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Leveraging the Anticoccidial Effectiveness and Hematological Consequences of Crude Methanol Leaf Extract from Psidium guajava Linn in Broiler Chickens Exposed to Eimeria tenella

Muhammad Y[1,4*], Suleiman MM[2], Jatau ID[3], Arifah AK4 and Abubakar A[5]

[1]*Department of Animal Health and Production, Binyaminu Usman Polytechnic, Hadejia, Jigawa State, Nigeria.*

[2]*Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna State Nigeria.*

[3]*Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna State Nigeria.*

[4]*Department of Preclinical Sciences, Faculty of Veterinary Medicine, 43400 Universiti Putra Malaysia, Serdang, Selangor, Malaysia.*

[5]*Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria.*

***Correspondence:**
Muhammad Y, Department of Animal Health and Production, Binyaminu Usman Polytechnic, Hadejia, Jigawa State, Nigeria, Phone +2347063249774.

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ABSTRACT

Background: *Coccidiosis presents a severe threat to the global poultry industry, adversely affecting both chicken health and productivity. The extensive use of synthetic anticoccidial drugs has contributed to the emergence of drug-resistant Eimeria strains, necessitating the exploration of natural alternatives that are safe, effective, and readily available.*

Objective: *This study focused on assessing the potential anticoccidial and hematological properties of the crude methanol leaf extract obtained from Psidium guajava in broiler chickens infected with Eimeria tenella.*

Methods: The research process began with the collection and identification of Psidium guajava leaves, from which active compounds were extracted using methanol. Safety tests included avian acute toxicity (LD50) and phytochemical screening, which confirmed the presence of secondary metabolites, indicating potential medicinal properties. The study then evaluated the anticoccidial efficacy of the Psidium guajava extract in 50 broiler chickens infected with Eimeria tenella. The chickens were divided into five groups: groups 1 to 3 received varying doses of Psidium guajava extract, group 4 received Amprolium (a standard anticoccidial drug), and group 5 received normal saline. The study spanned seven days, during which parameters such as feed intake, weight gain, and oocyst shedding were closely monitored. Following the study period, the chickens were euthanized, and various samples were collected for analysis.

Results: *Results indicated that the Psidium guajava extract was non-toxic, even at the highest tested dose of 5000 mg/kg, as demonstrated by the LD50 test. Chickens in the treatment groups exhibited milder clinical signs of coccidiosis, including reduced dullness, appetite loss, bloody droppings, and weight loss, compared to the untreated infected group. Treated and infected chickens showed significant improvements in feed intake, weight gain, and feed conversion ratios. Additionally, oocyst shedding, caecal oocyst values, and lesion scores were significantly ($P < 0.001$) lower in the treated groups, indicating the extract's effectiveness against Eimeria infection. Hematological parameters, including Packed Cell Volume (PCV), Hemoglobin (Hb), and Red Blood Cell count*

(RBC), showed significant (P < 0.05) improvements in treated chickens, with the highest values observed in the group receiving 600 mg/kg of Psidium guajava extract. White blood cell counts were also more favorable in the treated groups The also showed that the infected untreated broiler chickens (group 5) had significantly lower MCV, MCH, and MCHC values compared to those receiving Psidium guajava's crude methanol leaf extract (CME) at doses of 200, 400, and 600 mg/kg (groups 1, 2, and 3) or amprolium (group 4). Additionally, broiler chickens in groups 2 and 3, receiving 400 and 600 mg/kg of CME, exhibited significantly higher MCV, MCH, and MCHC values than those in group 1, which received 200 mg/kg of CME. In conclusion, this research highlighted Psidium guajava as a potential effective and safe anticoccidial agent for managing poultry coccidiosis.

Conclusions: *The study demonstrated enhanced chicken health and performance, positioning Psidium guajava as a promising candidate for further exploration in the poultry industry's disease management strategies. The findings suggest that this natural remedy could offer a viable alternative to synthetic anticoccidial drugs, aiding in the combat against drug-resistant Eimeria strains in poultry farming.*

Keywords

Broiler, *Eimeria tenella, Psidium guajava,* Phytochemical screening, Oocyst Count.

Introduction

Coccidiosis is a disease caused by an intracellular protozoan parasite belonging to the Eimeria genus within the Eimeridae family, the Eucoccidiorida order, and the Apicomplexa phylum [1]. Eimeria parasites colonise and infect the intestinal tract of various animals and birds. Infection with this parasite typically occurs when animals ingest feed or water contaminated with mature oocysts [2]. These parasites can infiltrate and replicate within the mucosal epithelia in different parts of a bird's gastrointestinal system through the oral route, resulting in significant gut damage, including inflammation, hemorrhage, and diarrhea. This, in turn, leads to morbidity and mortality in poultry [3]. The tissue damage caused by these parasites is responsible for the reduced absorption of nutrients, dehydration, and blood loss observed in coccidiosis [4]. Domesticated chickens are known to be susceptible to about nine different species of Eimeria, with *Eimeria brunette, Eimeria maxima, Eimeria necatrix*, and *Eimeria tenella* being the most pathogenic. On the other hand, *Eimeria acervulina*, *Eimeria mitis*, and *Eimeria mivati* are considered less pathogenic, and *Eimeria praecox* and *Eimeria hagani* are even less pathogenic [5].

Eimeria plays a significant role in avian species by causing severe enteritis, which results in substantial economic losses within the poultry industry [6]. It also contributes to the occurrence of secondary bacterial infections, such as clostridiosis, which can lead to severe necrotic enteritis [7]. However, infection with Eimeria species induces a long-lasting and robust immunity, and vaccines have been developed as an alternative to anticoccidial drugs. Nevertheless, vaccination itself can sometimes trigger severe hemorrhagic reactions [8], and there is a lack of a standardised protocol to evaluate highly effective vaccines. Abbas et al. [9], also noted that the use of ionophores, synthetic anticoccidial drugs, and vaccines are plagued by issues of resistance and adverse reactions, collectively affecting the performance of birds. Up to this point, Eimeria strains have developed resistance to nearly all known coccidiostats, and the development of new anticoccidials is unlikely due to stringent regulatory restrictions on in-feed drugs and increasing concerns among the general population regarding chemical residues in poultry products [10].

Numerous factors contribute to the development of coccidiosis, and these encompass a direct life cycle, transmission through the faeco-oral route, the presence of oocysts with resistance, a lack of cross-protection among Eimeria species, a high reproductive potential of oocysts, high stocking densities, and the presence of environmental conditions conducive to infectivity, including sporulation [11]. Eimeria oocysts can persist in the environment for several months and can be mechanically transported to poultry facilities through various means, such as the footwear of personnel, clothing, vehicle wheels, contaminated equipment, and dust [1]. Eimeria parasites have been observed in nearly every poultry facility globally. This widespread occurrence of chicken Eimeria makes eradicating the disease impractical, especially in birds kept under deep litter systems.

