Senna occidentalis leaf extract possesses antitrypanosomal activity and ameliorates the trypanosome-induced anemia and organ damage

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ABSTRACT

The *in vitro* and *in vivo* antitrypanosomal effects of the ethanol extract of *Senna occidentalis* leaf were investigated. The crude extract exhibited an *in vitro* activity against *Trypanosoma brucei brucei* as it completely eliminated parasites' motility within 10 minutes postincubation with 6.66 mg/ml of effective extract concentration. The extract was further used to treat experimentally *T. brucei brucei* infected rats at concentrations of 100 and 200 mg/kg body weight, beginning on day 5 post infections (p.i.). At the termination of the experiment on Day 11 p.i., the extract significantly ($P < 0.05$) kept the parasitemia lower than was recorded in the infected untreated rats. All the infected animals developed anemia, the severity of which was significantly ($P < 0.05$) ameliorated by the extract treatment. The infection caused significant ($P < 0.05$) increases in serum alanine and aspartate aminotransferases as well as serum urea and creatinine levels. However, treatment of infected animals with the extract significantly ($P < 0.05$) prevented the trypanosome-induced increase in these biochemical indices. Furthermore, the *T. brucei* infection caused hepatomegaly and splenomegaly that were significantly ($P < 0.05$) ameliorated by the extract administration. It was concluded that orally administered ethanol extract of *S. occidentalis* leaf possessed anti-*T. brucei brucei* activity and could ameliorate the disease-induced anemia and organ damage.

Key words: Anemia, antitrypanosomal effect, *Senna occidentalis*, *Trypanosoma brucei brucei*

INTRODUCTION

Pathogenic trypanosome infections of domestic animals are still a major scourge in sub-Saharan Africa and they largely account for the low livestock productivity of the continent,[1] thus making it an important priority for biomedical and public agencies, agricultural sector and the scientific community.[2] The disease caused by the *Trypanosoma brucei* subgroup is associated with anemia, hepatocellular degeneration and glomerulonephritis,[3] which is largely attributed to the large amount of free radicals and superoxides generated by the trypanosomes that attack membrane polyunsaturated fatty acids and proteins, resulting in cellular injuries and consequently affecting vital tissues and organs of the infected animals.[1,4,5] There is little or no hope for the production of antitrypanosomal vaccine in the near future because of

antigenic variation exhibited by the parasites. This coupled with the limitations of the present trypanocidal drugs such as toxicity, drug resistance, and being inconvenient to administer to local populations increase the need for urgent search of more effective plant-derived therapeutic agents against the disease.[6] More so, several semi-synthetic and synthetic drug derivatives were originally isolated from natural compounds.[7,8] Reports on ethnopharmacology revealed several medicinal plants as potent trypanocides.[9-12] These suggest the need for exploring medicinal plants for efficient and cheaper trypanocides.

Senna occidentalis (Linn.) (formerly *Cassia occidentalis*) is a weed of the leguminosae family, and is distributed throughout the tropical and subtropical regions of the world. It can be found in open pastures and in fields cultivated with cereals such as soybean, corn, sorghum and others; thus, during the harvest it is almost impossible to prevent this plant from mixing with the cultivated crops.[13,14] The antibacterial and antimalarial activities of leaves and rootbark extracts of this plant have been reported.[15-18] However, information on either *in vitro* and/or *in vivo* antitrypanosomal activities of any part of this plant has never been documented. In this article, we report

for the first time, the *in vitro* and *in vivo* antitrypanosomal activities of ethanolic extract of *S. occidentalis* leaves. We also evaluate the extract's ability to ameliorate the associated trypanosome-induced pathologic changes.

MATERIALS AND METHODS

Plant Material

Ripe *S. occidentalis* leaves were collected in August 2008 from the Botanical garden of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. *S. occidentalis* was identified at the species level at the Herbarium unit of the same department. The voucher herbarium specimen was deposited with number 1047. The leaves were thoroughly washed and air-dried for 2 weeks to a constant weight. The dried leaves were pounded to fine powder with mortar and pestle, and then stored in dry containers until needed.

