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ORIGINAL ARTICLE

AQUEOUS *ALLIUM SATIVUM* EXTRACT SUPPLEMENTATION DELAYS *TRYPANOSOMA BRUCEI* INFECTION IN ALBINO RATS

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

Trypanosomosis is one of the most important diseases of livestock and humans in sub-Saharan Africa. This study evaluated the effect of garlic (*Allium sativum*) aqueous extract supplementation on the course of trypanosomosis in albino rats, considering the phytochemical constituents of the extract, blood parameters, prepatent period, level of parasitaemia, and survival rate of the animals. Phytochemical evaluation of Garlic aqueous extract confirmed the availability of flavonoids, tannins and terpenoids while alkaloids and saponins were absent. All infected rats became parasitaemic 2 days PI (post infection) and climaxed 5 days PI. The post-treatment haematological parameters such as packed cell volume (PCV), haemoglobin (HB), and red blood cell count (RBC) in groups treated with Berenil® and Berenil® plus extract were significantly improved. Groups pretreated with aqueous extract before infection demonstrated delayed parasitaemia for 24 hours and increased survival for 2 days in those with 30 days of pretreatment. However, the extract supplement alone at the selected dosages had no inhibitory effect on *T. brucei* infection. This study concluded that aqueous extract of *Allium sativum* contains flavonoids, tannins, and terpenoids, which acted synergistically with diminazene aceturate against *T. brucei* infection.

Key words: albino rats; garlic; parasitaemia; phytochemistry; *Trypanosoma brucei*

INTRODUCTION

Trypanosomosis is an important insect-borne protozoan disease of animals and humans in sub-saharan Africa, especially in Nigeria. It is described as a disease transmitted biologically by tsetse fly and mechanically by other biting flies [1, 2]. Human African Trypanosomosis (HAT), or sleeping sickness, is caused by *Trypanosoma brucei gam-*

biense or *Trypanosoma brucei rhodesiense*, while American human Trypanosomosis or Chagas disease, is caused by *Trypanosoma cruzi* [3]. Animal African Trypanosomosis (AAT) is caused by different species of trypanosomes, including *Trypanosoma evansi, T. brucei, T. congolense*, and *T. simiae* and it hinders the growth and development of livestock production in endemic parts of Africa [4, 5].

Some of the clinical manifestations of the disease include intermittent fever, anemia, weight loss, infertility, lowered milk yield, abortion, nervous signs, and mortality in affected animals [1]. WHO in 2015 [3] reported that Chagas disease causes about 50,000 deaths annually, while Lejon et al., in 2003 [6], stated an estimated 300,000 to 500,000 Africans carry the disease while 60 million people are at risk of infection. Animals that recover from trypanosome infections are usually unproductive [4]. However, in some cases, infections may be subclinical or chronic showing no clinical signs, although stress factors such as overwork, parturition, malnutrition, and unfavourable environmental conditions may predispose animals to come down with the disease [7].

Efforts to prevent and control clinical trypanosomosis have partially failed due to drug resistance, which occours in an attempt to control the parasites in mammalian hosts, and the inability to find appropriate control measures for the various insect vectors of this disease. The compounds used clinically to control trypanosome infections were introduced in the country more than 50 years ago, but considerable toxicity and resistance have limited their use. This makes searching for efficacious alternative ethno-medicinal plants critical [8, 9].

Garlic, one of nature's wonder plants with its healing ability, exhibits anti-parasitic activity against major human intestinal parasites such as *Entamoeba histolytica, Ascaris lumbricoides* and *Giardia lamblia* [10]; lowers blood pressure [11]; lowers blood cholesterol [12]; and contains anti-tumor properties. It can also boost the immune system to fight off potential diseases and maintain health [13, 14]. Therefore, this study intends to investigate the effect of garlic (*Allium sativum*) aqueous extract supplementation on the course of trypanosomosis in albino rats.

MATERIALS AND METHODS

Plant collection and authentication

The fresh *Allium sativum* bulbs were purchased at Bodija Central Market, Ibadan, Nigeria, and authenticated at the Department of Botany, University of Ibadan.

