Effects of *Allium Sativum* Ethanolic Extract on *Trypanosoma brucei brucei* Parasites' Morphometric Parameters and Clinical Outcome in White Albino Laboratory Rats

* Ng'wena G. Magak[2], Odera Rosemary[1], Ngiywa M. Moses[1], Mwaniki D. Minjire[1] and Okumu Wilson[3]

1. *Department of Biological Sciences, Moi University, P.O. Box 1125-30100, Eldoret Kenya.*
2. *Department of Medical Physiology, School of Medicine, Maseno University, P.O. Box 333, Maseno Kenya.*
3. *Department of Medical Biochemistry, School of Medicine, Maseno University, P.O. Box 333, Maseno Kenya.*

Corresponding Author: Ng'wena G. Magak Email address: gngwena@hotmail.com

Summary

BACKGROUND

***Trypanosomosis* affects humans as well as wild and domestic vertebrates, yet has no successful prophylaxis, chemotherapy nor cure.**

OBJECTIVES

The study was to investigate the effects of *Allium sativum* extract on *Trypanosoma brucei brucei* parasites' *morphometric* parameters, *parasitemia* and the clinical outcome in white infected Albino laboratory rats in order to determine its *trypanocida*l effects.

METHODOLOGY

The study was conducted at the department of Biological Sciences Laboratory of the Moi University Eldoret. Thirty two (32) mature rats randomly divided into four groups (M, N, P and Q) were kept in four (4) cages in a well ventilated room, with adequate light supply in the day.

Sixteen (16) rats were infected with *T. b. brucei* (1.0 x 104 parasites per rat); eight (8) of which (Group N) were treated with the *A. sativum* ethanolic extract on day 5 and day 9 after infection, while the other eight (8)rats (Group Q) received saline treatment on the same days. Sixteen (16) non-infected rats (controls) were also divided into two groups of eight rats each (P and M) and treated as in group N and Q, respectively. The rats were obtained from University of Nairobi, Chiromo Campus.

RESULTS

All infected rats became parasitemic two days after infection and reached peak levels on day 4 and 5 post infection. Parasitemia in saline treated infected rats fluctuated between 4025.5 ± 0.05 - 5544.4 ± 0.05 parasites per 200WBC whereas in the extract treated rats parasitemia declined from 6976.6 ± 0.05 - 311.0 ± 0.05 parasites per 200WBC after the first treatment. Uninfected saline treated rats maintained normal Hb level (10.6g/L to 11.8g/L) as compared to the uninfected extract treated rats' whose Hb levels was at 13.41g/L to 14.36g/L.

The haemoglobin level changed to 8.0g/L four days after the infection in the group N rats before rising to 10.2g/L on day 8 post-infection following the extract treatments. Group Q rats' Hb declined to 6.43g/L by the end of the study. RBC count of the infected saline treated rats declined to 3.38 x 106/µL as compared to 4.93-7.61 x 106/µL in the normal rats by 11 days post-infection.

There was however no significant change in WBC, temperature and weight between the saline extract treated rats. The extract produced a shrinking effect on the parasite's body with some of the morphometric parameters appearing significantly (P<0.05) reduced as observed under a microscope with ocular and stage micrometer scale. The mean nucleus, posterior ends to nucleus centre, the nucleus centre to the anterior end and the body length were reduced from 2.41µm to 1.42µm(P=0.00), 4.42µm to 3.68µm(P=0.017) , 4.65µm to 4.18µm(P=0.001) and 8.58µm to 7.19µm(P=0.001) respectively.

CONCLUSION

In conclusion it was evident that, *A. sativum* ethanolic extract exhibited *Trypanocidal* effects that can be exploited to control clinical progression of *Trypanosomosis* in rats. In addition, the data presented demonstrates the plant extract had the potential to improve the red and white blood cell indices reducing *parasitaemia* following *T. b. brucei* infection. These findings suggest that, the garlic extract affected the plasma membrane of the parasites since shrinking was only possible with disrupted membrane biochemistry.

Key words: *Trypanosoma brucei brucei, Allium Sativum, Parasitemia, Morphometric* parameters,

[***Afr. J.*** Health Sci. 2020 33(1) : 1 - 13.]

Introduction

Trypanosomosis is one of the most crucial insect-transmitted tropical diseases that affect wild and domestic vertebrates as well as humans. Caused by protozoa parasites of the *genus Trypanosoma*. The disease is transmitted by tsetse flies of the *Glossina species* occurring in most tropical parts of Africa [14].

Pathogenic forms of *Trypanosoma* species parasites in the tropical areas include T*rypanosoma congolense, Trypanosoma evansi, Trypanosoma vivax* and *Trypanosoma brucei,* [6]. T*rypanosoma brucei rhodesience* and *Trypanosoma brucei gambiense* in particular are the causative agents of human sleeping sickness [10].

Trypanosomosis has been described as being the single most devastating disease in Africa in relation to poverty, loss of man hours and loss of agricultural production amounting to 3 billion pounds annually [9].

More than 60 million people with 300,000 – 500,000 thousands new infections reported per year [28, 25]. Some 25 million cattle in Africa, have been reported to be at the risk of infection with *Trypanosomosis* (Ghaffar, 2004).

The disease is endemic in 36 countries of Sub-Saharan Africa including, Kenya, Malawi, Ethiopia, Zaire, Zimbabwe and Botswana [28].

Generally the disease is characterized by intermittent fever, anemia, loss of condition, emaciation, and immunosuppression. It also causes reproductive dysfunction which include abortion and infertility, reversal of the sleep-wake cycles, endocrine dysfunction and nervous system disorders.

