# STUDIES ON GARLIC *(ALLIUM SATIVUM)* OIL AND ITS' EFFECT ON EXPERIMENTAL TRYPANOSOMOSIS

**BY**

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DECLARATION

I solemnly declare that this thesis is a record of my research work, and has not been presented for an award of higher degree in another University. Information and excerpts cited from the work of others have been duly acknowledged by means of references.

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CERTIFICATION

This thesis titled "Studies on Garlic (*Allium Sativum*) oil and its effect on experimental Trypanosomosis" meets the regulation governing the award of the degree of M.Sc Biochemistry Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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# DEDICATION

This thesis is dedicated to the Lord God Almighty.

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## ABSTRACT

Acute toxicity studies of garlic (Allium sativum) oil in rats and the use of this oil in the treatment of experimental infection of rabbits by 71 congolensea and 71 brucei were carried out. The effect of the oil on haemotological parameters, serum cholesterol and albumin and organ pathology in the infected and uninfected-but treated animals were assessed and compared with controls. Toxicity symptoms were observed in 1000-5000mg/kg dose range, and the median lethal dose ($LD_{50}$) of the oil was found to be 3800mg/kg. Infection of rabbits with 71 congolense or/1 brucei was established 11 days post infection (pi) and treatment thereafter with 100mg/kg garlic oil commenced daily for 6 weeks. Both infections resulted in anaemia, leucopenia, reduced serum albumin and elevated serum cholesterol levels ($P<0.05$) within 3-4 weeks pi. Treatment with garlic oil resulted in significant ($P<0.05$) alleviation of 71 congolense - induced anaemia, leucopenia, hypercholesteremia, hypoalbuminaemia and splenomegaly, and prevented hepatomegaly and fatty infilteration. Trypanosoma brucei - induced anaemia, leucopenia and hepatomegaly were not significantly ($P>0.05$) affected by garlic oil treatment, however, splenomegaly, hypercholesteraemia and hypoalbuminaemia were alleviated significantly ($P<0.05$). The results are discussed in relation to the diallyl disulphide, the active component in garlic oil, and the differences observed between rabbits treated with T. congolense and 71 brucei infection was in part attributed to the difference in their pathogenicity and the complexity of the factors involved.

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## CHAPTER ONE

INTRODUCTION.

Trypanosomoses are a group of allied protozoal diseases caused by species in the genus *Trypanosoma*. These are parasitic protozoa that cause several serious diseases of humans and domestic animals in tropical Africa and South America and hence one of the most important parasites from medical and veterinary points of view in Africa. Salivarian trypanosomes which cause African sleeping sickness in humans are *Trypanosoma rhodesiense* and *Trypanosoma gambiense* for East and West Africa respectively. The animal diseases include nagana in cattle (*Trypanosoma brucei*, *Trypanosoma vivax*, *Trypanosoma congolense*),surra in horses and camels (*Trypanosoma evansi*), and dourine in horses (*Trypanosoma equiperdum*).

Tsetse flies(Glossina species) are the vectors responsible for harboring and transmitting these parasites. Those harboring trypanosomes pathogenic to domestic animals are found in over 37% of the African continent. Many of these areas are potentially the most agriculturally productive (ILRAD,1990). The main trypanosome species involved (*Trypanosoma congolense*, *Trypanosoma vivax*, and *Trypanosoma brucei*) are extremely ubiquitous due to the widespread presence of suitable insect vectors. As a result of this, cyclical development of trypanosomes occurs in a number of different species of tsetse fly each of which is adapted to different climatic conditions and vegetation (Ford, 1970). Furthermore the trypanosomes are not only capable of infecting most domestic animal species, but also many species of wildlife (Machennan,1970, and Murray *et al*,1982). These species of wildlife do not suffer from severe clinical disease but become carriers and thus constitute an important reservoir of infection.

Meat losses due to trypanosomosis in sub-saharan Africa had been estimated at 5 billion US dollars annually, not counting losses in milk production, traction and manure (Mortelmans, 1986 ;

ILRAD, 1990). About 50 million people are at risk of contracting human trypanosomosis known as sleeping sickness, ( Kuzoe, 1993 ; ILRAD , 1993) and the disease constantly threatens to reach epidemic proportions. The disease if not treated is fatal due to the invasion of the central nervous system.

Over 3 million cattle die each year from various forms of the disease, and the rearing of domestic cattle, sheep and goats is impossible in over 10 million square kilometers of Africa (Fairlamb, 1982), and largely accounts for Africa's low livestock productivity.

Control of trypanosomoses presently has been with the use of trypanocidal drugs, control of tsetse fly population and rearing trypanotolerant livestock .Currently, chemotherapy of African trypanosomoses is not satisfactory largely due to the slow discovery of new drugs and the high cost of the existing ones. Most of the drugs currently in use have one or more toxic effects. Resistance against some of them with concomitant relapses of infection are frequent. Lack of financial return for the enormous investment required has contributed to the lukewarm attitude of the pharmaceutical industry towards further drug development (Fairlamb, 1982 ).

Anaemia is the main recognised feature of clinical trypanosomoses that is of importance in both man and domestic animals (Fiennes, 1954; Mackenzie and Cruskank, 1975; Mamo and Holmes, 1975 and Kobayashi *et al*, 1976). However the mechanism of this anaemia is still not very clear even though a number of hypotheses have been put forward to explain it. One of these hypotheses includes the role of free fatty acid (FFA) in precipitating the anaemia. Trypanosomes have been shown to generate potentially hemolytic factors on autolysis which results from host immune responses. This hemolysin has been shown to consist of a mixture of free fatty acids.

Tizard and his collegues ( 1978 a &b) have shown that massive trypanosome destruction occur

regularly as a result of host immune response. Apart from the FFA derived from autolysing trypanosomes, phospholipase A is also liberated. This group of hydrolytic enzymes are involved in the degradation of phospholipids which form about 50% of the total lipids in biological membrane. This enzyme is capable of catalyzing the hydrolysis of a fatty acid ester linkage in endogenous phosphatidyl choline or phosphatidyl ethanolamine to liberate more FFA. Tizard *et al*, (1977) observed that *Trypanosoma congolense* on autolysis possessed a hemolytic material which is composed of FFA and lysolecithin believed to be produced by the action of phospholipase on the host's phospholipids. These have been shown to be haemolytic due to their detergent - like properties which make them powerful surface acting agents and therefore membranolytic (Tizard *et al* 1978).

Although the role of FFA in *in vivo* haemolysis is in doubt because they bind to plasma albumin; this binding capacity could be exceeded, as could occur in infections with heavy parasitemias (Tizard *et al* 1978), moreso that albumin concentration is lowered during trypanosome infection (Anosa and Kaneko 1983 ).

Because of the problems associated with existing trypanocides, the use of combination therapy as a necessary strategy for effective treatment of the disease has become a promising therapeutic line of action. As search for new drugs continue, there is also need to assess locally available herbs for trypanocidal activity.

In view of the importance of garlic(*Allium sativum*) and its oil in traditional medicine both as prophylactic and therapeutic agent in many disease processes, the current study was under taken to investigates the role of the oil in the prevention of pathological lesions associated with trypanosomoses which are responsible for the death of infected animals, and also to see if the alleviation of these conditions by garlic oil could prevent the disease or the anaemia that is usually associated with it.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW.

### 2.1 African Trypanosomoses.

African trypanosomoses are a spectrum of diseases caused by the salivarian trypanosomes in both man and animals. It is refered to as sleeping sickness in man, nagana in cattle, surra in horses and camels, and dourine in horses.

The more important trypanosome species affecting man, domestic and experimental animals have been sub-divided into two groups; the haematinic group (*T. congolense, T. vivax*) which remains in the plasma, and the tissue - invading group (*T. brucei, T. evansi, T. equiperdum, T. gambiense* and *T. rhodesiense*) which is found extravascularly and intravascularly (Losos and Ikede, 1972). Because of their presence in blood, they produce numerous changes in its cellular and biochemical constituents.

#### 2.1.1 Pathogenesis of Trypanosomoses.

The haematological and other changes in trypanosome infections are determined by several factors which include the virulence of the parasite, the susceptibility of the host, the period of the infection during which samples are taken, the dose of the parasite (degree of challenge), the age of the animal, the physiological conditions of the animal (pregnant or not), the level of host resistence and the nature of infection (single or multiple) *et cetera*. Because of different combinations of these host parasite and other factors, the pattern of a trypanosome infection is therefore variable. Thus, per acute, acute, sub- acute and chronic infections are encountered, each with varied symptoms and severity, giving rise to three recognizeable phases of trypanosomoses (Anosa, 1988).

2.1.1.1 Phase 1: Acute crisis.

This begins with the first appearance of trypanosome in blood after incubation period. The parasitemia is high although fluctuating, with most days showing positive parasitemia.

In the per acute cases of trypanosomosis such as those produced in rodents by *T. brucei, T. vivax* and *T. congolense*, the infected animals usually die within one week, the parasitemia persisting (Isoun, 1975, Whitelaw *et al*, 1980; Taylor, 1968). In animals that show acute or chronic illness, this phase lasts several weeks and is characterized by fluctuating parasitemia. Death occurs commonly at this phase in acutely affected animals because of deleterious changes like severe pancytopenia. Animals destined to suffer chronic trypanosomosis also manifest an acute crisis but show less severe parasitemia and pathology than observed in animals with per acute or acute infection. This phase appears to be a period when the inadequately organized defence mechanisms of the infected animal are shocked by a vicious foe which evades the defence mechanisms of the host by manifesting antigenic variation. The host often succumbs unless the parasite is not very virulent.

2.1.1.2 Phase II.

Chronic crisis follows after the acute crisis. It is characterised by low frequency and intensity of parasitemia. The erythrocyte and the leucocyte values persist with minor fluctuations at the low levels attained at the end of phase I for periods ranging from a few weeks to several months. This period appears to be a time when the infected animal has fully mobilized it's defence mechanisms to a level that is adequate to depress parasite multiplication but yet insufficient to completely abort the infection or reverse the pathology that developed during phase I. With the tissue-invading trypanosomes, this is the time when they also establish extravascularly and are less numerous in the blood. Death could occur at this stage either as a result of the progressive pathology caused by the

parasite and/or due to secondary infections since affected individuals are immunodepressed, and can not mobilise commensurate immunologic response to non-trypanosome, bacterial, other protozoal and helminth infections.

2.1.1.3 Phase III: Recovery or post-crisis.

This period is characterised by aparasitemia or very low infrequent parasitemia which indicates the capitulation of the parasite to the host's defence mechanisms. The leucocyte, and erythrocyte levels begin to recover towards pre-infection levels, and other pathological changes are also slowly reversed.

2.2 Incidence (Prevalence) of Trypanosomosis.

Worldwide, regional and local distributions of the disease pattern are related to the presence of the insect vectors. *Trypanosoma equiperdum*, which is transmitted venereally is the only exception.

The regional and local incidence of trypanosomosis infection and its economic impact on livestock production is governed by many factors such as prevalence of carriers in domestic and wild animals, density of vector populations and local husbandry practices such as annual migration of livestock. In Africa, the most important forms of trypanosomoses and the impact on livestock production are caused by the salivarian species transmitted by tsetse flies which infest vast regions totaling about 10 million square kilometers where these flies greatly limit livestock production. All forms of domestic animals with the exception of poultry are susceptible to infections by one or more of the species.

One of the most important frequently observed features of the tsetse fly - transmitted trypanosomosis of cattle is multiple infection, occurring simultaneously or sequentially of *T.*

*congolense*, *T. vivax* and *T. brucei* which adds to the complexity of the situation affecting the methods which have to be used in diagnosis and chemotherapy.

## 2.3 Characterisation of Trypanosomes.

Identification of trypanosome strains is essential for an understanding of the epidemiology of trypanosomoses. One of the methods is the strain specific electrophoretic mobilities of enzyme forms i.e isoenzyme patterns within the trypanosome and isoelectric focussing (Allsopp and Gibsons, 1983).

In studies identifying trypanosomes, the digestion of kDNA fraction with selected restriction endonucleases followed by electrophoretic analysis of the fragments gave rise to patterns that could be used to characterize various populations of trypanosomes such as stocks, strains, and clones .

## 2.4 Classification.

Trypanosomes are parasitic protozoans of medical and veterinary importance. They belong to the genus *Trypanosoma* which is the only genus embracing all the known trypanosomes (Hoare, 1964). The mammalian trypanosomes in the genus *Trypanosoma* are divided into two groups - stercoraria and salivaria , according to their development in the vector and mode of transmission by either the Saliva or by faecal contamination of the wound caused by the bite of the vector. The two divisions differ mainly in the cause of development in their vectors. Group one comprises species whose development is completed in the posterior station of the vector and transmitted by contamination, while group two comprises of species whose development is completed in the anterior station and are transmitted by the innoculative method through the insect bite. These two major groups were designated with descriptive names stercoraria and salivaria, indicating the media (faecal or salivary) and the sites in which the flagelates complete the invertebrate developmental cycle.

The systematic position of *Trypanosoma* among the protozoa and their classification with reference to African species is as follows:

*Subphyllum:* Sarcomastigophora

*Superclass:* Mastigophora

*Class:* Zoomastigophora

*Order:* Kinetoplastida

*Sub order:* Trypanosomatina

*Family:* Trypanosomatidae

*Genus:* Trypanosoma.

Stercoraria.

The stercorarian trypanosomes always have a free flagellum, a large and not terminal kinetoplast, and have posterior end of their body pointed. Their multiplication in the mammalian host is discontinous. Development is completed in the posterior section of the insect vector, and are transmitted by feacal contamination. These trypanosomes are typically not pathogenic; for example *Trypanosoma theileri.*

Salivaria.

This group of trypanosomes may or may not have a flagellum. They have a terminal or subterminal kinetoplast, and the posterior end of the body is usually blunt. Multiplication in the mammalian host continues in trypamastigote stage while development in the vector (*Glossina*) is completed in the anterior section (except in mechanical vectors), and transmission is usually innoculative i.e passed on as a result of insect bite (except *Trypanosoma equiperdum*). These trypanosomes are pathogenic, for example *Trypanosoma brucei.*

2.4.2 Morphology.

Mammalian trypanosomes have a more or less spindle-shaped flattened body which is usually curved. The shape varies in different species. They have single vascular-type nucleus which contains a large achromatic karyosome and peripheral chromatin (as seen below) lining the membrane.

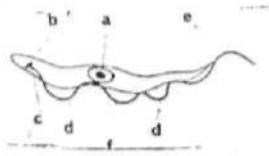



Figure 2.1.

**Structure of Trypanosome**. a= Nucleus; b= kinetoplast; c=Basal body; d, e=flagellum-marginal and free portions, f= undulating membrane. (From Hoare, 1949. As cited by Hoare, 1970).

The shape of the nucleus is typically round or elongated according to the width of the body. The organ of locomotion is represented by a single flagellum which starts behind the nucleus in the posterior part of the body where it emerges to run forward along the outer margin of a fold of the pellicle which extends throughout the length of the body and is known as undulating membrane. The base of the flagellum is formed by the basal body, sometimes called the blepharoplast. In close proximity to the basal body is a characteristic dark-staining organoid known as the kinetoplast, which is disc-shaped.

In addition to the major structural elements, trypanosomes may contain various cytoplasmic inclusions especially different kinds of volutin granules, the number of which varies during the course of infection and with the strain of the parasite. These granules may be associated with the immune reaction of the host.

The shape of tryapnosome changes during the different developmental stages of the life cycle in both the insect vector and in the mammalian host (see below).

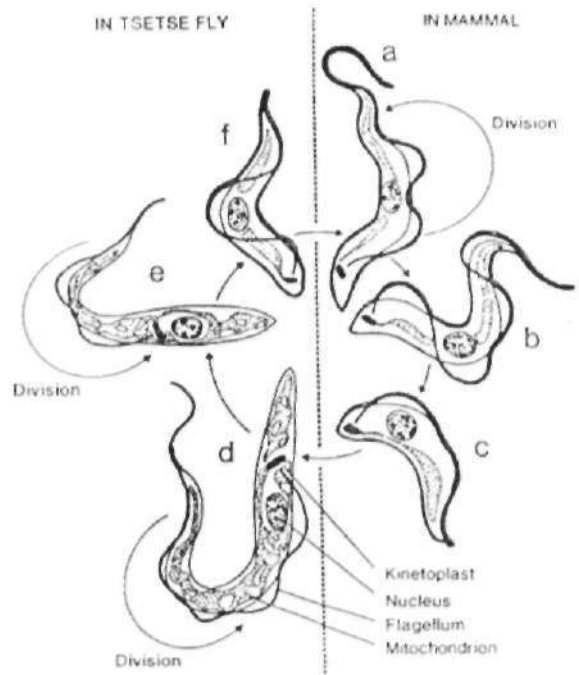



Figure 2.2

**The life cycle of *Trypanosoma brucei*.** In the mammalian host, slender forms of the parasite (a) multiply by binary fission until large numbers build up in the blood. The trypanosomes then transform first into intermediate forms (b) and then into stumpy forms (c) which are able to infect tsetse flies (insect vector).

In the midgut of the tsetse fly, procyclic forms (d) arise and undergo division after which they enter the proventriculus and later the salivary glands of the fly where they assume epimastigote forms (e). Further division of these parasites occur finally, giving rise to metacyclic forms (f) in the salivary glands of the insect which are able to infect mammals (From ILRAD Annual Report, 1990).

2.4.3 Transmission and Vectors.

African trypanosomes have complex transmission cycles involving an invertebrate host and a vertebrate host (either man, cattle, domestic animals and sometimes wild game which serve as infection reservoirs). The invertebrate host is an insect vector of the genus *Glossina* confined to the African continent. Certain species of *Glossina* contract infection with these parasites (trypanosomes) when they ingest blood from an infected mammalian host. Trypanosome infection is not in any way injurious to the fly (Baker, 1958), and has been reported that under comparable conditions, infected flies may even live longer than the uninfected ones.

Under suitable conditions, trypanosomes that are infested along with blood during feeding undergo a complete cycle of development in the fly and are transmitted to a fresh vertebrate host when the tsetse feeds on it.

The degree of risk of the exposure of livestock to *Glossina* - transmitted trypanosomosis depends on the species and density of the tsetse present; the infection rate in the tsetse, the species and strains of trypanosomes present; the feeding preferences of the flies and the extent to which these vectors are dependent on particular species of livestock as their source of food.

The tsetse fly pierces the skin of the mammalian host and ingest it's blood by means of proboscis. When ingested by the fly with the blood, the trypanosomes find themselves in the midgut where they continue to develop until they reach the hindgut and gradually advance forwards to the cardia. They continue to migrate forwards, first into the oesophagus and pharynx and then into the proboscis. *Trypanosoma congolense* completes its development here but *Trypanosoma brucei* and *Trypanosoma gambiense* enter the hypopharynx through the open anterior end after which they turn back again and find their way into the salivary ducts from where they invade the glands thereby

completing their development. Their final development in the *Glossina* takes place in the proboscis (*T. congolense*) or salivary gland (*T. brucei* and allied species) where they assume the epimastigote stage before giving rise to the metatrypanosomes. These parasites are then ejected with the fly's saliva during a subsequent blood meal (Fiennes, 1970).

The duration and efficiency of the invertebrate cycle are influenced by many factors such as the species of *Glossina*, parasite strain, climatic factors and the available host's susceptibility. In human African trypanosomosis, after the fly feeds on an infected person, they undergo this period of cyclical transmission until 18-35 days later, the infective forms appear in the salivary glands and the tsetse fly becomes capable of transmitting the disease. A tsetsefly once infected remains so for the rest of it's life. The average life span being 3 (Jackson, 1946) and 5 ( Saunders, 1962) weeks for males and females respectively.

Fly infection rate i.e the proportion of flies harboring infective forms of a given trypanosome specie are low for *T. brucei* group (rarely exceed 1%), while the infection rates are much higher with *T. congolense* and *T. vivax*, about 5 and 10% respectively (TDR, 1978). Both sexes of *Glossina* are exclusively blood sucking, and feed on quite a range of animal hosts. All tsetse species can potentially transmit trypanosomes, however, certain species are more efficient. *Glossina palpalis*, a riverine specie is regarded as the main vector of West African sleeping sickness while those of *Glossina morsitan* group are the most important vectors for East African sleeping sickness.

In addition to cyclical transmission by intermediate hosts, *T. congolense, T. vivax and T. brucei* (Hoare, 1947) can be transmitted to mammals directly by mechanical innoculators which are in some cases by tsetse flies or other blood sucking Diptera e.g Tabanid flies (Stomoxys) ( Hoare, 1947; Buxton, 1955). The success of mechanical transmission depends upon the length of survival

of the blood trypanosomes in the proboscis of the insect during the short interval between interrupted feeds. *Trypanosoma evansi* is exclusively transmitted mechanically by Tabanid flies and *Trypanosoma equiperdum* venereally by contact between the mammalian hosts.

