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RESEARCH ARTICLE

# Coccidiostat Activity of *Mahonia bealei* (Fort.) Leaves Extract against *Eimeria tenella* in Chickens

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**ABSTRACT**

The efficacy of *Mahonia bealei* (Fort.) leaves extract (MBLE) against coccidiosis in chickens was studied *in vivo*. For this purpose, a total of 120 Hyland brown chickens (one-day-old) were randomly divided into six groups, each comprising 20 chickens, viz. MBLE-L, MBLE-M, and MBLE-H; drug control group; infected-untreated group; and uninfected-untreated group. Except for the uninfected/untreated group, the chickens in all other groups received an oral inoculation of $1.0 \times 10^4$ pieces/feather sporulated oocysts of *E. tenella* on day 15. Then, for 7 days in a row, the drug control group was given sulfachloropyrazine sodium soluble powder (1.0 g/day) in drinking water, while the three treatment groups MBLE-L, MBLE-M and MBLE-H were given the MBLE @0.25, 0.5, and 1.0 g/day, respectively. The anticoccidial effects were evaluated by lesion score, body weight gain, oocyst output, and histopathological changes in the liver, kidney, and cecum of the chickens in each group. The results showed that no chickens died in all groups except 2 chickens died in the untreated group. The MBLE groups were able to decrease coccidia oocyst output, mitigate the impact of coccidian infection on chicken weight increase, and lessen pathological alterations in the liver, kidney, and cecum of infected chickens. Among these, the MBLE-H group demonstrated the greatest efficacy with an anticoccidial index of 159.17. Results showed that an extract from *Mahonia bealei* (Fort.) leaves extract had an anticoccidial effect on chickens infected with *E. tenella.*

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## INTRODUCTION

Coccidiosis is an infectious protozoan disease, caused by *Eimeria* (*E.*), in the intestinal epithelium of chickens with high morbidity and mortality. It is one of the most serious diseases hindering the development of poultry farming (Mohsin *et al.,* 2021; Jamil *et al.,* 2022). Symptoms of coccidiosis include slow growth, weight gain loss, and bloody feces, which cause huge economic losses to the poultry industry (Ojimelukwe *et al.,* 2018). Currently, the main methods of controlling coccidiosis are medication and vaccination ( Shivaramaiah *et al.,* 2014; Abbas *et al.,* 2019). While these methods are very effective in controlling the disease, issues with drug resistance, drug residues, worm strain variation, vaccine side effects, and vaccine safety have also surfaced (Ojimelukwe *et al.,* 2018; Pastor-Fernández *et al.,* 2019).

Compared to chemotherapeutic anticoccidial drugs, Chinese herbal medicine has unique advantages in preventing and controlling coccidiosis. It has a wide range of sources, low toxic side effects, low drug residues, and pathogens are not easily resistant to it. Therefore, it has broad application prospects in the research of anticoccidial drugs. (Kaingu *et al.,* 2017; Abbas *et al.,* 2020; Song *et al.,* 2020; Qaid *et al.,* 2021). Consequently, attempting to manage coccidiosis with Chinese medicine can surely offer a fresh perspective on the disease's management (Elmahallawy *et al.,* 2021; Jamil *et al.,* 2022; Abbas *et al.,* 2023). Numerous studies have documented the *in vivo* effectiveness of Chinese herbal extracts in the treatment of coccidiosis. For example, the acetone leaves extract of *Moringa oleifera Lam.* (Moringaceae) has anticoccidial properties on broiler chickens naturally infected with *Eimeria* species (Ola-Fadunsin and Ademola, 2013). The leaf extract of *Aloe secundiflora* may also improve the growth performance of chickens infected with *E. tenella* and reduce bloody diarrhea, mortality, oocyst production, and cecal lesions (Kaingu *et al.,* 2017), *Pomegranate peel* extract could reduce the excretion of Eimeria oocytes in the feces in broiler chickens (Khorrami *et al.,* 2022).

