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COMPARISON OF THE THERAPEUTIC EFFECTS OF NANO-ESSENCE OF MEDICAL HERB *ORIGANUM VULGARE* WITH THE KETOCONAZOLE 2% TOPICAL CREAM IN CATS INFECTED BY MICROSPORUM CANIS

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ABSTRACT

The use of herbal medicines for centuries to treat various diseases is known in many societies among of them Essences have well known properties. Transforming a drug to Nano sized particles adds some additional potency to that drug because it can effectively deliver the drug to a target site and thus increase the therapeutic effects, while reducing side effects. In this study, *Origanum vulgare* Nano-essence was used to treat dermatophytosis induced by *Microsporum canis* in an experiment on cats. Minimum inhibitory concentration of Nano-essence was 0/09 µl/ml. Treatment started 5 days after infection as 12 hours regimen until 40 days post infection. Both Ketoconazole and *Origanum vulgare* Nano-essence groups had complete cure at day 40. Results show that this *Origanum vulgare* Nano-essence is an effective drug to treat *M.canis* induced dermatophytosis.

Keywords: Nano-essence, *Origanum vulgare*, Ketoconazole, *Microsporum canis*, Cats

INTRODUCTION

Dermatophytosis is a dermatologic problem with zoonotic risk. On the basis of primary habits there are three classes of dermatophytes, geophilic, zoophilic and anthropophilic. Dermatophytosis has worldwide distribution and the causative agents are *Microsporum*, *Trichophyton* and *Epidermaphyton* spp. *Microsporum canis (M.canis)* is the most common cause of dermatophytosis in animals and human [1, 2, 3]. Every confirmed case of dermatophytosis should receive topical

therapy. Since there is a public health problem, an aggressive therapy should be designed to treat patients [3, 4]. Traditional topical antifungal drugs can treat dermatophytosis but there is no advantage one product over another. Besides they have variable side effects and fungal resistance is becoming common. Ketoconazole is one of these drugs. It has moderate degree of success against *M.canis* with systemic use. Clinicians use topical Ketoconazole to treat small lesions [5, 6]. Nano drugs are novel therapeutic agents. Their properties improved after Nano encapsulation. Chitosan is the main agent in this process and it adds many properties such as muco-adhesiveness, absorption enhancing and sustained-release characteristics to Nano sized drugs [7-11]. *Origanum vulgare* has been used as a culinary and medicinal herb for thousands of years. It has a beneficial effect upon the digestive and respiratory systems [12]. Locally Persian name is "Marzanjoush" which is growing in South Europe, Mediterranean and North regions of Iran. This herb has broad antifungal and antibacterial effects [13]. The essential oils that contain Thymol are effective against microbes, especially fungi [14-18]. The main antifungal components of *O.vulgare* are Thymol and Carvacrol [19]. The purpose of this study is to use the *Origanum vulgare* nanoessence to treat *M.canis* induced experimental dermatophytosis in cats.

MATERIALS AND METHODS

Animals: In this study, 36 male DSH cats with the same weight (ranging from 1.5 – 2 kg) and the same age (ranges between 6-12 moths). All animals were put in separate standard stainless steel cages in controlled condition (12 hours light period, relative humidity of 50±3% and temperature: 24±1°c). Animals were put in optimized condition and fed with basic diet for 1 week.

Drugs: *Origanum vulgare* essence was purchased from Barij Essence Pharmaceutical Company, (Kashan, Iran) and Nano encapsulation was prepared by Zist Shimi Azma Roshd Company (Tehran, Iran). Five milliliter of *Origanum vulgare* essence is sufficient to produce 1 liter of Nano-essence drug type. The product's reliability was confirmed by Fourier Transform Infrared Spectrometer and screening electron microscopy **(Figure 1, Figure 2)**. Ketoconazole 2% topical cream that was utilized in this study was purchased from Daroupakhsh pharmaceutical Company (Tehran, Iran).

