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Original Research Article

EFFECTS OF METHANOL EXTRACT OF *AZADIRACHTA INDICA (NEEM)* ON BROILER CHICKENS INFECTED WITH *EIMERIA* OOCYSTS

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ABSTRACT

Evaluation of anticoccidial effects of methanol extract of *Azadirachta indica (Neem)* on broiler chickens infected with *Eimeria* oocysts was investigated. The leaves were washed and air- dried to constant weight and dryness and then pulverized into fine powder. Extraction was done by cold maceration using hydromethanol mixture as solvent. A total of one hundred-day old Cobb 500 broiler chicks were used for the study. The birds were divided into six groups of sixteen birds per group. Group 1 – Infected/Untreated (Negative control); Group 2 - Infected/Treated with Amprolium (Positive control/standard – 125 mg/L); and Group 3 – Uninfected/Untreated (Normal control). Groups4, 5, and 6 received *Azadirachta indica* leaf extract (AILE) at 250, 500 and 1000 mg/L of water, respectively. The faecal oocyst counts of treated birds were significantly ($p<0.05$) lower when compared with that of the infected/untreated broiler chickens. Also, Hb, PCV, and RBC in the treated groups were significantly ($p < 0.05$) increased when compared with that of the infected/untreated group; while absolute eosinophil value was significantly ($p < 0.05$) lower in the treated groups when compared with that of the infected/untreated group. Live and relative dressed weights were not significantly ($p > 0.05$) affected; while relative defeathered weight of the infected birds treated with Amprolium drug and 250 mg/L of AILE were significantly ($p < 0.05$) increased when compared with the infected/untreated birds. This study demonstrated the potential of neem's leaves as a natural anticoccidial agent in broiler chicken production.

Keywords: Amprolium, Broiler, Coccidiosis, Eimeria, Neem

INTRODUCTION

The name poultry refers to all domestic birds such as chickens (domestic fowl), turkeys, ducks, geese, guinea fowls, ostriches, and others, which are mainly kept for the production of meat and egg for human consumption. Among these, chickens are the most important species, adapted globally to various climatic conditions where human being lives and play a significant role in supplying animal origin protein to improve human nutrition (Tallentire *et al*., 2018).

It has a high turnover rate and quick return on investment which has made it unique in the livestock enterprise. It contributes to faster growth in local economy through increased trade volume than many other agricultural livestock sectors. Despite the roles of poultry production in

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the livestock industry, the industry is faced with a number of challenges which has resulted in huge economic losses to the farmers. Parasitic infections have been a major setback or challenge to poultry farmers in Nigeria. Among the parasitic infections, coccidiosis has proved to be a major threat to poultry production. Coccidiosis is a widespread and economically significant parasitic disease in poultry, primarily caused by protozoan parasites of the genus *Eimeria* (Blake *et al.*, 2020; Peek & Landman, 2021). This disease affects the intestinal tract of birds, leading to severe health issues such as diarrhoea, weight loss, decreased feed efficiency, and increased mortality rates (Chapman *et al.*, 2020; Blake *et al.*, 2020). Coccidiosis is particularly detrimental in young birds and could lead to substantial economic losses due to decreased feed conversion efficiency and increased mortality (Chapman *et al.*, 2020). The global poultry industry suffers substantial economic losses due to coccidiosis, estimated to exceed $3 billion annually, due to decreased production and increased control costs (Abbas *et al.*, 2019).

Traditional management of coccidiosis has heavily relied on the use of anticoccidial drugs and vaccines (Chapman *et al.*, 2020; Peek & Landman, 2021). Anticoccidial drugs, including ionophores and synthetic chemicals, are commonly used for prophylactic and therapeutic purposes (Abbas *et al.*, 2019). However, the widespread use of these drugs has led to the emergence of drug-resistant strains of Eimeria, complicating the effective control of the disease (Blake *et al.*, 2020).

In Africa, there is increase in the incidence of coccidiosis especially in broiler chickens because the organism is ubiquitous; effective treatment is expensive or not readily available and control strategies to eliminate this intestinal protozoan parasite appear not to be in sight. Thus, poultry breeders in both rural and urban settlements in our society are now compelled to cut down production costs by embracing the use of plant preparations as alternative therapy for coccidiosis. These plants contain bioactive compounds that can be used directly or as precursors for the synthesis of useful drugs. Medicinal plants have been a cornerstone of traditional medicine systems and continue to be integral to modern pharmacology and therapy. They provide a natural source of therapeutic compounds that contribute to the prevention, management, and treatment of various health conditions.

