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Ameliorative effect of alcoholic extract of *Glycyrrhiza glabra* and diclazuril against experimental Eimeria stiedae infection in rabbits

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ABSTRACT

The aim of the current study was to assess the hepato-protective role of *Glycyrrhiza glabra* (*G. glabra*) on hepatic coccidiosis in experimentally infected rabbits. Forty New Zealand rabbits were used in this study. Rabbits were randomly divided into four groups: G1; rabbits supplemented with *Glycyrrhiza glabra* extract, G2; the protected group was administrated diclazuril at a dose of (1mg/kg B.W) for a week before infection, G3; was kept as infected + nontreated (CP) and G4 was kept non-treated and non-infected (CN). Animals in G1 were supplemented with a daily dose of 300 mg/kg BW which began one week before infection and continued daily till the end of the experiment. On the 7th day of the experiment, all groups except G4 were orally infected with 10^4 sporulated oocysts of *Eimeria stiedae* per rabbit using a stomach tube. Rabbits in all groups were examined for clinical signs, body weight, fecal oocysts count, hematological and biochemical parameters (liver, and kidney function), as well as histopathological lesion. The results showed a significant enhancement in clinical signs and body weight with a highly significant reduction in oocysts shedding, as well as a significant improvement in CBC and serum liver and kidney parameters in the botanical plant and diclazuril groups (G1, G2) when compared to infected control group. Finally, *G. glabra* has potential protective activity against *E. stiedae* in rabbits and could be applied as a safe and effective alternative product to protect rabbit from hepatic coccidiosis.

1. INTRODUCTION

Eimeria stiedae is a protozoal parasite that infects rabbits and causing hepatic coccidiosis (Omata et al., 2001). Coccidian parasites have the ability to decrease rabbits' high commercial value by producing direct and indirect losses due to acute illness, loss of weight, and high mortality and morbidity (El-Shahawi et al., 2012).

Chemoprophylaxis is a crucial strategy of traditional disease management efforts, which are costly (Dalloul and Lillehoj, 2005). Furthermore, long-term use of anti-coccidial drugs has resulted in drug resistance as well as food safety and public health concerns about drug residues in animal products, motivating researchers to focus on finding safer alternatives (El Banna et al., 2016). Plant products may provide an entirely different approach for coccidial control, to which resistance has not yet developed (Abbas et al., 2012), lowering farmer input costs and protecting animal health (Abu El-Ezz, 2005). The use of medicinal plant is the new trend in many countries to overcome the drawbacks of the chemical anti-coccidial drugs (Adamu and Boonkaewwan, 2014).

Glycyrrhiza glabra contains a high concentration of phytochemicals such as volatile oil, amino acids, amines, starch, flavonoids, saponins, isoflavonoids, tannins, vitamin B2, B3, B6 (Pandit et al., 2011). Although many studies have been performed to investigate the effects of licorice on a variety of diseases and microbes, only a few have examined their effect on coccidiosis (Hussain et al., 2017). Based on its reported antiviral, immunomodulatory, and antioxidant characteristics, *Glycyrrhiza glabra* was evaluated for its alleged role in the control of hepatic coccidiosis in rabbits (Omer et al., 2014).

The present study was designed to investigate the protective effect of alcoholic extract of *Glycyrrhiza glabra* against *Eimeria stiedae* infection in rabbits as compared to the effect of diclazuril.

2. MATERIAL AND METHODS

Ethical approval

The experiment was done at the Laboratory Animal Research Center, Faculty of Veterinary Medicine, Benha University. All procedures used in this experiment were followed the guidelines of the National Institute of Health and approved by the Institutional Animal Ethics Committee of the Faculty of Veterinary Medicine, Benha University, Egypt (Ethical No. BUFVTM 25-10-22).

2.1. Eimeria stiedae source

Eimeria stiedae strain used in this study was obtained from Dr. Nagwa El-Hawary, Parasitology Department, Faculty of Veterinary Medicine, Kafr El-Sheikh.

2.2. Propagation and isolation of Eimeria stiedae oocysts

Five rabbits were administered orally by Eimeria stiedae sporulated oocysts. After 20 days, these rabbits were

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slaughtered, and the livers and gallbladders were taken and crushed. The digested contents were sieved, centrifuged and repeatedly rinsed with saline solution before being checked under a microscope for *Eimeria stiedae* oocysts. The obtained oocysts were confirmed morphologically and kept in a 2.5% potassium dichromate solution until sporulation. The sporulated oocysts were counted by the McMaster technique and kept at 4ºC till needed (Levine, 1985).

2.3. Experimental animals

Forty New Zeeland rabbits aged 35-37 days and weighing 500–800 gm. During the experimental period, rabbits were housed individually in metal cages. Direct fecal analyses followed by flotation technique were done daily for two successive weeks to confirm that the rabbits were free from any coccidial infections before applying the intended experiments.

2.4. Preparation of botanical extract

2.4.1. The alcoholic extract of Glycyrrhiza glabra (licorice)

Was obtained from a local market and got authenticated by a botanist, where two Kg of the plant were mixed with 5500 ml ethanol (83%), then sonicated for 30 minutes and macerated and left for one day before filtration. The process was repeated 2 more times. Then the extracts were collected and dried under vacuum at 50°C. The resultant was dark red extracted residues weighing 156.22g, which were stored at 4°C for till use, according to Hussain et al. (2017) but who used an aqueous methanolic (70%) also the extract collected and dried by using freeze drier at -40°C)

2.5. Experimental design

The rabbits were divided randomly into four groups (ten rabbits each), the rabbits in G1 were supplemented in drinking water with *Glycyrrhiza glabra* extract in a daily dose of 300 mg/kg BW. Rabbits in G2were administrated diclazuril (Pharma Swede-Egypt) at a dose of (1mg/kg BW) in drinking water (Vanparijs et al., 1989). After one week, each rabbit in all groups except G4 were orally inoculated with 104 sporulated oocysts of *E. stiedae* using a stomach tube (Hassan et al., 2016).The treatment with plant extract continued daily post-infection and extended for 40 days. While rabbits in G3 were kept as non-treated-infected (control positive). Moreover, rabbits in G4 kept non-treated and non-infected (control negative).

