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Efficacy of crude neem seed kernel extracts against natural infestation of *Sarcoptes scabiei* var. *ovis*

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Abstract

This study was aimed to evaluate the efficacy of crude aqueous-methanol and aqueous extracts of neem (*Azadirachta indica*) seed kernel against sarcoptic mange of sheep. Crude aqueous-methanol (AME) and aqueous extracts (AE) of neem seed kernel (NSK) were prepared and formulated as 10% and 20% ointments (w/w), using Vaseline as vehicle. Forty-two lambs of Pak Karakul breed, having natural infection of sarcoptic mange were divided into seven experimental groups. Skin scrapings and clinical examination were carried out at scheduled intervals after treatment. Ivermectin (positive control) completely cleared infesting mites from animals after 10 days and 20% AME after 16 days. While, clinical mange was completely cured after 16 and 20 days with ivermectin and 20% AME, respectively, under field conditions. Only the higher concentration (20% AME) of NSK extracts completely cured the clinical mange, suggesting a dose-dependent response. Our results consolidate the belief that use of folk remedies can provide an effective and economic way of combating sarcoptic mange in sheep.

Keywords: Mange; Sheep; Neem; *Sarcoptes scabiei*

1. Introduction

Sarcoptic mange caused by the burrowing mites, *Sarcoptes scabiei* var. *ovis* (*S. ovis*), causes significant losses to sheep industry. Affected ewes produce less milk, production of live lamb per ewe decreases and affected lambs have a suboptimal growth rate (Fthenakis et al., 2000, 2001). Treatment of sarcoptic mange with various acaricides like Diazinon, Fenvalerate, Deltamethrin and Avermectin (Campbell, 1989; Merck, 1991) has been attempted with different grades of success. Rapid development of resistance (Clark et al., 1996; Synge et al., 1995), high cost, environmental contamination (Halley et al., 1993; O'Brien, 1999) and health hazards to humans during treatment of animal (Das, 1996) are, however, the major problems associated with the use of synthetic acaricides. In view of these problems, use of botanical acaricides against skin problems like mange is visualized.

Different indigenous plants, like *Cedrus deodara, Pongamia glabra, Jatropha curas, Diospyros scabra, Dobera glabra, Euphorbia abtyssinica,* and *Sterculia alexandri* have been tried against sheep mange (Teshale et al., 2004). Neem tree is one of the most extensively studied botanical sources of insecticidal compounds. Various parts of neem are being used in Ethnoveterinary practices for the treatment of mange mites in animals in Pakistan (personal communication). However, as far as ascertained, the claims of local healers/ farmers as to the effectiveness of neem in sarcoptic mange have not been studied. The objective of this trial was to evaluate the efficacy of neem seed kernel (NSK) extracts against natural *S. ovis* infection in sheep under field conditions, using standard parasitological procedures.

2. Materials and Methods

2.1. Preparation of ointment

Ripened neem fruit was collected directly from a neem tree and authenticated by a botanist in University of Agriculture, Faisalabad, Pakistan. After drying under shade, NSK was

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Table 1
Treatment assigned and mean lesion quality of different groups at start of experiment (D0)

Group no.	Treatment	Mean lesion quality (±S.D.)
1	AME 10%	3.33 ± 0.57
2	AME 20%	3.00 ± 1.00
3	AE 10%	3.00 ± 1.00
4	AE 20%	3.00 ± 1.00
5	Ivermectin (+ve control)	3.33 ± 0.57
6	Untreated control	3.00 ± 1.00
7	Vaseline	3.33 ± 0.57

AME 10% and 20% = 10% and 20% w/w ointment of aqueous-methanol neem seed kernel extract; AE 10 and 20% = 10% and 20% w/w ointment of aqueous neem seed kernel extract; ivermectin = at 200 μg kg^{-1} (s/c inj.) only once during experiment; untreated control = no treatment (lesions only cleaned with lukewarm water); vaseline = sham treatment; *N.B.* Ointments were applied to make a thin film over lesion.

