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# Antiprotozoal Effects of *Aloe vera* Leaves Extract against Experimentally Induced Coccidiosis in Broiler Chickens

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author SG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AH and GA managed the analyses of the study. Author MMI managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

The anti-coccidian properties of *Aloe vera* were investigated and its therapeutic effect was compared with that of conventional drugs (amprolium powder) on broiler chickens. The broiler was divided into four groups of 15 birds each. Group A served as control and were not infected while B, C, and D were infected with 0.2 ml of coccidian oocyte each orally at 4 weeks old. After the manifestation of the clinical signs of coccidiosis, on day 5 post-infection, the birds in group B were treated with 1.2 g/kg feed with crude extract of *Aloe vera*, while, group C with amprolium hydrochloride at 3.25 g/liter drinking water; and group D was not treated and served as infected control. All treatments were done once daily for seven days. The oocyte count obtained from faeces of group B and C broiler reduced significantly ($P<0.05$) from 120 oocytes at 5 days post-infection to 18. There was no reduction of oocyte count in-group D. The result showed that *Aloe vera* was able to reduce significantly ($P<0.05$) the number of the oocyte, in comparison to amprolium in broiler chickens infected with coccidiosis.

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## 1. INTRODUCTION

According to the world health organization (WHO), a medicinal plant is any plant in which one or more of its parts contains a substance that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs [1]. For years, medicine had depended exclusively on leaves, flowers, and barks of the plants, only recently that synthetic drugs came into use and many instances, these carbon copies of chemicals identified in plants traditionally the used of these secondary metabolites in plants preparation as sources of drugs are based on the experience of superstition passed from generation to generation.

In this regard, many plants and their constituents (secondary metabolites) including *Newbouldia laevis, Saccharum officinarum, Curcuma longa* [2,3,4] have been reported to have different medicinal properties. *A. vera* (*Aloe barbadensis Miller*) is the modifiers; Chickens most commonly used medicinal plant having historical importance. It is a succulent plant found in tropical and subtropical areas of many countries including Nigeria.

Major ingredients of *Aloe vera* include anthraquinones, polysaccharides, vitamins, enzymes and low molecular weight compounds [5] which gives *Aloe vera* its anti-inflammatory, immunomodulatory, wound healing, antiviral, antifungal, antitumor, antidiabetic and antioxidant effects [6]. Several studies have suggested that many benefits of *Aloe vera* are due to its polysaccharides contained in gel, which compose a large part of dry matter in this gel [7]. Almost 60% of dry matter of *Aloe vera* gel is composed of polysaccharides and active compound is acemannan which has immunomodulatory, antimicrobial and antitumor effects [5], antioxidants, wound healing, immune-modulatory and antidiabetic activities [8].

*Coccidiosis* is one of the most common and economically important diseases of Chickens worldwide. It is caused by a parasitic organism that damages the host's intestinal system, causing loss of production, morbidity and death. The animal becomes infected with coccidian organisms through the ingestion of food and water contaminated with coccidian. The coccidian penetrates the intestinal wall causing the cell to lyse and die.

A large number of anticoccidial drugs are used for the control of *coccidiosis* such as Sulphachlopyrazine sodium monohydrate (ESB3), amprolium, furazolidone, nitrofurazone worldwide. However, their excessive use has led to the development of drug resistance, residues in the tissues, organs and high economic cost. Such residues of veterinary drugs in food 'intended or unavoidable even at a low concentration constitute threats to human health and impact negatively on trade. Therefore, researchers are searching to identify the efficacy of different herbs and herbal by-products to decrease the huge losses caused by coccidiosis in the poultry business. Herbal plants and their byproducts may serve as remedies for coccidiosis because of their low toxicity and reduced cost of production, it is therefore based on this background that this study was conducted to evaluate and compare the therapeutic effects of *Aloe vera* with conventional anti-coccidian drugs (amprolium) in treating and controlling of *Coccidial* disease.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Plant Material

The plant of *Aloe vera* (leaves) was collected from Herbal Garden Yobe University. The plant part (leaves) was identified by a taxonomist in the Department of Biology, Yobe State University Damaturu.

