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Anti-Parasitic Efficacy of Some Essential Oils/Extracts Against Itch Mite, *Sarcoptes scabiei*

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The present study was designed to evaluate the acaricidal efficacy of some essential oils/extracts against the itch mite, *Sarcoptes scabiei* infesting the pet dogs. The essential oils of *Curcuma longa* and *Eucalyptus camaldulenses* and hexane extract of *Azadirachta indica* were topically applied to the skin lesions of the pet dogs suffering from *Canine scabies*. Three formulations of each essential oil/extract (20, 10 & 5 % w/w, oil in emulsifying wax) were prepared and each formulation was tested for its therapeutic efficacy on weekly basis. The observed efficacy of each formulation was compared with the efficacy of the standard drug Ivermectin which was injected subcutaneously at the dose of 200 µg/Kg body weight to a group of of affected dogs. No significant difference was found between the efficacy of 20 % formulation of hexane extract of *Azadirachta indica* and the efficacy of the standard drug Ivermectin after 14 and 21 days of treatment. *Azadirachta indica* is easily available, low-cost and eco-friendly so this natural remedy can easily substitute the much expensive and toxic synthetic acaricides.

Keywords: Acaicidal efficacy, Essential oils/extracts, *Canine scabies*.

INTRODUCTION

The itch mite, *Sarcoptes scabiei*, is an ectoparasite which causes a highly contagious skin infection in mammals. This infection is called sarcoptic scabies in humans and sarcoptic mange in other animals. *S. scabiei* infests a large number of wild, domestic and farm animals[1]. Although the disease is not life threatening, yet the parasitic infestation causes a lot of discomfort to humans and companion animals and results in gross economic losses when present in farm animals[2]. The close contact of humans to domestic and farm animals makes scabies one of the most encountered zoonotic disease[3].

A large variety of acaricides is available in the market to treat sarcoptic mange. The routinely used methods to treat this infection in pet animals generally consist of aerosol sprays, rinses and dips of acaricides which contain heavy metals and organochlorides. These acaricides are not eco-friendly and are harmful to the infested animal and its owner as well[4].

Ivermectin is the drug of choice for more than 30 years to treat human and animal scabies but resistance to drug and serious adverse effects have been reported in humans[5]. Ivermectin is also contraindicated in certain breeds of dogs[6]. Ivermectin excreted in the cattle dung is lethal for soil biota as well[7].

The acaricides of plant origin are much safer and cost-effective. They are biodegradable and eco-friendly[8]. Dimri and Sharma[9] used various essential oils to treat mange mites infection in sheep. Neem (*Azadirachta indica*), Turmeric (*Curcuma longa*) and *Eucalyptus* oils are widely used in pest management throughout Asia and Africa[10]. These oils are extensively used in Ayurvedic medicines due to their anti-fungal, antibacterial and antipyretic properties[11].

The use of these essential oils as acaricides is however limited. In the present study the essential oils obtained from *Eucalyptus camaldulensis* and *Curcuma longa* and *n*-hexane extracted neem (*Azadirachta indica*) oil were tested for their anti-parasitic efficacy against *S. scabiei*.

EXPERIMENTAL

The study was carried out from September, 2011 to December, 2012 in various public and private sector veterinary clinics of Lahore, Pakistan. This interventional study was designed to explore the therapeutic efficacy of some essential oils/ extracts against *Sarcoptes scabiei* infesting pet dogs.

Plants used for extraction of essential oils: The plants used to produce the essential oils/extracts in the current study were *Eucalyptus camaldulensis* and *Curcuma longa* and *n*-hexane extracted of neem (*Azadirachta indica*). These plants were selected on the basis of their ethno-botanical use in South Asia especially Pakistan and India. All plants were obtained from their natural habitat and authenticated by botanists in

Lahore College for Women University, Lahore, Pakistan. Parts of the plants required to extract oil were carefully separated, washed and dried at room temperature. The dried plants parts were crushed into small pieces, weighed and placed in the hydro-distillation flasks.The prepared materials were subjected to hydro-distillation for about 4 h. The essential oils obtained by hydro-distillation process were dried over anhydrous sodium sulphate and stored in dark colored glass bottles at 4 °C temperature until required for further use. A negligible quantity of *Azadirachta indica* (neem) oil was extracted by repeated hydro-distillation so, for this oil hexane extraction method was adopted.

Extraction of essential oils: Essential oils from the rhizome of *Curcuma longa* (Turmeric) and leaves of *Eucalyptus camaldulensis* were extracted by steam-distillation (Reverse Dean-Stark method) according to the British Pharmacopeia method (BP. 1988), while the oil from the seeds of *Azadirachta indica* was extracted by using hexane as a solvent.

