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Prevention of Nosocomial Infection, Hospital Laboratory Organization
and Advances in Blood Chemistry in Health and Disease

J. C. JONG, A. H. HARTO, A. R. C. J. VAN DER MEULEN



Microscopic image of blood cells



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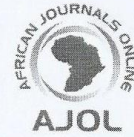
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The Effect of Garlic Extracts on Experimental *Trypanosoma brucei brucei* Infection of Rabbits.

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ABSTRACT

The anti-trypanosomal effect of aqueous and methanolic extracts of garlic were studied in *Trypanosoma brucei brucei* infected rabbits. With the establishment of infection, parasitaemia, anaemia, leucopenia, neutropenia and lymphocytosis developed. There was decrease in total serum protein, albumin and increase in cholesterol concentration. Treatment with aqueous and methanolic extracts of garlic had no significant effect on parasitaemia ($p > 0.05$). Anaemia, leucopenia, neutropenia and lymphocytosis persisted. Administration of garlic extracts significantly elevated ($p < 0.05$) total serum protein concentration, had no effect on serum albumin concentration and significantly decreased ($p < 0.05$) serum cholesterol concentration at weeks 3, 4, 7 and 8 post infection. It was concluded that the plant extracts had no direct curative effect on *T. brucei brucei* infection as there was no effect on parasitaemia. However, the extracts had positive effect in raising total serum protein, lymphocyte count and lowering serum cholesterol concentration.

Key word: *Trypanosoma brucei brucei*, aqueous, methanolic, garlic

INTRODUCTION

Control of African trypanosomiasis involves the use of methods that reduce the vector and drug treatment. The drugs used are however few, toxic and expensive (1). Besides, drug resistance has been reported to occur against all available trypanocides (2) and the introduction of new drugs into the market is virtually none existent. Anaemia is a consistent finding in trypanosomiasis (3), and an indicator of the disease in animals.

Several authors have reported the medicinal, antimicrobial, antiprotozoal and antifungal properties of garlic (4,5), an onion like plant of Asian origin. The present investigation was designed to study the anti-trypanosomal effect of garlic extracts on anaemia, total white blood cell and differential counts, total serum protein, albumin and cholesterol concentrations.

MATERIALS AND METHODS

Preparation of Aqueous garlic extract

Aqueous garlic extract was prepared as described by Ghannoun (5). Two hundred and sixty grams of fresh peeled garlic cloves was pounded and soaked in 2.6L of distilled water for 4 hours. This was filtered first using muslin cloth and then with Whatman No 1 filter paper. The filtered was stored over night at 4°C before freeze-drying and subjection to phytochemical screening.

Preparation of Methanolic Extract

Fresh garlic cloves (786.7g) were soxhlet extracted with 95% methanol and concentrated using rotatory evaporator. The resultant extract was oven dried at 40°C and stored in a dessicator containing silica gel.

*Corresponding author

Animals

Twenty-five male rabbits weighting between 1.5 to 2kg were purchased from the National Veterinary Research Institute, Vom. They were housed in cages and fed on growers mash (Bendel feed and flour Mills Ltd. Ewu), cabbage and water *ad libitum*.

Experimental Design

Trypanosoma brucei brucei from the Nigerian Institute for Trypanosomiasis and Onchocerciasis Research Institute, Vom was passaged once in a donor mouse. At peak parasitaemia, the mouse was sacrificed and trypanosome number estimated by the method of Horbert and Lumsden (6).

Twenty five rabbits were divided into five groups of five animals each in groups I-IV were each inoculated with 1.25×10^7 trypanosomes intra-peritoneally and parasitaemia was monitored daily by wet method of Walker (7). Group V rabbits served as uninfected untreated control. At the onset of parasitaemia, animals in group I were each given 2g/kg body weight of the methanolic extract orally for 2 days, while those in group II received 2g/kg body weight of the aqueous extract. Group III was treated with single

dose of 3.5mg/kg body weight of Berenil. Group V animals served as infected untreated control.

About 4ml of blood was collected from the lateral ear vein of each animal before inoculation for baseline data and thereafter weekly for the estimation of packed cell volume (PCV), Haemoglobin (Hb) concentration, total red blood cell (RBC) Count. Total and differential white blood cell (WBC) count as described by Dacie and Lewis (8). Serum was also prepared for the estimation of total protein (9), albumin (9) and cholesterol concentration (10).

