INVESTIGATION OF THE ANTITRYPANOSOMAL ACTIVITY OF *BUCHHOLZIA CORIACEA* SEED EXTRACT AGAINST A FIELD STRAIN OF *TRYPANOSOMA CONGOLENSE*

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Abstract

The antitrypanosomal activity of the methanol extract of *Buchholzia coriacea* seed against a field strain of *Trypanosoma congolense* was investigated using experimentally infected mice of both sexes. Monitoring of parasitaemia was by the rapid matching technique. When parasitaemia was approximately log 7.8 (63 x 10^6 parasites/ml), treatment with graded doses of the extract (250, 500 and 1000 mg/kg) was instituted for 5 consecutive days. Diminazene diaceturate (Dimivet® SKM Pharma Pvt. Ltd.) was given at 3.5 mg/kg i.p. to the positive control mice. No significant differences in body weights were observed. The rectal temperatures of infected mice showed fluctuations. The PCV of infected mice were significantly ($p < 0.05$) lower than those of the uninfected controls. There was no significant difference between the PCV of the extract-treated and untreated animals. Parasitaemia increased steadily in the extract-treated and untreated mice groups till all the animals died. Three days post-treatment with diminazene diaceturate parasitaemia was cleared. Six days later, there was a relapse of infection. By the end of the experiment, a 50 % relapse rate was recorded in the diminazene diaceturate-treated group. The methanol extract of *Buchholzia coriacea* seeds did not show any antitrypanosomal activity in mice infected with *Trypanosoma congolense* at the doses tested.

Key words: *Trypanosoma congolense*, *Buchholzia coriacea*, Antitrypanosomal activity.

Introduction

African animal trypanosomosis (AAT) is mainly caused by *Trypanosoma congolense*, *T. vivax* and *T. b. brucei.* It is one of the most important diseases of domestic livestock in sub-Saharan Africa. The disease is most important for cattle but also pigs, camels, goats and sheep are affected (Aderbauer et al., 2008). Infections of livestock as well as companion animals like dogs with *T. congolense* and *T. b. brucei* are very common especially, in South Eastern Nigeria (Omamegbe et al., 1984). Trypanosomosis is endemic in this part of the world. This is because of the typical rain forest ecology which favours the growth and spread of the tsetse flies responsible for the disease transmission. Trypanosomosis is a major setback to animal production in this area since virtually all livestock species are susceptible to one or more species of trypanosomes.

Control of trypanosomosis is mainly by chemotherapy however, few drugs are presently available. The available drugs are old, toxic and often too expensive for the rural farmers. There are often cases of relapse of infection after treatment and of growing parasite resistance. All these underscore the need for new, effective and inexpensive drugs for the treatment of trypanosomosis. Plants have always been among the common sources of medicaments, either processed as traditional preparations, or used to prepare pure active principles (Freiburghaus et al., 1996). In Africa, herbal treatment has a long tradition and still holds a strong position in medical care. In Nigeria, traditional healers use medicinal plants either alone or in combination to treat both human and animal trypanosomosis (Wurochekke and Nok, 2004). Several reports exist on the herbal treatment of sleeping sickness (Asuzu and Chineme, 1990; Asuzu and Anaga, 1991; Wurochekke and Nok, 2004).

The seeds of *Buchholzia coriacea* Engler (Capparaceae) are folklorically used in the treatment of feverish conditions in Eastern Nigeria (Nweze et al., 2009). They are chopped up and soaked overnight in the local gin. The infusion is drunk for the cure of such ailments as malaria in humans. Malaria and AAT are two important protozoan diseases which are endemic in Nigeria. The ethanolic extract of *Buchholzia coriacea* has been shown to have antitrypanosomal activity in mice experimentally infected with *Trypanosoma brucei brucei* (Nweze et al., 2009). At the dose of 1000 mg/kg i.p. for 3 consecutive days, the methanol extract was able to clear the parasites from peripheral blood circulation. *Buchholzia coriacea* leaves have been shown to have anthelmintic effects on *Fasciola hepatica* (Ajaiyeoba et al., 2001). The ethanolic extract of *Buchholzia coriacea* seed caused larval deaths of the infective stage larvae of *Haemonchus contortus* and *Heligmosomoides polygrus* at various concentrations *in vitro* (Nweze and Asuzu, 2006). Fractions prepared from the methanolic extract of *Buchholzia coriacea* stem back exhibited a high concentration-dependent antibacterial and antifungal activity comparable to standard antibiotics such as Ampicillin and Tioconazole (Ajaiyeoba et al., 2001).

