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In vivo* Trypanostatic Activity of *Tephrosia linearis* Extract on *Trypanosoma evansi

*¹Garba, M. H., ²Ampitan, T. A., ³AbdulAzeez, A., ⁴Tawakaltu, A-A., ⁵Fajobi, E. A. and ⁶Jeje, C. A.

¹Department of Biochemistry, Federal University Dutse, Jigawa State

²Department of Forestry Technology, Federal College of Wildlife Management, P.M.B. 268, New Bussa, Nigeria.

³Department of Biological Sciences, Federal University Dutse, Jigawa State

⁴Department of Biochemistry, Federal University of Technology, P.M.B. 65, Minna.

⁵Department of Basic Sciences, Federal College of Wildlife Management, P.M.B. 268, New Bussa, Nigeria

⁶Department of Computer Science, Federal College of Wildlife Management, P.M.B. 268, New Bussa, Nigeria

Abstract

In vivo antitrypanosomal activity of 70% methanol extract of *Tephrosia linearis* was evaluated. Mice infected with *Trypanosoma evansi* in different groups were administered 100, 200, 300 and 400 mg/kg body weight/day of the seventy percent (v/v) (methanol/water) crude extract of *T. linearis* intraperitoneally. The positive control group was treated with standard drug, berenil, while the negative control was infected and treated with normal saline. Doses of 300 and 400 mg/kg bw *i.p.* per day were found to significantly reduce the parasite loads and sustained the animals in the respective groups up to 3 and 4 weeks beyond the experimental period. There is drop in the percentage PCV during the first six days of the treatment but this was reversed in the subsequent days, except in the negative control group. The animals administered the highest effective (trypanostatic) dose of 400 mg/kg body weight for five consecutive days prior to infection were observed to develop infection 72 h post inoculation. The LD₅₀ determined was found to be 2800 mg/kg bw. It is obvious that, higher doses of 300 and 400 mg/kg bw has trypanostatic effect. Though, the extract could not show any prophylactic activity, further purification may possibly yield a fraction with trypanocidal effect.

Keywords: *Tephrosia linearis*, *Methanol extract*, *Trypanosoma evansi*, *Antitrypanosomal activity*, *In vivo*,

Introduction

Sleeping sickness, also known as Human African Trypanosomiasis (HAT), is a neglected disease that impacts 70 million people living in 1.55 million km² in sub-Saharan Africa. Since the beginning of the 20th century, there have been multiple HAT epidemics in sub-Saharan Africa, with the most recent epidemic in the 1990s resulting in about half a million HAT cases reported between 1990 and 2015 (Aksoy *et al.*, 2017). HAT is a disease caused

by haemoflagellate parasites called *Trypanosoma brucei*. These are kinetoplastid protozoa that devastate the health and economic well-being of millions of people in Africa. Severe side effects and the drug resistance issues plaguing current drugs has created a situation where a new chemotherapy is eagerly waited for. Recently, there has been emphasis on the use of medicinal plants worldwide (Kwofie *et al.*, 2016). The African trypanosomiasis

*Corresponding author: +2348134809595

Email address: mharunagarba@gmail.com

or sleeping sickness that occurs in equatorial Africa is in two forms both transmitted by the tse-tse fly (*Glossina*). East African or Rhodesian, sleeping sickness is an acute form of the disease caused by the subspecies *T. brucei rhodesiense*. West African, or Gambian, trypanosomiasis is a slower-developing chronic form of the disease caused by *T. brucei gambiense*. Both organisms can eventually invade the brain, causing mental deterioration, coma, and death. Other *Trypanosoma* species cause economically important diseases in livestock: *nagana*, *dourine*, *surra*, and *mal de caderas*.

Besides, great socioeconomic effects on the endemic areas by this disease are forecast if inadequate attention (both at the communal, national, and international levels) is not given (Conrad *et al.*, 2018). The diseases are reported by the WHO to be responsible for about 1 million new cases leading to approximately 30,000 deaths annually on a global scale (WHO report, 2019).