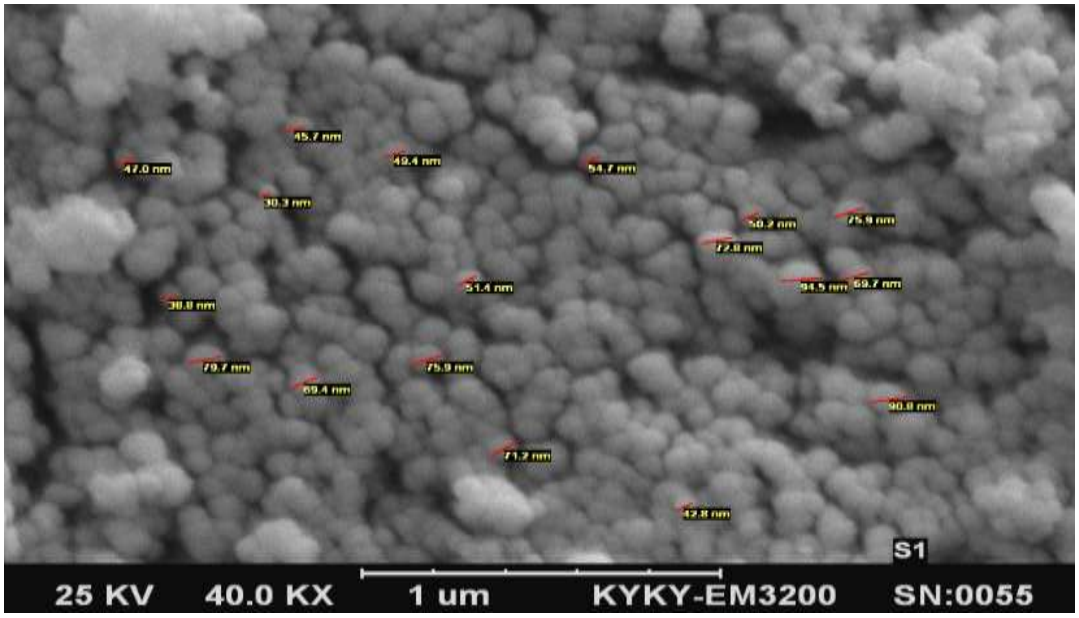

Figure 1: Loaded screening electron microscopy (SEM)

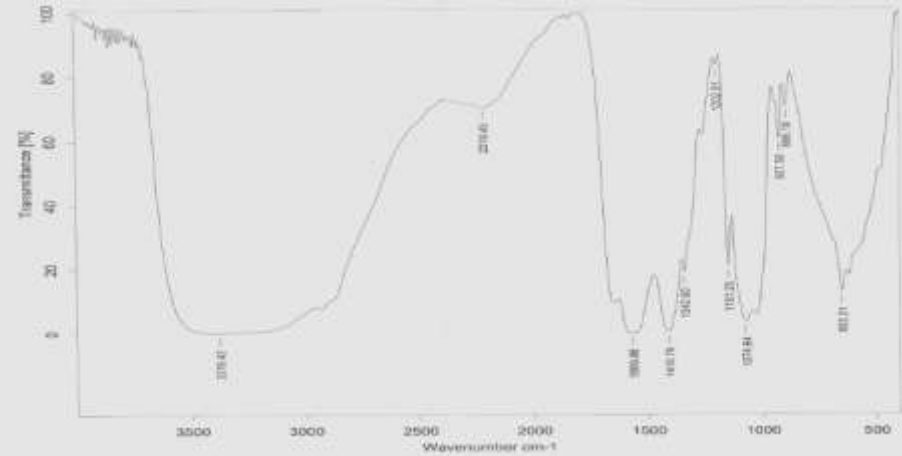

Figure 2: Loaded Fourier Transform Infrared Spectrometer (FTIR)

Test organism: *M.canis* standard isolate (PTCC 5069) and four 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 micro dilution M38-A protocol was used to determine MIC in vitro. Through the use of RPMI1640 medium, a 0.5-5x104 cells/ml suspension was obtained (20-22).

Animal infection: Cranial-dorsal portion (between the Shoulders) of every cat was scraped gently for as wide as 4 cm^2 with sterile scalpel blade [23, 24]. Such gentle skin traumatization makes the animal more susceptible to infection. Then Suspension containing 10^6 *M.canis* spores per milliliter inoculated to abraded site. The entire area was occluded with Vaseline® in order to keep the area closed 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 [25]. Animals divided to 3 groups randomly, negative control, *Origanum vulgare* Nano-essence (according to MIC) and Ketoconazole 2% topical cream treatment group. All cats except negative control group were diagnosed to have been infected on 5th day [24, 26, 27].