Congenital trypanosomosis due to transplacental infection has been reported in both *T. rhodesiense and T. gambiense* infections (Traub, *et al* 1978; Burke, *et al* 1974). There has also been a reported incidence of transfusion - induced African trypanosomosis (Hira and Husein 1979).

2.4.4 Trypanosome antigenic variation/molecular biology.

When tsetse fly introduces metacyclic forms of trypanosomes into the mammalian host, it initiates the pathogenesis of trypanosomoses. The trypanosomes manage to evade the host's immune response by means of antigenic variation.

Antigenic variation in trypanosomoses is the sequential expression of a series of antigens on the surface of the parasites. In a normal host, the initial population of parasites derived from a single cell uniformly expresses a single membrane surface antigen. Subsequent parasitemias rise and fall in waves each new parasite population carrying a new surface coat antigen immunologically distinct from previous antigens. Thus, a parasitemic peak is usually a mixture of variable antigen types in different proportions (McNeillage, *et al*, 1969; Van Meirvenne, *et al*, 1975). The host eliminates the major type or types of each remission leaving others to multiply and form the next peak. During multiplication, the parasites may compete, some growing faster than others, to establish the major variable antigen types of the next peak (McNeillage and Herbert 1968; Herbert, 1975; Van Meirvenne, *et al*, 1975). The immune response against each variant kills only the trypanosomes that possess that particular antigen and is invariably unable to affect the proportion of the population that has altered its antigenic identity.

The trypanosome is capable of replacing a surface coat that has been artificially removed (for example by capping) within 3 hours (Newton and Vickerman, 1979). The renewal is not affected by inhibitors of protein synthesis. A study has shown that at least 101 variable antigen types were produced by a clone of *T. equiperdum* (Newton and Vickerman, 1979). The control of antigenic variation and the processes that initiate the expression of the antigen have not been elucidated.

## 2.5 Metabolism of Trypanosomes.

Trypanosomes affect their host through their metabolic activities and inflict demages either by depleting essential nutrients and/or producing toxic metabolites. Damage to the host tissues in trypanosomoses though complex could be brought about through the metabolic activities of these parasites.

### 2.5.1 Carbohydrate metabolism.

A unique feature of all trypanomastidae is the presence of glycosomes, microbody - like organelle which are very important for the energy supply of blood stream forms of trypanosomes (Opperdoes and Borst, 1977; Opperdoes, 1983). Studies on the physiology and biochemistry of trypanosomes have been centred towards carbohydrate metabolism, changes in oxidative pathway in particular. This is as a result of the observation that trypanosome motility in blood depends upon adequate supplies of glucose (Schern, 1928), due to their low endogenous energy reserves (Opperdoes *et al.*, 1976).

The major end-products of glucose break down by trypanosomes are pyruvate and glycerol which are readily metabolised by the host to produce considerable amount of energy. Ageing trypanosomes however can metabolise 2- oxo-glutarate in place of glucose (Bowman and Flyan, 1977). Trypanosomes do not have functional mitochondria, cytochrome system (Hill, 1976) or

lactate dehydrogenase (Dixon, 1966), but the NADH +H$^+$ produced in glycolysis is re-oxidised by a system of glycerolphosphate oxidase which consist of glycerol - 3 - phosphate dehydrogenase and glycerol - 3 - phosphate oxidase. (Opperdoes and Borst, 1977).

2.5.2 Amino acid metabolism.

Exogenous amino acids are known to enter trypanosomes by both diffusion and specific transport systems (Voorheis, 1973; Jackson and Fisher, 1977), and there is evidence that blood stream forms of *T. brucei* are capable of ingesting and digesting proteins (Langreth and Balber, 1975). It was suggested by De Raadt and Seed (1977) that an important factor in determining when trypanosomes begin to develop in the cerebrospinal fluid (CSF) during an infection is the availability of ingestible proteins, amino acids and other nutrients. Further findings in agreement with this observation is that, the parasite may have access to the CSF throughout an infection (Peruzzi, 1928b) but are unable to grow until the CSF has been enriched with proteins and other nutrients from degenerating tissues.

There is evidence that accumulation of the end-products of amino acids metabolism by trypanosomes contributes to the characteristic pathology of African trypanosomoses (Newton, 1978). Transamination of tyrosine yields p-hydroxyphenyl pyruvate, high concentrations of which have been found in the urine but not in the blood of infected animals (Stibbs and Seed, 1975c, 1976). Deamination of tryptophan yields indole pyruvate which is further metabolised to indole lactate, indole acetate and indole ethanol (tryptophol) (Stibbs and Seed 1975a, b, d). Tryptophol causes lethargy (sleep), convulsions and death by respiratory depression when injected into mice and cats (Sabella *et al*, 1969) and it has been suggested that trypanosomes in extravascular sites of the central nervous system produce such effects. Tryptophol has also been reported to cause immunodepression (Ackerman and Seed, 1976b), and lyses red blood cells rapidly (Seed *et al*, 1978). Similar action on

(Ackerman and Seed, 1976b), and lyses red blood cells rapidly (Seed *et al*, 1978). Similar action on synaptic membrane may cause changes in the transmission of nerve impulses and give rise to behavioral changes and induce a sleep-like or comatose state which may explain the mental disturbances that are found often in 80 - 90% of trypanosomosis syndromes.

2.5.3 Purine metabolism.

Trypanosomes generally lack *de novo* pathway of purine nucleotide synthesis but they rely on the preformed bases and nucleotides from the hosts (salvage pathway) (Gutteridge and Gaborak, 1979; Bone and Steinart, 1956). These purines are acted upon by the salvage enzymes of the parasites and are trapped intracellularly as nucleotides to be incorporated into nucleic acids. Studies indicate that adenosine is the most important purine nucleotide for *T. congolense and T. brucei* (James and Born, 1981). Purines, mainly nucleosides, are continously released from tissues and myocardium (Rubio and Berne, 1969); also there is evidence of rapid turnover of adenosine in erythrocytes from normal blood (Hendersen and Lepage 1959; Adams and Harkness, 1973). It is therefore conceived that trypanosomes have access to the purine source available to red blood cells.

2.5.4 Metabolism of polyamines.

Polyamines (spermine and spermidine) are found in all bacteria and most animal cells. They are growth factors for microorganisms and serve to stabilise membrane structures of ribosomes. They are formed from putrescine and S- adenosyl methionine.

Biosynthesis of polyamines in trypanosomatids through putrescine probably follows the same route as in animals where putrescine is formed by decarboxylation of ornithine by the action of ornithine decarboxylase (Fairlamb, 1982). S- adenosyl methionine is first decarboxylated before reacting with putrescine to form spermidine. Spermidine and putrescine are the only polyamines in

blood stream *T. brucei* contained in significant quantities (Fairlamb, 1982).

The synthesis of polyamines in *T. brucei* can be inhibited by an enzyme - activated inhibitor of ornithine decarboxylase - alpha difluoromethyl ornithine (DFMO) whose administration to the drinking water of mice is curative to early *T. brucei* infections. Its use under the trade name Eflornithine against late stage sleeping sickness has proved curative (Peceny *et al.*, 1995)

2.5.5 Metabolism of trypanothione.

Trypanothione ($N^1$, $N^8$ - bis (glutathionyl) spermidine is uniquely found in trypanosomatids (hence the trivial name trypanothione), and is absent in mammalian cells (Fairlamb *et al* 1985). The function of the thiol group of glutathione as a cellular protectant against free radicals formed as by-products of oxygen metabolism in mammalian cells is being carried out mainly by trypanothione in trypanosomes and it accounts for the majority (80%) of glutathione in these cells (Fairlamb, 1988; 1990).

Mammalian glutathione peroxidase use glutathione as reductant in the removal of hydrogen peroxide and other intracellular peroxides. However, in African trypanosomes, glutathione peroxidase is absent and replaced by iso-functional trypanothione peroxidase (Henderson *et al*, 1987b). Mammalian cells also maintain their intracellular thiol disulphide balance by means of glutathione reductase (Schirmer *et al* 1989), an enzyme absent from trypanosomes (Fairlamb & Cerame 1985) and replaced by the analogous enzyme trypanothione reductase (Shames *et al*, 1986).

2.6 Anaemia in Trypanosomoses.

Anaemia is recognised as one of the important features of clinical trypanosomoses in both man and domestic animals (Fiennes,1954; MacKenzie and Cruskank, 1973; Mamo and Holmes, 1975; Kobayashi, *et al*, 1976) although some other important features include; elevation of body

temperature. Also respirations are rapid and laboured and there is significant loss in body weight (Stephen, 1970).

The severity of this anaemia depends on a number of factors like the species of the organism, isolate of the species as well as the species of host, and the acuteness or chronicity of the infection. Anaemia is a consistent finding in various trypanosome infection (Anosa, 1980).

In a haematological study on Nigerian cattle, it was discovered that trypanosomosis produced the most severe anaemia encountered among the animals of all blood protozoa and gastrointestinal helminth infections(Obi and Anosa, 1980). Similarly, experimental infections of rabbits with *T. bruce* produced a severe anaemia with a drop in the haemoglobin level to 6.0g/dl - half of the initial volume (Jenkins *et al*, 1980). Anemia was pronounced to be the primary cause of death by a group of some investigators (Mamo and Holmes 1975; Losos and Ikede, 1972).

The anaemia in trypanosomoses usually sets in during the first wave of parasitemia (Anosa, 1975; Anosa, 1980). Subsequent development of anaemia is determined by the frequency and intensity of the parasitemia. With each wave of parasitemia, the period between the waves are accompanied by a partial recovery of the erythrocyte values depressed during the growth phase. In per acute infection such as those produced by *T. brucei, T. congolense and T. vivax* in rodents (Isoun, 1975; Whitelaw *et al*, 1980; Taylor, 1968), the erythrocyte values drop progressively until death and the infected animals succumb to a single intense wave of parasitemia. Chronic crisis is characterised by less severe parasitemia, but it persists at the level attained during the acute phase.

The mechanism of this anaemia is unclear, but has been shown to involve sufficient increase in the rate of erythrocyte destruction with a consequent shortening of the erythrocyte lifespan (Tizard *et al* 1978a).

It is becoming obvious that the etiology of the anaemia in trypanosomoses is multifactorial with haemolysis, haemodilution, and disordered and/or compensotary erythropoiesis. A number of hypotheses have been put forward to explain the possible mechanisms for haemolysis during trypanosomoses. These include:-

1. Immune haemolysis

The evidence for immune sensitization of the red cells in trypanosome infections was supported by the observation that the host becomes immunosupressed as the waves of anaemia cease. Herbert and Inglis (1973) demonstrated that the attachment of trypanosome antigen to the red cell membrane renders them susceptible to phagocytosis by the reticuloendothelial system. Other studies confirmed that IgM and IgG directed against trypanosomes are adsorbed to host's erythrocytes (Amole *et al*, 1982; Kobayashi *et al*, 1976). While auto-antibodies specifically directed against the host's erythrocytes were lacking (Assoku, 1975). The development of anaemia was positively correlated with the percentage of IgM - positive RBC and with the antibody response, and in the later stages of infection, the arrest of parasitological crisis was accompanied by decrease in IgM - positive RBC and anaemia (Amole *et al*, 1982). Studies have also shown that some anti-RBC antibody was generated by host in *T. brucei* infection in mice (Kobayakawa *et al*, 1979). Compliment has also been found to be adsorbed to the erythrocytes of host (Rickman, and Cox, 1979; Assoku, and Gardiner, 1985).

Esievo (1979, 1980) has shown that *T. vivax* produces neuraminidase which hydrolyses neuraminic acid *in vivo*, and that neuraminidase activity is proportional to the number of trypanosomes. Esievo *et al* (1982) in another study showed that significant cleavage of erythrocyte sialic acid occur during acute trypanosomosis due to increased circulating neuraminidase resulting

from high parasitemia at this phase. This cleavage renders the erythrocytes more prone to phagocytosis by the reticuloendothelial system. Also Jancik *et al*, (1978) showed that treatment of red blood cells with neuraminidase removes the masking effect of the negative charges of sialic acid residues on the erythrocytes and the desialylated erythrocytes are opsonized by immunoglobulins and complements leading to recognition and subsequent destruction by the phagocytic cells of the liver and spleen.

2. Haemolytic factors.

As early as 1907, Landsteiner and Raubitchek observed that a hemolysin which might be lipid in nature and possibly a fatty acid was generated by *T. equiperdum*. Trypanosomes have been shown to generate potentially hemolytic factors on autolysis. Both *T. congolense* (Tizard and Holmes, 1976) and *T. brucei* (Huan, *et al*, 1975) have been shown to generate hemolytic substances when permitted to autolyse in saline at 2°C. In the case of *T. congolense*, the hemolysin has been shown to consist of a mixture of free fatty acids (FFA). Gas chromatography of this mixture shows palmitic, stearic and linoleic acids as the most predominant ones. Free fatty acid (FFA) are known to be hypoglycemic, mitogenic and cytotoxic. They have been grouped among the cytolytic surface active agents (Tizard *et al*, 1978a) which are capable of lysing washed rat and bovine erythrocytes (Tizard *et al*, 1978b; Lawrence and Moores, 1974; Tizard *et al*, 1977). Free fatty acid have also been shown to be immunosuppressive *in vitro* and *in vivo* (Meade, and Martin, 1975). Immunosuppression is characteristic of African trypanosomoses so that infected hosts frequently die of secondary infections (Hoffman *et al*, 1982).

Trypanosomes on autolysis also liberate phospholipases in addition to FFA. This group of hydrolytic enzymes are involved in the degradation of phospholipids which form about 50% of the

total lipids in biological membrane. It has been demostrated (Hambrey *et al*, 1981) that phospholipase A from *T. congolense* requires surface active agents for activity. Linoleate (0.5mM) has been found to be an effective activator. At high parasitemia, it was discovered that trypanosome - infected rabbbits displayed respiratory distress, a symptom of trypanosomoses in other species including man (Lebras *et al* 1979) which was believed to be the result of the hydrolysis of alveolar phospholipids or of lung surfactant by trypanosomal phospholipases. Free fatty acid does not normally exist in free state in the blood but complexed to serum albumin ( Spector, *et al* 1969). However during massive trypanosome destruction as a result of host immune response and the action of phospholipases on phospholipids, amount of FFA may be sufficient to exceed the binding capacity of albumin (Tizard, *et as* 1978c) and cause RBC lysis (Tizard *et al*, 1978). Akanji (1985) and Nok (1993) showed that serum FFA concentration rises continously with increase in parasitemia until the death of the animals.

3. Non specific reticulo endothelial activation.

The reticulo endothelial system shows intense proliferation during trypanosomoses which is evidenced from an increased rate of clearance of intravenously injected particles (Murray *et al*, 1974). In other cases where there is splenomegaly and hepatomegaly, phagocytic cells are known to consume erythrocytes in a non specific manner. Erythrophagocytosis is a marked feature in trypanosomoses.

4. Direct traumatic effect of trypanosomes on the red cells.

It has been demonstrated that *T. brucei* (Holwill, 1965) visibly distorted the red cells of the mice. Filmed sequences have shown the process of pinching out red cells by adherent trypanosomes in wet preparations of infected mouse blood. It is likely that this process affects the subsequent intergrity of the cell especially when parasitemia is at a high level.

2.7 Clinical manifestation.

Infection by pathogenic trypanosomes cause diseases which vary in severity depending on the virulence of the strain of trypanosome and susceptibility of the host. Strains of each species of trypanosomes are capable of producing (stimulating) a range of response including acute, chronic and a symptomatic carrier syndromes. Also there are very marked differences between species and breeds of animals in the susceptibility to the development of clinical disease.

In African livestock trypanosomoses, different species of trypanosome may infect several species of animals with different effects and degrees of severity. Trypanosomosis in the domestic pig for instance is characterised by massive parasitemia ending in death often within a period of 24 hours, but the same trypanosome in sheep causes a mild disease from which the animal may recover spontaneously while cattle are resistant even to a severe challenge. Therefore in animal trypanosomosis, the relative susceptibility of different breeds of animals, state of nutrition, occurence of other infections (bacterial, viral or helminth) stress due to vaccination regimen, trekking, thirst and temperature changes greatly influence the clinical manifestations.

However, the clinical manifestations produced by both human trypanosomes have certain features in common :(1)a primary lesion at the site of the infective tsetse bite (usually within 8-10 days after the bite), this is called the chancre. (2) Stages of parasite dissemination and invasion of the blood stream by the trypanosomes. (3) The later stage of central nervous system involvement.

Some signs and symptoms of early stage infection include fever, headache and splenic enlargement. Irritability, insomnia, and changes in personality or mood are characteristic early signs of human trypanosomosis. Others include anaemia, enlargement of lymph nodes, local oedema (face or eyelids). There may also be some endocrine dysfunction manifesting in form of sterility or abortion

in women. The onset is more gradual in the Gambian and may even be overlooked compared to the Rhodesian type of sleeping sickness where the infection tend to be more acute with death occuring within a week or two of onset.

The Gambiense disease is chronic and long term in character being sub-acute in the early stages followed by outbursts alternating periods of fever separated by remissions and may last from few days to a month or more. This blood-lymphatic stage may even last for years and patients at this stage seem to be unaware of any illness. This type of the disease may only be recognised in its final stages when the brain is affected.

The clinical picture of human trypanosomosis is complicated by the presence of secondary bacterial infection which is as a result of immunosuppression, such that infected host may even die of secondary infections. Human trypanosomosis are also not diagnosed early in many cases as the parasites are not continuously present in the peripheral blood and cerebrospinal fluid, the initial fever attacks are mistaken for malaria (Stephen, 1970).

2.8 Treatment of Trypanosomoses/Drug design.

The chemotherapy of trypanosomoses has not been satisfactory due to the paucity of new drugs and the high cost of existing ones coupled with toxicity and resistance problems associated with a lot of them, as well as the concomitant relapses of infection.

Research efforts to develop new drugs is now shifting towards a direct and rational approach in the field of biochemistry and molecular biology in order to identify striking differences between the biochemistry of the host and parasite that could be exploited as targets for new drugs.

A number of drugs in use for the treatment of early stages of infection are suramin, pentamidine and berenil. Most of these drugs are also used for prophylactic purpose for some period

of time. They are not used for later stages because of their inability to cross the blood brain barrier and get into the cerebrospinal fluid (CSF) once the infection reaches the nervous system.

Melarsoprol, the only drug effective against late stage African trypanosomosis is generally administered in the solvent propylene glycol, and if not given intra-veinously can cause severe tissue damage (TDR, 1984). Administration of melarsoprol in groundnut (Arachis) oil at the dose of 1.8mg/kg subcutaneously has been achieved with long term cures in monkeys with no relapse over a period of 600 days (TDR, 1984).

A number of compounds have been screened for trypanocidal properties (activities) among which four 2- substituted 5 - nitroimidazole given in combination with suramin brought a permanent cure of central nervous system (CNS) infection in mice, and the ornithine decarboxylase inhibitor - alpha diflouro methyl ornithine (DFMO) has been found to be effective in acute *T. b. brucei* animal infection and to act synergistically with other trypanocidal compounds (Bachi *et al,* 1982a and b) in the treatment of *T. b. brucei* CNS infection in rats (Onyeyili and Onwualu, 1991). In combination with bleomycin, DFMO killed berenil resistant trypanosomes in mice with CNS infection (Clarkson *et al* 1983). Another synthetic trypanocide is 1, 3, 5 triacetyl benzene tris (guanylhydrazone) TBG which has been screened and found to produce permanent cure of early *T. brucei* infection in mice at a single dose of 3mg/kg.

Improvement of chemotherapy of pathogenic trypanosomes depends on biochemical studies of the cellular composition, metabolism and function of these parasites and also determining the biochemical differences between host cells and trypanosomes which could be exploited to develop new drugs. Pathway for the generation of energy in the blood stream trypanosomes is a possible target of attack since inhibition of aerobic glycolysis by salicyl hydroxamic acid (SHAM) makes these

parasites switch over to anaerobic pathway through glycerol kinase reaction, the two pathways could be inhibited. This was achieved by the administration of SHAM and glycerol Hammond and Bowman, 1980). Trypanothione metabolism is one of the current drug target (Fairlamb, 1990) as effort has been shifted towards the identification of the target enzymes in the metabolic pathway. This metabolite is unique to trypanosomes and play the role of antioxidants (free radical scavenger). Trypanosomes could be killed by the inhibition of enzymes responsible for carrying out this function.

