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### The effect of honey propolis mixture against sarcoptic mange in rabbits

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#### ABSTRACT

**S**arcoptic mange is a highly contagious rabbit disease causes great economic losses. The present work was conducted with 80 adult rabbits (New-Zealand) in a two farms (private and governmental farm), where the infestation prevalence was 72.5%, affecting mouth, nose, ears and other surface body skin. Most affected rabbits were suffered from off food and body weight losses. The *in vivo* study aimed to estimate the efficacy of topically uses of honey Propolis mixture (Propolis in Egyptian fennel honey 20% wt/ vol) in treatment of naturally infested rabbits with *Sarcoptes scabiei* comparing with the already classic traditional treatment (ivermectin and sulfur ointment). Twenty (6–8 month old) of naturally infested rabbits with *S. scabiei* were randomly divided into four groups: the 1<sup>st</sup> group was control untreated, the 2<sup>nd</sup> group was honey propolis mixture topically treated once weekly, the 3<sup>rd</sup> group was Ivermectin 1% treated with dose of 0.3 mg/kg (double dose with 15 days interval) and the 4<sup>th</sup> group treated with sulfur ointment 20% topically once daily. Treatment course was designed up to three weeks and the treatment success was evaluated by clinical assessment, parasitological evaluation and histopathological examination of infested rabbits.

The obtained results showed no any cure parameter in all rabbits of both the 1<sup>st</sup> & the 3<sup>rd</sup> groups by the terminal end study (day 21), but all rabbits of the 2<sup>nd</sup> group honey propolis mixture showed marked acaricide efficacy (100%) with statistically highly significant difference ( $P < 0.001$ ) comparing with ivermectin 1% and significant difference ( $P < 0.05$ ) with sulfur ointment group. By the 2<sup>nd</sup> week post-treatment, where all rabbits 2<sup>nd</sup> group were mite free with full clinical cure and showing 100% reduction of mites. Rabbits became healthy and body weight gain. Also, they showed marked improvement in the epidermis with absence of mites in skin samples histopathologically. Three rabbits of the 4<sup>th</sup> group (60%) became cured by the terminal end study (day 21).

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On conclusion, our results demonstrated that topical application of honey propolis mixture in two consecutive doses (once weekly) were valuable, highly economic, safe therapeutic approach for scabies therapy in rabbits than both ivermectin 1% and sulfur ointment 20%. Moreover, other than the superiority of honey propolis mixture studied treatments, rabbits could be slaughtered just post treatment, while other chemical drugs need two weeks or more for withdrawal post treatment.

## INTRODUCTION

Sarcoptic mange is a highly contagious, pruritic skin condition in rabbits and is caused by *Sarcoptes scabiei* mite. Lesions of *Sarcoptic* mange are commonly seen in ears, nose, feet and perineal area (Kachhawa et al. 2013).

Overcrowded living conditions and poor hygiene are considered significant factors for infection with *Sarcoptes scabiei* mites (McCarthy et al. 2004).

The traditional treatment of sarcoptic mange includes external application of Organophosphates, pyrethroid compounds or amitraz. Also the ivermectin drug group (ivermectin, doramectin and selamectin) can be used for treatment of naturally infested rabbits (Kaplaywar et al. 2017). While certain studies reported clinical and *in vitro* evidence of ivermectin resistance in rather than *in vivo* of patients with multiple recurrences of crusted scabies who had previously received multiple doses of ivermectin (Curie et al. 2004). They mentioned that ivermectin resistance in scabies mites can develop after intensive use.

Apitherapeutic use of honey was used in wound dressing topically as it is or with modified formulations as honey chitosan-based or alginate-based hydrogel. While propolis used as topical dressing or its extract was used in ointment formulation (Abdul-Hafeez 2019) owing to the biological activities of propolis involved in wound healing and tissue regeneration correlates with its antimicrobial, anti-inflammatory and immunomodulatory properties (Martinotti and Ranzato 2015).