Animals that survive *Trypanosomosis* are often unhealthy and of very poor reproductive potential [6]. Although in some instances, infected animals show no signs of the disease they may succumb to the infection if stressed by work, pregnancy, milking or adverse environmental conditions [15].

Attempts to control and prevent *Trypanosomosis* had failed partly due to drug resistance and lack of appropriate vector control methods. Available chemotherapy approaches were aimed at inhibiting the multiplication of the *Trypanosomes* within the mammalian host in addition to boosting the host's immune system to overcome the infection. Chemotherapeutic drugs were toxic to the *Trypanosome* due to the blockage or disruptive effect on the parasites vital processes [16] including membrane formation, transcription, translation and also specific metabolic pathways. Chemotherapy only became more effective in a well fed and rested animal in which the immune system was not adversely affected by stress and lack of food [6].

Likewise, some studies had also reported that the drugs in use exhibited similar effects on the host's cells thus making them toxic to the host tissues [6, 4].

Plants have demonstrated remarkably diverse array of natural products, many of which have anti-microbial activities [11] and the use of plant materials particularly for treatment of specific diseases has a long history in Africa [24].

However, little had been reported about the treatment of *Trypanosomosis* using drug derived from plants. Garlic (*Allium sativum*) is one of the plants that had been reported to have anti-microbial activities. The active ingredients in garlic had shown to be numerous but arose mainly from sulphur-containing compound known as *diallyldisulphide* (DAD) [26].

DAD had a lipid-regulatory effect in lowering the levels of Cholesterol, Triglyceride, Low-density Lipoprotein (LDL) and Very Low –Density lipoprotein (VLDL). It also elevates High Density Lipoprotein (HDL) levels [12].

Although *A. sativum* had shown to have anti microbial activity, little information was available on its *Trypanocidal* effects. The present study was carried out to investigate the effects of *A. sativum ethanolic* extract on *Trypanosoma brucei brucei* through determination of any changes on the parasites morphometric parameters and clinical outcome in infected laboratory rats.

Materials and Methodology

The study Area

This laboratory based study was conducted at the department of Biological Sciences laboratory of Moi University, Eldoret. The study used adult white albino rats that were kept in four (4) cages in a well ventilated room, with adequate light supply in the day.

The Laboratory Rats

Thirty two (32) white albino adult laboratory rats used in this research were obtained from University of Nairobi, Chiromo Campus. After delivering the study rats to the research laboratory they were allowed two weeks for acclimatization during which they were accustomed to handling through regular weighing and *Phlebotomy*. All the rodents were fed with commercial mice pencils regularly and given drinking water *ad libidum*.

Trypanosoma brucei brucei Parasites

The *Trypanosoma brucei brucei* parasites were obtained from University of Nairobi, Chiromo Campus, Department of Biochemistry. The parasites were passaged into two adult rats which acted as donors (source) of parasites for the experimental rats, before being transported to the Moi University, Eldoret laboratory.

Blood Smears, Weights and Rectal Temperature

After the acclimatization period, blood smears of each rat was obtained daily by snipping of a minute piece of the tip of the tail and transferring the resulting drop of blood to a slide for microscopic examination before and after infection with the *Trypanosome* parasites. The blood obtained from the snipped tail of each rat was drawn into heparinized capillary tubes and then transferred to a coulter counter machine for analysis of RBCs and WBCs. The rats were also weighed daily using a digital weighing balance during the pre and post infection period. Rectal temperatures were taken daily before and after the infection by using a digital thermometer.

Extraction of Garlic (*Allium Sativum)* Extract

Five fresh bulbs were bought from the Eldoret municipal market, the cloves separated, peeled, and mashed using a mortar and pestle. About 30g of mashed sample of garlic cloves was weighed and put into 100ml of 85% (v/v) ethanol in a conical flask, mounted on a shaker and agitated for 3 hours at 45ºC. The contents were then centrifuged for 30 minutes at 5000rpm. The supernatant was dried in hot air (70ºC) to a constant weight of 8.55g, stored in a refrigerator, and was only reconstituted to a concentration of 17.6mg/2ml of water when required [22].

Experimental Design

Thirty two (32) adult albino white rats used in the study were divided randomly into four groups and kept in four different cages (eight rats per cage) labeled M (non-infected control rats that received saline treatment on day 5 and 9 in the post infection phase of

the study), N (*Trypanosome*-infected experimental rats that received *A. sativum* ethanolic extract treatment on the 5th and 9th day of post infection), P (non-infected control rats that received *A. sativum* ethanolic extract treatment on the 5th and 9th day in the post infection phase of the study) and Q (*Trypanosome*-infected experimental rats that received saline treatment on the 5th and 9th day during the post infection phase of the study. The treatment on the 5th and 9th day of post infection was administered intraperitoneally for each of the rats.

The infection process was done by passaging the parasite to the experimental animals and drawing blood (1.5ml) from the infected donor rat through cardiac puncture method. The blood was diluted by phosphate buffered saline solution (pH 7.4), and then each rodent was inoculated intraperitoneally by approximately 1.0 X 10^4 parasites on day zero (0).

Parasitaemia and Parasites' Morphological Parameters

In the direct method, the fresh blood was examined by microscopy using medium magnification. The parasitological diagnosis was done using the direct method of wet blood film and the thin blood smears. Thin blood smears were fixed by methanol and stained with Giemsa stain, using an oil immersion objective (40-50*x*) for scanning, and 100*x* for identification of *Trypanosomes* as well as the determination of the parasite densities. The smears were also used to determine morphometric parameters by using a microscope with ocular and stage micrometer scales. The morphometric parameters included the parasite length and the parasite nucleus sizes.

Data Analysis

The data was analyzed by the Levenes' test for equality of variance, and t-value for equal variance with significant level at 5% level and 95% confidence intervals.