## 2.9 Pharmacological properties of garlic oil

Garlic (*Allium sativum)* is a bulb commonly used as a condiment and food ascribed with many medicinal effects. The bulb has been used for more than 4000 years in traditional medicine against a variety of conditions such as cardiovascular disease, cancer, and infections (Srivastava *et al*, 1995). Garlic has also been used in the prophylaxis and treatment of gastro intestinal tract diseases, dysentries, typhoid fever and cholera, and has been reported that 1.4 - 1.6% solution of garlic extract inhibits the growth of *Mycobacterium tuberculosis in vitro* (Basu 1962). An oily extract of garlic was found to have trypanocidal activity on *T. b. brucei in vivo* (Nok *et al*, 1996). In another investigation, Umar and his collegues ( 1994) reported the ability of garlic oil to protect some organs against lipid infilteration which resulted from feeding on high fat diet and beer, therefore, its use has been recommended for preventing fatty infilteration of the liver and kidney.

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS.

### 3.1 MATERIALS

#### 3.1.1 Chemicals and Reagents

Diethyl ether, Petroleum spirit (40 . 60°C), sodium azide, Brij 35, Sulphuric acid were purchased from British Drug House (BDH) Chemicals Ltd., Poole England. Cholesterol standard, Bromocresol green (BCG), Bovine serum albumin (BSA) standard and Fearric chloride were purchased from Hopkins and Williams, Chadwell Health, Essex, England.

#### 3.1.2 Animal

Adult male albino rats weighing 46 - 214g were purchased from the Nigerian Institute for Trypanosomiasis Research, Vom, while mature male rabbits (cross breed of New Zealand White and local rabbits) weighing 2.0 - 2.8 kg were purchased from the National Veterinary Research Institute, Vom. These animals were acclimatized for two weeks period before commencement of the study.

#### 3.1.3 Animal Feed

Rabbit pellets was purchased from Pfizer Nigeria Limited, maize ophal (Dusa) was purchased from grinding mill at Aviation site II Zaria while rabbit grasses (*Trichodesma pulcherima)* and carrot leaves were obtained around Samaru village, Zaria.

#### 3.1.4 Garlic

Fresh garlic was purchased from Samaru village market, and commercially prepared garlic oil was obtained from Schiff products Inc. salt Lake city (UT) U.S.A.

#### 3.1.5 Trypanosome (Parasites)

*Trypanosoma congolense* Bassa strain and *Trypanosoma brucei* Lafia strain were obtained

from the Nigerian Institute for Trypanosomiasis Research, Vom.

3.1.6 Equipment

1. Mettler Balance (Mettler, P. 161) and Havard Trip Balance top loading Shandon scientific company Ltd, London.
2. Mortar and pestle (ceramic)
3. Rotatory evaporator Churchill England
4. Jenway spectrophotometer 6100
5. Microhaematocrit centrifuge Hawskey and Son Limited, England
6. Microscope Olympus England
7. pH meter WTW pH 422
8. Goldberge refractometer (TS meter) No 10400 American optical company, Buffalo, New York.

3.2 METHODS

3.2.1 Extraction of garlic oil

Garlic oil was extracted from raw cloaves of garlic (*Allium sativum*) with diethylether according to the method of Williams *et al.* (1985).

Raw cloaves of garlic were peeled, ground and soaked in diethylether for 48 hours after which the supernatant was decanted. The residue was re-extracted thrice. All the extracts were combined and the oil in the combined extract recovered by distilling off the solvent at 40°C.

The extracted oil was further clarified by mixing it with 20 volumes of redistilled petroleum ether (Bp 40 - 60°C) followed by centrifugation to separate the phases. The clear upper phase was

removed and the oil was prepared by distilling off the petroleum ether at 60°C.

3.2.2 Toxicity studies on garlic oil with rats

Acute toxicity determination was conducted in two step investigations according to Lorke (1983). The first step was carried out to determine the range of acute toxicity. Twelve male albino rats further acclimatized for 5 days were divided into 3 groups of 3 animals each. Group 1, 2 and 3 were orally administered 10, 100 and 1000 mg/kg body weight garlic oil in groundnut oil respectively. Group 4 received groundnut oil only.

They were observed frequently on the day of treatment. The nature and time of any adverse effect was noted, and where any animal died, the time of death was recorded, during the first day in hours and thereafter in days. Dead animals were autopsied and examined macroscopically for any pathological changes. Observations were carried out for 14 days and the experiment then terminated. All surviving animals were weighed, sacrificed, autopsied and examined for any pathological changes.

In the second and final investigation, 3 rats were divided into 3 groups of one animal each (groups 5 - 7). Based on the result from the first investigation, each animal in groups 5 - 7 were given a dose of garlic oil orally. These rats were observed according to the above procedure, and the studies terminated at the end of 14 days.

Recovery and body weight gain at the end of 14 days period of each investigation was taken as a sign of having survived the acute intoxication. The $LD_{50}$ was then calculated

3.2.3 Trypanosome count and rabbit innoculation

Eighteen matured healthy rabbits (male cross breed of NZW and local) weighing 2.0 to 2.8kg were divided into 6 groups of 3 animals each. They were housed in rabbit cages and supplied with feed and water ad libitum. The rabbits were acclimatized for 2 weeks and screened for parasites.

Haemoglobin (Hb), red blood cell count (RBC), leucocyte count (WBC), differential leucocyte count (DC) and serum albumin and cholesterol determinations.

*Trypanosoma brucei* and *Trypanosoma congolense* from liquid nitrogen were multiplied through innoculation of healthy mice. Massive parasitaemia was obtained after the mice were sacrificed and the number of trypanosomes per mililitre of blood estimated using Haematocytometer. Thereafter, animals in groups 1 and 2; were each innoculated intraperitoneally with one half millilitre normal saline containing $1 \times 10^4$ *Trypanosoma congolense*, and each rabbit in groups 3 and 4, same number of *Trypanosoma brucei*. Groups 5 and 6 served as uninfected garlic treated and uninfected untreated controls respectively.

3.2.4 Garlic oil treatment and sample collection

Blood was collected from infected rabbits daily to examine (for thin film) for the establishment of infection and then estimation of parasitaemia. Parasitaemia was estimated according to Herbert and Lumsden (1976) using the light microscope. Weekly blood collection was conducted for the determination of PCV, Hb, RBC, WBC and DC. Blood meant for biochemical analyses was allowed to clot and the serum used for albumin and cholesterol analyses. Daily collection of blood for the thin film was from the ear vein while the weekly collection was from the jugular vein.

Treatment with 100 mg/kg garlic oil was commenced 11 days post infection (PI) when the parasitaemia developed.

3.2.5 PCV determination

Hematocrit was determined by the microhaematocrit method. Blood was collected in heparinized capillary tubes. These were sealed at one end with crystaseal and centrifuged at 2,000g for 5 minutes

in micro-haematocrit centrifuge. The PCV was then read using the PCV reader and Hb estimated as PCV/3 according to Schalm *et al.*, 1975.

3.2.6 RBC, WBC and DC determinations

Total red blood cells (RBC) and white blood cells (WBC) counts were determined by coulter counter and differential count (DC) according to the standard procedure (Coles, 1974). Absolute values for leucocyte differential count were calculated.

3.2.8 BIOCHEMICAL ANALYSES

3.2.8.1 Total plasma protein (TPP), determination

Total plasma protein was determined using the Goldberge refractometer according to Schalm *et al.*, 1975. The capillary tube after the PCV reading was broken above the packed red blood cells, and the clear plasma portion used. A drop of plasma is placed on the prism of the TS meter and the total plasma protein concentration read directly on the protein scale in g/100ml.

3.2.8.2 Serum cholesterol

Cholesterol level was determined according to the method of Zlakis and Boyle (1959). In this technique, cholesterol reacts with concentrated sulphuric acid in the presence of ferric chloride to give a coloured compound that is read at 560 nm.

Procedure

One hundred microlitre of serum or cholesterol standard or water (for reagent blank) were placed in stoppered centrifuge tubes, and to each was added 4.9 ml solution of 10% ferric chloride and acetic acid (1:2 v/v). They were mixed and allowed to stand for 15 min. To 2.5 ml of this was added 1.5 ml of concentrated sulphuric acid. The tubes were cooled in a water bath and the absorbance read at 560 nm.

Total serum cholesterol per 100ml of serum was calculated as:

$$\frac{Au \times Cs}{As}$$

where $A_u$ = Absorbance of sample with unknown concentration of cholesterol.

$C_s$ = Concentration of standard

$A_s$ = Absorbance of standard

3.2.8.2 Serum albumin

Serum albumin concentration was estimated by the method of Doumas *et al.*, 1971 as modified by Spencer and Price, 1977.

The principle behind this method is that, the dye Bromocresol green (BCG) binds albumin to give a coloured complex that is read colorimetrically at 632 nm.

**Procedure**

Twenty microlitres of serum or albumin standard were added to 4.0 ml of working dye solution containing 0.5 mol/L succinate buffer pH 4.10, 10m mol/L BCG solution 40g/L sodium azide, and 250g/L Brij 3.5 (see appendix B). The contents were mixed and allowed to stand for 10 min. at 25°C and the absorbance at 632 nm read against a blank of working dye solution.

The albumin was estimated by the formula:

Serum albumin (g/L) was calculated as

$$\frac{\text{Reading of Unknown}}{\text{Reading Standard}} \times 40$$

where 40 = Concentration of albumin standard (40g/L)

3.2.9 Post mortem examination of rabbits

Post mortem examinations were carried out on dead rabbits and gross pathological lesions recorded. The study was terminated at the end of 11 weeks and all surviving rabbits (groups 1, 2, 5 and 6) were weighed sacrificed, autopsied and examined for pathological lesions. Organs such as heart, livers, kidneys, spleen and lungs of all the rabbits were weighed.

Comparison was made between garlic treated and untreated *T. congolense* (groups 1 and 2 respectively), *T. brucei* (groups 3 and 4 respectively) infected rabbits by student t test at 5% ($P<0.05$) level of significance.

# CHAPTER FOUR

## 4.0 RESULTS.

### 4.1 Characteristics of the extracted garlic oil.

Garlic oil extracted from raw cloaves of garlic was a semi liquid which was golden orange in colour. The oil was volatile and had a strong pungent odour. The average yield was 2.25 g/kg wet weight.

### 4.2 Toxicity studies.

The result for the two-step tests of the acute toxicity on garlic oil is summarised and shown in Table 4.1 Rats administered the oil up to 2900 mg/kg survived the two-week period of the tests and gained weight as shown in Table 4.1.

#### 4.2.1 Clinical symptoms of toxicity.

All rats treated with garlic oil from 1000 - 5000 mg/kg showed signs of dermal and oral irritations within 30 minutes of administration as indicated by rubbing of mouth and stretching of limbs. Other symptoms were, difficulty in breathing and disinclination to move. These were more prominent in the higher dose groups and resulted to 100% mortality in the 5000 mg/kg group.

#### 4.2.2 Gross pathology.

Animals dosed 10 - 100 mg/kg garlic oil showed no discernable gross pathological lesions, while those dosed in the range of 1000 - 5000mg/kg had congestions of the liver, lungs, kidneys and brain.

The median lethal dose ($LD_{50}$) of the oil was calculated to be 3800 mg/kg (that is, geometric mean of 2900 and 5000 mg/kg, doses for which 0/1 and 1/1 mortalities were found (Lorke 1983).

Table 4.1 Evaluation of acute toxicity of different doses garlic oil in rats

| Group | Dose (mg/kg) | Mean weight(g) of animal (s) | Mean weight gain (g) | Mortality ratio | % Survival |
|---|---|---|---|---|---|
| 1 | 10 | 197.4 | 10.6 | 0/3 | 100 |
| 2 | 100 | 213.5 | 7.1 | 0/3 | 100 |
| 3 | 1000 | 71.6 | 33.7 | 0/3 | 100 |
| 4 | Control | 81.8 | 45.3 | 0/1 | 100 |
| 5 | 1600 | 76.1 | 33.5 | 0/1 | 100 |
| 6 | 2900 | 46.3 | 22.1 | 0/1 | 100 |
| 7 | 5000 | 70.1 | 0* | 1/1 | 00 |

* = Died overnight before 24 hours.

Median lethal dose ($LD_{50}$) = Geometric mean of the doses where0% mortality was followed by 100% $\sqrt{2900 \times 5000}$ = 3800mg/kg

2.3 Percent Organ/body weight ratio.

The percent organ/body weight ratio is presented in Table 4.3. Comparison between control and different dose levels showed no significant difference ($P>0.05$) in kidneys, lungs, liver and heart weight per 100g body weight of 10 mg/kg dose. Significant increase ($<0.05$) was however observed in the heart of 100 mg/kg dose group, while the 1000 mg/kg group showed significant increases ($P<0.05$) in all the organs weighed (liver, heart, kidneys and lungs). Dose groups of 1,600 - 5000mg/kg were not compared as there was no measure of variability.

4.3 Experimental infection of rabbits with *T. congolense and T. brucei.*

Both *T. congolense and T. brucei* infected rabbits developed parasitaemia ten days post infection (Pi). (Figure 4.1a & b respectively).

4.3.1 *T. congolense* infection

The breed of rabbits used in this study (male cross breeds of New Zealand white and local rabbits) were susceptible to *T. congolence* (Bassa strain) infection (10 days Pi) but differ in degree of severity when compared with *T. brucei* (Lafia strain) infection. A summary of the nature of the infection is shown in Table 4.3. The study did not record any mortality, and was terminated at 11 weeks Pi.

4.3.1.1 Symptoms of infection.

Pyrexia was moderate and only at onset, while clinical signs such as nasal discharge, ocular lesions and respiratory distress were absent as shown in Table 4.3. The rabbits suffered poor weight gain. Anaemia was present but fluctuated.

Table 4.2: Effect of garlic oil treatment (10 - 5000 mg/kg) on percent organ/body weight ratio in rats.

| Organ | Percent organ/body weight ratio Dose (mg/kg) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 10 | 100 | 1000 | 1600 | 2900 | 5000 | Control |
| Kidney | 0.84±0.1[a] | 0.97±0.05[a] | 1.11±0.12[b] | 0.91 | 0.88 | 0.75 | 0.82±0.07[a] |
| Lungs | 1.03±0.12[a] | 1.18±0.06[a], | 1.67±0.29[b] | 1.10 | 0.73 | 1.94 | 0.92±0.05[a] |
| Liver | 4.37±0.3[a] | 4.90±0.16[a] | 6.27±0.73[b] | 5.38 | 4.83 | 4.02 | 4.76±0.07[a] |
| Heart | 0.37±0.1[a] | 0.48±0.06[a] | 0.57±0.07[b] | 0.37 | 0.29 | 0.43 | 0.33±0.04[a] |

Comparison of significant was done in rows.

Values in the same row with different supercripts differ significantly ($P<0.05$)

Table 4.3: Comparison between *T. congolense* and *T. brucei* infection of New Zealand White and local rabbit cross breeds

| | | *T. congolense* | *T. brucei* |
|---|---|---|---|
| 1. | Symptoms | Mild | Severe |
| 2. | Blood pathology (anaemia) | Present, fluctuating | Severe, progressive |
| 3. | Ocular lesions | Absent | Persistently present |
| 4. | Respiratory distress | Absent | Present and severe |
| 5. | Nasal discharge | Absent | Present and severe |
| 6. | Pyrexia | Moderate, at on set only | Present always |
| 7. | Recovery | Recovered with persistent and very low parasitemia | There was no recovery, all animals died on or by 8 weeks |
| 8. | Body weight | Slight body weight loss at iniial stage, subsequently, recovery resulted to gain in body weight | Progressive emaciation |

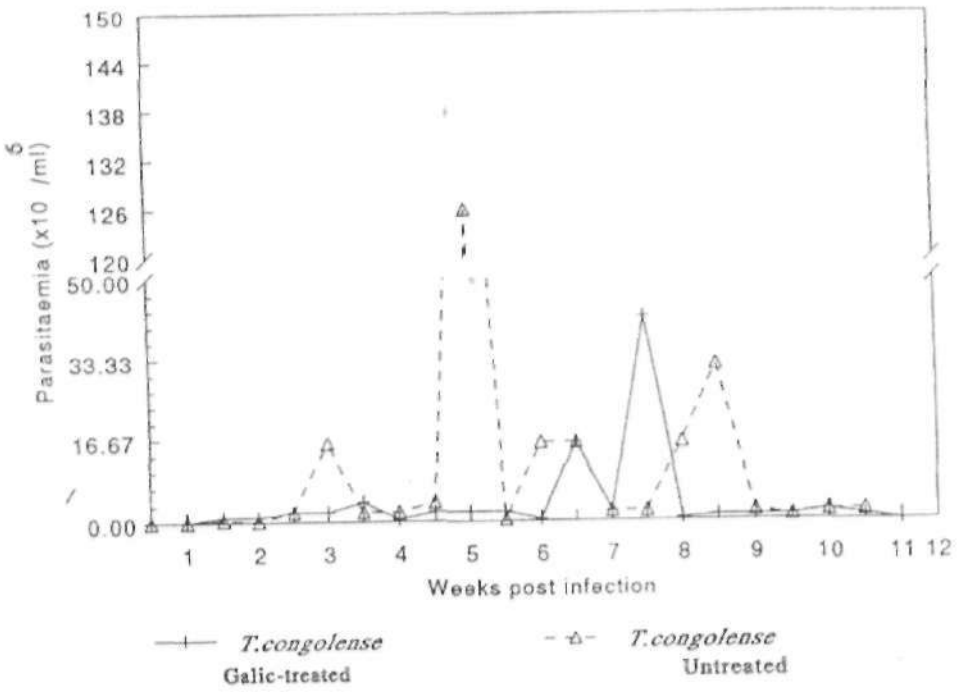



**Figure 4.1a**

Changes in parasitaemia of rabbits infected with T. congolense

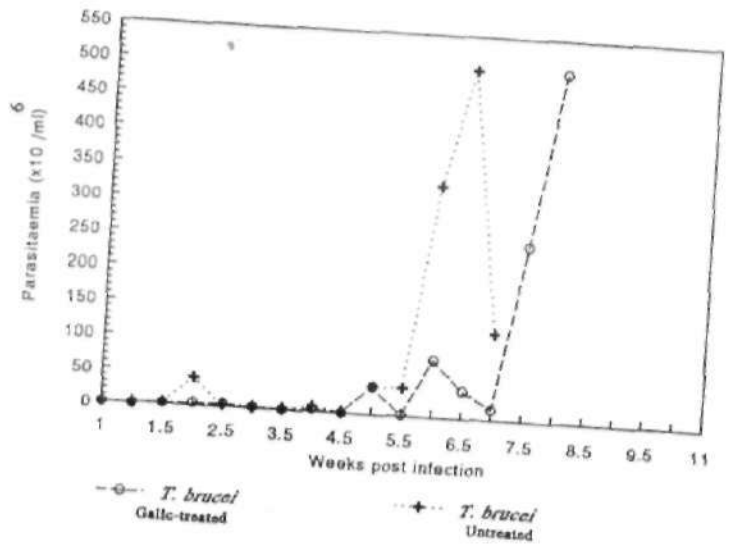



**Figure 4.1b**

Changes in parasitaemia of rabbits infected with T. brucei

4.3.1.2 Haematological changes.

The mean packed cell volume (PCV), haemoglobin concentration (Hb), and red blood cell count (RBC) of both garlic treated and untreated *T. congolense* infection are presented in Figures 4.2 to 4.4 respectively. Both treated and untreated rabbits had significantly ($P<0.05$) decreased PCV, RBC and Hb at 3 weeks Pi. The highest reductions occurred at 3 and 11 weeks Pi in treated and untreated groups respectively. The values fluctuated with some partial recoveries corresponding with periods of decline in the levels of parasitaemia (Figure 4.1a) particularly in garlic treated group. Garlic treatment resulted in significant increases ($P<0.05$) in PCV and Hb at 4, 9, and 11 weeks Pi, and in RBC at 4, 8, 9 and 11 weeks Pi when compared with untreated control.

4.3.1.3 Plasma proteins.

The changes in total plasma protein concentration are shown in Figure 4.5. Plasma protein concentration in treated and untreated groups were not significantly different ($P>0.05$) with the healthy controls except at week 11 Pi where significant ($P<0.05$) differences were observed between garlic treated and untreated groups compared with control. Enhancement in total protein was recorded at 4, 7, 9 and 11 weeks Pi in garlic treated and only at 11 weeks Pi in the untreated control.

4.3.1.4 Changes in white blood cell count (WBC).

Total leucocyte count in *T. congolense* infection of rabbits is shown in Figure 4.6. Leucopenia was observed by decreased WBC count in the treated and untreated rabbits at week 3 Pi. Leucopenia was highest at 3 and 8 weeks Pi in treated and untreated groups respectively.

