Plant Archives Vol. 21, Supplement 1, 2021 pp. 215-232 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
doi link : https://doi.org/10.51470/PLANTARCHIVES.2021.v21.S1.037

EXPLORING THE AMELIORATING ROLE OF *THYMUS VULGARIS AND ARTEMISIA ANNUA* POWDERS ON THE SIDE EFFECTS OF DICLAZURIL DRUG IN *EIMERIA TENELLA* EXPERIMENTALLY INFECTED BROILER CHICKENS

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ABSTRACT

Ameliorating effect of *Thymus vulgaris* and *Artemisia annua* powders on the adverse effects of diclazuril; an anticoccidial drug of *E. tenella* experimentally infected broiler chickens was investigated. Total of (105), one-day old cup broiler chicks were divided into seven groups each of 15 chickens: Group1 (G1) served as negative control. The other six groups were directly inoculated intra-crop with sporulated oocysts of *Eimeria tenella* on the 15th day of age. G2 was kept as the control positive. G3 treated with diclazuril (1ppm) in drinking water, G4 was fed ration mixed with *Thymus vulgaris* powder (5gm/kg^{-1}). G5 was treated with diclazuril and *Thymus vulgaris* powder, while G6 was fed ration mixed with *Artemisia annua* powder (3gm/kg^{-1}). G7 was treated with diclazuril and *Artemisia annua* powder. The experiment was continued for six weeks. The oocyst output, some biochemical parameters, hematological, immunological, drug residue, genotoxicity and histopathological changes were investigated. The results revealed that diclazuril had a worse effect on body weight, weight gain and feed conversion rate, while *Thymus vulgaris* ameliorates these worse effect, furthermore it failed to give complete coccidial clear, while *Artemisia annua* with diclazuril causes potent anti-coccidial effect. Diclazuril residues were detected in tissues. Administration of *Thymus vulgaris* and *Artemisia annua* had great value in decreasing diclazuril residues, enhance the immune profile and improved genotoxicity. In conclusion: administration of *Thymus vulgaris* and *Artemisia annua* powders with diclazuril can be successfully used in practice for ameliorating the adverse effects of diclazuril.

Keywords: *E. tenella*, Diclazuril, *Thymus vulgaris* powder, *Artemisia annua* powder, Broiler chickens.

Introduction

Avian coccidiosis is one of the most severe problems in poultry processing, as it has caused many losses in the poultry industry (Mathis, 2001). It was induced by *the Eimeria* species and causes various poultry syndromes including a decrease in body weight, enteritis, and bloody diarrhea (Peek, 2010). Also, it has effect on enzymatic activates and increase mortality rate. So it considers the most expensive disease facing the poultry industry in terms of both prevention and loss of performance of chicken (Xie *et al*., 2001).

Diclazuril is one of the benzenacetonitrile derivative with a broad spectrum activity against *Eimeria* species and was known to be the most effective anti-coccidial drug compared to the other chemical and ionophoric anti-coccidial agents in broiler (Conway *et al*., 2001), so it used intensively as a prophylactic anti-coccidial agent in poultry, (Awaad *et al*., 2003). It effects on asexual or sexual parasitic life cycle of coccidia prevents the excretion of oocysts causing disruption of their life cycle (Brander *et al*., 1991). The exact mechanism of action of diclazuril has been shown to cause disruption of trans-membrane potential of mitochondria and to induce ultrastructure changes in merozoites (Zhou *et al*., 2010)

Diclazuril had adverse effects on the growth performance in poultry (Bahadoran *et al*., 2014). Improper use of diclazuril inducing mutation of the parasites, evolution parasitic resistance that results in many economic losses in poultry industry (Abbas *et al*., 2014). Moreover; clear confirmation that diclazuril residues may be present in meat and affect consumers (Olejnik *et al*., 2009). Diclazuril had effect on immune system of poultry that because there was no protective immunity acquired as the bird is susceptible to infection by any infective oocytes in the litter (Reid, 1990). So, several poultry researchers all over the world are now actively examined the use of alternative plants and plant products to manage avian coccidiosis in a healthy, efficient and cheap way (Haq *et al*., 2011).

Addition of *Thymus vulgaris* with broiler ration had an excellent antibacterial, anti-coccidial and antifungal activity, thus improving the health of broilers (Haq *et al*., 2007), also it contain antioxidant agent which has role for growth, survival and maintenance productive health of the poultry (Franz *et al*., 2010). Also, it process coccidiostatic activity on *E. tenella,* when essential oil or crushed leaves, flowers and plant stems are introduced into the diets of chicken (Milhau *et al*., 1997). Moreover, it also rich in active compounds such as flavonoids which act as antioxidants therefore enhances the immune function (Cook and Samman,

1996). Adding *Thymus vulgaris* to chicken ration has no adverse influence on liver and kidney functions, (Zhu *et al*., 2016).

Artemisia annua is a member of the *Asteraceae* family. The most common species with anti-parasitic activity is *Artemisia annua*. It has many phytochemical ingredients; flavonoids and phenolic complexes compounds which can help birds to maintain commensal microflora and take up large amounts of nitrogen. It plays an important role in improving digestion, nutrient absorption and enhances innate and acquired immune response in poultry (Brisbin *et al*., 2008).

The anticoccidial effect of *Artemisia annua* against *E. tenella* infection in chicken was showed improvement in weight gain, feed conversion and reduction in lesion scores (Oh *et al*., 1995). Its mechanism of action was produced by inducing oxidative stress, which inhibits sporulation and the formation of cell walls in *Eimeria* organisms, leading to interference with the life cycle of *Eimeria* (Del Cacho *et al*., 2010).

This study was conducted to evaluate the ameliorating role of *Thymus vulgaris and Artemisia annua* plant powders on the side effects of diclazuril in chickens. In addition, to compare the effectiveness of these herbal plants with diclazuril on body weight, weight gain, feed conversion ratio (FCR), some serum biochemical parameters, hematological parameters, oocyst production, drug residues, the effect on the cell gene as well as histopathological pictures in *E. tenella* experimentally infected broiler chickens.

Materials and Methods

Chickens

Total of (105) one-day cup broiler chicks were purchased from Al-wady Poultry Company for the poultry industry, Egypt. They were divided into seven groups, 15 birds each reared under hygienic conditions, fed on formulated balanced ration without anti-coccidial additives and allowed free access to feed and water, as an approved programmer and the chicks were vaccinated against infectious bronchitis, Newcastle, and infectious bursal disease. All chickens were ethically treated according to Faculty of Veterinary Medicine, Beni-Suef University Committee for animal rights.

E. tenella oocysts:

E. tenella oocysts were obtained from Parasitology Department, Faculty of Veterinary Medicine, Beni-Suef University. Oocysts were collected from the duodenum of naturally infected chickens by the single oocyst isolation technique described by (Karim and Tress, 1990). The parasite was repeatedly passed in one-day old chicks. The oocysts were preserved in 2.5% potassium dichromate solution (Ali *et al*., 2014).

Drugs

Diclazuril (Diclacox liquid ®) was obtained from Arab Veterinary Industrial Company (AVICO) Jordan) 0.5% solution. It was administered at a concentration of (1 ppm) in drinking water. *Thymus vulgaris* and *Artemisia annua* powders were obtained from the Faculty of Pharmacy, Cairo University at dose 5gm/kg^{-1} and 3gm/kg^{-1} respectively.

Experimental design

The study was performed on (105) one-day-old cup broiler chickens; chickens were divided into 7 groups (15 chickens each). All groups except the first group were directly inoculated orally with 1ml solution containing about 1×10^3 of sporulated oocytes of *E. tenella* on the 15th days of age (Dalloul *et al*., 2003). The 1st group (G1) served as a negative control group (non- infected, non- medicated), the 2nd group (G2) control positive group (infected, non-treated), the 3rd group (G3) administrated diclazuril (1ppm) in drinking water (Conway *et al*., 2001). The 4th group (G4) fed on ration mixed with *Thymus vulgaris* powder (5gm/kg^{-1}) (Toghyani *et al*., 2010). The 5th group (G5) fed on ration mixed with *Thymus vulgaris* and diclazuril in drinking water, 6th group (G6) fed on ration mixed with *Artemisia Annua* powder (3gm/kg^{-1}) (Almeida *et al*. (2012) and the 7th group (G7) fed on ration mixed with *Artemisia Annua* powder besides of diclazuril in water. The experiment continued for six weeks.

Effects of Diclazuril, *Thymus vulgaris* and *Artemisia annua* on oocytes output in broilers chickens

The chickens were observed regularly for extreme clinical signs (bloody drop) that occurred at the 5th day post-infection. Fecal droppings (10gm) were collected daily from all chickens of all classes for 6 consecutive days from (6-11) days after infection (Farag *et al*., (2009), and oocysts were counted according to the Mc-Master technique in 1 gram of fecal matter (Georgi and Georgi, 1990). The percentage of oocyst reduction was determined according to the formula below:

$$\text{Reduction percentage} = (A-B)/A \times 100$$

Where, A is the mean number of oocysts in the positive control group and B is the mean number of oocysts in the treated group (Ali *et al*., 2014).

Effects of Diclazuril, *Thymus vulgaris* and *Artemisia Annua* on body performancein broilers chickens

At the end of each week, five chickens were selected randomly and the body weights were recorded. The body gain was calculated by subtracting the previous body weight and the next one.The feed intake was recorded and the FCR was calculated according to (Seddiek *et al*., 2008) using the following formula: FCR = Feed consumption in a given period/bodyweight gain at the same period. The experimental period lasted for six weeks.

Blood samples

At the end of the experiment, the first blood samples were taken from the wing vein of five birds in each group, mixed with EDTA anticoagulant (1mg/1ml) to be used in hematological studies. The second portion of the blood sample was taken at the age of 22 days by jugular puncture, and at the end of the experiment, into simple centrifuge tubes without anticoagulant, left to clot then centrifuged at 3000 r.p.m for 15 minutes, serum samples were kept at -20$^{C^o}$ until assayed for biochemical analysis.

Effects of Diclazuril, *Thymus vulgaris*and *Artemisia Annua* on blood picture in broilers chickens

Erythrocyte and complete leukocyte counts were performed according to Natt and Herrick (1952). The form of hemoglobin cyanide used to assess hemoglobin, as explained

by Varley (1980). The packed cell volume (PCV) was calculated according to (Schalm *et al*., 1975). The differential leukocytic count was performed according to (Schalm, 1961).

Effects of Diclazuril, *Thymus vulgaris* and *Artemisia Annua* on some biochemical parameter in broilers chickens

AST and ALT activities were estimated by using the colorimetric procedure described by Reitaman and Frankel (1957) while, alkaline phosphatase (ALP) was measured according to Belified and Gold berg, (1971). Serum uric acid was estimated using the colorimetric procedure as defined by Kageyama (1971) and serum creatinine was estimated according to Doolan *et al*. (1962).

Total serum protein was performed using the method of Gornall *et al*. (1949) , while the serum albumin was estimatedaccording to Doumas *et al*. (1971) method, estimation of globulin in serum by albumin substractions from total protein (Varley, 1980).

Effects of Diclazuril, *Thymus vulgaris* and *Artemisia Annua* on Haemagglution inhibition antibody titer (HIT) in broilers chickens.

Haemagglutination inhibition test (HI): was carried out in compliance with the protocol of (OIE 2002).

Comet assay (single-cell gel electrophoresis) SGE: for rapid genotoxicity evaluation as adopted the strategies suggested by Sasaki *et al*. (2002) for liver, duodenum, caecum tissue, tissue suspended in phosphate buffer saline.