The antimicrobial properties of the fresh seeds and extracts of *B. coriacea* were investigated by Ezekiel and Onyeoziri (2009). The fresh seed as well as the hexane and methanolic extracts showed antimicrobial activities against some food borne bacteria like *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Trichoderma viride* and *Aspergillus niger*. The zones of inhibitions ranged from zero to 62 mm depending on the susceptibility of the tested organism and the plant preparation used but the fresh seeds were found to be most active.

The aim of this study was to evaluate the antitrypanosomal activity of *B. coriacea* seed extract in mice experimentally infected with a field isolate of *T. congolense*.

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Materials and methods

Plant materials

Mature seeds of *Buccholzia coriacea* Engler. were collected in February, 2008 and identified by a taxonomist Mr A. O. Ozioko of Bioresources Development and Conservation Centre (BDCP), Nsukka. The seeds were pulverised into fine powder in a mill. The powdered plant materials were stored in sealed cellophane bags until extraction.

Preparation of extracts

Ground *B. coriacea* seeds were defatted with hexane at room temperature by cold maceration with intermittent shaking in a shaker for 72 h. The hexane extract was filtered out while the marc was allowed to dry. The marc was re-extracted using 80 % methanol in water. A ten-fold quantity of solvent in relation to plant material was used for all extractions. All extracts were filtered using size 1 filter papers (Schleicher & Schuell, Germany). The solvents were allowed to evaporate under a hood. For the methanol extract, nitrogen gas was used to evaporate the solvent. To further ensure that all the water was removed, the extract was freeze-dried (Edwards's high vacuum Crawley, England). The extract was stored at 4 ^{0}C until use.

Animals

Thirty-six in-bred mice of both sexes were obtained from the laboratory animal unit of the Department of Veterinary Obstetrics and Reproductive Diseases, University of Nigeria, Nsukka. They were housed under standard environmental conditions of temperature at 25 ^{0}C (± 3 ^{0}C) and 12 h light: 12 h dark cycle. They had an acclimatization period of 7 days before the start of the experiment. They were fed *ad libitum* with pelletized growers mash containing 18 % crude protein and had free access to drinking water.

Trypanosome stock

Trypanosoma congolense was obtained from stabilates stored in liquid nitrogen at the Nigerian Institute for Trypanosomiasis Research (NITR) Vom, Plateau State, Nigeria. The parasites were inoculated into rats. The inoculated rats were monitored daily for parasitaemia by examining microscopically at x 40 magnification a drop of blood collected from the tail. Blood collection was by nicking a tail vein with a sterile needle or lancet. Complete haemostasis was achieved before placing the animal back into the cage. Trypanosome counts were estimated by using the rapid matching technique of Herbert and Lumsden (1976). When parasitaemia reached log 8.1 (125 x 10^6 parasites / ml), 1 ml of the parasitaemic blood was collected via the periorbital plexus into a vacutainer tube containing 9 ml of normal saline and 0.2 ml of it was used to infect the experimental animals intraperitoneally.

