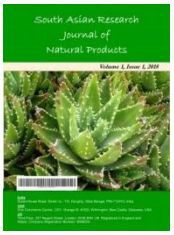

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Therapeutic Evaluation of *Combretum molle* (Combretaceae) (Bush Willow) Stembark Extract in Experimental Avian Coccidiosis

M. H. Garba[1*], M. L. Sherifat[2], A. O. Abdul-Majid[1], L. M. Hafsa[3], L. J. Bulus[1] and G. Yusuf[4]

[1]Department of Animal Production Technology, Federal College of Wildlife Management, P.M.B. 268, New Bussa, Niger State, Nigeria.
[2]Department of Basic Sciences, Chemistry Unit, Federal College of Freshwater Fisheries Technology, P.M.B. 1500, New Bussa, Niger State, Nigeria.
[3]Department of Basic Sciences, Mathematics Unit, Federal College of Wildlife Management, P.M.B. 268, New Bussa, Niger State, Nigeria.
[4]Department of Biological Sciences, Federal College of Education, P.M.B.39, Kontagora, Niger State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author MHG designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors LMH and MLS performed the statistical analysis, participated in the writing of the first draft of the manuscript. Authors AOA, LJB and GY managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The main thrust of this research work was to assess the potentials of the extract from the stem bark of *Combretum molle* (identified through ethno-botanical survey) using an appropriate solvent(s) *In vivo* against coccidial protozoa in experimental animals.
Methodology: The stem bark of the plant (*Combretum molle*) was collected, washed and dried at room temperature. It was then chopped into smaller fragments and pulverized using a grinder. Cold

**Corresponding author: E-mail: mharunagarba@gmail.com;*

extraction was performed using 70% methanol which was filtered after 24hrs and then dried using a rotary evaporator. Thirty-five (35), day-old broiler chicks were used. They were kept for acclimatisation period of 2 weeks in the research pen and fed with broilers starter and had unrestricted access to clean water *ad-libitum*. After the period of acclimatization, the birds were grouped into seven (7) with each group consisting of 5 birds. High standard of hygiene conditions was maintained throughout the course of the research work. Fresh faecal samples from the infected animals were collected. The infective dose of the culture (i.e 74,000 sporulated oocyst/ml) was administered orally using 1ml syringe. The treatment was carried out on three days interval in each group for 21 days (3 weeks). Birds on group 1, 2, 3 and 4 were administered 50 mg/kg bw, 100 mg/kg Bw, 150 mg/kg bw and 200 mg/kg bw *C. molle* extract respectively.

Results: *C. molle* stem bark extract at a dosage of 50 mg/kg bw, 100 mg/kg bw, 150 mg/kg bw and 200 mg/kg bw in the treatment of broiler chicks infected with *Eimeria spp* parasites shows reduction in the numbers of oocyst count in each group but the treatment of coccidiosis at a dose of 150 mg/kg bw and 200 mg/kg bw in broiler chicks prove to be more efficient. The assessment of haematological and serum biochemical parameters revealed that *C. molle stem bark extract* has no pathological effect on the experimental animals.

Conclusion: Stembark exract of *C. molle* could be employed/packaged as phytomedicine against Avian coccidiosis considering its efficacy against the infective oocyst and its potency as a prophylactic agent against the protozoa. Its non-toxicity to the experimental animals is also an added advantage that will further encourage its utilisation.

Keywords: Coccidiosis; Eimeria spp; broiler chicks; ethnobotany; Combretum molle; methanol extract.

1. INTRODUCTION

Coccidiosis is a parasitic disease of intestinal tract caused by single cell protozoan parasite belonging to genus *Eimeria*. It causes massive destruction of the epithelial cells, which leads to bloody diarrhoea, reduced weight gain and temporary reduction in litter production [1,2]. Seven species have been recognized to infect chicken and each species has its own characteristics according to the site of infection, immunogenicity and pathogenicity [3,4].

According to a recent estimate, coccidiosis may cost the US chicken industry about $127 million annually [5]. However, due to even lower nutritional efficiency and poor management practices in Africa, coccidiosis may cost the Africa chicken industry much more annually. Likewise, similar losses may occur worldwide particularly the underdeveloped and developing countries. Thus coccidiosis is probably the most expensive and wide spread infectious disease in commercial chicken systems.

