



Antitrypanosomal Activity of *Allium porrum* Ethyl Acetate Bulb Extract in *Trypanosoma brucei brucei* Experimentally Infected Wister Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SOO, AUY and MSA designed the study. Author SOO carried out the study, performed the literature search, statistical analysis and wrote the first draft of the manuscript. Authors AUY and MSA managed the analyses of the study and corrected the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The present study investigated the antitrypanosomal activity of crude ethyl acetate bulb extracts of *A. porrum* plant against *T. b. brucei*.

Methodology: Twenty five male albino rats were divided into five groups and infected with 10^6 /ml of *T. b. brucei*. Group 1 was infected and untreated while groups 2-4 were infected and treated with single intraperitoneal injection 50, 75 and 100 mg/kg of ethyl acetate extract of *A. porrum* while a single dose 3.5 mg/kg diaminazene aceturate was administered to group 5 when parasitaemia was established at 10^6 day 5 post infection.

Results: Clinical manifestations of trypanosomoses such as increased rectal temperature, weakness and dullness were observed in experimental rats in all the groups. There was decrease packed cell volume (PCV) and red blood cell count (RBC) in group 1-4 which was not significant ($p > 0.05$) while group 5 had a significant increase ($p < 0.05$) in PCV and non-significant increase

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($p > 0.05$) in RBC post treatment. There were feeble changes in the pre-treatment and post-treatment parasitemia level in the groups treated with ethyl acetate extract of *A. porrum* while there was significant clearance in parasitemia in the control group.

Conclusion: Interperitoneal injection of ethyl acetate extracts of *A. porrum* at the dose and concentration used in this study, has trypanosomal reduction activity in wister rats.

Keywords: *T. b. brucei*; *A. porrum*; ethyl acetate extract; Albino rat; trypanosomes.

1. INTRODUCTION

Trypanosomosis in man and animals is caused by protozoa of the genus *Trypanosoma* including *T. vivax*, *T. brucei* and *T. congolense* [1], resulting in anaemia, weight loss, decreased milk yield, abortion and death [2]. In Africa, 45-50 million cattle are estimated to be at risk of infection, with an estimated economic loss of up to US \$1.3 billion in cattle production [3]. In addition, Africa trypanosomosis is of great public health importance and still remains a disease with unsatisfactory medical control [4].

Chemotherapy and chemoprophylaxis using orthodox drugs such as diminazineaceturate and isomethamidium chloride, still remains the most effective methods of controlling the disease [5]. However, these trypanocides are expensive, toxic and have the tendency to elicit drug resistance [6]. In Nigeria, several plants have been used traditionally for the treatment of trypanosomiasis [7-8]. This has been associated to the presence of one or more biologically active principle in plant extract with trypanocidal or trypanostatic efficacy [9] and this may lead to development of new, save and more efficacious drug. Also *Allium sativum* a close relative of *A. porrum*, have been reported to have antitrypanosomal activities [10]. Therefore, this study was designed to evaluate the antitrypanosomal activity of bulb ethyl acetate extracts of *A. porrum* on *Trypanosoma brucei brucei*.

2. METHODOLOGY

2.1 Collection and Preparation of Plant Material

Fresh bulbs of *A. porrum* were purchased from the market at Ibadan, Oyo State, Nigeria. The bulb were taken to the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University, Zaria, where it was identified and a voucher specimen reference number (2196) was assigned.

2.2 Preparation of Extract

Plant extracts were prepared using Soxhlet extractor as described by [11]. Powdered *A. porrum* weighing 500 g was transferred into a cotton bag, and inserted into the thimble of soxhlet apparatus. Thereafter, 1.5 L of ethanol was poured into the thimble and heated to reflux until the solvent vapour travelled up a distillation arm and flooded into the chamber housing the thimble of solid. The non-soluble portion was discarded while the extracted material was concentrated by evaporation at room temperature on warm water bath (HH-S 21.6, double row six holes, HINOTEK China) to separate the solvent from the extract (oily liquid) at a temperature of 93.3°C.

