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INVESTIGATING THE TWO DIFFERENT CONCENTRATION OF MYRTUS COMMUNIS NANO-ESSENCE WITH TOPICAL TERBINAFINE CREAM IN EXPERIMENTAL DERMATOPHYTOSIS OF THE GUINEA PIG

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ABSTRACT

Dermatophytosis is a common contagious disease caused by fungi known as dermatophytes. Dermatophytes belong to a group of organisms that are able to break down the keratin in tissues such as the epidermis, hair, nails, feathers, horns and hooves. Microsporum canis (M.canis) is the most common cause of dermatophytosis in animals and human being.

In this study, 30 male guinea pigs were infected by M. Canis (by traumatization method) and treatment was done using Terbinafine 1% cream vs Chitosan nanoparticles containing M. Communis essence. Treatment duration was 40 days and it was started since 5 days post inoculation. The average of clinical score was calculated for each group. Microscopic examination and fungal culture of plucked hairs and scraped scales were also observed.

MIC ranges of Myrtus communis nano-essence was 3.2-6.5 µg/ml. The score in nano-essence groups began to reduce in comparison with Terbinafine group at day 5. The score inTerbinafine

group Scores until day 20 after treatment remained almost unchangedThat one negative point is terbinafine in the treatment process.This healing trend continued until day 40 of the treatment. Nano-essence comparing to non treatment group treated the infection significantly ($p<0.05$). Comparing Terbinafine and the nano-essence group with each other revealed a significant difference on days 10-25 ($p<0.05$). Three consecutive culture results for all animals were negative on days 30, 37 and 44in treatment and negative control groups. Both Terbinafine and Nano groups showed a healing trend, however the pace of healing was higher in nano groups. The average of clinical score in nano VS Terbinafine groups were not significantly different at the beginning of the experiment (4.9 ± 0.1 VS 4.1 ± 0.1) while this difference became significant through days 10-25. Nano-essence groups comes in to action with afaster performance than Terbinafine.The findings reveal that the nano-essence treatment group showed improvement in clinical symptomsfaster than the Terbinafine treatment group while its efficacy starts with a delay.

Keywords: nano-essence, Myrtus communis, Terbinafine, Microsporum canis and guinea-pig

INTRIDUCTION

Dermatophytes are significant due to their zoonotic potential and the concern of owners of pets with sometimes severe inflammatory skin diseases **[1]**. Dermatophytes are filamentous fungi, which are able to use as a source of carbon. Some of these organisms are true parasites; they develop in skin and hair and cause cutaneous lesions **[2,3]**. The disease is called dermatophytosis or ringworm and is recognized as one of the most common infectious dermatoses in pets. More than 20 different dermatophyte species have been isolated from dogs and cats **[4,5,6]**.

Antifungal treatment should be systematically recommended to shorten the course of the infection and toreduce dissemination of infective material into the environment. Infective material is composed of small piecesof hair covered by microscopic fungal spores (called arthroconidia)**[7,8]**. Infective material is easily spread and canremain viable in the environment for up to 18 months under optimal conditions of temperature and humidity. Infected animals (with or without clinical signs) and contaminated environments represent long term sources of exposure to other animals and owners. Systemic antifungals are supposed to speed up the resolution of the infection, whereas topical antifungals are required to reduce the

risk of transmission and environmental contamination **[9,10]**.

Terbinafine interferes specifically with fungal strol biosynthesis at an early step. this lead to a deficiency in ergosterol and to an intracellular accumulation of sequalene, resulting in fungal cell death.

Nano-particles have appeared as promising vehicles because of several attractive properties such as an increased surface-to-volume ratio, which offers high potential for macromolecule association and capacity to improve drug absorbtion **[24]**. Another advantage of administrating nanoparticles on the skin is reduction of epithelial resistance to drug transport, or the ability to carry the drug across the epithelium. Nanoparticles further enable the encapsulated molecules to retain their biological activity from the production steps to the final methods can be applied to produce nanoparticles using that excipient. These involve either hydrophilic or lipophilic environments that generally result in mild conditions or aggressive and time consuming processes respectively **[25]**.

Chitosan (CS), is one of the most commonly used natural polymers in the production of nanomedicine, and it is able to enhance absorption by increasing cellular permeability **[11, 12, 13]**.