Among the various natural products, *Azadirachta indica* (Neem) has attracted significant attention for its broad spectrum of biological activities, including its potential anticoccidial effects (Choudhary *et al.*, 2019; Elangovan *et al.*, 2021). Neem is rich in bioactive compounds such as azadirachtin, nimbin, and quercetin, which have been shown to exhibit antimicrobial, antifungal, antiviral, and antiparasitic properties (Saleem *et al.*, 2018b; Choudhary *et al.*, 2019).

This study was designed to investigate the anticoccidial activity of *Azadirachta indica* leaf extract (AILE) in broiler chickens experimentally infected with sporulated *Eimeria* oocyst.

MATERIALS AND METHODS

STUDY AREA

The study was conducted at the Teaching and Research farm of College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. Faecal oocyst count of the birds was carried out in the Department of Veterinary Parasitology and Entomology laboratory while the haematology and biochemical analysis were carried out in the Department of Veterinary Physiology and Pharmacology laboratory both in Michael Okpara University of Agriculture, Umudike.

EXPERIMENTAL BIRDS

One hundred broiler chickens (Cobb 500) were purchased from 'Zartech' farm in Jos. On arrival, the chicks were housed in deep litter brooding pen earlier prepared with litter material and heating to maintain optimal brooding temperature. They were stabilized by administration of glucose in their drinking water. The chicks were fed *ad libitum* with a commercial starter feed (HI PRO MAX^{R}), while a commercial finisher feed (PRO MAX^{R}) was used after the brooding. The birds were allowed access to good drinking water; necessary vaccinations were also administered.

EXTRACT PREPARATION

The leaves of *Azadirachta indica* were collected from the premises of Michael Okpara University of Agriculture, Umudike. The plant was identified by Dr. Nwajiobi Benson of Department of Forestry and Environmental Management, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The leaves were washed and air- dried to constant weight and dryness and then pulverized into fine powder using a contact mill. The leave powder was macerated in 1:5 w/v analytical grade methanol (MeOH) for 48- 72 hours and it was vigorously agitated every 2 hours. The mixture was filtered through Whatman filter paper into an already measured beaker at room temperature. The filtrate obtained was concentrated under reduced pressure using a rotary evaporator (Cole-Parmer type N-1110, China). The percentage yield (w/w) of the plant extract was calculated using the formula:

$$\% \text{ Yield} = \frac{\text{Weight of material extracted}}{\text{Weight of plant material}} \times \frac{100}{1}$$

The final product was refrigerated at 4°C till use.

ACUTE TOXICITY STUDY

This was done in 42 birds following the method of Lorke (1983). The study was carried out in two phases. In the first phase, four groups (A-D) consisting of 3 birds each were given distilled water (10 ml/kg) and graded doses (10, 100 and 1000 mg/kg) of the leaf extract of *Azadirachta indica* respectively. Birds in group A served as the normal control. The birds were observed for signs and symptoms of toxicity and mortality over a period of 24 hours.

In the absence of mortality, the second phase of the experiment commenced with four groups (A-D) and in which 3 newer sets of birds were allocated to each group. Treatments with distilled water (10 ml/kg) or increasing doses (1600, 2900 and 5000 mg/kg of the methanol extract of *Azadirachta indica* were administered respectively. Birds in group 1 served as the normal control. The birds were observed for 24 hours, post-administration. All treatments were given orally by gastric intubation. In each phase of the acute study, the birds were allowed for another 7 days to observe any delayed toxicity.

The median lethal dose (LD_{50}) of the extract was then calculated using the formula:

$LD_{50} = \sqrt{}$ (least dose with mortality × Highest dose without mortality)

EXPERIMENTAL DESIGN

The birds were randomly allocated to six experimental groups of sixteen birds per group. The groups were made up of three control groups and three test groups as follows:

Control group:

Group 1 A – Infected/Untreated (Negative control)

Group 1 B - Infected/Treated with Amprolium (Positive control/standard – 125 mg/L)

Group 1 C – Uninfected/Untreated (Normal control)

Test group:

Group 2 A – AILE (Low dose – 250 mg/L)

Group 2 B - AILE (Medium dose – 500 mg/L)

Group 2 C - AILE (High dose – 1000 mg/L)

Group 1 A was infected with *Eimeria* oocyst and given no treatment. Group 1 B was infected with *Eimeria* oocyst but treated with 125 mg/L of amprolium.

Group 1 C was not infected with *Eimeria* oocyst and was not given any treatment. Group 2 A was infected with Eimeria oocyst but treated with 250 mg/L of AILE. Group 2 B was infected with *Eimeria* oocyst but treated with 500 mg/L of AILE; while Group 2 C was infected with *Eimeria* oocyst but treated with 1000 mg/L of AILE.

EXPERIMENTAL INFECTION USING SPORULATED OOCYST

The oocysts were sporulated in 2.5% potassium dichromate. At day 21, the six groups of birds were infected same day with 3000 oocysts in 3ml of syringe orally. And a simultaneous trial was made on *Azadirachta indica* to determine its therapeutic effect on coccidiosis from the 9th day of infection for a span of 7 days.