2.6. Evaluation parameters

2.6.1. Clinical signs of hepatic coccidiosis

All the rabbits in different groups were clinically examined daily for recoding of clinical signs such as lack of appetite, dullness, and abdominal distention and dullness.

2.6.2. Fecal analysis and oocysts count

Fresh fecal samples were collected daily from each rabbit in each group into sterile separate containers. Each sample was microscopically examined till the end of the experiment to evaluate the pre-patent period and the number of *E. stiedae* oocysts per gram using McMaster counting chamber (Levine, 1985).

2.6.3. Body weight gain

The weight of each rabbit in each group was recorded at the beginning of the experiment and then weekly till the end of the experiment (40 DPI).

2.6.4. Gross lesion and histopathological examinations

At the end of the experiment, each rabbit was slaughtered and the liver was examined at PM for the presence of characteristic hepatic gross lesion of *E. stiedae*. For histopathological examination, liver specimens were immediately fixed in 10% neutral formalin, embedded into paraffin wax, sectioned to 5µm thickness, stained with hematoxylin-eosin stain and inspected microscopically according to (Culling, 1983).

2.6.5. Hematological and biochemical parameters analysis

For CBC analysis, blood samples were collected from each rabbit in each group into EDTA-coated tubes on the 7th and 40thdays of the experiment. Furthermore, the serum samples were also separated from the blood samples for biochemical assessment of liver and kidney function (Reithman and Frankel, 1957; Hewitt et al., 1989)

2.7. Statistical analysis

Statistical analysis was carried out using two-way ANOVA using SPSS, ver. 25. Data were treated as a complete randomization design according to (Steel et al., 1997). Multiple comparisons were carried out applying Duncan test. The significance level was set at $P< 0.05$

3. RESULTS

Clinical signs and mortalities (%)

Concerning the clinical signs, examination of G1 (*G. glabra* treated group) showed no rabbits in hepatic coccidiosis or any clinical abnormalities throughout the experiment compared to G2 (diclazuril group) which its animals remained healthy and showed normal appetite throughout the experiment, but slight dullness was exhibited at the end of the experiment. On the other hand, the rabbits in G3 (control infected group) showed signs of depression, rough coat, decrease appetite, loss of body weight, and abdominal distension. Rabbits in G4 (non-treated and non-infected group) remained normal without any signs of the disease. No Mortality were recorded in G1 and G4 during the entire experiment, only one death case was recorded in G2 and G3 on 32nd and on 40th DPI, respectively with mortality rate (10%)**.**

Body weight gain

Regarding body weight gain of rabbits, a significant increase ($P< 0.05$) in the body weight of rabbits was noticed in G1 which showed the best body weight gain (1668±116.16gm) at the end of the experiment compared to G2 (1533±92.89gm), G3 (1045±50.03gm) and G4 (1555±47.40gm) (Table 1).

Oocysts count

Oocysts shedding was observed in the feces, on 17th DPI, the results of oocysts count in both (G1, G2) showed a significant decrease in oocysts count from 17th to 32nd DPI compared to G3 (control infected group) except on day 24th G1 exhibited no significant difference compared to G3. Plant-treated group (G1) displayed a significant oocyst reduction ($P< 0.05$) in comparison to G2 (diclazuril group) on 23rd, 28th, 29th, 30th and 32nd DPI while on 22nd DPI there was no significant difference between G1 and G2 (Table 2)

Biochemical and hematological parameters

Concerning biochemical estimation of liver and kidney function in different groups on 40th DPI, a significant decrease ($P<0.05$) in creatinine and GGT was recorded in G1, G4 compared to G2, G3. Regarding urea level, no significant difference was recorded in different groups for urea level except G2 (diclazuril group) which showed

increase in urea level (68.93±2.47 mg/L) compared to other groups. AST in all groups (G1, G2, and G4) were showed a significant decrease compared to G3 (27.67±1.76 U/L). ALT level in all groups (G1, G4) recorded a significant decrease compared to G3 (22.67 U/L) except G2 which revealed no significant change (18.33) when compared to G4. No significant difference was recorded in different groups among albumin level. (Table 3).

Regarding hematological parameters, on the7th day, there were no change in hematological parameters among all groups while on day 40th DPI there is no significant difference($P<0.05$) in RBCS, MCV, MCH and MCHC among all groups but G1 showed significant increase in Hb (9.87±.20) and WBCS (8±1.01) compared to other groups. There is improvement in level PCV in G1 (28.47±.58) on day 40th DPI compared G2 (24±1.40) and G3 (24.13±.94). Moreover, G1 showed improvement in level of RBCS, Hb and PCV on day40th (at the end of the experiment) compared to 7th day. (Table 4).