Results

Parasitemia Levels

All infected rats became parasitaemic after the second day following inoculation with *T. b. brucei* parasites and the parasites were clearly visible in blood smears taken from all the experimental animals' tails ***(Figure 1).***

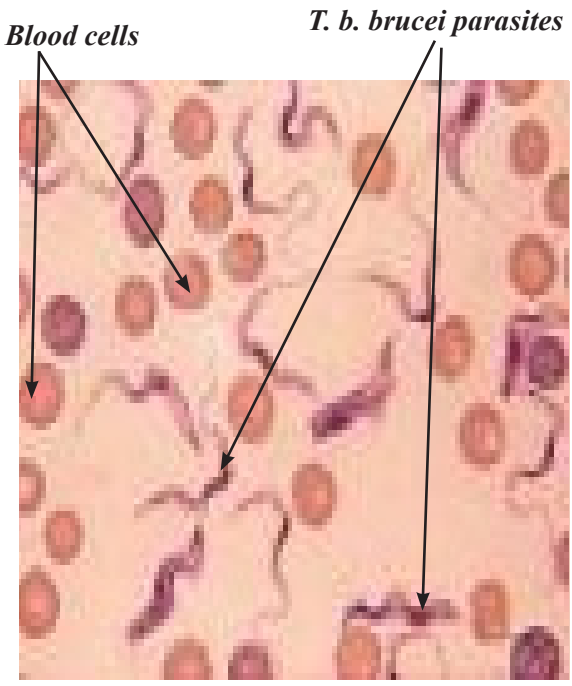

Figure 1 *Trypanosome parasites in a blood specimen from one of the infected rats on day 5 after the infection*

Parasitaemia peak (6996.3 parasites per 200 WBC in group N rats) occurred on day 5 post-infection.

Following the first administration of the *A. sativum* ethanolic extract on day 5 post-infection there was a drastic drop in *Parasitaemia* to 311.0 parasites per 200 WBC in the group N rats three days later (8th day post - infection) ***(Figure 2)***.

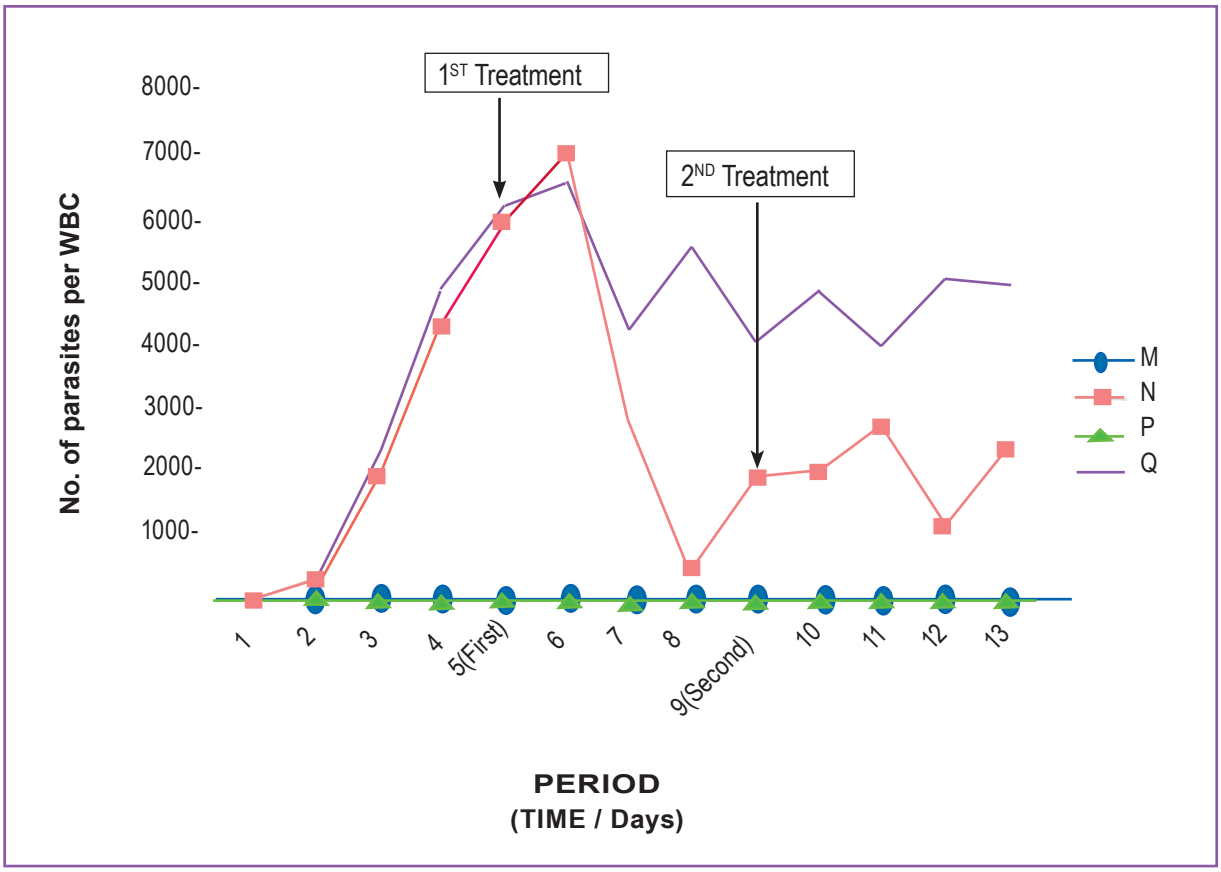

Figure 2: *Parasitaemia levels in rats infected with T. b. brucei*

By the 9th day of post-infection when the rats were due for the second treatment, *Parasitaemia* had started rising from (311.0 to 1800.0 parasites per 200 WBC) in the group N rats.

