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ORIGINAL ARTICLE

Anticoccidial effects of *Morinda lucida* acetone extracts on broiler chickens naturally infected with *Eimeria* species

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Abstract

Context: The development of drug resistance to *Eimeria* species in poultry is a reality on farms. This necessitates chemotherapeutic control alternatives, and plant secondary metabolities with activity is one of those potential solutions.
Objective: This study was designed to evaluate the efficacy of acetone leaf extract of *Morinda lucida* Benth. (Rubiaceae) against coccidiosis in broiler chickens.
Materials and methods: Acute toxicity of the plant was investigated using 30 one-day-old broiler chicks. Anticoccidial activity investigations were carried out in birds drenched with 2.0, 3.0, 4.0, 5.0 and 6.0 g/kg body weight (BW) of acetone extract of *M. lucida* leaves once a day for five days, with toltrazuril® and untreated controls. The activity was evaluated by means of oocyst inhibition in feces, fecal score, weight gain, mortality and hematological parameters.
Results: No sign of toxicity was observed during the acute toxicity test. Fecal oocyst counts decreased steadily in all the treatment groups with time. The anticoocidial efficacy of birds treated with 2.0, 3.0, 4.0, 5.0 and 6.0 g/kg BW of the extract was 91.2, 95.2, 98.7, 99 and 99.5%, respectively. The packed cell volumes, red blood cell counts and white blood cell of the treated birds were not significantly different ($p > 0.05$) from the untreated control. However, haemoglobin concentration and weight gain of the treated birds were significantly different ($p < 0.05$) from the untreated control.
Conclusion: This study is the first to demonstrate the anticoccidial effect of *M. lucida* on *Eimeria* parasites. *Morinda lucida* leaves could therefore find application in anticoccidial therapy.

Keywords

Chemotherapy, coccidiosis, oocyst, plant extract

History

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Introduction

Avian coccidiosis is one of the most important diseases of poultry worldwide. The parasites multiply in the intestine after tissue damage, lowered feed intake, poor absorption of nutrients from the feed, dehydration and blood loss (Seifert, 2006).

With the widespread use of anticoccidial drugs, resistance has developed to all the drugs introduced (Abbas et al., 2011a; Chapman, 1998). An additional, constraint in their use comes from the consumer and the ever-increasing need for the drug-free production of foods (Harper & Makatouni, 2002). However, the development of drug resistance by the causative parasites and the escalating cost of drug development have greatly reduced the commercial incentive to develop new chemical anticoccidials (Oluyemi & Roberts, 2000). Consequently, in recent years, the development of alternative, safer and environmentally friendly anticoccidial agents have become a priority in most parts of the world (Abbas et al., 2010, 2011b,c, 2012a,b; Zaman et al., 2012).

Morinda lucida Benth. (Rubiaceae) is a tropical West Africa rainforest tree also called Brimstone tree (Dalziel, 1973). In Nigeria, *M. lucida* is one of the four most used plants in the preparation of traditional medicines against fever. Decoctions and infusions or plasters of root, bark and leaves are recognized remedies against different types of fever, including yellow fever, malaria, trypanosomiasis and feverish condition during childbirth. The major constituents of *M. lucida* extract are various types of alkaloids, anthraquinones and anthraquinols (Adesogan, 1973). Two compounds (oruwalol and oruwal) and 10 anthraquinones have been isolated and characterized from the stem of the plant (Lawal et al., 2012). The acute oral toxicity result, LD_{50}, revealed *M. lucida* leaf extract to be non-lethal at 6.4 g/kg body weight (BW) in Wistar albino rats (Taofeeq et al., 2010). The possible use of *M. lucida* as anticoccidial was hypothesized on the basis of its wide use as an antimalarial agent by herbalists in Nigeria, and *Eimeria* species (causative agent of avian coccidiosis) belongs to the same phylum, Apicomplexa, with *Plasmodium* parasite (causative agent of malaria). In this study, we investigated the anticoccidial activity of the acetone leaf extract of *M. lucida* against *Eimeria* sp. in broiler chickens.

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Materials and methods

Parasites

The birds were naturally infected with mixed field species of *Eimeria*.