2.3 Phytochemical Screening of the Experimental Plant

Phytochemical analysis of the extracts were conducted to determine the presence of secondary metabolites such as saponins, alkaloids, glycosides, flavonoids, anthroquinones, tannins, volatile oils and triterpenoids, in the extract using standard procedures as described by [12].

2.3.1 Test for flavanoids

A portion weighing 1.0 g of crude ethyl acetate extracts each were dissolved separately in 2 cm³ of distilled water in a glass test-tubes and 2 cm³ of NaOH was added to give a yellow colour which turns colourless after addition of few drops of diluted Hydrochloric acid (HCl) solution indicating the presence of flavanoids.

2.3.2 Test for steroids

A portion (0.5 g) of crude ethyl acetate extracts each were dissolved separately in 2 cm³ of chloroform and concentrated Hydrogen tetraoxosulphate VI (H₂SO₄) was added; formation of red colour in the lower chloroform layer indicates the presence of steroids.

2.3.3 Test for alkaloids

Crude ethyl acetate extracts weighing 0.5 g each were separately mixed with 2 cm³ of 1% HCl and heated up gently to 45°C. Mayer's reagent was added to ethanolic extract mixture while Wanger's reagent was added to the ethyl acetate extract mixture. The turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

2.3.4 Test for terpenoids

Crude ethyl acetate extracts weighing 0.5 g each were dissolved separately in 2 cm³ chloroform and evaporated to dryness. To the mixtures, 2 cm³ of concentrated H₂SO₄ was added and heated for 2 minutes. Presence of grayish colour indicates the presence of Terpenes.

2.3.5 Test for saponins

Crude ethyl acetate extracts weighing 2 g each were boiled separately in 20 ml of distilled water in a water bath and filtered. Each of the filtrates measuring 10 mls were mixed with 5 ml of distilled water and shaken vigorously for a stable and persistent froth. The frothing was then mixed with 3 drops of olive oil, shaken vigorously and observed for the formation of emulsion which indicate presence of saponins.

2.3.6 Test for glycosides

2 g of the extract was dissolved in 5ml of the extracts. It was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlaid with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

2.3.7 Test for tannins

About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

2.4 Experimental Animals

Twenty five clinically healthy male albino rats aged 8-12 weeks weighing between 150-200

grammes were purchased from the animal house unit of Nigerian Institute for Trypanosomiasis Research (NITR) Kaduna and transported to Biological Science Laboratory, Nigerian Defence Academy, Kaduna, Nigeria. All the rats were kept in clean and ventilated cages for 2 weeks to acclimatize. Rats were fed commercially prepared feed and allowed water ad libitum. The rats were screened for the presence of haemoprotozoans parasites and were all confirmed negative. Stabilates of *Trypanosoma brucei brucei* were obtained from the Nigerian Institute for Trypanosomiasis Research (NITR) Kaduna. Parasites were passage into two healthy rats to maintain them until needed. The solid ethyl acetate extract (10 g) was dissolved in 10 ml Dimethylsulfoxide (DMSO) and then added to 240 ml of distilled water to make 40 mg/ml concentration.

The rats were randomly divided into five groups of five rats each and inoculated intraperitoneally with *T. b. brucei* (1x10⁶ per ml of blood). Group 1 was infected but treated with intraperitoneal injection of normal saline while Groups 2, 3, and 4 were infected and treated with single intraperitoneally administration of 50 mg/kg, 75 mg/kg and 100 mg/kg of the ethyl acetate extract respectively while group 5 was treated with 3.5 mg/kg Diminazene aceturate when parasitaemia was established at 1x10⁶ trypanosomes per ml of blood on day 5 post infection.

