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In Vivo Anti-Trypanosomal Activity of Ethanolic Root Extract of Carissa Spinarum (Wild Karanda) In Mice Infected With *Trypanosoma brucei brucei* Spp.

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ABSTRACT

Carissa Spinarum plant used for treatment of joints, muscle & chest pains by the massai people in Kenya is also implicated as cancer & antiviral supplement for HIV treatment in Tanzania. Acute toxicity and minimum inhibition concentration (MIC) using four (4) microorganism *(proteus, e.coli, staphylococcus aureus spp & enterobacter spp*) of ethanolic leaf extract was evaluated in mice. Phytochemical analysis of the extract was carried out while evaluation for *in vivo* anti-trypanosomal activity against federa strain of *Trypanosoma brucei brucei* across a four days suppressive, curative effect against established infection and prophylactic models of anti-trypanosomal studies were also established. The median lethal dose of the extract was determined to be ≥ 100mg/kg body weight. The extract (12.5, 25, 50mg / kg) exerted some dose dependent suppressive effects at the different levels of infections tested, with no significant curative effects recorded. However, further antitrypanosomal property can be explored for the management of *trypanosomiasis.*

KEYWORDS: *Carissa spinarum, antitrypanosomal, albino mice, trypanosome brucei brucei,*

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I. INTRODUCTION

African trypanosomes are protozoan parasites that cause sleeping sickness in humans and nagana in domestic livestock in sub-Saharan Africa. An epidemic involving several hundred thousand people that spread through Sudan, the Central African Republic, DRC and Angola in the 1990's, demonstrated how socially and economically devastating these diseases are [17]. Trypanosomes kill more than 3 million cattle annually and those animals that survive display low productivity due to the wasting effects of the disease [11]. The annual losses from trypanosomiasis in cattle amount to more than US $4.5 billion [7]. Trypanosomes, by influencing food production, natural resource utilization and the pattern of human settlement, are thus seen by the African Union as one of the greatest constraints to Africa's socio-economic development [12]. Sleeping sickness is known to have a prevalence of 300,000 – 500,000 [15] as well as three (3) million deaths to livestock occurring every year [15]. Despite this prevalence rate, few drugs are available for treatment and some known to be toxic, old, expensive and not readily available [6]. In addition, resistances to these major drugs as well multiple drug resistant populations have been described for different species of the parasite [1]. Relapses of unknown etiology are also reported with melarsoprol in recent epidemics. Hence, there is urgent need to seek for new sources of therapeutic agents [9].

II. MATERIAL AND METHOD

The plant was collected from Gombe, Gombe State, which is Northeastern zone of Nigeria and was identified at the Herbarium of Ahmadu Bello University, Samaru – Zaria, which is in the Northwestern zone of Nigeria with voucher Det: U.S Gallah 8/02/2012. All reagents and solvents used were of analytical grade.

Root part of the plant were harvested, dried under the shade or in open air in the laboratory. Dried materials were pounded in laboratory mortar into small particles. Fifty grams (50g) of the pounded dried plants materials were weighed and extracted with 3 X 150ml ethanol (70%) and allowed to macerate for 3 days, then filtered to obtain the extract which is then dried under electric fan and stored in a refrigerator at 4^{o}C until required.

Animals

Four (4) weeks old albino mice weighing between 18-20 g obtained from the Animal house of NITR, Kaduna were used for the study, they were housed in plastic cages with saw dust as beddings and given food and water ad libitum. Acclimatized for two (2) weeks before commencement of research.

Phytochemical Screening

The ethanolic root extract of Carissa spinarum was screened for the presence of secondary metabolites and constituents using conventional protocols for detecting the presence of steroids, alkaloids, lignin and phenols [8]; fatty acids, glycosides, triterpenoids and saponins [5]; tannins, leucoanthocyanins and emodins [20]; reducing sugars [19]; anthraquinons [2], flavonoids [16] and coumarins [18].

Determination of Parasitaemia

Parasitaemia was monitored in blood obtained from the tail of the mice, pre-sterilized with methylated spirit. The number of parasites was determined microscopically at X 400 magnification using the "Rapid Matching" method of Herbert and Lumsden [10]. Briefly, the method involves microscopic counting of parasites per field in pure blood or blood appropriately diluted with buffered phosphate saline (PBS, pH 7.2). Logarithm values of these counts obtained by matching with the table of Herbert and Lumsden is converted to antilog to provide absolute number of trypanosomes per ml of blood [3], [4].

Minimum Inhibition Concentration

Minimum Inhibitory Concentration (MIC) involves the lowest concentration of an antimicrobial that can inhibit the visible growth of the microorganism after the overnight incubation. In this case, four (4) common microorganism namely, e.coli, enterobacter, staphylococcus aureus app and proteus were subjected to inhibition properties with the ethanolic root extract of Carissa spinarum via serial dilution incubation of the extract with each microorganism (fig ii).