Control of coccidiosis mostly depends upon the chemoprophylaxis by using anti-coccidian drugs however, managerial skills are also important to get the maximum anti-coccidian effect of these drugs [6]. *Combretum molle* is a shrub or small tree up to 10 m high, rarely to 16 m, with a straight regular bole to 1 m girth, of savanna forest, from Senegal to West Cameroons, and widespread in tropical Africa. The stems are durable underground and are valuable for house-posts. The wood is brownish or yellowish-green, very hard and compact, strong and durable, but difficult to work. In Sudan, a leafy and stem bark decoctin is taken to treat fever, jaundice and bacterial infection. Aside of the use of the bark for tanning, its extract (stem bark) and leaves possessed significant activity against the chloroquine – sensitive Plasmodium falciparum strain NF54 and the leaf extract also totally inhited the enzyme HIV-1 reverse transcriptase [7].

The intensive use of anti coccidial drugs in poultry management has led to the development of resistance. It, therefore, becomes imperative to find some natural alternatives which improve the growth with either prophylactic or curative effect or both, against coccidiosis without health hazard to a human being.

The main thrust of this research work, therefore, is to assess the Anticoccidial potentials, Prophylactic effects and acute toxicity of the methanolic stem bark extract of *Combretum molle* (COMBRETACEAE) *In vivo,* against coccidial protozoa in experimental Broiler chicks.

2. MATERIALS AND METHODS

2.1 Experimental Site

The studies/research work was conducted at the teaching and research farm and the nutrition and Biochemistry laboratory of the Federal College of Wildlife Management, New-Bussa, Niger State.

2.2 Experimental Animals

Day old broiler chicks were purchased at Ibadan, Oyo State. They were kept in the research pen and fed with broilers feed and allowed 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

The ethno-botanical survey was carried out in the surrounding villages namely, Old/New Awuru, Koro, Popo, Kere, Lubaruru and Dogongari villages with the main aim of ascertaining from the local people (particularly the elderly ones), the plant species commonly utilised in the traditional management of coccidiosis. Part(s) utilised the method of preparation and period of harvest were also enquired from the interviewees. The identity of the plant was confirmed by Mr Musa Idris in the Department of Forestry, Federal College of Wildlife Management, New Bussa, Nigeria.

2.4 Plant Preparation

The stem bark of the plant *Combretum molle* (Bush willow) 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 and rotary evaporator was to remove the solvent.

2.5 Pharmacological Studies

2.5.1 Anti coccidian effect

A total number of thirty-five (35) broiler chickens were grouped into seven (7) treatments with each treatment consisting of five (5) chickens as thus:

Group I = 50 mg/kg bw
Group II = 100 mg/kg bw
Group III = 150 mg/kg bw
Group IV = 200 mg/kg bw
Group V = infected and not treated (given Normal saline)
Group VI = not infected and not treated (Normal)
Group VII = Infected and treated with the standard drug

2.6 Drugs

Toultrazuril obtained from Ministry of Livestock and Fisheries, Minna, Niger State, as an oral solution (2.5%) under trade name Tolacox^{R}.

2.7 Infection of Animals

This was carried out as described by Long [8]. Briefly, a fresh faecal sample containing *Eimeria* oocyst was collected from an infected bird and placed in a 500 ml capacity beaker containing distilled water and lightly macerated until completely disintegrated and dissolved. The compound microscope was used to carry out the oocytes count with subsequent dilution using Mac master chamber until an Oocyst count of 74,000 oocytes/ml was attained, which was used as an infective dose.

The infective dose of the culture (74,000 Oocytes/ml) was administered orally using a 1 ml insulin syringe which was introduced intra-crop. The treatment thereafter, commenced on day six (6) (prepatent period) in each group as indicated in section 2.5.1 for 21 days (3 weeks).

2.8 Prophylactic Activity Test

The test for prophylactic activity was done as described by Jocelyn et al. [9] and Ogbadoyi et al. [10].

2.9 Parasitological Examination (Oocytes Count)

A fresh faecal sample in each of the treatment groups I – VII (except VI) was collected. This was lightly macerated and subsequently washed and filtered through gauze and the final clear liquid sample containing the oocytes was poured into a beaker containing normal saline and kept for oocytes count using a compound microscope. The count was conducted at three days interval.