Natural products, such as extracts from plants, whether in the form of pure compounds or standardised extracts, offer an abundance of opportunities for discovering new drugs. This is primarily due to the unparalleled diversity of chemical compounds available. However, plant extracts typically consist of various bioactive compounds or phytochemicals with different polarities, making their separation a significant challenge when it comes to identifying and characterizing these bioactive compounds [12]. The process of extraction is a pivotal initial step in the analysis of medicinal plants, as it is essential for isolating the desired chemical constituents from the plant materials for subsequent separation and characterization. Numerous plant-based products have been documented for their efficacy in treating various animal diseases, including poultry coccidiosis. These natural remedies encompass a diverse range of botanical sources such as Artemisia annua and its active compound artemisinin [13], oregano [14], garlic [15], neem [16], various species of Aloe [17], green tea [18], sugar cane [19], turmeric [20], and a multitude of others [21]. These plant-derived substances, obtained from leaves, roots, stem bark, fruits, seeds, and powders, are known to contain a plethora of natural compounds exhibiting diverse pharmacological activities [22]. These compounds do not necessarily act directly to eliminate parasites but, instead, often exhibit immunomodulatory effects, possess antioxidative or anti-inflammatory properties. In doing so, they bolster the host organism's ability to combat infectious agents [9]. What sets natural products apart is their capacity to offer drug-like characteristics to molecules derived from combinatorial chemistry. This is evident in terms of functional groups, chirality,

and structural complexity [23]. Natural products, therefore, present a promising avenue for the development of novel therapeutic agents, with their multifaceted properties enabling a more holistic approach to disease management.

The persistent use of anticoccidial chemicals in poultry farming not only fosters the development of drug resistance but also raises concerns about the presence of drug residues in chicken tissues. Hence, the pursuit of a sustainable and herb-based approach to combat coccidiosis is of paramount importance [11]. In recent times, there has been a growing global interest in utilising herbal products as safer alternatives for controlling various diseases, primarily due to their reduced risk of resistance development [24-26]. The plant kingdom offers a vast array of potential solutions, with over 1200 plants reported to possess antiprotozoal properties [27,28]. Some of these herbal remedies find application in poultry diets, thanks to their dual benefits of promoting growth and stimulating the natural immune responses of birds [11].

Furthermore, the consistent rise in poultry meat consumption over the years, driven by its affordability and acceptance across diverse cultural and religious backgrounds [29], has prompted an increased interest from consumers in organic poultry production and the demand for natural, healthier products [30]. As a result, the use of natural remedies has emerged as a promising and environmentally sustainable alternative to traditional anticoccidial drugs [22]. This shift towards herbal solutions not only addresses concerns about drug resistance and residue but also aligns with the contemporary consumer demand for safer and more natural poultry products.

Various chemicals and ionophore-based feed additives with anticoccidial properties have been extensively employed in the poultry industry since 1939 as a means to combat the threat posed by Eimeria parasites, which have the potential to be harmful to poultry. These chemical interventions have played a pivotal role in the management of the disease. However, a concerning issue has surfaced over time.

The development of drug resistance, as highlighted by Abbas et al. [31], has become a significant challenge in the battle against coccidiosis. This means that Eimeria parasites are progressively becoming less responsive to the conventional drugs and treatments that were once effective. The emergence of drug-resistant strains of these parasites has resulted in a reduced effectiveness of these treatments, making it increasingly difficult to control coccidiosis in poultry populations [31]. Moreover, there is a growing concern related to the potential hazards posed to consumers through the presence of drug residues in poultry products. The prolonged and extensive use of these chemicals has led to residues persisting in poultry meat and eggs. Unless these residues are managed carefully, they can enter the food supply chain, ultimately reaching consumers. This poses a risk to public health, as the consumption of poultry products with drug residues may have adverse effects on consumers, including potential health risks [31].

Nevertheless, vaccination has long been regarded as the primary and effective alternative to synthetic anticoccidial drugs, primarily due to its capacity to elicit durable and robust immunity in poultry [3,32]. Regrettably, the use of anticoccidial vaccines is not without its challenges, as they can sometimes trigger severe hemorrhagic reactions, especially when poor management practices impact the performance and uniformity of flocks [33]. Currently, avian coccidiosis is managed using a combination of two main approaches: anticoccidial drugs and vaccines. Traditional anticoccidial drugs, including coccidiocides, coccidiostats, and ionophores, have been the cornerstone of coccidiosis control and treatment in modern poultry production [34]. While this strategy is cost-effective and has historically proven successful, the emergence of drug-resistant strains and increasing public demands for poultry products devoid of drug residues have spurred the development of alternative control methods [34]. In a comprehensive study conducted by Abbas et al. [8], it was revealed that the majority of the currently available anticoccidial drugs are of synthetic origin, and their use has been associated with adverse effects on bird health. These findings underline the growing need for more sustainable and safer approaches to manage coccidiosis in poultry, considering the welfare of both the birds and consumers.

Many poultry farmers often attribute positive benefits to certain natural or herbal products [35]. However, it is crucial to conduct clinical trials to substantiate the efficacy of a bioactive compound and validate these traditional claims, as emphasized by Sasidharan et al. [36]. Clinical trials are the systematic approach aimed at comprehensively understanding the pharmacokinetics, bioavailability, effectiveness, safety, and potential drug interactions associated with newly developed bioactive compounds. Furthermore, these trials involve a meticulous evaluation of the formulations, including extracts. Clinical trials are meticulously designed and executed to ensure the well-being of participants and to provide precise answers to specific research questions. These trials encompass the assessment of both immediate and long-term side effects, and their outcomes are thoroughly evaluated before a drug is widely adopted for use in patients, as described by Sasidharan et al. [36].

Hence, this study was aimed at assessing the anticoccidial efficacy, hematological and antioxidant effects of the crude methanol leaf extract derived from *Psidium guajava* in broiler chickens that have been exposed to *Eimeria tenella*.

Material and Methods

Location of the Research

The plant extraction and screening of the *Psidium guajava* extract were conducted within the Veterinary Pharmacology and Toxicology Department of the Faculty of Veterinary Medicine at Ahmadu Bello University, Zaria. Subsequently, *in vivo* studies to evaluate the anticoccidial properties of the extract were carried out in the Veterinary Parasitology and Entomology Department, also part of the Faculty of Veterinary Medicine at Ahmadu Bello University, Zaria, with geographical coordinates 11.010°N and 7.038°E.

Ethical Clearance

This study received ethical clearance from the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC), and it was assigned the approval number ABUCAUC/2022/015.

Plants Collection, Identification and Preparation

Fresh *Psidium guajava* leaves were collected in Jigawa State, Nigeria, and these samples were subsequently dispatched to the Herbarium located within the Department of Botany, Faculty of Life Sciences at Bayero University, Kano, Nigeria, for the purpose of botanical identification. During this process, a voucher number corresponding to the plant was allocated for reference. Upon returning to the laboratory, the freshly collected leaves were meticulously separated from the plants and subjected to a thorough washing, involving 2-3 cycles of rinsing with clean, running tap water. Subsequently, the leaves were allowed to dry naturally in the shade within the laboratory. After complete drying, the plant materials were processed into a powdered form using a mortar and pestle.

