Comparative Study On The Effect Of Propolis Extract And Toltrazuril In Rabbits Inoculated With *Eimeria Stiedae*

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ABSTRACT

Forty eight apparently healthy and coccidia-free New Zealand white male rabbits (mean body weight 757.60 ± 1.63 grams) were divided into four equal groups in well separated cages. The rabbits of the second, third and fourth groups were orally inoculated with 5 x 10^4 sporulated oocyst of *E. stiedae* by a stomach tube. The rabbits of first group was used as negative control; the rabbits of second group were inoculated and not treated (positive control). On week post inoculation (PI), the rabbits of third group were treated with 7 mg / kg b. wt. at a rate of 2.8 ml toltrazuril / 10 liter in drinking water continuously for 48 hr. While that the fourth group was treated with alcoholic extract of propolis 3 % in a dose of 1.5 ml / liter in drinking water for a week.

Clinical signs were recorded and the faecal samples were daily collected from the rabbits for 15 days between 11-25 days post infection, and then faecal oocyst outputs were counted. On the 25th day post *E. stiedae* infection, the red and white blood corpuscles were counted and haemoglobin (Hb) concentration was measured, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) enzymes activity, total protein, albumin, globulins, total cholesterol, serum urea and creatinine were determined. On the same day, bile samples were collected from the gall bladders and then billary oocysts / ml bile were counted. Post mortem lesions of livers were recorded and the liver gross lesions were scored in the sacrificed rabbits. Fresh liver tissue specimens of the sacrificed rabbits were fixed in 10 % neutral buffered formalin saline for the histopathological study. The body weights of rabbits were recorded at day of infection and then weekly consequently the body gain and feed conversion rate were calculated.

The rabbits exhibited decrease of the appetite, marked emaciation, semisolid faecal droppings and distension of the abdomen. Visible liver nodules were decreased by treatment with ethanol extracted propolis 3 % or toltrazuril 2.5 %. On the 25th day post *E. stiedae* inoculation, the rabbits treated with ethanol extracted propolis showed a significant increase of RBCs count and haemoglobin concentration (Hb) when compared with those of toltrazuril-treated group and the positive control. The total WBCs, heterophils counts and heterophil / leucocyte ratio were significantly decreased in rabbits treated with ethanol extracted propolis compared with that of rabbits treated with toltrazuril. The ALT, AST enzyme activities, total cholesterol, creatinine and urea were significantly decreased in rabbits treated with ethanol extracted propolis, meanwhile the albumin and total proteins levels were significantly increased in rabbits treated with ethanol extracted propolis compared with that of rabbits treated with toltrazuril.

From day 15 to 25 PI, the faecal oocyst outputs were significantly decreased in treated groups compared with positive control group. Similarly on the 25th day PI, the oocyst output in bile and the liver lesion scores were decreased in treated rabbits compared with that of positive control. The rabbits inoculated with *E. stiedae* and treated with ethanol extracted propolis showed an increase in both body weight and gain with improvement in FCR when compared with those treated with toltrazuril and the positive control. The histopathological changes showed that the rabbits treated with ethanol extracted propolis or toltrazuril showed fewer developmental stages and oocysts of *E. stiedae* in the epithelial linning of bile ductules with slight destruction of epithelial lining of bile ductules besides mild cystic formation.

It could be concluded that the anticoccidial efficacy of the ethanol extracted propolis was nearly similar to that of toltrazuril drug. On the other hand, the ethanol extracted propolis decrease the stresses on the infected rabbits with fewer side effects than toltrazuril resulted in improvement of growth performance.

INTRODUCTION

Hepatic coccidiosis caused by *Eimeria stiedae* in rabbits, is known to modify the morphological and physiological properties of liver resulting in structural and functional alterations similar to those appearing in human hepatic amoebiasis *(1)*. The development of resistance to the anticoccidial drugs is the major problem in poultry and rabbit industries. Anticoccidial drug-resistance occurs when the *Eimeria* multiply and / or survive in the presence of a therapeutic concentration of the anticoccidial drug which normally destroy the parasite or prevents its multiplication *(2)*.

Propolis (bee glue) has a long history of being used as a remedy, dating back to times of ancient Greece and Rome. Nowadays, it is still used for treatment of various diseases, and in products like health foods and biocosmetics because of its versatile biological activities *(3)*. Most Brazilian propolis has antibacterial, antimycotic and antiradical activities, depending upon its plant source and chemical composition. Propolis is the chemical weapon of bees against pathogenic microorganisms and the elements of weather *(4)*. However, different chemical constituents are responsible for the valuable activities of the different propolis types *(5)*. Typical propolis has approximately 50 constituents, primarily resins and vegetable balsams (50 %), waxes (30 %), essential oils (10 %) and pollens (5 %). Propolis is sticky at and above room temperature. At lower temperatures, it becomes hard and very brittle *(4)*. Isovlavonoides are important antimicrobial components of the red propolis, especially concerning the activity against *Candida albicans (4)*. This is not surprising, taking into consideration that petrocarpans are known for their antifungal activities *(6)*.

Recently a great attention has been paid to the natural medication. Propolis is a natural composite balsam, produced by honey bees from the gum of various plants. Bees combine the balsam with the bee wax, pollen and their own enzymes. Propolis is used by bees in their hives as antibiotics. Propolis has strong antibacterial activities *(7 , 8)*, antifungal activities *(9)*, as well as antiprotozoal activity and immune system booster *(10 , 11)*. Propolis has anti-inflammatory and hepatoprotective properties *(12 , 13)*.

