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Physiochemical Evaluation of Ethanolic Root Extract of Carissa Spinarum (Wild Karanda) on *Trypanosoma Brucei Brucei* (Federe Strain) Infected Mice.

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ABSTRACT: *Carissa Spinarum plant used as analgesic for treatment of joints, muscle & chest pains by the massai people of Kenya also for cancer & antiviral supplement for HIV treatment in Tanzania. Acute toxicity of the ethanolic Root extract of Carissa spinarum was evaluated in mice, Histology of heart, kidney and liver organs of mice indicating various level of inflammation were carried out. 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 vis-à-vis its toxicological effect can be explored for the management of trypanosomiasis.*

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

I. INTRODUCTION

Trypanosomiasis is one of the most important infectious kinds of livestock and humans of similar and etiology and epidemiology in sub-saharan Africa [18]. Besides death, it causes a heavy economic loss to livestock mainly in Africa, in man, the disease is called Sleeping sickness, while in animal, it's known as Nagana. Sleeping sickness is known to have a prevalence of 300,000 – 500,000 [13] as well as three million death to livestock occurring every year [11]. Despite this prevalence rate, few drugs, namely, melarsoprol, Suramin, pentamidine and efflornithine are available for treatment, which are 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 have also been reported for melarsoprol in recent epidemics. Hence, there is urgent need to seek for new sources of therapeutic agents [9].

With the above development, recent approaches to alternative therapeutic agents for treatment of trypanosomiasis have focused on plants and other natural products [7], thus in this research investigation, we would focus on assessing the phytochemistry of the plant, Carissa spinarum as well as the invivo antitrypanosomal activity of its ethanolic leaf extract.Carissa Spinarum also known as the conkerberry or Bush Plum is a large shrub, that belongs to the family of Apocynaceae, it's widely distributed in tropical region and in India, where it's abundantly occurs, it's commonly known as Wild Karanda due to its close relation with the (C. carandas), This shrub is found wild in most parts of India, especially in the dry foothills of the Punjab, the sub-Himalayan tract up to 4,000 feet in the trans-Indus territory and also on the coast of the southern Andamans [14]. The plant an erect thorny shrub, with forked branches, 2-3 metres in height; wood, very hard; bark, light brown to green, can be stripped off longitudinally by hand, exposing the white to light-green wood underneath; thorns, 3.2 cm long, brown to greenish at the base and deep brown towards the tip. Leaves are ovate, 4.5 cm long, 2.5 cm broad, leathery; venation, reticulate pinnate; margin, entire; petiole 3 mm long; leaves exuding white latex, when plucked from the stem.

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.
Leaf parts 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 [19]; reducing sugars [17]; anthraquinons [2], flavonoids [15] and coumarins [16].

Determination of Parasitaemia

Parasitaemia was monitored in blood obtained from the tail, 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 (fig ii) were subjected to inhibition properties with the ethanolic leaf extract of Carissa spinarum via serial dilution incubation of the extract with each microorganism.

Acute Toxicity Test

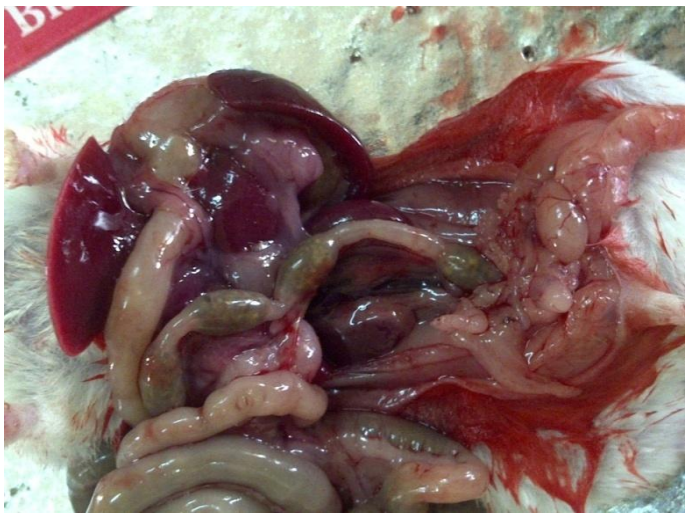
Acute toxicity test of Carissa spinarum root extract was carried out using the modified Lorke's method [12]. The study was carried in two phases, the first phase requires 9 (nine) mice randomized into 3 groups of three mice & each given intraperitoreally 10, 100 & 1000mg/kg body weight of the extract. 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 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, 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