On the current distribution, the disease incidence differs from one country to another as well as in different parts of a single country. Over 70% of the reported cases occurred in the Democratic Republic of Congo in the last 10 years. Endemic countries such as: Angola, Cameroon, Central African Republic, Chad, Congo, Guinea, Malawi, South Sudan and Zambia declared between 10 and 100 new cases in 2019, while Côte d'Ivoire, Equatorial Guinea, Gabon, Uganda, United Republic of Tanzania and Zimbabwe declared between 1 and 10 new cases. In the lesser endemic countries such as Burkina Faso, Ghana, Kenya and Nigeria, sporadic cases have been reported in the last 10 years. On the interesting note, countries like Benin, Botswana, Burundi, Ethiopia, Gambia, Guinea Bissau, Liberia, Mali, Mozambique, Namibia, Niger, Rwanda, Senegal, Sierra Leone, Swaziland and Togo have not reported any new cases for over a decade, though, transmission of the disease seems to have stopped in some of these countries. Still, due to some unstable social circumstances and/or difficult accessibility that greatly hinder surveillance and diagnostic activities, there are still some areas where it is difficult to assess the exact situation (WHO report, 2021).

Chemotherapeutic agents against HAT, namely, suramin, pentamidine, melarsoprol, and eflornithine (Simarro *et al.*, 2008), cause severe side effects (Bacchi, 2009), require lengthy parenteral administration, and are unaffordable for most patients and therefore, their continuous use is far from ideal. In addition to those concerns, the increase in drug resistance urges the need for the discovery of new chemotherapeutic agents against HAT (Wang, 1995).

With the absence of any vaccine targeting any parasites (except of course, the malarial vaccines) and resistance against the already existing anti-parasitic drugs, research efforts have been employed and encouraged towards the search for new, cheaper, potent and effective drugs to treat these diseases. Medicinal plants represent a potential source of new drugs. This is because natural products (NPs) from organisms such as animals, fungi and the higher plants have been known to be good sources of pharmacologically active compounds against several ailments, including parasitic infections (Conrad *et al.*, 2018).

Nigeria is naturally endowed with both savannah and tropical rainforest vegetations. These diverse floras offer a wide spectrum of unique medicinal plants. In Nigeria, the indigenous people are exploiting a variety of herbs for effective curing of various ailments (Mann & Ogbadoyi, 2012).

Tephrosia linearis (Willd.) Pers. (family *Leguminosae Papilionoideae*) is a more or less erect annual or short-lived perennial herb up to 130 cm tall; stems densely pubescent, the hairs appressed or, less often, spreading, white or yellowish. Leaf-rhachis, including a petiole of 2–7 mm, up to 5 cm long, prolonged 1–2 mm beyond the lateral leaflets; stipules triangular-acuminate, 2–4 mm long. Some local Nigerian names of this plant include: *Magimfa*, *jimfa* or *shibi* in Hausa, *lekki liddi* in fulfulde, *Koha* or *kuhwa* in Tiv, *orobeja* in Yoruba, *iwele* in Igbo, *oha* in Idoma etc. (Garba *et al.*, 2019). Many parts of the plants from this genus have been used traditionally for the treatment of diseases like rheumatic pains, syphilis, dropsy, stomach ache, diarrhoea, asthma, respiratory disorders, as well as being used as abortifacient, laxative diuretic, anti-inflammatory agent, etc (Dzenda *et al.*, 2007; Qureshi *et al.*,

2010). It is also used as tonic, laxative, antivenom, antiulcer, antidiarrheal, and in leprosy (Virupanagouda *et al.*, 2011).

In Nigeria, several ethnobotanical studies of Nigerian plants used in the traditional management of trypanosomiasis indicated significant *in vitro* and *in vivo* antitrypanosomal activity. It is against this background that *in vivo* antitrypanosomal activity evaluation of 70% methanol extract of *Tephrosia linearis*, a plant commonly available in North Central Nigeria, primarily to justify or otherwise the efficacy of this medicinal plant in the management of human trypanosomal infections was carried out.

Methodology

Experimental Site

The research was conducted at both the teaching/research farm and the Central Laboratory of the Federal College of Wildlife Management, New Bussa. The experimental station (New Bussa) sits at 9° 53'N, 9° 83'N and 4° 31'E, 4° 51'E (Garba *et al.*, 2020).

