STUDIES ON PROPOLIS EXTRACT AND SULFACLOZIN- NATRIC-MONOHYDRATE IN CHICKENS INFECTED WITH EIMERIA TENELLA

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ABSTRACT

One hundred and twenty (120), one day old Ross chicks (male and female) were divided into four equal groups. Groups (1-3) were inoculated with 1000 sporulated oocysts of E.tenella in the crop using a stomach tube at the age of 21 days. Group (1) was treated with 3% Ethanol Extracted Propolis (EEP) at a dose of 1ml / liter in the drinking water for 5 successive days. Group (2) was treated with 30% of Sulfaclozin natric-monohydrate (Esb_3) at a dose of 1g / liter in the drinking water for the same period. Group (3) was infected and not treated (positive control). While the group (4) was neither infected nor treated (negative control). The body weights and body weight-gains of the chickens were evaluated weekly. The daily oocyst outputs per gram of feces (droppings) were counted, using McMaster technique for ten successive days post inoculation (PI) starting from the 5^{th} to the 14^{th} day PI. The lesion scores of E.tenella were evaluated on the 7^{th} day PI. The ceca of the infected chickens were collected at the 6^{th}, 8^{th} and 10^{th} day PI. They were fixed in 10% neutral buffered formalin. Five micron thick paraffin sections were prepared and stained by hematoxylin and eosin, then examined microscopically. The developmental endogenous stages (schizonts, gametocytes and oocysts) were counted per microscopic field from stained sections on the end of the 5^{th} day of the treatment (at the 10^{th} day PI).

The overall mean of oocyst count per gram feces and mean number of developmental endogenous stages (schizonts, gametocytes and oocysts) of E.tenella in the cecal mucosa of Group (1) (infected with E.tenella and treated with Ethanol Extracted Propolis) were significantly decreased when compared with Group (3) (chickens infected with E.tenella and not treated), such a decrease was less significant than Group (2) (infected with E.tenella and treated with Sulfaclozin- natric- monohydrate). The cecal lesion scores of Group (1) were significantly decreased when compared with Group (3). However, it was not significantly changed when compared with Group (2). The mean body

weight-gain in Group (1) at the end of the 6th week of age, was significantly increased than the birds of Groups (2 and 3). Histopathalgically, the epithelial lining of the crypts of Lieberkuhn showed numerous second-generation schizonts, gametocytes and mature oocysts on the 8th and 10th days PI in Group (3). While Groups (1 and 2) showed a few second-generation schizonts, gametocytes and immature oocysts in the epithelial lining of the crypts of Lieberkuhn of the ceca. Slight necrosis and degenerative changes were encountered in the cecal epithelial cells, besides leukocytic infiltration in the lamina propria.

It could be concluded that the Ethanol Extracted Propolis (EEP) decreased the number of the oocysts in the feces (droppings) together with developmental endogenous stages and cecal lestons which led to improved gain in the body weight.

INTRODUCTION

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 (**Burdock, 1998**). Most Brazilian propolis are having antibacterial, antimycotic and antiradical activities, depending upon its plant source and chemical composition. This is due to the role that propolis plays in the hives. It is the chemical weapon of bees against pathogenic microorganisms and the elements of weather (**Trusheva et al, 2006**). However, different chemical constituents are responsible for the valuable activities of the different propolis types (**Bankova, 2005**). 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 become hard and very brittle (**Trusheva et al, 2006**). Isovlavonoides are important antimicrobial components of the red propolis, especially concerning the activity against Candida albicans (**Trusheva et al, 2006**). This is not surprising, taking into consederation that petrocarpans are known for their antifungal activities (**Renyaud et al, 2005**).

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. The bees combine it with the bee wax, pollen and their own enzymes. It is used by bees in their hives as antibiotics. Propolis has strong antibacterial activities (**Keskin et al, 2001 and Santos et al, 2002**), antifungal activities (**Ota et al, 2001**) and antiprotozoal activity and immune system booster (**Velikova et al, 2000 and Murad et al, 2002**). Propolis has anti-inflammatory and hepatoprotective proper-

ties **(Gonzales et al, 1995 and Miyataka et al, 1997)**. The anticoccidial activity of propolis on intestinal and hepatic coccidiosis of rabbits was studied by several authors **(Hollands et al, 1984; Hollands et al, 1988-a &-b; Moura et al, 1998 and El-Akabawy et al, 2004)**. Hollands et al. (1998-a &-b) 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, 1998** evaluated the antiprotozoal activity of hydroalcoholic propolis solution (HPS) and robenidene on intestinal Eimeria infections in rabbits, while **El-Akabawy et al (2004)** evaluated the effect of the aqueous solution of propolis and toltrazuril on E.stiedae in experimentally infected young New Zealand white rabbits.

The preparation of Esb_3 was applied at the 72nd hour from infection with E.tenella in chickens. It led to the degeneration of most of the second-generation schizonts and inhibited their further development **(Penev and Lozanov, 1983)**. The effect of the anticoccidial agents Esb_3, Trimedin, Coccistop and Coccibio were used in the control of E. adenoeides infection in turkeys. The best results were obtained by the use of Esb_3 and the Bulgarian preparation, trimedin. They inhibited the various endogenic developmental stages of the parasite **(Koinarski and Sherkov, 1987)**.

