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Aromatherapy of *Cinnamomum zeylanicum* Bark Oil for Treatment of Scabies in Rabbits with Emphases on the Productive Performance

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Abstract: The aim of this study was to investigate the possible antiparasitic effect of *Cinnamomum zeylanicum* bark oil to cure rabbits infected with sarcoptic mange and to study the general health status before and after treatment with reference to the productive performance. The study materials consisted of a total number of 48 adult female white Newzealand rabbits; 8 were normal healthy (G1) and 40 were naturally infected with sarcoptic mange, the later group was subdivided into 4 balanced sub groups; G2 was left without treatment and G3 was treated with Ivermictin, G4 was treated with EO 2.5% and G5 was treated with EO1.25%. Blood samples were taken from all groups for analyses of some immunological and biochemical parameters. Microscopic examination of dermal scrapings revealed *S. scabiei*. Serum biochemistry and the oxidative status of the animals were studied at the end of the experiment. Results revealed elevated γ glutamyl transferase, alanine amino transferase, aspartate amino transferase and urea in animals treated with Iverectin and EO2.5% suggesting hepato-renal toxic effect, while animals treated with EO.1.25% showed normal liver and kidney functions. Rabbits showed improved oxidative status after recovery and restored their reproductive performance. Depending upon these results, it is recommend to use *Cinnamomum zeylanicum* bark oil 1.25% for treatment of sarcoptic mange in rabbits. Essential oils should be used under guidance of a qualified aromatherapy practitioner. However, further investigatins on large number of rabbits still required.

Key words: Scabies · Sarcoptes scabiei · Rabbit · Cinnamomum zeylanicum · Essential oils

INTRODUCTION

The domestic rabbit when compared with other livestalk is characterized by early sexual maturity, relatively short gestation length, short generation interval and rapid growth [1,2]. The rabbit meat is white, fine grained, palatable, contained high good quality protein, low in fat and caloric contents and contains a higher percent of minerals than other meats [3]. Also, rabbit meat nearly has the same nutritive value as beef and comparable to that of broiler chicken with good meat-to-bone ratio and is acceptable to the consumer [3]. Therefore, rabbit production can play a considerable role in solving the problem of meat shortage in developing countries and particularly on the level of the small scale farmers.

Sarcoptic mange is an important parasitic disease caused by *Sarcoptes scabiei*. It causes heavy economic losses in rabbit farms because of the high morbidity and mortality rates [4]. Also, it is mostly associated with decreased feed consumption, digestibility and conversion rate with development of meningitis[5]. In the same time, rabbits in poor condition appear to be most susceptible for the disease and several other factors like stress, over crowding, poor nutrition, cold weather and immunosuppressants predisposes the animal for the disease [6,7]. Moreover, animal scabies can be transmitted to human being and constitutes health hazard [8]. Human scabies, or sarcoptic mange has global prevalence of 300 million infected individuals [9].

Scabies affected animals show severe pruritis with alteration of hepatic structure and function [10] with

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altered balance between pro-oxidant and antioxidant defense in hepatic tissues [11].

Several topical scabicides are available for treatment such as benzyl benzoate, lindane and permethrin. During the last few decades, Ivermectin was approved for the treatment of regular scabies after 4 to 8 weeks [12]. However, the intensive use of these drugs has led to the development of drug resistance in arthropods that constitutes a serious threat to many programs for ectoparasite control. There are 15000 adverse reports due to application of Ivermectin [13,14]. Because of limited safety data, Ivermectine should not be used in youngs or during pregnancy or lactation [15]. and consequently looking for a novel acaricide is of great importance. In view of these problems, use of botanical acaricides against skin problems like mange is visualized. The human folk medicine reports the activity of the leave's essential oil of cinnamon (*Cinnamomum zeylanicum)* against parasites and suggested its use for the treatment of *psoroptes cuniculi* [16,17]. Cinnamon is also included in many medicinal recipes that are used for lice and other skin parasites [16].

The current study was carried out as a trial to control the problem of *sarcoptis scabei* infection in rabbits using *Cinnamomim Zeylanicum* bark oil with special emphasis on Its impact on the performance of treated animals.