Plant material

The ethno-botanical survey was carried out in the surrounding villages namely, Old/New Awuru, Koro, Popo, Kere, Lubaruru and Dogongari villages around New-Bussa in Borgu Local Government Area of Niger State. The main aim was to ascertain from the local people (particularly the elderly ones), the plant species commonly utilised in the traditional management of trypanosomiasis. Part(s) utilised, method of preparation and period of harvest were also enquired from the interviewees. After the enquiries made, samples were collected from Kere village. The identity of the plant was confirmed by Mr Musa Idris in the Department of Forestry, Federal College of Wildlife Management, New Bussa, Nigeria. The plant was deposited at the Forestry Research Institute Herbarium with an assigned voucher number FIH/Garba/NBS/1647.

Preparation of the extract

The crude extract was prepared based on the method described by Garba *et al.* (2018). Briefly, fifty grams of the dried stem bark sample was

pulverised to powdered form and cold extracted in 400 ml of 70% v/v (methanol/water mixture). Extraction lasted for 48 h. The extract was filtered using muslin cloth and the solvent was removed and recovered using rotary evaporator. The extract was then transferred into a sterile universal bottle and stored at 4°C until required for use. The yield of the extract was 5.46 g/50 g or 10.92% of the whole sample extracted.

Phytochemical Analysis

The phytochemical analysis of the extract of *Tephrosia linearis* was carried out based on colouration and precipitation test as described by Trease and Evans (2002) and Sofowora (1982).

Acute Toxicity Studies

Acute toxicity studies of the extract were performed according to the Organisation of Economic Cooperation and Development guidelines (OECD, 2000) as described by Garba *et al.* (2019). Briefly, twenty (20) rats of average weight of 125-160 g were grouped into five (5) and simultaneously administered 400, 1000, 1600, 2200 and 2800 mg/kg bw of the *Tephrosia linearis* stem bark extract and then closely monitored for 24 hours.

Trypanosoma brucei brucei (*T. b. brucei*)

A stabilate of pleomorphic *T. evansi*, strain 8/18 was obtained from the Nigerian Institute for Trypanosomiasis Research, Vom, Jos, Nigeria.

Mice

Albino mice were purchased from the Department of Pharmacology, Ahmadu Bello University, Zaria, Nigeria. The experiment was conducted in compliance with the internationally accepted principle for animals care as contained in the Canadian Council on Animal Care guidelines on animal use protocol review (1997).

Infection of animals

Blood was collected by cardiac puncture with ethylenediamine tetraacetic acid (EDTA)-coated syringe from a heavily infected mouse and immediately diluted with physiological saline to serve as the inoculums. Healthy mice were infected intraperitoneally (*i.p.*) with 0.02 mL of the diluted blood containing 1×10^6 trypanosomes. Monitoring

of parasitaemia was done every 48 h by microscopic examination of blood sample taken from the tail of infected mouse pre-sterilised with methylated spirit.

Antitrypanosomal activity of crude extract

Four groups (A, B, C, D) each containing three mice were administered extracts at doses of 100, 200, 300 and 400 mg/kg body weight/day (*i.p.*). Three uninfected mice to which 400 mg/kg body weight per day was administered were in the fifth group (E) and served to determine toxicity at the highest dose of the extract. Another group (F) of three mice was infected but not treated with the extract serving as the negative control. For reference, a group (G) of three mice was infected and treated with the standard drug (445 mg diminazine diacetate + 555 mg phenazone/g, (Eagle Chemical Company LTD, Kaduna, Nigeria) a commercial trypanocidal drug.

Haematocrit determination

A small volume of blood was collected from the tail (presterilised with methylated spirit) of the experimental animals into a heparinised capillary tube, one end of which was sealed with plasticine and then spun for 5 min in a Micro-haematocrit centrifuge (Hawksley & Sons Ltd, UK). The packed cell volume (PCV) was determined with the aid of Hawksley Micro haematocrit reader which gave reading in percentage.

Blood and cerebrospinal fluid (CSF) infectivity test

One of the two mice that survived after the treatment with the crude extract was sacrificed six weeks post treatment and 0.02 mL of blood was drawn from the heart and sub-inoculated into two clean parasite-free mice and parasitaemia was monitored daily over a six weeks period. Inoculation of mice with CSF obtained from the second surviving mouse was done as per reported method of Garba *et al.* (2015).

Prophylaxis test

The test for prophylactic activity was done as described by Li *et al.* (2014). Three mice were each treated with the highest dose of the extract (400 mg/kg body weight) for five consecutive days before being infected with 1×10^6 trypanosomes cells. They were then routinely monitored for establishment of parasites.

Results

Phytochemical components of the extract

Phytochemical screening of the extract revealed the presence of polyphenols such as the flavonoids and tannins (Table 1).