Histopathological examination

Samples of tissue from scarified chicken from all experimental classes (liver, kidney, duodenum, and caecum) were fixed in a formalin buffer solution 10% for histopathological examination at7th day post-infection and at end of experiment (Claydan, 1971).

Effects of *Thymus vulgaris* or *Artemisia Annua* on diclazuril residue of liver, breast and thigh muscles of broiler chickens.

At the end of the experiment thigh, breast muscle and liver were taken and held at -20^{Co} for drug residue measurement using Liquid Chromatography (LC) according to Cortés Herrera *et al*. (2018) at the Animal Health Research Center, Dokki, Giza, Egypt.

Statistical analysis

The data obtained from the different groups of chickens were statistically analyzed for the mean and standard error using one-way variance analysis (ANOVA) using the approach defined in SPSS 14 (2006).

Results

Effects of Diclazuril, *Thymus vulgaris and Artemisia Annua* on body performance*in broilers chickens.*

Chickens infected with *E. tenella* (G2) showed significant decrease in body weight, body weight gain at 3rd week and feed consumption at 3rd and 4th week of age, while FCR showed no significant effect when compared to control group (G1). While, medication with diclazuril alone (G3) showed significant improved effect when compared to (G2), however it has significant decrease effect on body weight of chicken when compared to G5 and G7 at last two week. Chicken feed with *Thymus vulgaris* (G4) at 2nd week showed significant improved effect on body weight while after infection body weight, body weight gain at 3rd week and feed intake at 4th week showed significant decrease effect when compared to (G1) and diclazuril treated group (G3). Birds treated with *Thymus vulgaris* and diclazuril (G5) showed significant improvement in the body weight at 2nd, 3rd, 5thand 6th week of age, weight gain at 2nd, 6th week, FCR at 6th week and feed consumption at 4th and 6th week showed decreased effect in feed intake of chicken when compared to (G1) and diclazuril treated group (G3). Chicken feed with *Artemisia annua* (G6) showed no significant effect on body performance except at 4th week feed consumption showed significant decrease effect when compared to (G1) and diclazuril treated group (G3). Chicken treated with *Artemisia annua* and diclazuril (G7), showed no significant effect on body performance except at 4th week feed consumption showed significant decrease effect while body weight at 5th and 6th week,weight gain and FCR at 6th week showed significant improvement effect when compared to (G1) and diclazuril treated group (G3) as recorded in the table (1).

Table 1 : Effects of diclazuril, *Thymus vulgaris, and Artemisia Annua* on body performance *in broilers chickens* for 6 successive weeks, (Mean ± S.E), (n =5).

Time	Group	Bodyweight (g)	Body gain (g)	Feed intake(kg)	FCR %
1st week	G1	190.0±4.4^{a}	151.0±4.2^{a}	2.7±0.4^{a}	1.8±0.1^{a}
	G2	190.0± 6.3^{a}	151.0±7.01^{a}	2.7±0.4 ^{a}	1.8±0.08^{a}
	G3	208.6±2.4 ^{a}	169.6±3.3^{a}	2.7±0.4^{a}	1.6±0.1^{a}
	G4	196.0±5.09 ^{a}	157.0± 5.3 ^{a}	2.7±0.4 ^{a}	1.7±0.2^{a}
	G5	210.0±6.3 ^{a}	153.0±3.23 ^{a}	2.7±0.4^{a}	1.8±0.1 ^{a}
	G6	192.0±3.7 ^{a}	166.0±3.59 ^{a}	2.6±0.7 ^{a}	1.6±0.1 ^{a}
	G7	205.0±4.4^{a}	153.0±3.3 ^{a}	2.6±0.7 ^{a}	1.7±0.1^{a}
2nd week	G1	350.0±3.2^{b}	160.0±7.1^{b}	4.6±0.1^{a}	2.9±0.6^{a}
	G2	358.0±3.7^{b}	149.4± 2.5^{b}	4.5±0.3^{a}	3.01±0.3 ^{a}
	G3	346.6±1.9^{b}	150.6±4.3^{b}	4.6±0.1 ^{a}	3.05±0.3^{a}
	G4	388.0±3.74^{a}	178.0± 4.9^{b}	4.3±0.05^{a}	2.4±0.2^{a}
	G5	402.0±5.83^{a}	210.0±5.5^{a}	4.1±0.1 ^{a}	1.95±0.3 ^{a}
	G6	362.0±4.89^{b}	157.0± 6.6^{b}	4.6±0.1 ^{a}	2.9±0.1^{a}
	G7	366.0±3.2^{b}	161.0±3.3^{b}	4.3±0.04^{a}	2.67±0.2^{a}

3rd week	G1	718.0±10.2[b]	368.±11.6[a]	5.6±0.1[a]	1.5±0.12[a]
	G2	643.0±5.38[c]	285.0±5.0[b]	4.5±0.2[b]	1.57± 0.5[a]
	G3	715.0±6.325[b]	368.4± 5.6[a]	5.2±0.1 [a]	1.4±0.03[a]
	G4	652.0±17.72[c]	264.±15.7[b]	5.1±0.05[a]	1.9±0.4[a]
	G5	758.0±3.74[a]	356.0±8.8[a]	5.1±0.1 [a]	1.4±0.1[a]
	G6	720.0±6.325[ab]	358.± 10.7[a]	5.1±0.1 [a]	1.4±0.1[a]
	G7	754.0±2.5[ab]	388.0±7.5[a]	5.4±0.05[a]	1.4±0.1[a]
4th week	G1	1138.0±10.2[a]	420.±14.1[a]	6.7±0.1[a]	1.6±0.2[a]
	G2	1020.0±23.9[b]	377.±25.9 [a]	5.6±0.1[c]	1.5±0.2[a]
	G3	1148.0±15.9[a]	433.±15.9[a]	6.6±0.1 [a]	1.5±0.2[a]
	G4	1028.0±43.9[b]	376.±59.2[a]	6.1±0.05[b]	1.6±0.2[a]
	G5	1188.0±3.7[a]	430.0±6.3[a]	6.4±0.1[b]	1.5±0.2[a]
	G6	1142.0±5.8[a]	422.±11.6[a]	6.1±0.1[b]	1.4±0.2[a]
	G7	1172.0± 5.9[a]	418.0±7.4[a]	6.4±0.05[b]	1.5±0.1[a]
5th week	G1	1510.0±8.9[b]	372.0±8.0[a]	9.6± 1.4[a]	2.6±0.2[a]
	G2	1346.0±10.7[c]	326.± 19.4[a]	9.4±0.7[a]	2.9±0.1[a]
	G3	1500.0±3.16[b]	352.±13.2[a]	9.6±0.7 [a]	2.7±0.2[a]
	G4	1384.0±5.09[c]	356.±39.6 [a]	9.4±0.7 [a]	2.6±0.2[a]
	G5	1546.0±12.1[a]	358.±11.6[a]	9.4± 0.7[a]	2.6±0.2[a]
	G6	1510.0± 6.3[b]	368.±10.2 [a]	9.4±0.9 [a]	2.5± 0.3[a]
	G7	1546.±14.5 [a]	374.±20.7[a]	9.6± 1.3[a]	2.56±0.2[a]
6th week	G1	1650.0±13.7[b]	140.0±14[b]	10.3±0.1[a]	7.3±0.1[a]
	G2	1492.0±3.7[c]	146.0±8.4[b]	10.0±0.1[a]	6.8±0.06[a]
	G3	1680.0±7.7[b]	180.0±8.6[b]	10.2±0.2 [a]	5.6±0.1[a]
	G4	1502.0±4.9[c]	118.0±14.9[b]	10.2±0.1 [a]	8.6±0.1[a]
	G5	1778.0±5.8[a]	232.±8.9[a]	9.4±0.06[b]	4.1±0.6[b]
	G6	1670.0±4.5[b]	160.0±1.6[b]	10.6±0.1[a]	6.6±0.1[a]
	G7	1784.0±5.1[a]	238.±12.0[a]	10.3±0.1 [a]	4.3± 0.1[b]

Means with different letters (a, b, c) within the same column are significantly different at $P < 0.05$.

Effects of Diclazuril, *Thymus vulgaris* and *Artemisia Annua* on oocytes output in broilers chickens

Chickens experimentally infected with *E. tenella* and non-treated (G2) and *thymus vulgaris* (G4) showed more oocyst output compared to other groups. However effect of diclazuril alone (G3) or with *thymus vulgaris* (G5) on oocyst output has significantly improved effect when compared to infected non treated group (G2), with reduction %, is (71.26**,** 71.68 respectively). However, *Artemisia annua* when administrated alone or in combination with diclazuril (G7) significantly delayed oocyst output and the reduction ratio was (71.6, 87.53) respectively, as recorded in the table (2).

Table 2 : Effects of diclazuril*, Thymus vulgaris,*and *Artemisia Annua* on oocytes output in broilers chickens. (Mean× 10^4 oocysts/g feces ± S.E), (n =5).

Post infestation Days (PID)	Group1	Group 2	Group 3	Group4	Group 5	Group 6	Group7
6	0.0±0.0[c]	16.28±0.007[a]	9.52±0.17[b]	16.2± 0.1[a]	10.0± 0.1[a]	0.0±0.0[c]	0.0±0.0 [c]
7	0.0±0.0[d]	66.7±14.400[a]	20.6±1.7[b]	65.9±9.3[a]	20±3.2[b]	21±3.3[b]	12.2±1.96[c]
8	0.0±0.0[d]	180.5±0.173[a]	80.3±0.01[b]	179.9±.65[a]	79±0.26[b]	82.9±1.9[b]	30.2±0.1[c]
9	0.0±0.0[d]	130.7±0.070[a]	32.84±0.1[b]	130.6±0.2[a]	32.8±0.1[b]	36.6± 2.3[b]	20.2±0.1[d]
10	0.0±0.0[d]	86.5±0.0707[a]	8.7±0.070[b]	86±1.3[a]	8.2±0.1[b]	10.2±0.1[b]	3.2±0.07[c]
11	0.0±0.0[d]	65.2±0.0707[a]	4.7±0.070[b]	65.2±0.1[a]	4.4±0.1[b]	4.2±0.95[b]	2.2±0.07[c]
Overall mean	0.0±0.0[d]	90.86±0.00[a]	26.11±0.0[b]	90.63±0.0[a]	25.73±0[b]	25.8±0.0[b]	11.33±0[c]
Reduction%	--------	0.00	71.26	0.25	71.68	71.6	87.53

Means with different letters (a, b, c, d) within the same raw are significantat $P < 0.05$.

Effects of *Thymus vulgaris* or *Artemisia Annua* on diclazuril residue of liver, breast and thigh muscles of broilerchickens

Chickens treated with diclazuril (G3), showed the presence of diclazuril residues in the liver, thigh and breast muscle, and residues in the liver was higher than in muscle. While chickens feed *thymus vulgaris* with diclazuril (G5) or feed *Artemisia annua* with diclazuril (G7) both had improvement significant effect on decrease residue in the tissue of broiler, and (G7) showed highly significant improvement in decrease these residues when compare with diclazuril group (G3), as recorded in the table (3).

Table 3 : Effects of *Thymus vulgaris* or *Artemisia Annua* on Diclazuril residues inliver, breast ,and thigh muscles of broiler chickens. (Mean ± S.E), (n =5).

