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Effect of Aqueous Leaf Extract of *Stachytarphyta Jamaicensis* on Temperature and Parasitaemia of Mice Experimentally Infected with *Trypanosoma Brucei Brucei*

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ABSTRACT

Trypanosomabruceibrucei has negatively affected livestock health and production. Chemotherapy, the main means of controlling this disease is detrimental, unsafe, and unreliable due to reports of parasite resistance and adverse effect of the synthetic trypanocides. Thus, the study was aimed at evaluating the leaf of Stachytarphetajamaicensis on the Trypanosomabruceibrucei. Leaves of Stachytarphetajamaicensis were extracted using methanol and aqueous solvents. 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 of Trypanosomabruceibrucei parasites (blood diluted with normal saline to 2 (2.0 x 10^6) parasite per field) / ml intramuscularly/mouse and were treated with various concentrations of 100, 250 and 500mg/bw of aqueous leaf extract of Stachytarphyta 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. The study showed that treatment with aqueous leaf extract of S. jamaicensis resulted in significant reduction (P<0.05) in Parasitaemia and extension of survival days of the treated mice for additional 8 days against the infected untreated group. There was also a corresponding stability in body temperature values of group of mice infected with Trypanosomabruceibrucei but treated the the extract this was however, statistically significant (P<0.05) when compared with the infected untreated group. The plant extract have shown a positive efficacy based on the obtained data it can however be utilized as an alternative in the absence of synthetic chemical drugs.

Key word: *Stachytarphyta jamaicensis; Temperature; Trypanosomabruceibrucei;*

INTRODUCTION

Trypanosomiasis in animals is one of the major constraints of livestock production particularly in sub-Saharan Africa. The disease has been reported to cause reduction in milk production, weight gain and reproduction and eventually death of the affected animals (Gerald *et al*., 2010). It has also been reported that the disease causes the death of about 3 million cattle a year while 50-70 million animals are exposed to the infection (Ogbadoyi *et al*., 2007).

Trypanosoma b. Brucei resides in the subgenus *Trypanozoon. Trypanosoma b. Brucei* is an extremely polymorphic typanosome occurring as short, stumpy organisms without flagella, long slender organisms with distinct flagella, and intermediate forms that are usually flagellated. Horses, dogs, cats, camels and pigs are very susceptible to *T. b. Brucei* infection. Studies have shown, however, that this organism is widespread in East and West Africa and that it can cause serious disease and high mortality in cattle, sheep, and goats (Hoet *et al*., 2007).

Stachytarphetajamaicensis commonly called Blue flower, Light blue snake weed, Gervao, Rat tail Brazilian tea, verbena cimarrona, rooter comb, or blue porter weed or Snake weed is widely known for its high medicinal importance in traditional and folk medicinal systems in various countries. This plant has been reported to possess pharmacological effects due to the presence of various bioactive phyto chemicals. *S. jamaicensis* has also been reported to be extensively used by the elderly as a cooling tonic for the stomach. The leaf and stem extracts of this plant are usually prepared in the form of tea bag before being consumed. This cooling

tonic is however, consumed to stimulate the function of the gastrointestinal tract or to aid in digestive problems such as indigestion, acid reflux, ulcers, constipation, dyspepsia, and slow digestion (Idu *et al*., 2007). A study conducted by Okwu and Ohenhen (2010) on Isolation and Characterization of steroidal Glycosides from the leaves of *Stachytarphetajamaicensis* revealed that *S. jamaicensis* has often been used to treat allergies and respiratory conditions such as asthma, cold, the flu, bronchitis, and cough, as well as cirrhosis and hepatitis (Okwu and Ohenhen, 2010). The leaf extract of *S. jamaicensis* had also been used externally to clean cuts, wounds, ulcers, and sores (Okwu and Ohenhen, 2010). However, pharmacologically active compounds of this plant origin can provide an alternative to chemically synthesized drugs to which this parasite has become resistant. Thus, the need for this study which aimed at examining the leaf of *Starchetaphytajamaicensis* on Parasitaemia and weight of mice experimentally infected with *Trypanosomabruceibrucei.*

MATERIALS 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, and AkwaIbom State. The plant was identified by a Taxonomist in Department of Pharmacognosy and Natural Medicine, University of Uyo, AkwaIbom 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 , AkwaIbom State, Nigeria. The collected plant specimens were taken to Federal University of Agriculture, Department of Biological Sciences' Laboratory for extraction and futher 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 were maintained in accordance with the recommendations in the *Guide for the Care and Use of Laboratory Animals* (DHHS, NIH Publication No. 85-23, 1985). They were allowed to acclimatize the environment for 7 days before the commencement of the experiments. They had access to clean drinking water and feed (Vital Feed) *ad libitum* before and during the experiments.

