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Histopathological Effects of *Albizia lebbeck* Seeds Extract on Cecum Tissues of Broilers Infested Experimentally by *Eimeria tenella*

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Abstract

The study was aimed to investigate the pathological of cecum broiler chickens infected and treated by ethanolic Seed Extract of *Albizia lebbeck*. Sixty (Ross,310) broiler chicks were divided into 6 equal groups (10 birds / each), at age 17th day. A, control negative, B, control positive, C treated Amprolium1g/L in drinking water, and D, E, F (infected and treated groups) ethanolic *A. lebbeck* seed extract with at doses 100, 200 and 300 mg/Kg BW., respectively). All groups except group A chickens were experimentally infected with 500 sporulated oocysts of *Eimeria tenella*. The results showed that. In groups treated with the extract alone at a dose of 300 mg/kg, no particular histological abnormalities were seen, treated by Amprolium 1g/L in drinking water and control negative while, some lesions were observed in the groups treated at doses 100, mg/ Kg B. W. showed different stages of *E. tenella* maturation including schizont and microgametes were observed in crypt layer of cecum. *E. tenella*. Deferent stages of sexual and asexual of *E tenella* was observed within the necrotic epithelial cells' debris near the surface of cecum lumen and at doses 200 mg/Kg.BW showed *E. tenella* microgametes was observed within epithelial necrosis cystic change in the sub mucosa and of cecum severe lesions changes observed in the group of control positive.

Keywords: Histopathological, Cecal, crypt layer, Lesions, *Eimeria*, *Albizia*

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Introduction

Eimeriosis is illness caused by the parasite belong to the genus *Eimeria* which is recognized as the major parasitic illness of chickens **(Lopez-Osorio *et al*., 2020).** A major threat to poultry production is eimeriosis, a digestive system infection that is self-limiting (**Ameji *et al*., 2022**). *E. tenella* is the most pathogenic species of *the* genus that infects chickens. (**Xu, L.,*et al* .,2022**) Cecal eimeriosis is typically caused by *E. tenella*,. (**El-Shazly *et al*.,2020**)

When it replicates, it damages the epithelial cells of the ceca, which causes hemorrhagic diarrhea, poor growth, and compromised intestinal health in broilers (**Dung ,2020**).

The serious control of in poultry by using several alternatives and procedures that are successfully employed in various places throughout the world (**Attree *et al*.,2021**). Among the available options, a various botanical compounds had been demonstrated as excellent and commendable antieimerial with other therapeutic effects in poultry and livestock (**Felici, *et al*., 2023**). Although herbal medicines, are inexpensive and easy to obtain, they are become a popular option (**Mishra *et al*.,2023**), and they have an advantages being of natural origin, could be make *Eimeria* parasite control cheaper as a source of foreign revenue (**Aguiar-Martins *et al*., 2023**).

Albizia lebbeck (L.) Benth contains carbohydrates, potassium, copper, fatty amino acids, keto acids, alkaloids, flavonoids (geraldone, luteolin, and isocyanin, tannins, saponins (D-catechin, D-leucocyanidin, ferridlan-3-one, Y-sitosterol, lipquinins D, F, C, and H, echinocystic acid, flavones, vicinin II, and β-sitosterol), oleanan-type saponins (lipicosides A and B), glycoside (Albizinnin), and hemolysin. **(Obika and Obika ,2003)**.

Albizia lebbeck is a versatile plant that is extensively used as herbal medicine and animal feed in many countries. It is also used in folk medicines for abdominal tumors, boils, cough, eye diseases, flu, lung ailments, leprosy, and scrofulous swellings (**Gupta *et al*., 2006**). In Tanzania, it is used to cure eye issues, diabetes (**Sowe, 2023**), dysmenorrhea, malaria, and diarrhea. It is also used to augment domestic ruminant diets **(Ndemanisho *et al*., 2006).**

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Albizia lebbeck is a plant found in, tropical and subtropical Asia, India Bangladesh,and Africa (**Islam *et al*., 2018**). There is no information on the impact and antieimerial activities of *A. lebbeck* seed extract in broiler chickens, for that the purpose of this study was assess the cecal Histopathological changes in broiler infected with *E. tenella* oocysts and treated by this herbal.

Methodology

Eimeria tenella Oocysts Isolated

From the ceca of naturally infected chicks, *Eimeria tenella* oocysts were extracted and recovered using centrifugal floatation **(AL-Salhi *et al*.,2015; Sultan *et al*.,2021; Al-Zarkoushi and Al-Zubaidi, 2022).** The oocysts were then allowed to sporulate in a 2.5% potassium dichromate solution at room temperature **(Al-Saadoon and Al-Rubaie, 2018; Al-Zarkoushi and Al-Zubaidi, 2021).**

Plant seed Collection, Identification, and Extraction

The ethanol alcoholic extract was prepared by using the Soxhle device, where 50 g of dried plant seeds powder and 250 ml of ethanol alcoholic (%99). The extraction continued for 6 hours at 60 ^{o}C. The extraction of the raw seed was considered complete when the thimble's solution became transparent. Turned off the device. To eliminate the solvent, the extracted liquid and the solvent were submerged in a water bath and the extraction of a golden yellowish with high viscosity **(Shunmugadevi *et al*.,2022)**.