MATERIALS AND METHODS

Animals: A total number of 48 adult New Zealand White female rabbits (6 months old; 2.8 - 3.0 kg body weight) was included in this study. Rabbits were fed on a diet shown in Table 1. Eight of these animals were healthy, had no signs of any skin affection, kept in a separate room and allocated to the control group (G1). The other forty rabbits were reared in the same farm, naturally infested by *Sarcoptes scabiei*. The severity of the infestation was determined according to clinical and laboratory examination and the scoring system described by Ulutas *et al.* [18]. Skin scraping were taken from all rabbits and were, kindly examined at the Department of Parasitology, NRC, Egypt, under a stereo-microscope. Infected rabbits were allocated to four comparable groups for treatment The first group(G2) did not receive any treatment. The second group (G3) was injected with two doses of ivermectine at a dose 200µg/Kg body weight with 2 weeks intervals as prescribed. The third group (G4) received daily topical treatment with 2.5% *cinnamomum zeylanicum* bark oil (E.O 2.5%) diluted in a mixture composed of 98% of saline solution and 2% of olive oil on the affected areas in limbs and ear pinna of rabbits after cleaning with worm water and removal of the scales according to Fichi *et al.* [17]. The fourth group (G5) received every other day topical treatment of *cinnamomum zeylanicum* bark oil 1.25% (E.O.1.25%) diluted in the same mixture of G4. The food intake and body weight of the animals were recorded during the course of the experiment. Rabbits in all groups were followed up by skin scraping and examination under stereomicroscope till complete recovery. Does were introduced into cages of proved fertile healthy vigorous males and the reproductive behaviour of the females during the infection and after recovery were recorded. The breeding date was recorded, a few days later, the doe was placed back into the buck's cage to confirm conception. The does were palpated two weeks after exposing her to the buck and the marble-like embryos in the doe's belly were palpated. The number of female accept mounting, number of positive mounts were recorded for each group. Animals were left to kindle and the number of kits and their viability were recorded.

Table 1: Ingredients and chemical analysis of the experimental diet**

Ingredients	% of Diet
Yellow corn	32.0
Wheat bran	20.0
Soybean meal (44%)	18.0
Wheat straw	12.0
Alfalfa hay	5.0
Rice bran	5.0
Linseed straw	2.8
Sunflower meal	2.5
Lime stone	2.0
Sodium chloride	0.3
Vitamins and mineral premix	0.3
DL-Methionine	0.1
Total	100
Calculated analysis:	
DE(Kal/Kg)	2463
CP %	16.35
CF %	11.54

Vitamins and minerals premix per kilogram contains: Vit. A,10.000 IU; Vit.D3, 900 IU; Vit. E, 50.0mg; Vit. K,2.0mg; Vit. B1, 2.0mg; Vit.B2,6.0mg; Vit. B6, 2.0mg; Vit. B12, 0.01mg; Biotin, 0.2mg; Choline, 1200mg; Niacine, 50mg; Zinc, 70mg; Cu., 0.1mg; Mn., 8.5mg; Fe.,75.0mg; Folic acid, 5mg and Pantothenic acid, 20.0mg. ** According to NRC [19] for rabbits

Essential Oil Extraction: The essential oil of *Cinnamomum zeylanicum* bark used in this study was provided by the Medicinal and Aromatic Plants Division, Horticultural Research Institute,Agricultural Research

Center, Ministry of Agriculture, Egypt. The essential oil was obtained by steam-distillation for 3 hour using a Clevenger-type apparatus according to the method of Giary *et al.* [20]. The obtained essential oil was closed under nitrogen gas and stored in airtight glass vials covered with aluminum foil at -20°C.

Gas Chromatographic Analysis: GC analysis was performed by using Hewllet-Packard model 5890 equipped with flame ionization detector (FID). A fused silica capillary column DB5 (60 m×0.32 mm i.d.) was used. The oven temperature was maintained initially at 50°C for 5 min, then programmed from 50 to 250°C at a rate of 4°C/min. Helium was used as the carrier gas, at flow rate 1.1ml/min. The injector and detector temperatures were 220 and 250°C, respectively. The retention indices (Kovats index) of the separated volatile components were calculated with hydrocarbon (C8-C22, Aldrich Chemical Co.) as references.