Experimental animals

The protocol employed met the guidelines of the Good Laboratory Practice (GLP) regulations of World Health Organization. Apparently healthy white albino rats of both sexes weighing between 140 and 260 g were used for the work and were obtained from the Nigerian Institute of Trypanosomiasis and Onchorcerciasis Research, Kaduna, Nigeria. The animals were kept in well-ventilated laboratory cages with 12 hours day/night cycles. The rats were maintained on a commercial poultry feed (ECWA Feeds, Jos, Nigeria) and drinking water *ad libitum*.

Extract preparation

The ethanolic extract of *S. occidentalis* leaf was prepared by soaking 50 g of the powder in 150 ml of 95% ethanol and shaking on Wrist action shaker for 6 hours. The preparation was left to stand for another 24 hours and then filtered through a Whatmann's filter paper. The filtrate was concentrated to dryness at 40°C under reduced pressure on a rotary evaporator and stored in a refrigerator at −4°C until required.

Test organism

Trypanosoma brucei brucei (Federe strain) was obtained from Department of Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. Parasites harvested from the blood of a donor rat at peak parasitemia (10^9 parasites/ml) were diluted with phosphate buffered saline and then used for both the *in vitro* studies and infection of experimental animals.

In vitro Screening for Antitrypanosomal Activity

Different concentrations of the extract ranging from 2.5 to 20 mg/ml were prepared. The *in vitro* antitrypanosomal activity was assessed in triplicates in 96-well microtiter plates (Flow laboratories Inc., Mclean, VA, USA). In the wells of the microtiter plates, aliquots of 20 µl of each extract concentration were incubated with 40 µl of the infected blood, achieving effective extract concentrations of 6.66, 3.33, 1.67 and 0.83 mg/ml in the reaction mixtures. For control, the extract was replaced with phosphate buffered saline. Parasite count was then monitored on a glass slide (covered with a covering slip) and observed under a microscope at ×400 magnification. The percentage of motile parasites was counted at 10 minutes intervals for 1 hour. Cessation or drop in motility of the parasites in extract-treated blood compared to that of parasite-loaded control blood without extract was taken as a measure of antitrypanosomal activity.[11]

In vivo Activity of the Ethanol Extract of *S. occidentalis* Against *T. brucei brucei*

To investigate the effect of the extract on *T. brucei brucei* infection, 30 albino rats (140–260 g) of both sexes were divided into six groups of five rats in each and treated as follows.

Uninfected (normal) control: The rats in this group were neither infected with the parasites nor treated with the extract.

Extract control: This group of rats was also left uninfected but orally treated with 200 mg/kg body weight of the extract.

Infected untreated control: The five rats in this group were each infected by intraperitoneal injection of about 10^5 *T. brucei* per 100 g b.w. and were not further treated.

Infected + 100 mg/kg b.w.: Rats in this group were infected similar to that of the infected control group above. However, on day 5 post infections (p.i.), when parasitemia approximately reached 10^8 trypanosomes/ml, they were given daily oral treatment of 100 mg/kg b.w. of the extract.

Infected + 200 mg/kg b.w.: The rats in this group were also similarly infected but on day 5 p.i., when parasitemia approximately reached 10^8 trypanosomes/ml, daily oral treatment with 200 mg/kg b.w. of the extract was commenced.

Infected + diminal: Infection of the animals in this group with the parasites was carried out as described above and then treated with 80 mg/kg b. w. of Diminal® (Eagle Chemical Company Ltd, Ikeja, Nigeria; diminal contains 445 mg diminazene aceturate + 555 mg phenazone/g) starting from Day 5 p.i.

The parasites were then monitored daily using the

rapid matching counting method[19] and the experiment terminated on Day 11 p.i. The preinfection and terminal (on Day 11 p.i.) packed cell volumes (PCVs) of all groups of rats were determined by the microhematocrit method.

Serum biochemical parameters

Serum harvested from the blood of all animals during cardiac puncture on Day 11 p.i. was used to measure alanine aminotransferase and aspartate aminotransferase (ALT and AST) activities using commercial reagent kits (Randox Laboratories, Ireland), while urea and creatinine concentrations were determined by the diacetylmonoxime and Jaffe's reactions respectively, as described by Kaplan *et al.*[20] Liver, kidney and spleen of all the rats were also collected and weighed to ascertain the organ:body weight ratio for all groups of rats.