Plant extraction and Concentration

Fifty grams (50 g) of dried powdered *Psidium guajava* was subjected to maceration with 250 ml of 70% methanol in order to obtain raw methanolic extract. This procedure was repeated three times, and the resulting extract was subsequently filtered using Whitman filter paper. The extract was then concentrated under reduced pressure at 45°C to yield a dried extract, which was stored at 4°C until it was needed.

Phytochemical tests

The methanol leaf extract from *Psidium guajava* was subjected to testing to determine the presence of secondary metabolites, including carbohydrates, glycosides, flavonoids, tannins, alkaloids, saponins, steroids, and triterpenes. The evaluation was conducted using the techniques outlined in the methodology established by Trease and Evans in 2000.

Determination of Median Lethal Dose (LD_{50}) of the Extract

The median lethal dose (LD_{50}) of the methanol leaf extract derived from *Psidium guajava* was determined using the avian acute oral toxicity testing protocol established by the Organisation for Economic Cooperation and Development (OECD) in 2016. In summary, this involved the use of five (5) healthy birds, all three weeks old and of both sexes, to ensure uniformity in age. All the birds were administered a limit dose of 5000 mg/kg of the extract directly into their crop. Subsequently, they were monitored for a period of 14 days to observe any signs of toxicity or mortality.

Eimeria tenella isolate

The specific *Eimeria tenella* isolate utilised in this research, as characterised by Jatau et al. [37], was sourced from the parasite repository of the Department of Veterinary Parasitology and Entomology at Ahmadu Bello University in Zaria. To prepare for experimentation, oocysts were cultured in two-week-old chickens that were free from coccidian infections. Unsporulated oocysts were collected from the caecal contents on the seventh day post-infection, subjected to purification, and then preserved in a 2.5% potassium dichromate solution, which facilitated sporulation at 28°C. The sporulated oocysts were stored at 4°C until they were ready for use.

Experimental Birds

A total of fifty (50) day-old broiler chickens were procured from a well-regarded hatchery. These chickens were provided with commercial broiler starter feed and granted a two-week acclimatization period within the laboratory environment prior to their exposure to experimental infection with *Eimeria tenella* for drug testing. Furthermore, all the birds received vaccinations to safeguard against Newcastle disease (ND) and infectious bursal disease (IBD or Gumboro).

Drug Source and Preparations

A quantity of 250 mg of Amprolium HCl was procured from a reputable veterinary pharmacy. The drug's batch number and expiration date were duly observed and documented for reference.

In Vivo Chemotherapeutic Trial

Experimental Birds

The birds were allotted at random into 5 groups of 10 birds each. The first set of 5 groups were treated as follows:

- Group 1 received 200 mg/kg of CME of *Psidium guajava*
- Group 2 received 400 mg/kg of CME of *Psidium guajava*
- Group 3 received 600 mg/kg of CME of *Psidium guajava*
- Group 4 received 1.25 mg/kg of Amprolium (diluted to a concentration of 1.25g/L) in drinking water
- Group 5 received normal saline 5 ml/kg

Note that all treatments were via oral routes and for seven days

Determination of feed intake, weight gain and feed conversion ratio.

The average weight gains and feed conversion ratios for each group were calculated following the methodology outlined by Holdsworth et al. [38].

Daily Feed Intake (DFI)

To calculate this, a known quantity of feed was provided to the birds in the morning, and the amount left over was measured the following morning. The difference between the supplied and leftover feed was used to compute the daily feed intake using the following formula:

$$\text{DFI} = \frac{\text{Quantity of Feed Supplied (g)} - \text{Quantity of feed left over (g)}}{\text{Number of Birds}}$$

Body Weight Gain (BWG)

For this parameter, the actual body weight was subtracted from the weight recorded in the previous week. The formula is as follows:

BWG = Actual Body Weight (in grams) - Previous Weight (in grams)

Feed Conversion Ratio (FCR)

From a mathematical standpoint, the Feed Conversion Ratio (FCR) is determined by dividing the total mass of feed consumed by an animal (its feed intake) by the corresponding increase in the animal's body mass (body weight gain). Essentially, the FCR serves as a numerical representation of the quantity of feed necessary to promote a one-unit increment in the animal's body weight. To put it simply, it quantifies the amount of feed required for the animal to gain a specific weight increment.

FCR = Feed Intake ÷ Body Weight Gain

3.13.6 Determination of Oocyst Production Per Gram (OPG)

At day 7 following infection, all the birds, including those that were infected, treated, and the control group, were humanely euthanized by severing the jugular vein. The caeca from each bird were carefully collected, and two grams of caecal contents were specifically gathered from each bird. This was done to assess oocyst production per gram using the McMaster's counting method, as described by Subramanian et al.

Determination of gross lesions scores:

The caecal lesions were evaluated and scored using the technique outlined by Conway and McKenzie in 2005. The scoring system is as follows:

i. Grade 0: (no visible gross lesions).
ii. Grade 1: (a few scattered lesions).
iii. Grade 2: (numerous discrete lesions with notable bleeding).
iv. Grade 3: (extensively developed lesions with merging and some thickening of the caecal walls).
v. Grade 4: (extensive merging of lesions with significant thickening of the walls and the presence of large caecal cores).

Haematological Analyses

After the birds were humanely euthanized, around 3 ml of blood was collected from each bird using a 10 ml vial that contained EDTA as an anti-coagulant. This blood sample was subsequently utilized to evaluate a range of hematological parameters, which encompassed packed cell volume (PCV), total erythrocyte count, hemoglobin concentration, and erythrocytic indices. The assessment of these parameters adhered to the procedure delineated by Coles in 1986.

Data Analysis

Data obtained were expressed as means ± standard errors of mean (S.E.M), and were analysed using one-way analysis of variance (ANOVA) and compared with Tukey post-hoc test. Similarly, data for gross lesion score were analysed using Kruskal–Wallis test. Differences were considered statistically significant if P-value is less than or equal 0.05 ($P \leq 0.05$).

Results

Voucher number: BUKHAN 336

Extract yield

The percentage yield of the dried methanol leaf extract obtained from *Psidium guajava* was 67.67 %.

Phytochemical Screening test

he findings from the Phytochemical Screening examination are presented in tables 1. After being subjected to phytochemical screening tests following methanol extraction, all the plants showed the presence of secondary metabolites in the leaf extract of Psidium guajava.

Table 1: Phytochemical Screening test tube methods.

Compounds	Test	*Psidium guajava*
Carbohydrates	Molish test	+
	Fehling test	+
Saponin	Frothing test	+
Cardiac Glycoside	Kella-killian's test	+
Flavonoids	Shinoda test	+++
	N^+ hydroxide test	+++
Alkaloids:	Meyer's test	+
	Dragendroff's test	+
	Wagner test	+
Steroids and triterpenes	Liebermann-Burchad's test	+
Tannins	Ferric chlorid test	+
	Bromine water test	+
Anthraquinones	Bontragers's test	+

Acute toxicity study (LD_{50}) of *Psidium guajava* L

Following administration of limit dose of 5000 mg/kg, there was neither mortality nor signs of toxicity during acute toxicity trial. It is therefore, concluded that crude methanol leaf extract of *Psidium guajava* L is acutely safe at limit dose of 5000 mg/kg in broiler chickens. Hence the LD_{50} was assumed to be > 5000 mg/kg.