Tissue Group	Liver	Breast muscle	Thigh muscle
Group 3	0.40±0.025[a]	0.22±0.035[a]	0.24±0.003[a]
Group 5	0.25±0.023[b]	0.13± 0.006[b]	0.15±0.003[b]
Group 7	0.16±0.013[c]	0.09±0.002[b]	0.10±0.003[c]

Means with different letters (a, b, c) within the same column are significantly different at $P < 0.05$.

Effects of Diclazuril, *Thymus vulgaris* and *Artemisia Annua* on blood picture in broilers chickens

Chicken infected by *E. tenella* (G2), was showed significant decrease effect on blood pictures (RBCs, Hb and PCV) and marked increase of the total leucocytic count and eosinophil with decrease in lymphocyte count in comparison with control non-infected group (G1). While chickens medicated with diclazuril alone (G3) and/ or *thymus vulgaris* (G4, G5), *Artemisia annua* (G6, G7) has no significant difference effect on (RBCs, Hb, PCV). While chickens medicated with diclazuril alone (G3) showed significant decrease in lymphocyte and significant increase in eosinophil in comparison with control negative group (G1), also G4 and G5 showed significant increase in eosinophil, and significant improved effect of lymphocyte in comparison with diclazuril group (G3). However, they show improved significant effect in comparison with (G2). It was seen that the effect of *Artemisia annua*powder (G6) and when given with diclazuril (G7) on WBCs, and differential leucocytic count has no significant difference effect in comparison with control negative group (G1), as illustrated in the table (4).

Table 4: Effects of diclazuril, *Thymus vulgaris*, and *Artemisia Annua* on blood picture in broilers chickens. (Mean ± S.E). (n=5).

Parameter Group	RBCs (10^6/ul)	Hb (gm/ dl)	PCV %	WBCs (10^3/ dl)	Heterophil %	Lymphocyte %	Monocyte %	Eosinophil %
Group1	4.5±0.6[a]	26.23±2.0[a]	80.8±0.9[a]	3.6±0.3[b]	49± 2.12[a]	42.5±1.9[a]	6.1±0.64[a]	2.4±0.24[b]
Group2	1.6±0.2[b]	17.8±1.0[b]	66.2±2.9[b]	7.6±0.7[a]	54±4.3[a]	27±2.0[b]	9±0.16[a]	10±0.16[a]
Group3	4.4±0.2[a]	21.3±3.0[a]	74.7±2.3[a]	4.2±0.2[b]	53±1.9[a]	31±2.0[b]	7±0.32[a]	9±0.31[a]
Group4	4.2±0.4[a]	23.6±1.4[a]	72.4±3.2[a]	4.06±0.2[b]	40±4.7[a]	44± 2.4[a]	6±0.32[a]	10±0.40[a]
Group5	4.2±0.4[a]	23.8±1.2[a]	74.2±3.2[a]	4.6±0.4[b]	42±7.2[a]	43±3.4[a]	6 ±0.51[a]	9±0.50[a]
Group6	3.96±0.7[a]	22.8±1.96[a]	73.6±4.6[a]	4.2±0.3[b]	45±1.6[a]	43.2±2.9[a]	9±0.64[a]	2.8±0.33[b]
Group7	4.5±0.6[a]	25.2±1.59[a]	79.6±1.1[a]	4.9±0.3[b]	48±6.6[a]	44±4.0[a]	6 ±0.54[a]	2±0.27[b]

Means with different letters (a, b) within the same column are significantly different at $P < 0.05$.

Effects of Diclazuril, *Thymus vulgaris* and *Artemisia Annua* on Haemagglution inhibition antibody titer (HIT) in broilers chickens.

The result revealed that infected, non-treated group (G2) and diclazuril treated group (G3) at 7th day post infection and at the end of the experiment had significant decrease effect on HIT in comparison with control non-infected group (G1). Furthermore, it was seen that feeding broiler with *thymus vulgaris* powder (G4) or *Artemisia annua* powder (G6) and when feeding *thymus vulgaris* powder or *Artemisia annua* powder with diclazuril (G5, G6) showed improvement significant effect in comparison with (G3) and (G2), as illustrated in the table (5).

Table 5 : Effects of Diclazuril, *Thymus vulgaris* and *Artemisia Annua* on Haemagglution inhibition antibody titer (HIT) in broilers chickens. (Mean ± S.E), (n =5).

Time / Groups	7th day post infection	End of experiment
Group 1	4.4±0.24[a]	5.0±0.000[a]
Group 2	2.2±0.20[b]	3.0±0.57[b]
Group 3	2.2±0.55[b]	3.0±0.32[b]
Group 4	4.8±0.37[a]	4.8±0.37[a]
Group 5	4.5±0.22[a]	4.6±0.40[a]
Group 6	4.3± 0.20[a]	4.0±0.32[a]
Group 7	4.3± 0.25[a]	4.0±0.48[a]

Means with different letters (a, b) within the same column are significantly differentat $P < 0.05$.

Effects of Diclazuril, *Thymus vulgaris* and *Artemisia Annua* on Serum levels of total protein, albumin, globulin and A/G ratio in broiler chickens

Infected, non-treated group (G2) and diclazuril group (G3) had significant decreased effect on the total protein and albumin levels at 7th day post infection and at end of the experiment, in comparison with control non-infected group (G1). However, at 7th day post infection feeding *thymus vulgaris* powder (G4) alone showed decrease in total protein and globulin with increased in A/G, while when given with diclazuril (G5) has significant improved effect on total protein, Albumin in comparison with (G1). While at end of experiment, feeding *thymus vulgaris* powder (G4) has significant decrease effect on total protein in comparison

with (G1), and showed improvement effect when compared with diclazuril group (G3), while albumin, globulin and A/G showed no significant effect in comparison with (G1), and showed significant improvement effect when compared with diclazuril group (G3). *Thymus vulgaris* with diclazuril (G5) showed significant improved different effect on total protein in comparison with control and (G3). *Artemisia annua* (G6) and with diclazuril (G7) were showed no significant differential effect on total protein, Albumin, Globulin, and A/G at 7th day post infection and at the end of the experiment *Artemisia annua* (G6) showed significant decrease in total protein in comparison with control group (G1). Also it was showed improvement effect when compared with diclazurilgroup (G3), moreover (G7) at end of experiment showed improved significant effect on total protein in comparison with diclazuril group (G3), as illustrated in the table (6).

Table 6 : Effects of Diclazuril, *Thymus vulgaris* ,and *Artemisia Annua* on serum levels of total protein, albumin, globulin and A/G ratio in broiler chickens. (Mean ± S.E), (n =5).

Parameter	7th day post infection				End of experiment			
Group	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/ dl)	A/G	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/ dl)	A/G
Group1	4±0.63^{b}	2.5±0.32^{b}	1.5±0.23^{a}	1.6±0.03^{b}	5.0±0.30^{a}	2±0.09^{a}	3±0.22^{a}	0.6±0.1^{a}
Group2	1.9±0.1^{c}	1±0.07^{c}	0.9±0.34^{a}	1.1±0.05^{b}	2.8±0.41^{c}	0.9±0.1^{b}	1.9±0.39^{a}	0.5±0.1^{a}
Group3	2±0.3^{c}	1.1±0.16^{c}	0.9±0.20^{a}	1.2±0.07^{b}	2.9±0.43^{c}	0.9±0.06^{b}	2.0 ±0.44^{a}	0.5±0.2^{a}
Group4	1.9±0.4^{c}	1.6±0.24^{b}	0.3±0.21^{b}	5.3±0.08^{a}	3.8±0.12^{b}	1.3±0.2^{a}	2.5±0.43 ^{a}	0.5±0.3^{a}
Group5	6±0.64^{a}	3.8±0.40^{a}	2.2±0.29^{a}	1.7±0.08^{b}	4.9±0.29^{a}	1.4±0.24^{a}	3.5±0.23^{a}	0.4±0.1^{a}
Group6	3.7±0.3^{b}	2±0.32^{b}	1.7±0.38^{a}	1.2±0.03^{b}	3.8±0.17^{b}	1.3±0.33^{a}	2.5±0.49^{a}	0.5±0.3^{a}
Group7	3.4±0.2^{b}	2±0.06^{b}	1.4±0.42^{a}	1.4±0.09^{b}	4.3±0.15^{a}	1.3±0.04^{a}	3.0±0.13^{a}	0.4±0.2^{a}

Means with different letters (a, b, c) within the same column are significantly different at $P < 0.05$.

Effects of Diclazuril, *Thymus vulgaris* and *Artemisia Annua* on some biochemical parameter in broilers chickens

(a) Serum concentrations of uric acid and creatinine of broiler chickens

Diclazuril alone (G3), or in combination with either *thymus vulgaris* powder or *Artemisia annua* powder on kidney function of chicken infected with *E. tenella* on 7th day post infection and at the end of the experiment was illustrated in table (7). The results showed a significant increase in the level of uric acid and creatinine in (G2) in comparison with the control group. However administration of diclazuril alone or in combination with either *thymus vulgaris* powder or *Artemisia annua* powder improved these effects and the levels of uric acid and creatinine become the same as control non-infected group (G1).

Table 7 : Effects of Diclazuril, *thymus vulgaris*, and *Artemisia Annua* on serum concentrations of uric acid and creatinine of broiler chickens. (Mean ± S.E), (n =5).

Groups	7th day post infection		At end of experiment	
	Uric acid (mg/ dl)	Creatinine (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
Group 1	5.6±1.20^{b}	0.62± 0.17 ^{b}	5.67±0.88^{b}	0.45±0.28^{b}
Group 2	10±4.58^{a}	1.27±0.19^{a}	10.67±0.66^{a}	3.33±0.29^{a}
Group 3	5.3± 0.88^{b}	0.81±0.13^{b}	5.33± 1.20^{b}	0.43± 0.32^{b}
Group 4	5.0±0.57^{b}	0.89±0.18 ^{b}	5.33±0.33^{b}	0.36±0.25^{b}
Group 5	4.0±0.10^{b}	0.62±0.12 ^{b}	4.00±0.58^{b}	0.44± 0.08^{b}
Group 6	5.67±0.88^{b}	0.46±0.10 ^{b}	5.67± 1.33^{b}	0.19±0.06^{b}
Group7	5.3±0.66^{b}	0.39±0.06^{b}	5.67± 1.20^{b}	0.26±0.03^{b}

Means with different letters (a, b) within the same column are significantly different at $P < 0.05$.

(b) Serum levels of ALT, ALP, and AST of broiler chickens.

Experimentally infected chicken with *E. tenella* (G2) were showed significant effect on ALT, ALP, and AST at 7th day post infection and at end of experiment while, diclazuril treated group (G3) showed no significant effect on ALT, ALP, and AST at 7th day post infection and end of the experiment in comparison with control group and showed significant improvement effect in comparison with infected group (G2). Moreover, at 7th day post infection, chicken feeding with *thymus vulgaris* powder (G4) has significantly improved effect on ALT but at the end of the experiment, it showed no significant effect on ALT, ALP and AST. While, when feeding with diclazuril (G5) showed improvement effect on ALT, ALP and AST at 7th day post infection and at the end of the experiment in comparison with the control group (G1) and diclazuril group (G3). On the other side, addition of *Artemisia annua* powder to feed of chicken (G6) has no significant effect on ALT and AST; however it showed significant improved effect on ALP at 7th day post infection and the end of the experiment in comparison with the control group. While, group feeding *Artemisia annua* powder with diclazuril (G7) showed significant improved effect on ALP at 7th day post infection while at the end of the experiment ALT, ALP, and AST showed significant improved effect in comparison with the control group and diclazuril treated group (G3), as illustrated in the table (8).