Evaluation of extract for antitrypanosomal activity

Thirty-six mice weighing an average of 30 g were used for the experiment. They were divided into 6 groups of 6 mice each. Five groups were infected with *Trypanosoma congolense* obtained from the Nigerian Institute for Trypanosomiasis Research (NITR). They were infected intraperitoneally with 0.2 ml of blood containing an absolute number of 5 x 10^6 trypanosomes / mouse. By 12 days post infection, infection had established in all the animals except three. By 14 days post infection when parasitaemia was approximately log 7.8 (63 x 10^6 parasites/ml) treatment with graded doses of the extract (250, 500, 1000 mg/kg) was administered for 5 consecutive days to 3 different groups of mice (groups A, B and C). Diminazene diaceturate (Dimivet®) was given to mice in group D at a dose of 3.5 mg/kg intraperitoneally. Mice in group E were the infected untreated controls while those in group F were uninfected. Parasitaemia and packed cell volume (PCV) were monitored as already described at two days intervals. Body weight and temperature were monitored weekly. Body weight was measured by the use of a sensitive electronic weighing balance while temperature was assessed by means of a clinical thermometer per rectum.

Analysis of data

All data were subjected to statistical analysis using one-way analysis of variance (ANOVA) to determine significant difference between the means. Differences were considered significant at $P < 0.05$.

Results

There was no significant difference between the mean body weights of mice in all the experimental groups. This suggests that parasitaemia and / or treatment had no effect on the body weights of the experimental animals. This result is presented in Fig 1. Gain in body weight was gradual throughout the course of the experiment except for the animals in group D (Diminazene diaceturate-treated group) which lost weight after relapse of infection.

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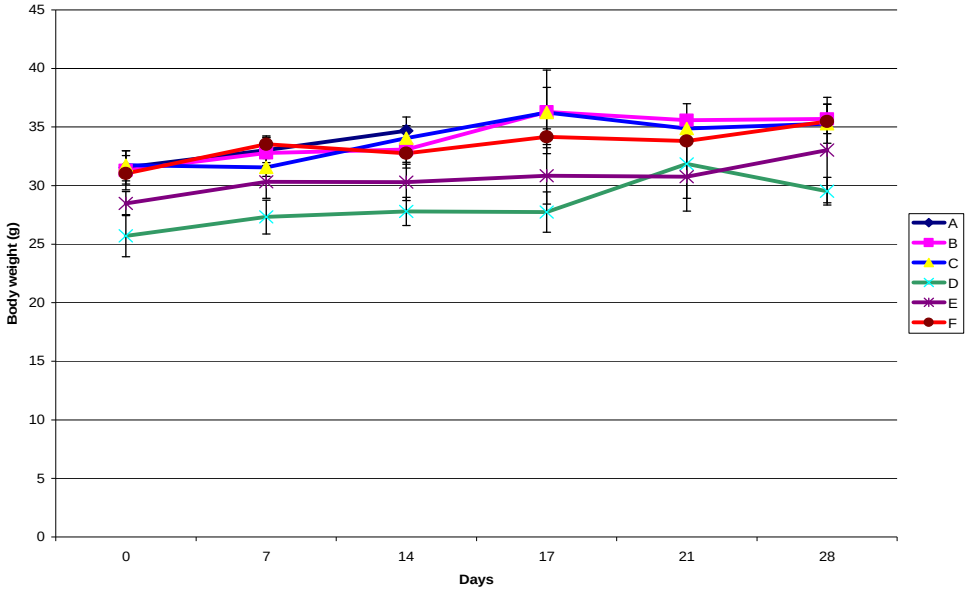

Figure 1: Mean group body weight (g) of *Trypanosoma congolense* infected mice treated with methanol extract of *B. coriacea* seed.
(A – 250, B – 500, C – 1000 mg/kg of *B. coriacea* methanol extract; D – diminazene diacaeturate 3.5 mg/kg; E – infected untreated; F- uninfected).