Moreover, the antimicrobial synergistic action of both honey and propolis is recommended *in vitro* (Hamouda et al. 2018 & Kwieci et al. 2020) and *in vivo* (Ibrahim et

al. 2022) since their combination stimulates the monocytes / macrophages to release cytokines important for wound healing, and promote neutrophils rather than B-lymphocytes (antibody production) protecting the healing tissue from pathogenic infection (Filipič et al. 2016). Honey and propolis increases migration and proliferation of fibroblasts to the wound enhancing collagen types I and III expression and degradation in wounds matrix (Muire et al. 2022).

Recently, attention has been focused on the anti-parasitic activity of propolis (Silva et al. 2017). Propolis has been demonstrated to have anti-parasitic properties against various intracellular and extracellular pathogenic protozoa. Its anti-parasitic properties remain poorly explored, especially regarding helminthes and arthropods (Paula et al. 2021).

For which, the present study was pointed to determine the potency of 20 % propolis honey mixture for treatment of contagious *sarcoptic* mange in naturally infested rabbits in comparison with sulfur ointment and 1% ivermectin.

## MATERIALS and METHODS

Between October 2022 and January 2023 skin scrapings of 80 New Zealand adult rabbits (6-9 month old) were collected from two farms (F1 private farm and F2 governmental farm). were subjected to detailed parasitological examination for the presence of mange mites. Skin scrapings were taken only from animals suspected for having clinical signs of mange as hair loss, scratching, and crusty or scaly skin lesions. Examination of each animal included different parts of the body such as head, face, neck, breast and tail. Records were also taken with regards to age, sex and breed of each examined animals. Samples were collected from the edges of lesions after moisturized with mineral oil and kept in a labeled plastic bottle

and then transferred to the laboratory (Fthenakis et al. 2000).

The samples were collected under consent of animal's owner with applying a protocol which approved by the Animal Welfare and Research Ethics Committee, Faculty of Veterinary Medicine, Assiut University, Egypt.

#### **Parasitological examination:**

Skin crusts were placed on glass petri dishes and incubated at 28°C, for one hour which encourages mites to move out of the crusts toward the heat source. For identification of the mites samples were heated with using 10% sodium hydroxide in water bath to dissolve debris. Skin scrapings were examined under dissecting microscope (Kotb and Abdel-Rady 2015), different stages of mites were identified according to the keys of Soulsby (1982).

#### **Prepare of propolis honey mixture:**

20 % Propolis honey mixture was prepared by adding 20 gm crude Chinese propolis powder retailed in bee products shops in 80 ml honey (Egyptian fennel honey) then used as topical application for treatment of naturally infested rabbits according to Abd El Aal (2005).

#### **Experimental design:**

For the *in vivo* assay (In the field trial), twenty of naturally infested rabbits with *S. scabiei* were divided into four groups (5 in each). The first group was left untreated group as the positive control group. The second group was treated topically with 20 % of honey propolis mixture, two doses one week in between. The third group was subcutaneously injected with ivermectin 1 % (Ivomec®, Bayer Korea Ltd) with dose rate 0.3 mg/kg body weight (double doses) according to Galdhar et al. (2015). The fourth group treated with 10% Sulfur topical ointment (Mega Pharm) daily topical application for three weeks. *In vivo* assay course was designed up to three weeks. For the supplementation of elemental zinc and essential vitamins, Zincovit (Apex laboratories Pvt. Ltd.) syrup was also administered orally five drops twice daily for 3 weeks.

Skin scrapings were collected from the infested or recovered regions of each animal

every 5 days up to day 21 and examined microscopically. The treatment success was evaluated by clinical assessment, parasitological evaluation and study histopathological changes of treated rabbits.

#### **Pathological examinations:**

Specimens from skin lesions of clinically infested and treated rabbits after 21 days post treatment were collected and fixed in 10% neutral buffered formalin solution, dehydrated in gradual ethanol (70-100%), cleared in xylene and embedded in paraffin. Five micron sections were prepared, routinely stained with hematoxylin and eosin (H&E) and examined microscopically (Suvama et al. 2013).

#### **Data analysis:**

The data were analyzed using the Statistical Package for Social Science version-15.0 (SPSS Inc., Chicago, IL). Association between prevalence and explanatory variables such as sex, age and rabbit breeds were carried out by using chi-square ( $\chi^2$ ) test. Significant difference was set at  $P < 0.05$  in all analyses.