Acute toxicity studies

The LD₅₀ determined after the administration 70% methanol extract to experimental rats, in an acute toxicity study was found to be 2800 mg/kg bw (Table 2).

Table 1: Phytochemical constituents of methanol stem bark extract of *Tephrosia linearis*

Phyto chemicals	Inference
Alkaloids	+
Anthraquinones	-
Flavonoids	+
Glycosides	+
Saponins	+
Terpenoids	+
Tannins	+
Phytosterols	+

+ = Present, - = Absent

Table 2: Effects of administration of various doses of the crude extract of *T. linearis* to healthy rats

Dosage	No of Animals	T/D	Observations
Normal Saline	4	4/0	No sign of toxicity, animals remained active even after the administration.
400 mg kg ⁻¹ bw	4	4/0	No sign of toxicity, animals remained active even after the administration.
1000 mg kg ⁻¹ bw	4	4/0	Looked a bit depressed, the breathing was slow and remained sluggish for a short while; became normal again.
1600 mg kg ⁻¹ bw	4	4/0	Sluggishness was observed, the breathing was slow and there was closing of the eyes and the furs stood erect but conditions returned to normal after about 24 h.
2200 mg kg ⁻¹ bw	4	4/1	One death was recorded about 13 h after the administration of the fraction and it took 27 h before the animals recovered fully from the sluggishness, depressed breathing, and erected fur.
2800 mg kg ⁻¹ bw	4	4/2	Two deaths were recorded about 17 h after administration of the extract and it took 48 h before the animals recovered fully from the sluggishness, depressed breathing, erect fur and closing of the eyes.

T/D = Total number of animals /Number of deaths recorded

Trypanocidal activity of 70% methanol extract

Considering the four different dose regimens administered to the experimental animals, none effectively cleared parasites from circulation. However, doses of 300 and 400 mg/kg bw *i.p.* per day (Figure 1) were found to significantly ($P < 0.05$) reduce the parasite loads and sustained the animals in the respective groups up to 3 and 4 weeks beyond the experimental period. Administration of these doses (300 and 400 mg/kg bw) of the extract to mice infected with *T. evansi* reduced the parasitaemia and sustained the tempo within nine days of continued administration. Three mice infected but not treated died before the eighth day post infection. One of the mice in the 300 and two in the 400 mg/kg body weight groups survived up to twenty-one and twenty-eight days post treatment. The mice administered 100 and 200 mg/kg body weight also died (due to high parasitaemia). Also, the group treated with the maximum dose (400 mg/kg body weight) but not infected did not die.

Percentage PCV

The result obtained for percentage PCV revealed a significant drop ($P < 0.05$) during the first six days of the treatment but this was reversed in the subsequent days, except in the negative control group (Figure 2).

Blood and CSF infectivity test

The blood and the CSF drawn from the surviving and low parasitaemic mice and inoculated into the healthy mice did actually induce/cause the development of infection six weeks after the subinoculation.

Prophylactic activity of extract

The animals administered the highest effective (trypanostatic) dose of 400 mg/kg body weight for five consecutive days prior to infection were observed to develop infection 72 h post infection (Figure 3). This indicated the inability of the extract to protect mice against infection.

