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RESEARCH PAPER

In-vivo anti-trypanosomal and antioxidant potential of aqueous extract from *Allium sativum* bulb in *Trypanosomal congolense* infected mice

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ABSTRACT

Trypanosomes are causative agent of trypanosomiasis, which is transmitted by tsetse fly. Trypanosomiasis is characterized by intermittent fever, anaemia, and frequent diarrhoea. The main purpose of the research was to ascertain the in vivo anti trypanosomal and antioxidant property of aqueous extract from *Allium sativum* bulb on *Trypanosoma congolense* infected mice. The acute toxicity study of *A. sativum* was also determined using Lorke's method, where different extract doses (10 mg/kg to 5000 mg/kg body weight (b/w)) were administered orally. The anti-trypanosomal, DPPH antioxidant activity and hematological studies were also deduced using standard methods. The acute toxicity recorded zero death both in phase I and phase II after a day (24 hours), and the lethal dose (LD50) was greater than 5000 mg/kg bw. Mean parasitemia count, packed cell volume (PCV) and body weight (kg) were monitored in anti-trypanosomal screening at doses of 100, 250, and 500 mg/kg bw (treatment group) including positive and negative control group which had been given standard drug (diaminazine aceturate) and water respectively. Among the treatment groups, 500 mg/kg bw was highly effective in all the screened parameters. However, the hematological screening after the treatment days (16 days) showed that, PCV (36.00±2.00b %; normal range: 35-45 %) and RBC (9.48±0.53c cells/µl of blood; normal range: 5.50-10.20×106 cells/µl of blood) recorded the highest values in extract dose of 500 mg/kg bw. DPPH antioxidant showed IC_{50} of 56.19±0.00d and 38.07±0.00a in garlic and vitamin C respectively. These results signify that *A. sativum* may have high efficacy as anti-trypanosomal agents, which could be used as an alternative drug in the management of trypanosomiasis.

Keywords: *Allium sativum*, African Trypanosomiasis, DPPH, Nigeria, Trypanosomal congolense

1. INTRODUCTION

Trypanosoma congolense are known as single cell parasite that is transmitted by tsetse flies.

They serve as agent that causes African Animal Trypanosomiasis (AAT) [1]. Trypanosomiasis leads to several diseases which are characterized by intermittent fever, anaemia, frequent diarrhoea and loss of conditions which eventually leads to death [2]. Despite series of studies on controls and preventions of trypanososmiasis, it is still the one of the limiting factors that greatly affects livestock and human in sub-Saharan Africa [3]. Among limitation factors targeting the control/prevention of trypanosomiasis are; improper clinical efficiency, toxicity of drugs and drug resistance [4-5]. Studies are still in progress on developing vaccines against AAT but presently, the use of chemotherapeutic agents is the main method of control it. Trypanososmiasis causes reduction in immune system which further leads to inability of the hosts to eliminate trypanosome even after the use anti-trypanosomal drugs [6-7]. The current trypanocidal drugs used for preventive and curative purposes are; diminazene aceturate and isometamidium chloride [8]. Unfortunately, parasites have developed resistance to these drugs, which creates a need for search of very potent but less toxic agents from available medicinal plants [9].

Garlic (*Allium sativum*) is a perennial flowering plant which is found under family Alliaceae and it grows up to 30-60 cm tall. Garlic has a strong and sharp smell with high varieties of flavours and textures when it is eaten raw or cooked [10]. Garlic has been in use for various diseases as medicine for several decades and it is one of the most common *Allium* species consumed worldwide [11]. It is used traditionally as antiseptic, expectorant, antihypertensive, stimulant, diuretic, and antiviral drugs for viral infections. Biological studies including antioxidant, antimicrobial, ant nociceptive and anti-trypanosomal activities have also been documented [12-13]. The main active ingredient in garlic bulb is allicin, which is responsible for the garlic's strong smell. This is formed as a result of reaction between non-protein amino acid (alliin) and an enzyme (alliinase) [12]. Allicin could be responsible for the therapeutic efficiency of garlic bulb [14]. Studies showed scanty information on the toxic effects of aqueous extract of garlic on mice. Therefore, this study will highlight the in vivo anti trypanosomal and DPPH antioxidant potential of aqueous extract of garlic bulb on *Trypanosoma congolense* infected mice.