## **RESULTS**

#### **Parasitological results:**

Out of 80 examined rabbits, 58 (72.5%) were infested with mange; there was significant difference ( $P > 0.05$ ) in prevalence between farm (1) 93.3% and farm (2) 60% (Table1). The distribution of mange lesions in infested rabbits was tabulated in Table (2). All examined males were infested 100%, while incidence of infestation in females was 69.01% (Table3). Most of infested rabbits 93.3 % had poor body conditions (Table3).

#### **Clinical signs:**

Most examined cases were dull, depressed, and had poor physical condition. In body mange (Fig 1 A); multifocal areas of alopecia and areas containing scales were seen scattered on legs (Fig 1 B), face, lips, nose (Fig 1 C), and eyelid. In most cases scratching of the lesions due to intense pruritis was observed. Exudation (Grayish or yellowish exudates) also crusts and ulceration was seen in few cases. In ear mange crusting of the external ear canal and excessive secretion of brownish waxy ma-

terial in affected ear was seen. In late cases, restlessness, irritation, and scratching, head

shaking and drooping of ears (Fig2 A & B) also foul-smelling discharges were noticed.

Table 1. Prevalence of Mange Mite in examined farms through skin scraping.

Location	No. Ex.	No. Positive	Prevalence (%)	X <sup>2</sup>	P value
F1**	30	28	93.3	= 3.23	P < 0.01
F2**	50	30	60		
Total	80	58	72.5		

\*\* High significant statistical difference between F1 and F2 chi<sup>2</sup> = 3.23, P < 0.01

Table 2 Prevalence of rabbit mange mites based on site of infestation.

	Site of Infestation	No	(%)
1	External ear	7	12.07
2	Body mange:		
	Face	9	15.5
	Leg	13	22.4
	Shoulder	29	50
	Total Body mange	51	87.9
3	Total	58	100

Table 3. Prevalence of mange mite in rabbits according to risk factors:

Risk Factors	N. of Positive	(%)
Age: Adult 6-8 M (80)	58	72.5
Sex: Male (9)	9	100
Female (71)	49	69.01
Body sanitary condition: Poor (30)	28	93.3
Moderate(50)	30	60.0

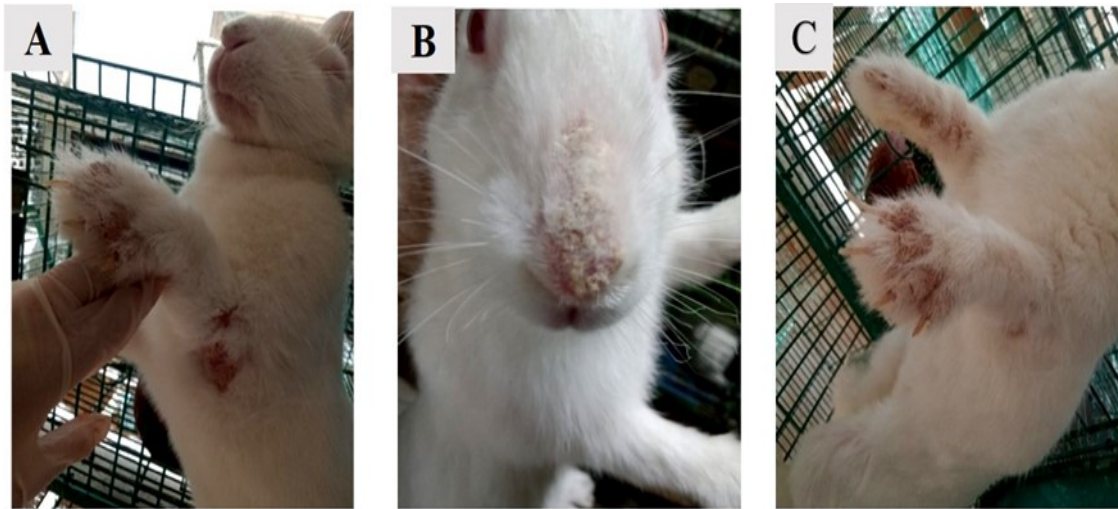


Fig. (1) Rabbits infested with *S. scabiei*  
 (A) Body surface (B) Nose (c) Legs of rabbits infested with *S. scabiei* before treatment

