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Invivo antitrypanosomal activity of methanolic stem extract of *strophanthus sarmentosus dc* on wistar white mice infected with *trypanosomal brucei brucei spp* (federe strain).

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Abstract
Strophanthus sarmentosus dc plant seeds are known to be mixed with other plants and boiled to produce poison arrow for hunters, it has also been implicated as a medicinal plant for treatment of gonorrhea and Leprosy in Senegal. Acute toxicity and minimum parasites inhibitions using four (4) microorganism *(proteus, e.coli, staphylococcus aureus spp & enterobacter spp*) of the methanolic leaf extract was evaluated in mice using Lorke's & serial dilution methods respectively. The methanolic leaf extract was evaluated for *in vivo* anti-trypanosomal activity against federa strain of *Trypanosoma brucei brucei* in albino mice. Four days suppressive, curative effect against established infection and prophylactic models of anti-trypanosomal studies were carried out. The median lethal dose of the extract was determined to be ≥ 10mg ⁄ kg body weight. The extract (2.5, 5, 10mg / kg) exerted some dose dependent suppressive effects at the different levels of infections tested, with no significant curative effects recorded. However, further antitrypanosomal property needs to be explored for the management of *trypanosomiasis.*

Introduction

African trypanosomiasis is a disease of medical and veterinary importance caused by extracellular hemoflagellates of the genus *Trypanosoma* [Mansfield, J. M. (1981]. Trypanosome-infected hosts are exposed to many parasite antigens during the course of infection. These antigens include invariant membrane, cytoplasmic, and nuclear antigens as well as a series of distinct, variable surface glycoprotein (VSG) molecules associated classically with this infection [,Mansfield, J. M. (1981), Mansfield, J. M. (1990)]. *Strophanthus sarmentosus* is an Apocynaceae plant common in Nigeria and is commonly referred to by some tribes as the following, the *BEROM* hwaàl ndɥ́m (poisonous medicine), (LB) *CHAMBA* me-ni , (FNH) *EDO* ò#víẹn-órà, (Ross) *FULA-FULFULDE (Nigeria)* awdi tooke (poisonous seeds) an epithet (JMD) maada (JMD) and tantsiyaari from Hausa (JMD). tooke, tookere = poison; the plant, an epithet (JMD) *GOEMAI* lauenne (JMD) *GWARI* obwa (JMD) *HAUSA* gama sowa (Ryan) gwasha (FNH) kwaŋkwánií (LB) tantsiya (JMD; ZOG) *IGBO* o#ta = a bow (JMD; AJC) o#ta nta = a hunting bow (NWT; JMD) *TIV* àgbùlCf1 (JMD) *YORUBA* agan olugbo (RJN) akan (RJN) ako-isa (Ross) ilagbà o#mo#dé, lãgba-o#mo#de = little child's horse-whip (Verger; JMD) ire (IFE) is#a (Oluakpata; IFE) is#akékeré = lesser (Ross; JMD) sagere (Millen) (Burkill, H.M. 1985).
Its Leaf is used as emetics; eye treatments, veneral diseases, analgesic, leprosy, insanity, rheumatism, and arthritis, also its known to be used as "arrow-poisons, which incidentally is the most common name for the plant.

Sleeping sickness in east and southern africa is caused by *Trypanosoma brucei rhodesiense* and by in west and central Africa by *Trypanosoma brucei gambiense*. Both protozoan species are morphologically indistinguishable, but have drastically different epidemiological features. Both are also known to affect the central nervous system. According to WHO reports, African sleeping sickness is the third most important contributor to the global burden of the parasitic diseases after malaria and schistosomiasis, if the Disability Adjusted Life Year (DALY) figures (i.e., loss of healthy life years by premature mortality and disability) are considered [P. Cattand, J. Jannin, and P. Lucas,2001]. More than 60 million people are at risk of infection from human African trypanosomiasis, with about 45,000 new cases reported annually. It is estimated that at least 300,000–500,000 people are presently infected. However, less than 4 million people are under surveillance and as such, it is estimated that less than 10% of the new cases are diagnosed and treated [WHO(1998)].