In Vivo Assay

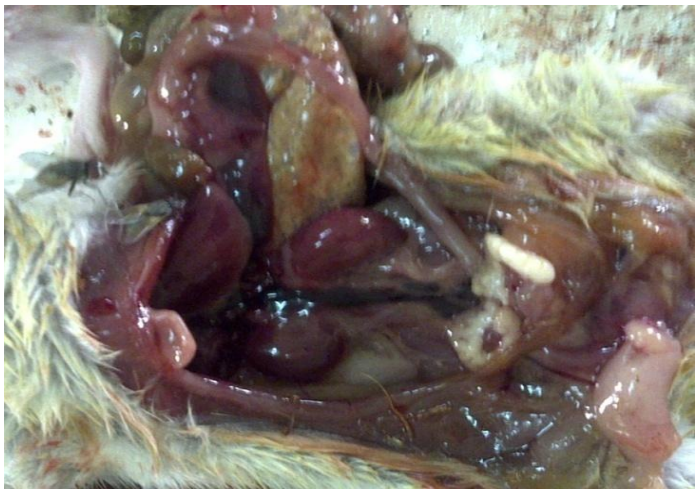
Following in vitro studies, Mice inoculated with *Trypanosoma brucei brucei* were intraperitoreally treated with 500 mg/kg body weight of the extracts when average parasitaemia was approximately two parasite per field for therapeutic & zero parasite per field for prophylactic. Preliminary investigation indicated relatively poor efficacy with 100 and 200 mg/kg doses of the extracts. 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.

Post Mortem

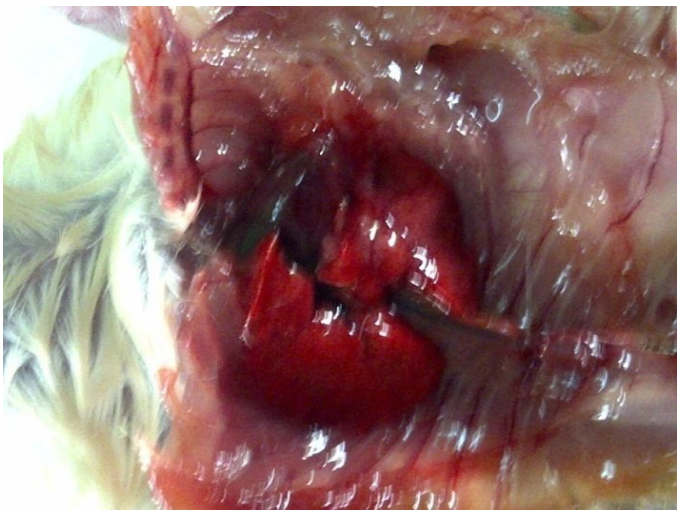
A post mortem was carried out on expired mice after treatment with the different dosage of *Carissa Spinarum* extract to determine which organs of the mice were actually affected by the extract and the organs photographed



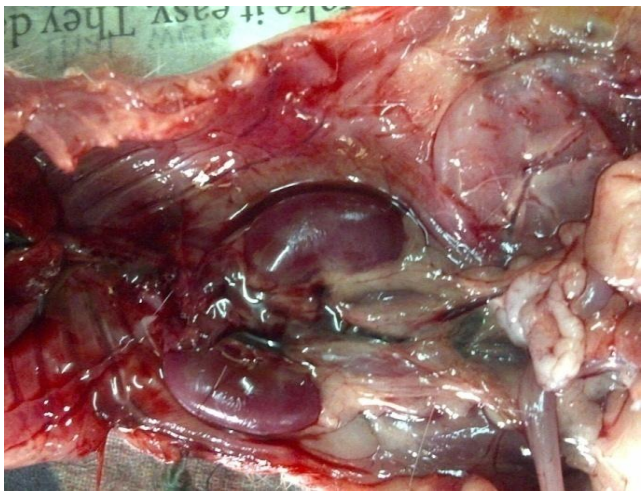
Carissa Spinarum Root extract 1000mg/kg dosage showing inflamed Livers