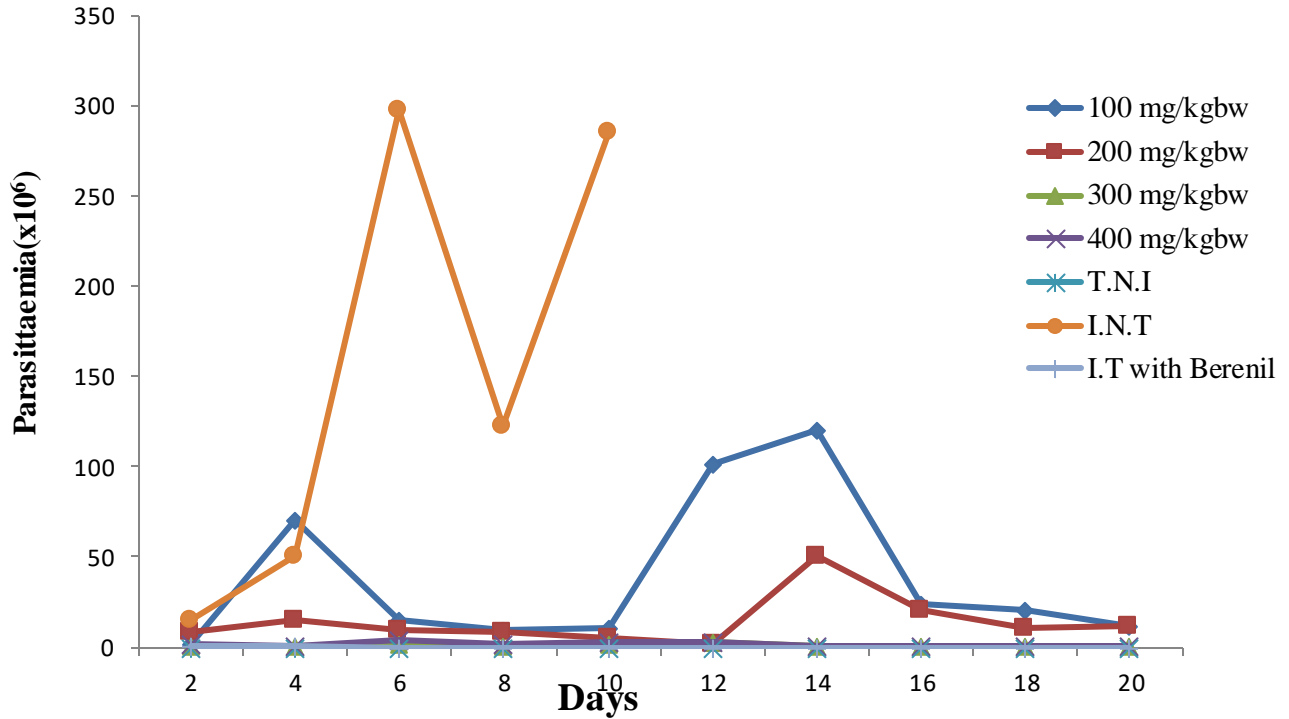


Figure 1: Trypanocidal effect of various doses of *Tephrosia linearis* extract.

I.N.T.: Infected not treated; T.N.I : Treated not infected; I.T.: Infected and treated.

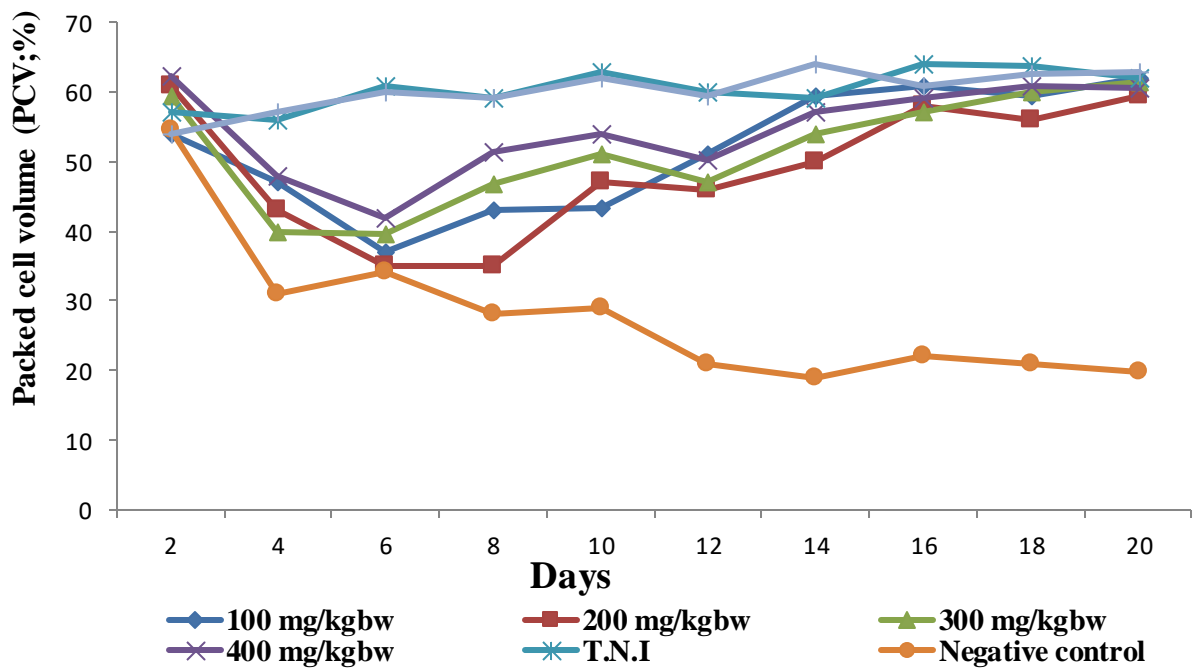


Figure 2: Mean PCV in group of mice treated with various doses of the *Tephrosia linearis* crude extract and standard drug (Berenil).

