Sarcoptes scabiei var. *cuniculi* mite-infected rabbit skin response to *Nerium oleander* leaf extract

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Abstract

Sarcoptes scabiei var. *cuniculi* is a global parasitic mite that infiltrates the skin and induces scabies. This study aims to assess the acaricidal activity of *Nerium oleander* (NO) methanolic extracts against *S. scabiei* var. in rabbits. Methanolic *Nerium oleander* extracts of three concentrations 10%, 25%, 50% w/v and a commercial acaricide, 1% ivermectin, were compared with rabbits that received no treatment (negative control), in addition to the infected group (positive control). A completely randomized experiment allocated thirty rabbits to six groups. All experimental groups except the control were infected for four weeks. Weekly counts of mange mites were conducted, the lesion index was scored, and the rubbing index calculated. Treatment was carried out from the fifth to the eighth week. The results showed that 50% *N. oleander* methanolic extracts on the skin worked better than the other extract concentrations ($P < 0.05$) within two weeks of treatment and were just as effective as ivermectin as an acaricide, which suggests that the response depends on the dose and time. We found that after 4 weeks, 25% *N. oleander* methanolic extract effectively eliminated clinical mange in rabbits. The results also showed a significant change in the tissue sectors after treatment. The results demonstrated the ability of methanolic *N. oleander* extracts to eliminate mange mites, presenting a cost-effective, secure, and eco-friendly solution for treating *Sarcoptes* mange in rabbits.

Acaricide, ivermectin, lesion, mange, rodent

Sarcoptes scabiei is now placed in the superfamily Sarcoptoidea and family *Sarcoptidae* along with many other ectoparasitic mites of mammals (Sharaf 2024). The family *Sarcoptidae* contains three subfamilies (*Sarcoptinae, Teinocoptinae,* and *Diabolicoptinae),* including 16 genera and 118 species, that are all inhabitants of the skin of mammals (Bochkov 2010). *Sarcoptes scabiei* var. *cuniculi* (*S. scabiei*) is an important ectoparasite in rabbits because of the possibility of zoonotic infection (Abd El-Ghany 2022); it is a pathogen that can cause infections on the skin surface (Seddiek et al. 2013). Sarcoptic mange is marked by severe itching, hair loss, redness, scales, and crusts on the head, neck, trunk, feet, ears, nose, and genitals, leading to a hypertensive response, significant reductions in body weight, productivity, wool yield, and mortality (D'Ovidio and Santoro 2021). *Sarcoptes scabiei* is more challenging to eradicate in rabbits than in other household animals (Wei et al. 2019). Two clinical forms are identified: a pruritic or hypersensitive variant predominantly affecting developing rabbits and a chronic hyperkeratotic variant marked by auditory crusts and a significant mite infestation, primarily impacting multiparous rabbits (Arlian and Morgan 2017). Females lay 40–50 or more eggs over a lifespan of 26–40 days, depositing them into tunnels created in the stratum corneum of the skin, resulting in severe pruritic rashes, hypersensitivity, and inflammation (Van Neste and Lachapelle 1981).

Scabies mites inhabit tunnels they create in the nonviable stratum corneum of mammalian epidermis. It was formerly thought that these mites consume lysed stratum corneum. Subsequent investigations indicated that the mites consume intercellular fluid (lymph)

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that permeates into the burrow surrounding their mouthparts as they excavate within the stratum corneum adjacent to the viable tissue of the lower epidermis (Arlian and Morgan 2017). Mites seem to excavate downward into the dermis to sustain their position when the basal layer of cells proliferates and the upper layer of the desiccated stratum corneum is displaced toward the skin surface (Sharaf 2024). Various commercial veterinary formulations (ivermectin, doramectin, abamectin, and eprinomectin) are employed to manage sarcoptic mange, including subcutaneous injections, oral administration, and topical applications such as pour-on, spot-on, spray, or dips. Most systemic medications target the nerve axons of mange mites by altering the kinetics of ligand-gated ion channels (Bernigaud et al. 2019; El-Saber Batiha et al. 2020). Generally, numerous chemical acaricides have limits, including resistance and toxicity (O'Brien 1999), environmental contamination, and persistence. The adverse consequences of chemical acaricides have initiated a quest for novel alternatives (Khater 2011).

Nerium oleander, commonly known as oleander, is a member of the *Apocynaceae* family. It mostly occurs in alluvial and rocky landscapes, alongside wadis in the northern Sahara and the Tassili and Hoggar mountains, and is sometimes planted as an attractive species for hedges in parks and gardens. The blooms flourish from spring to late summer; all fresh components are toxic: wood, bark, leaves, and flowers. The entire oleander plant possesses highly toxic and fatal glycosides and alkaloids (Zaid et al. 2022). Analysis revealed (GC-MS) that *N. oleander* leaves contain several biologically active compounds that have a lethal effect on the parasite that causes scabies (Murshed et al. 2024). They have several physiological effects: antibacterial, insecticidal (Dinan 2001), anti-inflammatory (Aggarwal and Shishodia 2006), and cytotoxic (Mijatovic et al. 2007). Their toxicity is specifically targeted at the neurological, reproductive, or digestive systems of pests (Kumar et al. 2012). Numerous researchers have examined the harmful effects of *N. oleander* on humans, animals, and some insects (Adome et al. 2003). This study aimed to investigate the acaricidal effectiveness of *N. oleander* extract (NOE) against the *S. scabiei* var. *cuniculi* mite in rabbits.