SAMPLE COLLECTION

Faecal samples were collected from the birds in each group on days 0, 7, 10, 13, 15 and 18 (from the start of treatment) into different sample bottles and examined for the presence of oocysts. Blood samples were collected from the jugular vein using 5 ml syringe. Blood collected from each bird was placed in two sample bottles. One of the sample bottles contained Ethylene diaminetetracetic acid (EDTA), while the second was a plain bottle. The blood in the EDTA bottles was used to estimate haematological parameters. The blood samples in the plain bottles were placed in a slanting position for 30 minutes to allow for serum separation, after which they were centrifuged at 2000 r.p.m for 10 minutes (Brar *et al*., 2000). The serum was harvested and used for biochemical analyses (Liver and Kidney function tests).

DETERMINATION OF THE FAECAL OOCYST COUNT

Faecal samples collected from the birds in each group on days 0, 7, 10, 13, 15 and 18 were examined for the presence of oocysts. The oocysts output was determined and expressed as per gram of faeces using McMaster counting technique.

CARCASS ANALYSIS

At the end of the 7th week of the experiment, three birds were randomly selected from each treatment, weighed and slaughtered for carcass analysis. Slaughtering was achieved by humanely severing the jugular vein. The feathers were loosened by immersing in hot water followed by de-feathering. The abdomen was cut open and viscera pulled out. The carcass was cut into parts (wing, back cut, breast, drum stick and thigh) and Weighed. The weights of different carcass parts were expressed as percentage of the live weight.

EVALUATION OF HAEMATOLOGICAL AND SERUM BIOCHEMICAL PARAMETERS

The haemocytometer method was employed in the red blood cell (RBC) count; haematocrit method (Brar *et al*.,2000) was used for Packed cell volume (PCV) estimation while haemoglobin (Hb) concentration was estimated with cyanomethemoglobin method using Drabkin's reagent (Brar

et al., 2000). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated as described by (Brar *et al*., 2000).

The total white blood cell (WBC) count was determined using hemocytometer method. The WBC was determined by counting the required number in the appropriate squares on the counting chamber under a microscope. The relative number of the neutrophil, lymphocyte, eosinophil, basophils and monocytes were estimated using haemocytometer method. The absolute numbers of the above cell types were calculated from the total WBC count. Absolute number = relative number × total WBC/100

A commercially available reagent kit (Randox Diagnostic Laboratories, United Kingdom) was used to evaluate the serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities, as well as serum total protein, albumin, total cholesterol, triglyceride, blood urea nitrogen and creatinine. The assays were carried out as instructed by the manufacturer.

DATA ANALYSIS

Descriptive and inferential statistics were used in this study to analyze collected data. All the data were subjected to Analysis of Variance (ANOVA) with Duncan multiple range post hoc test to separate means. Statistical significance was set at 95 % probability ($p < 0.05$)

RESULTS

EFFECT OF *AZADIRACHTA INDICA* LEAF EXTRACT AS A THERAPEUTIC AGENT IN BIRDS INFECTED WITH EIMERIA OOCYSTS

Amprolium (120 mg/L) produced Eimeria oocyst clearance of 82.9 % compared to 85.8%, 98.9%, and 90.2 % with extract at 250, 500 and 1000 mg/L respectively. Hence, Amprolium caused a significant ($p < 0.05$) reduction in oocyst count from 6,200.00 ± 379 on day 7 to 1,060 ± 70 at day 18 post treatment. Similarly, 250, 500 and 1000 mg/L of extract was able to reduce oocyst count from a range of 8000 -8,567± 296 to 1,133 ± 88, 950 ± 45 and 787 ± 95 respectively at the termination of the study. The mean number of the oocyst however, increased exponentially in the untreated birds across the study period (Table I).

