

**Evaluation the Anticoccidial Efficacy of Oregano Oil and Allicin against *Eimeria tenella* in Experimentally Infected Broiler Chickens**

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**ABSTRACT**

Cecal coccidiosis is a significant parasitic disease that affects poultry farms and is caused by *Eimeria tenella*. This study evaluated the anticoccidial efficacy of oregano oil and allicin against *Eimeria tenella* in experimentally infected broiler chickens. One hundred and twenty-one-day-old chicks were divided into six groups (20 chicks in each group): G1, non-infected non-treated (control negative group); G2, the infected non-treated (control positive group); G3 was treated with amprolium; G4 was treated with oregano oil, G5 was treated with allicin, and G6 was treated with a mixed dose of oregano oil and allicin. All groups were challenged with  $10^4$  *Eimeria tenella* sporulated oocysts at the 5<sup>th</sup> day old except the first group (G1). Body weight (B.W), food conversion rate (FCR), oocyst count, hematology, serum biochemical parameters, and histopathology were assessed throughout the experiment in all groups. Oregano oil and allicin significantly improved B.W., FCR, lesion score, and oocyst count. Additionally, histopathology showed that the oregano oil and allicin successfully treated the cecum thoroughly; the cecum was average in its histology with the absence of *E. tenella* developmental stages. In conclusion, this study proved that oregano oil and allicin have a protective and curative effect as anti-coccidial drugs of plant origin on *E. tenella*-infected chickens. Furthermore, the mixture of the two chemicals is a promising treatment for *E. tenella* infection in broiler chickens.

**Keywords:** Allicin; Cecum, Chickens; *Eimeria tenella*; Oregano.

**INTRODUCTION**

Coccidiosis is one of the most important parasitic diseases affecting the poultry industry due to the massive economic losses (Oluyemi and Roberts, 2000; El-Shazly et al., 2020). Different species of *Eimeria* are present with tropism to the intestinal tract. Cecal

coccidiosis caused by *E. tenella* sporulated oocysts that remain for long periods in soil and poultry litter and can contaminate food and water, resulting in continuous infection (Bhogal et al., 1988; Oluyemi and Roberts, 2000). It causes severe clinical symptoms such as bloody diarrhea, disturbed feeding, depression,

poor weight gain and conversion rates, and mortalities (James et al., 2009; Hector et al., 2020). The intensity of infection determines the severity of the disease, which ranges from mild to severe form with subsequent gross and microscopic lesions in the cecum (El-Shazly et al., 2020; Zhou et al., 2013).

Avian coccidiosis is mainly controlled by proper hygiene and anticoccidial drugs (El-Shazly et al., 2020). However, *Eimeria*-resistant strains emerge that limit the drug's efficacy (Chapman, 1997; Arab et al., 2006). Recently, consumers preferred poultry meat free from any drug residues (Harper and Makatouni, 2002). As a result, finding alternative and safe drugs to treat coccidia is needed (Youn and Noh, 2001; El-Shazly et al., 2020).

Aromatic plants and their derivatives are currently used since they are natural, eco-friendly, and safe products (Christaki et al., 2012). The anticoccidial action of Aromatic plants is attributed to their rich polyphenol content. Oregano oil is extracted from *Origanum vulgare*. Carvacrol and thymol represent 70% to 80% of oregano oil composition and are thought to have anticoccidial properties (Giannini et al., 2013).

Allicin, a sulfur-containing natural compound, is extracted from garlic (*Allium sativum* L.) with characteristic smell and taste and has many biological activities (Borlinghaus et al., 2014). Allicin has antifungal, antibacterial, antiprotozoal (Wahba, 2003; Salama et al., 2013), and antiviral effects (Mikaili et al., 2013). Moreover, allicin can help control body cholesterol and decrease blood pressure. The anticoccidial efficacy of garlic has been recorded in broilers (Elkhatam et al., 2014) and rabbits (Toulah et al., 2007).

The present study was designed to evaluate the protective effect of oregano oil and allicin alone or in combination against experimentally infected broiler chicks with *E. tenella* through examination

of weight gain, food conversion rate, gross lesion score, histopathology, and oocyst dropping count.