2.5 Assessment of Therapeutic Activity

Using a capillary tube, 1 ml of blood from each rat in all the groups was collected via the retro orbital sinus twice before infection (pre-infection), Days 3, 4, 5 (post infection post-infection) and Days 1-6 and twice weekly for two weeks after administration of treatment (post-treatment). Changes in hemathological parameters were determine as described by [13-15] while clinical signs, rectal temperature and live weight changes during the experiment were also recorded.

2.6 Determination of Parasitemia

The number of parasites was determined microscopically at X 400 magnification using the "Rapid Matching" method of Herbert and Lumsden [16]. Briefly, the method involves microscopic counting of parasites per field in pure blood or blood appropriately diluted with buffered phosphate saline (PBS, pH 7.2).

Logarithmic values of these counts obtained by matching with the table of Herbert and Lumsden [16] is converted to antilog to provide absolute number of trypanosomes per ml of blood.

2.7 Data Analysis

The Data obtained from the study were analyzed using computer Graph Pad Prism Software version 5. Results were presented in tables and expressed as mean $\pm$ standard error of mean (SEM). Test of significance was carried out using analysis of variance (ANOVA). Probability level of less than 0.05 ($p > 0.05$) was considered significant.

3. RESULTS AND DISCUSSION

The extract post evaporation yielded 183.35g. The Qualitative analysis of phytochemical constituents of crude ethyl acetate extract *A. porrum* (Table 1) showed that there is presence of alkaloids, flavanoids, saponins, terpenoids, glycosides and steroids in while tannins and anthracens were not detected.

Table 1. Qualitative analysis of phytochemical constituents of the ethyl acetate bulb extract of *Allium porrum*

Metabolites	Availability
Alkaloids	+
Flavanoids	+
Saponins	+
Terpenoids	+
Steroids	+
Tannins	—
Anthracens	—
Glycosides	+

+Represent presence of the chemical constituent in plant

- Represent absence of the chemical constituent in plant

All the rats in all the groups (1-5) exhibited clinical signs of trypanosomes infection with the onset of parasitaemia on day 5 before the commencement of *A. Porrum* extracts administration. The rats showed signs of dullness, weight loss and reduction of feed intake. The clinical manifestations of fever associated with rise in temperature loss of appetite coupled with weakness, emaciation and dullness of coat in *T. b. brucei* (Federe isolate) infected rats observed in this study are characteristic manifestations of trypanosomiasis in many hosts animals [17].

Clinical parameter showed (Table 2) that rats in groups 1-4 showed a non-significant ($p > 0.05$) decrease in post treatment mean weight (155.2 ± 4.43 g- 166.2 ± 2.58 g) compared to pre-infection mean weight (154 ± 7.87 g- 172.6 ± 4.52 g). Meanwhile, rats in group 5 treated with diaminazene aceturate had a non-significant ($p > 0.05$) increase (173.0 ± 4.33 - 176.3 ± 1.7) in mean post treatment weight. In all the groups (1-5), the mean rectal temperature showed a non-significant ($p > 0.05$) increase ranging from 1.0°C to 2.17°C when post infection and post treatment rectal temperatures were compared.

The reduced weight gain and emaciation observed in rats in this study may have coincide with the hypoproteinemia due to malabsorption of dietary protein resulting from decreased feed intake due to trypanosome infection [18]. Hypoproteinemia in trypanosomiasis has been associated with either increased trypanosomal uptake of albumin-bound fatty acids and lipoproteins, or increased catabolism by the host [19]. These factors probably could have contributed to the inconsistent decrease in the mean plasma protein observed in most of the groups. Proteinemia have been reported to result in muscle wasting that is commonly observed in trypanosomiasis [20]. Similarly, fever associated with rise in temperature is a common feature of trypanosomiasis [21] and this has been associated with inflammatory processes in parasitic diseases that leads to release of pyrogens [22].

