

ORIGINAL ARTICLE

Anti-trypanosomal activity of crude root extract of *Leptadenia hastata* (Pers) decne in Wistar rats infected with *Trypanosoma brucei brucei* and associated hematological changes

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ABSTRACT

Objective: An *in vivo* study was carried out to evaluate the possible anti-trypanosomal activity of *Leptadenia hastata* crude root extract with also its associated hematological changes particularly the packed cell volume (PCV) in experimental *Trypanosoma brucei brucei* infection using Wistar rats.

Materials and Methods: Thirty Wistar rats comprising of both males and females were categorized into six separate groups starting from A to F. Wistar placed in Group A and Group B were inoculated with *T. brucei brucei* and administered crude root extract of *L. hastata* at 100 and 200 mg/kg, respectively, as the treatment. Group C was infected with the parasite but untreated, while Group D was not infected with the parasite and was not treated. The remaining Groups E and F were inoculated with the parasite using diminazene diaceturate at 3.5 and 7.0 mg/kg, respectively. The extract was administered enterally when parasitemia was detected. Standard laboratory techniques were employed to determine parasitemia and PCV after collection of blood samples every 2 days via the tail vein.

Results: Infected Groups (A, B, C, E, and F) showed a pre-patent period 2 days post infection (P.I) with mean parasitic counts of 3.93 ± 2.38 , 2.46 ± 2.20 , 0.67 ± 0.77 , 4.60 ± 4.45 , and 1.53 ± 1.44 , respectively, which continued unabated in groups treated with the extract. The pack cell volume did not decline significantly in the in Groups A and B. Acute toxicity study revealed the absence of any clinical or behavioral changes suggesting toxicity.

Conclusion: There was no effect on parasitemia of Wistar rats infected with the parasite after administration of 100 and 200 mg, respectively, using the extract as the treatment. PCV of the groups infected remained fairly constant with the control groups throughout the study with the extract being non-toxic.

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Introduction

Trypanosomosis is a disease of vertebrate animals caused by parasitic protozoan of the Genus *Trypanosoma*. Infection with this parasitic protozoan causes intermittent fever, weight loss, and anemia. The infection is transmitted cyclically by biting insect, especially *Glossina* species (tse-tse fly). The major species are *Trypanosoma brucei*

brucei, *T. vivax*, *T. evansi*, and *T. congolense* which cause African Animals Trypanosomosis, while human African Trypanosomosis is mainly caused by the remaining sub species of the brucei group, namely, *T. brucei gambiense* and *T. brucei rhodenseinse* which causes West African and East African Sleeping Sickness [1,2]. It is estimated in Africa that large hectares of land approximately 7 million square

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kilometers of land would have been utilized for grazing if the scourge of trypanosomosis was effectively controlled. It is important to note that only about 30 million cattle are raised in zones where tse-tse was free from a possible population of 140 million [3]. Trypanosomosis has led to the death of about 2 million cattle yearly which has led to decrease in calving by 20% and milk production by over 20%, which has adversely affected farmers [4].

Leptadenia hastata is a valuable non-domesticated edible vegetable collected in the wild; typically grown in the tropical areas throughout Africa. The plant belongs to the phylum *Tracheophyta* and class *Magnoliopsida* [5,6]. Its ecological features, together with its particular geographic position, seem to have promoted its diversity, being found in the Northern area, it is considered Arab in origin. However, the fact that its potential as a vegetable is known to be of specific geographic communities, such as region of Mopti and West Africa [4]. *L. hastata* is called with different names according to the region. Some of the vernacular names are: Hagalhadjar (Arabic; Chad), Hayla (Kusume; Ethiopia), and Nzongne (Bambara; Mali). In Nigeria, it popularly referred to as Yadiya by Hausa community which are predominantly found in Northern Nigeria and also some parts of Niger [6–8].