2.10 Haematology

On day 21, blood samples from each treatment group were collected into labelled Ethylenediamine tetra acetic acid (EDTA), treated tubes for haematological analysis. The determination of the haematological parameters

was carried out using an automated haemato analyser (SpectruMedix 329LX) at the general hospital Minna, Niger State, Nigeria.

2.11 Serum Biochemistry

Blood samples were also collected into tubes without anticoagulant and the serum analysis on Blood glucose, alkaline phosphatase (ALP), Serum albumin, Total protein, Cholesterol and Blood urea was carried out using Radox analysis kit.

2.12 Acute Toxicity Studies

Doses of 600, 900, 1200 and 1500 mg kg^{-1}bw of the extract were orally administered to non-infected chicks and were observed for 72 hours.

2.13 Statistical Analysis

The data collected were subjected to one-way analysis of variance (ANOVA) in a completely randomized design (CRD) arrangement. The significant means were separated and compared using Duncan multiple range tests (DMRT).

3. RESULTS

The efficacy of the extract appeared to be concentration dependent. As the concentration increases, there is a corresponding increase in the activity of the extract as shown in Fig. 3.1. At the concentration(s) of 100, 150 and 200 mg/Kg bw, it became obvious that activity of the extracts in terms of oocytes count is higher than the standard drug.

3.1 Prophylactic Activity Test

Administration of a curative dose of 200 mgkg-1 bw of the stem bark extract of *Comberetum molle* to five different groups of healthy chickens for five consecutive days prior to infection appears to protect them from infection. The animals were observed not to have come up with the parasites after infection. The prophylactic activity displayed by the extract at a curative dose of 200 mg/kg bw is also an added advantage in the usage and progressive intensification of research to identify and develop the lead compound from the plant (Fig. 3.2).

One advantage that a drug may have over others also lies in its haemopoietic and immuno-modulatory role. It could be observed that there is no significant difference ($P \leq 0.05$) in the haemoglobin (Hb), packed cell volume (PCV), mean corpuscular haemoglobin concentration (MCHC) and the red blood count (RBC) between the normal control and the groups administered various doses of the extract.

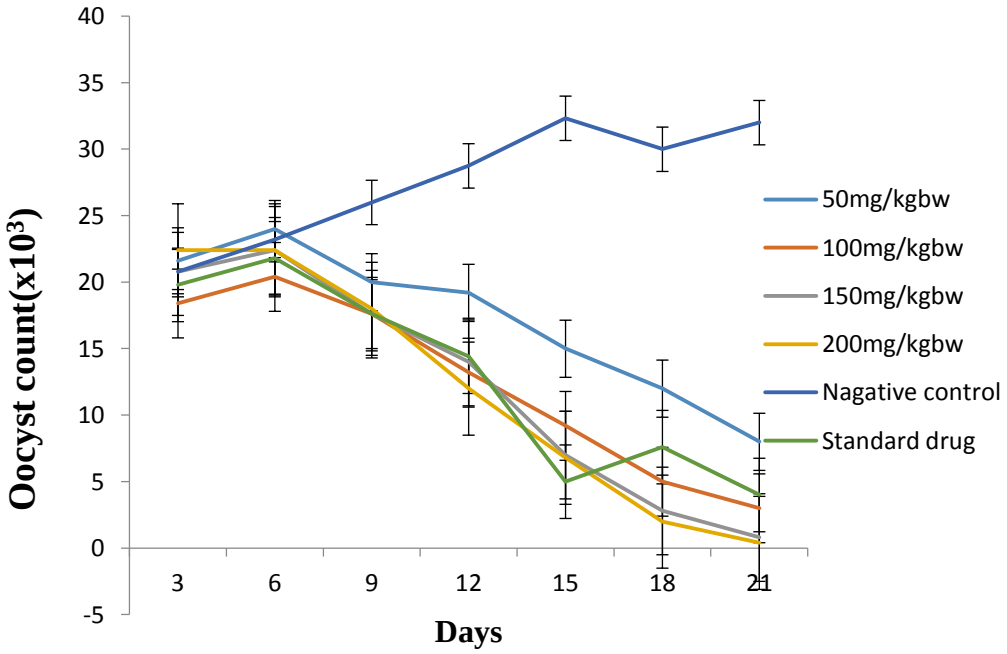

Fig. 3.1. Efficacy of the extract administer at dose of 50 mg, 100 mg, 200 mg, negative control and standard drug