Myrtus communis possess several pharmacologic, biological and medical activities including antiviral, antibacterial, anticandida, antimutagenic, antihemorrhagic, analgesic, anti-inflammatory, antioxidant, wound healing and anti-hyperglycemic. Although the chemical compositions of this herb variesaccording 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 **[14-17]**.

The purpose of this study is to use Myrtus communis nano-essence to treat dermatophytosis caused by M.canis under experimental conditions.

MATERIALS AND METHODS

In this study, 30 male guinea pigs (350-450 g) 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 to adopt to the situation. Myrtus communis essence was purchased from Barij Essence Pharmaceutical Company (Kashan, Iran) and 5 ml nano-essence was sufficient to produce 1 litre of nano-essence. To confirm the reliability of the product, Fourier Transform Infrared

Spectrometerand Surface Electron Microscopy (SEM) were used **(Figure 1 and 2)**. The Terbinafinehdydrochloridetopical cream 1% used in this study was purchased from Tehran Chemi Pharmaceutical Company (Tehran, Iran).M.canis standard isolate (PTCC 5069) and 4 field isolates were used to measure the minimum inhibitory concentration (MIC), and infection was caused by the standard isolate.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 0.5-5×104 cells/ml suspension was obtained **[18, 19, 20]**. An area of 2 × 2 cm on the back of each animal was clipped and gently scraped with the edge of a sterile scalpel20,21. Such gentle skin traumatization makes the animal more susceptible to skin infection. A suspension adjusted to a 0.5 McFarland turbidity standard (1-5×106 CFU/ml NaCl 0.9%) of M.canis was prepared and used to inoculate scratched skin area. It was administered on the mentioned area using a Pasteur pipette. The entire area was occluded with Vaseline® in order to keep the area closed just for 24hours **[21, 22]**. Experimental animals were divided into 4 groups (n=6 in each) randomly including:

Non-treated (NT): inoculation was done and NaCl 0.9% was used as placebo;

Negative control (NC): no inoculation and NaCl 0.9% was used as placebo;

Nano-essence (Nano): inoculation and administration of nano-essence;

Terbinafine hydrochloride treatment (Terbi): inoculation and administration of Terbinafine cream.

Treatment was started on day 5 after inoculation, when clinical features of M.canisinfection became most evident. Based on previous researches, we started topical treatment every 12 hours on the 5th day with both nano-essence and Terbinafine cream 1%. During the 40-day treatment, the nano-essence was

sprayed by a sprinkler on and around the infected area and Terbinafine cream was applied on and around the infected area, too. Changes in lesion area, erythema, ulceration and alopecia were monitored and recorded every 5 days. 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

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

4– Slightly erythematous skin; loss of hair; evident scaling.

5 – Extensive skin damage; redness; crusting, ulceration, loss of hair**[23]**.

Microscopic examination and fungal culture of plucked hairs and scraped scales were observed on days30, 37 and 44, respectively.

Data analysis

Kruskal-Wallis Test was used to analyse lesion scores in SPSS statistical package.

RESULTS

Chitosan nanoparticles containing M.communis were prepared by Zist Shimi Azma Roshd Company. SEM image shows that nanoparticles were spherical and their size was 150-200nm **(Figure 1)**.

MIC ranges of Myrtus communis in nano 1 group 6.5 µg/ml and nano 2 group 3.2 µg/ml. All the groups except the negative control found to be infectedwhen the treatment was going to begin (5 days after inoculation). Clinical lesion score on day 5 was 4.7±0.2 in each group, except the negative control group. The score was not significantly different among groups at the beginning of the experiment **(Figure 6).**

The score in nano-essence group began to reduce in comparison with Terbinafine group at day 5. This decreasing trend continued until day 40 of the treatment. Nano-essence and NT groups showed statistically significant difference on days 25, 30, 35 and 40 ($p<0.05$). A statistically significant difference was observed between the Terbinafine group and NT group. Comparing Terbinafine and the nano-essence group with each other revealed a significant difference on days 10, 15, 20, 25 ($p<0.05$). The cure trend can be seen in **(Figure 6).**

Three consecutive culture results for all animals was negative on days 30, 37 and 44 in treatment groups and negative control group **(Table 1)**. The treatment was ceased, when the second approve was achieved.

