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Research Article

Effect of methanolic leaf extract of *Stachytarpheta jamaicensis* on weight and packed cell volume of mice experimentally infected with *Trypanosoma brucei brucei*

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Abstract

Trypanosoma brucei brucei has detrimentally affected livestock and livestock production. The main means of controlling this disease is by the use of synthetic trypanocides but they are unsafe, unreliable because of reports of their toxic effect, and parasite resistant. Thus, the study was aimed at evaluating the effect of leaf of *Stachytarphyta jamaicensis* on the Weight and Packed Cell Volume (PCV) of mice experimentally infected with *T. brucei brucei.* Leaves of *S. jamaicensis* were extracted using methanol solvent. Thirty (30) mice weighing between 25-40g were divided into 6 groups (group 1- 6) of 5 mice each. Each Mouse was inoculated with 0.2 ml of blood containing an absolute number (2.0×10^6) of *T. brucei brucei* parasites (blood diluted with normal saline to 2 parasites per field / ml intramuscularly/mouse) and were treated with various concentrations of 100, 250 and 500mg/bw of methanol leaf extract of *S. jamaicensis*. Diminazine (Nozomil®) at standard concentration of 3.5mg/bw was used as control. Data collected were analyzed using analysis of variance (ANOVA) at $p<0.05$ level of significance. Result showed that treatment with methanol leaf extract of *S. jamaicensis* resulted in significant increase ($p<0.05$) in weight of the treated mice. There was also a continuous decrease in the PCV values of all the groups of mice infected but treated with the methanol leaf extract of *S. jamaicensis* but this was significantly higher ($P<0.05$) when compared with the infected untreated group. *S. jamaicensis* extract has demonstrated efficacy, which has suggested its anti-trypanosomal properties. It can however be considered as an alternative in the absence of synthetic chemical drugs.

Keywords: *Stachytarphyta jamaicensis;* Packed cell volume; Methanol leaf extract; Anti-trypanosomal property; *Trypanosoma brucei brucei*.

Introduction

Trypanosoma brucei brucei is one of the species of the genus *Trypanosoma* single-celled protozoan parasites. The genus causes parasitic infections known as African trypanosomiasis, which are transmitted primarily through the bite of infected tsetse flies. The disease adversely affects sub-Saharan Africa's entire economy by undermining the health of both humans and animals [1]. Animal African Trypanosomiasis (AAT), also called nagana, is a vertebrate animal disease caused by *T. b. brucei, T. congolense* and *T. vivax, T. evansi* and *T. equiperdum* [2]. Trypanosomes have a glycoprotein coat encoded by antigenically distinct genes, which enables the parasite to participate in an immune-evasive cycle of antigenic differentiation [2].

According to the Food and Agriculture Organization of the United Nations (FAO) [3], African animal trypanosomiasis is probably the only disease that has deeply affected the settlement and economic development of a significant part of the African continent. Saharan countries occupy approximately 9 million km2, an area that corresponds to roughly one-third of the total land area of Africa [4]. Trypanosomiasis affects an estimated 45 to 60 million cattle and tens of millions of small ruminants [5,6]. The FAO estimates that approximately three million cattle die from AAT

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each year. Trypanosomiasis also affects other important animals, such as camels [3].

Stachytarpheta jamaicensis is a medicinal plant commonly known as Blue Flower, Rat Tail or Snake Grass. Due to the presence of diverse bioactive phytochemicals, this plant has been reported to have pharmacological effects. Reportedly, this plant's leaf and stem extracts hold high pharmacological qualities in traditional and folk medicine. These were used to improve gastrointestinal tract activity or to assist with digestive issues such as indigestion, acid reflux, ulcers, constipation, dyspepsia, and poor digestion [7]. Research have shown that this plant's leaves have a beneficial impact in treating allergies and respiratory disorders such as asthma, cold, flu, bronchitis, and cough, as well as cirrhosis and hepatitis [8]. *S. jamaicensis* was also used outside for cleaning of cuts, wounds, ulcers and sores [8]. Studies on acute toxicity of S. jamaicensis has revealed that the plant extract possesses a wider safety margin above 5g/Bw in experimental albino rat [7]. However, this plant origin's pharmacologically active compounds can provide an alternative to chemically synthesized drugs. The objective of this study was to assess the effect of *Starchetaphyta jamaicensis* leaf on the Weight and Packed Cell Volume (PCV) of mice experimentally infected with *Trypanosoma brucei brucei*.

Material and methods

Collection and identification of plant materials

Mature *S. jamaicensis* plants were collected from their natural habitat in Ikot Nkim, Ibesikpo Asutan Local Government Area, Akwa Ibom State. The plant was identified by a Taxonomist in Department of Pharmacognosy and Natural Medicine, University of Uyo, Akwa Ibom State, Nigeria.Voucher number: UUPH 78(b) was given, the voucher specimen was kept in Herbarium unit, Department of Pharmacognosy and Natural Medicine, University of Uyo, Uyo, Akwa Ibom State, Nigeria. The collected plant specimens were taken to Federal University of Agriculture, Department of Biological Sciences' Laboratory for extraction and further experiments.

