Preliminary Studies on the Efficacy of *Aloe vera* (*Aloe barbadensis*) Extracts in Experimental *Trypanosoma brucei brucei* Infection of Mice

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Abstract

Extracts of *Aloe vera* were administered to experimentally-infected mice with the Nigerian strain of *Trypanosoma brucei brucei*. The thirty two (32) mice involved in the study were split into eight (8) groups of 4 mice each and treated intraperitoneally with the aqueous and ethanolic *Aloe vera* extracts of 40, 80, 20 mg/kg body weight of aqueous extracts and 3.5 mg/kg (Berenil®). The untreated (control) group recorded progressive increase in the level of parasitaemia, while the group treated with 3.5 mg/kg (standard trypanocidal drug) achieved aparasitaemia for 3 days post treatment. The toxicity of the extracts to the parasite was concentration-dependent. 80 mg/kg aqueous extract showed the most significant reduction in parasitaemia and effected the most dramatic post treatment reduction. This was followed by 40 mg/kg aqueous extract treatment regimen. The haematocrit values decreased with increasing concentrations of both the aqueous and ethanolic extracts.

Keywords: *Aloe vera*, trypanosomiasis, parasitaemia, mice, aqueous extract, ethanolic extract.

Introduction

Trypanosomiasis (sleeping sickness) is caused by the protozoan flagellates, trypanosomes that are transmitted to humans by the bite of various species of the haematophagous (blood-sucking) tse-tse flies (*Glossina* spp). The disease occurs in 36 sub-Saharan countries, between latitudes 14°N and 29°S within the limits of the geographical distribution of the tse-tse fly vector (WHO 1998). Human African trypanosomiasis is a major public health problem in endemic countries. Over 600 million people living in about 250 locations within the region of its distribution are estimated to be at risk of contracting the disease and more than 25000 people die from it annually. Besides, the infection kills about 3 million livestock yearly at an annual loss of US $600 million to US. $1.2 billion (WHO. 1998; Maurice, 2001); while about 10 million km^2 of African land mass are tse-tse infested (Chadenga, 1994). The case-fatality rate in untreated patients is 100% and the fear of the disease has led to abandonment of fertile lands. At the individual, family and community levels the disease is known to cause abortion, sterility and gynaecological disorders in women while it is responsible for physical growth and intellectual growth retardation in children (WHO 1969; Kuzoe; 2001). Aroke, *et al* (1998) reported that, in the past, *T.b. gambiense* sleeping sickness in children had an influence on their attainment of sexual maturity.

There has been a resurgence of interest in human African trypanosomiasis following observed recrudescence of the disease in endemic countries of sub-Saharan Africa. The disease occurs in two forms: the chronic form caused by *Trypanosoma brucei gambiense* sub-species, (which occurs in West and Central Africa) and the acute form caused by *Trypanosoma brucei rhodesiense* which occurs in Central and Southern Africa. The chronic infection lasts for years but the acute phase may last only for weeks before death occurs if treatment is not administered. Another sub-species, *Trypanosoma brucei brucei* only infects animals causing the disease called 'nagana' in livestock and is considered one of the most important infectious diseases holding back the development of livestock production in much of Africa. Natural immunity of humans to *T. b. brucei* infection has been attributed to the presence, in normal human serum of lytic factors for the parasites (Raper *et al*; 1996).

No vaccines exist against human African trypanosomiasis and the prospects of prophylactic immunization are poor since the parasites are capable of changing their surface coat periodically in the process of antigenic variation. Drugs remain the principal means of intervention. Five drugs are currently used against human African trypanosomiasis (Pepin and Milord, 1994), the drugs of choice depending on the infecting sub-species of *Trypanosoma brucei* involved and on whether the disease is diagnosed before or after the parasites have become established within the central nervous system (Barret, 2000) . Pentamidine and suramin are the drugs of choice for the early–stage of the disease, while melarsoprol is the drug of choice for the later–stage of the disease. Eflornithine, developed in 1990 has not been commercialized because of its prohibitive cost.

DL-α-difluromethylornithine (DFMO) developed in the late 1970s as an antitumour agent is effective against *T. b gambiense* forms but not active against *T. b. rhodesiense* infection. The cost of DFMO remains a limiting factor to its wider use (Doua, 2001). Besides, all these standard antitrypanocidal drugs are not easily available, possess adverse side effects and require hospitalization for administration by the parenteral route. This mode of treatment does not allow their use under rural conditions where health facilities are usually not adequate. As a result of these factors there is need for more studies to investigate and evaluate the chemotherapeutic potentials of plant materials for use in the control of trypanosomiasis.