Gas chromatographic–mass spectrometric analysis (GC/MS). The analysis was carried out by using a coupled gas chromatography Hewllet-Packard (5890)/mass spectrometry Hewllet-Packard-MS. The ionization voltage was 70 eV, mass rangem/z 39-400 amu. The GC conditions carried out as mentioned earlier. The isolated peaks were identified by matching with data from the library of mass spectra (NIST) and comparison with those of authentic compounds and published data. The quantitative determination was carried out based on peak area integration [21]. The quantitative determination was carried out based on peak area integration.

Analysis: Blood samples were collected from all rabbits at the end of the experiment, centrifuged and serum samples were collected for analysis of total protein, albumin, urea, creatinine, cholesterol, Aspartate amino transferase (AST), alanine amino transferase (ALT) as outlined by Henery [22]. Immunoglobulin [IgG; 23] γ glutamyl transferase [γ GT;24] values were analyzed. Oxidant/ antioxidant markers including malondialdehyde [MDA;25]; superoxide dismutase; [SOD;26]; reduced glutathione; [R-GSH;27] were calorimetrically assayed using chemical kits from Bio Diagnostic, Egypt.

Data were statistically elaborated with Analysis of Variance and the Test of Student–Newman–Keuls for multiple comparisons between groups [28] using Mc Graw Hill software[29]. Moreover, economic losses due to scabei were estimated, in a group of does (N=25) not receiving treatment, by multiplication of the number of affected animals by the expected losses / animal based on the effects of scabei infestation on rabbit live weight, price of consumed food, reduced kindling percentage and mortalities as outlined by Fthenakis *et al.* [30] and Milne [31].

RESULTS

Diseased does showed loss of weight, (Fig. 1) with reduced food intake, (Fig. 2), pruiritis, dullness and did not show any sexual behavior or accept male and finally die, while healthy does showed normal food intake and when introduced into male cage,showed good sexual behaviour, lay herself flat on the floor of the hutch, raise her tail ready for mating.

