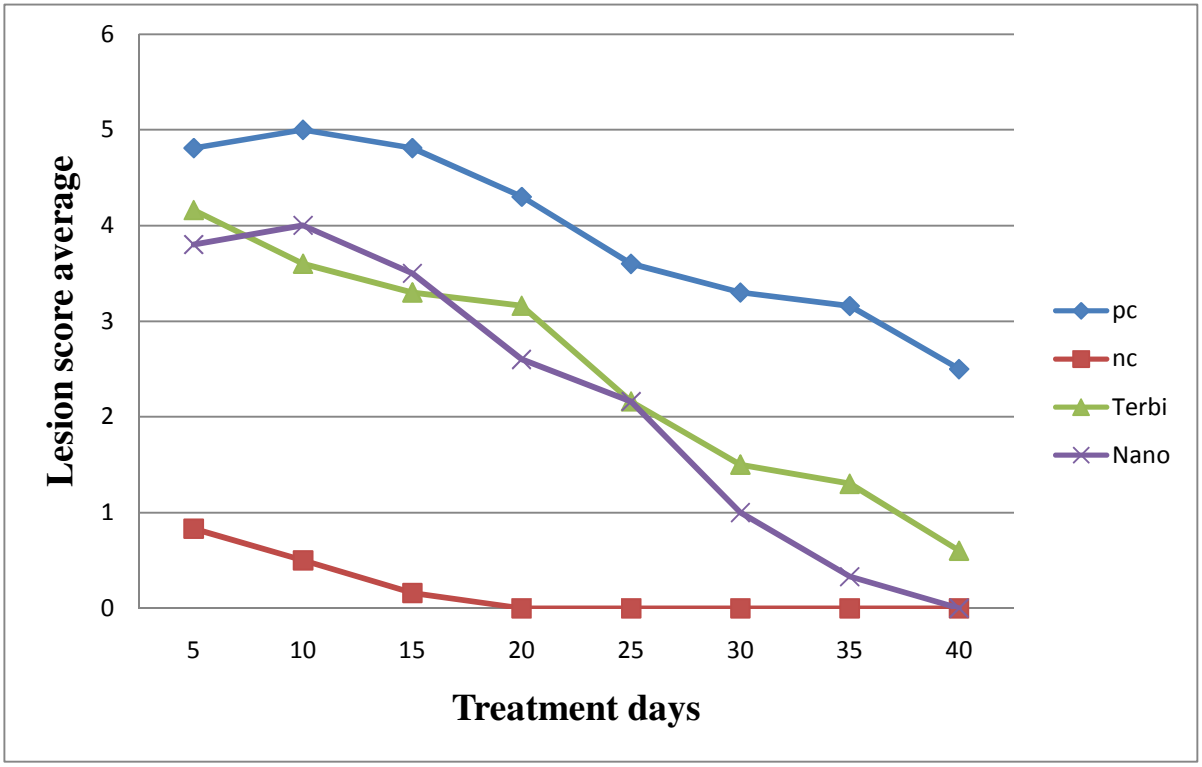

Figure-4
Clinical score average linear chart in different groups. Scores decreased from day 15 to day 40 in Nano-Essence. PC, positive control; NC, negative control; Nano, nano-essence; Terbi, Terbinafine