EFFECT OF *AZADIRACHTA INDICA* LEAF EXTRACT ON CARCASS CHARACTERISTICS OF BIRDS INFECTED WITH EIMERIA OOCYSTS

The result on the carcass characteristics of infected birds treated with graded doses of AILE, and the standard (Amprolium, 120 mg/L) drug, when compared with the infected/untreated birds is presented in Table II. The relative defeathered weight of the infected birds treated with Amprolium and the low dose (250 mg/L) of AILE were significantly ($p < 0.05$) increased when compared with the infected/untreated birds, and those treated with higher doses of the extract. Live and relative dressed weights were not significantly ($p > 0.05$) affected. The relative wing weight of the Amprolium standard drug, 250 mg/L, and 1000 mg/L AILE treated groups were statistically the same ($p > 0.05$), but were significantly ($p < 0.05$) higher when compared with the 500 mg/L extract, and the infected/untreated groups. The relative back cut weight of the infected birds treated with Amprolium, and AILE at 250, and 1000 mg/L were significantly ($p < 0.05$) lower than that of the infected/untreated birds, while the relative back cut of the birds treated with 500 mg/L AILE was significantly higher than infected/untreated birds. The values for relative breast weight of the infected birds treated with Amprolium standard drug, and the 250 mg/L, and 1000 mg/L of AILE were significantly ($p < 0.05$) lower when compared with the values observed for infected/ untreated group. The values for relative drum stick weights of the infected birds treated with graded doses of AILE were statistically the same ($p > 0.05$) with the value for normal birds(uninfected/untreated), but were significantly ($p < 0.05$) lower than the value reported for infected/untreated birds. However, the relative weight of thigh of the infected and treated birds were significantly ($p < 0.05$) heavier than those of the infected/untreated birds.

EFFECT OF NEEM (*Azadirachta indica*) LEAF EXTRACT ON HAEMATOLOGICAL PARAMETERS OF BIRDS INFECTED WITH EIMERIA OOCYST

The results of haematological parameters for haemoglobin (HB), packed cell volume (PCV), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) mean corpuscular haemoglobin concentration among the different experimental groups are presented in Table III. The values for Hb, PCV and RBC were significantly ($p < 0.05$) lower in the infected/untreated birds when compared with the normal and treated birds, which were within their normal ranges.

There were significant ($p < 0.05$) decrease in the MCH and MCHC of the normal, amprolium treated and AILE, 250 mg/L treated birds compared to the values recorded for infected/untreated. The values recorded for AILE 500 and 1000 mg/L were not significantly ($p>0.05$) different from infected/untreated.

0.05) when compared with the normal birds. Amprolium significantly ($p < 0.05$) decreased the relative number of heterophil. However, the relative number of eosinophil counted in the infected and treated birds were significantly ($p < 0.05$), low when compared with the mean count in the infected/untreated birds.

TABLE I: EFFECTS OF TTE ON FAECAL OOCYST COUNT OF BROILER CHICKENS INFECTED WITH *EIMERIA* SPP.

Treatment	DAY 7 PI	DAY 10 PI	DAY 13 PI	DAY 15 PI	DAY 18 PI
Infected/Untreated	7433.±744	8800±305	9333±145	9533±120	10000±115
Amprolium, 125 mg/L	6200±378*	4933±417*	3400±550*	2266±317*	1060± 70*
Uninfected/Untreated	0±0	0±0*	0±0*	0±0*	0±0*
AILE, 250 mg/L	8000±700	6233±753*	3900±450*	2333±145*	1133±88*
AILE, 500 mg/L	8566±296	6366±405*	3866±751*	1900±305*	950±45*
AILE, 1000 mg/L	8033±1092	4466±952*	2466±384*	1093±63*	786±94*

p<0.05 when compared with infected untreated group, AILE = *Azadirachta indica* leaf extract, PI = Post infection

TABLE II: EFFECTS OF AILE ON CARCASS CHARACTERISTICS OF BROILER CHICKENS INFECTED WITH *EIMERIA* SPP.

TREATMENTS	Infected/ Untreated	Amprolium, 125 mg/L	Uninfected/ Untreated	AILE, 250 mg/L	AILE, 500 mg/L	AILE, 1000 mg/L
Live wt (g)	2141.50±38	2088.50±28	1942.50±139	2155.00±113	2417.50±4.	2198.00±185
Defeathered (%)	93.23±2.02	97.01±1.54*	95.77±1.21*	96.17±1.09*	94.02±0.33	92.53±0.36
Dressed (%)	74.11±0.60	73.13±2.07	72.33±1.98	71.32±1.17	72.49±0.36	62.70±0.38*
Wing (%)	6.25±0.09	7.78±0.118*	7.73±0.37*	7.62±0.06*	6.64±0.53	7.22±0.23*
Backcut(%)	17.08±0.53	11.78±0.05*	11.24±0.20*	12.08±0.24*	22.52±0.31*	9.97±0.25*
Breast (%)	28.57±0.89	26.08±0.74*	27.00±2.13	24.81±1.05*	27.33±0.21	22.08±0.25*
Drumstick (%)	11.97±0.37	11.29±0.27	10.57±0.11*	10.63±0.43*	10.53±0.16*	9.98±0.09*
Thigh (%)	9.93±0.38	12.34±0.68*	13.00±0.21*	12.76±0.05*	7.97±0.27*	11.26±0.58*

***p < 0.05 when compared with the infected/untreated group; AILE = *Azadirachta indica* leaf extract**

EFFECT OF AILE ON LEUCOCYTE PROFILE OF BIRDS INFECTED WITH *EIMERIA* OOCYSTS

Table IV showed the results of the effects of neem leaf extract treatment on leucocyte profile of birds infected with *Eimeria* oocyts. The white blood cell count in the infected/untreated birds were significantly ($p < 0.05$) elevated when compared with the infected and treated birds. The relative differential lymphocyte was significantly ($p < 0.05$) low in infected/untreated birds, while the values for infected and treated birds were statistically the same ($p >$ The results presented in Table IV showed significant ($p < 0.05$) reduction in the absolute heterophil, monocytes, eosinophil and basophil amongst the infected birds treated with the standard drug and the graded doses of AILE when compared with values recorded for infected/untreated broiler chickens.

