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Effects of Methanolic Leaf Extract of *Lannea schimperi* on Some Organs Histopathology in Experimentally Induced Coccidiosis in Broiler Chickens

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Abstract

The most pathogenic species of *Eimeria* that infect chickens is *Eimeria tenella*. It is a major cause of coccidiosis, a self-limiting infectious disease of the digestive tract of chickens. In view of the resistance to the conventional anticoccidial drugs used in poultry, herbal extracts are often considered as safe alternatives. The present study was aimed at investigating the effects of methanolic leaf extract of *Lannea schimperi* on histopathological changes in the intestines, liver and kidneys of chicken infected with *Eimeria tenella*. Seven groups of 4 broilers chickens were used for the experiment. Groups I, II and III were treated with 25, 50 and 100 mg/ml of *L. schimperi* methanolic leaf extract respectively after experimental infection with *E. tenella*. Group IV was treated with amprolium after experimental infection with *E. tenella*, group V was infected but not treated, while, groups VI and VII were uninfected with *E. tenella* but treated with 25 and 100 mg/ml of *L. schimperi* extract respectively. Our findings revealed that the extract has reduced the growth and multiplication of *E. tenella* oocysts in all the treated groups compared to the infected/untreated group. No specific histopathological lesions were observed in groups treated with only the extract at both 25 and 100 mg/ml. However, some histopathological changes were observed in the group infected but treated with higher dose of the extract (Group III) and in the negative control group (Group V), these changes were not observed in the positive control group treated with amprolium. Our current findings suggests that the lesions observed could be due to the disease processes rather than treatment with the extract and methanolic leaf extract of *L. schimperi* could possess coccidiostatic potential that may require further scientific investigations.

Keywords: Methanolic Leaf Extract; *Lannea schimperi*; Broiler Chicken; Histopathology

Introduction

Coccidiosis caused by the apicomplexan protozoan *Eimeria* is recognized as the major parasitic disease of poultry [1,2]. The most pathogenic species of *Eimeria* that infect chickens is *Eimeria tenella*. Coccidiosis is a self -limiting infectious disease of the digestive tract and is a major threat to poultry production [3-5]. *Eimeria tenella* is located usually in the caecum causing caecal coccidiosis. Different stages of *Eimeria* penetrate the villus epithelial cells and invade the caeca, causing extensive destruction of caecal epithelium, bloody dropping, decrease in feed efficiency, decrease in body weight gain and finally death [6,7].

The conventional strategic ways of coccidiosis control have depended mainly on the use of anticoccidial drugs and, to a certain extent the use of live vaccines [1]. However, little is known about the use of safer herbal extracts alternatives in the control of avian coccidiosis. The plant *Lannea schimperi* is a tree up to 10-15 m tall; stunted bole short and low branching. The tree is distributed in Cameroon, Togo, northern Nigeria, Ethiopia, Tanzania etc. [8]. The fruits and seeds of *L. schimperi* are eaten fresh by children in Nigeria and throughout East Africa especially during rainy season [8]. Domestic animals feeds on the branchlets and leaves, while, the flowers probably serve as source of nectar for honey bees [8]. Methanolic leaf extract of *L. schimperi* has been reported to possess anticoccidial effect *in vitro* [9]. The median lethal dose of the plant methanolic leaf extract was reported to be 288.53 mg/kg in mice following intraperitoneal administration [10]. The emerging alternative way to combat coccidiosis is the natural products therapy [11]. Not fewer than four plant products are commercially available in the market which could be used as anticoccidial

feed additives in chickens and/or other animals, these products include Cocci-Guard (DPI Global, USA), a mixture of *Quercus infectoria, Rhus chinensis, and Terminalia chebula* (Kemin Industries, USA), *Apacox* (GreenVet, Italy) and *BP* formulation made up of *Bidens pilosa* and other plants (Ta-Fong Inc., Taiwan) [12-15]. Interestingly, investigation of compounds or their active ingredients contain in anticoccidial plants may inspire research and development of anticoccidial chemicals [11]. Halofuginone is a very good example of a successful synthetic halogenated derivative of febrifugine initially identified from antimalarial plant, Chang Shan (*Dichroa febrifuga*) [16]. However, there are several challenges regarding the anticoccidial use of natural products that need to be overcome prior to applications such as efficacy, safety, identification of active ingredients, mechanism of action, and cost-effectiveness of plant extracts and/or their active compounds [11].