In many parts of the world the use of plant products in treating various infections and disorders have been well documented. The *Aloe vera* plant, - a native of the semi arid and arid zones of Africa and America is a perennial herb closely related to the onion, garlic and asparagus plants. The herb, which has been domesticated, possesses thick succulent basal leaves with pale brown flowers (Ayensu, 1978; Sofowora, 1982; Iwu, 1993). For medical and therapeutic purposes the latex is the yellowish liquid extracted from underneath the outer epidermis of the leaf. The use of *Aloe vera* plant for medicinal purposes dates back to the biblical periods. The herb has been rediscovered and Davis and Kabani (2001) have recently demonstrated its use in the treatment of various medical problems. The antiarthritic activity of the anthraquinones found in the plant has been shown. (Davis and Agnew, 2000).

This study therefore aims to investigate the potency of the *Aloe vera* plant extracts in the treatment of *Trypanosoma brucei brucei* infection in mice. The findings are presented in this report.

Materials and Methods

Preparation of plant extracts: The *Aloe vera* plants (age 3 yrs) were obtained from a private *Aloe vera* farm in Nsukka Campus of the University of Nigeria. The stem of the Aloe sp (*Aloe barbadensis*) was cut into tiny pieces, air dried for 72 hours and the gel part scrapped out and used in the preparation of the aqueous and ethanolic extracts needed for the study. The aqueous extract was obtained by dissolving 400 g of Aloe gel with 200ml of distilled water, the mixture filtered and the filtrate serving as the extract. The ethanolic extract was prepared by dissolving 300g of Aloe plant gel with 300ml of 70% ethanol for 72 hours. The solution was centrifuged at 500 rpm for 5 minutes, allowed to stand for the alcohol to evaporate and the extract stored at 4 ^{o} C until use.

Source of the trypanosomes: An albino rat previously infected peritoneally with the strain of *Trypanosoma brucei brucei* and confirmed to be suffering from animal trypanosomiasis served as the reservoir of the parasites. 1 ml of the parasitaemic blood was obtained and dissolved in 1ml of phosphate buffer solution then mixed with the anticoagulant ethylene-diaminetriacetate acid (EDTA). The parasite load was checked and found to be 1.99 x 10 parasites per ml of blood. Dilution of blood was effected by mixing 0.1 ml from the sample with 99.1 ml of the phosphate buffer solution to give 100 ml.

Experimental animals and design: The experimental mice were obtained from the Faculty of Biological Sciences animal house. A total of thirty-two (32) mice of mean weight 20.1 ± 2.15g were used for the study and segregated into eight (8) groups of four (4) mice per group. The male mice were, *ab initio*, separated from the females to prevent possible copulation, which might influence the outcome of the result. Each mouse was infected with *Trypanosoma brucei brucei* by inoculation with 0.1ml of blood containing 1.99 x 10 trypanosome parasites per ml administered intraperitoneally. The mice were fed chicken growers' mash and water *ad libitum*.

Post–infection parasitaemia was checked after 4 days. Every mouse was screened to ensure that all were positively parasitaemic. Treatment commenced when the level of parasitaemia was at anti-log 8.1 to 9.0 which served as the reference point. Pre-treatment parasitaemia was recorded just prior to treatment, thereafter the various treatment doses were administered intraperitoneally for the groups as follows:

Group 1: All the mice were treated with 40 mg/kg body weight of the aqueous extract

Group 2: Each mouse was treated with 80mg/kg body weight of the aqueous extract.

Group 3: Every member of the group was given the standard trypanocide drug Berenil ® (Diminazene aceturate) at the dose of 3.5mg/kg body weight once.

Group 4: Each component member was given 20 mg/kg body weight of the aqueous extract.
Group 5: Each mouse in this group was treated with 40mg/kg body weight of the ethanolic extract.
Group 6 All the component members were treated with 80mg/kg body weight of the ethanolic extract.
Group 7: Every mouse in the group was treated with 20 mg/kg body weight of the ethanolic extract.
Group 8: This group served as control and did not receive any treatment.

