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Studies on Trypanocidal Efficacy of Aqueous Stem Bark Extract of *Terminalia avicennioides* (Guill and Perr) in Rats Infected with *Trypanosoma evansi*

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Abstract
The study was conducted to determine trypanocidal efficacy of aqueous stem bark extract of Terminalia avicennioides in rats infected with Trypanosomaevansi. The stem bark of the plant was obtained and dried under shade, grounded into powder using mortar and pestle, 100g of the dried powder was soaked in 1000ml of distilled water and left for 48hrs and sieved using muslin cloth and then filter paper to obtain the filtrate. Thirty adult albino rats were used, they were randomly divided into six experimental groups of five rats with each group made up of uninfected (negative control),infected not treated (positive control), infected and treated with 10mg of extract, infected and treated with 20mg of extract, infected and treated with 30mg of extract and infected and treated with standard drug diminazeneaceturate (3.5mg). The infected groups were inoculated intraperitoneally with 0.5ml of normal saline containing approximately $2x10^6$ Trypanosomaevansi. Treatment was carried out orally, once daily at the peak of infection (5 days-post infection) for a period of ten days. The 30mg of extract was found to be effective and had no significant difference at $P<0.05$with the standard drug. Therefore, stem bark of Terminalia avicennioides could be used for the treatment of African trypanosomiasis.

Keywords: Aqueous, Efficacy, Extract, Trypanocidal, Trypanosomiasis

INTRODUCTION
Trypanosomiasis is a parasitic disease caused by species of flagellate protozoa belonging to the genus Trypanosoma which inhabit the blood plasma and various body tissues and fluids, these parasites are found in many animals but seem to be pathogenic only for mammals, including man (Cadioli *et al*., 2006). Most Trypanosomes are transmitted by tsetse flies. Two tsetse-transmitted parasites, *T. bruceigambiense*and *T. bruceirhodesiense*, cause Human African Trypanosomiasis/sleeping sickness, which affects both Humans and Animals. The remaining tsetse-transmitted trypanosomes primarily affect animals and cause African Animal Trypanosomiasis. The most important Trypanosome species in Nigeria causing disease are *Trypanosomacongolense*, *T. vivax*, *T. bruceibrucei*, and *T. evansi*. Other species such as *T. simiae* and *T. godfreyi* can also cause African Animal Trypanosomiasis (Lukins, 2008)

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Animal trypanosomiasis occurs in most of the tropical regions and it is a complex debilitating, zoonotic protozoan disease of man and animal (WHO, 2008). Trypanosomiasis is a major factor limiting livestock production in large areas of humid and sub-humid Africa (Lukins, 2008). *Trypanosomaevansi*is responsible for a disease known as 'surra', and is the most widespread pathogenic Trypanosome globally (Cadioli *et al*., 2006), the symptoms of the disease include; fever directly associated with parasitemia, progressive anemia, weight loss, weakness, swellings of the lower parts of the body, plaques in the skin, hemorrhages and death if untreated. *Trypanosomaevansi* is a monomorphic haemoflagellate protozoan that has an elongated nucleus and a small circular terminal kinetoplast, which is sometimes missing in mutated wild strains that have followed drug treatment (Awulu *et al*., 2013). This flagellate is long and slender, it has length that varies between 15-34µm and a width between 1.5 to 2.2µm; like all parasitic trypanosomes, *Trypanosomaevansi* is covered by a dense protein layer consisting of a single protein called the variable surface glycoprotein (VSG). This acts as a major immunogen and elicits the formation of specific antibodies; the parasites are able to evade the consequences of these immune reactions by switching the VSG, a phenomenon known as antigenic variation (Holland, 2003).

Terminalia avicennioides is a tree plant widely distributed and commonly growing in the savannah region of West Africa (Burkill, 1985). The genus *Terminalia* belongs to the family Combretaceae consisting of about 514 species of which only 54 are accepted and recognized, 11 species are well represented in West Africa and have been used for various medicinal purposes (Azeez *et al*.,2015). *Terminalia avicennioides*is found in Nigeria with these Vernacular names: Nupe - Kpace, Gwari - Kpayi, Hausa -Baushe, Yoruba - Igiodan, Igbo – Edo and have been reported to possess antimicrobial activities (Mann, *et al*., 2008). The plant is known to be active against trypanosomes (Bulus *et al*.,2008).

