



Comparison of the Therapeutic effects of Nano-essence of Medical herb *Artemisia sieberi* with the ointment of Ketoconazole in guinea pig infected by *Microsporium canis*

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Abstract

Essences have well known properties. Transforming a drug to nano sized particles usually adds some additional potency to that drug. In this study we used *Artemisia sieberi* nano-essence to treat *Microsporium canis* induced dermatophytosis in guinea pig model. In vivo and In vitro methods were used to investigate the antifungal properties of nano-essence. Minimum inhibitory concentration of nano-essence was 0.3% to 2%. Treatment started 5 days after infection as 12 hours regimen until 45 days post infection. Both Ketoconazole and nano-essence groups had complete cure at day 40. Results show that this nano-essence is effective drug to treat *M.canis* induced dermatophytosis.

Keywords: Nano-essence, *Artemisia sieberi*, Ketoconazole, *Microsporium canis*, guinea-pig.

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 world wide distribution and the causative agents are *Microsporium*, *Trichophyton* and *epidermaphyton* spp. *Microsporium 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 common⁵. 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^{6,7}.

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 mucoadhesiveness, absorption enhancing and sustained-release characteristics to nano sized drugs⁸⁻¹².

Artemisia sieberi (*A.sieberi*) is an ancient medical herb that grows in China, Russia and desert part of Iran. Locally known as "Dermaneh", has antifungal, vermifugal, antibacterial and anticandidal effects. Although the chemical composition of this herb varies according to growing place but main components of

that is generally the same for all species those are α -thujone and β -thujone¹³⁻²⁵.

In present study we decided to use *A.sieberi* nano-essence to treat *M.canis* induced experimental dermatophytosis.

Material and Methods

Animals: In this study, 24 male Guinea pigs with the same weight (ranging from 350-400 grams) were obtained from Pasture institute (Tehran, Iran). All animals were put in separate polycarbonate 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: *A.sieberi* essence was purchased from Barij Essence Pharmaceutical Company, (Kashan,Iran) and Nano encapsulation was done by Zist Shimi Azma Roshd Company, (Tehran, Iran). 5 cc of *A.sieberi* essence is sufficient to produce 1liter of nano-essence. The product's reliability was confirmed by Fourier Transform Infrared Spectrometer and screening electron microscopy (figure-1 and figure-2). Ketoconazole topical cream that was utilized in this study was purchased from Iran Najo pharmaceutical Company (Tehran, Iran).

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

MIC determination: Microdilution broth, using Clinical: Laboratory Standards Institute (CLSI) M38-A protocol was used for MIC determination *in vitro*. Using RPMI1640 medium, a 0.5×10^4 cells/ml suspension was gained as described before^{5,26,27}.

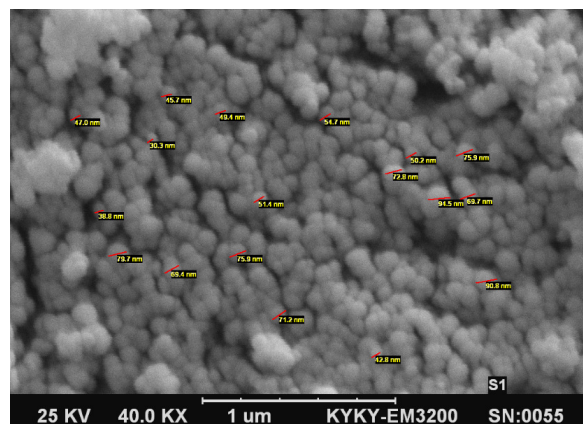


Figure-1
 Loaded screening electron microscopy (SEM)