The second treatment slowed down the *Parasitaemia* increment rate (2650.0 parasites per 200 WBC) between the 9th and 11th days in the group N rats before it declined to 1350.0 parasites per 200 WBC.

As for the group Q rats (infected saline treated) their highest pre-treatment parasitaemic peak also appeared on the 5th day of post infection with 5961.9 parasites per 200 WBC ***(Fig. 2).*** When the first saline treatment to the group Q rats was given on the same day the study recorded a further increase (6250.0 parasites per 200 WBC) in *Parasitaemia* on the 6th day.

However that dropped to 4232.1 parasites per 200 WBC one day later (day 7 post-infection) followed by fluctuations in *Parasitaemia* peaks until the end of the study. The *Parasitaemia* levels in that group of rats ranged from 5544.4 to 4025.5 parasites per 200 WBC after the 7th day of post-infection. *Parasitaemia* levels were significantly ($P<0.05$) reduced in group N rats that received *A. Sativum* ethanolic extract treatment (on the 5th and 9th days of post-infection) as compared to the group Q rats that only received saline treatment.

Morphometric Parameters of the *Trypanosoma brucei brucei* Parasites

The *A. sativum* ethanolic extract treatment caused the parasite's body to shrink. The mean

nucleus width (N) was reduced from 2.41*µm* to 1.42µm (P<0.05), mean posterior ends to nucleus centre (PN) from 4.42µm to 3.68µm(P<0.05), the mean nucleus centre to the anterior end(NA) from 4.65*µm* to 4.18µm(P<0.05) and the mean body lengths (BL), from 8.58*µm* to 7.19µm(P<0.05) ***(Fig. 3).***

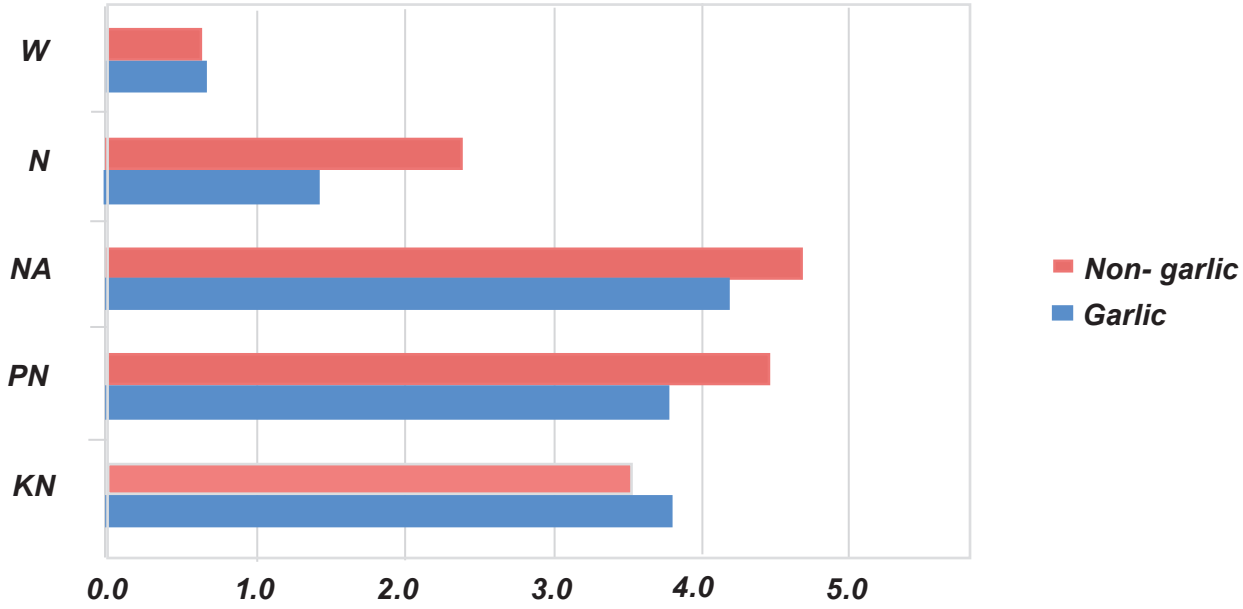

Figure 3: *Parasite Nucleus Sizes Parameters:*

Nucleus width (W), nucleus length (N), the length between the nucleus centre to the anterior end (NA), posterior end to nucleus center (PN), *Kinetoplast* to nucleus (KN)

Although the parasite lengths also reduced in specific cases (L-Body +*Flagella*, FF-Free *flagella*, BL-Body length) the changes were not statistically significant (P>0.05) compared the saline treated parasites except (P<0.05) in the case of body length (BL) ***(Fig 4).***

