Therapeutic evaluation of *Terminalia macroptera* (Guill. & Perr.) stembark extract in experimental Avian Coccidiosis

ABSTRACT

Seventy per cent (70%) of *Terminalia macroptera* stem bark, methanol extract was administered to four groups A, B, C and D of a day old broiler chickens with each group consisting of five (5) chicks. Different doses of 50, 100, 150 and 200 mg/kg bw corresponding to 0.5, 1.0, 1.5 and 2.0 ml of the dissolved extract were administered to the respective groups. Groups E, F and G were the other groups that were infected but not treated (Negative control), infected and treated with the Standard drug (Tolacox®) and Treated but not infected respectively. There was a significant difference (*P* ≤ 0.05) in the Oocyst count (x10^3) between the infected and treated with the extract compared to the negative control. While the negative control was observed to manifest watery and bloody stool, high lesion score and high faecal count, the trend seemed to be reversed by the administration of the various doses of the extract on daily basis for 28 consecutive days. The faecal count in the treated groups was found to fall down to 2.1, 4.0, 1.7 and 0.7 x 10^3/g in the 50, 100, 150 and 200 mg/kg bw doses administered respectively, when compared to the 36.9 x 10^3/g obtained in the negative control. Also, the efficacy of the extract was observed to be dose dependent, as the dose of 200 mg/kg bw was found to reduce the faecal count in the infected group to 0.7 as compared to the 0.8 x 10^3/g in the standard drug.

Keywords: Coccidiosis, *Emeria* spp, Oocysts, *Terminalia macroptera*, Phyto-medicine

1.0 Introduction

Avian coccidiosis is a complex intestinal disease caused by obligatory protozoan parasites belonging to genus *Eimeria*. It is a disease that wreaks havoc even in the developed countries of the world [1]. One recent estimate put it that, coccidiosis may cost the US chicken industry about $127 million and 1.4 billion Indian Rupees annually [2,3,4,35] and proportionally similar losses may occur in other parts of the world. What can be deduced from this estimate is the fact that, if such an enormous loss could be incurred in countries like the United State of America (USA), how much fatal and uneconomical could it be in the Asian and Sub Saharan Africa where sophistication in the health management practices is at the infant stage. Thus coccidiosis is probably the most expensive and widespread infectious disease in commercial poultry systems that have got to be arrested if substantial progress is to be made in ensuring enough protein supply, particularly in the developing countries of Sub Saharan Africa and Asia. The emergence of drug resistance has again unfortunately complicated the success thus far recorded with the chemoprophylaxis and anticoccidial feed additives in the control of this disease [5,6] and their potentially toxic effects on the animal health [7].
Another drawback inherent in the sustained use of drugs or antibiotic is the bio-accumulation of the residues in poultry products which may be potentially hazardous to consumers [8]. As observed by Ane et al. [9], vaccination of birds with live Eimeria oocysts, has been another approach for coccidiosis control but, in cases of poor management, severe reactions that in most cases affect the performance of flocks, mainly in broilers because of their rearing period has been found to be triggered by these attenuated organisms. As a result of this drawback of live vaccines, Abbas et al. [10, 11] reported on the use of attenuated vaccines (with reduced pathogenicity), but unfortunately observed that these are expensive to produce and hence increase the cost of production. The observed shortcomings, therefore, prompted the search for cost-effective alternative strategies for more effective and safer control of avian coccidiosis.

The use of botanicals over the years has played a major role in the control of avian coccidiosis, as they are not only natural products but may comprise new therapeutic molecules to which resistance has not yet developed [3]. The use of botanicals as anticoccidial remedies, therefore, holds promise as an alternative in the control of coccidiosis. This fact is well documented in the inventory of medicinal plants, listing over 20,000 species. In spite of the overwhelming influences and our dependence on modern medicine and tremendous advances in synthetic drugs, a large segment of the world population still like drugs from plants. In many of the developing countries, the use of plant drugs is increasing because modern life-saving drugs are beyond the reach of three-quarters of the third world’s population although many such countries spend 40-50% of their total wealth on drugs and health care. As a part of the strategy to reduce the financial burden on developing countries, it is obvious that an increased use of plant drugs will be followed presently and in the future [3].