Hydro-distillation method (Reverse Dean-Stark method): Plant material was placed in a round bottom 5 dm^3 flask and the flask containing the specific plant material was put into an isomantle for heating. Half of the flask was filled with plant material and distilled water was added to immerse the plant material completely. A reverse Dean Stark attached to a coil condenser was fitted on the mouth of the flask. Flask was heated continuously which resulted in the release of the essential oils from the plant material. Steam carrying oil passing through the condenser dropped into the Dean Stark apparatus after condensation. The oil thus collected floated on the top of the water layer. The water layer at the bottom was pushed into the flask through back arm and circulated again and again. After about 4 h of steam distillation, all the oil was extracted from the plant material used. At the end of the process, the layer of essential oils over water was separated by using separatory funnel. To remove the final traces of water anhydrous Na_2SO_4 was used. The collected essential oils were stored in sealed dark colored glass bottles at 4 °C. %age yield of essential oil obtained was calculated by the formula:

$$\text{Yield of essential oil (\%)} = \frac{\text{Weight of essential oil obtained}}{\text{Weight of fresh plant material}} \times 100$$

Hexane extraction of neem oil (Soxhlet's extraction method): Ripened fruit was collected from the neem tree to extract oil. The prepared plant material was put in the flask of the apparatus to extract the oil by using solvent extraction method. *n*-Hexane was used as a solvent to extract oil in Soxhlet's apparatus. The extracted oil was dried under vacuum to evaporate hexane and stored in colored glass bottle at 4 °C for evaluation of its miticidal activity.

Formulation of different concentrations of essential oils/extracts to find dose-response effect: Three formulations of each essential oil/extract were made by mixing them with emulsifying ointments as prescribed in British Pharmacopoeia 88 (BP-88), comprising 3 parts of Emulsifying wax, 5 parts of white soft paraffin and 2 parts of liquid paraffin. 20, 10 and 5 % w/w formulations were prepared from each oil. These formulations were used for therapeutic trials of the mangy dogs.

Experimental animals: A total of 120 adult German Shepherds were subjected to therapeutic trials. Of these 10 dogs were healthy and negative for sarcoptic mange (Group F).

TABLE 1
SUMMARY OF TREATMENT REGIME

Sr. No	Category	Sub groups (w/w %)	No. of dogs
1	Group A Treated with *A. indica* oil	A-1 20	10
		A-2 10	10
		A-3 5	10
2	Group B Treated with *C. longa* oil	B-1 20	10
		B-2 10	10
		B-3 5	10
3	Group C Treated with *E.camaldulensis* oil	C-1 20	10
		C-2 10	10
		C-3 5	10
4	Group D Treted with *Invermectin*	200 µg/Kg body weight	10
5	Group E Untreated positive control	—	10
6	Group F Untreated negative control	—	10
			Total = 120

The remaining 110 dogs showing the signs of pruritis and positive for sarcoptic mange were selected for therapeutic trials (Table-1). The mangy dogs were confirmed to be negative for any fungal or bacterial infection on the mite infested area by the veterinary doctor of University of Veterinary and Animal Sciences, Lahore, Pakistan. These dogs were randomly divided into 5 groups, *i.e.* Group A, B, C, D and E. Animals in the groups A, B and C were further divided into 3 subgroups to investigate the dose-response effect of the following oils:

1. *Azadirachta indica* (neem) oil; 2. *Curcuma longa* (Turmeric) oil; 3. *Eucalyptus camaldulensis* oil.

Animals in Group D were treated with Ivermectin at the recommended rate of 200 µg/Kg body weight. No treatment was given to the animals in Group E (untreated, positive control). Animals negative for sarcoptic mange were placed in Group F(negative control).

Evaluation of comparative efficacy of essential oils/extracts: A 28 days long experiment was designed to evaluate the comparative efficacy of essential oils/extracts. The comparative efficacy was evaluated on the basis of the clinical and parasitological responses of the infested dogs which were observed on weekly basis and compared with the animals treated with the standard drug Ivermectin. The observations were recorded up to 4 weeks. Negative skin scrapings, gradual disappearance of gross lesions, stoppage of itching, smoothening of skin surface and re-growth of normal hair were taken as criteria to assess the efficacy of essential oils/extracts. Persistence of lesions, positive skin scrapings and continuity of pruritis at the end of the study period was indicative of treatment failure. One week follow up period was included to see the recurrence.

Topical application of essential oils/extracts: Before application of the prepared formulation of essential oils/extracts, the lesions were cleaned with lukewarm water and cleaned area was allowed to dry for some time. The prepared ointment was applied topically using a cotton bud as an applicator. The prepared formulation was applied to the respective groups in sufficient quantity to make a thin film over the affected area. New cotton buds were used for each and every application. The prepared ointments were applied once daily for 7 days (1- day 7) and then 7 applications on alternative days for 2 weeks (9-21 day).