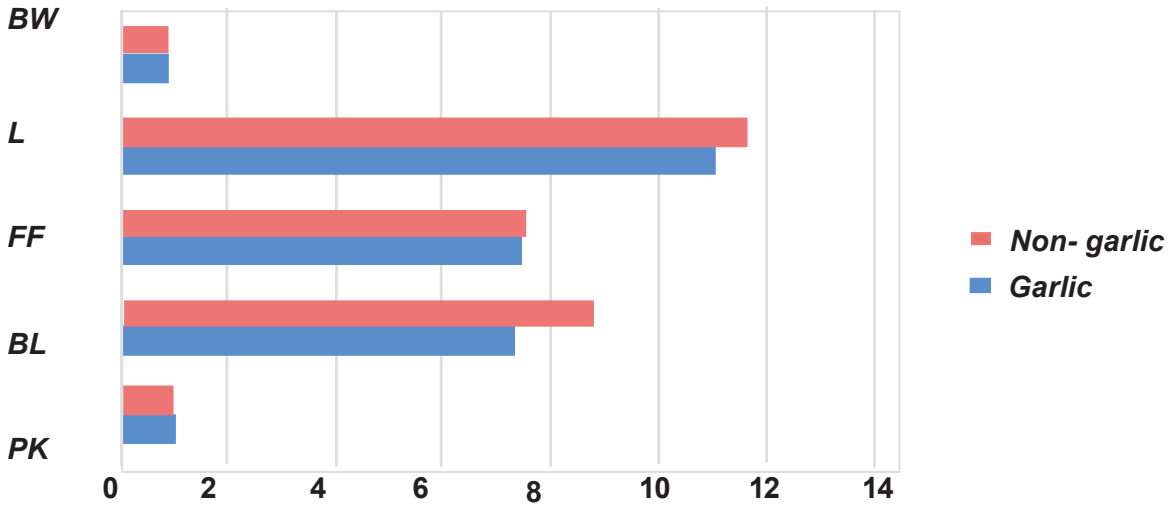

Figure 4: *Parasite length Parameters*

BW	Width of the body on the nucleus levels
L	Total length (body + *flagella*)
FF	Free *flagella* length
BL	Body Length
PK	Length of the posterior end to *Kinetoplast*

The width of the body on the nucleus level (BW) and the length of the posterior end to the *Kinetoplast* (PK) were not affected by the *A. sativum* ethanolic extract treatment.

Mean Hemoglobin Level

Mean Hb levels (g/dL) in the pre-infection period and in the group M rats during the treatment phase was 10.6-11.88g/dL ***(Table 1).***

Table 1: *Pre and Post-infection Mean Hemoglobin levels (gm/dL)*

Pre - Infection Mean *Hb* levels (gm/dL)		Post - Infection Mean *Hb* levels (gm/dL)		
		4th Day	8th Day	11th Day
M	11.8	10.6	11.2	11.8
N	11.38	8.0	10.2	9.2
P	11.88	13.43	13.41	14.36
Q	10.88	8.55	8.91	6.43

KEY: **M** - Uninfected and Saline treated rats.
N - Infected and *Allium sativu*m ethanolic extract treated rats.
P - Uninfected but *Allium sativum* ethanolic extract-treated rats.
Q - Infected and Saline treated rats.

In group N the Hb declined to 8.0g/dL but increased to 10.2 and 9.2g/dL following the treatment with the *A. sativum* ethanolic extract. In group Q animals the levels decline significantly ($P<0.05$) to 6.43g/dL by the time the study was terminated. On the other hand, Hb was significantly ($P<0.05$) elevated to 13.41 - 14.36g/dL in the group P rats during the treatment phase.

Mean Red Blood Cell count

The mean RBC count ranged between 5.2-6.8 x 10^6g/dL ***(Table 2)*** in all rats in the pre-infection and in the group M rats throughout the study period.

Table 2: *Pre- and Post-Infection Mean Red Blood Cell (RBC) Counts x 10^6*

Pre - Infection Mean Red Blood Cell (RBC) counts (x10^6)		Post - Infection Mean Red Blood Cell (RBC) counts (x10^6)		
		4th Day	8th Day	11th Day
M	6.7	5.2	6.8	5.2
N	5.96	6.6	6.08	5.0
P	5.46	7.61	6.8	6.67
Q	6.46	6.05	5.33	3.38

KEY: **M** - Uninfected and Saline treated rats
N - Infected and *Allium sativum* ethanolic extract treated rats
P - Uninfected but *Allium sativum* ethanolic extract-treated rats
Q - Infected and Saline treated rats

In group P rats the mean RBC count in the treatment phase (6.67-7.61x10^{6}g/dL) was not significantly different from the pre-treatment levels. Similar observations (6.6 vs 6.05 x10^{6}g/dL and 6.08 vs 5.33 x10^{6}g/dL) were made in group N and Q on day 4 and 8, respectively. However, on the 11 day of the post-infection period the mean RBC of the N group rats was significantly higher (3.38 5.0 vs 3.38x10^{6}g/dL) than that of group Q rats.

Mean White Blood Cell counts

The mean WBC count in all the normal rats ranged between 10.0-11.35 x10^3 ***(Table 3)***

Table 3 : *Pre- and Post-Infection Mean White Blood Cell (WBC) Counts (x 10^3)*

Pre-Infection Mean White Blood Cell (WBC) counts (x10^3/uL)		Post-Infection Mean White Blood Cell (WBC) counts (x10^3)		
		4th Day	8th Day	11th Day
M	10.0	10.6	10.8	11.0
N	10.85	15.4	11.5	12.7
P	10.35	14.4	14.3	10.2
Q	11.35	15.3	13.4	13.9

KEY: **M** - Uninfected and Saline treated rats.
N - Infected and *Allium sativu*m ethanolic extract treated rats.
P - Uninfected but *Allium sativum* ethanolic extract-treated rats.
Q - Infected and Saline treated rats.

In group P the levels increased from10.2 to 14.4 x10^3 whereas in group N there was an initial increase to 15.4 x10^3 before the levels declined to 11.5 x10^3 and another rise to only 12.7 x10^3. In the group Q rats there was an initial increase to 15.5 x10^3 and then a decline to 13.4-13.9 x10^3.