The present study was aimed at investigating the effects of methanolic leaf extract of *L. schimperi* on histopathological changes in the intestines, liver and kidneys of chicken experimentally infected with *E. tenella* oocysts.

Materials and Methods

Plant Collection, Identification, Processing and Extraction

The plant was collected fresh and identified by U.S Gallah of National Research Institute of Chemical Technology (NARICT) Zaria, Kaduna State, Nigeria. A voucher specimen was deposited numbered 0512 at the departmental herbarium. The cleaned fresh leaves were air dried and pounded with pestle and mortar into fine particles. The fine plant material was weighed and extracted by maceration for 72 hours in absolute methanol [17]. The extracts were filtered and evaporated to dryness; the dried extract was kept in capped bottles inside refrigerator at temperature of 4 °C until needed. Phytochemical analysis of the methanolic leaf extract of *Lannea schimperi* has been conducted previously [18].

Eimeria tenella Isolate

Coccidial oocysts of *Eimeria tenella* isolated from the caeca of naturally infected chicks were used for the study. Sufficient oocysts were recovered after propagation from the caeca of infected chicks using the centrifugal floatation technique [19]. The isolated oocysts were allowed to sporulate in 2.5% potassium dichromate solution at room temperature which was used for testing the efficacy of the different plant extract concentrations on the inoculated sporulated oocysts [20].

Infection of Experimental Chicken Groups

Chickens in groups one to five were infected orally with1 ml containing 1.0 x 10^3 sporulated oocysts of the *E. tenella* isolate. The study was approved by the University of Abuja Ethical Committee on Animal Use (UAECAU/2018/0002) and was conducted in accordance with the approved protocol.

Preparation of Drug Solutions

The dried extract was weighed and reconstituted in distilled water just before use during the experiment, a solution of 25, 50 and 100 mg/ml respectively were prepared by dissolving 25, 50 and 100 g of the dried methanolic leaf extract of *Lannea schimperi* into 1 liter (1000 ml) each of distilled water. Amprolium 250 WSP (KEPRO B.V./ Holland) was used, 1.5 g of amprolium was dissolved in 1 liter of water to produce a drug solution of 1.5 mg/ml.

Treatment of Experimental Chickens

Seven groups of four broilers chickens' age two weeks were used for the experiment. Groups I, II and III were treated with 25, 50 and 100 mg/ml of *L. schimperi* methanolic leaf extract respectively after infection with *E. tenella*. Group IV was treated with amprolium at the dose of 1.5 mg/ml after infection with *E. tenella*, group V was infected but not treated, while, groups VI and VII were treated with 25 and 100 mg/ml of *L. schimperi* methanolic leaf extract only without being infected with *E. tenella* oocysts. All treatments were given orally for seven days through drinking water.

Tissue Processing for Histopathology

Two chickens from each group were scarified on day 7 post infections (p.i.). Tissues from the caeca were fixed in 10% formal saline solution and in 50% alcohol for 4-6 hour and stored in 70% alcohol then dehydrated in 80%, 90%, 95% and absolute. Finally the specimens were embedded in paraffin wax, sectioned and stained with H&E. [21,22].