MATERIALS AND METHODS

Collection of Plant Samples

The leaves and stem bark of *Terminalia avicennioides* which is popularly known as Baushe in Hausa dialect was collected from Dajin Daraye (Daraye forest) of Wammakko Local Government Area, Sokoto State, the plant's identity was confirmed at the Herbarium of the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, with a voucher number UDUH/ANS/0237.

Preparation of Aqueous Extract

The stem bark of *Terminalia avicennioides* collected, was dried under shade for two weeks, and then grounded into powder using mortar and pestle. One hundred gram (100g)of the dried powder of the stem bark was weighed and soaked in 1000ml of distilled water, and were left for 48hrs and sieved, first with muslin cloth and then with whattmann size 15cm filter paper. The filtrate was dried at 25^0C using dry cabinet to obtain the concentrate. One gram (1g) of the concentrate was then dissolved in 100ml distilled water, this corresponds to 1000mg in 100ml or 10mg in 1ml as described by Bala (2005).

Experimental Animals

Adult albino rats of both sexes were obtained from Animal house of the Department of Biological sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. The rats were kept in cages in Parasitology laboratory of the department, and were allowed to acclimatize for 7 days before the study. All rats were fed with commercial pellets (Pfizer Nigeria Plc., Ikeja, Nigeria) throughout the period of the study. Fresh *Trypanosomaevansi* was obtained from blood of

infected rat at Veterinary Parasitology Department, Usmanu Danfodiyo University Sokoto state.

Experimental Design

Thirty adult albino rats of both sexes were used. The rats were randomly divided into six experimental groups of five rats as described by Bulus *et al*.,(2001); Bala (2005).They included uninfected and untreated negative control, infected untreated positive control, infected and treated with aqueous extract of the stem bark l0mg/ml/day, infected and treated with aqueous extract of stem bark 20mg/ml/day, infected and treated with aqueous extract of stem bark 30mg/ml/day and infected and treated with standard drug (Diminazeneaceturate 3.5mg/day).

The Animals were subjected to the same physical conditions. A clean environment was maintained throughout the course of the experiment.

Animal Inoculation

At the end of the acclimatization period, the experimental animals were inoculated with *Trypanosomaevansi* parasites, with the aid of needle and syringe (Onyeyili *et al*., 1994) and Bala (2005). One (1ml) of infected blood was taken from the donor rat with fulminating parasitemia and was diluted with 9ml phosphate buffered saline (pH 8.0). The trypanosomes were counted and thereafter 0.5ml of the diluted blood containing approximately 2.0×10^6 Trypanosomes per ml of blood was inoculated into the rats in each group except negative control group. Inoculation was done intraperitoneally as described by Onyeyili *et al*.,(1994) and Bala (2005) and was preceded by cleaning the area to be inoculated with cotton wool soaked in 70% alcohol. The same process (cleaning) was repeated after the injection to prevent secondary infection by micro-organisms.

Administration of the Aqueous Extract

The aqueous extract of the stem bark of the plant at different dose i.e 10mg/ml/day, 20mg/ml/day, 30mg/ml/day and standard drug 3.5mg/day were administered orally to the respective groups of the rats on daily basis, using an oral gavage tube for ten days, as described by Dozie (2015). The amount of extract administered was measured using the same oral gavage tube.

Determination of Parasitemia in Infected Rats

Parasitemia was monitored in blood obtained from the tail, pre sterilized with methylated spirit and was estimated in accordance with the rapid matching method of Herbert and Lumsden (1976). Briefly, the method employs a matching technique in which microscopic fields were compared with a range of standard logarithmic values. To count the number of parasites in blood, a drop of blood was obtained on slide from animal's tail and covered with a cover slip, the wet mount on the slide was observed under x 400 magnification (Boyt, 1984). The number of trypanosomes per microscopic field was then compared with those of the standard logarithmic Table provided by Herbert and Lumsden (1976). The logarithm values which matched the microscopic observation were then converted to antilogarithm, from where the absolute number of trypanosomes per ml of blood was obtained.