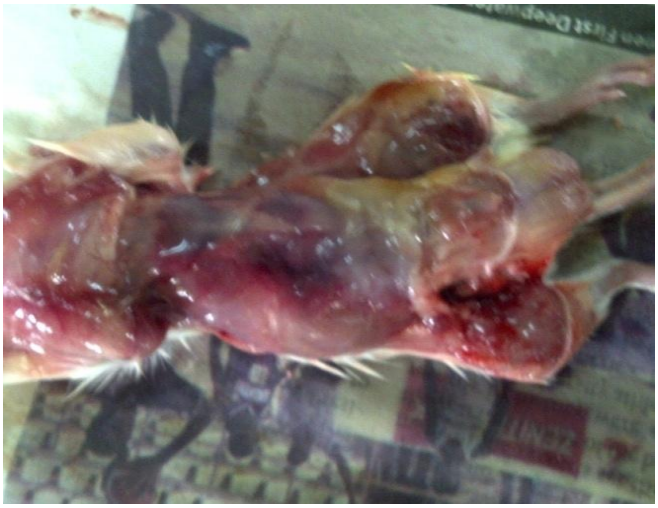
Carissa Spinarum Root extract 1000mg/kg dosage showing inflamed kidneys



Carissa Spinarum Root extract 100mg/kg dosage showing Liver



Carissa Spinarum Root extract 100mg/kg dosage showing Kidneys



Coloration of deposits observed underneath the skin of mice at dosage of 1000mg/kg of Carissa Spinarum.

Histopathological Examination

Histopathological examination of organs of treated expired animal was conducted on slides for Kidneys, Hearts and Livers at dosages of 100mg/ kg and 1000mg / kg of mice, where inflammation was observed.

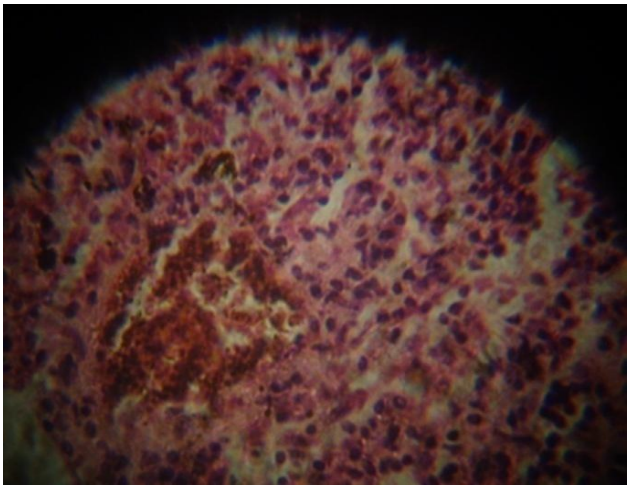


Fig I.Liver Slide At Dosage 1000mg/Kg Of Carissa Spinarum Indicates No Cirrhosis But Traces Of Fat Necrosis.

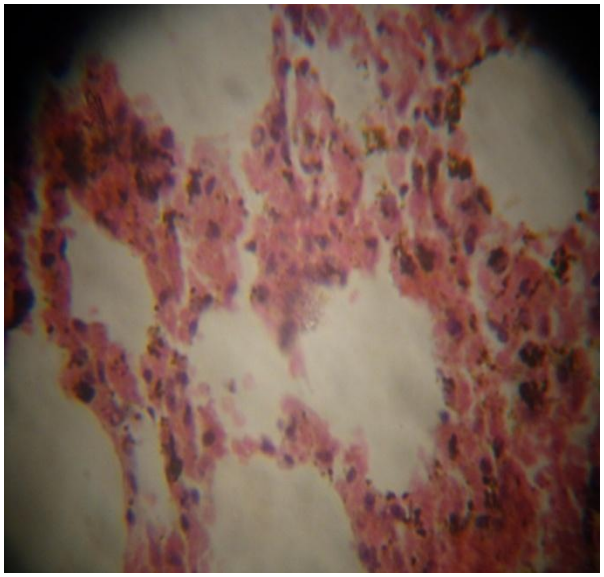


Fig ii.LIVER SLIDE AT DOSAGE 100mg/Kg of Carissa Spinarum indicates no cirrhosis.

