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Original Article

Anticoccidial and Biochemical Effects of Artemisia Brevifolia Extract in Broiler Chickens

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ABSTRACT

This study was conducted to evaluate the anticoccidial potential of *Artemisia brevifolia* extract (ABE) and its effect on biochemical parameters against experimental coccidiosis in broiler chickens. For this purpose, a total of 150 broiler chicks were procured and divided into 5 equal groups (30 birds per group). At one week of age, the first three groups (A, B and C) were orally fed with ABE at dose rates of 100, 200 and 300mg/kg respectively for seven consecutive days. Group D served as infected/medicated and was treated with Baycox® (1ml/L of water). Group E served as the infected/non-medicated control group. At two weeks of age, all groups were infected with 50,000 sporulated oocysts of *Eimeria tenella*. The anticoccidial potential was evaluated based on lesion score, oocyst score, fecal score, feed conversion ratio, and mortality (%). Furthermore, the effect of ABE on hematological parameters and serum chemistry was also evaluated by following standard procedures. The anticoccidial effects were compared with the standard drug Baycox® ($p>0.05$). Results of the study showed the anticoccidial activity of ABE in terms of lesion diminishing, oocyst and fecal scores, better feed conversion ratios, and mortality reduction (%). The ABE also improved hematological parameters and stabilized the serum enzymes (ALT, LDH, Creatinine) of infected chicks ($p>0.05$).

INTRODUCTION

Avian coccidiosis is caused by protozoa of genus *Eimeria*, which belongs to family *Eimeriidae* and has multiple species with complex life cycle stages (Abbas *et al.*, 2015, 2019; Khater *et al.,* 2020). *Eimeria* species mostly affect bird intestines and cause bloody diarrhea, poor feed conversion ratios, and high mortality. Among all *Eimeria* species, *E. tenella* and *E. necatrix* are the most pathogenic, causing high mortality in chickens. Naturally, coccidian oocysts are present everywhere and infection starts with the bird's ingestion of sporulated oocysts, which then produce millions of oocysts in the host's body (Fall *et al.,* 2016; Lin *et al.,* 2020; Zhang *et al.,* 2020). Coccidiosis causes heavy economic losses to the world's poultry industry annually (Chapman, 2014; Abbas *et al.,* 2017a). Synthetic anticoccidial drugs are generally used to control coccidiosis, including a variety of anticoccidial drugs. However, due to the irrational use of anticoccidial drugs, resistance has been developed and these drugs are losing their effectiveness (Abbas *et al.,* 2008, 2011a; Bachaya *et al.,* 2015; Abbas *et al.*, 2019). It takes time to find some alternative agents for coccidiosis control. In the past decade, several alternative approaches including use of plants and their products have been reported for their anticoccidial potential (Abbas *et al.,* 2011a, 2011b, 2011c, 2017b; Idris *et al.*, 2017; Sun *et al.,* 2019; Ayatollahi *et al.,* 2019; Tammam *et al.,* 2020). Because of frequent reports on parasites' resistance against chemical drugs (Saddiqi *et al.*, 20006; Abbas *et al.*, 2008, 2011a), the experimental use of plant extracts especially rich in antioxidants as antiparasitic agents has gained special importance (Abbas *et al.*, 2018, 2020; Ashraf *et al.,* 2020; Asrar *et al.,* 2020; Khater *et al.*, 2020; Aboubakr *et al.,* 2019; Fayyaz *et al.*, 2019; Elghobashy *et al.*, 2020; Gohar *et al.,* 2020; Hassan *et al.*, 2020; Salman *et al.*, 2020; Yasmin *et al.,* 2020; Zaman *et al.*, 2020). Antioxidant (phenols, flavonoids, tannins, and saponins) rich plants (Khaskheli *et al.,* 2020; Mahmoud *et al.,* 2020; Qamar *et al.,* 2020) are being used as an alternative strategy to treat coccidiosis (Abbas *et al.*, 2017b; Batool *et al.,* 2019; Yi *et al.,* 2020).