Preparation of extract

The plant's bulbs were air-dried at room temperature, pulverized, and 1000g of it soaked in 10 liters of distilled water for 24 hours, filtered through Whatman filter paper no1, and concentrated at 50^{o}C with a rotary evaporator. Thereafter, the garlic extract was preserved in the refrigerator until use. 1 gm of the dried extract concentrate was dissolved in 50 ml of distilled water to give a concentration of 20 mg/ml. To ensure that the active principles are preserved, fresh aqueous extracts were prepared every other day. Diminazene aceturate (Berenil$^{(R)}$) manufactured by Kepro Veterinary Company, Holland) was used for the positive control.

Test organism

Trypanosoma brucei brucei was obtained from the Nigeria Institute for Trypanosomiasis Research, Nigeria. The parasites were maintained in the laboratory by continuous passage in rats.

Experimental animals

50 adult male albino rats of about 6 weeks in age, weighing 100 – 150 g, were kept in the experimental animal housing unit of the Department of Veterinary Pharmacology and Toxicology, University of Ibadan. They were acclimatized for 14 days and randomly assigned to groups before the commencement of the experiment and fed with commercial Vital® feed and water *ad libitum.*

Phytochemical analysis of plant extract

Phytochemical screening of aqueous extract of *Allium sativum* was conducted as described by Trease and Evans [15].

Experimental design

The fifty (50) male albino rats of about 6 weeks in age were divided into 10 groups of 5 animals each. Group A: Negative control (no infection and no treatment), B: Infected without treatment, C: Infected and treated with a

single dose of Berenil (7 mg/kg) at day 5 post-infection, D: Infected and treated with Berenil (3.5 mg/kg) + aqueous extract (30 mg/kg) once at day 5 post-infection, E: Infected and treated with extract (30 mg/kg) for 5 days from day 5 post-infection, F: Infected and treated with extract (60 mg/kg) for 5 days from day 5 post-infection, G: 14 days pretreatment with extract (30 mg/kg) + Infection on day 15, H: 14 days pretreatment with extract (60 mg/kg) + Infection on day 15, I: 30 days pretreatment with extract (30mg/kg) + Infection on day 31, J: 30 days pretreatment with extract (60 mg/kg) + Infection on day 31.

Determination of prepatent period and parasitaemia

The level of trypanosome in the blood of the infected rats was monitored daily from the second day post-infection. Blood from the coccygeal vein was used to determine parasitaemia in wet mounts. The trypanosome counts were determined by examination of the wet mount microscopically at x400 magnification using the "rapid matching" method of Herbert & Lumsden [16], which involves microscopic counting of parasites per field in pure blood or blood appropriately diluted with buffered phosphate saline (PBS, pH 7.2).

Blood collection and analysis

The rats were anaesthetized with diethyl ether. Blood samples were collected from the orbital vein of each rat using sodium heparinized micro haematocrit tubes into separate lithium heparinized sample bottles to evaluate haematological and biochemical parameters. The experiment was terminated at day 21 post-infection and blood samples were collected from all surviving animals.

Statistical analysis

All data obtained from the study were presented as means ± standard error of the mean (SEM) and analysed using ANOVA, followed by the post-hoc Tukey's test to evaluate significant differences within the groups. SPSS (Statistical Package for the Social Sciences) was used for the analysis, and the probability level at 0.05 ($P \leq 0.05$) was considered significant.

Table 1. Phytochemical constituents of aqueous extract of *Allium sativum* bulb

Component	Test	Observation	Scoring
Alkaloids	Dragendorff's	Brownish-red colour	-
Tannins	Ferric chloride	Deep red colour	+
Flavonoids	Pew's	Red colour	+
Saponins	Frothing	Persistence foam	-
Terpenoids	Salkowski's	Reddish brown	+

+ indicate presence
- indicate absence

Table 2a. Mean haematological parameters of infected and treated animals with garlic extract and Berenil

