

**Preliminary Investigation on *In Vivo* Trypanocidal Activity of Hydroethanolic
Extracts of *Calotropis procera* and *Parkinsonia aculeata***



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ABSTRACT: Effect of extracts of *Calotropis procera* and *Parkinsonia aculeata* in the treatment of *Trypanosoma brucei brucei* infected rats was studied. The albino rats were treated for 7 days with 200 mg of the plant extracts intraperitoneally (ip) after establishment of parasitaemia. No significant ($p > 0.05$) changes in weights were observed in all the groups. The results showed that *Calotropis procera* (root) was 96.43% as effective as Diminazene aceturate (berenil) in clearing parasitaemia. Significant ($p < 0.05$) improvements in packed cell volume (PCV) were observed in the rats treated with *Calotropis procera* (root) and berenil. However, *Calotropis procera* (leaf) and *Parkinsonia aculeata* (leaf and stem bark) depressed red blood cell production. Total white blood cells (WBC) and differential count did not change significantly ($p > 0.05$) in *Calotropis procera* (root) and berenil (positive control drug) treated groups. These observations were reversed in leaf and stem bark and leaf extracts of *Calotropis procera* and *Parkinsonia aculeata*, respectively. In conclusion only the root extracts of *Calotropis procera* has demonstrated effective treatment of trypanosomiasis, possibly due to its phytochemical contents.

Key words: *Parkinsonia aculeata*, *Calotropis procera*, *Trypanosoma brucei brucei*, Haematological parameters

INTRODUCTION

Trypanosoma brucei brucei is a unicellular parasite transmitted by tse tse fly and is the causative agent of sleeping sickness in humans and related diseases in animals. The disease, Human African trypanosomiasis, is exclusively African and is more prevalent in the rural areas (Atougua and Costa, 1999). Trypanosomiasis has continued to contribute adversely to economic and social well being of sub-saharan Africans for several decades (Okochi *et al.*, 2003). Reports indicated that 66 million people in 36 African countries were afflicted and animal trypanosomiasis was responsible for 3 million cattle deaths each year (WHO, 1998, Truc, 2003).

Vaccination against African trypanosomiasis remains elusive and drugs resistance and toxicity still remain the major problems. In addition to emerging cases of drug resistance, the drugs (melarsoprol, suramin, pentamidine and eflornithine) with exception of eflornithine all have severe toxic side effects or are not readily available (Onyeyili and Egwu, 1995; Brun *et al.*, 2001). Evaluation of different chemicals/drugs and plant extracts show that they have

trypanocidal activity (Bodley and Shapiro, 1995; Freiburghaus *et al.*, 1998; Atawodi *et al.*, 2002; Okochi *et al.*, 2003; Atawodi and Alafiatayo 2007, Ogbunugafor *et al.*, 2007, Ibrahim *et al.*, 2008).

Jerusalem thorn (*Parkinsonia aculeata*), is a hardy specie and valued as an ornamental or shade tree. The leaves of the plant are used in northern Nigeria for the treatment of bacterial diseases, typhoid fever, diabetes and trypanosomiasis. *P. aculeata* has antibacterial activity (Hassan *et al.*, 2005), hepatoprotective action (Hassan *et al.*, 2008) and is effective in the treatment of diabetes related complications (Leite *et al.*, 2007). The plant contains tannins, flavonoids, saponins, glycosides, alkaloids, steroids and volatile oils (Hassan *et al.*, 2005).

Calotropis procera is a member of the family of Asclepiadaceae, commonly called Sodom apple and is used in indigenous treatment of convulsion, asthma, cough, diseases of domestic animals and wound healing (Aliero *et al.*, 2001). The leaf, root and stem extracts of the plant were reported to have antifungal properties (Hassan *et*

al., 2006). Reports are plentiful on the use of plants in the treatment of trypanosomiasis (Muelas-Serrano *et al.*, 2000, Atawodi *et al.*, 2003, Okochi *et al.*, 2003, Patric *et al.*, 2003, Taleb-Contini *et al.*, 2004, Wurochekke and Nok, 2004, Atawodi and Alafiatayo, 2007, Ibrahim *et al.*, 2008).

