OPEN ACCESS

RESEARCH ARTICLE

Spanish Journal of Agricultural Research
22 (3), e0502, 14 pages (2024)
ISSN-L: 1695-971X
https://doi.org/10.5424/sjar/2024223-20579

Effect of hydro-methanolic extract of *Mangifera indica* L. stem bark on body weight, pathological lesions, and hematology in experimental *Eimeria tenella*-infected broiler chickens

Garba M. Ugwuoke[1]* and Idika K. Idika[2]

[1]*Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.* [2]*Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.*

***Correspondence** should be addressed to Garba M. Ugwuoke: matthias.ugwuoke@unn.edu.ng

Abstract

Aim of study: To evaluate the weight gain, pathological lesions, and hematology in broilers treated with hydro-methanol extract of *Mangifera indica* stem bark (MISB) after experimental exposure to *Eimeria tenella* infection.

Material and methods: This investigation involved 56 three-week-old Ross 308 broilers, divided into 7 groups (A–G) of 8 birds each. Groups A–E were experimentally exposed to 25,000 oocysts of *E. tenella* orally. Groups A, B, and C were treated orally with graded doses of *M. indica* (250, 125 and 62.5 mg/kg, respectively), for seven consecutive days. Groups D (0.6 g/L sulfaquinoxaline, reference drug), E (infected non-treated), F (uninfected non-treated), and G (uninfected 125 mg/kg MISB-treated to validate effect of MISB on weight increase). After infection, blood and organs were extracted from each experimental group for hematology and pathology, and measurements of body weight gain and oocyst counts were made.

Main results: *M. indica* improved ($p<0.05$) weight gain in MISB-treated broilers (A, B, C, and G). On day 6 post-infection (dpi), lesions of coccidiosis caused by *E. tenella* were observed in groups A, B, C, D, and E. The reduction in oocyst per gram of feces in the MISB and sulfaquinoxaline-treated groups was similar ($p>0.05$) after medication. Reduced packed cell volume at 7 dpi in the broilers of groups A (22.5% ± 0.7), B (27.0 % ± 2.83), and C (25.7 % ± 0.71), improved at 14 dpi after medication.

Research highlights: *M. indica* improved weight gain, reduced oocyst shedding, and ameliorated cecal lesions in MISB-treated chickens.

Additional keywords: anemia; antiprotozoal compounds; coccidiosis; mango stem bark; parasite; phytochemicals; poultry.

Abbreviations used: BW (body weight); DMSO (dimethyl sulfoxide); EDTA (ethylenediamine tetra-acetic acid); HB (hemoglobin); MISB (*Mangifera indica* stem bark); NVRI (National Veterinary Research Institute); OPG (oocysts per gram of feces); PCV (packed cell volume); RBC (red blood cells); WBC (white blood cells).

Citation: Ugwuoke, GM; Idika, IK (2024). Effect of hydro-methanolic extract of *Mangifera indica* L. stem bark on body weight, pathological lesions, and hematology in experimental *Eimeria tenella*-infected broiler chickens. Spanish Journal of Agricultural Research, Volume 22, Issue 3, e0502. https://doi.org/10.5424/sjar/2024223-20579

Received: 03 Jul 2023. **Accepted**: 29 Jan 2024. **Published**: 04 Jun 2024.

Introduction

Nigeria relies heavily on agriculture, especially poultry products like meat and egg. Poultry farming in Nigeria is limited by various factors, among of which are diseases and their controls (Mares et al., 2023). Coccidiosis is one of the diseases commonly encountered by poultry farmers worldwide (Blake et al., 2020). Coccidiosis is facilitated by several elements, some of which include age of the bird, the high number of oocyst available, the compressed immunity of the birds, and pathogenicity of the *Eimeria* species (Adem & Ame, 2023). Others include the development of resistance to widely used commercial anticoccidial medications by *Eimeria* parasites, such as sulfaquinoxaline and diaveridine (Embazin-forte®), amprolium hydrochloride (Amprol®), and diclazuril (Diclacox®) (Monjur, 2023). Coccidiosis is a disease that commonly affects chicken operations and costs the worldwide poultry business about 3 billion US dollars per year (Rahmani et al., 2024). Coccidian of the apicomplexan parasite of the genus *Eimeria* (Family: *Eimeriidae*) with complex life cycle phases is incriminated as the causative agent of avian coccidiosis (Mares et al., 2023). These *Eimeria* species include *E. tenella, E. acervulina, E. brunetti*, *E. maxima*, *E. necatrix, E. mitis,* and *E. praecox* (Zhang et al., 2023). Among these species, *E. tenella* and *E. acervulina* are the most frequent and virulent species found in poultry that are associated with increased mortality, morbidity, and haemorrhagic lesion (Cheng et al., 2022). These pathological conditions decline the production of poultry and eventually cause economic losses (Pawestri et al., 2020). Fecal droppings from infected chickens may comprise sporulated oocysts of *Eimeria* species, which proliferate the cycle of transmission to other birds (Mares et al., 2023). The average prepatent period for *E. tenella* infection is six days, a period between the ingestion of sporulated oocysts, manifestation of clinical signs and oocysts detectable in fecal droppings (Cheru et al., 2023). Avian coccidiosis results in dehydration and an incapacity to absorb nutrients, which are the twin conditions that cause weight loss and bird mortality (Zhang et al., 2023). In the pathophysiology of various diseases, these clinical symptoms have also been thought to operate as risk factors. The immune system, for instance, may be weakened by *E. tenella* infection leaving birds more susceptible to secondary illnesses including *Escherichia coli, Clostridium*, and *Salmonella* (Abbas et al., 2020). Despite initiatives to reduce the disease's fatal consequences in terms of treatment costs, morbidity, and death, avian coccidiosis still has a high pathogenic index and causes enormous economic losses on a global scale (Khater et al., 2020).

The current medications and vaccines for the prevention and treatment of *E. tenella* infections are expensive, ineffective, and commercial anticoccidials are creating resistance in the *Eimeria* parasites (Zhang et al., 2023). These developments require alternative research in new agents such as plants for drug preparation against *Eimeria* species (Abbas et al., 2020). These commercial chemical agents are losing their effectiveness due to frequent and irrational use (Zhang et al., 2023). In industrialized nations of the world, regulatory authorities like the European Union are placing limitations against the use of coccidiostats and chemical agents in food animals, mostly due to the accumulation of toxic residues that build up during their passage through the food chain, posing possible risks to consumers (Mahmoud et al., 2023). In addition to resistant constraints in the food chain, consumers have also objected to their use, alluding to the risk of the toxic effects of these antimicrobials that may contaminate poultry products, compelling poultry farmers to seek for other alternatives (Saeed & Alkheraije, 2023).

The experimental use of herbs, particularly those rich in antioxidants, as antiparasitic agents has assumed distinctive significance (Abbas et al., 2020). Usually, plants and their components are ordinary products which are not expensive, are accessible and have shown the success of production of less harmful effects and with less or almost no deposition of residues in the treated individual or animal (Imran & Alsayeqh, 2022). Research has shown that antioxidant (alkaloids, phenols, flavonoids, tannins, and saponins) rich plants (Mahmoud et al., 2023) are being used as an alternative approach to treat avian coccidiosis (Saeed & Alkheraije, 2023).

Mangifera indica Linn (mango tree) belongs to the plant family known as *Anacardiaceae,* and originated in the region bounded by Bangladesh and northeastern India (Warschefsky & Wettberg, 2019). Mango trees are tropical plants used for food and medicine that grow well in arid environments (Ajayi et al., 2005). Numerous studies have been done on the various applications of mango fruits, peels, juice, and stem bark; however, there are few publications on the significance of *M. indica* stem bark extract and its potential for usage as a treatment for gastrointestinal protozoal infections. *M. indica* has been reported to have several pharmacological properties, including antimicrobial effects (Doughari & Manzara, 2008). *Mangifera indica* stem bark (MISB) contains flavonoids, phenols, alkaloids, saponins, and tannins (Somkuwar & Kamble, 2013). Extracts of mango leaves and stem barks have been used in conventional treatments for syphilis, nephritis, scabies, respiratory issues, bronchitis, diarrhea, diabetes, kidney disease, and urinary tract diseases (Shah et al., 2010). Mango stem bark and leaf extracts have been reported to have antioxidant, anti-inflammatory, and immunomodulatory properties (Maldonado-Celis et al., 2019). According to Zhang et al. (2017), the saponin in mango leaves has been known to increase weight gain, meat quality, plasma lipid metabolism, feed consumption efficiency, immunity, and provide microbial flora in broiler chickens.