## MATERIALS AND METHODS

### *Eimeria oocysts*

The sporulated oocysts of *E. tenella* were obtained from the Parasitology Department, Faculty of Veterinary Medicine, University of Sadat City, Egypt. The oocysts were collected from the caeca of naturally infected broiler chickens (Chapman and Shirley 2003). The collected oocysts of *E. tenella* were propagated and preserved (Long and Joyner 1976). The oocysts were stored in a 2.5% potassium dichromate solution for proper oocyst sporulation. Immediately before infection induction, the sporulated oocysts were washed several times with distilled water to remove the potassium dichromate solution.

### Drugs

Amprolium 20% was obtained from Adwia Company, 10<sup>th</sup> of Ramadan City, Egypt, under the commercial name Amprolium®. It was orally administered in drinking water at 1.25 gm/liter. Oregano oil 20% was obtained from Ropapharm International B.V., Netherlands, under the commercial name Ropadiar®. It was orally administered in drinking water at 2ml /3L. Allicin was purchased from Agrovvet Company, Egypt. It was orally administered in drinking water at 2 gm /L.

### Experimental design

One hundred and twenty-one-day-old chicks were divided into one control group and five infected groups (each group had 20 chicks). Group 1, non-infected non-treated (control negative group), Group 2, infected non-treated (control positive group). Group 3 was infected and treated orally with amprolium 20% for five days at a dose of 1.25 g/L for 18 hrs. Group 4 was infected and treated orally with oregano oil 20% for ten days at a dose of 2 cm/3L for 18 hrs. Group 5 was infected and treated orally with allicin for ten days

at a dose of 2 g/L for 18 hrs. Group 6 was infected and treated orally with a mixed half amount of oregano oil (0.5 ml) plus allicin (1.5 gm) /1.5 L for ten days for 18 hrs. Infected groups were challenged with  $10^4 E. tenella$  sporulated oocysts at the 5<sup>th</sup> day of age. Treatment started on the 6<sup>th</sup> day of age. All birds received water and feed *ad libitum*. Feed was free from anticoccidial drugs. The ethics committee approved the experimental design and animal procedures, Faculty of Veterinary Medicine, University of Sadat City.

#### **Mortality, Body Weight, and Feed Conversion Ratio**

The mortality rate and feed conversion ratio (FCR) were calculated in all groups. The number of dead birds was recorded, and the mortality percentage was determined according to the formula (Vetter and Matthews, 1999). FCR was determined by dividing the average body weight gain by feed consumption. Average B.W. was obtained by weighing individual chickens in each group at the 6<sup>th</sup>, 7<sup>th</sup>, 8<sup>th</sup>, 14<sup>th</sup>, and 21<sup>st</sup> days post-infection (dpi).

Mortality = (No. of dead birds/Total No. of birds) × 100

FCR = Feed intake in grams/Weight gain in grams

#### **The lesion score and cecal length**

Three chickens from each group were sacrificed at 6<sup>th</sup>, 7<sup>th</sup>, 8<sup>th</sup>, 14<sup>th</sup>, and 21<sup>st</sup> dpi to determine lesion scores and cecal length. According to Johnson and Reid (1970), the lesion scores were as follows: 0; no gross lesions, 1; mild lesions, 2; moderate lesions, 3; severe lesions, and 4; more severe lesions.

#### **Eimeria oocyst output count per gram of feces (OPG)**

Oocyst count was performed following the McMaster counting technique according to the method described by Long and Joyner (1976). Five fresh fecal samples were collected twice daily from the ground until OPG counting was performed at the 6<sup>th</sup>, 8<sup>th</sup>, and 15<sup>th</sup> dpi.

#### **Blood and serum samples**

Blood samples were collected from the wing vein at the 6<sup>th</sup>, 7<sup>th</sup>, 8<sup>th</sup>, 14<sup>th</sup>, and 21<sup>st</sup> dpi. One ml of blood was collected in EDTA-containing tubes for hematology, and 4 ml were put in a plain tube for serum collection. Serum samples were stored at -20°C until used to determine the biochemical parameters.