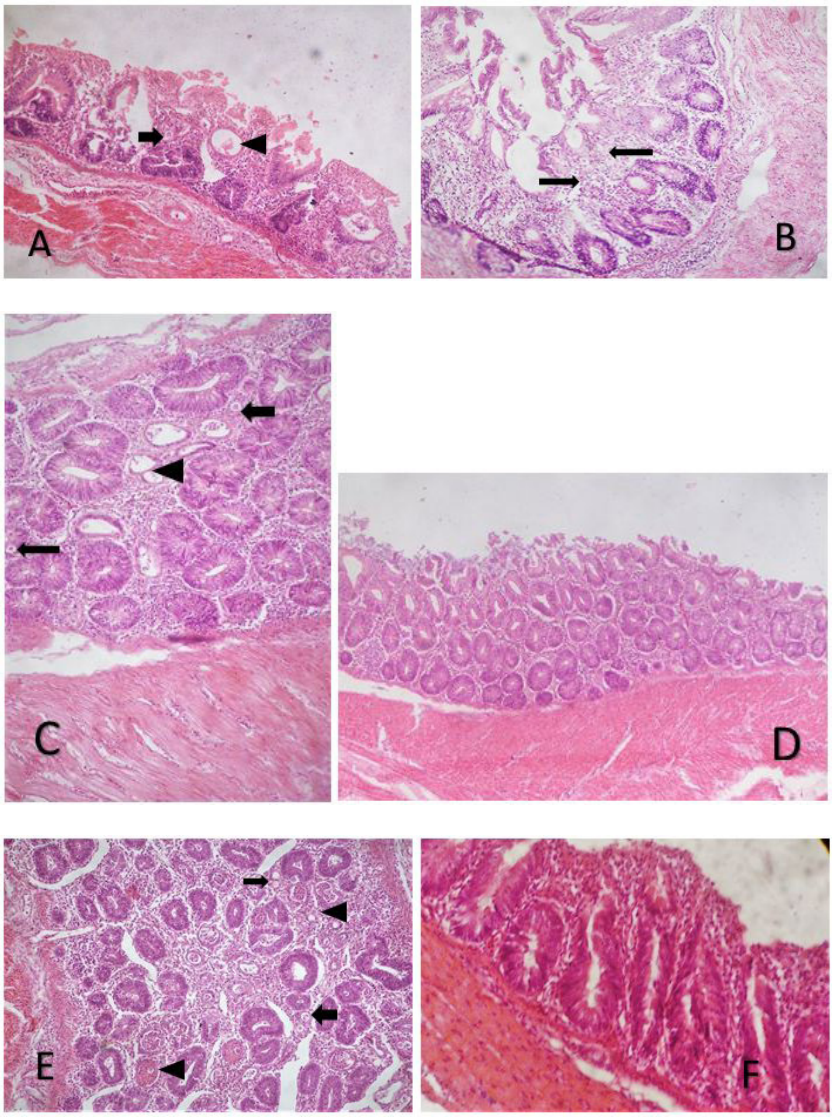
Microscopic Lesion Scoring (MLS)

Microscopic lesion scoring (MLS) system using adapted method of Godwin *et al.* was used to score lesion distribution in the caecal mucosa and submucosa [23]. Four random fields were evaluated for coccidial stages and lesions at × 20 of microscope objective lens (× 200 magnification), and scored as: 0 = no parasite/lesion, 1 = parasite/lesion in one field, 2 = parasite/lesion in two fields, 3 = parasite/lesion in three fields, 4 = parasite/lesions in four fields.

Results

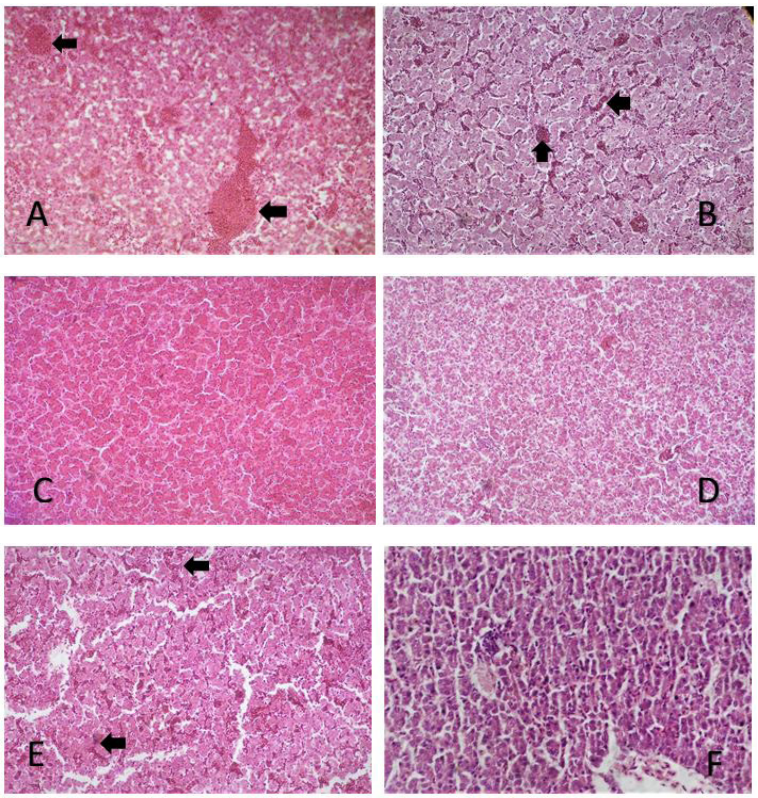
Histopathological changes observed in the intestinal caeca of infected chickens and treated with different concentrations of *L. schimperi* methanolic leaf extract include epithelial necrosis and developing parasite with cystic change in the submucosa (100