Hematological profiles, as well as gross and microscopic changes within tissues or systems, are significant markers of a person's biological function and can be used as indicators of their wellbeing. The outcomes may vary based on a person's health, stress levels, and toxicity (Khan & Zafar, 2005).

The current study aims to investigate the effects of mango hydro-methanol stem bark extract on mean weight gain, pathological lesions, and hematological profiles in broiler chickens experimentally infected with *Eimeria tenella* oocysts.

Material and methods

Broiler chickens used for the experiment

A total of fifty-six Ross 308 broiler chicks, both male and female, were acquired at one day of age from a reputable hatchery in Ibadan, Nigeria. The chicks were screened for *Eimeria* species and other parasites, and then vaccinated against Newcastle disease, infectious bronchitis, and infectious bursal disease at 1 week of age (Dakpogan et al., 2019). They were fed with a pelleted starter mash for broilers (Vital feed®), that does not include any coccidiostat. Feed and drinking water were provided at all time. They were reared as a single group from one-day-old till day 21 before they were randomly assigned to seven cages (n = 8 chicks per cage). The chicks were housed at the poultry unit of the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria, under appropriate conditions for animal rearing in terms of ambient temperature, light, and strict hygiene practices which were put in place within their surroundings before and during the trial.

Eimeria species samples collection and identification

The intestines of broiler chickens with suspected avian coccidiosis were taken from naturally infected chickens with *Eimeria* species in the Poultry Unit, Veterinary Research Farm, University of Nigeria. By fecal floatation procedure (Rani et al., 2021), two grams of feces were mixed with 60 mL of a 10% (w/v) NaCl solution and processed using the floatation method. A micropipette was used to introduce the suspension into the McMaster counting chamber; the number of oocysts was counted using a light microscope (MS001 Binocular) and suspended in 2.5% potassium dichromate for sporulation (Haug et al., 2006). Based on oocysts morphology, size and site of infection in the intestine, different species of *Eimeria* were identified (Gadelhaq et al., 2018). *E. tenella* was identified by its broad ovoid oocysts and absence of micropyle (Haug et al., 2006). The sporulated oocysts were counted using the modified McMaster technique. The slides were examined under a microscope at low (10x) and high magnification (40x) and sporulated *E. tenella* oocysts were resuspended in fresh 2.5% potassium dichromate solution and kept at 4°C until used (Wajiha et al., 2021).

Plant material and reference anticoccidial used for the experiments

In May 2022, fresh samples of mango stem barks were procured from their natural environment in Jos, Plateau state, Nigeria. The specimen of the plant was authenticated by Mr. AO Ozioko, a taxonomist with the Bioresources Development and Conservation Programme (BDCP), Nsukka, Nigeria. A voucher specimen (catalogue Number: UNN/VPP/2022/015) was kept in the institution's herbarium.

Sulfaquinoxaline (Embazin forte®) (Interchemie, Holland) used for the trials is a broad spectrum anticoccidial, water-soluble, saturated powdered form of sulfaquinoxaline sodium, usually administered to poultry in drinking water. For the purpose of this trial, sulfaquinoxaline was used as a reference anticoccidial at a dosage rate of 0.6 g/L in drinking water as recommended by the manufacturer and the design of the study (7 consecutive days for extract and reference drug) (Ugwuoke & Pewan, 2020; Ishaq et al., 2022).

Extraction of plant materials

The stem barks of *M. indica* were air-dried at ambient temperature and pulverized; 200 g of *M. indica* pulverized stem barks were extracted using cold maceration process in 70 % methanol, then filtered using Whatman® No.1 filter paper (Sigma-Aldrich, USA). The filtrate was concentrated using a rotary evaporator at 70°C (Akerele et al., 2008). The resulting solid residue was reconstituted in DMSO at stock concentration, and stored in the refrigerator at 4°C until used.

Qualitative and quantitative phytochemical screening of *M. indica* L. stem bark extract

The qualitative and quantitative phytochemical screening of *M. indica* L stem bark extract was conducted in the Department of Pharmacognosy and Drug Development of the University of Nigeria, Nsukka, Nigeria. The analysis was done to determine the active secondary metabolites present in *M. indica* L. stem bark extract. The phytochemical assays for flavonoids, alkaloids, tannins, terpenoids, saponins, phenols, and steroids were assessed according to established procedures (Trease & Evans, 2002).

Acute toxicity study of *M. indica* stem bark extract

This trial was conducted in two phases (Lorke's method, 1983). Phase 1: 9 chickens, each aged 3 weeks, were used in the trial's initial phase. The 9 birds were assigned into 3 groups of 3 each, and gastric gavage was used to provide graded doses of 10, 100, and 1000 mg/kg BW of *M. indica* to each group of birds. For 24 hours, the birds were checked for symptoms of toxicity and mortality.

Phase 2: When there was no evident sign of harmfulness after a duration time of 24 hours, 3 different chickens were administered orally with higher doses of *M. indica* (1600, 2900, and 5000 mg/kg BW, respectively), then observed for 24 hours for change in condition as well as mortality (Lorke, 1983). The median lethal dose (LD_{50}) of *M. indica* stem bark was calculated using the formula: $LD_{50} = \sqrt{D0 \times D100}$, where D0 = highest dose that gave no mortality; D100 = lowest dose that produced mortality.

In vivo anticoccidial assay of *Mangifera indica* stem bark extract

At 21 days of age, the birds were randomly separated into seven groups (A, B, C, D, E, F, and G) of 8 chickens per cage. They were weighed to establish full similarity with respect to BW. Preceding the exposure to the *E. tenella* infective stage, all trial chickens were screened and proved to be free from possible *Eimeria* species and other parasites. Groups A, B, C, D, and E were infected with 25,000 oocysts using a syringe attached to a gastric gavage on day 21 of age after grouping. On 6 dpi, the presence of the *E. tenella* parasite's infectious stage, or oocysts, was confirmed under a microscope in the chickens' feces. Using a syringe coupled to a gastric gavage, Groups A, B, C, and G received oral administration of each graded dose of MISB on day 7 dpi, which was determined based on the BW of each bird. Sulfaquinoxaline (reference drug) was administered in poultry drinking water at a dosage rate of 0.6 g/L (Group D). Group E was infected/non-treated, Group F uninfected/non-treated and Group G non-infected/treated with 125 mg/kg MISB. The treatments were administered for seven consecutive days and indices of coccidiosis such as clinical signs, fecal oocyst count (OPG), mean body weight gain (BWG) and pathology were assessed from the onset to the end of the trial while hematology was evaluated at 7 dpi and 14 dpi.

Counting of fecal oocysts (OPG)

Sequel to confirmation of infection at 6 dpi and following treatment at 7 dpi, samples of feces were obtained from fresh bird droppings in poultry cages and freshly eviscerated intestines of slaughtered chickens on days 1, 2, 3, 4, 5, 6, and 7, starting on day 2 post treatment in all experimental groups. The samples were then instantly analyzed microscopically using fecal floatation and the modified McMaster technique (Rani et al., 2021). Two grams of feces were mixed with 60 mL of a 10% (w/v) NaCl solution using floatation method (Rani et al., 2021). The suspension was instilled into the McMaster counting chamber using a micropipette, and oocysts (OPG) were counted using a light microscope (MS001 Binocular). Counted oocysts were obtained thus: OPG = oocyst count × dilution factor (fecal sample volume/counting chamber volume) (Rani et al., 2021).

Body weight (BW) and body weight gain (BWG)

On day 21 (the day birds were assigned into groups and infected), then at 7 dpi, the broilers were weighed (pre-treatment BW) and treated. The birds were again weighed on day 14 dpi (post-treatment BW), using an electronic chicken scale (Camry Emperors TL, China). Individual mean BW and mean BWG were computed, using the formula: BWG = TBW2 – TBW1; where BWG = body weight gain, TBW1 = pre-treatment body weight, and TBW2 = post-treatment body weight (Kaingu et al., 2017).