#### **Hematology**

Different hematological parameters, including red blood cell (RBC) count, hemoglobin, packed cell volume (PCV), platelets count, total leukocytic counts (TLC), differential leukocytic counts (DLC), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were measured according to Feldman et al. (2000).

#### **Serum biochemical parameters**

Serum concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), uric acid, and creatinine (Cr) were measured spectrophotometrically using Spinreact diagnostic kits (Spain) and following the manufacturer's instructions.

#### **Histopathological examination**

Three chickens were sacrificed from each group at 6<sup>th</sup>, 7<sup>th</sup>, 8<sup>th</sup>, 14<sup>th</sup>, and 21<sup>st</sup> dpi for a histopathology examination. Cecum samples from chickens were preserved in 10% neutral buffered formalin for three days. The formalin-fixed cecum was washed, dehydrated in ethyl alcohol, and then processed until paraffin sections of 4 μm thickness. Paraffin sections were cleared with xylene and stained by hematoxylin and eosin stain (H&E) for light microscopical examination (Bancroft and Layton 2013).

#### **Statistical Analysis:**

The statistical analysis used the Analysis of variance-test (ANOVA) followed by the Duncan Multiple Range test (DMRT) to determine the significant differences among different groups. The statistical analysis was made using the SPSS computer program.

## RESULTS

### 1. Clinical signs

The chickens showed typical signs of coccidiosis, including depression, dullness, ruffled feathers, bloody diarrhea, and weight loss. The clinical symptoms were severe in group 2, infected, and remained untreated. Clinical signs were alleviated with the addition of drugs to other infected groups.

### 2. Mortality, Body Weight, and Feed Conversion Ratio

The uninfected control group had no mortality. The mortality was observed

at 6<sup>th</sup> dpi in all infected groups. The untreated infected group had five dead chickens. The treated group had two dead chickens. The oregano oil-treated group had two dead chickens. The treated group had ones low chicken. Oregano oil plus allicin treated group had one dead chicken.

Compared to group 2, the B.W. was significantly improved in groups 3, 4, 5, and 6, with best results in group 6. There was no significance for FCR among the groups, as shown in Table. 1.

**Table 1:** The Effect of amprolium, oregano oil, allicin individual administration, and oregano oil plus allicin combination on mortality percentage, BW, and FCR in experimentally infected chicken with *E. tenella*.

Group	Mortality %	Body weight B. W	FCR
G1	(0/20)* 0%	1050.66±1.15 <sup>b</sup>	1.73
G2	(5/20)* 25%	601.33±1.33 <sup>d</sup>	2.07
G3	(10/20)* 10%	1050.33±2.60 <sup>b</sup>	1.42
G4	(10/20)* 10%	1060.33a±0.88 <sup>b</sup>	1.273
G5	(1/20)* 5%	1020.33±0.33 <sup>c</sup>	1.274
G6	(1/20)* 5%	1100.00±1.15 <sup>a</sup>	1.272

Groups.1: control negative; 2: control positive; 3: amprolium treated; 4: oregano oil treated; 5: allicin treated; 6: oregano oil plus allicin treated asterisk: number of dead to total chicken. Different superscripts differ at  $P \leq 0.05$ .

### 3. The lesion score and cecal length

No gross lesion was observed in the uninfected control group. Group 2 had extremely severe cecal lesions compared to other groups. Groups treated with amprolium, oregano oil, allicin alone, and a combination of oregano oil and allicin had gross lesions ranging from mild to moderate

in the cecum. The oregano oil plus allicin-treated group had the less gross lesions. The cecal length was shorter in G2 compared to the uninfected control group. Other treated groups with amprolium, oregano oil, and allicin had cecal length like the control uninfected group, as shown in Table 2.

**Table 2:** The effect of amprolium, oregano oil, allicin individual administration, and oregano oil and allicin combination on lesion score and cecal length in experimentally infected chicken with *E. tenella*.

