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Research Article

Potential of *Glycyrrhiza glabr*a (Licorice) Extract an Alternative Biochemical and Therapeutic Agent Against Coccidiosis in Broiler Chickens

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Abstract: To control coccidiosis, anticoccidials are generally used as feed additives. However, the frequent usage has given rise to the occurrence of resistant strains to available anticoccidial drugs. Botanicals may work as substitutes to anticoccidial drugs. The current research was designed to evaluate the efficacy of aqueous methanol extracts of *Glycyrrhiza glabra* (licorice) (roots) as anticoccidial in different concentrations i.e. (100, 200 and 300 mg/kg of body weight). For *in vivo* trial, 105-day-old broiler birds were grouped into seven equal groups (A, B, C, D, E, F and G). At the age of one week, groups A, B and C were orally treated with three doses of the *G. glabra* extracts (100, 200 and 300 mg/kg of body weight, respectively). Group D was medicated with Vitamin E and Group E worked as the infected medicated control group. Group F served as the infected non-medicated control group (PBS treated, negative control) and Group G was designated as the normal control group (non-infected and non-medicated group). At the age of the 14th day, all groups were infected orally with 60.000 sporulated oocysts of different *Eimeria* species. Though, comparable with reference drug G. glabra showed comparable anticoccidial efficacy against following parameters. i.e., feed conversion ratio, lesion score, fecal score and oocyst score, serum profile and hematological values showed no adversative effects (P<0.05) than infected non-medicated (negative control) group of aqueous methanol extract of *G. glabra* on the trial broiler birds. So, in this study the biochemical and therapeutic property of G. glabra extract found dose dependent manner against coccidiosis in broiler chickens.

Keywords: *Phytomedicine, Poultry, Alternatives, Disease*

Etlik Piliçlerde Koksidiyoza Karşı Alternatif Bir Biyokimyasal ve Terapötik Ajan Olarak *Glycyrrhiza glabra* (Meyan Kökü) Ekstraktının Potansiyeli

Öz: Koksidiyozun kontrolünde genellikle yem katkı maddesi olarak antikoksidiyaller kullanılır. Fakat, bunların sık kullanımı mevcut antikoksidiyal ilaçlara dirençli suşların ortaya çıkmasına neden olmuştur. Bitkisel ilaçlar antikoksidiyal ilaçların yerini alabilir. Bu çalışma, *Glycyrrhiza glabra* (meyan kökü)'nın (kökleri) sulu metanol ekstraktlarının farklı konsantrasyonlarda (örneğin; 100, 200 ve 300 mg/kg vücut ağırlığı) antikoksidiyal etkinliğinin değerlendirilmesi için tasarlandı. *In vivo* denemeler için, 105 günlük etlik piliçler yedi eşit gruba (A, B, C, D, E, F ve G) ayrıldı. Bir haftalıkken A, B ve C gruplarına oral olarak üç doz şeklinde (sırasıyla 100, 200 ve 300 mg/kg vücut ağırlığı) ekstrakt uygulandı. Grup D'ye Vitamin E uygulandı ve Grup E, ilaç uygulanmış enfekte kontrol grubu olarak yer aldı. Grup F, ilaçsız enfekte kontrol grubu (PBS ile tedavi edilen, negatif kontrol) ve Grup G, enfekte olmayan ve ilaçsız normal kontrol grubu olarak yer aldı. 14. günde, tüm gruplar farklı *Eimeria* türlerine ait 60.000 sporlu ookist ile oral yolla enfekte edildi. Referans ilaç ile kıyaslandığında *G. glabra*, yemden yararlanma oranı, lezyon skoru, dışkı skoru ve ookist skoru gibi parametreler yönünden karşılaştırılabilir antikoksidiyal etkinlik göstermesinin yanı sıra, serum profili ve hematolojik değerler de, *G. glabra*'nın metanol ektraktının deneme gruplarında, enfekte ilaçsız kontrol grubundan (negatif kontrol) daha olumsuz bir etki sergilemediğini ortaya koydu (P<0.05). Böylece bu çalışmada, *G. glabra* kök ekstraktının etlik piliçlerde koksidiyoza karşı doza bağlı olarak biyokimyasal ve tedavi edici özelliği saptanmıştır.