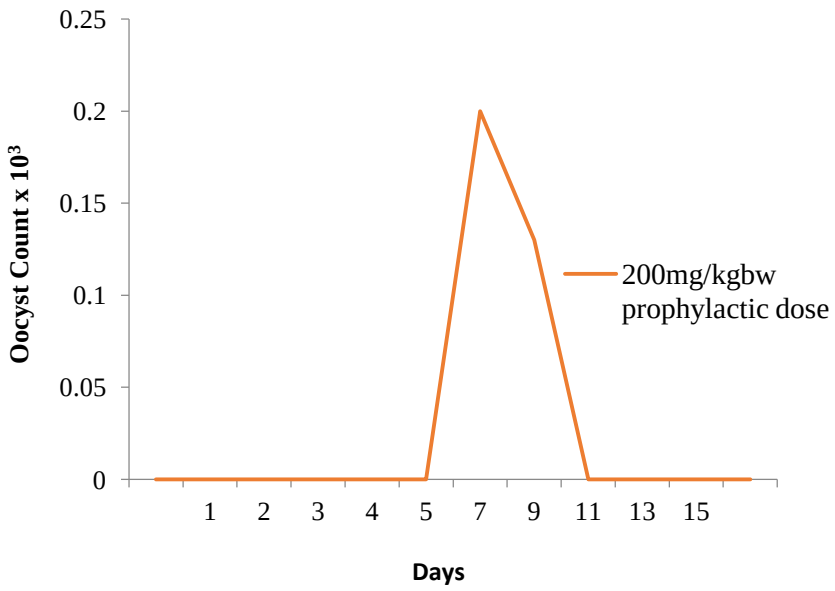

Fig. 3.2. Prophyactic activity of *Combretum molle* in experimental birds

While the higher concentration (P ≤ 0.05) in the Platelet counts PLC), Total white blood count (TWB), RDW, Lymphocytes counts, Monocytes,Eosinophil and basophil counts was observed in the animals administered the extract than the normal control, which is a clear indication of immuno-stimulatory/modulatory activity of the extract [11] (Fig. 3.3).

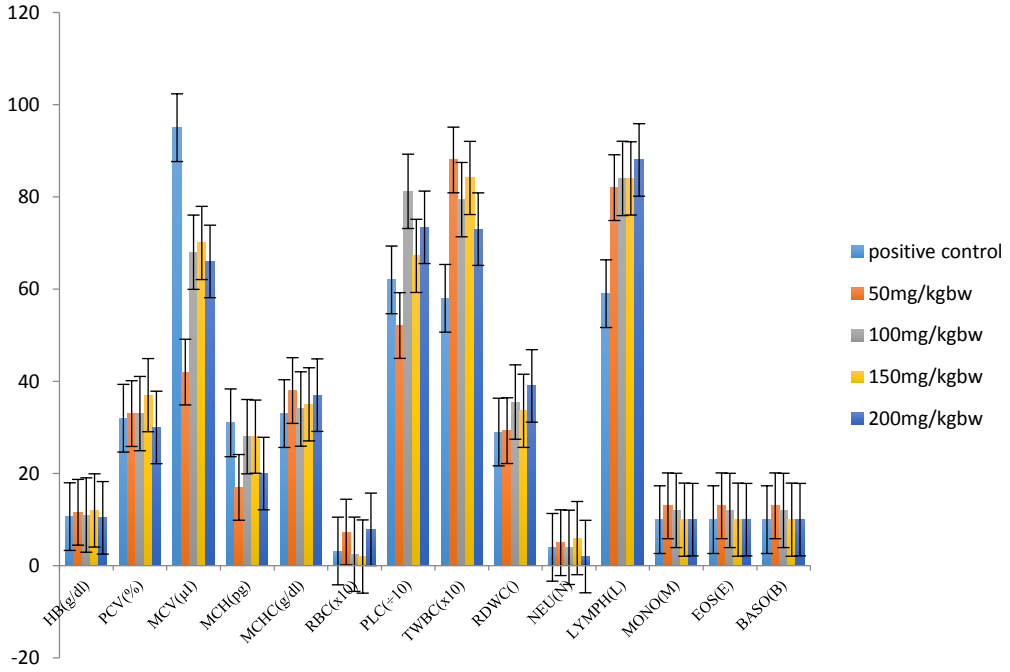

Fig. 3.3. Haematological parameters of broilers chickens treated with *Combretum molle*