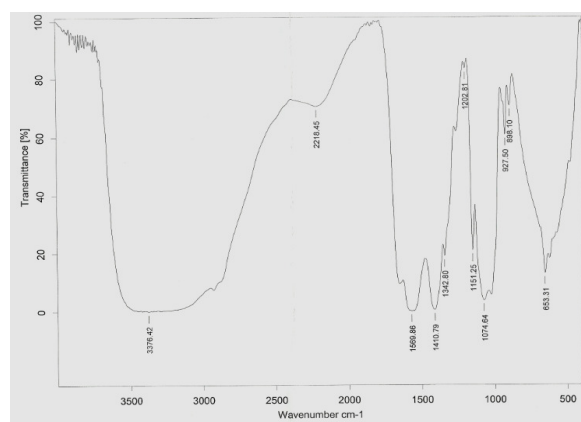


Figure-2
 Loaded Fourier Transform Infrared Spectrometer (FTIR)

Animal infection: Posterior dorsal portion of every animal was shaved gently for as wide as 4 cm². Shaved area was abraded with the back of sterile scalpel blade. Suspension containing 10⁶ *M.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 4 groups randomly, positive control, negative control, nano-essence (according to MIC) and Ketoconazole treatment group. All animals except negative control group were mycological positive at 5th day^{28,29,30}.

Treatment: According to statements in previous studies, 12 hours treatment regimen started at 5th day with nano-essence and Ketoconazole¹¹. In 45 days course of treatment, nano-essence was applied with sprinkler so that drug covered all the shaved and unshaved area around it. In negative and positive control groups, saline were used as a placebo during treatment.

Efficacy evaluation: Drug efficacy was evaluated by clinical lesion scoring and fungal culture. Modified lesion scoring is 5 degrees scoring system (0 to 4) indicated as: 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; and score 4, ulceration and scarring in addition to lesion 3. Scale and hair cultured at the day 30, 37 and 44 using scraping and plucking technique.

Data analysis: Mann-Whitney *U*-test (with SPSS for windows) was used to analyze lesion scores.

Results and Discussion

MIC: MIC ranges of *A.sieberi* nano-essence were 3 - 20 µl/ml (0.3%-2%). Forasmuch as the concentration of essence used for drug production process, MIC of *A.sieberi* essence will range from 0.15 to 1 %. This numbers are much lesser than stated before^{14,21,25}. For this study MIC was considered as 1%.

Lesion scoring: Gross findings are shown in figure-3. All animals except negative control were mycological positive when the treatment started. Clinical lesion score average on day 5 (treatment start) for all groups were between 2 and 3. The clinical lesion score average in nano-essence treatment group was higher than positive control group when treatment had started (figure-4). Decrease in score average happens gradually until all treatment receiving groups reach score 0 on day 40 except positive control group. Nano-essence and positive control groups show significant statistical difference on day 20, 25, 30, 35 and 40 ($p < 0.05$). This statistical difference is similar between Ketoconazole and positive control groups.

Intra group assessments shows clinical score reduction over treatment period for animals in drug receiving and negative control groups except positive control group (Figure-5 and figure-6). Comparing Ketoconazole and nano-essence treatment groups on various dates shows significant difference just on 10th day ($p < 0.05$).

Culture results: As it is shown on table-1, three consecutive culture results for all animals was negative on days 33, 40 and 47 in treatment groups and negative control group.

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

Group	Culture positive (%)		
	Day 33	Day 40	Day 47
PC	6/6(100)	5/6(83.3%)	5/6(83.3)
NC	0/6(100%)	0/6(0%)	0/6(0%)
nano	0/6(0%)	0/6(0%)	0/6(0%)
keto	0/6(0%)	0/6(0%)	0/6(0%)




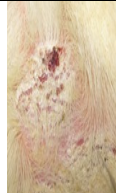



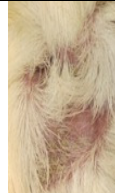




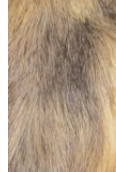


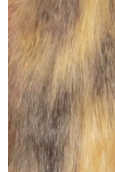







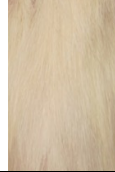

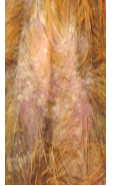






	Day5	Day 10	Day15	Day20	Day 25	Day30	Day35	Day40
PC								
NC								
Nano								
Keto								

