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Preliminary evaluation for anti-trypanosomal activity of aqueous stem bark extract of *Crossopteryx febrifuga* in *Trypanosoma congolense* - infected rats

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Abstract
Aqueous stem bark extract of *Crossopteryx febrifuga* was evaluated for anti-trypanosomal activity in rats. Twenty four rats divided into four groups (A, B, C, D) of six animals per group were used. Infection with *T. congolense* was established after a pre-latent periods of 10 and 11 days for curative (Group B) and prophylaxis (Group A) studies respectively. Both Group A and Group B were treated orally with 1500mg/kg of the crude extract for five consecutive days. Parasitemia, Packed Cell Volume (PCV), Red Blood Cells (RBC) counts and Differential leucocytes (WBC) counts were monitored throughout the studies. Phytochemical analysis of the crude aqueous extract was also carried out. Anaemia developed with establishment of infection in all the groups. Analysis of PCV, RBC and Differential WBC counts showed no significant difference (P>0.05) between treated and untreated animals. The crude extract contains saponins, tannins, reducing sugars, volatile oils and alkaloids as phytochemicals. It showed no significant prophylaxis or curative against *T. congolense* infection in rats as traditionally claimed. Although the plant may be used locally in combination with other plants or ingredients or even other orthodox drugs, further work is needed to fully assess the claim of the use of *Crossopteryx febrifuga* stem bark in the management or treatment of animal trypanosomiasis.

Keywords: *Crossopteryx febrifuga;* Trypanosomiasis; *Trypanosoma. congolense.*

Introduction

Trypanosomiasis is a disease caused by parasitic protozoans called trypanosomes that cause sleeping sickness in humans and related diseases in domestic animals. Some 35 million people and 25 million cattle in Africa are at risk of infection with trypanosomes (Turner, 1982). Although, chemotherapy and chemo prophylaxis have continued to form the most important and major aspect of the control and eradication of trypanosomiasis (Igweh and Onabanjo, 1989), the discovery and development of new drugs has been slow and far from satisfactory (Fairlamb, 1989). Resistance against existing trypanocides and toxicity are common occurrences (Onyekwelu, 1999). Thus, the need for new curative agents can not be over emphasized. Perhaps, local medicinal plants could provide such agents with potential remedy against

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both human and animal trypanosomiasis. One of such plants is *Crossopteryx febrifuga*, claimed to be used by Fulani herdsmen in Northern Nigeria, for the treatment of Animal trypanosomiasis (Okpara and Ali, 2002).

The present studies were therefore carried-out to evaluate the potential of *Crossopteryx febrifuga* in the treatment or management of *T. congolense* infected rats.

Experimental

Chemicals/reagents. Anhydrous sodium dihydrogen phosphate (NaH_2PO_4) and anhydrous disodium hydrogen phosphate (Na_2HPO_4), sodium chloride, all purchased from British Drug House (BDH). All reagents for phytochemical analysis were of Analar grade.

Collection of plant. The stem back of *C. febrifuga* (Hausa – Kashin Awaki; Fulani - Rimajegohi) was collected from Kaltingo LGA, Gombe State with the help of traditional medical practitioners. The plant was identified by Mr. Abdulkarim of the College of Forestry, Jos, Plateau State, Nigeria.

Preparation of aqueous extract. The extract was prepared according to the information obtained from the Fulani Herdsmen. The stem bark of *C. febrifuga* was air dried under ambient temperature and reduced to coarse powder using a pestle and mortar. Two hundred grammes (200g) of the powder was boiled in one liter of distilled water for 3 hrs. The aqueous extract obtained was decanted and filtered using muslin cloth and then Whatman's filter paper. The filtrate was carefully and slowly evaporated to dryness using hot water bath at 45°C. The dried extract was kept in a sealed container and refrigerated at 4°C until required.

Phytochemistry. The extract of *C. febrifuga* was tested for the presence of phytochemicals according to the method of Sofowora (1979). The pH was also determined using pH meter (Water proof pH scan 3, Eutech instruments)

Acute toxicity studies. The median lethal dose (LD_{50}) of the extract was determined by the method of Lorke (1983). This method involves two stages; stage one: 9 animals and dosages of 10mg/kg, 100mg/kg, 1000mg/kg are used. In stage two, 3 animals and dosages of 1600mg/kg, 2900mg/kg and 5000mg/kg are used. Thereafter, LD_{50} was calculated.

Experimental animals. Twenty four mature male albino rats were obtained from Parasitology Section, NITR, Vom. The animals were divided into four groups (A, B, C, D) of six animals each and allowed to acclimatize for two weeks. They were fed on Growers' mash (Vital Feeds, Nig. Ltd.) and water *ad libitum*.

Strain of parasite. Trypanosoma congolense (Karu strain) was obtained from Veterinary and Livestock Studies Division, NITR, Vom. The parasites were maintained in the laboratory by serial blood passage in donor rats.