Preparation of Treated Doses

The ethanolic seed extraction was weighed and reconstituted in distilled water for preparing the doses100, 200 and 300 mg/Kg.BW respectively **(Ahmed *et al*.,2014)** Amprolium 250 WSP was used by dissolved 1g in 1 litter of tope water (1 mg/ml).

Experimental Chickens

The experiment had six groups of 10 broiler hens, each aged 17 days. Group A (negative control), group B (positive control), group C (amprolium 1mg/ml in top water), and groups D, E, and F (Ethanolic A. lebbeck seed extract at dosages of 100, 200, and 300 mg/kg B.W), respectively. Except for group A (negative control), all groups received 500 sporulated E. tenella oocysts orally and were treated orally for seven days following infection.

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Pathological Examination

Gross Lesions

According t**o Reid and Johnsons (1970)** that examined previously known the methods for diagnosing cecal lesions. The ceca was identified, and the mucosal surface injury by degrees 0 to 4; 0 (no lesions) one (mild lesions with petechial hemorrhage on the surface of the cecal mucosal and mild change in wall color or cecum contents); two (moderate lesions with severe hemorrhage and light thickening of cecal wall); three (severe hemorrhage with blood clots and light thickening of cecal wall in cecal lumen)and four (severe hemorrhage with blood clot).

Histopathology Method

Cecal samples (approximately 3 cm in length) were collected from 36 chickens slaughtered from all groups at 14th days post infection. The tissue samples were gently rinsed with 0.1 M phosphate-buffered saline (PBS) at pH 7.2, and then fixed in 10% neutral buffered formalin before being transferred to the histopathology laboratory / College of Veterinary Medicine University of Baghdad. The samples were fixed, then passed through a tissue scroll device. The embedded in paraffin for preparation into fine blocks, and they were sectioned with a microtome to a size of 5µm; They were dewaxed and the tissue section was stained using haematoxylin and eosin (H and E) stain as describe (**Bancraft *et al*.,1990; Chang *et al*.,2021**; **Handi *et al* 2024**; **Mahmood,2004)**.

Statistical Analysis

Various variables' effects on research parameters were identified using the Statistical Analysis **System-SAS (2018)** application. To compare means significantly, we employed (Analysis of Variation-ANOVA). This research employed a chi-square test to compare percentages with a probability of 0.05 and 0.01.

Pathological Examination

Efficacy of the Ethanolic *A. lebbeck* seed extract on cecal lesions of *Eimeria tenella* infected broiler chickens

The effect of the A. lebbeck at a dose of 300 mg/Kg BW and the Amprolium treatment was that there was a significant difference (P ≤0.01). in the pathological effects in the ceca with the positive control group. There was also no

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significant difference (P ≤0.01). in the pathological effects between the A. lebbeck at a dose of 300 mg/Kg BW and the Amprolium treatment and the A. lebbeck at a dose of 200 and 100 mg/Kg BW. Now there was a significant difference (P ≤0.01) with the group the A. lebbeck at a dose of 200 and 100 mg/Kg BW with the positive control group. **Table (1)**

Table (1) Cecal Lesions in *Eimeria tenella* infected broiler chickens and treated with ethanolic *Albizia lebbeck* seed extract

Groups	Lesion scores of 6 chickens						Mean ±SD
A-(control negative)	0	0	0	0	0	0	0.0
B-(control positive)	+4	+2	+4	+3	+3	+2	3.00±1.4a
C-(treat with amprolum)	+1	+1	+1	+1	+1	+1	1.00±0.c
D-(Ethanolic *A. lebbeck* seed extract 100 mg/kg B.W)	+2	+1	+2	+3	+2	+1	1.83±0.10b
E (Ethanolic *A. lebbeck* seed extract 200 mg/kg B.W)	+1	+3	+1	+2	+1	+2	1.50±0.07. bc
F (Ethanolic *A. lebbeck* seed extract 300 mg/kg B.W)	+1	+1	+1	+1	+1	+1	1.00±0c
P-value	--	--	--	--	--	--	0.0091 **
** (P≤0.01).							

Different little letters indicate a major difference. Values are reported as the mean ± SD of lesions cores from 6 hens randomly chosen per group. Means with distinct superscript letters compared to the infected and untreated control in the same column show significant differences (P≤0.01).

