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In vivo effect of the aqueous extract of *Adansonia digitata* (Linn) fruit pulp on *Trypanosoma brucei brucei* infection in Wistar rats

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Abstract

This study investigated the effects of aqueous extract of fruit pulp of *Adansonia digitata* on albino rats infected with *Trypanosoma brucei brucei*. Acute toxicity test was conducted on the extract and then analysed for some phytoconstituents. Thirty-five adult rats were divided into seven groups of five rats each. Group A were the non-infected control group while groups B, C, D, E, F and G were inoculated with 1x 10^6 trypanosomes per 100 g body weight (BW). At day 6 post infection (6 PI), groups C and D were treated with diminazene aceturate and vitamin C at dose rates of 3.5 mg/kg BW intra peritonealy once and 200 mg/kg BW orally for 3 days respectively, while groups E, F, G were orally treated for 3 days with 40, 80, and 160 mg/kg BW of the extract respectively. The rats were monitored for parasitaemia, PCV and body weight. The LD_{50} of the extract was greater than 9000 mg/kg. The phytochemical analysis revealed 3.51% flavonoid, 0.07% alkaloid, 0.10% saponin and 0.03.0% oxalate in the extract. While there was progressive increase in parasitaemia from day 6 PI in groups E and F throughout the study, parasitaemia decreases and was completely cleared by day 8 and 11 PI in groups C and G respectively. PCV of group A was not significantly different ($p>0.05$) from that of F and G. There was significant difference ($p<0.05$) in the PCV of Group A and that of groups B and C and highly significantly different ($p<0.01$) with that of D and E. Significant ($p<0.05$) body weight increase of rats in groups D, E, F and G at day 10 PI was observed. Thus, fruit pulp of *A. digitata* at a dose of 9000 mg/kg was not toxic to rats, and contains active compounds with potential *In vivo* anti-trypanosoma activity.

Keywords: *Adansonia digitata,* Albino rats, Animal trypanosomosis, Phytochemicals, *Trypanosoma brucei brucei*

Introduction

The disease African animal trypanosomosis, affects most domestic animals and is caused by blood dwelling protozoan parasites of the genus *Trypanosoma.* The disease is considered the most important livestock disease after Contagious Bovine Pleuro Pneumonia (CBPP) and remains a major obstacle to livestock production in Nigeria (PATTEC, 2001; Jeremy, 2014). African animal trypanosomosis

(AAT) is transmitted cyclically by tsetse flies (*Glossina* species) and mechanically by biting flies such as tabanids and Stomoxys, of these tsetse transmitted trypanosomes, *T. congolense, T. vivax and T. brucei* comprise the major disease agents that affect livestock (Abebe, 2005).

The most significant clinical symptoms of trypanosomosis are intermittent fever, anemia, enlargement of superficial lymph nodes, abortion, infertility, reduced milk yield, reduced weight gain and lowered work output and high mortality occurring in some animals during acute phase of the disease if left untreated (Inabo & Fathuddin 2011; Ohaeri & Eluwa 2011). Shaw (2009) predicted that eradication of trypanosomosis from Africa would increase the overall agricultural production to 4.5 million US dollar per year.

Over the years, field control of animal trypanosomosis relied mostly on two broad strategies: use of chemotherapeutic drugs and vector control (Steveding, 2008). Currently the chemotherapy of African trypanosomosis is unsatisfactory because of toxicity and cost especially in developing countries (Welburn *et al.,* 2001). Attention is now focused on the search for non-toxic and readily available natural products without parasite resistance for the treatment of trypanosomosis (Ibrahim *et al*., 2014).

Adansonia digitata also known as Baobab is a large iconic multi-purpose tree found in the savannas of Africa, locally known as `*Kuka*` in Hausa and `*Ose*` in Yoruba. Various parts of the plant (e.g. leaf, bark, fruit pulp), have traditionally been used as immune-stimulant, anti-inflamatory, analgesic, insect repellent and pesticide (Rahul *et al*., 2015). Their uses in the treatment of diarrhoea and dysentery in many African countries have been confirmed as a substitute for imported western drugs (El-Rawy *et al*., 1997). Previous work by Manfredini (2002) suggested antitrypanosomal potential of the root extract of *A. digitata*. Hence, the aim of the current work is to investigate the *in vivo* activity of the aqueous extract of fruit pulp of *A. digitata* against *T. brucei brucei* in albino rats.