Experimental animals

Thirty mice of both sexes were obtained from The Laboratory Animal Unit of Nigerian Institute for Trypanosomiasis Research (NITR), Vom, Plateau State, Nigeria. The animals have been treated as specified in the Guide for the Care and Use of Laboratory Animals (DHHS, NIH Publication No. 85-23, 1985). They were allowed to acclimatize the surroundings 7 days before the experiments began. Prior to and during the experiments they had access to clean drinking water and food (Vital Feed) ad libitum.

Preparation of methanol extract

Leaves of *S. jamaicensis* were washed thoroughly to remove sand and other foreign products, using distilled water. They were cut into tiny pieces and dried for two weeks on the laboratory bench. After drying, they were pulverized to obtain a powdery specimen using wooden mortar and pestle along with manual grinder with a periodic sieving using rubber sieve. Pulverized specimens were placed in clear, clean plastic jars which had been labelled. For extraction, the cold extraction method mentioned in Evans (2002) and Sofowora (2006) was adopted[9,10]. 250g of dry powder specimen was macerated (soaked or immersed) in 2.5 litres (2500ml) of methanol at room temperature (27± 2^{o}C). For the maceration, volume of solvents were sufficient enough to completely immerse the corresponding extractions of the dry powder. Maceration was 72hours with periodic stirring. Maceration or cold extraction was preferred because care was taken to preserve phytochemical components of the plant specimens that are easily destroyed by heat.

After maceration, each extract (the whole volume) was filtered repeatedly, thrice using sterile muslin cloth and twice using sterile filter funnel and Whatman No. 1 filter paper with non-absorbent cotton wool. In each case, the filtrate was decanted to remove the marc before further filtration. After filtration, each liquid extract (entire volume of supernatant) was concentrated. Methanol was first evaporated using a rotary evaporator at a temperature of 40^{o}C before it was allowed to concentrate at room temperature.

Weight of dry extracts obtained was determined using an electronic weighing balance (Model number: Labtech® BL2OOO1). Concentrated extracts were packed separately in sterile, labelled, cellophane bags and kept in a desiccator in the air conditioned freeze-drying unit for later use.

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Trypanosoma brucei brucei Stock

Trypanosoma brucei brucei was obtained from the stabilizations established at the Nigerian Institute for Trypanosomiasis Research (NITR), Vom, State Plateau.

Continuous passage in rats kept the parasite in the laboratory until necessary. Passage was deemed appropriate when parasitamea ranged from 16-32 parasites per area. In passage, in 0.1-0.2 ml of blood / PBS solution 1×10^3 parasites were introduced intramuscularly into rats. Approximately 0.1-0.2 ml of the blood (diluted with PBS containing approximately 1×10^3 parasite / ml) was injected into clean mice that were acclimatized for a week under laboratory conditions[11].

Experimental mice groupings and infection

For the experiment, thirty mice weighing between 25-40 g have been used. They had been divided into six groups of 5 mice each. Five groups were infected with *Trypanosoma brucei* isolate (Federe strain), obtained from the Nigerian Institute for Trypanosomiasis Research (NITR) Vom, Plateau State, Nigeria. (It was originally isolated from cattle during an outbreak that occurred in Federe in Jos East Plateau State Local Government Area in 1997 and was cryopreserved in liquid nitrogen; it is known to have been severely passed in albino rats). The mice were inoculated with 0.2 ml of blood containing an absolute number of intramuscular trypanosomes (blood diluted with normal saline to 2 parasites per field)/ml. Parasitaemia has been identified in all mice by the four days after infection. Treatment with graded doses of methanol leaf extracts by six days' post infection. For 3 consecutive days, (100, 250 and 500 mg / kg respectively) was administered orally to 3 different groups of mice (1–3). Studies on acute toxicity of S. jamaicensis by Idu et al. (2007) has revealed that the plant extract possesses a wider safety margin above 5 g/Bw in experimental albino rat [7]. This, this ensured the safety usage of this plant. In group 4, mice were administered intraperitoneally to diminazene aceturate (Nozomi ®) at the normal dose (3.5 mg/kg).

Detailed grouping of mice was as follows

Group 1: *T. brucei* infected mice were treated with 500 mg/kg of methanolic leaf extract of *S. jamaicensis*

Group 2: *T. brucei* infected mice were treated with 250 mg/kg of methanolic leaf extract of *S. jamaicensis*

Group 3: *T. brucei* infected mice were treated with 100 mg/kg of methanolic leaf extract of *S. jamaicensis*

Group 4: *T. brucei* infected mice were treated with Diminazene aceturate (Nozomil®) at standard dose (3.5 mg/kg) given intraperitoneally.