Histopathologically, the ceca infected with E.tenella, showed erosions and desquamation of the mucosal cells. These lesions were slight in the neck region, more severe in the dilated portion, most severe in the mid region, and moderate in the distal area **(Witlock et al, 1975)**. During the infection with E.tenella, early fenestration was seen in the epithelium followed by its disruption. The crypts were easily seen as the disease progressed and in some cases the epithelium became denuded. The infective organism may inhibit the replacement of the degenerating epithelium **(Bayer et al, 1976)**. A twofold increase in both the mucosal and muscular thicknesses was described **(Witlock, 1982)**. The first- and second-generation schizonts of E.tenella showed extensive cecal degenerative changes that finally resulted in a complete loss of the parasitic stage. The degeneration was characterized by loss of the internal structure and the appearance of many intracytoplasmic vacuoles, besides incomplete merogony. The merozoites themselves showed similar degenerative changes including the presence of numerous small vacuoles in the cytoplasm **(Maes et al, 1988)**. Hemorrhage was a major lesion of E.tenella infections, associated with the disruption of the cecal mucosa by the developing parasite **(Allen, 1997)**. The intracellular cycle of E.tenella in chicken intestinal cells involved the maturation of schizonts within the epithelial cell lining the crypt lumens of the ceca. After invasion, these cells detach themselves from the epithelial layer and migrate into the underlying connective tissue, where maturation of the second-generation schizonts takes place. However, the detached epithelial cells, that harbor the parasite and localize in the lamina propria did not undergo apoptosis despite the fact that

they were parasitized and located in an inappropriate microenvironment **(del Cacho et al, 2004)**.

The aim of this work was to evaluate the anticoccidial activity of propolis extract through the parasitological and histopathological parametres as well as the growth performance and lesion scoring in chickens infected with a field strain of E.tenella.

MATERIALS AND METHODS

Birds :

One hundred and twenty (120), one-day-old Ross chicks (male and female) were purchased from Cairo Poultry Company and leg bangled. The chicks were divided into four equal groups (30 chicks per each). The birds were allotted in separate units of metal wire-floored battery after arranging them using the ranking method **(Gardiner and Wehr,1950)**.

Ration and Water :

Chickens were fed on commercial standard balanced ration from Cairo Poultry Comp. It contained crude protein (21%), crude fat (2.7%), crude fibers (2.7%) and metabolizing energy not less than 2950 Kcal /kg ration. The ration and fresh water were offered to the chicks ad-libitum. The ration was sterilized in hot air oven at 65 C^{o} for 18 hr to destroy the probable accidental sporulated oocysts of Eimeria which may contaminate the rations. The water was boiled then cooled before offered to the birds.

Oocyst inoculation :

Oocysts of a field strain of E.tenella were collected from the ceca of naturally infected chickens by the single oocyst isolation technique described by **Karim and Tress (1990)**. The E.tenella oocysts have been previously recognized and identified in the Poultry Diseases Dept., Faculty of Vet. Med., Moshtohor, Benha Univ., since 1999. The parasite was repeatedly passed in one day old chicks every three months. Sporulated oocysts of E.tenella were preserved in 2.5% potassium dichromate solution. Groups (1-3) were inoculated directly intracrop (using stomach tube) with 1000 sporulated oocysts of E.tenella on the 21^{st} day of age. Ten grams of the feces (droppings) were collected daily for ten successive days post inoculation (PI), starting from the 5^{th} to the 14^{th} day PI. The collected droppings were preserved in potassium dichromate solution (2.5%) till counted, using the McMaster technique **(Georgi and Georgi, 1990)**.

Treatments :

The drugs used in this study were Propolis adapted from Ministry of Agriculture, Agricultural Research Center, Bee rearing Dept., and Sulfaclozin- natric- monohydrate (Esb_3 30%, NOVARTS Company). Ethanol Extracted Propolis (EEP) was prepared at a concentration of 3% according to **Biavatti et al (2003)**. Groups (1-3) were the experimental groups which were orally infected with 1000 sporulated oocysts of E.tenella. Group (4) was the control. The experimental birds were treated for five consecutive days starting from the 5th day from infection. Group (1) was infected and treated with 3% EEP at a dose of 1ml /liter in the drinking water for 5 successive days. Group (2) was infected and treated with 30% of Sulfaclozin- natric- monohydrate at a dose of 1g /liter in the drinking water for the same period. Group (3) was infected without treatment. While Group (4) was neither infected nor treated. The scores of E.tenella lesions were evaluated according to **Johnson and Reid (1970)**. The body weights and body weight-gains were evaluated weekly, calculated and tabulated for discussion.

Histopathological studies :

The ceca of the infected chickens with E.tenella were collected at the 6th, 8th and 10th day PI. One cm. of the ceca was removed immediately after killing the infected birds, slit opened longitudinally and fixed in 10% neutral buffered formalin for 10 days. Five micron thick paraffin sections were stained with H&E **(Bancroft et al, 1996)** and examined microscopically. The developmental endogenous stages (schizonts, gametocytes and oocysts) were counted per microscopic field from stained sections on the end of the 5th day of the treatment (at the 10th day PI).