To our knowledge, *in vivo* trypanosomal activity of *Calotropis procera* (root and leaf) and *Parkinsonia aculeata* (leaf and stem bark) have not been documented. The present study evaluates the *in vivo* trypanosomal activity of *Calotropis procera* and *Parkinsonia aculeata* and highlights the problems of growing resistance and drug toxicity encountered in the treatment of trypanosomiasis.

MATERIALS AND METHODS

Chemicals

All chemicals used were purchased from BDH Chemical Company, Poole, England and were of analytical grade.

Plant material

Calotropis procera (root and leaf) and *Parkinsonia aculeata* (stem bark and leaf) were collected around Usmanu Danfodiyo University Campus, Sokoto, Nigeria. The plants were identified at the Botany Unit of the Department of Biological Sciences, Usmanu Danfodiyo University Campus, Sokoto and Voucher specimens were deposited in the herbarium of the department.

Preparation of plant extracts

The portions collected were air-dried under the shade and pulverized into coarse powder. The powdered (40 g) leaf, stem and root of the plants were extracted with 50 % ethanol-water (1000 mL of 1:1) at room temperature for 48 hours. The extracts were filtered through Whatman filter paper (No. 1) and concentrated by removing the solvents completely under reduced pressure (Guntupalli *et al.*, 2006 and Hassan *et al.*, 2008). The yields of the extracts were 7.9 (leaf) and 20.80 % w/w (stem bark) of *Parkinsonia aculeata* and 23.85 (root) and 42.50 % w/w (leaves) of *Calotropis procera*. The residues were reconstituted in sterile distilled water for antitrypanosomal studies.

Animals and parasite

Forty -two Albino rats (Wister strains) of either sex weighing 170-205 g were purchased from animal house, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. The rats were divided into 7 groups of 6 animals each. They were allowed free access to food and water for one week to acclimatize and maintained on standard animal feeds (Bendel feeds and flour mills, Edo state, Nigeria). The standard orogastric cannula was used for oral administration of the extract. The parasites *T. brucei brucei* were obtained from Nigerian Institute for Trypanosomiasis and Onchocerciasis Research Centre, Kaduna. They were maintained by continuous serial passage in the laboratory animals (albino rats) until required. Experiment was performed according to ethical guidelines for the investigation of experimental pain in conscious animals as reported by Zimmerman (1983).

In vivo trypanocidal assay

In vivo trypanocidal activity test of the ethanolic extracts was performed according to previously established protocols (Ogbunugafor *et al.*, 2007). The rats were inoculated with 10^6 trypanosomes per mL of blood intraperitoneally (i.p.) using phosphate buffered saline as diluent. Group 1 served as Negative control (uninfected and untreated), group 2 served as positive control (infected and untreated) and group 3 was infected and treated with a reference drug diminazine acetate (Berenil). Groups 4 and 5 (infected groups) were test groups and were administered 200 mg/kg body weight of extracts of *Calotropis procera* (root and leaf) and *Parkinsonia aculeata* (stem bark and leaf) respectively for 7 days. The reference drug (3.5 mg/kg) was administered for 7 days too. Assessment of the activity of the extracts was done based on survival of the animals. Parasitaemia levels, using Rapid Matching Method of Hebert and Lumsden (1976), were monitored from day 5 to day 28 or until death of the rats. Parasitaemia levels were compared with infected untreated animal group. The weights of the animals were monitored daily on laboratory weighing balance.

Measurement of haematological parameters

The packed cell volume (PCV), white blood cell count (WBC) and differential counts (lymphocytes and neutrophils) were estimated based on the method of Dacie and Lewis (1991).

Statistical analysis

The data were analyzed using one way analysis of variance or t-test, Graph Pad InStat Software (San Diego, USA).

