Anticoccidial evaluation of a traditional chinese medicine—*Brucea javanica*—in broilers

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ABSTRACT The traditional Chinese medicinal plant *Brucea javanica* has received much attention for its significant antiprotozoal effects in recent years; however, little is known about its potential anticoccidial functions. In the present study, a series of experiments was conducted to investigate the prophylactic and therapeutic effects of ethanol extract from *B. javanica* on coccidiosis induced by *Eimeria tenella* in broiler chickens. Chickens infected with *E. tenella* were treated with *B. javanica* extract and compared either with broilers treated with the anticoccidial halofuginone hydrobromide (Stenorol) or with control groups that consisted of infected-unmedicated and uninfected-unmedicated broilers. The experiments revealed that the *B. javanica* extract could significantly ($P < 0.05$) reduce bloody diarrhea and lesion scores. Additional, OPG output in these plant extract treated groups was reduced in comparison with non-treated groups ($P < 0.05$). However, there was no evidence to show that the extract could promote BWG. Histological data showed that the number of second-generation schizonts in the medicated groups was substantially less than that in the infected-unmedicated control. In summary, our work showed that *B. javanica* extract exerted considerable anticoccidial effects, supporting its use as a promising therapeutic in controlling avian coccidiosis.

Key words: *Brucea javanica*, ethanol extract, anticoccidial effect, broiler chicken, *Eimeria tenella*

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INTRODUCTION

Coccidiosis, caused by the parasites of the genus *Eimeria* (phylum Apicomplexa), is one of the most severe poultry diseases worldwide. Coccidiosis causes large economic losses up to $3 billion dollars worldwide and about $30-60 million dollars in China every year (Dalloul and Lillehoj, 2006; Hao et al., 2007; Michels et al., 2011). The high mortality and morbidity in chicken, the accompanied expenditure on medication, and the subsequent poor performance of surviving birds all contribute to the financial burden.

In recent years, a number of phytogenic products (pure compound or mixed extract) has been reported to be effective candidates in protecting chickens from coccidian damage. De Pablos et al. (2010) found that maslinic acid (purified from the oil of *Olea europaea* L.) significantly decreased the infection rate at 120 h post-infection. Kim et al. (2013) reported that dietary supplementation with *Curcuma longa* could enhance coccidiosis resistance by increased BWG, reduced fecal oocyst shedding, and decreased gut lesions. Tanweer et al. (2014) reported that the methanolic extract of wild rue (*Peganumhar mala* L.) increased BW, while reducing cecal lesion and leucocyte infiltration in broiler chickens. Moreover, a natural product, betaine, also was found to reduce the impact of coccidian challenge (Augustine et al., 1997; Amerah and Ravindran, 2015). Previously, our research group also confirmed that *Dichroa febrifuga* extract could significantly reduce bloody diarrhea and intestinal lesion caused by *E. tenella* infection (Zhang et al., 2012). Therefore, using plant products offers an alternative route to improve the performance of the clinical and subclinical infected birds.

Brucea javanica (L.) Merr (Simaroubaceae), a traditional Chinese medicinal plant, is distributed widely throughout the tropical and subtropical zones of Asia. The ripe seeds of this plant, named Ya-dan-zi, are included in the Chinese Pharmacopoeia for the treatment of dysentery, malaria, warts, and corns. The oil extracted from *B. javanica* has been used as a commercially available anti-tumor drug in China for years. Extensive work has shown that *B. javanica* possesses a variety of biological activities. During the 1980s, the methanol extract of *B. javanica* was found to exert potent anti-malarial activities against chloroquine-resistant *Plasmodium falciparum* (O'neill et al., 1985; Pavanand et al., 1986; O'Neill et al., 1987). Further studies showed that quassinoids as the characteristic

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metabolites of *B. javanica* acted effectively against several kinds of parasites, including *Entamoeba histolytica*, *Giardia intestinalis*, *Toxoplasma gondii*, and *Ttrypanosoma* (Wright et al., 1993; Hout et al., 2006). Additionally, Hall et al. (1983) found that brusatol and other related quassinoids could reduce the release of hydrolytic enzymes and stabilize the lysosomal membranes leading to an anti-inflammatory effect.

The above data support *B. javanica* as being a promising candidate to treat coccidiosis. In the present study, different levels of ethanol extracts of *B. javanica* were evaluated for coccidiosis treatment in broilers.