Artemisia brevifolia, known as worm wood in English and as Afsanteen locally, bears diverse pharmacological and therapeutic properties against different parasitic and bacterial diseases, due to the action of various antioxidant compounds (Allen *et al.,*1997; Zeb *et al.,* 2018). Based on the diverse therapeutic properties of *Artemisia brevifolia*, the present study was planned to assess the anticoccidial activity of its extract against *Eimeria* infection in broiler chickens.

MATERIALS AND METHODS

Plant Material

Artemisia brevifolia (afsanteen) leaves were procured from a local market. The plant material was dried out under a covered place and then ground into powder using an electric mill. The aqueous methanolic extract of *Artemisia brevifolia* was prepared using Soxhlet's apparatus (Velp Italy) (Abbas *et al.*, 2017b). The prepared *Artemisia brevifolia* extract was stored at 4°C in a refrigerator until further use.

Parasite

The *Eimeria* parasite was collected from outbreak cases in Faisalabad and guts of naturally infected chicken with *Eimeria*. Oocysts of *E. tenella* were collected from caeca of chickens obtained from coccidiosis outbreaks in Faisalabad, Pakistan. The sporulation of oocysts was conducted in a potassium dichromate solution (2.5%) at 25-29℃ with 60-80% humidity level (Ryley *et al.,* 1976). The sporulated oocysts were counted using the modified McMaster technique. The slides were examined under microscope at low (10x) and high magnification (40x).

Experimental Design

The experiment was approved by the Board of studies committee members, Department of Parasitology, University of Agriculture, Faisalabad, Pakistan. A total of 150 broiler chicks were purchased and divided into 5 equal groups (30 birds per group). At one week of age, groups (A, B and C) were orally fed with *Artemisia brevifolia* extract at 100, 200, and 300 mg/kg respectively for seven consecutive days. All doses of plant extract were dissolved in PBS and fed orally using a soft plastic tube attached to a 0.5 ml sterile syringe. Group D served as infected/medicated, being treated with Baycox® added to the drinking water (1 ml/L of water). Group E served as the infected control group. At the same day, all groups were orally infected with 50,000 sporulated oocysts of *Eimeria tenella*.

Evaluation Of Anticoccidial Activity

The anticoccidial potential of *Artemisia brevifolia* extract (ABE) was evaluated on the basis of parameters such as mortality (%), which was calculated till the end of experiment, with death cause being confirmed by postmortem analysis. Feed Conversion rate (FCR) was evaluated on the 30th day of the experiment (Abbas *et al.,* 2019), while lesion scores were recorded on the 14th (Johnson & Reid, 1970). An oocyst score ranging from 0 to 5 was also recorded on the 14th day of trial, calculated through a microscopic examination of caecal scrapings (Hilbrich, 1978). A fecal score (1 to 5) was recorded between the 4th and 6th day after the infection (Youn *et al.,*1993). Score of 1 indicated normal feces and score of 5 indicated the presence of severe diarrhea or profuse blood. These parameters were monitored in order to estimate the intensity of disease.

Serum Chemistry

Serum samples were analyzed using different imported kits (Merck, Germany) for serum enzymes levels (ALT, LDH, Creatinine) to check the toxic effects of plant extract and cellular injuries, if any (Abbas *et al.,* 2019).

Hematological Parameters

Blood and serum samples were collected at the 40th day of experiment. Collected blood samples were analyzed for Packed cell volume (PCV) by the microhaematocrit method. Hemoglobin level (Hb) was checked using Sahli's apparatus (Benjamin, 1978). Erythrocyte (RBCs) and leukocyte (WBCs) counting was done using an haemocytometer (Natt & Herrick, 1952).

Statistical Analysis

Statistical analysis was done through the ANOVA technique using the SAS software (SAS, 2004). The data was considered statistically significant with *p*-value <0.05.

