Comparison of the Therapeutic Effects of Nano-essence of *Artemisia sieberi* with the Terbinafine 1% in Animal Model Dermatophytosis

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ABSTRACT

Background and aims: One of the most important world wide zoonotic infection is dermatophytosis which caused by dermatophytes fungi. Dermatophytosis treatment minimizes the time course of the infection and the potential of spread to other animals and humans. This study aims to use the Artemisia sieberi nano-essence to treat dermatophytosis caused by *Microsporum canis* under experimental conditions in guinea pigs.

Methods: In this study, guinea pigs randomly divided into 7 groups; negative control, positive control, terbinafine hydrochloride 1% and 4 different nano-essence concentrations treatment. 5 day after *Microsporum canis* suspension inoculation to abraded skin, treatment program for every nano-essence dilutions and terbinafine was started every 12 hours for 40 days.

Results: Comparison of all nano-essence and terbinafine treatment groups on various days till 40th day revealed no significant differences. Three consecutive culture results for all animals were negative on days 33, 40, and 50 in nano-essence groups, but 16% of terbinafine groups was positive in fungi culture on day 33.

Conclusion: The results showed the nano-essence treatment groups showed improved clinical symptoms faster than the terbinafine treatment group over a shorter treatment period.

Keywords: Guinea pig, Terbinafine, Artemisia sieberi, *Microsporum canis*, Nano-essence.
INTRODUCTION

Recently fungal infections increase in human and animals, because limited antifungal agents were available or there is old compounds and toxic for treatment.\textsuperscript{1,2} Infection of the superficial keratinized skin layer and appendages (hair, nails, feather, horns) of animal, birds and humans with dermatophytes is called the term "Ring worm".\textsuperscript{3} Dermatophytes depending on host preferences and natural habits were divided in three groups: anthropophilic, zoophilic and geophilic and this groups have three genera including \textit{Epidermophyton}, \textit{Trichophyton} and \textit{Microsporum}.\textsuperscript{4} One of the most common agent that use for dermatophytosis treatment is an allylamin compound "Terbinafine" that competitively inhibits epoxidase in fungus so cause depleting ergosterol.\textsuperscript{5}

Nanotechnology is an advance technology with fast revolution in sub-micron or nano-meter sized objects.\textsuperscript{6} Applying nanotechnology to pharmaceutical and medical sciences cause significantly improve diagnose and therapeutic procedures of diseases via drug nano-delivery and reducing drug’s toxicity.\textsuperscript{7,8,9} Nanomaterials have special size and shape which can be solely and individual properties such as functions and effects.\textsuperscript{10} Nanoparticles because of their small size, biocompatibility and biodegradability can be able to taken up by cells, delivered then clear from body without side effects.\textsuperscript{11,12}

Chitosan is a high-molecular-weight linear non-toxic biopolymer derived by the deacetylation of chitin. The second most natural polymer in nature after cellulose, found in the cell walls of fungi.\textsuperscript{13}

\textit{Artemisia} is one plant genus known as folk medical herb with about 500 species and about 34 species found in all over Iran. \textit{Artemisia sieberi} is named in Iran "Dermaneh" and in English named "Woodworm" use for their therapeutic properties such as diuretic, anti-inflammatory, antiparasitic, antifungal, anticaldial, insecticidal, vermicidal and antiprotozoal effects. Bioactive chemical composition of this herb include alkaloids, phenolic compounds, vitamins and minerals is vary according to growing place but main components of that is the same for all species those are \textit{\textalpha{}}-\textit{thujone} and \textit{\textbeta{}}-\textit{thujone}.\textsuperscript{14-17}

METHODS

Animals

42 healthy male guinea pigs with the same weight range (ranging from 300-350 grams) were obtained from Pasture institute (Tehran, Iran). All animals were kept in controlled environmental conditions (12 hours light period, relative humidity of 50±1\% and temperature: 25±1°C) in separate polycarbonate cages. Animals were put in optimized condition and fed with basic diet for one week.
Drugs  
*Artemisia sieberi* essence was purchased from Barij Essence Pharmaceutical Company, (Kashan, Iran) and nano encapsulation was done by Zist Shimi Azma Roshd Company (Tehran, Iran). 1 liter of nano-essence was produced from 5 ml of *Artemisia sieberi* essence. The product’s reliability was confirmed by Fourier transform infrared spectrometer and screening electron microscopy (Figure 1 and Figure 2). The terbinafine hydrochloride 1% cream used in this study was purchased from Tehran Chemie Pharmaceutical Company (Tehran, Iran).

Test organism  
*Microsporum canis* standard isolate (PTCC: 5069) was used to measure minimum inhibitory concentration (MIC) and infection was induced by standard isolate.

Minimum inhibitory concentration (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 0.5-5×10^4 cells/ml suspension was obtained.

Animal infection  
We shaved posterior dorsal portion of every animal gently for as wide as 4 cm^2 (2cm× 2cm). With the back of sterile scalpel blade, shaved area was abraded then suspension containing 10^6 *Microsporum canis* spores per milliliter inoculated to abraded site. The entire area was occluded with Vaseline® in order to keep the area closed just for 24 hours. Animals divided to 7 groups (6 animal per each group) randomly in negative control, positive control, four different nano-essence’s concentrations treatment and 1% terbinafine hydrochloride treatment groups. All animals except negative control group were mycological positive at 5th day.