Anahtar sözcükler: *Bitkisel ilaç, Kümes hayvanları, Alternatifler, Hastalık*

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Introduction

Avian coccidiosis is mostly produced with single cell parasitic protozoa of genus *Eimeria* which belongs to phylum *Apicomplexa* having complex life cycle [1]. *Eimeria* species affected birds mostly show symptoms of enteric damage resulting into bloody diarrhea, reduced weight gain and mortality which lead to the economic losses in industry [2]. Additionally, links have been reported between Emirian infection and higher intestinal colonization with bacterial foodborne pathogens such as *Clostridium perfringens* and *Salmonella enterica* serovars Typhimurium and Enteritidis. Thus, increasing risk of zoonotic food borne diseases and to food security. There are seven species of *Eimeria* which have been recognized which cause coccidiosis in chickens by residing in different sections of intestine. The most virulent species is *E. tenella* that causes cecal coccidiosis in chickens. Infection starts with the ingestion of sporulated oocyst [3]. According to an estimation in US poultry industry, coccidiosis causes a loss of $127 million annually and this can be expected to cause likewise loses world-wide [4,5]. Due to ubiquitous nature of coccidian oocysts, they sporulate rapidly and millions of new oocysts emerge from each single sporulated oocyst of *Eimeria* which make very difficult to keep birds protected from coccidiosis [6].

Synthetic chemicals and anticoccidials are generally added in feed and water to control coccidiosis. Sulfanilamide was the first anticoccidial used as a treatment against coccidiosis in poultry birds, and a variety of anticoccidial feed additives and antibiotics, have also been developed and used. However, with frequent use of these drugs, anticoccidial drug resistance has emerged in *Eimeria* species by the passage of time [7]. Efficacy of these chemical agents is becoming questionable with the advent of these drug-resistant parasite strains. Adverse effects of these drugs are also reported on the health status of birds and humans, due to the drug residual effects in meat leading to the reduced consumption by humans [8,9].

It's the need of hour to look in indigenous, cost effective and potent resources for eradication and control of coccidiosis. Therefore, we must select alternatives for the operative and designated control of coccidiosis [10,11]. The experimental work showed that probiotics could be a good hope due to its antioxidant, immunomodulatory and growth-promoting effect against poultry coccidiosis alone or in combination with vaccines, including IMP1C based vaccine [12-14]. In the last few years, some botanicals gave us promising hope for their use as anticoccidials [15,16] and immunomodulatory effects [17-20]. Studies also evaluate that plants methanolic extracts possess best anticoccidial, anthelminthic and antioxidant activities due to presence of medicinally important phytochemicals [21,22].

Some botanicals have been described as promising anti-coccidials and immunomodulators [23,24]. Because of the restrictions on the synthetic anticoccidials due to their resistance against *Eimeria* species in poultry, antioxidant-rich plant extracts have attained promising future importance [25]. Botanicals high in antioxidant chemicals such phenols, flavonoids, tannins, and saponins are being used to treat coccidiosis as an alternate method [26]. *Glycyrrhiza glabra* plant is well renowned for its therapeutic effects like anti-allergic, anti-inflammatory, spasmolytic, laxative, antistress, antidepressant, antiulcer, estrogenic, immunomodulator, and antidiabetic substance in livestock and public health. It is also used to treat cough, throat infections, mouth and stomach infections [27]. Therefore, the present research was designed to evaluate the anti-coccidial benefits of aqueous methanolic extract of *G. glabra* (roots) against a simulated mixed *Eimeria* infection in chickens based on the available literature, which included antioxidant capabilities of botanicals.

Material and Methods

Ethical Statement

This research was conducted with approval from ethical committee of University of Agriculture Faisalabad (Approval No: 144) Faisalabad, Pakistan.

Plant Material

Glycyrrhiza glabra roots were purchased from a local market. A botanist from the Botany Department (University of Agriculture, Faisalabad-Pakistan) recognized and confirmed the plant material. In an electric mill, dried plant material was ground into powder. Using Soxhlet's equipment at 80°C, an aqueous methanolic (70%) extract of *G. glabra* was obtained by using rotary evaporator at 35°C under decreased pressure. The extracted material was freeze dried and stored at 4°C until used.