Group 5: Infected mice – non-treated (positive control).

Group 6: Non infected – non-treated (negative control).

Determination of live weight

Using weighing balance (sensitive electronic weighing balance with model number: Labtech ® BL2OOO1), mice in all groups were weighed in grams (g) at a 3 day's interval. Mean weights from each replication were also reported for determining growth rates against a mouse's predicted normal growth curve.

Determination of packed cell volume

Coles' (1968) microhaematocrit procedure was used for evaluating PCV. In short, mice tail blood was collected to one third of the tube into sodium heparinized capillary micro-haematocrit tubes. With plastiseal, the unfilled end of the tube was sealed by pushing the capillary tube down into it. The capillary tube was then centrifuged 5 minutes at 10,000 revolutions per minute (rpm) in a microhaematocrit centrifuge. Using a haematocritic reader the PCV was read off [12].

Data analysis

Input and management of data including PCV levels and body weight using Microsoft Excel (version 2007). Analysis was conducted using version 21 of SPSS, and data values obtained were summarized and expressed as mean ± standard deviation. P values below 0.05 have been deemed significant.

Result and discussion

Effect of treatment on daily live weight of mice infected with T. brucei and treated with different concentrations of methanol leaf extracts of S. jamaicensis.

The result of weight of mice infected with *T. brucei* and treated with *S. jamaicensis* is

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presented in Table 1. The first day of inoculation of T. brucei into the experimental mice was counted as Day 1. In all the infected group (Group 1 – 5), the infection of the mice with the *T. brucei* resulted in all the infected mice exhibiting a reduction in their various live body weight on the 3rd day post infection and this continued progressively till Day 6 of the experiment. However, when various doses of extract of *S. jamaicensis* was administered on the 6th day (day 6) post infection there was a regression in the weight of the treated mice. Compared with the uninfected control group (G6), this was however not statistically important ($p>0.05$).

In comparison, the uninfected control (Group 6) group's progressive weight gain was observed throughout the experiment period. However, on the third day after treatment (Day 9) through day 12, a significant increase ($p< 0.05$) in mice weight after administration of the extracts was observed in the Diminazene treated group (Group 4), Group 1 and Group 2 as compared to the infected untreated group 5 which showed a significant decrease in weight ($p < 0.05$).

The infected untreated group of mice (Group 5) showed a progressive decrease in live body weight followed by mortality of all the mice in the group on the 9th day post infection days. After the infusion of the extracts, all the treated groups (1-3), including the Diminazene treated group administered with different doses of the extracts were able to demonstrate a decrease in weight values around the sample.

Effect of treatment on packed cell volume (PCV) (%) of mice infected with T. brucei and treated with different concentrations of methanol leaf extracts of S. jamaicensis

The result of packed cell volume (PCV) of mice infected with *T. brucei* and treated with *S. jamaicensis* is presented in Table 2. There was a significant decrease ($p < 0.05$) in PCV of all the infected group (Group 1 – 5) on the 6th day post infection when compared with group 6 (Uninfected control group). However, among the treated groups, (group 1-4), Group 2 (44.50±2.33) showed a significant reduction ($p < 0.05$) in the mean PCV values. PCV value was however, significantly higher ($p < 0.05$) in other treated groups when compared to the infected untreated group (group 5) on 4th day (day 9) post treatment through the end of the experiment. Mean PCV values was significantly low ($p < 0.05$) (42.50±00) in the infected untreated group (Group 5) on Day 9 of the experimental Days.

However, there was a progressive reduction in PCV values across the treated group which was significantly different ($p < 0.05$) from the uninfected control group and Diminazene group as recorded on day 14 and 17.

A progressive increase in PCV values of the Diminazene treated group of mice was observed all through the post treatment period of the experiment. Group 5 which served as the infected untreated group recorded a progressive statistically significant drop ($p < 0.05$) in PCV values from Day 6 till the death of all the mice in the experimental group. In contrast, Group 6 which served as the uninfected group (Group 6) exhibited and maintained a stable level of PCV all through the period of the experiment.

The study result shows a progressive reduction in the infected group's weight of mice (Group 1 – 5) on the 3rd day post-infection prior to extract administration on the 6th day (day 6) post-infection period. However, this is a symptom usually associated with trypanosome infection in a non-human host such as rodents, among other signs as neurological symptoms, dependent edema, cardiac lesions, vomiting, keratitis, lacrimation, loss of appetite, irregular fever, anaemia and other clinical signs. This manifestation may be evidence of accumulated tissue / cell damage associated with rapid parasite replication and depends on various factors such as parasite species, viability, infectivity, virulence, tropism, host age, nutritional status and immunological competence. Gerald *et al*. (2010) [2] and Barrett *et al*. (2003)[13] documented the correlation of the adverse impact and appearance of certain signs of *Trypanosoma* infection that results in mortality of severely infected animal host.