Materials and Methods

Experimental animals

Thirty-five Laboratory bred male albino rats weighing between 100-150g (8-12 weeks old) were purchased from the Animal House unit of NITR, Vom, Plateau State. The rats were allotted into 7 groups; each group containing 5 rats and were kept in clean cages, in a well-ventilated room. The rats were fed with pelleted grower feed obtained from a commercial feed outlet (Vital Feeds Plc, Plateau State, Nigeria) and water was given *ad-libitum*. The rats were acclimatized for two weeks before commencing the experiment.

Ethical statement

The animals were maintained and used at the Nigerian Institute for Trypanosomiasis Research (NITR), Vom, following the guidelines of NITR Ethical Committee. The study was carried out in accordance with the principles of Laboratory Animal Care.

Plant materials

Fruits of *Adansonia digitata* were plucked from the tree behind the Department of Parasitology and Entomology, Ahmadu Bello University, Zaria. Samples of the fruit and leaves of the plant were sent to the Herbarium section of the Department of Botany, Ahmadu Bello University, Zaria for authentication, after which a voucher specimen number 2512 was allocated for reference purpose.

Preparation of aqueous extract of Adansonia digitata

The fruit was cracked open using a hammer, and the fruit pulp was detached from the seed using mortar and pestle. The pulp was then separated from the seeds and fiber by sieving. A total of 280 g of the fruit pulp was weighed and placed in a conical flask, seven litres of distilled water was added and left for 72 hours on the laboratory bench. It was then filtered using 850 nm and 150 nm sieve respectively. The third stage of filtration was done using Whatman filter paper no.1 and cotton wool was placed in the filter paper to get a pure solution. It was then frozen and dried using freeze-drying machine (ILSHIN freeze dryer with concentrator, Ilshin Lab. Co. Ltd, Netherlands).

Toxicity acute test

The extract was tested for acute toxic effect using method described by Lorke (1983). The test was conducted in two phases. In phase one, nine rats weighing between 100 and 150 g were randomly selected and used for the experiment. The nine rats were divided into three groups of three animals each. Groups 1, 2 and 3 were given 10, 100, 1000 mg/kg of the aqueous extract respectively. All the animals were observed for 24 hours for any sign of toxicity or death. In phase two of the trial, which depended on the outcome of the first trial, three healthy rats were grouped into three containing one animal each. Rats

in groups 1, 2 and 3 were orally given 3000, 6000 and 9000 mg/kg of the aqueous extract respectively.

Phytochemical screening
Phytochemical analysis of the fruit pulp of *Adansonia digitata* was performed according to the method described by Sofowora (1993) and Evans (1998).

Source of other drugs used
The standard antitrypanosomal agent diminazene aceturate (Berenil®, Intervet South Africa (Pty) Ltd) and Vitamin C 200 (Animal Health, Venter Holland) used in this study were obtained from a reputable Veterinary drugs distributor in Jos, Plateau state, Nigeria.

Inoculum
Stabblate of *Federe* strain of *Trypanosoma brucei brucei* was obtained from the Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna and passage into healthy rats to maintain the parasite

Preparation of inoculum from donor rats
Two donor rats were inoculated intraperitoneally with approximately $X10^6$ *Trypanosoma brucei brucei* (*Federe* strain) per 100 g BW. Parasitaemia was monitored every 24 hours using blood obtained from the tail tip. At high parasitaemia, the rat was sacrificed and blood obtained by cardiac puncture using 2 ml syringe. Blood was dispensed into EDTA bottle. The amount of parasite per ml of the suspension was estimated for subsequent experiments. Estimation of parasitaemia was done by examining wet blood smear at × 40 magnification using the rapid matching methods of (Herbert and Lumsden (1976). This 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 a slide by pricking the tip of the tail with a sterile needle and covered with a cover slip. The wet mount was then observed under × 40 magnification. The number of trypanosomes per microscopic field was compared with the table of logarithmic values. The logarithmic values which matched the microscopic observation were converted to antilogarithm from where the absolute number of trypanosomes per ml of blood was obtained.