RESULTS

The phytochemical screening of both extracts revealed the presence of saponins and reducing sugars as presented in Table 1. In addition, the methanolic extract also contained alkaloids while the aqueous extract had cardiac glycosides. The breed of rabbits used was susceptible to *T. brucei brucei* infection which was chronic with several parasitaemic peaks. Despite treatment with either aqueous or methanolic garlic extracts, parasitaemia peaks (Figure 1, 2 and 3) developed and animals died by week 8 post infection (P1) except the berenil-treated control.

Table 1 Qualitative analysis of phtochemicals contents of aqueous and methanolic extracts of garlic.

Phytochemical Compound	Occurrence in extract:	
	Aqueous	Methanolic
Anthroquinones		
Free	- ^a	-
Combined	-	-
Tannins	-	-
Phlobatannins	-	-
Cardiac glycosides	+ ^b	-
Reducing sugars	+ ^c (trace)	+
Saponin	+	+
Alkaloids	-	+

Occurrence: ^a - Negative, ^b + present., ^c ± trace

Haematological parameters

The mean PCV, RBC Count and Hb concentration of rabbits treated with aqueous and methanolic extracts are shown in Figures 4, 5, and 6 respectively. Packed cell volume (PCV), RBC Count and Hb concentration decreased significantly ($p > 0.05$) with establishment of infection. Treatment with either of the garlic extracts recorded an insignificant increase ($p > 0.05$) in PCV, and RBC count at week 3 PI when compared with infected untreated control. Treatment with Berenil restored the PCV, RBC count and Hb concentration to pre infection level. Administration of methanolic extract had no effect on Hb concentration, while the increase

recorded at weeks 3 and 4 (PI) after administration of aqueous garlic extract was not significant ($p > 0.05$). The effect of garlic extracts on total and differential leucocyte count is shown on Figures 7, 8 and 9. Leucopenia developed at week 1 for all infected groups. Differential leucocyte count revealed neutropenia, and lymphocytosis. There were inconsistent changes in monocytes and eosinophils count. Despite treatment with aqueous or methanolic garlic extract, the leucopenia, neutropenia and lymphocytosis persisted as also observed in infected untreated control. On the contrary, treatment with Berenil alleviated leucopenia, neutropenia and lymphocytosis.

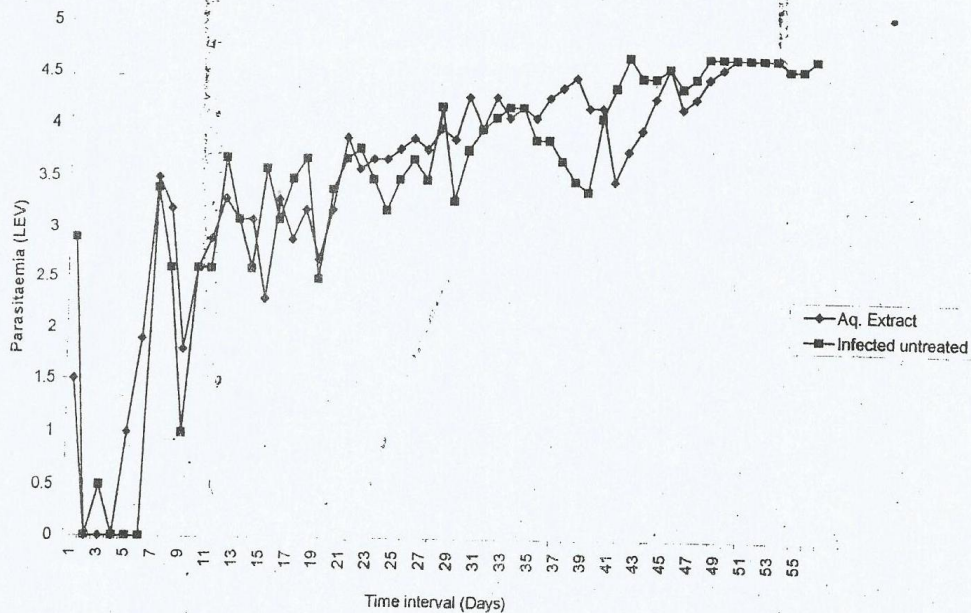


Fig. 1 : Parasitaemia of *T.b. brucei* - infected rabbits treated with aqueous garlic extract