Preparation of Methanolic and Aqueous Extracts

Leaf of *S. jamaicensis* was washed thoroughly, using distilled water, to remove sand and other foreign materials. They were chopped into tiny pieces, and both parts were air-dried on laboratory bench, for two weeks. After drying, they were pulverised using wooden mortar and pestle along with manual grinder with a periodic sieving using rubber sieve to obtain a powdery specimen. Pulverized specimens were stored in dry, sterile, labelled plastic jars. Cold extraction described by Harborne (1984) and Sofowora (2006) was used adopted for the extraction.

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Test Organisms

Trypanosomabruceibrucei was obtained from stabilates maintained at the Nigerian Institute for Trypanosomiasis Research (NITR), Vom, Plateau State**.**

The parasite was maintained in the laboratory by continuous passage in rats until required. Passage was considered necessary when parasitaemia was at the range of 16 – 32 parasites per field. In pass aging, 1 X 10^3 parasites were introduced intramuscularly into rats in 0.1 - 0.2 ml blood/PBS solution. For several passages, approximately 80% blood solution (v/v) was obtained by cardiac puncture into 1ml syringe containing 0.2 ml EDTA (1% w/v). About 0.1 - 0.2 ml of the blood collected as described above or blood (diluted with PBS to contain approximately 1 X 10^3 parasite/ml) was injected into clean mice which were acclimatized under laboratory condition for one week.

Groupings and Infection of the Experimental Mice

Thirty mice weighing between 25 - 40g were used for the experiment. They were divided into

6 groups of 5 mice each. Fivegroups were infected with *Trypanosomabrucei* isolate (Federe strain) which was obtained from the Nigerian Institute for Trypanosomiasis Research (NITR) Vom, Plateau State, Nigeria. (It was originally isolated from cattle during an outbreak that occurred at Federe in Jos East Local Government Area of Plateau State in 1997 and cryo preserved in liquid nitrogen; The stabilate is known to have been passaged severally in albino rats). The mice were inoculated with 0.2 ml of blood containing an absolute number of trypanosomes (blood diluted with normal saline to 2 parasites per field) / ml intramuscularly. By four days post infection, Parasitaemia was established in all the mice. By six days post infection, treatment with graded doses of both methanolic and aqueous leaf extract of *S. jamaicensis*(100, 250 and 500 mg/kg respectively) were administered orally for 3 consecutive days to 3 different groups of mice Group (1-3). Diminazeneaceturate (Nozomi®) at standard dose (3.5mg/kg) was given intraperitoneally to mice in group 4.

Mice in group 8 were infected but not treated.

Those in group 9 were not infected at all.

Detailed Grouping of Mice were as Follows

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

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

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

Group 4: *T. Brucei* infected mice were treated with Diminazeneaceturate (Nozomil®) at standard dose (3.5 mg/kg) given intraperiton-eally.

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

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

Determination of Body Temperature

Body temperature was determined per rectum by the use of a digital clinical thermometer (MODE: GF-MT502). Before taking the temperature, the thermometer was properly disinfected using a cotton wool containing methylated spirit. The bulb end was thereafter lubricated with water to reduce friction against the mucous membranes during insertion. Once inside the rectum, the thermometer was slanted to make sure the bulb made contact with the rectal mucous membranes thus avoiding taking the temperature of the faces and was allowed for 1minutesto make the "bib" sound approximately for 60sec before taking the reading.

Quantification of Parasitaemia

The level of Parasitaemia was monitored in each mouse every day by the rapid matching technique as described by Herbert and Lumsden (1976). Briefly, a drop of mouse tail blood was examined first at x10 then x40 magnification using a table microscope. The numbers of trypanosomes in each field were counted. Each counting per field were matched with log figures obtain from the reference table. The log figures were converted to antilog values which were subsequently converted to absolute numbers. This gave the number of trypanosomes per milliliter. Where no trypanosomes were seen in blood, the buffy coat layers were also examined.