PARAMETERS	GROUP A	GROUP B	GROUP C	GROUP D
PCV	41.5 ± 0.3	37.7 ± 2.2 [a]	39.0 ± 1.0	46.5 ± 1.5
HB	13.7 ± 0.2	12.6 ± 0.7 [a]	13.0 ± 0.5	15.6 ± 0.7
RBC	6.8 ± 0.2	6.2 ± 0.5 [a]	6.4 ± 0.2	7.7 ± 0.4
WBC	5437.5 ± 1494.1	3933.3 ± 233.3[a]	3475.0 ± 225.0	5325.0 ± 325.0
PLATELET	114750.0 ±14783.9	90666.7 ±5607.5	84000 ± 28000	118500.0 ± 15500.0
LYMPHOCYTE	56.8 ± 1.6	64.3 ± 1.9	63.5 ± 4.5	70.5 ± 3.5
NEUTROPHIL	39.0 ± 1.8	32.7 ± 1.5[a]	32.0 ± 7.0	25.5 ± 2.5
MONOCYTE	2.0 ± 0.4	1.7 ± 0.3	2.0 ± 1.0	2.5 ± 0.5
EOSINOPHIL	2.3 ± 0.3	1.3 ± 0.9	2.5 ± 1.5	1.5 ± 0.5

Treatment was administered once at day 5 post-infection. All values are expressed as mean ± standard error of mean. Statistical significance is reported at $\alpha^{0.05}$ and indicated with [a] in the table. **GROUP A**: No infection and no treatment; **GROUP B:** Infected with no Berenil and extract treatment; **GROUP C**: Infected and treated with Berenil (7 mg/kg) once on day 5 post-infection; **GROUP D**: Infected and treated with Berenil (3.5 mg/kg) + extract (30 mg/kg) once on day 5 post-infection (PCV: Packed cell volume, HB: Haemoglobin, RBC: Red blood cell count, WBC: White blood cell count)

RESULTS

Phytochemical analysis of *Allium sativum* extract

Phytochemical analysis of the aqueous extract of *Allium sativum* detected flavonoids, tannins, and terpenoids while alkaloids and saponins were not detected (Table 1).

Effects of infection and treatment on blood parameters

Following infection with *Trypanosoma brucei,* the PCV (Packed cell volume), HB (Haemoglobin), and RBC (Red blood cell) count were reduced in infected-untreated animals compared to the uninfected control. By day 15 post-treatment, the red cell indices (PCV, HB and RBC) were significantly ($P < 0.05$) improved in groups C and D treated with 7 mg/kg Berenil and 3.5 mg/kg Berenil + 30 mg/kg extract, respectively (Table 2a).

Haematological parameters for groups G, H, I, and J (pretreated and infected) and groups E and F (infected and treated with only the extract) are not recorded because all experimental animals died before the experiment was terminated on day 21 PI.

Following infection with *Trypanosoma brucei*, the plasma total protein, albumin, and globulin were reduced, whereas liver enzymes AST (Aspartate transferase), ALT (Alanine aminotransferase), and ALP (Alkaline phosphatase) were increased in infected groups compared to the uninfected control group. However, on day 21 post-infection, the plasma concentrations of liver enzymes (ALT, AST, and ALP) were reduced in groups C (Infection + treatment with 7 mg/kg Berenil once on day 5 post-infection) and D (Infection + treatment with 3.5 mg/kg Berenil + 30 mg/kg extract once at day 5 post-infection (Table 2b).

Effect of garlic extract on *T. brucei* infected rats

The parasitaemia in the positive control group (infected untreated) was >5.4 on day 2 PI to >8.7 on day 9 PI when all animals in this group died, whereas the negative control group (uninfected), whose animals survived, had no parasitaemia throughout the experiment (Table 3, Figure 1).

Pretreatment with *Allium sativum* extract for 14 (60 mg/kg) and 30 days (30 mg/kg and 60 mg/kg) before infection delayed the prepatent period for 24 hours. The treatment of infected rats with garlic extracts at 30 mg/kg and 60 mg/kg had no inhibitory effect on *Trypanosoma brucei* parasitaemia. There was no significant difference between the parasitaemia levels in the group treated with garlic extract and the positive control group (infection only) with no extract treatment.

The parasitaemia level reduced significantly in groups C (infected + 7 mg/kg Berenil once on day 5 PI), and D (infected + 3.5 mg/kg Berenil + 30 mg/kg extract once on day 5 PI), from >7.8 to <5.4 within 24 hours and then 0 within 48 hrs post-treatment. Both groups had no evidence of relapse throughout the experiment at day 21 PI. The *T. bruccei* parasitaemia levels observed in all the experimental groups are presented in Table 4.