Cecal lesions and internal organs evaluation

In each experimental group (A, B, C, D, E, F, and G) four chickens were arbitrarily selected and euthanized by cervical dislocation at the end of the trial to inspect gross lesions in ceca and other segments of the intestines. Following evisceration, the ceca and ileum mucosa were examined to see if there were any obvious ceca mucosal injuries such as change in mucosal surface color, ballooned ceca, wet brown feces and ceca filled with blood and tissue debris. Internal organs were carefully removed, checked and weighed (Nghonjuyi et al., 2015) using an electronic balance (Camry Emperors TL, China). The organs examined and measured include; heart, liver, lungs, spleen, gizzard, proventriculus, ceca, kidneys, cecal weight and length. Gross lesions were graded on a scale of 0 to 4 based on the lesion scoring method (Dacie & Lewis, 2001): grade 0, not affected by the infection; 1, minor lesion with mucoid brown feces; 2, significant damage with ballooning of ceca; 3, feces stained with blood; 4, very dangerous and massive, droppings, liter stained with blood and debris of tissues in a distended cecum. Sample tissues of the duodenum, jejunum, ileum, cecum, and rectum were also obtained for histopathology. According to Durry & Wallington (1976), sample tissues were well-maintained in 10% neutral buffered formalin within 24 hours. Samples were cleaned in xylene after the fixation procedure, imbedded with paraffin wax after being dried out in ethanol, sliced in 5 mm thickness and stained with H & E differential stain. Mounted stained tissues were examined using a light microscope (MS001 Binocular).

Hematological evaluation

Blood samples from each experimental group were collected on days 7 and 14 dpi for hematological evaluation. Each set of bird group (A to G) had four blood samples obtained and the sample mean calculated. The wing vein was punctured to induce bleeding, and blood collected into test tubes containing EDTA, an anticoagulant and used immediately for determination of PCV (packed cell volume), and HB (hemoglobin concentration), using the microhematocrit method (Coles, 1986). The red blood

Table 1. Induction of coccidiosis and medication of broiler chickens (*in vivo* assay)

Groups of broilers	Infective stage	Treatment (7 dpi)
A (infected/MISB treatment)	25,000 oocysts of *E. tenella* (d21 after grouping)	250 mg/kg of MISB
B (infected/MISB treated)	25,000 oocysts of *E. tenella* (d21 after grouping)	125 mg/kg of MISB
C (infected/MISB treatment)	25,000 oocysts of *E. tenella* (d21 after grouping)	62.5 mg/kg of MISB
D (infected/Sulfaquinoxaline medicated water)	25,000 oocysts of *E. tenella* (d21 after grouping)	0.6 g/L Sulfaquinoxaline in poultry drinking water
E (infected/non-treated)	25,000 oocysts of *E. tenella* (d21 after grouping)	Non-medicated control
F (uninfected/non-treated)	Non-infected	Non-medicated control
G (non-infected/MISB treated)	Non-infected	125 mg/kg of MISB

MISB*: Mangifera indica* stem bark

cells (RBC) and white blood cells (WBC) counts were performed using the method of Schalm et al. (1975).

Study data statistics

SPSS statistics version 29 for Windows was used to analyze all of the study's data. The general linear model (GLIM) and Repeated Measures ANOVA were used to process the data. The Duncan's multiple range test was used to distinguish between different means, and Crawley (1993) defined significance as $p<0.05$.

Results

Qualitative and quantitative phytochemical screening of hydro-methanolic mango stem bark extract

The qualitative and quantitative phytochemical screening showed the presence of saponins (6.94 ± 0.03 µg/mg), alkaloids (6.36 ± 0.05 µg/mg), phenolic compounds (1.09 ± 0.08 µg/mg), tannins (1.94 ± 0.02 µg/mg), flavonoids (7.14 ± 0.03 µg/mg), and terpenoids (0.7 ± 0.01 µg/mg).

Acute toxicity

Sequel to the administration of graded doses (1600, 2900 and 5000 mg/kg bw respectively) of the methanolic extract of *Mangifera indica* L. to broilers, no clinical signs of toxicity or death were observed in all medicated groups after 24 h. Therefore, the LD_{50} of the methanolic extract of *Mangifera indica* L. stem bark extract in broiler chickens appears to be greater than 5000 mg/kg body weight.

Efficacy of mango stem bark on mean oocyst count (OPG) of broilers infected with *Eimeria tenella*

Lesions of *E. tenella*-induced coccidiosis were seen in groups A, B, C, D, and E at 6 dpi. Following medication at 7 dpi, (Table 1), the reduction in OPG amongst MISB and sulfaquinoxaline-treated groups were similar ($p>0.05$). The progressive reduction in OPG and other clinical signs of coccidiosis showed that MISB demonstrated a significant reduction ($p<0.05$) in parasite density with corresponding increase in extract dose levels as opposed to the infected/untreated control groups (Fig. 1). The extract significantly ($p<0.05$) suppressed the parasites *in vivo* in a dose-dependent manner: 250 mg/kg reduced OPG to 160 ± 18.71 (98.75%), 125 mg/kg to 190 ± 10.00 (98.74%), 62.5 mg/kg to 280 ± 37.42 (98.38%), infected/untreated posted 16840 ± 386.78 (0% OPG reduction). The reference drug (Embazin forte®) caused complete suppression (100%), similar to the highest MISB dosed group of birds. The depletion of *E. tenella* parasites in the ceca was confirmed by the microscopic examination of McMaster slides of fecal samples prepared at 14 dpi, indicating increased suppression of parasites in all the groups treated with MISB and the reference drug group of broilers compared to the negative control group E. There

was no significant difference ($p>0.05$) between the mean OPG of groups A, B, C and Embazin forte® treated (Group D) broilers at the end of the study (Fig. 1). There were no clinical lesions of coccidiosis in the broilers of groups F (non-infected/non-treated control) and G (non-infected/MISB-treated control).

Effectiveness of *Mangifera indica* stem bark extract (MISB) on average weight and average weight gain of the broiler chickens

Body weight gain amongst the MISB-medicated groups (A, B, C and G) showed a dose-dependent pattern, though, at the end of the experiment, mean BW among the infected/MISB-treated groups (A, B, and C), and the sulfaquinoxaline-treated D group (Table 2) did not vary significantly ($p<0.05$) when compared to the infected-non-treated control. Group A recorded the highest weight gain amongst the infected-MISB treated A, B, and C groups, and sulfaquinoxaline-treated D group. Group E (infected–non-treated control) had the lowest weight gain of (0.41 ± 0.12 kg). Group F (non- infected/untreated) had a weight gain of (1.03 ± 0.13 kg). Amongst the MISB-treated groups, G (non-infected/MISB-treated) had the highest BWG (1.90 ± 0.15 kg) which is significantly ($p<0.05$) higher than the control group F (Table 2), while the lowest BWG among MISB treated group was recorded in group C. The weight gains of the extract-treated groups (A, B, C, and G) and sulfaquinoxaline-treated group D, were significantly ($p<0.05$) higher than the weight gain of the infected-non-treated control group E (Table 2).

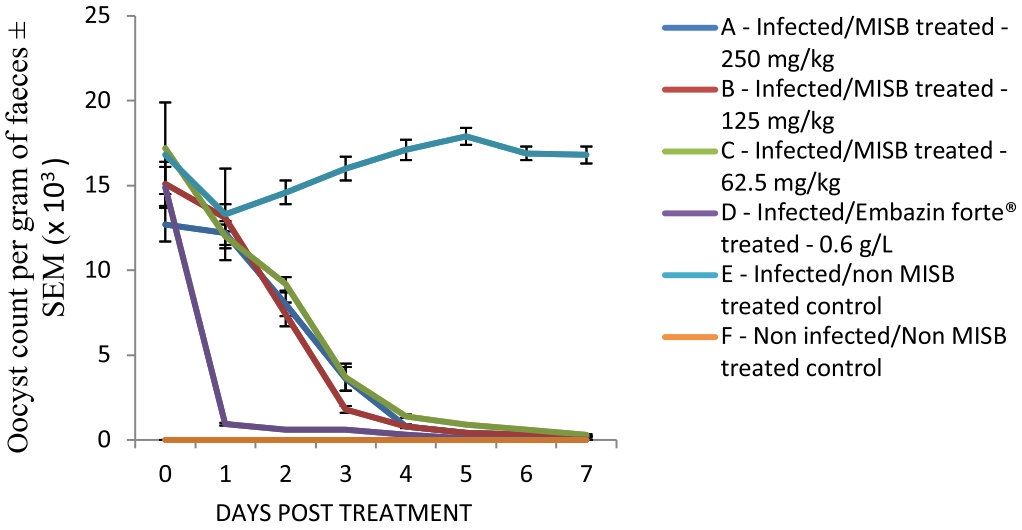

Figure 1. Efficacy of *Mangifera indica* stem bark and sulfaquinoxaline on mean oocyst count (OPG) of broilers infected with *Eimeria tenella*

Table 2. Effectiveness of *Mangifera indica* stem bark (MISB) extract on mean body weight (BW) and mean weight gain (BWG) of broiler chickens after graded dose treatments

Variables (kg)	Groups						
	A	B	C	D	E	F	G
Mean BW (pretreatment BW, 7 dpi)	0.77±0.17	0.80±0.20	0.60±0.12	0.97±0.33	0.90±0.15	0.90±0.06	0.72±0.07
Mean BW (post-treatment BW, 14 dpi)	1.76 ± 0.03^{b}	1.67± 0.04^{b}	1.33 ± 0.15^{a}	1.65±0.31^{b}	1.31±0.42^{a}	1.93±0.23^{c}	2.62±0.18^{c}
Mean BWG	0.99±0.17^{b}	0.87± 0.16^{b}	0.73 ± 0.12^{b}	0.68±1.23^{b}	0.41±0.12^{a}	1.03± 0.13^{b}	1.90±0.15^{c}

Values are presented as mean ± SEM. Means with different superscript letters compared to the infected- untreated control in the same row are significantly different ($p<0.05$).

