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Anticoccidial Effects of *Magnolia officinalis* Extract on *Eimeria tenella* in Chicken

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Abstract: Anticoccidial effects of *Magnoliae officinalis* Extract (MOE) were evaluated in chickens following oral infection with *Eimeria* (E.) *tenella*. This study was conducted on the 3 days old chickens (n = 30). Those animals were divided with 3 groups; MOE 0.5% treated/infected (n = 10), MOE untreated/infected (n = 10) and non-infected control (n = 10). Chickens were fed a standard diet supplemented with or without MOE for 1 week prior to infection with *E. tenella* (10,000 sporulated oocysts per chicken). The effects of MOE on *E. tenella* infection were assessed by two parameters, fecal oocyst shedding and body weight gain. The MOE-fed chickens produced significantly reduced fecal oocysts ($p<0.05$) when compared to the *E. tenella*-infected group fed standard diet. Also, MOE-based diet, improved body weight loss caused by *E. tenella* infection. The data demonstrated that MOE had remarkable anticoccidial activities against *E. tenella*. This finding might have implications for the development of anticoccidial drug.

Key words: Anticoccidial activity, *Eimeria tenella*, Eimeria, *Magnoliae officinalis*, magnolol

INTRODUCTION

Coccidiosis is induced by *Eimeria* species infection and an important parasitic disease of poultry (Dalloul and Lillehoj, 2006). Losses include mortality, morbidity and cost of preventative or therapeutic drugs and/or vaccination. In addition, many of the in-feed medications commonly used for prevention of infections with *Eimeria* species have become less effective because some strains of parasites have developed reduced susceptibility to anticoccidials (Wilson and Fairbairnz, 1961). This suggests that coccidiosis is likely to have a greater impact on the profitability of broiler meat production in the future (Wilson and Fairbairnz, 1961).

Magnolia officinalis is grown in Asian countries such as China, Thailand and Korea. Its bark has been used for many years in traditional Chinese medicines and Japanese remedies for the treatment of a variety of mental disorders including depression (Nakazawa *et al*., 2003). It is well known that *M. officinalis* is a major component in herbal formulations such as Banxia-houpu decoction and Saiboku-to used as remedies for depression and other illnesses, e.g., coughing, asthma, liver disease, shoulder pain, urinary problems and diarrhea (Wang *et al*., 2005). Magnolia extract, produced primarily from the dried stem, root or branch bark of *M. officinalis* (Chang and But, 1986) is a constituent of dietary supplements and topically applied cosmetic products. It contains the active compounds magnolol and honokiol which have various pharmacological activities including anti-inflammatory effects (Wang *et al*., 1995), antimicrobial activity (Chang *et al*., 1998; Ho *et al*., 2001), antioxidant activity and free radical-scavenging activity (Lo *et al*., 1994). The inhibitory effects of *M. officinalis* Extract (MOE) against *Listeria monocytogenes*, *Streptococcus faecalis*, *Escherichia coli*, *Salmonella typhimurium*, *S. aureus* and *Bacillus anthracis* were reported (Hu *et al*., 2011).

Although, a variety types of natural products have been investigated in search for alternative controls of coccidiosis in chickens (Dalloul and Lillehoj, 2006), the effects of MOE on Eimeria infection has not been studied. The present study is aimed to investigate the anti-apoptotic activities by *E. tenella* infection to MDBK cells *in vivo*.