Group	Lesion Score	Cecal length
G1	0	12.99±0.075 <sup>a</sup>
G2	4	7.66±0.034 <sup>e</sup>
G3	1.8	12.66±0.11 <sup>b</sup>
G4	1.1	11.66±0.092 <sup>d</sup>
G5	1.3	12.66±0.13 <sup>b</sup>
G6	1	12.33±0.075 <sup>c</sup>

Groups.1: control negative; 2: control positive; 3: amprolium treated; 4: oregano oil treated; 5: allicin treated; 6: oregano oil plus allicin treated. Different superscripts differ at  $P \leq 0.05$ .0; no gross lesions, 1; mild lesions, 2; moderate lesions, 3; severe lesions, and 4; more severe lesions.

**4. Eimeria Oocyst output count per gram of feces (OPG)**

The uninfected control group had no OPG. In G2, *E. tenella* oocysts were detected in feces at the 6<sup>th</sup> dpi and peaked at the 21<sup>st</sup> dpi. In G3, G4, G5, and G6, *E. tenella*

oocysts were detected in feces at 6<sup>th</sup> dpi and declined at 8<sup>th</sup> and 21<sup>st</sup> dpi. The treated group with amprolium, oregano oil, allicin alone, and oregano oil with allicin combination had significantly lower OPG ( $P \leq 0.05$ ), as shown in Table. 3

**Table 3:** The Effect of amprolium, oregano oil, allicin individual administration, and oregano oil and allicin combination on oocysts output per gram feces in experimentally infected chicken with *E. tenella*.

Group	6 <sup>th</sup> dpi	8 <sup>th</sup> dpi	15 <sup>th</sup> dpi
G1	0	0	0
G2	8.85	13.38	21.61
G3	8.81	5.12	5.46
G4	9.85	7.69	2.89
G5	7.08	5.85	2.96
G6	7.73	6.95	2

**5. Hematological parameters**

There was a decrease in RBCs, Hb, and PCV% between all groups and G1 ( $P \leq 0.05$ ). Conversely, a significantly high result was noticed in G3, G4, G5, and G6 when compared with G2 ( $P > 0.05$ ), with more significance in G6. The leucocytic

count showed significant elevation in TLC (heterophil and monocyte count) and a significant reduction in lymphocyte count in all groups when compared with G1 ( $P \leq 0.05$ ). On the other hand, these parameters were high in G3, G4, G5, and G6 when compared with G2.

**Table 4:** The Effect of amprolium, oregano oil, allicin individual administration, and oregano oil and allicin combination on hematological parameters in experimentally infected chicken with *E. tenella*.

Group	Hb g/dl	RBCs X 10 <sup>6</sup>	PCV %	Platelets %	TLC X 10 <sup>3</sup> /μl
G1	9.86± 0.045 <sup>a</sup>	2.08±0.02 <sup>b</sup>	135.33±2.9 <sup>a</sup>	20.33±0.24 <sup>b</sup>	122.03±0.59 <sup>b</sup>
G2	6.23± 0.037 <sup>d</sup>	1.75±0.011 <sup>d</sup>	118.33±1.66 <sup>b</sup>	14.33±0.17 <sup>e</sup>	135.66±0.28 <sup>a</sup>
G3	8.46± 0.33 <sup>bc</sup>	1.95±0.015 <sup>c</sup>	130.33±3.92 <sup>a</sup>	16.33±0.25 <sup>d</sup>	106.23±0.21 <sup>e</sup>
G4	8.83±0.056 <sup>bb</sup>	2.08±0.015 <sup>b</sup>	134.00±1.15 <sup>a</sup>	18.66±0.34 <sup>c</sup>	111.50±0.09 <sup>d</sup>
G5	9.20±0.11 <sup>b</sup>	1.95±0.032 <sup>c</sup>	134.33±3.48 <sup>a</sup>	29.33±0.055 <sup>a</sup>	112.86±0.12 <sup>c</sup>
G6	9.03± 0.09 <sup>b</sup>	2.34± 0.02 <sup>a</sup>	135.33±3.52 <sup>a</sup>	28.66±0.7 <sup>a</sup>	104.70± 0.18 <sup>f</sup>

Groups.1: control negative; 2: control positive; 3: amprolium treated; 4: oregano oil treated; 5: allicin treated; 6: oregano oil plus allicin treated. Different superscripts differ at  $P \leq 0.05$ .