Collection of plant and extraction

Morinda lucida leaves were obtained from Ibadan, south-western Nigeria, in May and June 2012 and identified and authenticated by Prof. Ayodele AE in the herbarium of the Department of Botany, University of Ibadan, Nigeria, where a voucher specimen was deposited with voucher number UIH22346. The leaves were air-dried. The dried leaves (1100 g) were reduced to a fine powder by grinding with a milling machine and extracted. Extracts were prepared by maceration with intermittent shaking for 72 h in 70% acetone with a 10:1 solvent to dry weight ratio (1000 g:10 L) (Eloff, 1998). The extracts were filtered using Whatman No. 1 filter paper and a funnel, and the acetone removed under a stream of air and stored in a dark container at 4 °C until use.

Diets and management

One hundred one-day-old Anak broiler chickens (Zartech®, Ibadan, Nigeria) were purchased and fed *ad libitum* on a proprietary broiler ration (Top feeds®, Ibadan, Nigeria) without coccidiostat additives and also given access to water *ad libitum*. They were routinely vaccinated against Newcastle and Gumboro diseases.

Birds and experimental design

The preliminary acute toxicity study was conducted by using 30 one-day-old broiler chickens that were divided into five groups of six chickens each. Each bird in groups A–E was individually drenched with the graded doses of the acetone extract of *M. lucida* to be tested. The chickens were observed for 24 h for any sign of toxicity (eye blinking, panting, lethargy, salivation and incordination), including change in behavior or death.

A total of 70, one-day-old Anak broiler chickens of both sexes were reared as a single group on used litter. At 14 days of age, all experimental birds were adequately infected with field strain of *Eimeria* spp. and fecal samples were assessed (in triplicate) for the number of oocyst per gram (OPG) of feces prior to treatment, which ranged from 21 300 to 23 100.

Birds were randomly assigned to 7 treatment groups (A–G) according to the oocyst level in feces with 10 birds each. Group A was the untreated group (negative control) where the birds received no treatment, group B (positive control) was treated with toltrazuril (Vazuril® Batch No. 1074, Vapco product, Jordan) orally at a dose rate of 1 ml/L (7 mg/kg) for two days as prescribed by manufacturers, groups C–G were individually drenched on day 15 with 2.0, 3.0, 4.0, 5.0 and 6.0 g/kg BW, respectively, of acetone extract of *M. lucida* leaves once a day for five days. A treatment period of five days was selected as this was the estimated period of oxidant insult induced by the coccidian parasite (Koinarski et al., 2005).

Data collection

Pooled daily oocyst counts per group were undertaken. Individual oocyst counts were not feasible because the birds were housed in group. A total of 10 g of feces was collected daily beginning from a day before treatment until the seventh day post treatment. The modified McMaster technique as described by Vassilev (2002) was used to estimate OPG. This was repeated three times and the mean value was calculated.

Birds from each treatment group were weighed individually on a daily basis throughout the treatment period and the mean BW gains were calculated. The mortality was determined using the formula: percent mortality = (total number of dead chicks in the group/initial number of birds in group) × 100 and evaluated for death rate and hazard analysis after being exposed to the infectious agent in the absence and presence of the herbal extract.

From day 0–7 post treatment (i.e., day 15–21 of birds), the extent of bloody/tan 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/tan color in total feces, respectively. Blood samples (2 ml) were collected from the wing venipuncture from three chickens in each group at the onset of the study, day 3 of the study and after the study. The PCV was determined by the microhaematocrit method (Goldenfarb et al., 1971). The values found were expressed as a percentage of the total blood volume. Red blood cell (RBC) and white blood cells (WBC) were counted using a haemocytometer (Jain, 1986). Haemoglobin (Hb) concentration was determined by the standard cyanometahemoglobin method as described by Jain (1986) and its values were expressed in g/ml of blood.

Statistics

The relative bioactivity of the various concentrations of the extracts and the positive and negative controls were assessed by comparing the oocyst inhibition values by one-way ANOVA and Tukey's multiple comparison test. Weight gains and haematological parameters for each group for the different time points were also compared using one-way ANOVA and Tukey's multiple comparison tests to ascertain if weights differed significantly over the growth period. All data analyses were performed using GraphPad Prism version 5.0 for Windows (San Diego, CA).