Table 8 : Effects of Diclazuril, *Thymus vulgaris* and *Artemisia Annua* on serum levels of ALT, ALP, and AST of broiler chickens (Mean ± S.E), (n =5).

	7th day post infection			At end of experiment		
Parameter Groups	**ALT (U/ml)**	**AST (U/ml)**	**ALP (U/ml)**	**ALT (U/ml)**	**AST (U/ml)**	**ALP(U/ml)**
Group 1	14±2.5^{b}	91±8.70^{b}	289. 7±5.48^{b}	20±0.88^{b}	100±8.81^{b}	338.0±19.5^{b}
Group 2	22±1.3^{a}	170±12.25^{a}	387.0±53.41^{a}	35±6.12^{a}	180±37.42^{a}	429.33±24^{a}
Group 3	14±2.4^{b}	100±5.77^{b}	300.0±11.55^{b}	18±2.00^{b}	120±20.0^{b}	352.0±21^{b}
Group 4	4.5±0.50^{c}	92±6.01^{b}	253.3±14.5^{b}	16.0±2.5^{b}	90 ±10.0^{b}	337.3±12^{b}
Group 5	5.8±1.06^{c}	40±6.33^{c}	233.3±8.82^{c}	6.0±0.33^{c}	23±1.23^{c}	217.3±8.9^{c}
Group 6	13±2.00^{b}	94.6±2.91^{b}	248.3±20.58^{b}	18±0.58^{b}	125±11.18^{b}	300.0±10.7^{b}
Group 7	11± 1.00^{b}	91.3±5.93^{b}	240.0±25.86^{c}	7.0± 2.45^{c}	23.4 ± 1.03^{c}	212.3±3.9^{c}

Means with different letters (a, b, c) within the same column are significantly different at $P < 0.05$.

Histopathological findings

Different body organs such as (liver, duodenum, caecum, and kidney) from all groups were investigated for the microscopic examination at 7th day post infection and the end of the experiment. In liver normal central vein (C.V.) with normal hepatic cord and normal portal area with normal hepatic structure as observed in G1, G6 and G7 at 7th day post infection. However, congestion, moderate follicular degeneration, dilation of sinusoid, focal coagulation necrosis and focal aggregation of inflammatory cell appeared in G2, G3, G4 and G5 which slightly return to normal at the end of the experiment (Fig. 1, 2). The duodenum normal intact villi and epithelium observed at the G1 and G7. While in G2, presence of large number of developmental stage of *Eimera*, massive infiltration of inflammatory cells, degeneration and necrosis in epithelial cells and obstruction in villus epithelium were observed at 7th day post infection and at the end of the experiment. Slight congestion, mild infiltration of inflammatory cell especially eosinophil in G3 but multiple different stages of *Eimeria* was observed in G4 and destruction of intestinal villi. Fusion and corrugation of villi, with infiltration of inflammatory cells showed in G6 and slight infiltration of inflammatory cell showed in G5 at 7th day post infection but back to normal at the end of the study (Fig. 3, 4). Caecum (Fig. 5, 6). G1 normal intact villi and epithelium. G2 showed presence of large number of developmental stage of *Eimera,* massive infiltration of inflammatory cells.G3 infiltration of inflammatory cell and presence of developmental stage of *Eimera.* G4 and G5 showed multiple different stages of *Eimeria* were observed. G6 showed very few developmental stages of *Eimeria*. G7 showed moderate infiltration of inflammatory cellsat 7th days post infection. At the end of experiment G1, G3 and G7 showed normal intact villi and no *Eimera* stages. G2 and G4 showed presence of large number of developmental stage of *Eimera,* massive infiltration of inflammatory cells.G5 and G6 showed infiltration of inflammatory cells. Kidney normal renal structure appeared in both G1 and G7. Sever congestion of cortex and medulla blood vessels, with normal epithelium lining and degenerative change in renal tubules observed in G2-G6 at the both slaughtered (Fig. 7, 8).

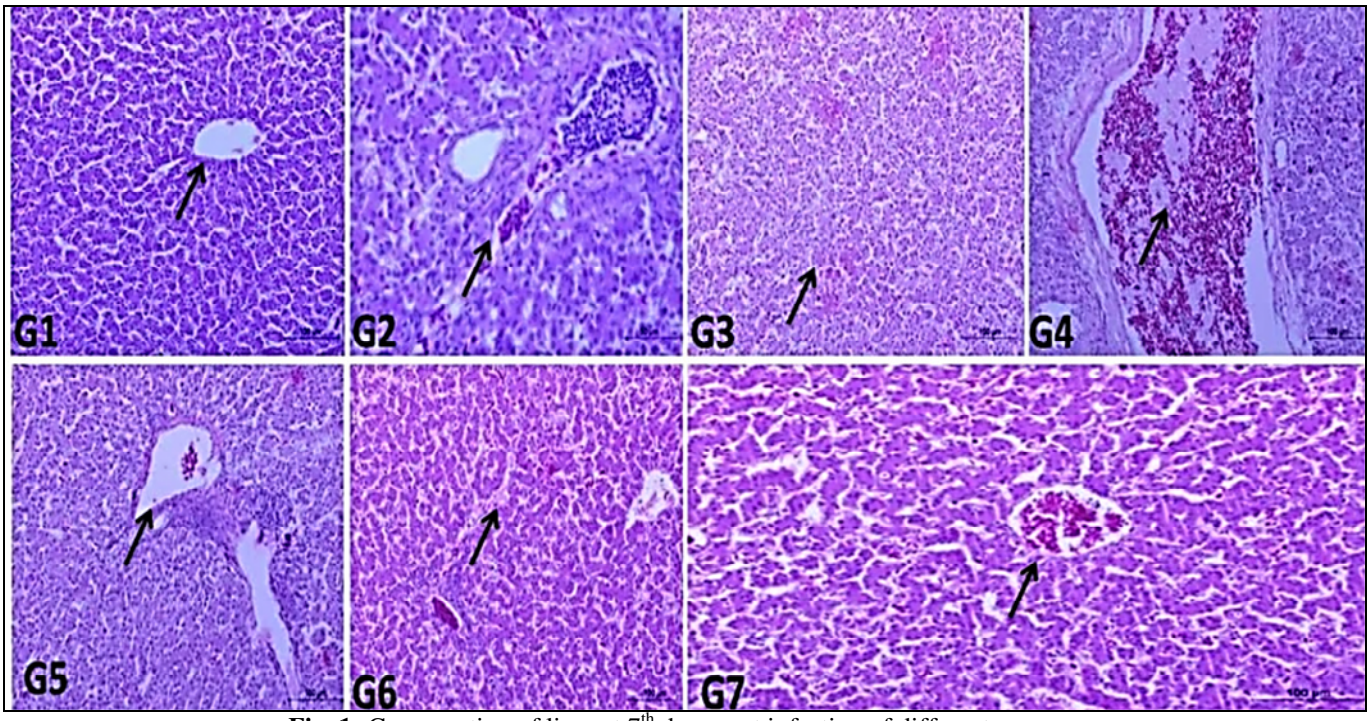

Fig. 1: Cross section of liver at 7th days post infection of different groups.

G1, G6 and G7 showed normal central vein (C.V.) with normal hepatic cord and normal portal area with normal hepatic structure (H&E x 100). G2, G3, (G4, H&E x 200), and G5 showed congestion, moderate follicular degeneration, dilation of sinusoid, focal coagulation necrosis and focal aggregation of inflammatory cell (H&E x 100).

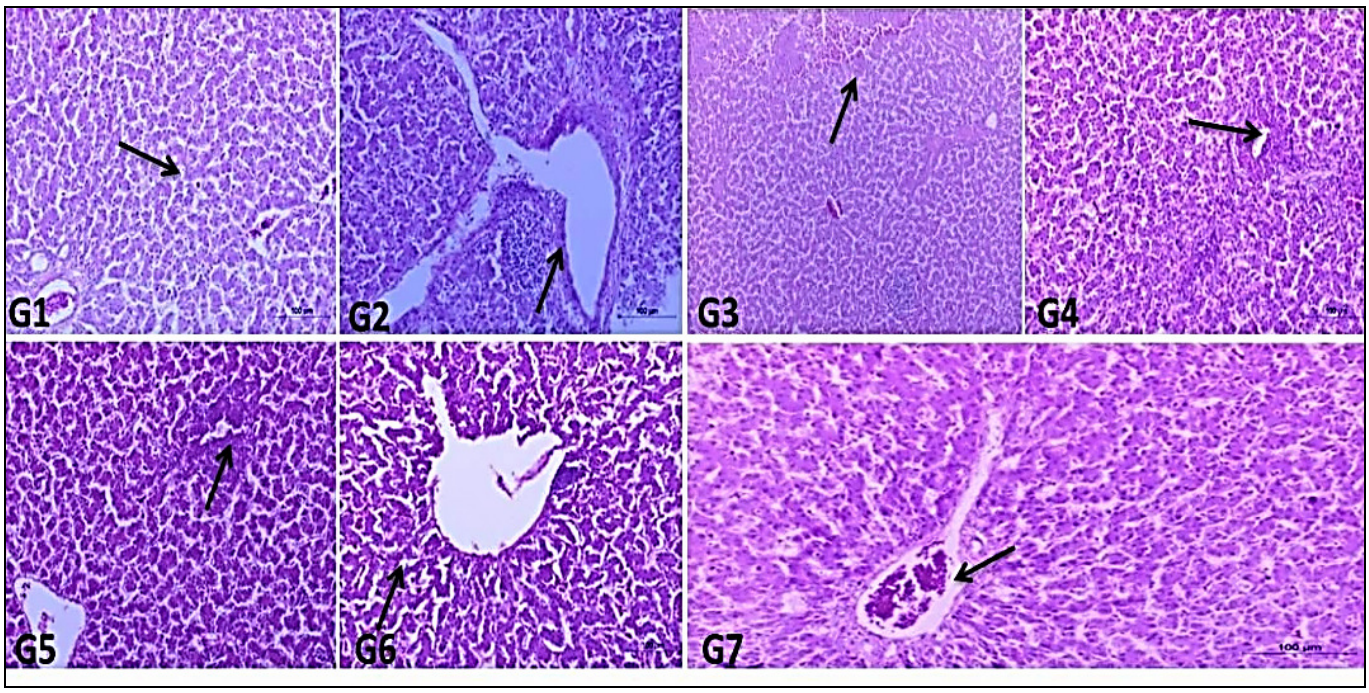

Fig. 2: Cross section of liver at end of experiment of different groups.