Traditional Chinese medicine has made extensive use of *Mahonia bealei* (Fort.) Carr. Which is formally recognized in the Chinese Pharmacopoeia (2010) (Hu *et al.,* 2016). Research has demonstrated that *Mahonia bealei* (Fort.) Carr.'s roots, stems, and leaves contain a range of biological activities, including antioxidant, anti-inflammatory, antifungal, and anti-Alzheimer's disease properties (Hu *et al.,* 2019; Huang *et al.,* 2021; Yang *et al.,* 2021). However, the anticoccidial efficacy of *Mahonia bealei* (Fort.) Carr. Leaves on poultry has not been reported. The present study was designed to examine and evaluate the efficacy of *Mahonia bealei* (Fort.) leaves extracts on the *E. tenella* infection in chickens *in vivo*.

## MATERIALS AND METHODS

**Preparation of *Mahonia bealei* leaves extract:** The leaves of *Mahonia bealei* were collected from Jiangsu Province (China) and identified by Professor Wu (College of Veterinary Medicine, Nanjing Agricultural University, Nanjing, China). The active ingredients of *Mahonia bealei* (Fort.) leaves (MBLE) were extracted by water decoction method (Hu *et al.,* 2009). The leaves were dried in a drying oven at 60 ℃, crushed in a wall breaker, and passed through a 160-mesh sieve. Then 100 g of the leaves powder was weighed, 5 times the volume of pure water was added to the leaves powder, soaked for 24 hours, boiled on high heat and then adjusted to low heat for 1 hour, and the residue was filtered through a 50mesh sieve to keep the liquid; The above procedure was repeated after 500 mL of pure water was added to the powdered leaves residue. After being combined, the filtrate was concentrated to create 1 g/mL in 100 mL, diluted in a gradient to make 0.5 g/mL and 0.25 g/mL, and then kept in a refrigerator at 4°C.

**Preparation of eimeria oocysts:** Thirty Hyland Brown chickens, aged eight days, were chosen, and given an oral inoculation with $1\times10^4$ sporulated *Eimeria* oocysts. The chickens' feces were constantly collected for 6-8 days following infection and floated in saturated saline, as described previously (Kumar *et al.,* 2014). The oocysts were counted using the McMaster technique (Rashid *et al.,* 2018) after being concentrated and incubated at 28 °C in a 2.5% potassium dichromate solution. The sporulated oocysts were then diluted to $1\times10^4$ oocysts/mL and kept at 4°C for further use (Fig. 1).

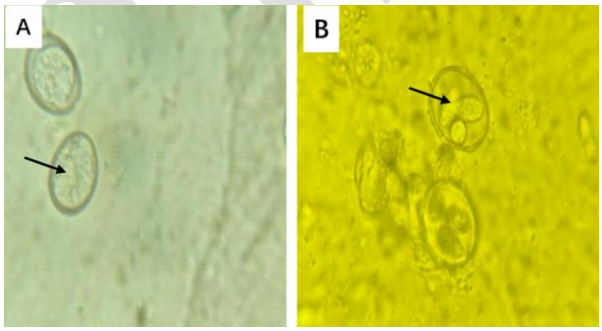


**Fig. 1:** The unsporulated oocysts (A) and sporulated oocysts (B) under light microscope at 1000×

**Experimental design:** One hundred and twenty (one day old) Hyland brown chickens free of coccidiosis were purchased from Nanjing Tegeili Planting Professional Cooperative (Nanjing, China). The chickens were split up into six groups of twenty each at random on day ten, including an uninfected-untreated group (non-infected/non-medicated), an infected-untreated group (infected/non-medicated), a drug control group and three MBLE treated groups. On day fifteen, the chickens in the MBLE-L, MBLE-M, and MBLE-H groups were administrated with 0.25 g, 0.5 g, and 1 g /day of MBLE per chicken by gavage for seven days in a row, respectively, the chickens in the drug control group were treated with sulfachloropyrazine sodium soluble powder (an anticoccidial drug, made in China) in drinking water at a dosage of 1 g/L, once a day for 7 successive days. The chickens, except for the uninfected-untreated group, were orally infected with $1\times10^4$ sporulated oocysts. During the experiment, the following parameters, such as clinical symptoms, lesion score, oocyst counts, and anti-coccidiosis index (ACI) were measured.