TABLE III: EFFECTS OF AILE ON BLOOD PARAMETERS OF BROILER CHICKENS INFECTED WITH *EIMERIA* SPP.

Treatments	Hb(g/dL)	PCV(g/dL)	RBC(g/dL)	MCV(g/dL)	MCH(g/dL)	MCHC(g/dL)
Infected/untreated	11.13±0.29	22.00±1.15	2.49±0.12	88.33±0.28	44.82±1.08	50.75±1.35
Amprolium, 125 mg/L	13.13±0.29*	31.67±1.45*	3.59±0.16*	88.20±0.05	36.66±0.87*	41.57±1.01*
Uninfected/untreated	13.27±0.37*	31.33±2.33*	3.59±0.25*	87.30±0.50	37.19±1.46*	42.62±1.88*
AILE, 250 mg/L	12.60±0.31*	28.00±1.53*	3.20±0.17*	87.58±0.24	39.55±1.30*	45.15±1.48*
AILE, 500 mg/L	12.53±0.07*	26.67±0.33	3.03±0.08*	88.07±1.18	41.41±0.85	47.01±0.34
AILE, 1000 mg/L	12.40±0.20*	26.33±0.88	2.97±0.12	88.81±0.67	41.90±1.27	47.15±1.09

***p < 0.05 when compared with the infected/untreated group; AILE = *Azadirachta indica* leaf extract, Hb = haemoglobin, PCV = packed cell volume, RBC = red blood cell, MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration**

TABLE IV: EFFECTS OF AILE ONLEUCOCYTE PROFILE OF BROILER CHICKENS INFECTED WITH *EIMERIA* SPP.

Treatments	Infected/ untreated	Amprolium, 125 mg/L	Uninfected/ untreated	AILE, 250 mg/L	AILE, 500 mg/L	AILE, 1000 mg/L
Total WBC ($\times10^3/\mu L$)	32.98±2.04	24.65±1.09*	20.40±0.53*	21.35±0.39*	23.13±0.91*	23.00±0.86*
Relative Lymphocyte (%)	40.67±0.88	55.33±1.45*	61.33±1.45*	51.00±0.58*	53.33±0.67*	52.67±1.20*
Relative Heterophil (%)	41.00±1.53	34.33±2.33*	31.00±0.58*	38.67±0.67	38.00±1.53	39.33±1.76
Relative Monocyte (%)	6.00±0.58	4.67±0.33	5.33±0.67	7.00±0.58	5.00±0.58	6.00±0.00
Relative Eosinophil (%)	11.67±0.88	5.67±0.67*	2.33±0.33*	3.33±0.88*	3.33±0.67*	2.00±0.58*
Absolute lymphocyte ($\times10^3/\mu L$)	13.40±0.80	13.67±0.96	12.52±0.59	10.89±0.20	12.33±0.39	12.12±0.63*
Absolute heterophil ($\times10^3/\mu L$)	13.51±0.87	8.41±0.22*	6.32±0.12*	8.26±0.29*	8.82±0.69*	9.03±0.44*
Absolute monocyte ($\times10^3/\mu L$)	1.99±0.26	1.16±0.12*	1.08±0.11*	1.49±0.10*	1.15±0.09*	1.38±0.05*
Absolute eosinophil ($\times10^3/\mu L$)	3.86±045	1.41±0.23*	0.48±0.07*	0.71±0.19*	0.77±0.15*	0.46±0.14*
Absolute basophil ($\times10^3/\mu L$)	0.22±0.11	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.07±0.07*	0.00±0.00*

***p < 0.05 when compared with the infected/untreated group; AILE = *Azadirachta indica* leaf extract, WBC = white blood cell**

EFFECT OF AILE ON SERUM BIOCHEMICAL PARAMETERS OF BIRDS INFECTED WITH *EIMERIA* OOCYSTS

The effects of AILE on the total protein, albumin, globulin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (ASP), total cholesterol, triglyceride, high density lipoprotein (HDL), very low-density lipoprotein (VLDL), and low-density lipoprotein (LDL)of broiler chickens infected with *Eimeria* spp. are presented in Table V.