Experimental Design
Thirty-five acclimatized adult male rats were divided into seven groups (A, B, C, D, E, F and G) of five rats each. Rats in group A were not infected with the parasite and served as the non-infected control group while rats in group B, C, D, E, F and G were inoculated with approximately $X10^6$ of the *T. brucei brucei* strain per 100 g BW. At the onset of parasitaemia (6 days post infection) all the infected rats except for group B were treated as follows: Rats in groups C and D were treated with diminazene aceturate and vitamin C 200 at dose rates of 3.5 mg/kg body weight (BW) intraperitonealy once and 200 mg/kg BW orally for 3 days respectively, while groups E, F, G were orally treated for 3 days with 40, 80, and 160 mg/kg of the extract respectively. Therapeutic effects of the extract on infected animals were determined by monitoring daily parasitaemia, packed cell volume and body weights.

Packed cell volume, parasitaemia and body weight determinations
The packed cell volume (PCV) was determined a day before the experimental infection (day 0) and at the termination of the experiment (day 10 PI) using standard technique as described by Rehman *et al*. (2003). Estimation of parasitaemia was done daily by examining wet blood smear at × 40 magnification using the rapid matching methods of Herbert and Lumsden (1976). The body weights of each rat were determined on day 0 and day 10 PI using a top loading weighing scale (Ohaus(R) triple beam weighing scale of 500 g capacity, OHAU corporation, USA) and recorded.

Data analysis
Data generated was analyzed using descriptive statistics. Results were expressed as mean ± standard deviation (SD). One- way ANOVA with Turkey's multiple comparison test was performed using GraphPad Prism Version 4.00 for windows. A value of $p < 0.05$ was considered significant. The results obtained were presented in table and charts.

Results
Acute toxicity study on fruit pulp of Adansonia digitata
The acute toxicity test revealed that extract of the fruit pulp of *A. digitata* is safe even at the highest dose of 9000 mg/kg as all rats administered the extract did not exhibit signs of toxicity such as piloerection, rapid respiration, tremors, abnormal gait, photosensitization, convulsion and death (Table 1).

Phytochemical analysis

The result obtained from the quantitative phytochemical screening of aqueous extract of *Adansonia digitata* fruit pulp is shown in Table 2. The result showed that the extract contains high proportion of flavonoid (3.51%) followed by saponin (0.10%) then alkaloid (0.07%) with least amount of oxalate (0.03%).

Effect of treatment with aqueous extract of fruit pulp of Adansonia digitata on parasitemia

Pre-experimental screening of the rats by wet film preparation technique revealed no parasite in blood. The parasites were detected in all the infected groups 6 days post infection (pre-patent period). The effect of the extract on parasitaemia in the treated rats presented in figure I showed that the extract activities on the parasitaemia were dose dependent. All the groups affected were treated at patency (6 days post infection). No parasitaemia was observed in group A rats as they were uninfected/untreated (negative control).

In group B (infected/untreated) rats, mortality was observed from day 8 and at day 10 post infection, there was mortality in the group due to high parasitaemia. In group C that was infected and treated with 3.5 mg/kg of diminazene aceturate (standard drug), it was observed that parasitaemia was present on the 6^{th} and 7^{th} day post infection (1-day post treatment), but the rats became aparasitaemic thereafter for the rest of the experimental period.

In group D rats (infected/treated with vitamin C) parasites were detected at 5 days post infection and there was a progressive increase in parasitaemia in two of the rats while the other 3 rats died between 9^{th} and 10^{th} post infection. In groups E and F, (groups infected and treated with 40 mg/kg and 80 mg/kg), respectively, a progressive parasitaemia was observed and all rats died between the 9^{th} and 10^{th} day post infection. In group G, (infected/treated with 160 mg/kg of the extract) Parasites were detected on day 5 post inoculation and there was reduction from the 8^{th} day post infection and treatment. All the rats in this group became aparasitaemic 4 days post treatment (day 10 post infection).

Effect of treatment with aqueous extract of fruit pulp of Adansonia digitata on Packed Cell Volume

Packed cell volume (PCV) of rats in this study range from 42% -50%. Group A (uninfected and untreated) had the highest PCV value which was not significantly different ($p>0.05$) from groups F and G (infected and treated with 80 mg/kg and 160 mg/kg of the extract respectively). However, there was significant difference ($p<0.05$) in the PCV values of Group A with that of groups B (infected and untreated) and C (infected and treated with diminazine aceturate) and significantly different ($p<0.01$) with that of groups D (infected and treated with Vitamin C) and E (infected and treated with 40mg/kg of the extract) (Table 3).