Packed volume of cells (PCV) has decreased similarly to that associated with parasitaemia [16,17]. Anaemia is a known clinical manifestation of trypanosomosis. Packed cell volume is common and a critical feature in African trypanosomiasis pathogenesis

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contributing to morbidity and mortality [14,16,17] and manifested in PCV reduction similar to that observed in infected mice in this experiment.

Infected mice treated with *S. jamaicencis* extract in the study displayed significantly higher rates of PCV compared with negative (infected untreated) control group. This may be related to enhanced erythrocyte haemolysis resistance. The ability to protect erythrocytes from haemolysis may be attributed to polyphenols present in extracts of *S. jamaicencis.* Kagira *et al*. (2006) reported the effectiveness of phenolic compound on *T. brucei rhodesiense* infection haematology in vervet monkeys[14]. Moreover, the presence of phenolic compounds in the plant extracts assessed in the study may have also contributed to the prolonged days of survival reported in the infected and treated classes. Sikkema *et al*. (1995) and Udo et al (2020) confirmed that phenols interact with cytoplasmic membranes and change their cation permeability leading to impairment and death of crucial processes in parasite cells [15,16].

Table 1. Mean live weight (grams) (mean±sd) of mice infected with *T. brucei* and treated with different concentrations of methanol leaf extracts of *S. jamaicensis*

	DAY 1	DAY 3	DAY 6	DAY 9	DAY 12	DAY14	DAY 16
Group 1	30.70±5.20	28.03±5.75	26.87±5.75	28.07±5.49a	31.23±8.90a	35.33±10.45^{ab}	32.60±0*^{ab}
Group 2	34.47±8.55	31.43±8.08	29.33±8.48	30.37±8.51a	30.47±10.85^{a}	24.20±0*^{a}	22.00±0*^{ab}
Group 3	27.30±2.11	24.50±1.90*	22.10±1.97*	23.30±1.59*a	23.55±0.63*^{a}	24.10±0a	-*^{b}
Group 4	28.27±2.58	25.97±2.38	24.37±2.40*	25.27±2.25a	26.40±2.07^{a}	27.57±1.80^{a}	28.53±1.65*^{a}
Group 5	27.90±2.36	25.57±1.86	23.46±1.70*	-*^{b}	-*^{b}	-*^{b}	-*^{b}
Group 6	29.00±2.78	29.33±2.80	30.53±3.25b	31.33±2.96^{a}	32.90^{a}	34.17±2.05^{a}	34.29±2.29^{ab}

Values are presented as mean ± standard deviation (SD).Values indicated by asterisk down the group are statistically different compared to the Non- infected control (GRP6) at P<0.05 whereas all values indicated by the superscript (a) down the group are statistically different compared to the Infected control group (GRP5) and values indicated by superscript (b) are statistically different compared to the Dimi group (GRP4).

Table 2. Mean PCV (%) (Mean±SD) of Mice Infected with *T. brucei* and Treated with Different concentrations of Methanol Leaf extracts of *S. jamaicensis*

	DAY 1	DAY 6	DAY 9	DAY14	DAY 17
Group 1	51.43±1.35	49.46±0.71	45.97±3.88*	42.83±2.84^{a}	37.35±1.63*^{ab}
Group 2	50.30±3.03	47.36±3.42*	46.99±3.95	43.75±2.33^{a}	35.80±0.00*^{ab}
Group 3	51.90±0.26	49.22±0.49*	48.74±0.59	39.75±1.06^{a}	-*^{b}
Group 4	52.40±0.17	49.33±0.58*	47.00±5.28	36.70±2.44^{a}	51.46±0.45^{a}
Group 5	51.36±1.18	48.92±0.73*	42.50±0.00*	-*^{b}	-*
Group 6	52.30±0.20	52.20±0.26ab	52.40±0.26a	52.33±0.49^{a}	52.10±0.16^{a}

Values are presented as mean ± standard deviation (SD).Values indicated by asterisk down the group are statistically different compared to the Non- infected control (GRP6) at P<0.05 whereas all values indicated by the superscript (a) down the group are statistically different compared to the Infected control group (GRP5) and values indicated by superscript (b) are statistically different compared to the Dimi group (GRP4).

Conclusions

Stachytarpheta jamaicensis has demonstrated a promising efficacy on the weight and PCV of mice infected with *T. brucei brucei* thus indicating that the plant contains bioactive components which exhibits trypanocidal properties. However, this plant can also be considered in the management of trypanosomiasis.

Conflict of interest

Authors have declared no conflict of interests.

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