The anticoccidial activity of propolis on intestinal and hepatic coccidiosis of rabbits was studied by several authors *(14-18)*. Hollands *et al (15 ,16)* found that the coccidiostatic effect of 3 % alcoholic propolis solution was superior to that of two sulphonamides in rabbits infected with *E. magna, E. media* and *E. perforans*, Moura *et al (17)* evaluated the antiprotozoal activity of hydroalcoholic propolis solution (HPS) and robenidene on intestinal *Eimeria* infections in rabbits, while El-Akabawy *et al (18)* evaluated the effect of the aqueous solution of propolis and toltrazuril on *E. stiedae* in experimentally infected young New Zealand white rabbits.

Toltrazuril can thus be used for supplemented control with in-feed anticoccidials or as a primary anticoccidial with non medicated feed. The drug prevented mortality in chickens infected with field isolate of *E. tenella* with complete absence of oocyst production in faeces *(19)*. Toltrazuril most completely eliminated all coccidial lesions and dramatically reduced oocyst shedding after its administration in drinking water at the rate of 7 mg / kg b. wt. for continuous 48 hr *(20)*. The use of toltrazuril for two consecutive days in drinking water between 10 and 14 days of age would be the best time for good coccidiosis control *(21)*.

Toltrazuril in a dose of 75 or 150 ppm in drinking water gave the best protection when administered for 48 hrs especially at 4 and 5 days PI *(22)*. Toltrazuril characterized by short application periods, rapid mode of action and

significant reduction of oocyst shedding *(23)*. The effect of toltrazuril could be due to the long-existing tissue levels of the product and its metabolites as well as its specific effect on the second generation of schizonts *(24)*.

Propolis supplementation at 100 and 150 mg / kg diet is beneficial for improving the performance and immunity in commercial laying hens. Propolis significantly decreased the heterophils count and increased lymphocyte count when compared with the control group *(25)*. In birds, the heterophils are phagocytic cells whose main function is protection against invading microorganisms whereas primary functions of lymphocytes involve cell-mediated and humeral immunity.

In rabbits, ALT, AST enzyme activities were decreased in treated rabbits with aqueous extracted propolis compared with toltrazuril-treated ones *(18,26)*. In chickens, propolis significantly increased plasma total protein and globulin compared to the control group *(25, 27,28)*, whereas there was no significant difference among treated groups for plasma albumin. Propolis significantly decreased cholesterol, ALT and AST levels compared to the control group *(25)*. On the other hand ALT and AST activities returned to the control level after administration of propolis in rats infected with *Staph. aureas* and *E. coli (29)*. Propolis enhances protein biosynthesis *(30, 31)*.

Therefore, the present study was designed to investigate the efficacy of ethanol extracted propolis on *E. stiedae* infection in the rabbits compared with toltrazuril drug .

MATERIAL AND METHODS

Animals

Fifty five (55) apparently healthy and coccidia-free New Zealand white male rabbits (average body weight of 757.60 ± 1.63 grams) were used. The rabbits were put under observation with daily faecal examination for one week for excluding any animal infected with *Eimeria* or any illness. Seven rabbits were slaughtered after a rest time (one week) for post mortem examination to ensure that the rabbits are free from coccidiosis. The rest 48 rabbits were then divided into four equal groups each of twelve in a separate wire-floored metal cage and given pelleted feed and previously boiled then cooled drinking water *ad libitum*.

Coccidia parasite

The oocysts of *E. stiedae* were collected from the gallbladder and liver lesions of naturally infected rabbits with hepatic coccidiosis. The collected oocysts were suspended in 2.5% potassium dichromate solution and incubated at 28°C for 4 days (the period for maximum sporulation), and then the oocysts were washed 2-3 times with distilled water by centrifugation for 10 minutes at 1500 rpm. The *Eimeria* oocysts were propagated in two *Eimeria*-free rabbits (4-5 weeks old) to obtain sufficient number of *E. stiedae* oocysts. The oocysts were allowed to sporulate, preserved in 2.5% potassium dichromate solution and then stored at 4-8°C until used. Before inoculation, the oocysts were washed by centrifuge and counted by haemocytometer *(32)*. Faecal and biliary oocysts of *E. stiedae* were counted by McMaster technique *(33 , 34)* allover the experiment.

Drugs

1-Toltrazuril 2.5 %, BAYCOX® (IFT Co.): Toltrazuril is used in a dose of 7 mg / kg b. wt. (2.8 ml toltrazuril / 10 liter) continuously for 48 hr in drinking water (according to the producer).

2-Propolis: Propolis used in this study was collected by scrapping off the frames of bee hives located on Kaliobia governorate, Egypt. The samples were kept at -10°C until used. Ethanol Extracted Propolis (EEP) was prepared at a concentration of 3 % according to Salomao *et al (35)*. After 24 hr, the extracts were filtered, evaporated to dry under vacuum at 40°C and stored in a desiccator at 4°C in clean and tightly closed dark bottles until used *(36)*.

Methods

Each rabbit of the second, third and fourth group was orally inoculated with 5 x 10^4 sporulated oocyst of *E. stiedae* by a stomach tube *(34)*. The rabbits of the first group were left

non-infected, non-drug treated and served as a negative control. The rabbits of the second group were inoculated with *E. stiedae* oocysts and not treated (Positive control). On the other hand, the rabbits of the third group were treated with 7 mg / kg b. wt. of toltrazuril at a rate of 2.8 ml / 10 liter continuously for 48 hr (2 consecutive days) in drinking water, while the rabbits of the fourth group were treated with alcoholic extract of propolis 3 % in a dose of 1.5 ml / liter in drinking water for a week according to Salomao *et al (35).*