Efficacy of *Mangifera indica* on the average weight of some internal organs of *Eimeria tenella* infected broiler chickens

The mean weight of the heart, liver, lungs, spleen, proventriculus, and ceca did not differ significantly ($p>0.05$) across any of the experimental groups (A to G), despite the fact that the treated groups' weight values varied in a dose-dependent manner. On the contrary, significant ($p<0.05$) decrease in the weight of the gizzard, weight of kidney, and cecal length of the infected-non-treated group E was recorded (Table 3).

Efficacy of M. *indica* on hematological profile of *Eimeria tenella* infected broiler chickens

There were no significant ($p>0.05$) differences observed among the mean PCV, HB concentrations, RBC, and WBC in all the infected groups (A, B, C, D, and E) prior to treatment at 7 dpi. Accordingly, following the treatment with the extract of *M. indica* at 7 dpi, there was an observed significant ($p<0.05$) increase in the mean PCV, HB concentrations, and RBC of all the infected and treated groups as compared to that of infected-non-treated control group E. As regards WBC, which increased during infection, no significant ($p>0.05$) difference was observed among the WBC of all the infected groups (A, B, C, D, and E) prior to treatment at 7 dpi. However, following the commencement of treatment at 7 dpi, there was an observed significant ($p<0.05$) decrease in the WBC counts of the treated groups while those of the infected-non-treated control group increased when compared to the WBC and lymphocyte percentages of group F and G broilers. The lymphocyte percentages of all the experimental groups were found to be non-significant ($p>0.05$) prior to treatment at 7 dpi as well as at 14 dpi following medication (Table 4).

Gross and histopathological lesions

The ceca of chickens in Groups A, B, and C, treated with MISB extract were improved with little alterations in the color of the mucosal surfaces (Fig. 2). Furthermore, a number of broilers from groups B and C, displayed wet chocolate-brown feces when euthanized and their ceca cut open. Group D broilers treated with sulfaquinoxaline had very few lesions and a modest alteration in wall surface. Group E (infected/non-treated) control had considerable bleeding along with blood and tissue debris in their ceca. Broilers of group F (uninfected/non-treated), and Group G (uninfected/treated with 125 mg/kg MISB) did not have any noticeable coccidiosis lesions on their ceca. The cecal lesions of the infected birds (A, B, C, and D) that received MISB or sulfaquinoxaline treatment were significantly ($p<0.05$) enhanced above the scores of birds infected but non-treated (Group E) with MISB or sulfaquinoxaline (Table 5). The uninfected/non-treated (F) revealed a normal architecture of the cecum. Wet chocolate feces were noticed in the ceca among recovering birds (Group

Table 3. Effectiveness of *Mangifera indica* stem bark extract (MISB) on mean weight of some internal organs and cecal lengths of broiler chickens infected with *E. tenella* and treated with graded doses of *Mangifera indica* and sulfaquinoxaline.

Variables	Groups						
	A	B	C	D	E	F	G
Heart weight (g)	6.4±5.7^{a}	6.9±1.3^{a}	7.3±0.9^{a}	6.6±0.5^{a}	5.8±0.5^{a}	7.6±1.5^{a}	7.5±1.6^{a}
Gizzard weight (g)	30.2±3.2^{b}	33.2±0.3^{b}	28.3±7.1^{a}	35.9±3.9^{b}	26.6±1.3^{a}	34.4±2.4^{b}	35.2±2.4^{b}
Liver weight (g)	30.0±5.7^{a}	28.8±2.5^{a}	28.3±6.7^{a}	28.6±2.6^{a}	25.3±0.7^{a}	31.2±2.7^{a}	30.3±2.7^{a}
Spleen weight (g)	1.8±0.8^{b}	1.9±0.7^{b}	1.4±0.4^{a}	1.7±0.9^{b}	1.5±0.1^{a}	2.1±0.6^{b}	2.3±0.4^{b}
Lungs weight (g)	6.5±0.5^{a}	5.7±0.8^{a}	4.6±2.0^{a}	4..8±0.6^{a}	4.6±0.5^{a}	5.3±1.2^{a}	6.0±1.2^{a}
Proventriculus weight (g)	6.8±1.1^{a}	7.5±1.5^{a}	8.0±1.4^{a}	6.9±0.9^{a}	6.3±1.0^{a}	8.2±2.0^{a}	7.5±3.0^{a}
Kidney weight (g)	0.3±0.0^{a}	0.2±0.2^{a}	0.4±0.0^{a}	0.5±0.2^{b}	0.2±0.1^{a}	0.2±1.0^{a}	0.4±2.0^{a}
Cecal weight (g)	6.4±0.5a	8.3±0.8^{a}	5.8±0.8^{a}	6.7±2.3^{a}	5.3±0.4^{a}	7.3±1.2^{a}	7.4±1.3^{a}
Cecal length (cm)	16.8±0.6^{b}	16.2±0.4^{b}	15.2±1.1^{a}	15.9±0.4^{a}	14.0±1.7^{a}	17.3±1.6^{b}	17.5±1.6^{b}

Values are represented as mean ± SEM. Means with different superscript letters compared to the infected-untreated control in the same row are significantly different ($p<0.05$).

Table 4. Hematological values (mean ± SEM) of broilers infected with *E. tenella* and treated with *Mangifera indica* stem bark (MISB) extract and sulfaquinoxaline

Groups	Hematological values			
	Weekly hematological variables[1]	**Before infection**	**During infection (7 dpi)**	**Post-treatment (14 dpi)**
A	HB (g/dL)	12.10±37	8.30±0.01^{a}	11.35±0.11^{b}
	PCV (%)	32.00±2.83	22.50±0.70^{a}	34.75±2.47^{b}
	RBC (× 10^{6}/L)	2.51±0.13	3.4±0.78^{a}	2.50±0.04^{a}
	WBC (× 10^{9}/L)	12.65±0.71	34.80±0.57^{b}	14.78±0.25^{a}
	Lymphocytes (%)	78.2±2.60	87.0 ± 2.6^{b}	86.2±2.10^{b}
B	HB (g/dL)	10.05±1.34	9.45±2.47^{a}	13.83±0.95^{b}
	PCV (%)	32.00±1.41	27.00±2.83^{a}	33.50±2.48^{b}
	RBC (× 10^{6}/L)	2.99±0.08	3.09±1.76^{a}	3.20±0.26^{a}
	WBC (× 10^{9}/L)	14.35±0.35	36.30±0.14^{b}	12.80±0.64^{a}
	Lymphocytes (%)	86.0±2.30	85.0 ± 3.60^{b}	90.1±2.40^{b}
C	HB (g/dL)	12.10±0.28	8.50±0.28^{a}	12.62±1.10^{b}
	PCV (%)	34.50±2.12	25.70±0.71^{b}	30.00±0.35^{c}
	RBC (× 10^{6}/L)	2.78±0.21	2.32±0.17^{a}	3.04±0.41^{a}
	WBC (× 10^{9}/L)	13.95±0.92	33.75±0.72^{b}	12.48±1.24^{a}
	Lymphocytes (%)	72.3±3.50	89.3±1.20^{b}	87.4±1.30^{b}
D	HB (g/dL)	9.10±0.28	8.80±1.13^{a}	13.23±1.52^{b}
	PCV (%)	30.50±0.71 2.89±0.42	28.50±0.71^{b}	33.50±0.71^{c}
	RBC (× 10^{6}/L)	14.0±0.14	2.46±0.20^{a}	3.40±0.81^{a}
	WBC (× 10^{9}/L)	79.2±2.20	16.80±0.14^{b}	13.08±0.81^{a}
	Lymphocytes (%)		88.0±0.70^{b}	85.3±3.00^{b}
E	HB (g/dL)	12.05±1.34	10.00±1.41^{a}	12.93±1.24^{a}
	PCV (%)	32.00±1.41 2.69±0.08	28.00±1.41^{b}	30.50±0.71^{b}
	RBC (× 10^{6}/L)	13.35±0.35	2.36±1.48^{a}	2.90±1.04^{a}
	WBC (× 10^{9}/L)	76.1±0.40	28.35±0.07^{b}	28.08±0.81^{b}
	Lymphocytes (%)		85.4±1.60^{b}	86.4±2.30^{b}
F	HB (g/dL)	9.30±0.28	6.90±1.27^{a}	12.85±1.52^{b}
	PCV (%)	32.50±0.71	29.20±2.12^{b}	29.50±0.71^{b}
	RBC (× 10^{6}/L)	2.46±0.04	2.35±0.64^{a}	3.52±0.76^{a}
	WBC (× 10^{9}/L)	13.20±0.57	16.75±0.49^{b}	13.75±1.13^{a}
	Lymphocytes (%)	68.3±2.10	87.1±3.10^{b}	86.3±3.20^{b}
G	HB (g/dL)	9.50±0.57	7.50±0.28^{a}	12.30±0.28^{b}
	PCV (%)	30.30±3.54	26.60±0.71^{b}	30.70±0.71^{c}
	RBC (× 10^{6}/L)	2.70±0.56	2.32±0.17^{a}	3.54±0.80^{a}
	WBC (× 10^{9}/L)	12.20±0.28	17.65±0.72^{b}	14.20±0.57^{b}
	Lymphocytes (%)	77.2±3.50	87.8±3.10^{b}	86.2±0.30^{b}