Figure-3

Time manner gross findings in different groups infected with *M.canis*. PC, positive control; NC, negative control; Nano, nanoessence; Keto, Ketoconazole

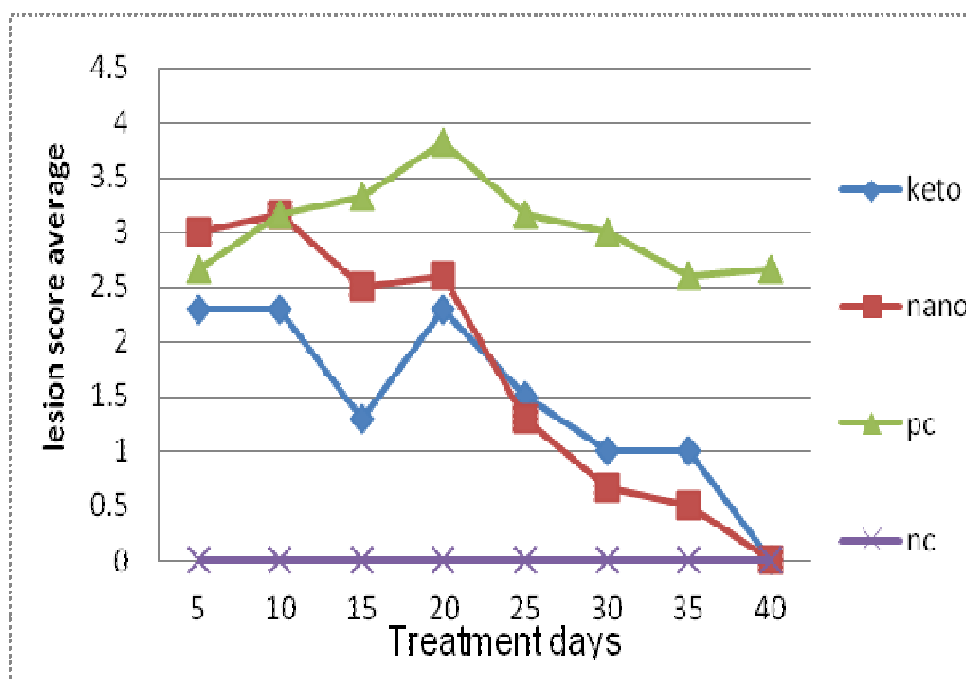


Figure-4

Clinical score average linear chart in different groups. Note the decrease in scores in day 20. PC, positive control; NC, negative control; Nano, nanoessence; Keto, Ketoconazole