**6. Serum biochemical parameters**

There is a significant decrease in serum ALT, AST, urea, and creatinine of all groups compared to G2 ( $P \leq 0.05$ ), with

improvement in G5 and G6. No statistical differences between G3, G4, G5, and G6 result with that of G1, as shown in Table 5.

**Table 5:** The Effect of amprolium, oregano oil, allicin individual administration, and oregano oil and allicin combination on serum biochemical parameters in experimentally infected chicken with *E. tenella*.

Group	ALT (U/L)	AST (U/L)	Urea (mg/dl)	Creatinine (mg/dl)
G1	12.67± 1.45 <sup>b</sup>	208.67±1.45 <sup>b</sup>	8.33± 0.33 <sup>b</sup>	0.23±0.01 <sup>b</sup>
G2	25.33± 0.33 <sup>a</sup>	408.33±1.66 <sup>a</sup>	15.00± 0.57 <sup>a</sup>	0.44±0.01 <sup>a</sup>
G3	12.33± 0.33 <sup>b</sup>	209.33±0.33 <sup>b</sup>	8.33±0.33 <sup>b</sup>	0.23±0.01 <sup>b</sup>
G4	11.66± 0.66 <sup>b</sup>	200.33±0.33 <sup>c</sup>	7.66± 0.33 <sup>b</sup>	0.25±0.005 <sup>b</sup>
G5	12.00± 0.57 <sup>b</sup>	202.33±0.88 <sup>c</sup>	7.33±0.33 <sup>b</sup>	0.23±0.01 <sup>b</sup>
G6	11.33± 0.66 <sup>b</sup>	207.33±1.45 <sup>b</sup>	8.33± 0.33 <sup>b</sup>	0.25±0.005 <sup>b</sup>

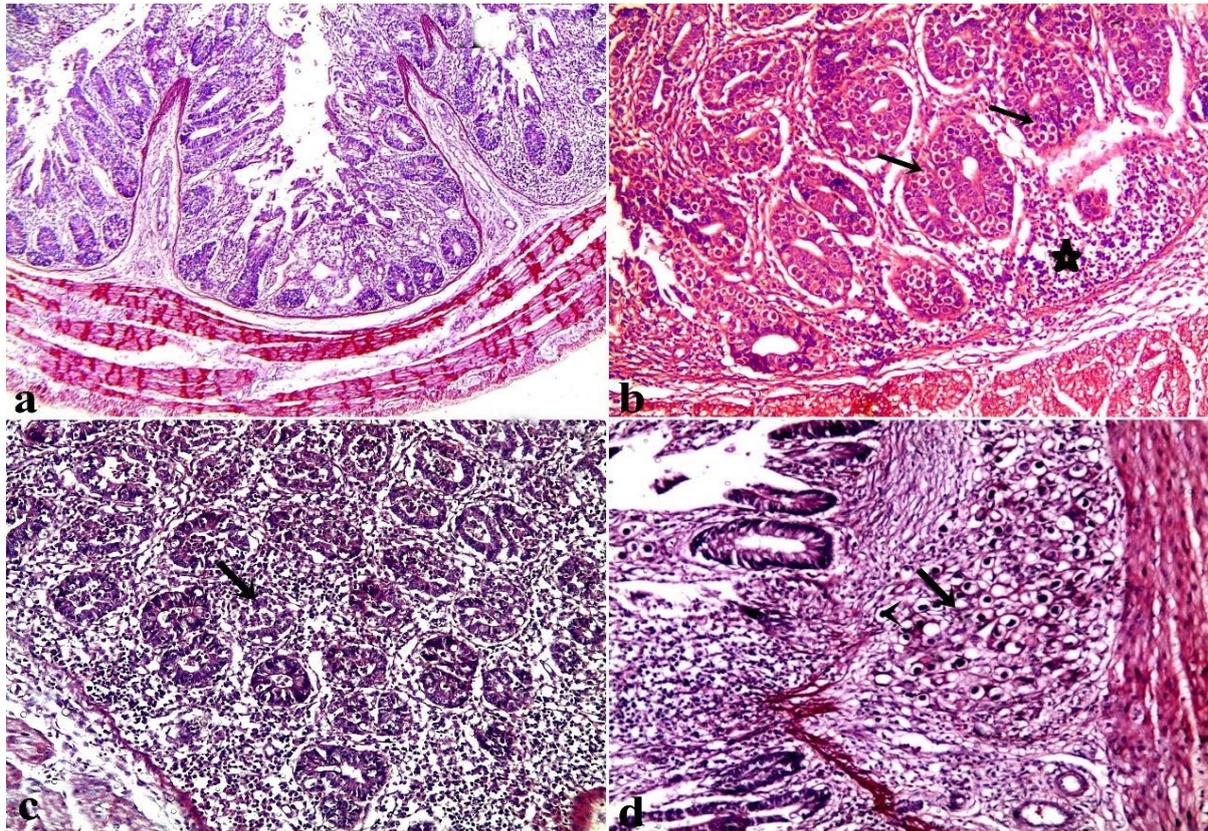
Groups: 1: control negative; 2: control positive; 3: amprolium treated; 4: oregano oil treated; 5: allicin treated; 6: oregano oil plus allicin treated. Different superscripts differ at  $P \leq 0.05$ .