Table 2b. Mean plasma biochemical parameters in infected and treated animals with garlic extract and Berenil

PARAMETERS	GROUP A	GROUP B	GROUP C	GROUP D
TOTAL PROTEIN	7.3 ± 0.1	7.1 ± 0.2	7.5 ± 0.9	7.6 ± 0.4
ALBUMIN	3.0 ± 0.2	2.5 ± 0.2	3.2 ± 0.5	3.3 ± 0.3
GLOBULIN	4.3 ± 0.1	4.6 ± 0.1[a]	4.3 ± 0.4	4.4 ± 0.2
AG RATIO	0.7 ± 0.1	0.5 ± 0.0	0.7 ± 0.1	0.8 ± 0.1
AST	41.3 ± 0.5	44.7 ± 1.5	42.0 ± 3.0	42.5 ± 1.5
ALT	30.3 ± 0.8	32.7 ± 0.9	31.0 ± 3.0	32.5 ± 0.5
ALP	108.8 ± 5.5	124.0 ± 2.0[a]	101.0 ± 2.0	115.0 ± 9.0
BUN	16.4 ± 0.3	16.9 ± 0.3	17.1 ± 0.5	16.8 ± 0.4
CREATININE	0.7 ± 0.1	0.7 ± 0.0	1.0 ± 0.3	0.8 ± 0.0

Treatment was administered once at day 5 post-infection. All values are expressed as mean ± standard error of mean. Statistical significance is reported at $\alpha^{0.05}$ and indicated with [a] in the table. **GROUP A**: No infection and no treatment; **GROUP B:** Infected with no Berenil and extract treatment; **GROUP C**: Infected and treated with Berenil (7 mg/kg) once on day 5 post-infection; **GROUP D**: Infected and treated with Berenil (3.5 mg/kg) + extract (30 mg/kg) once on day 5 post-infection. (AG RATIO: Albumin Globulin ratio, AST: Aspartate transferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, BUN: Blood Urea Nitrogen).

Table 3. Survivability of animals infected and treated with garlic extract and/or Berenil

Days	A	B	C	D	E	F	G	H	I	J
1 (PI)	0/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
2	0/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
3	0/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
4	0/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
5(Treatment)	0/5	4/4	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
6	0/5	4/4	2/5	2/5	3/3	3/3	3/3	3/3	4/4	4/4
7	0/5	2/2	0/5	0/5	1/1	2/2	1/1	1/1	3/3	2/2
8	0/5	1/1	0/5	0/5	1/1	2/2	0/0	1/1	2/2	2/2
9	0/5	0/0	0/5	0/5	0/0	0/0	0/0	0/0	2/2	2/2
10	0/5	0/0	0/5	0/5	0/0	0/0	0/0	0/0	1/1	0/0
12	0/5	0/0	0/5	0/5	0/0	0/0	0/0	0/0	0/0	0/0
15	0/5	0/0	0/5	0/5	0/0	0/0	0/0	0/0	0/0	0/0
17	0/5	0/0	0/5	0/5	0/0	0/0	0/0	0/0	0/0	0/0
19	0/5	0/0	0/5	0/5	0/0	0/0	0/0	0/0	0/0	0/0
21	0/5	0/0	0/5	0/5	0/0	0/0	0/0	0/0	0/0	0/0

PI: Post-infection, Numerator = Number of rats positive for *T. brucei,* Denominator = Number of surviving rats in the group; **A**: No infection and no treatment; **B:** Infected with no Berenil and extract treatment; **C**: Infected and treated with Berenil (7 mg/kg) once on day 5 post-infection; **D**: Infected and treated with Berenil (3.5 mg/kg) + extract (30 mg/kg) once on day 5 post-infection; **E:** Infected and treated with extract (30mg/kg) for 5 days; **F:** Infected and treated with extract (60mg/kg) for 5 days; **G:** 14 days pretreatment with extract (30 mg/kg) + Infection; **H**: 14 days pretreatment with extract (60 mg/kg) + Infection; **I**: 30 days pretreatment with extract (30 mg/kg) + Infection; **J:** 30 days pretreatment with extract (60 mg/kg) + Infection