**Evaluation of anticoccidial effect:** After calculating the survival and mortality rates for each group on day 22, the relative weight gain rate was calculated. To determine the caecum lesion score and perform a histological study, all the chickens were slaughtered in the interim and bloody diarrhea was also observed. The relative weight gain (RWG) was calculated according to the following formula: Relative weight gain (RWG) rate (%) is the ratio of the average weight gain of chickens in each drug treatment group to the average weight gain of chickens in the uninfected-untreated control group. Feces were collected from 5 to 8 dpi in chickens, and the oocysts/gram of cecal contents (OPG) were counted by McMaster's method. The cecum was dissected and scored for cecum lesions, which ranged from 0 to 4 (Haug *et al.,* 2006). The anti-coccidiosis index (ACI) was computed using the following equation: ACI = (Relative weight gain rate + Survival rate) − (Lesion value + Oocyst value). The criteria for determining the efficacy were: ACI <120, poor efficacy; 120≤ACI≤160, moderate efficacy; 160≤ACI≤180, good efficacy; ACI>180, excellent efficacy.

**Histopathological observations:** After dissection, the kidney, liver, and cecum tissue samples were collected and fixed in 10% formalin, the tissue samples were embedded in paraffin and the tissue sections were prepared by microtome in 5 µm thickness. All of the sections were stained with hematoxylin and eosin (HE) for histopathological examination.

**Statistical analysis:** The experimental data were expressed as mean ± standard deviation and SPSS 23.0 software was used to evaluate each outcome. The experimental data was subjected to variability analysis using One-way analysis of variance (ANOVA) with a significant difference between groups identified at $P<0.05$.

## RESULTS

**Clinical symptoms:** The chickens in the untreated-uninfected group exhibited normal health status throughout the trial, and no coccidia oocysts were found in their excrement. Nonetheless, the chickens in the infected-

untreated group had more severe clinical symptoms, including depression, increased water intake, and a marked decrease in appetite, particularly on day 6 post-infection. They also expelled a significant volume of bloody feces. In the infected-untreated group, two chickens perished after six days of infection. After the dead chickens were dissected, it was observed that the cecum was enlarged and filled with blood and thickened wall (Fig. 2), indicating that the modeling was successful. The chickens in the drug control group and all MBLE groups showed varying degrees of bloody stools with milder symptoms, and no chicken deaths were observed.

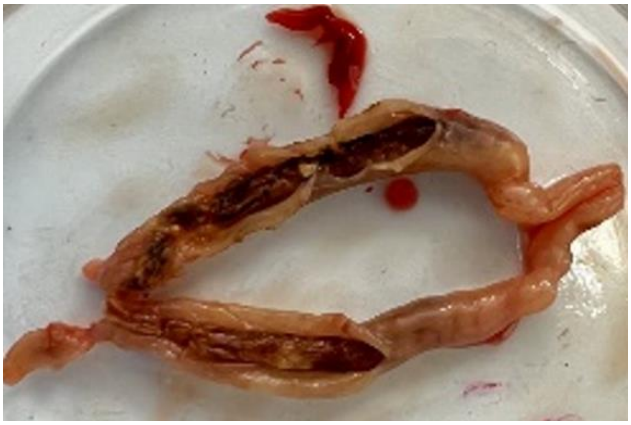

**Fig. 2:** The gross pathological examination of the cecum of *E. tenella*-infected chickens showing congestion of the cecum with bloody contents.