Terminalia macroptera Guill. & Perr. (Combretaceae) is a tree which grows in Western Africa from Senegal to Cameroon, and occasionally as far as Sudan. The tree is mostly found in Guinean and Sudanese-Guinean savannas, preferably in moist areas and clayey ground, where it is stated to be common [12,13]. Terminalia spp. range from small and medium-sized shrubs or trees to large deciduous forest trees, ranging in height from 1.5 to 75 m tall [14,15]. Traditionally, the plant has been used in combination with Anogeissus leiocarpa for colouring cotton fabric yellow or ochre. This is then used for treatment of newly circumcised children due to its putative antimicrobial effect [16]. The roots of the plant are regarded as an efficient antimicrobial remedy and are sold in markets in Guinea-Bissau [17]. In Burkina Faso, the plant is employed against malaria [18]. The bark is used against diarrhoea and dysentery in Nigeria [12]. A decoction of the leaves is used against hepatitis, ringworm and skin diseases [18]. The leaves are also used in gastritis, colic and hypertension, against fever, leprosy and tuberculosis [13]. The timber of T. macroptera is regarded as strong and resistant, and is popular as building material and in furniture [19].

Chemical investigations of T. macroptera has led to the identification of flavonoids triterpenoids, ellagitannins and related phenolics [20,21,17,22]. This research work therefore set to evaluate the efficacy or otherwise of the methanolic extract of Terminalia macroptera against induced coccidiosis in broiler chickens with a view to come up with a coccidiocidal/static phyto component that is locally available, affordable and accessible to the farmers, as opposed to the often inaccessible and unaffordable synthetic drugs against this menace that hinders flock growth and expansion.

2.0 Materials And Methods

2.1 Experimental site:
The studies/research work was conducted at the teaching and research Farm of the Federal College of Wildlife Management, New-Bussa, Niger state.

2.2 Experimental animals
Day old broiler chicks were purchased at Ibadan. They were kept in the research pen and fed with broilers feed with unrestricted access to clean water *ad-libitum*. High standard of health/sanitary conditions was maintained throughout the course of the research work.

2.3 Plant materials
Ethno-botanical survey was carried out in the surrounding villages viz: Old/New Awuru, Koro, Popo, Kere, Lubararu and Dogongari in Borgu Local Government Area of Niger state, Nigeria, with the main aim of ascertaining from the local people (particularly the elderly ones), the plant species(s) commonly utilised in the traditional management of coccidiosis. Part(s) used the method of preparation and period of harvest were also enquired from the interviewees.

2.4 Plant Preparation:
The stem bark of the plant (*Terminalia macroptera*) was washed with clean water and dried at room temperature and then chopped into smaller fragments and pulverised using a grinder. Cold extraction was performed using 70% methanol.

2.5 Drugs:
Toultrazuril was purchased from State Veterinary Clinic, Bosso, Minna as oral solution (2.5%) under trade name Tolacox®.

2.6 Pharmacological Studies

2.6.1 Anticoccidial effect
Total number of 35 chickens were grouped into seven (7), each group consisting of 5 chickens as thus:
- **Group I** = 50mg/kgbw
- **Group II** = 100mg/kgbw
- **Group III** = 150mg/kgbw
- **Group IV** = 200mg/kgbw
- **Group V** = infected and not treated (negative control)
- **Group VI** = not infected and not treated (Normal control)
- **Group VII** = treated with the standard drug

2.7 Infection of animals
Fresh faecal sample containing *Eimeria* oocyst was collected from the infected animal and Microscope was used to carry out the oocyst count and subsequent dilution to 74,000 Oocyst/ml as an infective dose. The infective dose of the culture (i.e 74,000 Oocyst/ml) was administered orally using cannulation tube. Then the treatment was carried out in each group for 21 days (3 weeks).

2.8 Parasitological examination (Oocyst Count)
A fresh faecal sample was collected from freshly evacuated faeces. This was lightly macerated and subsequently washed and filtered through gauze and the final clear liquid sample containing the Oocyst was poured into a beaker containing normal saline and kept for oocyst count using compound microscope. The count was carried out at 3 days interval using haematocytometer and compound microscope.

2.9 Prophylactic activity Test:
The test for prophylactic activity was done as described by (Jocelyn et al. [23] and Ogbadoyi et al. [24]).

2.10 Acute Toxicity Studies:
Oral administration of the extract to non-infected chicks at various doses of 400, 800, 1200 and 2000mg kg⁻¹bw reveals that it is acutely toxic at a dose of 1600mg kg⁻¹ body weight and above and the LD50 has been found by extrapolation to be 2000mg kg⁻¹bw (Table3.1).