mg/ml) (Figure 1A), epithelial necrosis and developing parasite (50 mg/ml) (Figure 1B), developing parasite and cystic change in the submucosa (25 mg/ml) (Figure 1C). In the infected/untreated chickens (negative control) developing parasites and cystic changes in the submucosa were observed (Figure 1E). No lesions were seen in the intestinal caeca of the chickens infected and treated with amprolium (Figure 1D) as well as those not infected but treated with the extract at concentrations of 25 and 100 mg/ml (Figure 1F).



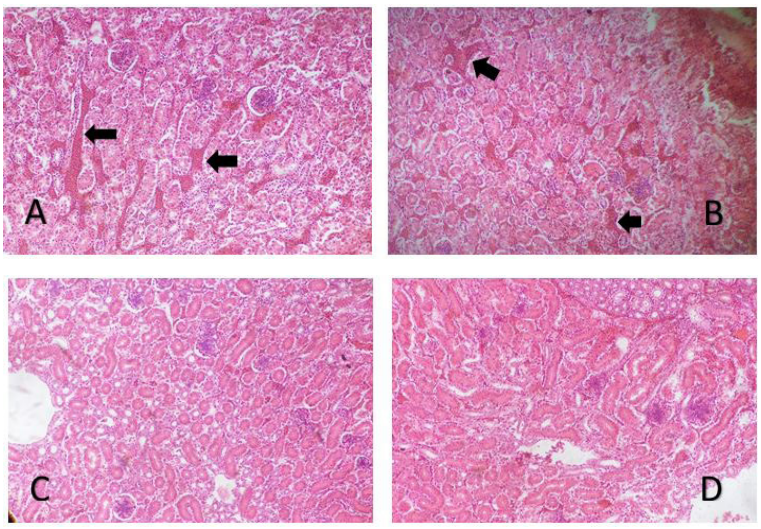
Key:
Figure 1A: Photomicrograph of caecum of chicken infected with *E. tenella* and treated with 100 mg/ml of *L. schimperi* extract (Group III). Note epithelial necrosis and developing parasite (arrow) with cystic change in the submucosa (arrowhead). H&E × 230
Figure 1B: Photomicrograph of caecum of chicken infected with *E. tenella* and treated with 50 mg/ml of *L. schimperi* extract (Group II). Note epithelial necrosis and developing parasite (arrows). H&E × 230
Figure 1C: Photomicrograph of caecum of chicken infected with *E. tenella* and treated with 25 mg/ml of *L. schimperi* extract (Group I). Note developing parasite (arrows) and cystic change in the submucosa (arrowhead). H&E × 230
Figure 1D: Photomicrograph of caecum of chicken infected with *E. tenella* and treated with amprolium (positive control) (Group IV). Note absence of parasite. H&E × 230
Figure 1E: Photomicrograph of caecum of chicken infected with *E. tenella* (negative control) (Group V). Note developing parasite (arrows) and cystic change in the submucosa (arrowheads). H&E x 230
Figure 1F: Photomicrograph of caecum of chicken treated with 100 mg/ml of *L. schimperi* extract (Group VI & VII). Note absence of lesions. H&E × 230.
Figure 1: Effects of treatment with graded doses of the plant extract and amprolium on caecal histology in experimentally induced coccidiosis in broiler chickens