MATERIALS AND METHODS

Animals and Parasites

For each of the 3 experiments, a batch of one-day-old male broiler chickens (Mei-Ling) was purchased from a local company (Zhejiang GuangDa ZhongQin CO. LTD., Hangzhou, China) and reared in a controlled coccidian-free house until use. The birds were kept in clean stainless-steel wire cages and were offered a basal diet based on corn (64.60%), rice polishing (3.00%), meat meal (5.00%), soybean meal (23.70%), soybean oil (1.64%), DL-methionine (0.25%), L-lysine (0.08%), salt (0.35%), limestone powder (1.13%), dibasic calcium phosphate (0.15%), and vitamin-mineral mixture (0.10%). The diet provided approximately 3,100 kcal/kg metabolizable energy, 20% crude protein, 0.92% calcium, 0.41% available phosphorus, 0.21% sodium, 0.59% methionine, 1.15% lysine, 0.83% threonine, and 0.26% tryptophan. Feed and water were available on an ad libitum basis. Heating was supplied by electric heater bulbs and temperature was maintained at 28 to 32°C by air conditioning. All procedures performed in the studies involving animals were in accordance with the ethical standards of the Institutional Animal Care and Use Committee (IACUC) at Zhejiang University.

The *E. tenella* (Shanghai strain) oocysts used were kindly provided by Dr. Bing Huang, Shanghai Veterinary Research Institute, China Academy of Agricultural Sciences. The oocysts were propagated in two-week-old coccidian-free chickens before use. The unsporulated oocysts collected from the cecal content on the seventh day post-infection was purified and preserved in 2.5% potassium dichromate solution to induce sporulation at 28°C. The sporulated oocysts were kept at 4°C until use.

Preparation of B. javanica Premix

The mature seeds of *B. javanica* used in this study were purchased from Beijing TongRenTang Group CO. LTD. (Beijing, China). A dry powdered sample (500 g) was extracted with petroleum ether (2,000 mL, 3 times) in a traditional refluxing apparatus for 3 h. Filtrates were removed to get rid of the oil constituents. Residues were then further extracted with ethanol (2,000 mL, 3 times). The ethanol filtrates were combined and evaporated under reduced pressure at 55°C to obtain a crude de-oiled *B. javanica* extract. Finally, the *B. javanica* premix was prepared with the addition of cornstarch until a total of 500 g was achieved.

Experimental design

Experiment 1 In this preliminary experiment, 50 chicks having the same weight and size were selected and weighed (recorded as initial BW before parasitic challenge). The birds were randomly assigned to 5 groups (10 birds per group). Chickens in groups 1, 2, and 3 were infected and treated with *B. javanica* extract equivalent to 2.5, 5 g raw herbs per kilogram feed or 3 mg halofuginone hydrobromide (Zhejiang Esigma Animal Health CO.LTD, Hangzhou, China) per kilogram feed, respectively. Groups 4 and 5 served as infected-unmedicated and uninfected-unmedicated controls, respectively. All the birds were orally infected with one mL *E. tenella* suspension containing 25,000 sporulated oocysts except those in group 5.

Experiment 2 In order to study the anticoccidial effects of a higher dose, the birds were randomly assigned to 6 groups (10 birds per group). Chickens in groups 1, 2, 3, and 4 were infected and treated with *B. javanica* extract equivalent to 2.5, 5, 10 g raw herbs per kilogram feed or 3 mg halofuginone hydrobromide per kilogram feed, respectively. Groups 5 and 6 served as infected-unmedicated and uninfected-unmedicated controls, respectively. All the birds were orally infected with 26,000 sporulated oocysts except those in group 6.

Experiment 3 Based on the results of the previous 2 experiments, experiment 3 increased the number of experimental animals. Two hundred and forty 12-day-old chicks were randomly assigned to 6 groups (groups 1 to 6). Each group was subdivided into 2 subgroups (cage A and cage B) with 20 birds per subgroup. Birds in groups 1, 2, and 3 were provided with a gradual increase of *B. javanica* premix (equivalent to 2.5 g, 5 g, or 10 g raw herbs per kilogram feed, respectively) 2 d before challenge. Group 4 was treated with 3 mg halofuginone hydrobromide per kilogram feed on the d challenged with *E. tenella*, while group 5 and group 6 served as infected-unmedicated and uninfected-unmedicated control, respectively. At 2 wk, except for those in group 6, all the birds were orally infected with one mL *E. tenella* suspension containing 28,000 sporulated oocysts. Clinical signs and mortality were examined and recorded each d post infection.

