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Research Article

Effect of n-Butanol Root Extract of *Leptadenia hastata* on Experimental *Trypanosoma brucei brucei* Infection and Packed Cell Volume (PCV) Changes in Albino Rats

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Abstract

This study was conducted to determine the effect of n-butanol root extract of *Leptadenia hastata* and Packed cell volume (PCV) changes in albino rats. Thirty (30) albino rats comprising of both sexes were divided into six (6) groups (A - F) with each group containing five (5) rats. Group A and B were infected with *Trypanosoma brucei brucei* and treated with the extract at 100mg/kg and 200mg/kg respectively. Group C was infected but untreated control, while Group D was uninfected and untreated. Group E was infected with *Trypanosoma brucei brucei* and treated with diminazene aceturate at 3.5mg/kg. The proximate content and phytochemical properties of the extract were determined. After inoculation blood samples were collected daily to determine the parasitaemia and PCV according to standard laboratory technique. The proximate content analysis revealed the presence of dry matter, crude fiber, crude protein, moisture and ash, while alkaloids, carbohydrates, cardiac glycosides, saponin and terpenoids were the phytochemical constituents. Infected groups showed a prepatent period of 2 days with parasitaemia value increasing significantly (P>0.05) in groups (A and B) treated with extract in respective of the dose with the PCV fairly constant. The result of this study shows that the extract has no anti-trypanosomal activity but has haematinic potential.

Keywords: Effect, *Leptadenia hastata*, packed cell volume, parasitaemia, haematinic.

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INTRODUCTION

Leptadenia hastata (*L.hastata*) is an edible non- domesticated plant which is found in the wild throughout Africa especially in tropical dry lands, it is valuable herb with creeping latex, glabescent leaves with racemes flower as well as follicle fruit [1]. L. hastate is a wild food which helps in food security especially during seasonal changes and is used medicinally in many areas of Africa, This include its use as antimicrobial, antidiabetic, antiadrogenic and antioxidant activity [2]. Local names for plant vary according to location and the local dialect such as Yadiya (Hausa) in Northern Nigeria and Hagalhadar (Arabic) in Chad [3]. It commonly used by the Hausa speaking communities as spice in source [1]. Also in Nigeria, local healers use the plant in the treatment of scabies, hypertension and catarrh [4]. The plant is widely used in Senegal for prostrate and rheumatic complaints [5]. In Burkina Faso, it is locally used for sexual potency (Chewing the leaves), treatment of trypanosomosis (decoction of leaves) and wound healing (application of latex) [6]. *L hastata* leaves in combination with *Erythrina senegalensis* is used to treat onchocercosis in Mali. [7]. It is estimated that about 7million square kilometers of land would be suitable for livestock production if the menace of trypanosomsis was controlled effectively [8]. Trypanosomosis is a disease of veterbrates caused by protozoan parasite of the genus Trypanosoma which is transmitted via the bite of tse-tse fly (Glossina) which infects the susceptible host. The disease is of zoonotic importance.The major species of Trypanosomes in domestic animals include *T.vivax, T.congolense* and *T. brucei*. Not all trypanosomes are potential disease producers, but some of those which affect animals in Africa are *T. vivax*, *T. congolense* and *T. brucei* [9, 10]. In man; *T. b. rhodesiense*, causes acute sleeping sickness in east Africa and *T. b. gambiense*, chronic sleeping sickness in West Africa [11-13]. However, it was observed that these trypanosomes were also harbored by antelopes, cattle and pigs which act as

reservoirs of infection [14, 15]. Although the incidence of sleeping sickness has been greatly reduced, some 20,000 cases still occur across the continent and around 70% in Nigeria annually [16].

Drug resistance and varying reports of relapse have been reported with the use of various trypanocides in recent years, which create the need to search for alternatives trypanocides. This search is towards exploring the potential of traditional pharmacopoeia and medicinal plants. This study was designed to investigate the in-vivo antitryposonal potential of *leptadenia hastata* and its effect on haematological parameter (PCV).

MATERIALS AND METHOD

Collection and Processing of Experimental Materials

Fresh samples of the roots of *L. hastata* were collected from Dalori village of Konduga local government in Borno state, Nigeria. The plant was identified and authenticated by a plant taxonomist in the University of Maiduguri were a voucher specimen number was given. The root of the plant was cleaned and air dried under the shade for several days and finally pulverized into powder.