Also, there were significant differences in differential leukocyte counts in all groups on the 7th day. On 40th DPI, values of neutrophils, eosinophils and monocytes recorded no significant difference between G1 (Glycyrrhiza glabra treated group) and G4 (CN). Furthermore, all groups showed no significant difference in level of lymphocytes and basophils. (Table 5)

Gross lesion of the liver

Concerning gross lesion of livers, on 40th day of the experiment, the livers of rabbits in G1were slightly enlarged without obvious nodules, while in G2 liver were moderately enlarged as compared to the livers in G3 (CP) which showed extensive enlargement of the liver with a pale appearance and studded whitish nodules with distended gall bladders. In G4 (CN), the rabbit's livers appeared normal without any abnormal changes (Figure 1).

Histopathological Findings

Regarding to microscopic findings of rabbit in G4, the examined liver exhibited normal tissue architecture with the normal structure of bile ducts in the portal areas (Figure 2 A). Comparing with the livers of different groups, it was cleared that the livers in G1 pretreated with *Glycyrrhiza glabra* extract showed bile ducts with subtle intra-epithelial coccidian developmental stages surrounded by minimal inflammatory cells (Figure 2 D) and mild mucosal hyperplasia in gall bladder with few intra-epithelial coccidian developmental stages (Figure 2 E). In G2,livers showed diffuse advanced hydropic degeneration of the hepatic cells while bile duct surrounded by slight lymphohistiocytic inflammatory cells with focal periportal hepatic necrosis invaded with inflammatory cell (Figure 2 F and G). Generally by the end of the experiment, the pathological changes recorded in the livers of all groups were significantly improved when compared with G3 (CP) which showed impaction of the bile duct with large number of the developmental stages of coccidia associated with marked periductal lymphohistiocytic inflammatory cells infiltration. Moreover, there was marked necrosis of the biliary epithelium accompanied with presence of numerous macro-gametocytes of coccidial parasites (Figure 2 B, C).

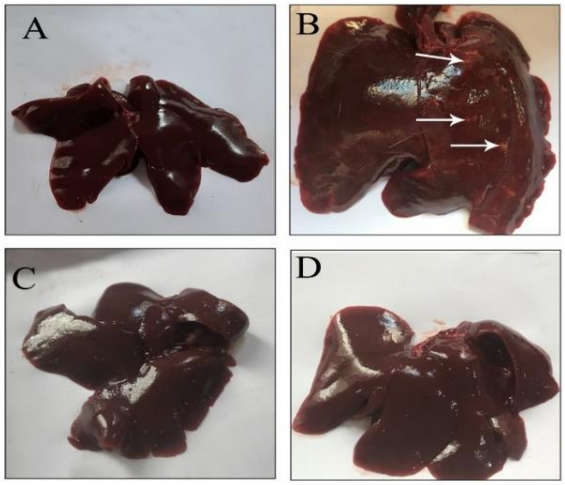

Fig (1) The Gross appearance of the liver of different experimental groups infected by *Eimeria stiedae*. (A): liver of rabbits in control negative, (B): liver of rabbits in control positive white arrows refers to typical nodules in the liver of rabbits caused by *E. stiedae*, (C): liver of rabbits in licorice treated group, (D): liver of rabbits in diclazuril group.

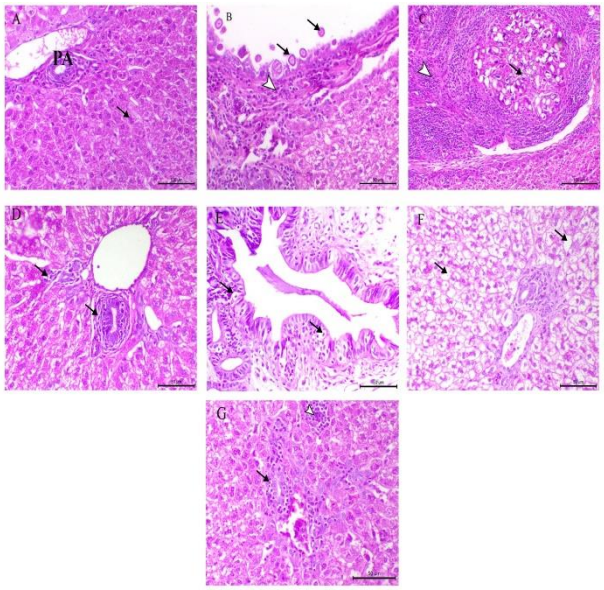

Fig (2) Histopathology of the liver section of rabbits stained by H&E in different experimental groups. (A): control negative (PA: portal area), (B, C): control positive group, (D, E): treated with licorice, (F, G) diclazuril group. G4 showed normal liver (A).In G3 (control positive) showed marked necrosis of the biliary epithelium accompanied with presence of numerous macro-gametocytes of coccidial parasites (B).Moreover there was impaction of the bile duct with large number of the developmental stages of coccidia associated with marked periductal lymphohistiocytic inflammatory cells infiltration(C).G1 pretreated with *Glycyrrhiza glabra* extract showed bile ducts with subtle intra-epithelial coccidian developmental stages surrounded by minimal inflammatory cells (D) also showed mild mucosal hyperplasia in gall bladder with few intra-epithelial coccidian developmental stages (E). In G2, livers showed diffuse advanced hydropic degeneration of the hepatic cells (F) while bile duct surrounded by slight lymphohistiocytic inflammatory cells with focal periportal hepatic necrosis invaded with inflammatory cell (G).

Table 1 Body weight (gm) of rabbits in different groups.

