

## ***In Vivo* Anti-Trypanosomal Activity Of Methanolic Leaf Extract Of *Strophanthus Sarmentosus dc* In Mice Infected With *Trypanosomal brucei brucei spp.***

Onotu. C.S<sup>1</sup>, Abedo. J.A<sup>1</sup>, Anchua R.G<sup>1</sup>, Andrew T<sup>1</sup>, Baraya K.Y<sup>1</sup>, Sambo F<sup>1</sup>,  
Daniel J<sup>1</sup>

<sup>1</sup>Department of Vector & Parasitology Studies,  
Nigerian Institute for Trypanosomiasis Research,  
PMB 2077, Kaduna.

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**ABSTRACT :** *Strophanthus sarmentosus dc* plant also known as poison arrow because of its earlier use by hunters as a sedative in arrows has been implicated as a medicinal plant for treatment of various ailments. Acute toxicity of the methanolic leaf extract was evaluated in mice using Lorke's method followed by minimum inhibitory concentration using four (4) microorganism (*proteus*, *e.coli*, *staphylococcus aureus spp* & *enterobacter spp*) by serial dilution method. The methanolic leaf extract was evaluated for *in vivo* anti-trypanosomal activity against federe strain of *Trypanosoma brucei brucei* in albino mice. Four days suppressive and curative effects against established infection and prophylactic model of anti-trypanosomal studies were carried out. The median lethal dose of the extract was determined to be  $\geq 10\text{mg/kg}$  body weight. The extract (2.5, 5, 10mg / kg) exerted some dose dependent suppressive effects at the different levels of infection tested, with both prophylactic and curative evaluations against established control indicating some suppression to trypanosome parasites.

**KEYWORDS :** *Strophanthus sarmentosus dc*, antitrypanosomal, albino mice, trypanosome *brucei brucei specie*

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### **I. INTRODUCTION**

Trypanosomiasis can be said to be one of the most important infectious kinds of livestock and humans disease of similar etiology and epidemiology in sub-saharan Africa [18]. Its known to bring about death to its host with an observed expiration after infection thus bringing about heavy economic loss to livestock mainly in Africa. In human, the disease is called Sleeping sickness, while in animal, it's known as Nagana. It's a zoonotic disease because of its animal to human transmittability capability. It has a preferred vectorial acceptability with the Glossina Tsetse fly and caused by the protozoan trypanosoma. Sleeping sickness is known to have a prevalence of 300,000 – 500,000 in man, [13] causing immense debilitation and if untreated leads to coma and an eventual expiration of the subject. Putatively, its responsible for three million death to livestock occurrence every year [11]. Despite this alarming prevalence rate, few drugs are known to exist for the treatment and control of this disease namely, melarsoprol, Suramin, pentamidine and efflornithine, 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. These very unfavourable variables have brought about the urgency to seek for new sources of therapeutic agents [9] with emphasis on availability and cost effectiveness. Thus from the above development, recent approaches to alternative therapeutic agents for treatment of trypanosomiasis have become more focused on plants and other natural products [7].

## II. MATERIALS AND METHOD

The plant was collected and supplied by a cattle rearer at the Ladduga Grazing Reserve in Kachia Local Government Area of Kaduna which is Northwestern 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 12/10/2011. 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 methanol (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°C until required.

**Animals :** Albino mice of four (4) weeks, 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. Two weeks of acclimatization was observed before commencement of research.

**Phytochemical of Extract :**Phytochemical screenings of extract of *strophanthus sarmentosus dc* indicate presence of glycosides and saponin

**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].

## III. 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 spp and proteus (fig ii) were subjected to inhibition properties with the methanolic leaf extract of *Strophanthus sarmentosus dc* via serial dilution incubation of the extract with each microorganism.

**Acute Toxicity Test :** Acute toxicity test of *strophanthus sarmentosus dc* methanolic leaf 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 intraperitoneally 10, 100 & 1000mg/kg body weight of the extract. The mice were observed for usual 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 intraperitoneally, 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:

$$LD50 = \sqrt{\text{minimum toxic dose} \times \text{maximum tolerated dose}}$$

**In Vivo Assay :** Following in vivo studies, Mice inoculated with *Trypanosoma brucei brucei (federe strain)* were intraperitoneally treated with 500 mg/kg body weight of the extracts and 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.

## IV. RESULTS

A behavioral sign of toxicity was observed in all mice administered with various doses. Mortality of 2 (two) mice recorded in the first 4 (four) hours at 1000mg of extract / kg body and 1 (one) mice mortality at 100mg / kg body weight was observed & total mortality of all mice within the 100mg / kg & 1000mg / kg body weight after several hours. The median lethal dose LD50 was determined to be  $\geq 10\text{mg} / \text{kg}$  body weight.

(fig ii)MINIMUM INHIBITORY CONCENTRATION (MIC) OF METHANOLIC LEAF EXTRACTS OF *STROPHANTHUS SARMENTOSUS DC* PLANT.