Statistical analysis :

The data, obtained in present study, were analysed according to **Duncan (1955) and Snedecor and Cochran (1969)** using the computer software program called SPSS (2001, Ver., 11) and the means were compared using the level of significance at 0.05%.

RESULTS

Table (1) shows the daily mean and overall mean of oocyst count per gram feces of chickens infected with E.tenella and treated with Ethanol Extracted Propolis (EEP) or Sulfaclozin- natric-monohydrate for five consecutive days. The mean oocyst output of Group (1) was insignificantly increased when compared with Groups (2 and 3) at the 13th and 14th day PI at $P \leq 0.05$. The overall mean of oocyst count per gram feces of Group (1) was significantly decreased when com-

pared with Group (3). On the other hand, it was not significantly decreased when compared with Group (2) at $P \leq 0.05$.

Table (2) shows the mean number of developmental endogenous stages (schizonts, gametocytes and oocysts) of E.tenella in the cecal mucosa of Groups (1 and 2) on the 5th day from treatment. The mean number of developmental endogenous stages of E.tenella in the cecal mucosa of Group (1) was significantly decreased when compared with Group (3), however it was insignificantly decreased when compared with Group (2) on the 5th day from treatment at $P \leq 0.05$.

Table (3) shows the mean lesion-scoring in the ceca of Groups (1,2 and 3). On the 7th day PI, the mean lesion-scoring of Group (1) was significantly decreased when compared with Group (3). However, it was not significantly changed when compared with Group (2) at $P \leq 0.05$.

Table (4) shows the weekly mean of body weight-gains (in grams) for Groups (1 and 2) after treatment with EEP and Esb_3 for 5 consecutive days (starting from the 5th day to the 10th day PI). At the age of 4 and 5 weeks (1 and 2 week PI), the mean body weight-gain in Group (1) was significantly increased when compared with Group (3), but was not significantly changed when compared with Group (2) at $P \leq 0.05$. Meanwhile at the 6th week of age, the mean body weight- gain in Group (1) was significantly increased when compared with Group (2) at $P \leq 0.05$.

Most examined birds (ten) of Group (3) showed severe cecal congestion, mucoid hemorrhages and cecal cord. While, the ceca of Groups (1 and 2) appeared slightly congested with scanty mucus.

Microscopically, numerous second-generation schizonts, gametocytes and immature oocysts were seen in the epithelial lining of the crypts of Lieberkuhn of the ceca, besides necrosis of the epithelial cells and leukocytic infiltration in the lamina propria in Group (3) at the 6th day PI (fig.1). There were few number of the second-generation schizonts, gametocytes and immature oocysts in the epithelial lining of crypts of Lieberkuhn, besides slight necrosis and degenerative changes in the epithelial cells in the presence of and leukocytic infiltration in the lamina propria in Groups (1 and 2) (figs.2&3). At the 8th and 10th day PI, the epithelial lining of the cecal crypts of Lieberkuhn of the ceca in Group (3) showed numerous different developmental stages of E.tenella (second-generation schizonts, gametocytes and mature oocysts), besides necrosis and erosions of the mucosa together with leukocytic infiltration in the lamina propria (figs.4&7). Groups (1 and 2) showed few different developmental stages, slight necrosis and degenerative changes in the epithelial cells, besides leukocytic infiltration (figs.5,6,8&9).

DISCUSSION

The overall mean of oocyst output per gram feces for Group (1), was markedly decreased when compared with Group (3) (chickens infected with E.tenella without treatment), whereas it was nearly similar to that of Group (2). This may be due to the inhibitory effect of EEP and Esb_3 on the invasive forms of E.tenella, leading to their destruction in the cecal mucosa. This agreed with the findings of **Moura et al (1998)** who reported that the hydroalcoholic propolis solution reduced the fecal oocyst counts of intestinal Eimeria species in New Zealand white rabbits similarly to robenidine. The reduction percentage of the oocyst output from Group (1) was nearly similar to that of Group (2). Such reductions were 86.20% and 85.35%, respectively. The obtained results were excellent when compared with Group (3). Similar results were reported by **Hollands et al (1998-a) and Hollands et al (1998-b)** who found that the coccidiostatic effect of 3% alcoholic propolis solution was superior to that of two sulphonamides (2% sulphamethazine, and 0.1% sulphaquinoxalline) in rabbits infected with E.magna, E.media and E.perforans. Our results were similar to the findings of **El-Akabawy et al (2004)** who reported a great reduction in the fecal oocyst outputs as well as the clinical sings, gross and microscopic lesions in the rabbits infected with E.stiedae and treated with aqueous solution of propolis and toltrazuril.