MATERIALS AND METHODS

Preparation of *Magnoliae officinalis* extract: The dry bark of *Magnolia officinalis* was purchased from an Oriental Pharmacy (Iksan, Korea) was according to the standard as mentioned in Korean Pharmacopoeia and Korean Herbal Pharmacopoeia which are the official compendia of standard. The procedure for preparing MOE was as follows. The air-dried bark of *M. officinalis* (1 kg)

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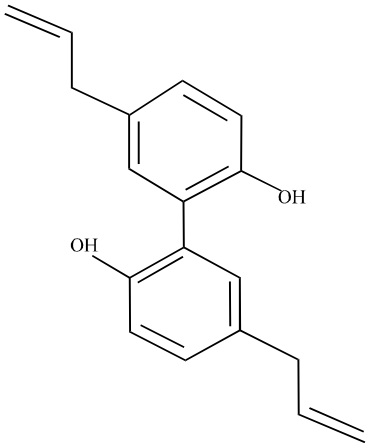

Fig. 1: Magnolol, a biphenyl neolignan made from the bark of *Magnoliae officinalis*

was cut into pieces and extracted twice with 70% (v/v) ethanol (three times as much as the weight of the dried plants) for 3 h at 100°C. After filtration through a 400-mesh filter cloth, the filtrate was refiltered through filter paper (Whatman, No. 5) and concentrated on a rotary evaporator (EYELA, Tokyo, Japan) and the concentrated filtrate was evaporated to dryness under vacuum with freezing dryer (Labconco, USA). Finally, the solid residue was collected, placed in sealed bottles and stored at -20°C.

Ultra-Performance Liquid Chromatography (UPLC) Analysis of MOE: Magnolol (5, 5'-di-2-propenyl-(1,1'biphenyl)-2,2'-diol) which was used for the standard material of MOE composition was purchased from the Korean Food&Drug Administration (Cheongwon-gun, Korea). Figure 1 shows the chemical structures of magnolol. The magnolol composition of MOE was analyzed by UPLC. Waters ACQUITY UPLC System (Waters Corp., Milford, USA) was used for UPLC System. The column was C18 type ACQUITY UPLC BEH (2.1×50 mm, 1.7 μm, Waters Corp., Milford, USA). A Waters Nova Pack C-18 column (ACQUITY UPLC BEH (2.1×50 mm, 1.7 μm, Waters Corp., Milford, USA) was employed. The wavelength of the UV detector was set at 300 nm. The column temperature was set at 30°C with a flow rate of mobile phase at 0.6 mL min^{-1} (0.1% H_3PO_4/Acetonnitrile).

Experimental animals: This study was conducted on the 3 days old chickens (n = 30) in the animal facility of Center for Animal Resources Development, Wonkwang University, Korea. Animals were acclimatized and kept in an animal facility room with regulated temperature (28±2°C), humidity (50±5%) and light/dark cycle (12/12 h). The animals were fed commercial post-broiler diet without antibiotics and coccidiostat (Hanil Feed Co., Yongin, Korea) and tab water *ad libitum*. The chickens were kept in wire-floored grower cages during study period. All studies were performed in accordance with the Guide for Animal Experimentation by Wonkwang University and approved by the Institutional Animal Care and Use Committee of Wonkwang University (Approval No. WKU11-007). All efforts were made to minimize pain or discomfort of animals used.

Experimental design: Anticoccidial effects of MOE were evaluated in chickens following oral infection with *E. tenella*. This study was conducted on the 3 days old chickens (n = 30). Those animals were divided with 3 groups; MOE 0.5% treated/infected (n = 10), MOE untreated/infected (n = 10) and non-infected control (n = 10). Chickens were fed a standard diet supplemented with or without MOE for 1 week prior to infection with *E. tenella* (10,000 sporulated oocysts per chicken). The effects of MOE on *E. tenella* infection were assessed by two parameters, fecal oocyst shedding and body weight gain.

Inoculation of eimeria oocysts: Oocysts of *E. tenella* were cleaned by flotation on 5.25% sodium hypochlorite and washed three times with phosphate buffered saline. *E. tenella* was provided kindly by Professor Wongi Min at Gyeongsang National University in Korea. Chickens were treated orally by gavages using a 24 gauge, mouse stainless steel animal feeding tube (Popper and Sons, Inc., New York, USA) attached to a 3 mL syringe. The oral infectious dose of has been approximated 10^4 oocysts of *E. tenella* in 1 mL of saline. The control chickens (n = 10) received saline through the same route.