Effect of crude methanol leaf extract of *Psidium guajava* in broiler chickens experimentally infected with *E. tenella*

Clinical and d postmortem signs observed

The clinical signs in the broiler chickens (infected and treated as well as infected untreated) with *Eimeria tenella* oocysts were dullness, inappetance, somnolence, drowsiness, ruffled feather, emaciation/loss of weight, blood mixed faeces in droppings and frank blood. These signs were mild in the treatment groups (1-4) when compared with infected untreated group. The signs started to disappear from 5-7 days' post infection in all the treatment groups (1-4) when compared with infected untreated group (5).

Effects of crude methanol leaf extract of *Psidium guajava* on weight gain in broiler chickens experimentally infected with *E. tenella*

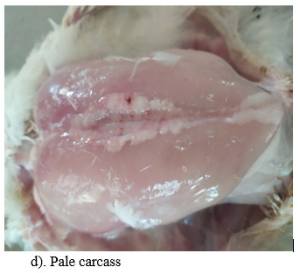
Table 2 illustrates the impact of using crude methanol leaf extract from *Psidium guajava* on the weight gain of broiler chickens that were experimentally infected with *Eimeria tenella*. The mean weight gain showed a significant increase ($P < 0.001$) in all the infected and treated groups (1-4) when compared to the infected groups that did not receive treatment (group 5). Additionally, there was a significant difference ($P < 0.005$) in the mean weight gain between the birds that received 600 mg/kg of *Psidium guajava* crude methanol leaf extract (group 3) and those that received 0.025 mg/kg of amprolium (group 4) when compared to the birds

that were administered 200 mg/kg and 400 mg/kg of *Psidium guajava* crude methanol leaf extract (groups 1 and 2). There were no significant differences (P > 0.005) in weight gain between the birds that received 200 mg/kg and 400 mg/kg of *Psidium guajava* crude methanol leaf extract (groups 1 and 2). Similarly, there was no significant difference (P > 0.005) in weight gain between the birds that received 600 mg/kg of *Psidium guajava* crude methanol leaf extract (group 3) and those that received 0.025 mg/kg of amprolium (group 4).

a). Bloody diarrhea

b). Balloon caecum filled with blood with haemorrhages

c). Mortality



d). Pale carcass

Table 2: Effects of crude methanol leaves extract of *Psidium guajava* on weight gain in broiler chickens experimentally infected with *E. tenella.*

Groups	Treatments (mg/kg)	Mean initial weight (kg)	Mean final weight(kg)	Relative weight gained (%)
1	CME200	0.701± 0.024	1.115± 0.334^{b}	59.10
2	CME400	0.724± 0.025	1.166±0.392^{b}	61.04
3	CME400	0.729 ± 0.018	1.264±0.304^{a}	73.39
4	AMP 0.025	0.742 ± 0.017	1.276±0.320^{a}	71.97
5	NS 5 mL	0.677±0.343	0.947±2.570^{c}	4.43

Mean values with different alphabet are statistically different (P<0.05).

Effect of the crude methanol leaf extract of *Psidium guajava* on Total Feed Intake and Feed Conversion Ratio in broiler chickens experimentally infected with *E. tenella*

Table 3 illustrates the impact of using crude methanol leaf extract from *Psidium guajava* on the total feed intake and feed conversion ratio in broiler chickens that were experimentally infected with *Eimeria tenella*. The average values for total feed intake exhibited a significant increase (P < 0.001) in broiler chickens that were administered 0.025 mg/kg of amprolium (group 4) when compared to birds that were given 200, 400, and 600 mg/kg of *Psidium guajava* extract (group 1, 2, and 3) and those that received a normal saline solution (group 5). Similarly, the average values for total feed intake showed a significant increase (P < 0.001) in broiler chickens that received 400 and 600 mg/kg of *Psidium guajava* extract (group 2 and 3) when compared to those that received 200 mg/kg of *Psidium guajava* extract (group 1) and those that received normal saline (group 5). There was no significant difference (P > 0.05) in the average values for total feed intake in broiler chickens that received 200 mg/kg of *Psidium guajava* extract (group 1) when compared to those that received normal saline (group 5).

The feed conversion ratio in broiler chickens that were experimentally infected with *Eimeria tenella* showed significant difference (P < 0.001) between the birds that received 600 mg/kg of *Psidium guajava* crude methanol leaf extract (group 3) and those that received 0.025 mg/kg of amprolium (group 4) when compared to those that were given 200 and 400 mg/kg of Psidium guajava extract (group 1 and 2) and those that received normal saline (group 5). Additionally, there was a significant difference (P < 0.001) in the average values of feed conversion ratio in broiler chickens that received 200 and 400 mg/kg of *Psidium guajava* extract (group 1 and 2) when compared to those that received normal saline (group 5).

Table 3: Mean ± SEM of the crude methanol leaves extract of *Psidium guajava* on Total Feed Intake and Feed Conversion Ratio in broiler chickens experimentally infected with *E. tenella*.

Groups	Treatments (mg/kg)	Survival rate (%)	Total feed Intake (kg)	Total weight gain (kg)	Feed Conversion Ratio
1	CME200	100	1.85^{c}	1.12^{b}	1.7^{b}
2	CME400	100	2.33^{b}	1.17^{b}	1.9^{b}
3	CME600	100	2.54^{b}	1.26^{a}	2.0^{a}
4	AMP 0.025	100	2.92^{a}	1.28^{a}	2.3^{a}
5	NS 5 mL	90	1.74^{c}	0.95^{c}	2.8^{c}

Mean values with different alphabet are statistically different (P<0.05).

Effect of crude methanol leaf of *Psidium guajava* on Oocyst Shedding in broiler Chickens Infected *E. tenella*

Table 4 presents the impact of *Psidium guajava* crude methanol leaf extract (CME) on Oocyst Shedding in broiler chickens experimentally infected with *Eimeria tenella*. The oocyst counts per gram of faeces increased from the fifth to the sixth day after infection in both the infected and treated broiler chickens (groups 1-4) as well as in the infected but untreated ones (group 5). The average values of oocyst shedding exhibited a significant increase (P < 0.001) in the infected and untreated broiler chickens (group 5) from day 4 to day 6 post-infection when compared to the infected and treated broiler chickens (group 1-4). Similarly, there was a significant decrease (P < 0.001) in the average values of oocyst shedding in broiler chickens that received 600 mg/kg of *Psidium guajava* CME (group 3) and those that received 0.025 mg/kg of amprolium (group 4) from day 4 to day 6 post-infection when compared to those that received 200 and 400 mg/kg of *Psidium guajava* extract (group 1 and 2). There was no significant difference (P > 0.005) in the average values of oocyst shedding between broiler chickens that received 600 mg/kg of *Psidium guajava* CME (group 3) and those that received 0.025 mg/kg of amprolium (group 4) from day 4 to day 6 post-infection. Similarly, there were no significant difference (P > 0.005) in the average values of oocyst shedding in broiler chickens that received 200

and 400 mg/kg of *Psidium guajava* CME (group 1 and 2) from day 4 to day 6 post-infection.

