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Original Article

Plant extracts: An Eco-friendly Approach to Parasite Management in Rabbit production

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Abstract:

Rabbits are one of the most important animals in the world, mite infestations are commonly spread among rabbits through direct skin contact or contact with the environment, causing severe economic losses. the study aimed to evaluate the effects of two plant extracts (neem and pomegranate peels) against *Sarcoptes scabiei* infection in rabbits. a total of 20 rabbits of locally two different breeds were grouped into 5 groups, where: the 1st group served as a control healthy. The 2nd group was neem treated, the 3rd group was the pomegranate peel treated, and the 4th group was the Ivermectin treated, the last group was the infested non treated. Growth performance, immunological parameters, antioxidant assay and tissue histopathology of the rabbit groups were evaluated to determine the efficacy of both plant extracts on *S. scabiei* infection. The parasitological examination of the three treated groups revealed the absence of *S. scabiei* in all rabbits under treatment, and there is significant improvement in immunological state and histopathological picture when compared with the infested non treated group. Conclusively, pomegranate peel and neam extracts proved their competence as a natural product against *Sarcoptes scabiei* infestation

Key words: *Sarcoptes scabiei*, rabbits, neam, pomegranate peel

Introduction

Rabbits are one of the most important animals in the ecological balance, rabbit is a good source of high protein meat with low fat and, in addition to the meat flavor and ease of digestion; it is recommended for persons with cardiovascular diseases because of its nutritional value and dietetic properties. On the other hand, rabbits are characterized by their high reproductive performance, short gestation, and growth rate is fast, these criteria allow rabbits to cover the shortage of protein in developing countries (Para *et al*., 2015). This animal is infected with many diseases, especially external and internal parasites, which affect productivity. For instance, mite infestations are commonly spread among rabbits through direct skin contact or contact with the environment (Thakre *et al*., 2017).

Sarcoptic mange is a highly contagious skin disease that affects the face, ears, and legs in rabbits (Kumar *et al*., 2018). Poor hygienic condition and overcrowding are important factors for *Sarcoptes scabiei* infestation in rabbits, this disease can cause severe pruritis, crust formation. Severe morbidity and economic losses occur without treatment of the infested cases (Rao et al., 2020).

External application of organophosphates, and injection of Ivermectin are effective methods against mange of rabbits (Abdallah *et al*., 2023). Meanwhile, the side effects, drug residual and resistance development from their extensive uses lead to the search for a new effective with low side effects acaricide. The neem plant (*Azadirachta indica*) is one of the most important medicinal plants that increased in Asia and Africa, it is used in the medication of many parasitic diseases that affect animals. Neem plant contains many proteins and trace minerals *(Seddiek et al.,* 2013), and improves the immune system of animals when exposed to infection with animal parasites. Neem extracts are used to control intestinal worms and dermatitis in animals (Chhabra and Pathak, 2011). Pomegranate (*Punica granatum*) is an important commercial fruit crop that is extensively cultivated in the Middle East. Pomegranate is a biologically unique and potent source of many of the body's physiological factors having significant effects on human and animal health. It has been used for thousands of years to cure a wide range of diseases across different cultures and civilizations (Akram *et al.*, 2020). This study aimed to evaluate the effects of the two plant extracts (neem and pomegranate peels) against *Sarcoptes scabiei* infection in rabbits.

Material and Methods:

Animals and management

25 rabbits of two different breeds were used in this experiment from a farm located in the College of Agriculture at Suez Canal University in the Ismailia governorate, Egypt. The farm was naturally infested with *Sarcoptes scabiei*. The ages of animals ranged from 16 to 25 weeks with an average body weight of 2.08 ± 0.15 kg. Animals were divided into five groups. The first group was healthy and free of any external parasites or skin diseases. The four other groups suffered severely from acute parasitic mange. Animals were individually housed in galvanized wired cages (50 x 50 x 40 cm), where feed and water were provided *ad libitum*. Animals were fed on a basal pelleted ration containing yellow corn, soybean meal, corn gluten, minerals and vitamins premix, and molasses. The calculated chemical components of the diet were 17% crude protein, 2.8% fat, 12.8% crude fiber, and 2600 KCal digestible energy/kg diet (NRC, 1977). A lighting system was 14 hours' light/10 hours' dark in the rabbitry during the experimental period. All animals were kept continuously under the same managerial and environmental conditions during the experimental period.

