Anticoccidian effects of the *Artemisia absinthium* L. extracts in broiler chickens

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SUMMARY

Herbs are common resources in, both pure products and traditional drug production. This study aimed to investigate the occurrence of artemisinin in *Artemisia absinthium L* .and test the anticoccidian effects of plant extract in broilers. Anticoccidial effects of the plant extract were tested on chicken challenged with *Eimeria tenella.* The *in vivo* investigation was carried out on 150 heavy line broilers (*Arbor acres*) of both sexes. One hundred fifty, 21 day old chickens were divided into five groups consisting of 30 chicks each. At the age of 21 day, the chicken from first four groups (A to D) were infected with *Eimeria tenella* sporulated oocysts at a dose of 20.000 per chicken. The negative control consisted of group E chickens that were not infected or treated. The infected chickens were treated with *absinthium* extracts at the dosages of 1, 2 and 3 mg/kg per day. The results indicated that *Artemisia absinthium* extract can reduce the severity of coccidial infection induced by *Eimeria tenella.* The anticoccidial effects of *A. absinthium* extracts caused significant decrease in output number of oocysts per gram of faeces in chickens challenged with *Eimeria tenella.*

Keywords: broilers, *Eimeria tenella, Artemisia absinthium L.* extract, anticoccidian effects

INTRODUCTION

Coccidiosis is a common parasitic disease of broiler chickens caused by single-celled protozoan parasites of the genus *Eimeria* which are commonly referred to as coccidian (Cervantes,2008). Many anticoccidian drugs have been developed and introduced in the poultry industry all over the world. The increasing resistance of avian coccidian (protozoa) to anticoccidian drugs currently used by the poultry industry has stimulated the search for new methods of control (Allen et al., 1996). Therefore, there is need to find out the safe alternatives for the control of avian coccidiosis. In this context, a number

of plants and herbal products have been found to be effective for a broad range of parasites such as protozoa, arthropods and helminths (Akhtar and Rifaat, 1985; Jiang et al., 1985; Klayman, 1985). In addition to this knowledge, we have investigated *Artemisia absinthium* as a potential source of compounds with anticoccidian activity.

The genus *Artemisia* belongs to the family *Compositae* (*Asteraceae)* with over 300 species spread worldwide. In the past 8-10 years, new medicinal benefits were reported for several *Artemisia* species (spp.) due to the anti-parasitic effects of some artemisinin-based compounds and the high antioxidant capacity of crude extracts of some plants of this genus (Ferreira, 2009). The essential oil obtained from *Artemisia absinthium* wild plant show antibacterial, antifedant, antipyretic, fertility increasing, cytostatic and antimalarial activities (Kaul et al., 1976; Khattak et al., 1985). Although the chemical composition of *Artemisia absinthium* has not been fully characterized, a number of compounds including terpenes as limonene, myrcene, α and β thujone (Vostrowsky et al., 1981), the sesqueterpene, caryophellene (Tucker and Maciarello, 1993) and sabinyl acetate and chrysanthenyl acetate (Chilava et al., 1983) have been identified. Artemisinin is a secondary or natural plant metabolite identified as a sesquiterpene lactone endoperoxide (Klayman et al., 1984). Several methods have been used for the extraction of artemisinin from the plant. Extraction can be performed using various solvents: non polar, such as hexane (Klayman et al., 1984), polar, such as ethylacetate or ethanol (Rodriques et al., 2006), aromatic solvents such as toluene (Marchaud et al., 2007) hydrofluorinated solvents (hydrofluorocarbons (HFC-134a) (Lapkin et al., 2006), supercritical carbon dioxide (Marcel et al., 1997) or ionic liquids (Lapkin et al., 2006). For the purpose of this research the artemisinin content of the *Arthemisia absinthium* was extracted by hydro-distillation in a specially designed apparatus according to several European pharmacopoeias (German Pharmacopoeia, 1986; German Pharmacopoeia, 1993; Pharmacopoeia 6th ed., 2008). Our choice method, hydro-distillation, is the oldest and still widely used in many countries. It allows for easy separation of, resulting essential oils. Testing anticoccidian activity of Artemisia plants extracts on chickens has been intensified in recent years. Tests were conducted on different breeds of chickens, using different concentrations of extracts in correlation to the number of artificially entered *Eimeria* oocysts (Allen et al., 1997; Arab et al., 2006; Dragan et al., 2010). It was found that anticoccidial activity of Artemisia plant extracts depends on the number of oocysts and the type of Eimeria, and not on the broiler breed. This study aimed to evaluate the anticoccidian effects of the *Artemisia absinthium L.* extract in chickens challenged with species of *Eimeria tenella.*

MATERIAL AND METHODS

Chickens and Housing

The experimental protocol was approved by the local Ethics Committee and the principles of animal protection strictly followed. Experiments under *in vivo* conditions were performed on 150 broilers of both sexes of the heavy Arbor acres line. Age of parent flock was between 35 and 45 weeks, when the average weight of eggs was about 60 g. All chicks were received as one day old and raised in a clean and disinfected room under standard conditions. Birds were fed standard basal diet. They had access to water and food *ad libitum* and faecal samples were taken daily to monitor the possibility of infection. Temperature and lighting regimens were in accordance with the recommendation of the breeder. The initial room temperature 32-33° C was reduced weekly by 1°C to a final temperature of 28°C.