The major challenge in the treatment of trypanosomosis using standard drugs designed for the treatment of the infection are the reports of resistance to the drugs and relapse during the course of treatment, with series of drug trials it is likely that a better alternative, which would be effective, affordable, and no toxicity, will be discovered [9,10]. The poor economy and poverty level of farmers in many developing countries where animal rearing is a major source of occupation also forms the basis for the search of a cheaper alternative for the treatment of the disease. The search for alternative drugs for the treatment of the infection has been directed to ethnopharmacology. Medicinal plants, which are known to possess some medicinal properties used traditionally with some active principles, are explored for possible success by scientist conducting various research [11]. *L. hastata* is used in the traditional medicine and plays an essential role in treating hypertension, catarrh and skin disease in Nigeria [12], trypanosomosis, sex impotence, wound healing, and against milk drying [13,14]. The plant is effective against bacteria [15] and also in the treatment of diabetes [16], anti-fungal activity [15], and anti-inflammatory effect [17]. In Mali, *L. hastata* is used effectively in the treatment of onchocercosis [18]. A key factor in the use *L. hastata* is its safety and is considered non-toxic [14]. Despite the use of the plant traditionally in various infections, this study is geared toward evaluating the potential anti-trypanosomal activity, toxicity, and possible hematological properties through in experimental

infection through *in vivo* approach, in particular the root of the plant since there is paucity of information.

Materials and Methods

Ethical approval

The approval for the research was from the committee of Animal welfare and Central Research from Faculty of Veterinary Medicine in the University of Maiduguri, Nigeria. All the animals were handled according to international guidelines for conducting biomedical research and also considering their welfare [19,20].

Identification of the plant and sample collection

An expert Prof. S. S. Sanusi in the field of plant taxonomy from the Department of Biological Science identified the plant with subsequent deposition of the Herbarium specimen in the laboratory. Fresh root of *L. hastata* was obtained from Dalori, a village in Konduga Local Government close to the state capital Maiduguri. After the collection, the root samples were made clean and kept under the shade to dry and later pulverized into powdery foam called the “plant material.”

Plant material extraction

The powdery form of the extract weighed 11.2 kg and a Soxhlet apparatus was used to extract the from the plant material using 85% ethanol in distilled water [21]. A rotary evaporator was used to concentrate the extract at reduced pressure.

Phytochemical screening

The secondary plant metabolites, such as carbohydrates, flavonoids, alkaloids, saponin, tannins glycosides, and terpenoids were determined as described by Harborne [22].

Trypanosomes

T. brucei brucei strain was inoculated in donor rats and was transported to the laboratory where the laboratory animals were stabilized prior to presaging before inoculation in experimental animals. These strains were obtained from Nigerian Institute for Trypanosomosis and Onchocerciasis Research Jos, Nigeria. Parasitic techniques to monitor parasitemia were determined by methods as described by Herbert and Lumsden [23].

Laboratory animals

Thirty adult Wistar rats comprising of males and females were purchased from a reliable laboratory animal breeder in Maiduguri. The Wistar rats were kept in locally constructed cages which were covered with wire mesh for the

purpose of ventilation in the Research Animal Laboratory. Prior to commencement of the research, the laboratory animals were allowed to acclimatize for a period of 2 weeks before the onset of experimental trials, with the provision of adequate feeding with growers mash obtained from vital feed and also water *ad libitum*.

Experimental drug

Diminazene diacetate (Iverzene®) which contains diminazeneaceturate and Phenazone granules was procured commercially from the market and was reconstituted according to the manufacturer's instruction.

Acute toxicity test

Acute toxicity was calculated as described by Aliu and Nwude [24].

Inoculation of infection

After successful repeated serial passage of the *T. brucei brucei* in albino rats in the Veterinary Parasitology Laboratory, parasitemia was established and became patent in the inoculated Wistar rats. After obtaining blood samples from the tail vein, these blood samples from donor rats were kept in a petri-dish where it was diluted with glucose saline. Rats in infected groups were inoculated with about 0.5 ml of blood containing 1.0×10^6 *T. brucei brucei* through intra-peritoneal route, quantified using serial dilution as reported [23].