Evaluation of Anticoccidial Effects

The anticoccidial efficacy of *B. javanica* extract was evaluated by bloody diarrhea, oocysts per gram of feces, BWG, lesion scores, and survival rate. The anticoccidial index was calculated according to Ma

et al. (2011) using the following formula:

$$\text{ACI} = (\text{relative ratio of BW gain} + \text{survival rate}) - (\text{lesion scores} + \text{oocyst value})$$

An ACI value above 180 was determined as an excellent anticoccidial effect, with 180 to 160 as marked, 160 to 140 as moderate, 140 to 120 as slight, and below 120 as inactive (Morisawa et al., 1977).

On the fourth to seventh d post infection, the chickens were examined for bloody diarrhea by counting bloody feces 2 times a d. The extent of bloody diarrhea was assigned to one of 5 degrees (from 0 to 4) according to the mean pieces of bloody feces in each group. Briefly, 0 represents normal feces; 1 stands for one piece; 2, 2 pieces; 3, 3 pieces; and 4, 4 or more pieces of bloody feces, respectively (Morehouse and Baron, 1970). The mean pieces of bloody feces are rounded to the nearest integer.

All the feces of each group were collected from d 4 to 7 post infection. The oocysts were counted using a hemocytometer and the method was simplified according to previously described (Holdsworth et al., 2004). Briefly, one g of sample from each well of mixed feces was dispersed in 10 mL water. The number of oocysts in 10 μl suspension was counted under an optical microscope. The results were expressed as OPG output. The oocyst value for ACI was calculated according to Rose and Mocket (1983) and Du and Hu (2004) using the following formula: Oocyst value = (OPG output of each group) / (OPG output of the infected-unmedicated control) × 100.

The survival rate was recorded as (number of survival birds) / (initial number of birds) ×100. At the end of the experiment (d 7), all the birds in experiments 1 and 2 and those in cage A of experiment 3 were sacrificed and weighed to obtain the final BW. The BWG was recorded as the final BW minus the initial BW. The relative ratio BWG was calculated as (average BW gain in each group) / (average weight gain of uninfected-unmedicated group) × 100.

The cecum of each bird was removed and examined. Lesion scores were marked from 0 to 4 depending on the severity of the gut. Zero represents normal status and 4 corresponds to both the most severe and dead chickens. In experiment 3, the length of the cecums was also measured and recorded as mean ± SEM cm.

Tissue Section Preparation

In experiment 3, 2 birds in cage B were sacrificed by cervical dislocation each d from d one to d 7. The cecal tissue samples were collected and fixed in 10% neutral formalin solution (4°C). The samples were then embedded in paraffin, sectioned, and stained with hematoxylin and eosin according to routine protocol. The physiological and pathological features of cecal tissues were observed and photographed using an Olympus IX71 microscope and an Olympus DP72 camera with Olympus DP2-BSW software.

Statistical Analysis

All the data were statistically analyzed by using the software SPSS 19.0 on Windows XP operating system. BWG and OPG were carried out using one-way analysis of variance and were expressed as mean ± SD or mean ± SEM. The bloody diarrhea score and lesion score of each group were compared by the nonparametric Kruskal-Wallis H test followed by Dunn's multiple comparison test for statistical differences; the results were presented as median. Comparison with P-values equal or lower than 0.05 were considered significantly different.

RESULTS

Experiment 1

Initial experiments with low dosages (2.5, 5 g raw herbs per kilogram feed) of *B. javanica* exhibits a certain anticoccidial effect. Administration of the *B. javanica* extract to the infected chickens was shown to be associated with significant ($P < 0.05$) milder bloody diarrhoea (Figure 1A). The groups medicated with *B. javanica* extract showed significantly ($P < 0.05$) lower lesion scores than the infected-unmedicated control (Figure 1C). However, as shown in Figure 1B, the extract did not increase the BWG effectively. Additionally, the ACI value was only 131, corresponding to a slight anticoccidial effect (Figure 1D).

Experiment 2

A higher dose of *B. javanica* extract was introduced in experiment 2. The results were similar to experiment 1, which showed reduced bloody diarrhoea and lower lesion scores in medicated groups (Figure 2A and C). The BW of all 3 medicated groups did not increase significantly compared to the infected-unmedicated control (Figure 2B). The ACI value of 146 was obtained, which was determined as a moderate anticoccidial effect (Figure 2D). The results confirmed that supplementation of feed with *B. javanica* extract can reduce the impact of coccidian infection.