Materials and Methods

Ethical statement

The research was conducted in agreement with the 'Guide for the Care and Use of Laboratory Animals'. The research complied with the animal use rules of King Saud University and met the standards set by the National Committee of Bio-Ethics (NCBE) in Saudi Arabia. The Royal Decree numbered M59 was issued on 14/9/1431H. The Research Ethics Committee of King Saud University (REC) (KSU-SE-22-38) sanctioned all experimental techniques, guaranteeing the rights and welfare of animals, minimizing stress, and preventing harm or suffering.

Preparation of extract and ointment

The NOE was prepared using leaves obtained from the public parks in the city of Riyadh, Saudi Arabia, and the botanical identity of the plant was confirmed by a taxonomist at the Department of Botany, University of King Saud. The leaves were dried at 40 °C, ground into a powder, and then extracted with 200 ml of methanol (70%) for 24 h at 4 °C. The obtained extract was concentrated and dried in a rotary vacuum evaporator (Yamato RE300, Tokyo, Japan) until a thick, sticky material formed. The extract was formulated as an ointment (10%, 25%, and 50%), using Vaseline as a vehicle (w/w). We used distilled water to dissolve the extract.

Experimental animals

A total of 30 rabbits (*Oryctolagus cuniculus*, mean weight of 1,200–1,700 g) were purchased from farms around the city of Riyadh, Kingdom of Saudi Arabia. All rabbits were uninfected with the *S. scabiei*, as was confirmed by skin scraping examination. They were housed in the Zoology Department, Faculty of Science, King Saud University, with free access to water and standard food (a commercial pellet diet). In addition, two rabbits naturally infected with *S. scabiei* were purchased to be used to infect the rabbits to be tested with the extract, and were confirmed to have infection by skin scraping examination.

Experimental design and treatment strategy

We carried out the infection by mixing two infected rabbits with 25 rabbits free of mange for two weeks while keeping five rabbits without infection as a control group. We examined the skin of each rabbit to confirm

the infection, then divided the animals into five groups, each containing 5 rabbits: a negative control group (uninfected), an infected group as a positive control (untreated), a group treated with concentrations of 10%, 25%, and 50% w/v of extracts with ointment, and a group treated with 1% ivermectin (ARABCOMEO—Arab Company for Medical Products, Obour City, Industrial Area, Cairo, Egypt) subcutaneously. The experiment was conducted for eight weeks. We clipped the hair around the affected parts before applying the ointment and then applied enough ointment to form a thin film over the affected area. The treatment was done with an extract every two days for four weeks, while ivermectin was given at a dose of 100 µg/kg three times within a week interval.

Histopathology of the skin

Tissue specimens of skin (skin biopsy) were collected as per the protocol described by Iqbal et al. (2006). Briefly, the areas were disinfected using gauze dipped in alcohol. A disposable needle was used to pull an area of skin. A disposable scalpel blade was used to cut a piece of skin pulled up by the point of the needle. Pieces of skin were fixed in 10% neutral buffered formalin, dehydrated in ethanol, embedded in paraffin, and then sectioned in 5 µm thickness. Sections were then stained with Mason's trichrome (Solarbio Life Sciences, Beijing, China). To evaluate the skin histological injury, we used a semi-quantitative score system (Giamarellos-Bourboulis et al. 2006). Skin areas were assessed for the dermis and epidermis and inflammatory cell areas (0, absent; 1, slight; 2, moderate; and 3, pronounced) and for the increase in the number of apoptotic cells, macrophages, necrotic cells, and pigment presence (0, absent; and 1, present). The scoring of each section of the rabbit skin tissue represented a mean score of five separate sections of high microscopic power fields.

Statistical analyses

Weekly mite counts were used for each treatment to calculate the acaricide efficacy ratio:

Acaricide efficacy = 1 - (treated mite count/untreated control mite count)

The disparities were examined utilizing the one-way analysis of variance (ANOVA) approach in SPSS Vision 21 statistical program. We utilized Duncan test to compare the mean values of various substances. A probability of < 0.05 was deemed significant ($P \leq 0.05$). We included the mean values alongside the standard deviation (SD).