Experimental design

Thirty adult Wistar rats were categorized into six groups randomly starting from Group A to Group F with the group having a total five rats in cages, respectively. Intra-peritoneal inoculation of infected groups comprising of A, B, C, D, and E was done with the dilution of infected blood from donor rats with glucose saline at a pH of 7.2. Groups A and B were administered 100 and 200 mg of the extract, respectively, as the treatment after inoculation of infection. Rats in Group C were inoculated with the infection but not treated, while those in Group D were not infected and no treatment was administered. Groups E and F were infected and treated with 3.5 and 7.0 mg/kg of diminazene diacetate (Iverzene), respectively.

Detection of parasitemia

Following inoculation of the respective groups with strains of the trypanosome, all the infected groups became parasitemic 2 days after successful inoculation of infection via the intra-peritoneal route. Blood from the tail vein of Wistar rats were analyzed by wet films with the estimation of the degree of parasitemia by using the rapid matching technique [23] every day for the period of the experiment.

Rapid matching technique for the examination of parasitemia

The number of parasites in each field under the microscope was matched with the standard reference pictures according to Herbert and Lumsden [23]. The count in each field was matched to logarithmic a figure which was converted to numbers to reflect parasitic counts per milliliter.

Determination of packed cell volume

Determination of packed cell volume (PCV) was carried out as described by Coles [25]. Capillary tubes were filled with blood obtained from experimental Wistar rats via the tail vein. The tube ends were sealed using plasticine, while excess blood wiped off using cotton wool. The filled tubes were placed in a slot in a micro hematocrit centrifuge machine (Hawsley, England) with sealed ends outward. The samples were centrifuged at a revolution of 1,200 gm in 5 min. PCV value was read using micro hematocrit reader (Hawsley, England) and then expressed in percentage (%).

Data analysis

Data obtained from the study were presented as mean \pm standard deviation (SD) using Analysis of Variance and $p < 0.05$ was considered as significant [26].

Results

Phytochemical screening

Table 1 presents the phytochemical screening of *L. hastata* crude root extract which shows the presence of alkaloids, some carbohydrates (e.g., ketoses), cardenolides, cardiac glycosides, flavonoids, saponin, and terpenoids.

Parasitemia

The mean parasite count of Wistar rats experimentally infected with *T. brucei brucei* with their controls is presented in Figure 1. In Group A (infected/treated with crude root extract of *L. hastata* orally at 100 mg/kg) and Group B (infected/treated with crude extract root of *L. hastata* orally at 200 mg/kg), a uniform prepatent period of 2 days was observed, mean parasitic count of 3.93 ± 2.38 and 2.46 ± 2.20 was observed, respectively, day 2 (P.I). The parasitic counts continued to appreciate significantly ($p < 0.05$) without abating despite instituting therapy at day 2 (P.I). A parasitemic count of 259.67 ± 3.57 was seen by day 5 post infection. When all the Wistar rats died as a result of the infection in Group A. In Group B, the parasitic count continued to appreciate to a significant level ($p < 0.05$) until 4 days post infection, with the subsequent death of rats in the group.

In Group C of Wistar rats (infected/untreated control), a mean parasitic count of 0.67 ± 0.11 was observed after a prepatent period of 2 days. The parasitic count continued to appreciate at significant level ($p < 0.05$) until when the rats in the group died 4 days post infection.

In Group D (uninfected/untreated), no parasite was detected throughout the study period.

In Group E (infected/treated with diminazene diaceturate at 3.5 mg/kg) and Group F (infected/treated with diminazene diaceturate at 7.0 mg/kg), a mean parasitic count of 4.60 ± 4.45 and 1.53 ± 1.44 was observed after a pre-patent period of 2 days, this continued to appreciate at a significant level ($p < 0.05$) to day 3 post infection. Following treatment with diminazene diaceturate, parasitic count began to decline significantly ($p < 0.05$) until they could no longer be detected in peripheral circulation after 6 days post infection.

Packed cell volume changes

The mean value of PCV in Wistar rats infected with *T. brucei brucei* with their control is presented in Figure 2.

