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Comparative Studies on Commercial Livecox® Vaccine and *Artemisia* Extract Against Experimentally Induced Coccidiosis in Broiler

Shaimaa M. Abdel- Fattah, Eman S. El-Ashry*, Reham A. El-Shafei and Magdy S. Amer
Pharmacology Department, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt.

Abstract

Coccidiosis, a parasitic disease affecting chickens, poses a significant economic threat to poultry farmers worldwide, causing global economic losses. Therefore, seventy-five chicks one day old were divided into five equal groups, where group (1) represented the negative control, and groups (2–5) were infected with *Eimeria tenella* at a dose of 50.000 at 21 days and then received 200 ppm *Artemisia* extract (group 3), 1ml/100 chicks in the group (4) and 200ppm *Artemisia* extract with 1ml/100 chicks livecox vaccine in the group (5), respectively. Livecox® vaccine was found to have a considerable positive impact on protection against cecal coccidiosis, as evidenced by the following outcomes: reduced mortality rates, improved feed conversion and body weight, decreased oocyst output, improved the IgM and IgG, and decreased lesion formation. The current study revealed that two potential treatments for cecal coccidiosis were Livecox® vaccination and *Artemisia* liquid extract as they decreased oocyst count and cecal lesions and subsequently improved changes in some hematological (Hb, PCV%, WBCs and differential leukocytic counts) and biochemical parameters (IgM and IgG). Before using the extract in medical applications, additional investigations are necessary to determine the chemical components, recommended dosages, and mechanism of action.

Keywords: Broiler, *Artemisia brevifolia, Eimeria tenella,* Livecox vaccine.

Introduction

Coccidiosis, a parasitic disease that affects chickens, poses a significant reduction economic to poultry farmers worldwide, causing global economic losses exceeding $3 billion annually in the poultry industry. The infection leads to various negative health issues in birds, including death, nutrient absorption problems, poor feed conversion, stunted growth in broiler chickens, and decreased production of eggs in laying hens [1]. The severity of these impacts is evident in regions like Egypt, where coccidiosis has been linked to mortality rates of approximately 11.83% and income losses nearing 21.67% [2].

Protozoan parasites of the genus *Eimeria*, which inhabit chickens' digestive systems, can cause significant damage to the intestinal lining. This damage disrupts essential functions such as feeding, digestion, and nutrient absorption, ultimately leading to reduced weight gain, hemorrhage, and a heightened susceptibility to other bacterial infections [3].

Nine species of *Eimeria* have been identified in chickens, highlighting the widespread nature of this parasitic threat [4].

Eimeria tenella is a species of protozoan parasite that specifically targets the ceca, a part of the digestive tract, in chicken. Infections with *E. tenella* can lead to several severe symptoms such as weight loss, bloody diarrhea, high rates of illness and mortality, and significant damage to the cecal lining. This damage can manifest as small hemorrhages (petechiae), thickening of the cecal wall, larger areas of bleeding (ecchymosis), and the accumulation of blood and cheesy material within the ceca [5].

Poultry coccidiosis is primarily managed through preventative measures, with anticoccidial drugs playing a central role. These drugs, often added directly to chicken feed, work by disrupting the life cycle of *Eimeria* parasites, effectively inhibiting their ability to multiply and cause disease [6]. However, this reliance on anticoccidial drugs presents several challenges.

*Corresponding authors: Eman S. Elashry, E-mail: dr_emansaleh@mans.edu.eg Tel.: 01063036423
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These include mandatory withdrawal periods before slaughter to ensure drug residues are not present in meat, the potential for parasites to develop resistance to commonly used medications, and the Possible persistence of drug residues in poultry products intended for human consumption [7]. The incidence of *Eimeria* infections has been effectively managed with the use of anti-coccidial medications. However, the continuous and extensive usage of these drugs has led to the worldwide emergence of drug resistance. Consequently, the general population is more aware of the detrimental consequences of drug residues in chicken products [8].

Over the last 10 years, various alternative methods that involve using plants and their products have been documented for their ability to combat coccidial infections [9]. Many findings indicate that parasites are acquiring resistance to pharmacological medications [10], Experimental use of plant extracts, especially those rich in antioxidants, as antiparasitic medicines has gained much importance [11].

Artemisia brevifolia, commonly nicknamed wormwood in English and locally known as Afsanteen, exhibits a diverse array of pharmacological and therapeutic properties against specific parasite and bacterial diseases. These effects are ascribed to the active involvement of various antioxidant compounds [12].

Vaccines have been historically employed in the poultry sector for over five decades, mostly in the context of broiler breeders and layer flock replacement [13]. Various strategies have been developed globally to protect hens from coccidiosis by administering small amounts of sporulated oocysts in vaccines, irradiated sporulated oocysts [14], sporozoites [15], merozoites, recombinant merozoite antigen [16], recombinant refractile body antigen [17] and sonicated oocyst [18]. In general, vaccinations against *Eimeria* spp. are believed to activate the host immune system [19]. In chickens, coccidial vaccinations reduced oocyst shedding, lesion score, bloody dropping, and death rates while increasing feed consumption rate (FCR) and body weight gain (BWG). The live attenuated vaccine had the greatest impact in these areas [20].

The present study aimed to evaluate the potential anticoccidial effect of commercial Livecox ® vaccine and *Artemisia* Extract in *E. tenella*-infected chicken.

Material and Methods

Plant materials and commercial drug

Artemisia brevifolia (afsanteen) leaves were procured from Agriculture Research Center (ARC) in Kafr El-Sheikh Governorate, middle north of the Nile Delta Region. The researchers extracted compounds from *Artemisia brevifolia* by drying the plant material in a sheltered area. Then, they used an electric mill to grind the dried material into a powder. Finally, they obtained an aqueous methanolic extract using a Soxhlet apparatus (Velp Italy) [21]. After extraction, *Artemisia brevifolia* extract was stored in a refrigerator at 4°C until further analysis. Livecox vaccine supplied by BioPharma Pharmaceuticals and veterinary drugs, company at dose 1ml/100 birds

Isolation and preparation of Eimeria tenella oocysts:

The study utilized a field strain of *Eimeria tenella* obtained from commercial poultry shops in Kafr El-Sheikh city. To induce sporulation, the oocysts were stored in a 2.5% potassium dichromate solution at 25-29°C with a humidity level of 60-80%. The sporulated oocysts were then refrigerated (2–5°C) until use. At 21 days old, each bird was challenged with 50,000 *E. tenella* oocysts [22].