Results

Figure 1 shows the mean rubbing index over time for each treatment. After four weeks, the rubbing index for the non-treated group was 0.94, whereas for the ivermectin treatments it was zero. In addition, the rubbing index for the rabbits treated with 50% NOE was 0.7 in weeks five and six, and 0.5 in weeks six and seven. Up until the fifth week, there was no difference ($P > 0.05$) in the rubbing index between the 10% and 25% NOE treatments. In the sixth week of the trial, the rubbing indices of these two treatments were different (Fig. 1).

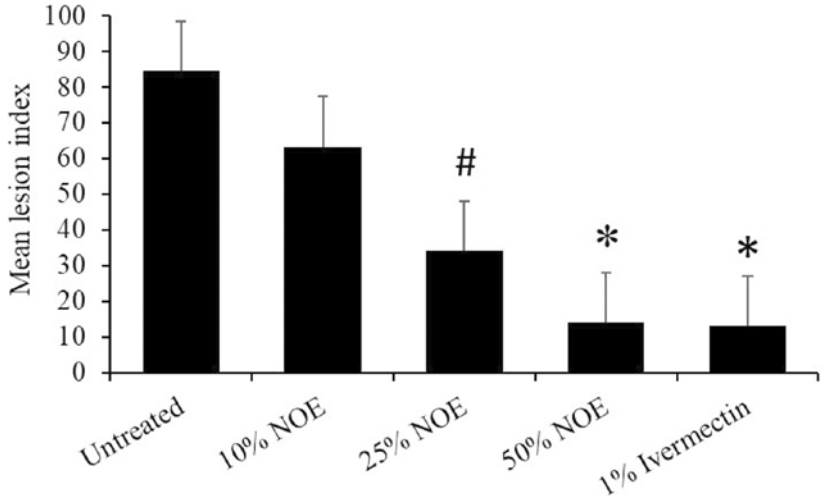

Fig. 1. Mean lesion index in rabbits given topical applications of methanolic *Nerium oleander* extract (NOE) and 1% ivermectin acaricides.

#, * Significant differences compared to untreated control ($P < 0.05$)

During the first and second weeks of the trial, the mean number of mange mites counted was identical ($P < 0.05$) for all treatments (Plate V, Fig. 2). From three to six weeks of the experiment, however, mange mite counts were much lower in the ivermectin and 50% of NOE treatments. From three to six weeks, there was no difference in the mange mite counts between these two treatments. The number of mites seen from the third week onward was the same for the 10%, 25%, and 50% NOE treatments. They did, however, have more mites than the other treatments ($P<0.05$). The mean number of mites sampled per rabbit increased from around 13.3 to 19.7 in the untreated group.

Previous investigations that evaluated the *S. scabiei* var. *cuniculi* mite in infested rabbits led to the selection of ivermectin and a variety of NOE dosages (10%, 25%, and 50 %). Figure 3 depicts the mortality of *S. scabiei* var. *cuniculi* mites exposed to 10%, 25%, and 50% of ivermectin from oleander leaf methanol extracts and the control. The study found a significant influence of oleander extract concentrations on mortality ($P < 0.05$). The findings of this investigation revealed that all concentrations of oleander leaf extracts have an acaricidal effect on *S. scabiei* mortality percentage. The results showed that the mean mortality varied according to the treatment time and NOE concentrations utilized in the study. The results showed that the mortality ranged from 11% after one week at a 25 mg/kg concentration to 96% after four weeks at a 50% dose plus ivermectin (Plate V, Fig. 3).

After four weeks, the highest mean mortality was 10% at a 10 mg/kg concentration and 55% at a 25 mg/kg concentration (Table 1; Fig. 3). The mite mortality rate ranged from 11.1% at 10 mg/kg to 96 and 95% at a 50% concentration and ivermectin after 4 weeks (Table 2). No death was reported in the untreated group for the whole treatment period, or at a dose of 10 mg/kg in the first week.

Table 1. Mortality (mean ± standard error) of *Sarcoptes scabiei* var. *cuniculi* mites in naturally infested rabbits treated with *N. oleander* extract at various periods (1–4 weeks).

	Mortality (%)			
N. oleander concentration (mg/kg)	Week 1	Week 2	Week 3	Week 4
Untreated	0.0 ± 0.0 ^{c}	0.0 ± 0.0 ^{c}	0.0 ± 0.0 ^{c}	0.0 ± 0.0 ^{c}
10%	0.0 ± 0.0 ^{c}	5.0 ± 1.0 ^{abc}	7.3 ± 2.7 ^{b}	10.0 ± 4.7 ^{b}
25%	11.1 ± 2.0 ^{b}	23.0 ± 4.3 ^{ab}	38.3 ± 4.4 ^{ab}	55.0 ± 2.2 ^{bc}
50%	26.7 ± 11.0 ^{b}	59.3 ± 4.4 ^{ab}	76.7 ± 3.7 ^{ab}	95.0 ± 2.5 ^{a}
Ivermectin 1%	40.0 ± 4.7 ^{ab}	61.3 ± 11.5 ^{ab}	83.3 ± 9.9 ^{ab}	96.0 ± 2.6 ^{a}

Means with different superscripts in columns or rows show significant differences ($P < 0.05$). Those with the same superscripts are not significantly different ($P > 0.05$).