The parasitemia level during the experiments (Table 3) showed that there was a significant increase ($p = .05$) in mean parasitemia of rats in group I ($1.1-2.21 \times 10^6$) while rats in group 5 treated with diaminazeneaceturate showed significant ($p = .05$) clearance of parasitemia post treatment. However, rats of groups 2-5 treated with crude ethyl acetate extract showed slight non-significant ($p > 0.05$) increase in mean parasitemia post treatment. The trypanocidal efficacies of many plants have been associated with the presence of one or more biologically active principles [23]. Studies have shown that plant extracts which contained either alkaloids, flavonoids, phenolics and/or terpenes showed trypanocidal activity [24-25]. These compounds were found to be abundant in the ethyl acetate extracts of *A. porrum* used in this study. The weak/feeble activity of the components therefore may be due to the strain/species of the parasite used for the study since these constituents have been reported to have higher activity against

Table 2. Mean live weights (g) and rectal temperature (°C) of rats experimentally infected with *T. b. brucei* and treated with different concentration of crude ethyl acetate bulb extract of *A. porrum*

Clinical parameters	Treatment groups	Pre-infection	Post-infection	Post-treatment
Live weight	1	154.6±7.87	163.6±1.99	155.2±4.43
	2	159.8±4.84	169.6±2.44	163.1±3.50
	3	167.2±3.31	172.1±2.49	163.7±2.08
	4	172.6±4.52	172.2±2.85	166.2±2.58
	5	173.0±4.33	176.3±2.25	176.3±1.71
Rectal temperature	1	36.3±0.34	38.10±0.37	39.40±0.81
	2	38.54±1.94	37.40±0.28	39.57±0.39
	3	36.32±0.24	37.35±0.37	39.24±0.73
	4	36.26±0.24	37.91±0.45	39.43±0.57
	5	36.70±0.30	36.45±0.20	37.66±0.55

Table 3. Mean parasitemia of rats experimentally infected with *T. b. brucei* and treated with different concentration of crude extract of *A. porrum*

Group	Post-infection	Post-treatment
GRP 1	1.1x10 ^{6a}	2.21x10 ^{6b}
GRP 2	1.05x10 ⁶	1.30x10 ⁶
GRP 3	1.0x10 ⁶	1.32x10 ⁶
GRP 4	1.01x10 ⁶	1.38x10 ⁶
GRP 5	1.1x10 ⁶	0.00

a, b= value with different superscript are significant at P<0.05

Table 4. Hemathological changes in rats experimentally infected with *T. b. brucei* and treated with different concentration of crude ethyl acetate bulb extract of *A. porrum*

Hemathological parameters	Treatment groups	Pre-infection	Post-infection	Post-treatment
Packed cell volume (%)	1	41.40±1.03 ^a	41.10±1.11	31.50±5.55 ^b
	2	46.60±2.71	39.90±0.66	39.00±0.66
	3	40.80±1.39	41.60±1.13	39.10±0.70
	4	43.40±3.41	40.70±0.65	38.60±0.60
	5	40.12±0.21 ^a	40.90±1.35	45.85±2.13 ^b
Red blood cell count (x10 ³ /μL)	1	11.38±0.27 ^a	9.62±0.31	7.93±1.35 ^b
	2	10.4800±0.17	10.25±0.33	10.00±0.25
	3	10.5000±0.22	10.52±0.23	9.69±0.23
	4	10.5600±0.18	10.29±0.25	9.58±0.31
	5	10.83±0.11	10.22±0.31	10.68±0.23
Total white blood cell count (x10 ³ /μL)	1	9.10±0.44	9.08±0.27	9.31±1.83
	2	8.76±0.22	9.63±0.48	11.21±0.43
	3	8.36±0.29	10.12±0.33	11.85±0.74
	4	8.58±0.22	9.72±0.32	11.65±0.48
	5	8.74±0.13	8.77±0.35	10.28±0.48
Total plasma protein (g/dl)	1	6.04 ± 0.34	6.06 ± 0.13	4.40 ± 0.71
	2	5.44 ± 0.25	5.56 ± 0.28	5.59 ± 0.24
	3	6.64 ± 0.28	5.58 ± 0.23	5.68 ± 0.19
	4	5.80 ± 0.25	6.20 ± 0.22	5.50 ± 0.21
	5	6.80 ± 0.32	5.74 ± 0.20	5.92 ± 0.19

a, b= value with different superscript are significant at P<0.05

T. cruzi and *T. evansi* [26]. In addition, palmitic acid was identified by gas chromatographic mass spectrometry (GCMS) to be the most abundant in

the ethyl acetate extract of *A. porrum* and this has been reported to be ineffective against *T. b. brucei* [26].