Clinical observation and weight measurements: During the study period, the animals were checked twice daily for morbidity and mortality. Further, researchers compared clinical signs and body weight changes of experimental animals. Body weights were individually measured for 2 weeks before infection and for 10 days post-infection.

Fecal sampling and oocyst counting: Fecal materials were collected from 6-10 days post-infection. The fecal samples were analyzed for the presence of coccidial oocysts using a standard fecal flotation technique (Lee *et al*., 2011). Briefly, 5 mL from each sample was pelleted by centrifugation at 1500×g for 5 min. The resulting pellet was resuspended in saturated sodium chloride (aqueous), passed through a 1 mm mesh size sieve to remove coarse fecal debris. The resulting filtrate was used in a standard gravity vial fecal flotation using 22×22 mm coverslips.

After flotation, the coverslip was mounted on a slide and examined in its entirety for the presence of coccidial oocysts. Total number of oocysts was calculated using the following equation:

$$\text{Total No. of oocysts} = \frac{\text{Oocyst count} \times \text{Dilution factor} \times \left(\frac{\text{Fecal sample volume}}{\text{Counting chamber volume}}\right)}{\text{No. of birds per cage}}$$

Statistical analysis: Differences in mean oocyst production and mean weight gain between the 4 groups were tested by using one-way analysis of variance (ANOVA; GraphPad InStat; GraphPad Software Inc., San Diego, CA) and considered significant at $p<0.05$.

RESULTS AND DISCUSSION

The extract yield of dry bark of *M. officinalis* with 70% ethanol was 30%. Researchers analyzed MOE composition by UPLC. The retention time of magnolol in the specified UPLC condition was 7.886 min. The concentration of magnolol in MOE was 10.21±0.10%. Figure 2 shows the UPLC chromatograph of MOE.

The effects of MOE on *E. tenella* infection were assessed by two parameters, fecal oocyst shedding and body weight gain. MOE-fed chickens produced significantly reduced fecal oocysts ($p<0.05$) when compared to the *E. tenella*-infected group fed standard diet. Also, MOE-based diet, improved body weight loss caused by *E. tenella* infection.

The results showed that compared to untreated controls, chickens treated with MOE had significantly decreased fecal oocyst shedding and showed strong anticoccidial activities ($p<0.01$).

As shown in Table 1, oocyst shedding was significantly higher in the inoculated chickens than in the control chickens ($p<0.05$). The number of fecal oocysts shed was highest on day 7 post-inoculation (Table 1).

Table 1: Results of shedding oocysts number count in the feces of studied chickens

	Body weights (g)/Days post infection				
Groups	6	7	8	9	10
Control	0±0*	0±0*	0±0*	0±0*	0±0*
Infected control[a]	13.5±1.80	58.1±1.91	23.2±1.40	7.5±1.08	1.6±0.70
MOE+Eimeria[b]	8.1±1.29*	23.9±1.20*	12.7±0.95*	2.2±1.03*	0±0*

Moreover, body weight gain was lesser in the animals in the inoculated group than in the animals in the control group (Table 2).

Coccidiosis of domestic fowl is a worldwide disease caused by obligatory intracellular protozoa of the genus *Eimeria*. It is responsible for important economic losses in poultry production. *E. tenella* is important pathogen causing avian coccidiosis in laboratory avian animals and known to affect influencing experimental results obtained with contaminated animals (Dalloul and Lillehoj, 2006; McDougald, 2003). The disease is characterized by enteric lesions of variable extent and severity reducing the absorptive function of the intestinal mucosa thus leading to weight loss, diarrhea, poorer feed conversion and a higher mortality in the affected flocks (Stotish *et al*., 1978).