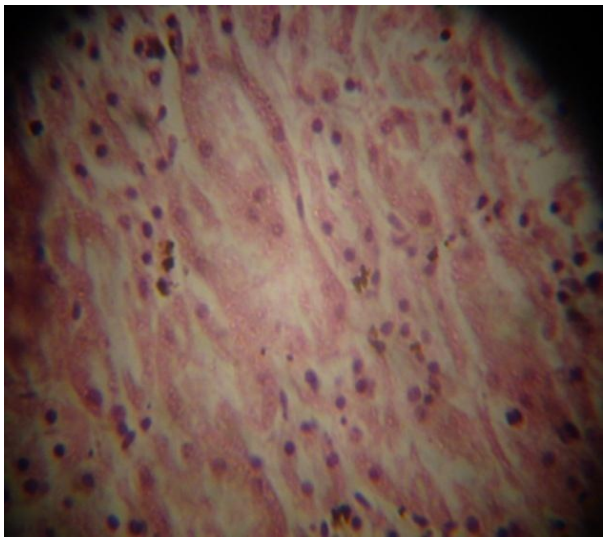


Fig iii. Kidney slide at dosage 1000mg/kg of Carissa Spinarum indicate trace sign of Pyelonephritis.

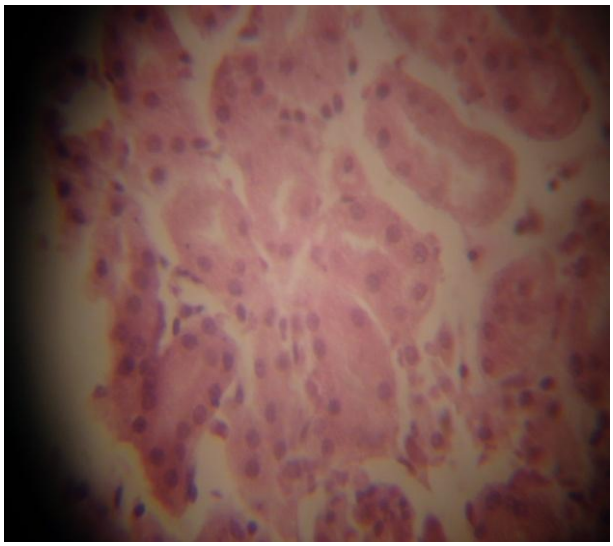


Fig iv.Kidney slide at dosage 100mg/kg of Carissa spinarum indicates Pyelonephritis.

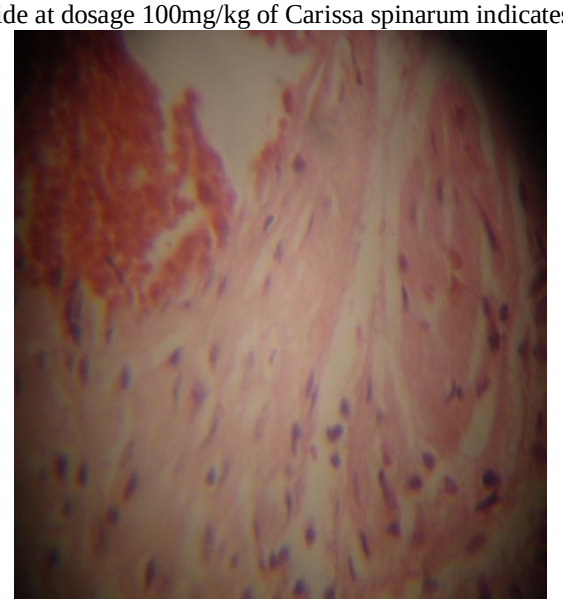


Fig v. Heart Slide dosage at 1000mg/kg of Carissa Spinarum indicates Myocardial Infarction (Heart Attack).

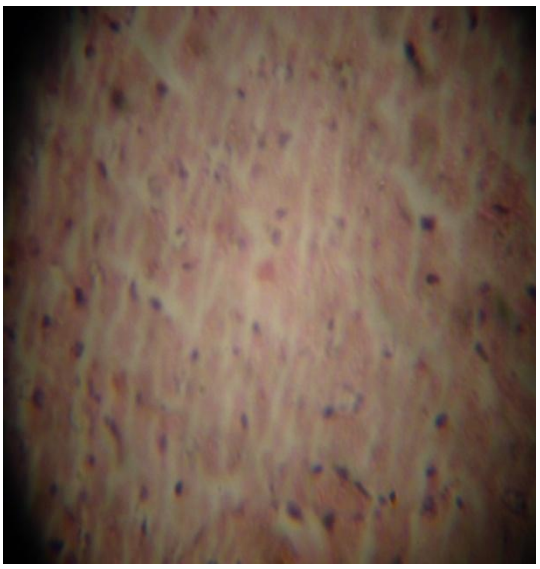


Fig vi. Heart Slide dosage at 100mg/kg of Carissa Spinarum with slight signs of Myocardial Infarction.