G1, G6 and G7 showed normal central vein (C.V.) with normal hepatic cord and normal portal area with normal hepatic structure (H&E x 100). (G2, H&E x 200), G3, showed, moderate follicular degeneration, dilation of sinusoid, and focal aggregation of inflammatory cell (H&E x 100). G4 and G5 slightly return to normal at the end of the experiment (H&E x 100) **(G1)** negative control (non-infected and non-treated), **(G2)** control positive (infected and non-treated), **(G3)** infected and treated with diclazuril, **(G4)** infected and fed ration mixed with *thymus vulgaris* powder**, (G5)** infected and treated with diclazuril and *thymus vulgaris* powder**, (G6)** infected and fed ration mixed with *Artemisia annua* powder**. (G7)** infected and treated with diclazuril and *Artemisia annua* powder**.**

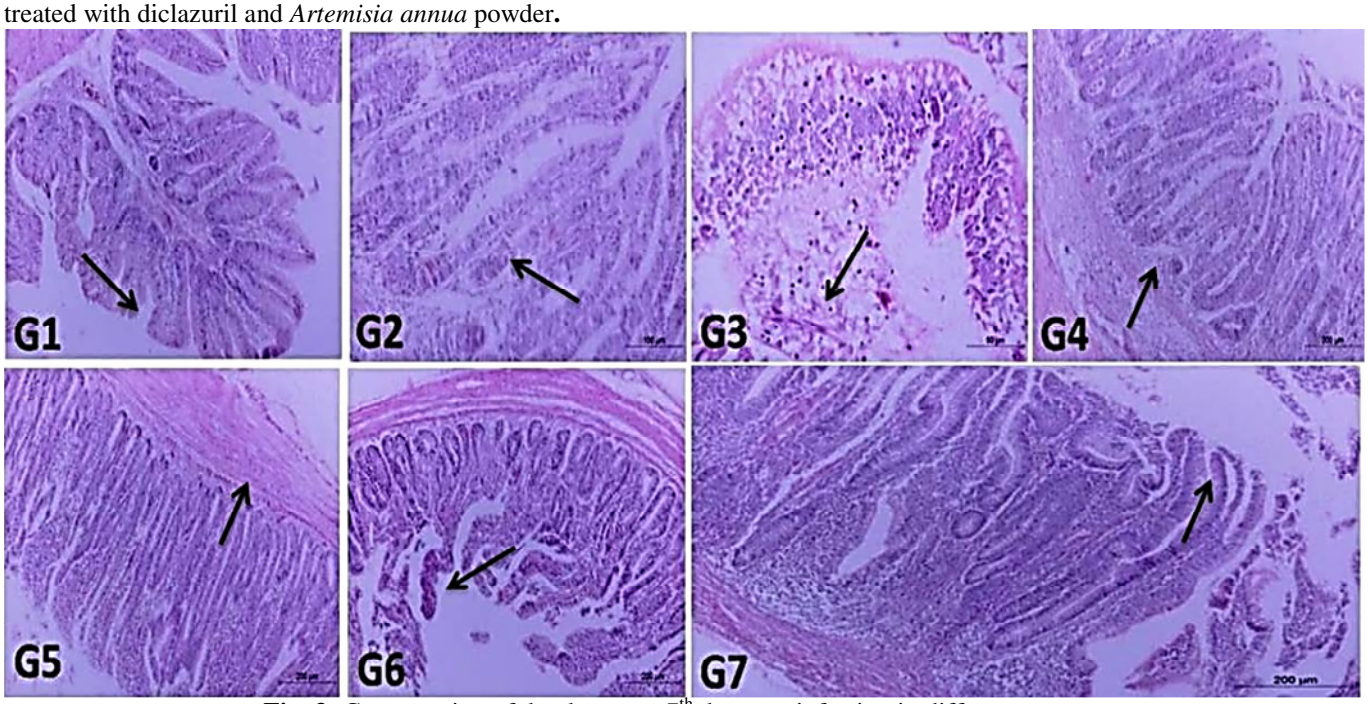

Fig. 3: Cross section of duodenum at 7th day post infection in different groups.

G1 (H&E x 100), and G7 (H&E x 200) showed normal intact villi and epithelium. G2 showed presence of large number of developmental stage of *Eimera,* massive infiltration of inflammatory cells, degeneration and necrosis in epithelial cells and obstruction in villus epithelium (H&E x 100). G3 showed slight congestion, mild infiltration of inflammatory cell especially eosinophil (H&E x 100). G4 multiple different stages of *Eimeria* were observed (H&E x 200). G5 showed slight infiltration of inflammatory cell (H&E x 200). G6 showed fusion and corrugation of villi, with infiltration of inflammatory cells (H&E x 200).

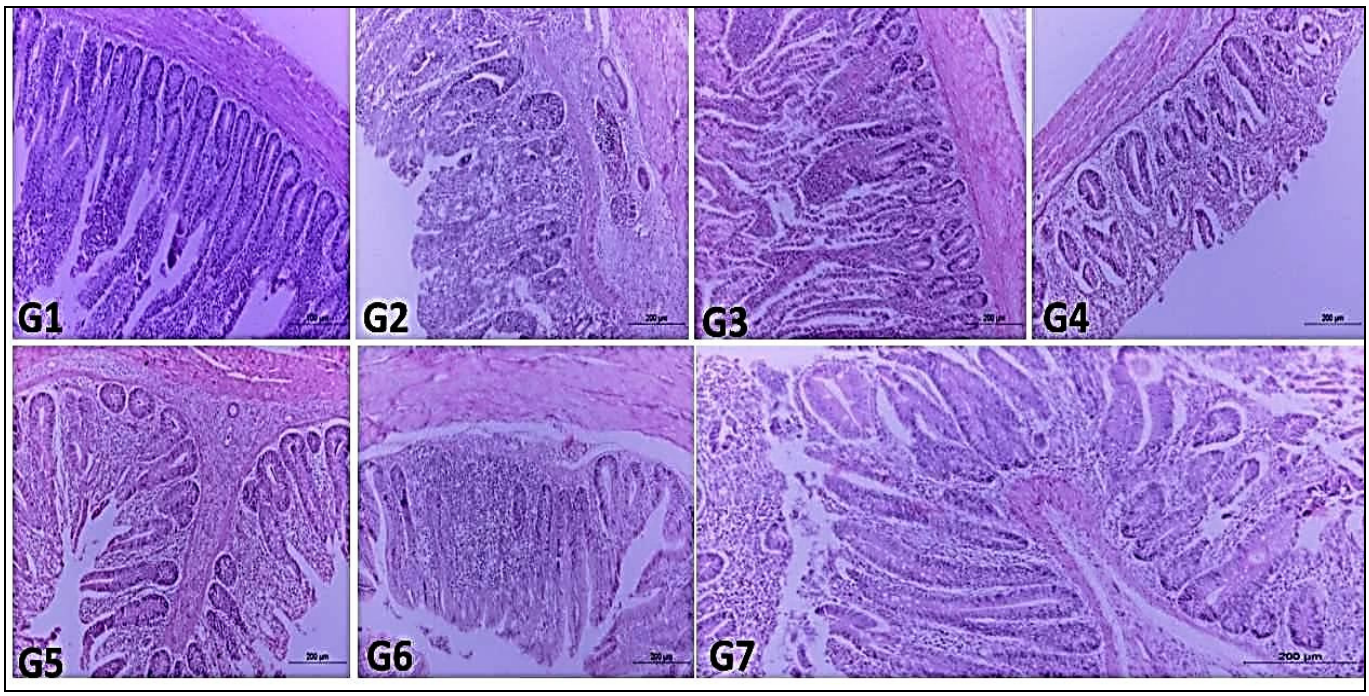

Fig. 4: Cross section of duodenum at end of experiment of different groups.
(G1, H&E x 100), G5, G6 and G7 showed normal intact villi and epithelium (H&E x 200). G2 showed presence of large number of developmental stage of *Eimera* (H&E x200). G3 showed infiltration of inflammatory cell (H&E x 200).G4 destruction of intestinal villi (H&E x 200).
(G1) negative control (non-infected and non-treated)**, (G2)** control positive (infected and non-treated), **(G3)** infected and treated with diclazuril,**(G4)** infected and fed ration mixed with *thymus vulgaris* powder**,(G5)** infected and treated with diclazuril and *thymus vulgaris* powder**, (G6)** infected and fed ration mixed with *Artemisia annua* powder**. (G7)** infected and treated with diclazuril and *Artemisia annua* powder**.**

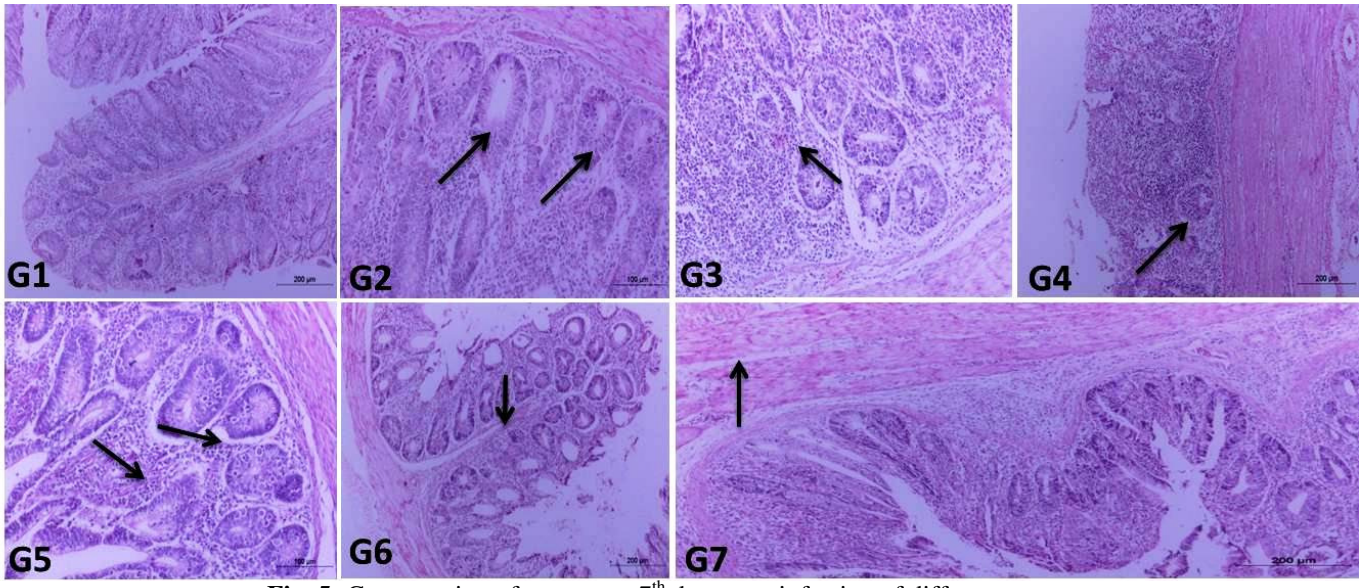

Fig. 5: Cross section of caecum at 7th days post infection of different groups.
G1 showed normal intact villi and epithelium (H&E x 200). G2 showed presence of large number of developmental stage of *Eimera,* massive infiltration of inflammatory cells (H&E x 100).G3 infiltration of inflammatory cell and presence of developmental stage of *Eimera* (H&E x 100).(G4, H&E x 200) and (G5, H&E x 100) showed multiple different stages of *Eimeria* were observed. G6 showed very few developmental stages of *Eimeria* (H&E x 200). G7 showed moderate infiltration of inflammatory cells (H&E x 200).