Experimental design. Blood was collected from the tail of a heavily infected donor rat and immediately diluted with sterile phosphate saline buffer (pH 7.4). Parasites in the diluted blood which served as the inoculum were counted using a microscope and estimated by the method of Herbert and Lumsden (1976). Group A (prophylaxis) was treated orally with the extract at 1500mg/kg body weight for five days consecutively and then inoculated intraperitoneally with 0.1ml of the inoculum containing 1×10^5 parasites. Group C (prophylaxis control) was given normal saline for five consecutive days. Group B (curative) was inoculated intraperitoneally with 0.1ml of the inoculum containing 0.1ml of the parasite. When the parasites begin to appear in the peripheral blood which indicated the establishment of infection, treatment commenced with the extract at 1500mg/kg body weight for five days consecutively. Group D (curative control) was given normal saline for five days simultaneously with group B treatment.

Determination of hematological parameters. Packed Cell Volume (PCV), Red Blood cells (RBC) counts and Differential leucocytes (WBC) counts were determined weekly. PCV was by microheamatocrit method, RBC counts using haemocytometer and Differential WBC counts as described by Schalm *et al.* (1975).

Results

Phytochemical analysis. The phytochemical analysis of the crude aqueous extract showed that it contains saponins (saponin glycosides), reducing sugars, tannins, volatile oils and alkaloids. It was also acidic with pH of 5.30.

Acute toxicity studies. The media lethal dose (LD_{50}) for the oral administration of the extract was found to be above 5000mg/kg body weight. No immediate or late sign of toxicity was observed, thus the extract was considered safe (Lorke, 1983).

Prophylaxis studies. After treatment for 5days and then inoculation with the parasites, animals were examined daily for parasitemia. Infection developed after a pre-latent period of 11 days in both treated group and untreated control. The animals died from 9-13 and 4-11 days post infection (Pi) for treated and untreated groups respectively. No significant difference (P>0.05) was observed in the RBC count, PCV and Differential WBC counts between treated and untreated groups (Table 1, 3 and 5). However, a slight but insignificant (P>0.05) increase in monocytes was observed in the treated group when compared to the control (Table 5).

Curative studies. Infection developed after a pre-latent period of 10 days in both treated and untreated groups. Treatment for 5 days commenced 10 days post inoculation and did not change the pattern of parasitemia as the parasites continued to multiply until all the animals died. The animals died between 9-11 and 8-13 days post-infection for treated and untreated groups respectively. There was no significant difference (P>.05) observed in the RBC counts, PCV and Differential WBC counts between the treated animals and untreated control (Table 2, 4 and 6). However, a slight but insignificant increase in lymphocytes was observed after the start of treatment.

Table 1: Prophylactic effect of *C. febrifuga* on RBC of *T. congolense* infected rats.

Post inoculation days	RBC Count X 10^{12}/L (mean ± S.D)		P
	Group A	Group C	
0	5.20±0.7	5.90±0.2	>0.05
5	5.79±0.5	6.15±0.3	>0.05
12	4.92±0.4	4.63±0.3	>0.05
19	3.06±0.1	2.74±0.1	>0.05

Key: Group A = Treated infected, Group C = Untreated infected control.

Table 2: Curative effect of *C. febrifuga* on RBC of *T. congolense* infected rats.

Post inoculation days	RBC Count X 10^{12}/L (mean ± S.D)		P
	Group B	Group D	
0	5.05±0.20	4.98±0.30	>0.05
6	5.41±0.50	5.40±0.30	>0.05
13	4.39±0.40	3.37±0.70	>0.05
20	-	5.01±0.00	

Key: Group B = Infected treated, Group D = Infected untreated control

Table 3: Prophylactic effect of *C. febrifuga* on PCV of *T. congolense* infected rats.

Post inoculation days	Packed Cell Volume- (mean ± S.D)		P
	Group A	Group C	
0	46.3±2.5	38.0±4.9	>0.05
5	37.0±1.4	45.7±2.1	>0.05
12	40.3±3.3	45.7±2.1	>0.05
19	29.0±1.0	29.0±1.0	>0.05

Key: Group A = Treated infected, Group C = Untreated infected control

Table 4: Curative effect of *C. febrifuga* on PCV of *T. congolense* infected rats.

Post inoculation days	Packed Cell Volume- (mean ± S.D)		P
	Group A	Group C	
0	49.3±0.90	45.3±2.5	>0.05
6	41.0±5.4	37.3±3.8	>0.05
13	41.7±1.70	40.7±5.2	>0.05
20	-	38.0±0.0	

Key: Group B = Infected treated, Group D = Infected untreated control

Table 5: Prophylactic effect of *C. febrifuga* on differential WBC counts of *T. congolense* infected rats.