Experiment 3

Bloody Diarrhea All the infected birds showed clinical signs of coccidiosis such as huddling together, ruffled feathers, and depression. Mortality was absent in all groups except for 2 chickens in cage B of the infected unmedicated control (group 5). Bloody diarrhea was observed from the fourth to seventh d after *E. tenella* challenge in groups 1, 2, 3, and 5

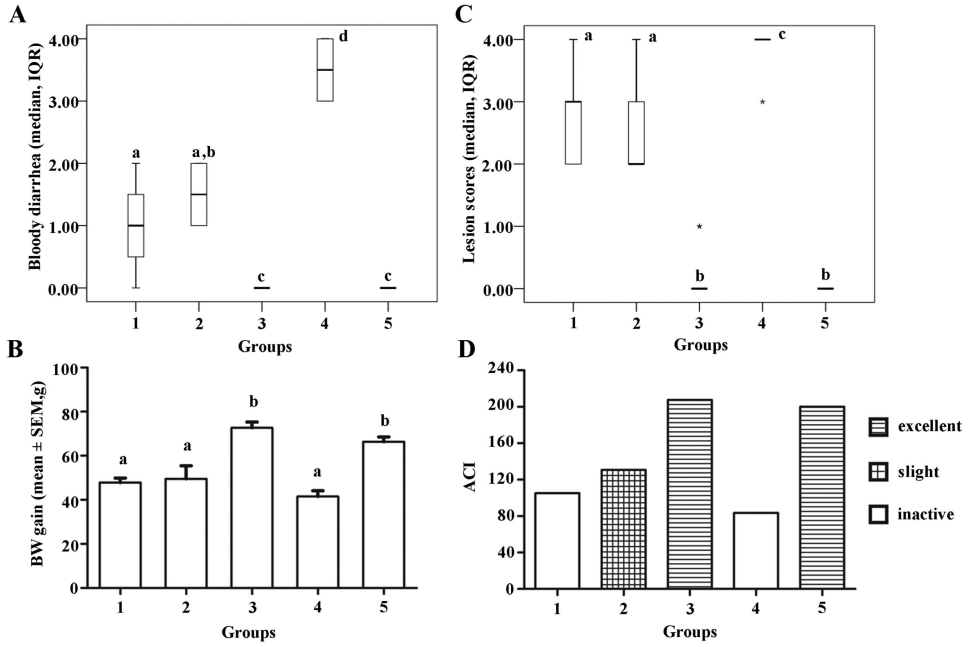

Figure 1. The birds were randomly assigned to 5 groups (10 birds per group). Chickens in groups 1, 2, and 3 were infected and treated with *B. javanica* extract equivalent to 2.5, 5 g raw herbs per kilogram feed or 3 mg halofuginone hydrobromide per kilogram feed, respectively. Groups 4 and 5 served as infected-unmedicated and uninfected-unmedicated controls. **A** Bloody diarrhea score (median, IQR) of each group on fourth to seventh d after challenge with *E. tenella*; **B** Effects of *B. javanica* extract on BWG (mean ± SEM, g); **C** Lesion scores (median, IQR) of cecum examined on the seventh d post challenge; **D** Anticoccidial index (ACI) of each group. Letters above or near the columns mean statistical difference ($P < 0.05$).