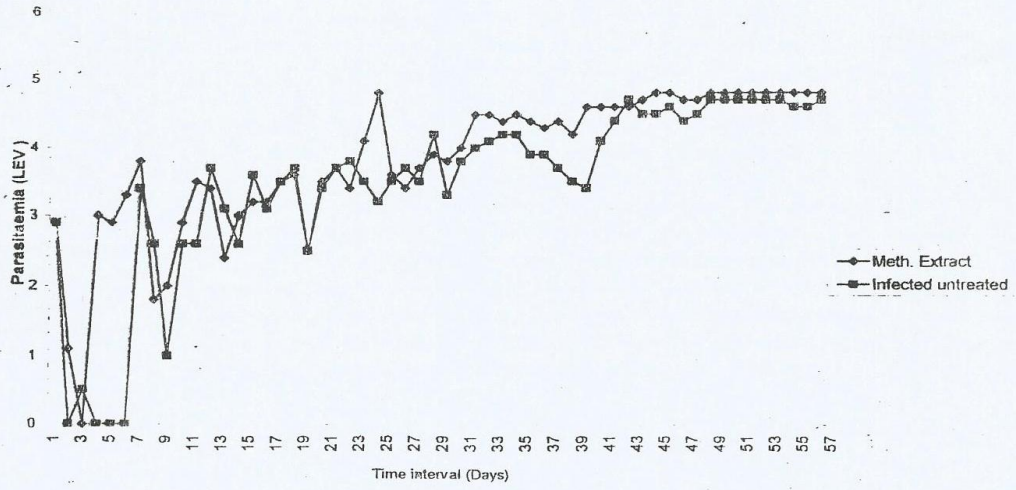


Fig. 2 : Parasitaemia of *T. b brucei* - infected rabbits treated with methanolic garlic extract

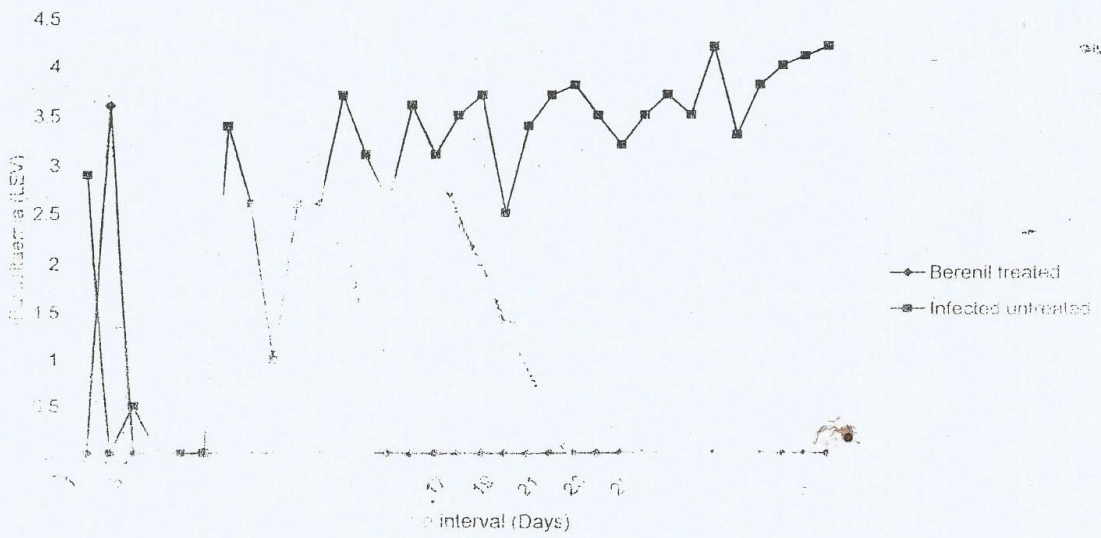


Fig. 3 : Parasitaemia (LEV) of *T. brucei* - infected Rabbits treated with Berenil