Statistical analysis

The results were presented as mean $\pm$ standard deviation and students' *t*-test was used to compare paired means and a difference was considered statistically significant when $P < 0.05$.

RESULTS

In vitro Screening

The ethanol extract of *S. occidentalis* exhibited an *in vitro* anti-*T. brucei brucei* activity in a dose-dependent fashion. While no parasitic motility was observed at 10 minutes postincubation with 6.66 mg/ml of the effective extract concentration, 75% of the parasites were still motile in the control with 18% being active even at 60 minutes postincubation [Figure 1].

In vivo Trypanosuppressive Action of the Extract

Figure 2 presents the effect of different intraperitoneal doses of the extract on the course of *T. brucei brucei*
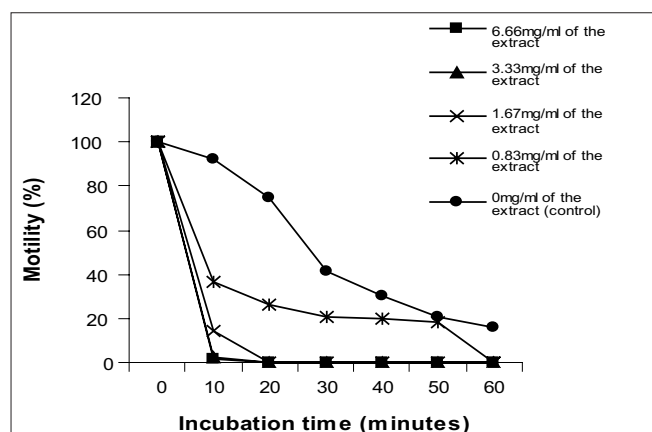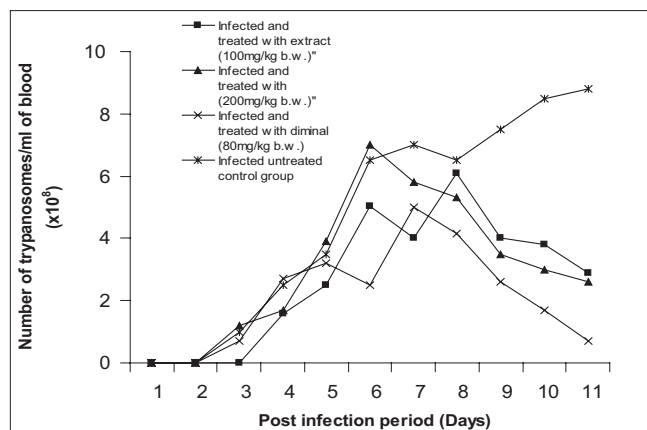

Figure 2: *In vivo* antitrypanosomal activity of different doses of ethanolic extract of *S. occidentalis* leaves

infection in rats. Compared with the infected untreated rats, treatment with the extract suppressed the multiplication of the parasites in a dose-dependent manner; whereas a higher trypanosuppresive effect was observed in the diminal- treated infected group on Day 11 p.i.

Effect of the extract on the parasite-induced anemia and organ damage

The *T. brucei brucei* infections in this work caused significant ($P < 0.05$) drop in PCV of infected rats, indicative of anemia. However, the anemia observed in the infected untreated controls is significantly ($P < 0.05$) more severe than that of the extract- and diminal-treated animals [Table 1]. The results of the indices on hepatic and renal functions analyzed in this experiment are presented in Table 2. Infection, without treatment, caused significant increases ($P < 0.05$) in the activities of serum ALT and AST which were significantly ($P < 0.05$) ameliorated by the administration of 200 mg/kg b.w. of the extract and diminal. Trypanosome-induced increases in both serum urea and creatinine concentrations were also significantly ($P < 0.05$) prevented by the extract and diminal treatments. More so, the hepatomegaly and splenomegaly observed as increase in liver:body weight and spleen:body weight ratios, respectively, in the infected animals were significantly ($P < 0.05$) higher in the untreated rats than in the extract-treated [Table 3] rats. The kidney:body weight ratio was affected by neither the infection nor the extract administration.