NO.	TEST ORGANISM	LEAVE EXTRACT(ml)			Ethanol
		10~1	10~2	10~3	
1.	<i>E.choli</i> spp.	-	-	-	1.0mm
2.	<i>Enterobacter</i> spp.	-	-	-	1.5mm
3.	<i>Proteus</i> spp.	3.95mm	3.25mm	-	-
4.	<i>Staphylococcus aureus</i> spp.	-	-	-	-

(fig iii)Acute toxicity test for *strophanthus sarmentosus dc* methanolic leaf extract in albino mice

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

EFFECTS OF METHANOLIC LEAF EXTRACT OF *STROPHANTHUS SARMENTOSUS DC* ON PARASITAEAMIA FOR 7 DAYS.

Fig v. PROPHYLACTIC TREATMENT FOR METHANOLIC LEAF EXTRACT OF *STROPHANTHUS SARMENTOSUS DC* PLANT.

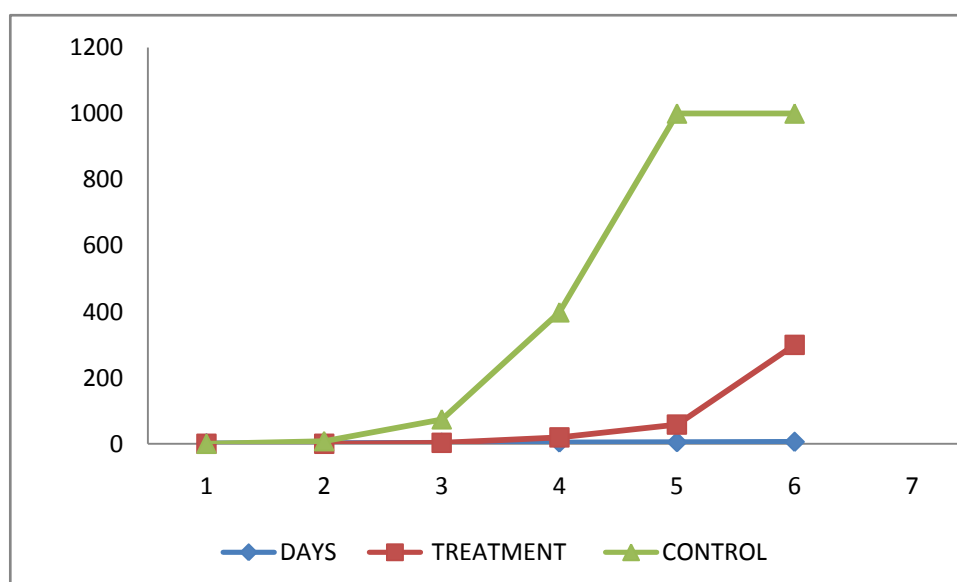
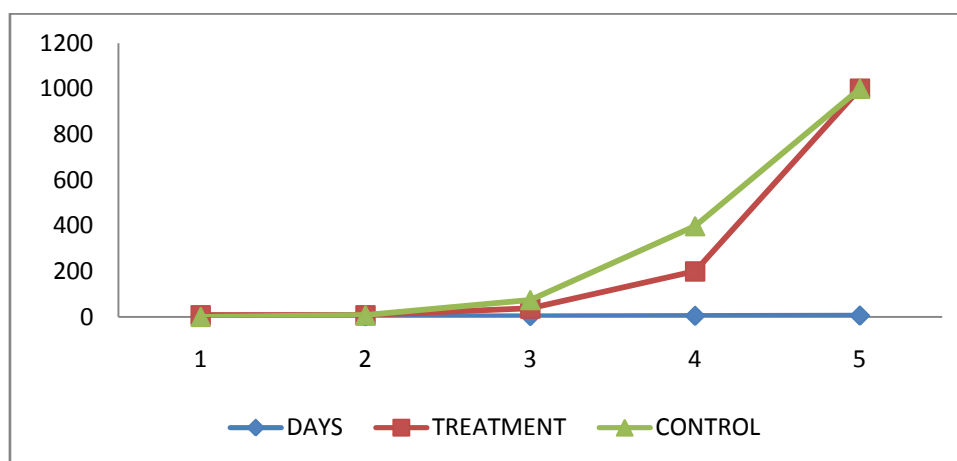


Fig vi. CURATIVE TREATMENT FOR METHANOLIC LEAF EXTRACT OF *STROPHANTHUS SARMENTOSUS DC* PLANT



## V. DISCUSSION

That the anti-trypanosomal effect observed under *in vivo* condition following administration of ethanolic leaf extracts of *carissa spinarum* (fig v) 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 within 7 days of infection, while most survived beyond the 7 days signifying the prophylactic properties of the extract. The Minimum Inhibition Concentration of the extract against the four microorganism indicate very little inhibition which only occurred with proteus spp (fig ii). The phytochemical analysis indicate the absence of alkaloids & saponins (fig i), which in most cases are positive indicators of non antitrypanosomal activity, however, the presence of other substance like phenol, reducing sugars, Glycosides, steroids, etc may indicate different (fig i). Tannin on the other hand is an antinutrient and may be responsible for the enlarged kidneys observed in the mice from high dosage and thus observed in the low potassium in blood of the mice. 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-trypanosomal 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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