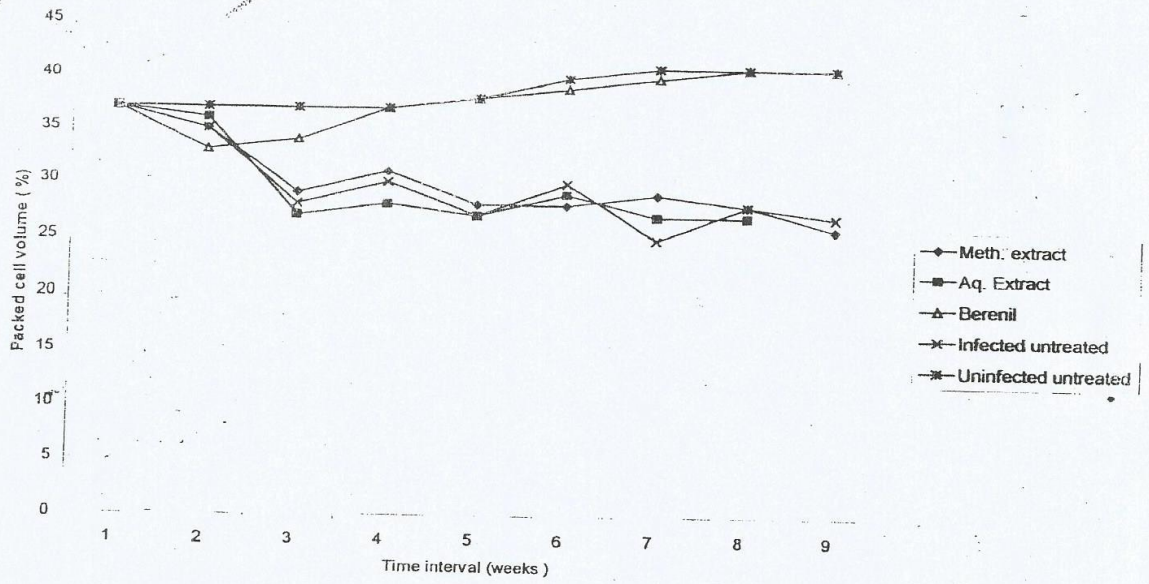


Fig. 4: Effect of methanolic and aqueous garlic extracts on PCV of *T. b. brucei* - infected rabbits

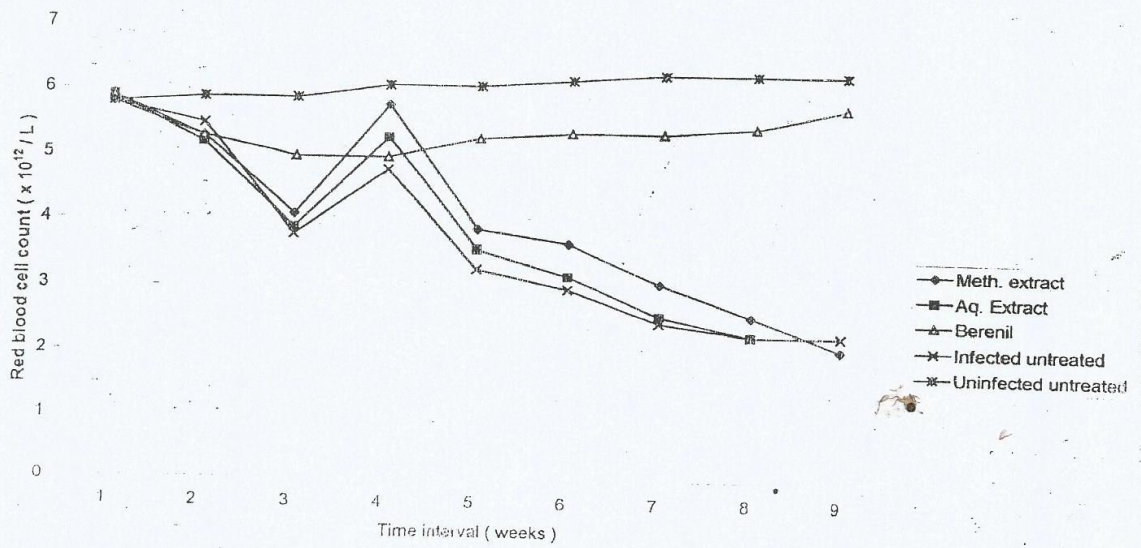


Fig. 5: Effect of methanolic and aqueous garlic extract on RBC of *T. b. brucei* - infected rabbits