The number of sporulated oocysts was determined using the modified McMaster technique. The slides were then examined under a microscope at both low (10x) and high (40x) magnification.

Experimental animals

Seventy-five chicks were divided into five equal groups. Five chick groups were divided as follows:

- G1 (negative control group): Non-challenged, non-treated.
- G2 (positive control group): Infected by 50,000 sporulated oocysts of *E. tenella* at day 21 of age [22] but non-treated.
- G3: Infected by 50,000 sporulated oocysts of *E. tenella* at day 21 of age then given *Artemesia* extract at a dose of 200 mg/kg orally for 5 successive days [23]**.**
- G4: Vaccinated by Livecox® Vaccine on day 4 in drinking water, then challenged by 50,000 sporulated oocysts of *E. tenella* at day 21 of age [26].
- G5: Vaccinated by Livecox® Vaccine on day 4 in drinking water, then challenged by 50,000 sporulated oocysts of *E. tenella* at day 21 of age [22] then given *Artemesia* extract at a dose of 200 ppm orally for 5 successive days [23].

This section will detail the criteria used to evaluate the preventive efficacy of the assessed plant extracts and vaccine:

Clinical signs

Observations for signs of cecal coccidiosis began on day 23 post-challenge (PC) and continued throughout the experimental period.

Mortality rate

Every day, the deaths were noted for each group, and the percentage of mortality was calculated.

Score

The cecum from the three birds that were slaughtered on the 7th and 21st days after infection was used to make it. The cecum was removed and a system of scores was implemented, ranging from 0 to 4, with the following categories: no gross lesion (0), mild lesion (1), moderate lesion (2), severe lesion (3), and extremely severe gross lesions (4).

Growth performance:

The chicks were weighed at the start till the end of the experiment. Their feed intake (FI), feed conversion ratio (FCR), body weight (BW), and body weight gains (BWG) were documented according to Sharaban et al., 2021 [20].

Evaluation of oocyte reduction percentage:

From day 5 to day 10 post-infection, three fresh fecal oocyst samples were taken daily from each group, from various regions of the litter scattered over the battery tare, for oocyst count. The collected fecal samples were kept in plastic containers labeled with group identification and date. They were then brought to the laboratory to be counted as the mean number of oocysts per gram of feces (OPG) for each group after being concentrated by the flotation technique and counted using the McMaster counting technique according to Levine 1988 [24**]**. Based on the number of oocysts per gram of feces in the treated and control groups, the reduction % of oocysts was calculated.

Hematological examination

A Neubauer hemacytometer slide was used to assess the erythrocyte and leukocyte counts, and the Natt-Herrick solution was used as a diluent stain [25]. A commercial kit was used for the cyanmethemoglobin technique to measure the hemoglobin (Hb) content. Before determining the absorbance, the Hb test samples were centrifuged to eliminate any radioactive material that may have become distributed. Using a micro-capillary reader and the microhematocrit centrifugation method (10,500 ×g for 5 min), the packed cell volume (PCV) was determined according to Coles, 1967 [26]. Diff-Quik stain was used to stain blood smears for differential leukocyte count.

Biochemical analysis

The following blood samples were taken for analysis on days 7, 14, and 21 after infection from three randomly selected birds in each group and placed in test tubes without an anticoagulant agent for collection of serum samples: Serum IgG and IgM levels were assessed according to Smith et al, 1994 [27].

Histopathological examination

On the 7th and 21st after the challenge, histopathological liver and cecal specimens were obtained. They were then preserved in 10% formalin, dried in a graded alcohol concentration, cleaned with xylene, and embedded in paraffin. The embedded tissues were sectioned at a thickness of 5 µm, then stained with hematoxylin and eosin (H&E) stain and seen under a microscope [28].

Statistical Analysis:

The data was shown as mean ± standard error or SE. At a significant level of $P<0.05$, the mean values of the various groups were compared using a one-way analysis of variance (ANOVA)-Tukey test. Statistical analysis was performed using the method cited in Petrie and Watson 1999 [29] and computerized using SPSS.

Results

Clinical signs and mortality rate

Chickens in groups treated with Livecox® vaccine (groups 4) and Livecox® vaccine and *Artemisia* extract (group 5) displayed minimal clinical symptoms and remained generally healthy throughout the study. Their appetite remained relatively normal. Conversely, chickens in the infected and untreated group (group 2) and those treated solely with *Artemisia* extract (group 3) exhibited characteristic symptoms of coccidiosis. These symptoms included depression, lethargy, ruffled feathers, weight loss, reduced activity, decreased food intake, and bloody diarrhea. This decline in health was progressive and led to death within 3 to 5 days post-infection in some cases. Specifically, one chick from the *Artemisia*-only treated group (group 3) and three severely affected birds from the infected and untreated group (group 2) died. Notably, no deaths occurred in groups 4 and 5 (Table 1).

Lesion score

While groups treated with *Artemisia* extract (G3) and Livecox® vaccine (G4) didn't show significant differences in lesion scores, the group receiving both the vaccine and the extract (G5) had the lowest scores. As expected, the infected and untreated group (G2) presented the highest lesion scores. Table 1 provides a detailed overview of the lesion scores observed in the ceca of all experimental groups.

Growth performance

Groups treated with *Artemisia* extract (G3), Live cox® vaccine (G4), and the combination of both (G5) all showed significant improvements in growth performance metrics compared to the infected and untreated group (G2) by weeks 5 and 6. These metrics included body weight, body weight gain, feed consumption, and feed conversion ratio.