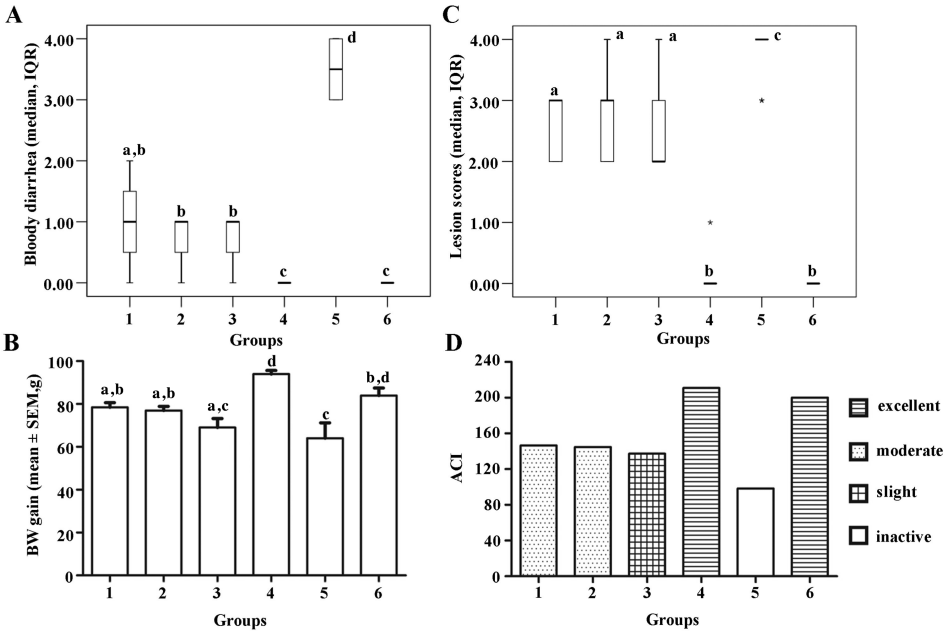

Figure 2. The birds were randomly assigned to 6 groups (10 birds per group). Chickens in groups 1, 2, 3, and 4 were infected and treated with *B. javanica* extract equivalent to 2.5, 5, 10 g raw herbs per kilogram feed or 3 mg halofuginone hydrobromide per kilogram feed, respectively. Groups 5 and 6 served as infected-unmedicated and uninfected-unmedicated controls. **A** Bloody diarrhea score (median, IQR) of each group on fourth to seventh d after challenge with *E. tenella*; **B** Effects of *B. javanica* extract on BWG (mean ± SEM, g); **C** Lesion scores (median, IQR) of cecum examined on the seventh d post challenge; **D** Anticoccidial index (ACI) of each group. Letters above or near the columns mean statistical difference ($P < 0.05$).

(Figure 3A). However, none was observed in the groups treated with halofuginone hydrobromide (group 4) and the uninfected-unmedicated control (group 6) throughout the whole experiment (Figure 3A). All 3 groups medicated with *B. javanica* extract showed significantly lower lesion scores than the infected-unmedicated control (group 5) ($P < 0.05$). Among all medicated groups, groups 1 and 2 showed similar lower lesion scores, while group 3 displayed the lowest score as the extract dosage increased.

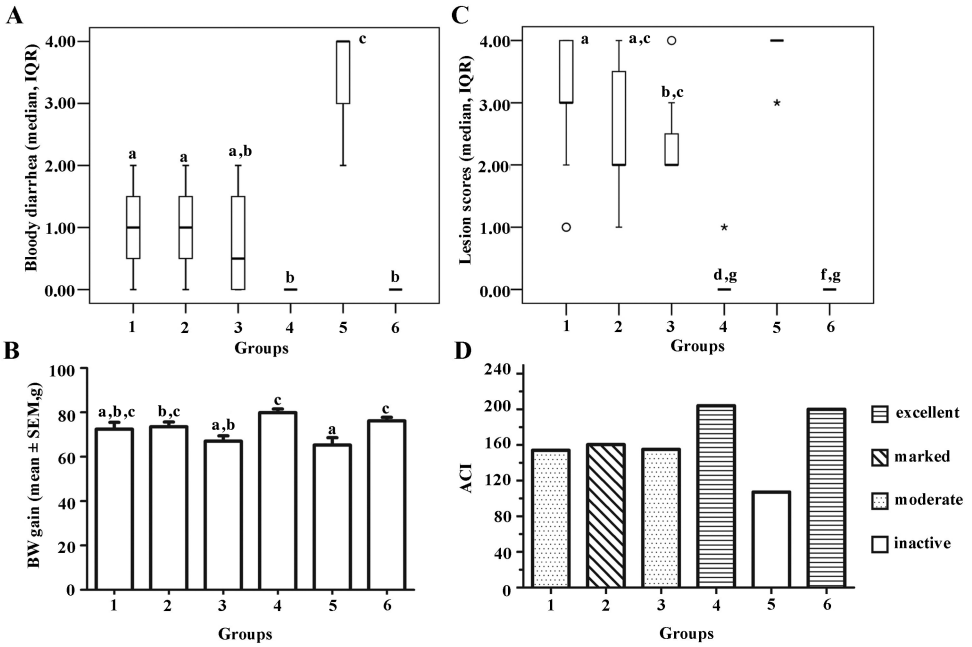

Figure 3. The birds were randomly assigned to 6 groups (20 birds per group). Chickens in groups 1, 2, 3, and 4 were infected and treated with *B. javanica* extract equivalent to 2.5, 5, 10 g raw herbs per kilogram feed or 3 mg halofuginone hydrobromide per kilogram feed, respectively. Groups 5 and 6 served as infected-unmedicated and uninfected-unmedicated controls. **A** Bloody diarrhea score (median, IQR) of each group on fourth to seventh d after challenge with *E. tenella*; **B** Effects of *B. javanica* extract on BWG (mean ± SEM, g); **C** Lesion scores (median, IQR) of cecum examined on the seventh d post challenge; **D** Anticoccidial index (ACI) of each group. Letters above or near the columns mean statistical difference ($P < 0.05$). Asterisks (∗) indicate extreme values; circles (○) represent outliers.