Ambient temperature and relative humidity inside the rabbitry were recorded daily during the experimental period by using a digital

thermometer and hygrometer. The temperature-humidity index (THI) was estimated according to the thermal comfort level of an animal environment according to (Marai *et al.*, 2002). The calculated mean value of THI was 26.70 during the entire experimental period. The obtained value was less than 27.8 and therefore classified as absence of heat stress.

Experimental design

Animals were divided randomly into 5 equal experimental groups (5 each): the 1st group served as a control healthy. The 2nd group was neem treated, the 3rd group was the pomegranate peel treated group, and the 4th group was the Ivermectin treated (Iver. Group) treated with Ivermectin (Ivomec® Super, Merial, USA). Each animal in the Iver. group was subcutaneously (S.C.) injected with 0.5 ml Ivermectin (two times, 10 days apart), the last group is the infested non treated.

Rabbit clinical examination and productive performance

Rabbits were clinically examined, and their live body weights were recorded at zero-day, 2nd and 3rd weeks of treatments using a digital balance, and samples (skin scraping and blood) were taken from skin lesions. Rectal body temperatures were recorded at the end of experimental period.

Parasitological examination and skin scraping:

Rabbits were selected for parasitological investigation after clinical examination, samples were taken from skin lesions using a dull scalpel blade dipped in physiological solution, and skin scrapings were collected from the skin of both distal forelimbs. Dermis, epidermis, scales, and hair were all included in the samples that were taken (Sajid *et al*., 2017).

Each sample was dissolved in 5 ml of potassium hydroxide (KOH) 10% solution. After centrifuging and stirring the mixture, the supernatant was discarded. A few drops of each sample's solution were placed on a slide for a light microscope inspection at x 40 magnification. According to (Ilić *et al.*, 2018), an infestation is rated as Low (++), Medium (+++), and High (++++) when it occurs in populations of 2-4, 5-7, and 8–10, respectively.

All mite samples were removed from the examination of rabbits, and they were mounted in Hoyer's medium on microscope slides before being viewed under a phase contrast (Olympus, BHA) microscope. With the aid of the Adobe Illustrator software (2020), key structures were depicted. Using a phase contrast microscope's eyepiece and a graded ocular micrometre, measurements of the size of mite structures are provided in micrometres.

Histopathological changes

Specimens of the skin of sacrificed rabbits from all groups were fixed in 10% neutral formalin for histopathological examination. They were dehydrated in graded alcohol concentration, cleared with xylene, and embedded in paraffin, the sections were cut at 4–5 µm thickness using a rotatory microtome and finally stained by haematoxylin and eosin according to the method described by (Deo and Deshmukh, 2018).

Clinicopathological changes

Blood sampling: At zero day, 2nd and 3rd weeks of treatments, blood samples were collected in a plain tube and then centrifuged at 3000 rpm for 20 minutes to obtain clear serum to be used for estimation of biochemical parameters**.**

Serum biochemical parameters:

Serum samples were analyzed for Malonaldialdehyde (MDA), Catalase Activity Assay (CAT), and Total Glutathione (GSH) using a commercial kit provided by Cell Biolabs, Inc. (USA) in accordance with Armstrong *et al.,* (1998), Sandhu and Kaur, (2002), and Maté *et al.,* (1999), respectively.

The Rabbit C-reactive protein (CRP), Rabbit Interleukin 1β (IL 1β) and rabbit Tumor Necrosis Factor α (TNFα) were estimated by

CUSABIO (USA) according to the manufacturer's directives.

Statistical analyses

Data of live body weight, changes in body weight, rectal temperature and temperature humidity Index (THI) were statistically analyzed using One-Way ANOVA of SPSS(IBM Corp, 2017). Duncan's Multiple Range was used to clarify the differences between means.

MDA, CAT, GSH, CRP, IL 1β and TNFα MLA were statistically analyzed using style: Developer. COSTAT Statistical Software.