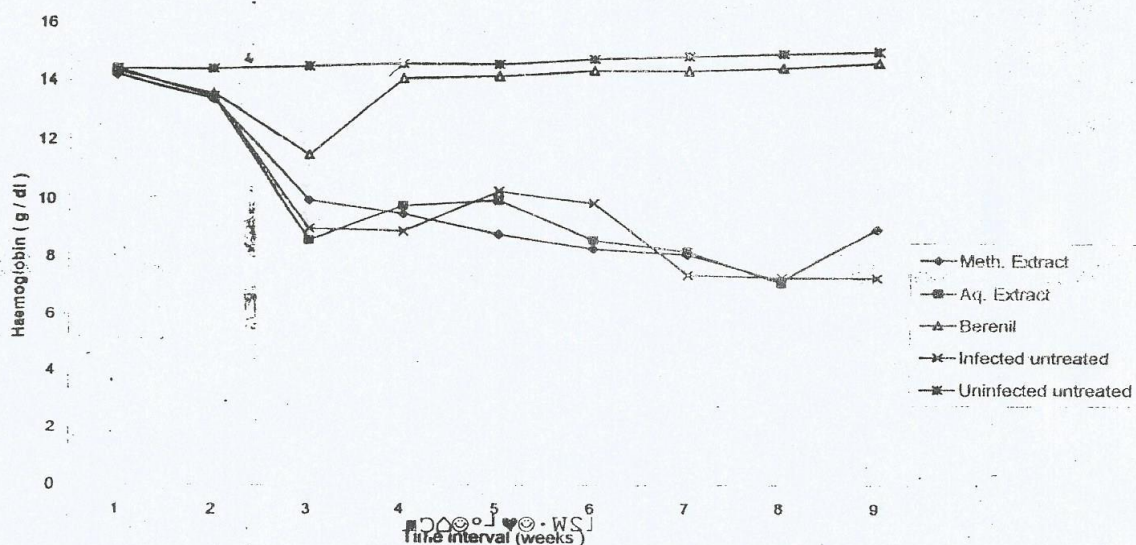


Fig. 6: Effect of methanolic and aqueous Garlic extracts on mean Haemoglobin conc. of *T. b. brucei* - infected rabbits

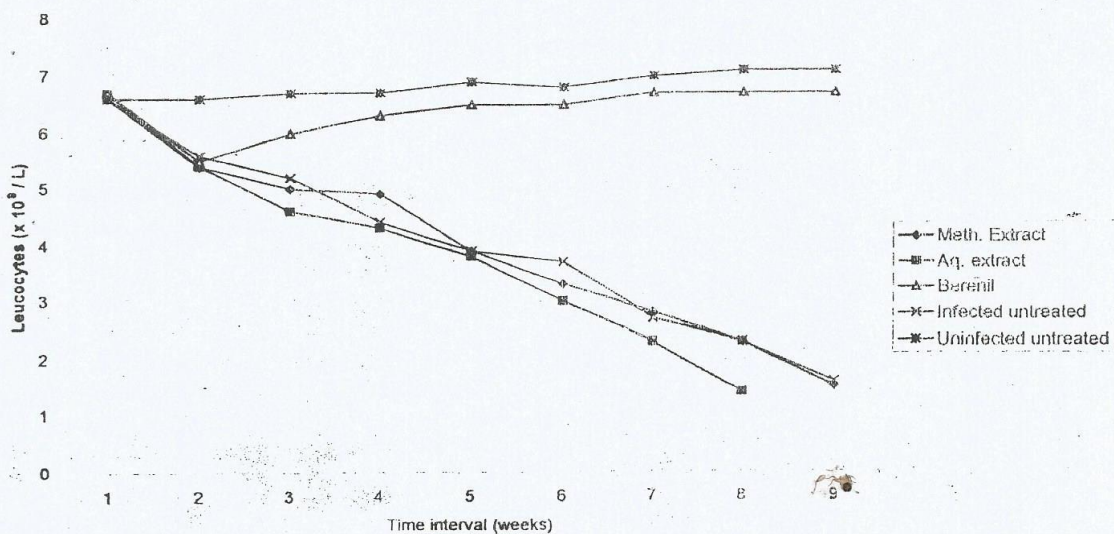


Fig. 7: Effect of Methanolic and aqueous garlic extract on Total Leucocyte count of *T. b. brucei* - infected rabbits