Group A is infected and treated with crude root of *L. hastata* orally at 100 mg/kg, Group B infected and treated

with crude extract of *L. hastata* orally at 200 mg/kg, and Group C is infected/untreated group). The pre-infected values of 48.80 ± 8.93 , 47.40 ± 6.84 , and 49.20 ± 5.67 were fairly within the same range ($p > 0.05$) through the entire period of the study period till day 5 for Group A and day 4 for Groups B and C (P.I) when all the infected rats died.

Wistar rats from Group D (uninfected/untreated). Pre-infection value of 53.60 ± 4.93 was constant ($p > 0.05$) during the period of the research throughout the study period.

In Group E (infected and treated with diminazene diaceturate at 3.5 mg/kg), the pre-infection values of 49.80 ± 0.44 remained fairly constant till day 7 post infection or 5 days post treatment, when a decline which was significant ($p < 0.05$) was seen, while in Group F (infected/treated with diminazene diaceturate at 7.0 mg/kg), the pre-infection value of 48.60 ± 6.22 was constant during the entire study.

Table 1. Phytochemical analysis of crude ethanolic root extract of *L. hastata*.

S/No.	Group constituents	Test	Results
1.	Alkaloids	Dragendorff's Mayer's	+ +
2.	Antraquinones Combined antraquinones	Borntreger's	-
3.	Carbohydrates General test Monosaccharide Free reducing sugar Combined reducing sugar Ketoses Pentoses	Molisch's Barfoed's Fehling's Fehling's Salivanoff's	+ - + + + -
4.	Cardenolides	Keller-Kiliani's	+
5.	Cardiac glycosides Salkowski's Lieberman-Buchard's	L-Buchard's L-Buchard's	+ +
6.	Flavonoids	Shinoda's Ferric chloride Lead acetate NaOH	+ + - +
7.	Phlobatannins		-
8.	Saponins	Frothing's	+
9.	Soluble starch		-
10.	Tannins	Ferric chloride Lead acetate	- -
11.	Terpenoids		+

Legends: + = Present, - = absent.

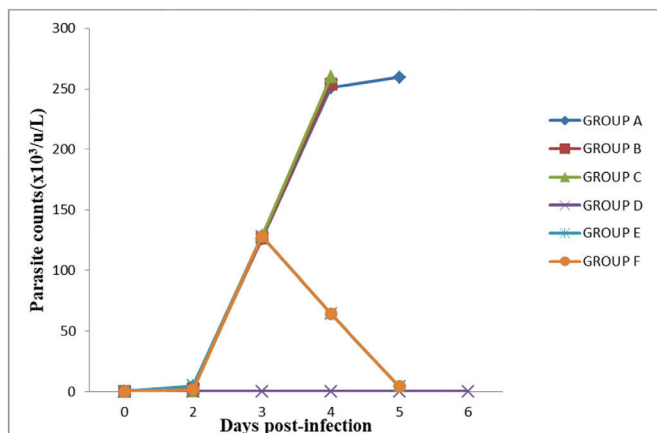


Figure 1. Anti-trypanosomal activity of crude ethanolic extract of *L. hastata*.

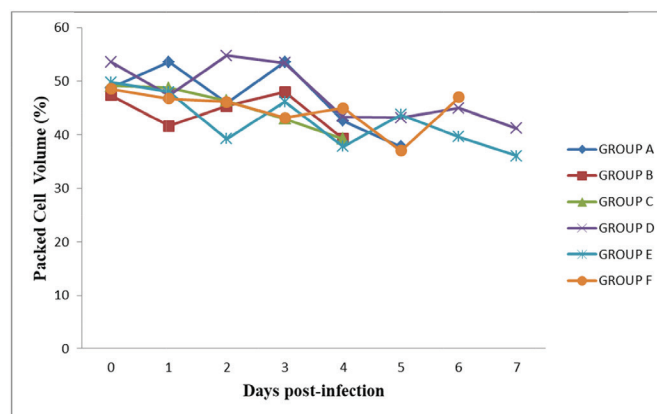


Figure 2. Effect of crude root ethanol extract of *L. hastata* on the packed cell volume of Wistar rats infected with *T. brucei brucei*.