HB = Haemoglobin, PCV = Packed Cell Volume, RBC = Red Blood Count, TWBC =TOTAL White blood Count, MCV = Mean cell volume, MCHC = Mean corpuscular haemoglobin concentration, MCH = Mean corpuscular haemoglobin, N=Neutrophils, L=Lymphocytes, M=Monocyte, E=Eosinophils, B=Basophils

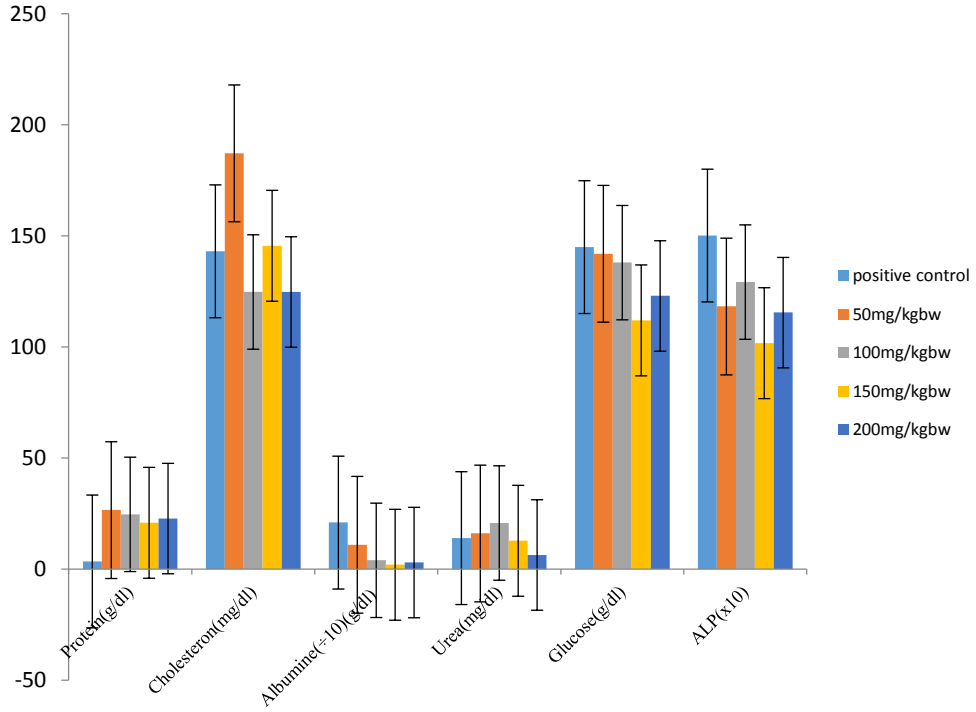

Fig. 3.4. Serum biochemical characterisctics of broilers treated with *Combretum molle*
ALP = Alkaline phosphatase

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

Dosage	No of animals	T/D	Observations
Distilled H20 or Normal Saline	4	4/0	No sign of toxicity, animals remained active even after the administration.
300 mgkg-1bw	4	4/0	No sign of toxicity, animals remained active even after the administration.
600 mgkg-1bw	4	4/0	Looked a bit depressed, the breathing was slow and remained Sluggish for a short while became normal again.
900 mgkg-1bw	4	4/0	Sluggishness was observed, the breathing was slow and there was the closing of the eyes and the feathers stood erect but conditions returned to normal after about 24h.
1200 mgkg-1bw	4	4/0	Sluggishness was observed, the breathing was slow and there was the closing of the eyes and the feathers stood erect but conditions returned to normal after about 24h.
1500 mgkg-1bw	4	4/0	Sluggishness was observed, the breathing was slow and there was the closing of the eyes and the feathers stood erect but conditions returned to normal after about 48h.