2.11 Phytochemical analysis
Phytochemical analysis of the 70% methanol extract of the stem bark of *Terminalia macroptera* was carried out as described by Innalegwu et al. [25].

2.12 Statistical Analysis
The data collected were subjected to one way analysis of variance in a completely randomized design (CRD) arrangement. The significant means were separated and compared using Duncan multiple range test.

3.0 Results
In the present study, and after challenging the chicks with mixed Eimeria especially in the control non treated group, birds showed severe form of coccidiosis represented by a high mortality rate (46.7%), lesion score was 3.57 and fecal score was 4.8 (meaning severe watery diarrhea with obvious amount of voided blood in litter). Administration of 0.5ml of T. macroptera methanol extract reduced mortality to 8.7%. Worthy of note is the efficacy comparable to the standard drug (Tolacox⁶) displayed by the extract at 50mg/kgbw. While the standard drug cleared the Oocyst down to 0.8 on the 28th day, the extract was able to lower its level to 2.1 x 10³/g on the same day. What this implies is that, further purification of the crude extract might yield a drug molecule that could compete favourably with the standard drug (Fig. 3.1). However, it was observed that when the extract was administered at the dose of 100mg/kgbw (1.0ml), the Oocyst count of 4.0 x 10³/g was found which is higher than what was observed in the case 50g/kgbw. This, based on our understanding and postulations might not be unconnected to the physiological and immunological differences between the animals in the two groups. However, the reasons advanced for these discrepancies might well be beyond our hypothetical assumptions, therefore, further research
employing genetic/molecular tools might certainly resolve this dogma. Interestingly, low lesion score and faecal counts were observed in both the two concentrations administered compared to the positive control (Fig. 3.1). Of greater interest is the fact that administration of 150mg/kgbw to the experimental chickens reduced the Oocyst count by 95.4%, that is from 36.9 down to $1.7 \times 10^{3}$ (Figure 3.1) while it was found to be 47.0% as effective as the standard drug. With the exemption of the 100mg/kgbw dose, the efficacy of the extract is dose dependent, as the dose of 200mg/kgbw was observed to not only reduced the Oocyst count by 99.8%, that is, from 36.9 down to $0.7 \times 10^{3}$ and well comparable with, and a bit more efficacious than the standard drug, as the Oocyst count was found to be 0.7 compared to $0.8 \times 10^{3}$ obtained in the case of the standard drug. The lesion score and faecal count was found to be low in both cases.

![Graph showing coccidiocidal activity of various concentrations of Terminalia microptera extract](image.png)

**Fig. 3.1: The coccidiocidal activity of various concentrations of Terminalia microptera extract**

### 3.2 Prophylactic activity Test:

Administration of a curative dose of 200mgkg-1bw of the stem bark extract of *Terminalia macroptera* to a group of five healthy chickens for five consecutive days prior to infection
did not protect them from infection. The animals were observed and found to come up with the parasites 96h after infection.

3.3 Acute Toxicity Studies:

Oral administration of the extract to non-infected chicks at various doses of 400, 800, 1200 and 2000mg kg-1bw reveals that it is acutely toxic at a dose of 1600mg kg-1 body weight and above and the LD50 has been found by extrapolation to be 2000mg kg-1bw (Table 3.1).

Table 3.1. Effects of administration of various doses of the crude extract to healthy chickens.

<table>
<thead>
<tr>
<th>Dosage</th>
<th>No of Animals</th>
<th>T/D</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled H2O or Normal Saline</td>
<td>4</td>
<td>4/0</td>
<td>No sign of toxicity, animals remained active even after the administration.</td>
</tr>
</tbody>
</table>

Figure 3.2: Prophyactic activity of Teminalia macroptera in experimental birds
400mgkg⁻¹bw 4 4/0 No sign of toxicity, animals remained active even after the administration.

800 mgkg⁻¹bw 4 4/0 Looked a bit depressed, the breathing was slow and remained Sluggish for a short while became normal again.

1200 mgkg⁻¹bw 4 4/0 Sluggishness was observed, the breathing was slow and there was closing of the eyes and the feathers stood erect but conditions returned to normal after about 24h.

1600 mgkg⁻¹bw 4 4/1 One death was recorded about 13 h after the administration of the fraction and it took almost 27h before the animals recovered fully from the sluggishness, depressed breathing, and erected feather.