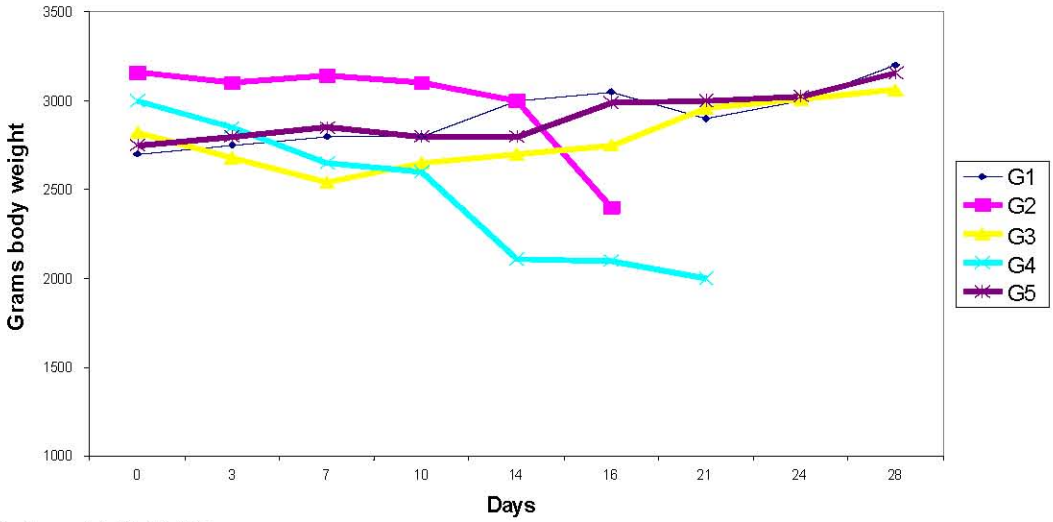

Fig. 1: Body weight/rabbit/day

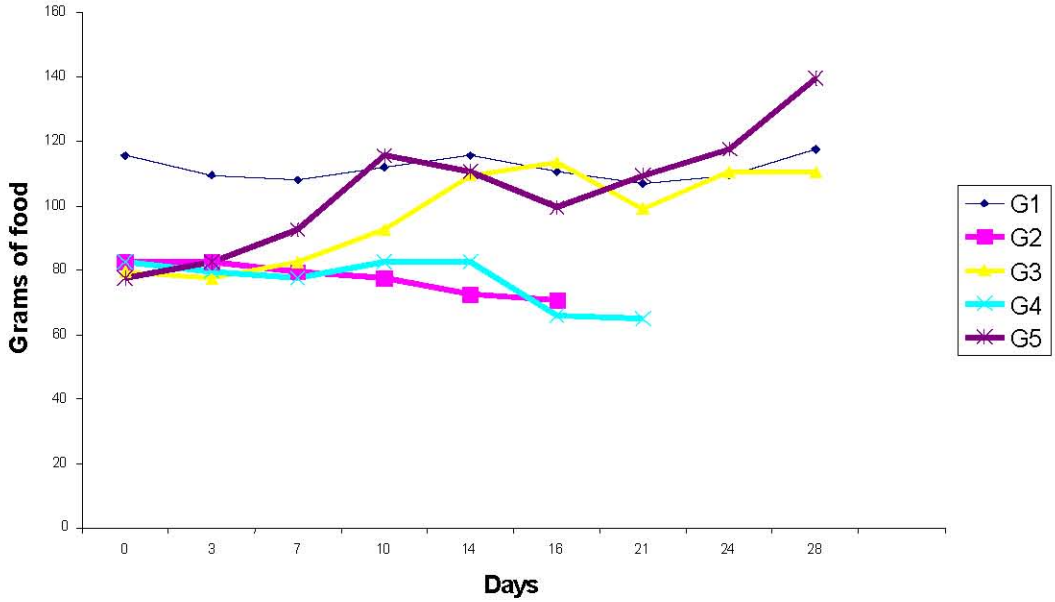

Fig. 2: Food intake/ rabbit/day

Table 2: Chemical Composition of bark Oil from *Cinnamoum Zylanicum*

Peak No.	KI[a]	Conc.[b]	Compound	Identification[c]
1	906	0.20	α-Thujene	KI &MS
2	913	0.55	α-Pinene	KI &MS&St
3	929	0.08	Camphene	KI &MS
4	1031	0.33	Limonene	KI &MS&St
5	1146	0.06	Terpinene-4-ol	KI &MS
6	1163	0.07	α-terpineol	KI &MS&St
7	1223	0.13	(Z)-cinnamaldehyde	KI &MS
8	1278	57.37	(E)-cinnamaldehyde	KI &MS
9	1282	0.72	Anethole	KI &MS
10	1297	0.79	Cinnamyl alcohol	KI &MS
11	1330	0.62	Eugenol	KI &MS&St
12	1349	13.26	α-Copaene	KI &MS
13	1375	0.18	(Z)-Caryophellene	KI &MS
14	1390	0.43	(E)-β- Caryophellene	KI &MS
15	1420	3.42	(E)-Cinnamylacetate	KI &MS
16	1424	0.18	α-Humulene	KI &MS
17	1427	0.06	δ-Cadinene	KI &MS&St
18	1436	1.30	α-Muurolene	KI &MS
19	1487	0.29	α-Cadinene	KI &MS
20	1490	3.96	α-Amorphene	KI &MS
21	1505	5.82	O-methoxycinnamic aldehyde	KI &MS
22	1571	0.57	Globulol	KI &MS
23	1602	0.28	Torreylol	KI &MS
24	1621	0.43	10-epi-Eudesmol	KI &MS
25	1630	0.64	Trans-cadinol	KI &MS
26	1642	0.61	α-Cadinol	KI &MS
27	1690	0.72	Epi-α-Bisabolol	KI &MS
28	1731	0.05	Benzyl benzoate	KI &MS
29	1937	1.80	Methyl palmitate	KI &MS
30	21.40	0.05	Methyl oleate	KI &MS