2. MATERIALS AND METHODS

2.1. Authentication and collection of sample

Garlic (*A. sativum*) bulbs were obtained in September, 2019 from Kure market, in Bosso Local Government of Niger State, Minna and it was further authenticated at Department of Plant Biology, Futminna, Niger State.

2.2. Experimental animals

The animals used for the screening were purchased from National Institute for Research, Jos Plateau State, Nigeria. The animals i.e. the albino mice (25.69±2.17 g) were acclimatized for 6 days at the Animal Holding Unit, Department of Biochemistry, for subsequent use. All experiments were performed on animals

using standard methods and in conformation with accepted rules for laboratory animal care.

2.3. Trypanosoma congolense

Trypanosoma congolense known as parasite was procured from National Institute for Research, Jos Plateau State, Nigeria, which was subsequently maintained in the Animal Housing Unit of the Department of Biochemistry by asynchronous transfer in mice.

2.4. Preparation of sample

The preparation of garlic sample was done according to Gupta *et al.,* (2015) with slight modification [12]. The cloves of garlic were separated, sliced, and dried after it was peeled off from the outer layer. The cloves were dried using freeze-dryer (lyophilized) at 23°C and grounded repeatedly with electric blending machine. The grounded garlic bulbs were stored in refrigerator before extraction process.

2.5. Preparation of garlic extract

The preparation of garlic sample was done according to Luckins (2002) with slight modification [15]. About 220 g of the grounded garlic bulbs was measured and soaked in 2 L distilled water for 2 hours. The mixture was then heated in water bath for another 2 hours at 50 °C. However, the mixture was allowed to cool for about 10 hours followed by filtration using Whatman No 1 filter paper. The solution was concentrated at 50 °C on rotary evaporator. The concentrated sample was then further freeze-dried for total removal of moisture.

2.6. Test organism preparation

Trypanosoma congolense parasite was maintained by asynchronous passage in mice until it was needed. 20 mL of NaCl (0.98%) was used to dissolve 0.2 mL of blood from infected mice, and the mixture was further injected into clean mice [16].

2.7. Acute toxicity (LD_{50}) determination

Two phases was employed in the determination of acute toxicity of garlic aqueous extract, by Lorke's method [17]. In the first phase, six (6) mice were grouped into three (3) of two (2) mice each and they were all given *A. sativum* extract orally using cannula from 300 mg/mL stock solution prepared at dose of 10, 100 and 1000 mg/kg bw (group 1,2 and 3 respectively). After 24 hours of extract administration, no death was recorded and this led to the second phase where three (3) groups of one (1) mouse each were used at dose 1,600, 2900 and 5000 mg/kg bw (group 1, 2 and 3 respectively). The mice were also observed for death and behavioural changes (sign of toxicity) within 24 hours.

2.8. In vivo screening for trypanocidal activity

Trypanocidal activity of garlic aqueous extract was done based on the method described by Luckins, (2002) [15].

2.8.1. Animal infection

Blood was obtained from the tail of highly infected mouse using EDTA coated insulin syringe. The inoculums were prepared by diluting the blood with normal saline (0.9% NaCl). Acclimatized/healthy mice with body weight ranging from 17-40 g were infected

Table 1: Toxicity (acute) of Aqueous Extract of Allium sativum (Garlic Bulb)

Group(s)	Dose (mg/kg) bw	No of animals	Mortality
Phase 1			
1	10	2	0/2
2	100	2	0/2
3	1000	2	0/2
Phase 2			
1	1600	1	0/1
2	2900	1	0/1
3	5000	1	0/1

(0/2, 0/1) means 0 = number of mortality recorded, 2 & 1 = number of animal used

intraperitoneally with 0.2 mL of the inoculums containing the parasite (trypanosomes).