Treatment  
Treatment was started on day 5 after inoculation, after clinical features of infection were most evident. According to previous researches, we started topical treatment every 12 hours on the 5th day with 4 concentrations of nano-essence and terbinafine 1% 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 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 7 days.

Efficacy evaluation  
Drug efficacy was evaluated by fungal examination and fungal culture and evaluation according clinical lesion scoring. Modified lesion scoring is 5 degrees (0 to 4) scoring system indicated as follow: Score 0: No visible lesion; Score 1: Only hair loss; Score 2: Well defined redness with few scales;
Score 3: Well defined redness with large scale; Score 4: Ulceration and scarring in addition to lesions of score 3. Scales and hairs by using scraping and plucking technique, removed for microscopic examination and cultured at the day 33, 40 and 50.

Statistical analysis
Kruskal-Wallis statistic test was used to analyze lesion scores in SPSS (ver. 22) software.

RESULTS

Minimum Inhibitory Concentration (MIC)
In this study MIC of Artemisia sieberi nano-essence were determined as 3 μg.ml⁻¹.

Lesion scoring
Clinical gross findings at different days are shown in Figure 3. Clinical lesion scoring at the start of treatment (day 5 after inoculation) for all groups was expected and the averages of the scoring at the same time, shown in Figure 4. When all groups except negative control were mycological positive (day 5) treatment was started.

Culture results
Three consecutive culture results for all animals was negative on days 33, 40 and 50 in treatment groups and negative control group (Table 1).
Four different concentrations of nano-essence; Nano-essence 1:3 μg.ml⁻¹, Nano-essence 2:6 μg.ml⁻¹, Nano-essence 3:1.2 μg.ml⁻¹ and Nano-essence 4:2.4 μg.ml⁻¹ was considered. Comparison of the nano-essence 1, 2, 3, 4 and terbinafine 1% treatment groups on various dates shows no significant differences on all treatment duration (p=0.121). Intra group assessments shows clinical score reduction over treatment period for animals in drug receiving (Figure 5 - Figure 9).

DISCUSSION
Recently alternative herbal medicine substitute with modern serious medicine because plants which use for therapy purposes have the best effects and much lesser adverse effects and more safe.
Table 1. Number and percentage of culture positive animals in each group

<table>
<thead>
<tr>
<th>Days</th>
<th>Positive control</th>
<th>Negative control</th>
<th>Terbinafine</th>
<th>Nano-essence 1</th>
<th>Nano-essence 2</th>
<th>Nano-essence 3</th>
<th>Nano-essence 4</th>
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<td>Day 33</td>
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<td>6/6 (100%)</td>
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Various herbal extracts have been use according to their antifungal properties. 27,28 Antimycotic specially anti dermatophytic effects of various herbs test in vitro and in vivo or both conditions. Mitrocarpus scaber,29 Catharanthus Roseus,30 Curcuma longa, Quercus sessilifolia,31 were used for treatment of fungal infections. Other research Myrtus communis nano-essence, Origanum vulgare nano-essence and Artemisia sieberi nano-essence have been used to treating dermatophytosis in animal models.32,33,34

In present study we decided to use Artemisia sieberi nano-essence to treat Microsporum canis induced dermatophytosis in guinea pigs. Microdilution broth is well known and widely used method in mycology, using CLSI M38-A protocol was used for MIC determination. By using this technique, nano-essence MIC was determined 3 μg.ml⁻¹, this is similar to the other study.34 In this study we decided to utilize nano-essence to add some additional properties to Artemisia sieberi essence in treatment of Microsporum canis induced dermatophytosis. Therapy started from
5th day after inoculation and lasted every 7 days until day 40 after inoculation. All animals except positive control, in day 40 were clinically cured.

**CONCLUSIONS**

The aim of this study is to use the *Artemisia sieberi* nano-essence to treat dermatophytosis caused by *Microsporum canis* under experimental conditions in guinea pigs.

According to the results no significant differences shown between all 4 nano-essence and terbinafine treatment groups. However, the clinical scores in all nano-essence treatment groups less than terbinafine treatment group. The findings reveal that the nano-essence treatment groups showed improved clinical symptoms faster than the terbinafine treatment group over a shorter treatment period. It is concluded that nano-essence of *Artemisia sieberi* was effective and could be a replacement for terbinafine ointment to treat dermatophytosis but generalization of results in animals and human patients needs further clinical trials.

**CONFLICT OF INTEREST**

All authors disclose any financial and personal relationships with other people or organizations and the authors declare that there are not any potential conflicts of interest.

**Authors’ Contributions**

**ACKNOWLEDGEMENT**

**REFERENCES**


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Fig.3. Time manner gross finding in different groups infected with *Microsporum canis*
Therapeutic of medical herb's nano-essence

Fig.4. Clinical score average linear chart in different groups

Fig.5. Nano-essence 1 intergroup clinical lesion scores
Fig. 6. Nano-essence 2 intergroup clinical lesion scores

Fig. 7. Nano-essence 3 intergroup clinical lesion scores
Therapeutic of medical herb’s nano-essence

Fig. 8. Nano-essence 4 intergroup clinical lesion scores

Fig. 9. Terbinfine intergroup clinical lesion scores