Day	Groups			
	G1	G2	G3	G4
0	1179.00±73.91^{Bbc}	1368.00±70.63^{Aa}	832.00±58.99^{Ca}	1379.00±62.42^{Ab}
7	1107.22±92.90^{Bc}	1582.00±85.28^{Aa}	906.00±107.73^{Ba}	1472.00±59.62^{Aab}
14	1243.75±91.53^{Bbc}	1533.50±88.05^{Aa}	1005.50±62.26^{Ca}	1491.00±52.06^{Aab}
21	1219.29±112.31^{Bbc}	1610.56±92.43^{Aa}	988.50±54.76^{Ca}	1506.00±50.23^{Aab}
28	1426.00±79.43^{Aab}	1521.11±87.90^{Aa}	988.50±55.86^{Ba}	1525.00±50.82^{Aab}
35	1490.00±107.66^{Aab}	1419.38±83.58^{Aa}	880.00±52.87^{Ca}	1537.00±49.58^{Aab}
40	1668.00±116.16^{Aa}	1533.33±92.89^{ABa}	1045.00±50.03^{Ca}	1555.00±47.40^{ABa}

Values with different superscripts (a, b, c) within the same column differed significantly at P<0.05.
Values with different superscripts (A, B, C) within the same row differed significantly at P<0.05.

Table 2 Daily oocyst count (mean ±SE) in different groups of rabbits.

Day post infection (DPI)	Groups			
	G1	G2	G3	G4
17	3.80 ± 0.10^{aB}	2.56 ± 0.18^{deE}	3.92 ± 0.08^{efA}	0 ± 0^{aF}
18	3.58 ± 0.08^{bcdB}	2.52 ± 0.21^{eC}	4.50 ± 0.03^{bA}	0 ± 0^{aD}
19	3.77 ± 0.10^{aB}	2.70 ± 0.03^{cdE}	5.01 ± 0.07^{aA}	0 ± 0^{aF}
20	3.78 ± 0.08^{aB}	2.35 ± 0.17^{fE}	4.98 ± 0.02^{aA}	0 ± 0^{aF}
21	3.34 ± 0.09^{efB}	2.63 ± 0.23^{deE}	4.10 ± 0.04^{deA}	0 ± 0^{aF}
22	2.65 ± 0.34^{gCD}	2.59 ± 0.29^{deD}	3.87 ± 0.05^{fgA}	0 ± 0^{aE}
23	2.58 ± 0.20^{ghE}	2.83 ± 0.13^{cD}	3.55 ± 0.12^{iA}	0 ± 0^{aF}
24	3.66 ± 0.05^{hiAB}	2.71 ± 0.36^{cdD}	3.73 ± 0.20^{abA}	0 ± 0^{aE}
25	3.77 ± 0.05^{aA}	2.86 ± 0.22^{cC}	3.67 ± 0.04^{hiA}	0 ± 0^{aE}
26	3.68 ± 0.17^{abB}	2.81 ± 0.07^{cD}	3.91 ± 0.06^{fgA}	0 ± 0^{aE}
27	3.44 ± 0.11^{deB}	2.35 ± 0.35^{fD}	3.95 ± 0.04^{efA}	0 ± 0^{aE}
28	3.34 ± 0.11^{efC}	3.70 ± 0.09^{aB}	4.33 ± 0.04^{cA}	0 ± 0^{aF}
29	2.44 ± 0.23^{hE}	3.65 ± 0.12^{abB}	4.19 ± 0.04^{cdA}	0 ± 0^{aF}
30	2.68 ± 0.50^{gD}	3.62 ± 0.13^{abB}	3.75 ± 0.04^{ghA}	0 ± 0^{aF}
31	3.51 ± 0.06^{cdC}	2.00 ± 0.17^{gE}	3.90 ± 0.03^{fgA}	0 ± 0^{aF}
32	3.24 ± 0.11^{fC}	3.49 ± 0.03^{bB}	3.85 ± 0.04^{fgA}	0 ± 0^{aE}

Values with different superscripts (a, b, c) within the same column differed significantly at P<0.05.
Values with different superscripts (A, B, C) within the same row differed significantly at P<0.05.
G1: Glycyrrhiza glabra treated group, G2: diclazuril group, G3; Positive control, G4: Negative control

Table 3 Liver and kidney function tests of rabbits in different groups

Parameter	Day	Groups			
		G1	G2	G3	G4
Creatinine (mg/dL)	7th day	1.40 ± 0.12^{Aba}	1.27 ± 0.09^{ABb}	1.50 ± 0.06^{Aa}	1.20 ± 0.10^{ABa}
	40th DPI	1.10 ± 0.06^{Ca}	1.83 ± 0.09^{Aa}	1.53 ± 0.12^{Aba}	1.20 ± 0.12^{Ca}
Urea	7th day	43.93 ± 8.24^{Aa}	40.70 ± 1.65^{Ab}	43.93 ± 2.03^{Aa}	35.17 ± 2.54^{Aa}
(mg/L)	40th DPI	37.90 ± 2.44^{Ca}	68.93 ± 2.47^{Aa}	50.40 ± 5.93^{BCa}	37.80 ± 2.42^{Ca}
AST	7th Day	11.00 ± 0.58^{Cb}	13.00 ± 0.00^{BCb}	17.67 ± 0.67^{Ab}	12.00 ± 0.58^{BCb}
(U/L)	40th DPI	19.67 ± 2.33^{Ba}	21.00 ± 1.15^{Ba}	27.67 ± 1.76^{Aa}	17.33 ± 0.67^{Ba}
ALT	7th day	8.67 ± 0.67^{Cb}	10.67 ± 0.33^{Bb}	15.00 ± 0.58^{Ab}	7.67 ± 0.33^{Cb}
(U/L)	40th DPI	17.33 ± 2.03^{Ba}	18.33 ± 1.45^{Aba}	22.67 ± 1.86^{Aa}	13.33 ± 0.33^{Ba}
GGT	7th day	10.67 ± 1.20^{Ba}	12.33 ± 0.88^{ABb}	16.00 ± 1.15^{Ab}	11.67 ± 0.88^{Ba}
(U/L)	40th DPI	12.67 ± 1.45^{Ca}	19.33 ± 2.03^{Aba}	22.67 ± 2.03^{Aa}	12.33 ± 1.20^{Ca}
Albumin	7th day	3.27 ± 0.03^{Ba}	3.30 ± 0.00^{Ba}	3.47 ± 0.09^{Aa}	3.27 ± 0.03^{Ba}
(g/L)	40th DPI	3.47 ± 0.13^{Aba}	3.47 ± 0.09^{Aba}	3.50 ± 0.12^{Aba}	3.37 ± 0.12^{Ba}