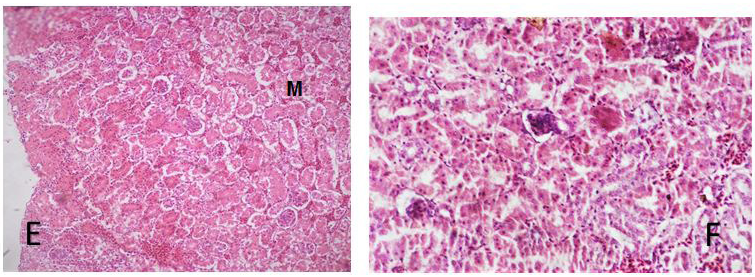
Pathological changes observed in the liver of infected chickens and treated with different concentrations of the extract were hepatic congestion (100 mg/ml) (Figure 2A), mild hepatic congestion (50 mg/ml) (Figure 2B), mild hepatic congestion was also observed in the negative control (Figure 2E). No lesions were seen in the liver of the chickens infected and treated with amprolium (Figure 2D) and 25 mg/ml concentration of the extract (Figure 2C) as well as the chickens not infected but treated with the extract at concentration of 100 mg/ml (Figure 2F).



Key:
Figure 2A: Photomicrograph of liver of chicken infected with *E. tenella* and treated with 100 mg/ml of *L. schimperi* extract (Group III). Note hepatic congestion (arrows). H&E × 230
Figure 2B: Photomicrograph of liver of chicken infected with *E. tenella* and treated with 50 mg/ml of *L. schimperi* extract (Group II). Note mild hepatic congestion (arrows). H&E × 230
Figure 2C: Photomicrograph of liver of chicken infected with *E. tenella* and treated with 25 mg/ml of *L. schimperi* extract (Group I). Note absence of lesions. H&E × 230
Figure 2D: Photomicrograph of liver of chicken infected with *E. tenella* and treated with amprolium (Group IV). Note absence of lesions. H&E × 230
Figure 2E: Photomicrograph of liver of chicken infected with *E. tenella* (negative control) (Group V). Note mild hepatic congestion (arrows). H&E × 230
Figure 2F: Photomicrograph of liver of chicken treated with 100 mg/ml of *L. schimperi* extract (Group VI & VII). Note absence of lesions. H&E × 230
Figure 2: Effects of treatment with graded doses of the plant extract and amprolium on liver histology in experimentally induced coccidiosis in broiler chickens

Lesions observed in the kidneys of chickens infected and treated with different concentrations of the extract were renal congestion (100 mg/ml) (Figure 3A) and mild renal congestion (50 mg/ml) (Figure 3B). In the infected untreated chickens mild renal congestion was observed (Figure 3E). No lesions were seen in the kidneys the chickens infected and treated with amprolium (Figure 3D) and 25 mg/ml concentration of the extract (Figure 3c) as well as the chickens not infected but treated with the extract at concentration of 100 mg/ml (Figure 3F).





Key:
Figure 3A: Photomicrograph of kidney of chicken infected with *E. tenella* and treated with 100 mg/ml of *L. schimperi* extract (Group III). Note renal congestion (arrows). H&E × 230
Figure 3B: Photomicrograph of kidney of chicken infected with *E. tenella* and treated with 50 mg/ml of *L. schimperi* extract (Group II). Note mild renal congestion (arrows). H&E × 230
Figure 3C: Photomicrograph of kidney of chicken infected with *E. tenella* and treated with 25 mg/ml of *L. schimperi* extract (Group I). Note absence of lesions. H&E × 230
Figure 3D: Photomicrograph of kidney of chicken infected with *E. tenella* and treated with amprolium (positive control) (Group IV) Note absence of lesions H&E × 230
Figure E: Photomicrograph of kidney of chicken infected with *E. tenella* (negative control) (Group V). Note mild congestion of medulla (M). H&E × 230
Figure 3F: Photomicrograph of kidney of chicken treated with 100 mg/ml of *L. schimperi* extract (Group VI & VII). Note absence of lesions. H&E × 230
Figure 3: Effects of treatment with graded doses of the plant extract and amprolium on kidney histology in experimentally induced coccidiosis in broiler chickens