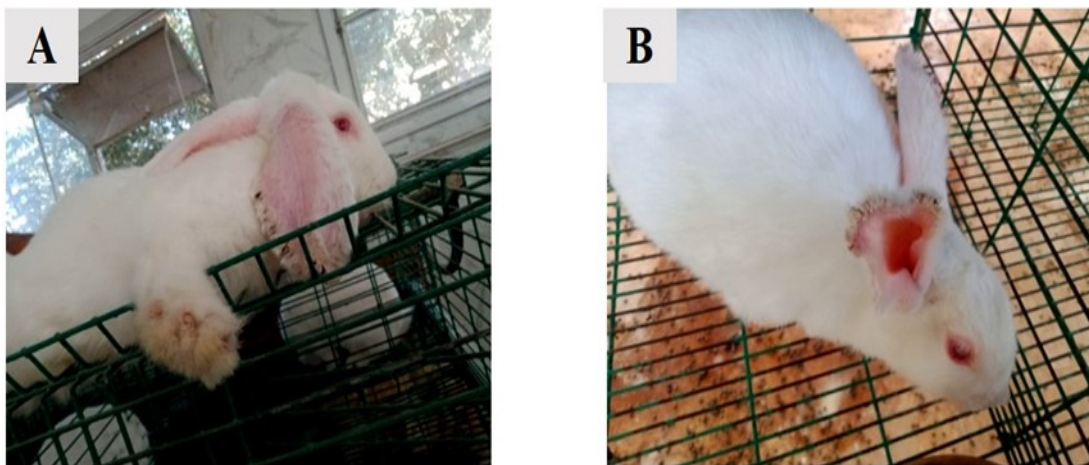


Fig. (2) (A&B) Ears of infested rabbits with *S. scabiei* before treatment.

### Morphologically

Microscopic examination revealed the presence of three stages of mange; adult stages (Fig 3), larva (Fig 4) and egg (Fig 5). Adult mite dorsally showed rounded, tortoise-like body armed with semi-spherical scales. And ventrally, mite provided with four short pairs of legs (two pairs of legs extended forwards with a sucker on the tip, while the other two pairs extended backwards. Male was usually smaller in size than female, their last segment

(tarsus) of legs I, II and IV end with a small sucker-like pad, while in female the last segment (tarsus) of legs III and IV terminate in a long seta. Larva was morphologically similar to the adult stage but had three pairs of short legs. Eggs are ovoid in shape with two rounded ends usually contains developing larvae. Based on these morphological characteristics and on the keys to arthropod parasites, our specimens were identified belonging to *Sarcoptes sp.* mange.

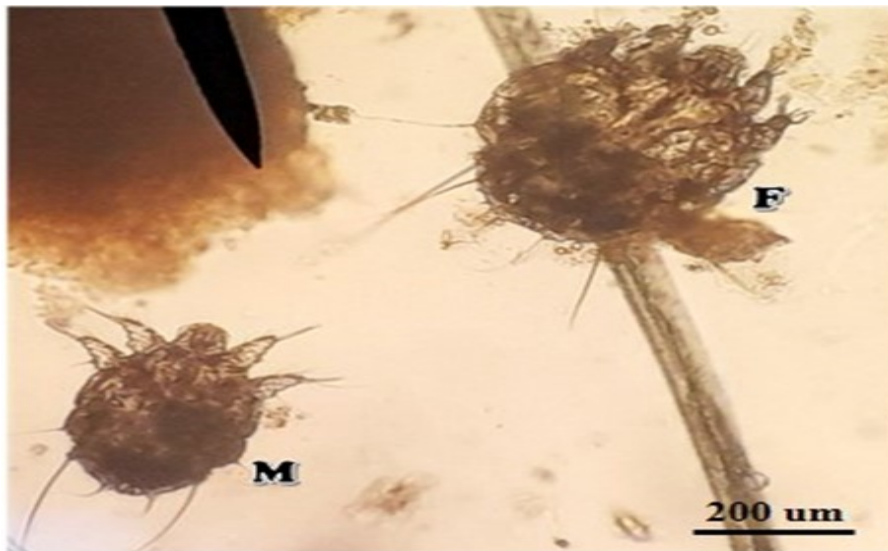


Fig. (3) Adult male (M) and female (F) of *Sarcoptic* mange ventral view Scale bar = 200 μm



Fig. (4) *Sarcoptic* larva ventral view. Scale bars = 100 μm.