RESULTS AND DISCUSSION

All the groups administered with *Artemisia brevifolia* extract (ABE) exhibited better feed conversion ratio (FCR) at graded doses (Figure 1). The best FCR was observed in the group treated with the highest dose of plant extract. There was no statistical analysis for FCR due to the group feeding of chicks. A lower mortality (%) was observed in ABE administered groups and the lowest mortality was observed in the groups treated with the highest dose of ABE (Figure 2). Lower lesion scores were observed in ABE treated groups (Table 1). Groups treated with ABE showed reduced lesion scores, with no significant difference to the Baycox® treated group ($p>0.05$) and significantly different to the infected/non-medicated group ($p<0.05$).

All ABE administered groups showed reduced oocyst scores in infected chicks. However, the oocyst scores of groups treated with a higher dose of ABE was non-significantly different ($p>0.05$) from Baycox® (Table 2). The fecal score of ABE treated groups was significantly different to those treated with standard medicine (Baycox®) (Table 3). However, the best results were obtained in the group administered with the highest dose of ABE. *Artemisia brevifolia* extract showed improved serum chemistry in infected birds (Table 4). At a higher dose of ABE, the serum enzyme values were non-significantly different ($p>0.05$) from the Baycox® group.

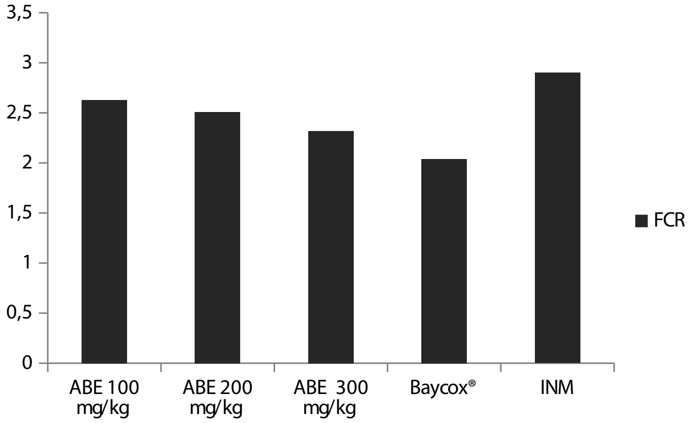

Figure 1 – Feed conversion ratios (FCR) of chickens infected with *Eimeria tenella* and treated with different doses of *Artemisia brevifolia* extract.

ABE: Artemisia brevifolia extract.

INM: Infected/non-medicated *Because of group feeding, statistical analysis was not achievable.

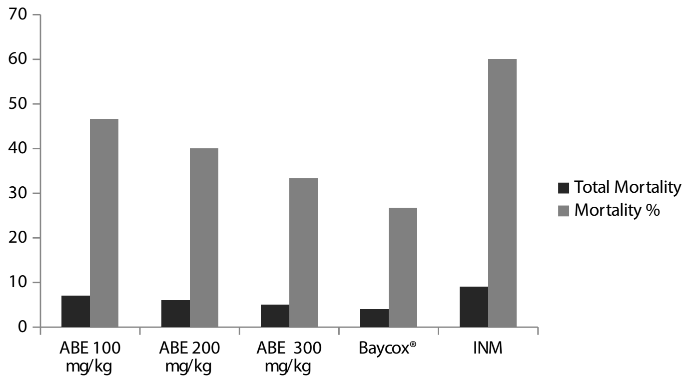

Figure 2 – Mortality rates (%) of chickens infected with *Eimeria tenella* and treated with different doses of *Artemisia brevifolia* extract.

ABE: Artemisia brevifolia extract; INM: Infected/non-medicated.

Table 1 – Lesion scores of chickens infected with *Eimeria tenella* and treated with different doses of *Artemisia brevifolia* extract.