**Lesions score and oocyst value:** The examination results of lesions score, and oocyst value are displayed in Table 1. MBLE-M, MBLE-H, and the drug control group exhibited significantly reduced mean lesion scores than the infected-untreated group ($P$ <0.05). The MBLE-M, MBLE-H, and drug control groups had the lowest values of coccidia oocysts, particularly the MBLE-H group, which had the lowest oocyst ratio at 35.07. The infected-untreated group had a larger oocyst value than the other treatment groups.

**Relative weight gain rate:** The results of the relative weight gain rate of chickens are shown in Table 2. The average starting weight of the chickens in each group was not statistically different, but the average final weight of the chickens varied dramatically, particularly in the case of the infected-untreated group, where the chickens' relative weight gain was 43.15% lower than that of the uninfected-untreated group. The MBLE-H group had the greatest relative weight gain rate of 87.97%, which was numerically higher than that of the drug control group and significantly higher than that of the infected-untreated group. However, overall, there was no significant difference in the relative weight gain rate of the chickens between the treated groups. This implied that the relative weight gain rate of the sick chickens may be recovered using MBLE.

**Anti-Coccidiosis index:** The anticoccidial indices of the MBLE-L, MBLE-M and MBLE-H groups were 126.03, 146.73, and159.17, respectively (Table 3), which were higher than that of the infected-untreated control group (74.81). All anticoccidial indices shown by MBLE groups were within the range of 120-160, indicating that the anticoccidial effect of MBLE was moderate. Among them, the MBLE-H group showed the highest anticoccidial activity.

**Histopathological examination:** In the untreated-uninfected group, the cecum's structure was evident, and no visible lesions were seen (Fig. 3A). On the other hand, the cecum of infected-untreated group's displayed obvious lesions. Their intestinal mucosa contained a significant number of *Eimeria* stages, while, certain intestinal glands were necrotic, and the gland lumen contained basophilic material (Fig. 3B). When compared to the untreated group, the drug control group (Fig. 3C) and the MBLE-H group (Fig. 3F) had fewer oocysts in their intestinal mucosa. Conversely, when compared to the MBLE-H group, the MBLE-L group (Fig. 3D) and the MBLE-M group (Fig. 3E) had a greater number of developmental stages of *Eimeria* parasite.

In the untreated-uninfected group, the liver's structure was normal, and no visible lesions were seen (Fig. 3G). Hepatocyte vacuolar degeneration and inflammatory cell infiltrates were observed in the confluent area of the liver in the uninfected-untreated group (Fig. 3H). But the drug control group (Fig. 3I) and the MBLE groups (Fig. 3J-L) showed less hepatocyte vacuolation degeneration, with smaller area of inflammatory foci than the infected-untreated group. Similarly, a decrease in inflammatory cell infiltration in the confluent area of the chickens' liver was observed in former groups than the latter one.

In the untreated-uninfected group, the kidney's structure was normal with firmly organized tubules, and no other abnormalities were obvious (Fig. 3M). In contrast, the renal tubules of the infected-untreated chickens had vacuolar degeneration of the renal tubular epithelial cells and were somewhat loosely organized (Fig. 3N). No obvious lesions were observed in the drug control group (Fig. 3O) and MBLE-H (Fig. 3R), while slight vacuolar degeneration of renal tubular epithelial cells was observed in MBLE-M group (Fig. 3Q) and MBLE-L group (Fig. 3P).

## DISCUSSION

Herbs or herbal compounds have been shown in earlier research to be useful in treating or preventing coccidiosis in chickens (Zaman *et al.*, 2012). Research has shown that the components of Chinese medicine can paralyze and poison intestinal parasites and have certain anti-worm effects (Qaid *et al.*, 2021). For instance, sugar cane extracts, coumarin and *Aloe vera* extracts have been shown to have some therapeutic effects against *E. tenella* (Awais *et al.*, 2011; Michels *et al.*, 2011; Akhtar *et al.*, 2012; Kaingu *et al.*, 2017).