III. RESULTS

Phytochemical Screening

Results obtained from the phytochemical screening of the ethanolic root extract of Carissa spinarum revealed the presence of Anthraquinons, Emodins, Glycosides, Lignins, Phenols, Reducing sugar, Steroids and Tannins, while Alkaloids, Coumarins, Fatty acids, Flavonoids, Leucoanthocyanins, Saponin and Triterpenoids were absent (fig i).

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

Serological test consisting of kidney function test was conducted on mice administered with extract at 100mg / kg body weight as post mortem indicated highly inflamed kidneys in mice administered with extract. Results show a high deficiency in potassium at 2.2mmol as against the normal range of 3.4 – 5.3mmol (fig iv).

(Table 1) Phytochemical composition of ethanolic Root extract of Carissa spinarum

Phytochemical Components	***Inference***
Alkaloids	Absent
Anthraquinons	Present
Coumarins	Absent
Emodins	Present
Fatty acids	Absent
Flavonoids	Absent
Glycosides	Present
Leucoanthocyanins	Absent
Lignins	Present
Phenols	Present
Reducing sugars	Present
Saponin	Absent
Steroids	Present
Tannins	Present
Triterpenoids	Absent

(Table 2)MINIMUM INHIBITORY CONCENTRATION (MIC) OF ETHANOLIC ROOT EXTRACTS OF *CARISSA SPINARUM* PLANT.

NO	*TEST ORGANISMS*	*LEAVE EXTRACT (10-1mm) 0.5ML*	*LEAVE EXTRACT (10-2) 0.5ML*	*LEAVE EXTRACT (10-3) 0.5ML*	*CONTROL (ETHANOL) 0.5ML*
1	E.COLI	-	-	-	-
2	ENTROBACTER	-	-	-	-
3	PROTEUS	20.5mm	18mm	-	-
4	STAPHYLOCOCCUS AUREUS	-	-	-	-

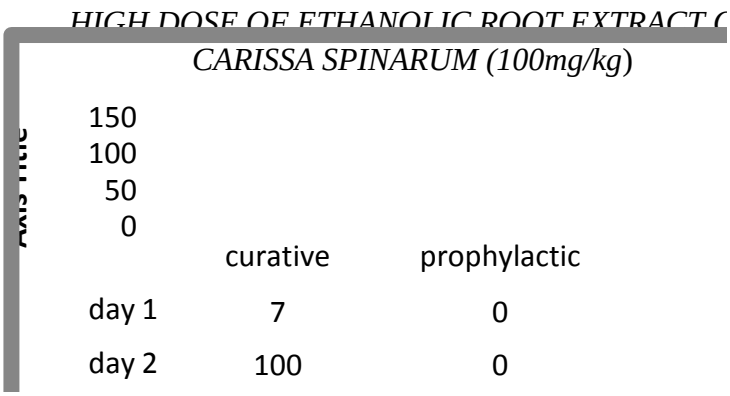
(Table 3)Acute toxicity test for Carissa spinarum ethanolic root extract in albino mice

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

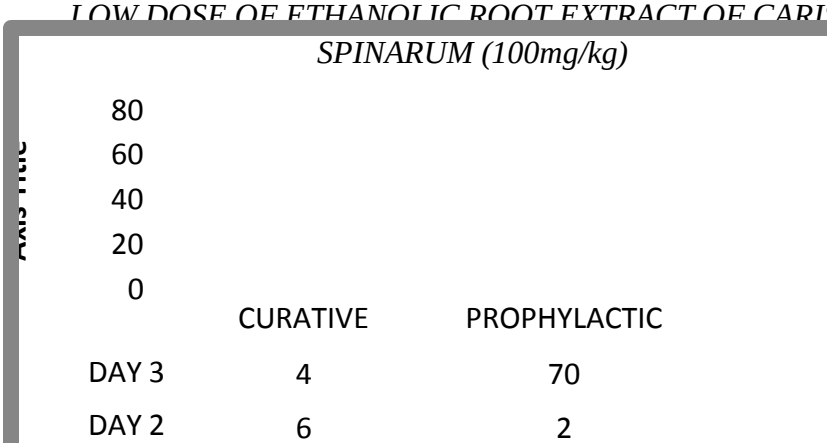
(Table 4)Serological result performed on kidney of mice administered with Carissa spinarum ethanolic root extract.