The results presented in Fig. 4 show the relationship between the effect of the extract at different concentrations and its comparison with ivermectin during the treatment for four weeks. The concentration of 50 mg/kg was proven to have an effectiveness equivalent to the reference treatment. For the relationship between the effect of time and concentrations, it was found that the effectiveness of the extract increased with the increase in the treatment period, which in the fourth week reached a mite mortality rate of 95% for the concentrations of 50 ml/kg NOE and ivermectin, respectively (Plate VI, Fig. 5).

The lesions were marked by minute red papules and widespread erythema, initially appearing around the mouth (lips, nostrils, and feet) before disseminating to other facial regions and then to the head and ears. Post-treatment, all these symptoms exhibited improvement (Plate VI, Fig. 6).

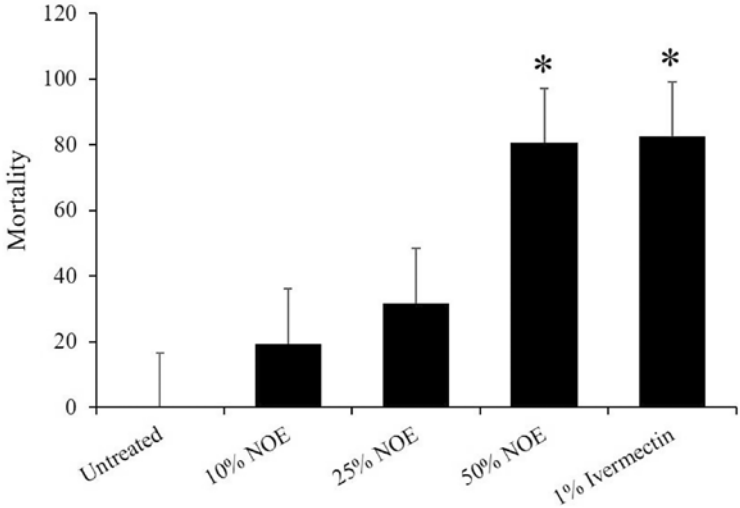

Fig. 4. Effect of different concentrations of *Nerium oleander* extract (NOE) and ivermectin on the mortality rate of *Sarcoptes scabiei* var. *cuniculi* treated for different time periods (1–4 weeks).

Histopathological changes

In the control group, skin appeared separated and had normal structure. The infection of rabbits with *S. scabiei* var. *cuniculi* resulted in a clear histopathological change, revealing hyperkeratosis, acanthosis, and complete destruction of the epidermis. The extracts successfully replicated the pathological changes caused by the parasite (Plate VII, Fig. 7). The skin histology score was calculated as approximately fivefold when compared to the control non-infected group, and after treating rabbits with *N. oleander* leaf extract, the score decreased to about twofold (Fig. 8).

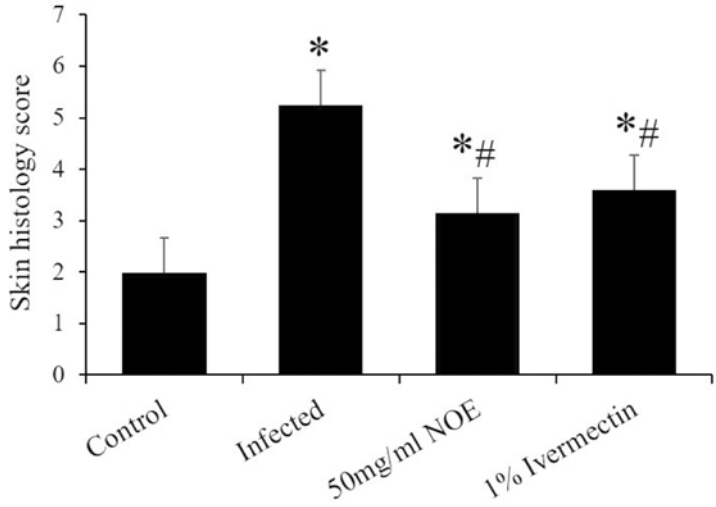

Fig. 8. Effect of *Nerium oleander* extract (NOE) on skin histology score of rabbits infected with *Sarcoptes scabiei* var. *cuniculi*. *, # Significant differences ($P < 0.05$) compared to control and infected groups, respectively.

During infection with *S. scabiei* var. *cuniculi*, the skin increases in volume. This explains the decrease in skin capsule thickness (Plate VIII, Fig. 9) resulting from the stretching of the skin's cellular parenchyma. Moreover, the infection-induced splenomegaly in rabbits increased the immune response and increased the number of cells. Our results demonstrated that NOE could significantly reduce the dermatomegaly caused by the parasite. The NOE contains a significant natural component that may aid in improving impaired skin histological changes.