### 2.2 Separation of Gel from the Leaf

The mucilaginous leaf gel was separated from *A. vera* leaves within 3-4 h post collection to avoid aero deterioration of gel contents according to the method described by Jiang et al. [9]. Gently, the prewashed *A. vera* leaves were incised longitudinally with the help of a sharp sterilized knife followed by gentle scraping of gel using a spatula. The gel was homogenized, filtered through cheesecloth and stored in screw-capped jars at 4°C till further use.

Screening of Phytochemical Components: Phytochemical components were analyzed qualitatively [10].

Broilers: Sixty day-old broiler, were used for the study.

Feed: Broiler starter (Commercial feed, Vital) was used from day old to four weeks of age while broiler finisher was used from 5 to 8 weeks of age.

Coccidia oocyst: Were obtained from the Pathology laboratory of the National Veterinary Research Institute, Vom.

### 2.3 Rectal Temperature Measurement

The rectal temperatures of the birds were measured with the aid of a digital clinical thermometer (Cocet China). The thermometer was inserted into the cloaca of each bird through the rectum and kept tilted to the mucous membrane of the rectum, till the sound of the alarm from the thermometer is heard indicating the end of the reading. The rectal temperatures of the birds were recorded during the treatment period.

### 2.4 Bodyweight Measurement

The birds were weighed at four weeks, shortly before the experimental infection and weekly until the end of the experiment. The body weights were recorded with the aid of a digital weighing scale.

### 2.5 Experimental Infection and Treatments of Birds

At four weeks, birds in groups B, C and D received 0.2 ml of coccidian oocyte orally containing 120, coccidial oocyte each. Group A served as control and was not infected. Approximately 5 grams of feces were collected from all groups for oocytes count. After 5 days, the post-infection fecal sample was collected daily to identify the presence of oocyst. Group B was treated on 8 days post-infection with 1.25 crude extract powder of *Aloe vera* in four liters of drinking water, while group D was not treated and left as infected control. The treatment was done once daily for seven (7) consecutive days.

#### 2.5.1 Oocyst count

One gram of feces from each group was collected during the treatment period and taken to the microbiology laboratory of the University for Oocyst Count at once daily for seven (7) days of the treatment period.

### 2.6 Feed Conversion Efficiency (Fce)

The amount of the feed consumed by the bird per day/ bird was calculated using the formular

$$FCE = \frac{feed\ consumption\ (gm)}{body\ weight\ (gm)}$$

### 2.7 Statistical Analysis

The data obtained was analyzed using student t-test, P value less than 0.05 was considered significant.

## 3. RESULTS

The phytochemical active components of *A. vera* were qualitatively analyzed and the results are presented in Table 1. An analysis of Tannin compounds brownish-green color developed to indicate the presence of Tannin. Similarly based on the presence or absence of color change indicates positive and negative results. In this screening process Tannin, Saponin, Flavonoids, and Terpenoids revealed positive results in the plant extracts.

Table 2 shows the live weight gain of the birds at four (4) weeks, the average weight gain for the group A was 0.74 Kg, group B was 0.73 Kg, group C was 0.74 Kg and Group D was 0.73 Kg respectively. However, at 5 weeks, group A gained 0.16 Kg; group B gained 0.11 Kg; group C gained 0.11 Kg and group D gained 0.01 Kg.

The result in weight gain during the study period was significantly ($P<0.05$) higher in the control group that was not infected and not treated. The birds' weight gain was lower ($P<0.05$) compared to the other groups. This indicated that the coccidial infection was capable of reducing the weight gain in birds. This may be due to the destructive nature of the parasite in the gastrointestinal tract, which is responsible for feed absorption.