Values with different superscripts (a, b, c) within the same column differed significantly at P<0.05.
Values with different superscripts (A, B, C) within the same row differed significantly at P<0.05.

Table 4 Hematological parameters in different groups

Parameter	Days	Groups			
		G1	G2	G3	G4
RBCs	7th Day	2.57 ± 0.07^{Ab}	2.67 ± 0.03^{Aa}	2.70 ± 0.10^{Aa}	2.57 ± 0.03^{Aa}
($x10^6$/µl)	40th DPI	3.37 ± 0.09^{Aa}	2.83 ± 0.17^{Aa}	2.90 ± 0.25^{Aa}	2.83 ± 0.17^{Aa}
HB	7th Day	7.56 ± 0.26^{Ab}	7.87 ± 0.13^{Aa}	7.90 ± 0.25^{Aa}	7.80 ± 0.36^{Aa}
(g/dL)	40th DPI	9.87 ± 0.20^{Aa}	8.30 ± 0.50^{BCa}	7.93 ± 0.45^{BCa}	8.43 ± 0.26^{BCa}
PCV	7th Day	21.77 ± 0.77^{Ab}	22.83 ± 0.47^{Aa}	22.80 ± 0.71^{Aa}	21.67 ± 0.33^{Aa}
(%)	40th DPI	28.47 ± 0.58^{Aa}	24.00 ± 1.40^{BCa}	24.13 ± 0.94^{BCa}	25.37 ± 1.64^{ABCa}
MCV	7th Day	84.93 ± 0.63^{Aa}	82.27 ± 3.93^{Aa}	81.17 ± 3.42^{Aa}	86.07 ± 0.13^{Aa}
(fl)	40th DPI	84.67 ± 0.52^{Aa}	84.07 ± 0.47^{Aa}	84.67 ± 0.78^{Aa}	85.17 ± 0.44^{Aa}
MCH	7th Day	29.43 ± 0.23^{Aa}	28.07 ± 1.83^{Aa}	27.63 ± 1.63^{Aa}	27.50 ± 1.55^{Aa}
(pg)	40th DPI	29.27 ± 0.17^{Aa}	29.23 ± 0.33^{Aa}	29.30 ± 0.31^{Aa}	29.37 ± 0.27^{Aa}
MCHC	7th Day	34.60 ± 0.00^{Aa}	32.87 ± 1.73^{Aa}	32.87 ± 1.73^{Aa}	34.60 ± 0.00^{Aa}
(%)	40th DPI	34.60 ± 0.00^{Aa}	34.60 ± 0.00^{Aa}	34.60 ± 0.00^{Aa}	34.60 ± 0.00^{Aa}
WBCs	7th Day	6.93 ± 0.48^{Aa}	7.40 ± 1.25^{Aa}	8.73 ± 1.47^{Aa}	6.53 ± 0.87^{Aa}
($x10^3$/µl)	40th DPI	8.00 ± 1.01^{Aa}	3.53 ± 0.30^{Cb}	4.70 ± 0.46^{BCa}	3.20 ± 0.10^{Cb}
Platelets	7th Day	283.33 ± 33.21^{ABa}	356.67 ± 67.15^{ABa}	199.33 ± 46.86^{Ba}	358.33 ± 65.21^{ABa}
($x10^3$/µl)	40th DPI	235.33 ± 33.12^{ABa}	299.67 ± 15.39^{Aa}	277.33 ± 54.03^{Aa}	288.00 ± 4.36^{Aa}

Values with different superscripts (a, b, c) within the same column differed significantly at P<0.05.
Values with different superscripts (A, B, C) within the same row differed significantly at P<0.05.