Parasite

Natural *Eimeria* infected chicken guts were obtained from several poultry selling sites and different poultry farms of district Faisalabad, Pakistan. Microscopically, the GIT material was observed using the method described by [28]. Sporulation of oocysts was achieved by incubating them in a 2.5 percent potassium dichromate solution for 48 hours at 24-30°C with 60-80 percent humidity. The sporulation was tracked using a light microscope at a magnification of 40x to examine sporocysts.

Management

A total of 105-day-old broiler chicks were purchased from a local market in Faisalabad and kept on floor pens using standard management procedures. For the first two weeks, all chicks were fed broiler starter diet, then finisher ration used till last day. Anticoccidial ingredients were not included in the standard feed. Feed and water were freely

available, and chicks were vaccinated against Newcastle Disease, Infectious Bronchitis, Infectious Bursitis [29]. During the first week of life, the temperature was kept at 85-90°F; however, it was dropped by 5°F on a weekly basis. Throughout the experiment, light was delivered for 24 h at a time. The experiment was carried out for 42 days.

Experimental Design

Chicks (n=105) were separated into seven equal (n=15) groups for the anticoccidial experiment. On day 14 of the experiment, all groups except G were orally infected with 60.000 sporulated oocysts of mixed *Eimeria* species. At the same day, Groups A, B and C were orally given *G. glabra* extract at 100, 200 and 300 mg/kg of body weight. Group D was treated with Vitamin E at the dose rate of 87 mg/kg of body weight. Group E was treated with Toltrazuril (Baycox® Bayer, Leverkusen, Germany) at the dose rate of 1 mL/ liter of water. Group F was treated with PBS (negative control) and Group G remained (Normal control). This procedure continued for day 14, 15 and 16 of experiment.

Feed Conversion Ratio

FCR was calculated on the 30th day of the experiment through total feed consumed/total weight gain.

Oocyst, Lesion and Fecal Score

After 7 days of post infection, 6 birds from each group were slaughtered and score of oocyst (0-5), lesions (0-4) and fecal were observed by [30-32].

Hematological Analysis

Packed cell volume determinations (PCV), red and white blood cell count and hemoglobin (Hb%) level were assessed by using hematology analyzer FMI- 6180 (Jiangsu, China) by following the standard method as reported.

Serum Chemistry

Toxicity level of *G. glabra* roots in infected chickens was observed by measuring different levels of serum enzymes like aspartate aminotransferase (AST), alanine transferase (ALT), lactate dehydrogenase (LDH), urea, and creatinine. All these procedures were performed by given guideline of Kits producing company (Diagnostic Ltd. UK).

Statistical Analysis

Statistical significance was determined using SAS statistical analysis software utilizing Duncan's multiple range and analysis of variance [33].

Results

The better FCR was observed in groups treated with GGE at different doses but, excellent result was observed at higher dose. Feed conversion ratio of groups treated with GGE at the dose rate of 300 mg/kg of body weight, was comparable to Toltrazuril and Vitamin E *(Table 1)*.

The oocyst score in all treated groups was minimum ($P<0.05$) compared to infected un-medicated (negative) control group. Among GGE treated groups, minimum oocyst score was recorded in chickens treated with GGE at 300 mg/kg of body weight followed in increasing order by groups treated with 200 and 100 mg/kg of body weight. Oocyst score of groups treated with GGE at the dose rate of 300 mg/kg of body weight, Toltrazuril and Vitamin E treated groups was significantly comparable *(Table 2)*.

The fecal score in all treated groups was minimum ($P<0.05$) compared to infected un-medicated (negative control) control group. Among GGE treated groups, minimum fecal score was recorded in chickens treated with GGE at 300 mg/kg of body weight followed in increasing order by groups treated with 200 and 100 mg/kg of body weight. Fecal score of groups treated with GGE at the dose rate of 300 mg/kg of body weight, Toltrazuril and Vitamin E treated groups was almost similar *(Table 3)*.