Acute Toxicity Test

Acute toxicity test of Carissa spinarum root extract was carried out using the modified Lorke's method [14]. The study was carried in two phases, the first phase requires 9 (nine) mice randomized into 3 groups of three mice & each given intraperitoreally 10, 100 & 1000mg/kg body weight of the extract. The mice were observed for signs of toxicity which included but not limited to paw licking, salivation, stretching of the body, weakness, sleep, respiratory stress, coma & death in the first four (4) hours of extract administration and subsequently daily for hours. In the second phase, another fresh set of 9 (nine) mice were randomized into 3 groups of three mice again & administered with 1600, 2900 & 500mg/kg of the extract intraperitoreally based on the result of the first phase (fig iii), further observation of signs of toxicity & mortality for the first 4 (four) critical hours and daily afterwards. The oral median lethal dose was calculated using the formula:
LD50 = √ minimum toxic dose x maximum tolerated dose

Anti-trypanosomal activity Assay.

Following in vivo studies, Mice inoculated with *Trypanosoma brucei brucei federe strain* were intraperitoreally treated with 10 mg/kg body weight of the extracts when average parasitaemia was approximately two parasite per field for therapeutic & zero parasite per field for prophylactic. Preliminary investigation indicated relatively poor efficacy with 2.5 and 5 mg/kg doses of the extracts (fig iii). The treatment continued daily with continuous monitoring of parasitaemia for 4 days. After withdrawal of treatment, parasitaemia was also monitored daily until the 5th day and thereafter monitoring was reduced for surviving animals. Three animals were used per treatment group. An infected but untreated mouse was included as a negative control.

III. RESULT

Phytochemical screening of the ethanolic root extract of the plant Carissa spinarum indicated the presence of the following compounds; Terpenoids, Flavonoids, Alkaloids, Tannins, Saponins and Steroids(fig i).

Behavioral signs of toxicity was observed in all mice administered with various doses and mortality of 3 (three) mice recorded in the first 4 (four) hours at 1000mg of extract / kg body weight & subsequent loss of 2 (two) mice at dosage of 100mg extract per Kg weight of mice after several hours. The median lethal dose LD50 was determined to be ≥ 10mg / kg body weight (fig iii).

Post mortem from toxicity mortalities of mice indicated visible enlarged kidneys for all doses and further enlarged spleen, fluids in peritoneal, haemorrhage in Liver, severe haemorhage & inflamed lungs & accumulated fluid in subcuteaneous for dosage induced mortalities of 1000mg extract per Kg weight of mice (fig va-vd).

(fig i) Phytochemical composition of ethanolic Root extract of Carissa spinarum

Phytochemical Inference	Components
Alkaloids	**Present**
Coumarins	**Absent**
Flavonoids	**Present**
Leucoanthocyanins	**Absent**
Reducing sugars	**Absent**
Saponin	**Present**
Steroids	**Present**
Tannins	**Present**
Terpenoids	**Present**

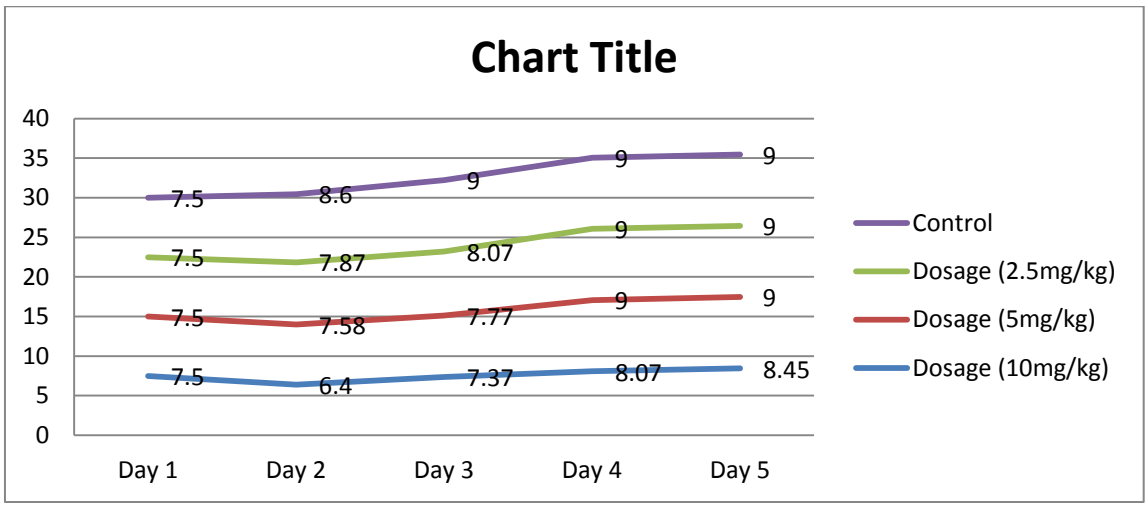
(Fig ii) Minimum Inhibitory Concentration (Mic) Of Ethanolic Root Extracts Of *Carissa Spinarum* Plant.