Table 5 Differential leukocyte count of rabbits in different groups

Parameter	Days	Groups			
		G1	G2	G3	G4
Neutrophils (%)	7th Day	15.67 ± 1.45^{Aa}	14.67 ± 0.88^{Aa}	15.33 ± 1.20^{Aa}	14.67 ± 0.88^{Aa}
	40th DPI	9.00 ± 1.15^{Abb}	11.00 ± 1.15^{Aba}	11.33 ± 1.76^{Aa}	7.33 ± 0.88^{Bb}
Lymphocytes (%)	7th Day	77.67 ± 1.45^{ABb}	78.00 ± 0.58^{Aa}	76.33 ± 2.03^{Aba}	73.33 ± 0.33^{ABb}
	40th DPI	85.67 ± 1.86^{ABCa}	83.67 ± 2.03^{Bca}	82.33 ± 2.33^{BCa}	81.00 ± 1.53^{Ca}
Monocytes (%)	7th Day	5.67 ± 0.33^{Aa}	6.00 ± 0.58^{Aa}	5.33 ± 0.33^{Aa}	6.00 ± 0.58^{Aa}
	40th DPI	3.67 ± 0.33^{ABb}	4.33 ± 0.33^{Aa}	3.67 ± 0.33^{ABb}	2.67 ± 0.33^{Bb}
Eosinophils (%)	7th Day	1.00 ± 0.00^{Ba}	1.00 ± 0.00^{Ba}	2.00 ± 0.58^{Ba}	1.33 ± 0.33^{Ba}
	40th DPI	1.00 ± 0.00^{ABa}	1.00 ± 0.58^{Aba}	2.33 ± 0.33^{Aa}	0.33 ± 0.33^{Ba}
Basophiles (%)	7th Day	0.00 ± 0.00^{Aa}	0.33 ± 0.33^{Aa}	1.00 ± 0.58^{Aa}	0.33 ± 0.33^{Aa}
	40th DPI	0.00 ± 0.00^{Ba}	0.00 ± 0.00^{Ba}	0.00 ± 0.00^{Ba}	0.00 ± 0.00^{Ba}

Values with different superscripts (a, b, c) within the same column differed significantly at P<0.05.
Values with different superscripts (A, B, C) within the same row differed significantly at P<0.05.

4- DISCUSSION

Numerous studies have demonstrated the excellent anti-coccidial potential of botanicals and their antioxidant compounds, exhibiting that they are the best substitute for synthetic anti-coccidial medications (Gotep et al., 2016).Plants with high antioxidant content have also demonstrated impressive immune-modulating effects (Abbas et al., 2017; Abbas et al., 2017b).

In the present study, the use of Glycyrrhiza glabra for treatment of *Eimeria stiedae* in experimentally infected rabbits declared that the botanical *Glycyrrhiza glabra* extract has a potent anti-coccidial effect which improves weight gain, general performance of rabbits, drop in oocysts count, decrease liver lesion and not affect liver and kidney enzymes.

Regarding oocysts count of *Eimeria stiedae* among infected rabbits, G. glabra's good result as an anti-coccidial plant and it displayed a significant oocysts reduction in comparison to G2 (diclazuril group) and G3 (Control infected group)from 17th day and continued till the end of the experiment (day 32) and this agree with Abu El Ezz et al. (2020) and Hussain et al. (2017). This result could be attributed to the presence of potent antioxidants and bioactive components in *G. glabra* extract, which has an efficient and protective effect against the *Eimeria stiedae* parasite, potentially limiting the growth and development of *Eimeria stiedae* stages and resulting in a reduction in oocysts formation and appearance in the feces.

Concerning body weight, rabbit in G1that received Glycyrrhiza glabra extract recorded the heaviest body gain on day 40th when compared with other groups, this agreed with Khalaji et al. (2011) and Seddiek and Metwally (2013).This could be due to alcoholic extract of *Glycyrrhiza glabra* contain (glycyrrhizic acid) that enhance food intake and outperform the body weight which in respect with Ocampo et al. (2016) who recorded that the broilers supplemented with licorice (60 µg/mL in water) had higher body weight gain (BG), final body weight, better FCR, and the lowest mortality rate compared to the non-treated controls or might have been related to G. glabra extract's ability to act as an antioxidant (Antioxidants are a crucial component of the host body's defensive system and assist in reducing the oxidative stress produced by reactive oxygen species (Alagawany et al., 2019).

Gross lesion and histopathological changes of the liver of sacrificed rabbit in G3 showed extensive enlargement with a pale appearance and whitish nodules with distended gall bladders this finding was aligned with those of Al-Naimi et al. (2012) and Kardena et al. (2015). Conversely, rabbits in (G1, G2) had almost a normal liver at PM examination and the microscopical architecture of the liver indicating its protective effects of (G. glabra and Diclazuril) against *E. stiedae* infection which were in line with results previously recorded by Kardena et al. (2015).

Moreover, it was noticed that the liver lesions were completely ameliorated in histopathological hepatic damage by G1and G2 and the findings of the present study were consistent with the previous research (Allam et al., 2020; Seddiek and Metwally, 2013).

This indicated that licorice extract recorded a decrease in the pathological reaction following *Eimeria stiedae* infection mainly by stimulating the cellular immune responses by the activation of host defense potentiators, through maturation, proliferation or differentiation of the immune cells. Also, licorice's bioactive substances include saponins, glycyrrhizic acid, liquiritigenin, triterpenes (glycyrrhizin), and flavonoids (isoflavonoids and liquiritin), all of which have anti-inflammatory and antioxidant properties (Asan-Ozusaglam and Karakoca, 2014; Yatoo et al., 2018).

Generally, coccidiosis altered blood and biochemical parameters in addition to liver enzymes activities ALT, AST and GGT in infected non-treated. The recorded data of the current study revealed lowered serum enzyme (AST and ALT) values in G1 and G2 as compared to control positive group that could be related to cell destruction due to the parasitic infection, which caused the escape of enzymes into the bloodstream (Hanada et al., 2003),especially high levels of GGT and AST might be a sign of the broken epithelial covering of the bile duct due to increased numbers of parasite oocysts (Sanyal and Sharma, 1990). While the improvement of blood serum enzymes in (G1, G2) could be attributed to the recovery of hepatic cells and the lining epithelium of bile ducts (the primary site of infection), inhibition of the parasite sexual stages development and normalization of many biochemical parameters' levels, including liver enzymes following pretreatment. These results are similar to that of Abdel Maged et al. (2013) and Çam et al. (2008). The current observations revealed that the recorded values of tested biochemical parameters were nearly similar to those of control non infected group after pretreatment by licorice extract which approve its anti-coccidial efficacy and hepato-protective effects. In addition to, herbal extract improved the general health of rabbits where the values of hematological parameters were nearly similar to those recorded value in non -infected control group.