Sampling and methods of investigations

- Any clinical signs on the rabbits of all groups were recorded along the experimental period.
- Faecal samples were daily collected from the rabbits of all groups for 15 days between 11-25 days post infection (PI), and the oocysts were counted in 1 gm of faecal matter by the Mc Master technique *(33).*
- On the 25th day post inoculation, blood samples were collected from three rabbits of each group and divided into two portions, the first portion was taken on heparin for red and white blood corpuscle count and haemoglobin (Hb) concentration *(37).* The second portion was kept for serum separation for determination of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) enzymes activity *(38),* total protein *(39),* albumin *(40),* globulins (the difference between total protein and albumin), cholesterol *(41),* urea *(42)* and creatinine *(43).* This rabbits were then sacrificed and the post mortem examination and liver lesion scores were recorded *(44).*
- Bile samples were then collected from the gall bladders of the sacrificed rabbits of all groups and the oocysts of *E. stiedae* were counted per 1 ml bile *(34).*
- Fresh liver tissue specimens of the sacrificed rabbits on the 25th day post infection were fixed in 10% neutral buffered formalin for the histopathological examination *(45).*
- The body weights of rabbits of all groups were recorded at the day of infection and then weekly with feed consumption till the end of experiment, and the body weight gain and feed conversion rate were calculated *(46).*
- The obtained data were statistically analysed by Duncan multiple range test *(47)* using a computer program *(48).*

RESULTS

Clinical signs

The infected rabbits showed a gradual decrease of the appetite in the first week, marked emaciation on the 3rd week, semisolid faecal droppings in some cases and distension of the abdomen by the end of the experimental period. One rabbit from the positive control (infected, non-treated) died in the 3rd week post infection.

Oocyst output in faeces

All the infected (treated and non-treated) groups of rabbits began to excrete oocysts of *E. stiedae* in their faeces from the 15th day post inoculation (PI). The daily oocyst output per gram of faeces increased until the 23rd day PI (the peak of oocyst production) with significant increase in group 2 (inoculated and non treated) than the treated groups, then began to decrease from the 24th day PI. However, there were no significant changes of faecal oocyst output between group 3 (toltrazuril-treated) and group 4 (propolis-treated) (Table, 1).

Haematological parameters

On the 10th day post last drug treatment (25 day post infection), the rabbits treated with ethanol extracted propolis 3 % showed a significant increase of RBCs count and haemoglobin concentration (Hb gm/dl) when compared with those of toltrazuril-treated group and the positive control (inoculated and not treated). The total WBCs count, heterophils count and heterophil/ leucocyte ratio were significantly decreased in rabbits treated with ethanol extracted propolis compared with that of rabbits treated with toltrazuril at $P \leq 0.05$ (Table 2).

Some serum biochemical constituents

On the 25th day post infection, the albumin and total proteins concentrations were significantly increased in rabbits of group 4 (treated with ethanol extracted propolis 3%) when compared with that of rabbits of group 3 (treated with toltrazuril 2.5%). On the other hand, ALT and AST enzyme activities, the total serum cholesterol, creatinine and urea were significantly

decreased in rabbits of group 4 compared with that of group 3 at P≤0.05 (Table 3).

Post mortem findings

The infected rabbits showed gastro-intestinal distension with gases, visible and sometimes protruded liver nodules which decreased by treatment with ethanol extracted propolis 3 % or toltrazuril 2.5 %.

Oocyst output in the bile

On the 25th day PI, there was a statistically significant decrease of oocyst output in the bile of rabbits treated with either propolis (group 4) or toltrazuril (group 3) when compared with that of positive control rabbits (group 2). However, there were no significant changes of billary oocyst output between group 3 and group 4 (Table 4).

Liver lesion scores

On the 25th day PI, there was a significant decrease in the liver lesion score in rabbits treated with propolis or toltrazuril when compared with that of rabbits inoculated and not treated (Table 5).

Histopathological findings

The histopathological changes are recorded in Table (6)

Growth performance

At the end of expirement, the rabbits inoculated with *E. stiedae* and treated with ethanol extracted propolis (3%) showed a significant increase in both body weight and weight gain and a significant decrease in FCR when compared with those treated with toltrazuril (2.5%) and the positive control. Hence, the rabbits treated with propolis showed a significant improvement in their growth performance than that treated with toltrazuril (Table 7).

Table 1. Oocyst output per gram faeces / rabbit (x10^3) post inoculation with 5 x 10^4 sporulated oocysts of *E. stiedae*. (Mean ± SE, n=12)

Days PI	Oocyst output per gram faeces / rabbit (x 10^3) at groups			
	(1)	(2)	(3)	(4)
11	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
12	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
13	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
15	0.00 ^{c} ± 0.00	32.67 ^{a} ± 1.10	6.92 ^{b} ± 0.34	7.52 ^{b} ± 0.26
16	0.00 ^{c} ± 0.00	44.83 ^{a} ± 1.21	10.33 ^{b} ± 0.31	10.73 ^{b} ± 0.37
17	0.00 ^{c} ± 0.00	65.58 ^{a} ± 1.05	11.75 ^{b} ± 0.33	12.17 ^{b} ± 0.21
18	0.00 ^{c} ± 0.00	86.33 ^{a} ± 0.94	13.33 ^{b} ± 0.28	14.25 ^{b} ± 0.37
19	0.00 ^{c} ± 0.00	100.67 ^{a} ± 1.33	17.75 ^{b} ± 0.41	18.00 ^{b} ± 0.56
20	0.00 ^{c} ± 0.00	132.33 ^{a} ± 1.82	26.75 ^{b} ± 1.00	29.42 ^{b} ± 1.51
21	0.00 ^{c} ± 0.00	159.75 ^{a} ± 2.94	37.83 ^{b} ± 1.07	39.52 ^{b} ± 0.69
22	0.00 ^{c} ± 0.00	231.75 ^{a} ± 3.90	40.42 ^{b} ± 0.81	41.52 ^{b} ± 0.87
23	0.00 ^{c} ± 0.00	348.00 ^{a} ± 3.92	38.50 ^{b} ± 1.08	38.78 ^{b} ± 0.94
24	0.00 ^{c} ± 0.00	298.33 ^{a} ± 3.38	28.75 ^{b} ± 1.19	29.42 ^{b} ± 1.26
25	0.00 ^{c} ± 0.00	252.33 ^{a} ± 2.76	11.25 ^{b} ± 1.57	13.58 ^{b} ± 1.64
Overall mean	0.00 ^{c} ± 0.00	159.33 ^{a} ± 6.84	22.14 ^{b} ± 1.65	23.17 ^{b} ± 1.72
Production %	0	100	13.90	14.54
Reduction %	0	0	86.10	85.46