Animal trypanosomiasis is caused by a wider number of trypanosome species and carried with higher prevalence by a greater number of glossina species with high vectorial capability, thus invariably the greater epidemic across the African continent with direct economic consequences. The epidemiological trend indicates that the disease is widespread in 36 sub-Saharan countries for HAT (Human African Trypanosomiasis) and 37 countries for AAT (Animal African Trypanosomiasis) covering over 9 million square km between 14 degree north and 20

degree south latitude, approximately one third of African's total land area (NITR Annual Report, 1989; Molyneux et al., 1996; Steverding, 2008), trypanosome infections that threaten livestock have a 100 to 150-fold higher prevalence than the HAT [A. M. Jordan 1976].About 60 million people and 50 million cattle are at risk of being infected with a standing prevalence of 500,000 infections and 66,000 human as well as three (3) million livestock deaths occurring annually. Less than 15% of cases in humans are diagnosed and treated (Cecchi and Mattioli, 2009; Wellcome Trust, 2005; Kamuanga, 2003; WHO, 2000; Kristjanson, 1999), these above statistical analysis should give very ample reason for worry.

Materials and Methods

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 $4^{o}C$ 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 methanolic stem extract of *Strophanthus Sarmentosus dc* was screened for the presence of secondary metabolites and constituents using conventional protocol for detecting the presence of fatty acids, glycosides, triterpenoids and saponins [Anene B.M., Onah D. N., Nawa Y. (2001)].

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 [Ayoola, G.A., F.M. Lawore, T. Adelowotan, et al. (2008)]. Briefly, the method involves microscopic counting of parasites per field in pure blood or blood appropriately diluted with phosphate buffer 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 [Hursey, B.S., (2001)], [Brun R., Schumacher R., Schmid C., et al. (2001)].

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 methanolic stem extract of *Strophanthus Sarmentosus dc* via serial dilution incubation of the extract with each microorganism.

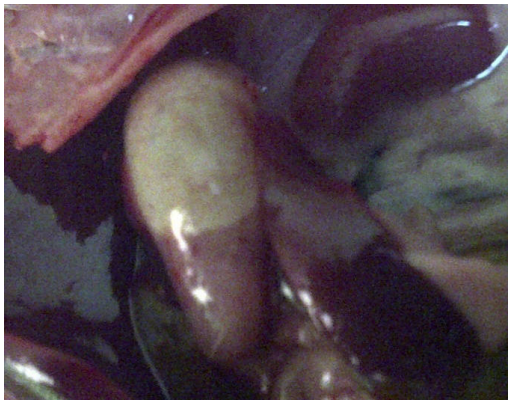
Acute Toxicity Test

Acute toxicity test of *Strophanthus Sarmentosus dc* stem extract was carried out using the modified Lorke's method [Pepin J. and Meda. H.A.(2001)]. 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

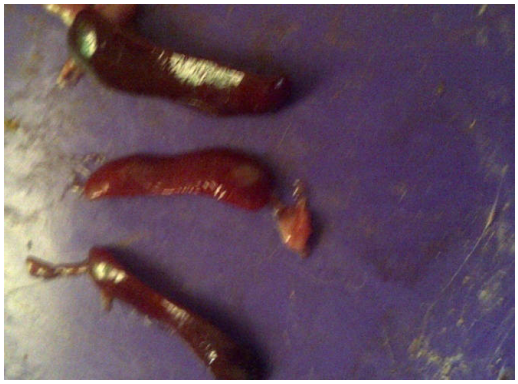
extract administration and subsequently daily for hours. In the second phase, another fresh set of 9 (nine) mice were randomized into 3 groups of three mice again & administered with 1600, 2900 & 500mg/kg of the extract intraperitoreally based on the result of the first phase, further observation of 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, where the average safe dosage is determined to be 5mg/kg of mice.