During dermatomegaly, the skin capsule decreases in thickness when compared to the non-infected skin. The decrease in thickness was about twofold compared to the control group. Both NOE and ivermectin were able to ameliorate the induced decrease in skin capsule thickness (Fig. 10).

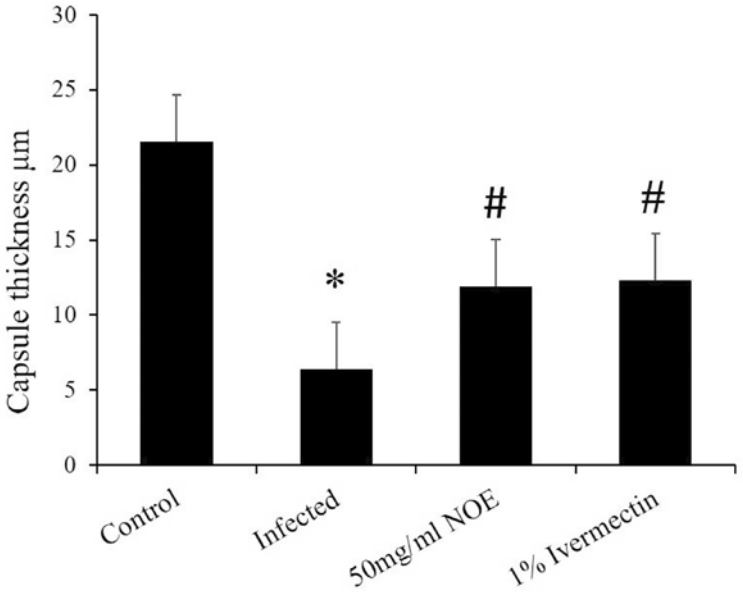

Fig. 10. Effect of *Nerium oleander* leaf extract (NOE) on thickness of skin capsule of rabbit infected with *Sarcoptes scabiei* var. *cuniculi*. *, # Significant differences ($P < 0.05$) compared to control and infected groups, respectively.

Discussion

Sarcoptes scabiei var. *cuniculi* is a very common dermatological condition and represents a major public health burden globally (Hay et al. 2014). The insufficiency of current therapeutic options and the emergence of mite drug resistance have led to significant effort in developing a new, safe, and effective drug for scabies. Plants have been used for medicinal purposes since ancient times. In the current study, we assessed the acaricidal activity of NOE against *S. scabiei* var. *cuniculi* in rabbits. When NOE ointment was applied *in vivo* to naturally infected rabbits by dipping the infected parts in the ointment three times a week for four consecutive weeks, it effectively killed the mites. Meanwhile ivermectin was administered at a dose of 100 µg/kg body weight, three times within a week interval. Improvement was observed in the skin of the rabbits treated with NOE ointment and ivermectin compared to the positive control. The reason could be that the NOE ointment contains chemical compounds that effectively kill mites in the

skin of infected rabbits, thereby removing the pathogen. This results in the skin returning to its natural state and the rabbits returning to their healthy states. Morsi et al. (2022) conducted a study where NOE demonstrated significant activity against adult *S. mansoni* worms, leading to their high mortality. Additionally, NOE is crucial in eliminating various insects. The water-based leaf NOE can kill both adult and young *Culex tritaeniorhynchus* and *Culex gelidus* (Kumar et al. 2012). The phytochemical screenings done by Barbosa et al. (2008) and Chaudhary et al. (2015) have shown that NOE contains different amounts of terpenoids, alkaloids, flavonoids, saponins, tannins, and carbohydrates. Researchers have found these compounds effective in killing ticks and many parasites. Also, the results revealed that rabbits treated with NOE ointment and ivermectin showed an increase in feed intake. These findings support many studies concluding that the treatment of sarcoptic mange in rabbits led to improved feed conversion and daily gain (Ademola et al. 2014). This may be due to the absence of the intense pruritus associated with sarcoptic mange, which interferes with production, weight gain, and wool and fibre quality (Aboelhadid et al. 2016). Mange is a disease that induces high levels of irritation and restlessness, leading to a reduction in feed intake or even the cessation of feeding (Uni et al. 2001). Scaglioni et al. (2018) reported mange to be mostly associated with decreased feed consumption. Patel et al. (2003) discussed how diseased, untreated animals exhibited poor reproductive performance due to a decline in their nutritional status and health.