The serum levels of total protein and globulin were not affected significantly ($p > 0.05$) by the *Emeria* inoculation, whereas, the serum albumin was significantly ($p < 0.05$) decreased in the infected/untreated birds when compared with the infected birds treated with 250 mg/L of AILE.

The total cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL) of the infected/untreated birds were significantly ($p < 0.05$) decreased, whereas, the triglyceride and very low density lipoprotein (VLDL) of the infected/untreated broiler chickens were significantly ($p < 0.05$) increased when compared with the infected/treated birds.

There were significant ($p < 0.05$) differences in Alkaline phosphatase (ALP), Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) liver enzymes activities between the infected/untreated and the infected and treated with the graded doses of AILE. The infected birds treated with the graded doses of the extract recorded significant ($p < 0.05$) increases in the levels of ALP, ALT and AST when compared with infected/untreated group.

The kidney assay revealed significant ($p < 0.05$) increases in the level of urea in birds infected and treated with graded doses of AILE when compared with the infected/untreated birds, whereas, the creatinine level of birds treated with the standard drug, 250 mg/L, and 500 mg/L doses of the extract were significantly ($p < 0.05$) higher than the mean level of the untreated birds.

DISCUSSION

The result of the acute toxicity test indicated that AILE has a high safety margin and was well tolerated by the birds even at 5000 mg/kg dose, it induced no toxic manifestation or mortality. *Azadirachta indica* extract has been reported by Ilesanmi *et al*. (2017) to be safe and non-toxic even at 5000

TABLE V: Effects of AILE on the serum biochemical parameters of broiler chickens infected with *Eimeria* spp

Treatments	Infected/ untreated	Amprolium, 125 mg/L	Uninfected/ untreated	AILE, 250 mg/L	AILE, 500 mg/L	AILE, 1000 mg/L
Total protein (g/dl)	2.74±0.01	2.73±0.08	2.90±0.10*	2.83±0.12	2.87±0.03	2.81±0.01
Albumin (g/dl)	1.39±0.04	1.33±0.03	1.58±0.02*	1.50±0.02*	1.39±0.03	1.42±0.02
Globulin (g/dl)	1.35±0.04	1.40±0.10	1.32±0.10	1.33±0.10	1.48±0.01	1.39±0.02
Cholesterol(mg/dl)	97.23±3.55	89.05±1.20*	112.64±3.31*	122.02±3.01*	119.61±2.14*	104.93±4.83
Triglyceride(mg/dl)	143.49±1.27	114.92±4.48*	127.30±3.74*	122.54±3.36*	139.37±0.84*	71.11±0.84*
HDL (mg/dl)	44.55±0.65	48.46±0.86	51.71±1.49*	66.34±0.56*	64.72±1.42*	59.84±5.67*
VLDL (mg/dl)	28.70±0.25	22.98±0.90*	25.46±0.75*	24.51±0.67*	27.87±0.17	14.22±0.17*
LDL (mg/dl)	23.98±3.30	17.61±0.46	35.47±3.40*	31.17±2.54	27.03±0.96	30.87±7.23
ALP (U/L)	113.34±0.23	113.43±0.25	113.88±0.08	114.74±0.09*	114.82±0.08*	114.82±0.28*
ALT (U/L)	4.88±0.23	5.52±0.32	7.20±0.09*	6.32±0.14*	6.02±0.13*	5.33±0.25
AST (U/L)	87.05±2.63	94.85±6.38	87.70±0.75	93.55±4.13	124.75±1.88*	93.55±4.13
Urea (mg/dl)	4.22±0.35	5.43±0.60	5.63±0.20	10.65±0.53*	10.45±0.20*	11.05±0.72*
Creatinine (mg/dl)	0.48±0.02	0.97±0.06*	0.44±0.08	0.67±0.03*	0.82±0.15*	0.54±0.02

***p<0.05 when compared with infected untreated group, AILE = *Azadirachta indica* leaf extract, AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, ALP = Alkaline phosphatase, HDL = High Density Lipoprotein, LDL = Low Density Lipoprotein, VLDL = Very Low Density Lipoprotein**

mg/kg. Also, Sani *et al.* (2020), reported that Neem is well-tolerated by albino rats, with no adverse effects reported at therapeutic doses up to 5000 mg/kg. The reports from above studies supported the acute toxicity findings in the present research.

Azadirachta indica extract progressively reduced *Eimeria* oocysts count in the infected birds from the treated groups, with 85.83, 88.89, 90.21% clearance at the concentrations of 250mg/L; 500mg/L and 1000 mg/L respectively, even better than the positive control (Amprolium® treated) group with 82.90% clearance of the Oocysts.