### 7. Histopathological findings

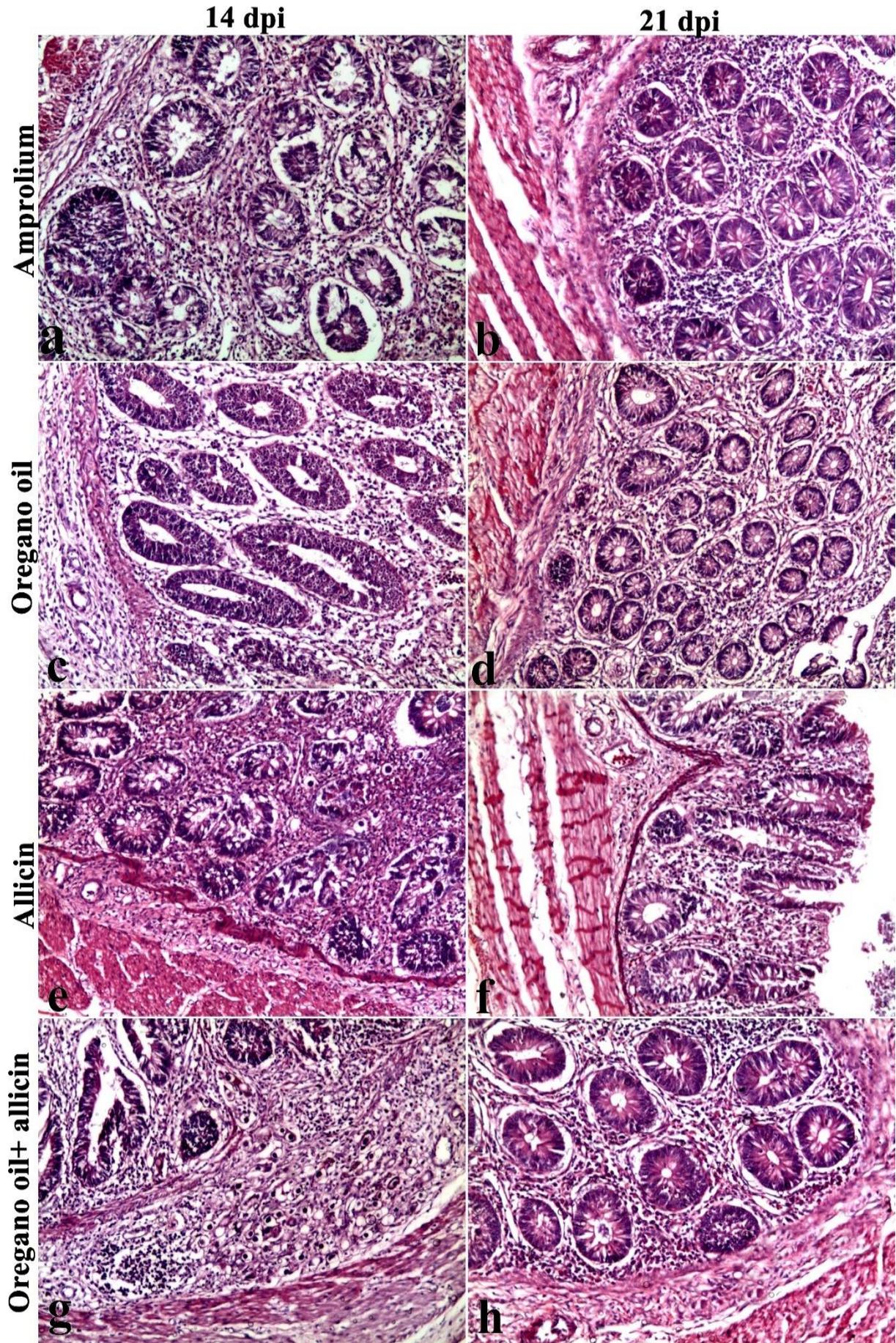
A gross examination of the infected chicken's cecum showed congestion and hemorrhage from the external serosal surface. Internally, the cecal mucosa was inflamed and contained brownish to reddish bloody content.