Challenge infection of chickens

The birds were randomly divided into non-infected and infected groups. The animals in infected groups were challenged with *E. tenella* oocysts. The broilers were challenged with sporulated oocysts, collected from infected chicken farms and prepared in the laboratory. The challenge infection of 10-day-old chickens via oocysts was performed by oral administration of oocyst suspension.

Parasite and dose

Coccidial oocysts of *E. tenella spp.* were obtained from the guts of infected chickens and administered in broiler chickens by oral suspension. The oocysts were preserved in 2.5% potassium dichromate solution to induce sporulation and kept in a refrigerator at 2-5°C until use. Each bird was challenged with 20,000 oocysts of *E. tenella* at the age of 21 day.

Preparation of Artemisia absinthium L. extract

The plant (*Artemisia absinthium L.)* was obtained from the Institute for Medicinal Plant Research „Dr Josif Pančić“, Belgrade, Serbia. The extract was prepared from dried plant material by hydro-distillation (Woerdenbag and Pras, 2001). The amount of 50.0 g of freshly powdered plant *A. absinthium* was distilled in a 1,000 cm^3 flask, with 500 cm^3 of water as distillation liquid and 0.50 cm^3 xylene as the collection liquid. The distillation rate was 2-3 cm^3 per minute and the distillation time was 2 hours. The distillation was performed using Clevenger type apparatus. The extract was dried over anhydrous calcium chloride and stored in sealed vials at 2°C before the analysis by HPLC (Arab et al., 2006).

Experimental protocol

This experiment was conducted to evaluate the effects of *Artemisia absinthium* extracts on coccidian infection caused by *E. tenella.* Four groups of chickens (A to D) 21 days old, were challenged with 20,000 *E. tenella* oocysts per chick by oral administration. The birds in group E were the negative control (non-infected). Twenty-four hours after infection, chickens in B, C and D groups received 1, 2 and 3 mg/kg *Artemisia absinthium* extract per day respectively, via oral administration. The extract was given to the chickens three times a day for 5 days. The oocyst output was measured daily in each group, during the period from 6th to 9th day after the infection. The medication with herbal extracts was started according to the following schedule.

Group A: Infected, un-medicated control;
Group B: *Artemisia absinthium* extract: 1 mg/kg per day;
Group C: *Artemisia absinthium* extract: 2 mg/kg per day;
Group D: *Artemisia absinthium* extract: 3 mg/kg per day;
Group E: Uninfected, un-medicated control.

The means of OPG (oocysts per gram of faeces) output in treated groups were compared with non-treated control groups to evaluate the effects of the plant extract on avian coccidiosis induced by *E. tenella.* Bloody diarrhoea was investigated from 4^{th} to 6^{th} day after the challenge. The extent of bloody diarrheal score was assigned one of the four degrees, from 0(-) to 3(+++). Zero was the normal status, whereas 1, 2 and 3 corresponded to 33, 33-66, 66-99% blood in total faeces, respectively.

Statistical analysis

Data obtained from analytical tests and *in vivo* experiments were expressed as mean ± SEM from at least thirty experiments (anticoccidian effects). The mean value for each group was analysed and compared with other groups using Student's *t*-test. *P*-Values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The essential oil from leaves of *Artemisia absinthium* plant were extracted by hydro-distillation method and analysed by HPLC. The essential oil yield (cm^3/100 mg of dried substances) was 0.57%. The principal constituent of the *Artemisia absinthium* extract was the α-zhujone (39.69%). The yield of essential oil obtained by hydro-distillation was 1.3 times higher than the yield obtained by extraction with toluene (0.44%) (Mannan et al., 2010), but 8 times

less than the yield achieved by supercritical fluid extraction with CO_2 (Martin et al., 2011). Since the distillation extraction procedure is simple and fast and also provides a satisfactory extract yield, it can be used for the extraction of essential oils from the plant *Artemisia absinthium*.

Bloody diarrhoea in all experimental groups, except the uninfected control group, was observed from the fourth to sixth day after the infection with *E. tenella.* The intensity of bloody diarrhoea in all chicken groups is shown in Table 1.