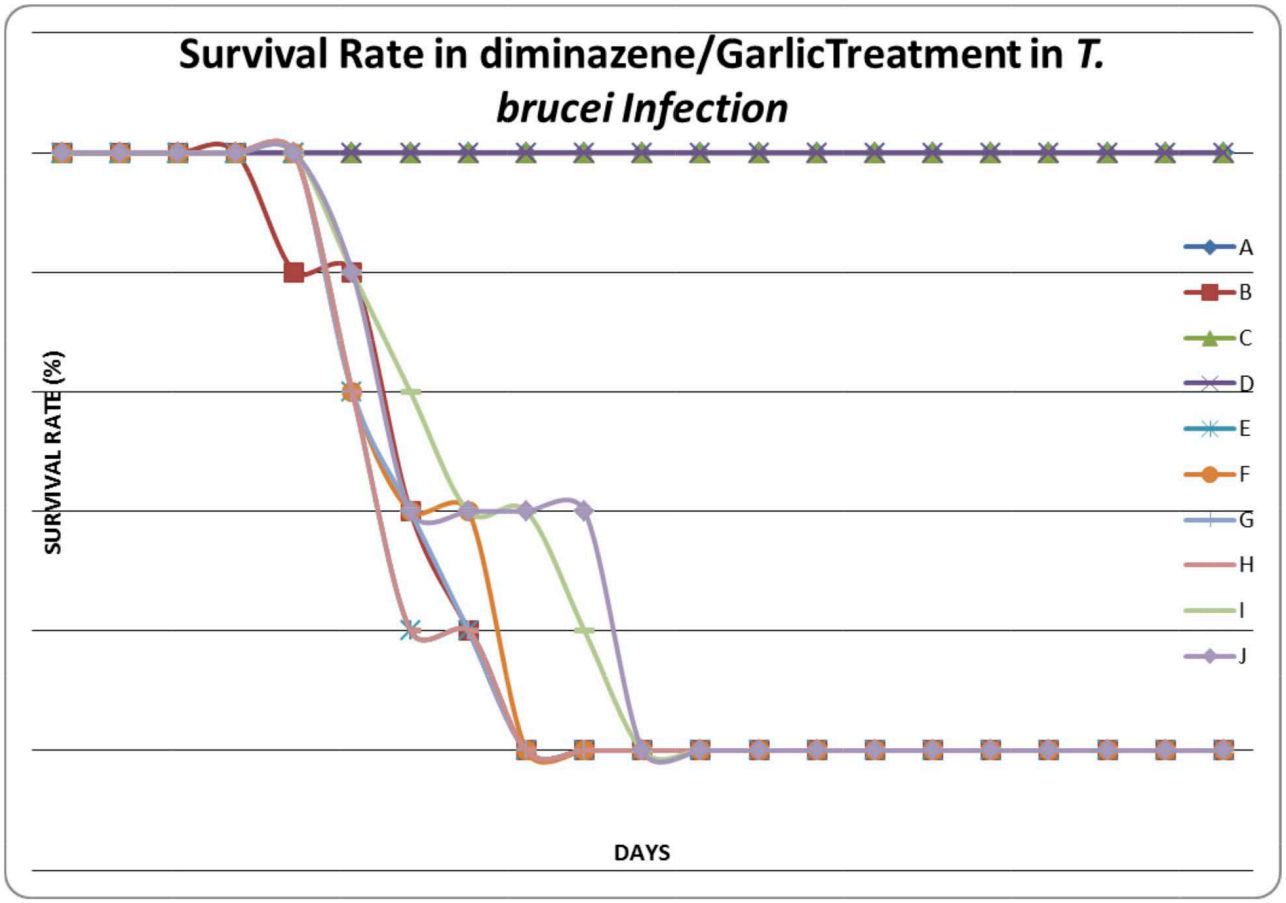

Fig. 1. Survival rate of animals infected and treated with diminazene and/or garlic extract

A: No infection and no treatment; **B:** Infected with no Berenil and extract treatment; **C:** Infected and treated with Berenil (7 mg/kg) once on day 5 post-infection; **D:** Infected and treated with Berenil (3.5 mg/kg) + extract (30 mg/kg) once on day 5 post-infection; **E:** Infected and treated with extract (30mg/kg) for 5 days; **F:** Infected and treated with extract (60 mg/kg) for 5 days; **G:** 14 days pretreatment with extract (30 mg/kg) + Infection; **H:** 14 days pretreatment with extract (60 mg/kg) + Infection; **I:** 30 days pretreatment with extract (30 mg/kg) + Infection; **J:** 30 days pretreatment with extract (60 mg/kg) + Infection