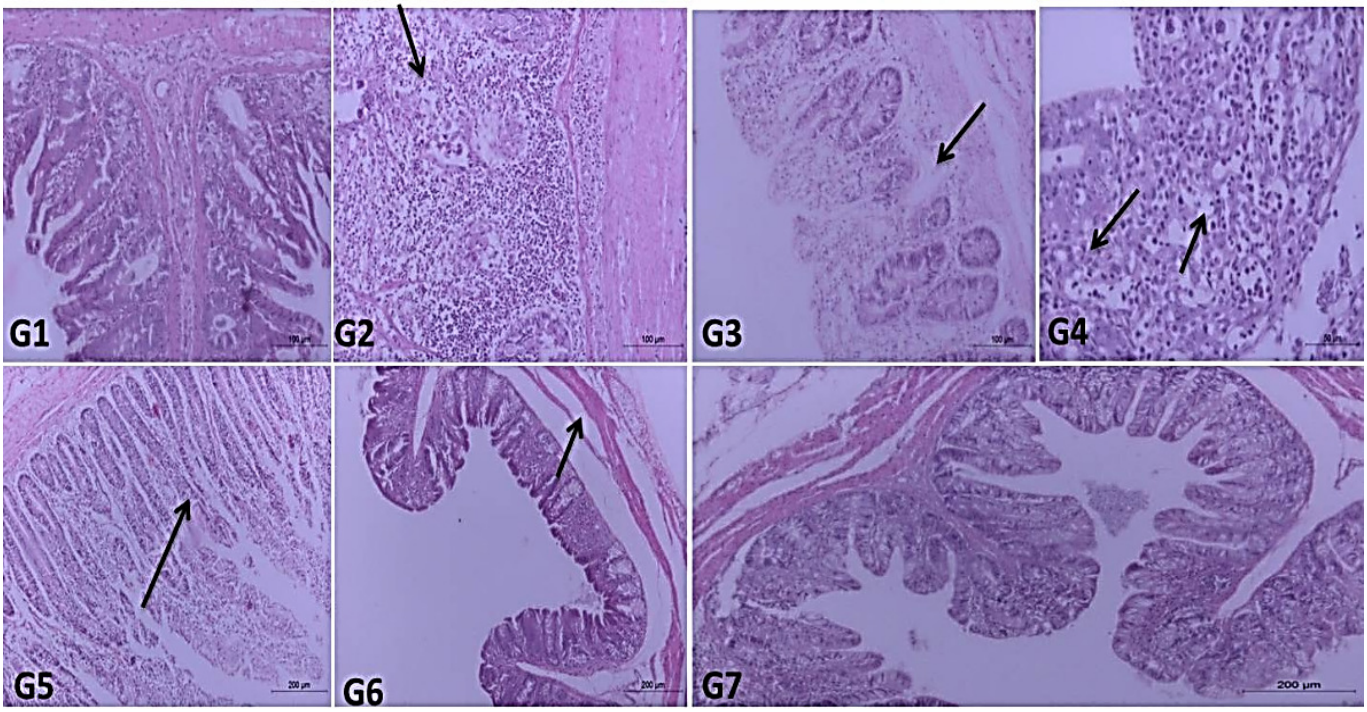

Fig. 6: Cross section of caecum at end of experiment of different groups.

G1, G3 and (G7 H&E x 200) showed normal intact villi and no *Eimera* stages (H&E x 100). G2and G4 showed presence of large number of developmental stage of *Eimera,* massive infiltration of inflammatory cells (H&E x 100).G5 and G6 showed infiltration of inflammatory cells (H & E x 200).

(G1) negative control (non-infected and non-treated),**, (G2)** control positive (infected and non-treated), **(G3)** infected and treated with diclazuril,**(G4)** infected and fed ration mixed with *thymus vulgaris* powder**,(G5)** infected and treated with diclazuril and *thymus vulgaris* powder**, (G6)** infected and fed ration mixed with *Artemisia annua* powder**. (G7)** infected and treated with diclazuril and *Artemisia annua* powder**.**

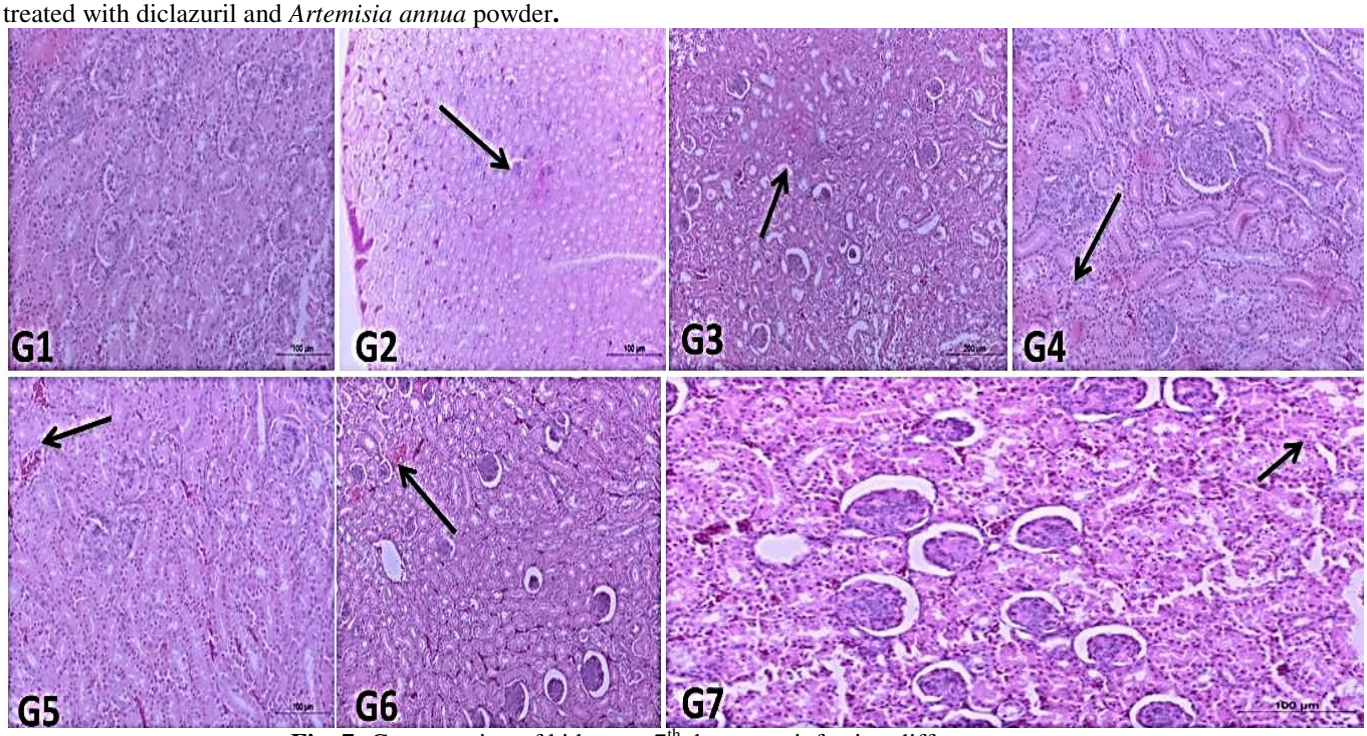

Fig. 7: Cross section of kidney at 7th days post infection different groups.

Normal renal structure appeared in both G1 and G7 (H&E x 100). Sever congestion of cortex and medulla blood vessels, with normal epithelium lining and degenerative change in renal tubules observed in G2 (H&E x 100), (G3, G4 H&E x 200), (G5, G6 H&E x 100).

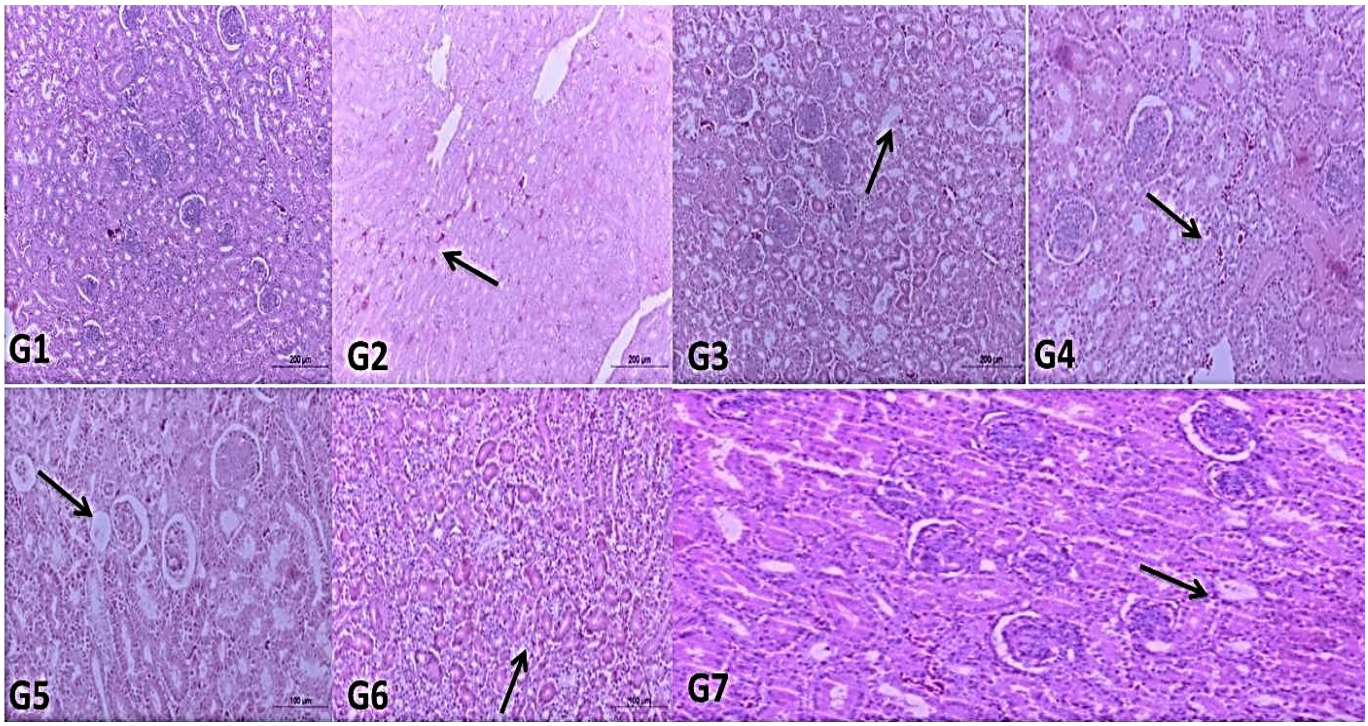

Fig. 8: Cross section of kidney at end of experiment of different groups.

Normal renal structure appeared in both G1 and G7 (H&E x 200). Sever congestion of cortex and medulla blood vessels, with normal epithelium lining and degenerative change in renal tubules observed in G2, G3, G4 (H&E x 200), and G5, G6 (H&E x 100).

(G1) negative control (non-infected and non-treated)**, (G2)** control positive (infected and non-treated), **(G3)** infected and treated with diclazuril, **(G4)** infected and fed ration mixed with *thymus vulgaris* powder**, (G5)** infected and treated with diclazuril and *thymus vulgaris* powder**, (G6)** infected and fed ration mixed with *Artemisia annua* powder**. (G7)** infected and treated with diclazuril and *Artemisia annua* powder**.**

Cell Gene' DNA'' estimation (Genotoxicity) (Comet assay)

Infected, non-treated group (G2) and diclazuril group (G3) showed positive genotoxicity in liver, intestine and caecum. While when given *thymus vulgaris* powder with or without diclazuril (G4, G5) cause negative genotoxicity in the gene of these tissues. However thymus powder alone (G4) caused damage only in the caecum gene. Furthermore, the administration of *Artemisia annua*powder with or without diclazuril (G6, G7) causes negative genotoxicity in the gene of these tissues when compared with control negative group (G1) as illustrated in figures (9,10,11) respectively.

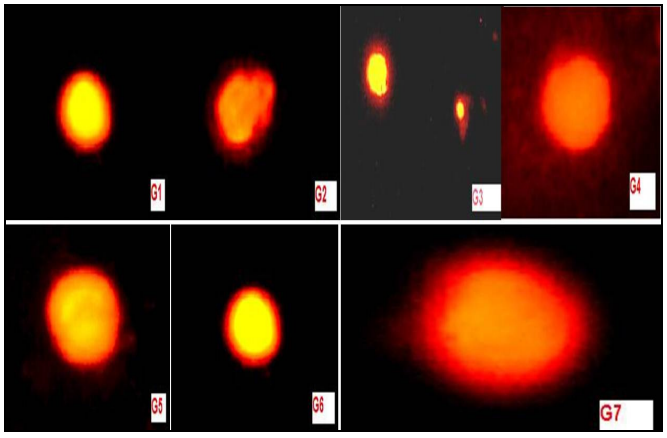

Fig. 9: Comet assay of liver in different groups.