However, differences were observed in week 4. During this week, group G5 (vaccine and extract) showed significant improvements across all growth performance measures compared to G2. Groups G3 and G4, showing no significant improvement in body weight or weight gain, showed significant increases in feed consumption and feed conversion ratio compared to the untreated group (G2). Detailed data for each growth performance metric are presented in Fig 1, 2, 3, and 4.

Oocyst count

All treatment groups (G3, G4, and G5) exhibited a significant reduction in oocyst count compared to the control-positive group (G2), as detailed in Table 2.

Hematological parameters

Furthermore, these treatment groups demonstrated a significant increase in erythrocytic counts, hemoglobin concentration, and packed cell volume compared to the infected and untreated group (G2). These improvements were observed on days 7, 14, and 21 post-challenge, with the most favorable results observed in group G5 (combined vaccine and *Artemisia* extract treatment) (Table 3).

Differential leucocytic count

On the 7th day post challenge

The study found no significant changes in neutrophil percentages for groups treated with *Artemisia* extract (G3), Livecox® vaccine (G4), or the combined treatment (G5). However, these treatment groups (G3, G4, and G5) all exhibited a significant increase in lymphocyte percentages compared to the control-positive group. Conversely, these groups showed a significant decrease in monocyte, eosinophil, and basophil percentages compared to the untreated, infected group. (Table 4).

On the 14th day post challenge

G3 and G4 revealed a non-significant change in lymphocyte % and a significant decrease in neutrophils %, monocyte%, eosinophils %, and basophils %, Meanwhile in G5, there was a significant increase in lymphocyte % but a significant decrease in neutrophils %, monocytes %, eosinophils %, and basophils % when compared with the control-positive group (Table 5).

On the 21st day post challenge

Comparing G4 and G5 with the control-positive group, the lymphocytic count showed a significant increase, otherwise, there was a significant decrease in neutrophils %, and monocytes % (Table 6).

Blood biochemical parameters

The current study revealed a significant decrease in serum immunoglobulin M (IgM, gm/dl) and the serum Immunoglobulin G (IgG, gm/dl) levels in G3, G4, and G5 treated groups in comparison with G2 on the 7th & 14th days post-challenge while on the 21st day post-challenge, there were non-significant changes in serum immunoglobulin M (IgM, gm/dl) and the serum Immunoglobulin G (IgG, gm/dl) in G3 but a significant increase in serum immunoglobulin M (IgM, gm/dl) and the serum Immunoglobulin G (IgG, gm/dl) in G5 and G4 compared to G2 (Table 7).

Histopathology results

At 2nd sacrifice (21 days post-infection)

At necropsy, G1 revealed that the cecum was of normal size with no obvious nodules or foci (Fig. 5) also the cecum of G2 showed cystic catarrhal enteritis associated with invasion of the glandular structures with different coccidial stages within the lining epithelium, mucin retention cyst and interstitial inflammatory cells infiltration, also presence deep abscess associated with massive necrosis with the presence of coccidial oocysts and surrounded with massive fibrosis (Fig. 6 & 7). In G3, there were deep abscesses associated with massive necrosis with the presence of coccidial oocysts and surrounded with massive fibrosis (Fig. 8). Moreover, the cecum of G4 showed a decrease in the necrotic changes within the mucosal lining associated with the coccidial stages within the epithelial lining of the intestinal crypt with mild interstitial inflammatory cells infiltration and marked the enteritis lesions accompanied with focal interstitial degenerated coccidial oocysts and with normal intestinal glands (Fig. 9) and in the cecum of G5 gives normal hepatocytes and minimal periportal inflammation associated with slight infiltration of mononuclear cells mostly lymphocytes and macrophages (Fig. 10).

Discussion

Coccidiosis is a continuous health problem in the chicken business, particularly in intensive production systems. Since the annual expenses of treatment and prophylaxis approach 2 billion euros globally, it is the most significant poultry illness economically [30].

Since *E. tenella* causes hemorrhagic diseases and has a high infection-related mortality rate, it has been identified as the most pathogenic species [31]. Prophylactic use of anticoccidial medications in food was the main strategy for controlling coccidiosis. Since their widespread usage has unavoidably resulted in the emergence of medication resistance, alternate methods of controlling avian coccidiosis have been researched. The new methods involve modifying the immune system of chickens, using natural products, probiotics, live vaccines, and better farm management techniques [7].

In this study, chickens of group 4 (Livecox® vaccine) and group 5 (Livecox® vaccine and *Artemisia* extract) medicated groups showed mild clinical signs, and no deaths were recorded. However, chickens in the infected non-treated group (G2) and *Artemisia* extract medicated group (G3) were exposed to the typical symptoms of coccidiosis. There was a lower mortality rate in G3 (*Artemisia* extract treated group) than in G2 (infected non-treated group). Groups treated with the Livecox® vaccine and/or *Artemisia* extract (G3, G4, and G5) showed a reduction in lesion scores and were significantly different from the infected/non-medicated group (G2). In comparison to the group that was not treated (G2), the chickens in groups G3, G4, and G5) who received the LiveCox® vaccine and/or Artemisia extract showed improvements in feed consumption, feed conversion, and body weight and demonstrated low fecal oocyst count.

Similar results were obtained from previous studies as Hussain et al., 2021 and Allen et al., 1997 [23, 12] who revealed that, the anticoccidial activity of *Artemisia brevifolia* extract, *A. annua*, and live vaccine in different parameters, including, oocyst count, feed conversion ratio, and mortality (%).

Several antioxidants (phenols, flavonoids, conyzorium, methexnebilitin, and quercetin) compounds found in *Artemisia brevifolia*, could be responsible for anticoccidial action. These compounds can reduce coccidiosis by interfering with Eimeria's life cycle [23].

Coccidian immunity, achieved through active or passive immune responses, can be observed through bird health and performance improvements. These improvements include reduced severity of intestinal lesions, decreased shedding of parasite oocysts, and better overall growth [8].

