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In vitro Trypanocidal Activity of Aqueous Leaves Extract of *Allium cepa linn* in Experimentally Infected Wistar Rats

[1]Biu, A. A., [2]Auwal, M. S., [2*]Maina, U. A., [2]Salihu, S. I., [2]Kashimbe, I. B., [3]Buratai, L. B., [4]Ibrahim, A., [5]Lawan, F. A. and [3]Ruth, P. A.

[1]Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria
[2]Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria
[3]Department of Biochemistry, Faculty of Science, University of Maiduguri, Nigeria
[4]Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria
[5]Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria

[*] *Author for Correspondence: mainaumar62@unimaid.edu.ng*

ABSTRACT

The aqueous leaf extract of *Allium cepa* L. was evaluated for its phytochemistry, acute toxicity, efficacy and *in vitro* trypanocidal activity using standard procedures. A total of 12 Wistar rats were grouped into 4 groups of 3 rats each and were intraperitioneally administered with graded doses of 100, 200, 400 and 800 mg/kg bw of the extract. All groups of Wistar rats were observed for 24 hours for sign of toxicity and mortality. Qualitative phytochemical analysis of the aqueous leaf extract revealed the presence of alkaloids, flavonoids, anthraquinones, steroids, saponins, reducing sugars, monosacharides, glycosides, tannins and ketones, while phlabotannins was absent. The median lethal dose (LD_{50}) of the plant extract was determined as 600 mg/kg. Clinical signs exhibited in the treated Wistar rats were dose dependent. The *in vitro* trypanocidal efficacy of the extract of *A. cepa* on *Trypanosoma brucei* showed lethality at 800 mg/ml.

KEYWORDS: Acute toxicity*; Allium cepa;* Leaves extract; *Trypanosoma brucei brucei;* Trypanocidal efficacy

INTRODUCTION

African Trypanosomosis, also known as African sleeping sickness is a disease caused by trypanosomes that are protozoans transmitted by the bite of haematophagus flies producing a chancre on the skin followed by swelling of lymph nodes and subsequently invasion of blood stream, where it multiplies so fast within 4 – 6 hours post infection in susceptible species (Soulsby, 1982; Jennings, *et al.,*1983; Mbaya, et, al., 2009. Rise in trypanosome number in the blood results in destruction of red cells and platelets (Yusuf *et al.,*2013). Anaemia, low immunity and chronic cachexia ensue and many animals die of cardiac failure (Igbokwe and Anosa., 1989). *Trypanosoma brucei* a haemo-protozoon parasite transmitted by tsetse-fly causes the disease Trypanosomosis that leads to serious losses in cattle, sheep and goats (Gibson *et al.,*1978).

The parasites in the midgut of tsetse fly transforms into procuclic trypomastigotes that culminates into epimastigotes that finally reaches the fly's salivary glands and continue it's multiplication by binary fission (Griffin, 1983). They are about 23 species of these flies in sub-saharan Africa that are actively involved in the transmission of trypanosomosis which is primarily made of *Glossina morsitans, G. palpalis, G. fusca* (Krinsky, 2019).

Animal trypanosomosis in Nigeria is widely distributed from mangrove forest to Sudan savannah region due to presence of the tse-tse flies in this area (Isaac *et al.,* 2017). The economic importance of the disease includes high morbidity, mortality rate and cost of treatment (Bukachi *et al.,*2017). The current chemotherapy of this disease relies on synthetic drugs such as suramin, pentamidine, melasoprol, eflorithine, arsobal and mel B, pentamidine, diminazene aceturate (berenil), melarsopol (arsobal) and suramin (Sofowora, 1996).

It has been observed that natural products derived from plants and plant products offer novel possibilities for new drugs that are active against trypanosomes (Hoet *et al.,*2004). Onion a biennial plant with the botanical name *Allium cepa* L. is a member of the *Alliceae* with about 4000 species in existence and is the second most important biennial horticulture crop all over the world. Other species in existence includes species such as *A. sativum*, *Allium stulosum*, *A. tuberosum*, *A. schoenoprasum, A. ursinum* and *A. porrum* (Fenwick *et al.,*1985; Randle and Lancaster, 2002).