The mean number of the developmental endogenous stages (schizonts, gametocytes and oocysts) of E.tenella, in the cecal mucosa of chickens infected with E.tenella and treated with Ethanol Extracted Propolis (Group 1) and Sulfaclozin- natric- monohydrate (Group 2) on the 5th day post treatment were nearly similar. This showed that the anticoccidial efficacy of EEP and Esb_3 were almost similar. Meanwhile, the mean number of each was highly decreased when compared to that of chickens infected with E.tenella and not treated (Group 3). They apparently inhibited the second-generation schizonts and merozoites of E.tenella. This agrees with the findings of **Genev and Lozanov (1983) and Kolnarski and Sherkov (1987)** who found that the Esb_3 inhibited various endogenic developmental stages after infection with E.tenella in chickens and E. adenoeides in turkeys, respectively.

The mean cecal lesion-scoring on the 7th day PI, in Groups (1 and 2) was nearly similarly decreased when compared with Group (3). The revealed data were similar to the findings of **El-Akabawy et al (2004)** who reported that the aqueous propolis extract caused a great reduction in the gross and microscopic lesions in the rabbits infected with E.stiedae.

The mean gain of the body weight, in grams in Groups (1 and 2) were nearly similar at the 4th and 5th week of age, whereas the gain was higher than that of Group (3). Such gain was higher in Group (1) than Groups (2 and 3) at the end of the 6th week of age. The improvement in the gain of the body weight of Group (1) could be attributed to the anticoccidial effect besides the an-

timicrobial and antifungal effects of the Ethanol Extracted Propolis, so it was the most preferable material for the treatment of coccidiosis. This agrees with that of **El-Akabawy et al (2004)** who reported that the infected rabbits with E.stiedae and treated with aqueous extract of propolis (prophylactic or therapeutic), showed improved the body gain that due to prevention of the gross hepatic lesions with few degenerated oocysts in the bile ducts.

Maes et al (1988) found that the first- and second- generation schizonts of E.tenella showed extensive degenerative changes in the cecal mucosa which was characterized by loss of internal structure, appearance of many intracytoplasmic vacuoles, and incomplete merogony. Hemorrhage was a major pathological manifestation of E.tenella infections, associated with disruption of the cecal mucosa by the developing parasite **(Bayer et al, 1976 and Allen, 1997)**. In the present study, Groups (1 and 2) showed few second-generation schizonts, gametocytes and immature oocysts in the epithelial lining of the cecal crypts of Lieberkuhn besides slight necrosis and degenerative changes in the mucosa with leukocytic infiltration in the lamina propria on the 6th day PI when compared with Group (3). Meanwhile, Groups (1 and 2) showed few different developmental stages (second-generation schizonts, gametocytes and mature oocysts) together with slight mucosal necrosis, degenerative changes and leukocytic infiltration on the 8th and 10th day PI when compared with Group (3). This may be due to inhibitory effect of the EEP on the developmental endogenous stages of E.tenella in the cecal epithelial cells, which resembled that of Group (2), in addition to its antibacterial and antimycotic effects. Our results agree with those of **Penev and Lozanov, (1983); Kolnarski and Sherkov (1987); El-Akabawy et al (2004) and del Cacho et al (2004)**.

It could be concluded that the Ethanol Extracted Propolis was better than the Sulfaclozinnatric- monohydrate (Esb_3) in the treatment of coccidiosis in chickens.

Table (1): Daily oocyst output per gram feces of chickens infected with *E.tenella*. (Mean ± SE, n=12) (oo. x 10^3)

Group / Days PI	*E. tenella* infected chickens			Control	LSD at P≤ 0.05
	treated with Ethanol Extracted Propolis (EEP) (1)	treated with Sulfaclozin-natric-monohydrate (Esb_3) (2)	without treatment (3)	not infected and not treated (4)	
5 days	0.92^{b} ±0.23	1.36^{b} ±0.31	3.15^{a} ±0.49	0.00^{c} ±0.00	1.3636*
6 days	6.92^{b} ±0.67	7.96^{b} ±0.53	22.92^{a} ±2.13	0.00^{c} ±0.00	7.4545*
7 days	15.58^{b} ±0.96	17.18^{b} ±1.13	59.23^{a} ±4.69	0.00^{c} ±0.00	15.5833*
8 days	24.50^{b} ±1.42	27.36^{b} ±1.43	143.08^{a} ±10.03	0.00^{c} ±0.00	24.5000*
9 days	15.25^{b} ±1.18	14.27^{b} ±0.91	96.62^{a} ±7.30	0.00^{c} ±0.00	14.2727*
10 days	8.58^{b} ±0.98	8.55^{b} ±0.92	80.00^{a} ±5.11	0.00^{c} ±0.00	71.4167*
11 days	4.92^{b} ±0.50	5.46^{b} ±0.51	54.31^{a} ±4.61	0.00^{b} ±0.00	48.8531*
12 days	1.67^{b} ±0.36	1.73^{b} ±0.39	40.23^{a} ±3.67	0.00^{b} ±0.00	38.5641*
13 days	0.83^{b} ±0.17	1.09^{b} ±0.21	24.62^{a} ±2.40	0.00^{b} ±0.00	23.5245*
14 days	0.58^{b} ±0.15	0.46^{b} ±0.16	17.31^{a} ±1.17	0.00^{b} ±0.00	16.7244*
Overall mean	7.98^{b} ±0.74	8.47^{b} ±0.79	57.82^{a} ±4.03	0.00^{c} ±0.00	7.9750*
Reduction %	86.20%	85.35%	0.00%	-------	-------

- (*): Significance at P≤0.05. •n= 12
- LSD: Least significance difference among means at P≤0.05.
- Means with different alphabetical superscripts in the same row are significantly different at P≤0.05. •Data were analysed by One Way ANOVA.
- NB: oocyst count in feces for 10 consecutive days.