In the present study, hematological values (including RBCs, WBCs, PCV, and Hb) in medicated groups with Livecox® vaccine and/or *Artemisia* extract were significantly improved than the infected/non-medicated group. These results matched Hussain et al., 2021 [23].

The results showed that G3, G4, and G5 evoked a significant increase in lymphocyte and a significant decrease in leukocyte count, neutrophils %, monocyte%, eosinophils %, and basophils % compared with positive control. Before, a study by Hussain et al., 2021 [23] showed that treating chickens infected with *E. tenella* with *Artemisia Brevifolia* extract significantly affected their white blood cell counts compared to infected chickens who didn't receive the extract. This finding is important because *E. tenella* infection is known to disrupt normal white blood cell levels, causing leukocytosis, lymphopenia, and heterophilia [32]. Also, Dar et al., 2014 [33] observed a significant increase in the overall leukocyte count in broiler chickens with *E. tenella* infection. Since hematopoietic cells are vital for defense and homeostasis, acute or chronic inflammatory diseases is the cause of monocytosis or heterophilia in birds [34].

In the current study, Results demonstrated significantly elevated antibody titers (IgG & IgM) in vaccinated groups (G4 & G5) on the 21st day post-challenge compared with the control group. These results matched with previous study in which higher IgA, IgG, and IgM [35] were detected in vaccinated chickens.

The histopathological examination showed that *E. tenella* infection was accompanied by a marked cecal lesion (G2). Suprihati and Yunus, 2018 [36] discovered that *E. tenella* displayed a significant quantity of oocysts in the cecum's lamina propria, whole epithelial desquamation, severe bleeding, and lamina muscularis edema. Free radicals are produced by a variety of cell types in the GI tract mucosa because of regular cellular metabolism. On the other hand, excessive generation of reactive oxygen species damages proteins within cells and breaks down the GI tract barrier, increasing intestinal permeability and causing inflammation. Additionally, too many reactive oxygen species causes polymorphonuclear leukocytes leading to additional damage to the tissue [37]. Reactive oxygen species are produced more frequently in cases of coccidiosis because of the parasite's activity and the host's cellular reaction, which lowers the levels of antioxidant enzymes and GSH in intestinal lining cells [38].

Our study revealed that medicated groups (G3, G4, and G5) showed a reduced cecal lesion. Protective immunity effectively blocks both the parasite's ability to reproduce (measured by oocyst production) and the development of clinical signs of disease in challenged birds. Antioxidants can lessen the cytotoxic effects of reactive oxygen species, which minimizes the damage

that parasite invasion causes to intestinal tissue [39], and their antioxidant components either delay or prevent *E. tenella* from multiplying [40].

Conclusion

The current study revealed that two potential treatments for cecal coccidiosis are Livecox ® vaccination and Artemisia liquid extract as they decreased oocyst count and cecal lesions and subsequently improved changes in the assessed hematological and biochemical parameters. Before using Artemisia extract in medical applications, additional investigations are necessary to determine the chemical components, recommended dosages, and mechanism of action.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study follows the ethics guidelines of the Faculty of Veterinary Medicine, Mansoura University, Egypt (ethics approval number; Ph.D/ 106).

TABLE 1. Mortality (%) and lesion score (1-4) in control and treated groups. (M ± S.E) N = 15

Group	Group1 (non-infected non treated)	Group2 (infected non treated)	Group3 (*Artemisia* extract treated)	Group4 (Live cox® treated)	Group5 (Live cox® + *Artemisia* extract treated)
Mortality	0	3	1	0	0
Percent	0 %	20 %	6.6 %	0 %	0 %
Lesion score (7th day)	0^{e}	3.67 ± 0.33^{a}	2.33 ± 0.30^{b}	2.00 ± 0.58^{b}	1.33 ± 0.33^{c}
Lesion score (21st day)	0^{e}	3.69 ± 0.33^{a}	1.33 ± 0.32^{b}	1.30 ± 0.33^{b}	0.33 ± 0.30^{c}

Pars carry different letters are significant at level ($P<0.05$)

TABLE 2. Oocysts count (x10³/gm feces) in control and treated groups. (M ± S.E) N = 15

Group	oocysts count (x10³ / gm feces) Time along the experimental period					
	5th day	6th day	7th day	8th day	9th day	10th day
G1 (Control negative)	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00^{e}
G2 (Infected non-treated)	5.27±0.09^{a}	27.23±0.1^{a}	49.27±0.33^{a}	93.98±0.07^{a}	63.51±0.22^{a}	34.92±0.07^{a}
G3 (*Artemisia* treated)	3.30±0.03^{b}	21.22±0.11^{b}	32.43±0.23^{b}	64.99±0.08^{b}	27.88±0.05^{b}	18.84±0.06^{b}
G4 (Live cox® vaccine treated)	2.29±0.05^{c}	5.56±0.09^{c}	15.50±0.06^{c}	33.06±0.08^{c}	13.33±0.09^{c}	4.66±0.09^{c}
G5 (*Artemisia* + Live cox® vaccine treated)	1.29±0.08^{d}	3.55±0.09^{d}	7.75±0.08^{d}	18.03±0.04^{d}	9.15±0.03^{d}	2.05±0.03^{d}

Pars carry different letters are significant at level (P<0.05)

TABLE 3. Effects of Artemisia extract and Livecox vaccine after their administration on RBCS Count (10⁶/mm³), Hemoglobin concentration (mg%), and Packed cell volume (%) of control and treated groups. (M ± S.E) (N = 3)