Mean Rectal Temperatures

The post-infection mean rectal temperatures of the infected and *Allium sativum* ethanolic extract-treated rats (group N) was 38.08-38.11^{0}C. The infected and saline treated rats (group Q) on the other hand had a post-infection mean rectal temperature of 38.21-38.29^{0}C which was higher but apparently not significantly different (P = 0.548) from the mean rectal temperatures of the group N rats ***(Table 4).***

Table 4: *Pre- and Post-Infection Mean Rectal Temperatures*

Pre - Infection Mean Rectal Temperatures(^{0}C)		Post - Infection Mean Rectal Temperatures (^{0}C)		
		4th Day	8th Day	11th Day
M	37.1^{0}C	37.1^{0}C	37.1^{0}C	37.1^{0}C
N	37.2^{0}C	37.98^{0}C	38.08^{0}C	38.11^{0}C
P	37.1^{0}C	37.1^{0}C	37.2^{0}C	37.3^{0}C
Q	37.2^{0}C	37.91^{0}C	38.21^{0}C	38.29^{0}C

KEY: **M** - Uninfected and Saline treated rats.
N - Infected and *Allium sativu*m ethanolic extract treated rats.
P - Uninfected but *Allium sativum* ethanolic extract-treated rats.
Q - Infected and Saline treated rats.

Mean Body Weight Changes

The mean post-infection body weight for infected and *A. sativum* ethanolic treated rats (group N) was 198.12g on the 8th day and 187.24g on the 11th day of post - infection. The group Q rats that only received saline treatment after infection had mean post-infection body weights of 197.55g on the 8th day and 186.16g on 11th day of post - infection. Despite the non - infected rat groups that exhibited body weight gains (M - 30.52g and P - 9.99g) in the post - infection phase of the study the infected rats group showed loss in body weights (N =-9.16 and Q = -12.87g). Weight loss in the *A. sativum* treated group N rats was less as compared to the group that received only saline treatment.

Table 5: *Pre - and Post - Infection Mean Body Weights (gm)*

Pre - Infection Mean Body weight (gm)		**Post-Infection Mean Body weight (gm)**			**Mean body weight gain at the end of the study**
		4 Days	8 Days	11 Days	
M	190.04	190.07	206.47	220.56	30.52
N	196.40	201.66	198.12	187.24	-9.16
P	226.09	227.17	240.11	236.08	9.99
Q	199.03	200.42	197.55	186.16	-12.87

KEY: **M** - Uninfected and Saline treated rats
N - Infected and *Allium sativum* ethanolic extract treated rats
P - Uninfected but *Allium sativum* ethanolic extract-treated rats
Q - Infected and Saline treated rats

Discussion

Pathogenesis of any parasitic infection was driven by an increase in *Parasitaemia* levels with concomitant destruction of the host's tissues by the parasite, its products or host products induced by the parasite's presence.

The present study observed an increase in *Parasitaemia* in all the infected rats during the first five days indicating that the *Trypanosomes* underwent a rapid multiplication following the *peritoneal* inoculation.

The presence of the parasites in host's body naturally leads to activation of the immune system resulting in production of antibodies specific to the Variant Surface *Glycoproteins* (VSG) of the parasites [1].

The drop in *Parasitaemia* after the fifth day of infection could be accounted for by the host's immune response acting on the parasites' through *macrophages* and *phagocytes* [6].

However, *Trypanosomes* were reported to be among the parasites that had perfected the mechanisms of evading the host's immune response through Variation of their Surface *Glycoproteins* (VSGs) [1, 5].

Therefore, the apparent fluctuations seen in *Parasitaemia* levels are suggestive of different parasite population arising with new VSGs. Each population tends to multiply rapidly and cause another peak of *Parasitaemia*, before the host's immune response is raised against its specific VSGs.

The first *Parasitaemic* peak was observed only one day after the treatment and this was followed by a sharp drop in *Parasitaemia* levels. The findings that *Allium sativum* ethanolic extract treated laboratory rats' *Parasitaemia* dropped to levels much lower than those of the Saline treated rats suggest that, treatment improved the strength of the rats' immune system. These observations were an indication of *Trypanocidal* activity of *Allium sativum* which could occur through a boost to the immune system or direct effects on the parasite.

The plant was reported to have a sulphur-containing compound *Diallydisulphide* (DAD) [26], which has a high lipid regulatory effect. The compound was associated with lowering the levels of cholesterol *triglyceride*, Low Density *Lipoproteins* (LDL), Very Low Density *Lipoprotein* (VLDL), and elevate High Density *Lipoproteins* (HDL) [12].

The reduction of steroid levels by DAD was reported to decrease the growth of parasites in vivo due to early arrest in the synthesis of membrane lipids [22] This could bring about the death of the unwanted cells (parasites) thus providing a possible mechanism for the decline in the *Parasitaemia* level in the garlic extract treated rats.

Other experiments conducted on the synthesis of steroids and the inhibition of phospholipase A2 demonstrated that the theraupetic effects of the *A. sativum* ethanolic extract are due to its ability to inhibit lipid metabolism in *Trypanosome* [22]. With insufficient lipids in the case of *A. Sativum* treatment, cell membranes of the parasites will not be formed accordingly and the surface coat for antigenic variations will also be disrupted thus hindering the development of new antigenic variations to avoid the host's immune responses against the parasites antigens. This mechanism might make it difficult for the parasite to keep on changing its VSGs thus limiting the parasite's capacity to evade the host's immune responses.

The extract's effects on the plasma membrane could also be supported by the evidence from morphometric measurements. It was observed that the *A. sativum* ethanolic extract had shrinking effect on the lengths between *Kinetoplast* to nucleus, posterior end to nucleus, nucleus centre to the anterior end, the body length and the nucleus length. These findings suggest that the garlic extract affected the plasma membrane of the parasites as shrinking is only possible with disrupted membrane biochemistry.

African *Trypanosomes* were known to acquire lipids through their ability to hydrolyse host's membrane. Lipids eg. *lechthium* by the release of phospholipases [17] whose products i.e free fatty acids and *lysophospholipids* are utilized by *lysophosphotidyl transferase* system of the parasites in the synthesis of lipids. In addition, the blood stream parasites required exogenus Cholestrol mediated by plasma LDL.