Researchers attribute the pathogenic effects of these mites to their burrowing activity, the mechanical damage they cause during excavation, the irritant action of their secretions and excretions, allergic reactions to some of their extracellular products, and especially the release of cytokines (Liu et al. 2010). The histopathological images of skin treated with 50% of *N. oleander* ointment revealed improvements in the dermis and epidermis, accompanied by a gradual modulation, a decrease in inflammatory cells, and the absence of mites. The death of the mites and the cessation of irritation, inflammation, and pruritus may contribute to this improvement, which in turn stops skin destruction, hyperkeratosis, and scale formation. Meanwhile, slowly curable changes in the skin layers were shown in the skin treated with ivermectin during the course of the treatment; few inflammatory cells were observed at the end of the study. The skin healed faster and better after treatment with 20% of *N. oleander* ointment. This may be because the *N. oleander* plant has glycosides and alkaloids that are very harmful to mites and can kill them (Barbosa et al. 2008). The killing of mites results in a reduction in their number, allowing the skin to return to its natural composition. Additionally, the glycerin in the ointment aids in moisturizing the skin and restoring its natural texture.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgement

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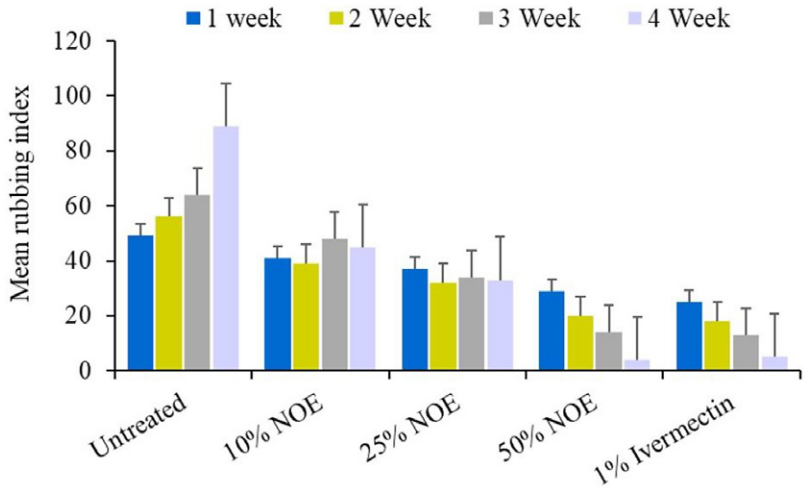

Fig. 2. Mean rubbing index in rabbits given treatments of methanolic *Nerium oleander* extract (NOE) and 1% ivermectin-based acaricides for 1–4 weeks

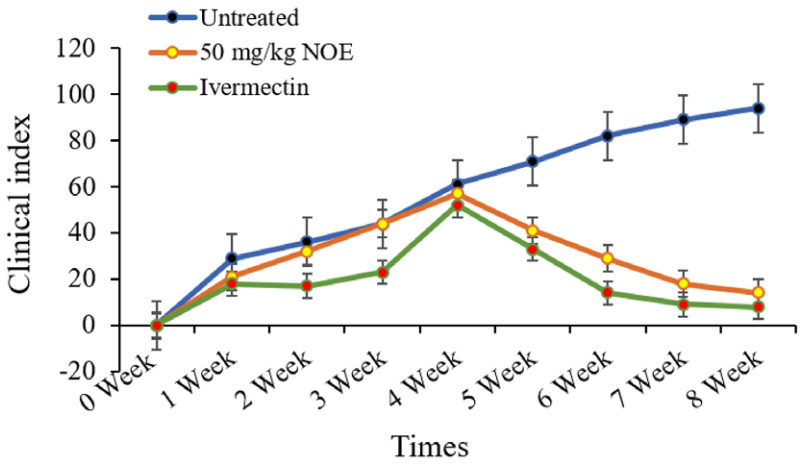

Fig. 3. A rabbit's clinical index (mean ± SD) at the start of the infestation (Week 0), four weeks into the infestation (Week 4 = D0), and over the whole post-treatment period is displayed for each rabbit treated with ivermectin and *Nerium oleander* extract (NOE). The areas of skin affected by the lesion (0, healthy skin; 1, lesions surface < 10% of body surface; 2, lesions surface 10–20% of body surface; 3, lesions surface 20–30% of body surface; 4, lesions surface > 30% of body surface) are used to calculate the clinical index.

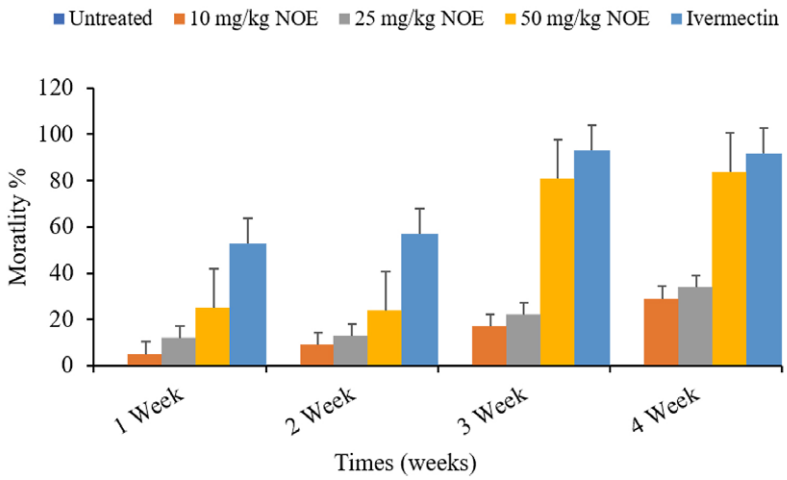

Fig. 5. The effect of the treatment period on the mortality of *Sarcoptes scabiei* var *cuniculi* mite treated with *Nerium oleander* extract (NOE) and ivermectin at various time periods (1–4 weeks).