obtained and crude aqueous-methanol (AME) and aqueous (AE) extracts were prepared. AE was prepared by boiling 0.5 kg of ground NSK in 1 L of water in a 2 L capacity boiling flask for 30 min. After filtration, new solvent was added and the above said procedure was repeated for a total of three times. AME was prepared with cold maceration method. Briefly, ground plant material (1 kg) was soaked in 3 L solvent (aqueous-methanol 30:70) for 3 days. After filtration, new solvent was added and the above procedure was repeated for a total of three times. AME and AE were obtained after evaporating the solvent with rotary evaporator, until a thick sticky material formed. All the extracts were formulated as ointment (10% and 20%), using Vaseline as vehicle (w/w).

2.2. Animals and treatment

A 22-day (D) long experiment was carried out on Livestock Research Station, Rakh Kherwala, District Layyah, Punjab, Pakistan. Forty-two sheep (Pak Karakul breed) of almost same age group and weight were selected from a herd of 600 animals. All the sheep were confirmed for having natural infection of *S. ovis* by skin scraping examination. Sheep were randomly divided into seven groups ($n = 6$) and assigned to different treatments (Table 1) randomly, in a completely randomized design.

Before application of ointment, hair around the affected parts were clipped, lesions were cleaned with lukewarm water and allowed to dry for some time and then ointment was applied in sufficient quantity to make a thin film over the affected area. All the groups were treated for a total of seven times, on alternate days (D0–14), except the positive control group (treated with ivermectin). Positive control group was treated with ivermectin at 200 μg kg^{-1} (s/c inj.), only once at the start of experiment (D0). Response to the treatment was monitored on alternate days at the time of drug application in terms of mean recovery response (MRR) and % reduction in mites. Statistical analysis was done by the ANOVA and Tukey's test using SAS software (SAS, 1998) and differences were considered as statistically significant when $P < 0.05$.

2.3. Clinical examination

Clinical examination of sheep was carried out on D0 and then onward on alternate days up till D22. At the start of trial, quality of lesion of individual animal was described using grade codes from 1 to 4 indicating an increasing severity of skin reaction (given below), and mean lesion quality (MLQ) of each group was calculated. After treatment (from D0 till end of trial) recovery in individual animals was described with grade codes from 0 to 4 (given below) and MRR of each group was calculated (Sharma et al., 1997) to compare the effect of treatment.

	Grade codes
Description of lesions	
Reddening of skin	1
Bare, exposed, moist lesions with serious exudation	2
Dry lesions with scab formation and loss of hairs	3
Thick, wrinkled skin with hyperkeratinization	4
Description of recovery	
No response	0
Dryness of lesions and loss of itching	1
Start of shrinkage of lesions and hair growth	2
Marked hair growth with smooth skin surface	3
Complete recovery	4

2.4. Parasitological examination

Skin scrapings were taken from the part of the lesions bordering healthy tissue by scraping 1 $in.^2$ area from two different body sites by the method described by Fthenakis et al. (2000). Samples were examined within 12 h of collection. Scrapings from two sites were collectively placed into a test tube with 5 mL of distilled water and 10% KOH and heated until hair and epidermal scales were dissolved. They were centrifuged at 10 g for 10 min. The sediment was suspended in distilled water and re-centrifuged. Sediment was examined under a microscope and mites were identified with the help of morphological keys (Soulsby, 1982). Total numbers of mites present were counted and %age efficacy of each treatment was calculated (Khan et al., 1998).

$$\%\text{age efficacy} = \frac{\text{No. of mites before treatment} - \text{No. of mites after treatment}}{\text{No. of mites before treatment}} \times 100$$

3. Results and discussion

Difference in MLQ of different groups before treatment (D0), was statistically non-significant ($P > 0.05$). Products prepared from crude NSK extracts were found safe for sheep as they did not show the sign of irritation or restlessness at the time of application or afterwards. Effect of different treatments on diseased animals is presented in Table 2.