Fig. (5) *Sarcoptic* egg contain developing larva

### Ant-mange Efficacy Trials:

Clinical and macroscopic investigation of treated rabbits showed improvement in skin lesions beginning from the 3<sup>rd</sup> day post treatment in the 2<sup>nd</sup> group (20% propolis honey mixture) manifested by reduction or absence of scales, absence of different mite stages (adult, larvae and eggs) and healing nearly completed after 15 days (Fig. 6) as treatment success (100%). There is significant statistical difference between different treatments in second week and high significant statistical difference between different treatments in third week (table 4).

Contrarily, all rabbits of the 1<sup>st</sup> group (untreated group control +ve) and third group (1% ivermectin treated rabbits); Signs of mange infestation were became more severe, no signs of improvement (clinically or parasitologically) as treatment success (0%). But rather, poor general condition and progressive increase in lesions of mites were noted in the 3rd week post-treatment.

On other hand, two rabbits of the 4<sup>th</sup> group (daily topical application of sulfur ointment) were cured by the day 15 post treatment as 40% treatment success which raised by the day 21 of the experiment to 60%.

Cured rabbits in both 2<sup>nd</sup> group and 4<sup>th</sup> group showed marked improvement in all general conditions and clinically manifested by absence of scales, new hair growth and smooth skin in addition to, complete absence of exudates from ear canal. After day21, skin scrapings were found to be negative for adult mites and its developmental stages in 2<sup>nd</sup> group and 60% of 4<sup>th</sup> group. Meanwhile, in 1<sup>st</sup> and 3<sup>rd</sup> groups, dead and live mites were found during microscopic examinations until the 21dayof treatment.

**Pathologically:**

Rabbits of both groups (1&3) showed sub-epidermal lymphocytic dermatitis and extravasated erythrocytes, destruction and ulceration of the epidermis, excess keratin production with necrotic debris, developmental stages of parasites penetrate deeper in underlying structure of the ear skin, surrounded by lymphocyt-

ic infiltration in sub-epidermal tissue. Also showing parasitic elements embedded within hyperplastic epidermal cells (Fig.7A-D& G-H).

While with those rabbits of the 2<sup>nd</sup>group (20% propolis honey mixture treated rabbits), healing was noted in three rabbits after 7 days of treatment nearly complete healing observed of all animals (100%) of this group after the 14<sup>th</sup> day of treatment. In rabbits of this group show regeneration in epidermal cells layer covering mature fibrous tissue, with remnants of dead parasitic element in skin dermis and hypodermis surrounded by minimal lymphocytic infiltration (Fig.7 E-F). Rabbits of the 4<sup>th</sup> group (sulfur ointment group), we noticed little improvement of the epidermis, mature fibrous tissue with little inflammatory cells aggregation in the dermal layer with reduction of parasitic elements (Fig.7 I-J).

Table 4. The efficacy treatment of *S. scabiei* infested rabbits treated with either 20% Propolis honey mixture or 1%Ivermectin, or Sulfur ointment.

Study groups	First week *				Second week **				Third week **			
	Non Infested		Infested		Non Infested		Infested		Non Infested		Infested	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Positive control (G1)	0	0	5	100	0	0	5	100	0	0	5	100
Propolis honey ointment 20 %( G2)	3*	60	2	40	5 **	100	0	0	5 **	100	0	0
1% Ivermectin (G 3)	0	0	5	100	0	0	5	100	0	0	5	100
Sulfur ointment 10% (G4)	0	0	5	100	2	40	3	60	3	60	2	40
Statistical analysis												
chi2	7.5				10.18				10.18			
P value	0.0235				0.0062				0.0062			

Significant statistical difference between different treatments  $P < 0.05$   
 High significant statistical difference between different treatments  $P < 0.01$ .