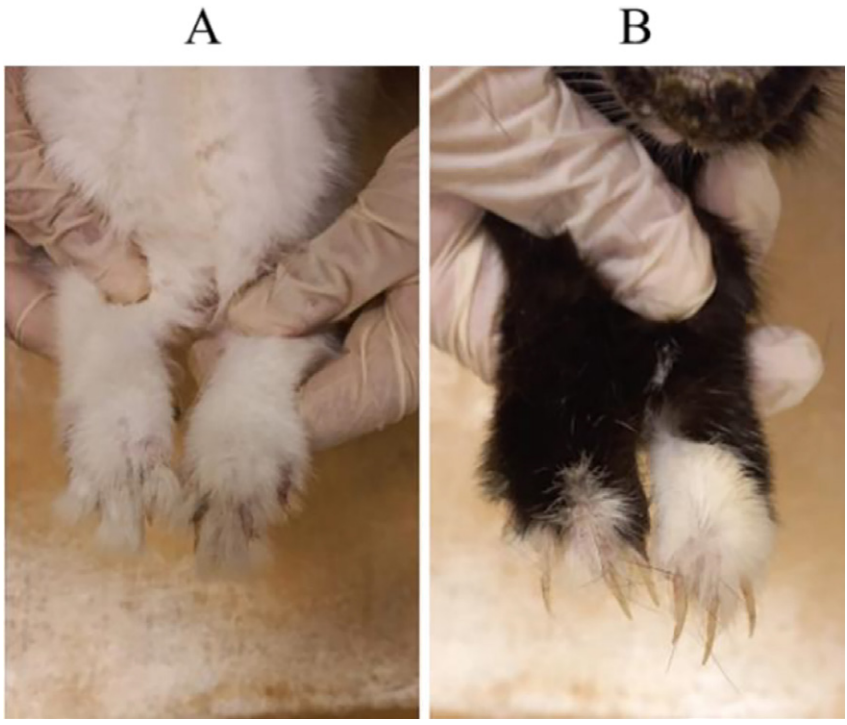

Fig. 6. Illustrative image before treatment (A) and after treatment (B).