2.8.2. *Administration of crude extract to infected mice*

About 20 albino mice were separated into five (5) groups of four (4) mice each. Trypanosome (*T. congolense*) was used to infect all the mice. Positive and negative control groups were given diaminazine aceturate (3.5 mg/kg bw; from 110 mg/mL stock solution) and infected but not treated respectively. Three (3) days after infection, parasites were found in the mice and treatment started the same day. Group 1, 2 and 3 were given aqueous extract of garlic (100 mg/mL) orally at doses 100, 250 and 500 mg/kg bw respectively for 16 days (Garbriel et al., 2009).

2.8.3. *Mean parasitaemia determination*

Blood obtained from the tail vein of infected mice was screened using microscope to determine the mean level of parasite. Small drop of blood was placed on a grease free slide and it was covered with a cover slip. The slides were further viewed on microscope using ×40 objectives lens. This was monitored from the second day of parasite infection till the last day of the treatment days (16 days), to ascertain increase or decrease in level of the parasite and effects of the extract [18].

2.8.4. *Packed cell volume (PCV) determination*

Packed cell volume is the fraction of red cell in the total blood volume. Blood was obtained from the tail vein of the mice and this was collected into a heparinized capillary tube. The blood was centrifuged for 5 minutes at 11,000 rpm after which the percentage of the red cell was taken with PCV reader [19].

2.8.5. *Body weight determination*

The weight of the mice was taken using weighing balance throughout the period of treatment. This was done by making the mice dizzy (spun) for accurate measurement of the weight.

2.8.6. *Haematology sample preparation*

Blood samples of the survived mice were taken after the 16 days treatment by sacrificing the mice. The sacrifice was done using blade on their cervical region and their blood samples were collected into EDTA sample bottle for further haematological analysis. The haematological analysis was done using standard method [20].

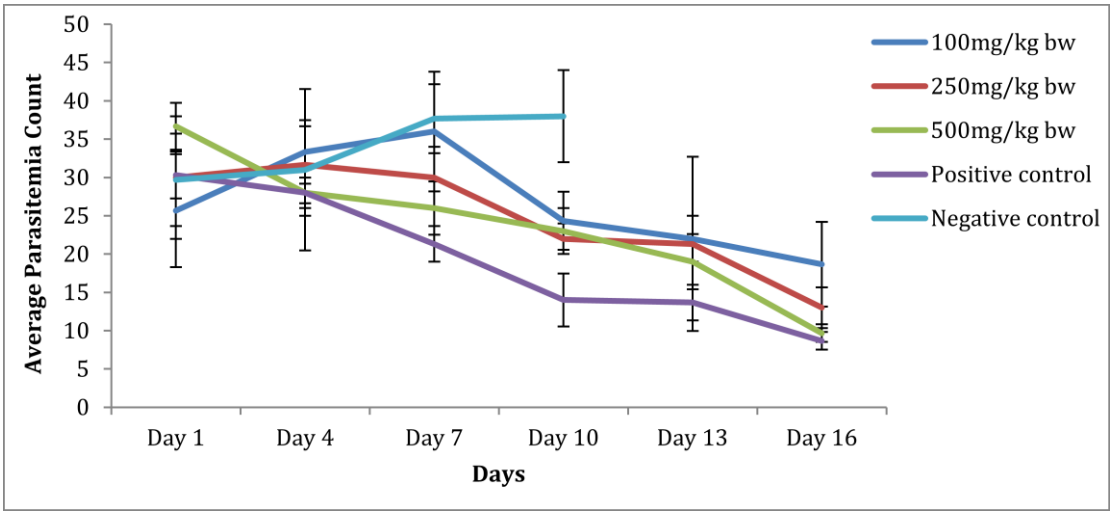

Figure 1: Effects of *Allium sativum* Aqueous Extract on Level of Parasitemia on *Trypanosoma congolense* Infected Mice