[a]:Kovat index at DB5 column, [b]:Conc: relative peak area, [c]: MS; Mass spectra; St: identified by co-injection standard compounds

Table 3: Effect of *Cinnamomum zeylanicum* on some serum biochemical values of does affected with *sarcoptic scabei*

Scabies (Means±SE)	G1	G2	G3	G4	G5
Total protein (g/dl)	7.06±0.04^{a}	5.16±0.09^{b}	7.20±0.08^{a}	5.56±0.17^{c}	6.36±0.05^{d}
Albumin (g/dl)	3.58± 0.03^{d}	2.06±0.05^{b}	3.96± 0.04^{a}	2.70±0.05^{c}	3.46±0.05^{d}
Globulin (g/dl)	3.50±0.08^{c}	3.95±0.10^{d}	3.22±0.09^{ac}	2.74±0.21^{b}	3.00±0.10^{ab}
IgG (mg/dl)	545.46±13.20^{a}	780.55±8.22^{c}	660.64±7.33^{b}	552.5±9.11^{a}	543±15.2^{a}
Cholesterol (mg/dl)	130.20±1.20^{a}	86.40±5.55^{b}	131.00±3.37^{a}	101.6±0.74^{c}	120.00±0.70^{d}
äGT (U/L)	10.97±.057^{a}	15.89±.050^{b}	20.24±.037^{c}	24.90±.058^{c}	14.20±.017^{b}
ALT (IU/ml)	23.80±1.46^{a}	45.40±1.12^{c}	67.80±4.02^{b}	57.20±0.96^{b}	30.60± 3.37^{d}
AST (IU/ml)	22.00±.057^{b}	28.40±1.60^{a}	49.20±1.98^{c}	39.60±0.50^{d}	30.80±0.58^{a}
Urea (mg/dl)	29.20±0.37^{a}	31.60±1.16^{a}	47.20±2.26^{b}	38.80±0.37^{c}	29.60±0.50^{a}
Creatinine (mg/dl)	0.68±0.06^{a}	1.22±0.07^{b}	0.88±0.06^{c}	0.92±0.08^{c}	0.68±0.04^{a}
R-GSH(mmol / L)	18.80±0.58^{a}	7.00±1.30^{b}	18.00±0.54^{a}	9.40±0.24^{c}	16.40±0.50^{a}
SOD (U/ml)	21.00±0.83^{a}	18.40±0.50^{a}	28.40±0.12^{b}	26.80±0.58^{b}	38.80± 0.15^{d}
MDA (mmol/ml)	30.60±0.24^{d}	39.80±0.66^{a}	14.40±1.02^{b}	20.20±0.48^{c}	11.00±0.44^{b}

Table 4: Some reproductive performance parameters in Does affected with sarcoptic mange and treated with Ivermectine and *Cinnamomum zeylanicum* bark oil **

Groups	R%	F%	C%	V	M	K%
G1	38.40	75.00	75.00	1.20	8.00	95.00
G2*	00.00	00.00	00.00	00.00	00.00	00.00
G3	30.00	66.00	60.00	2.00	6.00	92.00
G4*	00.00	00.00	00.00	00.00	00.00	00.00
G5	40.00	79.00	70.00	1.40	7.80	93.00

R% : Daily percentage of females accept mounting F%: the percent of positive mounts. C% concepton rate V: average number of required services for conception M: average number of obtained kits per birth. K%: percent of viable born cubs

* = Data were not available due to mortality

** Parameters were taken after complete recovery of the animals

Table 5: The production losses incurred from scab and proportion of a flock infested

Number of weeks from introduction of infestation	Total loss /rabbit (LE)*	Proportion of flock infested at the end of period
0-4	70	60%
4-8	90	99%

* The financial loss estimated in Egyptian pound for each infected rabbit left without treatment. it is calculated according to body weight loss, reduced fertility, the cost of previously consumed food and deaths

Complete recovery of the skin lesions took place after 18-23 days of treatment with E.O. while those does treated with ivermectin got rid of infection after 21-37 days. Animals treated with EO 1.25% and Ivermectin showed normal sexual behaviour whereas does were ready for mating which took approximately 25 seconds. The number of positive mounts were recorded in Table 4. The number of services required for conception in does treated with Ivermectin (G3) was more than those treated with E.O.1.25% (G5). Conception rate was 60% in Ivermictin (G3), 70% in EO 1.25 (G5) groups Vs. 75% in the control group (G1). The number of viable kits were less in G3 than in G5 (Table 4).