Body Weight Gain The mean BWG of each group is presented in Figure 3B. There were no significant differences among the initial weights of each group of chickens (data not show). Although BWG of group 2 showed a significant increase ($P < 0.05$) compared to that in the infected-unmedicated control (group 5), groups 1 and 3 did not show a significant increase (Figure 3B). However, both groups 1 and 2 showed no statistical significant difference ($P > 0.05$) when compared with the uninfected-unmedicated control (group 6). No significant difference was found among the medicated groups (groups 1, 2, and 3) ($P > 0.05$). In summary, our data indicated that *B. javanica* extract, at the current dose used, did not have an effect on BW.

Lesion Scores The cecum of each bird in cage A was removed and examined as shown in Figure 4A. *E. tenella* primarily infects the cecum. Severe pathological features were observed in the infected-unmedicated control (group 5) cecums such as atrophy, erosion, wall thickening, and occupancy by dark blood clotting. The degree of atrophy is measured by the length of each cecum. As presented in Figure 4B, cecums from group 5 displayed the highest degree of atrophy. Cecal morphology and shrinking were significantly ($P < 0.05$) improved in the medicated groups (groups 1, 2, and 3). The cecums in both uninfected-unmedicated control and the halofuginone hydrobromide group showed normal morphology.

The intestine was dissected to further examine the inner wall of the cecum and the intestinal feces. Combining all the pathological features, the lesion scores were finally recorded in Figure 3C. It was clear that *B. javanica* extract has significantly ($P < 0.05$) alleviated the undesirable cecal lesions caused by coccidiosis. The beneficial effects of the extract were not obvious ($P > 0.05$) in groups 1 and 2 with lower doses, while group 3, given the highest dosage, exhibited the least pathological features.

Oocysts Per Gram Output The mean OPG output of each group from d 4 to d 7 is presented in Table 1. Fecal output of oocysts was absent during the first 4 d after challenge. From the fifth day onward, OPG output differences between the medicated and unmedicated chickens were observed. Although the lowest dosage group (group 1) did not show significant difference ($P > 0.05$) compared to the infected-unmedicated control (group 5), much lower oocysts numbers ($P < 0.05$) were found in the other 2 groups treated with the higher dosage (groups 2 and 3). The inhibition rate of group 1, group 2, and group 3 was 55.24, 68.60, and 71.41%, respectively. A relationship was seen in which oocysts output reduces with increasing extract dose.

Anticoccidial Index As shown in Figure 3D, the infected-unmedicated control group showed the lowest ACI value (107). Among the medicated groups, groups 1 and 3 showed moderate anticoccidial effect with ACI indexes of 154 and 155, respectively, while the ACI index of group 2 reached 161, representing a marked anticoccidial effect. As expected, the control group treated with halofuginone hydrobromide showed the highest ACI value (204).