Results

Yield of extract and preliminary acute toxicity test

The acetone extract of *M. lucida* gave a yield of 120 g (10.90% w/w). The preliminary acute toxicity test conducted revealed that there was no sign of toxicity (eye blinking, panting, lethargy, salivation and incordination) neither was there mortality in any of the treated groups of broiler chicken, including those drenched with the highest dose (6.0 g/kg) of acetone extract of *M. lucida* leaves.

Oocyst output in feces

The acetone extract of *M. lucida* leaves demonstrated an inhibitory effect on the shedding of oocyst in feces in boiler chickens in a concentration-dependent manner (Figure 1). The group treated with toltrazuril gave the highest oocyst

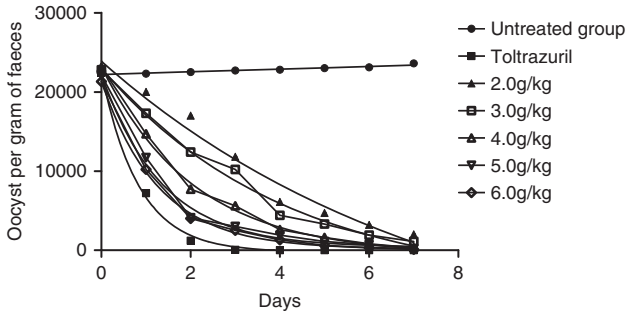

Figure 1. Oocyst production over time for each of the treatment groups.

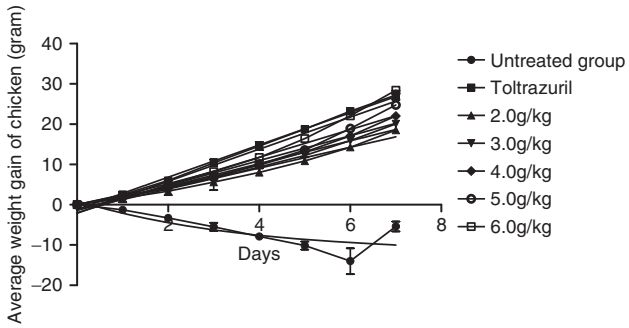

Figure 2. Average weight gains per treatment group over time.

inhibitory effect on oocyst shed in feces (100%) indicating that it is more effective than the other treatment groups, although not to a significant level ($p > 0.05$). In the untreated group (control), there was an increase in oocyst shed in feces. The group treated with 2.0 g/kg BW of the extract produced the least effect on oocyst shed in feces (91.2%), while the groups treated with 3.0, 4.0, 5.0 and 6 g/kg BW of the extract produced 95.2, 98.7, 99.1 and 99.5% inhibition effect on oocyst shed in feces, respectively. The oocyst shed in all the treated groups significantly ($p < 0.05$) decreased compared with the untreated control.

Weight gain

Changes in the BW of broiler chickens infected with species of *Eimeria* before, during and after treatment are shown in Figure 2. The BW of the untreated group decreased by 14.0%, while the group treated with toltrazuril increased by 27.0% and the group treated with 2.0, 3.0, 4.0 5.0 and 6.0 g/kg BW of acetone extract of *M. lucida* leaves increased by 18.0, 20.0, 22.0, 25.0 and 28.0%, respectively. The weight gain observed in all the groups treated with of the *M. lucida* shows a significant difference ($p < 0.05$) compared with the untreated group. However, there was no significant difference ($p > 0.05$) between the group treated with 6.0 g/kg BW of acetone extract of *M. lucida* and the other treated groups. One bird died on day 6 and another died on day 7 in the untreated group.

Faecal score

Bloody/tan color diarrhea was observed in all the treatment groups at the onset of treatment as shown in Table 1. In the group treated with toltrazuril, the extent of bloody/tan color diarrhea was milder than those observed in other groups. The colored diarrhea change observed in the groups treated with acetone extract of *M. lucida* leaves was in a concentration-dependent manner.

Table 1. Fecal score of broiler chickens naturally infected with mixed species of *Eimeria* and treated with toltrazuril and acetone extract of *M. lucida* leaves.