T/D = Number of deaths recorded from the total number of animals in each group

4. DISCUSSION

Findings made from this study showed higher efficacy of the crude extract of *Combretum molle* compared to the standard drug Toultrazuril (Tolaclox^{R}) at a concentration of 100, 150 and 200 mg/kgbw (Fig. 3.1) this is a confirmation of the previous reports that organism are gradually developing resistance to most of the drugs currently in use. It could also be observed that the efficacy of the extract is concentration dependent. What this interesting finding reveals

is the fact that, further purification of this extract, may possibly yield a fraction that will be more efficacious at a lower dosage, than the currently available drugs. This will certainly open a new window of opportunity for the rural farmers to have a better alternative that will be less costly, readily available and more accessible. Though we cannot say with certainty, the pharmacokinetics of this extract, this interesting result might not be unconnected with some phyto-components reviewd by Sudipta et al. [12] which include: CombreteneA, Combretene B, 2α,3β-,6β--trihydroxy-23-, galloylolean-12-en-28-oate, Combregenin, Arjungenin, Arjunglucoside I, Combreglucoside, Sericoside, Mollic Acid, Mollic acid 3β-O-glucoside, Mollic acid 3β--O-arabinoside, Ellagitannin and Punicalagin identified to be associated with this plant [13,14,15,16,17,18,19,20,21,22,23,24,25]. These phyto-components were shown to individually or in synergy with some other molecules display high antiprotozoal, antifungal, antihelmintic, anti-inflamatory and anti molluscidal activities against range of pathogenic organisms [12]. Therefore, the high anti-coccidial effect might be as a result of similar or interplay of the mode of actions exhibited in other pathogens by these phyto chemicals.

This novel prophylactic property displayed by this plant extract has not been displayed by plants such as *Aloe vera, Aloe spicata, Myrothamnus flabellifoilius, Lannea stullmannii, Capsicum annum (Pepper), Parinaria curatellifolia, Albizia gummisera, Albizia adianthifolia and Soot investigated by* Marizvikuru et al. [26]. Though *Lannea stullmannii was found to possess a similar activity* [27]. It has to be added into the feed at a high ratio of 1:4 which may not bring down the cost of production.

An interesting observation made in relation to the plasma protein concentration due to the administration of this extract is the significant increase ($P \leq 0.05$) across all the groups administered the extract compared with the normal control (Fig. 3.4). Since the animals were allowed access to drinking water *ad libitum,* this condition may not be attributed to the dehydration but rather paraproteinemia that may probably be induced by the extracts as a result of their ability to induce B- lymphocytes or plasma cells to produce these monoclonal proteins that exist as heavy chain subtypes (IgG, IgA, IgG, and less commonly IgD or IgE) and light chain subtypes (kappa or lambda) [28]. The exact opposite of protein concentration was observed with the serum albumin concentration (Fig. 3.4) probably due to successive replacement of the protein albumin by the paraproteins in the groups administered the extract. It is pertinent to state that, there is no significant difference ($P \leq 0.05$) in the cholesterol, urea, glucose and alkaline phosphatase concentration between the normal control and the groups administered the extracts. This observation will certainly go a longway to show the relative safety of chronic administration of this extract.

On the acute toxicity of this extract, Table 3.1 clearly indicates that it is relatively safe at a dose of 1500 mg/kgbw, this corroborates the findings of Ademola and Eloff [36], that found it to be safe on acute administration to albino mice at a dose of 849–900 mg/KgBw.

5. CONCLUSION

Taken the results from this study together, the methanol stem bark extract of *Combretum molle* appears to be more efficacious than the conventional drug Toultrazuril (Tolacox$^{R)}$ at the doses of 100, 150 and 200 mg/KgBw. Its Prophylactic potentials is also a relief and succour to the poor rural farmers that suffers most dilapidating damages caused by this disease. The low chronic toxic effect (except paraproteinemia) and its non-acute toxicity even at a dose of 1500 mg/KgBw exhibited by this extract will certainly encourage its deployment in the management of this disease. However, further research on the potency of the partially purified (fractionated) extract will shade more light on the active component and its possible mode of action.

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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