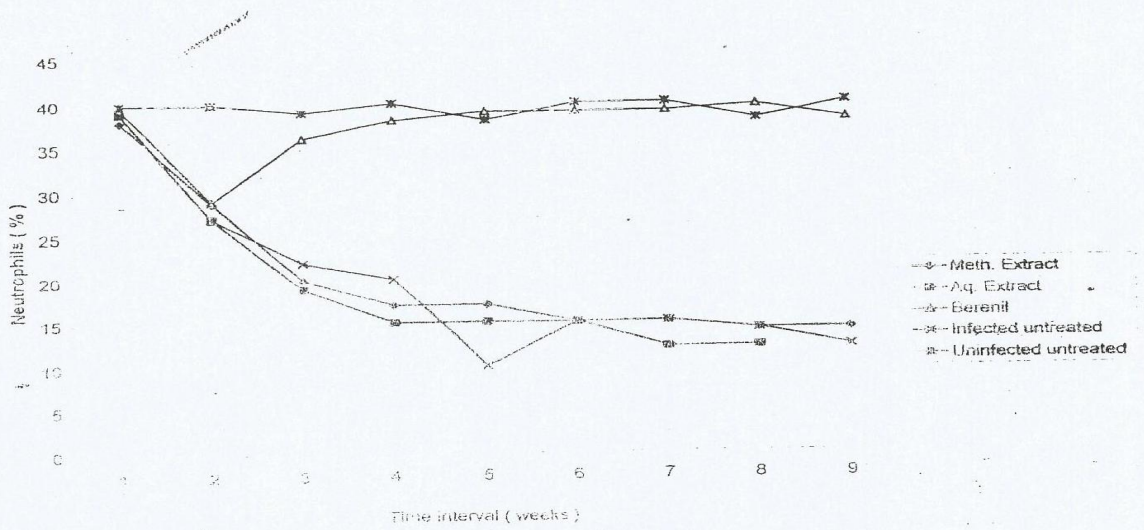


Fig. 8 : Effect of methanolic and aqueous garlic extract on Neutrophils of *T. b brucei* - infected rabbits

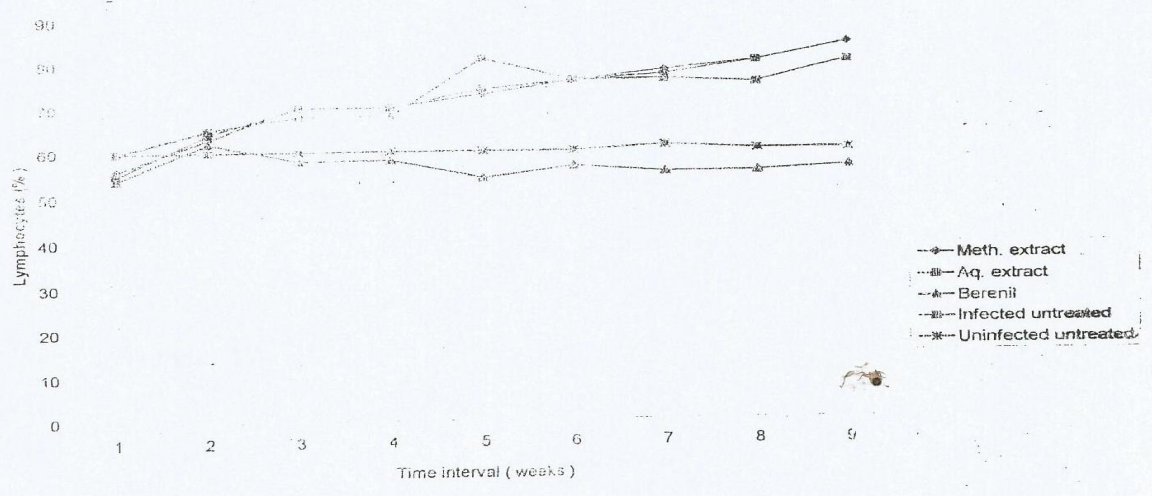


Fig. 9 : Effect of methanolic and aqueous garlic extract on Lymphocytes of *T. b brucei* - infected rabbits