Data were analyzed by One Way ANOVA.
Means with different alphabetical superscripts in the same row are significantly different using Duncan test at P ≤ 0.05.
Group (1): Negative control (Non- inoculated and non-treated).
Group (2): Positive control (Inoculated and non-treated).
Group (3): Inoculated and treated with Toltrazuril (2.5%).
Group (4): Inoculated and treated with Ethanol Extracted Propolis (3%).

Table 2. Some haematological parameters in rabbits on the 25th day post inoculation with 5 x 10^4 sporulated oocysts of *E. stiedae*. (Mean ± SE, n=5)

Group / Parameters	(1) -Ve control (Non-inoculated and non-treated)	(2) +Ve control (Inoculated and non-treated)	(3) Inoculated and treated with Toltrazuril (2.5%)	(4) Inoculated and treated with Ethanol Extracted Propolis (3%)
Erythrocytes (RBCs) (cell x10^6/ml)	$6.01^{a} \pm 0.13$	$4.56^{b} \pm 0.10$	$4.81^{b} \pm 0.16$	$5.85^{a} \pm 0.05$
Haemoglobin (Hb) (gm/dl)	$13.97^{a} \pm 0.19$	$11.71^{b} \pm 0.07$	$11.68^{b} \pm 0.33$	$13.77^{a} \pm 0.10$
Total Leucocytes (L) (WBCs) (cell x10^3/ml	$6.16^{a} \pm 0.12$	$5.74^{b} \pm 0.07$	$4.75^{c} \pm 0.09$	$4.17^{d} \pm 0.13$
Heterophils (H)	$3.98^{a} \pm 0.26$	$3.15^{b} \pm 0.19$	$3.19^{b} \pm 0.10$	$2.35^{c} \pm 0.09$
H / L Ratio	0.52	0.60	0.74	0.51

Data were analyzed by One Way ANOVA. L = Total Leucocytes (WBCs). H = Heterophils
Means with different alphabetical superscripts in the same row are significantly different using Duncan test at $P \leq 0.05$.

Table 3. Some biochemical parameters in rabbits on the 25th day post inoculation with 5 x 10^4 sporulated oocysts of *E. stiedae*. (Mean ± SE, n=5)

Group / Parameters	(1) -Ve control (Non-inoculated and non-treated)	(2) +Ve control (Inoculated and Non-treated)	(3) Inoculated and treated with Toltrazuril (2.5%)	(4) Inoculated and treated with Ethanol Extracted Propolis (3%)
Total Protein (gm/dl)	$5.29^{a} \pm 0.03$	$4.48^{d} \pm 0.50$	$4.96^{c} \pm 0.02$	$5.11^{b} \pm 0.03$
Serum albumin (gm/dl)	$2.76^{a} \pm 0.02$	$2.35^{c} \pm 0.02$	$2.57^{b} \pm 0.03$	$2.73^{a} \pm 0.02$
Serum globulin (gm/dl)	$2.54^{a} \pm 0.04$	$2.13^{b} \pm 0.05$	$2.39^{a} \pm 0.03$	$2.44^{a} \pm 0.10$
ALT (IU/L)	$12.36^{d} \pm 0.05$	$19.43^{a} \pm 0.11$	$16.02^{b} \pm 0.07$	$13.94^{c} \pm 0.19$
AST (IU/L)	$23.69^{d} \pm 1.21$	$45.38^{a} \pm 1.55$	$33.52^{b} \pm 1.01$	$29.14^{c} \pm 0.79$
Total cholesterol (mg/dl)	$126.21^{c} \pm 0.28$	$152.78^{a} \pm 0.99$	$1390.08^{b} \pm 0.36$	$128.54^{c} \pm 2.18$
Serum Urea (mg/dl)	$50.09^{c} \pm 0.54$	$71.10^{a} \pm 2.18$	$57.67^{b} \pm 0.65$	$52.25^{c} \pm 0.25$
Serum Creatinine (mg/dl)	$0.846^{c} \pm 0.010$	$1.234^{a} \pm 0.015$	$0.942^{b} \pm 0.024$	$0.868^{c} \pm 0.007$

Data were analyzed by One Way ANOVA.
Means with different alphabetical superscripts in the same row are significantly different using Duncan test at $P \leq 0.05$.

Table 4. Oocyst output per ml bile / rabbit (x10^4) on the 25th day post inoculation with 5 x 10^4 sporulated oocysts of *E. stiedae*. (Mean ± SE, n=12)

Group / Parameters	(1) -Ve control (Non-inoculated and non-treated)	(2) +Ve control (Inoculated and non-treated)	(3) Inoculated and treated with Toltrazuril (2.5%)	(4) Inoculated and treated with Ethanol Extracted Propolis (3%)
Oocyst output per 1ml of bile (x10^4)	0.00 ^{c} ± 0.00	49.20 ^{a} ± 1.53	6.00 ^{b} ± 0.37	6.90 ^{b} ± 0.43
Production %	0	100	12.20	14.03
Reduction %	0	0	87.81	85.98

Data were analyzed by One Way ANOVA.
Means with different alphabetical superscripts in the same row are significantly different using Duncan test at $P \leq 0.05$.