2.8.7. *Antioxidant property of garlic extract by DPPH method*

Extract of 0.2 mg was weighed into vial bottle and 2 mL of methanol was added, which made it to be 100 µg/mL. Other concentrations; 50, 25, and 12.5 µg/mL were also prepared by diluting the stock solution (100 µg/mL) with methanol. Vitamin C was used as the standard along the garlic extract sample and 2 mL of 0.4% of 2, 2-diphenyl-1-picrylhydrazyl was to both the standard and the extract. The absorbance of the mixtures was taken at wavelength 517 nm after they were allowed to stay in dark environment for 20 minutes. However, methanol was used as a blank while taking the absorbance with UV-spectrophotometer.

$$\%\text{Inhibition}=\{[(AB_{control})-(AB_{extract})]/(AB_{control})\} \times 100$$

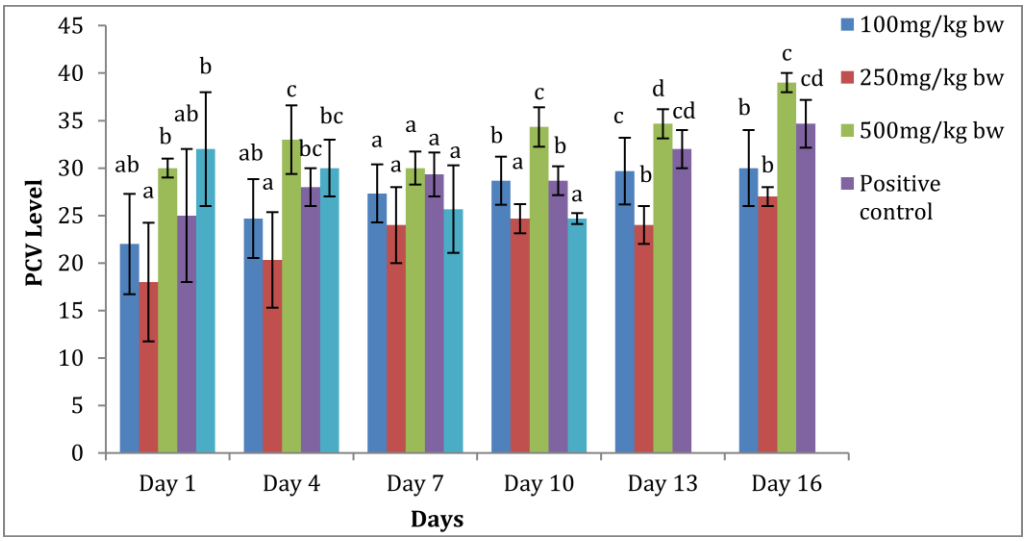

Figure 2: Effects of *Allium sativum* Extract on PCV Level on *Trypanosoma congolense* Infected Mice

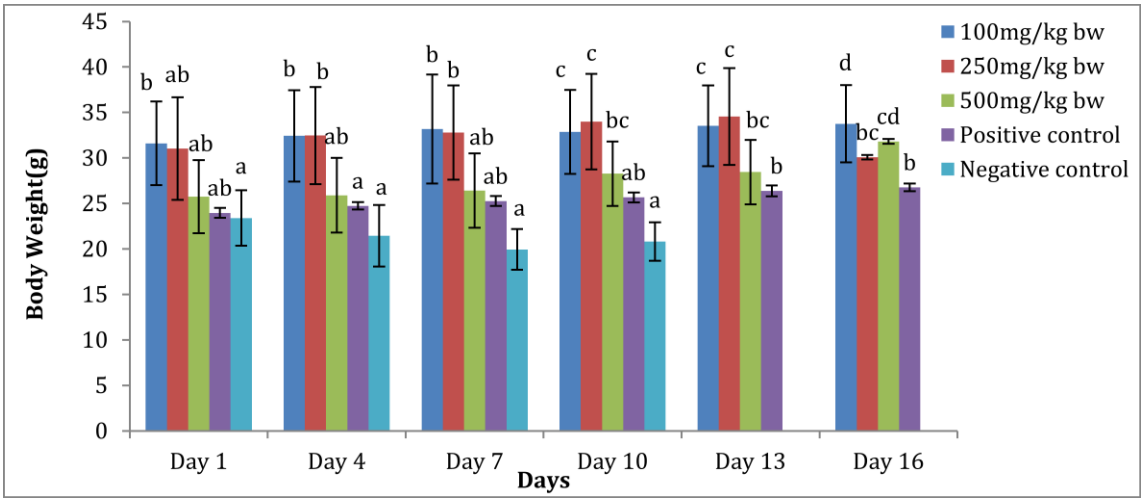

Figure 3: Effects of *Allium sativum* Extract on Change in Body Weight on *Trypanosoma congolense* Infected Mice