Table 1: Number and percentage of culture positive animals in every group

	Positive culure (%)		
group	**Day 30**	**Day 37**	**Day 44**
NT	**(100%)6/6**	**(100%)6/6**	**(83.3%)5/6**
NC	**(0%)0/6**	**(0%)0/6**	**(0%)0/6**
Nano1	**(0%)0/6**	**(0%)0/6**	**(0%)0/6**
Nano2	**(0%)0/6**	**(0%)0/6**	**(0%)0/6**
Terbi	**(0%)0/6**	**(0%)0/6**	**(0%)0/6**

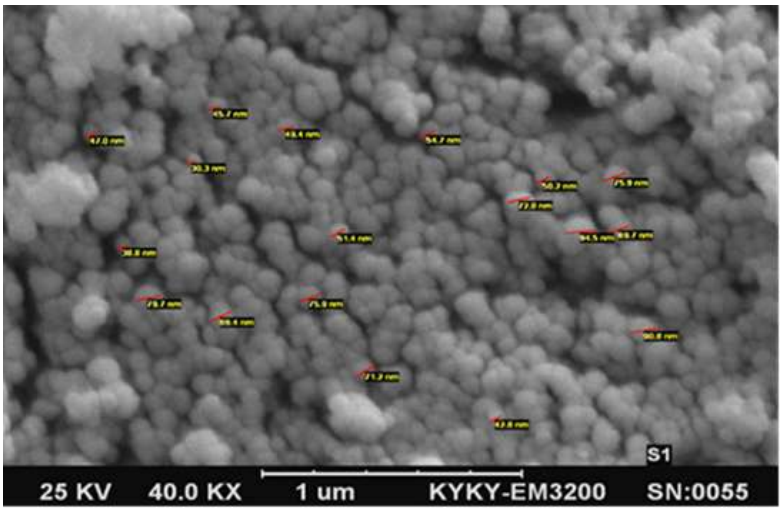

Figure 1: Surface electron microscopy (SEM) of chitosan nanoparticles containing myrtus communis essence

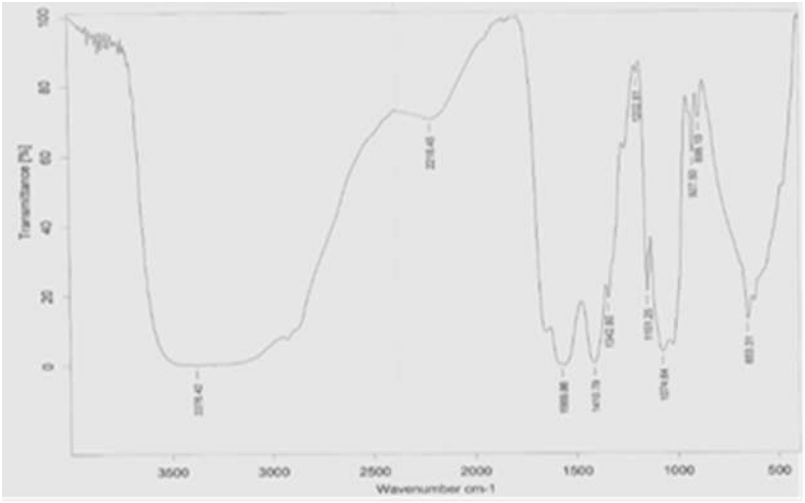

Figure 2: Loaded Fourier Transform Infrareded Spectrometer(FTIRs) of chitosan nanoparticles containing myrtus communis essence

