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Anticoccidial effects of the *Houttuynia cordata* extract on experimental animal infection model

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Abstract

Coccidiosis is caused by infection of *Eimeria* species and an significant parasitic disease in poultry. Various kinds of natural products have been studied to find alternative treatments for coccidiosis in chickens, but the effect of *Houttuynia cordata* on *Eimeria* infection has not been investigated. The aim of this study is to study the anticoccidial effect of *H. cordata* extract (HCE) in chickens after oral infection by *Eimeria tenella*. Anticoccidial effects of the HCE was evaluated in chickens after oral infection with *E. tenella*. This study was performed on three-day-old chicks (n = 30). These animals were divided into 3 groups; HCE 0.2% treated/infected (n = 10), HCE untreated/infected (n = 10) and non-infected control (n = 10). The effect of HCE on *E. tenella* infection was assessed by two parameters; fecal oocysts shedding and body weights gain. the chicks fed HCE significantly reduced fecal oocysts when compared to the *E. tenella*-infected group fed standard diets ($p<0.05$). Furthermore, the HCE-based diet improved weight loss due to *E. tenella* infection. Our data shows that HCE had significant antiprotozoal activity against *E. tenella*. These findings may have implications for the development of anticoccidial drugs.

Keywords: anticoccidial activity; antiprotozoa; *Eimeria tenella*; eimeria; *Houttuynia cordata* extract

Coccidiosis is caused by infection with *Eimeria* species and an significant parasitic disease in poultry [1]. Losses include mortality, morbidity and cost of prophylactic or therapeutic drugs and/or vaccination. Also, many medications such as in-feed drugs commonly added to feed to prevent infection with *Eimeria* spp. have become less effective because some parasite strains have reduced susceptibility to anticoccidial agents [2]. This suggests that coccidiosis may have a greater impact on the profitability of future broiler production [2].

Traditionally, *H. cordata* Thunb has been used as an herbal medicine for the treatment of inflammatory diseases such as ulcerative colitis in humans [3]. Previous studies have shown that *H. cordata* extracts exhibit antiviral and antibacterial [4, 5], antiallergic [6], antioxidant and anti-mutagenic activity [7]. The main components of *H. cordata* extract include methyl nonyl ketone, β-myrcene, β-pinene, α-pinene, α-terpineol and n-decanoic acid. And the anti-inflammatory effects of *H. cordata* have also been proven [8]. Thogh various types of natural products have been investigated to find alternative controls for coccidiosis in chickens [1], the effects of *H. cordata* on

Eimeria infection has not been investigated.

The aim of this study is to study the anticoccidial effect of *H. cordata* extract (HCE) in chickens after oral infection by *E. tenella*.

The dried lump of *H. cordata* was purchased from an Oriental medicine pharmacy (Iksan, Korea), and it meets the official standards of Korean Pharmacopoeia and Korean Herbal Pharmacopoeia. The procedure for preparing HCE is as follows. Naturally dried *H. cordata* masses (100 g) were cut into pieces and extracted twice for 3 h using 50% (v/v) ethanol (6 times the weight of the dried plants at 80°C. After filtration through a 400-mesh filter cloth, it was filtered again with filter paper (No. 5, Whatman, Maidstone, UK) and concentrated with a rotary evaporator (EYELA, Tokyo, Japan) and the concentrated filtrate was vacuum-dried to dryness under vacuum with freezing dryer (Labconco, Kansas City, MO, USA). Finally, the solid residue was collected, bottled, sealed and stored at –20°C.

This study was performed on three-day-old chickens (n = 30) in the animal facility of Center for Animal Resources Development, Wonkwang University, Korea. Animals were acclimatized and housed in an animal facility with controlled temperature (28 ± 2°C), humidity (50 ± 5%), and light-dark cycle (12/12 h). These chicks were provided with a commercial post-broiler feed (Hanil Feed, Yongin, Korea) and tap water that did not include antibiotics and coccidiostat and tab water *ad libitum*. The chicks stayed in wire-floored cages during the study period. All studies were conducted in accordance with the guidelines for animal experiments and were approved by the Institutional Animal Care and Use Committee of Wonkwang University. Every effort was made to minimize the pain or discomfort of the animals participating in the experiment.

The research team evaluated the anticoccidial effect of HCE after oral infection in chicks by *E. tenella*. This study was performed on three-day-old chicks (n = 30). Three-day-old chicks were classified into three groups; HCE 0.2% treated/infected (n = 10), HCE untreated/infected (n = 10) and non-infected control (n = 10). We have determined the following subsequent doses of HCE as a concentrated additive to the recommended feed. Chicks were inoculated with or without HCE for a week prior to infection with *E. tenella* (10,000 sporulated oocysts per a chick). The effectiveness of HCE on *E. tenella* infection were assessed by two parameters, fecal oocysts numbers and weights gain.