Several *Allium spp* mentioned above have been used as remedy against variety of clinical conditions diseases such as common cold, anthelmintic, aphrodisiac, carminative, emmenagogue, vertigo, migraine, expectorant, tonic and for the treatment of bruises, bronchitis, cholera, colic, headache, fever, high blood pressure, adjuvant therapy for diabetes jaundice, pimples, dropsy and sores (Bora and Sharma, 2009).

Fresh Onion juice is often recommended in folk medicine in various countries for pain and swelling management after bee or wasp stings, which are followed by an allergy-induced reaction of the skin. The observed inhibitory effects of onion extracts on this kind of cutaneous reactions led to the discovery of anti-inflammatory and antiasthmatics, thiosulfinates and cepaenes (Kahrizi *et al.,*2012; Elberry *et al.,*2014).

This study was therefore designed to determine the possible application of aqueous leaves extract of *Allium cepa* L. as an alternative chemotherapeutic agent in the management and treatment of Trypanosomosis in human and livestock species.

MATERIAL AND METHODS

Plant collection and Identification

Allium cepa leaves was obtained from Gomboru Market in Maiduguri, Borno State and authenticated by a botanist in the Department of Biological Sciences, Faculty of Science University of Maiduguri, Nigeria.

Plant Processing

The fresh leaves of *A. cepa* was rinsed in clean tap water and dried under shade, ground into fine powder using pestle and mortar and sieved. The processed material yielded 1000g of the leave product.

Plant Extraction Process

The 1000g of *dried A. cepa* leaves were soaked in sufficient quantity of double distilled water, kept for 48hours and shaken at 6hours interval. After which it was sieved and filtered then evaporated in an oven at 50 ºC. The dried product is grounded into powder yielding 73.18g resulting in 7.318 % yield.

Phytochemical Analysis

Test for tannins, alkaloids, saponins, steroids, flavonoids, free anthraquinones, glycosides, ketones, reducing sugars and carbohydrates was done through standard procedure (Trease and Evans, 1987).

Experimental Animals

Total of 12 Wistar rats, aged between 12- 16 weeks and weighed 110- 160g were obtained and used for the study from the animal breeding house behind DG 118 at the Faculty of Veterinary Medicine, University of Maiduguri, Borno State. The rats were kept in a polyacrylic cage and fed grower mash (Vital Feed Nigeria Limited) and drinking water given *ad libitum*.

Ethical statement

The experiment was conducted following the OECD guidelines on the use of experimental animals (Demers *et al.,*2006).

Trypanosome Stock

Trypanosoma brucei brucei for this study was obtained from the Department of Veterinary Microbiology, University of Maiduguri, Nigeria. The parasites were identified as *T. brucei* based on morphology and negative blood inhibition infectivity test (BIIT) and stabilized by four passages in rats before storage in liquid nitrogen. The stabilates were passaged twice in donor rats. Tail blood from the donor rats was diluted with phosphate buffered glucose saline (PBSG) pH 7.2. Each mouse was infected intra-peritoneally with blood from the donor rats containing about 1.5 $x10^6$ trypanosomes. The initial detection of parasitaemia was done by wet mount and haematocrit buffy-coat methods (Jennings, *et al.,*1983).

Experimental Design

Acute Toxicity Study

Total of 12 Wistar rats were grouped into 4 of 3 Wistar rats each. They were intraperitoneally treated with graded doses of 100, 200, 400 and 800mg/kg body weight of aqueous leaves extract of *Allium cepa*. All the groups were observed for 24 hours for clinical signs of toxicity and death (Kojima *et al.,* 1993).