RESULTS AND DISCUSSION

The body weight of the animals pre-infection ranged from 175.90±31.50 to 197.70±25.50, after infection and treatment the body weights ranged from 153.90±38.90 to 195.80±28.50. Generally, at the end of study period body weights decreased within groups but were not statistically significant ($p>0.05$) indicating that treatments had no untoward effects on food consumption and utilization (Table 1).

Table 2 presents the effect of extracts of *Parkinsonia aculeata* and *Calotropis procera* on haematological profiles, parasitaemia and survival days of *Trypanosoma brucei brucei* infected rats. The PCV, WBC and neutrophil levels of the infected treated rats were significantly reduced compared to uninfected (normal) rats. This phenomenon was reversed in infected animals group treated with berenil (diminazine acetate) but not in animals treated with leaf and stem bark of *P. aculeata*, and leaf of *Calotropis procera*. *T. brucei brucei* induces anaemia in animals such as cattle, dogs, rats and mice (Jenkins *et al.*, 1980). Mwangi *et al.*, (1995) have reported this parasite to induce anaemia in animals with significant reduction in packed cell volume (PCV) and haemoglobin levels.

The hydroethanolic root extracts of *Calotropis procera* show *in vivo* trypanocidal activity. The extract (200 mg/ml) inhibited the growth of *T. brucei brucei* at the end of 7 days treatment. The activity was 96.43% compared to diminazine acetate (which produced complete inhibition) (Table 2). There was no observed trypanocidal activity in leaf and stem bark and leaf of *Parkinsonia aculeata* and *Calotropis procera*, respectively.

Trypanocidal activity was not reflected in longer periods of survival days because the crude extracts consist of a mixture of many substances and these substances(s) with possible antitrypanosomal activity may be present only in a small concentration. The isolation of the active ingredients should help to prepare extract/drugs with higher concentrations of active ingredients.

The levels of parasitaemia in rats treated with berenil and the root extract of *Calotropis procera* were significantly ($p<0.05$) reduced (from 21.40±1.50 to 8.90±0.95 parasitaemia/ml $\times 10^6$) compared with infected untreated animals. This suggests that the root extract of the plant has trypanocidal activity. The root extracts of *Calotropis procera* was able to produce good effects in reducing the mortality of the parasite infected rats and prolonging the life span of the animals. This could be a welcome development in the field of trypanosomiasis control where drug resistance and toxicity still remain the major problems. Resistance has been reported against drugs such as melarsoprol, suramin, and pentamidine coupled to severe toxic side effects or lack of ready availability (Onyeyili and Egwu, 1995; Brun *et al.*, 2001).

Table 1: Effect of extracts of *Parkinsonia aculeata* and *Calotropis procera* on body Weights (g) of the experimental rats

Groups	Pre-infection	Post-infection/treatment
Negative control (uninfected and untreated)	179.90±28.90	195.80±28.50
Positive control (infected and untreated)	181.80±41.90	153.90±38.90
Berenil (treated with diminazine acetate)	197.70±25.50	191.60±26.30
<i>Calotropis procera</i> (root)	187.80±40.80	177.90±36.50
<i>Calotropis procera</i> (leaf)	175.90±31.90	115.79±38.20
<i>Parkinsonia aculeata</i> (stem bark)	179.80±43.40	157.90±40.70
<i>Parkinsonia aculeata</i> (leaf)	180.0±25.30	170.00±22.10

Values are mean ± standard deviation and were not significantly ($p>0.05$) different by using student t-test.