Fluctuations in WBC count followed the same pattern as the erythrocytes (PCV, Hb & RBC). Garlic treatment restored the total leucocyte count within normal range by 8 weeks Pi ($7.0 \pm 1.2 \times 10^9$/L) which differ significantly ($P<0.05$) from the count recorded for the untreated group ($3.8 \pm 0.44$

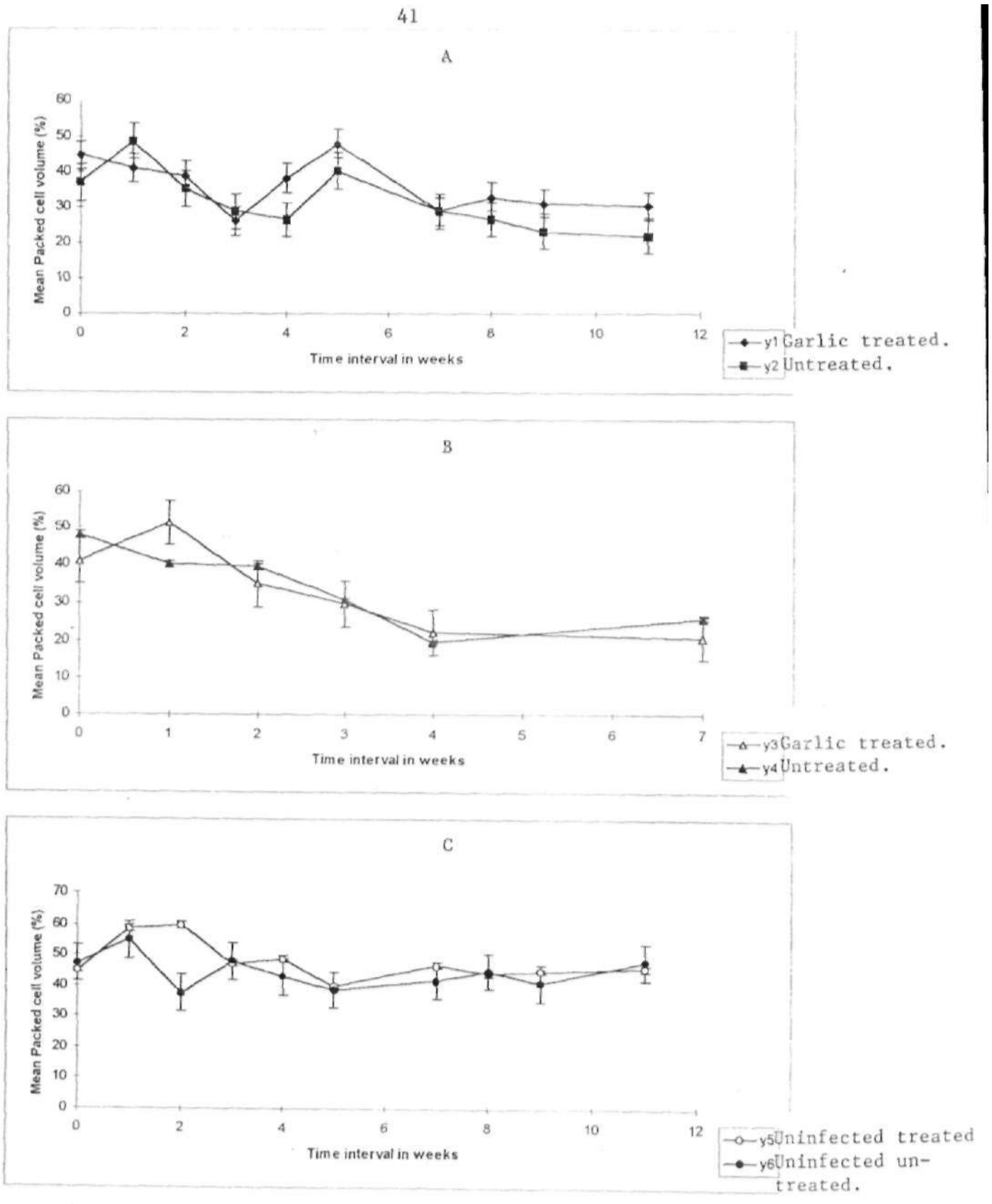



**Figure 4.2**

Effect of garlic oil on mean Packed cell volume of rabbits infected with T. congolense (A), and T. brucei (B) and Controls (C).

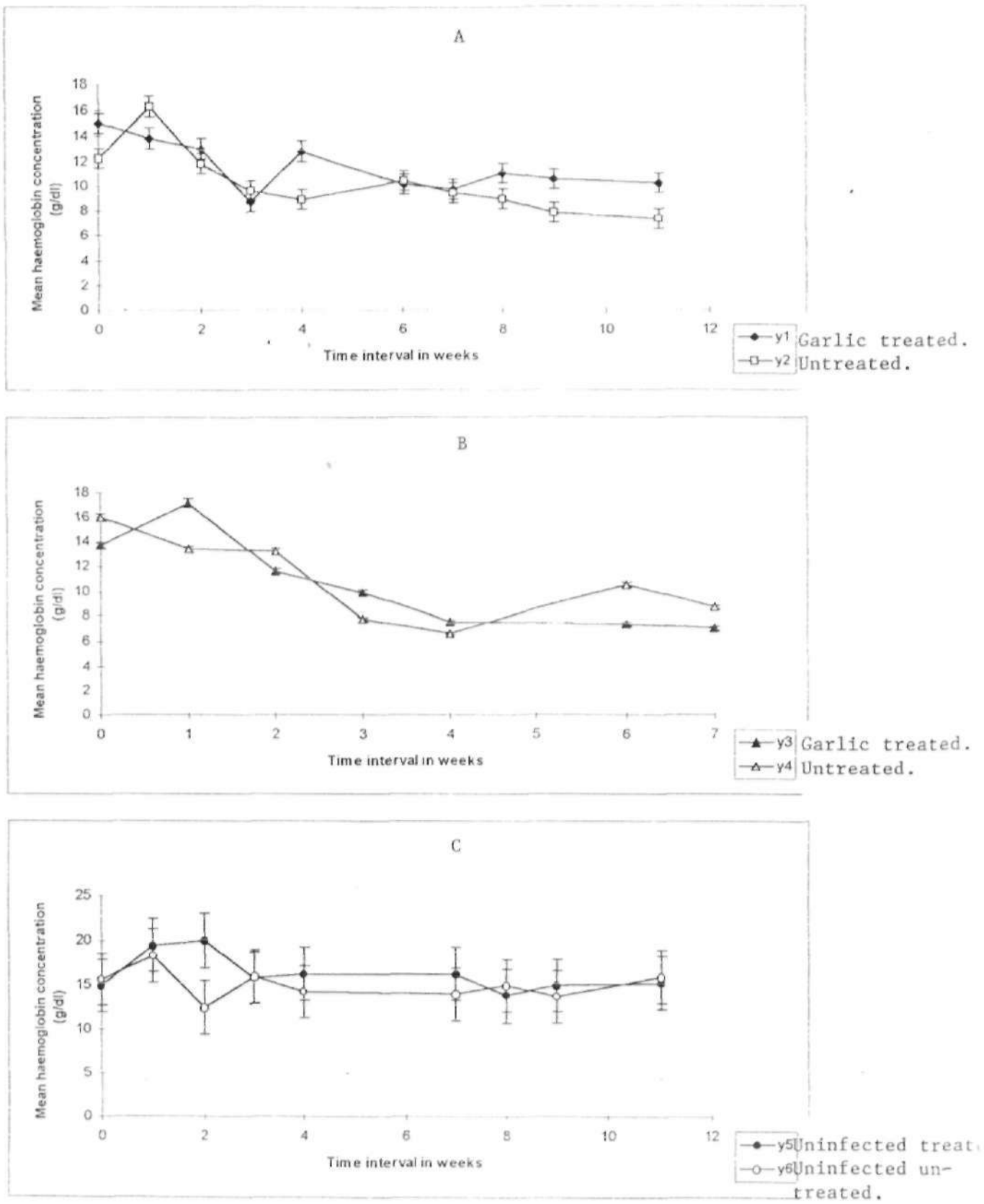



**Figure 4.3**

Effect of garlic oil on mean haemoglobin concentration (g/dl) of rabbits infected with T. congolense (A) and T. brucei (B) and controls (C)

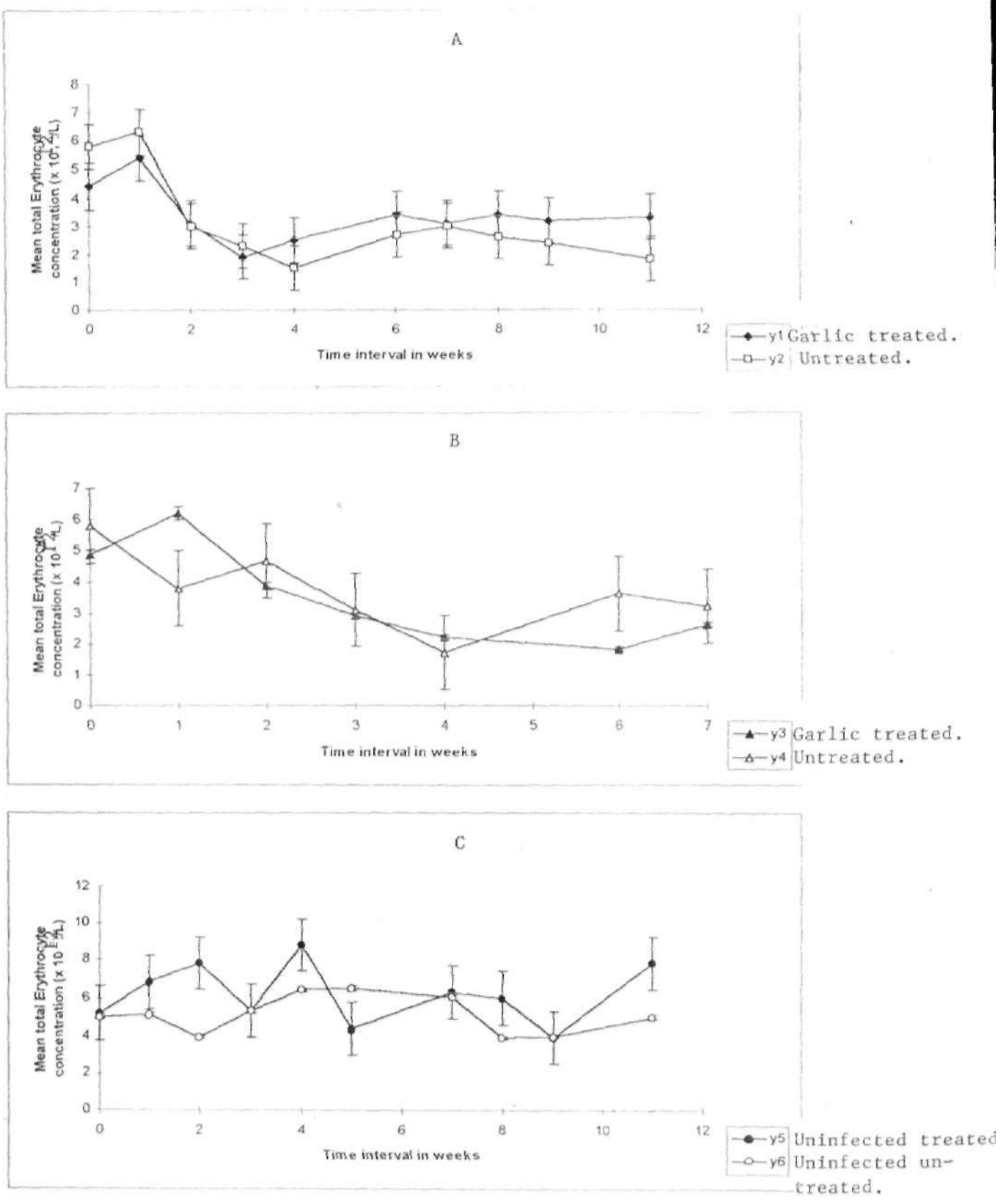



**Figure 4.4**

Effect of garlic oil on total red blood cell count (X10 $^{12/L}$) of rabbits infected T. congolense (A) T. brucei (B) and controls (C)

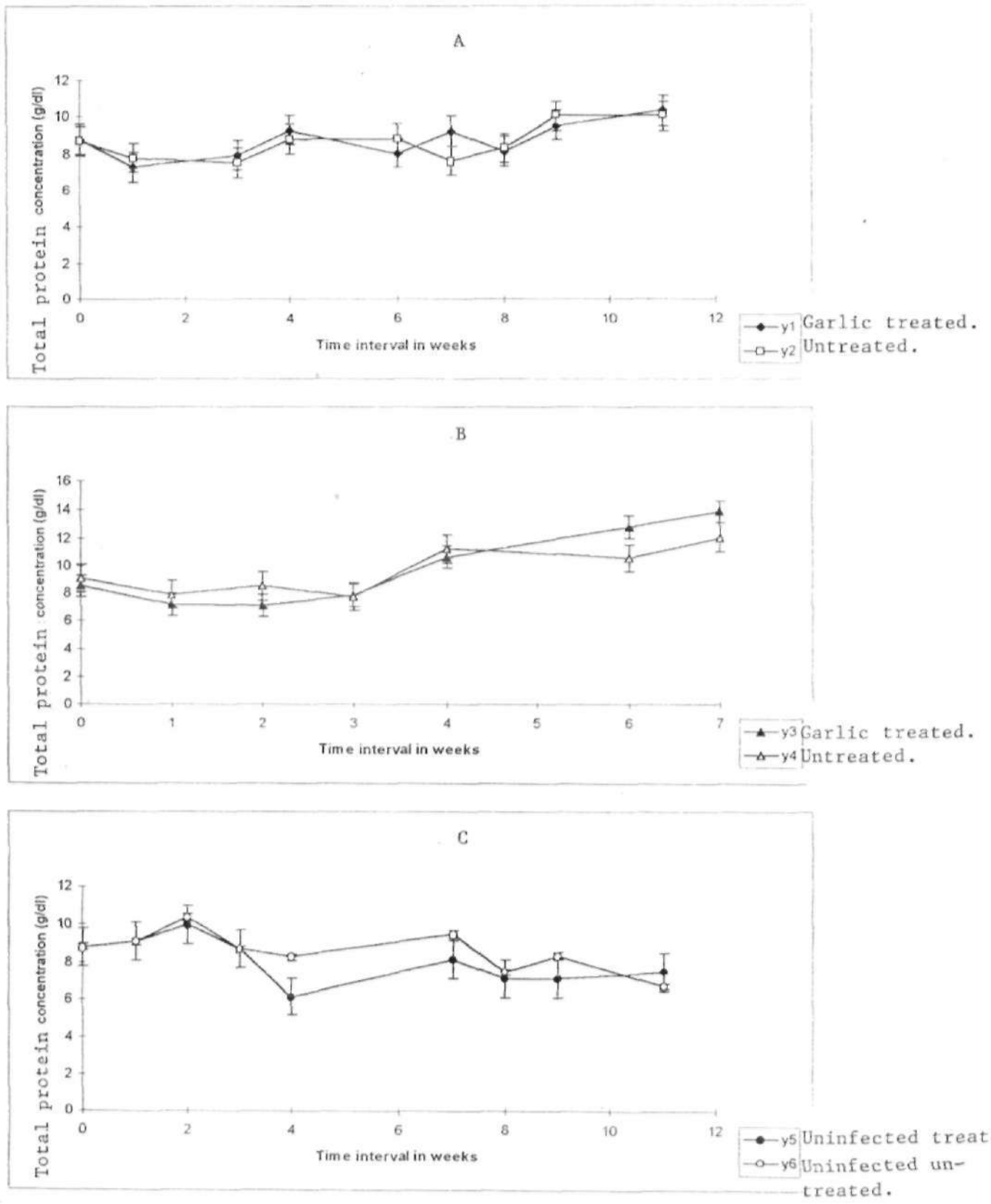



**Figure 4.5**

Effect of garlic oil on total plasma protein concentration of rabbits infected with T. congolense (A) and T. brucei (B) and controls (C)

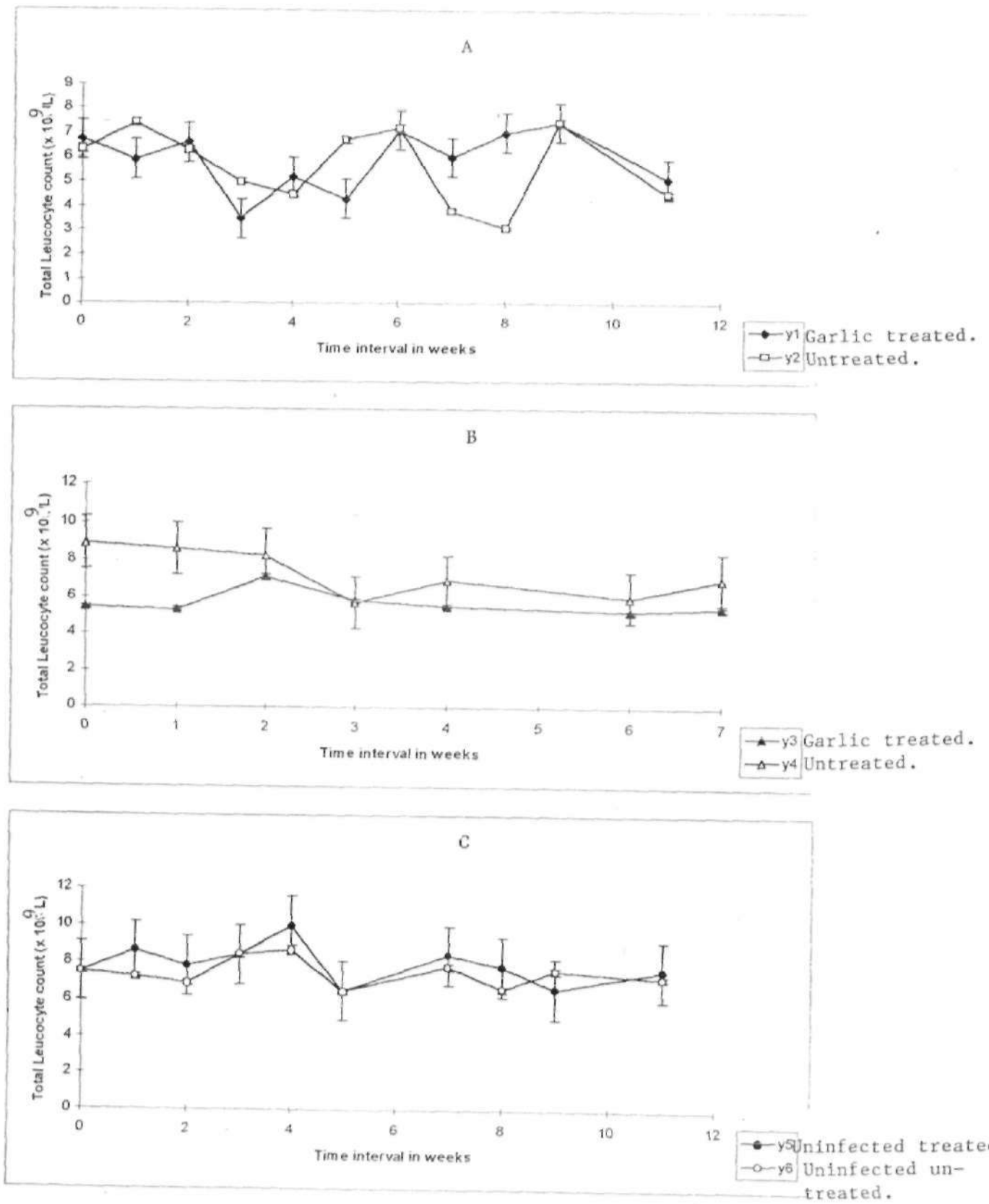



**Figure 4.6**

Effect of garlic oil on total leucocyte count ($X10^{9/L}$) of rabbits infected with T. congolense (A) and T. brucei (B) and controls (C)

x $10^9$ /L. Leucopenia was observed at the termination of the study 11 weeks Pi in both groups.

Differential leucocyte counts as presented in Figures 4.7 to 4.10 show changes in neutrophils, lymphocytes, monocytes and eosinophils respectively, but basophils were not detected in this rabbit cross breed. Neutrophils and lymphocytes significantly decreased ($P<0.05$) by 3 and 4 weeks Pi, in all the groups accounting for the leucopenia recorded at these periods. Garlic administration restored the lymphopenia and neutropenia at 6 weeks Pi, while significantly lower counts of these cells were recorded at 7 and 8 weeks in the untreated group when compared with garlic treatment. Neutrophil counts were significantly lower in untreated control at the termination of the study (11 weeks Pi). Lymphopenia was observed in both treated and untreated groups at the 11 weeks terminal period of the study which account for the terminal leucopenia observed.

Changes in monocytes and eosinophils were not consistent.