Treatment with ivermectin: The positive dogs belonging to Group D was treated with Ivermectin at 200 µg/Kg body weight by subcutaneous injection only once at the start of the therapeutic trials (day 1). If not cured after 14 days, a second dose was administered at the same rate.

Treatment given to negative and positive controls: The positive dogs belonging to group E were treated with emulsifying ointment (without any essential oil/extract) after cleaning the lesion with lukewarm water. The negative dogs belonging to group F were left without any treatment.

Mite examination: The skin scrapings of the treated dogs were collected from edges of the lesions in clean sterilized Petri dishes. These samples were brought to the laboratory for the examination. Petri dishes containing scrapings were warmed at 38 °C for approximately 2 min and then examined under the stereoscopic microscope for the presence or absence of the mites.The negative scrapings were transferred to the test tubes containing 10 mL of 10 % KOH solution and were heated for 5 min in a beaker containing boiling water. The tubes were centrifuged for 5 min at 2000 rpm and the supernatant was discarded. About 5 mL of water was added to the sediment and the tubes were centrifuged again. The supernatant was again discarded and the sediment was examined microscopically for the presence of mites and their developmental stages[12]. The skin scrapings were examined microscopically on weekly basis in all the groups.

RESULTS AND DISCUSSION

The results of therapeutic trials showing percentage of dogs recovered are summarized in Table-2.

TABLE-2
OUTCOME OF THERAPEUTIC TRIALS

Category	Sub groups	Treatment	Dose (w/w %)	7th day (%)	14th day (%)	21st day (%)	28th day (%)
Group A	A-1	Neemoil	20	—	70	90	90
	A-2		10	—	50	50	60
	A-3		5	—	—	10	20
Group B	B-1	Turmeric oil	20	—	60	60	70
	B-2		10	—	30	40	40
	B-3		5	—	—	—	20
Group C	C-1	Eucalyptus oil	20	—	30	50	60
	C-2		10	—	10	30	40
	C-3		5	—	—	—	10
Group D	D	Ivermectin	200 µg/Kg body weight	—	70	90	100
Group E	E	Untreated positive control	—	—	—	—	—
Group F	F	Untreated negative control	—	100	100	100	100

Group A (treated with neem oil; *Azadirachta indica*)

Sub group A-1: (Treated with 20 % w/w formulation of Hexane extracted oil of *A. indica*). At day 7, dryness of lesions was observed in all the subjects but the skin scrapings were still positive for sarcoptic mites. At day 14, 70 % of the subjects showed stoppage/loss of itching & smoothening of skin. The skin scraping showed negative results. At day 21, 90 % of the subjects were negative for skin scraping tests. The lesion quality was much improved with regrowth of hair. At day 28, 90 % of the subjects showed complete recovery with smoothening of the skin surface and marked hair regrowth.

Sub group A-2: (Treated with 10 % w/w formulation of Hexane extracted oil of *A. indica*). At day 7, a slight reduction in itching and dryness of skin lesions was observed. At day 14, 50 % of the treated dogs were negative for sarcoptic mites at this stage. At day 21, 50 % of the subjects negative for manage showed a marked improvement in lesion quality. The shrinkage of lesions was evident at this stage. At day 28, 60 % of the treated dogs showed signs of complete recovery. The remaining 40 % of the subjects were still positive for sarcoptic manage.

Sub group A-3: (Treated with 10 % w/w formulation of Hexane extracted oil of *A. indica*). Although there was some improvement in the lesions quality but 90 % subject were still positive for mites up to day 21st. At the end of the study; at day 28th, 20 % of the treated dogs were negative for Sarcoptic mites.

Group B (treated with essential oil of *Curcuma longa*)

Sub group B-1: (Treated with 5% w/w formulation of essential oil of *C. longa*). At day 7, a marked improvement was seen in the lesion quality but skin scrapings were still positive for mites in all the subjects. At day 14th, 60 % of the treated dogs showed negative skin scrapings and partial recovery of the lesions. At day 21st, the lesions quality showed a marked improvement with the dryness and shrinkness of the margins and loss of itching was also noted.

At day 28th, 70 % of the treated dogs showed complete recovery while 30 % were still positive for manage.

Sub group B-2: (Treated with 10 % w/w formulation of essential oil of *C. longa*). At day 7th, no considerable improvement in the lesion quality was observed and all the treated dogs were positive for skin scrapings. At day 14th, slight improvement in the lesion quality was observed and 30 % of all the treated dogs were negative for skin scrapings. At day 21st, moderate improvement in the lesion quality was observed with 40 % of the total treated dogs were negative for skin scrapings.