There is virtually no variation between the groups in different Leukocytic count especially G1 which showed no significant difference in level of neutrophils, eosinophils and monocytes compared to G4(CN).According to Castro and Duszynski (1984), who reported that rats infected with *E. nieschulzii* interferes with the directed migration of leukocytes to sites of inflammation and does not affect hematopoiesis. That is the reason why the granulocyte count (lymphocytes and monocytes) in peripheral blood is normal or slightly increased in coccidial rabbits.

The results of the hematological examination showed no significant difference on level of eosinophil between (G1,G2) and(G3)this is may be due to eosinophilia in rabbits rarely occurs but it may be associated with parasitism eosinophilia which occurs as a pathophysiologic response to infection with parasites through participation in the immune response by discharging their cytotoxic granular contents onto the parasites, which kill them (Capron, 1991; Rothenberg, 1998).

5. CONCLUSION

This study was carried out to evaluate the protective effect of *Glycyrrhiza glabra* supplementation against induced hepatic coccidiosis in rabbits. Supplementation with alcoholic extract of Glycyrrhiza glabra demonstrated effective anti-coccidial results on *Eimeria stiedae* infection in rabbits associated with improving bodyweight gain and in reducing liver lesions, mortality and the numbers of oocysts shed with no side effects on liver and kidney functions.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

6. REFERENCES

1. Abbas, A., Iqbal, Z., Abbas, R., Khan, M., Khan, J., Hussain, K., Mahmood, M., Rizwan, H., 2017a. Immunomodulatory effects of Camellia sinensis against coccidiosis in chickens. Journal of Animal & Plant Sciences, 27, 415–421.
2. Abbas, A., Iqbal, Z., Abbas, R.Z., Khan, M.K., Khan, J.A., 2017b. Immunomodulatory activity of Pinus radiata extract