Group	RBCS Count (10⁶/mm³)			Hemoglobin concentration (mg %)			Packed cell volume (%)		
	Days post infection			Days post infection			Days post infection		
	7th day	14th day	21st da	7th day	14th day	21st day	7th day	14th day	21st day
G1 (Control negative)	4.1±0.09a	4.05±0.33a	4.00±0.10a	7.97±0.1^{a}	7.63±0.20^{a}	7.83±0.20^{a}	35.97±1.19^{a}	39.91±0.40^{a}	33.1±0.36^{a}
G2 (Control positive)	2.43±0.26b	2.67±0.09c	2.96±0.09c	4.81±0.38^{c}	5.47±0.18^{c}	6.05±0.09 ^{c}	26.54±1.82^{b}	23.76±0.38^{c}	28.68±0.28^{c}
G3 (*Artemisia* extract medication)	3.02±0.18b	3.11±0.09bc	3.27±0.14bc	6.6±1.22^{ab}	6.24±0.19^{b}	7.03±0.07 ^{b}	32.24±2.69^{a}	29.57±0.31^{b}	31.04±1.28^{bc}
G4 (Live cox® vaccine medication)	2.62±0.07b	3.25±0.11b	3.4±0.15b	6.51±0.25^{ab}	6.37±0.23^{b}	7.23±0.20^{b}	31.64±0.52^{ab}	29.67±1.08^{b}	32.83±1.58^{ab}
G5 (Live cox® vaccine +*Artemisia* extract medication)	2.42±0.26b	3.64±_0.08ab	3.79±0.03a	7.4±0.30^{a}	7.11±0.10^{a}	8.02±0.12^{a}	33.62±1.51^{a}	32.54±0.44^{a}	35.67±0.23^{a}

The different letters in the same column are significant at level (P<0.05)

TABLE 4. Effects of Artemisia extract and Livecox vaccine after their administration on WBCS Count, Neutrophils, Lymphocytes, Monocytes, Basophils, and Eosinophils of control and treated groups. (M ± S.E) N = 3

Group	WBCS Count (103/mm³)			Neutrophils (%)			Lymphocytes (%)			Monocytes (%)			Basophils (%)			Eosinophils (%)		
	Days post–challenge			Days post–challenge			Days post–challenge			Days post–challenge			Days post–challenge			Days post–challenge		
	7th day	14th day	21st day	7th day	14th day	21st day	7th day	14th day	21st day	7th day	14th day	21st day	7th day	14th day	21st day	7th day	14th day	21st day
G1 (control negative)	10.05± 0.51c	10.02± 0.32c	10.06± 0.07c	22.76± 1.52b	20.98± 0.44d	21.37± 0.35d	71.65± 1.95a	64.04± 10.30a	74.44± 0.57a	3.9±0 .38b	3.68± 0.09b	3.63± 0.23b	0.18± 0.04c	0.08± 0.01c	0.1± 0.01b	1.43± 0.18c	1.36± 0.07c	1.41±0. 06bc
G2 (control positive)	24.7±1. 78a	22.68± 0.74a	18.22± 0.21a	58.44± 2.57a	45.49± 0.72a	43.89± 0.07a	30.87± 2.38d	42.93± 0.48b	44.97± 0.09c	7.06± 0.64a	9.03± 0.46a	8.81± 0.14a	0.62± 0.1a	0.46± 0.05a	0.48± 0.03a	3.01± 0.48a	2.37± 0.13a	2.31±0. 25a
G3 (*Artemisia* extract treated)	15.47± 0.54b	15.3±0 .41b	13.33± 0.53b	44.51± 2.57ab	39.06± 0.60b	37.06± 0.72b	47.93± 2.65c	54.41± 0.92ab	56.19± 0.97bc	5.13± 0.27b	7.34± 0.24b	4.58± 0.57b	0.49± 0.05b	0.21± 0.01b	0.13± 0.01b	1.94± 0.32b	1.95± 0.08b	1.77±0. 07b
G4 (Live cox® vaccine treated)	14.87± 0.81b	14.22± 0.18b	13.25± 0.30b	41.42± 7.78ab	35.34± 3.15bc	35.48± 1.49b	48.00± 4.65c	55.05± 1.24ab	68.22± 8.97ab	5.11± 0.50b	4.27± 0.18b	4.38± 0.46b	0.47± 0.07b	0.21± 0.02b	0.12± 0.01b	1.99± 0.55b	1.79± 0.03b	1.79±0. 05b
G5 (*Artemisia* extract + Live cox® vaccine treated)	11.29± 0.68c	11.01± 0.81c	9.63±0 .32c	42.53±1 2.29ab	29.91± 2.10c	30.89± 0.47c	61.62± 3.04b	66.14± 1.27a	64.21± 1.18ab	4.7±0 .57b	3.62± 0.18b	3.66± 0.51b	0.24± 0.05c	0.15±0 .01bc	0.1±0. 01b	1.77± 0.11b	1.38± 0. 1c	1.28±0. 01c

The different letters in the same column are significant at level (P<0.05)

TABLE 5. Effects of Artemisia extract and Livecox vaccine after their administration on Immunoglobulin M (IgM) and Immunoglobulin G (IgG) levels (ng/ml) of control and treated groups. (M ± S.E) N = 3

Group	Immunoglobulin M (IgM) (ng/ml)			Immunoglobulin G (IgG) (ng/ml)		
	Days post–challenge			Days post–challenge		
	7th day	14th day	21st day	7th day	14th day	21st day
G1 (control negative)	70.01+_7.47e	72.39+_7.06e	73.73+_4.42c	150.90+_8.91e	150.18+_10.32e	151.83+_9.04c
G2 (control positive)	341.19+_8.48a	285.19+_6.12a	218.72+_10.78b	551.49+_7.01a	501.78+_10.06a	335.10+_12.34b
G3 (Artemisia extract treated)	217.59+_3.85b	203.61+_4.36b	190.05+_4.14b	350.33+_6.60b	319.64+_2.40b	320.75+_3.39b
G4 (livecox vaccine treated)	188.96+_3.93c	155.26+_2.85c	241.16+_14.79ab	286.61+_4.51c	260.70+_9.52c	336.90+_4.36b
G5 (Artemisia extract+ livecox vaccine treated)	98.13+_3.61d	93.58+_4.42d	267.23+_6.69a	198.51+_4.35d	178.54+_4.46d	415.46+_7.39a