Table 4: Effect of CME OF *Psidium guajava* on Oocyst Shedding in broiler Chickens Infected *E. tenella*.

Days Post Infection				
Treatments (mg/kg)	**Day 4 (10^4)**	**Day 5 (10^4)**	**Day 6 (10^4)**	**Day 7 (10^4)**
CME200	0.177 ± 0.0192^b	2.356 ± 0.0924^b	6.115 ± 0.1907^b	2.636 ± 0.0440^b
CME400	0.140 ± 0.0261^b	0.824 ± 0.0212^b	4.162 ± 0.1771^b	1.488 ± 0.0427^b
CME600	0.074 ± 0.0087^a	0.292 ± 0.0174^a	3.242 ± 0.1036^a	0.232 ± 0.0356^a
AMP 0.025	0.032 ± 0.0102^a	0.100 ± 0.0651^a	2.236 ± 0.2789^a	0.048 ± 0.0049^a
NS 5 mL	1.328 ± 0.0432^c	3.573 ± 1.1390^c	7.113 ± 0.2046^c	5.768 ± 0.0885^c

Mean values with different alphabet are statistically different (P<0.05).

Effect of the crude methanol leaf extract of *Psidium guajava* on Lesion Score in broiler chickens experimentally infected with *E. tenella*

Table 5 depicts the impact of using crude methanol leaf extract from Psidium guajava on lesion scores in broiler chickens that were experimentally infected with *Eimeria tenella*. The mean values of lesion scores showed a significant increase ($P < 0.05$) in the infected but untreated broiler chickens (group 5) when compared to the infected and treated broiler chickens (group 1-4). Similarly, there was a significant decrease ($P < 0.05$) in the mean values of lesion scores in broiler chickens that received 600 mg/kg of *Psidium guajava* CME (group 3) and those that were given 0.025 mg/kg of amprolium (group 4) when compared to those that received 200 and 400 mg/kg of *Psidium guajava* extract (group 1 and 2). There was no significant difference ($P > 0.05$) in the mean values of lesion scores between broiler chickens that received 600 mg/kg of *Psidium guajava* CME (group 3) and those that were given 0.025 mg/kg of amprolium (group 4). Similarly, once again, there were no significant difference ($P > 0.05$) in the mean values of lesion scores in broiler chickens that received 200 and 400 mg/kg of *Psidium guajava* CME (group 1 and 2).

Table 5: Mean ± SEM of the crude methanol leaves extract of *Psidium guajava* on Lesion Score in broiler chickens experimentally infected with *E. tenella*.

Group	Treatments (mg/kg)	Mean lesion score
1	CME200	2.56 ± 0.1979^b (2 – 3)
2	CME400	1.94 ± 0.0595^b (2 – 3)
3	CME600	1.80 ± 0.4231^a (2 – 3)
4	AMP 0.025	1.20 ± 0.2000^a (2 – 3)
5	NS 5 mL/kg	3.84 ± 0.1030^c (3 – 4)

Mean values with different alphabet are statistically different (P<0.05).

Effect of the crude methanol leaf extract of *Psidium guajava* on caecal oocyst contents in broiler chickens experimentally infected with *E. tenella*

Table 6 illustrates the impact of using crude methanol leaf extract from *Psidium guajava* on caecal oocyst values in broiler chickens that were experimentally infected with *Eimeria tenella*. The effect of *Psidium guajava* crude methanol leaf extract on caecal oocyst count varied depending on the dose. The average caecal oocyst values showed a significant increase ($P < 0.05$) in the infected but untreated broiler chickens (group 5) in comparison to the infected and treated broiler chickens (group 1-4). Similarly, there was a significant reduction ($P < 0.05$) in the average caecal oocyst values in broiler chickens that received 600 mg/kg of *Psidium guajava* CME (group 3) and those that were administered 0.025 mg/kg of amprolium (group 4) when compared to those that received 200 and 400 mg/kg of *Psidium guajava* extract (group 1 and 2). There was no significant difference ($P > 0.05$) in the average caecal oocyst values between broiler chickens that received 600 mg/kg of *Psidium guajava* CME (group 3) and those that were given 0.025 mg/kg of amprolium (group 4). Similarly, once again, there was no significant difference ($P > 0.05$) in the average caecal oocyst values in broiler chickens that received 200 and 400 mg/kg of Psidium guajava CME (group 1 and 2).

Table 6: Mean ± SEM of the crude methanol leaves extract of *Psidium guajava* on caecal oocyst contents in broiler chickens experimentally infected with *E. tenella*.

Groups	Treatments (mg/kg)	Caecal Oocyst Values
		OPG ($\times 10^5$)
1	CME200	4.540 ± 2.2920^b
2	CME400	3.746 ± 0.0382^b
3	CME600	2.704 ± 0.0531^a
4	AMP 0.025	2.867 ± 0.0508^a
5	NS 5 mL/kg	9.320 ± 0.1140^c

Mean values with different alphabet are statistically different (P<0.05).

Effect of the crude methanol leaf extract of *Psidium guajava* on haematological parameters in broiler chickens experimentally infected with *E. tenella*

Figure 1 illustrates the effect of *Psidium guajava* crude methanol leaf extract on hematological parameters in *Eimeria tenella*-infected broiler chickens. The lowest values for Packed Cell Volume (PCV), Hemoglobin (Hb), and Red Blood Cell count (RBC) were found in untreated infected broiler chickens (group 5), with values of 18.36 ± 1.339, 9.23 ± 0.338, and 1.317 ± 0.038, respectively. Conversely, the highest values for PCV, Hb, and RBC were observed in broiler chickens infected and treated with 600 mg/kg of *Psidium guajava* (group 3), with values of 33.84 ± 1.780, 16.74 ± 0.866, and 2.587 ± 0.074, respectively. These findings indicate a positive impact of the extract on hematological parameters in infected broiler chickens.

Effect of the crude methanol leaves extract of *Psidium guajava* on erythrocytic indices; MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin) and MCHC (mean corpuscular haemoglobin concentration) in broiler chickens experimentally infected with *E. tenella*.

In this study, figure 2 presents findings regarding the impact of crude methanol leaf extract (CME) of *Psidium guajava* on erythrocytic indices, specifically Mean Corpuscular Volume

(MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) in broiler chickens infected with Eimeria tenella. The infected untreated broilers (group 5) exhibited a significant decrease ($P < 0.05$) in the mean values of MCV, MCH, and MCHC compared to broiler chickens in groups 1, 2, and 3, which received 200, 400, and 600 mg/kg of CME of *Psidium guajava*, as well as those in group 4, which received 0.025 mg/kg of amprolium.