Coccidiosis typically prevents chickens from gaining body weight by decreasing feed intake, digestibility, and absorption of numerous minerals (Qaid *et al.*, 2021). At this time, weight gain is thought to be the more sensitive factor when it comes to coccidiosis and anticoccidial therapy (Gerhold *et al.*, 2016). The sluggish and depressed behavior, untidy feathers of the infected chickens in this study could be attributed to disturbed environmental

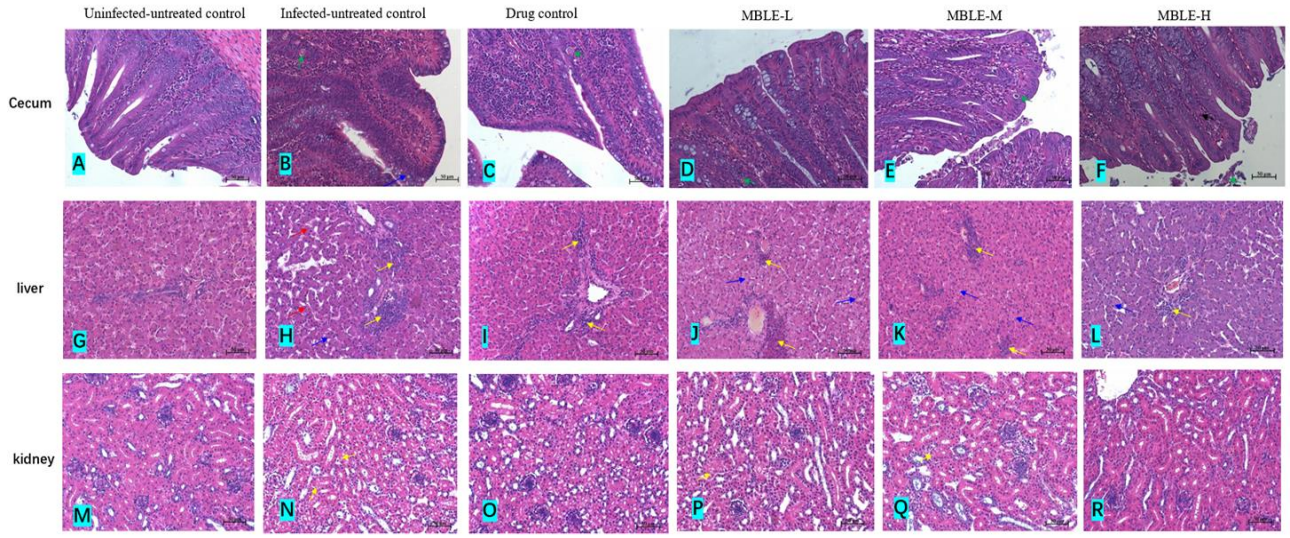


**Fig. 3:** Histopathological examination of cecum (A-F), liver(G-L), and kidney(M-P) (Scale bar = 50 μm); Fig. 3A-F shows the histopathological examination of cecum. No obvious lesions in the uninfected-untreated group (Fig. 3A); an increased number of cupped cells in the mucosal layer (Fig. 3B, denoted by a blue arrow), and a large number of worm eggs in the mucosa of infected-untreated group (Fig. 3B, denoted by a green arrow); few oocysts in cecum mucosa cells, and a small number of inflammatory cells infiltrated in the submucosa layer of drug control group (Fig. 3C), MBLE-L group (Fig. 3D), MBLE-M group (Fig. 3E), and MBLE-H group (Fig. 3F); Fig. 3G-L shows the histopathological examination of liver. No obvious changes in the liver of uninfected-untreated group (Fig. 3G); hepatocyte necrosis (Fig. 3H, denoted by yellow arrows), inflammatory cell infiltration (Fig. 3H, denoted by blue arrows) and hepatic blood sinusoidal stasis in infected-untreated group (Fig. 3H, denoted by red arrows); less inflammatory area degeneration of hepatocytes and a small amount of vacuolar degeneration in drug control group (Fig. 3I), MBLE-L (Fig. 3J) group, MBLE-M (Fig. 3K) group, and MBLE-H group (Fig. 3L); Fig. 3M-R shows the histopathological examination of kidney. No obvious lesions were seen in the uninfected-untreated group (Fig. 3M); a slightly looser arrangement of renal tubules and vacuolar degeneration of renal tubular epithelial cells (Fig. 