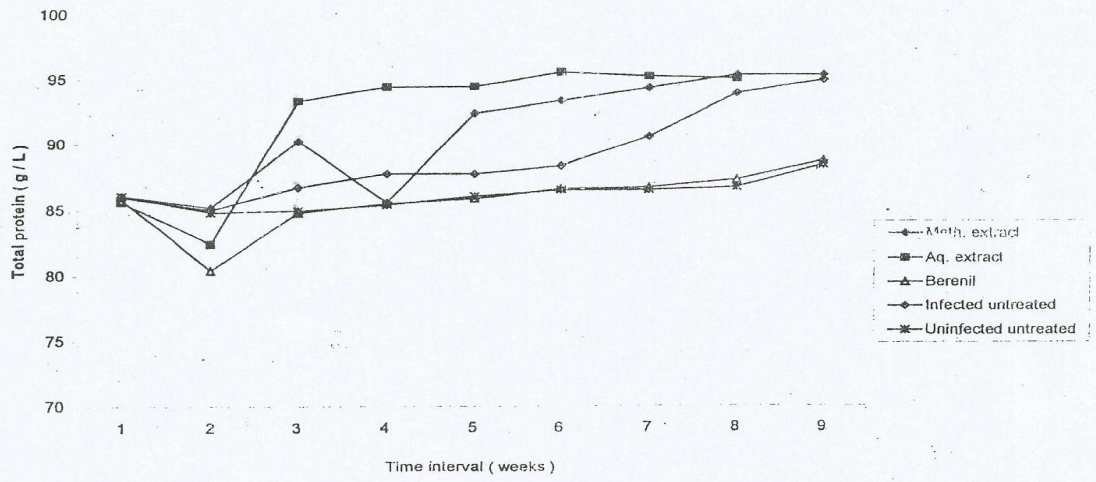


Fig. 10 : Effect of methanolic and aqueous garlic extract on Total protein conc. of *T.b brucei* infected rabbits

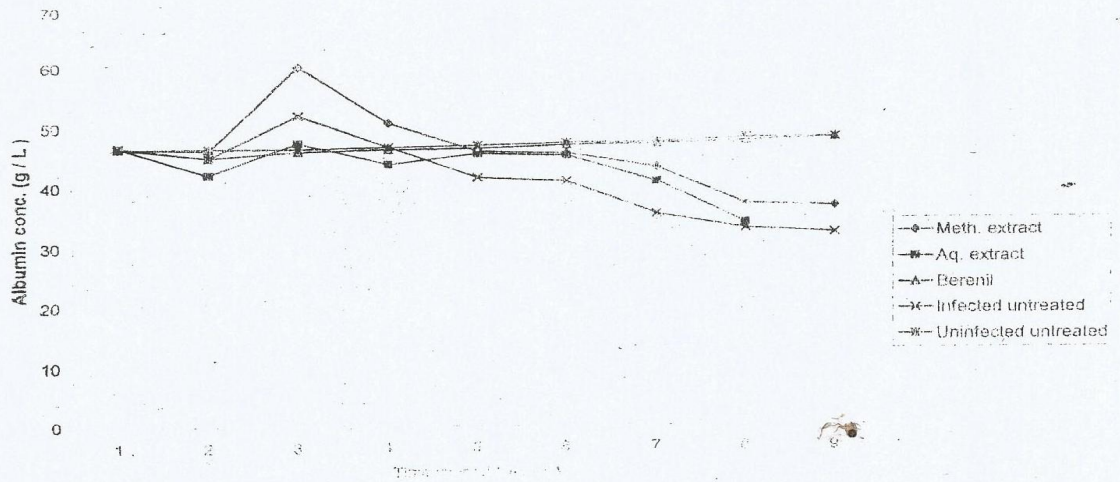


Fig. 11 : Effect of methanolic and aqueous garlic extract on Albumin conc. of *T.b brucei* infected rabbits

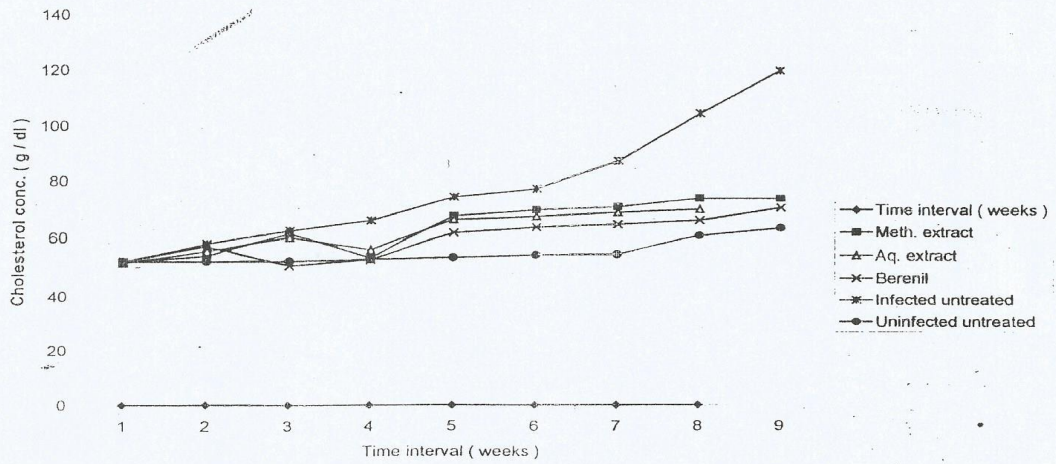


Fig. 12 : Effect of methanolic and aqueous garlic extract on serum Cholesterol conc. of *T. b. brucei* infected rabbits

Biochemical parameters

The effect of garlic extracts on total serum protein, albumin and cholesterol concentrations are shown in figures 10, 11, and 12 respectively. There was decrease in total serum protein and albumin concentration at week 1 (P1). Treatment with garlic extracts significantly elevated ($p < 0.05$) the total serum protein concentration when compared with Berenil and uninfected untreated control, while the albumin concentration was insignificantly reduced ($p > 0.05$) except at week 3 (P1) when compared with infected untreated control. Serum cholesterol concentration increased with infection (Figure 12) Treatment with aqueous or methanolic extracts significantly reduced ($p < 0.05$) serum cholesterol concentration at week 3, 6, 7 and 8 (P1) when compared with infected untreated control.