Table 4. Mean Parasitaemia in infected and treated animals with garlic etract and/or Berenil

Days	A	B	C	D	E	F	G	H	I	J
1 (PI)	0	0	0	0	0	0	0	0	0	0
2	0	6.0	6.3	6.0	0	6.0	6.0	0	0	0
3	0	6.6	6.9	6.9	6.3	6.6	6.9	6.3	6.0	6.6
4	0	7.2	7.2	7.2	6.6	6.9	7.5	6.9	6.6	6.9
5(Treatment)	0	7.5	7.8	7.8	7.2	7.5	8.1	7.2	6.9	7.2
6	0	7.8	<5.4	<5.4	7.5	8.1	8.4	7.8	7.2	7.5
7	0	8.4	0	0	8.1	8.4	8.7	8.1	7.5	7.8
8	0	8.7	0	0	8.7	8.7	Dead	8.7	7.8	8.1
9	0	Dead	0	0	Dead	Dead	-	Dead	8.1	8.7
10	0	-	0	0	-	-	-	-	8.4	Dead
11	0	-	0	0	-	-	-	-	Dead	-
15	0	-	0	0	-	-	-	-	-	-
17	0	-	0	0	-	-	-	-	-	-
19	0	-	0	0	-	-	-	-	-	-
21	0	-	0	0	-	-	-	-	-	-

All values are expressed as the mean of base 10 logarithm for the concentration of the parasites per milliliter of blood for reference point. PI: Post-infection; **A**: No infection and no treatment; **B:** Infected with no Berenil and extract treatment; **C**: Infected and treated with Berenil (7 mg/kg) once on day 5 post-infection; **D**: Infected and treated with Berenil (3.5 mg/kg) + extract (30 mg/kg) once on day 5 post-infection; **E:** Infected and treated with extract (30 mg/kg) for 5 days; **F:** Infected and treated with extract (60 mg/kg) for 5 days; **G:** 14 days pretreatment with extract (30 mg/kg) + Infection; **H**: 14 days pretreatment with extract (60 mg/kg) + Infection; **I**: 30 days pretreatment with extract (30 mg/kg) + Infection; **J:** 30 days pretreatment with extract (60 mg/kg) + Infection

DISCUSSION

This study explores a comprehensive phytochemical analysis of the aqueous extract of *Allium sativum* (garlic) and its effects on *T. brucei* infection in white Wistar rats. According to this study, phytochemical constituents of *A. sativum* indicated the presence of flavonoids, tannins, and terpenoids. These findings are consistent with previous studies that have identified similar phytochemicals in garlic, which are known for their various pharmacological properties. Also, these relatively cheap and efficient medicinal components have been reported to have remarkable biological activities, and their therapeutic effects have been substantiated for various ailments [17].

Moreover, Huzaifa et al. [18] reported the presence of alkaloids, flavonoids, saponins, tannins and cardiac glycosides in the aqueous extract of garlic bulbs, while Boukeria et al. [19] observed the presence of saponins, alkaloids, and traces of glycosides in *A. sativum* varieties. These findings are consistent with previous studies that have identified similar phytochemicals in garlic, which are known for their various pharmacological properties [20].

Following infection with *T. brucei,* the PCV, HB, and RBC counts were reduced in infected rats compared to the uninfected control, indicating anaemia, a consistent manifestation associated with trypanosomosis [1], which could be due to hemolysis and bone marrow suppression. However, the red cell indices, i.e. PCV, HB and RBC counts were significantly improved in groups C and D treated with Berenil® plus garlic aqueous extract to a level similar to the observation in the uninfected control group. This showed that aqueous extract of garlic was not contraindicated with Berenil®. The animals treated with only the extract before or after the establishment of infection died before the experiment was terminated, suggesting the inability of the extract to inhibit the multiplication of Trypanosome parasites or red cell destruction by *T. brucei* at the selected concentration for this work.