Data analyses

The data obtained from the study (parasitemia) were summarized as means, ± standard error of mean and the differences between the means were determined at $P<0.05$ level of significance using one-way analysis of variance (ANOVA) followed by Duncan Multiple Range Comparison Test Using SPSS Version 20**.**

RESULTS AND DISCUSSION

Table 1: Effects of aqueous stem bark extract of *Terminalia avicennioides* on Trypanosome Infected and Treated Rats

	Parasite Load ($\times 10^6$)		
Group	Before Infection	Peak of Infection	After Treatment
Negative Control	0.00±0.00	0.00±0.00^c	0.00±0.00^d
Positive Control	0.00±0.00	19.00±4.58^a	101.40±0.68^a
10mg of Extract	0.00±0.00	14.60±2.48^a	3.40±0.51^b
20mg of Extract	0.00±0.00	9.40±1.54^b	2.00±0.45^c
30mg of Extract	0.00±0.00	7.80±0.58^b	0.40±0.24^d
D/aceturate 3.5mg	0.00±0.00	7.20±0.73^b	0.00±0.00^d

Note: Values in the column with different superscript Letters are significantly different ($P<0.05$).

The results of parasite load before infection shows that there is no significant difference in the level of parasitemia in rats of all the groups because they are not infected with trypanosomes (Table 1). In the same Table, at the peak of infection (5 days-post infection), the results revealed that there is significant difference among the groups indicating that the infection rate between the groups varies. However, after treatment there is no significant difference among negative control (0.00), 30mg of extract (0.40±0.24) and the standard drug (0.00±0.00) this shows that 30mg of extract and standard drugs are positively effective on trypanosome than the extracts of 10mg (3.40±0.51) and 20mg (2.00±0.45) respectively.

The results suggested that all the infected rats developed peak parasitemia within five days and that once infectivity is established, there was continued increase in parasitemia within the untreated group. This finding agrees with the work of Akinwale *et al* (1999), Okochi *et al* (2003) and Bala (2005). For the untreated groups, the level of parasitemia continued to rise until all the rats died. However, for the treated groups, the level of parasitemia decreased significantly resulting in total cure for Diminazeneaceturate (standard drug) treated group, although the *Terminalia* treated group was not completely cured at 10mg/ml and 20mg/ml but 30mg/ml of extract has no significant difference with standard drug.

Crude extracts of *Terminalia avicennioides* tested was able to significantly reduce trypanosomes (parasitemia) in infected rats. The positive control group shows a progressive increase in the level of parasitemia while the group treated with the standard drug showed a complete eradication of the parasite in the host, this finding agreed with the comparison of the activities of the tested extracts of *Terminalia avicennioides* with commercial diminazeneaceturate of Atawodi (2005).

While it is possible that parasites could still be found in other organ(s) even after their disappearance from the blood (Anosa, 1991), it seems that their presence and rapid proliferation in the blood are very important in the disease process. Any chemical substance that is able to delay the appearance of the parasites in the blood will concomitantly delay the manifestation of some pathological lesions (Atawodi, 2005). Sustained suppression of this parasitemia would have, no doubt, alleviated the anemia observed with infected rats since parasitemia is one of the several factors implicated in the mechanism of anemia in human and animal Trypanosomiasis (Atawodi *et al*.,2011).This study has also provided evidence that *T. avicennioides* stem bark extracts exhibits trypanocidal effect 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 results also revealed the activity of the aqueous extract of *Terminalia avicennioides* was at least five fold weaker, since the curative dose of

standard drug (diminazeneaceturate) for drug sensitive trypanosome populations in rat is estimated to be below 10mg/kg, this also agreed with the findings of Eisler *et al* (2001), and is consistent with several report made on other medicinal plant extract (Atawodi, 2005; Maikai and Kobo, 2008 and Ene *et al.*,2009). These differences between the activity of the extracts and diminazeneaceturate were not surprising, as the crude extracts consist probably of a mixture of many substances, the substance(s) with possible trypanocidal activity being present only in a small concentration. The isolation of the active ingredients should help to prepare extracts/drugs with higher concentrations of active ingredients.

CONCLUSION

The 30mg of the aqueous extract is the most effective. Therefore, current study established that stem bark of *Terminaliaavicennioides* could have potential trypanocidal activity which can be considered as potential source for new drugs in chemotheraphy of African trypanosomiasis. However, the aqueous extracts obtained from the stem bark of *Terminaliaavicennioides* have a weak activity in comparison with conventional trypanocides, as has observed in this study.

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