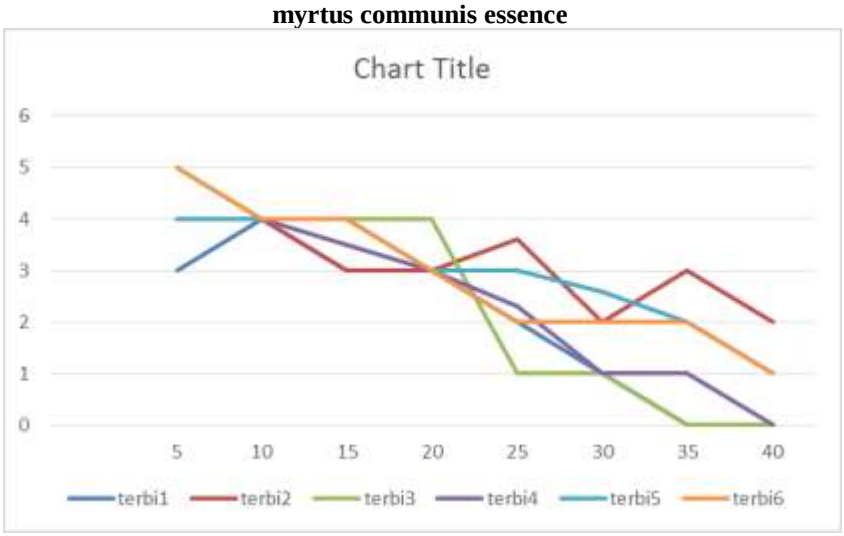

Figure 3: Terbinafine intergroup clinical lesion scores

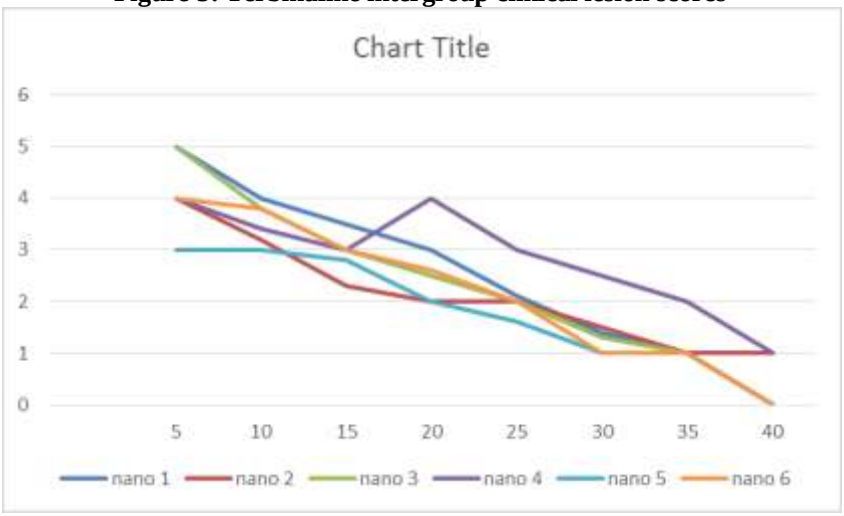

Figure 4: Nano-1 intergroup clinical lesion scores. Nano1-6 represents animal numbers. Note the score at which most of animalswere when treatment started and dramatic decrease at the days 10

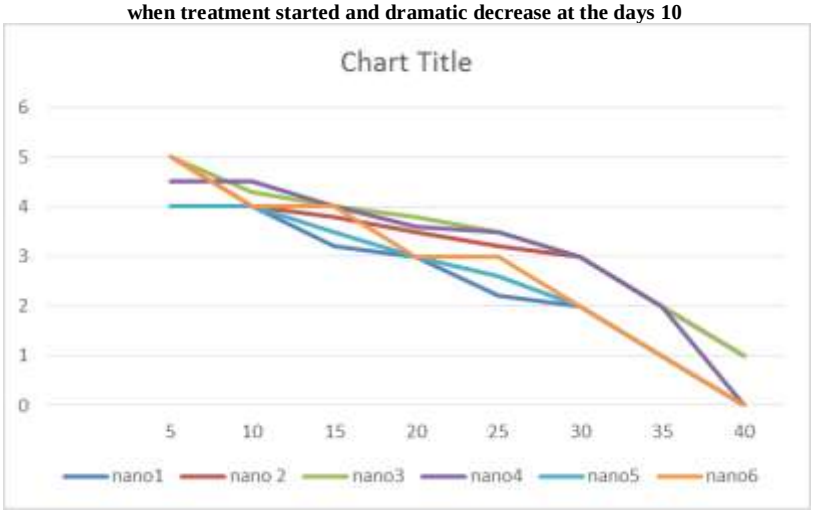

Figure 5: Nano-2 intergroup clinical lesion scores. Nano1-6 represents animal numbers. Note the score at which most of animalswere when treatment started and dramatic decrease at the days 10