Unfortunately and inspite of the complete recovery of the skin lesions, all animals treated with E.O.2.5%, especially those having heavy infestation still off food, lost weight, lost condition, did not accept male, the same as in G2,. The mortality rate was high in G2 and G4 towards the end of the experiment so that the reproductive parameters were not available for these groups in Table 4.

Gas Chromatography – Mass Spectrometry (GC/MS) analysis of cinnamon bark volatiles is presented in 2. Thirty compounds were positively identified. The analysis showed that cinnamaldehyde (57.37%) was the main compound of the extracted oil. Bioactive monoterpenes, e.g. eugenol and terpineol, were successfully identified. Additionally, some sesquiterpenes were detected e.g. Epi-α-Bisabolol.

Table 3 shows some serum biochemical values of the diseased untreated group G2 showed significant ($p < 0.05$) elevation in total globulin, IgG, γGT, ALT, AST, urea and creatinine indicating the hepato-renal harmful effect of scabies on rabbit. Diseased animals showed high level of oxidants MDA and low level of antioxidants R-GSH and

SOD as well as cholesterol, total protein and albumin in comparison to normal healthy animals. Animals treated with ivermactin and EO showed lower level of serum IgG if compared to diseased animals. Cholesterol level showed elevation in treated animals if compared with diseased animals.

Liver enzymes γGT, ALT and AST and urea showed elevation in both diseased and treated groups with Ivermactin and EO 2.5% suggesting hepatotoxic effect, while the condition was better in those treated with EO 1.25%. The oxidative status is greatly improved in treated animals if compared to diseased ones.

The estimation of the production losses was calculated in Table 5 on the basis of the body weight loss, the cost of the previously consumed food, the loss of fertility and the estimated loss of new individuals and finally the loss of the animals due to death. The flock losses were calculated from the equation:

The expected losses in the farm = the number of affected animals × expected losses/ rabbit.

It is worthy to mention that the cost of treatment will be less in G5 than G3 as the price of Ivermectine is higher and the course of treatment is longer if compred to EO 1.25%.

DISCUSSION

A FAO Expert Consultation on Rural Rabbit Production emphasized that if the high rate of growth in meat consumption in future years will to be met, much of the increase in production may to come from short-cycle animals kept by small-scale farmers such as rabbits [32].

In the current investigation, the chemical composition of the essential oil of *C. zeylanicum* bark indicated that the cinnamaldehyd represent the main component. Also α-Thujene, α Pinene, Camphene, Limonene, Terpinene-4-ol, α terpineol, Globulol, 10-epi-Eudesmol, Torreylol, Trans-cadinol, α -Cadinol, Epi-α-Bisabolol and Eugenol, identified in our results, have antioxidant activity and may play an important role in treatment of scabies. Camkerten *et al.* [33] suggested a tight relationship between the sarcoptic mange infestation and the oxidant/ antioxidant imbalance in dogs. This observation was in agreement with this study whereas the oxidative status of the treated animals is improved after recovery.

It was reported that the composition of the essential oil of C. zeylanicum is quite variable, depending on the locality of growth and the organ used. In the same time, many authors reported eugenol as the main component of the oil of the leaves and cinnamaldehyde for the oil from the bark [34,35].

Essential oils are volatile, natural, complex compounds characterized by a strong odour and are formed by aromatic plants as secondary metabolites. Known for their antiseptic, bactericidal, virucidal and fungicidal and acaricidal medicinal properties [36].