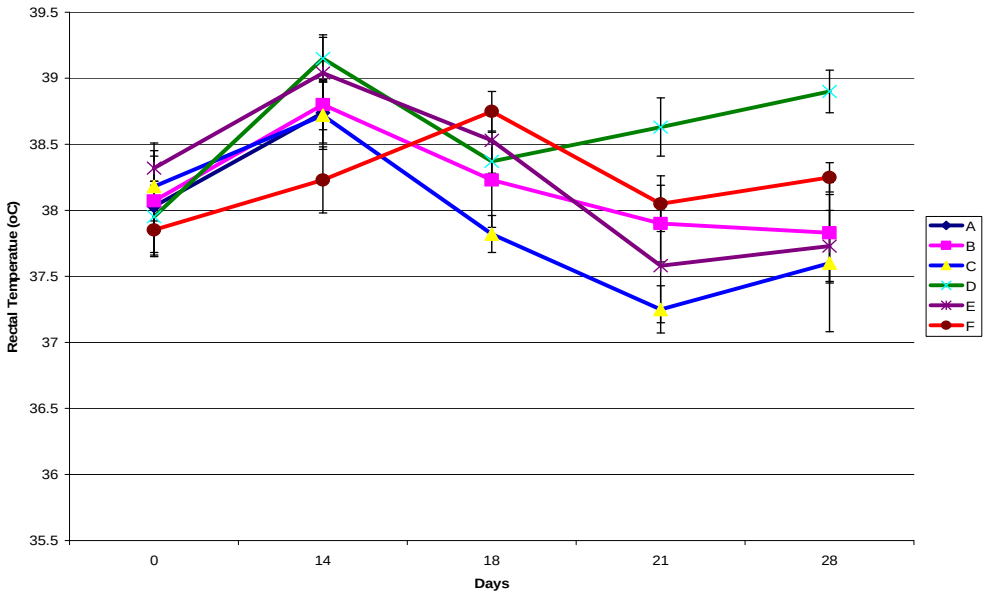

Figure 2: Mean group rectal temperature (^{o}C) of *Trypanosoma congolense* infected mice treated with methanol extract of *B. coriacea* seed**.**
(A – 250, B – 500, C – 1000 mg/kg of *B. coriacea* methanol extract; D – diminazene diacaeturate 3.5 mg/kg; E – infected untreated; F- uninfected).

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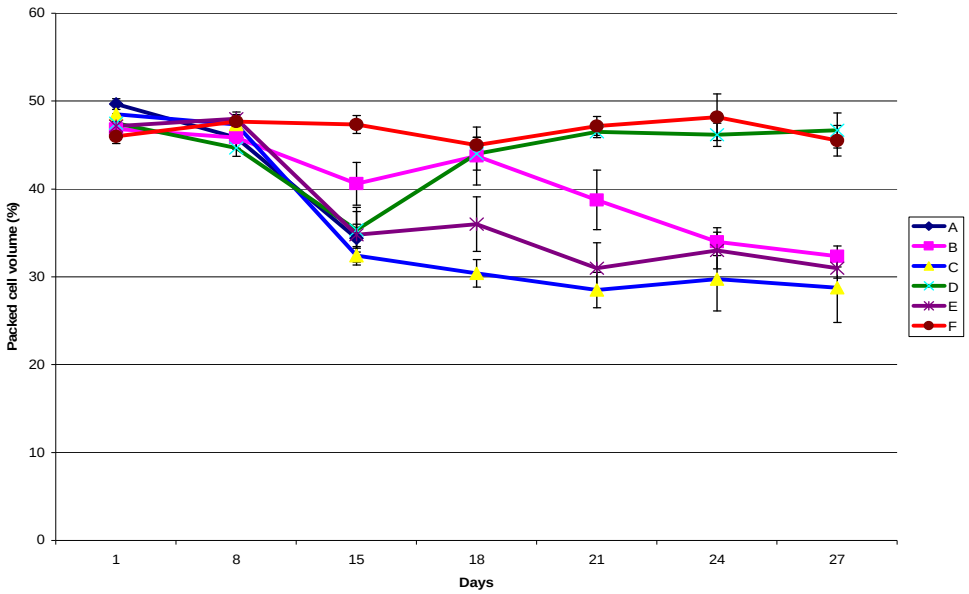

Figure 3: Mean group packed cell volume (%) of *Trypanosoma congolense* infected mice treated with methanol extract of *B. coriacea* seed.
(A – 250, B – 500, C – 1000 mg/kg of *B. coriacea* methanol extract; D – diminazene diacaeturate 3.5 mg/kg; E – infected untreated; F- uninfected).