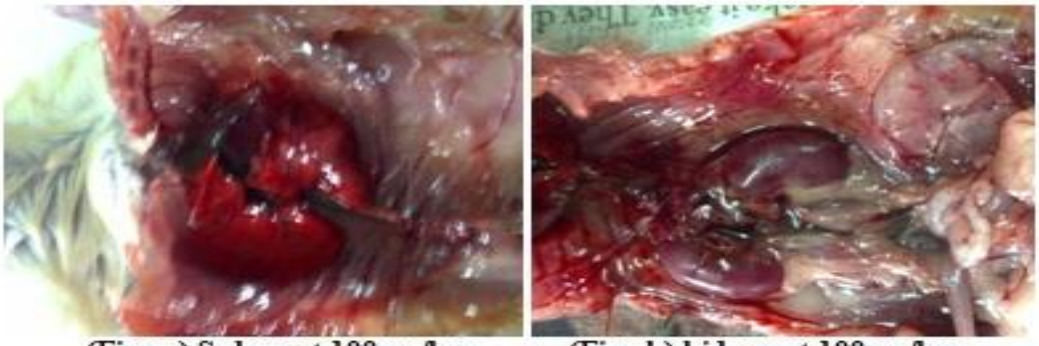
NO	TEST ORGANISMS	ROOT EXTRACT (10-1mm) 0.5ML	ROOT EXTRACT (10-2) 0.5ML	ROOT EXTRACT (10-3) 0.5ML	CONTROL (ETHANOL) 0.5ML
1	E.COLI	-	-	-	-
2	ENTROBACTER	-	-	-	-
3	PROTEUS	16.8mm	17mm	-	-
4	STAPHYLOCOCCUS AUREUS	-	-	-	-

(Fig iii)Acute toxicity test for *Carissa spinarum* ethanolic root extract in albino mice

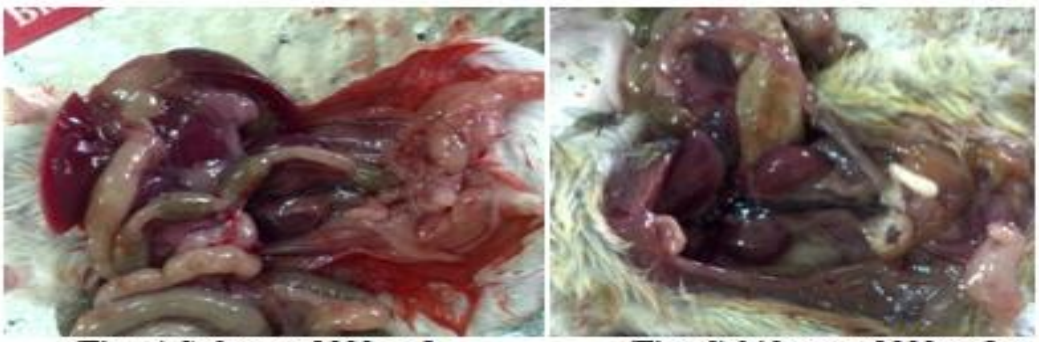
Dose (mg/kg)	Total mice	Mortality
10	3	-
100	3	2
1000	3	3
1600	3	3
2900	3	3
5000	3	3

(Fig iv) Effect of Ethanolic Root Extract Of Carissa Spinarum On Parasiteamia For 5 Days.





(Fig va) Spleen at 100mg/kg (Fig vb) kidney at 100mg/kg



(Fig vc) Spleen at 1000mg/kg (Fig vd) kidney at 1000mg/kg

IV. DISCUSSION

That the anti-trypanosomal effect observed under *in vivo* condition following administration of ethanolic leaf extracts of carissa spinarum (as seen above) is attributable to the extracts, appears to be confirmed by the death of all members of the control group that were infected with the parasite but left untreated in less than 7 days of infection, while most survived beyond the 7 days signifying the prophylactic properties of the extract.

The Minimum Inhibitory Concentration indicates that inhibition of common microorganism occurred with only Proteus while the other three (3) showed no extract inhibition.

The phytochemical analysis indicate the presence of alkaloids & saponins, which in most cases are positive indicators of antitrypanosomal activity, Tannin on the other hand is an antinutrient and may be responsible for the enlarged kidneys observed in the mice from high dose (fig va-vd).

The weakness observed in the mice of different groups with continuous administration of the extracts; even after parasites were eliminated from the blood stream suggest that the extracts may have some cumulative toxic effects at the high dose used. However, put together, these results suggest that *Carissa spinarum* possess significant anti-trypanonosomal effect to warrant further detailed studies utilizing bioassay-guided fractionations under varied pharmacological conditions in order to unequivocally establish its therapeutic efficacy.

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