In vitro Trypanocidal Study

Two hundred milligrams (200mg) of the aqueous leaves extract of *Allium cepa* L. was weighed and dissolved in 5 m1 of phosphate buffered saline solution yielding the concentration of 40 mg/ml. A serial dilution of this stock solution was done using phosphate buffered saline solution to obtain descending concentrations of 0.078, 0.156, 0.313, 0.625, 1.25, 2.5, 5, 10, 20 and 40mg/ml, respectively and a tube containing 2.0 ml of parasites suspended in phosphate buffered saline solution was used as a control. Evaluation of the *in vitro* trypanocidal efficacy of the extract was performed in test tubes using 2 *u1* of *T. brucei* infected blood from albino rats and was inoculated into each test tube containing various concentrations of the extract incubated at 37°C. Parasites count was then monitored using glass counting chamber covered with a cover slip and observed under a microscope at x40 magnification. The numbers of motile parasites were counted at 30, 60, 90 and 120 minutes' interval. The percentage mortality of trypanosome parasites was then calculated using this formula given below:

$$\% \text{ Mortality } = \frac{parasite\ count\ of\ control - parasite\ count\ of\ the\ treated}{parasite\ control} x100$$

Statistical Analysis

Data on mortality counts of the parasites were analysed by Graph Pad Instat Biostatistic Version 3 Inc®, U.S.A., 2002 software using one way analysis of variance (ANOVA) where values are expressed as Mean $\pm$ SD and p value $\leq$ 0.05 were considered significant.

RESULTS

Phytochemical constituents

The phytochemical screening result for bioactive substance in aqueous leaves extract *of Allium cepa* revealed the presence of alkaloids, flavanoids, anthraquinones, steroids, saponins, reducing sugars, monosacharides, glycosides, tannins and ketones, whereas phlabotannins was absent as indicated in Table1.

Table 1. Qualitative phytochemical constituent of aqueous leaves extract *of Allium cepa.*

Phytochemical constituents	Test	Inference
Alkaloids	Dragendorff's	+
Flavonoids	Ferric chloride	+
Anthraquinones	Borntrigger's	+
Steroids	Lierbrman-Buchard	+
Saponins	Frothing	+
Reducing sugar	Benedict's	+
Monosacharides	Barfoed's test	+
Tannins	Ferric chloride	+
Glycosides	Killer William test	+
Ketones	Salivanoff's test	+
Phlobatannins	Goldbeaters test	-

Key: + Present - Not detected

Median Lethal Dose

The median lethal dose (LD_{50}) of the extract is presented in Table 2. The extract at the dose of 800mg/kg produced 100% mortality while the LD_{50} was 600 mg/kg. No mortality was observed at graded doses of 100, 200, 400mg/kg of the extract. Clinical signs observed following the administration of the extract doses to the rats were sluggishness, awkward posture, loss of appetite, starry hair coat and death within 24 hours.

Table 2. Median Lethal Dose (LD_{50}) of aqueous leaves extract *of Allium cepa* in Wistar rats

Group (n=3)	Plant extract (mg/kg)	Dose difference (DD)	Number dead	Mean dead (MD)	DD × MD
A	100		0		
		B – A = 100		0	0
B	200		0		
		C –B = 200		0	0
C	400		0		
		D – C = 400		1.5	600
D	800		3		
Total					600

$$LD_{50} = LD_{100} - \frac{DD \times MD}{n} = 800 - \frac{600}{3} = 600\text{mg/kg bw}$$

In Vitro Trypanocidal Activity

The mean evaluation of trypanocidal activity of aqueous leaves extract of *Allium cepa* following *in vitro* exposure of *Trypanosoma brucei* to various concentrations is presented in Table 3. There was significant ($P<0.05$) reduction in mean parasite count ($x10^6$) of *Trypanosoma brucei* exposed to graded concentrations of 0.078, 0.156, 0.313, 0.625, 1.25, 2.5, 5, 10, 20 and 40 mg/ml of the extract. There were no parasites observed at 1.0, 2.0 and 4.0mg/ml concentration of the extract (Freiburghaus *et al.,*1996).

Table 4 shows the *in vitro* effect aqueous leaves extract of *Allium cepa* on *Trypanosoma brucei* activity. There was 100% inhibition of *Trypanosoma brucei* parasites at the extract concentration of 10, 20 and 40mg/ml, respectively.