Groups	0	+1	+2	+3	+4	Mean±SD
ABE@ 100 mg/kg	-	1	2	2	1	2.50±0.5b
ABE @ 200 mg/kg	–	1	2	3	–	2.33±0.5b
ABE @ 300 mg/kg	–	2	2	2	–	2.00±0.5c
Baycox®	–	3	2	1	–	1.66±0.5c
Infected/non-medicated	–	–	1	2	3	3.33 ±0.5a

ABE = Artemisia brevifolia extract

Table 2 – Oocyst scores of chickens infected with *Eimeria tenella* and treated with different doses of *Artemisia brevifolia* extract.

Groups	0	+1	+2	+3	+4	+5	Mean±SD
ABE@ 100 mg/kg			1	2	2	1	3.5 ± 0.5[b]
ABE @ 200 mg/kg		1	1	2	2		2.8 ± 0.75[b]
ABE @ 300 mg/kg	1	1	3	1			1.6 ± 0.5[c]
Baycox®	2	2	2				1.00 ± 0.5[c]
Infected/non-medicated				2	2	2	4.00 ± 0.50[a]

ABE = Artemisia brevifolia extract

Table 3 – Fecal scores of chickens infected with *Eimeria tenella* and treated with different doses of *Artemisia brevifolia* extract.

Groups	4th day	5th day	6th day
ABE @ 100 mg/kg	3.21±0.8[b]	3.30±0.7[a]	2.89±0.6[b]
ABE @ 200 mg/kg	3.15±0.5[b]	3.22±0.7[a]	1.90±0.5[bc]
ABE @ 300 mg/kg	3.00±0.5[bc]	3.08±0.75[bc]	1.81±0.6[c]
Baycox®	1.71±0.8[c]	1.66±0.5[c]	1.37±0.5[c]
Infected/non-medicated	3.80±0.44a	3.89±0.5[a]	3.00±0.88[a]

ABE = Artemisia brevifolia extract.

Table 4 – Serum enzyme values of chickens infected with *Eimeria tenella* and treated with different doses of *Artemisia brevifolia* extract.

Groups	ALT	LDH	Creatinine
ABE@ 100 mg/kg	14.90 ± 1.23[b]	575.76 ±16.74[b]	0.36 ±0.03[a]
ABE @ 200 mg/kg	12.90 ± 1.71[b]	556.91±18.30[b]	0.28 ±0.04[b]
ABE @ 300 mg/kg	12.80 ±1.14[c]	533.03 ±24.35[c]	0.26 ±0.03[c]
Baycox®	11.50 ±1.11[c]	518.50 ±20.13[d]	0.17 ±0.01[c]
Infected/non-medicated	25.61 ± 2.32[a]	889.94 ±22.16[a]	0.54 ±0.03[a]

ABE = *Artemisia brevifolia* extract; ALT = Alanine transaminase; LDH = Lactate dehydrogenase.

ABE also improved the blood parameters of the infected groups (Table 5). Hematological values (including PCV, RBCs, WBCs, and Hb) were significantly different from the infected/non-medicated group (p<0.05), and were non-significantly different from Baycox®(p>0.05).

Previous and recent research reports have shown that plants rich in antioxidant compounds (phenols, flavonoids, tannins, and saponins) are being used as alternative agents to treat avian coccidiosis and have shown promising anticoccidial effects (Allen *et al.,*1997; Jang *et al.,* 2007; Molan *et al.,*2009; Abbas *et al.,* 2020). Based on the results obtained from the current research, it may be concluded that *Artemisia brevifolia* extract has anticoccidial activity in terms of different parameters, including lesion score, fecal score, mortality (%), and FCR. The ABE also improved the hematology and serum chemistry of infected chicks. Results were non-significantly different to standard medicine (Baycox®) (p>0.05). The results of this study revealed that *Artemisia brevifolia* at 100, 200, 300 mg/kg showed dose-dependent anticoccidial activity against *Eimeria tenella* infection in broiler chickens. Similar types of dose dependent trends have also been reported in previous studies on the evaluation of anticoccidial potential of different herbal extracts (Dkhil *et al.,* 2011; Yang *et al.,*2015; Wang *et al.*, 2016; Abbas *et al.,* 2019, 2020). In one study, ethanolic extract from *Carica papaya* showed remarkable effect on the weight gain in broiler chickens infected with *Eimeria* (Nghonjuyi *et al.,*2015). In another study, oral administration of *Ageratum conyzoides* extract showed a positive effect in reducing lesion score and oocyst count, while also improving hematological parameters in challenged treated birds (Nwezeand & Obiwulu, 2009).