Analysis of Data

Data including the Parasitaemia levels and body weight were entered and managed using Microsoft Excel (version 2007). Analysis was performed using SPSS version 21 and Values of data obtained were summarized and expressed as mean ± standard deviation. P values less than 0.05 were considered significant.

RESULT

Effect of Treatmento Daily Live Temperature (^{O}c) Of Mice Infected with T. Brucei and Treated with Different Concentration of Aqueous Leaf Extracts of S. Jamaicensis

The result of temperature of mice infected with *T. brucei* and treated with Aqueous Leaf extracts of *S. jamaicensis* is presented in Table 1. There was a significant increase ($P < 0.05$) in temperature of mice in the infected group (G1 – G3) on third day (DAY 3) post infection as compared with the uninfected group (Group 6). However, the temperature of the treated mice significantly dropped ($P < 0.05$) (ranging from 38.53±0.40 to 36.55±0.64) on 3rd day (Day 9) post treatment period though with degree of variations across the various treated groups upon administration of the tested extract.

However, there was no significant decrease or increase ($P > 0.05$) in the temperature mean values of the treated groups as compared with

the control group on Day 12 of the experiment. A significant fall ($P < 0.05$) in mean temperature value was recorded in Group 3 with a mean temperature value of 36.85±1.06. However, the infected untreated group (Group 5) recorded a significant increase ($P < 0.05$) in temperatures of the mice in the group on the 3rd day post infection but however recorded a continuous drop in temperature from day 6 post infection and significantly decrease ($P < 0.05$) to 32.3°C at day 9 pending the death of all the mice in the group. On the other hand, the uninfected control group (Group 6) maintained a stable body temperature ranging from 38.00±10 to 38.23±0.06 all through the period of the experiment.

Table2. *Mean group Temperature (°C) (Mean±SD) of Mice Infected with T. brucei and Treated with Different concentrations of Aqueous Leaf extracts of S. jamaicensis*

	DAY 1	DAY 3	DAY 6	DAY 9	DAY 12	DAY14	DAY 16
GROUP 1	38.20±20	38.92±0.18*	38.75±0.19	37.46±1.21a	37.40±0.00a	38.80±0.14a	-*
GROUP 2	38.30±0.26	39.66±0.49*	39.46±0.50*	38.40±0.40a	37.70±0.20a	38.06±0.70a	-*
GROUP 3	38.03±0.15	39.26±0.15*	39.03±0.05	38.50±0.99a	36.85±1.48a	30.00±0.00a	-*
GROUP 4	38.10±0.10	39.33±0.21*	39.13±0.15*	38.16±0.15a	38.13±0.11a	38.06±0.11a	38.13±0.15ab
GROUP 5	38.20±0.20	39.73±0.72*	39.53±0.80*	32.30±0.00*b	-*b	-*b	-*
GROUP 6	38.13±0.11	38.00±0.10ab	38.06±0.11ab	38.23±0.06a	38.30±0.10a	38.10±0.10a	38.20±0.20ab

Values are presented as mean ± standard deviation (SD).Values indicated by asterisk down the group are statistically different compared to the Non- infected control (GRP9) at P<0.05 whereas all values indicated by the superscript (a) down the group are statistically different compared to the Infected control group (GRP8) and values indicated by superscript (b) are statistically different compared to the Dimi group (GRP7).

Effect of Treatment on Parasitaemia of Mice Infected with T. Brucei and Treated with Different Concentrations of Aqueous Leaf Extracts of S. Jamaicensis

The effect of S. jamaicensis aqueous leaf extracts on Parasitaemia quantification in infected and treated mice is presented in Table 2. Parasitaemia was established (Prepatent period) in all the infected groups by Day 4 of the post infection period. Despite the treatment given to mice in all the treated groups, Parasitaemia increased steadily from Day 4 to Day 6 (Table 2). However, there was a reduction in Parasitaemia values across the treated groups (G1 – 3) on the 4th Day (Day 9) of treatment when aqueous leaf extracts was administered to the mice though this was not statistically significant ($P > 0.05$) except in the Deminazene treated group (group 4). A steady increase in Parasitaemia was observed in group 4 (infected control group) from 4th Day post infection through Day 9 and this was significantly higher ($P < 0.05$) (491.10) when compared to other groups.