DISCUSSION

Herbal medicines from onions have gained global recognition due to its medicinal and economic values. Extensive use of herbal products from onions and other plants throughout the world has raised multiple concerns, hence the quality, safety and efficacy of plant and their products has to be scientifically assessed before application in any form of therapy. Onion (*Allium cepa* Linn.), a member of the family *Alliaceae*, is the second most important horticultural crop all over the world. This plant is an important source of phyto-constituents used as food, and a flavoring agent in soup (Bora and Sharma, 2009). Onions possess high level of antioxidant activity attributed to phytoconstituents such as flavonoids, quercetin, kaempferol, myricetin, pigments such as anthocyanins and organonosulphur compounds in the plant. The most important among the Sulphur compounds in the plant are the cysteine derivatives such as non-volatile amino acids, S-alk (en) yl-substituted cysteine sulphoxides and their decomposition products such as thiosulfinates and polysulfides.

Table 3. *In vitro* activity of aqueous leaves extracts of *Allium cepa on T. brucei* Count per Million (x 10^6)

Conc. of extract (mg/ml)	Parasite count per minutes post inoculation (MPI) (x 10^6)			
	30	**60**	**90**	**120**
S	4.9 ± 0.09 (4.75-4.95%)	5.0 ± 0.13 (4.85 -5.15%)	5.2 ± 0.13 (5.05-5.35%)	5.3 ± 0.18 (5.05 – 5.45%)
0.078	4.6 ± 0.06 4.55-4.70	4.8 ± 0.07 4.75 – 4.89	4.8 ± 0.03 4.80-4.85	4.9±0.06 4.85-5.00
0.156	4.1± 0.02 3.80 – 4.25	4.3± 0.34 4.00 – 4.80	4.5 ± 0.23 4.25 – 4.80	4.4 ± 0.42 4.10 – 4.70
0.313	3.8 ± 0.09 3.65 – 3.80	4.1± 0.26 3.85 – 4.45	4.2± 0.23 4.00 – 4.55	4.2 ± 0.42 3.95 – 4.85
0.625	4.0 ± 0.09 3.95 – 4.15	4.1± 0.11 4.00 – 4.25	4.3± 0.06 4.20 – 4.35	4.2± 0.33 3.95 – 4.65
1.25	3.3 ± 0.23 3.00 – 3.55	3.6± 0.49 3.25 – 4.30	3.8± 0.51 3.40 – 3.65	3.7 ± 0.61 3.40 – 4.65
2.5	3.4 ± 0.05 3.35 – 3.45	3.6 ± 0.06 3.55 – 3.65	3.5 ± 0.11 3.40 – 3.65	3.3± 0.10 3.25 – 3.45
5	1.8 ± 0.11 1.65 – 1.90	1.9 ± 0.09 1.85 – 2.05	1.7 ± 0.17 1.55 – 1.95	1.6 ± 0.13 1.45 -1.75
10	0.02 ± 0.11 0.01 – 0.03	0.00 ± 0.00 0 -0	0.00 ± 0.00 0 -0	0.00 ± 0.00 0 -0
20	0.00 ± 0.00 (0– 0)	0.00 ± 0.00 (0 -0)	0.00 ± 0.00 (0 -0)	0.00 ± 0.00 (0 -0)
40	0.00 ± 0.00 (0 -0)	0.00± 0.00 (0 -0)	0.00 ± 0.00 (0 -0)	0.00± 0.00 (0 -0)

Table 4. *In vitro* efficacy of *Allium cepa* leaf aqueous extract on *Trypanosoma brucei* (% inhibition)