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 *Origanum vulgare* Nano-essence and Ketoconazole 2% topical cream [10]. During the 40 days treatment period, the *Origanum vulgare* Nano-essence was sprayed by a sprinkler on and around the infected region. In addition, in negative control group, 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: 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: score 0: No signs of infection, hair fully re-grew. Score 1: Skin is Healthy; half-length long hair; no scaling. Score 2: Hair re-grew on entire lesion surface; little scaling. Score 3: No redness; little scaling; hair started to re-grow; few bald patches. Score 4: Slightly erythematous skin; loss of hair; evident scaling. Score 5: Extensive skin damage; redness; crusting, ulceration, loss of hair [28]. Microscopic examination and fungal culture were performed of plucked hairs and scraped scales on days 33, 40 and 45 respectively.

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

RESULTS

MIC: In this study, MIC was considered as 0/09 µl/ml for *Origanum vulgare* Nano-essence.

Lesion scoring: Gross findings are shown in Figure 3. All animals except negative control were mycological positive when the treatment started. Clinical lesion score averages on day 5 (start of the treatment) for all of the groups, except the negative control group, were between 4 and 4.5. The average of the clinical lesion scores in the *Origanum vulgare* Nano-essence treatment group was close to the Ketoconazole 2% group when the treatment had started. Decrease in score average happens gradually until all treatment receiving groups reach score 0 on day 40 **(Figure 4)**.

A Kruskal-Wallis H test showed that there was a statistically significant difference in lesion score between the different drug treatments, $\chi^2(2) = 10.291$, $p = 0.006$, with a mean rank lesion score of 11.67 for Nano-essence, 12.73 for Ketoconazole 2% and 4.00 for negative control group.

Intra group assessments shows clinical score reduction over treatment period for animals in drug receiving **(Figure 5, Figure 6)**.