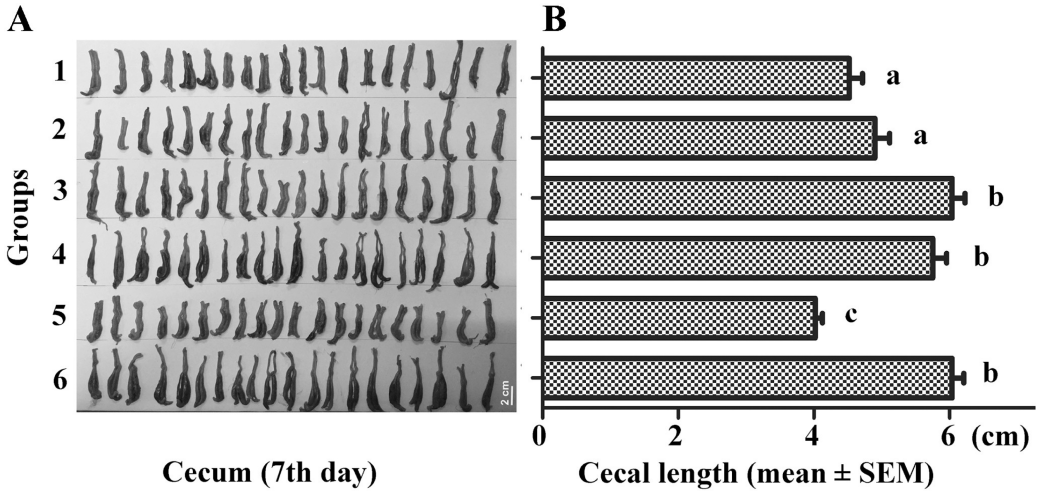

Figure 4. The cecum of each bird was collected and measured. **A** Cecums collected from each group on seventh d after challenge. **B** The cecal length was measured and expressed as mean ± SEM cm. Letters above the columns mean statistical difference ($P < 0.05$). Scale bar, 2 cm.

Table 1. Comparison of mean OPG output calculated in the feces of different groups and inhibition rate.

Groups	OPG (mean ± SD, × 10^5)[1]	Inhibition rate (%)[2]
1 *B. javanica* premix (2.5 g/kg)	11.99 ± 3.00^{a,b}	55.24
2 *B. javanica* premix (5 g/kg)	8.41 ± 2.15^{a}	68.60
3 *B. javanica* premix (10 g/kg)	7.66 ± 1.87^{a}	71.41
4 halofuginone hydrobromide	0.00 ± 0.00^{c}	100.00
5 infected-unmedicated	26.79 ± 6.76^{b}	0.00
6 uninfected-unmedicated	0.00 ± 0.00^{c}	-

[1]Mean OPG output was calculated from d 4 to d 7.

[2]Inhibition rate (%) = (OPG in infected-unmedicated group - OPG in experiment group) / OPG in infected-unmedicated group × 100%.

$^{a-c}$Column with different letters mean statistically different ($P < 0.05$).

Histological Examination Light microscopic inspection of the hematoxylin-eosin staining sections showed that the epithelial cells of the cecums in the challenged groups were subjected to different degrees of damage (Figure 5).

Cecum tissues of the chickens in the uninfected-unmedicated control group demonstrated a normal appearance with finger-shaped outgrowths (Figure 5A), as well as the group treated with halofuginone hydrobromide (data not show). A large number of schizonts (Figure 5B and C) was found in the sections of the infected-unmedicated control group on the fourth d. However, only a small amount of these second-generation schizonts (Figure 5E) was found in group 2, and no schizonts were observed in group 1 (Figure 5D) or group 3 (Figure 5F).

DISCUSSION

Anticoccidial drugs such as halofuginone, a synthetic halogenated derivative of a natural quinazolinone alkaloid found in *Dichroa febrifuga* (a traditional Chinese medicinal plant), were developed from natural products and widely used in coccidiosis control. We initially planned to find a plant product with anticoccidial properties that could potentially be developed further into a new anticoccidial drug. After a preliminary screen of a variety of Chinese herbs we focused on *Brucea javanica*. This plant is rich in quassinoids, terpenoids, triterpene saponins, alkaloids, and flavonoids, as well as some steroids, peptide, and fatty acids (Zhao et al., 2014). These diverse compounds bestow this plant with a variety of biological activities. In the current study, our data confirmed that extract from *Brucea javanica* yields significant anticoccidial effects.

Coccidiosis is a well-known infectious disease. It causes severe bloody diarrhea in broiler chickens by destroying intestinal epithelial cells. Typical symptoms of avian coccidiosis were observed in the present study such as bloody diarrhea, huddling together, ruffled feathers, and depression. Similar signs were observed by Jordan et al. (2011) and Tanweer et al. (2014). We found that the *B. javanica* extract could significantly reduce the bloody diarrhea of the challenged chickens. Reduced bleeding can protect the infected chickens from secondary bacterial infection, inflammatory response, and the absorption of toxic substances (Bozkurt et al., 2013). The mode of actions of this product as an anti-diarrheic medicinal plant may result from pharmacological effects such as a reduction in intestinal motility or from a direct anticoccidial activity. This observation is supported by similar findings of Sawangjaroen and Sawangjaroen (2005).

The number of oocysts in the feces is an important factor for the spreading of coccidiosis in intensive farming (del Cacho et al., 2010). In the present study, *B. javanica* extract reduced the OPG output in the treated groups significantly, suggesting that *B. javanica* extract may play a key role in the control of large-scale outbreaks of avian coccidiosis on chicken farms.