Discussion

Phytochemical analysis of the Crude root extract of *L. hastata* showed some pharmacological active substances. These substances include alkaloids, saponins, cardenolides, cardiac glycosides, terpenoids, and flavonoids. However the contradicts the findings of Haruna et al. [4] and Bello et al. [16] were both of the researchers reported alkaloids, steroids, flavanoids, tannins, terpenoids, and anthraquinone in their study. This could probably be result of various method employed in screening of the plants, source of the plant (Location) and could also be dependent on the particular part of the plant employed in the research. The metabolites are known to play various pharmacological roles in the body. Saponins are known to bind cholesterol, block its uptake by the intestine, thus facilitating its excretion as well as the coagulation of red blood cells [27]. Saponins as indicated by previous studies have no toxicity report; especially in blood when administered orally as their bio-transformation is fast easily become hydrolyzed [28]. A lot of anti-trypanosomal medicinal plants in Nigeria flora have been proven to possess anti-trypanosomal potentials to combat the disease as in the case of *L. hastata* leaf has been reported to possess such potentials [29]. Alkaloids are known to possess some very active pharmacological effects, especially in mammals which has lead to its use in most drugs derived from natural environment particularly plants. Studies have shown that flavonoids have pharmacological properties that are effective against bacteria, fungi, and inflammation [30]. This shows its ethnomedical importance in the traditional medicine.

However in this study, the extract administered as the treatment in Wistar rats infected with *T. brucei brucei* did not show any significant effect on the parasitemia as it continue to significantly increase from day 2 post infection to reach the peak by day 4 and by day 5 when all the rats died in groups treated so also the group used as a negative control. This could probably be as a result of the presence or absence of some pharmacological active substances from the photochemical screening of the root and the leaf, as the leaf has been reported to have some anti-trypanosomal activity. In a study by Haruna et al. [4], various factions of different metabolites revealed that saponins are the most active against trypanosomes proliferation showing it is the most potent anti-typonosomal metabolite of *L. hastata* leaf. The quantity of saponin was very high in *L. hastata* leaf. This study did not quantify the various metabolic substances.

Blood vascular system is a target for the most compounds with toxic properties which reflects in both physiological and pathological pathways in mammals [31]. One of the key clinical manifestations of trypanosomosis is anemia [32]. Crude extract of *L. hastata*-treated groups

showed that the PCV remained fairly constant as with the diminazene diacetate-treated groups and the positive control; this could not for long as all the Wistar rats treated with the extract died by day 6 post infection. Similarly, a significant increase in PCV and hemoglobin count was reported by Abubakar et al. [33] in rats treated with ethanol extract of *L. hastata*. Since anemia is a cardinal sign of trypanosomosis, this may be an indication that the plant extract could boost blood production and could be used as a possible hematinic agent in the treatment of anemia.

Conclusion

The *in vivo* studies showed that the root extract of *L. hastata* had no effect on parasitemia of Wistar rats inoculated with the infection and administered 100 and 200 mg/kg of the extract as the treatment. However, the PCV remained fairly constant during the study, suggesting the possibility of extract being a hematinic agent with no clinical or behavioral changes as a result of toxicity. Further studies should be conducted using higher doses of the extract and with quantitative determination of phytochemical metabolites with multiple treatments to ascertain the effect of the extract and the possible hematinic activity using the same or different animal model.

Acknowledgments

The authors express their gratitude to Prof. S. S. Sanusi of the Department of Biological for his role in identifying the nomenclature of the plant and also all the laboratory staff who provided technical support and assistance during the course of the research.

Conflict of Interest

The authors declare that no conflict of interest exist in the study.

Authors' contribution

Samson Anjikwi Malgwi, Albert Wulari Mbaya, Mohammed Kyari Zango and Falmata Kyari were involved in the designing of the study and laboratory investigation. Kyari Abba Sanda played keyed role in the preparation and extraction of the extract. Gamgong Dennis was in-charge of the laboratory animals with their feeding and administration of extract. All the other authors were involved in reviewing the manuscript.

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