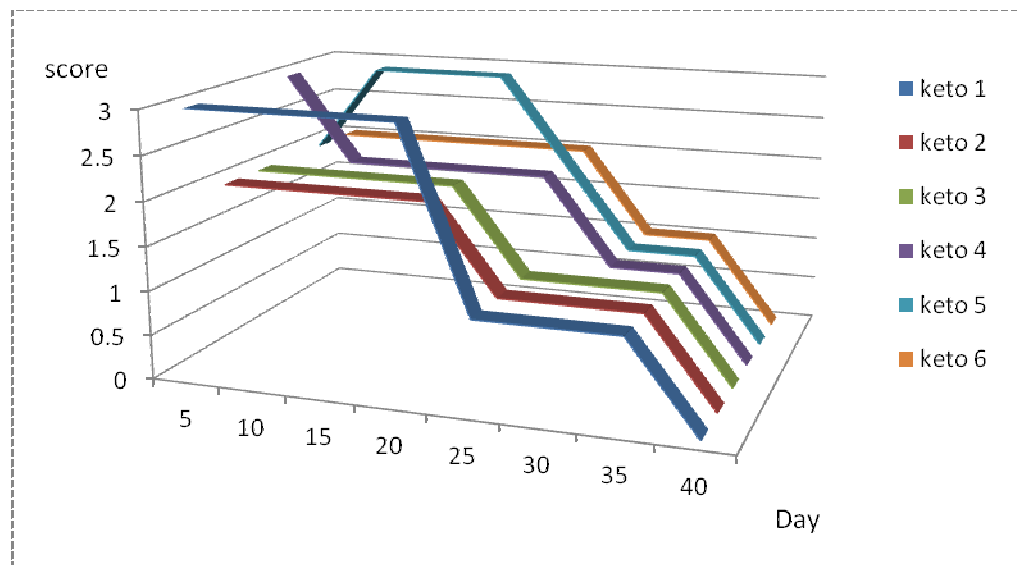


Figure-5

Ketoconazole intergroup clinical lesion score keto1-6 represents animal numbers, Note the reduction of scores from day 20

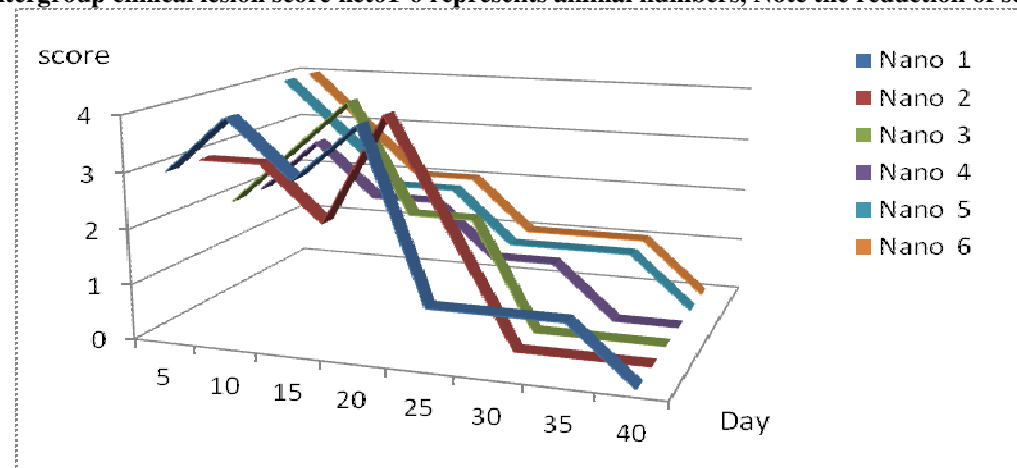


Figure-6

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

Discussion: Different topical and systemic drugs have been used to treat dermatophytosis. From standpoint of clinical practice, topical medications are cornerstone of treatment.

Various herbal extracts have been tested for their antifungal effects. In present study we decided to use *A.sieberi* nano-essence to treat *M.canis* induced dermatophytosis in guinea pigs.

Microdilution broth, using CLSI M38-A protocol was used for MIC determination. This is well known and widely used method in mycology. Using this technique, nano-essence MIC was determined at the range of 0.3% to 2%.

Chitosan and its properties were discussed in previous studies. In this study we decided to utilize nano-essence to add some

additional properties to *A.sieberi* essence in treatment of *M.canis* induced dermatophytosis.

Therapy started from 5th day after infection and lasted until day 40 after infection. All animals in day 40 were clinically cured except positive control group. Clinical score average in 5th day after infection in all groups was between 2 and 3 except negative control group. It should be noted that, there was a drastic decrease in clinical lesion score average between 10th and 15th day post infection in Ketoconazole treatment group that is accompanied by an increase at following 5 days while there was very little changes in nano-essence treatment group at these days. So that clinical score average in nano-essence treatment group at 25th day was lesser than that of Ketoconazole receiving group, although there was not any statistically significant difference. Reduction in clinical score averages continues until

40th day after treatment in both treatment groups, although there is not any significant difference between nano-essence and Ketoconazol receiving groups. As it is shown on linear chart, there is a decreasing manner in clinical score from day 20 to day 40 after infection in positive control, Ketoconazole and nano-essence receiving groups but this reduction in nano-essence group is much more severe than these groups Because the clinical lesion scores average in nano-essence receiving animals were higher than that of animals in positive control group when treatment had started. This declining trend in clinical score average from 30th day post infection, is followed by 3 consecutive negative culture result in 100% of nano-essence and Ketoconazole receiving animals compared to 100% positive culture result in 33rd and 83.3% in 40th and 47th day in positive control group.