The mechanism by which neem leaf extract exerted its anticoccidial effect could be multifaceted, involving direct anti-parasitic activity and modulation of the host's immune system (Alzohairy, 2016; Singh *et al.,* 2020). Azadirachtin, a bioactive compound isolated from Neem leaf extract has been shown to disrupt the lifecycle of Eimeria parasites by inhibiting their replication and development within the host (Elangovan *et al.,* 2021). Additionally, the plant extract was also documented to possess immunomodulatory properties which have enhanced the host's ability to mount an effective immune response against the parasites, reducing the severity of the infection via Oocysts clearance (Naidu *et al.,* 2012; Singh *et al.,* 2020). The antioxidant properties of the plant extract could have also played a crucial role in mitigating the damage caused by coccidiosis (Girish & ShankaraBhat, 2008; Tchodo *et al.*, 2024).

While the live weight, dressed weights, breast and drum stick weight of the infected/treated birds were not significantly ($p > 0.05$) affected compared with the infected and untreated birds, the carcass characteristics result demonstrated positive and significant impact on relative defeathered, wing, back cut, and the thigh weight of the infected and treated with the lower doses of the extract which were significantly ($p < 0.05$) increased, comparable with the enhanced carcass characteristics of the birds infected and treated with the standard (Amprolium) drug. This enhanced carcass characteristics in the extract-treated group, has also laid more credence to the recovery of the *Eimeria* infected birds treated with (AILE). Abiola *et al.* (2024) reported that Neem leaf significantly improved the carcass characteristics of broiler chickens with an enhanced appetite, comparable to those obtained with synthetic drugs (Naidoo *et al.,* 2020). Elangovan *et al.* (2021) from their study reported that neem-treated groups showed a significant reduction in oocyst count and an improvement in overall health (carcass qualities) compared to control groups. By scavenging free radicals and reducing oxidative stress, it has been reported that the AILE helps in maintaining the integrity of the intestinal lining and supports overall gut health, which is essential for optimal nutrient absorption and growth in poultry (Kumar *et al.,* 2021). This is a strong support to the findings in the present study.

Blood which is a vital special circulatory tissue is composed of cells suspended in a fluid intercellular substance (plasma) with the major function of maintaining homeostasis. Haematological components, which consist of RBC, WBC, MCV, MCH and MCHC are valuable in monitoring feed toxicity especially with feed constituents that affect the blood as well as the health status of farm animals (Isaac *et al.*, 2013; Salami *et al.*, 2021). The Hb, PCV, and RBC were significantly ($p < 0.05$) decreased in the negative control (Infected/untreated) group indicative of anemia as supported by Fabiyi (2020) which may be due to blood loss. The significant ($p < 0.05$) reduction in the red cell count of the infected/untreated birds could be as a result of significant destruction of red cell, and the mortality recorded in this group could be attributed to complications arising from red cell destruction caused by the parasites. The significant ($p < 0.05$) increases in the MCH and MCHC mean values is suggestive of macrocytic and hyper-chromic type of anemia in the infected/untreated birds. However, AILE exhibited both anticoccidia activity by destroying the causative organism, and also exhibited an ameliorative ability in preventing further destruction of the red cells by parasites, an activity comparable with the standard drug. This could also be as a result of the plant's anti-inflammatory and gastro-protective properties mitigating the intestinal damage caused by *Eimeria* infection (Saleem *et al.,* 2018b; Choudhary *et al.,* 2019) and enhancing the gut health, in turn combating the disruption of the intestinal epithelium. Earlier work by Nayaka *et al.* (2013) on AILE showed that the extract improved hematological parameters in broilers, and this is in agreement with the finding of the present study.

The major functions of the WBC and its differentials are to fight infections, defend the body by phagocytosis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response. Thus, animals with low WBC are exposed to high risk of disease infection, while those with high counts are capable of generating antibodies in the process of phagocytosis and have high degree of resistance to diseases and enhance adaptability to local environmental and disease prevalent conditions (Wu, *et al.*, 2020; Huang *et al.*, 2025). The increased number of eosinophil recorded in the circulation of the infected/untreated birds could be due to the compromise in the normal body function of the birds as a result of increased numbers of *Eimeria* parasite. This finding is supported by Fabiyi, (020), who described the parasite's ability to generally invade the intestinal lining and cause tissue damage, thereby triggering eosinophilic involvement which plays a key role in fighting parasitic infections and in modulating allergic reactions. When compared with the groups treated with AILE, the significant ($p < 0.05$) decrease in eosinophil recorded could be due to the antiprotozoal properties of Neem (Saleem *et al.*, 2018a; Choudhary *et al.*,

2019), the ability to regulate the immune response, with tendency to limit tissue damage while promoting parasite clearance causing eosinophilia (Nayaka *et al.*, 2012).