There was no increase in the size of the cecum compared between the positive control group and the infected and treated groups. There were pathological effects represented by enlargement (thickening) specific to the positive control group, and there was a decrease in these pathological effects gradually proportional to the increase in the dose of the *A. lebbeck* as well as the amprolium treatment, and *A. lebbeck* dose 300 mg/kg group showed slight pathological effects. The positive control group showed the presence of bleeding, blood clots, and thickening of

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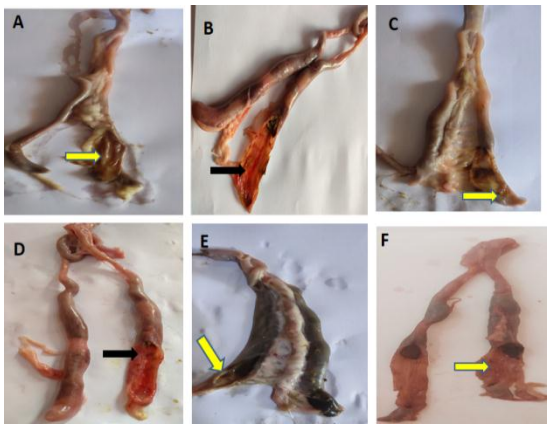
the cecal wall, and this bleeding, blood clot, and wall thickening were inversely proportional to the increase in dose of *A. lebbeck* and also to the amprolium treatment. (Figure, 1)



Figure,1 Gross of Cecal Lesions in *Eimeria tenella* Infected haematological and thick wall Cecal in Chickens (Figure,1-A) Ceca of control negative, was normal (Figure,1- B) Ceca of control positive were other typical pathologic traits of lesions such as wall thickening, erosion, and dark blood clotting. (black arrow) (Figure,1-C) Ceca of treat with amprolium was normal (yellow arrow) (Figure,1-D) Ceca, ethanolic *A.lebbeck* seed extract 100 mg/Kg BW showed wall thickening and dark blood clotting(black arrow) (Figure,1-E) Ceca from ethanolic *A. lebbeck* seed extract 200 mg/Kg BW showed slight thickening and little bleeding(yellow arrow) (Figure,1-F) Ceca from ethanolic *A. lebbeck* seed extract 300 mg/Kg BW showed normal (yellow arrow).

4-4- Pathological Lesions

The histopathological lesions of control negative (non - infected or-treated) group showed normal cecal mucosa, sub mucosa, muscular, muscular layers, glandular layers and villi serosa. **(Figure2-A)** Control positive (infected and non - treated) group showed a different stages of *E. tenella* maturation including schizont and microgametes were observed in crypt layer of cecum. *E. tenella* microgametes was observed within the necrotic epithelial cells' debris near the surface of cecum lumen (**Figure 2-B)** In group of *E. tenella* infected

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treated group Amprolium1g/ Ltreated Normal cecal histological architecture. **(Figure2- C)** In group of *E. tenella* infected treated group a dose at 100 mg/ Kg BW showed different stages of *E. tenella* maturation including schizont and microgametes were observed in crypt layer of cecum. *E. tenella* microgametes was observed within the necrotic epithelial cells' debris near the surface of cecum lumen, and showed E. tenella microgametes was observed within epithelial necrosis cystic change in the sub mucosa and of cecum. **(Figure2-D)** In group of *E. tenella* infected cecum of GE in a dose 200 mg / Kg BW showed *E. tenella* macrogametes was observed between villi of cecum (**Figure3- E)** and at dose 300 mg/ Kg BW showed normal cecal histological architecture (**Figure3- F).**