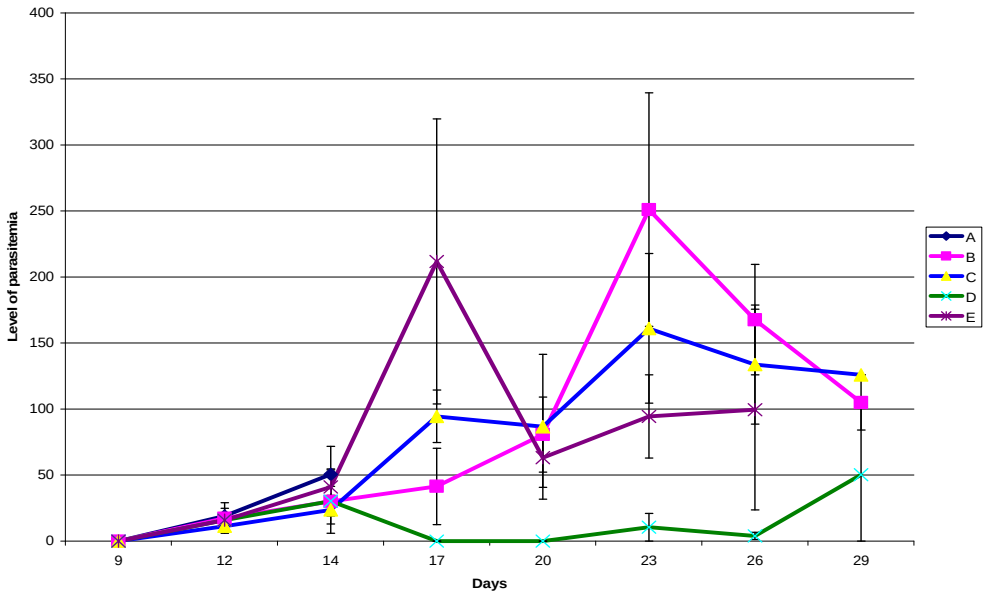

Figure 4: Mean group parasitaemia (10^6) of *Trypanosoma congolense* infected mice treated with methanol extract of *B. coriacea* seed**.**
(A – 250, B – 500, C – 1000 mg/kg of *B. coriacea* methanol extract; D – diminazene diacaeturate 3.5 mg/kg; E – infected untreated; F- uninfected).

The rectal temperatures of the animals were fluctuating throughout the experiment. The result is presented in Fig 2. There was a sharp rise in body temperature for all the groups except group F (the uninfected controls) on day 14 which coincided with the onset of parasitaemia on day 12 post infection. This observed difference though was not statistically significant.

The result of the packed cell volume (PCV) is shown in Fig 3. There was a sharp drop in PCV of all the experimental groups except the uninfected controls from days 8 to 15 post infection. By day 15, this difference in PCV

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between the infected and uninfected groups was significant ($P < 0.05$). This drop in PCV coincided with the establishment of infection and onset of parasitaemia which was observed as from day 12 post infection. By day 18 post infection, there was no significant difference between the PCV of groups D (the Diminazene diaceturate-treated group), F (uninfected control) and B (untreated control). As from day 21 post infection the PCV of the mice in group B began to go down steadily till the end of the experiment. There was no statistical difference between the PCV of mice in group B and the extract-treated groups from day 24 to the end of the study.

Parasitaemia was observed in all the infected groups 12 days post infection. The level of parasitaemia increased in all the infected groups but in a fluctuating manner except for the mice in group D (Diminazene diaceturate-treated group). After the institution of therapy on day 14 post infection, there was zero parasitaemia in group D mice 3 days post treatment. But after 9 days post treatment there was a 50 % relapse rate recorded. The other 50 % of the group D animals remained cured till the termination of the experiment. For the extract-treated groups, parasitaemia was on the increase till all the animals died off. The result showing levels of parasitaemia is presented in Fig 4.