Group	A	B	C	D	E	F	G
Day				($n = 10$)			
0	++	+++	++	++	++	++	++
1	++	+	+++	++	++	+++	++
2	++	+	++	++	++	++	+
3	+++	−	++	++	++	+	+
4	+++	−	+	+	+	+	+
5	++	−	+	+	+	+	−
6	++	−	+	+	+	−	−
7	+	−	+	−	−	−	−

A, Untreated group; B, treated with toltrazuril; C, 2.0 g/kg BW of *M. lucida*; D, 3.0 g/kg BW of *M. lucida*; E, 4.0 g/kg BW of *M. lucida*; F, 5.0 g/kg BW of *M. lucida*; G, 6.0 g/kg BW of *M. lucida.*

Haematology

The mean PCV, Hb, WBC and RBC are shown in Table 2. The mean Hb concentration after treatment of the broiler chickens with the various doses of acetone leaf extract of *M. lucida* was significantly ($p < 0.05$) higher than the untreated group, while the PCV, WBC and RBC were not significantly ($p > 0.05$) different compared with the untreated control.

Discussion

In the preliminary acute toxicity test, *M. lucida* proved to be safe at 6 g/kg BW. The use of *M. lucida* was hypothesized on the basis that it possess anti-malaria properties (Tona et al., 1999), and *Eimeria* belongs to the same phylum Apicomplexa, as *Plasmodium* parasite, the causative organism of malaria. The usual biological similarities in organisms exemplified by taxonomic classification were expected to manifest in a similar selective toxicity profile of phytochemicals in *M. lucida* toward *Plasmodium* and *Eimeria* species.

The group treated with 6.0 g/kg BW of *M. lucida* leaves extracts produced the highest percentage of *Eimeria* oocyst reduction on the 7th day of our study. However, the percentage reduction is lower than the group treated with toltrazuril, although the difference was not significant ($p > 0.05$). The anticoccidial effect of *M. lucida* could be attributed to its antioxidant properties. Ogunlana et al. (2008) reported the antioxidant properties of *M. lucida* and that the antioxidant constituents are the phenolic compounds. Allen et al. (1998) state that plants with antioxidant effect possess anticoccidial activities. The slight difference in the oocyst reduction between the synthetic drug used and the plant extracts can be explained by the presence of small concentration of the active ingredient in the plant extracts compared with the synthetic anticoccidial where the chemical compounds are isolated in pure form. The higher percentage oocyst reduction observed in the group treated with 6.0 g/kg BW over the other groups treated with the extracts explains the dose-related response. Naidoo et al. (2008) who used *Artemisia afra* leaves at 150 mg/kg BW and *Tulbaghia*

Table 2. Hematological parameters of broiler chickens naturally infected with mixed species of *Eimeria* before, during and after treatment with toltrazuril and acetone extract of *M. lucida* leaves.

Groups (10 birds)[a]	A	B	C	D	E	F	G
Parameters							
PCV[b] (%)	22.6 ± 0.28	22.9 ± 0.14	22.2 ± 0.14	23 ± 0.00	22.5 ± 0.14	22.5 ± 0.14	22.1 ± 0.00
PCV[c] (%)	19.4 ± 0.28	24.7 ± 0.14	22.4 ± 0.00	23.2 ± 0.14	22.8 ± 0.14	23 ± 0.00	22.9 ± 0.00
PCV[d] (%)	17.5 ± 0.42	26.3 ± 0.14**	23 ± 0.14	23.5 ± 0.14	23.9 ± 0.00	24 ± 0.14	24.2 ± 0.14
Hb[b] (g/dl)	6.5 ± 0.14	7.1 ± 0.14	7.1 ± 0.14	7.7 ± 0.14	7.3 ± 0.14	8.1 ± 0.14	7.9 ± 0.00
Hb[c] (g/dl)	5.3 ± 0.14	7.6 ± 0.00	7.7 ± 0.14	7.6 ± 0.00	7.7 ± 0.14	8.4 ± 0.14	8.6 ± 0.14
Hb[d] (g/dl)	4.8 ± 0.14	8.4 ± 0.28**	8.7 ± 0.14**	8.2 ± 0.14**	7.5 ± 0.14*	8.8 ± 0.14***	8.7 ± 0.14***
RBC[b] (×106/μL)	2.5 ± 0.42	2.6 ± 0.00	2.6 ± 0.00	2.4 ± 0.14	2.1 ± 0.14	2.5 ± 0.00	2.7 ± 0.14
RBC[c] (×106/μL)	2.7 ± 0.14	2.7 ± 0.14	2.9 ± 0.14	2.7 ± 0.00	2.6 ± 0.00	2.6 ± 0.14	2.8 ± 0.14
RBC[d] (×106/μL)	1.4 ± 0.57	3.7 ± 0.14	3.2 ± 0.00	2.9 ± 0.00	2.7 ± 0.14	2.8 ± 0.14	3.1 ± 0.14
WBC[b] (×103/μL)	15.8 ± 0.14	15.4 ± 0.14	15.6 ± 0.14	15.9 ± 0.00	15.6 ± 0.00	15.4 ± 0.14	15.05 ± 0.07
WBC[c] (×103/μL)	16.4 ± 0.14	15.1 ± 0.14	14.7 ± 0.14	16.5 ± 0.14	12.9 ± 0.07	15.5 ± 0.14	14.8 ± 0.07
WBC[d] (×103/μL)	17.7 ± 0.14	14.4 ± 0.07	14.8 ± 0.07	15.5 ± 0.14	15.3 ± 0.00	15.05 ± 0.07	14.5 ± 0.07