DISCUSSION

The antibacterial and antimalarial activities of *S. occidentalis* leaf extracts have been demonstrated.[15,16] So far, information on the trypanocidal action of this plant does not appear in the literature. This study is the first attempt to demonstrate the *in vitro* and *in vivo* activities of an extract of *S. occidentalis* against *T. brucei* and also the ameliorative

Figure 1: *In vitro* antitrypanosomal effects of ethanolic extract of *S. occidentalis* leaves

Table 1: Effect of oral administration of different doses of ethanolic leaf extract of *S. occidentalis* on PCVs (mean ± SD) in *T. brucei brucei* infected rats (n = 5)

	Uninfected (normal) control	Extract control	Infected untreated control	Infected + 100 mg/kg b.w. of the extract	Infected + 200 mg/kg b.w. of the extract	Infected + diminal (80 mg/kg)
Preinfection PCV (%)	43.20 ± 1.14	43.00 ± 1.14	44.40 ± 1.49	42.40 ± 2.79	43.8 ± 1.38	45.6 ± 82
Terminal PCV (%)	47.00 ± 1.20	45.00 ± 1.12	34.60 ± 1.50	36.50 ± 2.19	37.4 ± 1.75	41.6 ± 1.52
% Change in PCV	+9.25 ± 2.26[a]	+5.12 ± 2.7[a]	−21.70 ± 2.70[b]	−13.60 ± 7.98[c]	−14.6 ± 7.98[c]	−8.77 ± 16.4[d]

Values with different superscripts along a row are statistically significant ($P < 0.05$); *positive signs (+) indicate increases and negative signs (−) indicate decreases

Table 2: Effect of oral administration of different doses of ethanolic leaf extract of *S. occidentalis* on hepatic and renal function (mean ± SD) of *T. brucei brucei* infected rats (n = 5)

Biochemical parameters	Uninfected (normal) control	Extract control	Infected untreated control	Infected + 100 mg/kg b.w. of the extract	Infected + 200 mg/kg b.w. of the extract	Infected + diminal (80 mg/kg)
Aspartate aminotransferase (IU/l)	18.31 ± 1.52[a]	20.26 ± 2.92[a]	32.07 ± 2.51[b]	28.33 ± 2.08[b]	24.50 ± 1.20[c]	19.05 ± 1.01[a]
Alanine aminotransferase (IU/l)	30.21 ± 2.05[a]	35.66 ± 3.50[a]	47.66 ± 4.51[b]	38.33 ± 3.21[a,c]	36.33 ± 2.30[a,c]	32.35 ± 5.29[a,c]
Urea (mM)	91.33 ± 6.02[a]	130.00 ± 5.21[b]	227.91 ± 6.43[c]	136.05 ± 6.13[b]	132.00 ± 5.29[b]	126.81 ± 5.90[b]
Creatinine (mM)	34.34 ± 4.70[a]	39.35 ± 4.93[a]	131.68 ± 14.70[b]	80.27 ± 3.60[c]	62.32 ± 8.73[d]	74.33 ± 1.23[e]

Values with different superscripts along a row are statistically significant ($P < 0.05$)

Table 3: Effect of oral administration of different doses of ethanolic leaf extract of *S. occidentalis* on relative organ weights (Mean ± SD) of *T. brucei brucei* infected rats (n=5)

Groups	Liver:body weight ratio ($\times 10^{-2}$)	Spleen:body weight ratio ($\times 10^{-2}$)	Kidney:body weight ratio ($\times 10^{-2}$)
Uninfected (normal) control	3.70 ± 0.98[a]	0.84 ± 0.43[a]	0.85 ± 0.37[a]
Extract control	3.5 ± 0.78[a]	1.0 ± 0.39[a]	1.04 ± 0.39[a]
Infected untreated control	5.7 ± 0.24[b]	2.19 ± 0.38[b]	1.20 ± 0.32[a]
Infected + 100 mg/kg b.w. of the extract	4.3 ± 1.36[c]	1.43 ± 0.15[c]	1.13 ± 0.25[a]
Infected + 200 mg/kg b.w. of the extract	5.2 ± 0.28[d]	1.6 ± 0.09[d]	1.13 ± 0.42[a]
Infected + diminal (80 mg/kg)	5.3 ± 0.35[e]	1.30 ± 0.16[c]	1.16 ± 0.56[a]

Values with different superscripts within a column are statistically significant ($P < 0.05$)

effects of the extract on the disease-induced pathologic changes.