G2 and G3 show positive genotoxicity in the gene of liver but there is negative genotoxicity in the gene of G4, G5, G6 and G7.

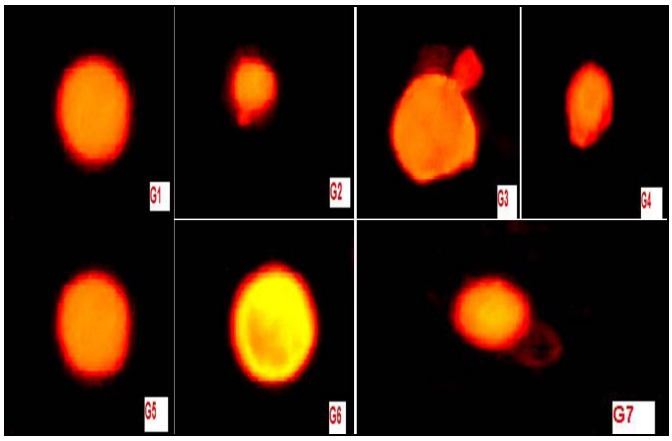

Fig. 10: Comet assay ofduodenum in different groups.

G2 and G3 show positive genotoxicity in the gene of duodenum but there is negative genotoxicity in the gene of G4, G5, G6 and G7.

(G1) negative control (non-infected and non-treated), **(G2)** control positive (infected and non-treated), **(G3)** infected and treated with diclazuril,**(G4)** infected and fed ration mixed with *thymus vulgaris* powder**, (G5)** infected and treated with diclazuril and *thymus vulgaris* powder**, (G6)** infected and fed ration mixed with *Artemisia annua* powder**. (G7)** infected and treated with diclazuril and *Artemesia annua* powder**.**

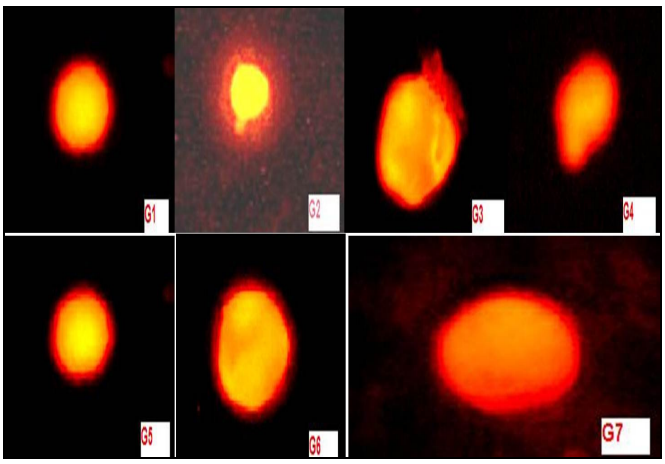

Fig. 11: Comet assay of caecum in different groups. G2, G3 and G4 show positive genotoxicity in the gene of caecum but there is negative genotoxicity in the gene of G5, G6 and G7.

(G1) negative control (non-infected and non-treated), **(G2)** control positive (infected and non-treated), **(G3)** infected and treated with diclazuril,**(G4)** infected and fed ration mixed with *thymus vulgaris* powder**,(G5)** infected and treated with diclazuril and *thymus vulgaris* powder**, (G6)** infected and fed ration mixed with *Artemesia annua* powder**. (G7)** infected and treated with diclazuril and *Artemesia annua* powder**.**

Discussion

The present research was conducted to assess the side effects of diclazuril; An anticoccidial drug in the treatment of *E. tenella* experimentally infected broiler chickens and try to decrease these side effects by using herbal plants as *thymus vulgaris* and *Artemisia annua*.

Effects of Diclazuril, *Thymus vulgaris* and *Artemisia Annua* on body performance in broilers chickens

The weekly evaluation of growth performance revealed that experimentally *E.tenella* infectionin positive control group showed significant decrease in body weight, body weight gain at 3rd week and feed consumption at 3rd and 4th week of age, while FCR showed no significant effect when compared to control group (G1). This result could be explained by our histopathological findings in the intestine which showed massive infiltration of inflammatory cells, degeneration and necrosis in villus epithelium. The growth disorders in chicken infected with coccidiosis and decrease in feed intake. That the explained on basis of loss of intestinal epithelial cells during oocyst infection due to negative effects on the digestion and absorption of nutrients Arczewska and Swiatkiewicz (2012), Rehman *et al*. (2010) and Sharma, *et al*., (2013).

Medication with diclazuril alone showed improved effect when compared to infected and non-treated group after infection with *E. tenella*, however the combination with *thymus vulgaris* in G5 and *Artemisia annua* in G7 showed more improved effect compared to diclazuril alone at last two weeks. This result was supported by histopathological findings in the intestine, which showed fusion of most villi, heavy mononuclear cells infiltration between villi and this finding agreement with Hossein *et al*. (2010) who stated that diclazuril was found to decrease villous length, width and surface area, mainly in duodenum and jejunum which impaired intestinal nutrient absorption and reduced enteric function due to use of anticoccidial drug. This means that diclazuril alone has negative effects on the growth efficiency of the chicken compared withG5and G7. Also this finding agreed with Pirali-kheirabadi *et al*. (2008) reported that chickens fed with 200 ppm diclazuril during *Eimeria* infection; apparently had more mean body weight and gained more weight than birds of other infected groups.

Chicken feed with *Thymus vulgaris* powder (G4) at 2nd week showed significant improved effect on body weight while after infection body weight, body weight gain at 3rd week and feed intake at 4th week showed significant decrease effect, while chickens treated with *thymus vulgaris* and diclazuril (G5) showed significant improvement in the body weight at 2nd, 3rd, 5thand 6th week of age, weight gain at 2nd, 6th week, FCR at 6th week and feed consumption at 4th and 6th week showed decreased in feed intake of chicken when compared to control negative group and diclazuril treated group at end of experiment. This finding also agreed with findings obtained by Asmaa and El-Tahawy, (2018) and Hashemipour *et al*. (2013) who indicates that the addition of either *thyme* or their oils to diets improves the growth, FCR, economical efficiency, production index of broiler compared to the un-supplemented control.

Chicken feed with *Artemisia annua* (G6) showed no significant effect on body performance except at 4th week feed consumption showed significant decrease effect when compared to control negative group (G1) and diclazuril treated group (G3).This was in line with Ebiamadon *et al*. (2008), who stated that there were no major variations between the growth efficiency of broiler chickens supplied with commercial anticoccidial drugs by drinking water and those provided by *Artemisia*. Also Almeida *et al*. (2012) clarified that in *A. annua*, *artemisinin* induces a bitter dietary taste that contributes to a decline in chicken palatability. However, feeding chickens with *Artemisia annua* powder in combination with diclazuril (G7) showed no significant effect on body performance except at 4th week feed consumption showed significant effect while body weight at 5th and 6th week, weight gain and FCR at 6th week showed significant improvement effect when compared to diclazuril group (G3). This may be due to that *Artemisia annua* powder decreases the harmful impact of infection, has anticoccidial impact and plays a significant role in enhancing the body's metabolic and health status of chicken. This result confirmed with the histopathological finding ofintestine, which revealed normal intestinal villi; this finding was accepted with findings obtained by Wan *et al*. (2017). In contrast the result obtained by Pop *et al*. (2017) who reported higher body weight and average daily intake of food in broilers fed *Artemisia* diets than in the control group. The differences could be explained by the different climatic conditions under which feeding experiments (hot and humid seasons) were conducted.

Effects of Diclazuril, *Thymus vulgaris* and *Artemisia Annua* on oocytes output in broilers chickens

It was noted that chickens experimentally infected with E. *tenella* and non-treated (G2) showed more oocyst output as compared to G1. This finding was coincident to Hady and Zaki (2012) who investigated that the *E. tenella* affected broiler chickens, showing more lesion ratings, body weight

gains loss, bloody diarrhea, and more oocyst excretions compared to the control group.

Regarding diclazuril's impact on oocyst production, it was induced significant oocyte output reduction compared to G2 (infected non treated group). These results agreed with Zhou *et al*. (2010), who explained that diclazuril induced significant action in suppressing the coccidia development cycle in chickens. The effect of *thymus vulgaris* on oocyst output is not significant compared to G2 and the reduction ratio was 0.25. However, *thymus vulgaris* with diclazuril was significantly improved the reduction ratio (71.68). This variation explained by Anna and Sylwester (2013), who stated that *thymus* does not always specifically target *Eimeria* parasites while, it had positive effects on healing after infection as it has immunomodulatory activity, antioxidant or anti-inflammatory property and possessing a beneficial effect on compensatory growth and final output levels without reducing fecal oocysts excretion. However, *Artemisia annua* when administrated alone (G6) or in combination with diclazuril (G7) significantly reduced oocyst output and increase the reduction ratio to (71.6, 87.53) respectively. This effect may be due to its content of*artemisin* which was slowly absorbed with prolonged interaction on the parasites in the digestive tract, so it had an advantage for treatment of coccidiosis. *Artemisia annua* powder causes the coccidia to die by alkylation of coccidial proteins Messai *et al*. (2014).

Effects of *Thymus vulgaris* or *Artemisia Annua* on diclazuril residue of liver, breast and thigh muscles of broiler chickens

Our results revealed that diclazuril residues were detected in the chicken's liver, thigh muscle ,and breast muscle with a higher percentage in the liver than the muscle. Scan (1997) and Zhang *et al*. (2019) declared that the liver was contained high diclazuril residues than the muscle. Feeding ration containing *thymusvulgaris* powder and diclazuril, significantly reduced diclazuril residues in the liver and muscles of chicken this was coincident with Abdulkarimi *et al*. (2011) who explained that *thymus vulgaris* enhances liver function which increases drug elimination from tissues owing to bile acid conjugation of the target to a polar molecule of low molecule weight. Feeding chicken ration containing *Artemisia annua* powder and diclazuril (G7) significantly reduced the diclazuril residue when compared to the diclazuril group (G3). Those because of antioxidant capacity of *A. annua* which remove any harmful radical substance as drug radical and could reduce the harmful effects of oxidation. Wan *et al*. (2016).

Effects of Diclazuril, *Thymus vulgaris* and *Artemisia Annua* on blood picture in broilers chickens

Chickens inoculated with *E. tenella* and non-treated had significant reduced effect on (RBCs, Hb and PCV) compared to the control non-infected group. These result similar to that obtained by Adamu *et al*. (2013) who found that chicken infected with *E. tenella* showed reductions in hematocrit (PCV) and (Hb) that resulted in anemia.

Concerning the effect of diclazuril on RBCs, Hb and PCV, it had no significant effect compared to the control non-infected group (G1) and other groups, but significantly improved compared to the control positive group (G2).This similar to Kamal *et al*. (2020) showed improvement of these parameters RBCs, Hb and PCV% compared to infected untreated (positive control), as a consequence of interference with the multiplication of *E. tenella* resulting in diminishing the bleeding after administration of lomefloxacin or diclazuril to broiler chickens.