[a]See legend of Table 1 for definitions of A–G.
[b]Day 0 before treatment.
[c]Fourth day post treatment.
[d]Seventh day post treatment.
*Significant.
**Moderately significant.
***Highly significant.

violacea leaves at 35 mg/kg BW obtained 66.7 and 44.7% oocyst reduction, respectively, at day 7 of their study.

The acetone extract of *M. lucida* leaves at different concentrations had a good dose-related response on the improvement of BW in the broiler chickens. There was no significant difference ($p > 0.05$) between the BW gain of the group of chickens treated with toltrazuril and all the other treated groups. The increase in weight gain can be attributed to the decrease in the number of *Eimeria* oocyst in the intestine. However, the untreated group lost 14.0% of their initial weight; this could be as a result of the damaging effect caused by the coccidial parasite in the intestine of the bird which impaired the absorption of food and water. The improvement in BW of birds treated with the *M. lucida* could also be attributed to the decrease in oocyst shed in feces, which translates to reduction of damage to the intestinal epithelium by the sporozoites of the parasite. The reduction in the damage to the intestinal lumen of the birds treated with the extract could be responsible to the decrease in blood/tan diarrhea observed in the treated groups. On the 5th day of our study, there was the absence of bloody/tan colored in the feces of the group of birds treated with 6.0 g/kg BW of *M. lucida*.

One bird each died in the untreated group on the 6th and 7th day and postmortem examination suggests coccidiosis due to *Eimeria* species. Mortality due to infection with *Eimeria tenella* and field isolate of *Eimeria* species has been reported by Guo et al. (2007).

The hematological parameters of broiler chickens infected with species of *Eimeria* show a decrease in the PCV, RBC, Hb and an increase in WBC on day 0. The decrease in PCV, RBC and Hb could be due to the blood loss associated with the disease. Ellakany et al. (2011) reported a significant decrease in packed cell volume, Hb concentration and lymphocyte percentage in *E. tenella* infected broilers. Broiler chickens treated with acetone extract of *M. lucida* had an increase in the values of the hematological parameters post treatment. The improvements in the hematological parameter can be attributed to the daily reduction in the oocyst shed in feces. *M. lucida* could also possess hemopoetic properties. Asuzu and Chineme (1990) reported that *M. lucida* leaf extract increase the packed cell volume and Hb concentration in mice infected with *Trypanosoma brucei brucei*.

Conclusion

Since *M. lucida* is reported to be safe and widely available and can be easily cultivated, it could therefore serve as a useful alternative product for the control of avian coccidiosis in poultry production. However, further spectroscopic studies on the active principles and the development of quality assurance protocols involving the use of reference substance of plant origin for this extract is warranted. Unambiguous structure elucidation of the active principles could provide leads for drug discovery and suitable bioactive marker compounds for standardization of the extract as a phytomedicine.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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