2000 mgkg⁻¹bw 4 4/2 Two deaths were recorded about 17 h after administration of the extract and it took almost 48h before the animals recovered fully from the sluggishness, depressed breathing, erect fur and closing of the eyes.

3.4 Phytochemical analysis
Phytochemical analysis of the 70% methanol extract of the stem bark of *Terminalia macroptera* showed that it contained Alkaloid, Flavonoid, Tannins, Saponins, Phenols, Cardiac glycosides, Anthraquinones, Steroids, Phlobatannins and Terpenes.

4.0 Discussion
More than thirty (30) different ailments (in humans) have reported to be cured by the traditional healers through the use of this plant in countries such as Ghana, Mali, Nigeria,
Senegal Sudan and Uganda [25]. An interesting finding from this study is the ability of the methanol extract from this plant to suppress Coccidian bacterial growth in experimental chickens at low dose of 50mg per kilogram body weight of the crude extract. Considering the inability of most of our rural farmers to purchase standard drugs (due to wanton poverty), unavailability, inaccessibility of (these drugs) and low literacy level to appreciate the importance of regular treatment of birds in the flock as a preventive management practice, all culminated into shortage of protein supply in the sub-Saharan Africa. Therefore, availability of extract from local plant resources with efficacy closely comparable to the standard drug will bring the much needed succour in the management of deadly poultry diseases such as the coccidiosis. As can be observed from figure3.1, the efficacy of the crude extract appeared to be concentration dependent, as the dosage of 150 and 200mgkg\(^{-1}\)bw seems to compare favourably with the standard drug at adequate dosages, the efficacy of the extract finally comes close to the standard treatment (i.e.) after 28 days interval, but, yet, remains less efficient at shorter time intervals.

It therefore means that the isolated pure compound when eventually obtained may be a drugable candidate, efficacious enough to allow its standardization and packaging for use as phyto-medicine for the treatment of Avian coccidiosis.

A strong relationship always exist between the anti-cancer potency, Cardiovascular effect and Anti diabetic effect of an extract and the equivalent attention it will receive from drug manufacturers, who would otherwise be unwilling to invest in development of drugs for a disease of the rural poor in Africa for lack of financial incentives, to invest in its development into drug when it also has anti-cancer activity or indeed activity against any other disease that is also a major public health problem in the rich and developed nations.

There are reports of anticancer activity of various extracts of *T. macroptera* [1,26,27] including that of methanol extract of *T. macroptera* bark by a group in India[28]. The use of extracts of *T. macroptera* in the treatment of diabetes has also been documented [29,30]; Konczak et al.[31] has also reported on the efficacy of root, stem bark and leaf extracts from *T. macroptera* against cardiovascular disorders. We are therefore encouraged by these preliminary findings hoping that eventually a point where these findings would be translated into products will be reached and would bring the much needed succour to the teeming population of peasant and poultry farmers in rural Africa whose flock size has been kept at the peasant/insignificant level by this devastating disease (coccidiosis). Despite the fact that, the extract seems not to exhibit prophylactic activity (Figure 3.2), the acute toxicity studies clearly shows that, the extract is only mildly toxic to the experimental birds; even at an acute dose of 1600mgkg\(^{-1}\)bw only 25% mortality was recorded (Table 3.1). What this interesting finding on its toxicity implies is that, since *Terminalia spp* were reported to possess high concentration of antioxidants such as tannins and flavonoids [32] which again, in higher concentration display pro-oxidant effect [26,33,34], administration of this extract may aside of clearing the causative bacteria, also bio-accumulate within the bird’s system which when eventually consumed by humans will certainly be released into their system and hopefully exert similar antioxidant effect on the healthy cells and pro-oxidant effect (when in high concentration) on the tumour cells and hence provide two-fold effect of protein supply and cancer control. Therefore, findings from this study on the efficacy and none toxicity of this extract on the experimental birds will pave way for further search into the possibility of the birds so treated with extract from the *T. macroptera* to be both source of protein and a nutraceutical providing a very good source of anti-oxidants.
4.1 Conclusion

The results when considered together, could be concluded that methanol extract of *Terminalia macroptera* cures experimental coccidiosis and it is potentially useful as an anticoccidiosis phyto-agent.

Ethical approval

Authors hereby declare that "principles of Laboratory animal care" (nih publication no. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

References


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