Table (2): Number of developmental endogenous stages (schizonts, gametocytes and oocysts) in the cecal mucosa of chickens infected with *E.tenella*. (Mean ± SE, n=10)

Group / Days post treatment	*E. tenella* infected chickens			Control	LSD at P≤ 0.05
	treated with Ethanol Extracted Propolis (EEP) (1)	treated with Sulfaclozin-natric-monohydrate (Esb_3) (2)	without treatment (3)	not infected and not treated (4)	
The end of the 5th day from treatment	21.80^{b} ±2.39	23.20^{b} ±1.73	82.00^{a} ±3.02	0.00^{c} ±0.00	21.8000*

- (*): Significance at P≤0.05. •n= 10
- LSD: Least significance difference among means at P≤0.05.
- Means with different alphabetical superscripts in the same row are significantly different at P≤0.05. •Data were analysed by One Way ANOVA.
- NB: Number of developmental endogenous stages / microscopic field counted, on the end of the 5th day of treatment.

Table (3): Mean lesion-scoring in the ceca of chickens infected with *E.tenella*. (Mean ± SE, n=10)

Group / Days PI	*E. tenella* infected chickens			Control	LSD at P≤ 0.05
	treated with Ethanol Extracted Propolis (EEP) (1)	treated with Sulfaclozin-natric-monohydrate (Esb_3) (2)	without treatment (3)	not infected and not treated (4)	
7th day PI	1.60^{b} ±0.27	1.60^{b} ±0.27	3.60^{a} ±0.16	0.00^{c} ±0.00	1.6000*

- (*): Significance at P≤0.05. •n= 10
- LSD: Least significance difference among means at P≤0.05.
- Means with different alphabetical superscripts in the same row are significantly different at P≤0.05. •Data were analysed by One Way ANOVA.
- NB: Lesion scoring in the ceca on the 7th day PI.

Table (4): Body weight-gain (in grams) in chickens infected with *E.tenella* and treated with Ethanol Extracted Propolis (EEP) or Sulfaclozin- natric- monohydrate (Esb_3). (Mean ± SE, n=30)

Group / Age in weeks	*E. tenella* infected chickens			Control	LSD at P≤ 0.05
	treated with Ethanol Extracted Propolis (EEP) (1)	treated with Sulfaclozin-natric-monohydrate (Esb_3) (2)	without treatment (3)	not infected and not treated (4)	
1	101.50^{a} ±1.46	102.40^{a} ±1.99	101.13^{a} ±1.74	102.27^{a} ±1.77	------
2	157.93^{a} ±2.39	157.20^{a} ±2.91	156.10^{a} ±2.67	156.03^{a} ±2.22	------
3	299.93^{a} ±2.93	302.93^{a} ±2.79	300.50^{a} ±2.36	302.17^{a} ±2.46	------
4	384.57^{b} ±3.54	379.70^{b} ±3.57	217.43^{c} ±3.46	516.77^{a} ±2.59	132.2000*
5	398.83^{b} ±4.69	404.67^{b} ±3.51	306.17^{c} ±3.94	440.00^{a} ±4.21	11.1667*
6	463.67^{b} ±3.95	434.33^{c} ±4.80	230.17^{d} ±3.33	642.33^{a} ±7.48	28.8333*

- (*): Significance at P≤0.05. •n= 30
- LSD: Least significance difference among means at P≤0.05.
- Means with different alphabetical superscripts in the same row are significantly different at P≤0.05. •Data were analysed by One Way ANOVA.
- NB: Treatment for 5 consecutive days starting from the 5th day to the 10th day PI.

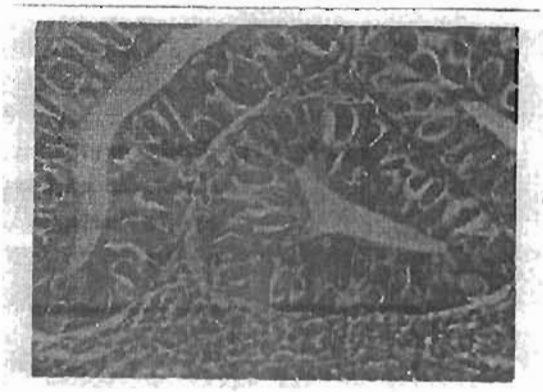

Fig. (1) : Group (3), cecum showing numerous second-generation schizonts, gametocytes and immature oocysts in the epithelial lining of the cecal crypts of Lieberkuhn and necrosis of the epithelial cells with inflammatory cell infiltration in the lamina propria. H&E, X 400.