Intra group clinical score assessments shows logical decrease over treatment period. Assessments in nano-essence group indicates that 33% of these animal were in score 4 when trial started but none of the animals in Ketoconazole receiving groups were in this level at the same day. None of the animals in Ketoconazole treatment groups reached score 4 during trial but 33% of animals in nano-essence group were in score 4 on day 20. These differences are expressed on clinical score average. Although there is a dramatic decrease between 20th and 25th day post infection in both treatment receiving groups, it should be considered that nano-essence receiving animals had higher score at the beginning of trial so their score reduction are contemplated more prominent. Generalization of results in animals and human patients needs further clinical trials.

Conclusion

It is concluded that nano-essence of *A.sieberi* could be a replacement for Ketoconazole ointment to treat dermatophytosis but generalization of results in animals and human patients needs further clinical trials.

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References

1. Baldo A., Mondo M., Mathy A., L. Cambier, E.T. Bagut, V. Defaweux, F. Symoens, N. Antoine and B. Mignon, Mechanisms of skin adherence and invasion by dermatophytes, *Myc*, **55**, 218-223 (2013)
2. Fontenelle R., Morais S.M., Brito H.S.E, Brilhante R. S.N., Cordeiro R.A., Lima Y.C., Brasil N.V.G.P.S., Monteiro A.J., Sidrim J.J.C and Rocha M.F.G., Alkylphenol Activity against *Candida* spp. and *Microsporium canis*: A focus on the antifungal activity of thymol, eugenol and O-Methyl Derivatives, *Molecules*, **16**, 6422-6431 (2011)
3. Scott D.W., Miller W.H. and Griffin C.E., Miller and Kirk's Small Animal Dermatology, **6**, 1528 (2001)
4. Medleau L. and Hnilica K., Small animal dermatology, **2**, 526 (2006)
5. Lee S. and Han J., Antifungal effects of Eugenol and Nerolidol against *Microsporium gypseum* in a guinea pig model, *Bio. and Pharma.Bul*, **30**, 184-188 (2007)
6. Foster A. and Carol F., BSAVA manual of small animal dermatology, **2**, 300 (2003)
7. Rochette F., Engelen M. and Vanden Bosche H., Antifungal agents of use in animal health – practical applications, *J Vet Pharmacol Ther*, **26**, 31-53 (2003)
8. Dash M., Chiellini F., Ottenbrite R.M. and Chiellini E., Chitosan—A versatile semi-synthetic polymer in biomedical applications, *Progr.in Poly.Sci*, **36**, 981-1014 (2011)
9. Kong M., Chen X., Xing K. and Park H., Antimicrobial properties of chitosan and mode of action: A state of the art review, *Inter.J.of Food Micro*, **144**, 51-63 (2010)
10. Pillai C.K.S., Paul W. and Sharma C., Chitin and chitosan polymers: Chemistry, solubility and fiber formation, *Prog. Polym. Sci.*, **34**, 641-678 (2009)
11. Sinha V.R. and Singla K.A., Chitosan microspheres as a potential carrier for drugs, *Int. J Pharma*, **274**, 1-33 (2004)
12. Siripatrawan U. and Harte B., Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract, *Food Hydro*, **24**, 770-775 (2010)
13. Abad M., Ansuategui M. and Bermejo P., Active antifungal substances from natural sources, *ARKIVOC*, **2**, 116-145 (2007)
14. Abad M., Bedoya L., Apaza L. and Bermejo P., the *Artemisia* L. Genus: A Review of Bioactive Essential Oils, *Molecules*, **17**, 2542-2566 (2012)
15. Chen W., Vermaak I. and Viljoen A., Camphor—A Fumigant during the Black Death and a Coveted Fragrant Wood in Ancient Egypt and Babylon—A Review, *Molecules*, **18**, 5434-5454 (2013)
16. Chiasson H., Belanger A., Bostanian N., Vincent C. and Poliquin A., Acaricidal properties of *Artemisia absinthium* and *Tanacetum vulgare* (Asteraceae) essential oils obtained from three methods of extraction, *J. Econ. Entomol*, **94**, 167-171 (2001)
17. Dehghani Bidgoli R. and Pessarakli M., Heshmati G.A., Comparison of Essential Oils in Aerial Parts of *Artemisia Sieberi* from Kashan Province of Iran, *J. of Agri. Sci. and Tech*, **14**, 392-396 (2012)