The different letters in the same column are significant at level (P<0.05)

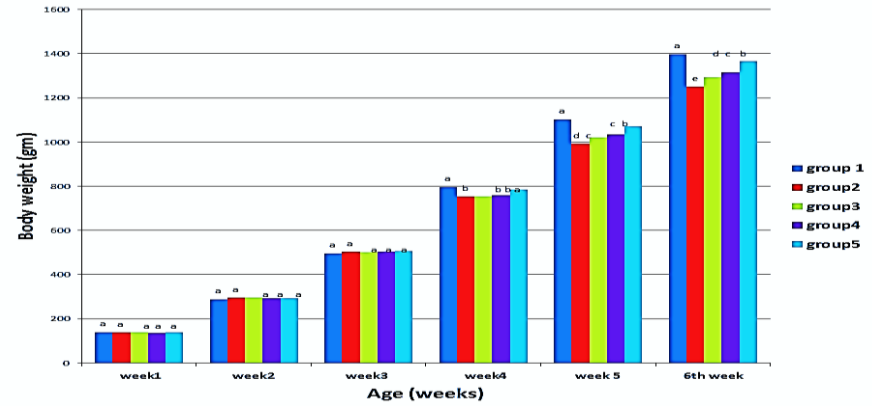

Fig 1. The effect of the orally administrated Artemisia extract (200mg/kg) and livecox vaccine (1ml/100 birds) on body weight (gm) of medicated broilers. Group 1 (control negative); Group 2(control positive); Group 3 (artmesia extract medicated); Group 4 (livecox vaccine medicated); Group 5 (artmesia extract and livecox medicated). Pars carrying different letters are significant at level (P<0.05)

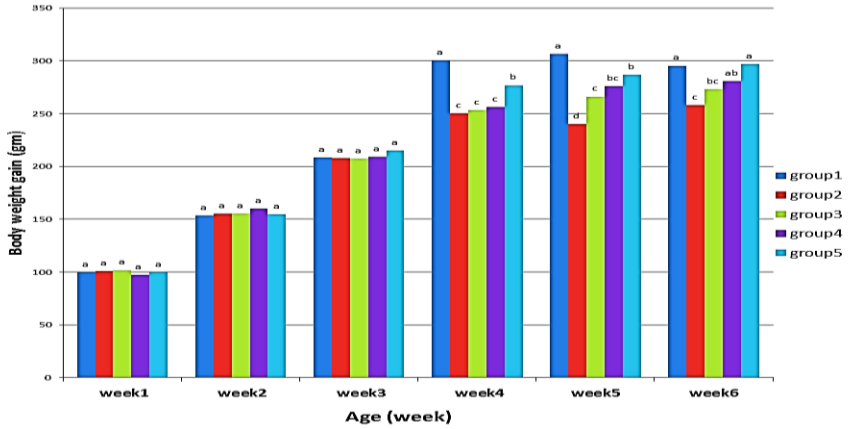

Fig 2. The effect of the orally administrated Artemisia extract(200mg/kg) and livecox vaccine (1ml/100 birds) on body weight gain (gm) of medicated broilers.Group1 (control negative); Group 2(control positive); Group3 (artemisia extract medicated); Group 4(livecox vaccine medicated); Group 5(artemisia extract and livecox medicated).Pars carrying different letters are significant at level (P<0.05)

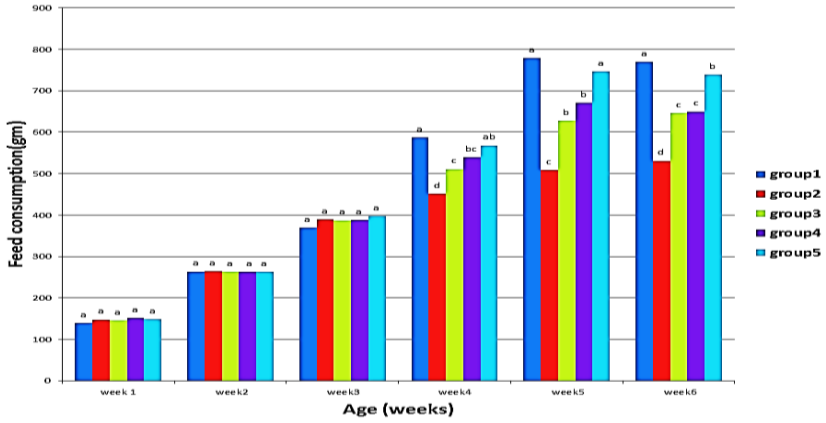

Fig 3. The effect of the orally administrated Artemisia extract(200mg/kg) and livecox vaccine (1ml/100 birds) on feed consumption (gm) of medicated broilers.Group1 (control negative); Group 2(control positive); Group3 (artemisia extract medicated); Group 4 (livecox vaccine medicated); Group 5 (artemisia extract and livecox medicated).Pars carrying different letters are significant at level (P<0.05).