4.3.1.5 Serum changes.

Figures 4.11 and 4.12 show weekly averages of serum albumin and cholesterol concentrations respectively.

Infection with *T. congolense* resulted in significant decreases ($P<0.05$) in albumin concentration of both groups prior to administration of garlic oil,i.e before the appearance of parasitaemia (Figure 4.1). Treatment with garlic oil increased the concentration significantly above ($P<0.05$) those in untreated rabbits at 2, 3 and 5 weeks Pi.

Cholesterol level increased with the establishment of *T. congolense* infection significantly ($P<0.05$) 2 weeks Pi. Treatment with garlic oil however reduced the levels significantly ($P<0.05$) at 2, 3 and 6 weeks Pi when compared with untreated control.

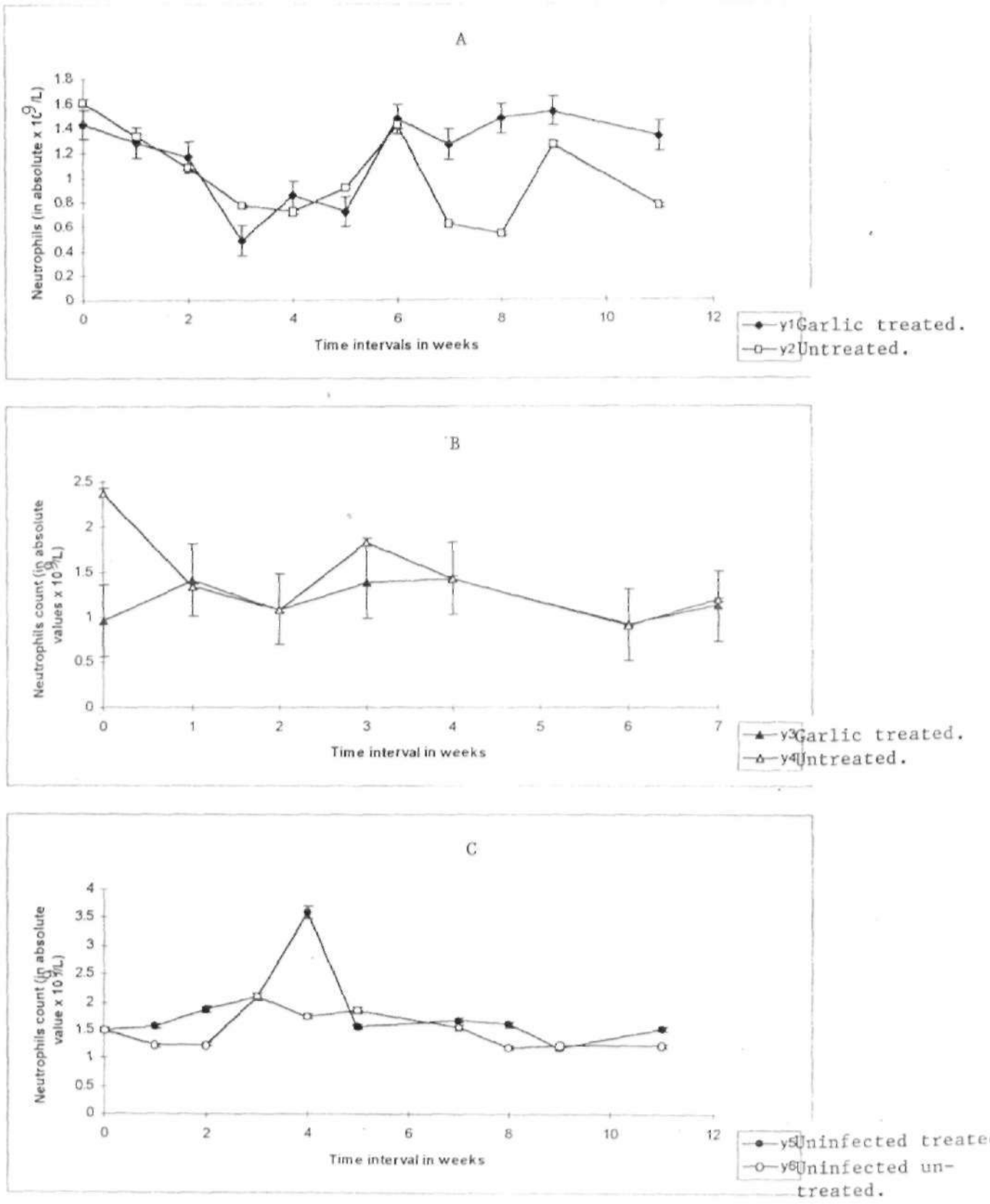



**Figure 4.7**

The effect of garlic oil on neutrophils count (in absolute value ($X10^{9/L}$) of rabbits infected with T. congolense (A) and T. brucei (B) and controls (C)

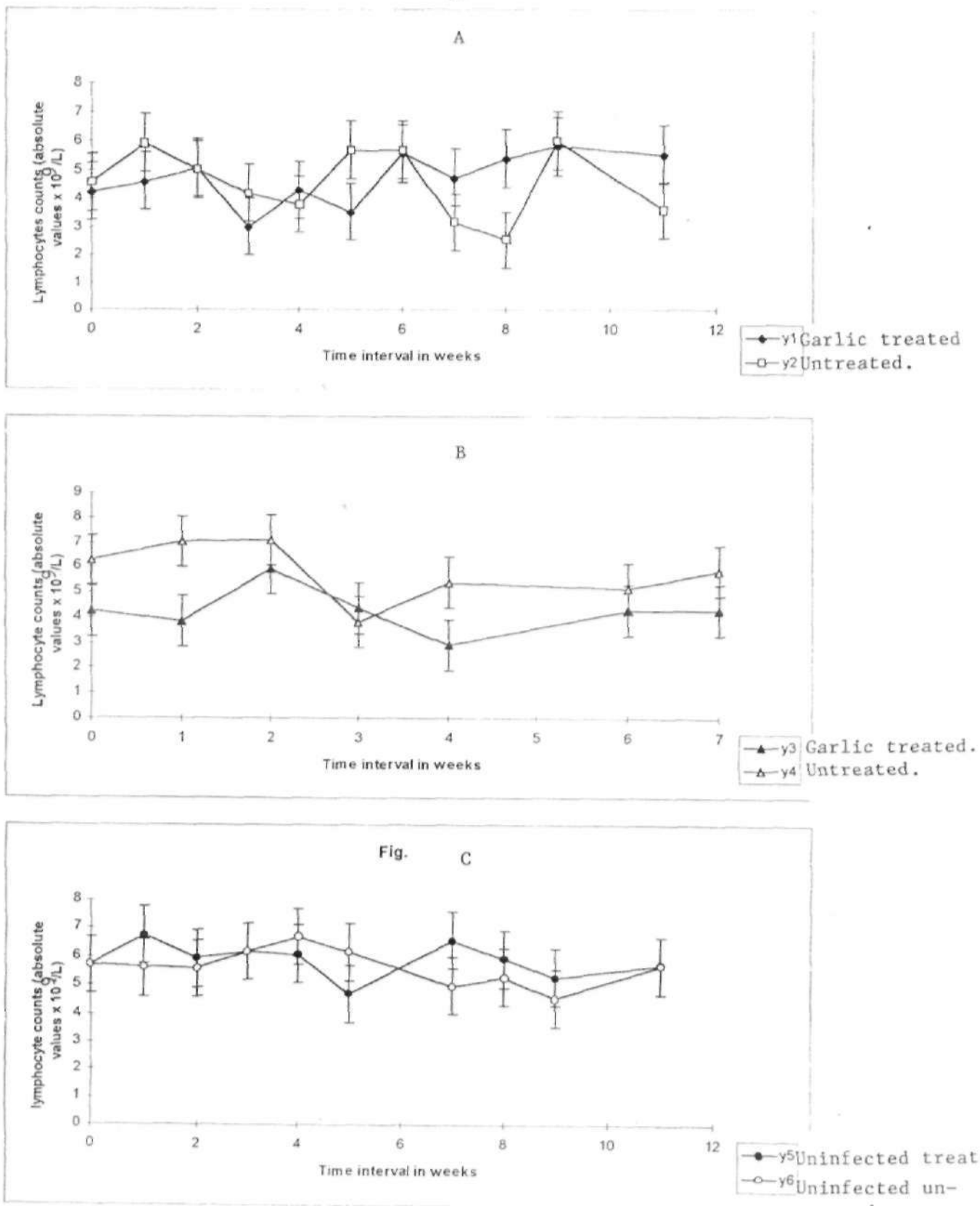



**Figure 4.8**

Effect of garlic oil on Lymphocyte count (absolute values X10 $^{9/L}$) of rabbits infected with T. congolense (A) and T. brucei (B) and controls (C)

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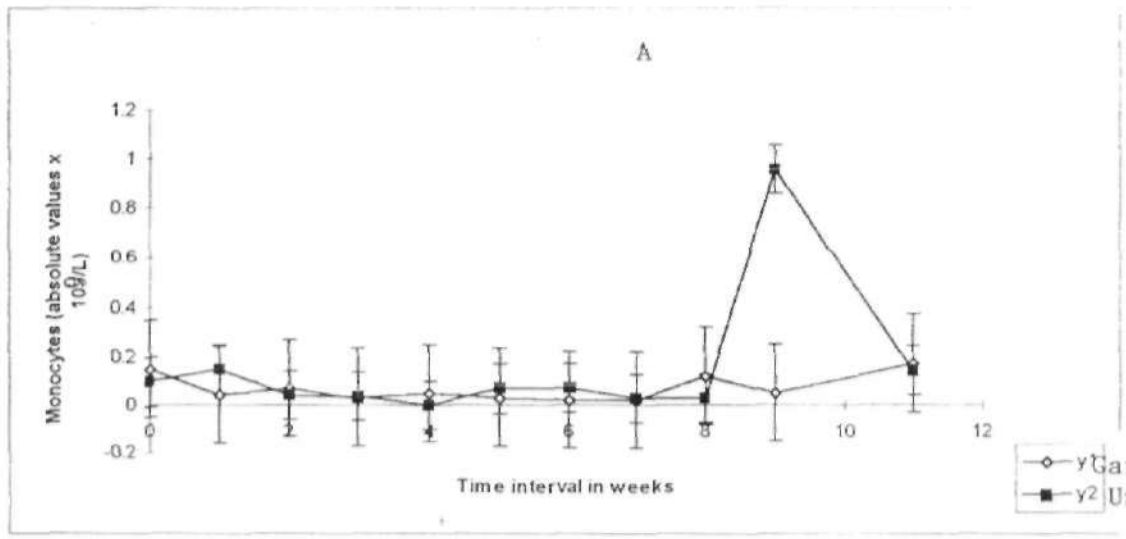



**Figure 4.9**

Effect of garlic oil on Monocytes count (absolute values $X10^{9/L}$) of rabbits infected with T. congolense (A) and T. brucei (B) and controls (C)

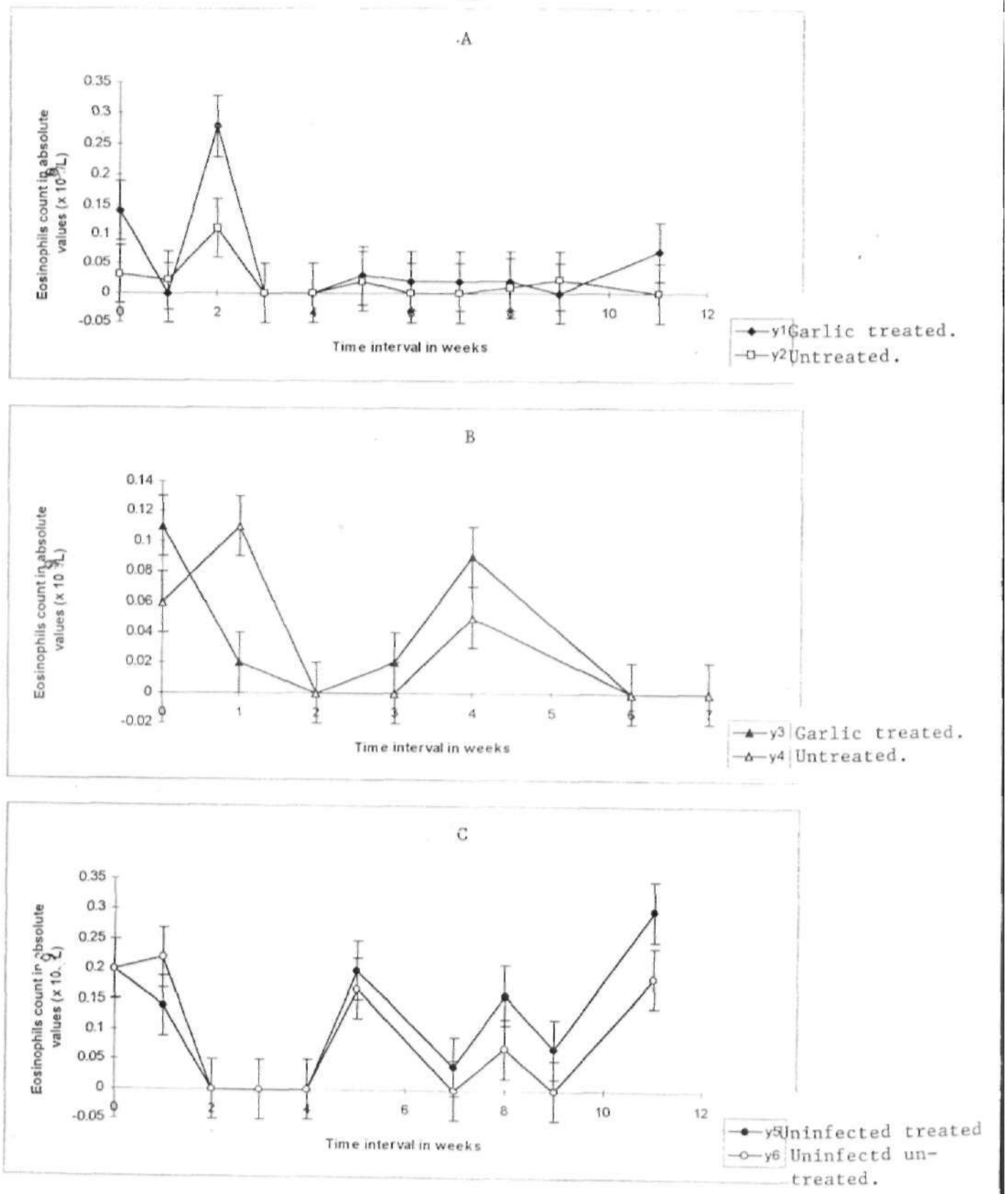



**Figure 4.10**

Effect of garlic oil on eosinophils count (absolute values $X10^{9/L}$) of rabbits infected with T. congolense (A) and T. brucei (B) and controls (C).

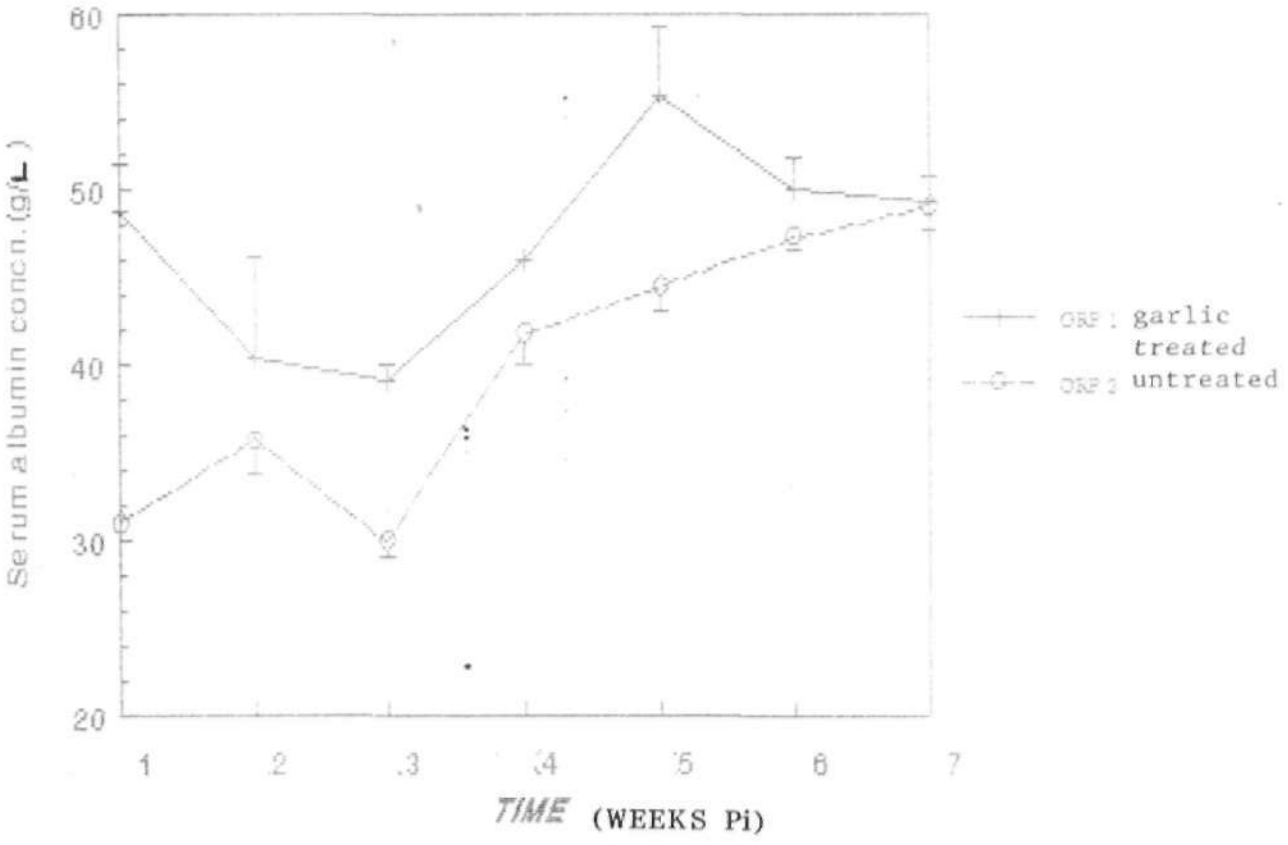



Figure 4.11a.

Effect of garlic oil on serum albumin concen. (g/L) of rabbits infected with T. congolense

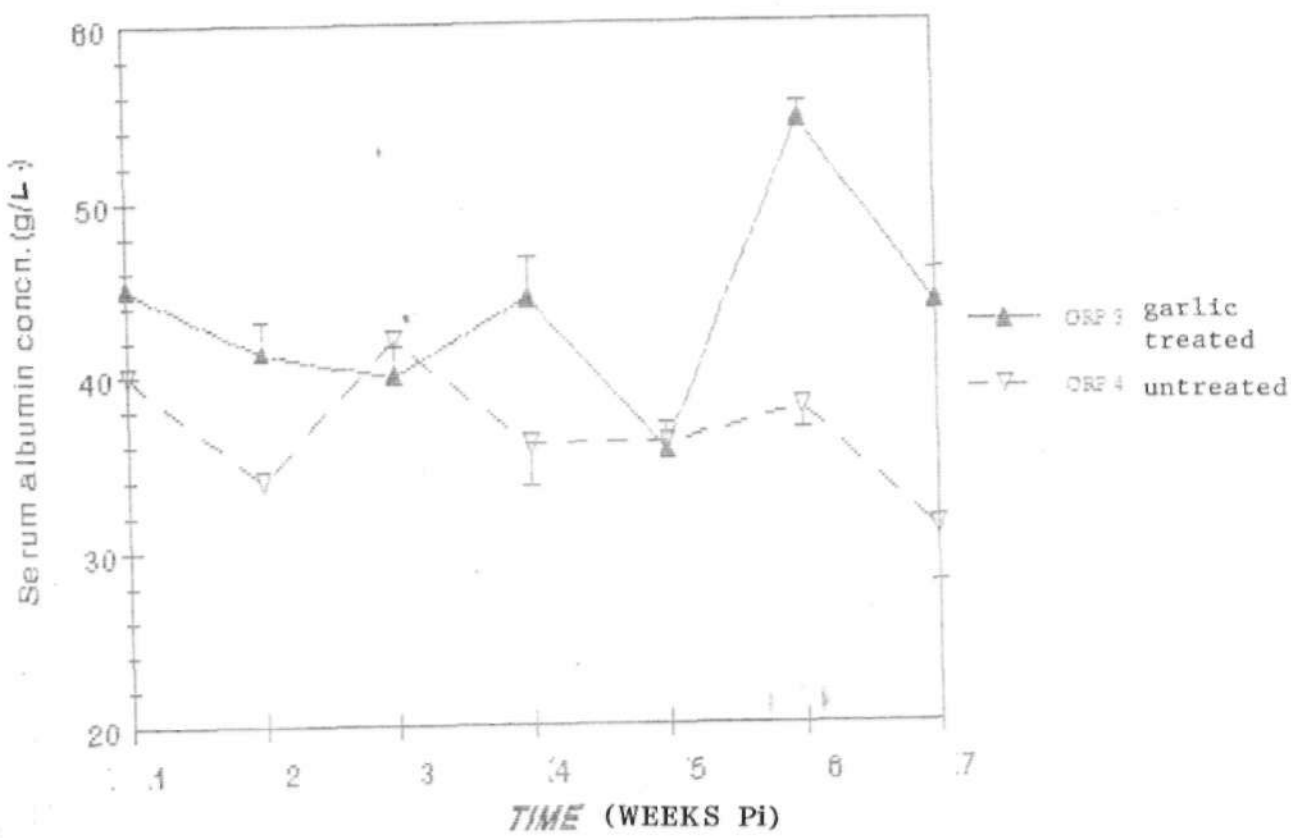



Figure 4.11b.