Post Mortem

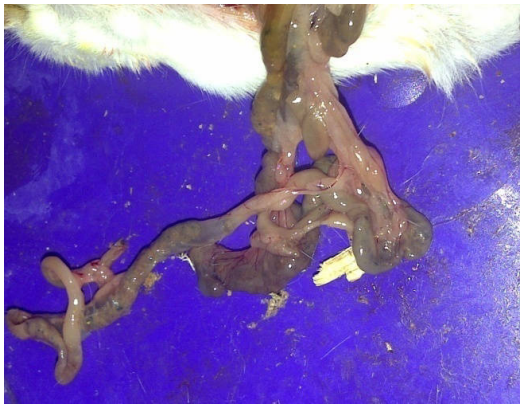
A post mortem was carried out on expired mice after treatment with the different dosage of *strophanthus sarmentosus dc* extract to determine with organs of the mice where actually affected by the extract and the organs photographed.



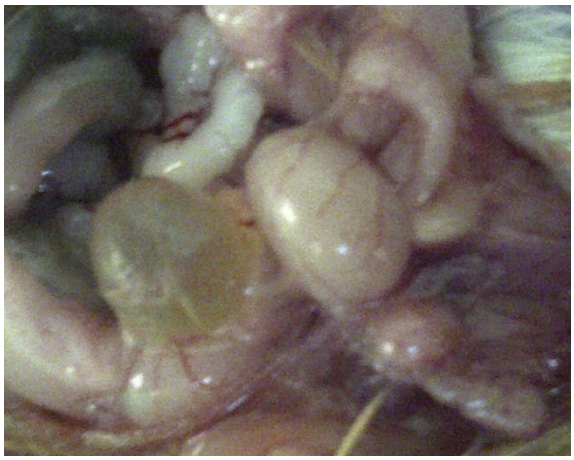
i. Inflammation of the Spleen is observed at 10mg/kg dosage.



ii. The higher the toxicity, the more the inflammation observed in the spleen.



iii. Intestines are indicative of extract poisoning.



iv. Bladders and testis are indicative of inflammation due to extract.

In Vivo Assay

Mice inoculated with *Trypanosoma brucei brucei (federe strain)* were intraperitoreally treated with 5mg/kg body weight of the extracts when average parasitaemia was approximately two parasites per field for therapeutic & zero parasite per field for prophylactic. Preliminary investigation indicates efficacy with tolerance of extract at dosage 1.25mg/kg of mice. 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.

RESULTS

Phytochemical Screening

Results obtained from the phytochemical screening of the methanolic stem extract of *Strophanthus Sarmentosus dc* revealed the presence of Glycosides and Saponins.

Behavioral signs of toxicity like mobility and sedation was observed in all mice administered with various doses and mortality recorded in the first 4 (four) hours at 5mg 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.

Table 1. Minimum inhibitory concentration (MIC) of methanolic stem extract of *strophanthus sarmentosus dc* plant.

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

Table 2. Acute toxicity test for *Strophanthus Sarmentosus dc* methanolic stem 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