Table 5. Liver lesion scores in rabbits on the 25th day post inoculation with 5 x 10^4 sporulated oocysts of *E. stiedae*. (Mean ± SE, n=12)

Group (1)	Group (2)	Group (3)	Group (4)
-Ve control (Non-inoculated and non-treated)	+Ve control (Inoculated and non-treated)	Inoculated and treated with Toltrazuril (2.5%)	Inoculated and treated with Ethanol Extracted Propolis (3%)
0.00 ^{c} ± 0.00	3.83 ^{a} ± 0.17	2.53 ^{b} ± 0.10	2.60 ^{b} ± 0.08

Data were analyzed by One Way ANOVA.
Means with different alphabetical superscripts in the same row are significantly different using Duncan test at $P \leq 0.05$.

Table 6. The histopathological findings in rabbits on the 25th day post inoculation with 5 x 10^4 sporulated oocysts of *E. stiedae* and treated with Toltrazuril or Ethanol Extracted Propolis.

Group	Lesions of liver on the 10th day post treatment cessation (25th day PI)
(1)	Normal histological structure
(2)	- Numerous developmental stages and oocysts of *E. stiedae* in the epithelial linning of bile ductules. (Fig., 1-a). - Severe cystic formation, hyperplasia of bile ductules which were completely occluded with numerous developmental stages of *E .stiedae* in their lumens. (Fig.1-b).
(3)	- Few developmental stages and oocysts of *E. stiedae* in the epithelial linning of bile ductules. (Fig., 2-a). - Mild cystic formation of bile ductules. (Fig.2-b).
(4)	- Few developmental stages and oocysts of *E. stiedae* in the epithelial linning of bile ductules. (Fig., 3-a). - Slight destruction of epithelial lining of bile ductules with mild cystic formation. (Fig.3-b).

Group (1): Negative control (Non-inoculated and non-treated).
Group (2): Positive control (Inoculated and non-treated).
Group (3): Inoculated and treated with toltrazuril (2.5%).
Group (4): Inoculated and treated with Ethanol Extracted Propolis (3%).

Table 7. Growth performance parameters in rabbits post inoculation with 5 x 10^4 sporulated oocysts of *E. stiedae*. (Mean ± SE, n=12).

Days PI	Growth performance parameters	Group (1)	Group (2)	Group (3)	Group (4)
Initial Body weight	Body weight (gm)	757.83 ^{a} ± 4.16	756.00 ^{a} ± 3.51	757.33 ^{a} ± 3.39	759.25 ^{a} ± 3.34
Day of inoculation	Body weight (gm)	1267.08 ^{a} ± 3.92	1267.92 ^{a} ± 3.56	1270.00 ^{a} ± 2.89	1269.58 ^{a} ± 3.05
7	Body weight (gm)	1387.50 ^{a} ± 4.71	1327.92 ^{b} ± 2.91	1325.42 ^{b} ± 2.73	1330.83 ^{b} ± 6.24
	Body gain (gm)	120.42 ^{a} ± 6.14	68.33 ^{b} ± 8.40	53.75 ^{b} ± 4.89	54.58 ^{b} ± 2.78
	FCR	1.57 ^{b} ± 0.03	2.13 ^{a} ± 0.03	2.15 ^{a} ± 0.02	2.19 ^{a} ± 0.03
14	Body weight (gm)	1565.67 ^{a} ± 7.94	1385.42 ^{c} ± 4.58	1441.25 ^{b} ± 3.80	1447.92 ^{b} ± 3.50
	Body gain (gm)	167.08 ^{a} ± 10.29	69.17 ^{c} ± 8.77	117.50 ^{b} ± 5.17	124.58 ^{b} ± 5.24
	FCR	1.71 ^{c} ± 0.03	3.03 ^{a} ± 0.05	2.31 ^{b} ± 0.04	2.21 ^{b} ± 0.02
21	Body weight (gm)	1615.83 ^{a} ± 5.57	1433.75 ^{d} ± 4.85	1516.67 ^{c} ± 3.45	1542.50 ^{b} ± 5.42
	Body gain (gm)	105.00 ^{a} ± 5.19	49.17 ^{c} ± 6.39	75.42 ^{b} ± 4.43	85.25 ^{b} ± 7.54
	FCR	1.91 ^{d} ± 0.03	3.27 ^{a} ± 0.04	2.24 ^{b} ± 0.04	2.41 ^{c} ± 0.03
28	Body weight (gm)	1689.58 ^{a} ± 6.42	1511.25 ^{d} ± 5.23	1563.75 ^{c} ± 5.15	1628.33 ^{b} ± 4.41
	Body gain (gm)	108.75 ^{a} ± 8.38	35.42 ^{c} ± 10.05	62.08 ^{b} ± 7.47	95.17 ^{a} ± 6.77
	FCR	1.89 ^{c} ± 0.03	3.34 ^{a} ± 0.05	2.26 ^{b} ± 0.05	1.99 ^{c} ± 0.04

Data were analyzed by One Way ANOVA.

Means with different alphabetical superscripts in the same row are significantly different using Duncan test at $P \leq 0.05$.

Group (1): Negative control (Non- inoculated and non-treated).

Group (2): Positive control (Inoculated and non-treated).

Group (3): Inoculated and treated with toltrazuril (2.5%).

Group (4): Inoculated and treated with ethanol extracted propolis (3%).