Plant extract

Creation of crude (aqueous neem and ethanolic pomegranate peel) extract:

According to the approach outlined below by Mamoon-ur-Rashid **et al.,** (2011):the dried *Azadirachta indica* tree leaves and pomegranate peel were prepared, and then each was ground and homogenized in an electric blender with distilled water for *Azadirachta indica* leaves and 100% ethyl alcohol for the pomegranate peel. Filtration of the homogenate was done using triple-folded gauze that had been sterilized. Before usage, evaporation of the solvent was carried out using a rotary vacuum evaporator, serial dilation of the extracts 5, 10, 15, 20, 30, and 40% was prepared and tested in vitro while 40% of both extracts was applied topically to the scabies areas of the infected rabbits.

Evaluation of toxicity in vitro:

The toxicity tests (LC50 and LT50) against *Sarcoptes scabiei* larvae and nymphs were conducted using extracts that exhibited the strongest acaricidal activity. 72 hours were spent in complete incubation (Du et al., 2008). Even when stimulated with a needle, the larval mites remained immobile, and (Nardoni and Mancianti, 2022) judged these behaviours to be signs of mortality.

Results

1. **Clinical observations and efficacy of the treatment trials**

The infected group showed clinical signs of pruritis, crust formation, as well as an increase in skin sickness in addition to wrinkling in the affected region and ulcers, while, the treated groups with (neam, pomegranate peel, and Ivermectin) displayed fewer clinical signs (Fig. 1, 2, 3).

2. **Growth performance parameters**

revealed that all infected groups showed slow growth rates during the whole experimental period. The average body weight change for the three infected groups was 146.67 ± 0.04 g, while the average change in live weight during the experimental period for the control group was 338.20 ± 0.07 g. There is clear decrease in growth rates of infected groups and it also appears that the growth performance of groups treated with neem or pomegranate peel preparations did not differ significantly from those treated with the traditional Ivermectin treatment. At the end of the experimental period, the rectal body temperature differences between groups (37.7±0.07, 37.9±0.17, 38.2±0.9 and 37.7±0.13℃ for Control, Ivermectin, neem and pomegranate peel groups, respectively were not statistically significant and all obtained values were within the normal range of rabbits.

3. **Parasitological examination**

From the parasitological examination of the rabbits were infested with *Sarcoptes scabiei* (Fig. 5). The species taxonomically significant structures were illustrated with the help of adobe illustrator software (2020) (Fig. 6) and the identification was done according to the morphological identification key of (Soulsby, 1968).

Examination of a skin scraping on the 14th and 28th day of treatment revealed the absence of *S. scabiei* in all rabbits under treatments (Table1).

4. Histopathological results

The histopathological examination as shown in (Fig.7&8), revealed a nearly normal histological picture of the skin of the drug group, while, in pomegranate peel group there

was mildly thickened epidermis, with orthokeratosis but no evidence of mite bodies, neam group showing inflammation with the remnant of dead mite bodies, the inflammation degree is less when compared with the infested non treated group which showing sever inflammation with mite bodies appearance.

5. Clinico-pathological finding

The level of serum immunological parameters and serum antioxidants showed a significant increase in the infested group when compared with the non-infested group. While, in treated groups; the first week after treatments the parameters and the antioxidant levels showed significant decreases when compared with the infested group, especially in neam group and Ivermectin group. After two weeks the measured parameters showed significant decreases, as in drug group the measurement became nearer to the normal measurements (Table 2).

Discussion

Rabbits have been regarded as key livestock, which are increasingly being raised in many countries worldwide (Arul Prakash *et al.*, 2017), animals have been intentionally released as a source of meat and fur production. However, diseases are the major challenges facing productivity as well as the sustainability of rabbit farming.

Many strategies have been applied to control mange in rabbits, in this study; three treated groups were designed for mange treatment, the first group was treated with ivermectin. Ivermectin's efficacy in treating mange in rabbits is well-established globally (Desoky and El-Sheikh, 2014). However many factors can impact the treatment approach for *S. scabiei* infection, including the development of drug resistance due to the repeated use of the same drug with the same mode of action on the same infected animals (Khan *et al.*, 2022). Additionally, the use of chemical acaricides can result in toxicity and environmental contamination. Thus searching for new types of acaricides should be taken into consideration in future research work (El-Ghany and Wafaa, 2022), so neam and pomegranate peel were used in the second and third treatment groups and comparing its effect with the drug group.