3. RESULTS AND DISCUSSION

Toxicity (acute) test of garlic extract at different doses; 10, 10, 100, 1600, 2900 and 5000 mg/kg bw showed that the extract was not toxic as no death was recorded (Table 1) within 24 hours of the study (phase I and phase II). This is similar to the work of Raphael *et al.,* (2009), where no lethal case was recorded after given dose of 5000 mg/kg body weight to mice, although the animals were inactive (change in physiological behavior) for some hours [21].

3.1. Anti-trypanosomal activity

The Anti-trypanosomal essay of the garlic

Table 2. Effects of Garlic extract on haematological parameters on infected mice

Parameters	100 mg/kg	250 mg/kg	500 mg/kg	Positive control
HB (g/di)	6.27±1.07^{a}	11.57±7.31^{a}	12.10±0.40^{a}	10.35±3.25^{a}
PCV (%)	18.50±3.50^{a}	22.00±2.00^{a}	36.00±2.00^{b}	34.50±5.50^{b}
MCV (Fi)	48.00±2.00^{a}	55.00±1.00^{ab}	54.50±3.50^{ab}	58.50±8.50^{b}
MCH (pg)	14.00±2.00^{a}	12.50±1.50^{a}	12.00±2.00^{a}	14.50±0.50^{a}
MCHC (g/di)	30.00±2.00^{b}	28.00±2.00^{b}	21.50±2.50^{a}	30.50±1.50^{b}
RBC (×10%)	4.25±0.25^{a}	6.30±0.80^{ab}	9.48±0.53^{c}	7.00±2.10^{b}
PLC (×10%)	408.5±249.50^{b}	60.50±5.50^{a}	330.00±18.00^{b}	361.50±76.50^{b}
TWBC (×10%)	12.85±5.25^{a}	10.25±2.25^{a}	13.75±2.35^{a}	11.10±2.00^{a}
Neutrophils (%)	14.00±6.00^{ab}	10.50±2.50^{ab}	17.50±2.50^{b}	7.50±3.50^{a}
Lymphocytes (%)	64.00±1.00^{a}	80.00±4.00^{b}	69.50±8.50^{a}	82.83±5.35^{b}
RDWC (%)	34.70±0.00^{a}	44.80±1.60^{ab}	54.50±1.30^{b}	47.70±12.20^{b}
Eosinophils (%)	12.00±5.00^{a}	11.50±3.50^{a}	14.50±4.50^{a}	8.00±1.00^{a}

Values are Mean ± Standard Deviation of determination of three replicates. Superscripts with different values on the same rows are p<0.05 (significantly different).

Table 3. Antioxidant Property (DPPH) of Aqueous Extract of Allium sativum

Concentrations (ug/mL)	*Allium sativum* (% inhibition)	Standard (Vitamin C) (% inhibition)
12.5	38.87 ± 1.03[a]	40.40 ± 0.10[b]
25	52.17 ± 0.03[b]	49.53 ± 0.01[c]
50	51.65 ± 0.03[b]	57.01 ± 2.57[d]
100	53.20 ± 0.03[c]	60.32 ± 0.03[e]
IC_{50}	56.19 ± 0.00[d]	38.07 ± 0.00[a]

Values are Mean ± Standard Deviation of determination of three replicates. Superscripts with different values on the same columns are p<0.05 (significantly different).