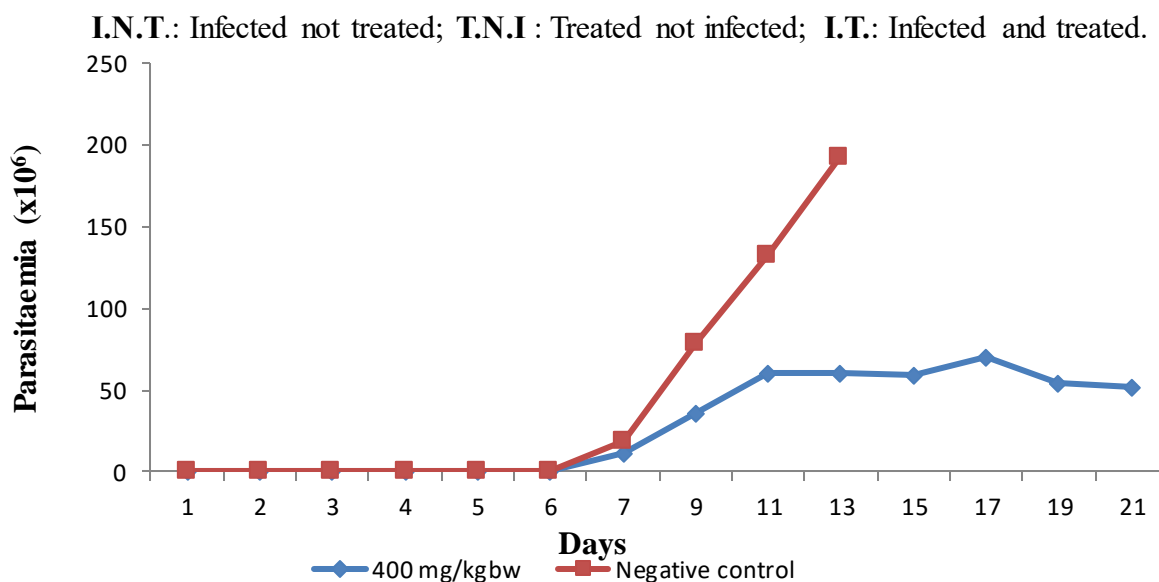


Figure 3: Prophylactic activity of the crude extract of *T. linearis*.

I.N.T.: Infected not treated.

Discussion

The genus *Trypanosoma* are kinetoplastid protozoa that devastate the health and economic well-being of millions of people in Africa through the disease human African trypanosomiasis (HAT). Notable among the Nigerian plants used in the traditional management of trypanosomiasis on which ethnobotanical studies were carried out include the extracts of *Afrormosia laxiflora*, *Anogeissus leiocarpus*, *Annona senegalensis*, *Cochlospermum planchonii*, *Khaya senegalensis*, *Piliostigma reticulatum*, *Prosopis africana*, *Securidaca longepedunculata* and *Terminalia avicennioides*, which were all reported to have distinctively exhibited significant trypanocidal activity (Mann & Ogbadoyi, 2012). The results from this study will surely add to the list, as it has not been reported to be ethno-pharmacologically studied, with regards to its potency as an anti trypanosomal agent.

Results obtained from this study revealed that the methanol extract of *Tephrosia linearis* has trypanostatic effect within the limit of the higher doses administered. Though, none of the dosage regimens cleared the parasites completely from circulation, it suffices to say that doses of 300 and 400 mg/kg bw *i.p.* per day (Figure 1) were found to significantly reduce the parasite loads and sustained

the animals in the respective groups up to 3 and 4 weeks beyond the experimental period. Administration of these doses (300 and 400 mg/kg bw) of the extract to mice infected with *T. evansi* reduced the parasitaemia and sustained the tempo within the first nine days of continued administration. Three mice infected but not treated died before the eighth day post infection. One of the mice in each of the 300 and two in the 400 mg/kg body weight groups survived up to twenty-one and twenty-eight days post treatment, but in this case with higher parasitaemia. The mice administered 100 and 200 mg/kg body weight also died due to high parasitaemia). Also, the group treated with the maximum dose (400 mg/kg body weight) but not infected also did not die indicating the non-toxicity of the extract at this concentration.