Sarcoptic mites belong to the family sarcoptidae and are highly transmittable and burrowing parasites. *Sarcoptes scabiei* is an important ectoparasite in rabbits because of the prospect of zoonotic infection and extensive losses in weight, productivity, wool, and fiber quality (Ilić *et al.*, 2018). In Egypt, mange in rabbits is second to coccidiosis impotence, with high losses reported. *Sarcoptes scabiei* var. cuniculi causes mange infestation in rabbits, affecting their ears, nose, feet, and areas around the genitalia, resulting in hypertensive reaction, body weight loss, and death (Ilić *et al.*, 2018).

Sarcoptes scabiei can cause direct damage to the skin and subcutaneous tissues, causing inflammation. The infected animals showed pruritis, crust formation, an increase in skin sickness in addition to wrinkling in the affected region and ulcers, these symptoms were also recorded with (Abdallah *et al.*, 2023). Additionally, The clear decrease in the growth rate of the infested groups may be attributed to the stress caused by parasite infection, (Elshahawy *et al*., 2016) mentioned that the behavior of ectoparasites can indirectly harm rabbits by causing disturbances, increasing behaviors like rubbing, and reducing time spent on feeding or rumination leading the decrease in the growth rate, these results confirmed by the clinico-pathology findings and the histopathological pictures, the infected rabbits showed an increase in the level of serum immunological parameters and antioxidants when compared with the non-infested group and the treated groups during the treatment. On the other hand, the treated groups showed significant improvement in histopathological picture when compared with the infested non treated

group which showed severe inflammation with the presence of mite bodies, the pathology of the *Sarcoptes scabiei* is the original cause of the inflammation and increasing level of serum antioxidants (Panigrahi *et al.*, 2016), meanwhile, there is no significant difference between the growth performance of groups treated with neam or pomegranate peel preparations and the traditional Ivermectin treatment, may be attributed to the nearest effect of the three treatments against the parasites and these results agreed with mortality rates of the mites which is nearly equal in the three treated groups comparing with the control group.

Conclusion

Pomegranate peel and neam extracts proved their competence as a natural product against *Sarcoptes scabiei* infestation in comparison with the synthetic drug. Therefore, further studies are recommended with different concentrations of both extracts to detect the ideal concentration and dose against the *sarcoptes* mite.

Ethics

This study was approved by the Ethics Committee of Faculty of Agriculture, Suez Canal University, Egypt (Approval No. 198827).

Conflict of interest

The authors declare that they have no conflict of interest.

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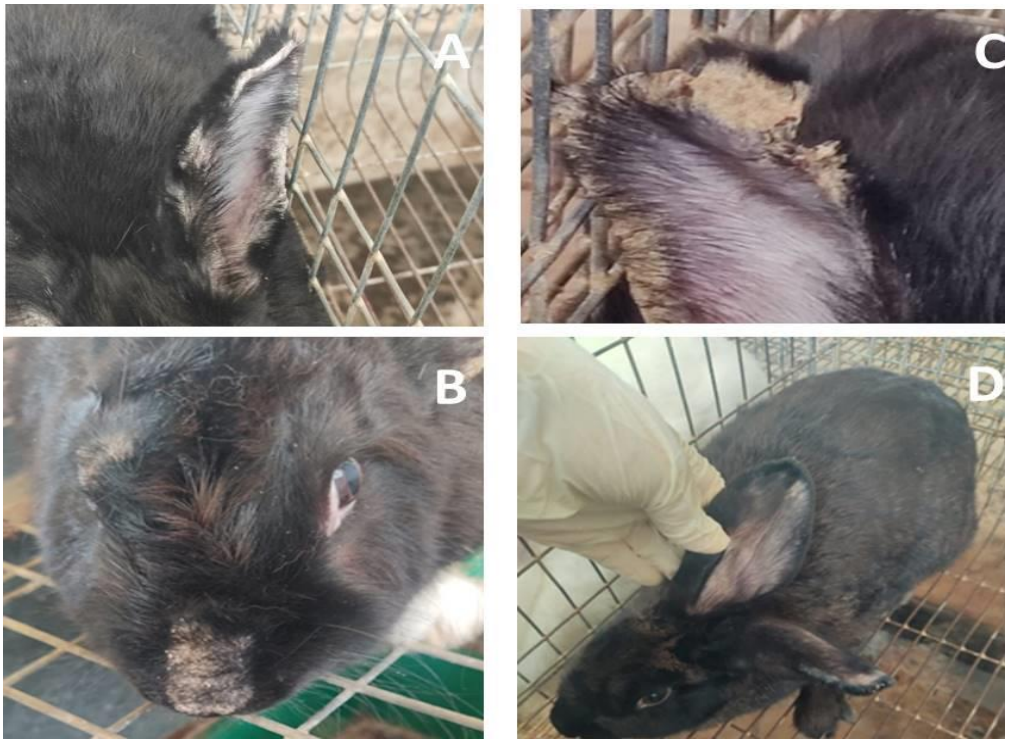