against coccidiosis in broiler chicken. Pak Vet J, 37, 145-149.
3. Abbas, R., Colwell, D., Gilleard, J., 2012. Botanicals: an alternative approach for the control of avian coccidiosis. World's Poultry Science Journal, 68, 203-215.
4. Abdel Maged, A., Ahmed, N.E., Ramadan, M., Elashrey, M., 2013. Biochemical effects of antiprotozoa on gastrointestinal tract enzymes and related hormones in rabbits. Benha Veterinary Medical Journal, 25, 113-124.
5. Abu El-Ezz, N., 2005. Comparative efficacy of Nigella sativa seeds and Diclazuril on coccidiosis in chickens. J Egypt Vet Med Assoc, 65, 137-147.
6. Abu El Ezz, N., Aboelsoued, D., Hassan, S., Abdel Megeed, K., El-Metenawy, T., 2020. Therapeutic effect of Moringa oleifera and Thymus vulgaris oils against hepatic coccidiosis in experimentally infected rabbits. Tropical Biomedicine, 37, 1018-1028.
7. Adamu, M. and Boonkaewwan, C., 2014. Effect of Lepidium sativum L.(garden cress) seed and its extract on experimental Eimeria tenella infection in broiler chickens. Agriculture and Natural Resources, 48, 28-37.
8. Al-Naimi, R., Khalaf, O., Tano, S., Al-Taee, E., 2012. Pathological study of hepatic coccidiosis in naturally infected rabbits. Al-Qadisiyah Journal of Veterinary Medicine Sciences, 11, 63-69.
9. Alagawany, M., Elnesr, S.S., Farag, M.R., Abd El-Hack, M.E., Khafaga, A.F., Taha, A.E., Tiwari, R., Yatoo, M.I., Bhatt, P.M.G., Dhama, K., 2019. Use of Licorice (Glycyrrhiza glabra) Herb as a Feed Additive in Poultry: Current Knowledge and Prospects. Animals, 9(8), 536.
10. Allam, T., AbdelGaber, M., Thabet, N., AbouLaila, M., Elkhatam, A., 2020. Clinicopathological effects of diclazuril prophylaxis and treatment on rabbits experimentally infected with Eimeria stiedae. Damanhour Journal of Veterinary Sciences, 4, 20-28.
11. Asan-Ozusaglam, M. and Karakoca, K., 2014. Evaluation of biological activity and antioxidant capacity of Turkish licorice root extracts. Romanian Biotechnology Letters, 19, 8994-9005.
12. Çam, Y., Atasever, A., Eraslan, G., Kibar, M., Atalay, Ö., Beyaz, L., İnci, A., Liman, B.C., 2008. Eimeria stiedae: experimental infection in rabbits and the effect of treatment with toltrazuril and ivermectin. Experimental parasitology, 119, 164-172.
13. Capron, M., 1991. Eosinophils and parasites. Annales de parasitologie humaine et comparee, 66, 41-45.
14. Castro, G.A. and Duszynski, D.W., 1984. Local and Systemic Effects on Inflammation during Eimeria nieschulzi Infection 1: Anti-Inflamation In E. Nieschulzi INFECTION. The Journal of protozoology, 31, 283-287.
15. Culling, C.F., 1983. Hand book of Histological and Histochemical Techniques, 3rd ed. . Butterwoth, London. Boston.
16. Dalloul, R.A., Lillehoj, H.S., 2005. Recent advances in immunomodulation and vaccination strategies against coccidiosis. Avian diseases, 49, 1-8.
17. El-Shahawi, G., El-Fayomi, H., Abdel-Haleem, H., 2012. Coccidiosis of domestic rabbit (Oryctolagus cuniculus) in Egypt: light microscopic study. Parasitology research, 110, 251-258.
18. El Banna, H., Atef, M., Nabil, G., 2016. Anti-coccidial activity of Moringa oleifera plant. Anim. Vet. Sci, 4, 19-25.
19. Gotep, J., Tanko, J., Forcados, G., Muraina, I., Ozele, N., Dogonyaro, B., Oladipo, O., Makoshi, M., Akanbi, O., Kinjir, H., 2016. Therapeutic and safety evaluation of combined aqueous extracts of Azadirachta indica and Khaya senegalensis in chickens experimentally infected with Eimeria oocysts. Journal of Parasitology Research ,(8), 1-9.
20. Hanada, S., Omata, Y., Umemoto, Y., Kobayashi, Y., Furuoka, H., Matsui, T., Maeda, R., Saito, A., 2003. Relationship between liver disorders and protection against Eimeria stiedai infection in rabbits immunized with soluble antigens from the bile of infected rabbits. Veterinary parasitology, 111, 261-266.
21. Hassan, K.M., Arafa, W.M., Mousa, W.M., Shokier, K.A., Shany, S.A., Aboelhadid, S.M., 2016. Molecular diagnosis of Eimeria stiedae in hepatic tissue of experimentally infected rabbits. Experimental parasitology, 169, 1-5.
22. Hewitt, D., Innes,D.,J., J., Savory, Wills, M.,R., 1989. Normal biochemical and hematological values in New Zealand white rabbits, Clinical Chemistry, 35, 1777-1779.
23. Hussain, K., Iqbal, Z., Zahid Abbas, R., Kasib Khan, M., Kashif Saleemi, M., 2017. Immunomodulatory activity of Glycyrrhiza glabra extract against mixed Eimeria infection in chickens. International Journal of Agriculture & Biology, 19(4), 928–932.
24. Kardena, I.M., Ketut, B., Ida, B., Oka, W., Ida, B., Made, O., Siswanto, F., 2015. Pathological case study of rabbit hepatic coccidiosis in tabanan, bali. Proceeding-Kuala Lumpur International Agriculture, Forestry and Plantation, Hotel Putra, Kuala Lumpur, Malaysia
25. Khalaji, S., Zaghari, M., Hatami, K., Hedari-Dastjerdi, S., Lotfi, L., Nazarian, H., 2011. Black cumin seeds, Artemisia leaves (Artemisia sieberi), and Camellia L. plant extract as phytogenic products in broiler diets and their effects on performance, blood constituents, immunity, and cecal microbial population. Poultry science, 90, 2500-2510.
26. Levine, N.D., 1985. Veterinary Protozoology, 1st edition, Iowa State University Press, Ames.
27. Ocampo, C.L., Gómez-Verduzco, G., Tapia-Perez, G., Gutierrez, O.L., Sumano, L.H., **2016**.Effects of glycyrrhizic acid on productive and immune parameters of broilers. Brazilian Journal of Poultry Science 18:435–442.
28. Omata, Y., Satake, M., Maeda, R., Saito, A., Shimazaki, K., Uzuka, Y., Tanabe, S., Sarashina, T., Mikami, T., Yamauchi, K., 2001. Reduction of the infectivity of Toxoplasma gondii and Eimeria stiedai sporozoites by treatment with bovine lactoferricin. Journal of Veterinary Medical Science, 63, 187-190.
29. Omer, M.O., AlMalki, W.H., Shahid, I., Khuram, S., Altaf, I., Imran, S., 2014. Comparative study to evaluate the anti-viral efficacy of Glycyrrhiza glabra extract and ribavirin against the Newcastle disease virus. Pharmacognosy research, 6(1), 6-11.
30. Pandit, S., Ponnusankar, S., Bandyopadhyay, A., Ota, S., Mukherjee, P.K., 2011. Exploring the possible metabolism mediated interaction of Glycyrrhiza glabra extract with CYP3A4 and CYP2D6. Phytotherapy Research, 25, 1429-1434.
31. Reithman, S. and Frankel, S. A., 1957. Colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology, l28, 56–63.
32. Rothenberg, M.E., 1998. Eosinophilia. New England Journal of Medicine, 338, 1592-1600.
33. Sanyal, P. and Sharma, S., 1990. Clinicopathology of hepatic coccidiosis in rabbits. Indian Journal of Animal Sciences, 60, 924-928.
34. Seddiek, S.A. and Metwally, A.M., 2013. Anticoccidial activity of black cumin (Nigella sativa) in rabbits. Assiut Veterinary Medical Journal, 59, 85-96.
35. Steel, R., Torrie, J., Dickey, D., 1997. Principles and procedures of Statistics: A Biometrical Approach, 3rd ed.,. McGraw-Hill, New York, NY.
36. Vanparijs, O., Marsboom, R., Desplenter, L., 1989. Diclazuril, a new broad spectrum anticoccidial drug in chickens. 1. Dose titration studies and pilot floor pen trials. Poultry science, 68, 489-495.
37. Yatoo, M., Gopalakrishnan, A., Saxena, A., Parray, O.R., Tufani, N.A., Chakraborty, S., Tiwari, R., Dhama, K., Iqbal, H., 2018. Anti-inflammatory drugs and herbs with special emphasis on herbal medicines for countering inflammatory diseases and disorders-a review. Recent patents on inflammation & allergy drug discovery, 12, 39-58.