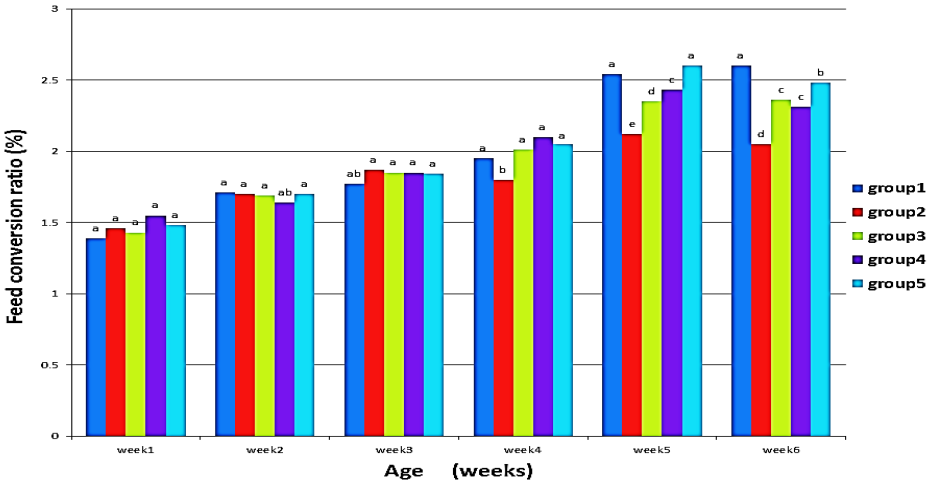

Fig 4. The effect of the orally administrated Artemisia extract (200mg/kg) and livecox vaccine (1ml/100 birds) on feed conversion ratio (%) of medicated broilers.Group1(control negative); Group 2(control positive); Group3 (artemisia extract medicated); Group 4(livecox vaccine medicated); Group 5 (artemisia extract and livecox medicated).Pars carrying different letters are significant at level (P<0.05)

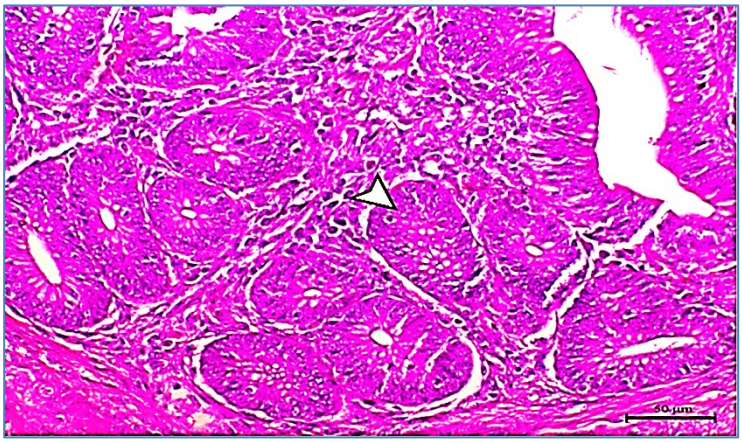

Fig. 5. The cecum of a normal broiler, on the 7th day post-infection showed normal mucosal lining consisting of normal glandular structures within the submucosa (arrowhead), H&E, X200, bar= 50 µm.

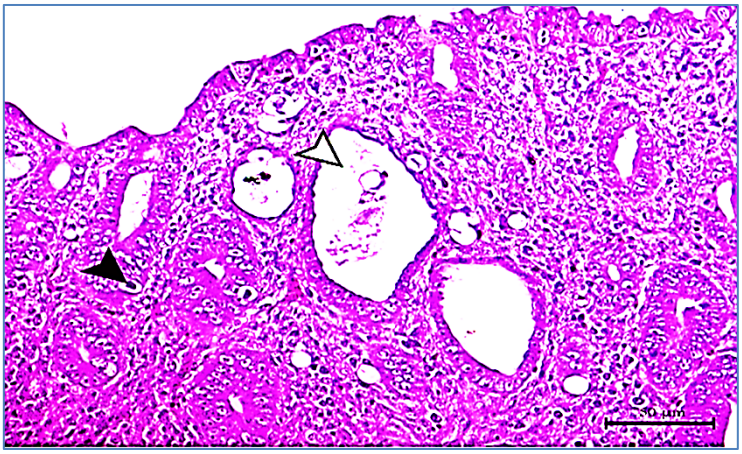

Fig. 6. The cecum of the broiler in G2 (challenged non treated group) on the 21st day post-infection, showed deep abscess associated with massive necrosis with the presence of coccidial oocysts (black arrowhead) and surrounded with massive fibrosis (white arrowhead), H&E, X200, bar= 50 µm.

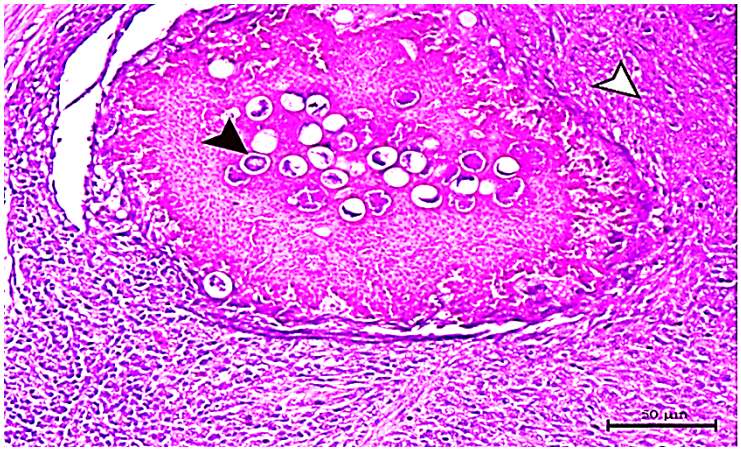

Fig 7. The cecum of the broiler in G2. (challenged non treated group) on the 21st day post-infection, showed cystic catarrhal enteritis associated with invasion of the glandular structures with different coccidial stages within the lining epithelium (black arrowheads), mucin retention cyst (white arrowhead), and interstitial inflammatory cells infiltration, H&E, X200, bar= 50 µm.