LDL was an obligatory requirement for the growth of procyclic *Trypanosome* [3]. But the DAD effect on the LDL reduced the ability of the *Trypanosoma brucei brucei* to aquire the lipids. This retarded the growth of the parasites and brought about shrinkage of the morphometric parameters mentioned above.

In the case, the *Trypanocidal* effects of *A. sativum* could be partly attributed to the effects of the sulphur - containing compound *diallydisulphide* (DAD) [26], which affects the membrane structure and functions.

Data presented in this report also showed that *A. sativum* treatment caused an improvement on the WBC, Hb and RBC levels particularly following the first treatment on the 5th day after infection with the *T. b. brucei.*

The study demonstrated fluctuations in White Blood Cell counts in all *T. b. brucei* infected laboratory rats with WBC peak levels being synchronized with the *Parasitaemia* peaks. The increase in the number of WBC was probably an indication that, *T. b. brucei* parasites stimulated the rats' anon - specific immune response resulting in *leucopoiesis* (ILRI, 1992).

The findings were in agreement with the previous studies which reported that, due to rapid multiplication of the metacyclic *Trypomastigotes* injected into the animal's body, the successive increase in the *Parasitemia* level and the host rapidly developed a strong immune response [6].

The immune response in this case was triggered by the parasites' surface coated antigens depending on the strength the initial *Parasitaemia* rise could be followed by an immediate decline due to parasite clearance from circulation by the immune cells [16].

A study carried out on mice, exposed the major initial mechanisms for removal of *Trypanosomes* in vivo by the parasites being agglutinated and opsonized by antibodies for *Phagocytosis* by white blood cells including *monocytes, macrophages, neutrophils,* and *eosinophils* [1].

This phenomenon accounts for the initial increase in the WBC counts. Heamoglobin (Hb) levels changed from 8.0g/dL to 10.2g/dL after treatment with garlic in the *T. b. brucei* infected rats but they were very low in the rats that did not receive *A. sativum* ethanolic extract treatment.

In the case of RBC levels, the rats that did not receive *A. sativum* ethanolic extract treatment following *T. b. brucei* infection also had very low (3.38×10^6 / dL) RBC count as compared to the groups that received the treatment ($5.0\text{-}6.6 \times 10^6$ /dL). *A. sativum* ethanolic extract alleviated blood pathology, which resulted from *T. b. brucei* infection.

Haemoglobin is naturally present within the RBCs and is very essential in the transportation of oxygen and carbon (IV) oxide in the body. As blood circulates, oxygen is transported from the lungs to the body tissues whereas carbon (IV) oxide is transported from the tissues to the lungs. Oxygen is required by the body tissues for metabolic reactions that lead to generation of energy in the form of ATP [7]

The study on the Hb and RBC levels following *T. b. brucei* infection agree with the previous studies that reported decline in the RBCs number and Hb levels with resultant anemic condition [6] which was one of the most important reported pathologic features in *Trypanosomosis.* The reduction of RBCs in the *T. b. brucei* infection might have been due to the recognition of the RBCs as 'foreign' and attacked by the host's own *reticuloendothelial* system because their surface membrane was altered in some way by the presence of the parasites [10].

The destruction might be increased by toxic substances emanating from the *Trypanosomes* which cause haemolysis in addition to the actual parasitization of the cells by the *Trypanosomes* while in circulation [6].

When this occurred the Hb levels in the blood also declined and the infected animals became anemic. *Trypanosomosis* - associated anaemia usually coincides and progresses with the onset of *Parasitaemia*, findings from infected and untreated mammals reported that, anemia generally determined the severity of *Trypanosomosis* [6, 13, 19].

Anemia, together with loss of appetite has been associated with loss in body weight in chronic infections [20]. Studies have suggested that the decline in RBC counts and Hb levels reduces the blood's oxygen carrying capacity thus causing a general body weakness.

This occurs together with repeated bouts of fever, as it was the case in the study. The infected animals were subjected to severe muscle protein degeneration resulting in loss of body weight. The

findings of the study were in agreement with the report. Since *Hyperthermia* (increase in the body temperature) and decrease in body weights following *T. b. b.* infection was observed. The *hyperthermia* development in *Trypanosomosis* was probably due to *Pyrogenic* (toxins) substances of parasite origin. Those substances were not eliminated easily from circulation and when they were carried to the *Hypothalamus* they ended up causing thermodysregulation [21, 23].

Management and treatment of *Trypanosomosis* had largely been done through chemotherapy but most drugs had been reported to have side effects while others were resisted by the parasites [6].

The data presented in this report gives evidence of possible *Trypanocidal* effects of *A. sativum* extract treatment. The treatment caused reduction in *Parasitaemia* and on specific *Trypanosome morphometric* parameters in addition to preventing the occurrence of drastic anemia in the rats following *T. b. brucei* infection. *A. sativum* ethanolic extract alleviated the anaemic condition in *T. b. brucei* infected laboratory rats by boosting their Hb and RBC levels.

References

1. **Barrett M.P., Burchmore R.J., Stich A.** (2003). "The *Trypanosomiasis*". *Lancet* 362 (9394) : 1469 – 80.

2. **Black S., Vandeweed, V.** (1989) Serum lipoproteins are required for multiplication of *T.b.brucei* under axenic culture conditions. *Mol Biochem Parasitol* 37:62-72.

3. **Coppens I., Baudhuin P., Opperdoes F.R., Courtoy P.J.** (1988) Receptors of high density *Lipoprotein* on haemoflagellates of *T.b.brucei* and involvement in the growth of parasites. Proc *Natl Acad Sci USA* 85:6753-6757.