Effect of garlic oil on serum albumin concen (g/L) of rabbits infected with T. congolense

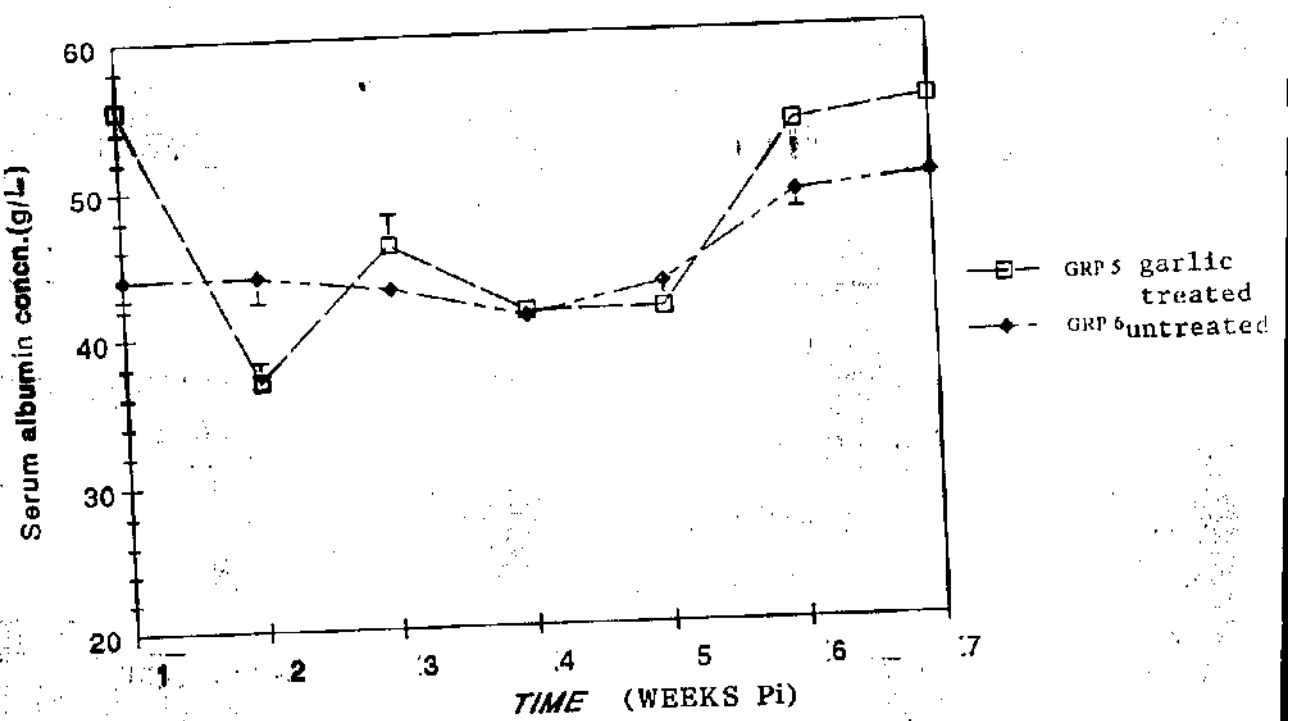



Figure 4.11c

Changes in serum albumin concentration (g/L) of healthy rabbits

4.3.1.6 Gross pathology.

The gross pathological lesions observed in the *T. congolense* infected rabbits were pale mucous membranes, enlargement of adrenal glands and testicles in both treated and untreated rabbits. Hepatomegaly with fatty infilteration was observed only in the group not treated (control). Splenomegaly was observed in both groups to a lesser extent in garlic treated group but severe (spleen size ten times the healthy control) in the untreated group.

4.3.1.7 Percent organ/body weight ratio.

Percent organ/body weight ratio for *T. congolense* is presented in Figure 4.13 and Table 4.18 (Appendix). Significant increases were seen ($P<0.05$) in the spleen, lungs and heart of garlic treated rabbits; and liver, heart and spleen of the untreated group when compared with healthy control. Splenomegaly was significantly reduced ($P<0.05$) in the garlic treated rabbits when compared with the untreated control.

4.3.2 *T. brucei* infection.

*Trypanosoma brucei* (Lafia strain) was highly infective to this rabbit breed. Parasitaemia was established 10 days Pi like *T. congolense*.

4.3.2.1 Symptoms of infection.

Infected rabbits manifested severe clinical signs of *T. brucei* infection. These were pyrexia, occular lesions, nasal discharge and respiratory distress and severe anaemia. There was also progressive emaciation with reduced activeness. The study lasted 8 weeks Pi following the death of the last animal in the garlic treated group as a result of severe pathology associated with *T. brucei* infection. All the rabbits in untreated control died by 7 weeks Pi.

4.3.2.2 Haematological changes.

Changes in mean PCV, Hb and RBC with *T. brucei* infection are shown in Figures 4.2 to 4.4. Severe and progressive decreases in these parameters were recorded and did not correspond with the level of parasitaemia in circulation (Figure 4.16b). Even when parasitaemia was low in some rabbits, or undetected by wet film, these values remained low (week 4 Pi).

Reductions of 58% and 56%, were recorded at 4 weeks Pi in PCV and Hb of garlic treated and untreated *T. brucei* infected rabbits respectively. RBC count decreased to less than half the normal pre infection level at 6 and 4 weeks Pi in treated and untreated groups respectively. Improvement in these parameters were observed at the terminal stages in both groups. Garlic treatment appeared not to have positive effect in these parameters particularly at 6 weeks Pi.

4.3.2.3 Plasma protein.

The result for weekly total plasma protein concentration with *T. brucei* infection is shown in Figure 4.5. Significant elevations ($P<0.05$) were recorded at 4 weeks Pi in untreated group; and 6 and 7 weeks Pi at garlic treated groups. Comparison between treated and untreated groups showed no significant difference ($P>0.05$) in their overall total protein concentration.

4.3.2.4 Changes in leucocytes.

White blood cell count changes following infection with *T. brucei* in the rabbit is presented in Figure 4.6. Leucopenia in both treated and untreated groups set in at 3 weeks Pi as observed with *T. congolense* infection. Garlic treatment slightly raised the count above (not significant) the untreated control. Slight recoveries were recorded in WBC at terminal stage of the infection in both groups as observed with PCV, Hb and RBC.

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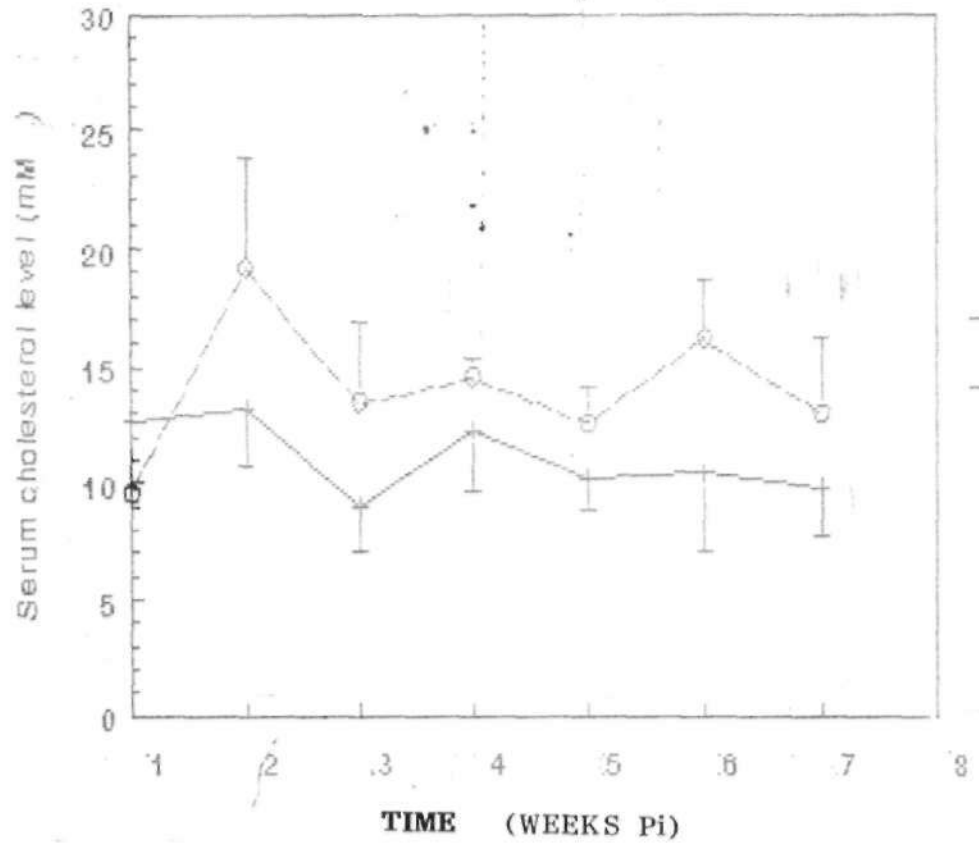



**Figure 4.12a**

Effect of garlic oil on serum cholesterol level (mM) of rabbits infected with T. congolense.

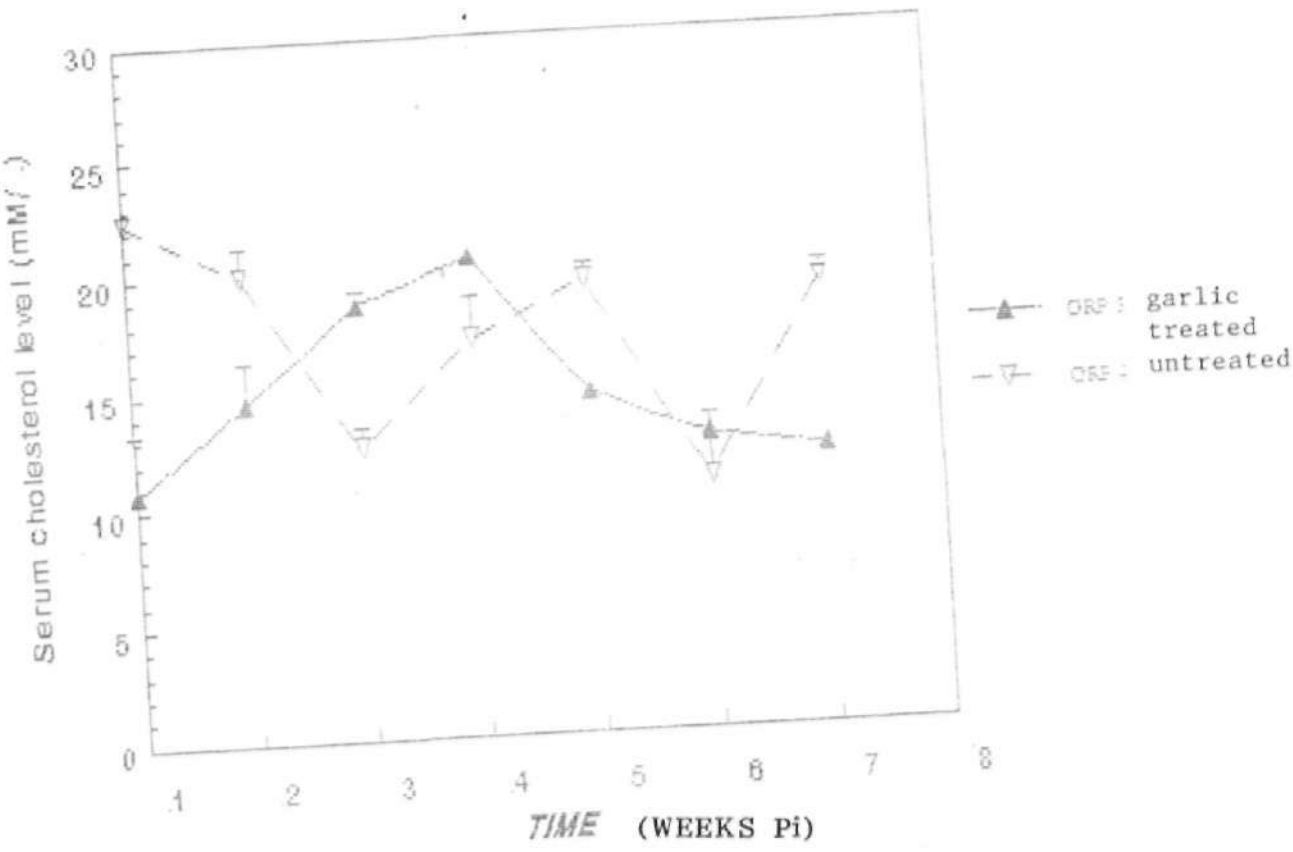



Figure 4.12b.

Effect of garlic oil on serum cholesterol (mM) of rabbits infected with T. brucei.

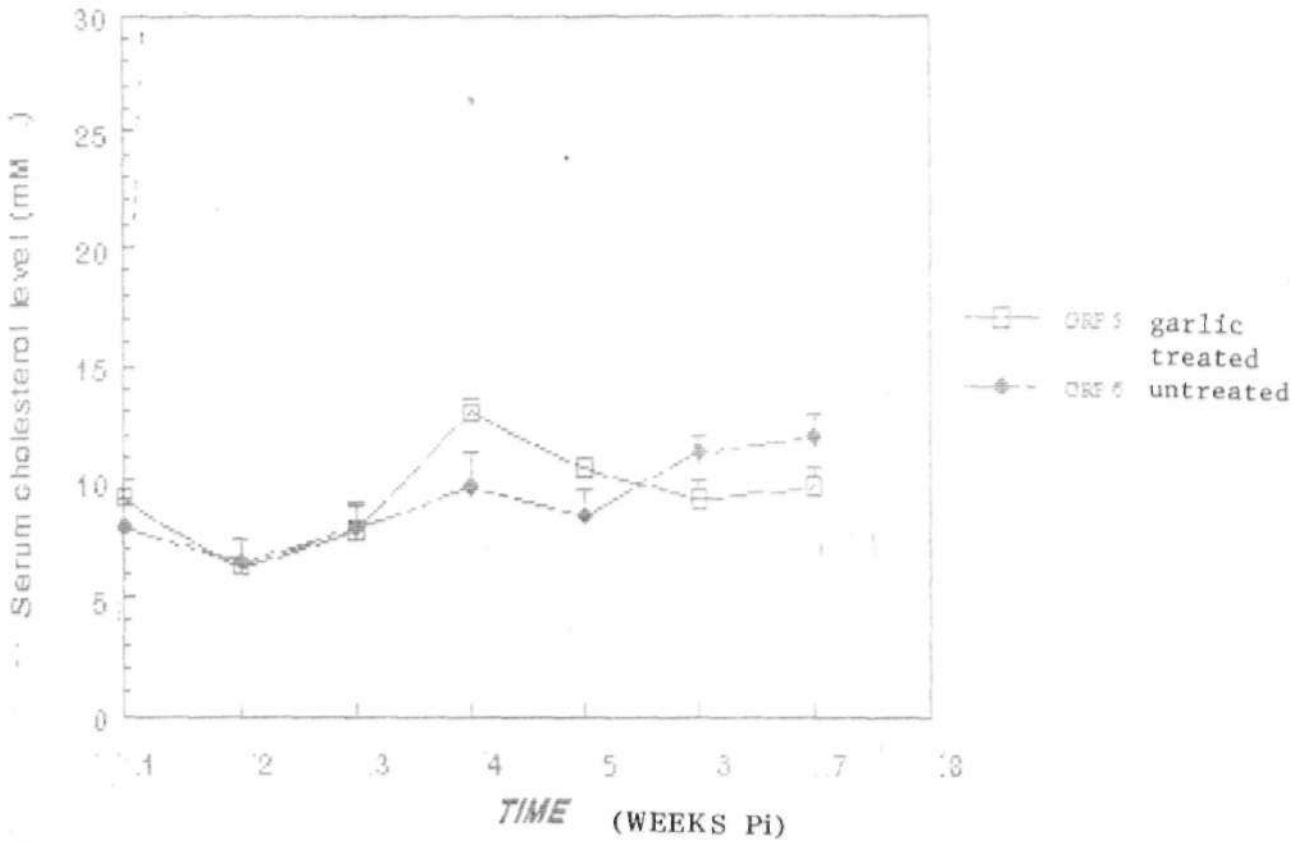



Figure 4.12c.

Effect of garlic oil on *serum cholesterol* (mM) healthy rabbits

Differential leucocyte count in absolute values (Figures 4.7B to 4.10B) represent neutrophils, lymphocytes, monocytes and eosinaphils respectively with *T. brucei* infection. Monocytes and eosinophils did not show significant differences ($P>0.05$) between treated and untreated groups, and also healthy controls. However, significant decreases ($P<0.05$) were observed at 6 weeks Pi in neutrophils count of garlic treated and untreated groups, and elevation in lymphocytes (lymphocytosis) at 7 and 14 days Pi in untreated group, lymphopenia (reduction) persisted with garlic treatment.

4.3.2.5 Serum changes.

Serum changes with *T. brucei* infection in albumin and cholesterol are shown in Figures 4.11B and 4.12B respectively. Albumin concentration was reduced following the infection by 7 days Pi. Significantly higher levels ($P<0.05$) were recorded with garlic treatment at 2, 4,6 and 7 weeks Pi, periods characterised by higher levels of parasitaemia in untreated than treated control groups (Figure 4.1b).

Hypercholesteraemia in both treated and untreated rabbits was recorded at week 4 and 1 respectively. The increase as a result of the infection was significantly ($P<.05$) reduced with garlic administration at 6 and 7 weeks Pi.

4.3.2.6 Gross pathology.

The summary of the gross pathological lesions observed at post mortem examination is compared between *T. brucei and T. congolense* infections and controls as shown in Table 4.3. Both carcasses of garlic treated and untreated *T. brucei* infected rabbits showed pale mucous membranes. There was enlargement of adrenal glands, testicles,liver and spleen. Splenomegaly was significantly reduced ($P<0.05$) in garlic treated rabbits when compared with untreated group.

4.3.2.7 Percent organ/body weight ratio.

Percent organ/body weight ratio of *T. brucei* infected rabbits is presented in Figure 4.1 ( Table 4.18.) Comparison with healthy control showed significant increases ($P<0.05$) in kidneys, lungs, liver, heart and spleen weights of both garlic treated and untreated rabbits. However, splenomegaly was significantly reduced ($P<0.05$) with garlic treatment when compared with the untreated control group.