extract shows mean parasitemia count wit significant different (p<0.05) among the groups treated and that of the positive and negative control groups. Constant elevation in the level of parasite was noted in negative control group till day 10 when they all died due to lack of treatment. This is similar to the work of Maikai *et al*., (2007), in which all the negative control animals died on 6th day [22]. There was no complete clearance of the parasite in positive control (Figure 1), but reduce it to a very minute level. 100 mg/kg bw group experienced increase in level of parasite till day 7 but later decreased, and it was able to contain the parasites till the last day of the treatment (day 16). While there was a decrease in parasite level in 250 mg/kg bw group from day 4 till day 16. High anti-trypanosomal activity was seen in 500 mg/kg bw group (Figure 1) when compared to other groups. This is related to the work of Raphael *et al*., (2009), who reported that, anti-trypanosomal activity of garlic was dose dependent i.e. increase in dosage increases the efficacy of the garlic extract [21].

As observed in the change in body weight of screened animals, aside the treatment groups and the positive control, there was a decline in health status of negative control group (Figure 3) till day 10 where they all died off. But there was also a decline in body weight in 250 mg/kg bw from day 13, while 500 mg/kg bw recorded high increase in change in body weight (Figure 3). However, all the other groups except the negative control witnessed a promising increase in PCV level as observed (Figure 2). There was highest increase in PCV level of positive control group followed by 500 mg/kg bw (Figure 2) while slight increase was observed in 100 mg/kg bw group. The results were similar to other studies giving conformation where highest PCV level was recorded in the highest dose [21].

3.2. Hematological studies

In the hematological studies (Table 2) of the survived animals after the treatment days, Hb, MCH, TWBC and Eosinophils counts of the positive control group and treated groups showed p>0.05 (no significant difference). While in the RBC count, treatment group and positive control group recorded a significant different (p<0.05) with the highest RBC recorded (9.48±0.53c cells/µl of blood) in 500 mg/kg body weight.

Also this shows ability of trypanosome to cause different forms of anemia. Anemia severity is said to be linked to level of parasitaemia and it is also a feature of trypanosome infections [23]. Reactive oxidative stress can be generated by trypanosomes that attack the membrane of red blood cell, including its oxidation and subsequently hemolysis. Anti-trypanosomal activity of the garlic extract might be as a result of secondary metabolites that generate free radicals to disrupt the metabolism of the parasites [22]. The release of nitric oxide and cytokines initiates the pathological effects of trypanosome. The bioactive components (alliin and its derivates) of garlic have ability to neutralize toxic compound thereby prolonging the life span of the organism [24]. Incomplete clearance of the parasite at the end of the treatment days might be due to low amount of active ingredients in garlic and more so, late commencement of treatment gives rooms for the parasites to fully establish it in the host [25].

3.3. Anti-oxidant activity

The antioxidant activity was determined by DPPH essay of garlic aqueous extract showed that, there was $p<0.05$ (significant difference) in % inhibition of vitamin C (standard) and the garlic extract (Table 3), with the exception 25 µg/ml and 50 µg/ml concentrations of garlic where no significant different ($p>0.05$) was recorded i.e. 52.17±0.03b µg/ml and 51.65±0.03b µg/ml respectively. Moreover, there was an increase in the % inhibition of the standard as the concentration was increasing (i.e. from 12.5 µg/ml – 100 µg/ml) with inhibition concentration (IC50) of 38.07±0.00a µg/ml. Increase in *A. sativum* % inhibition was also noted but with a slight diversion between 25 µg/ml an 50 µg/ml, with inhibition concentration (IC_{50}) of 56.19±0.00a µg/ml. Therefore, the theory is that, the lower the IC_{50}, the better the antioxidant property.

4. CONCLUSION

There are some selective changes in some of the screened parameters. However, since high anti trypanosomal activities was observed in 500 mg/kg bw, garlic bulbs can be considered relatively safe and can be explored by oral healing at this capacity. Efficacy of garlic as anti trypanosomal agents might be due to the presence of secondary metabolites which forms free radicals and interferes with metabolism of trypanosomes. Further research at varying concentration is encouraged and starting treatment early may also lead to complete clearance of the parasites.

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6. CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

7. SOURCE/S OF FUNDING

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