Discussion

In this experiment, the observed prepatent period of *Trypanosoma congolense* in mice was 12 days. In a similar experimental inoculation of *T. congolense* in mice, Nok (2002) recorded a prepatent period of just 3 days. Generally, *T. congolense* has a longer prepatent period than *T. brucei*. For example, balb/c mice were infected intraperitoneally with 2 x 10^6 *T. b. brucei* organisms and by two days post infection (PI) they were already parasitaemic (Kubata et al., 2005). Length of prepatent period is determined by the strain of the parasite in question and host immune status. Different strains of *T. congolense* differ in their pathogenicity (Bengaly et al., 2002).

There was no significant difference observed between the body weight measurements of infected and uninfected mice in this experiment. Also there was no significant difference between infected mice treated with *B. coriacea* seed extract and those treated with the standard drug, diminazene diaceturate. This is not surprising since the infection had an acute course. Emaciation is a feature of chronic infections of animals with trypanosomes. Infected animals suffer from anaemia and emaciation and most die if untreated (Brun and Lun, 1994).

Animals infected with trypanosomes characteristically exhibit fever in addition to other non-specific host defence mechanisms (Kluger, 1986). Pyrexia in trypanosomosis is caused by trypanolytic crisis which enhances red blood cells damage and destruction leading to anaemia (Anosa, 1988). This was shown in the present study by the occurrence of febrile peaks during periods of parasitaemia with attendant fall in packed cell values (Figs 2, 4 and 3). The elevation in body temperature results in an enhancement of the immune response by increased mobility and activity of the white blood cells. It is also postulated that the high body temperature itself is detrimental to the trypanosomes (Zwart et al., 1990). Other workers have also reported anaemia in animals as a result of *T. congolense* infection (Abenga et al, 2005; Bengaly et al., 2002; Dargie et al., 1979).

From the result of mean group parasitaemia (Fig. 4) the methanol extract of *B. coriacea* seed had no antitrypanosomal effect in *T. congolense* infected mice at the tested doses. Parasitaemia was on the increase till the infected animals all died. Diminazene diaceturate showed 100 % therapeutic efficacy in the treated mice at the standard dose of 3.5 mg/kg but with a 50 % relapse rate after 9 days. This highlights one of the aforementioned constraints of trypanosome therapy which is the occurrence of relapse even in a case where treatment is effective. This study with the methanol extract of *B. coriacea* seed highlights the fact that a drug can have different activities on different species of the same organism. Whereas this extract showed antitrypanosomal activity against *T. brucei* infection in mice, it has proved ineffective against *T. congolense* infection at the same doses.

There have also been reports on the different susceptibilities of *T. b. brucei* and *T. congolense* to chemotherapeutic agents. In a study of the antitrypanosomal activities of 34 different alkaloids, *T. congolense* was found to be less susceptible than *T. b. brucei* (Merschjohann et al., 2001). In another study involving the antitrypanosomal activity of niclosamide, *T. congolense* was found to be 10 times less susceptible than *T. b. brucei* (Merschjohann and Steverding, 2008).

The susceptibility of different trypanosome species to different chemotherapeutic agents vary. A good example of this selective activity is the case of α- DFMO which is active against *T. b. gambiense* but refractory to *T. b. rhodesiense* (Brun et al., 2001). This is so because DFMO is a covalent inhibitor of ornithine decarboxylase (ODC), the enzyme that commits ornithine to polyamine synthesis. *Trypanosoma brucei rhodesiense* is insensitive to DFMO due to a faster turnover of ODC (Iten et al., 1997). The innate insusceptibility of *T. b. rhodesiense* is said to be a case of drug tolerance, not resistance (Maser et al., 2003).

In conclusion, the methanol extract of *B. coriacea* did not show any antitrypanosomal activity in mice infected with *T. congolense* at the tested doses.

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