[1] HB = hemoglobin (g/dL), PCV = packed cell volume (%), RBC = red blood cells (x 10^{6}/L), WBC = white blood cells (x 10^{9}/L). Values are presented as mean ± SEM. Means with different superscript letters compared to the infected-untreated control in the same column are significantly different ($p<0.05$).

Table 5. Cecal lesion scores in *Eimeria tenella* infected broiler chickens treated with methanolic stem bark extract of *Mangifera indica* and sulfaquinoxaline (Embazin-forte® 0.6 g/L)

Groups	4 broilers lesion scores[1]	Mean ± SEM[2]
A	+ 2 + 1 + 1 + 1	1.25 ± 0.5^{a}
B	+ 1 + 1 + 1 + 2	1.25 ± 0.7^{a}
C	+ 2 + 1 + 2 + 1	1.5 ± 0.4^{a}
D	+ 2 + 1 + 1 + 1	1.5 ± 0.3^{a}
E	+ 3 + 3 + 2 + 4	3.0 ± 1.2^{b}
F	+ 0 + 0 + 0 + 0	0.0 ± 0.0^{a}
G	+ 0 + 0 + 0 + 0	0.0 ± 0.0^{a}

[1] See text. [2] Values are presented as mean ± SEM of lesion scores of broilers selected randomly per group. Means with different superscript letters compared to the infected-untreated control in the same column are significantly different ($p<0.05$)

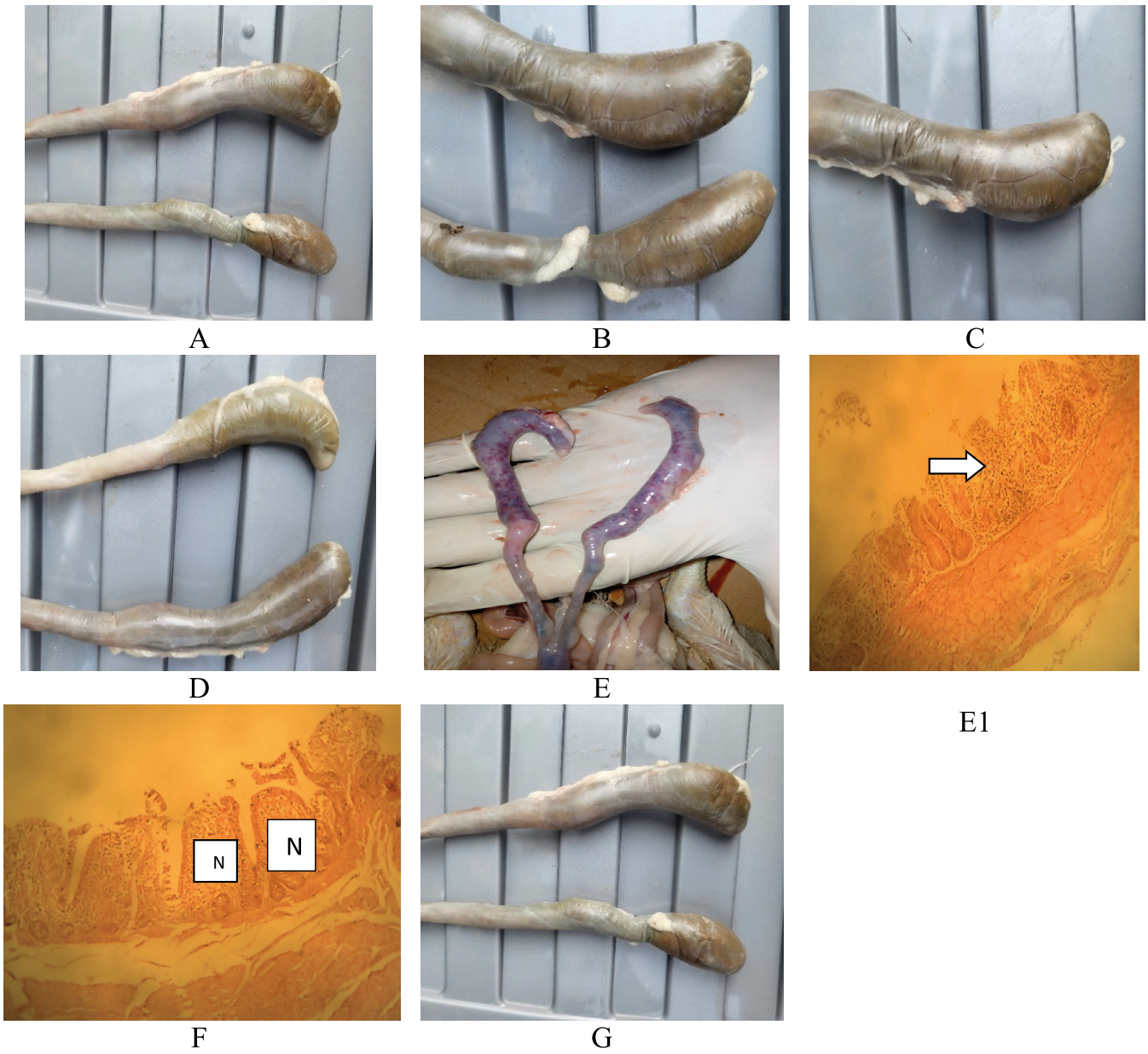

Figure 2. Gross and histopathological lesions in the cecum of the broiler chickens (14 dpi). A: Gross lesions of cecum of a broiler (infected/treated) with 250 mg/kg of MISB, showing normal fecal consistency after MISB medication. B: Gross lesions of cecum of a broiler (infected/treated) with 125 mg/kg of MISB, showing a distended cecum filled with mildly wet feces after MISB medication. C: Gross lesion of cecum of a broiler (infected/treated) with 62.5 mg/kg of MISB, showing mildly wet brown feces after MISB medication. D: Gross lesion of cecum of a broiler (infected/treated) with 0.6 g/L of sulfaquinoxaline, showing normal fecal consistency following treatment. E: Gross lesion of cecum of a broiler (infected/non-treated) showing ballooned ceca, desquamated blood, and tissue debris (14 dpi). E1: Microscopic lesion of cecum of a broiler (infected/non-treated) showing erosion of epithelial cells (arrow) and developmental stages of *E. tenella* in the epithelial mucosa (hematoxylin & eosin × 400). F: Microscopic lesion of cecum of a broiler (uninfected/untreated) (normal control), presenting no abnormalities (NN) (hematoxylin & eosin × 100). G: Gross lesion of cecum of a broiler (un-infected/treated) with 125 mg/kg of MISB (control), showing normal fecal consistency.

B) during post-mortem on day 14 dpi (Fig. 2). Epithelial desquamation, lymphocytic infiltration, edema and parasites' developmental stages, or schizonts were among the microscopic alterations seen in the intestinal tissues of infected/non-treated chickens (Group E).