Moreover, there was a decrease in Parasitaemia values across the treated groups (1-3) on Day 9 (95.91±16.54 , 130.80±20.10 and 198.55±4.19 respectively) through Day 12 and this was statistically significant ($P < 0.05$) when compared with the control group (Table 2). However, there was a rise in Parasitaemia values across the extracts treated group (G 1 – 3) on Day 14 resulted in complete elimination of the treated mice on Day 17 of the experiment. Parasitaemia was significantly higher in group all the treated groups on Day 14 of the experiment. The Deminazene treated group (Group 4) was able to achieve a complete reduction in Parasitaemia on Day 14 of the experiment.

Table4. *Mean (±SD) group Parasitaemia (106) of Mice Infected with T. brucei and Treated with Different concentrations of Aqueous Leaf extracts of S. jamaicensis.*

	DAY 1	DAY 4	DAY 6	DAY 9	DAY 12	DAY14	DAY 17
GROUP 1	0	28.30±20.5	148.60±33.90*	103.50±23.90*ab	95.91±16.54*ab	294.68±10.08*ab	-
GROUP 2	0	30.20±20.2	161.70±35.90*	135.40±21.00*ab	130.80±20.10*ab	308.40±98.40*ab	-
GROUP 3	0	58.14±12.30*	188.10±13.70*	171.80±25.90*ab	198.55±4.19*ab	407.70±14.70*ab	-
GROUP 4	0	33.70±15.73	173.00±27.90*	21.10±17.80a	2.98±1.88	0	0
GROUP 5	0	19.66±10.73	121.70±23.90*	491.10±0.00*b	-	-	-
GROUP 6	0	0	0ab	0a	0	0	0

Values are presented as mean ± standard deviation (SD).Values indicated by asterisk down the group are statistically different compared to the Non- infected control (GRP9) at P<0.05 whereas all values indicated by the superscript (a) down the group are statistically different compared to the Infected control group (GRP8) and values indicated by superscript (b) are statistically different compared to the Dimi group (GRP7).

Key: - represent complete mortality of mice in the group.

DISCUSSION

Temperature of mice in the infected group rose on third day post infection as compared with the uninfected group. Fever has been reported to be a primary clinical signs in African Animal Trypanosomiasis (Zwart *et al*., 1990). The elevation in body temperature could probably be as results of an enhancement of the immune response by increased mobility and activity of the white blood cells. Zwart *et al*. (1990) postulated that the high body temperature itself is detrimental to the trypanosomes. Bizimana *et al*. (2006) reported intermittent fever to relatively affect mice induced with *Trypanosomabrucei*.

Aqueous and methanolic leaf extract of *S. jamaicensis* induced trypanocides effect as evident in the suppressed Parasitaemia and extension of survival days which was dose and extract dependent in the treated groups of mice infected with *T. brucei*. This could probably be due to inhibition or impairment of invasion and /or replication and development of *T. Brucei* parasites species in the blood, lymph nodes and spleen, and cerebrospinal fluid of mice treated with the extracts leading to relatively destruction and elimination of the trypomastigote form of trypanosome. In other words, the effect of the extracts may be on the trypomastigote stages of the parasite especially in the blood as well as those of lymph. It could also be attributed to the effect of antioxidant present in both the plants evaluated in the study. Antioxidants are reported to be responsible for the control of parasitic infections during oxidative stress and lipid peroxidation in the blood. Allen *et al*. (1998) reported that antioxidant-rich plants is lethal to parasites due to induced oxidative stress and neutralization of reactive oxygen species which makes it effective in treating protozoan infections. According the author, antioxidant compounds are known to reduce the severity of trypanosoma infections by ameliorating the degree of blood lipid peroxidation. Lipid peroxidation has been reported to be one of the best indicators of the reactive oxygen species (ROS) that induced systemic biological damage of parasite (Popova and Popov, 2002).

The antioxidant system has a cellular protective action against oxidative stress of cell, organs and tissue damage that result from parasitic invasion (Chuenkova *et al.*, 1989; Das *et al*., 1996; Dede *et al*., 2002). It has also been reported to play a role in the protection of the phagocytic leukocytes against their own products and oxygen radicals. Reduced glutathione, an important antioxidant enzyme, reacts with peroxides to remove toxic substances and radicals, since it possesses active sulphydryl group (Novak *et al*., 1991). Although some investigations have revealed that parasitic infection causes change in lipid peroxidation parameters (Kaya*et al.*, 2007;Cam *et al*., 2008).

CONCLUSION

Thus, the plants extract having shown to possess a recommendable ability based on the obtained data hence it can however be used alternatively in the absence of synthetic chemical drugs.

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