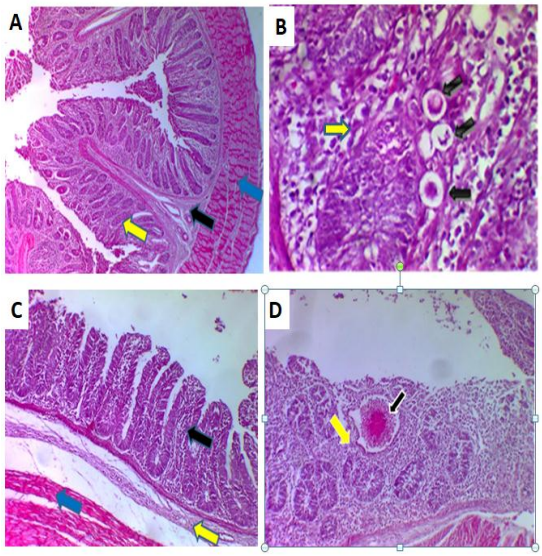

Figure 2: (A) Photomicrograph of cecum of control negative group. normal cecal showed mucosa, (black arrow) sub mucosa, (yellow arrow) muscular layers, (blue arrow) glandular layers and villi, (histological architecture). (H and E. 10X). B- Photomicrograph of *Eimeria tenella* infected cecum of control positive group. Different stages of *Eimeria tenella* (maturation including schizont, microgametes microgametes) observe (black arrow) observe in cryptlayer of cecum. within the necrotic epithelial cells' debris near the surface of cecum lumen (yellow arrow) (H and E. 40X).C- Photomicrograph of *Eimeria tenella* infected cecum of Amprolium 1g/ Ltreated group showed mucosa, (black arrow) sub mucosa, (yellow arrow) muscular layers, (blue arrow) of cecal (H andE. 10X) D: Photomicrograph of Eimeria tenella infected cecum of treated group in a dose 100 mg/ Kg B.W. Albizia lebbeck Ethanolic seed extract Eimeria tenella observe (black arrow) observe in crypt layer of cecum with presence of inflammatory cells surrounding epithelial cells (yellow arrow) (H and E

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A:10X)

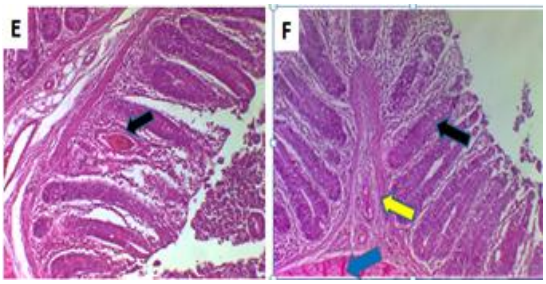

Figure 3: E: Photomicrograph of Eimeri tenella infected cecum of treated group in a dose 200 mg/ Kg B. W. Ethanolic A. lebbeck seed extract.

Eimeria (black arrow) surrounded by necrotic epithelia cell of cecum (.H and E. 10X). F: Photomicrograph of Eimeria tenella infected cecum of treated group. in a dose 300 mg/ Kg B. W. Ethanolic A. lebbeck seed extract showed mucosa, (black arrow) sub mucosa, (yellow arrow) muscular layers, (blue arrow) of cecal (Normal cecal histological architecture) .(HandE.10X)

Discussio

One of the conventional methods for determining the degree of cyto-architectural changes and degenerative changes in tissues is histopathological examinations **(Mikail *et al.*, 2019).** Results from studies done on hens infected with *E. tenella* corroborate the presence of epithelial necrosis in the caeca **(Abdel Wasae *et al.*, 2017).** The groups that were infected and treated with Amprolium and *A. lebbeck* at a concentration of 300 mg/kg BW did not show any lesions in the ceca. Similarly, the groups that were not infected but treated with the ethanolic plant extract of *A. lebbeck* did not show any lesions in the ceca. This suggests that the histopathology lesions observed could be a result of the disease itself. On the other hand, the groups that were infected and untreated did not observe any multiple developmental stages of *E. tenella* parasites in the ceca, and neither did the groups that received the highest concentration of treatment (300 mg/kg BW). The absence of parasite developmental stages in the caeca of chickens infected with Amprolium suggests that the lesions observed in the ceca are most likely caused by invasion. The group treated with lower concentrations of the extract showed fewer lesions and fewer parasite developmental stages, suggesting that the extract may have antieimerial activity. Increasing the concentration of the plant extract by a factor of many or isolating the antieimerial compound could lead to even greater activity. We further corroborate the assertion made by **Mikail *et al.* (2019)** that the ethanolic leaf extract of L. schimperim has potential antieimerial properties in vitro. Past studies have shown the possible

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antieimerial properties of some plant extracts; for example, Pletrothechus spp. was found to lessen the extent of damage to the cecum tissues of broiler chickens infected with E. tenella oocysts in one experiment. Another study by Abdel **Wasae *et al.* (2017**) found that some plant extracts have antieimerial action against *E. tenella* in experimentally infected broiler chickens. **Habibi *et al.* (2016)** also shown this in vitro and in vivo. Additionally, **Muthamilselvan et *al.* (2016)** found that various plant species had antieimerial effects. While this study found that Amprolium was more effective in stopping the invasive developmental stages of *E. tenella* from multiplying in tissues, the extract from seeds had more anticoccidial activity in the groups that received it at doses of 300, 200, and 100 mg/kg body weight, respectively, compared to the infected/control negative group. of sick hens reveals that the illness has a minor impact, and there are no discernible alterations in their body weight.

Ethical clearance

The ethical permission number 403was in 20/2/2023 was receive the local committee of animal care/ College of Veterinary Medicine / University of Baghdad.

Statement Novelty:

This is the first work that Ethanolic *Albizia lebbeck* seed extract had beneficial effects on *E. tenella* -induced patent coccidiosis in broiler chickens through reducing Oocysts shedding, histopathological lesions and reduce cecal lesions. Further

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