4. **De Koning H.P.** (2001). Transporters in African *Trypanosomes* role in drug action and resistance. *Int. J. Parasitol.* 31, 512-22.

5. **El-Sayed N.M., Hegde, P., Quackenbush J., Melville S.E., Donelson J.E.,** (2000). The African *Trypanosome* genome. *Int J Parasitol* 30,329-45.

6. **FAO.** (2006). A field guide for the diagnosis, treatment and prevention of African animal *Trypanosomiasis*. FAO of the UN. *Rome. Pp* 1-18,13-17.Nations (FAO). Rome.

7. **Hayward G**. (2006) Transport in Animals, *Macmillan Secondary Biology, Book 2,* Nairobi Kenya. Pp 31.

8. **Herb N' Health.** (2004). Garlic Ginger http:// herbsnhealth.homestead.com/garlic ginger html.

9. **Hursey B.S.** (2000). PAAT: The program Against African *Trypanosomiasis*. *Trends Parasitol PO4 (Special edition).*

10. **ILRI.** (2005). *Trypanosomiasis. ILRAD report* Nairobi, Kenya. (of 08/09/2005).

11. **Kayser O., Kiderlen A.F., Groft S.L.** (2002) Natural products as potential antiparasitic drugs In: Atta-ur - Rahman (Ed.) Studies in N*atural products Chemistry, Vol 26. Elsevier,* UK, pp 779-848.

12. **Lau B.H.S** (1989). Anticoagulant and lipid regulating effect on garlic *(Allium sativa).* In Spriller G.A, Cala J (eds) New protective rules for selected nutrients in human nutrition. *Liss, New York.* pp 293-325.

13. **Losos G.T.** (1986). Infectious tropical diseases of domestic animals, Longman Scientific and Technical, in association with the International Development Research Center, Canada, pp 183-212.

14. **Luckins A.G.** (2006). Methods for diagnosis of Trypanosomiasis in Livestock. Centre for Tropical Veterinary Medicine, East Bush, Roslin Midlothiah EH25 9RG. UK.

15. **Luckins A.G.** (1998). *Trypanosome evansi* in Asia. *Parasitol. Today* 4;137-142.

16. **Mehlhorn H.** (1988). Parasitology in Focus, Facts and Trends. Springer-Verlag N.Y. Berlin Heidelberg. pp 773-791.

17. **Mellors A.** (1990) Metabolism of phospholipids in *Trypanosoma brucei*, Report of a meeting on the development of drugs against African *Trypanosomiasis* and *Chagas*' disease, Geneva, Switzerland, 12-13 March. *Tropical Disease*

Research/WHO, Geneva, p 8.

18. **Mikail H.G.** (2009). *Journal of Herbs, Spices and Medical Plants.* Department of Phamacology and Toxicology, Facult of Veterinary Medicine, University of Abuja, Nigeria. Vol 15 issue of 3 July 2009, pg.265-271.

19. **Murray M., Dexter T.M.** (1988): Anaemia in Bovine African *Trypanosomiasis*: *a review Acta Trop*., 45 389 – 432.

20. **Mutayoba B.M., Ekersall P.D., Jeffcoate I.A., Gray CE., Holmes P.H.** (1995). Effects of *Trypanosoma* congolense on pituitary and adrenocortcal function on sheep. Changes in adrenal gland and cortisol secretion. *Research in Veterinary Sciences.* 58:174-179.

21. **Ng'wena A.G.M., Patel N.B., Wango E.O.** (1997) Plasma lutenizing hormone (LH) in Response to Gonadotroppin-Releasing hormone Agonist and Clonidine in *Trypanosoma* congolence infected female goats. *Brain Research Bulletin* 44(5):591-595.

22. **Nok A.J., Williams S., Onyemewe P.C.** (1996) *Allum sativum*-induced death of African *Trypanosoma. Parasitol Res* (1996) 82: 634-637.

23. **N. J. Masimba, N. G. Magak, M. Ndiema, R. Odera, L. Chemwolo, M. D. Minjire, D. K. Ndungu**(2012): Disruption of reproductive cycles in rats (*Rattus norvegicus*) acutely infected with *Trypanosoma brucei brucei(T.b.b)* is associated with *Hyperthermia*, ovarian and pituitary gland tissue degenerations. *African Journal of Health Sciences* 22:232-237.

24. **Samwelsson, G, Farah, M.H., Claeson, P, Hagos, M, Thulin, M, Hedberg, O, Warfa, A.M.,Hassan, A.O., Elmi, A.H., Abdulrahman, A.D.** (1991). Inventory of plants as traditional medicine in Somalia 1. Plants of the families *Acanthaceae -chenopodiaceae.J. ethnopharmacol.* 35:25-63.

25. **Seed,J.R.,** (2001) African *Trypanosomiasis* research: 100 years of progress but Question and problems still remain *Int. J. Parasitol*. 31 : 434 - 442.

26. **Srivastava K.C., Bordia A.S. Verma S.K.** (1995) Garlic (*Allium sativum*) for disease prevention. *S.Africa J.Sci* 91:69-77.

27. **Turay A.A, Nwobu G.O., Okogun G.R.A., Igwe C.U., Adeyeye K., Aghatise K.E., Okpala H.O., Tatfeng Y.M.** (2005). A comparative study on the susceptibil of male and female albino mice to *Trypanosoma brucei brucei.* Department of Medical Laboratory Science, Faculty of Pathological Sciences, College of Medicine, Ambrose Alli University, Ekpoma, Edo State; Nigeria.

28. **World Health Organization** (2004). **Anon,** 2004 Communicable Diseases surveillance and Response, *WHO,* Geneva.