The mechanisms of action of the essential oils particularly at the antimicrobial level were recently investigated. Because of the great number of constituents (20-60 compounds), essential oils seem to have no specific cellular targets [37]. As typical lipophiles, they pass through the cell wall and cytoplasmic membrane, disrupt the structure of their different layers of polysaccharides, fatty acids and phospholipids and permeabilize them. Cytotoxicity appears to include such membrane damage. In bacteria, the permeabilization of the membranes is associated with loss of ions and reduction of membrane potential, collapse of the proton pump and depletion of the ATP pool [38]. Moreover, it was evident that essential oils can coagulate the cytoplasm [39] and damage lipids and proteins[40,41]. In the case of oxidative stress and bioenergetic failure. permeabilization of outer and inner mitochondrial membranes leads to cell death by apoptosis and necrosis [42,43]. It seems that chain reactions from the cell wall or the outer cell membrane invade the whole cell, through the membranes of different organelles like mitochondria and peroxisomes. These effects suggest a phenolic-like prooxidant activity [41,44]. Essential oils also include antioxidants such as terpenoid and phenolic components. The antioxidant property of essential oils and components has been very often verified *in vitro* by physicochemical methods [45- 47]. A tight relationship was recorded between the sarcoptic mange infestation and the oxidative stress [33]. This is in agreement with this study whereas the oxidative status of the treated animals got improved after recovery [48]. Tarigan and Huntley [48] mentioned that immunoglbulins (IgG and IgE) increased in goats serum after mite challenge. Following Ivermectin treatment, the mite infestation was rapidly abrogated and was followed by a marked decline in IgG and IgE. and this was in agreement with the results of this study. Also, Dimri and Sharma [11] traced the effect of sacoptic mite on liver function, the condition is aggravated with the Ivermictin and E.O. 2.5% treatments while it was better with EO 1.25%.

From the reproductive point of view, results revealed lower conception rate and average number of kits/delivery in G3 than G1 and G5 and this coincide with the results of

Paasch and Haustein [15] who mentioned the negative effect of Ivermectine on reproductive performance of animals specially pregnant ones. Diseased untreated animals showed poor reproductive performance as a result of declined nutrional and health status, loss of blood due to infection also it was reported that scabies induced marked changes in blood component of farm animals, elevated prolactine and consequently ovarian inactivity and failure of pregnancy [7]. The high mortality observed in this group of rabbits was previously recorded by Kemp *et al.* [49]. Small amounts of cinnamon have been used for thousands of years as a spice without any reports of side effects. By contrast, no reliable data are available on the effects of the daily continuous administration of the high concentration. However, cytotoxic activities of essential oils or their major components, were recently demonstrated in mammalian cells *in vitro* [50].

Besides the high coumarin levels (produced as a metabolite) that may be harmful for liver cells, the cinnamaldehyde levels in cinnamon may possibly constitute a risk for health. Animal experiment studies indicated that taking cinnamaldehyde during pregnancy could lead to damage to the unborn offspring. European Food Safety Authority EFSA [52] and Federal Institute for Risk Assessment [53] recommends that products of this kind should carry warnings, something which has only rarely been the case up to now. The formerly mentioned data may explain the clinical signs of toxicity in the group of rabbits treated with EO2.5% and we suggest to test *in vivo* less concentrations of C. zeylanicum bark oil because they could be equally efficacious but safer for treated animals.

From the economical point of view, Results recorded in Table 5 declared the production losses incurred due to scabei and in the absence of effective control. The morbidity and mortality of the disease is high if left without treatment or control measure [31]. The spread of scabei period coincides with the mating period in the rabbit production cycle. Scab infestation at this time reduced kindling percentage [54,30]. In particular we have shown the importance of risk due to rapid spread of disease and the resulting financial losses shown in Table 5. It is concluded that the most effective action is to prevent disease entering your farm. Isolate and treat any diseased or incoming animals [55]. However, failure to prevent scab increases the risk of disease introduction to neighbouring farms, the model demonstrates the need for greater use of partnership between animal breeders in disease prevention as discussed in the Animal Health and Welfare Strategy (AHWS).

In conclusion, Scabies infection threats animal productivity. Use of chemical drugs may affect the reproductive performance of the animals and results in drug resistance. Cinnamon essential oil showed good curative effect for the skin lesions but it is recommended to be used with caution and under aromatherapy practitioner consultation.

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