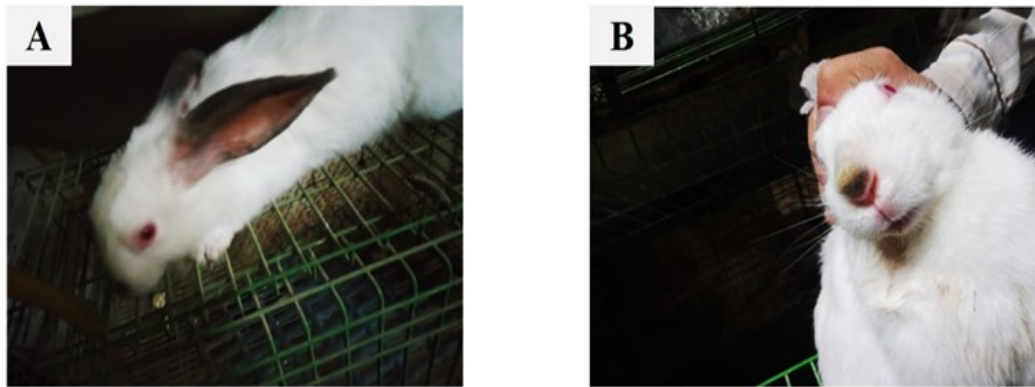


Fig. (6) (A&B) Ear and nose of rabbit infested with *S. scabiei* post treatment with 20 % propolis honey ointment