Preliminary Proximate and Phytochemical Screening

The active components were isolated at the Department of Pharmacological Chemistry and Pharmacognosy, university of Maiduguri according to standard method. [17], While the proximate analysis was also processed according to standard methods [18].

Extraction of Plant Material

The air dried ground powdered plant material (11.2kg) was extracted exhaustively with 85% ethanol in distilled water using Soxhlet apparatus [19]. The combined ethanolic extract was concentrated to dryness at a reduced pressure using rotary evaporator. This was later suspended in distilled water and then partitioned with n-Butanol.

Source of Trypanosomes

Trypanosoma brucei brucei, Federe strain used for this study was obtained from the Nigeria Institute for Trypanosomosis and Onchocerciasis Research (NITOR) in Jos, Nigeria. It was identified as *T. brucei brucei* based on morphology and negative Blood Inhibition Infectivity Test (BIIT) and stabilized by four passages in rats before storage in liquid nitrogen. The stabilates were passaged twice in donor rats. Tail blood from the donor rats was diluted with phosphate buffered saline glucose (PBSG) pH 7.2. The rabbits were infected intra-peritoneally with blood from the donor rats containing 1.5 $x10^6$ trypanosomes. The initial detection of parasitaemia was by the wet mount and haematocrit buffy-coat methods [20] while the degree of parasitaemia was estimated by the rapid matching method [21]

Experimental Animals

Twenty five (25) healthy albino rats of both sexes were purchased from the laboratory Department of Veterinary Anatomy, University of Maiduguri. These rats were maintained in plastic rats cages with feed and water provided ad libitum. The rats were allowed to acclamatise for two weeks prior to the commencement of the research. All experiments were carried out in accordance with international guidelines for the use of animals for biomedical research and welfare [20, 22]

Experimental Inoculation

After successful repeated serial passage of the *Trypanosoma brucei brucei* in albino rats, parasitaemia was established and became patent in inoculated rats. The donor rats were bleed via the tail vain in to the petridish, the blood was diluted with phosphate buffered glucose saline (pH 7.4). Each rat in the infected groups was inoculated with 0.5ml of blood containing10^6 via the intra-peritoneal route.

In-vivo Experimental Design

Twenty five adult albino rats of both sexes were randomly separated into five groups (A-F) of five rats each, in clean plastic cages. Group A and B were infected with Trypanosoma brucei brucei and treated with 100mgkg and 200mg/kg respectively. Group C was infected but untreated control, while Group D was uninfected and untreated. Group E was infected with *Trypanosoma brucei brucei* and treated with diminazene aceturate at 3.5mg/kg.

Monitoring of Parasitaemia and Packed Cell Volume.

The experimental rats were monitored daily. The detection of parasitaemia was done initially by wet mount and haematocrit buffy coat examination using tail blood [23]. While the degree of parasitaemia was estimated by the rapid matching technique [21] Packed cell volume (PCV) was determined by microhaematocrit method daily [24].

Statistical Analysis

Data generated from the study were expressed as mean ± standard deviations (S.D) using two-way analysis of variance (ANOVA) and $p < 0.05$ was considered significant [25].

RESULTS

The proximate content of the root extract of *Leptadenia hastata* is presented in Table-1. The proximate content analysis revealed the presence of dry matter (95.5%), carbohydrate (55.20%), crude fiber (30%), crude protein (6.3%), moisture content (4.1%), ash (4.0%) and other extracts (1.5%) respectively.

Table-1: Proximate Content of the Crude Root Extract of *Leptadenia hastate*

Content	Percentage
Dry matter	95.5
Carbohydrates	55.0
Crude fiber	30
Crude protein	6.3
Moisture content	4.9
Ash	4.0
Other extracts	1.5

The phytochemical constituents of root extract of *Leptadenia hastata* is presented in Table-2.

The pytochemical analysis of the extract revealed the presence of alkaloids,anthraquinones, carbohydrates (free reducing sugars, combined reducing sugars, ketoses and cardenolides), cardiac glycosides, phlobatannins, saponins and terpenoids.