Discussion

Previous studies have revealed that MISB contains medicinally significant biologically active substances known as phytoconstituents which contribute to the unique

characteristics of the plant and its antimicrobial potentials (Indriyani et al., 2023). One of MISB's distinctive qualities as a medicinal plant is the presence of certain chemical compounds, which have been shown to have considerable activity in treating *E. tenella* infections (Zhang et al., 2023). According to Chen et al. (2023), flavonoids have a contributory effect on arachidonic acid metabolism and are known to be an outstanding antimicrobial agent against a wide range of microorganisms. This action may be due to the ability of flavonoids to form complexes with extracellular and soluble proteins and to complex with microbial cell walls (Sebghatollahi et al., 2022). Tannins are also documented to be active antioxidants, antimicrobial and anti-carcinogenic agents (Lopez-Corona et al., 2022; Indriyani et al., 2023). Terpenoids can be added to proteins to improve their attachment to the cell membrane in a process identified as isoprenylation. They also play a role in traditional herbal preparations and are active against pathogenic microorganisms (Indriyani et al., 2023). Although phenols are typically employed to produce disinfectants, some also have the ability to disrupt estrogenic or endocrine systems (Kauffmann & Castro, 2023). They are also the active component in spices that contribute to its flavor, taste and medicinal properties (Kazeem et al., 2020). Therefore, it may be concluded that a combination of the aforementioned characteristics of M. indica's phytochemical constituents led to the plant's anticoccidial efficacy (Chen et al., 2023). Further investigation disclosed that the activity mechanism of plant extracts like *M. indica* against microbes produced by phenolic compounds and their derivatives was through the denaturation process of microbial cell proteins (Galovičová et al., 2022).

M. indica's capacity to decrease *E. tenella*'s internal replication is an important foundation of the *in vivo* model (Rani et al., 2021). Our study's finding on the start of treatment within the period between exposure to infective stage of the parasite and manifestation of clinical signs was decisively designed to target the parasite's infectious stage (Ishaq et al., 2022). This ranges between 4 -7 days, mainly as indicated by the existence of infectious stages (oocysts) in feces (Abbas et al., 2020). Thus, many anticoccidials were designed to target the complex life cycle of the species, *Eimeria,* as well as its sexual and asexual stages of reproduction (Zhang et al., 2020). Several researchers have used this practice to enhance extract's efficacy (Ishaq et al., 2022). In this investigation, there were considerable levels of *in vivo* anticoccidial efficacy as demonstrated by MISB which is similar to those of other researchers following similar practices. The anticoccidial effectiveness of MISB was assessed using the OPG as an indicator (Rani et al., 2021). In comparison to the other treated groups, the maximum dose of MISB (250 mg/kg) considerably decreased OPG expression in the chickens. MISB extract medication resulted in a significant decline in daily output of droppings between 2 to 7 days post-treatment. The reduction in oocyst shedding may be the cause of this decline. Reduced oocysts expression in the feces can diminish gastrointestinal lining deterioration and hence, decline bleeding (Abbas et al., 2020; Ishaq et al., 2022). Our findings sustain those of Zhang et al. (2020) who reported a similar effect using *Camellia sinensis* (green tea) extract in broiler chickens.

It's possible that the infected and non-treated broilers' fecal oocyst output is rising but varying because MISB or sulfaquinoxaline medication was not administered to them (Wajiha et al., 2021). Sulfaquinoxaline-treated group showed the largest post-treatment percentage OPG reduction in oocyst expression in feces. Using sulfaquinoxaline, oocysts are destroyed at diverse stages by the drug's inhibition of dihydrofolate synthetase in their structures (Murshed et al., 2023). This is in line with the findings of Desalegn & Ahmed (2020), who suggested that prolonged use of common commercial anticoccidial agents to treat coccidiosis would have contributed to anticoccidial resistance, which is the reason why sulfaquinoxaline is unable to totally suppress parasite replication, and oocyst production in feces.

The significant ($p<0.05$) weight gain in group G broilers that were non-infected but dosed with MISB extract compared to the non-infected/non-treated control group indicates that MISB extract improves weight gain in broiler chickens. It's worthy to note that groups B and G were assigned equal doses of medication (125 mg/kg of MISB) in order to validate the specific effect of MISB on weight gain in broiler chickens. This effect may be due to phytochemicals (mangiferin and saponins). Mango saponins are known to enhance growth rate and weight gain in broilers fed mango leaves extract (Zhang et al., 2017). The absence of either MISB or sulfaquinoxaline medication may be the cause of the lethargic weight improvement in the infected and non-treated control. Regardless of this, there was no significant ($p>0.05$) distinction in weight increase amongst the untreated/ infected group E, and the therapeutic groups A, B, C, and D. Ugwuoke & Pewan (2020) reported similar findings in their study in terms of mean weight increase using stem bark extract of *P. biglobosa* in experimental *E. tenella* infection in broiler chickens. Infected group with *E. tenella* that were not treated gained the least weight. This result may be related to the strain's unfavorable effects on food assimilation, digestion, and absorption. According to the current study, the infected and not medicated bird's BW significantly declined; however, after receiving MISB or sulfaquinoxaline medication, there was visible improvement in growth rate among treated chickens. This shows that MISB has activity against the parasite and is potent enough to reverse the effect of the disorder on BW (Wajiha et al., 2021). In our study, MISB increased the growth rate of broilers (Group G). This finding was entirely a new effect because, to the best of our knowledge, no study has investigated MISB effect on growth performance in poultry and its efficacy on *E. tenella* infected broilers. In the present study, the positive response of BW to MISB treatment indicated its beneficial role in broiler growth. In addition, the feed efficiency was

marginally improved in MISB treated group of broilers. These results may be attributed to the health-promoting (antioxidant, analgesic, antimicrobial, anti-inflammatory, and antifungal) properties of the MISB extract (Diso et al., 2017).

A considerable reduction in mortality (%) was observed in MISB dosed groups while the lowest mortality was observed among the groups treated with the highest dose of MISB extract. Decrease in lesion scores were also observed in MISB-treated groups. Groups treated with MISB showed less lesion scores, with no significant difference to the sulfaquinoxaline treated group ($p > 0.05$) and significantly different to the infected/non-medicated group ($p<0.05$). Briefly, as established in our trial, the decreased oocyst shedding rate, higher survivability, reduction in bloody diarrhea and improvement in cecal lesions in the *M. indica* treated groups compared to the infected-non-treated group suggest the involvement of some immune effectors like tannin, phenols, and flavonoids which are found in *M. indica* (Kazeem et al., 2020). It was reported that by reducing the body's concentration of free radical molecules, antioxidant substances are known to shield the body from their damaging effects. It's possible that the reduction in oocysts expression are promoted by the free radical scavenging activities of the phenolic compounds contained in MISB extract, which also brought about the improvement in cecal lesion that was observed (Moryani et al., 2021). The cecum is the organ most commonly damaged by *E. tenella* infection, and the present investigation found that the infected-non-treated group's cecal weights and lengths significantly decreased. The substantial decrease in cecal weights and lengths observed in the infected-non-treated group may be brought on by the onset of intestinal absorptive malfunctions connected to *Eimeria* infection. It can also be due to their ability to bind to extracellular and soluble proteins on the parasite's cell membrane thereby preventing the progress of the *E. tenella* complex life cycle in the host (Moryani et al., 2021). Contrasted with the organ weights in the other groups, there were minor increases in Group G's organ weight, maybe as a result of MISB treatment without *E. tenella* infection.