The lesion score in all treated groups was minimum ($P<0.05$) compared to infected un-medicated control group. Among GGE treated groups, minimum lesion score was recorded in chickens treated with GGE at 300 mg/kg of body weight followed in increasing order by

Table 1. *Feed conversion ratio (FCR) in different treatment groups*

Treated Groups	Feed Consumed	Final Weight	Feed Conversion Ratio
GGE 100 mg/kg	1698.20	651.9	2.60
GGE 200 mg/kg	1730.10	706.5	2.44
GGE 300 mg/kg	1783.7	778.2	2.29
Vitamin E, 87 mg/kg	1764.6	803.25	2.19
Toltrazuril, 1 mL/L	1750.18	805.17	2.17
Infected Group	1679.94	588.5	2.85
Normal Group	1836.71	939.4	1.95

GGE: *Glycyrrhiza glabra extract; * Statistical analysis was not possible because of group feeding of chicken*

Table 2. *Oocyst score (n=6) in different treatment groups*

Treated Groups	Oocyst Score						Mean±SD
	0	+1	+2	+3	+4	+5	
GGE 100 mg/kg	2	2	0	2	-	-	2.01±0.74^{b}
GGE 200 mg/kg	2	1	1	2	-	-	1.4±0.72^{bc}
GGE 300 mg/kg	0	3	2	1	-	-	1.49±0.74^{b}
Vitamin E, 87 mg/kg	1	3	1	1	-	-	1.30±0.75^{c}
Toltrazuril, 1 mL/L	2	1	3	-	-	-	1.16±0.85^{c}
Infected Group	-	-	1	1	2	2	2.73±0.31^{a}
Normal Group	-	-	-	-	-	-	-

GGE: *Glycyrrhiza glabra extract;* ***0:*** *No oocysts;* ***+1:*** *1-10 oocysts per field of microscope;* ***+2:*** *11-20 oocysts per field of microscope;* ***+3:*** *21-50 oocysts per field of microscope;* ***+4:*** *51-100 oocysts per field of microscope;* ***+5:*** *more than 100 oocysts per field of microscope; Means with different letters are significantly different (P<0.05)*

Table 3. *Fecal score (n=6) in different treatment groups*

Treated Groups	4th Day	5th Day	6th Day
GGE 100 mg/kg	1.79±0.74^{b}	2.60±0.54^{b}	0.97±0.13^{b}
GGE 200 mg/kg	1.60±0.56^{b}	2.55±0.52^{b}	0.59±0.51^{b}
GGE 300 mg/kg	1.43±0.52^{bc}	1.63±0.52^{b}	0.52±0.54^{bc}
Vitamin E, 87 mg/kg	1.29±0.52^{bc}	1.31± 0.52^{c}	0.41±0.52^{bc}
Toltrazuril, 1 mL/L	1.23±0.50^{bc}	1.29±0.52^{c}	0.36±0.52^{bc}
Infected Group	3.95±0.41^{a}	3.43±0.50^{a}	2.63±0.62^{a}
Normal Group	0.01±0.0^{a}	0.00±0.0^{e}	0.00±0.0^{e}

GGE: *Glycyrrhiza glabra extract; Means with different letters are significantly different (P<0.05)*

Table 4. *Lesion score (n=6) in different treatment groups*

Treated Groups	Lesion Score					Mean±SD
	0	+1	+2	+3	+4	
GGE 100 mg/kg	-	2	2	2	-	1.54±0.53^{b}
GGE 200 mg/kg	1	2	2	1	-	1.32±0.51^{bc}
GGE 300 mg/kg	2	3	1	-	-	1.33±0.53^{bc}
Vitamin E, 87 mg/kg	2	3	1	-	-	1.15±0.53^{c}
Toltrazuril, 1 mL/L	3	1	2	-	-	1.10±0.21^{c}
Infected Group	-	-	1	1	4	3.79±0.50^{e}
Normal Group	-	-	-	-	-	-

GGE: *Glycyrrhiza glabra extract;* ***0:*** *No gross lesion;* ***+1:*** *Very few;* ***+2:*** *More numerous;* ***+3:*** *Large amount;* ***+4:*** *Blood; Means with different letters are significantly different (P<0.05)*

groups treated with 200 and 100 mg/kg of body weight. Lesion score of groups treated with GGE at the dose rate of 300 mg/kg of body weight, Toltrazuril and Vitamin E treated groups was comparable *(Table 4)*.