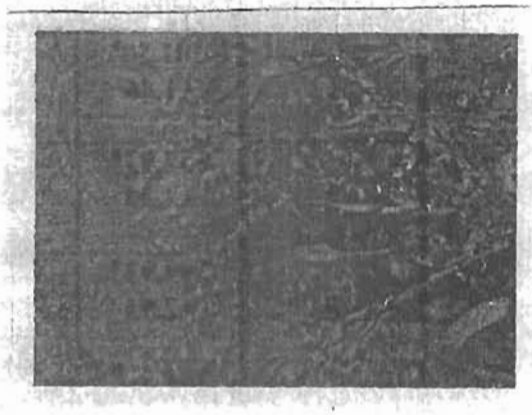

Fig. (2): Group (1), cecum showing few second-generation schizonts and gametocytes in the epithelial lining of the cecal crypts of Lieberkuhn, besides slight necrosis, degenerative changes in the mucosa and leukocytic infiltration in the lamina propria. H&E, X 400.

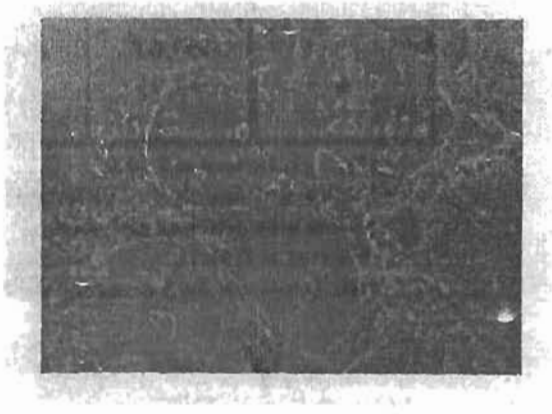

Fig. (3): Group (2), cecum showing few second-generation schizonts and gametocytes in the epithelial lining of the cecal crypts of Lieberkuhn, besides slight necrosis, degenerative changes in the mucosa and leukocytic infiltration in the lamina propria. H&E, X 400. At 6thday PI

Fig. (4): Group (3), cecum showing numerous different developmental stages of E.tenella (second-generation schizonts, gametocytes and mature oocysts) besides necrosis and erosions of the mucosa together with inflammatory cell infiltration in the lamina propria. H&E, X 400.

Fig. (5): Group (1), cecum showing few different developmental stages, slight necrosis, degenerative changes in the mucosa and leukocytic infiltration. H&E, X 400.

Fig. (6): Group (2), cecum showing few different developmental stages, slight necrosis, degenerative mucosal changes and leukocytic aggregation. H&E, X 400.

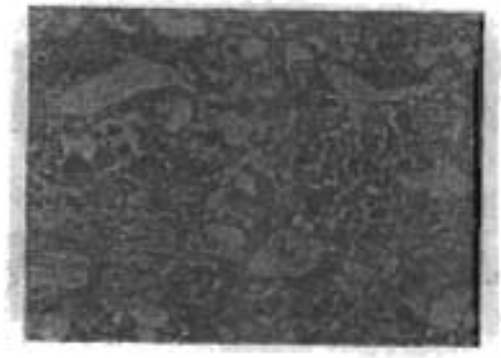

Fig. (7) : Group (3). cecum showing numerous different developmental stages of E.tenella (second-generation schizonts, gametocytes and mature oocysts), necrosis and erosion of the mucosa with extensive infiltration of inflammatory cells in the lamina propria. H&E, X 400.

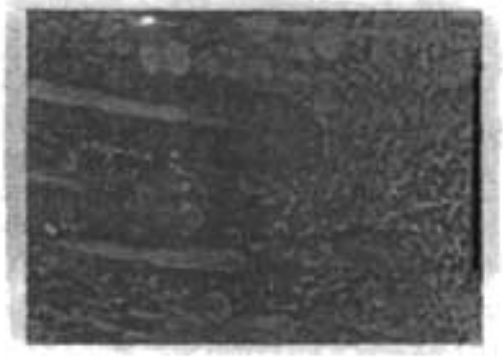

Fig. (8) : Group (1), cecum showing few different developmental stages, slight necrosis, degenerative changes in the mucosa and leukocytic infiltration. H&E, X 400.

Fig. (9) : Group (2), cecum showing few different developmental stages, slight necrosis, degenerative mucosal changes and leukocytic aggregation. H&E, X 400. At 10th day PI

REFERENCES

Allen, P. C. (1997) : Nitric oxide production during E. tenella infections in chickens. Poult. Sci., 76 (6): 810-813.

Bancroft, G. D.; Steven, S. A. and Turnal, N. (1996) : Theory and Practice of Hstopathological Technique. 4th, ed., Churchill, Livingstone, Edinburgh. London, Melbourne and New York.

Bankova, V. (2005) : Recent trends and important developments in propolis research. Evidence Based Complementary and Alternative Medicine, 2: 29-32.

Bayer, R. C.; Chawan, C. B. and Bryan, T. A. (1976) : Cecal mucosal response to coccidiosis in growing chickens. Poult. Sci., 55 (3): 1020-5.

Biavatti, M. W.; Bellaver, M. H.; Volpato, L.; Costa, C. and Bellaver, C. (2003) : Preliminary studies of alternative feed additives for broilers: Alternanthera brasiliana extract, propolis extract and linseed oil. Revista Brasileira de Ciencia Avicola, 5 (2): 2-9.