Table 1. Bloods diarrhoea of chickens treated with *Artemisia absinthium* extract and challenged with *Eimeria tenella*

Groups	Blood in faeces (days after infection)				
	3	4	5	6	7
Infected, un-medicated control	-	+	+++	+	-
1 mg/kg *Artemisia absinthium* extract	-	+	++	+	-
2 mg/kg *Artemisia absinthium* extract	-	+	++	-	-
3 mg/kg *Artemisia absinthium* extract	-	+	+	-	-
Uninfected, un-medicated control	-	-	-	-	-

Administration of *Artemisia absinthium* extract to the infected chickens was shown to be associated with the reduction of oocyst output. The summary of statistical values obtained from 30 chickens in each test group is shown in Table 2.

Table 2. Oocyst excretions and mortality of chickens treated with *Artemisia absinthium* extract and challenged with *Eimeria tenella*

Test group	Average oocyst count g^{-1}		Mortality rate %
	Before treatment	After treatment	
Infected control	2,867	34,800	12(30)
1 mg/kg *Artemisia absinthium* extract	3,670	13,093	6(30)
2 mg/kg *Artemisia absinthium* extract	2,866	10,060	5(30)
3mg/kg *Artemisia absinthium* extract	4,000	1,336	5(30)
Uninfected control	-	-	-

After the challenge with *E. tenella*, the bloody diarrhoea and numbers of excreted oocysts in faeces were investigated during two weeks. Bloody diarrhoea in all the experimental groups, except the uninfected control group, was recorded during 4-6 days after the infection with *E. tenella.* But the extent of bloody diarrhoea in the groups treated with 3 mg/kg *Artemisia absinthium* extract was milder than in the other groups. Excreted oocysts in the groups treated 1 and 2 mg/kg *Artemisia absinthium* extract were relatively lower than in the infected control group. In the groups treated with 3 mg/kg *Artemisia*

absinthium extract, the peak excretion of oocysts was delayed for about 1 or 2 days relative to the control infected group. However, the non-treated chickens infected with *E. tenella* showed significant excretion of oocysts in faeces (Table 2).

The oocysts output and mortality rate were lower in all the treated groups as compared to the infected un-medicated control group. Comparing the medicated groups, the birds treated with 3 mg/kg *Artemisia absinthium* extract showed better results in terms of oocyst count per gram of faeces and mortality rate as compared to those treated with 1 and 2 mg/kg *Artemisia absinthium* extract. The counts were zero in uninfected groups. The parasite was not completely suppressed by any of the treatments.

Table 3 shows the numbers of oocysts collected each day from the faeces of broilers on days 6-9 after the infection.

Table 3. Effects of *Artemisia absinthium* extract on coccidian infection induced by *Eimeria tenella*

Test group	OPG output at given post-infection day				Total number of excreted oocysts
	Day 6	Day 7	Day 8	Day 9	
E. tenella infected group (untreated)	9,455	9,818	9,091	2,636	31,000
E. tenella infected group (treated with 1 mg/kg *A. absinthium* extract	9,090	8,727	9,091	2,073	28,981
E. tenella infected group (treated with 2 mg/kg *A. absinthium* extract	9,820	9,454	2,364	1,090	22,728
E. tenella infected group (treated with 3 mg/kg *A. absinthium* extract	5,091	4,000	18	16	9,125*

Values are from 30 birds in each group;*Significant difference ($p < 0.05$) between treated and untreated groups.

As shown in Table3, the oocyst output obtained from the fourth group (infected with *Eimeria tenella* and treated with 3 mg/kg *Artemisia absinthium* extract) decreased significantly from 5091 on day 6 to only 16 as counted on the day 9. However, in the untreated birds group, the OPG values were in the range of 9455-2636. There was no significant difference between non-treated broilers infected with *Eimeria tenella* and infected treated group with 1 mg/kg *A. absinthium* extract in terms of OPG values (Table 3).

Artemisia absinthium extract reduced oocyst output in broilers infected with *Eimeria tenella.* It was found that doses of 2 and 3 mg/kg per day were optimum for coccidian infection treatment. Based on these results, it is concluded that the extract *A. absinthium* at a dose of 3 mg / kg of feed for broilers reduces the number of oocysts in broilers infected with *E. tenella* and can be used for the prophylaxis of moderate coccidiosis. We believe that the applied dose of extract does not depend on the breed of chickens, but may

depend on their age. The young chickens are more receptive for coccidiosis, but administering *A. absinthium* extract at a dose of 3 mg/kg can successfully treat the disease.

Conclusions

This study demonstrated that the evaluated plant, *Artemisia absinthium,* showed anticoccidian activity. Maximum coccidiostatic effect was observed with *Artemisia absinthium* extract of 3 mg/kg per day, significantly reducing bloody diarrhoea as compared to the other infected groups receiving 1 and 2 mg/kg *Artemisia absinthium* extract. *Artemisia absinthium* could be a potential source of protection agents against coccidiosis.

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