Microscopically, the cecum of uninfected control chickens was normal without any change in its histology (Fig. 1a). Compared to the control group, the cecum of positive control group (G2) was invaded by different developmental stages of *E. tenella*. At the 7<sup>th</sup> dpi, *E. tenella* was found in the cecal glands associated with submucosal inflammatory cells infiltration (Fig. 1b). At the 14<sup>th</sup> dpi, the glandular epithelium was necrosed and contained *E. tenella* with submucosal inflammation around the glands (Fig. 1c). At 21<sup>st</sup> dpi, aggregates of *E. tenella* in the submucosa with edema and inflammatory cells infiltration (Fig. 1d). While *E. tenella*

infected chickens and treated with different drugs showed the following: Amprolium treated group at 14<sup>th</sup> dpi showed the absence of *E. tenella* with the presence of submucosal inflammation. At 21<sup>st</sup> dpi, the cecum was infection-free with normal histology (Fig. 2 a and b). The oregano oil treated group at 14<sup>th</sup> dpi showed cecal glands severely infected with *E. tenella* with inflammation. At 21<sup>st</sup> dpi, the cecum showed typical histological features (Fig. 2 c and d). The allicin-treated group showed *E. tenella* stages with the presence of submucosal inflammation. At 21<sup>st</sup> dpi, allicin treated the cecum completely (Fig. 2 e and f). Oregano oil plus allicin treated group at 14<sup>th</sup> dpi showed *E. tenella* stages in the submucosa with infiltrating inflammatory cells. At 21<sup>st</sup> dpi, chickens showed normal cecal glands and the absence of *E. tenella* (Fig. 2 g and h).



**Fig. 1:** Microscopic lesions of *E. tenella* in the cecum of chickens. (a) The uninfected control group showed normal cecal musculosa, submucosa, and mucosa histology. H&E. X4. (b) The infected group at 7<sup>th</sup> dpi showed heavy infection of the cecal glands with *E. tenella* (arrows) with severe inflammation in the submucosa (star). (c) The infected group at 14<sup>th</sup> dpi showed severe degeneration and necrosis of the cecal glands (arrow) with the presence of *E. tenella* and inflammation extended between glands in the submucosa. (d) The infected group at 21<sup>st</sup> dpi showed degenerated stages of *E. tenella* in the submucosa (arrow) with inflammation and absence of *E. tenella* from the cecal glands. H&E. X10.



**Fig. 2:** Microscopic lesions of *E. tenella*-infected chickens received treatment. (a) Amprolium treated group at 14<sup>th</sup> dpi showed the absence of *E. tenella* with submucosal inflammation. (b) Amprolium treated group at 21<sup>st</sup> dpi showing normal histology of treated chickens cecum. (c) Oregano oil treated group at 14<sup>th</sup> dpi showing cecal glands severely infested with *E. tenella* with presence inflammation. (d) Oregano oil treated group at 21<sup>st</sup> dpi showing typical histological features of the cecum. (e) The allicin-treated group at 14<sup>th</sup> dpi showed *E. tenella* stages with submucosal inflammation. (f) The treated group at 21<sup>st</sup> dpi showed the absence of *E. tenella* with normal cecal histology. (g) Oregano oil+allicin treated group at 14<sup>th</sup> dpi showed *E. tenella* stages in the submucosa with infiltrating inflammatory cells. (h) Oregano oil+allicin treated group at 21<sup>st</sup> dpi showing normal glands and absence of *E. tenella* from the cecum. H&E.X10