Conc. of extract (mg/ml)	Parasite count in minutes post inoculation (MPI) (x 10^6)			
	30	**60**	**90**	**120**
PBS	-	-	-	-
0.078	4.6 ± 1.27 (3.09-6.06 %)	3.8± 2.62 (0.00-5.94%)	6.9±1.95 (5.83-9.35%)	6.6± 3.00 (2.97-11.01%)
0.156	16.9 ± 4.43 (12.63-22.45)	13.8± 4.91 (6.80-17.53)	13.9± 2.52 (10.28-15.84)	17.4± 3.24 (13.76-21.15)
0.313	22.7 ± 1.66 (21.05-24.74)	18.1± 3.38 (13.59-20.79)	18.6± 2.52 (14.95-20.79)	19.8± 6.48 (11.01-25.24)
0.625	17.2 ± 1.63 (15.79-19.39)	17.9± 1.95 (15.46-19.80)	18.0± 1.80 (15.48-20.00)	20.96± 0.31 (14.63-24.30)
1.25	32.6 ± 4.52 (28.28-38.78)	28.6± 8.48 (16.51-34.65)	27.3± 8.30 (14.95-32.38)	29.1± 9.77 (14.58-35.51)
2.5	30.3 ± 0.93 (29.47-31.63)	28.0± 2.22 (24.74-29.70)	32.4 ± 3.04 (29.70-36.45)	36.8± 2.85 (33.65-40.37)
5	63.2 ± 2.47 (61.05-65.98)	61.2± 1.91 (58.59-63.11)	66.8± 3.87 (36.45-71.03)	70.3± 2.22 (67.29-72.48)
10	99.6 ± 0.20 (99.39-99.80)	100± 0.00 (100–100)	100± 0.00 (100–100)	100± 0.00 (100–100)
20	100± 0.00 (100–100)	100± 0.00 (100–100)	100± 0.00 (100–100)	100± 0.00 (100–100)
40	100± 0.00 (100–100)	100± 0.00 (100–100)	100± 0.00 (100–100)	100± 0.00 (100–100)

These sulphur compounds and flavonoids possess, antioxidant, antidiabetic, anti-inflammatory, anticancer, antimicrobial, antihyperlipidaemic, anticholesterolaemic, fibrinolytic, antiatherosclerotic, anticataractogenetic, antiplatelet aggregation, immunomodulatory, neuroprotection and various other biological activities (Bora and Sharma, 2009).

The median lethal dose (LD_{50}) of the extract was calculated to determine the margin of safety and toxicity of the extract which was found to be 600mg/kg. The extract is found to be toxic due to clinical signs of sluggishness, awkward posture, loss of appetite, starry hair coat and death within 24hours as observed in Wistar rats treated with 800mg/kg of the extract. No mortality was observed in rats administered the doses of 100, 200 and 400mg/kg body weight of the extract, respectively which indicates low toxicity at low doses.

In vitro efficacy of the extract on *Trypanosoma brucei* at different concentrations was conducted, where 100% inhibition and mortality of *Trypanosoma brucei* organisms at 10, 20 and 40mg/ml was recorded. This agrees with Sepulveda and Cassel's findings which indicated that natural plants products exhibit their trypanocidal effect by acting either on the respiratory centre or cellular defence mechanism of trypanosomes by inducing oxidative stress

related effects. Natural products possess structures capable of generating chemicals that may cause perioxidative damage to trypanothine reductase enzymes that is very sensitive to alterations in redox balance, it is also known that some natural products act by binding to kinetoplast DNA of trypanosomes leading to mortality of *Trypanosoma brucei* organisms (Alli and Gower Jr, 2010).

Concerning the antimicrobial activity of *Allium sativum* which also belong to same family with *A. cepa*, it has been established by several studies to be more active than *Allium cepa*, which agrees with the literature from Benmalek *et al.,* (2013). In this study, we could only confirm the known antimicrobial activity of *A. cepa* as indicated in the results above where trypanosome was completed inhibited upon the introduction of *A. cepa* leaves extracts at 10, 20 and 40mg/ml concentration. This is in confirmation with the study conducted by Li *et al.,* (2015), where he established the synergistic therapeutic efficacy of *A. sativa* in combination with other antimicrobial agents.

In conclusion, *Allium cepa* aqueous leaf extract exhibited *in vitro* trypanocidal activity which had positive correlation with the concentration levels of the product used in this study. The extract is shown to be toxic at high doses and possess bioactive components with trypanocidal properties. *In vivo* studies on trypanocidal effect of *Allium cepa* leaves on *Trypanosoma brucei* is recommended

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Author Contribution

AAB designed the research work. AMS and UAM collected samples and conducted the Laboratory work. SSI and UAM reviewed literatures, prepared and proofread the manuscript. KIB and IA were responsible for the log book and calculating results while LFA and RPA conducted the data analysis. All authors have read and agreed with the content of the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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