Table-2: Phytochemical constituents of n-Butanol portion of the Root extract of *Leptadenia hastata*

S/No.	Group constituents	Test	Result
1.	**Alkaloids**	Dragendorff's	+
		Mayer's	+
2.	**Antraquinones**		
	Combined antraquinones	Borntrager's	-
3.	**Carbohydrates**		
	General test	Molisch's	+
	Monosacharide	Barfoed's	-
	Free reducing sugar	Fehling's	+
	Combined reducing sugar	Fehling's	+
	Ketoses	Salivanoff's	+
	Pentoses		-
4.	**Cardenolides**	Keller-Kiliani's	+
5.	**Cardiac glycosides**		
	Salkowski's	L-Buchard's	+
	Lieberman-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.

The mean parasitic count of albino rats infected with Trypanosoma brucei brucei with their controls is presented in Table-3.

In group A (infected / treated with n- butanol root extract of *L.hastata* at 100mg/kg) a mean parasitic count of 1.53±0.64 was observed after a prepatent period of 2 days post infection. The parasite count continue to appreciate significantly ($p>0.05$) without abating despite instituting therapy at day 2 post infection to 253.00±1.00 by day 4 P.I. All the rats died of the infection by day 5. So also similar fate was observed in group B (infected/treated with n-butanol root extract at 200mg/kg), the mean parasitic count rose significantly to the peak value of 253.33±2.57 by day 4 post infection.

In group C (infected/untreated control), a mean parasitic count of 0.67±0.11 was observed by day 2 post infection which continued to appreciate significantly ($p>0.05$) to day 4 post infection with a value of 260.00±2.57. The rats died by day 5 post infection, while group D (uninfected/untreated control) no parasite was detected throughout the study. In group E (infected/treated with diminazene acetutate at 3.5mg/kg) a mean parasitic count of 4.60±4.45 was

recorded by day 2 P.I. the parsitaemia continued to appreciate significantly ($p<0.05$) till day 4 post infection but decline by day 5 after instituting therapy by day 2 P.I. It is important to note that all treatment was done by day 2 post infection.

Table-3: Effect of n-Butanol Extract of *Leptadenia hastata* on Mean Parasitic Count (x10/uL) of Albino Rats Experimentally Infected with *Trypanosoma brucei brucei* and their controls

Groups (n=5)	Days post-infection				
	0	2	3	4	5
Group A	0^a	1.53 ± 0.64^a	129.00 ± 1.53^b	253.00 ± 1.00^c	*****
Group B	0^a	1.73 ± 1.36^a	129.66 ± 1.53^b	253.33 ± 2.57^c	*****
Group C	0^a	0.67 ± 0.11^a	128.33 ± 1.52^b	260.00 ± 2.57^c	*****
Group D	0^a	0^a	0^a	0^a	0^a
Group E	0^a	4.60 ± 4.45^a	128.00 ± 2.00^b	64.33 ± 1.52^c	4.10 ± 0.10^a

Numbers with different superscripts in rows and columns differed significantly ($p<0.05$)

Keys:

Group A: Infected/treated with n-butanol extract of *L.hastata* at 100mg/kg by day 2 post infection.
Group B: Infected/treated with n-butanol extract of *L.hastata* at 200mg/kg by day 2 post infection.
Group C: Infected/untreated control
Group D: Uinfected/untreated control
Group E: Infected treated with dimiazene aceturate at 3.5mg/kg by day 2 post infection.
*****: Number of albino rats that died.

The mean PCV of albino rats infected with *Trypanosoma brucei brucei* with their control is presented in Table-4. In group A (infected/treated with n-butanol root extract of *L. hastata* at 100mg/kg). The pre-infection values of 50.60±5.98 slightly decline. However this decline was not significant and remained fairly constant ($P>0.05$) throughout the study, until day 5 P.I when all the rats died of the infection. In group B (Infected/treated with n-butanol root extract of *L.hastata* at 200mg/Kg). The pre-infection value of 52.60± 7.43 remained fairly constant until day 4 P.I, when a significant decline ($p>0.05$) was observed. All rats died of the infection by day 5 P.I.