At day 28th, although some improvement was observed in lesion quality but the number of the infected dogs, as confirmed by skin scrapings, remained similar to that observed on day 21st.

Sub group B-3: (Treated with 5 % w/w formulation of essential oil of *C. longa*). Moderate improvement in the lesions quality was observed at day 21 but 90 % of the treated dogs were positive for manage mites and only 10 % were negative at this stage. At the day 28, 20 % of the treated dogs were negative for manage mites and signs of skin recovery were evident in these subjects.

Group C (treated with essential oil of *Eucalyptus camaldulensis*)

Sub group C-1: (Treated with 20 % w/w formulation of essential oil of *E. camaldulensis*). At day 7, very little improvement in lesion quality was noted. All of the treated dogs were positive for manage at this stage. At day 14, 70 % of the

treated dogs were still positive for manage. The remaing 30 % treated animals which were negative for manage mites, showed a slight improvement in skin quality and reduction in itching. At day 21, 50 % of the subjects and at day 28, 60 % of the subjects showed negative skin scraping test. Smoothening of skin and scanty hair growth at the margins of the lesions was also observed.

Sub group C-2: (Treated with 10 % w/w formulation of essential oil of *E. camaldulensis*). At day 14, only 10 % of the treated dogs showed negative scraping test. At day 21, 30 % animals were negative for manage mites. At day 28, 40 % of the dogs were negative for manage mites.

Sub group C-3: (Treated with 5 % w/w formulation of essential oil of *E. camaldulensis*). The least recovery was noticed in this subgroup. All the treated animals remained positive for sarcoptic mites up to day 21. Up to this stage, the lesions were moist and itchy. At the end of the study period only 10 % of the treated dogs were negative for manage mites.

Group D (treated with ivermectin at a dose of 200 µg/kg body weight)

At day 7, although the skin scrapings of all the treated dogs were positive for mange mites at this stage dryness on lesions was observed in all the treated mangy dogs. At day 14, shrinkage of lesions and their growth at the margins of the lesions was observed in all the treated dogs and 70 % dogs were negative mange mites. 30 % dogs which were still positive for mange mites were given the second dose of ivermectin at the rate of 200 µg/kg body weight. At day 21, smoothening of skin surface and marked hair growth was observed in all the treated dogs. Skin scrapings were negative for mange mites in all the subjects at this stage. At day 28, all of the dogs were free of mange lesions and showed normal hair growth of the previously infested area indicative of complete recovery.

Group E (untreated positive control): 10 mangy dogs selected for this category were left untreated. All the animals showed severe itching, serous exudation and scab formation followed by alopecia, thickening & hyper keratinization of skin during the 28 days of study period. The number of the mites in skin scrapings increased gradually per unit area of skin. At the end of the study period 12-15 mites/inch^2 were noted in these untreated dogs.

Group F (untreated negative control): These animals remained healthy throughout the study period. They showed no signs of sarcoptic mange such aspruritis or lesions on skin and in these animals normal hair growth on the scarified area was observed.

Essential oils/extracts have been used in traditional medicines and cosmetics in Pakistan and India since old ages due to their fungicidal, bactericidal, virucidal and insecticidal properties. However their use as acaricidesis limited, modern acacides of synthetic origin normally have a single active compound against targeted pest and the pest easily develops resistance against this single ingredient by detoxifying it[14]. The essential oils/extracts on the other hand, normally have 35-40 biologically active ingredients. Neem oil extracted from flowers and fruit has 45 active ingredients[15]. It is virtually impossible for a pest to develop resistance against such a large number of constituents.

Neem oil is extensively used asinsecticide and acaricide. Azadirachtin is the primary biologically active ingredient of this oil which is mainly responsible for its biocidal activity. Topical application of neem oil to control scabies on sheep was reported by Hirudkar *et al.*[16]. They reported 87.8 % efficacy of 50 % neem oil preparation. The synergistic effect of neem and Karanj oil was studied by[17] and the combination was reported to be 100 % efficacious to treat scabies in pigs. Hagawa *et al.*[18] used a paste of 50 g of *Curcuma longa* powder in 25 g of neem oil to treat sarcoptic mange in sheep. This paste was applied once daily and 100 % cure rate was reported after 15 days of topical application[19]. Used a paste of turmeric and neem to treat 814 patients and reported a cure rate of 97 % after 2 weeks of treatment. *Eucalyptus* oil is commonly used as a repellent for different insect species such as mosquito. *Eucalyptus citriodora* was reported to be an excellent repellent for termites by Manzoor *et al.*[19], but the insecticidal/acaricidal activity of *Eucalyptus* oil is yet to be discovered in the current study the efficacy of 20 % neem oil was not significantly different from the efficacy of ivermectin after 14 and 21 days of treatment. All the other formulations were not so efficacious.

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