The amount of the feed consumed by the birds is shown in Table 3. The result showed that the feed intake of broilers from the day-old to four weeks of age, in group A was 22.4 Kg, in group B was 22.3 Kg, while in group C was 22.2 Kg and in group D was 22.5 Kg. While from 5 to 8 weeks, group A was 37.5 Kg, group B was 33.5 Kg, group C was 32.2 Kg and group D was 30.8 Kg. The feed intake was hampered by constant diarrhea noticed in the infected birds, especially those not treated has resulted in a sharp decline

in feed consumption and consequently causes a reduction in the weight gain of the birds. The significantly (P<0.05) higher feed consumption and increase in the live weight gain of up to 177.6% in the control group is a result of it not being infected by the protozoa and thus were healthy and unaffected during the research period.

The mortality rate and the number of oocytes counted per slide during the treatment period are shown in Table 4. The initial oocyte counted showed that no oocyte in group A (Control), while groups B, C, and D were infected with an oocyte. On the first oocyte count, group A was negative (no oocyte) and group B reduced to 2+ (++), while group C and D have 3+ (+++) each, respectively. One the second oocyte count, group A was still negative, group B and C were 2+ (++) while group D was 3+ (+++). On the third oocyte count, group A remained negative, group B was 2+ (++), group C was 2+ (++) and group D was 3+ (+++). On the fourth count group A remained negative, group B was 1+ (+), group C was 2+ (++) and group D was 3+ (+++). No mortality was recorded in groups A, B, and C,

**Table 1. Qualitative analysis of *Aloe vera* components**

| Sceondary metabolites | Test | Results |
|---|---|---|
| Tannin | Ferric chloride | + |
| Phlobatannins | HCl | - |
| Saponin | Frothing test | + |
| Flavonoids | Shinoda | + |
| Trepenoids | Salkowsi | - |
| carbohydtrates | Molish | + |

**Table 2. Live weight gain in (Kg) for treatment and weekly live weight gain in (Kg) of the Broiler post infection period**

| Group | Week 4 | Week 5 | Week 6 | Week 7 | Week 8 | Weight gain | %Weight gain |
|---|---|---|---|---|---|---|---|
| A Control (N = 15) | $0.74^{a}$ | $0.90^{a}$ | $1.51^{a}$ | $1.56^{a}$ | $1.61^{a}$ | 0.87 | 177.6 |
| B(N = 15) | $0.73^{a}$ | $0.84^{b}$ | $1.33^{b}$ | $1.48^{b}$ | $1.53^{b}$ | 0.8 | 109 |
| C(N = 15) | $0.74^{a}$ | $0.85^{b}$ | $1.34^{b}$ | $1.46^{b}$ | $1.54^{b}$ | 0.8 | 108 |
| D (N=15) | $0.75^{a}$ | $0.82^{b}$ | $1.12^{ac}$ | $1.31^{c}$ | $1.47^{c}$ | 0.72 | 95 |

*Abc; = Value relating to the same column with different superscript alphabet are significant (P<0.05)*

**Table 3. The amount feed in (Kg) consumed by the broilers at different stages of growth and treatment**

| Group | Broiler starter from 1st week to 4th week | Broiler finisher from 5th week to 8th week |
|---|---|---|
| A Control (N = 15) | $22.4^{a}$ | $37.5^{a}$ |
| B (N = 15) | $22.3^{a}$ | $33.5^{b}$ |
| C (N = 15) | $22.2^{a}$ | $32.2^{b}$ |
| D (N=15) | $22.5^{a}$ | $30.8^{c}$ |

*Abc; = Value relating to the same column with different superscript alphabet are significant (P<0.05)*