Fig.1.The skin of treated rabbits (neam group) (A,B,C before treatment)& (D after treatment)

Fig. 2. The skin of treated rabbits (pomegranate peel Group) (A,B,C before treatment) & (D,E,F after treatment)

Fig.3. The skin of treated rabbits (Ivermectin Group) (A, B before treatment) & (C after treatment)

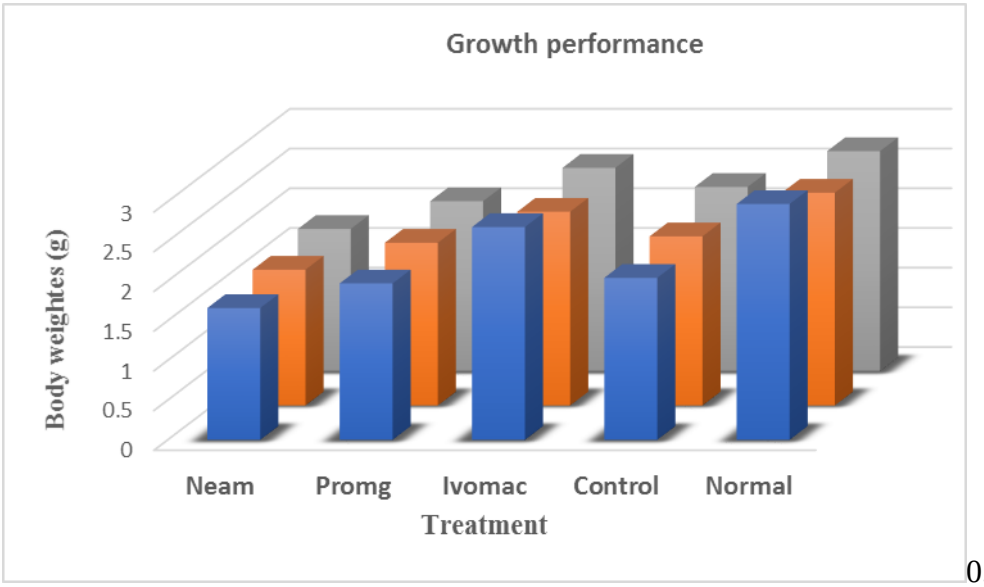

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Fig. 4. Body weight changes of rabbits treated against mange parasite *(Sarcoptes scabiei)* compared to control & Normal group

Fig.5. Light microscopy (LM) of permanent slide *Sarcoptes scabiei* adult (ventral view).