Oocysts of *E. tenella* were cleaned by flotation on 5.25% sodium hypochlorite and washed three times with phosphate buffered saline. Chicks were treated orally by gavages using a 24 gauge, mouse stainless steel feeding tube (Popper & Sons, New York, NY, 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 chicken (n = 10) was provided with saline solution through the same route.

The disease rate and mortality rate of animals were confirmed twice a day during the study period. In addition, the clinical symptoms and weight gain changes in experimental animals were compared. Body weights were separately measured for 10 days after infection.

Feces were collected 6 to 10 days after infection. The fecal samples were analyzed for the presence of coccidial oocysts using standard fecal flotation techniques [9]. Briefly, 5 mL from

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each sample was centrifuged at 1,500 × g for 5 min. The produced pellets were redeposited in saturated sodium chloride (aqueous), passed through a 1 mm mesh size sieve to remove coarse fecal debris. The producesg filtrates were used for standard gravity vial stool suspension using a 22 mm × 22 mm cover slip. After floating, the coverslip was mounted on the slide and the entire presence of the coccidium follicle was examined. The total number of oocysts was calculated using the following formula: [total number of oocysts = oocyst count × dilution factor × (fecal sample volume / counting chamber volume) / number of birds per cage].

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

The extract yield of *H. cordata* by 50% ethanol was 21.50%. The HCE composition was analyzed by LC. The concentration of decanoyl acetaldehyde in HCE was 121.6 ug/g.

The chicks treated with HCE showed a significant reduction in fecal oocyst excretion and strong anticoccidial activity compared to the untreated control group ($p<0.01$).

As shown in Table 1, oocyst shedding was significantly higher in inoculated chickens than in controls ($p<0.05$). The number of excreted fecal oocysts was highest at 7 days after inoculation (Table 1). Moreover, the weight gain was less in the animals of the inoculated group than the animals of the control group (Table 2).

Coccidiosis in poultry is a global disease caused by absolute intracellular protozoa of the genus *Eimeria*. This disease causes considerable economic loss to poultry production. *E. tenella* is an important pathogen that causes avian coccidiosis in laboratory avian animals and is known to affect experimental results from contaminated animals [1, 10]. The disease is characterized by intestinal lesions of varying extent and severity, which reduce the absorptive function of the intestinal mucosa, resulting in weight loss, diarrhea, low feed conversion and

Table 1. Results of the number of oocysts from the feces of the studied chicks

Group	Oocysts numbers (× 10^6) / Days post infection				
	6	7	8	9	10
Control	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*
Infected control[1)]	12.3 ± 1.64	61.9 ± 5.63	36.1 ± 4.82	21.6 ± 4.03	15.8 ± 3.01
HCE + *Eimeria*[2)]	8.6 ± 2.22*	25.5 ± 3.63*	13.0 ± 3.09*	2.4 ± 1.43*	0 ± 0*

[1)] The chicks inoculated with *Eimeria tenella* oocysts.

[2)] Chicks inoculated with *Eimeria* live oocysts and treated with 0.2% *Houttuynia cordata* extract (HCE).

*Significantly difference with *Eimeria* infected control chicks ($p<0.05$).

Table 2. Results of changes in body weight of studied chicks

Group	Body weights (g) / Days post infection				
	1	3	5	7	10
Control	118.1 ± 1.73	138.5 ± 3.50*	179.9 ± 2.08*	219.9 ± 2.88*	252.4 ± 2.41*
Infected control[1)]	118.6 ± 2.37	127.0 ± 2.45	142.3 ± 2.75	178.9 ± 3.14	184.7 ± 4.06
HCE + *Eimeria*[2)]	117.4 ± 1.84	136.3 ± 1.06*	178.5 ± 1.72*	216.0 ± 3.37*	249.0 ± 2.91*

[1)] The chicks inoculated with *Eimeria tenella* oocysts.

[2)] Chicks inoculated with *Eimeria* live oocysts and treated with 0.2% *Houttuynia cordata* extract (HCE).

* Significantly difference with *Eimeria* infected control chickns ($p<0.05$).

high mortality in affected flocks [11].

Results of the study show that HCE has a strong anticoccidial effect on *E. tenella*. HCE contains a various components (terpenoids, hydrocarbons, esters, alcohols, ketones, aldehydes, acids, phenols, ethers and mixed compounds) and flavonoids (quercitrin, isoquercitrin, aphzeline, hyperin, rutin) [12]. Among compounds contained in the extracts, aldehydes such as lauryl aldehyde and decanoyl acetaldehyde (houttuynin) have antimicrobial effects against Gram positive bacteria and antifungal effects [5, 13].

This study estimated the anticoccidial effect of HCE in chickens after oral infection by *E. tenella*. Chicks fed HCE significantly reduced fecal oocysts when compared to *E. tenella*-infected group fed standard feed ($p<0.05$). In addition, the HCE-based diet improved weight loss due to *E. tenella* infection. Our experimental results showed that HCE has significant antiprotozoal activity against *E. tenella*. This finding may have implications for the development of anticoccidial drugs.

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