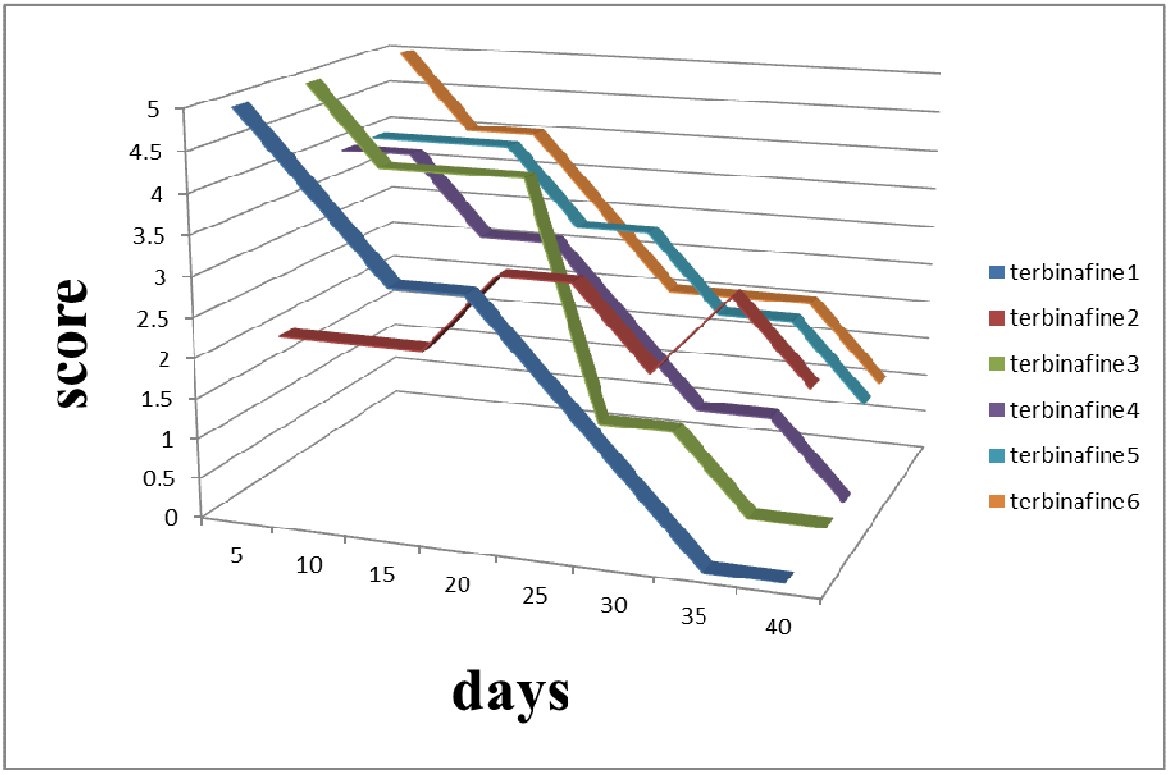

Figure-5
Terbina fine intergroup clinical lesion scores

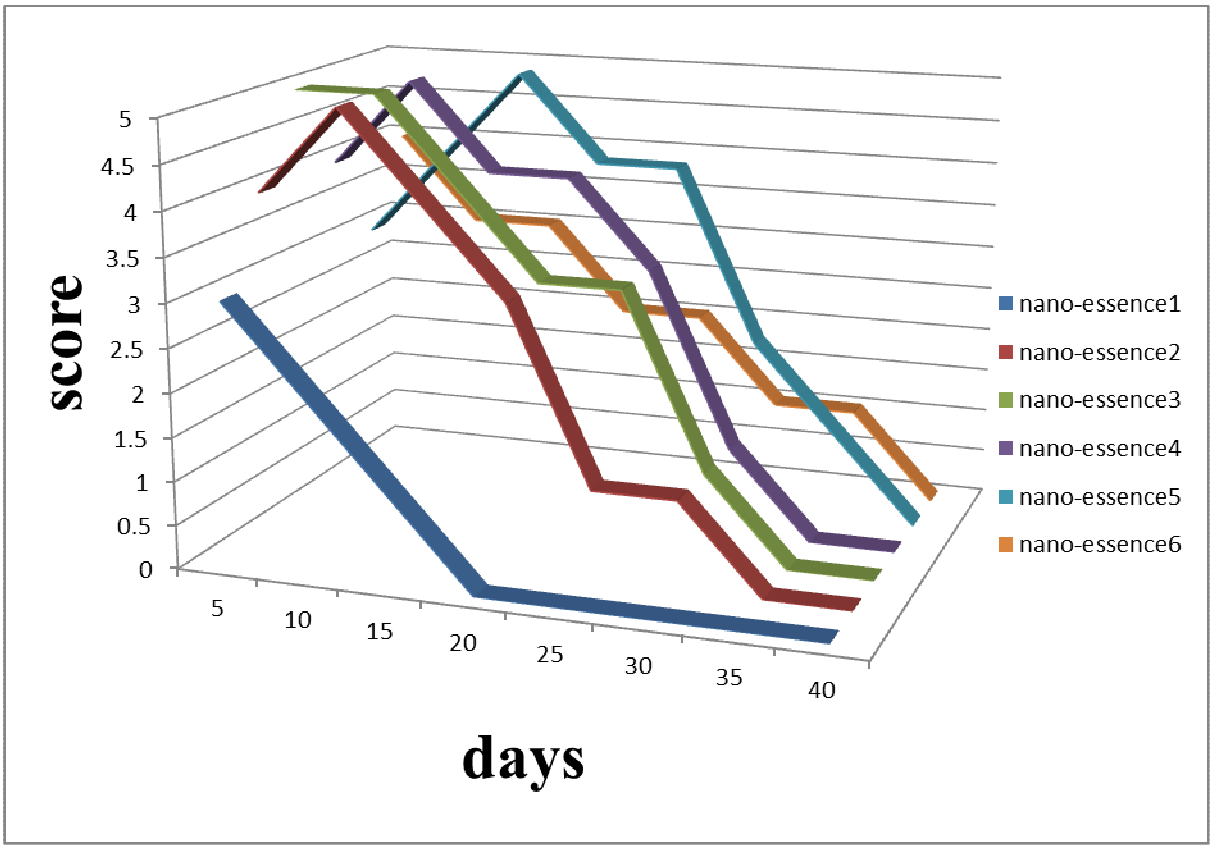

Figure-6
Nano-essence intergroup clinical lesion scores. Nano1-6 represents animal numbers.Note the score at which most of animals were when treatment started and dramatic decrease at the days 15 and 20

Table-1
Number and percentage of culture positive animals in every group, PC, positive control; NC, negative control; nano, nano-essence

Culture positive (%)			
Days / Groups	30	37	44
Control positive	6/6(100%)	6/6(100%)	6/5(83.3%)
Contotol negative	0/6(0%)	0/6(0%)	0/6(0%)
Nano-essence	0/6(0%)	0/6(0%)	0/6(0%)
Terbinafine	0/6(0%)	0/6(0%)	0/6(0%)

Conclusion

It is concluded that nano-essence of *M.communis* could be a replacement for Terbinafine cream to treat dermatophytosis but generalization of results in animals and human patients needs further clinical trials.

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