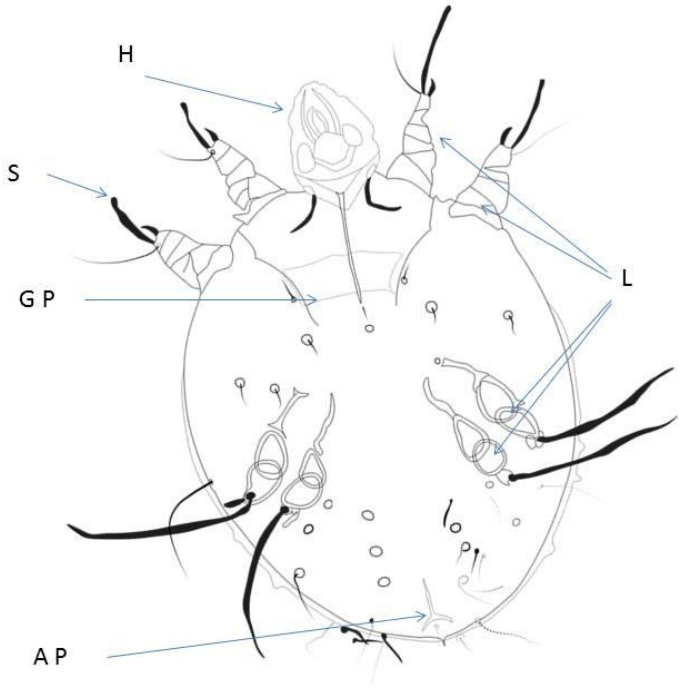

Fig. 6. Whole-body illustration of *Sarcoptes scabiei*
AP: anal aperture, GP: genial aperture, S: sucker, L: legs, H: head

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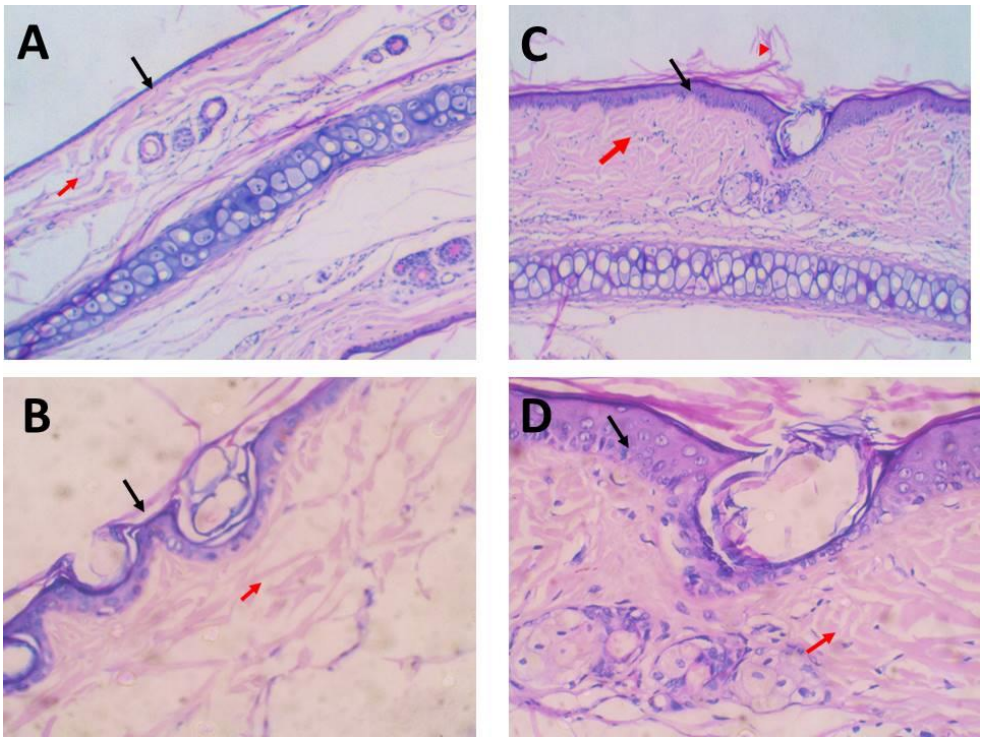

Fig. 7. **(A.10x &B.40x)** Ivermectin treated group skin, showing uniform epidermis (Black arrow), with underlying dermis (Red arrow), and chondroid tissue (Black arrowhead), **(C.10x &D.40x)** Skin of PPME treated group shows uniform mildly thickened epidermis (Black arrow), with orthokeratosis (Red arrowheads), with no evidence of mite bodies. The underlying dermis shows no significant inflammation (Red arrow), and chondroid tissue (Black arrowhead) is seen.