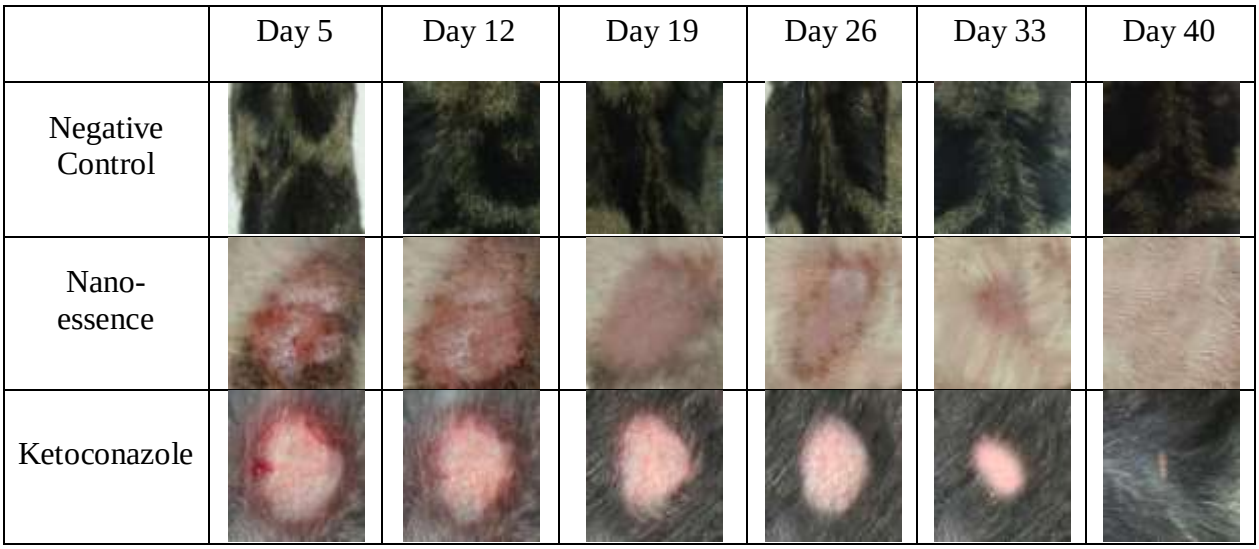

Figure 3: Time manner gross findings in different groups infected with *M.canis*

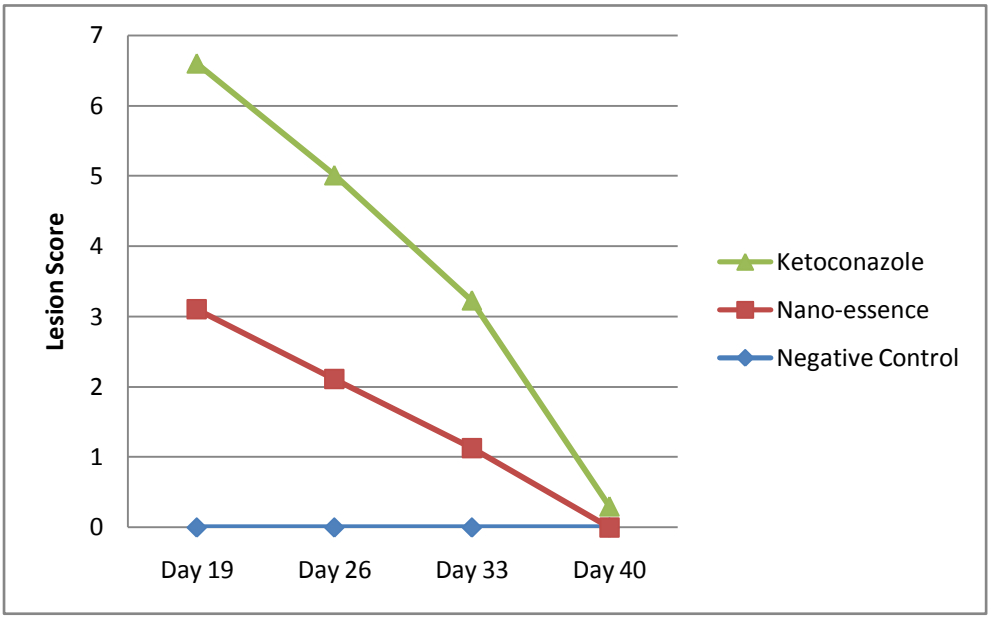

Figure 4: Clinical score average linear chart in different groups.