Furthermore, broiler chickens in groups 2 and 3, which received 400 and 600 mg/kg of CME of *Psidium guajava*, showed a significant increase ($P < 0.05$) in MCV, MCH, and MCHC compared to those in group 1, which received 200 mg/kg of CME of *Psidium guajava*. Notably, there was no significant difference ($P > 0.05$) in the mean values of MCV, MCH, and MCHC between broiler chickens in group 3 (receiving 600 mg/kg of CME of Psidium guajava) and those in group 4 (receiving 0.025 mg/kg of amprolium).

Effect of the crude methanol leaf extract of *Psidium guajava* on total and differential White Blood Cell count in broiler chickens experimentally infected with *E. tenella*.

Figure 3 shows the impact of *Psidium guajava* extract on white blood cell counts in Eimeria tenella-infected broiler chickens. Untreated infected chickens (group 5) had the highest counts, while those treated with 600 mg of *Psidium guajava* extract (group 3) had the lowest counts. Infected untreated chickens had significantly higher counts than those treated with the extract (groups 1, 2, 3) or amprolium (group 4). There was a significant decrease in counts in broilers receiving 400 and 600 mg/kg of the extract (groups 2 and 3) compared to 200 mg/kg (group 1). No significant differences were observed between broilers receiving 600 mg/kg of the extract (group 3) and those given amprolium (group 4).

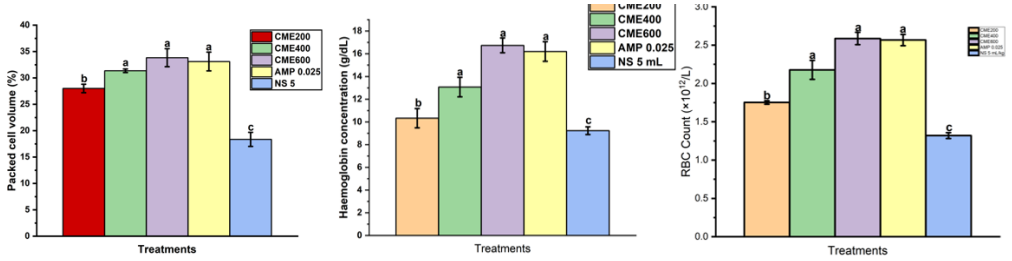


Figure 1: Effect of the crude methanol leaf extract of *Psidium guajava* on haematological parameters in broiler chickens experimentally infected with *E. tenella.*

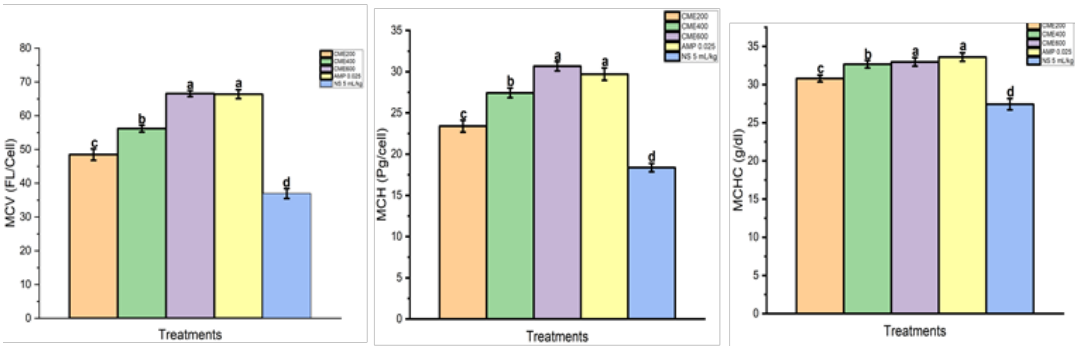


Figure 2: Effect of the crude methanol leaves extract of *Psidium guajava* on erythrocytic indices; MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin) and MCHC (mean corpuscular haemoglobin concentration) in broiler chickens experimentally infected with *E. tenella.*

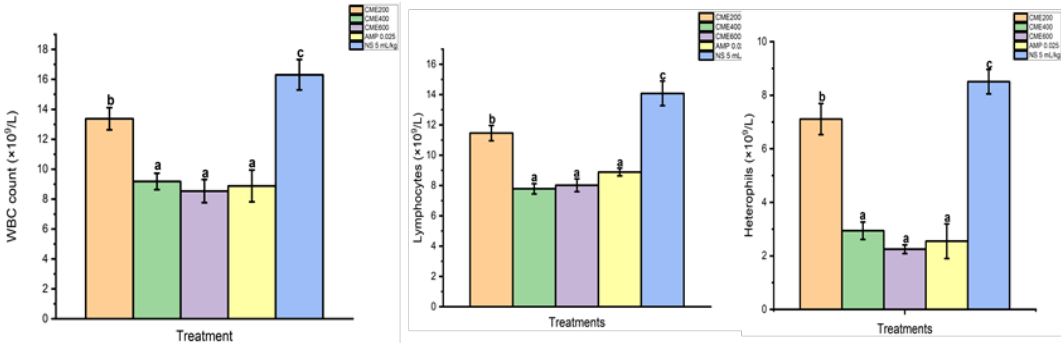


Figure 3: Effect of the crude methanol leaf extract of *Psidium guajava* on total and differential White Blood Cell count in broiler chickens experimentally infected with *E. tenella*.

Discussions

In this study, avian acute oral toxicity assessment was carried out in broiler chickens exposed to a limit dose of 5000 mg/kg of *Psidium guajava* methanolic leaf extract, and no signs of toxicity or mortality was observed. This suggests that the LD_{50} (lethal dose for 50% of the population) for the extract exceeds 5000 mg/kg, indicating its relative non-toxicity. According to OECD, 2016 guidelines, substances with LD_{50} values falling between 2000 mg/kg and 5000 mg/kg when administered orally are generally considered safe. These findings align with previous research by Sekhar et al. [39], Roy et al. and Atik et al. [40], which also demonstrated high LD_{50} values for *Psidium guajava* extracts, reinforcing the safety of the substance.

In broiler chickens infected with *Eimeria tenella* oocysts, whether they were treated or left untreated, various clinical symptoms were observed. These included lethargy, reduced appetite, drowsiness, ruffled feathers, weight loss, the presence of blood spots in their droppings, and frank blood in their feces. The severity of these symptoms were lower in the treatment groups (groups 1-4) compared to the untreated group (group 5). In the treated groups (1-4), these symptoms began to diminish between days 5 and 7 post-infection, while group 5, which remained untreated, continued to display severe symptoms. The pattern of clinical findings is consistent with a study by Yamssi et al. [41], where broiler chickens infected with *Eimeria tenella* and treated with a methanol leaf extract of *Psidium guajava* exhibited similar signs. The weight loss, weakness, drowsiness, and lethargy were attributed to the significant loss of body fluids due to diarrhea. Hambesha et al. also reported similar results in their study, supporting the findings of the present study. Additionally, prior research by Ogbe et al. [42] and Conway et al. [43] confirmed a substantial reduction in the body weight of broiler chickens infected with *Eimeria tenella* oocysts. It's important to note that no mortality was observed in the groups of infected chickens that received treatment (groups 1-4), indicating the effective suppression of oocyst development by the extract. In contrast, one mortality was recorded in the group of infected chickens that did not receive treatment (group 5), aligning with the results reported by Razzaq et al. [44], who noted the highest mortality rate among untreated infected broilers.