3N, denoted by yellow arrows) were observed in the infected-untreated group of chickens (Fig. 3N); Slight vacuolar degeneration of renal tubular epithelial cells (denoted by yellow arrows) was observed in chickens of MBLE-L (Fig. 3P) group and MBLE-M (Fig. 3Q), No obvious lesions in the drug control group (Fig. 3O) and MBLE-H group (Fig. 3R).

**Table 1:** Cecal lesion and oocyst value of chickens in each group

| Group | Mean lesion score (0-4) | Lesion value (0-40) | Oocysts ratio (%) | Oocyst value (0-40) |
|---|---|---|---|---|
| MBLE-L | 2.63±0.74$^{cd}$ | 26.3 | 50.13 | 20 |
| MBLE-M | 2.13±0.23$^{bc}$ | 21.3 | 45.03 | 10 |
| MBLE-H | 1.88±0.23$^{b}$ | 18.8 | 35.07 | 10 |
| Drug control | 2.00±0.19$^{b}$ | 20 | 46.38 | 10 |
| Infected-untreated control | 2.87±0.13$^{d}$ | 28.7 | 100 | 40 |
| Uninfected-untreated control | 0.00±0.00$^{a}$ | 0 | 0 | 0 |

Different letters mean significant difference ($P < 0.05$).

**Table 2:** Relative weight gain rate of chickens in each group

| Group | Average initial weight (g) | Average final weight (g) | Average weight gain (g) | Relative weight gain rate (%) |
|---|---|---|---|---|
| MBLE-L | 108.00±2.87 | 147.30±4.72$^{ab}$ | 39.29 ±4.60$^{ab}$ | 72.33±8.47 $^{ab}$ |
| MBLE-M | 104.97±5.25 | 147.36±4.62$^{ab}$ | 42.39±2.48 $^{b}$ | 78.03±4.56$^{b}$ |
| MBLE-H | 110.58±2.68 | 158.36±5.42$^{bc}$ | 47.79±4.37$^{bc}$ | 87.97 ±8.05$^{bc}$ |
| Drug control | 108.87±3.00 | 152.16±3.72$^{abc}$ | 43.29 ±1.52$^{b}$ | 79.69±2.80 $^{b}$ |
| Infected-untreated control | 112.04±1.34 | 142.92±2.53$^{a}$ | 30.88±2.71 $^{a}$ | 56.85±3.54 $^{a}$ |
| Uninfected-untreated control | 109.87±1.75 | 164.19±1.85$^{c}$ | 54.32±1.97 $^{c}$ | 100.00±3.62 $^{c}$ |

Relative weight gain rate (%) is the ratio of the average weight gain of chickens in each treatment group to the average weight gain of chickens in the uninfected-untreated control group. Different letters mean significant difference ($P < 0.05$).