Avian coccidiosis is characterized by anemia since the infection can result in significant blood loss in affected chickens (Murshed et al., 2023). The considerable drop in PCV, HB, and RBC which was noticed in the infected birds during infection may be related to cecal bleeding. One possible cause of this bleeding is mechanical mucosal destruction; considerable volumes of blood can escape through capillaries due to parasite burden in the intestinal lumen expanding following reproduction or the histamine released during tissue damage (Mohamed et al., 2021). Hence, PCV, HB, and RBC were evaluated in this study to assess the ability of MISB to prevent further bleeding due to increasing invasion of the cecal mucosae by *E. tenella* parasites (Murshed et al., 2023). It is important to note that MISB prevented a significant decline in PCV, HB, and RBC in the medicated broilers. The decline in PCV, HB, and RBC were observed to be overturned following treatment with either MISB or sulfaquinoxaline in contrast to the non-treated/infected control. In comparison, WBC was found to be considerably lower in the treated groups after treatment. According to Hidanah et al. (2018) marginal WBC were probably activated as a result of parasitic activities in gut, as evidenced by the sustained rise in leukocytes of the negative control at 14 dpi. This study found that the infected/non-treated group had mild leukocytosis, although this difference was not statistically significant ($p>0.05$) from the WBC proportion in some treated groups. According to Hidanah et al. (2018), some factors that contribute to the rise in WBC include parasitic infections, acute injury, viral and other microbial infections. The most prevalent type of phagocytic leukocyte, heterophils, are essential to the inborn immune status of individual animal, because they mediate sudden injuries, diseases and metabolic conditions (Banjoko et al., 2020). Latimer & Bienzle (2010) showed that prevalence of heterophils in hematopoietic cells can decline as a result of existing infectious conditions. Comparing the elevated WBC to increased lymphocytes in the negative control, the rise in lymphocyte count was not statistically significant. Increased lymphocyte counts are a sign that there is an existing disease or illnesses caused by infectious agents (Latimer & Bienzle, 2010). Investigation on hematology profile of birds infected with *Eimeria* species and treated with extract is similar to this study and several other studies that have shown similar discoveries (Aljedale & Al-Malki, 2020).

In conclusion, *Mangifera indica* did not produce unfavorable effects in the treated broilers and exhibited excellent anticoccidial capabilities against *E. tenella*-induced coccidiosis. The improvement of the treated broilers' hematological indicators and the decrease in cecal lesions may be due to the crude MISB extract's strong antioxidant activity. This anticoccidial activity is highly comparable to the reference anticoccidial, sulfaquinoxaline (Embazin forte®). The results also suggest that MISB would make a good dietary supplement for improvement of growth rate in broilers and could be used as a feed additive in broiler chicks for the prevention of avian coccidiosis. This finding could encourage further research in the application of MISB as a feed additive in farm animals and as prophylaxis for avian coccidiosis. Further trials are required to unravel the specific active compound(s) responsible for the anticoccidial efficacy, improvement of weight gain, hematological markers, and its specific mechanism of action. This may aid its validation as an alternative remedy for the management of avian coccidiosis and loss of weight that characterize *E. tenella* infection.

Acknowledgments: The authors acknowledge Dr. Pewan SB (National Veterinary research institute, Plateau State, Nigeria) for the supply of *Eimeria* oocysts used in this research work.

Competing interests: The authors have declared that no competing interests exist.

Authors' contributions: Garba M. Ugwuoke: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Visualization, Writing – original draft, Writing – review & editing. **Idika K. Idika:** Project administration, Software, Supervision, Validation.

All authors read and approved the manuscript.

Funding: The authors received no specific funding for this work.

References

Abbas RZ, Iqbal Z, Khan A, Sindhu ZUD, Khan JA, Khan MN, et al., 2020. Options for integrated strategies for the control of avian coccidiosis. Int J Agr Biol 14: 1014-1020.

Adem DM, Ame MM, 2023. Prevalence of poultry coccidiosis and its associated risk factors in and around Haramaya District, Ethiopia. Vet Med Open J 8(1): 9-17. https://doi.org/10.17140/VMOJ-8-172

Ajayi DA, Adeneye JA, Ajayi FT, 2005. Intake and nutrient utilization of west African dwarf goats fed mango (Mangifera indica), ficus (Ficus thonningii), gliricidia (Gliricidiasepium) foliages and concentrates as supplements to basal diet of guinea grass (Panicum maximum). World J Agric Sci 1(2): 184-189.

Akerele JO, Obasuyi O, Ebomoyi MI, Oboh IE, Uwumarongie OH, 2008. Antimicrobial activity of ethanol extracts and fractions of the seeds of Garcinia kola Hackel (Guttiferae). Afr J Biot 7(2): 169-172.

Aljedale MM, Al-Malki ES, 2020. Anticoccidial activities of Salvadora persica (arak) Zingiber officinale (ginger) and Curcuma longa (turmeric) extracts on the control of chicken coccidiosis. J King Saud Univ Sci 32: 2810-2817. https://doi.org/10.1016/j.jksus.2020.07.002

Banjoko OJ, Adebayo IA, Osho IB, Olumide MD, Fagbiye OOA, Ajayi OA, et al., 2020. Evaluation of varying levels of Carica papaya leaf meal on growth carcass hematological parameters and its use as anticoccidial for broiler chicken. Niger J Anim Sci 22(3): 229-241.

Blake DP, Knox J, Dehaeck B, Huntington B, Rathinam T, Ravipati V, et al., 2020. Re-calculating the cost of coccidiosis in chickens. Vet Res 51(1): 115. https://doi.org/10.1186/s13567-020-00837-2

Chen S, Wang XJ, Cheng Y, Gao HS, Chen XH, 2023. A review of classification, biosynthesis, biological activities and potential applications of flavonoids. Molecules 28(13): 4982. https://doi.org/10.3390/molecules28134982

Cheng PP, Wu PYT, Guo SS, Ma XY, Fei CZ, Xue FQ, et al., 2022. RPA assay coupled with CRISPR/Cas12a system for the detection of seven Eimeria species in chicken fecal samples. Vet Parasit 311: 109810. https://doi.org/10.1016/j.vetpar.2022.109810

Cheru H, Tamrat H, Hailemelekot M, Cassini R, Belayneh N, 2023. Epidemiology and identification of Eimeria species affecting poultry in East Gojjam Zone, North West Ethiopia. J Vet Med Sci 9(5): 2160-2167. https://doi.org/10.1002/vms3.1243

Coles GC, 1986. Anthelminthic resistance in sheep. Vet Clinic North Am: Food Anim Pract 2(2): 423-432. https://doi.org/10.1016/S0749-0720(15)31253-6

Crawley M, 1993. GLIM for ecologists. Blackwell Sci, Oxford.

Dacie JV, Lewis SM, 2001. Practical Hematology, 9th Ed. Churchill Livingstone, London. pp: 1-5.

Dakpogan HB, Mensah S, Attindehou S, Chysostome C, Aboh A, Naciri M, et al., 2019. Anticoccidial activity of Carica papaya and Vernonia amygdalina extract. Int J Biol Chem Sci 12: 2101. https://doi.org/10.4314/ijbcs.v12i5.12

Desalegn AY, Ahmed MR, 2020. Anticoccidial activity of Aloe debrana and Aloe pulcherrima leaf gel against Eimeria oocysts. J Parasit Res 1: 1-10. https://doi.org/10.1155/2020/8524973

Diso S, Ali M, Mukhtar S, Garba M, 2017. Antibacterial activity and phytochemical screening of Mangifera indica (Mango) stem and leaf extracts on clinical isolates of methicillin resistant Staphylococcus aureus. J Adv Med Pharm Sci 13: 1-6. https://doi.org/10.9734/JAMPS/2017/31127

Doughari JH, Manzara S, 2008. In vitro antibacterial activity of crude leaf extracts of Mangifera indica Linn. Afr J Microbiol Res 2: 067-072.

Durry KAB, Wallington EA, 1976. Histological technique. Histology 4: 48-58.

Gadelhaq SM, Arafa WM, Abolhadid SM, 2018. In vitro activity of natural and chemical products on sporulation of Eimeria species oocysts of chickens. Vet Parasit 251: 12-16. https://doi.org/10.1016/j.vetpar.2017.12.020

Galovičová L, Borotová P, Vukovic NL, Vukic M, Kunová S, Hanus P, et al., 2022. The potential use of Citrus aurantifolia L. essential oils for decay control, quality preservation of agricultural products, and anti-insect activity. Agron 12: 735. https://doi.org/10.3390/agronomy12030735

Haug A, Williams RB, Larsen S, 2006. Counting coccidial oocysts in chicken faeces: A comparative study of a standard McMaster technique and a new rapid method. Vet Parasit 136(3-4): 233-242. https://doi.org/10.1016/j.vetpar.2005.11.024

Hidanah S, Sabdoningrum EK, Wahjuni RS, Chusniati S, 2018. Effects of meniran (Phyllan-thus niruri L) administration on leukocyte profile of broiler chickens infected with Mycoplasma gallisepticum. Vet World 11: 834-839. https://doi.org/10.14202/vetworld.2018.834-839

Imran A, Alsayeqh A. 2022. Anticoccidial efficacy of Citrus sinensis essential oil in broiler chickens. Pak Vet J 42: 461-466. https://doi.org/10.29261/pakvetj/2022.082

Indriyani NN, Jamaludin A, Nandang P, Sarifah N, Euis J, 2023. Bioactive components and their activities from different parts of Citrus aurantifolia (Christm.) Swingle for food development. Foods 12(10): 2036. https://doi.org/10.3390/foods12102036