In group C(Infected/untreated control), the pre-infection value of 49.20±5.67 declined slightly which was not statistically significant until day 4 P.I or day 2 post treatment, when a significant decline($p>0.05$) was observed, however the infected rats died on day 5 P.I. In group D (uninfected/untreated). The value remained fairly constant throughout the study while in group E (infected treated with diminazene aceturate at 3.5mg/kg), the pre-infection values of 49.80±0.44 remained fairly constant until day 7 P.I or day 5 P.T when a significant decline was observed.

Table-4: Effect of n-Butanol Extract of *Leptadenia hastata* on Mean Packed Cell Volume (%) Changes of Albino Rats Experimentally Infected with *Trypanosoma brucei brucei* and their Controls

Groups (n=5)	Days post-infection							
	0	1	2	3	4	5	6	7
Group A	50.60 ± 5.98^a	43.20 ± 8.55^a	50.00 ± 4.02^a	47.00 ± 5.52^a	41.40 ± 4.66^a	*****	-	-
Group B	52.60 ± 7.43^a	49.40 ± 7.53^a	43.80 ± 6.41^a	48.60 ± 6.22^a	41.40 ± 7.09^b	*****	-	-
Group C	49.20 ± 7.43^a	48.80 ± 8.93^a	46.40 ± 4.18^a	43.00 ± 4.18^a	39.00 ± 3.93^a	*****	-	-
Group D	53.60 ± 0.44^a	47.40 ± 6.84^a	54.80 ± 9.88^a	53.40 ± 4.56^a	43.40 ± 3.78^a	43.20 ± 3.89^a	45.00 ± 7.28^a	41.20 ± 1.87^a
Group E	48.60 ± 0.44^a	48.00 ± 9.46^a	39.20 ± 7.36^a	46.20 ± 8.25^a	37.80 ± 3.78^a	43.80 ± 5.68^a	39.60 ± 3.58^a	36.00 ± 1.87^b

Numbers with different superscripts in rows and columns differed significantly ($p<0.05$)

Keys:

Group A: Infected/treated with n-butanol extract of *L.hastata* at 100mg/kg by day 2 post infection.
Group B: Infected/treated with n-butanol extract of *L.hastata* at 200mg/kg by day 2 post infection.
Group C: Infected/untreated control
Group D: Uinfected/untreated control
Group E: Infected treated with dimiazene aceturate at 3.5mg/kg by day 2 post infection.
*****: Number of albino rats that died.

DISCUSSION

The proximate composition of *L.hastata* root extract in this study revealed the presence of dry matter, carbohydrates, crude protein, crude fiber, moisture content, ash and other extracts in varying amount as was found in leaves powder of *L.hastata* [26]. The phytochemical screening of the *L. hastata* root showed the presence of active compounds such as alkaloids, carbohydrate, cardiac glycosides, saponins, terpenoids. This was also reported by Pal *et al*., [27] and Abubakar *et al*., [26]. These constituents are known to be biologically active; they serve as therapy in various medical ailments [6]. Saponins are known to play critical roles to bind cholesterol, block its uptake by the intestine thus facilitating its excretion from the system faster, as well as the coagulation of erythrocytes [28]. Alkaloids generally exert pharmacological activity particularly in mammals and are most commoly used as drugs are alkaloids from natural sources. Flavonoids have anti fungal and antibacterial and anti inflammatory property activity [26]. This shows its ethnomedical importance in traditional medicine. *L. hastata* is being used locally for the treatment of trypanosomosis [6, 30]. However in this study the n butanol root extract of *L.hastata* did not show any positive result as a potent anti-trypanosomal agent. Despite instituting therapy the parasitaemia levels kept increasing unabated. The haemopoietic system is one of the most responsive targets for the toxic compounds and an important manifestation of physiological and pathological status in man and animals [31]. The n-butanol extract of the root of L. hastata treated groups showed that the PCV remain fairly constant as with the diminazene treated groups and the positive control. Similarly a significant increase in PCV and haemoglobin count was reported by Abubakar *et al*., [26] in rats treated with ethanol extract of *L.hastata*. since anaemia 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 haematinic agent in the treatment of anaemia.

CONCLUSION

From the findings of this study, the root extract of *L.hastata* did show any antrypanosomal activity against *Trypanosoma brucei brucei* but poses haematinic effect especially against the pack cell volume (PCV).

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Conflict of Interest

The authors declare that no conflict of interest exists.

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