The infection also led to reductions in plasma total protein, albumin, and globulin levels, accompanied by increases in liver enzymes (ALT, AST, and ALP), indicative of hepatic damage and dysfunction. Post-treatment, the groups receiving Berenil alone or in combination with garlic extract showed a significant reduction in these liver enzyme levels by day 21 post-infection, pointing to a res-

toration of liver function. This highlights the therapeutic potential of Berenil, both alone and in combination with garlic extract, in mitigating liver damage induced by trypanosomiasis.

Also, it was observed that albino rats pretreated with aqueous extract for 30 days survived for 2 days more than those pretreated for 14 days and those that were not pretreated. Comparing the effect of pretreatment with the extract alone on the establishment of parasitaemia and survivability of *T. brucei*-infected rats is limited due to a lack of data. Nevertheless, Shubha et al. [21] and El-katcha et al. [22] have reported the immune-stimulating potential of garlic, and this may contribute at least in part to the delay in the establishment of parasitaemia till day 3 in all extract-pretreated rats and the increased survivability (additional 2 days) of *T. brucei*-infected rats pretreated for 30 days.

Treatment of infection with extract alone has no significant effect on survivability, whereas treatment with a combination of 30 mg/kg of extract and 3.5 mg/kg of diminazene aceturate effectively improved the survivability of animals. Pretreatment with *Allium sativum* aqueous extract couldn't effectively prevent the establishment of infection, though it delayed the establishment of parasitaemia for 1 day longer than groups that were not pretreated. Also, treatment with extract alone could not efficiently reduce parasitaemia levels in already established infection, while treatment with 30 mg/kg of extract combined with 3.5 mg/kg of diminazene aceturate was as effective as treatment with only 7 mg/kg of diminazene aceturate in reducing parasitaemia to 0 within 48 hours post-treatment with no evidence of relapse.

This study emphasised the findings of Peni et al. [23], who stated that diminazene aceturate efficacy in managing *Trypanosoma brucei* infection is enhanced when combined with sub-therapeutic doses of *Allium sativum* bulb extract. This synergistic effect of garlic with diminazene aceturate in the treatment of trypanosomosis can be attributed to several mechanisms, some of which include its antioxidant effects, modulation of the host's immune system, and enhanced trypanocidal impact primarily due to its sulfur compound named allicin, which can enhance the Berenil mechanism of action. Arreola et al. [14] previously reported that aqueous garlic extract exerts antioxidant actions by scavenging reactive oxygen species (ROS) and enhancing cellular antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. The immunomodulatory potential of phytochemicals has been largely attributed to their dynamic regulation of molecules such as cytokines and chemokines. This explains the therapeutic effect associated with the different bioactive molecules identified in the garlic plant.

While our study provides valuable insights, we acknowledge some limitations that suggest directions for future research. The sample size was small and might limit the generalizability of findings. In addition, the dosage used had no curative effect when used alone, though it delayed the onset of Trypanosomosis. Future investigation could explore the potential curative effect of the extract at a higher dosage and the possible mechanism of action of garlic as an antitrypanosomal agent. However, these limitations do not diminish the significance of our findings but rather highlight opportunities for more comprehensive future investigations.

CONCLUSION

This study concluded that aqueous extract of *Allium sativum* contains flavonoids, tannins, and terpenoids, which explain the synergy with diminazene aceturate against *T. brucei* infection. Also, 14 days of pretreatment of animals with extract delayed parasitaemia for 24 hours, and 30 days increased survivability for 2 more days, whereas the extract alone at low doses was not curative against *T. brucei* infection. This combination therapy provides a more effective approach to treatment. It may reduce the severity of *Trypanosoma brucei* infection, which may be complicated by already existing drug resistance, toxicity, and high cost of purchase.

Ethical Approval

The experiment was carried out with strict adherence to the international regulations guiding the use of animals for research purposes as outlined in the University of Ibadan Animal Care Use and Research Ethics Committee protocol. Ethical clearance was obtained from the Animal Care Use and Research Ethics Committee (ACUREC), University of Ibadan, with file number UI-ACUREC/17/0034.

Data Availability Statement

The raw data of this study will be made available upon request without any reservation.

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Declaration of Interests

There is no conflict of interest.

Authors' Contributions

Adeoye conceptualised the design while Ojo and Olakojo were also involved in the literature search, data processing and manuscript writing.

Generative AI statement

Generative AI technologies were not employed in writing this manuscript.

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