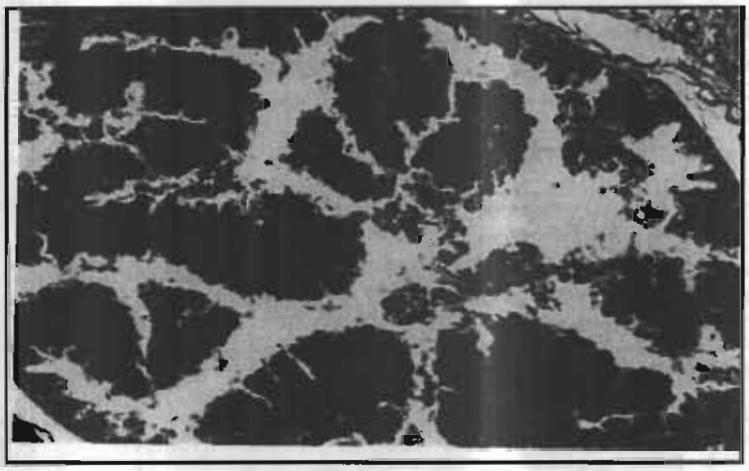

Fig. 1-a. Photomicrograph of the liver section of a rabbit inoculated with *E. stiedae* and not treated (positive control) on the 25th day PI, showing numerous developmental stages of *E. stiedae* in the epithelial lining of severely destructed bile ductules.(H & E, x 400).

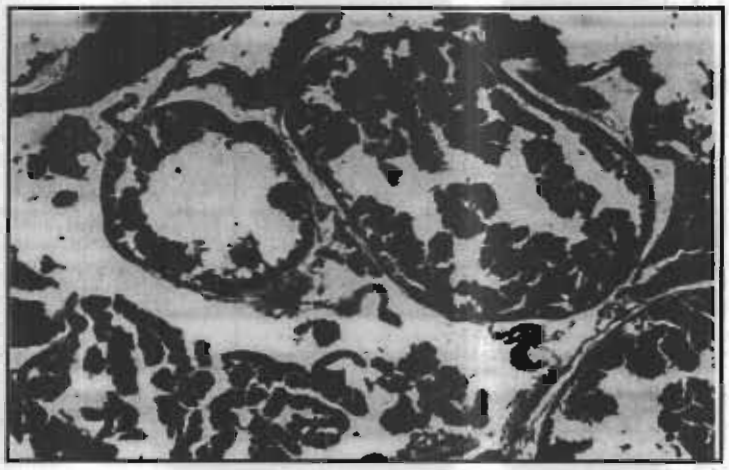

Fig. 1-b. Photomicrograph of the liver section of a rabbit inoculated with *E. stiedae* and not treated (positive control) on the 25th day PI, showing great number of cystic formation of bile ductules causing pressure atrophy of the surrounding hepatic parenchyma.(H & E, x 40).

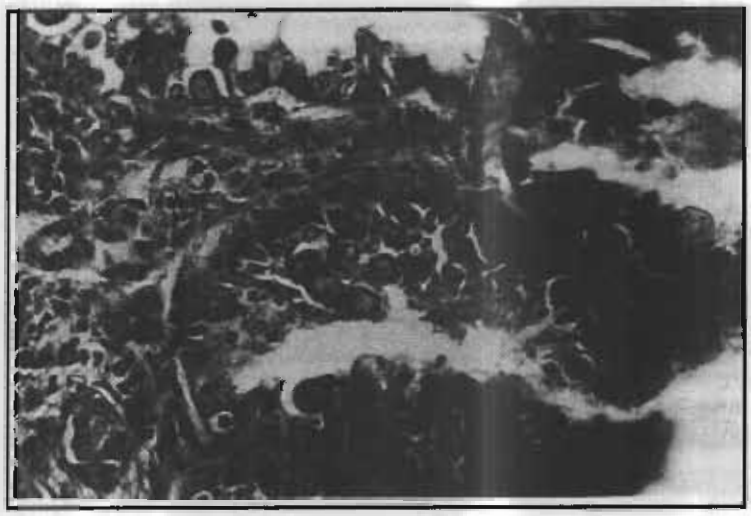

Fig. 2-a. Photomicrograph of the liver section of a rabbit on the 25th day PI with *E. stiedae* and treated with toltrazuril (2.5%) showing few number of different developmental stages of the parasite in the moderately destructed bile ductules.(H & E, x 400).

Fig. 2-b. Photomicrograph of the liver section of a rabbit on the 25th day PI with *E. stiedae* and treated with toltrazuril (2.5 %) showing few number of cystic formation of bile ductules surrounded by fibrous connective tissue.(H & E, x 40).

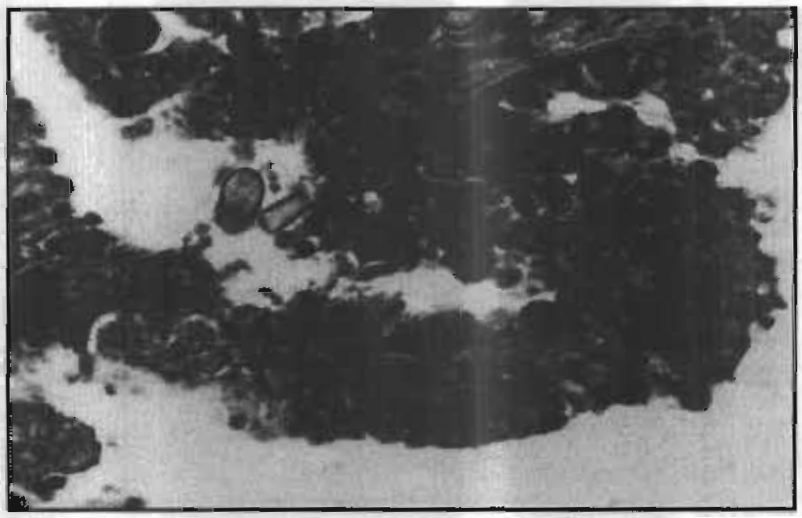

Fig. 3-a. Photomicrograph of the liver section of a rabbit on the 25th day PI with *E. stiedae* and treated with ethanol extracted propolis (3 %) showing few number of different developmental stages of the parasite without destruction of the epithelial lining of bile ductules.(H & E, x 400).