Compound	*Normal Range (mmol)*	*Reading (mmol)*
Urea	1.7 – 9.1	1.9
Sodium	136 – 148	137
Potassium	3.4 – 5.3	2.2
Chloride	95 – 111	96
Bicarbonate	22 – 32	24
Creatinine	9 – 124	25

(Table 5a)Effects of Ethanolic Root Extract of Carissa Spinarum on Parasiteamia for 3 Days



(Table 5b)



IV. DISCUSSION

The anti-trypanosomal effect observed under *in vivo* condition following administration of ethanolic root 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 (Table 5a & 5b), also histopathological slides indicates that the mice expired at high dosage of 1000mg/kg of the extract from Myocardial Infarction (Heart Attack).

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 as also evidenced from the deposit of colorations seen underneath the mice skin during post mortem examinations. 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.

V. ACKNOWLEDGEMENT

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REFERENCES

[1] Rizk AM, Constituents of plant growing in Qatar. I. a chemical survey of sixty plants. Fitoterapia, (1982); 52: 35-42.
[2] Kristjanson, P.M., B.M. Swallow, G.J. Rowlands, et al. (1999) Measuring the costs of African animal trypanosomosis, the potential benefits of control and returns to research. Agric. Syst., 59: 79-98.
[3] Hursey, B.S., 2001. The programme against African trypanosomiasis-aims, objectives and achivements. Trends Parasitol., 17: 2-3.
[4] Brun R., Schumacher R., Schmid C., et al. (2001) The phenomenon of treatment failures in Human African Ttrypanosomiasis. Trop. Med. Intl. Health, **6**(11): 906-914.
[5] Anene B.M., Onah D. N., Nawa Y. (2001) Drug Resistance in pathogenic African trypanosomes: What hopes for the future? Vet. Parasitol, 96: 83-100.
[6] Gutteridge NE. (1985) Existing chemotherapy and its limitations. Brit. Med. Bull, **41**(2): 162-168.
[7] Budd LT (1999) DFID-funded tsetse and trypanosomiasis research and development since 1980 (V. 2. Economic analysis). London: Department for International Development.
[8] Lorke D (1983). A new approach to practical acute toxicity testing. Arch. Toxicol. 54: 275-287
[9] Gibbs, R.D., 1974. Chemotaxonomy of Flowering Plants. Vol. 1, McGill-Queen's University Press, Montreal and London.
[10] Ayoola, G.A., F.M. Lawore, T. Adelowotan, et al. (2008) Chemical analysis and antimicrobial activity of the essential oil of Syzigium aromaticum (clove). Afr. J. Microbiol. Res., 2: 162-166.
[11] Satyanarayana, K. and Rehse, K. (1998), Organic Azides. Arch. Pharm. Pharm. Med. Chem., 331: 207–210. doi: 10.1002/(SICI)1521-4184(199806)331:6<207::AID-ARDP207>3.0.CO;2-5
[12] Pepin J. and Meda. H.A.(2001).The epidemiology and control of human African trypanosomiasis Advances in Parasitol. 49: 71-132.
[13] ASEAN countries. Standard of ASEAN herbal medicine, Vol.1 Jakatra: Aksara Buena Printing, (1993): 116-28.
[14] Mhlanga, J D M. (1999) Sleeping sickness: perspectives in African trypanosomiasis. Sci Progress, 79: 183-187.
[15] Peach K and Tracey MV, Modern methods of plant analysis. Vol.3, Springer Verlag, Berlin, 1956.
[16] Herbert, W.J. and W.H.R. Lumsden, 1976. Trypanosomabrucei, a rapid "matching" method for estimation of host's parasitemia. Exp. Parasitol., 40: 427-431.
[17] Atawodi S.E, Ameh D. A., Ibrahim S., et al. (2002) Indigenous knowledge system for treatment of trypanosomiasis in Kaduna state of Nigeria. J. Ethnopharmacol, **79**(2): 279-282.
[18] Atawodi S.E., Bulus T. Ibrahim S., et al. (2003) In vitro trypanocidal effect of Methanol extract of some Nigerian Savannah Plants. Afri J. Biotechnol, 2(9): 317-321.
[19] Kabayo JP: Aiming to eliminate tsetse from Africa**.** *Trends Parasitol* 2002, **18:**473-475