The observed *in vitro* antitrypanosomal activity of *S. occidentalis* leaves' ethanolic extract is not surprising since previous reports[10,21-23] have clearly demonstrated that plants of different families could possess antitrypanosomal activity. Since an extract with high *in vitro* activity may show no *in vivo* antitrypanosomal activity and vice versa, due to the biotransformation of plant materials that may convert active therapeutic molecules to inactive ones, we further tested the extract for *in vivo* activity so that a conclusive statement can be made on the antitrypanosomal action of this plant.

The *S. occidentalis* ethanolic extract trypanosuppressive activity in a dose-dependent manner supports earlier reports that some plant extracts contain potent trypanocides.[9-12] Although the exact mechanism for the observed *in vivo* trypanosuppressive effect is not known, it is obvious that the

extract contains some phytochemicals that could interfere with the survival of the parasites *in vivo*. However, this work did not involve structural elucidation but the presence of alkaloids, flavonoids, tannins and anthraquinones have been reported in this extract[24] and previous reports attributed the antitrypanosomal activity of a number of tropical plants to the flavonoids (azaanthraquinone), highly aromatic planar quaternary alkaloids, barbarine and harmaine.[25,26] It is thus possible that the observed trypanosuppressive action of this extract could be due to the presence of one or more of these bioactive compounds.

The acute anemia recorded in the *T. brucei* infected rats is a consistent feature of trypanosome infections[5,27] and the extract treatment was able to significantly ($P < 0.05$) ameliorate the disease-induced anemia. Perhaps the ability of the extract to lower the parasitemia could translate into the amelioration of the trypanosome-induced anemia since the degree of parasitemia has been linearly linked to the severity of anemia.[27] It is also possible that this extract

possesses some antioxidant activities that could scavenge *T. brucei* generated free radicals which are implicated in the development of anemia during trypanosomiasis.[3,5,27-29] We have previously reported the amelioration of trypanosome-induced anemia and organ damage by some antioxidant vitamins.[3,5]

While previous reports focus on establishing the *in vitro* and/or *in vivo* antitrypanosomal action of medicinal plants only, this investigation further assesses the ability of the extract to reduce the trypanosome-associated pathologic changes. The trypanosome-induced hepatocellular damage, monitored by hepatic cell leakage of ALT and AST, was invariably eliminated by the extract treatment. Furthermore, the damage to renal structures as reflected by an increase in serum concentrations of urea and creatinine during infection was also significantly ($P < 0.05$) prevented by the extract administration. These could all be attributed to the ability of this extract to reduce the degree of parasitemia and/or due to the presence of some antioxidant activities in it, consequently decreasing the damage to hepatic and renal cells. The extract-treated uninfected rats did not show much difference in the biochemical parameters from the normal rats. This is indicative of the relative safety of the extract administration in the animals.

Hepatomegaly and splenomegaly consistently reported[5,30] in trypanosomiasis were also observed in this investigation. The enlargement of liver and spleen is caused by the activation and expansion of the reticuloendothelial system during trypanosome infection. The extract administration also prevented, to a lesser extent, the disease-induced hepatomegaly and splenomegaly. This observation could further lend credence to the above results on biochemical parameters and that the ethanol extract of *S. occidentalis* could ameliorate trypanosome-associated organ damage.

We concluded that some ethanol-extractable phytochemicals from *S. occidentalis* leaves possess both *in vitro* and *in vivo* antitrypanosomal activities and tend to ameliorate the trypanosome-induced anemia and organ damage. The findings as a whole further support the fact that cheap, easily available and relatively safe/nontoxic trypanocides could be developed from a tropical plant. We are presently working on detailed bioassay-directed fractionation to identify and structurally elucidate the bioactive compound(s) responsible for the results observed herein.

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