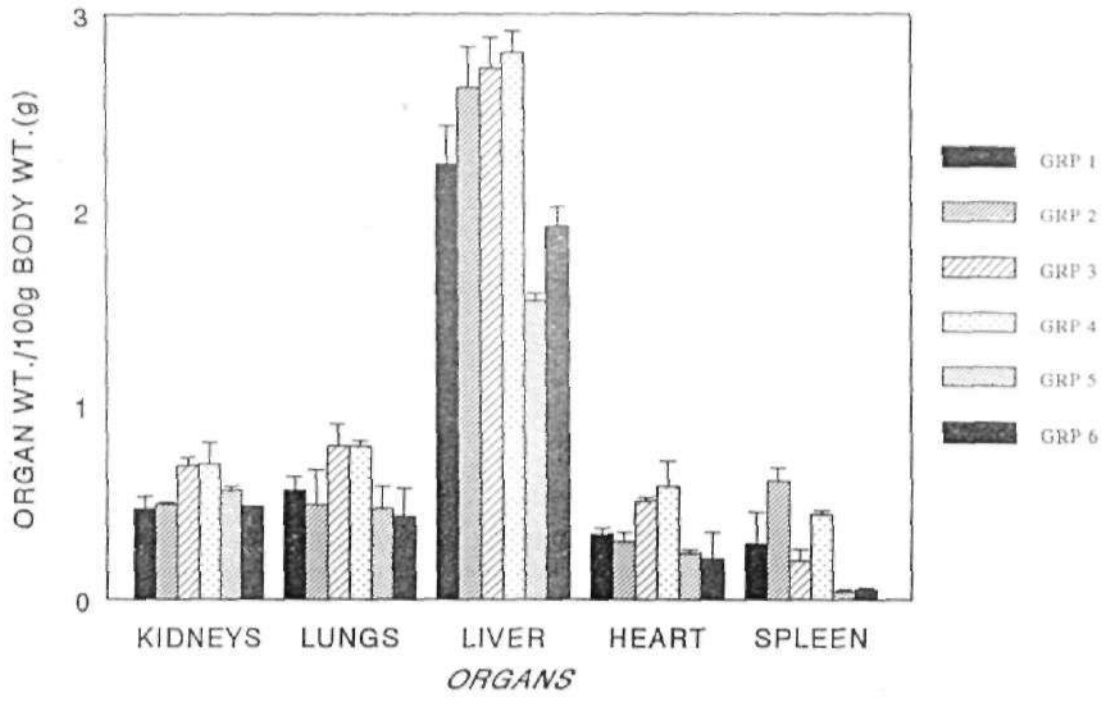



**Figure 4.13**

Percent organ/body wt ratio of garlic treated (100mg/kg) and untreated rabbits infected with T. congolense (Grp 1, Grp 2 respectively) and T. brucei (Grp 3 and Grp 4 respectively). Grp 5 and 6 represent uninfected garlic treated and uninfected untreated controls respectively.

## CHAPTER FIVE

### 5.0 DISCUSSION.

### 5.1 Acute toxicity.

The result of acute toxicity test with garlic oil indicates that it is only slightly toxic to rats. Chemical substances with $LD_{50}$ in the range of 1000 - 5000 mg/kg are regarded as slightly toxic (VanGelder *et al* 1976; Lorke, 1983). The observed clinical signs of toxicity agree with the report of Behl *et al* (1993) that garlic oil is an irritant poison when taken in excess. There has also been reports of contact dermatitis (Blevmink, 1972; Martindale, 1993) and skin irritation (Behl *et al*, 1993) associated with the use of garlic. Other toxic effects reported include fatalities in children (British pharmaceutical codex, 1953; Behl *et al* 1993). The use of whole garlic in feed has been reported to cause anaemia (Umar *et al*, 1996).

Toxicity resulting from the use of garlic either as a spice or as commercially available preparations (oil/tablet) is not expected since the dose at which toxic symptoms were seen are much higher than the dose recommended of the commercial preparations (2 - 4 mg/kg/adult human). When also used as a spice, garlic is sparingly used because of its effect on breath (Kafaru, 1994) and its strong pungent odours, more so that the yield of the oil from extraction is very small (2.25 g/kg). The oil in garlic is known to be volatile, a yield of 0.1-0.3% (1-3g/kg) has been reported in British pharmaceutical Cordex (1953), which differ from that obtained by Williams *et al* (1985).

The anaemia attributed to the consumption of whole garlic (raw) could be due to other components in garlic other than the oil which chemically contains diallyl-disulphide. Some of these include hydrocyanic acid, phosphorus, and some enzymes (African pharmacopoeia (1985).

Congestion of the liver, kidneys, lungs and brain were the main lesions observed in the higher dose groups suggesting a direct effect of the oil on these tissues.

5.2 Disease pattern.

The cross breeds of New Zealand white (NZW) and local rabbits used in this experimental infection were found to be more susceptible to *T. brucei* than *T. congolense*. Although the prepatent period for the two species was the same, *T. brucei* elicited higher parasitaemia particularly at the terminal stage of the infection (Fig. 4.1b) where it developed without abatement. Also, the *T. brucei* infected rabbits showed acute disease with death of all the rabbits recorded by the end of 7 and 8 weeks (for untreated and garlic oil treated groups respectively). The difference in length of survival between treated and untreated *T. brucei* infected rabbits was not significant ($P>0.05$). In the case of *T. congolense* infection, the rabbits had less intense parasitaemia with fluctuations. The infection with *T. congolense* was chronic, and all the rabbits both treated and untreated survived the 11 week period of the study. Maintenance of very low parasitaemia was observed at 10 weeks Pi in both groups. The indication of recovery from this *T. congolense* infection was also manifested by improvement in body weight, and change of skin fur, suggesting the parasites low infectivity to the rabbit breed resulting in the development of asymptomatic carrier stage. This shows that the immune mechanism of these rabbits was mobilised to a level that is adequate to depress *T. congolense* multiplication but not *T. brucei*, and that treatment with garlic oil particularly in *T. brucei* infection did not change the pattern of the disease significantly. Earlier reports (Weitz, 1970) observed that the course and nature of trypanosomoses depend on the interaction between the parasite and the host, giving rise to clinical disease ranging from asymptomatic carrier to rapidly progressive fatal diseases.

5.3 Clinical signs.

Clinical signs of trypanosomoses were more prominent in *T. brucei* infection and almost absent in *T. congolense*. Ocular lesions, nasal discharge and respiratory distress were observed at 3 weeks Pi and subsequently throughout the period of infection in both garlic treated and untreated *T. brucei* infection, suggesting that garlic oil administration although recorded lower levels of parasitaemia (Fig 4.1b) had no effect on the clinical picture of the disease, and also the severity. These signs persisted and became severe even at low level of parasitaemia, a point suspected as the time when the parasites established extravascularly and were less numerous in the blood (weeks 4.5 - 7.0 for treated, and 5.5 for untreated groups), confirming *T. brucei* as both plasma and tissue - invading trypanosome exerting some direct effect on the tissues infested. Similar view was held in other infections with *T. brucei* sub-group that the number of organisms present in the tissues does not correlate with the number in the blood (Morrison *et al*, 1978). *Trypanosoma congolense* although infective to the rabbit cross breed could not exhibit the above mentioned clinical signs, except for pyrexia which manifested at onset (Table 4.3), an indication that the defence mechanism of these rabbits is more efficient against *T. congolense* species. Different species and strains of trypanosomes vary in virulence, those with greater virulence inducing more severe symptoms of infection as also observed and reported in *T. brucei* infection of rabbits (Jenkins *et al*, 1980; McCrorie *et al*, 1980).

5.4 Anaemia.

Anaemia occurred in both *T. brucei* and *T. congolense* infections. The changes observed in the mean values of PCV, Hb and RBC count differ between the two infections as shown in Figures 4.5 - 4.7. While PCV, Hb, and RBC fluctuated with rises and falls (in waves) in the T. congolense

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5.4 Anaemia.

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infected groups, gradual and more progressive decreases were observed in the *T. brucei* infected groups. The anaemia in both infections set in at 3 weeks Pi with significant drops ($P<0.05$) in PCV Hb and RBC count when compared with pre-infection values and also the healthy control groups (Figs. 4.5 to 4.7). The variation in the pattern of reduction between the two infections could be attributed to the differences in virulence between the 2 species. The level of anaemia in *T. congolense* showed direct relationship with the extent of parasitaemia, improved/partial recoveries in PCV, Hb and RBC were observed at periods of low parasitaemias and sharp decreases in these parameters at periods of high parasitaemia. *Trypanosoma brucei* - infected rabbits manifested reductions which progressed until death, even at periods of low parasitaemias. Saror (1979) and Anosa and Isoun (1980) reported that animals with acute infection usually develop higher levels of parasitaemia and greater anaemia than those with chronic infection. Infection with *T. brucei* in this study developed more intense parasitaemia.

With garlic treatment in *T. congolense* infection, partial recoveries of PCV, Hb and RBC were recorded at weeks 4 & 8; 4 & 8; and 4, 6, & 11 Pi respectively (Figs 4.5 - 4.7) corresponding with periods of low parasitaemias, marked with increased plasma protein concentration and leucocyte count (specifically lymphocyte elevation). These improvements could be associated with enhancement of the animal's defense mechanisms as reported by Anosa and Kaneko (1983) that highly tolerant deer mice had elevated plasma protein and leucocytosis (due to lymphocytosis). Similar recovery was however not observed in garlic treated *T. brucei* infection, and no significant difference ($P>0.05$) was observed in the level of anaemia when compared with untreated control. This could result from the inability of garlic oil to penetrate and reach the various tissues and organs of the animals where the parasites established extra-vascularly, suggesting its possible concentration

in the blood. The mechanism of trypanosome- induced anaemia is multifactorial (Jenkins and Facer 1985). The anaemia usually sets in during the first wave of parasitaemia (Jenkins *et al*, 1980) and is subsequently determined by the intensity of the parasitaemia. Significant alleviation of the anaemia in garlic-treated *T. congolense* infection was recorded at 4, 9 and 11 weeks Pi ($P<0.05$). The main active principle in garlic oil is diallyldisulphide (Williams *et al*, 1985) which has the ability of reacting with thiol group systems including proteins, enzymes and amino acids, thus, the availability of SH group compounds and enzymes may be reduced by the action of garlic oil (Williams *et al*, 1985). The enzymes of importance in proposed mechanism of the anaemia in trypanosomoses are the phospholipases and sialidases, and could be liable to inhibition by garlic oil administration. The sialidases catalyse the removal of erythrocyte surface sialic acid thereby making them prone to attack by the reticuloendothelial system of the body (Esievo *et al*, 1982), while the phospholipases catalyse the hydrolysis of fatty acid ester linkage in endogenous phosphatidyl choline or phosphatidyl ethanolamine liberating hemolytic free fatty acid and surface acting lysolecithin (Tizard *et al*, 1978a). Even though the relative contribution of each factor in the aetiology of the anaemia has not been well defined, other factors responsible in the genesis of anaemia in *T. brucei* infections could be more important in the overall aetiology than haemolysis.

5.5 Leucocytes.

As indicated by Fig 4.9, leucopenia developed significantly ($P<0.05$) at 3 weeks Pi in both *T. congolense and T. brucei* infected rabbits. Significant improvement in WBC count was observed with garlic treatment in *T. congolense* infection from week 4 Pi. This improvement could possibly result in the improvement of defense machinery accounting for the alleviation recorded of the anaemia in this garlic treated group in agreement with several studies that alleviation of leucopenia preceeding

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alleviation of anaemia (Anosa and Isoun, 1980).

Garlic oil is reported to have antimicrobial effect (Martindale, 1993) and its role in preventing secondary infection by opportunistic non trypanosome parasites is suspected. Garlic oil is also ascribed with many medicinal effects and is used in traditional medicine. Leucopenia was observed in *T. brucei* suggesting progressive pathology induced on tissues invaded by *T. brucei* to account for most of the disease condition rather than the suppression of the defence mechanism. The significant differences observed between treated and untreated *T. congolense* infected rabbits at 7 and 8 weeks Pi might also be linked with the ability of the oil to reduce the level of freefatty acid (Williams *et al*, 1985) known to be immunosuppressive ( Meade and Martin, 1975) thereby shielding the immunosuppressive effect of FFA generated by autolyzing trypanosomes.

The observation in *T. brucei* infection was different (Fig 4.9). Leucopenia was to a lesser degree particularly in untreated control. The persistence of leucopenia with garlic treatment in the *T. brucei* infection was quite unexpected, and possible explanation to this is not available at present. It is obvious that the pathology associated with *T. brucei* infection could be more of the tissue lesions than immunosuppression.

Differential leucocyte counts indicated significant differences ($P<0.05$) between the treated and untreated control groups in neutrophils and lymphocytes (Fig 3 4.10 - 4.11). Neutropenia observed at 3 and 4 weeks Pi in both treated and untreated *T. congolense* infection respectively was alleviated with garlic administration by 6 weeks significantly ($P<0.05$) above the group not treated. Phagocytosis of *T. congolense* by neutrophils has been reported (Kissling, *et al*, 1982, Wellde *et al*, 1974) and has also been observed by transmission electron micrograph in mice infected with *T. brucei* (Anosa, 1983; Anosa and Kaneko, 1983) which could account for the differences observed between

garlic oil treated and untreated rabbits in parasitaemia since garlic oil administration elevated neutrophils count significantly above ($P<0.05$) the group not administered. Proposed mechanism of neutropenia include decreased neutrophils production as a result of inhibition and splenic sequestration ( Kaaya *et al*, 1980) These views were supported by the observation of depression of myeloid colony at periods with marked leucopenia and neutropenia in the serum of cattle infected with *T. congolense*; and the fact that splenomegaly results in neutropenia. Anosa and Kaneko (1984) reported that neutrophils increased from 0.4% in spleens of control mice to 1.7% in mice infected with *T. brucei* whose spleens were enlarged 25.9 fold. It is therefore interesting to note that splenomegaly was significantly reduced ($P<0.05$) in the garlic oil treated *T. congolense* infection than the untreated control (Table 4.18). Increase in total splenic granulocyte population in mice infected with *T. congolense* whose spleen was enlarged by 20 fold has also been reported (Morrison *et al*, 1981). Neutrophil phagocytosis by macrophages has also, been observed in the spleens and other tissues of sheep infected with *T. congolense* (Mackenzie and Cruskshank, 1973). Therefore the ability of garlic oil to alleviate spleen enlargement and consequently its ability to trap neutrophils could be responsible for the boosting up of the defense mechanism and enhance *T. congolense* clearance from circulartion.

Lymphopenia observed in this studies set in at 3 and 4 weeks Pi in treated and untreated *T. congolense* infected rabbits respectively. However, restoration to normal range was recorded in both groups at 6 weeks Pi. The lymphocyte count was significantly higher and different ($P<0.05$) from untreated group at 7 and 8 weeks Pi (Figure 4.11), periods marked with low parasitaemia in the garlic treated group. Lymphopenia observed persistently in the untreated group could be as a result of increased demands for lymphocytes for transformation into *T.* lymphocytes as the predominant

inflammatory cell types as a result of intense antigenic variation (Anosa 1988 I) resulting from the occurrence of more peaks of parasitaemia at these periods (Fig 4.1a) indicating antigenic variation. More parasitaemic peaks were observed to occur in untreated than garlic oil treated *T. congolense* infection. The persistent occurrence of leucopenia (due to lymphopenia in garlic treated; and a combinationof lymphopenia with neutropenia in the untreated group) suggest the non clearance of the parasites completely from the system even though parasitaemia became low and infrequent.

In *T. brucei* infection however, leucocyte changes were inconsistent (Fig 4.9B) as also reported in monkeys infected with *T. b. rhodesiense* (Sadun *et al*, 1973). Peaks of parasitaemia did not correlate with the actual severity of the clinical signs of the disease. These observations may explain the inconsistencies observed in both erythrocytes and leucocytes. The possible factors responsible may not be far from the differences in pathogenicity between *T. brucei* and *T. congolense* infections.

5.6 Biochemical changes.

Total plasma protein as recorded in both *T. congolense* treated and untreated groups (Fig 4.8) showed no significant differences ($P>0.05$) from the healthy controls. Other literature reports are in support of this observation in various trypanosome infections involving different species of animals (Smithers and Terry, 1959; Moon *et al*, 1968; Jaktar *et al*, 1973; Verma and Gautam, 1977; Verma and Gautam 1983). Periods recording enhanced (not significantly different) plasma protein levels (weeks 4, 7, 9 and 11) in the garlic treated *T. congolense* infected group corresponds with low parasitaemia (Fig 4.1) and improved lymphocyte count.

Elevated plasma protein was however recorded from 4 weeks Pi until death in treated and untreated *T. brucei* infected rabbits (Fig. 4.8). Same observation was reported in *T. brucei* infection

of rabbits (Facer, 1976) and *T. brucei* infected deer mice and was interpreted as indication of development of tolerance to the infection since it was observed in the apparently more resistant group that did not show anaemia (Anosa and Kaneko, 1983). The fact that the elevation in the protein concentration persisted until death of all the rabbits infected with *T. brucei* does not seem to agree with the speculation of enhancement of tolerance to the infection, rather, sudden increase in parasitaemia observed towards the terminal end of the infection (Fig. 4.1b) could be responsible for the improved leucocyte count and elevated protein concentration in an attempt to increase the immune factors (proteins) against the parasites. Also, elevation in plasma protein at the terminal end indicates a direct relationship between total protein concentration and the severity of infection. This view has been supported by Anosa (1988II), and that probably explains the inconsistent protein levels reported by different investigators who may be working with infections of differing severities.

The results obtained for serum albumin indicate significant increases ($P<0.05$) with both *T. congolense* and *T. brucei* infections 7 days Pi (Fig 4.14). Treatment with garlic oil in both infections significantly increased ($P<0.05$) the albumin concentration above the control values. Several trypanosome infections recorded decreased albumin concentrations (Smithers and Terry, 1959; Jarktar *et al*, 1978; Whitelaw *et al*, 1980; Anosa and Kaneko, 1983) largely due to plasma expansion since it was found that the hepatocytes which synthesise albumin do not develop severe pathology (Anosa, 1988b). Depression of albumin with elevation of globulin levels are consistent changes in serum protein levels. The mechanism by which garlic oil increase the albumin concentration could not be explained (Williams *et al*, 1985).

Serum cholesterol level was observed to increase significantly with both trypanosome infections (Fig 4.15). Significant reductions ($P<0.05$) were however observed with garlic treatment.

Hypercholesteraemia is a common finding in trypanosomoses (Diehl, 1974; Venkatesan and Ormerod, 1976) which is a high risk factor in heart pathology (Kamio, 1976). Increase in cholesterol content of lipid bilayer of membrane could affect the normal transport function of the membrane. The role garlic oil plays in reducing serum cholesterol is associated with the exchange reaction of its disulphide with thiol containing multienzyme complex of fatty acid synthesis (Co A) and the rate limiting enzyme for cholesterol synthesis (HMG Co A reductase) deactivating them, thereby reducing the rate of FFA and cholesterol synthesis (Williams *et al* 1985).

5.7 Tissue lesions.

Percent organ body weight ratio indicates (Fig. 4.13) significant increases ($P<0.05$) of the kidneys, lungs, liver, heart and spleen of *T. brucei* infected groups, consequently some possible pathological effect of the parasites on these organs. The significant reduction ($P<0.05$) in percent spleen body weight ratio observed in all the garlic oil treated rabbits is indicative of the protective role of garlic oil on the spleen. There was not significant difference ($P>0.05$) in percent kidneys, liver, lungs and heart per body weight ratio with garlic treatment, implying that the treatment did not protect these organs and could be the reason why there was no difference in the severity of the clinical signs of *T. brucei* infection between treated and untreated groups (Table 4.3).

Garlic oil treated *T. congolense* infected rabbits showed significant increases ($P<0.05$) in spleen, lungs and heart weight compared with healthy control. The untreated control however showed increases in liver, heart and spleen. Garlic oil treatment prevented hepatomegaly associated with *T. congolense* infection, and significantly reduced ($P<0.05$) the extent of splenomegaly (Table 4.18). Fatty infilteration was observed in the enlarged liver of the untreated *T. congolense* infected rabbits. Garlic oil has been useful in decreasing the levels of lipid in the liver of normal (Augusti,

1976), high lipid, high cholesterol diet (Sodimu *et al*, 1964) and ethanol (Lieber, 1968) or ethanol and high lipid (Umar *et al*, 1987; Shoetan, *et al*, 1994) fed rats. Garlic oil has been reported to protect the liver and kidney from fatty infilteration resulting from high fat and alcohol feeding (Umar *et al*, 1994). The mechanism by which it does so is related to the reaction of its disulphide with NADPH (Black 1962) which is necessary for lipid synthesis, reducing the synthesis and enhancing lipid oxidation (Umar *et al*, 1994). Both hepatomegaly and splenomegaly are consistent observations in African trypanosomoses especially in laboratory rodents (Greenwood and Whittle, 1980; Mansfield, 1982). Splenomegaly was associated with erythrocyte and trypanosome phagocytosis. The spleen size of infected animals could reach ten times the normal, as observed in the *T. brucei* and *T. congolense* infected untreated rabbits (Table 4.18), and also reported in *T. brucei* infection of rabbits (Greenwood and Whittle, 1980). Hepatomegaly has been reported in both *T. brucei* (Anosa and Kaneko, 1983) and *T. congolense* (Morrison *et al*, 1982) and other trypanosome infections. Hepatosplenomegaly observed in this study is directly related to the severity of the anaemia; an observation also reported by Anosa and Kaneko (1980). Anaemia was very severe in intact rabbits but mild in splenectomized rabbits that were infected with *T. brucei* (Jenkins *et al*, 1974). Histological evidences have shown the presence of erythrophagocytosis in the liver and spleen (Woodruff *et al*, 1973; Valli, *et al*, 1978).

The enlargement of the liver, spleen, heart and lungs observed in this study agree with the results of Valli and his co-workers (1978) with *T. congolense* infection, and Losos and Mwambu (1978).