Burdock, G. A. (1998) : Review of biological properties and toxicity of bee propolis. Feed Chem. Toxicol., 36: 347-363.

del Cacho, E.; Gallego, M.; Lopez-Bernad, F.; Quilez, J. and Sanchez-Acedo, C. (2004) : Expression of anti-apoptotic factors in cells parasitized by second-generation schizonts of E.tenella and E.necatrix. Vet. Parasitol., 125 (3/4): 287-300.

Duncan, D. R. (1955) : Multiple range and multiple F tests. Biometrics,11:31-42.

El-Akabawy, L. M.; Zayan, K. A.; Tantawy, A. A. and Omar, R. E. M. (2004) : Anticoccidial efficacy of propolis and toltrazuril against E.stiedae in New Zealand white rabbits. Zag. Vet. J., 32 (1): 129-145.

Gardiner, J. L. and Wehr, E. E. (1950) : Selecting experimental groups of chicks by weight. Proc. Helminthol. Soc. Washington, 17: 25-26.

Georgi, J. and Georgi, M. (1990) : Parasitology for Veterinarians. 5th Edition, W.B. Saunders Company, Philadelphia, London, Toranto, Montereal, Sydney, Tokyo.

Gonzales, R.; Corch, I.; Remirez, D.; Rodringuez, S.; Amcheta, O.; Merino, N.; Gonzales, A. and Pascual, C. (1995) : Hepatoprotective effects of propolis extract on carbon tetrachloride-induced liver injury in rats. Phytoth. Ras., 9: 114-117.

Hollands, I.; Mlyares, C. and Sigarroa, A. (1988-a) : Comparative analysis of the action of propolis, sulphaquinoxaline and sulphamerazine in rabbits with coccidiosis. Revista Cubana de Ciencias Veterinarias, 19 (2): 99-104.

Hollands, I.; Miyares, C. and Pimienta, R. (1988-b) : Quality control of propolisina (alcoholic extraction of propolis) used as a coccidiostat, by means of a biological method. Revista Cubanas de Ciencia Veterinarias, 19 (4): 319-326.

Hollands, I.; Miyares, C.; Sigarroa, A. and Perez, A. (1984) : Efficacy of propolis against infection by intestinal Eimeria in rabbits. Revista Cubana de Ciencias Veterinarias, 15 (2): 157-163.

Jonhson, J. and Reid,W. M. (1970) : Anticoccidial drugs: Lesion scoring techniques in battery and floor-pen experiments with chickens. Experimental Parasitology.28: 30-36.

Karim, M. J. and Tress, A. J. (1990) : Isolation of five species of Eimeria in Bengldish. Tropical Animal Health Production, 22: 153-159.

Keskin, N.; Hazir, S.; Basser, K. H. and Kurkcuoglu, M. (2001) : Antibacterial activity and chemical composition of Turkish propolis. Zeitschrift fur Naturforschung (C), 56: 1112-1115.

Koinarski, V. and Sherkov, Sh. N. (1987) : Comparative trial of the action of coccidiocidal drug agents to control coccidiosis in turkeys due to E. adenoeides. Vet. Med. Nauki., 24 (7): 88-91.

Maes, L.; Coussement, W.; Vanparijs, O. and Marshboom, R. (1988) : In vivo action of the anticoccidial diclazoril (Clinacox) on the developmental stages of E.tenella: a histological study. J. Parasitol., 74 (6): 931-938.

Miyataka, H., Nishiki, M., Mastsumoto, H., Fujimoto, J., Matsuka, M. and Satoh, T. (1997) : Evaluation of propolis: 1- Evaluation of Brazilian and Chinese propolis by enzymatic and physio-chemical methods. Biol. Pharm. Bull., 20: 496-501.

Moura, L. P. P.; Scapinello, C.; Martins, E. N.; Franco, S. L. and Ribbeiro, M. C. M. (1998) : Effect of hydroalcoholic propolis solution and robenidine on faecal oocyst counts of Eimeria species in New Zealand white rabbits. Revista Brasileira de Zootecnica, 27 (2): 325-330.

Murad, J. M.; Calvi, S. A.; Soares, A. M.; Bankova, V. and Sforcin, J. (2002) : Effect of propolis from Brazil and Bulgaria on fungicidal activity of macrophages against Paracoccidioides brasiliensis. J. Ethnopharm., 79: 331-334.

Ota, C.; Unterkircher, C.; Fantinato, V.; Shimizu, M. T. (2001) : Antifungal activity of propolis on different species of Candida. Mycoses, 44: 375-378.

Penev, P. M. and Lozanov, L. (1983) : Action of sodium sulfachlorpyrazine (Esb_3) on the endog-

enous development of E. tenella in the experimental infection of chickens.Vet.Med.Nauki., 20 (5-6):72-79.

Reynaud, J.; Guilet, D.; Terreux, R., Lussignol, M. and Walchshofer, N. (2005) : Isoviavonolds in non leguminous families; an update. Nat. Prod. Rep., 22: 503-515.