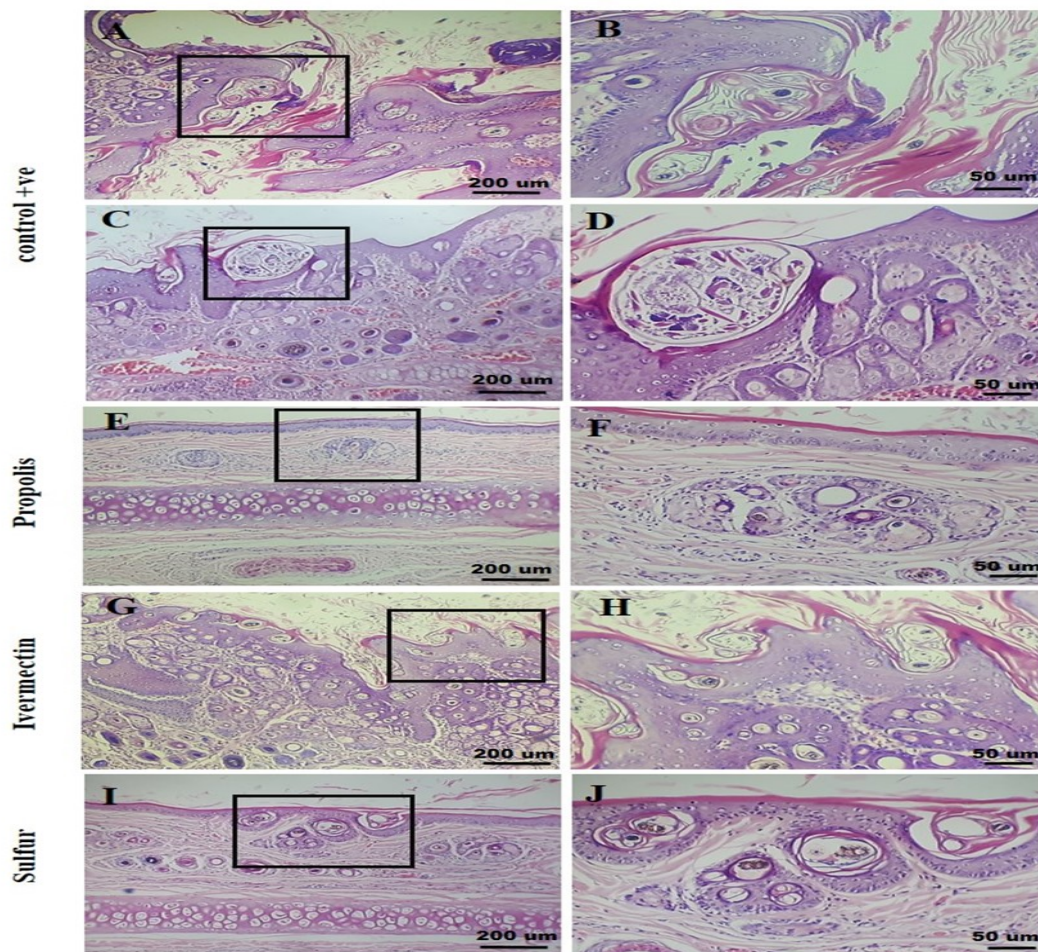


Fig. (7): Photomicrographs of rabbit skin stained with hematoxylin and eosin. (A-D) control + ve mange infested group showing cross section in epidermal burrows and parasitic element embedded within hyperplastic epidermal cells, mixed with thickened keratin, necrotic debris and sloughed epithelium on tissue surface, sub-epidermal lymphocytic dermatitis and extravasated erythrocytes. (E-F) propolis treated group showing regenerated epidermal cells layer covering mature fibrous tissue and skin adenexa containing remnant of dead parasitic element surrounded by minimal lymphocytic infiltration. (G-H) Ivermectin treated group showing destruction and ulceration of the epidermis, excess keratin production with necrotic debris and developmental stages of parasites penetrates deeper in underlying structure of the ear skin, surrounded by lymphocytic infiltration in sub-epidermal tissue. (I-J) Sulfur treated group showing an improvement of the epidermis, reduction of parasitic elements, mature fibrous tissue with little inflammatory cells aggregation in the dermal layer. Scale bars = 200  $\mu\text{m}$  & 50  $\mu\text{m}$ .



## DISCUSSION

*Sarcoptes scabiei* that infests the skin of its host by burrowing into the epidermis causing scabies in humans and sarcoptic mange in domestic animals (Niedringhaus et al. 2019). It is a common parasitic disease in rabbits infest both young as well as adult one causing high losses that is difficult to eradicate (Sparsa et al. 2006). Scabies is a significant human public health threat and may be associated with secondary complications in humans such as pyoderma, rheumatic heart disease, and acute post-streptococcal glomerulonephritis (Abd El-Ghany 2022). Most of the available treatment options for scabies fail to completely control it, since there are several limitations for uses of these drugs including genotoxic and cytotoxic effects; in addition, their widespread use induces resistance (Coles and Dryden, 2014).

Propolis could be used as an efficient and safe natural therapy to control rabbit mange (Mohamed et al. 2017).

In the present work examination of skin scraping revealed that the overall prevalence of mite spp. infestation among rabbits was 72.5% and *Sarcoptic scabiei* mange was the only mite recovered from samples collected. *S. scabiei* was reported in previous studies among rabbits as reported in India (Soundararajan & Iyue 2005), Kenya (Aleri et al. (2012) Zimbabwe (Gono et al. 2013) and Qena province, Egypt (Elshahawy et al. 2016). Some reports suggested that *S. scabiei* mange was described as more commonly found in some parts of the world, such as Africa (Scott et al. 2001).

There is a significant statistical difference in prevalence of mange in the present study between F1 (private farm) and F2 (governmental farm). This might be due to the poor management and less sanitary conditions of the private flock where rabbits could be not exposed to sunlight, and were kept in compacted cage (Abu-Samra et al. 1981) in comparison with balanced ration and sanitary care in the governmental farm.

Regarding to sex, male rabbits were found to have higher prevalence (100 %) than females (69.01%). Likewise Elshahawy et al. (2016) found male rabbits were more susceptible to the mite infestation than female. Schalk and Forbes (1997) mentioned that high levels of parasitism in males than for females especially for arthropod infestation may as result sex biases because estrogens stimulate immunity, whereas androgens suppress immunity.

Clinically diseased rabbits were characterized by presence of scales, crusts, alopecia, pruritis, intermittently scratching of lesions and later hemorrhagic crusts. These manifestations may be due to by burrowing activity of mites during excavation, and irritant action of their secretions and excretions (Darzi et al. 2007).