Table 1: Observation of rats following oral administration of fruit pulp of *Adansonia digitata* for acute toxicity study

Signs/ Observations	Control	Dose (mg/kg)					
		10	100	1000	3000	6000	9000
Pilorection	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Respiration	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Tremors	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Gait	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Reactivity to Light	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Reactivity to Environment	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Convulsion	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Death	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Table 2: Some phytochemical constituents of aqueous extract of fruit pulp of *Adansonia digitata*

S/N	Phtochemical constituents	Quantity (%)
1	Alkaloids	0.07
2	Flavonoids	3.51
3	Oxalate	0.03
4	Saponin	0.10

Effect of treatment with aqueous extract of fruit pulp of Adansonia digitata on weight gain

The weight of the infected and untreated rat (group B) decreased from 82.26g (initial weight) to 79.92g (final weight) and group C infected and treated with diminazene aceturate also decreased from 84.76g (initial weight) to 82.26g (final weight) while that of control uninfected and non-treated (group A) increased from 77.78g (initial weight) to 82.10g (final weight) so also there was increase in weight in the remaining groups that is, group D, E, F and G.

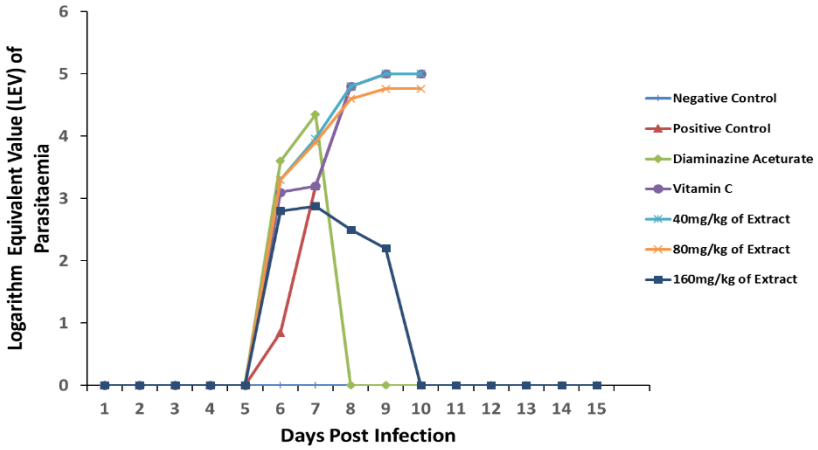

Figure 1: Course of parasitaemia of rats infected *with T. b. brucei* and treated with different concentration of aqueous extract of fruit pulp of *Adansonia digitata*

There was no significant difference ($p > 0.05$) in weight across the groups A, B and C while Groups (D treated with Vitamin C); E, F and G treated with 40 mg/Kg, 80 mg/Kg and 160 mg/Kg of the extract respectively, had higher significant difference in weight ($P < 0.05$). (Table 4).

Discussion

In the present study, *Trypanosoma brucei brucei* parasites became detectable in blood collected from the tail of experimental rats five days after infection. Upon invasion of the mammalian system trypanosomes proliferate quickly to establish its population in infected host resulting in characteristic waves of parasitaemia every three to five days (Lundkvist, 2001; Pentreath & Kennedy, 2004).

The present work has shown that fruit pulp of *Adansonia digitata* contains phytochemicals, such as saponins, flavonoids, alkaloids and oxalate, which may have potentials for the treatment of some disease conditions especially trypanosomosis and cancer (Manfredini *et al*., 2002). This agrees with the observation of earlier authors who found sterols, saponins and triterpenes in the fruit pulp of *A. digitata* (Anani *et al*., 2000). Plants that contain flavonoids have been shown to have anti trypanosomal activity (Tarus *et al*., 2002). In addition, several authors have identified the presence of flavonoids, saponins, tannins, cardiac glycosides in plants that showed trypanocidal activities (Nok, 2002; Nok, 2005; Atawodi *et al*., 2011, Nwodo *et al.,* 2015) which could also be responsible for the anti trypanosomal activity observed in this study.