There have been no previous reports on the trypanocidal activity of the whole plant extract of *T. linearis*. But traditionally, many plants from this genus have been used for the treatment of diseases like rheumatic pains, syphilis, dropsy, stomach ache, diarrhoea, asthma, abortifacient, respiratory disorders, laxative, diuretic, and inflammation etc. (Dzenda *et al.*, 2007; Qureshi *et al.*, 2010). It is also reported to be used as tonic, laxative, antivenom, antiulcer, antidiarrhoeal, and in leprosy (Virupanagouda *et al.*, 2011).

Of interest in the result obtained in this study is the ability of the extract to suppress and maintain a very low level of parasitaemia throughout the course of treatment and thereafter, a gradual increase in the level of the parasites in the blood up till the third and the fourth weeks that the animals died due to high parasitaemia. What this goes to indicate is the fact that the active ingredient in the extract probably has a very high affinity for the plasma protein/albumin which, in this study, prolonged its plasma availability through conferring on it, a long clearance time/period. This property is of great significance in the sense that, this extract can be packaged as phytomedicine, particularly in the rural areas of the sub-Saharan Africa. These packaged drugs can be administered at recommended doses to suppress the parasites proliferation, thereby avoiding the unfortunate incidence of its crossing the blood-brain barrier at chronic stage, and so, prolonging the terminal stage of the disease until a more efficacious chemotherapeutic agent is available.

This study has provided evidence that *T. lineari* stem bark extract exhibits trypanostatic effect which is often associated with reduction in anaemia and promote weight gain in experimental African trypanosomiasis (Mamo & Holmes, 1975). Anaemia is the most outstanding clinical and laboratory feature of African trypanosomiasis (Bizimana *et al.*, 2006) and also the primary cause of death (Ogbadoyi *et al.*, 1999). Trypanostatic effect of the plant extracts were explained with corresponding increase in PCV which prolong the lifespan of treated animals by reducing the parasite load or neutralizing the toxic metabolites produced by trypanosomes (Abubakar *et al.*, 2005).

The mechanism by which this plant extract exerted its trypanostatic activity is unknown for now since the active ingredients were not isolated in this study. However, previous studies have indicated that a number of plants contain constituents that have been demonstrated to be clinically effective against many protozoan diseases (Mothana *et al.*, 2014). The existing trypanocidal drugs are known to exert their therapeutic actions through a variety of mechanisms depending on their chemical nature. Thus, while arsenic compound-based drugs poison

the parasites cell by their action on glucose catabolism through glutathione oxidation, suramin targets glycolytic enzymes in the glycosomes. Pentamidine and other diamidines disrupt the kinetoplast and may also interfere with polyamine synthesis. Yet others, for example eflornithine, are selective inhibitors of ornithine decarboxylase, thereby depleting the biosynthesis of polyamines such as spermidine, a precursor of trypanothione (Virginie *et al.*, 2003). That the active extract may be of peculiar polarity (considering the physicochemical property of the methanol solvent used for the extraction) is an indication that the bioactive constituents of the extract may belong to a variety of phytochemicals that will exert their trypanostatic action by one or more of the already identified mechanisms of action for trypanocidals. This is consistent with earlier reports which attributed the trypanocidal activity of certain plant extracts to the highly aromatic planar quaternary alkaloids, berberine, and harmine whose antiprotozoal action is through intercalation with DNA (Garba *et al.*, 2015).

The results of the present study confirmed that the use of medicinal plants in folk medicine contributes significantly to primary health care, and that natural products are potential sources of new drugs for the treatment of important tropical diseases caused by trypanosomes. The high activity values obtained for this plant render it a possible candidate for the isolation of anti-trypanosomal compounds which could develop into new lead structures for drug development. Therefore, the trypanocidal effects of this extract will require further isolation of trypanocidal compounds which requires State-of-the-Art instrumentation; particularly for further bioactive fractionation and characterization using chromatographic and spectroscopic techniques.

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