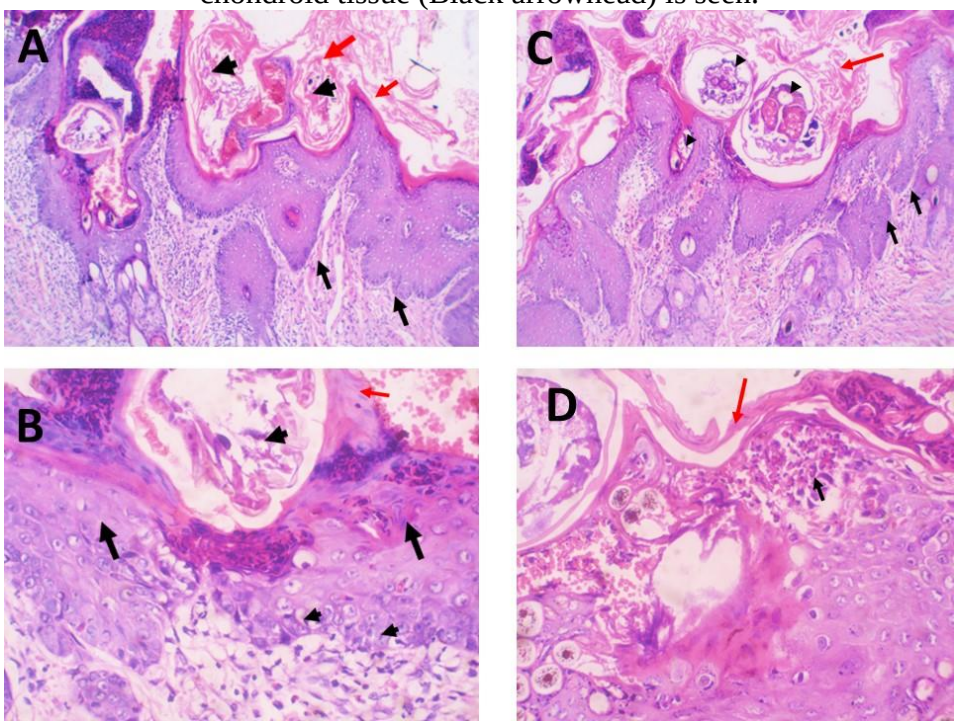

Fig. 8. **(A)** Skin of **neam** treated group, tissue shows remnants idiosoma (parts body of mites) (Black arrowheads) within keratin layer. Epidermis shows acanthosis with epidermal hyperplasia and elongation of rete ridges (Black arrows) and hyperkeratosis (Red arrows). There is chronic inflammation within dermis (Red arrowheads) (H&E, 10x)**, (B)** Higher magnification of the previous figure shows reduction in number of eosinophils within epidermis (Blak arrows) and (H&E, 40x)
(C.10x &D.40x) Skin tissue of infested non treated group shows idiosoma (body of mites) (Black arrowheads) within keratin layer. Epidermis shows acanthosis with epidermal hyperplasia and elongation of rete ridges (Black arrows) and hyperkeratosis (Red arrows).

Table (1): *Sarcoptus scabiei* in vitro mortality % after treatment with varied quantities of crude aqueous neem extract, Pomegranate peel extract, and the prescribed dose of ivermectin

Time post treatment h									
	24h			48h			72h		
	AV±SE	D /T	Mo%	AV±SE	D/T	Mo%	AV±SE	D/T	Mo%
Negative Control	1.67± 0.33	1.7/80	2.08%	5.33±0.88	5.3/80	6.70%	7.00±1.73	7.1/80	8.80%
Ivo	71.00±0.58	71/80	88.75%	79.70±0.33	79.7/80	99.60%	80.00±0.00	80/80	100%
Neem	69.33±1.76	69/80	68.66%	78.33±0.88	78/80	97.90%	80.00±0.00	80/80	100%
Promg	73.30±1.76	73/80	91.66%	80.00±0.00	80/80	100%	80.00±0.00	80/80	100%
LSD	4.210			2.105			2.824		

Values within a column followed by different lowercase letters were significantly different (P≤0.05), while values within a column followed by the lowercase letters were not significantly different (P≤0.05)
LSD*=least significant difference at P≤0.05
D/T=(number of dead mites/total number of mites)
MO%=Mortality%
AV ±SE = Average number of dead mites ±Stander Error
IV0 = ivermectin, Neem= aqueous leaf extract of neem, Promg=Pomegranate peel extract

Table (2): The effect of mange and their treatments on some serum immunological parameters and serum antioxidants:

	CRP	IL-ß1	TNF-α	CAT	MDA
infestation	41.09 ^{a}±0.97	31.72 ^{a}±0.45	352.16 a±2.15	4.21 h±0.03	5.46a ±0.03
control	8.21^{g} ±0.03	15.11 ^{e}±0.67	123.93 h±1.30	6.72 a±0.03	2.68g ±0.02
Neem 1	31.43^{c} ±1.01	23.53 ^{c} ±0.39	274.83 c±3.07	4.89 f±0.04	4.89 c±0.02
Pomegranate 1	34.44 ^{b}±0.62	26.07 ^{b}±0.77	304.9 b±3.82	4.57 g±0.03	5.14b±0.04
Ivomac 1	26.59^{d} ±0.55	20.14^{d} ±0.62	230c ±0.73	5.21d±05	4.17 e±0.04
Neem 2	22.28^{e} ±0.46	19.52 ^{d} ±0.54	215.7 b±2.58	5.43c±0.02	3.96 d±0.05
Pomegranate 2	26.88 ^{d}±0.62	23.79 ^{c}±0.55	247.3d±2.51	5.02e±0.12	4.26d±0.04
Ivomac 2	13.83 ^{f} ±0.72	16.63 ^{e}±0.40	151.63g±1.90	6.16b±0.04	3.18f±0.06
LSD 0.05	2.059	1.685	7.313	0.115	0.114

Values in the same column followed by different superscripts are differ significantly (P≤0.05).
CRP: C-Reactive Protein; IL-1ß: Interleukin-1ß; TNF-α: Tumor necrosis alpha; CAT: Catalase , MDA:Malondialdehyde;
(1:Before treatment , 2:After treatment).

ملخص عربي

المستخلصات النباتية: نهج صديق للبيئة لإدارة الطفيليات في إنتاج الأرانب

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تهدف الدراسة إلى التخفيف من انبعاثات الغازات الدفيئة عبر استخدام أساليب صديقة للبيئة ، مثل استخدام مستخلصات نبات النيم وقشر الرمان في مكافحة الطفيليات الخارجية للأرانب ، ودراسة تأثير هذه المركبات الطبيعية على الإنتاجية ، وتركز الدراسة على التحديات التي تواجه تربية الأرانب ، وتحديداً تأثير الإصابة بالجرب على صحة الأرانب وإنتاجيتها ، حيث تم إنشاء ثلاث مجموعات علاجية ، إحداها باستخدام إيفيرميكتين كعلاج معترف به على نطاق واسع ، والمجموعتين الأخريين باستخدام مستخلصات نبات النيم وقشرالرمان كبدائل ، تم التركيز على الجرب كطفيل خارجي هام في الأرانب مما يؤدي إلى مشاكل صحية وخسائر اقتصادية ، شكلت الإصابة ضررًا مباشرًا على الجلد والأنسجة تحت الجلد ، مما أدي إلى التهاب، حكة، تكوين قشور، وأعراض أخرى. تشير الدراسة إلى تراجع في معدل النمو لدى المجموعات المصابة، يعزى إلى التوتر الناجم عن الإصابة بالطفيل ، تؤثر سلوك الطفيليات بشكل غير مباشر على الأرانب من خلال التسبب في اضطرابات، وتقليل وقت الأكل، والتأثير على معدلات النمو.

يكشف الفحص الهستوباثولوجي عن التهاب شديد في المجموعة الغير معالجة ، في حين تظهر المجموعات المعالجة تحسنًا كبيرًا ، يقل مستوى المناعة ومضادات الأكسدة في الأرانب المصابة مقارنة بالمجموعات غير المصابة أثناء العلاج ، ومع ذلك لا يوجد فرق كبير في أداء النمو بين المجموعات التي تم علاجها بمستخلصات النيم أو قشر الرمان وبين المجموعة التي تم علاجها بإيفيرميكتين ، مما يشير إلى فعاليتهما المؤكدة ضد الطفيليات.

وفي الختام تسلط الدراسة الضوء على ضرورة البحث عن البدائل نظرًا للمخاوف المتعلقة بمقاومة الأدوية المستخدمة مثل الإيفيرميكتين ، وكذلك التلوث البيئي ، وتظهر مستخلصات نبات النيم وقشر الرمان نتائج واعدة في إدارة إصابات الجرب في الأرانب، مما يقدم خيارات مستدامة لعلاج الجرب في المستقبل.