Ishaq AN, Sani D, Abdullahi SA, Jatua D, 2022. In vitro anticoccidial activity of ethanolic leaf extract of Citrus aurantium L. against Eimeria tenella oocysts. Sokoto J Vet Sci 20: 37-43. https://doi.org/10.4314/sokjvs.v20i5.4

Kaingu F, Liu D, Wang I, Tao J, Waihenya R, Kutima H, 2017. Anticoccidial effects of Aloe secundiflora leaf extract

against Eimeria tenella in broiler chicken. Trop Anim Health Prod 49: 823-828. https://doi.org/10.1007/s11250-017-1267-y

Kauffmann AC, Castro VS, 2023. Phenolic compounds in bacterial inactivation: A perspective from Brazil. Antibio 12(4): 645. https://doi.org/10.3390/antibiotics12040645

Kazeem MI, Bankole HA, Oladokun TI, Bello AO, Maliki MA, 2020. Citrus aurantifolia (Christm.) Swingle (Lime) fruit extract inhibits the activities of polyol pathway enzymes. eFood 1: 310-315. https://doi.org/10.2991/efood.k.200824.001

Khan TA, Zafar F, 2005. Hematological study in response to varying doses of estrogen in broiler chickens. Int J Poult Sci 4(10): 748-751. https://doi.org/10.3923/ijps.2005.748.751

Khater HF, Ziam H, Abbas A, Abbas RZ, Raza MA, et al., 2020. Avian coccidiosis: Recent advances in alternative control strategies and vaccine development. Agrobiol Rec 1: 11-25. https://doi.org/10.47278/journal.abr/2020.003

Latimer KS, Bienzle D, 2010. Determination and interpretation of the avian leukogram. In: Schalm's Veterinary Hematology, 6th ed; Weiss D, Wardrop KJ (Eds.). Blackwell Publ Ltd Ames, pp. 345-357.

Lopez-Corona AV, Valencia-Espinosa I, González-Sánchez FA, Sánchez-López AL, Garcia-Amezquita LE, Garcia-Varela R, 2022. Antioxidant, anti-inflammatory and cytotoxic activity of phenolic compound family extracted from raspberries (Rubus idaeus): A general review. Antioxidants 11(6): 1192. https://doi.org/10.3390/antiox11061192

Lorke D, 1983. A new approach to acute toxicity testing. Arch Toxicol 53: 275-287. https://doi.org/10.1007/BF01234480

Mahmoud K, Morsy MA, Abd El-Lateef HM, Marzok M, El-Beltagi HS, Al Khodair KM, et al., 2023. A century of "anticoccidial drugs": bibliometric analysis. Front Vet Sci 10: 1157683. https://doi.org/10.3389/fvets.2023.1157683

Maldonado-Celis ME, Yahia EM, Bedoya R, Landázuri P, Loango N, Aguillón J, et al., 2019. Chemical composition of mango (Mangifera indica L.) fruit: Nutritional and phytochemical compounds. Front Plant Sci 10: 1073. https://doi.org/10.3389/fpls.2019.01073

Mares MM, Saleh A, Rewaida A, Mutee M, 2023. Morphological and molecular characterization of Eimeria spp. infecting domestic poultry Gallus gallus in Riyadh City, Saudi Arab. Micro 11(3): 795. https://doi.org/10.3390/microorganisms11030795

Mohamed ERA, Elazab MF, El-Habashi N, Elhawary N, Mokhbatly AA, 2021. Anticoccidial effect of Origanum majorana aqueous extract on Eimeria tenella-infected chicken. Trop Biomed 38: 62-72. https://doi.org/10.47665/tb.38.1.011

Monjur A, 2023. Intestinal parasitic infections in 2023. Gastroenterol Res 16(3): 127-140. https://doi.org/10.14740/gr1622

Moryani AA, Rajput N, Naeem M, Shah AH, Jahejo AR, 2021. Screening of the herbs and evaluation of their combined effects on the health and immunity of coccidiosis challenged broiler chickens. Pak Vet J 41: 228-234.

Murshed M, Aljawdah HMA, Mares M, Al-Quraishy S, 2023. In vitro: The effects of the anticoccidial activities of Calotropis procera leaf extracts on Eimeria stiedae oocysts isolated from rabbits. Molecules 28(8): 3352. https://doi.org/10.3390/molecules28083352

Nghonjuyi NW, Kimbi HK, Keambou CT, Manka'a CN, Toukala JP, Juliano RS, et al., 2015. Assessment of anti-coccidial efficacy of ethanolic extract of Aloe vera leaf in Kabir chicken in Cameroon. J Adv Parasit 2: 23-29. https://doi.org/10.14737/journal.jap/2015/2.2.23.29

Pawestri W, Nuraini DM, Anditya M, 2020. The estimation of economic losses due to coccidiosis in broiler chickens in Central Java, Indonesia. IOP Conf Ser: Earth Environ Sci 411: 012030. https://doi.org/10.1088/1755-1315/411/1/012030

Rahmani A, Ahmed Laloui H, Kara R, Dems MA, Cherb N, Klikha A, Blake DP, 2024. The financial cost of coccidiosis in Algerian chicken production: A major challenge for the poultry sector. Avian Pathol 1-12. https://doi.org/10.1080/03079457.2024.2336091

Rani Z, Abbas RZ, Abbas A, Saeed Z, Rehman T, Hussain KM, et al., 2021. In vitro and in vivo anticoccidial effects of butyric acid and its impact on blood and serum chemistry of broiler chickens. Kafkas Univ Vet FakDerg 27: 583-588.

Saeed Z, Alkheraije KA. 2023. Botanicals: A promising approach for controlling cecal coccidiosis in poultry. Front Vet Sci 10: 1157633. https://doi.org/10.3389/fvets.2023.1157633

Schalm OW, Jain NC, Caroll EJ, 1975. Veterinary Haematology, 3rd Ed., Lea & Fabiger, Philadelphia, pp. 15-218.

Sebghatollahi Z, Ghanadian M, Agarwal P, Ghaheh HS, Mahato N, Yogesh R, 2022. Citrus flavonoids: Biological activities, implementation in skin health, and topical applications: A review. ACS Food Sci Technol 2: 1417-1432. https://doi.org/10.1021/acsfoodscitech.2c00165

Shah KA, Patel MB, Patel RJ, Parmar PK, 2010. Mangifera indica (mango). Pharmacogn Rev 4: 42-48. https://doi.org/10.4103/0973-7847.65325

Somkuwar DO, Kamble VA, 2013. Phytochemical screening of ethanolic extracts of stem, leaves, flower and seed kernel of Mangifera indica L. Int J Pharma Biosci 4(2): 383-389.

Trease GE, Evans WC, 2002. Phytochemicals: In: Pharmacognosy, 15th Ed. Saunders Publ, London, pp: 42-393.

Ugwuoke GM, Pewan S, 2020. Effect of methanol extract of Parkia biglobosa root bark on organ and carcass weight and histopathological changes in Eimeria tenella infected broiler chickens. Anim Res Int 17: 3587-3595.

Wajiha G, Qureshi NA, Afridi R, 2021. Comparative analysis of egg adapted vaccines and salinomycin against coccidiosis in chicks. Microbiol Pathol 123: 454-60. https://doi.org/10.1016/j.micpath.2018.08.005

Warschefsky EJ, Wettberg EJB, 2019. Population genomic analysis of mango (Mangifera indica) suggests a complex history of domestication. New Phytologist 222(4): 2023-2037. https://doi.org/10.1111/nph.15731

Zhang K, Li X, Na C, Abbas A, Abbas RZ, Zaman MA, 2020. Anticoccidial effects of Camellia sinensis (green tea) extract and its effect on blood and serum chemistry of broiler chickens. Pak Vet J 40: 77-80. https://doi.org/10.29261/pakvetj/2019.119

Zhang L, Zhang H, Du S, Song X, Hu D, 2023. In vitro transcriptional response of Eimeria tenella to toltrazuril reveals that oxidative stress and autophagy contribute to its anticoccidial effect. Int J Mol Sci 24(9): 8370. https://doi.org/10.3390/ijms24098370

Zhang YN, Wang J, Qi B, Wu SG, Chen HR, Luo HY, et al., 2017. Evaluation of mango saponin in broilers: effects on growth performance, carcass characteristics, meat quality and plasma biochemical indices. As-Austr J Anim Sci 30(8): 1143-1149. https://doi.org/10.5713/ajas.16.0847