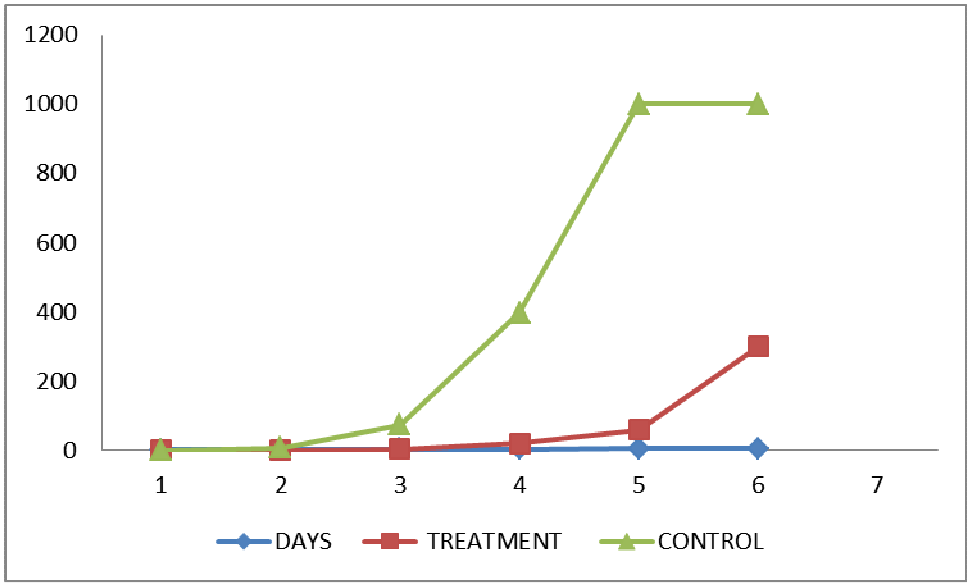


Figure (i). Prophylactic treatment for methanolic stem extract of *strophanthus sarmentosus dc* plant.

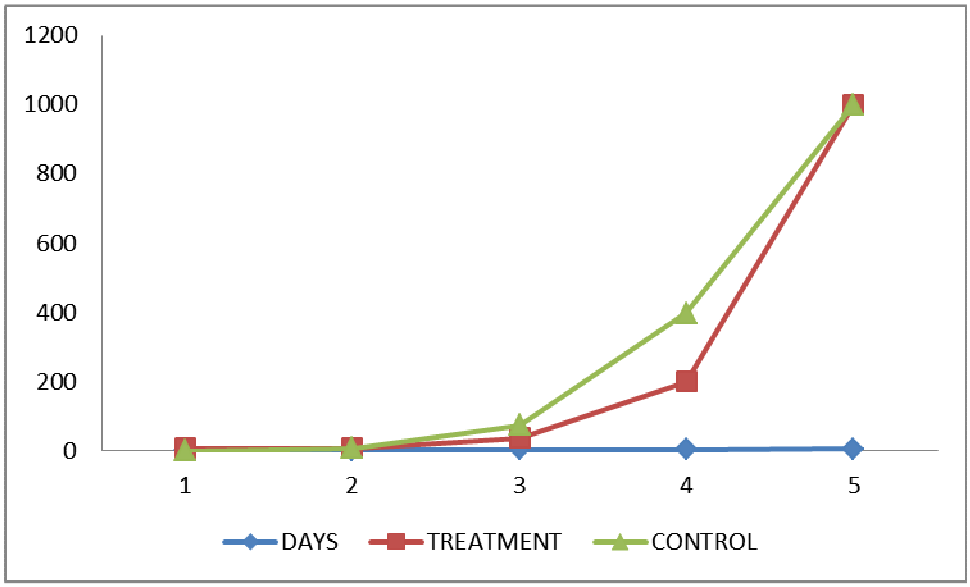


Figure (ii) Curative treatment for methanolic stem extract of *strophanthus sarmentosus dc* plant.

DISCUSSION

Anti-trypanosomal effect observed under *in vivo* condition following administration of methanolic stem extracts of *strophanthus sarmentosus dc* (from above graphical representation) 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 4 days of infection, while most survived beyond the 5th days signifying the prophylactic properties of the extract whilst for curative treatment parasitaemia was observed to be controlled in the inception of treatment but subsequent with little differential.
The Minimun Inhibition Concentration of the extract against the four microorganism indicated no inhibition on all four (4) microorganism tested.
The phytochemical analysis indicates the presence of glycosides and saponins, with glycosides signifying a positive indicator due to its usage in the production of cortisone for treatment of rheumatoid arthritis etc, hence its observed trypanosomal activity, saponins on the other hand indicates different.
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 even low dosage as indicative from the inflammations of the spleen, bladder and testis of the mice and extremely lethal at high dose as high mortality was observed with dosage of 5mg/kg. Animals were most tolerant of dosage of 1.25mg/kg of the extract. However, put together, these results suggest that *Strophanthus sarmentosus dc* 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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