Table 2. Effect of extracts of *Parkinsonia aculeata* and *Calotropis procera* on Heamatological profiles, parasitaemia and survival days of *Trypanosoma brucei brucei* infected rats

Plant/Drug	PCV (%)	WBC (%)	Lymphocytes (%)	Neutrophils (%)	Parasitaemia/ml (x10 ⁶)	Survival days
Negative control (uninfected and untreated)	+ 40.50±1.40 ++ 39.50±1.40	8.90±0.14 8.70±0.42	36.00±1.50 37.70±0.40	35.00±1.40 34.50±0.70	0.00 0.00	-
Positive control (infected untreated)	+ 42.50±0.70 ++ 32.00±2.10	6.40±0.14 7.10±0.30	61.20±1.13 71.60±0.56	38.10±1.13 28.50±0.40	15.50±3.60 599.3±4.30	8-12 (5)
Berenil (treated with diminazine aceturate)	+ 44.50±0.70 ^{b,c} ++ 42.50±0.70 ^b	7.10±0.14 7.33±0.07	67.80±0.28 ^{d,c} 63.90±0.50	27.50±0.71 ^{c,d,a} 36.40±1.60 ^b	19.50±3.60 0.00	10-28 (0)
<i>Calotropis procera</i> (root)	+41.50±0.70 ^b ++39.25±0.4 ^b	7.90±0.07 ^a 8.05±0.07 ^a	64.50±1.40 ^{c,d} 62.75±2.40 ^{c,d,b}	30.00±1.40 ^{a,b} 34.00±1.13 ^b	21.40±1.50 ^{a,b,c,d} 8.90±0.95 ^{a,b,c,d}	10-15 (0)
<i>Calotropis procera</i> (leaf)	+42.00±1.40 ^a ++35.50±0.70 ^{b,a}	7.90±0.14 6.80±0.28	63.90±0.14 ^{c,d} 75.00±1.40 ^{a,c,d}	33.40±1.40 28.90±0.14	16.90±0.50 ^{b,c,d} 308.70±2.50 ^{a,b,c,d}	8-10 (2)
<i>Parkinsonia aculeata</i> (stem bark)	+ 40.50±0.85 ^b ++38.30±3.00 ^a	6.48±0.14 ^{d,c} 7.19±0.14	64.30±5.50 ^{d,c} 79.00±3.00 ^{a,d,c}	34.80±3.00 ^b 28.10±2.00 ^{a,d,c}	12.90±1.00 ^{b,c,d} 75.50±3.70 ^{a,b,d,c}	9-12 (2)
<i>Parkinsonia aculeata</i> (leaf)	+ 45.00±5.70 ^b ++35.80±2.70 ^a	6.70±0.07 7.70±0.40	70.50±2.10 ^{c,d} 76.80±0.28 ^{c,d}	29.80±0.40 ^{c,a} 26.07±1.00 ^{a,d,c}	7.10±3.20 ^{a,b,c,d} 117.00±2.00 ^{c,d,a,b}	9-12 (2)

a= Significant (p<0.05) vs positive control before treatment, b= Significant (p<0.05) vs positive control after 7 days of experiment, c= Significant (p<0.05) vs negative control after 7 days of experiment, d= Significant (p<0.05) vs negative control before the experiment, by using t-test, Graph Pad Instant Software (San Diego, USA). += before treatment, ++=after 7 days of treatment. Numbers in parenthesis are animals that died.

The mechanism (s) by which the root extract of *Calotropis procera* produces trypanosome clearing activity is yet to be precisely determined. Thus, the root extract may exert its effect by strengthening the body's immune system, invigorating the defense mechanism in the infected animals. It is expected that the root extract may probably show more activities against other species of trypanosomes when used at higher concentrations.

Calotropis procera is medicinally versatile as apart from antitrypanocidal and antifungal activities it also has antihelmintic activity (Larhsini *et al.*, 1999, Al-Qarawi *et al.*, 2001; Shivkar and Kumar 2003, Iqbal *et al.*, 2005). The results obtained in this study demonstrate that the root extract of *Calotropis procera* has trypanocidal activity against *T. brucei brucei* and its active principle(s) reported (Hassan *et al.*, 2006) may be responsible for this activity. This plant may offer some potential for new trypanocidal drug preparations, a welcome relief in the area of trypanosomiasis treatment.

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