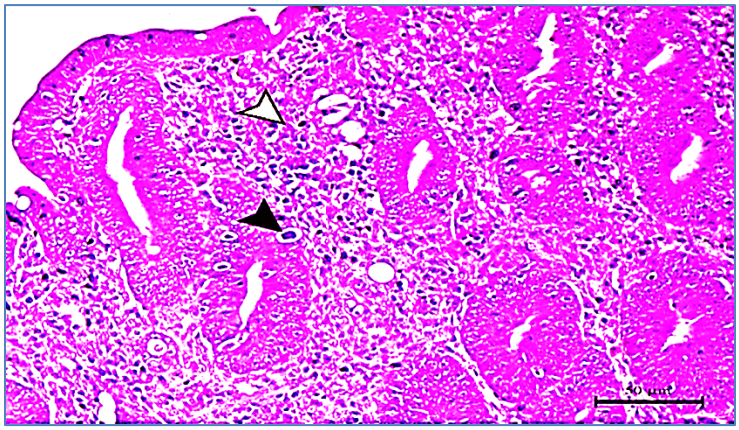

Fig. 8. On the 21st day post-infection, the intestine (cecum) of the broiler in G3 (diseased and treated with *Artemisia* extract) showed a decrease in the necrotic change within the mucosal lining associated with the coccidial stages within the epithelial lining of the intestinal crypt (black arrowhead) with mild interstitial inflammatory cells infiltration (white arrowhead), H&E, X200, bar= 50 µm.

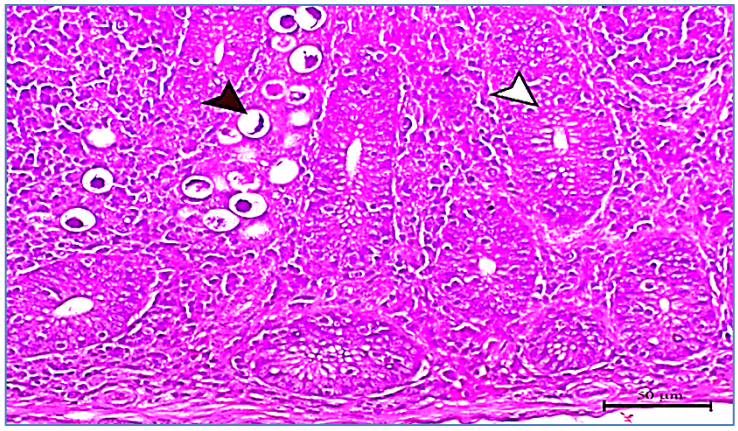

Fig. 9. The cecum of the broiler in G4 (diseased treated Live cox® vaccine), on the 21st-day post-infection, showed marked enteritis lesions accompanied by focal interstitial degenerated coccidial oocysts (black arrowheads) and with normal intestinal glands (white arrowhead), H&E, X200, bar= 50 µm.

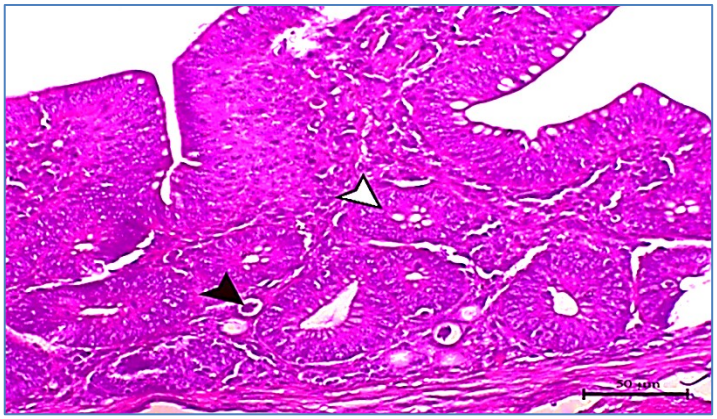

Fig. 10. The cecum of the broiler in G5 (diseased and treated with *Artemisia* extract + Live cox® vaccine), on the 21st-day post-infection, showed a marked decrease in the intraepithelial parasitic stages, and most of them have degenerated between the glands (black arrowhead) and most of the intestinal glands are normal (white arrowhead), H&E, X200, bar= 50 µm.

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دراسات مقارنة على اللقاح التجاري لايف كوكس ومستخلص الشيح ضد دجاج اللحم المصابة معمليا بالكوكسيديا

شيماء محمد عبد الفتاح، ايمان صالح العشرى، ريهام احمد الشافعى ومجدي صلاح عامر

قسم الفارماكولوجي، كلية الطب البيطري، جامعة المنصورة، مصر.

الملخص

تسلط هذه الدراسة الضوء على إمكانية استخدام لقاح الكوكسيديا (لايف كوكس) وخلاصة نبات الشيح كعلاجات لداء الكوكسيديا الأعوري، وهو مرض طفيلي يسببه طفيلي كوكسيديا الاعورين في الدجاج. تم تقسيم خمسة وسبعين كتكوتًا عمرها يوم واحد إلى خمس مجموعات، حيث كانت إحدى المجموعات بمثابة المجموعة الضابطة السلبية، وتم إصابة المجموعات الأخرى بطفيلي الكوكسيديا. تلقت بعض المجموعات المصابة علاجات مثل لقاح الكوكسيديا أو خلاصة الشيح أو مزيجًا من الاثنين. تشير النتائج إلى أن لقاح الكوكسيديا كان له تأثير وقائي كبير ضد داء الكوكسيديا الأعوري من خلال: خفض معدلات الوفيات، وتحسين معدلات تحويل العلف، وزيادة الوزن، وتقليل عدد البيوض (مما يشير إلى انخفاض العبء الطفيلي)، وتحسين المؤشرات البيوكيميائية وتقليل تكوّن الآفات في الأعور. وبالمثل، أظهرت خلاصة الشيح تأثيرات إيجابية، خاصة عند استخدامها مع اللقاح، مما عزز من مقاومة الدواجن للعدوى. تشير الدراسة إلى أن كلًا من لقاح الكوكسيديا وخلاصة الشيح هما علاجات واعدة لتقليل عدد البيوض، والآفات الأعورية، وتحسين الحالة الصحية العامة في الدجاج المصاب بالكوكسيديا.ومع ذلك، يتثني قبل التطبيق الواسع لخلاصة الشيح في مجال تربية الدواجن أو في التطبيقات الطبية، المزيد من الأبحاث التطبيقية لفهم التركيب الكيميائي، والجرعات المثلى، وآلية عملها بشكل أفضل.

الكلمات الدالة: كوكسيديا الاعورين، دجاج اللحم، الشيح، لقاح اللايف كوكس.