The evaluated hemathological parameters above (Table 4) showed that PCV decreased significantly ($P=0.05$) from $41.40 \pm 1.0\%$ pre infection to $31.50 \pm 5.5\%$ post treatment in rats of group 1 while there was slight non-significant decrease in PCV of rats in groups 2-4 when pre-infection and post treatment values were compared ($P>0.05$). However, there was a significant ($P=0.05$) increase in mean PCV of group 5 from $40.12 \pm 0.21\%$ pre-infection to $48.90 \pm 2.13\%$ post treatment. The RBC counts decreased significantly ($P=0.05$) from $11.38 \pm 0.27/\mu\text{L}$ pre-infection to $7.93 \pm 1.35/\mu\text{L}$ post-treatment in rats in group 1 while there was slight non-significant decrease in post-infection RBC counts ($0.48\text{--}1.07/\mu\text{L}$) of rats in group 2-5 compare to pre-infection values ($P>0.05$). However, there was a non-significant increase in RBC counts of rats in group 5 administered diaminazene aceturate from $10.22 \pm 0.3/\mu\text{L}$ post infection to 10.68 ± 0.23 post-treatment ($P>0.05$). Decreased packed cell volume and red blood cell count (anemia) recorded in rats of groups 1-5 in this study may be attributed to intravascular hemolytic activity of *T. b. brucei* which is commonly seen in most trypanosoma infection [27]. Other factors that may responsible include oxidative damage as a result of reduced glutathione on the membrane of the red cells and erythrophagocytosis which have been reported to contribute to reduced red blood cell count in trypanosome infection [27].

Also in all the groups (1-5) there was a non-statistically significant ($p>0.05$) increase in WBC counts in a range of $0.21\text{--}3.49/\mu\text{L}$ when the pre-infection and post treatment counts were compared. With the exception of rats in group 1 and 5 which showed a clear decrease in mean total plasma protein of 1.64 g/dl and 0.88 g/dl respectively, the rats in group 2-4 showed inconsistent pattern of increase and decrease ($p>0.05$) when pre-infection, post-infection and post treatment indices were compared. The observed increase in total white blood cell (leukocytes) counts in this study could be as result of a physiological increase in lymphocytosis and eosinophilia. These cells are required for immunological response against invading parasites and modulation of allergic inflammatory response which play a crucial role in immunopathogenesis of trypanosomosis [28,29]. In previous trypanosomosis studies, leucocytosis and lymphocytosis were observed in trypanosome infected rabbits [29] and in trypanotolerant breeds of animals [30]. It is possible that malabsorption of dietary protein by

host due to infection could have been responsible for the fluctuation in total plasma protein in rats in the present study [30]. This is because most of the rats infected with *T. b. brucei* in this study lost appetite which resulted in poor feed intake which may directly or indirectly affect protein uptake.

4. CONCLUSION

In conclusion, phytochemical constituents of ethyl acetate extracts of *A. porrum* detected in this study include alkaloids, flavanoids, saponins, terpenoids, glycosides and steroids. Administration of ethyl acetate extracts of *A. porrum* at the tested concentration does not show obvious ameliorative effects on the physiological and haematological changes due to experimental *Trypanosoma b. brucei* infection. However, there was feeble reduction in parasitemia level when compared the rats in untreated group.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "principles of laboratory animal care" (NIH publication no. 85-23, revised 1985) were followed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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