For each mouse, the level of post-infection parasitaemia, and the haematocrit/packed cell volume (PCV) were monitored and recorded daily.

Counting the trypanosomes: The technique adapted for counting the trypanosomes was the rapid matching procedure as described by Herbert and Lumsden (1976). A drop of blood (approximately 20/μl) was placed on a clean, grease-free glass slide. A cover slide (22 x 7 mm) was placed in such a way that the entire blood was then examined under the x 400 magnification of a compound binocular microscope. The number of trypanosomes in each field was counted and each count matched with the log figures obtained from the reference tables. The log tables were subsequently converted to anti-log and absolute numbers reflecting the number of trypanosomes per ml of blood.

Haematological examination and statistical analysis: Blood was collected from the tail of each mouse into heparinised capillary tubes held at 45° to the tail to enable the blood flow up the tube by capillarity. Both ends of the capillary tube were sealed with plasticine, placed in a microhaematocrit and centrifuged for 5 minutes and the haematocrit value read off the microhaematocrit reader. Statistical analysis was done using the analysis of variance (one way ANOVA) at 5 % significance level.

Results and Discussion

The effects of different concentrations of crude aqueous and ethanolic extracts of *Aloe vera* plant of the mean parasitaemic level of mice infected with *T b brucei* are presented in Table 1. Generally there is an initial increase in the level of parasitaemia in all the groups of the experimental mice during the first four days before treatment.

At the commencement of treatment at day 5, the mean parasitaemia in group 3 (containing the trypanocide "Berenil") decreased markedly while varying for all other groups except group 8 (control) which recorded continuous increase in parasitaemia. Generally, the level of mean parasitaemia decreased continuously in group 3 treated with the standard trypanocide Berenil (diminazene aceturate) to the extent that no trypanosomes were detected in the blood of the infected mice 3 days post treatment.

The different concentrations of both the aqueous and ethanolic extracts resulted in the decrease in the mean-parasitaemia level (albeit to varying degrees). Aqueous extracts at 80mg/kg body weight exerted the most dramatic post-treatment reduction of the parasite load, followed by the 40mg/kg aqueous extract, 80mg/kg ethanolic extract achieved the same effect more gradually. Analysis of this result showed that there is no statistically significant difference between the trypanocidal effects of the aqueous and ethanolic extracts (χ^2 = 0.016; df = 1; P < 0.05). However, between treatment significant difference in the levels of parasitaemia exists (χ^2 = 10.71, df 5, p > 0.05).

The effects of *Aloe vera* extracts on the mean packed Cell Volume (PCV) of mice infected with *T. b brucei* are shown in Fig. 1. The haematocrit decreased with increasing concentrations of both the aqueous and ethanolic extracts. In compassion with the untreated control mice (group 8), the haematocrit values were significantly reduced in the treatment groups (χ^2 = 1.21; df = 1, P> 0.05). Mice in the control group (8) showed the most rapid reduction of the haematocrit while those treated with the trypanocide "Berenil" (group 3) showed the least reduction in the packed cell volume. Also the inter-treatment group differences in heamatocrit values was statistically significant (χ^2 = 10.56, df = 5, p > 0.05). The differences of the mean in the various extract groups may be due to experimental error.

The results of the study provide empirical evidence that the aqueous and ethanolic extracts of the *Aloe vera* plant possess antitrypanocidal potentials. The different doses of the intraperitoneal injections generally give rise to different and increased plasma concentrations of the extracts which indicates that absorption of the extract from the gastrointestinal tract may be both rapid and extensive; - factors which may favour *Aloe vera* as a candidate antitrypanocidal herb. The absorption may have been enhanced by the presence of anthraquinone found in the gel (Davis and Agnew, 2000).