The result of the acute toxicity test revealed that extract of the fruit pulp of *A. digitata* is safe even at 9000 mg/kg and this is in line with (Ramadan *et al*., 1993) which says the pulp is safe at more than 8000 mg/kg. As such, the fruit pulp of *A. digitata* could therefore be a suitable candidate as anti trypanosoma agent if eventually confirms to be efficacious, since most of the current anti trypanosoma agents have problems of toxicity especially at high dose. In this study, it was observed that significant weight gain in the groups infected and treated with the extract which was not observed in other infected groups. Chadare *et al.* (2009) and Diop *et al.* (2005) reported that the leaves, the seeds and the pulp from baobab are rich in nutrients while Nkafamiya *et al.* (2007) and Kinuthia *et al*. (2017) also reported that baobab fruit pulp contains minerals and vitamins, such as Zinc, Iron and vitamin D3 that improve appetite and nutrients absorption in supplemented animals. The possible presence of these nutrients in the fruit pulp extract might be responsible for the weight increase observed in the animals administered the extract in this study. Rafiu *et al*. (2017) similarly reported significant increased feed intake, weight gain, feed conversion ratio and nutrient digestibility in broiler chickens supplemented with fruit of *Adansonia digitata*. The drop-in feed intake and a decrease in body weight observed in the positive control and diminazene aceturate-treated group is similar to what was

Table 3: Packed Cell Volume of Wistar rats infected with *Trypanosoma brucei brucei* and treated with different concentration of aqueous extract of fruit pulp of *Adansonia digitata*

Group	Initial PCV (Day 0 PI)	Final PCV (Day 10PI)	p- value
A	48.8 ± 1.304	50.436 ± 1.717	0.079
B	48.8 ± 2.168	43.902 ± 4.002	0.034[b]
C	49.6 ± 2.966	42.782 ± 5.338	0.026[b]
D	44.6 ± 3.435	42.722 ± 3.667	0.000[a]
E	48.2 ± 2.302	45.186 ± 3.048	0.001[a]
F	48.4 ± 2.302	45.186 ± 3.048	0.066
G	46.8 ± 0.169	48.658 ± 2.527	0.215

Level of significance along rows a = p<0.01; b = p < 0.05

Table 4: Mean body weight (g) of Wistar rats infected with *Trypanosoma brucei brucei* and treated with different concentration of aqueous extract of fruit pulp of *Adansonia digitata*

Group (n=5)	Initial Weight (g) (Day 0 PI)	Final Weight (g) (Day 10PI)	P-values
A	77.78 ± 12.438	82.10 ± 11.487	0.329
B	82.26 ± 7.708	79.92 ± 7.509	0.582
C	83.86 ± 9.243	82.62 ± z8.590	0.669
D	99.66 ± 7.519	100.74 ± 7.549	0.000[a]
E	102.84 ± 5.879	111.12 ±5.832	0.000[a]
F	105.64 ± 7.556	114.10 ± 11.803	0.000[a]
G	115.36 ± 10.459	129.98 ± 12.062	0.000[a]

Level of significance along rows: a = p <0.05

previously reported in humans suffering from African trypanosomosis described by Dumas & Bisser (1999) which could also be attributed to the lack of appetite and depletion of food reserve in the host by the parasite (Adamu *et al*., 2009).

The decrease in the PCV values observed in the infected rats apart from group G (treated with 160 mg/kg of the extract) indicated anaemia. Anaemia has been reported to be the most important pathogenic features of trypanosomosis, which is apparently caused by the destruction of the red blood cells by the parasite (Cadioli *et al.*, 2006). However, the higher PCV values observed in the group treated with 160 mg/kg of the extract is in line with report of Yusuf *et al.* (2012) who reported that treatment with flavonoid rich methanolic extracts of *Vernonia amygdalina* leaf improved oxidative status of *T. brucei* infected animals resulting in decreased parasitaemia, prevention of anemia and protecting against liver damage.

The diseases caused by *Trypanosoma brucei* sub group has been reported to cause in addition to anemia, hepatocellular degeneration and glomerulonephritis (Umar *et al*., 2008). This is largely attributed to the large number of free radicals and superoxide generated by the *trypanosomes* that attack membrane polyunsaturated fatty acids and proteins, resulting to cellular injuries and consequently affecting vital tissues and organs of infected animals (Umar *et al.*, 2001).

The result obtained from the *in vivo* studies indicated that the effect of the extract on the parasite was dose dependent evidenced by the complete clearance of parasitaemia in rats treated with the highest dose 160 mg/kg body weight. Our findings concurred with the findings of Manfredini (2002) who reported that extracts of baobab roots eliminate parasitaemia in *Trypanosoma congolense* infection of albino rats.

In conclusion, this work has shown that the fruit pulp of *A. digitata* is non-toxic and contains active compounds with potentials of *in vivo* anti-trypanosoma activity and amelioration of some pathological effects (anaemia and weight loss) caused by *T. brucei brucei* in infected rats.

Conflicts of Interest

The authors declare no conflict of interest.

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