The cecum is one of the important digestive organs in chickens. When the epithelial cells of the cecum are

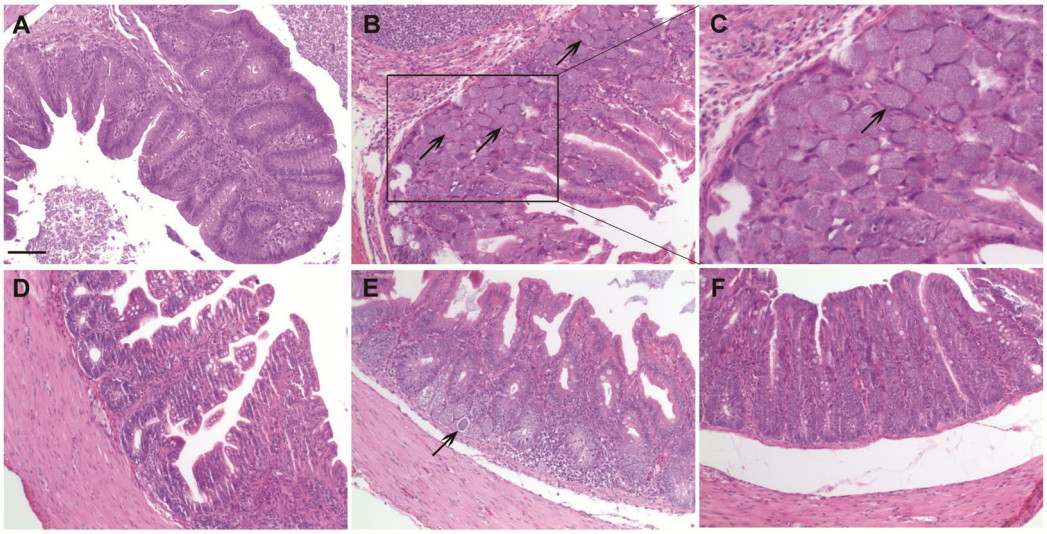

Figure 5. Cecal sections of birds in medicated groups demonstrated none or fewer second-generation schizonts (black arrows) on d 4. **A** Normal cecal morphology from uninfected-unmedicated control (group 6); **B** Cecal section from infected-unmedicated control (group 5) demonstrated a number of second-generation schizonts; **C** Zoomed image of schizonts; **D** No schizont was observed in cecal section from group 1; **E** Only a few schizonts were observed in cecal section from group 2; **F** No schizont was observed in cecal section from group 3. Scale bar, 50 μm.

destroyed by *Eimeria*, the host suffers from malabsorption leading to poor weight gain (Witlock, 1982; Blake and Tomley, 2014). Supplementation with *B. javanica* extract reduced the impact of coccidian challenge on the macroscopic lesions of cecums. A degree of mild atrophy was observed in the medicated groups; however, it was more serious in the infected-unmedicated control. Results from cecal sections showed that the extract could greatly reduce the coccidiosis burden. These suggest that the in vivo development of *E. tenella* is suppressed or delayed by the ingredients present in the extract. Therefore, apart from targeting the parasites directly, the *B. javanica* extract could play an instrumental role in improving the conditions of the infected chickens by its organ-protective properties (Masood et al., 2013; Wunderlich et al., 2014).

Some limitations exist in addition to the important discoveries revealed by these studies. For example, although the data on weight gain do not seem to support this plant in promoting BW, it is possible that an overdosed extract may exert detrimental impacts on the animals. Thus, any medicinal dosage should be monitored carefully to avoid undesirable effects on BW. More work should be carried out to estimate the extract's effects on weight.

The synthetic halofuginone used as a control in the current study exhibited excellent anticoccidial effect. The challenged chickens treated with halofuginone showed no clinical symptoms of coccidiosis, such as bloody diarrhea and oocysts output in feces. The excellent anticoccidial effect could partially be attributed to halofuginone hydrobromide's being a pure compound. This is consistent with the work reported by Naidoo et al. (2008). As the *B. javanica* extract contains a rich pool of chemical constituents, it is likely that they play a combined effect in the infected chickens. To further evaluate the anticoccidial efficacy of *B. javanica*, pure components should be isolated and identified. This will help us to better understand the relationships between the various individual components and their respective anticoccidial effect to allow better drugs development.

In summary, we conclude that the ethanol extract of *B. javanica* possesses significant anticoccidial properties at the concentrations tested. Due to the wide distribution, economical nature, and ease of use of the plant, *B. javanica* could serve as a powerful alternative anticoccidial agent. Eventually, this study provides a new strategy for the control of avian coccidiosis.

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