The possible explanation in support of the result from this work is the suggestion made by Anosa (1988 II) that since most of the haemolytic mechanisms known in trypanosomosis are

trypanosome dependent, and that haemolysis as a mechanism of inducing anaemia is only important when trypanosomes are numerous in the blood, the only mechanism which could possess the potential to persist after trypanosomes have decreased as in chronic crisis recorded in this study for *T. congolense* infection are hypersplenism and RBC fragmentation due to glomerulo-nephritis and splenomegaly. This view is supported by the fact that the kidneys weight of all the *T. congolense* infected rabbits were normal (Fig 4.18) and hence the level of anaemia recorded from PCV, Hb and RBC count was less severe; thus the only mechanism responsible at this stage is splenomegaly. It is therefore interesting to also note that the final values for these parameters when comparing garlic treated and untreated groups related directly with the extent of splenomegaly (Figs 4.5 - 4.7).

# CHAPTER SIX

## 6.0 CONCLUSION AND RECOMMENDATION.

### 6.1 CONCLUSION.

Garlic oil at a dose of 100mg/kg is considered safe, but slightly toxic at 1000 - 5000 mg/kg dose range where it could produce both dermal and oral irritation and also pathology of the liver, lungs, kidneys and brain.

The rabbit cross breeds of New Zealand white and local stock are more susceptible to *T. brucei* than to *T. congolense*, infection. *Trypanosoma brucei* is highly infective to this rabbit cross breed.

Several factors influence the nature, and severity of haematological, biochemical and tissue pathological changes in different trypanosome species. Different species of trypanosomes vary in virulence, those with greater virulence induce more severe changes/alterations in haematological, biochemical and tissue pathologies.

Animals with acute (sub chronic) infection develop higher levels of parasitaemia and greater anaemia than those with chronic infection.

Treatment of experimental *T. congolense* infection resulted in the alleviation of leucopenia by elevating neutrophils and lymphocytes counts. Also, the severity of splenomegaly was reduced and consequently the anaemia. The mechanism by which garlic oil achieved these is not precisely known but probably by elevating total serum albumin level known to bind haemolytic and immunosuppressive FFAs thereby abolishing these effects. A proposed mechanism of the enhancement of neutrophils count is the protection of the spleen against enlargement since splenomegaly results in neutropenia.

It is therefore concluded that treatment of experimental *T. congolense* infection of rabbits with garlic oil could protect the liver, and also has some apparent potential of boosting the animals defence mechanism, protecting against splenomegaly, red cell destruction and consequently anaemia and leucopenia/ immunosuppression that are common features of animal trypanosomiasis.

It is also concluded that although treatment of experimental *T. brucei* infection with garlic oil has positive effect in raising albumin and lowering cholesterol levels, it does not have effect on haematological, and clinical picture of the disease. It however has an apparent potential of protecting the spleen. The difference observed between treated rabbits with *T. brucei* and *T. congolense* infection was in part attributed to the difference in their pathogenicity and the complexity of the factors involved.

6.2 Recommendations/suggestions.

Further work should be carried out to:

7. Determine the median lethal dose of garlic oil in other species of laboratory and domestic animals.
2. Determine the mechanism by which garlic oil raises serum albumin.
3. Establish the exact role of splenomegaly in trypanosomoses and the possible mechanism of action of garlic oil in preventing/reducing it.
4. Establish the mechanism by which this rabbit crossbreed seem to develop tolerance to *T. congolense* infection.

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## APPENDIX.

A. Geometric mean of 2900 and 5000 where 0/1 and 1/1 lethality were found

$$G_m = \sqrt[x]{n_1 n_2 \text{---} n_x}$$
$$= \sqrt[2]{2900 \times 5000}$$
$$= 3807.89 \quad \text{approx. } 3800 \text{ mg/kg.}$$

B. PREPARATION OF REAGENTS.

For albumin determination.

1. Stock, succinate buffer, pH 4.10, 0.5 mol/L
   10g sodium hydroxide and 56g succinic acid were dissolved in 800ml water. pH was adjusted to 4.10± 0.05 at 20°C with 1mol/L sodium hydroxide and made up to 1 litre with water. This was stored at 4°C.

2. Stock BCG dye solution, 10m mol/L.
   1.75g BCG (indicator grade) was dissolved in 5ml 1mol/L sodium hydroxide and made up to 250ml with water.

3. Stock sodium azide.
   40g sodium azide was dissolved in 1litre water.

4. Stock Brij 35, 250g/L
   25g solid Brij 35 was warmed in water to dissolve, and was made up to 100ml with water.

5. Working dye solution.
   To 2 litres of water in 5 litres volumetric flask was added:
   (i) 500ml stock succinate buffer
   (ii) 40 ml stock dye solution, removing all traces of the dye from the pipette walls by washing with water.
   (iii) 12.5 ml stock azide
   (iv) 12.5 ml stock Brij 35.

The volume was made up with water and mixed thoroughly. The pH was adjusted to 4.15 ± 0.05 at 20°C with 1mol/L sodium hydroxide.

Reagents for cholesterol determination.

1. 10% Ferric chloride: Acetic acid (1:2 v/v).
2. Concentrated sulphuric acid.

Table 4.5

Packed cell volume (%) of garlic oil treated (*) and untreated rabbits infected with T. congolense and (1*&2) T. brucei (3*&4) and controls (5*&6) (Mean ± SEM)

| Time Interval (weeks) | GROUPS | | | | healthy controls | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| | 41.0 ±5.5 | 48.7 ±0.7 | 51.3 ±.7.0 | 40.3 ±2.7 | 45.0 ±0.9 | 55.0 ±0.4 |
| 2 | 39.0 ±6.0 | 35.3 ±7.8 | 35.0 ±2.9 | 39.7 ±8.1 | 60.0[c] ±0.2 | 47.7[c] ±1.2 |
| 3 | 26.3 ±2.2 | 29.0 ±6.1 | 29.7 ±1.8 | 30.7 ±1.8 | 47.5 ±0.5 | 48.0 ±0.7 |
| 4. | 38.3[a] ±0.7 | 26.7[a] ±2.2 | 22.3 ±1.5 | 19.7 ±1.5 | 49.0 ±0.8 | 43.0 ±1.0 |
| 6 | 30.0 ±2.7 | 31.3 ±0.3 | 21.7[b] ±0.9 | 31.5[b] ±5.5 | 40.0 ±1.3 | 38.7 ±1.3 |
| 7 | 29.0 ±4.0 | 29.0 ±1.5 | 25.5 ±0.5 | 26.0 - | 47.0 ±0.8 | 42.0 ±0.5 |
| 8 | 33.0 ±0.6 | 26.7 ±0.6 | ** | ** | 44.0 ±0.3 | 45.0 ±0.1 |
| 9 | 31.3[a] ±1.9 | 23.3[a] ±3.0 | | | 45.0 ±0.6 | 41.0 ±0.8 |
| 11 | 30.5[a] ±5.5 | 22.0[a] ±4.2 | | | 46.0 ±0.1 | 48.0 ±0.1 |

1 & 2 ;3 & 4 and 5&6 were compared
Values with same superscript within a row differ significantly ($P<0.05$).
** Rabbits alldied.

Table 4.6:

Mean haemoglobin concentration (g/dl) of garlic treated (*) and undtreated rabbits infected with T. congolense (1*2)T. brucei (3*4) and controls (5*6) Mean ± SEM

| Time Interval (weeks) | GROUPS | | | | | |
|---|---|---|---|---|---|---|
| | | | | | healthy controls | |
| 1 | 1 | 2 | 3 | 4 | 5 | 6 |
| | 13.8<br>±1.8 | 16.3<br>0.3 | 17.1<br>±1.1 | 13.4<br>±1.1 | 19.5<br>±0.5 | 18.3<br>±0.3 |
| 2 | 13.0<br>±2.0 | 11.8<br>±2.6 | 11.6<br>±1.0 | 13.2<br>±2.7 | 20.0[c]<br>±0.2 | 20.5[c]<br>±0.8 |
| 3 | 8.7<br>±0.7 | 9.6<br>±2.0 | 9.9<br>±0.6 | 10.2<br>±0.6 | 15.8<br>±0.4 | 16.0<br>±0.7 |
| 4 | 12.8[a]<br>±0.2 | 8.9[a]<br>±0.7 | 7.5<br>±0.4 | 6.6<br>±0.5 | 16.3<br>±0.3 | 14.3<br>±0.2 |
| 6 | 10.1<br>±0.8 | 10.4<br>±0.1 | 7.3[b]<br>±0.2 | 10.5[b]<br>±1.9 | 13.3<br>±0.5 | 12.8<br>±0.9 |
| 7 | 9.7<br>±1.3 | 9.4<br>±0.3 | 8.5<br>±0.2 | 8.6<br>- | 16.3<br>±1.0 | 14.0<br>±1.8 |
| 8. | 11.0<br>±0.2 | 8.9<br>±0.3 | ** | ** | 13.8<br>±0.7 | 15.0<br>±0.5 |
| 9 | 10.5[a]<br>±0.6 | 7.8[a]<br>±1.0 | | | 15.0<br>±0.1 | 13.7<br>±0.6 |
| 11 | 10.2[a]<br>±1.9 | 7.3[a]<br>±0.9 | | | 15.0<br>±0.3 | 13.7<br>±0.9 |

** Rabbits all died.
1 & 2 3 & 4 and 4 & 6 were compared.
Values with same superscript within a row differe significantly (P<0.05)

Table 4.7:
Total erythrocyte count (RBC) ($x10^{12}/L$) of garlic treated (*) and untreated rabbits infected with T. congolense (1* & 2) T. brucei (3* & 4) and controls (5* & 6) (Mean ±SEM)

| Time Interval (weeks) | GROUPS | | | | | |
|---|---|---|---|---|---|---|
| | | | | | healthy controls | |
| | 1 | 2 | 3 | 4 | 5 | 6 |
| 1 | 5.4 ±0.86 | 6.3 ±0.12 | 6.2[b] ±1.62 | 3.8[b] ±0.56 | 6.8 ±0.32 | 5.1 ±0.45 |
| 2 | 3.1 ±0.05 | 3.0 ±0.15 | 3.9 ±0.87 | 4.7 ±0.79 | 7.8 ±0.1 2 | 6.9 ±0.91 |
| 3 | 1.9 ±0.12 | 2.3 ±0.59 | 2.9 ±0.41 | 3.1 ±0.07 | 5.3 ±0.33 | 5.3 ±0.42 |
| 4 | 2.5[a] ±0.09 | 1.5[a] ±0.26 | 2.2[b] ±0.44 | 1.7 ±0.19 | 8.8 ±0.05 | 7.4 ±0.47 |
| 6 | 3.4 ±0.50 | 2.7 ±0.65 | 1.8[b] ±0.12 | 3.6[b] ±0.36 | 6.4 ±0.23 | 6.5 ±0.25 |
| 7 | 3.1 ±0.23 | 3.0 ±0.09 | 3.1 ±0.48 | 3.2 | 6.3 ±0.14 | 6.0 ±0.20 |
| 8 | 3.4[a] ±0.20 | 2.6[a] ±0.23 | ** | ** | 6.0 ±0.61 | 5.9 ±0.4 |
| 9 | 3.2[a] ±0.17 | 2.4[a] ±0.26 | | | 3.9 0.82 | 4.0 ±0.77 |
| 11 | 3.3[a] ±0.30 | 1.8a ±0.50 | | | 7.8 ±0.34 | 6.8 ±0.32 |

1 & 2; 3 & 4 and 5 & 6 were compared.
Values with same superscript within a row differ significantly ($P<0.05$).
**All rabbits died.

Table 4.8:

Total plasma protein concentration (g/dl) of garlic treated (*) and untreated rabbits infected with T. congolense (1*& 2) T. brucei (3* & 4) and controls (5 & 6) (mean ±SEM)

| Time Interval (weeks) | GROUPS | | | | | |
|---|---|---|---|---|---|---|
| | | | | | healthy controls | |
| | 1 | 2 | 3 | 4 | 5 | 6 |
| 1 | 7.3 ±0.30 | 7.8 ±0.7 | 7.2 ±0.5 | 7.9 ±0.7 | 9.1 ±3.0 | 9.1 ±0.4 |
| 2 | 7.9 ±0.2 | 7.8 ±0.6 | 7.1 ±0.7 | 8.5 ±0.5 | 10.0 ±0.1 | 10.4 ±0.2 |
| 3 | 7.9 ±0.9 | 7.5 ±0.4 | 7.8 ±0.7 | 7.7 ±0.5 | 8.7 ±0.6 | 8.7 ±0.5 |
| 4 | 9.3 ±0.4 | 8.8 ±0.3 | 10.6 ±0.4 | 11.2 ±0.7 | 6.2 ±0.8 | 8.3 ±0.3 |
| 6 | 8.0 ±0.3 | 8.8 ±0.5 | 12.8 ±0.2 | 10.5 ±0.5 | 9.8 ±0.1 | 8.5 ±0.7 |
| 7 | 9.2 ±1.2 | 7,6 ±0.7 | 13.3 ±0.2 | 12.0 - | 8.2 ±0.9 | 9.5 ±0,4 |
| 8 | 8.1 ±0.5 | 8.3 ±0.7 | ** | ** | 7.2 ±0.5 | 7.5 ±0.6 |
| 9 | 9.5 ±1.0 | 10.0 ±0.3 | | | 7.2 ±0.4 | 8.4 ±0.2 |
| 11 | 10.3 ±0.8 | 10.0 ±0.9 | | | 7.5 ±0.4 | 6.8 ±0.4 |

1 & 2; 3 & 4 and 5&6 were compared.
No significant difference ($P<0.05$) between the paired groups.
** Rabbits all died.

Table 4.9:

Total leucocyte counts ($x10^9/L$) if garlic treated (*) and untreated rabbits infected with T. congolense (1*&2) T. brucei (3*&4) and control (5* & 6) (Mean ± SEM)

| Time Interval (weeks) | GROUPS | | | | healthy controls | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| 1 | 5.9 ±0.9 | 7.4 ±2.8 | 5.3[a] ±0.27 | 8.6[b] ±2.7 | 8.6 ±0.25 | 7.2 ±0.69 |
| 2 | 6.6 ±0.64 | 6.3 ±0.44 | 7.1 ±1.1 | 8.3 ±0.44 | 7.8 ±0.21 | 6.9 ±0.50 |
| 3 | 3.5[a] ±0.32 | 5.0[a] ±1.0 | 5.8 ±1.15 | 5.7 ±0.68 | 8.4 ±0.9 | 8.4 ±0.46 |
| 4 | 5.2 ±0.34 | 4.5 ±0.37 | 5.5 ±0.66 | 6.9 ±0.84 | 10.0 ±0.42 | 8.7 ±0.11 |
| 6 | 7.1 ±1.2 | 7.2 ±0.83 | 5.2 ±0.78 | 6.0 ±1.0 | 6.5 ±0.38 | 6.5 ±0.35 |
| 7 | 6.0[a] ±1.4 | 3.8[a] ±0.44 | 6.1 ±0.75 | 7.0 - | 8.4 ±0.23 | 7.8 ±0.15 |
| 8 | 7.0[a] ±1.2 | 3.1[a] ± 0.44 | ** | ** | 7.8 ±0.33 | 6.6 ±0.61 |
| 9 | 7.4 ±0.67 | 7.4 ±0.33 | | | 6.6 ±0.55 | 7.6 ±0.23 |
| 11 | 5.1 ±0.40 | 4.5 ±0.42 | | | 7.6 ±0.35 | 7.2 ±0.24 |

1 &2; 3&4, and 5&6 were compared.
Values with same superscript within a row differ significantly ($P<0.05$)
** Rabbits all died.

Table 4. 14:

Serum albumin level (g/dl) of garlic treated (*) and untreated rabbits infected with T. congolense (1:&2) T. brucei (3*&4) and controls (5*&6) (Mean & SEM).

| Time Interval (weeks) | GROUPS | | | | healthy controls | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| 1 | 48.7<br>±2.7 | 41.0<br>±0.0 | 45.0<br>±3.0 | 40.0<br>±2.0 | 55.0<br>±11.5 | 44.0<br>±1.3 |
| 2 | 40.3[a]<br>±5.8 | 35.7[a]<br>±1.9 | 41.3[b]<br>±1.8 | 34,0[b]<br>±0.0 | 37.0[c]<br>±1.3 | 44.0[c]<br>±1.7 |
| 3 | 39.0[a]<br>±1.0 | 30.0[a]<br>±1.0 | 40.0<br>±1.5 | 42.0<br>±0.6 | 46.0<br>±2.1 | 43.0<br>±0.2 |
| 4 | 46.0<br>±0.0 | 41.8<br>±1.9 | 44.3<br>±2.3 | 36.0<br>±2.5 | 41.3<br>±0.3 | 41.0<br>±0.7 |
| 5 | 55.3[a]<br>±3.9 | 44.5[a]<br>±1.4 | 35.5<br>±1.5 | 36.0<br>±0.4 | 41.3<br>±1.7 | 43.0<br>±0.5 |
| 6 | 50.0<br>±1.7 | 47.3<br>±0.0 | 54.5[b]<br>±0.9 | 38.0[b]<br>±1.3 | 53.7<br>±0.5 | 49.0<br>±1.2 |
| 7 | 49.3<br>±1.3 | 49.0<br>±1.4 | 44.0<br>±1.8 | 31.0**<br>- | 55.0<br>±0.2 | 50.0<br>±0.7 |

Groups 1 & 2; 3 & 4, 5&6 were compared by student t test.
Values with same superscripts within a row differ significantly ($P<0.05$)
** Only one animal left.

Table 4.15:

Serum cholesterol level (mM) of garlic treated (*) and untreated rabbits infected with T. congolense (1* &2) T. brucei (3* &4) and controls (5&6) (Mean ± SEM)

| Time Interval (weeks) | GROUPS | | | | | |
|---|---|---|---|---|---|---|
| | | | | | healthy controls | |
| | 1 | 2 | 3 | 4 | 5 | 6 |
| 1 | 12.7 ±2.9 | 9.6 ±0.3 | 10.8[a] ±2.5 | 22.5[b] ±13.5 | 9.3 ±0.3 | 8.0 ±1.2 |
| 2 | 13.2[a] ±2.4 | 19.2[a] ±4.2 | 14.6[b] ±1.8 | 20.0[b] ±1.2 | 6.3 ±0.3 | 6.5 ±1.0 |
| 3 | 9.0[a] ±2.0 | 13.5[a] ±3.4 | 18.6 ±0.6 | 12.5 ±0.9 | 7.9 ±1.0 | 8.0 ±1.1 |
| 4 | 12.3 ±2.7 | 14.6 ±0.8 | 20.5 ±0.5 | 17.0 ±1.8 | 13.0 ±0.6 | 9.8 ±1.5 |
| 5 | 10.2 ±1.4 | 12.6 ±1.6 | 14.5 ±0.5 | 19.3 ±0.7 | 10.6 ±0.4 | 8.5 ±1.1 |
| 6 | 10.4[a] ±3.4 | 16.2[a] ±2.5 | 12.5[b] ±0.9 | 20.5[b] ±1.8 | 9.2 ±0.8 | 11.3 ±0.6 |
| 7 | 9.8 ±2.1 | 13.0 ±3.2 | 11.8 ±0.3 | 19.0** - | 9.8 ±0.8 | 12.0 ±0.9 |

1&2; 3&4 and 5&6 were compared.
Values with same superscript within a row differ significantly ($P<0.05$).
** Rabbits all died.

Table 4.18:

Percent organ body weight ratio of garlic treated (*) rabbits infected with T. congolense (1*&2), T. brucei (3* & 4) , and controls (5*& 6) (Mean ±SEM)

| Organ | GROUPS | | | | healthy controls | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Kidney | 0.47 ±0.06 | 0.49 ±0.01 | 0.68** ±0.04 | 0.70** ±0.11 | 0.56 ±0.02 | 0.48 ±0.5 |
| Lungs | 0.56** ±0.04 | 0.49 ±0.14 | 0.79** ±0.11 | 0.79** ±0.03 | 0.47 ±0.12 | 0.43 ±0.0 |
| Liver | 2.24 ±0.19 | 2.62** ±0.21 | 2.72** ±0.16 | 2.80** ±0.11 | 1.54 ±0.04 | 1.92 ±0.12 |
| Heart | 0.34** ±0.03 | 0.30** ±9.05 | 0.51** ±0.02 | 0.58** ±0.13 | 0.24 ±0.02 | 0.21 ±0.14 |
| Spleen | 0.29a** ±0.16 | 0.61b** ±0.07 | 0.20c** ±0.06 | 0.44d** ±0.02 | 0.04 ±0.01 | 0.05 ±0.02 |

** Significant increase compared with healthy control ($P<0.05$)
Superscripts a & b; c & d differ significantly ($P<0.05$)