18. HAQ I., Mannan A., Ahmed I., Hussain I., Jamil M. and Mirza B., antibacterial activity and brine shrimp toxicity of *Artemisia Dubai* extract, *Pak. J. of. Bot*, **44**, 1487-1490 (2012)
19. Liu Z., Chu S.S. and Liu R., Chemical Composition and Insecticidal Activity against *Sitophilus zeamais* of the Essential Oils of *Artemisia capillaries* and *Artemisia mongolica*, *Molecules*, **15**, 2600-2608 (2010)
20. Mahboubi M., Iranian medicinal plants, *J. of Micro.Biotech. And Food Sci*, **2**, 2388-2405 (2013)
21. Mahboubi M. and Farzin N., Antimicrobial activity of *Artemisia sieberi* essential oil from central Iran, *Ira.J. of Microb*, **1**, 43-48 (2009)
22. Moghadamnia A. and Akhavan Tafi A., Evaluation of the effect of *Artemisia Sieberi* mouthwash 1% on denture stomatitis, *Cas. J. of Med*, **2**, 47-49 (2010)
23. Monzote L., Alacron O. and Setzer W., Antiprotozoal Activity of Essential Oils, *Agri.Cons. Sci*, **77**, 167-175 (2012)
24. Pirbalouti G., Momeni A. and Bahmani M., Ethnobotanical study of medicinal plants used by Kurd tribe in Dehloran and Abadan districts, Ilam province, 2013, *Afr J Tradit Complement Altern Med*, **10**, 368-385 (2013)
25. Upadhyay R.K., essential oils antimicrobial antihelminthic antiviral anticancer and anti-insect properties, *J. Appl. Biosci*, **36**, 1-22 (2010)
26. Rodrigues C., Miranda K.C., Fernandes O.F.L., Soares A.J. and Silva M.R.R., In vitro susceptibility testing of dermatophytes isolated in Goiania Brazil against five antifungal agents by broth microdilution method, *Rev. Inst. Med. Trop. S. Paulo*, **51**, 9-12 (2009).
27. Singh J., Zaman M. and Gupta A.K., Evaluation of microdilution and disk diffusion methods for anti fungal susceptibility testing of dermatophytes, *Mycoses*, **45**, 595-602 (2007)
28. Ghannoum M.A., Long L., Cirino A.J., Miller A.R., Najafi R., Wang L., Sharma K., Anderson M. and Memarzadeh B., Efficacy of NVC-422 in the treatment of dermatophytosis caused by Trichophyton mentagrophytes using a guinea pig model, *Inter. J.of dermat*, **52**, 567-571 (2013)
29. Neves Cavalcanti J., Guerra J. and Gamble W., Histopathologic and mycologic aspects of experimental infection of guinea pigs with *Microsporium canis*, *Braz. J. vet. Res. anim. Sci.*, **39**, 238-242 (2002)
30. Shimamura T., Kubota N. and Shibuya K., Animal Model of Dermatophytosis, *J. of Biomed. and Biotech*, **2012** 1-11 (2012)