**Table 4. Mortality rate and the number of oocyst counted per slide post infection and during the treatment**

| Group | Initial oocyte count | 1st oocyte count | 2nd oocyte count | 3rd oocyte count | 4th oocyte count | Mortality |
|---|---|---|---|---|---|---|
| A Control (N = 15) | -ve | -ve | -ve | -ve | -ve | 0 |
| B (N = 15) | $+++^{+}$ | ++ | ++ | ++ | + | 0 |
| C (N = 15) | $+++^{+}$ | +++ | ++ | ++ | ++ | 0 |
| D (N=15) | $+++^{+}$ | +++ | +++ | +++ | +++ | 3 |

*Group A = Control i.e no infection; Group B = Infected under treatment with Aloe Vera extract; Group C = Infected under treatment with amprolium; Group D = Infected without treatment*
*-ve = No oocyte; + = 1 – 20 number of oocyte per slide; ++ = 21 – 50 number of oocyte per slide; +++ = 51- 100 number of oocyte per slide; ++++=100 and above number of oocyte*

while in group D (infected without treatment) there were three (3) mortality rates recorded. The oocyte count in group B treated with Aloe Vera declined from 120 post-infection to 18 oocyte post-treatment, while in group C treated with amprolium, the oocyte count declined from 120 to 28 oocyte post-treatment. In group D infected and not treated, the oocyte count increased from 120 to 132 oocyte.

## 4. DISCUSSION

*Aloe vera* is considered as one of the promising candidates having biological response modifying effects in different animal models [3]. The therapeutic efficacy of *A. vera* components had been reported in different animal models and human beings with promising results [11]. The plant has been reported to cure variety of conditions including fever, burns and wound healing, gastrointestinal disorders, sexual vitality and fertility problems, inflammation, ulcer, arthritis, cancer, immunosuppression, AIDS, and coccidiosis [3,12,13]. It is also considered an effective tool to enhance immunity in broiler chicks and increasing microvilli density [3,9,14].

The result of the oocyte count post-infection in our study have shown that the oocyte used were viable and capable of causing coccidiosis in the broilers. The response of the broiler infected with coccidial oocyte and treated with *Aloe vera* indicated that the extract was able to suppress the infection process from 120 oocytes post-infection to 18 oocyte post-treatment. *Aloe vera* chemical constituents are known to inhibit the growth of *eimeria* species at sporogony and merogony stages. Akhtar et al. [3] have reported that the oral administration of aloe vera extract (ethanol and aqueous) has significantly lowered the oocyst count in feces with compared to control group, they found that the broilers that received aqueous extract of aloe vera pulp had the lowest mean score lession in caeca and intestine in comparison to the control and the group that received ethanol extract of aloe vera pulp.

Yim et al. [14] reported that dietary supplementation of *A. vera* has significantly lowered the gut lession score and reduce fecal oocyst shedding of *E. maxima* broiler chickens, they further suggested that reduced oocyst shedding is a protective role against *Eimeria* infection in aloe-based chicken diets could be associated more with cell-mediated responses than antibody responses.

The *Aloe vera* post-treatment in this study didn't reduce the coccidian parasite completely but manage to bring the infection level to a point where a bird could start building immunity and resist the future infection of *Eimeria* species, it may be the few of tannins which is a secondary metabolite in the extract of the plants that are known to penetrate the wall of the oocyst and damages the cytoplasm is not sufficient enough, since tannin is known to inactivate endogenous enzymes responsible for sporulation [15]. Mwale et al. [12] state that an increase in aloe vera and aloe spicata content significantly decrease coccidian oocyst count.

Post-treatment with conventional drugs (amprolium powder at 3.25 g/liter) has lowered the oocyst count in feces from 120 oocysts to 28 oocysts. Amprolium is a thiamine antagonist and due to its close structural similarity, it blocks the thiamine receptors. The blockage of the receptors prevents Coccidian from utilizing thiamine and as a result, thiamine is unavailable to coccidian (Competitive inhibition of thiamine uptake). This vitamin (thiamine pyrophosphate) is a cofactor of several decarboxylase enzymes that play a role in cofactor synthesis. It is the only agent that can be used in laying birds both for the prevention and treatment of outbreaks. At higher doses, thiamine deficiency can occur in the host but it can be prevented by the addition of thiamine Vinay et al. [16].