The PCV percentages, RBCs and WBCs counts in all treated groups were significantly higher (P<0.05) than infected un-medicated control group. Among GGE treated groups, maximum hematological values were recorded in chickens treated with GGE at 300 mg/kg of body weight followed in decreasing order by groups treated with 200 and 100 mg/kg of body weight. The PCV percentages, RBCs and WBCs counts of groups treated with GGE at the dose rate of 300 mg/kg of body weight, Toltrazuril and Vitamin E treated groups were comparable *(Table 5)*.

These serum enzyme (ALT, LDH, Urea and Creatinine) values in all treated groups were minimum (P<0.05) compared to infected un-medicated control group. Among GGE treated groups, minimum serum enzyme values were recorded in chickens treated with GGE at 300 mg/kg of body weight followed in increasing order by groups

Table 5. Hematological Values in different treatment groups

Treated Groups	PCV%	Hb g/dL	RBC10^6/µL	WBC 10^3/µL
GGE 100 mg/kg	22.61±1.68^b	11.99±1.06^b	2.99±0.76^b	19.82±2.84^c
GGE 200 mg/kg	24.11±1.41^b	12.30±1.44^b	3.13±0.86^b	22.51±2.61^{bc}
GGE 300 mg/kg	25.50±1.76^a	13.22±1.28^a	3.96±0.71^a	23.56±1.53^b
Vitamin E, 87 mg/kg	26.00±2.21^a	12.10±0.73^a	4.10±0.71^a	24.89±2.78^b
Toltrazuril,1 mL/L	27.12±2.23^a	12.88±0.64^a	4.28±0.71^a	25.51±2.79^b
Infected Group	19.15±1.15^b	9.45±0.82^c	1.95±0.21^c	15.00±5.04^d
Normal Group	29.32±1.03^a	13.39±1.23^a	4.89±0.58^a	25.51±3.26^a

***GGE:** Glycyrrhiza glabra extract; **PCV:** Packed cell volume; **Hb:** Hemoglobin level; **RCB:** Red Blood Cell; **WBC:** White Blood Cell; Means with different letters are significantly different (P<0.05)*

Table 6. Serum Enzyme Values in different treatment groups

Treated Groups	ALT	LDH	Urea	Creatinine
GGE 100 mg/kg	12.91±0.92^a	549.01±17.82^b	12.90±0.81^b	0.33±0.04^b
GGE 200 mg/kg	11.88±0.97^b	519.93±22.43^b	11.99±0.95^b	0.21±0.03^b
GGE 300 mg/kg	11.70±1.27^b	493.21±21.12^b	8.32±1.02^b	0.15±0.03^c
Vitamin E, 87 mg/kg	10.90±1.27^b	512.23±20.22^b	9.20±1.02^c	0.17±0.03^c
Toltrazuril, 1 mL/L	11.21±1.13^b	495.43±20.13^b	7.30±0.48^c	0.15±0.03^c
Infected Group	24.72±2.32^a	865.94±23.16^a	20.18±1.12^a	0.45±0.05^a
Normal Group	8.90±1.86^c	475.43±16.67^c	5.29±0.48^c	0.11±0.02^d

***GGE:** Glycyrrhiza glabra extract; **ALT:** Alanine transaminase; **LDH:** Lactate dehydrogenase; Means with different letters are significantly different (P<0.05)*

treated with 200 and 100 mg/kg of body weight. Serum enzyme values of groups treated with GGE at the dose rate of 300 mg/kg of bodyweight, Toltrazuril and Vitamin E treated groups were significantly comparable *(Table 6)*.

Discussion

Botanicals high in antioxidant chemicals such as phenols, flavonoids, tannins, and saponins are being employed as an alternate technique to treat coccidiosis [25-27]. The current study found that GGE has anticoccidial potential in terms of improved feed conversion ratio, reduced oocyst, lesion scores, and fecal score in a dose-dependent manner. The Toltrazuril and Vitamin E treated groups had similar results (P>0.05) on these metrics. Previous studies evaluating the anticoccidial potential of several plant extracts have also revealed similar dose-dependent results [34-36].