**Table 3:** The anticoccidial index of each group

| Group | Survival rate (%) | Relative weight gain rate (%) | Lesion score (0-40) | Value of oocyst (0-40) | anti-coccidiosis index (ACI) |
|---|---|---|---|---|---|
| MBLE-L | 100.00 | 72.33 | 26.3 | 20 | 126.03 |
| MBLE-M | 100.00 | 78.03 | 21.3 | 10 | 146.73 |
| MBLE-H | 100.00 | 87.97 | 18.8 | 10 | 159.17 |
| Drug control | 100.00 | 79.69 | 20 | 10 | 146.69 |
| Infected-untreated control | 86.67 | 56.84 | 28.7 | 40 | 74.81 |
| Uninfected-untreated control | 100.00 | 100 | 0 | 0 | 200 |

homeostasis in the intestine, which would impair feed intake and metabolism and prevent weight gain (Karalazos *et al.,* 2011). MBLE attenuated histopathological alterations in the cecum, and therefore, relative weight gain rates were improved in MBLE groups. The best effect was observed in the MBLE-H group with an average weight gain of 47.79 g, a relative weight gain of 87.97% and the low bloody diarrhea. One often-used metric to assess a drug's anticoccidial activity is the anticoccidial index (Song *et al.,* 2020). In this trial, the ACI of the MBLE-H group was 159.17, which was in the range of 120-160, indicating that the anticoccidial efficacy shown by MBLE was moderate with a comparable anticoccidial effect to the drug control.

Chicken coccidiosis damages the renal tubules, and the cause of death in infected chickens may be associated with the problem of renal tubular dysfunction. Parasitism and reproduction of coccidia in the chicken intestine is the main cause of cecum lesions, which cause necrosis and detachment of intestinal mucosal epithelial cells, accompanied by breakage and disintegration of intestinal villi (Abd-El Rahman *et al.,* 2022). The liver is the largest digestive gland in the digestive system, and coccidia development in this organ in chicken may lead to cellular swelling and vacuolar degeneration. In this experiment, the pathological changes of cecum, the liver and kidney of the chickens in MBLE groups were lighter than that in the infected-untreated group, which indicated that MBLE had a certain protective effect on the cecum, liver, and kidney of the infected chickens and could reduce the pathological damage in these organs by *E. tenella*. Among these groups, the high dose group of MBLE had shown best anticoccidial activity.

Plants containing antioxidants may attenuates coccidia infection by increasing the degree of intestinal lipid peroxidation because coccidia infection is linked to the death of host cells, which is related to oxidative stress and lipid peroxidation in the intestinal mucosa (Abbas *et al.,* 2013; Idris *et al.,* 2017; Akhter *et al.,* 2021). Similarly, the extracts of various other plants with antioxidant properties have shown anticoccidial activity. For instance, the antioxidant characteristics of curcumin can be utilized to eradicate intracellular parasites (Chan *et al.,* 2005). Zingiber officinale containing antioxidant active ingredients resulted in effective reduction of oocyst production in infected coccidian broilers (Naidoo *et al.,* 2008). The above results suggested that the antioxidant active ingredients from plants have good effects on coccidiosis control. Studies have confirmed that aqueous extract of *Mahonia bealei* (Fort.) Carr. Leaves is rich in flavonoids and polyphenols which protect oxidative damage to proteins caused by hydroxyl radicals (Hu *et al.,* 2019; Huang *et al.,* 2021).

**Conclusions:** This study describes the anticoccidial activity of MBLE against *E. tenella*. The results showed that certain concentrations of MBLE could reduce the mortality in chickens infected with *E. tenella* by decreasing the development of coccidia oocysts and reducing the pathological damage of coccidia to the cecum, liver, and kidney of chickens. Taken together, this study supports a theoretical basis for seeking new anticoccidial drugs. Certainly, to explore the anticoccidial mechanism and active components of MBLE responsible for anticoccidial activity, further studies are suggested.

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**Declaration of competing interest:** The authors declare no conflict of interest with this research.

**Authors' contribution:** P Zhang conceived and designed the study. W Lei, and P Zhang evaluated of anticoccidial effect. P Zhang, M Xue, and D Guo did the histopathological observations, and P Zhang analyzed the data. P Zhang and J Gong wrote the article, and D Guo revised the paper.

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