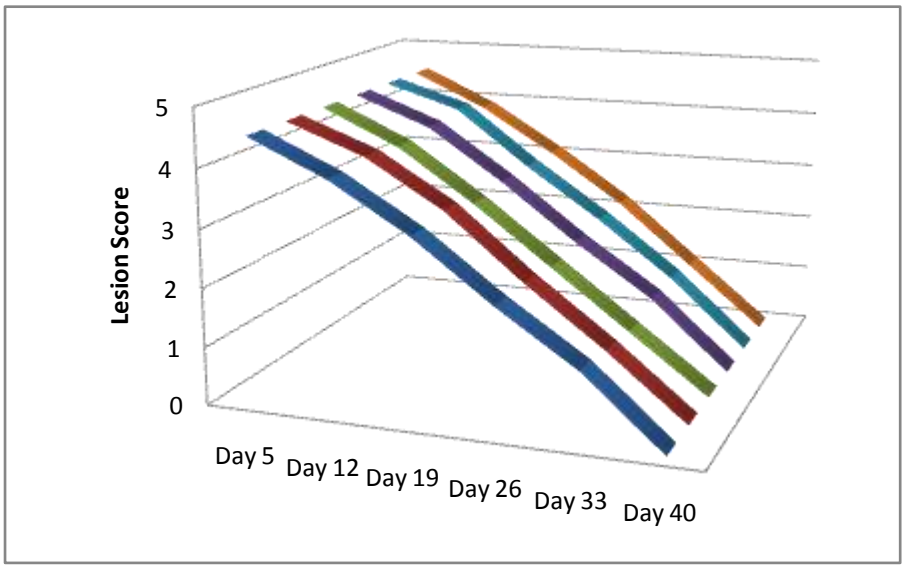

Figure 5: Nano-essence intergroup clinical lesion scores

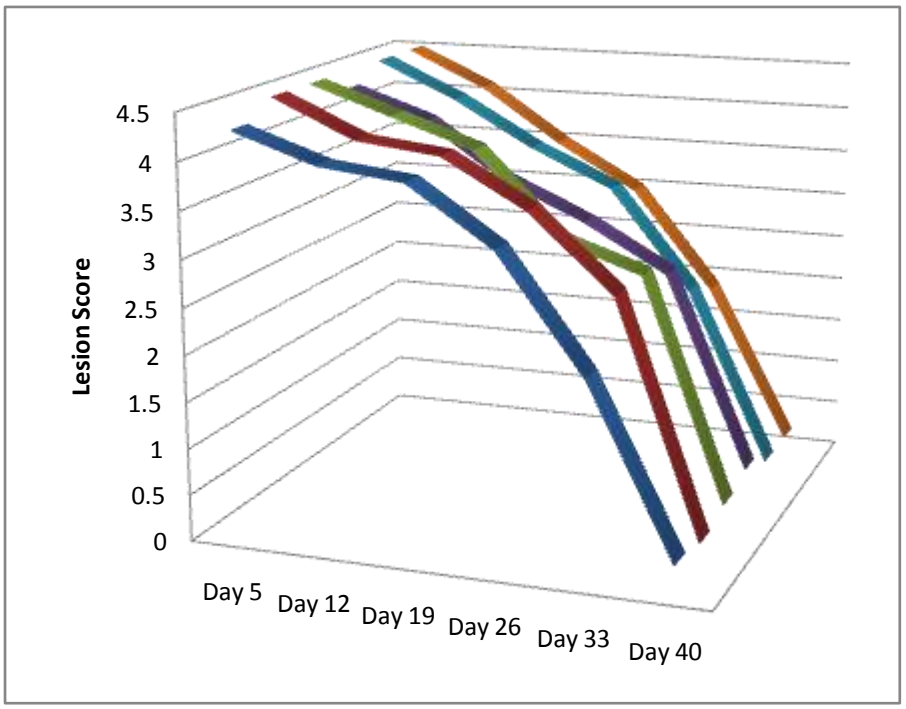

Figure 6: Ketoconazole intergroup clinical lesion scores

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

Group/Day	Day 33	Day 40	Day 50
Negative Control	0/6(0%)	0/6(0%)	0/6(0%)
Nano-essence	0/6(0%)	0/6(0%)	0/6(0%)
Ketoconazole	2/6(33%)	0/6(0%)	0/6(0%)

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 *Origanum vulgare* Nano-essence was used to treat dermatophytosis caused by *Microsporum canis* in cats. Micro dilution broth using CLSI M38-A protocol was used for MIC determination. This technique is widely used in mycology laboratory. MIC was determined at the 0/09 µl/ml. Chitosan is used in production of many Nano drugs and its properties were discussed in previous studies. In this study, we utilized Nano technology to affix additional properties to *Origanum vulgare* essence in treatment of *M.canis* induced dermatophytosis. All cats in this study were successfully infected then 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 clinically improved successfully by day 40.

Clinical lesion score averages on day 5 (start of the treatment) for all of the groups were between 4 and 4.5 except negative control group. The average of clinical lesion scores on day 12 in the *Origanum vulgare* Nano-essence treatment group was 3.9 and the average of the Ketoconazole 2% treatment group on day 12 was 4.0. On days 19, 26, 33 and 40 after the start of treatment, the average of clinical lesion scores in the *Origanum vulgare* Nano-essence treatment group was 3.10, 2.11, 1.13, 0.0 respectively, whereas the average of Ketoconazole 2% treatment group on the same days was 3.5, 2.9, 2.1, 0.3 respectively. This declining trend in the average of clinical scores in all groups was followed by 3 consecutive negative fungal cultures in 100% of the animals in both *Origanum vulgare* Nano-essence treatment and Negative groups whereas the fungal cultures were negative on day 33 and 40 for Ketoconazole 2% treatment group.

Effects of different herbal products with antimycotic properties have been determined in clinical trials in different animal species [29-31].

Mugnaini et al. was used essential oils of *Origanum vulgare* in 2013 to treating dermatophytosis in sheep. They stated that treatment of dermatophytosis caused by Trichophyton spp. with essential oils of *Origanum vulgare* was successful and better than conventional treatments [29].

Myrtus communis and Artemisia sieberi Nano-essences have been used by Mashhady Rafie et al. in 2013 and 2014 in guinea pig to treating dermatophytosis. Both studies demonstrated that Nano-essences showed better results with regard to the

speed of resolution [30, 31].

According to the results obtained from the groups, they revealed that the *Origanum vulgare* Nano-essence treatment group showed improved clinical symptoms faster than the Ketoconazole 2% treatment group over a declared treatment period. Further clinical trials are needed to generalize of these results in animals and human patients.

CONCLUSION

It is concluded that Nano-essence of *Origanum vulgare* could be a replacement for Ketoconazole 2% cream to treat dermatophytosis but generalization of results in animals and human patients needs further clinical trials.

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