Regarding the impact of the treatment with the crude methanol leaf extract from *Psidium guajava*, the mean values for total feed consumption, weight gain, and relative weight gain in broiler chickens infected with *Eimeria tenella* significantly ($P < 0.001$) increased in a dose-dependent manner in treatment groups (1-4). Similarly, the mean values for the feed conversion ratio in these broiler chickens decreased significantly ($P < 0.001$) in a dose-dependent manner in treatment groups (1-4) but increased significantly ($P < 0.001$) in the infected untreated broiler chickens. In contrast, the infected untreated broiler chickens showed a significant decrease ($P < 0.001$) in total feed intake, weight gain, and relative weight gain. The increase in feed conversion ratio in the infected untreated broilers (group 5), agrees with the findings of Renaudeau et al. [45], who suggested that reduced available energy in the body could lead to decreased body weight or increased feed conversion in infected untreated broilers. The reduced feed intake in the untreated infected groups, resulting in decreased weight gain and an increased feed conversion ratio, may be attributed to the unappetizing taste of the feed material due to coccidiosis, because of the findings of Kudo et al. who reported that *Eimeria tenella* has the ability to affect the integrity of taste buds in chicken oral cavities, affecting their ability to detect tastes of various dietary substances, as noted by Rajapaksha et al. [46]. However, the increase in total feed consumption, weight gain, and relative weight gain, coupled with a reduction in the feed conversion ratio, in broiler chickens can be linked to the improved taste and desirability of their diet. This enhancement can be attributed to the presence of phenolics and various phytoconstituents, which are known for their capacity to boost the performance and responsiveness of taste receptors in the oral cavities of chickens. Consequently, it is reasonable to assume that broiler chickens are benefiting from an enhanced sense of taste, which encourages them to consume more feed [46]. This increased consumption, in turn, leads to greater weight gain and overall growth, while the improved feed conversion ratio suggests that the feed is being utilized more efficiently by the chickens. Several researchers have identified the presence of flavonoids, phenolics, saponins, anthraquinones, terpenoids, alkaloids, and cardiac glycosides in the crude methanol leaf extract of *Psidium guajava* [47]. These compounds were associated with the improved feed intake, weight gain, relative weight gain, and feed conversion ratio. Mahmoud et al. [48] demonstrated that incorporating dried guava leaves into diets significantly improved body weight, weight gain, feed conversion ratio, and overall health status of broiler chicks, although it had no impact on feed consumption. Conversely, Rahman et al. reported the effects of guava extract on feed intake, showing that the inclusion of guava leaf meal in broiler diets significantly affected feed intake and decreased mortality rates as the quantity of guava leaf meal in the broiler diet increased. The enhanced feed conversion ratio in the groups treated with *Psidium guajava* leaf extract could also be attributed to the reduction of intestinal inflammation and injuries by the extract, as reported by other researchers [49].

The shedding of oocysts in faeces exhibited a significant increase ($p < 0.001$) between the fifth and sixth days after infection in both infected and treated broiler chickens (groups 1-4) and infected, untreated ones (group 5). Notably, there was a significant decrease ($p < 0.001$) in the average oocyst shedding in broiler chickens that received 600 mg/kg of *Psidium guajava* CME (group 3) and those given 0.025 mg/kg of amprolium (group 4) between days five and six post-infection when compared to those that received 200 and 400 mg/kg of *Psidium guajava* extract (group 1 and 2). No significant difference ($p > 0.005$) was observed in the mean oocyst shedding between broiler chickens that received 600 mg/kg of *Psidium guajava* CME (group 3) and those that received 0.025 mg/kg of amprolium (group 4) during the same time frame. Similarly, there was no significant difference ($p > 0.005$) in the average oocyst shedding between broiler chickens receiving 200 and 400 mg/kg of *Psidium guajava* CME (group 1 and 2) from days five to six post-infection. The efficacy of the test substance in reducing fecal oocyst shedding of *Eimeria tenella* is of interest.

These positive effects may be attributed to the phytochemical components of the plant, as suggested by Anyanwu and Dawet [50]. Plant chemicals, such as those found in *Psidium guajava* leaves, have been found to be effective against protozoa, including *Eimeria tenella*. Specific phytochemicals, like saponins and flavonoids, previously identified in *Psidium guajava* leaves, have been shown to bind to sterol molecules on the cell membrane of *Eimeria tenella* and destroy them, as reported by Taura et al. [51] and Offor [52]. The reduction in fecal oocyst shedding observed in this study aligns with the plant's activity against *Eimeria tenella* and other related organisms, such as plasmodium spp [53], highlighting its potential in managing protozoan parasitic infections. The mean values of oocyst shedding significantly increased ($p < 0.001$) in infected, untreated broiler chickens (group 5) between days five and six post-infection when compared to infected and treated broiler chickens (groups 1-4). In this study, broiler chickens were exposed to 10,000 doses of *Eimeria tenella* oocyst, resulting in a significant increase in oocyst shedding between days five and six post-infection ($p < 0.001$). This finding supports the idea put forth by Walker et al. [54] that smaller inoculation doses of *Eimeria tenella* can lead to higher oocyst shedding if left untreated. Williams [55] also demonstrated that higher inoculation dose levels can linearly increase oocyst yields until reaching a point of maximum production, and doses exceeding this maximum can actually reduce oocyst yields in broilers. However, no significant proliferation of oocysts was observed between days seven and eight post-infection in any of the groups in the current study ($p > 0.05$).

In this research, a noticeable and statistically significant ($P < 0.05$) increase in the severity of gross pathology was observed in untreated broiler chickens that were infected (group 5) when compared to broiler chickens that were both infected and treated (groups 1-4). In group 5, enlarged caeca with clotted blood, haemorrhagic spots on the caecal wall, signs of inflammation, necrotic patches, and dilated caeca with solidified caecal contents were observed in nearly all of the broiler chickens. Upon opening the caeca, the characteristic bloody mass of caecal coccidiosis was once again found in group 5. Additionally, a colour change from red to mottled reddish or milky white was noted in group 5 due to the presence of oocysts. These findings are consistent with previous research conducted by Sourabh et al. [56] and Hambesha et al., as they also observed enlarged caeca with clotted blood, hemorrhages throughout the caecal mucosa, and a shift in coloration from reddish to milky white.