DISCUSSION

Phytochemical screening of aqueous and methanolic garlic extracts indicated the present of cardiac glycosides in the former and alkaloids in the latter with saponins and reducing sugars in both (Table 1). All these may play some major role in the pharmacological properties of garlic as reported by some workers including Ellimore and

Fekldberg (11). The *in vitro* trypanocidal activity of alkaloids against *T. brucei brucei* had been reported (12) although extrapolation of such activity cannot be made *in vivo*.

The garlic extracts at the dose level used did not seem to have significant effect on parasitaemia (Fig. 1-3). This could be as a result of the plasma threshold concentration which was not sustained in the treated animals, early in the infection process. Though the prepatent period in both treated and untreated controls was averagely 8 days, there was no significant difference ($p > 0.05$) in the survival rate of both garlic treated and untreated control. The Berenil treated and uninfected untreated control survived till the termination of the experiment.

Despite treatment with garlic extracts, the anaemia which developed post infection as indicated by decreased PCV, RBC Count and Hb concentration (Fig. 4-6) persisted until death of all the animals. Similar result was obtained when 100mg/kg garlic oil was administered over a long period of time (13). The leucopenia (Fig. 7) which develop at week 1 has been reported in several trypanosome infections (14,15). The leucopenia progressed despite the increase in lymphocytes in both garlic treated and untreated control. This was due to neutropenia as indicated by Figure 8. There

was no significant decrease ($p > 0.05$) in the neutrophils of garlic treated when compared with infected untreated control.

The lymphocytosis (Fig. 9) observed in the garlic treated and untreated control was significant when compared with Berenil treated and uninfected untreated control. Lymphocytosis has been reported in *T. brucei* infection of tolerant deer mice (16) and rabbits (17) and is associated with tolerance. Garlic maybe acting in the sustenance of this tolerance by this rabbits breed. Despite the increase in lymphocyte count, leucopenia in this work persisted as a result of persistent neutropenia.

The decreased total serum protein (Fig. 10) observed at week 1 post infection was significantly elevated ($p < 0.05$) after treatment with garlic extracts when compared with Berenil treated and uninfected untreated control. Such decrease has been reported in *T. rhodesiense* infections of moneky (18).

Infection with *T. brucei brucei* produced a decrease in albumin concentration. Treatment with garlic extracts brought an insignificant increase ($p > 0.05$) at week 2 when compared with infected untreated control. Decrease in albumin concentration has been a consistent observation in several studies on trypanosome infections (19, 20) largely due to plasma expansion since the hepatocytes that synthesize albumin have been found not to develop severe pathology (21).

Increase in total serum protein concentration without significant increase in albumin levels could be as a result of increase in the globulin fraction. This result is quite unexpected because of the fact that garlic / its oil was reported to increase serum albumin significantly (22, 13). The difference observed in this work could be as a result of non-sustenance of garlic in the system due to the few days of treatment.

Serum cholesterol level was observed to increase with infection (Fig. 12). Treatment with garlic extracts decreased the cholesterol level significantly ($p < 0.05$) at week 3, 6, and 7 when compared with infected untreated control. Hypercholesterolaemia is a common observation in trypanosomiasis (23) and a high risk factor in heart pathology. The hypocholesterolaemic effect of garlic was first reported by Lampic (24). The mechanism by which garlic decreases serum cholesterol is associated with the ability of its oil

which is chemically diallyl disulphide to exchange its disulphide with thiol (RSH) compounds such as co-enzyme A, the multienzyme complex of fatty acid synthesis and HMG CoA reductase, the rate limiting enzyme for cholesterol synthesis making them inactive (25).

In conclusion, the aqueous and methanolic extracts had no direct curative effect on *T. brucei brucei* infection of rabbits as there was no effect on parasitaemia. However, the extracts had positive effect in raising total serum protein, lymphocyte count and lowering serum cholesterol concentration.

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