In the present work the histopathological examination of mange infested rabbit skin and ears revealed; hyperkeratosis, epidermal hyperplasia, folliculitis, and dermatitis associated with external ear thickening in addition to parasitic element embedded within hyperplastic epidermal cells. Findings of the present study are similar with that recorded by Mohamed et al. (2017) who notice hyperkeratosis, and sub-epidermal dermatitis, excess keratin production, with necrotic debris and sloughed epithelia mixed with developmental stages of the parasite either on the skin surface or within epidermal burrows of infested rabbits. Development of these lesions of mange could be related to direct mechanical stimulation of the mange that induce allergic inflammatory reaction associated with excessive itching feeling lead the animal rubbing ear to objects and damage it (Tehrani et al. 2011).

Recently, as result of scabies drug resistance, multiple studies had been conducted to produce a new drug safe, effective with better activity against eggs and to cover the whole life cycle of the mite "14-days". Through the present study, we tried to evaluate the efficacy of 20% propolis honey mixture as a topical application for scabies therapy. Which is a non-toxic anti-microbial natural product, can reduce biofilm generation and result in accelerat-

ed healing (Oryan et al. 2018). The anti-parasitic effect of propolis is related to their ability to increase the fluidity and permeability of the plasma membrane of the parasites leading to cell lysis (Camargos et al. 2014).

In the present study, we found that only two doses of topical application of 20% propolis honey mixture (single dose/ week) were effectively killed *S. scabiei* mites. Treated rabbits in this group showed marked improvement in all general conditions and the clinical manifestations of rabbits were subsiding rapidly after two weeks. The pathological findings of this group referred to regenerated skin, which characterized by regenerated epidermal cells layer covering mature fibrous tissue and skin (dermis & hypodermis) containing remnant of dead parasitic element surrounded by minimal lymphocytic infiltration.

Acaricidal effect of propolis extract (10 % propolis ointment) was studied previously and proved as topically uses for three successive days (Mohamed et al. 2017) or as twice a day for ten days (Metwally et al. 2018) both studies concluded complete recovery of rabbits was done after 10 -15 days of treatment and these depended on the solvent of extraction (70 % or 40 % ethanol, or water), concentration of propolis, and contact time. According available literatures it is the first time of use mixture of crude propolis (not extracted) with honey as acaricidal agent. Garedeew (2003) mentioned that addition of propolis extract to honey contributed to a significant increase in the content of polyphenolic compounds, including flavonoids, phenolic acids and ferulic acid reached the highest level which leads to remarkable reduction in metabolic rates of mites.

Regarding treatment rabbits with sulfur ointment (group 4) in the present work, there was a gradual increase in recovery rates, being 40% in the second week and reaching 60% in the third week. This result was agreement with Abdelaziz et al. (2020) who recorded the efficacy of sulfur ointment in treatment mange in rabbits at 2<sup>nd</sup> week was 73.8% and at 4<sup>th</sup> week was increased to 98%.

Concerning to ivermectin treatment in present work, we found complete resistance in all the rabbits treated with double doses of 1% ivermectin, as none of them were cured, and the general condition of the animals was worse. Currie et al. (2004) reported in clinical and in vitro evidence of crusted scabies resistance for ivermectin treatment of in two patients.

Otherwise, ivermectin may not be ovicidal because of inadequate penetration of the thick eggshell, as well as possibly being ineffective against the younger stages of the parasite inside the egg because, at that point in the life cycle, the parasite's nervous system has not yet developed (Usha and Gopalakrishnan 2000). Furthermore, Currie and Carapetis (2000) concluded that the intensive ivermectin use over a period of several years leads to the emergence of ivermectin resistance in *S. scabiei* which reflects the failure to control the current epidemics of scabies infestation.

## CONCLUSION

We concluded that 20% Propolis honey mixture has highest efficacious (100 %) among tested compounds in treatment of mange in rabbits followed by Sulfur ointment 60%, while there is clinical evidence of ivermectin resistance in treated rabbits. Thus, 20% Propolis honey mixture can be used as an efficient and safe natural agent for treatment of mange in rabbits with avoidance the problems of chemical drugs.

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