Table1: Effect of *Aloe vera* extracts on the parasitaemia (mean ± SE) in mice infected with *Trypanosoma brucei brucei*

Day/ Group	1	2	3	4	5	6	7	8
1	5. ± 0.3	4.2± 2.8	5.8± 2.3	5.4± 0.0	5.5± 0.2	5.9 ± 0. 3	5.4 ± 0.0	5.4 ± 0.2
2	6.2± 0.9	6.3± 0.3	6.9 ± 0.3	6.9± 0.6	6.9± 1.1	7.1± 0.6	7.1±0.6	6.0±0.2
3	6.7±0.7	7.8± 0.3	7.3± 0.7	7.3± 0.7	6.7± 1.0	7.6± 0.7	7.6± 0.7	6.8± 0.7
4	7.5± 0.7	7.8± 0.2	7.9± 0.5	8.0± 0.6	7.5± 0.7	8.0± 0.4	7.9± 0.5	7.3± 0.4
5	8.0± 0.6	8.1± 4.1	7.9± 0.1	8.2± 0.5	7.8± 0.5	7.9± 0.5	7.9±0.5	7.7± 0.5
6	8.2± 4.1	7.8± 3.9	7.7± 0.2	7.9± 0.5	7.7±0.8	8.0± 04	8.3±04	8.3± 0.2
7	7.6± 3.8	7.8± 3.9	7.6± 0.4	7.6± 0.3	7.7± 05	7.2± 0.8	8.0±0.5	8.0± 0.3
8	7.3± 3.7	7.7± 3.9	6.9± 0.0	7.1± 0.6	7.1± 0.6	7.2± 0.8	7.1± 3.6	8.6± 0.4
9	7.2± 3.7	7.5± 3.8	0	7.1± 0.8	7.1± 0.9	7.3± 0.6	7.5± 0.3	8.9± 0.2
10	6.3± 3.2	6.3±3.2	0	5.9± 0.2	6.5± 0.6	6.4± 3.3	6.3± 0.6	8.8± 0.2
11	5.8± 3.0	5.8± 3.0	0	5.9± 0.2	5.9± 0.2	6.0± 2.9	5.6± 28	9.0± 0.0

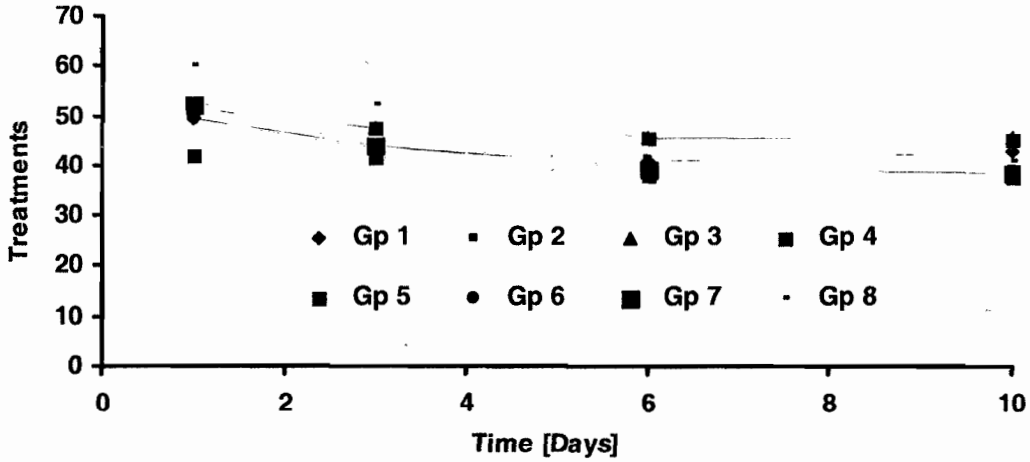

Figure 1: The effects of the Aloe vera extract on the mean Packed Cell Volume (PCV) of mice infected with T.b. brucei.

The decrease in the levels of parasitaemia recorded for the different experimental groups could be compared with that of the standard trypanocidal drug, diminazene aceturate ("Berenil"). The positive reductions of parasitaemia are indications of the antitrypanosome effects of the various concentrations of *Aloe vera*. Analysis shows a significant difference in the levels of parasitaemia between treatment (P<0. 05).

The decrease in the haematocrit indicates a reduction in the number of circulating red cells. It may also occur if the plasma volume increases (Anosa 1988). The latter inference is more likely in the study since the trypanosomes are extracellular parasites and the effect of the extracts are as a result of the increased plasma concentrations of the extractions.

In conclusion, this study shows that the extracts of *Aloe vera*_possess anti-trypanosome effect on mice experimentally infected with *Trypanosoma brucei*. Since the current trypanocidal drugs present adverse side effects, and most are toxic, not readily available and need hospitalization for administration, studies aimed at producing alternative trypanocides should be an area of practical interest and *Aloe vera* appears to be a promising candidate herb.

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