The caecal lesion score and caecal oocyst count demonstrated a significant decrease ($P < 0.05$) in broilers that were infected and received treatment (groups 1-3) when compared to broilers that were infected but left untreated (group 5). This outcome is consistent with the findings reported by Hambesha et al., who attributed the severe caecal lesions observed in untreated, infected broilers to the destruction of the caecal epithelium and underlying connective tissue of the mucosa caused by the proliferative stages of *Eimeria tenella*. This damage could result in hemorrhage within the caecal lumen, catarrhal inflammation, and diarrhea. The mild caecal lesions observed in our study may be due to the protective effects of the crude methanol leaf extract of *Psidium guajava* on the caecal mucosa and submucosa, thereby preventing damage caused by *Eimeria tenella*. This protective effect is likely attributed to the presence of certain bioactive compounds in the crude methanol extract of *Psidium guajava* known to have an effect against *Eimeria tenella*. Additionally, our study found that untreated broilers infected with *Eimeria tenella* (group 5) exhibited a noticeable increase in crypt depth within the ceca, as evident in the caecal scrapings used to determine caecal oocyst counts. This heightened crypt depth was particularly prominent in the untreated, infected broilers (group 5). The precise reasons behind this increased crypt depth resulting from *Eimeria tenella* infection remain unclear, as mentioned by Choi et al. [57]. Therefore, this rise in crypt depth likely contributed to the significant ($P < 0.05$) increase in the oocyst count within the caecal contents. One hypothesis is that *Eimeria tenella* may deepen caecal crypts (mucosal layer) to create a more suitable habitat within the ceca. Alternatively, it's possible that crypts were deepened to enhance the absorption of volatile fatty acids, which are essential nutrients for the parasite. This is noteworthy because volatile fatty acid production is restricted during *Eimeria tenella* infection, as noted by Choi et al. [57]. However, it's worth considering that increased crypt depth could impede the production and absorption of volatile fatty acids in the ceca, as suggested by Choi et al. [57]. Another plausible explanation for the increased crypt depth in broilers infected with *Eimeria tenella* is that the birds may have deepened the crypts to function similarly to villi as a defense mechanism in the proximal caeca. This adaptation might be aimed at reducing vulnerability to further infections, such as bacterial infections, in *Eimeria tenella*-infected caeca, as proposed by Choi et al. [57].

The results of this study revealed a dose-dependent decrease in hematological parameters, such as PCV (packed cell volume), Hb (hemoglobin), and RBC (red blood cell) counts, in broiler chickens that were both infected and treated with the crude methanol extract of *Psidium guajava* (groups 1-3), as compared to infected but untreated broiler chickens (group 5). These findings were in accordance with the reference values established by Williams, which indicated that coccidiosis induced by *Eimeria tenella* resulted in a more significant reduction in PCV, Hb, and RBC levels. Therefore, the outcomes of this study were consistent with those reported by Hambesha et al., who observed lower PCV, Hb, and RBC counts in broiler chickens infected with *Eimeria tenella* when compared to uninfected controls. Choi et al. [57] also noted a notable decrease in PCV, Hb, and RBC counts in untreated broilers infected with *Eimeria tenella.* Additionally, Yamssi et al. [41] demonstrated a substantial improvement in PCV, Hb, and RBC levels in broiler chickens that were experimentally infected with *Eimeria tenella* and subsequently treated with the crude methanol extract of *Psidium guajava*. Anemia, characterized by reduced PCV, Hb, and RBC levels, is a common erythrocyte abnormality in birds. Birds with a PCV below 28% are typically considered anemic [41], and the decrease in RBCs can be attributed to blood loss in the gastrointestinal tract, known as external blood loss anemia. The hallmark of avian coccidiosis is anemia, as the infection can lead to severe blood loss in the infected chickens.

The significant ($P < 0.05$) decrease observed in the mean values of PCV, Hb concentration, and total RBC in infected but untreated broiler chickens (group 5) at 7 days' post-infection may be linked to caecal hemorrhage. This hemorrhage could be a result of mechanical breakage of mucosal capillaries caused by pressure from caecal epithelial cells expanding after schizogony or the release of histamine during tissue damage, which increases the permeability of blood vessels, allowing for the escape of large quantities of blood, as suggested by Hambesha et al.

The average measurements of MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), and MCHC (mean corpuscular hemoglobin concentration) exhibited a significant ($P < 0.05$) increase in infected, untreated broilers (group 5) when compared to broiler chickens in groups 1, 2, and 3, which received doses of 200, 400, and 600 mg/kg of *Psidium guajava* CME, as well as those in group 4, which received 0.025 mg/kg of amprolium. Several previous studies that investigated changes in red blood cell parameters following experimental infections in animals have documented instances of anemia [58]. Our findings align with the observations reported by Freitas et al. [59], who recorded a significant decrease in MCV, MCH, and MCHC following exposure to sporulated oocysts. Similarly, Hana et al. [60] identified anemia in rabbits suffering from coccidiosis caused by *Eimeria magna*, attributing it to damage inflicted on the intestinal mucosal epithelium and blood vessels by the coccidia. Results of this study supported findings of Cam et al. regarding PCV, Hb, RBC count, as well as MCV, MCH, and MCHC, all of which exhibited a decline in the infected, untreated groups. This study reinforces the existing understanding of anemia in the context of chronic inflammatory diseases [61] and the inflammatory response during Eimeria infection [59]. According to the findings of this study, the type of anemia observed in infected, untreated broilers (group 5) appears to be microcytic and hypochromic. This could be attributed to various factors, including a diminished iron content in the diet, inadequate absorption of iron from the gastrointestinal tract, instances of acute and chronic blood loss, or the recovery process following significant trauma or surgery [62]. Moreover, the mean values of MCV, MCH, and MCHC displayed a significant improvement ($P < 0.05$) in broiler chickens treated with 200, 400, and 600 mg/kg of *Psidium guajava* CME (groups 1, 2, and 3). This enhancement can be attributed to the extract's ability to prevent or suppress the detrimental effects of *Eimeria tenella*, as the extract contains bioactive compounds with diverse activities against protozoan parasites.

Conclusion

In summary, this study provides valuable insights into the effects of *Psidium guajava* methanolic leaf extract on avian acute oral toxicity and its potential for managing *Eimeria tenella* infection in broiler chickens. The extract exhibited no signs of toxicity, reinforcing its safety and non-toxic nature. In the context of *Eimeria tenella* infection, treated groups showed milder clinical symptoms, reduced mortality, and symptom improvement between days 5 and 7 post-infection, compared to untreated groups. The extract positively influenced parameters such as feed consumption, weight gain, and feed efficiency, with dose-dependent improvements. It significantly reduced oocyst shedding and protected the caecal mucosa, while untreated groups exhibited severe lesions and increased crypt depth. Hematological parameters were affected by the infection, and the extract mitigated these effects, particularly microcytic and hypochromic anemia. In conclusion, *Psidium guajava* methanolic leaf extract holds promise for managing *Eimeria tenella* infection in broiler chickens, with further research needed to explore its mechanisms and optimal dosages for effective coccidiosis control in poultry.

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