Feeding *thymus vulgaris*, *Artemisia annua* powder and/or diclazuril to chickens had no significant effect on RBCs, Hb and PCV when compared to (G1). This finding is due to the antioxidant effect of thymus component, these agreed with Anna and Sylwester (2013) whoconcluded that the *thymus* herbal plant have a favorable impact on compensatory growth and, blood parameters and thus, on the final performance indices. Jatau *et al*. (2014) who stated that *Artemisia annua* reduces the level of oxidized red blood cells membranes and reduce the decomposition of these cells as result of *Eimeria* infection.

Chickens experimentally infected with *E. tenella* and non-treated (G2), showed marked increase of the total leucocytic count and eosinophiles with a decrease in lymphocytescount compared to control group. Fatma *et al*. (2008) showed a significant increase in the total number of leukocytes, heterophiles, monocytes and eosinophils associated by decrease number of lymphocyte and might be due to the stress of infections. Diclazuril had improved significant this effect in comparison with infected non treated group (G2), but showed increased in eosinophil and significantly decreased lymphocyte compared to (G1) (Hirani *et al*., 2018).

Thymus vulgaris powder and/or diclazuril (G4, G5) has no significant difference in the effect of WBCs, heterophil, monocyte and basophil but there was a significant increase in the effect of eosinophil compared to the control non-infected group (G1) and improved the significant effect of lymphocyte in comparison with diclazuril group (G3). This result was supported by histopathological findings in the intestine; indicated presence of infiltration of inflammatory cells, there was a massive infiltration of different stages of *Eimeria* in caecum as an eosinophil, a sign of parasite presence. The result was accepted with Irizaary-Rovira (2004) who stated that the elevated eosinophils count, was associated with intestinal parasitic infections and with parasites tissue migration.Also the result was accepted with Fallah and Mirzaet (2016), who reported that flavonoid-rich *thyme* extended the action of vitamin C that acts as antioxidants and thus had a positive impact on enhancing the cellular, humeral immune function and blood composition of broilers. Furthermore feeding ration containing *Artemisia annua* powder and/ or diclazuril to chicken (G6, G7) has no significant difference effect when compared with control non-infected group (G1).These results may be due to the antioxidant and anti-inflammatory effects of *Artemisia annua* resulting in better humeral response, Wan *et al*., (2016).

Effects of Diclazuril, *Thymus vulgaris,* and *Artemisia Annua* on Haemagglution inhibition antibody titer (HIT) in broilers chickens.

Infected non-treated group (G2) and diclazuril treated group were showed significant decrease in HIT at 7th day post- infection and the end of the experiment in comparison with the control non-infected group (G1). This result agreed with Kaneko *et al*. (1997), who reported that immune response in coccidia infected chickens, was reduced due to acute stress induced by an infection that results in cortisol secretion and protein catabolism. Hirani *et al*. (2018)

reported that diclazuril inhibited the immune response in the body and affect the lymphoid organs .Chickens treated with *thymus vulgaris*, *Artemisia annua* powder and/or diclazuril did not have a significant difference but showed significant improvement effect in comparison with G2 and G3. This may be due to flavonoid-rich *thyme*, which acts as antioxidants can increase, immune function (cellular and humeral immunity) Hosseini *et al*. (2013). *Artemisia annua* has antioxidant and anti-inflammatory properties resulting in improvment immune response as stated by Brisibe *et al*., 2009.

Effects of Diclazuril, *Thymus vulgaris* and *Artemisia Annua* on Serum levels of total protein, albumin, globulin and A/G ratio in broiler chickens

Experimentally infected, non-treated group (G2) had significant decrease in total protein. Kumar-Mondal *et al*. (2011) stated that severe hemorrhage occurs in *E. tenella* infection resulting in significant loss of plasma protein accompanied by excessive interstitial fluid movement resulting in acute hypoproteinemia in poultry and due to acute stress induced by cortisol secretion causeprotein catabolism. The significant decrease in total protein and albumin induced by diclazuril was in agreement with Abdel-Hafez, (2004) who observed that there was decrease in total protein, albumin and gamma-globulins in diclazuril treated group at 1st and 2nd week post vaccination with new castle vaccine that due to the negative effect of diclazuril on the lymphoid organs which is considered the main part in synthesis of gamma globulins and decreased the total serum proteins.

Chickens fed *thymus vulgaris* (G4) alone showed a decrease in total protein and globulin with increased in A/G while when given with diclazuril (G5) has improved significant reducing effect on total protein, Albumin in comparison with control non-infected group (G1). While at end of experiment feeding *thymus vulgaris* powder (G4) has significant decrease effect on total protein in comparison with (G1), and showed improvement effect when compared with diclazuril group (G3), while albumin, globulin and A/G showed no significant effect in comparison with (G1), and showed significant improvement effect when compared with diclazuril group (G3), however when given *thymusvulgaris* with diclazuril (G5) showed significant improved effect on total protein in comparison with diclazuril group (G3). These results were parallel to data obtained by Youssef *et al*. (2017) who reported that essential *thyme* oil and *thyme* powder significantly increased serum total protein indicate an enhancement in broiler general health status.

Feeding ration mixed with *Artemisia annua* (G6) and diclazuril (G7) were showed no significant differential effect on total protein, Albumin, Globulin, and A/G at 7th day post infection and at the end of the experiment *Artemisia annua* (G6) showed significant decrease in total protein in comparison with control group (G1), and showed improvement effect when compared with diclazurilgroup (G3), moreover (G7) at end of experiment showed improved significant effect on total protein in comparison with diclazuril group (G3).This finding was agreed with the results obtained by Gholamrezaie *et al*. (2013) who stated that there were significant increase in total protein, gamma and alpha globulins during the 2nd and the 3rd weeks in *artemisia annua* treated groups which may be due to their immunostimulant effect.

Effects of Diclazuril, *Thymus vulgaris* and *Artemisia Annua* on some biochemical parameter in broilers chickens.

(a) Serum concentrations of uric acid and creatinine of broiler chickens.

Infected non treated group (G2) showed significant increasing in uric acid and creatinine as stated previously by Fatma *et al*. (2008) as serum content of creatinine and uric acid in affected groups of birds were greatly increased and this could be due to an injury of kidney parenchyma due to the damaging effect of *Eimeria*parasite. Diclazuril had an improvement effect which confirmed by histopathological finding of the kidney that showed normal structure with only a slight degenerative change in the tubules Ines Varga *et al*. (2017) reported that uric acid and creatinine did not vary between diclazuril treated group when compared with non-infected non-treated chickens. *Thymus vulgaris* and/ or diclazuril has no significant difference effect similar to Saleh *et al*. (2014) who reported that serum uric acid was not influenced by the introduction of*thymus vulgaris*powder to feed.Moreover *Artemisia annua*and/ or diclazuril have no significant difference and the histopathological finding showed normal structure with only very mild degenerative change in the tubule. Because of its protective effects, *Artemisia annua*does not affect the histopathology of liver, kidneys and intestines as stated by Ebrahimi *et al*. (2013).

(b) Serum levels of ALT, ALP, and AST of broiler chickens

Chickens of group (G2) showed significant increase in ALT, ALP and AST levels when compared with (G1). Fatma *et al*. (2008) reported that there was a substantial increase in serum ALT and AST activities in infected chickens' classes, and this could be due to decreased liver function and damage to liver parenchyma related to the damaging effect of *Eimeria* parasite. Concerning, the effect of diclazuril (G3) on ALT, ALP and AST, it was showed significant improvement effect in comparison with infected non treated group (G2). These results confirmed by histopathological finding of the liver that showed normal structure with only very moderate congestion. EFSA (2014) was reported that diclazuril treated group did not show any significant difference of all examined biochemical parameters (Serum AST, ALT, total protein, albumen, globulin) when compared with non-infected non-treated chickens. Chicken feeding *thymusvulgaris* with diclazuril (G5), had significant improvement effect on liver function and the histopathological finding of liver was showed normal hepatocyte, with a moderate number of eosinophil, due to *Eimeria* infection, as it is an important sign of parasitic infestation. These result agreed with Xiaolei *et al*. (2016) who reported that the introduction of *thyme* to broiler foods had reduced the activity of ALT, AST and ALP so thyme addition in the feed of broiler has no adverse impact on liver activities.

Furthermore, at the end of the experiment chicken feeding *Artemisia annua* (G6), was showed no significant difference in these parameters, whereas when used with diclazuril (G7), there was significant improvement effect in ALT, ALP, and AST in comparison with (G1) and (G3) groups. This result was consistent with histopathological

findings of liver that, showed normal hepatocyte and slight congestion. Our results agreed with Payam *et al*. (2018) who reported that there are no substantial variations in ALT and AST levels between the *Artemisia* groups of nutritional treatments relative with that of the control group in chicken.

Cell Gene' DNA'' estimation (Genotoxicity) (Comet assay)

The infected non-treated group was showed positive genotoxicity in the gene of tissues (liver, intestine, and caecum) in comparison with the control non-infected group. This finding was established with Ferrazzi *et al*. (1991) who claimed that free radicals were generated in poultry tissue caused by exposure to intense stress as an infection that caused serious damage to cell nucleic acids (DNA). Diclazuril caused positive genotoxicity in the gene of tissues in comparison with (G1). This was agreed with Sasaki *et al*., (2002) who stated that comets are produced after drug penetration cells resulting in alterations to the cellular genetic code resulting in unusual cell damage due to the terrible influence of drugs on the cell gene and damages to DNA. *Thymus vulgaris* feeding with or without diclazuril (G4, G5) causes negative genotoxicity in the gene of this tissue (liver, intestine), while the *thymus vulgaris* group alone (G4) causes positive genotoxicity to the gene of caecum as a result of the infection of *Eimeria tenella* which causes damage to the cell gene of caecum which considers the target tissue of *E. tenella*. This result was agreed with Abdulkarimi *et al*. (2011) who reported that *thyme* extraction, specifically the *thymus* oil phenolic and terpenic compounds, protects from DNA destruction and has beneficial antioxidant effects. Furthermore feeding *Artemisia annua* powder to broiler with or without diclazuril (G6, G7) caused negative genotoxicityof examined tissue gene. This could be due to its antioxidant properties. The finding is followed by Dragan *et al*. (2013) who reported that various biologically active *Artemisia* components, such as purines coumarin, flavonoids, steroids and phenols have numerous biological functions, and protecting cell genes.

Conclusion

In conclusion,

- Supplementing broilers with *thymus vulgaris* powder with or without diclazuril improve body weight performance, But it hasn't any anticoccidial effect when used alone, slight decrease drug residue in tissues and show significant improved effects of all examined blood and biochemical parameters (liver and kidney function), as well as increased values of total protein and serum albumin.
- Supplementing broilers with *Artemisia annua* powder with or without diclazuril it has a potent anticoccidial effect; furthermore it has a great value in decrease drug residue in tissue of broiler and reveals no alteration in cell gene, showed no significant difference effect in body performance, when compared with control one, except only at last two week of experiment showed significantly improved effect, Also did not show any significant difference of all examined blood and kidney function, as well as no significant effect on total protein, serum albumin and A/ G ratio. While ALT, AST, ALP showed significant improved effect when used with diclazuril at end of experiment when compared with non-infected non-treated chickens.
- *Thymus vulgaris* powder and diclazuril can be successfully used in practice as a natural feed additive for broiler chickenwithadjuvant with diclazuril has anticoccidial effect.
- Supplementing broilers with *Artemisia annua* powder alone or with diclazuril caused potent anticoccidial effect.
- *Thymus vulgaris* and *Artemisia annua* powder decrease diclazuril residue in tissue of broiler chicken meat.

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