Post inoculation days	Differential WBC X 10^9/L (mean ± SD)							
	Group A				Group C			
	Neu	Lym	Eos	Mono	Neu	Lym	Eos	Mono
0	4.40±0.5	4.42±0.6	0.13±0.06	0.04±0.55	3.46±0.25	5.07±0.33	0.32±0.10	0.11±0.08
5	4.59±0.55	4.22±0.37	0.14±0.12	0.05±0.70	5.58±0.21	3.41±0.21	0.00±0.00	0.00±0.00
12	4.77±0.10	3.77±0.09	0.33±0.09	0.10±0.07	4.79±0.12	3.88±0.28	0.28±0.10	0.03±0.04
19	4.89±0.01	3.74±0.06	0.20±0.11	0.15±0.05	4.80±0.09	3.75±0.15	0.36±0.04	0.06±0.04

Neu = Neutrophils, Lym = Lymphocytes, Eos = Eosinophils, Mono = Monocytes;
Group A= Treated infected, Group C = Untreated infected control.

Table 6: Curative effect of *C. febrifuga* on differential WBC counts of *T. congolense* infected rats.

Post inoculation days	Differential WBC X 10^9/L (mean ± SD)							
	Group B				Group D			
	Neu	Lym	Eos	Mono	Neu	Lym	Eos	Mono
0	3.44±0.36	5.38±0.46	0.21±0.09	0.05±0.06	4.39±0.80	4.20±0.80	0.28±0.05	0.13±0.09
5	4.99±0.26	3.85±0.37	0.10±0.07	0.04±0.06	5.07±0.33	3.74±0.36	0.13±0.18	0.04±0.06
13	4.31±0.70	4.52±0.74	0.13±0.09	0.03±0.04	5.01±0.10	2.70±1.76	0.13±0.09	0.07±0.09
20								

Neu = Neutrophils, Lym = Lymphocytes, Eos = Eosinophils, Mono = Monocytes;
Group B = Infected treated, Group D = Infected untreated control.

Table 7: Effect of *C. febrifuga* extract on the course of parasitaemia in *T. congolense* – infected rats.

Days post infection	Parasitaemia X 10^6/ml (mean ± S.D)											
	1	2	3	4	5	6	7	8	9	10	11	12	13
Group B	0.05±0.1	2.5±0.5	6.0±2.9	20.1±8.2	31.7±10.3	53.3±25.0	30.5±10.0	Dead					
Group D	0.7±0.4	12.5±2.5	13.7±8.9	45.0±5.0	55.0±7.0	80.5±27.0	40.0±28.0	60.0±0.0	60.0±0.0	Dead			

Note: Treatment commenced day 1 of infection. Group B = Infected treated, Group C = Infected untreated.

Discussion

Treatment with *C. febrifuga* extract showed no significant effect on the number of parasites as the parasitemia continued to progress after infection was established. The parasitemia was milder in treated animals compared to untreated controls (Table 7). In both prophylaxis and curative studies, the pre-latent periods of treated groups and untreated controls were the same. This shows the absence of prophylaxis against *T. congolense* infection. The PCV and RBC counts of the treated groups continued to drop till all the animals died as a result of anaemia. Expectedly, the drops in PCV and RBC counts correspond with the periods of progressive increase in parasitemia. The development of anaemia towards the terminal stage of the disease is thought to be as a result of the numerous increases of circulating trypanosomes causing lysis of the erythrocytes by the lashing action of the parasites flagellae (Esievo *et al.*, 1982). The breakdown of plasma protein, and the activities of the enzymes neuraminidase and phospholipases produced by trypanosomes may have also been involved in this process, since they are reported to be released towards the late stage of infection (Esievo *et al.*, 1982). The slight monocytosis in prophylaxis group was accompanied by mild neutrophilia, lymphopaenia and eosinopaenia. Mono cytosis, lymphopaenia, and eosinophania are all consistent findings in trypanosomiasis (Emeribe and Anosa, 1991).

The observed monocytosis is associated with the increased demands to remove particulate matter such as dead trypanosomes and lysed red blood cells from circulation (Nwosu and Ikeme, 1992).

Conclusion

Within the frame of this work, the aqueous stem bark extract of *C. febrifuga* has not shown significant anti-trypanosomal activity in rats infected with *T. congolense*. This seems to be contrary to the claims of the Fulani herdsmen. However, it is likely that the herdsmen use it in combination with other plant, ingredients or even orthodox drugs, whereby the action of one plant or ingredient may potentiate that of the other synergistically to produce a desired effect. Thus, caution and care should be employed while seeking information on medicinal plants from local herdsmen or traditional herbalists, because such information obtained may be misguided or incomplete.

Nevertheless, further work will be needed to fully assess the claim of the use of *C. febrifuga* stem bark extract in the management or treatment of animal trypanosomiasis as this is a preliminary investigation.

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