The beneficial effect of GGE on these parameters could be attributed to the antioxidant chemicals found in this plant, which may help to alleviate the oxidative stress caused by coccidiosis. It is also suggested that supplementation of herbs mixture at the dose 2 mL/L to the coccidiosis challenged broiler chickens overall improved the health and immunity by regulating the mRNA expression of immunity-related toll-like [37]. Furthermore, plant extract inhibited the development of the *Eimeria* life cycle in the host cell before oocysts were discharged in chicken faces, resulting in lower *Eimeria* oocyst excretion and infection severity [38].

Glycyrrhiza glabra is a famous plant with peeled or unpeeled roots and stolon that is generally known as licorice (English) and mulethi (Hindi) is enriched with olatile oil, amino acids, amines (glucose and sucrose 5-15 percent sugars), starch, saponins and flavonoids, tannins. It has a long history of use as a demulcent, expectorant, anti-allergy, anti-inflammatory, spasmolytic, moderate laxative, anti-stress, anti-depressive, antiulcer, liver protector, estrogenic, emmenagogue, and antidiabetic. Bronchitis, dry cough, respiratory infections, catarrh, tuberculosis, genitourinary illnesses, urinary tract infections, abdominal pain, gastric and duodenal ulcers, stomach inflammation, and mouth ulcers are all treated with it. Licorice has been shown in recent investigations to have steroid-like properties. Eczema, peptic ulcers, duodenal and stomach ulcers, and dental plaque have all been found to benefit from it [39]. The ancient civilizations of India, Rome, Greece, Egypt, and China all used this plant in their pharmacopoeia. Glycyrrhizin, glabranin A&B, glycyrrhetol, glabrolide, isoglabrolide, glabridin, formononetin, glabrone, neoliquiritin, hispaglabridin A&B, herianin, umbelliferone, onocerin, p-amyrin, stigmasterol are the primary bioactive elements of *G. glabra* [40]. The substance "glycyrrhizin," produced from *Glycyrrhiza glabra*, and its derivatives have anti-oxidant and anti-inflammatory properties. CD4+ T-cell

and tumour necrosis factor-mediated cytotoxicity are both inhibited by glycyrrhizin. Glycyrrhizin acts as a membrane stabilizer [41].

All results point to the possibility of testing its immunomodulatory and anticoccidial properties. The anticoccidial and liver protective properties of a methanolic extract of *Azadirachta indica* and *Carica papaya* leaf extract against *Eimeria* infection in mice and chicken were investigated in another study. Infected mice and chicken were given a methanolic extract showed an anticoccidial effect and stabilized blood enzyme values at different dose rates (ALT, AST), indicating hepatoprotective properties [42]. Infected chickens were given aqueous extracts of *A. indica* and *Khaya senegalensis* at 400 mg/kg, which had a favorable effect on blood enzyme levels [24]. All of this research imply that plant-derived extracts may be less hazardous when used to treat coccidiosis in chickens.

The anticoccidial effects of *G. glabra* aqueous methanolic extract in broiler chickens were discovered in this study. *G. glabra* showed anticoccidial effectiveness against coccidiosis in a dose-dependent manner. It suggests that extracts obtained from *G. glabra* may be less harmful in managing coccidiosis in chickens. Characterizing the active components of *G. glabra* that are involved in increasing the anticoccidial potential against avian coccidiosis will require more research.

Availability of Data and Materials

Research and Supporting data will be available from the author (Kashif Hussain) on request.

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Competing Interest

The author declared that there is no conflict of interest.

Ethical Statement

This research was conducted with approval from ethical committee of University of Agriculture Faisalabad (Approval No: 144) Faisalabad, Pakistan.

Author Contributions

KH and AFA conceived and designed the experiments; AA and RZA analyzed the data and drafted the manuscript; AR and WZ performed experiments and acquired data. TUR and SM search the data and help in *Eimeria* identification. All authors read and approved the final manuscript.

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