Santos, F. A.; Bastos, E. M. A.; Uzeda, M.; Carvalho, M. A. R.; Farias, L. M.; Moreira, E. S. A. and Braga, F. C. (2002): Antibacterial activity of Brazilian propolis and fractions against oral anaerobic bacteria. J. Ethnopharmacology, 80: 1-7.

Snedecor, G. W. and Cochran, W. G. (1969) : Statistical methods, sixth Ed., Iowa State University Press, Asmes, Iowa, USA.

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Trusheva, B.; Popova, M.; Bankova, V.; Simova, S.; Marcucci, M. C.; Miorin, P. L.; Pasin, F. R. and Tsvethova, I. (2006) : Bioactive constituents of Brazilian red propolis. Evidence Based Complementary and Alternative Medicine, 3 (2): 29-32.

Velikova, M.; Bankova, V.; Tsvethova, I.; Kukumgiev, A. and Marcucci, M. C. (2000): Antibacterial ent-kaurence from Brazilian propolis of native stingless bees. Fioterapia. 11: 693-696.

Witlock, D. R. (1982) : Changes in cecal composition with E. tenella infection. Poult. Sci., 61 (1): 57-61.

Witlock, D. R.; Lushbaugh, W. B.; Danforth, H. D. and Ruff, M. D. (1975) : Scanning electron microscopy of the cecal mucosa in E. tenella infected and uninfected chickens. Avian Dis., 19 (2): 293-304.

الملخص العربى

دراسات على مستخلص البرويوليس والسلفاكلوزين - ناتريك - مونوهيدرات فى الدجاج المعدى بالأيميريا تينيللا

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فى هذه الدراسة أستخدمت مائة وعشرون (١٢٠) كتكوت عمر يوم من سلالة روص (ذكور وإناث)، وقسمت الكتاكيت إلى أربعة مجموعات متساوية (٣٠ كتكوت لكل مجموعة)، تم تجريع كتاكيت المجموعات الأولى، الثانية، والثالثة مباشرة داخل الحوصلة بجرعة ١٠٠٠ حويصلة متجرثمة من عترة الأيميريا تينيللا لكل كتكوت عند عمر ٢١ يوم، عولجت كتاكيت المجموعة الأولى بالمستخلص الكحولى للبرويوليس (صمغ عسل النحل) بتركيز ٣٪ وبجرعة ١سم٣/ لتر فى مياه الشرب لمدة ٥ أيام متتالية، وعولجت كتاكيت المجموعة الثانية بدواء سلفاكلوزين - ناتريك - مونوهيدرات "ESb3" (٣٠٪) بجرعة ١جم/لتر فى مياه الشرب لمدة ٥ أيام متتالية وتركت المجموعة الثالثة بدون علاج (مصابة وغير معالجة)، أما كتاكيت المجموعة الرابعة أعتبرت مجموعة ضابطة سلبية (غير مصابة وغير معالجة)، تم عد حويصلات الأيميريا فى رزق الفراخ لمدة عشرة أيام متتالية إبتداء من اليوم الخامس وحتى اليوم الرابع عشر بعد العدوى، وتم تحديد درجة الإصابة فى الأعورين فى الفراخ عند اليوم السابع من العدوى، وتم وزن الفراخ والعلف المستهلك إسبوعياً، تم أخذ عينات نسيجية من الأعورين عند اليوم السادس، الثامن والعاشر من العدوى وذلك لعمل الدراسات الهستوباثولوجية عليها، وأوضحت النتائج أن الفراخ المعدية بطفيل الأيميريا تينيللا والمعالجة بالمستخلص الكحولى للبرويوليس قد بينت نقص معنوى فى عدد حويصلات الأيميريا فى رزق الفراخ وعدد الأطوار الخلوية المختلفة (الشيزونتات، الجاميتات والحويصلات) للطفيل وكذلك درجة الإصابة فى الأعورين، كما بينت النتائج زيادة معنوية فى وزن الجسم المكتسب للطائر، وكذلك تحسين كفاءة خلايا الأعورين الطلائية عند مقارنتها بالمجموعة الضابطة الإيجابية(المجموعة المعدية وغير المعالجة)، وكانت هذه النتائج تقريباً متشابهة مع نظيرتها المعالجة بدواء سلفاكلوزين - ناتريك - مونوهيدرات (لاتوجد فروق معنوية بينهما)، وتبين من الفحوص الهستوباثولوجية قلة الإلتهابات فى الطبقة المبطنة للأعورين فى المجموعات المعالجة بالبرويوليس مقارنة بالمجموعة المصابة والغير معالجة مما يوضح كفاءة البرويوليس فى تقليل الإصابة بالكوكسيديا، وخلاصة هذه الدراسة أن المستخلص الكحولى للبرويوليس (٣٪) له كفاءة عالية فى علاج الكوكسيديا تضاهى كفاءة سلفاكلوزين - ناتريك - مونوهيدرات "ESb3" (٣٠٪) حيث أنه يقلل عدد حويصلات الأيميريا فى الزرق ويقلل درجة الإصابة فى الأعورين كما أنه يحسن من أداء الفراخ بزيادة وزنها ويحسن من أداء خلايا الأعورين، وربما لاتوجد مقاومة دوائية "Drug resistance" ضده من الكوكسيديا.