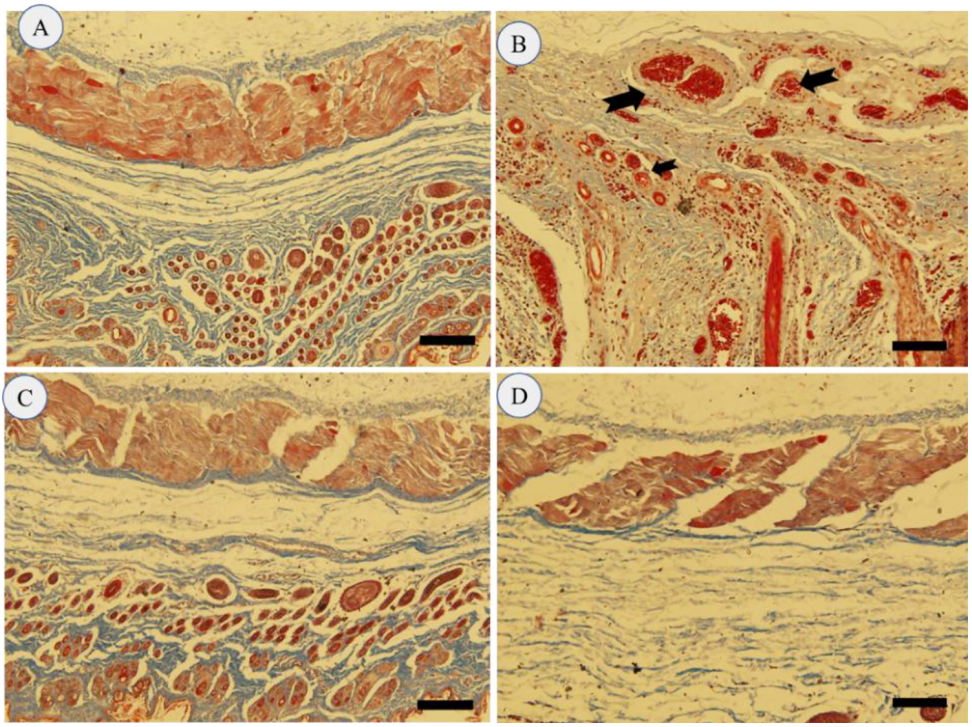

Fig. 7. Effect of *Nerium oleander* extract (NOE) on skin histopathology of rabbits infected with *Sarcoptes scabiei* var. *cuniculi*. (A) Control non-infected rabbits appearing with normal structure. (B) Infected rabbits with displayed destruction of the epidermis and congestion of blood vessels (arrow) and enlarged dermatomegaly. (C) Group of infected rabbits treated with NOE with improved structure nearly normal dermis and epidermis. (D) Group of infected rabbits treated with ivermectin and apparently with improved structure and a curable dermis and epidermis with minimal infiltrations of inflammatory cells. Sections stained with Mason's trichrome. Scale = 10 µm.

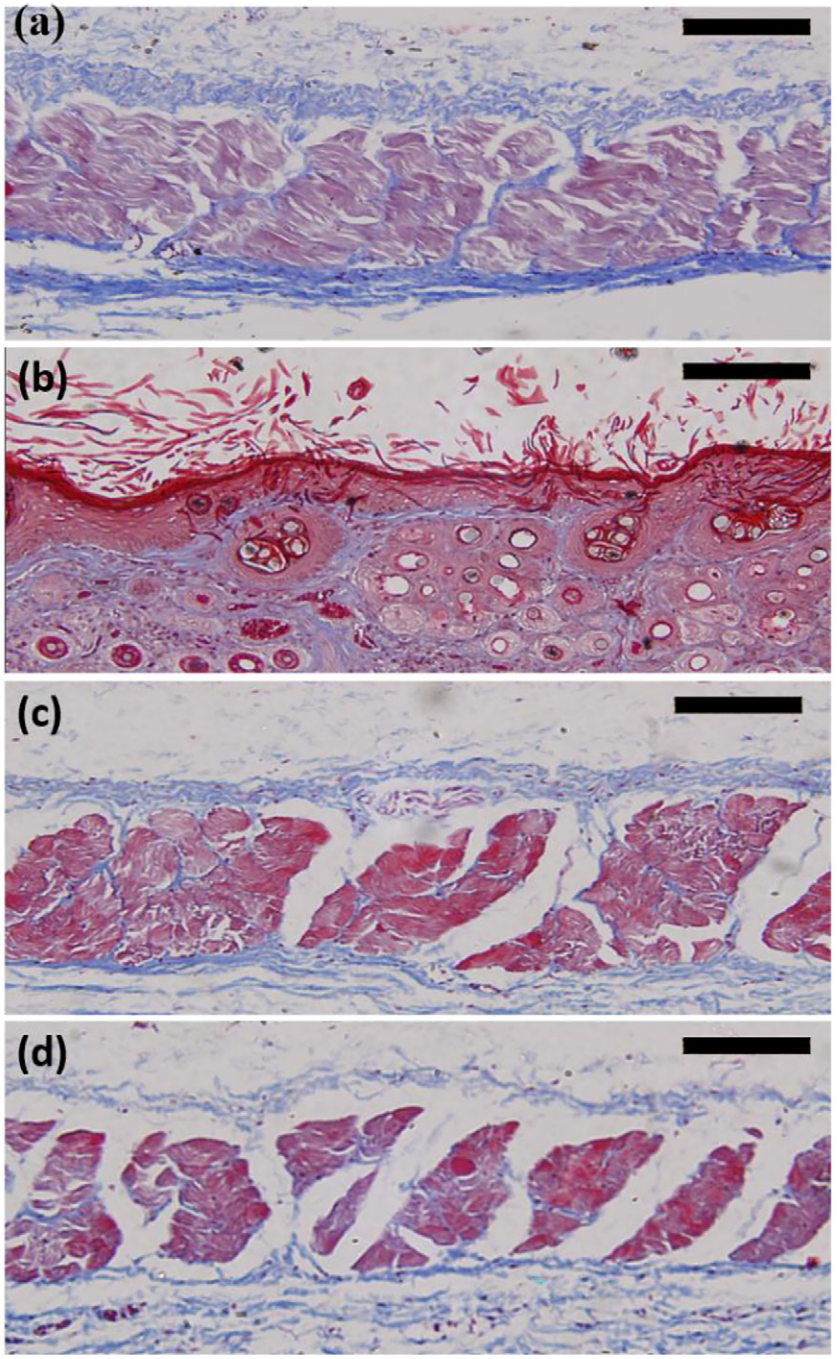

Fig. 9. Sections in skin showing capsule thickness. (a) Control non-infected rabbit. (b) *Sarcoptes scabiei* var. *cuniculi* infected rabbit. (c) Infected rabbit treated with *Nerium oleander* extract. (d) Infected rabbit treated with 1% ivermectin. Sections stain Mason's trichrome. Scale = (10 µm).