Also, this antiparasitic effect of the plant could be attributed to the active compounds like quercetin which have been reported to stimulate immune cells, which aided the clearance of the invading parasites, including *Eimeria* species (Mlcek*et al.*, 2016).

Plasma proteins are mainly synthesized by hepatic cells. Albumin carries drug particles and hormones throughout the body, and also helps in tissue growth and healing, while globulin aids in fighting infections and transportation of nutrients (Oleforuh-Okoleh *et al.*, 2015). From this study, the significant increase in total protein of broilers treated with AILE might be due to quick somatic growth and evidence of recovery, since anabolic process and growth are well known factors that increase serum protein levels in healthy animals (Adebomi *et al.*, 2025). This finding of the present study gives credence to an earlier work of Mousa *et al.* (2017) who reported that addition of *Moringa oleifera* in diets of broiler chickens had significant effect on serum total protein and albumin.

Aspartate amino transferase and ALT are involved in assessing the health status of the liver. They are found primarily in the liver with small quantities in the pancreas, heart, muscles, RBC, and kidney (Adebomi *et al.*, 2025). The AST and ALT levels in the serum can be used in the diagnosis of acute myocardial infarction and acute liver disease. Alanine amino transferase is a good mirror of the overall body's enzymatic and metabolic processes (Kamil *et al.*, 2024). The significant increases in the liver biomarkers of the untreated/infected birds could suggest that the parasites induced hepatic injury or damage (Adebomi *et al.*, 2025). Also, higher activities/concentration of both ALT and AST are associated with bile duct problems (Moriles *et al.*, 2024). However, the AILE ameliorated the harmful effect of these parasites on the liver by preventing the rise of these biomarkers. Urea is essential in assessing the health status of the kidneys. It is a product of protein metabolism in the liver and a significant increase in serum urea level, above the normal range is an indication of renal failure, dehydration; diet high in protein and harmful effect of the parasites on the kidney (Wachtel-Galor & Benzie, 2011). In addition, an increase in blood creatinine levels, twice the normal range is a possible indication of kidney damage. The outcome of urea and creatinine of infected/untreated revealed the deleterious effect on the kidney (Sitasiwi *et al.*, 2018), whereas, those treated with AILE yielded an ameliorative effect comparable with the standard antiprotozoan drug used in this study. This therapeutic activity of *Azadirachta indica* on *Eimeria* induced liver and kidney damages could be attributed to the antioxidant properties of the plant which play a crucial role in mitigating the damage caused by coccidiosis by scavenging free radicals and reducing oxidative stress, thereby aiding the liver and kidney functions. This is in agreement with the study by Girish & ShankaraBhat, (2008) on the plant's (Neem) antioxidant properties which play a crucial role in scavenging free radicals induced by coccidiosis.

The blood lipid test index indicative of the total cholesterol shows HDL and LDL of the negative control (infected/untreated) group which were significantly ($p < 0.05$) decreased when compared with the groups treated with AILE. The lipid profile results obtained in this study showed that treatment with AILE prevented the significant increase in serum triglyceride, LDL and VLDL, but caused an increased ($p < 0.05$) level of serum HDL in a concentration dependent manner. This could imply that treatment with AILE against *Eimeria* parasites modulated the lipoprotein synthetic capability of the liver in such a way that relatively more HDL was synthesized by the liver, while more LDL and VLDL were catabolized by the liver. The significant increase in LDL and VLDL levels observed in the untreated birds was as a result of the adverse effect of the parasites on lipid synthesis. This could be due to the parasite's involvement in the affected organs such as the liver, blood vessels of the intestine, etc. leading to reduction in the pH of the intestinal lumen, making it more acidic. This can significantly impact digestive processes, and nutrient absorption showing that the dosage of *Eimeria* caused reduction in lipid absorption. Higher values of HDL and LDL were recorded in groups treated with AILE indicative of this plant beneficial effect on gut health, which could give rise to allowing the good cholesterols. Whereas, the triglyceride and VLDL of the negative control (infected/untreated) group were significantly ($p < 0.05$) increased when compared with the infected birds treated with AILE. This is due to the destruction of the normal function and integrity of the liver and intestine. This demonstrated a dose-dependent anti-lipophilic effect of AILE.

CONCLUSION

The study demonstrated neem's potential as a natural anticoccidial agent in broiler chicken, showing its benefits in terms of growth, health indices, and reduction in the severity of illness by reducing the oocysts count progressively. Adopting neem leaf extract as a natural medicine in chicken production could reduce dependency on synthetic pharmaceuticals, minimize resistance difficulties, and promote sustainable farming techniques. It will also restore carcass qualities lost to *Eimeria* infection, with an excellent potential to become part of an integrated coccidiosis management program, which would be especially beneficial for organic and antibiotic-free chicken production.

CONFLICT OF INTEREST

Authors declare no conflict of interests.

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