Recently, Zhang *et al.* (2020) have reported a similar type of anticoccidial effects with the use of *Camellia sinesis* (green tea) extract in broiler chickens. *Camellia sinensis* extract reduced the *Eimeria* infection by reducing mortality (%), oocysts count, and lesion score in experimentally infected broiler chickens, with effects that were non-significantly different to those of the standard anticoccidial drug Toltrazuril® (p>0.05).

In another recent study, the anticoccidial effects of *Trachyspermum ammi* (Ajwain) have been observed

Table 5 – Hematological values of chickens infected with *Eimeria tenella* and treated with different doses of *Artemisia brevifolia* extract.

Groups	PCV%	Hb g/dl	RBC10^6/μL	WBC 10^3/μL
ABE@ 100 mg/kg	15.33±1.63[c]	9.58±1.35[c]	2.88±0.48[b]	20.01±3.54[b]
ABE @ 200 mg/kg	17.20±1.47[c]	10.44±0.59[c]	3.13±0.61[a]	19.86±2.31[b]
ABE @ 300 mg/kg	18.19±2.31[b]	10.90±1.01[a]	3.20±0.48[a]	21.16±2.94[b]
Baycox®	24.23±1.75[b]	12.00±0.11[a]	3.05±0.65[a]	23.77±1.86[a]
Infected/non-medicated	19.15±1.47[c]	8.20±0.55[d]	2.00±0.24[c]	16.85±3.98[c]

ABE = Artemisia brevifolia extract.

in broiler chickens: *Trachyspermum ammi* was supplemented in the feed of chickens, which reduced lesion and oocyst scores of infected groups (Abbas *et al.,* 2019).

Artemisia brevifolia is enriched with various antioxidant compounds (phenols, flavonoids, conyzorium, methexnebilitin, and quercetin), essential oils and some other important derivatives, such as artemisinin, which are commonly used to cure lethal diseases such as malaria and cancer (Zeb *et al.,* 2018).

The anticoccidial potential of *Artemisia brevifolia* based on different parameters may be due to the action of different antioxidant compounds present in the plant, which can decrease coccidiosis by interfering with the life cycle of *Eimeria.* During this study, the stabilization of different serum enzyme values in plant administered groups indicates that the extract has no toxic effect. ALT and LDH are refer to hepatotoxicity, while creatinine is refers to nephrotoxicity. *Artemisia brevifolia* extract also improved hematological parameters (PCV, RBCs, WBCs and Hb) in infected chicks. Plants rich in antioxidants have gained special experimental importance as antiparasitic agents. Based on the aforementioned studies, it may be concluded that plants-derived extracts like that of *Artemisia brevifolia* may be helpful in controlling avian coccidiosis.

CONCLUSION

The present study showed the anticoccidial effects of *Artemisia brevifolia* extract in broiler chickens and showed it to be dose-dependent. It suggests that *Artemisia brevifolia* derived antioxidant compounds may be helpful in controlling avian coccidiosis and treating chickens' internal injuries. However, further studies are needed to characterize the active novel compounds of *Artemisia brevifolia* that are involved in enhancing the anticoccidial potential against avian coccidiosis.

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