## DISCUSSION

Cecal coccidiosis is a parasitic disease caused by *E. tenella* that causes severe economic losses to the poultry industry (Bhogal et al., 1988; Oluyemi and Roberts, 2000). Due to the emergence of new strains of *Eimeria* that are resistant to antibiotics (Chapman, 1997; Arab et al., 2006). Also, there is a need for safe chicken products that are free from drug residues (Harper and Makatouni, 2002). We aimed to evaluate the effect of oregano oil and allicin in treating chicken experimentally infected with *E. tenella*.

In this study, the plant-origin drugs' effect on the broiler chickens' body weight was like those reported (Symeon et al., 2010; Abou-elkhair et al., 2014; Zhang et al., 2021). The use of individual preparation of oregano oils and allicin or mixture between them to treat chickens infected by *E. tenella* was beneficial with improvement of chicken's performance by increasing the body weight, the feed intake, and the food conversion at 21<sup>st</sup> dpi, as previously that revealed reported by Dakrouy et al. (2014); Dias G. et al. (2015); Sidiropoulou et al. (2020). The results showed that oregano oils and allicin had a good effect on the cecal morphology and the *E. tenella* oocyst output as recorded by El-Khtam et al. (2014); Dakrouy et al. (2014); Sidiropoulou et al. (2020). Also, oregano oil and allicin significantly reduced cecal lesion scores and fecal oocyst count as compared to the control infected group, which agrees with

(Remmal et al., 2011; El-Khtam et al., 2014; Sidiropoulou et al., 2020). Oregano oil and allicin may inhibit the invasion and replication of *Eimeria* in the gut tissues of chickens.

Oregano oil and allicin significantly affected hematological parameters like Hb, RBCs, PCV, and WBCs, which this agrees with (Jameel et al., 2014). On the other hand, our results disagreed with Elagib et al. (2013), who reported that no significant effect was found on Hb, RBCs, PCV, and WBCs. This current study indicates that oregano oil and allicin are immunostimulatory, mobilizing the immune system and empowering the body's defense against infectious agents.

The histopathological effects of oregano oil and allicin and the mixed dose of them in this study were significant; where succeeded in treating the cecum completely, the cecum showed normal histological features, and chickens showed normal cecal glands and the absence of *E. tenella*, which in harmony with Silva et al. (2009); Ali et al. (2019). Cecal epithelial cells were invaded by 1st generation merozoites, which free themselves from crypt epithelial cells and extend to the lamina propria. In the lamina propria, 1st generation merozoites developed into 2nd generation schizonts, as El-Shazly et al. (2020) reported. Treated groups with amprolium, oregano oil, and allicin had damaged and necrosed *E. tenella* stages, and a reduction in oocysts count, as

observed by El-Shazly et al. (2020). Cecal mucosal damage is a predisposing factor for clostridial necrotic enteritis as dead tissue, damaged mucosa, blood, and plasma proteins escape to the intestinal lumen can favor the condition for Clostridium infection (Van Immerseel et al. 2004; Williams 2005; El-Shazly et al., 2020). Oregano oil and allicin may interfere with *Eimeria* replication and proliferation in the intestine, limiting clostridium or other bacterial infections.

In conclusion, this study proved that oregano oil and allicin have a protective and curative effect on *Eimeria*-infected chickens as anti-coccidial drugs of plant origin, which enhance body weight and immunity. Furthermore, the mixture of the two chemicals is a promising treatment for *E. tenella* infection in broiler chickens.

#### **Ethical approval**

This study was approved by the Faculty of Veterinary Medicine, University of Sadat City ethics committee with approval number (VUSC-030-1-21).

#### **Acknowledgments**

We are very grateful to the pathology department technician for their great help preparing histopathological sections.

#### **Conflicts of Interest**

The authors declare no conflict of interest.

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