The results of this study showed that MOE had a strong anticoccidial effect on *E. tenella*. MOE contains a large amount of polyphenolic compounds (magnolol, honokiol, tetrahydromagnolol, isomagnolol, etc.). Earlier studies have shown that phenolic compounds can affect microbial growth by altering microbial cell permeability and permitting the loss of macromolecules inside the cell. Once the phenolic compounds have crossed the cell membrane, interactions with membrane enzymes and proteins will cause an opposite flow of protons affecting

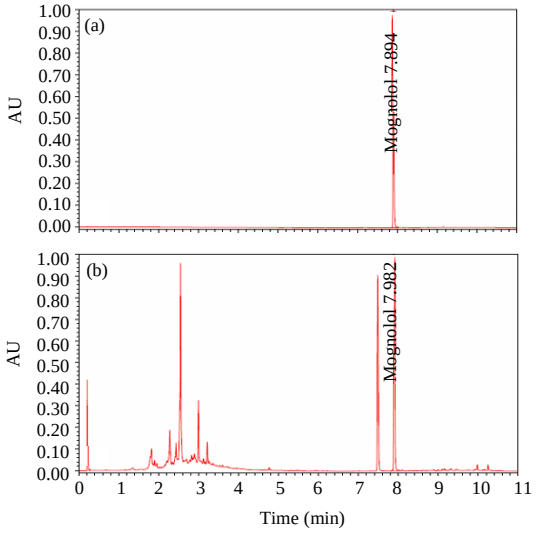

Fig. 2: UPLC chromatogram of Magnolol in the test material; a) Standard mixtures; b) Extract of the bark of *Magnoliae officinalis*

Table 2: Results of body weight changes of studied chickens

	Body weights (g)/Days post infection				
Groups	1	3	5	7	10
Control	119.9±1.52	137.5±1.43*	169.8±1.32*	217.6±1.43*	251.7±2.06*
Infected control[a]	117.9±1.66	126.5±1.51	151.4±1.65	177.5±1.84	210.8±1.75
MC+Eimeria[b]	119.4±1.26	135.8±1.14*	167.6±1.17*	214.8±1.84*	247.9±2.08*

[a]The chickens inoculated with *Eimeria tenella* oocysts. [b]The chickens inoculated with Eimeria live oocysts and treated with 0.5% *Magnoliae officinalis* extract (MOE). *Significantly difference with Eimeria infected control chickens ($p<0.05$)

cellular activity. It has also been suggested that cell membrane of *Pseudomonas aeruginosa* causing an increase in permeability (Bernheim, 1972). This theory was substantiated by rapid swelling of *P. aeruginosa* cells due to phenol (Puupponen-Pimia *et al*., 2001). Davidson (1993) reported that phenolic compounds may act on microbial cell walls or membranes, proposing that they inhibit microbial growth by altering microbial cell permeability which leads to the loss of intracellular molecules such as proteins, DNA, RNA and ATP. Phenolic compounds could also interact with membrane proteins causing damage to their structure and functionality. Conner and Beuchat (1984) suggested that the antimicrobial activity of essential oils against yeasts could be a result of the disturbance of several enzymatic systems involved in energy production and the synthesis of structural components.

The data demonstrated that MOE had remarkable anticoccidial activities against *E. tenella*. This finding might have implications for the development of anticoccidial drug. This study is the first to demonstrate anticoccidial effect of MOE on Eimeria parasites.

CONCLUSION

Anticoccidial effects of *Magnoliae officinalis* Extract (MOE) were evaluated in chickens following oral infection with *Eimeria* (E.) *tenella*. The MOE-fed chickens produced significantly reduced fecal oocysts ($p<0.05$) when compared to the *E. tenella*-infected group fed standard diet. Also, MOE-based diet, improved body weight loss caused by *E. tenella* infection. The data demonstrated that MOE had remarkable anticoccidial activities against *E. tenella*. This finding might have implications for the development of anticoccidial drug.

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