Result from caecal microscopic lesion scores showed absence of lesion/developmental stage of the parasites in the group treated with aprolium (Table 1). Relatively more number of lesions/developmental stages of the parasites were seen in the infected untreated group as well as the group treated with the lowest dose of the plant extract (25 mg/ml) (Table 1). The groups treated with 50 and 100 mg/ml of the plant extract showed relatively lower number of lesion/parasite developmental stages (Table 1). Similarly, no lesions were seen in the caeca of chickens not infected but treated with lower and higher doses of the plant extract (25 and 100 mg/ml) (Table 1).

Experimental groups	Caecal lesion scores
Group I	+ 4
Group II	+ 3
Group III	+ 3
Group IV	0
Group V	+ 4
Group VI & VII	0

Key:
Group I: Infected and treated with 25 mg/ml of *L. schimperi* extract
Group II: Infected and treated with 50 mg/ml of *L. schimperi* extract
Group III: Infected and treated with 100 mg/ml of *L. schimperi* extract
Group IV: Infected and treated with 1.5 mg/ml of amprolium
Group V: Infected untreated (Negative control)
Group VI: Uninfected and treated with 25 mg/ml of *L. schimperi* extract
Group VII: Uninfected and treated with 100 mg/ml of *L. schimperi* extract
Table 1: Showing microscopic lesion scores of caeca of the different experimental chickens groups

Discussion

Histopathological investigation remains one of the standard procedures for evaluating the extent of tissue degenerative changes and cyto-architectural alterations. The epithelial necrosis recorded in the caeca agrees with the report of Abdel- Wasae *et al.,* [3] for histopathological studies conducted in chickens infected with *Eimeria tenella* oocysts. All the lesions seen in the caeca, liver and kidneys were not recorded in the group infected and treated with amprolium and those not infected but treated only with the methanolic leaf extract of *L. schimperi* suggesting that the pathology recorded could be due to the disease process rather than the toxic effect of the plant extract administered.

Similarly, relatively fewer developmental stages of the coccidian parasites were seen in the intestinal caeca of chickens infected and treated with higher concentrations of the plant extract, whereas, multiple developmental stages were seen in the infected/ untreated group as well as the group treated with lower concentration of the plant extract. No developmental stage of the parasite was seen in the intestinal caeca of chickens infected and treated with amproilum, this further suggest that the lesions observed in the intestinal caeca could likely be due to invasion by the parasitic developmental stages. More so, the reduction in number

of lesions and developmental stages of the parasite seen in the groups treated with the higher concentrations of the extract is an indicator of its potential anticoccidial activity. Supposedly, an increase in the concentration of the plant extract to many folds or isolation of the potential anticoccidial compound may yield greater activity. Earlier reports by Mikail *et al.,* revealed the potential anticoccidial effect of the *L. schimperi* methanolic leaf extract *in vitro,* therefore, our current finding further supports this claim [9]. Earlier researches have revealed the potential anticoccidial effects of some plant extracts. Herbal plants such as Plectranthus spp has been shown to repaired some lesion and decreased some destruction in caecum tissue of broiler chickens experimentally infected with *E. tenella* oocysts [3]. So also, *in vitro* and *in vivo* trials by Habibi *et al.,* illustrated anticoccidial activity of some plant extracts against oocysts of *E. tenella* in experimentally infected broiler chickens [24]. Furthermore, in a review by Muthamilselvan *et al.,* the anticoccidial effects of several plants species have been shown [11].

Although the current study revealed that amprolium was comparatively more effective in arresting tissue multiplication of the invasive developmental stages of Eimeria parasite, however the extract was shown to possess some anticoccidial activity in the extract-treated group, relative to the infected/untreated group. So also, an insignificant change in the body weight of infected chickens suggests that the disease process was a moderate one.

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