The average weight gain by the birds at four (4) weeks for each group was 0.74 Kg, 0.73 Kg, 0.74 Kg and 0.73 Kg for A, B, C and D respectively. However, at 5 weeks, group A gained 0.16 Kg; group B gained 0.11 Kg; group C gained 0.11 Kg and group D gained 0.01 Kg. The result in weight gain during the study period was significantly ($P<0.05$) higher in the control group that was not infected and not treated. The birds' weight gain was lower ($P<0.05$) compared to the other groups. This indicated that the coccidial infection was capable of reducing the weight gain in birds. This may be due to the destructive nature of the parasite in the gastrointestinal tract, which is responsible for feed absorption. Odo et al. [17] has reported that higher weight gain in birds fed 5% level of inclusion *Aloe vera* than those of 10% and explain that *Aloe vera* in large quantity exerts powerful purgative effects than in smaller quantity.

The amount of the feed consumed by the birds from the day-old to four weeks indicated that group A has the highest FCE intake of 22.4 kg

and low FCE was recorded in group C at 22.2 kg. While from 5 to 8 weeks, group A has FCE of 37.5 Kg, group B 33.5 Kg, group C was 32.2 Kg while group D has 30.8 Kg. The feed intake was hampered by constant diarrhea noticed in the infected birds, especially those not treated has resulted in a sharp decline in feed consumption and consequently causes a reduction in the weight gain of the birds. The significantly ($P<0.05$) higher feed consumption and increase in the live weight gain of up to 177.6% in the control group is a result of it not being infected by the protozoa and thus were healthy and unaffected during the research period. Khan et al. [18] experimented on birds fed with a diet supplemented with 1% or 2% *Aloe vera* leaves and found that bird's weight gain has increase and feed intake FCR has increased than the control group without supplement.

An experiment was conducted by Darabighare et al. [19] to compare the effects of *Aloe vera* gel mixed with feed and Antibiotics growth promoter (virginiamycin), the results indicated that AGP has better growth performance compared to the performance of the group that received *Aloe vera* gel at 1.5%, 2%, and 2.5% respectively, and control group, while no significant difference was observed between AGP and the 2% *Aloe vera* gel group in terms of body weight gain and FCR. This may be supported by another study by Mmerole [20] that reported that dietary inclusion of *Aloe vera* leaves powder in broilers at 1% has no significant difference in body weight gains as compared to the control group.

Based on the treatment, group D which was infected and not treated was characterized by slow growth rate, decreased feed intake, hampered by constant diarrhea noticed in the infected birds, especially those not treated has resulted in a sharp decline in feed consumption and consequently causes a reduction in the body weight gain of the birds which ultimately lead the death (mortality) of three birds out of the fifteen experimental animals in group D, this low mortality rate (20%) may be attributed to the resistant nature of the breed used for the experiment, although the average mortality rate as a result of the Coccidian infection is mostly 50% Vinary et al. [18].

## 5. CONCLUSION

Several factors may lead to the partial reduction of oocyst count result observed in this work, as noted by Sofowara [10] practices used in traditional medicine include the collection of certain plants only at certain seasons using cold extraction, instead of hot extraction for some herbs. using fallen dead leaves of certain plant rather than fresh ones etc have rationalized as been due to seasonal, diurnal, or age variation, inactive constituents of plant and the thermal ability of the active ingredients of certain plants, thus the resistance formed by this protozoan after post-treatment with *Aloe vera* gel extract at this work may be due to the absence of the potent secondary metabolites on the leaves which may be acting synergistically with one another.

## ETHICAL APPROVAL

As per international standard ethical approval has been collected and preserved by the authors.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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