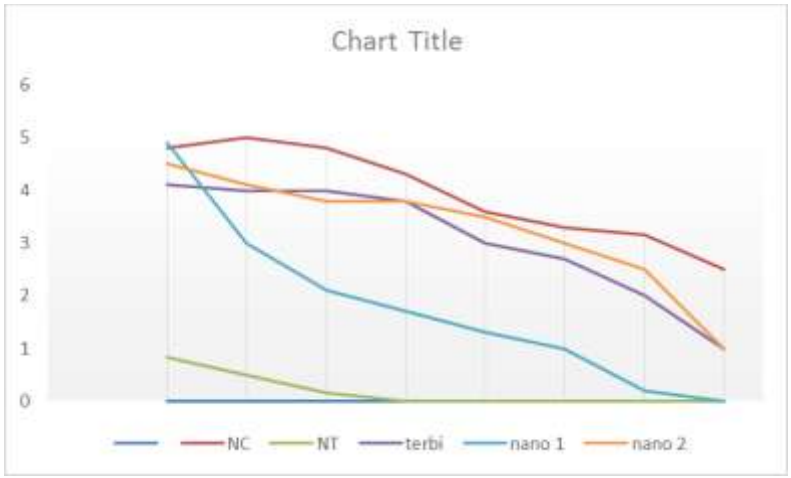

Figure 6: Clinical score average in different groups. Scores decreased from day 5 to day 40 in Nano-Essence 1,2. NT,non-treatment; NC, negative control; Nano, nano-essence; Terbi, Terbinafine

Figure 7: Time manner gross finding in different groups infected with M.canis. NT,non treatment .NC, negative control;Nano1-2 , nano-essence; Terbi, Terbinafine.

DISCUSSION

Healthy animals often have self-limiting infections that resolve within a few months, but treatment can speed recovery, prevent the lesions from spreading, and decrease the risk of transmission to people or other animals. Drugs available to treat dermatophytosis in animals include topical antifungal creams or shampoos, and systemic antifungals. Topical drugs are unable to eliminate dermatophytes from within hairs and hair follicles, but they may be effective against organisms in superficial sites (e.g., in the skin), and they can decrease contamination and transmission to others. The optimal treatment in small animals is combined topical and systemic treatment.

Minimum inhibitory concentration of nano-essence groups 3.2-6.5µg/mlwas.Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 1–1000 nm. Nanoparticles able to enhance the topical and transdermal penetration of drugs has become essential.Chitosan is safe, non-toxic and can interact with polyanions to form complexes and gels **[26].**

All guinea pigs in this study were completely infected (except negative control group) and treatment was began on the 5th day post inoculation. Treatment duration was 40 days (BD) and all animals except the NT group were clinically treated at the end of the study as seen in.In Nano 1 group the average score at the beginning of treatment (day 5) was 4.9±0.1 and the score was reduced gradually in the following days after applying the topical nano essence **(Figure 4).** This reduction was significantly different in days 10 to 15 and 15 to 20. Nano 2 group the average score at the beginning of treatment (day 5) was 4.5±0.1 and the score was reduced gradually in the following days after applying the topical nano essence **(Figure 5)** although to a slight slope in comparing to nano 2 group.This reduction was significantly different in days 10 to 15 and 15 to 20. To evaluate data more quantitatively, 18% and 8% reduction was occurred in days 15 and 20 , respectively and the score was 2.1 and 1.7 in the mentioned days. On the other hand, the score did not show any significant reduction in terbinafine group during days 5 to 15 and a light and insignificant reduction was occurred in day 20 (4% in comparison to day 5). It supports the complain of higher anti fungal effect of nanoessence comparing to Terbinafine. According tothe score was gradually reduced in Nano 2 group with a smoother slope **(Figure 6)**. The reason must be the lower concentration of nanoessence that was administered. Since the healing trend happened more slowly it is obvious that it depends to formulation concentration.

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, asm opposed to 100% positive culture observed on days 30 and 37, 83.3% on day 44 in the positive control group **(Table 1).**

The findings reveal that the nano-essence treatment group showed improvement in clinical symptoms faster than the Terbinafine treatment group.Generalization of results in animals and human patients needs further clinical trials.

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