Fig. 3-b. Photomicrograph of the liver section of a rabbit on the 25th day PI with *E. stiedae* and treated with ethanol extracted propolis (3 %) showing very few cystic formation of bile ductules which surrounded by connective tissue.(H & E, x 40).

DISCUSSION

The hepatic coccidiosis of rabbits, caused by the sporulated oocysts of *E. stiedae*, remains one of the dangerous and common disease of Egyptian rabbits causing economic losses in rabbit production. The resistance of coccidia against many anticoccidial drugs causes an important problem in controlling the disease. The ethanol extracted propolis (one of natural origin) has been proven to be effective in controlling coccidiosis ***(14-16)***.

The current study revealed a significant reduction of the oocyst output in either the bile or faeces and a significant reduction of the liver lesion scores in rabbits treated with 3 % ethanol extracted propolis or toltrazuril when compared with that of the positive control rabbits. The reduction percent of faecal oocysts was 85.46 % and 86.10 % in propolis- and toltrazuril-treated rabbits, respectively. Also, the reduction percent of bile was 87.81 % and 85.98 % in propolis- and toltrazuril-treated rabbits, respectively. Our results agreed with those of Hollands *et al* ***(14)*** who used 3% alcoholic solution of propolis and reported 92.2% reduction in the number of oocysts per gram of intestinal coccidiosis in rabbits, and that recorded by El-Akabawy *et al* ***(18)*** who found that the aqueous solution of propolis has a similar effect with toltrazuril drug on *E. stiedae* infection in young rabbits. Ethanol extracted propolis decreased the number of the oocysts of *E. tenella* in the feces together with developmental endogenous stages and cecal lesions which led to improved gain in the body weight in chickens ***(49)***. Meanwhile, it has been found that the coccidiostatic effect of 3% alcoholic propolis solution was superior to that of two sulphonamides in rabbits infected with *E. magna, E. media* and *E. perforans* ***(15,16)*** The anticoccidial activity of hydroalcoholic propolis solution (HPS) was better than robenidene on intestinal *Eimeria* infections in rabbits ***(17)***.

The haematological study on the 10th day post last drug treatment (25 day post infection) showed that the rabbits treated with ethanol extracted propolis 3 % showed a significant increase of RBCs count and haemoglobin concentration (Hb) when compared with those of toltrazuril-treated group and the positive control (inoculated and not treated). This may be due to the direct anticoccidial effect of ethanol extracted propolis besides its indirect activities (antimicrobial, antifungal, anti inflammatory and hepatoprotective). Similar was recorded results ***(25)***. On the other hand, the total WBCs and heterophils counts as well as heterophils / leucocytes ratio were significantly decreased in rabbits treated with ethanol extracted propolis compared with that of rabbits treated with toltrazuril. This may be due to the propolis decreased the heterophils count related to the total leuckocytic count and this decreased the stresses effects on the infected rabbits when compared with the toltrazuril-treated and positive control groups. The propolis supplementation at 100 and 150 mg / kg diet is beneficial for improving the performance and immunity in commercial laying hens and significantly decreased the heterophils count when compared with the control group in birds ***(25)***.

In the present study, the hepatic degenerative and necrotic lesions reflected on serum parameters of infected and non-treated rabbits (group 2) through a significant increase in AST and ALT activities with hypoproteinaemia and hypoalbuminamia. Previous studies ***(50,51)*** demonstrated similar results. Levels of ALT and AST enzyme activities were significantly decreased in treated rabbits with propolis when compared with those of toltrazuril-treated ones. Propolis decreased ALT and AST serum levels in rabbits ***(18,26)***. The activity of ALT and AST enzyme returned to the control level after administration of propolis in rats infected with *S. aureos* and *E. coli(29)*. In our results, toltrazuril showed a significant increase in ALT and AST enzyme activities compared with the negative control and propolis treated-rabbits. In calves experimentally infected with *Neospora canimium* showed similar findings ***(52)***. Meanwhile, the high levels of AST and hypoalbuminaemia in case of rabbit coccidiosis were improved till reached within normal value after 28 days post treatment with toltrazuril ***(53)***. Total cholesterol, urea and creatinine showed a

significant decrease in rabbits inoculated with *E. stiedae* and treated with propolis when compared with toltrazuril-treated ones. Similar results were obtained in laying hens *(25)*.

Regarding to the body weight, weight gain and FCR, Table 6 showed an increase in both of body weight, weight gain and an improvement in FCR in rabbits of group 4 (treated with propolis) when compared with those of group 3 (treated with toltrazuril). This may be either due to the direct anticoccidial effect of propolis on *E. stiedae* on intestinal coccidiosis in rabbits *(14-17)* or to the indirect effects as strong antibacterial *(7,8)*, antifungal *(9)*, immune system booster *(10,11)* and anti-inflammatory and hepato-protective agent *(12, 13)*.

The present histopathological studies showed that the rabbits treated with ethanol extracted propolis 3% showed few developmental stages and oocysts of *E. stiedae* in the epithelial linning of bile ductules and slight destruction of epithelial lining of bile ductules with mild cystic formation. These findings are nearly similar to those in rabbits treated with toltrazuril 2.5%. While they were all the best when compared with those of positive control (infected and non-treated) which showed numerous developmental stages and oocysts of *E. stiedae* in the epithelial linning of bile ductules and severe cystic formation, hyperplasia of bile ductules which were completely occluded with numerous developmental stages of *E .stiedae* in their lumens. In New Zealand white rabbit similar effect was recorded *(18)*.

It could be concluded that the ethanol extracted propolis has nearly similar anticoccidial efficacy to that of toltrazuril drug. On the other hand, the propolis decreased ALT, AST activities and cholesterol in the infected rabbits with fewer side effects than toltrazuril. Propolis also relief the stresses caused by *E. stiedae* infection through reduction of heterophils / leuckocytes ratio toward the normal. Consequently the propolis resulted in improvement of growth performance (body weight, weight gain and FCR).

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الملخص العربى

دراسة مقارنة على تأثيرمستخلص البروبوليز والتولترازوريل فى الأرانب المعدية بالأيميريا ستيدى

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استخدمت فى هذه الدراسة 48 من ذكور الأرانب النيوزيلاندى البيضاء السليمة والخالية من الكوكسيديا (بمتوسط وزن 757.6 جرام) حيث قسمت الأرانب إلى أربعة مجموعات متساوية فى أقفاص منفصلة. أرانب المجموعات الثانية والثالثة والرابعة تم عدواها بحويصلات الأيميريا ستيدى المتجرثمة (5 X 10^4 لكل أرنب) بإستخدام أنبوب اللى المعدى. المجموعة الأولى من الأرانب استخدمت كضابط سلبى للتجربة (غير معدية وغير معالجة)، والمجموعة الثانية تم عدواها بحويصلات الأيميريا ستيدى المتجرثمة ولم تعالج (ضابط إيجابى), عند أسبوع من العدوى المجموعة الثالثة عولجت بدواء التولترازوريل بجرعة 7 مجم / كجم وزن حى (بمعدل 2,5 ملليلترتولترازوريل على 10 لتر ماء) فى مياه الشرب لمدة 48 ساعة متواصلة. بينما المجموعة الرابعة عولجت بالمستخلص الكحولى للبروبوليز(3 %) بجرعة 1,5 ملليلتر على 1 لتر ماء لمدة أسبوع فى مياه الشرب. الأعراض تم تسجيلها طوال فترة التجربة, وعينات البرازجمعت يوميا ولمدة 15 يوما إبتداء من اليوم الحادى عشر وحتى اليوم الخامس والعشرين بعد العدوى, وتم تحديد عدد الحويصلات لكل 1 جم براز. فى اليوم الخامس والعشرين من العدوى بالأيميريا ستيدى, عينات الدم تم تجميعها من ثلاثة أرانب لكل مجموعة بعد الذبح لعد كرات الدم الحمراء والبيضاء وقياس الهيموجلوبين, ولتجهيز عينات السيرم لقياس مستوى كل من ALT AST , والبروتين الكلى والألبيومين و الجلوبيولين والكوليستيرول الكلى واليوريا والكرياتينين. عند نفس اليوم, عينات السائل المرارى جمعت من الحويصلة المرارية بعد الذبح وذلك لتحديد عدد الحويصلات لكل 1 ملليلتر سائل مرارى. وبإجراء الصفة التشريحية على الكبد بعد الذبح تم تحديد درجة الإصابة فى الكبد. وتم تثبيت قطع صغيرة من الكبد فى الفورمالين (10 %) للدراسة

الهستوباثولوجية. كما تم وزن الأرانب عند اليوم الأول للعدوى وكذلك أسبوعيا وتم حساب الأوزان المكتسبة ومعدل التحويل الغذائى.

أوضحت النتائج الأعراض على الأرانب متمثلة فى ضعف الشهية, هزال, إسهال, و إنتفاخ فى البطن. وبإجراء الصفة التشريحية إتضح قلة عدد ال Nodules على الكبد بوضوح فى الأرانب المعالجة بمستخلص البروبوليز أو التولترازوريل عند مقارنتها بالمجموعة الضابطة الإيجابية. عند اليوم الخامس والعشرين من العدوى (أى بعد عشرة أيام من آخر جرعة علاج) قد زاد كل من عدد كرات الدم الحمراء ومستوى الهيموجلوبين فى الأرانب المعالجة عند مقارنتها بالمجموعة المعالجة بالبروبولييز والمجموعة الضابطة الإيجابية, بينما قل العدد الكلى لكرات الدم البيضاء و الهتيروفيلز ومعدل الهتيروفيلز / كرات الدم البيضاء فى الأرانب المعالجة بمستخلص البروبوليزمقارنة بتلك المعالجة بالتولترازوريل. وقد قل مستوى كل من ALT, AST والكوليستيرول الكلى واليوريا والكرياتينين فى الأرانب المعالجة بمستخلص البروبوليز بينما إرتفع مستوى الألبيومين والبروتين الكلى فى الأرانب المعالجة بمستخلص البروبوليز وذلك عند مقارنتها بالأرانب المعالجة بالتولترازوريل.

قد تبين من النتائج عند اليوم الخامس والعشرين من العدوى قلة عدد الحويصلات فى كل من السائل المرارى والبراز وكذلك قلة درجة الإصابة فى الكبد للأرانب المعالجة بمستخلص البروبوليز أو التولترازوريل (بدون فرق معنوى بينهما) عند مقارنتها بالمجموعة الضابطة الإيجابية. كما تبين من النتائج أن الأرانب المعالجة بمستخلص البروبوليز كانت الأفضل فى اوزانها والأوزان المكتسبة كما تحسن معدل تحويلها الغذائى بالمقارنة بتلك المعالجة بالتولترازوريل أو المجموعة الضابطة الإيجابية. وبالدراسة الهستوباثولوجية تبين أن قلة أطوار النمو وحويصلات الأيميريا ستيدى فى الخلايا المبطنة للقنيات المرارية مع تدمير طفيف فيها بالإضافة إلى mild cystic formation فى الأرانب المعالجة بمستخلص البروبوليز أو التولترازوريل عند مقارنتها بالمجموعة الضابطة الإيجابية.

نستنتج من هذه الدراسة أن مستخلص البروبوليز الكحولى تقريبا متشابه فى كفاءته كمضاد للكوكسيديا مع دواء التولترازوريل, مع قلة تأثيراته الجانبية وتقليله للضغوط Stresses على الأرانب المصابة مما أدى إلى التحسن الملحوظ فى كفاءة النمو.