INVESTIGATION OF

ANTITRYPANOSOMAL ACTIVITIES OF

Tridax procumbens and *Morinda lucida*

BY

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(Ph.D/SSSE/2001/067)

A Thesis submitted to the Postgraduate School of Federal University of Technology, Minna, in partial fulfilment of the requirement for the Degree of DOCTOR OF PHILOSOPHY

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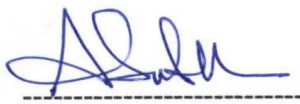
July, 2011

DECLARATION

I hereby declare that this research project/thesis is my original work and to the best of my knowledge has not been presented in any form for the award of degree or any other certificate in any other institution.



08/08/11

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CERTIFICATION

This thesis titled: "INVESTIGATION OF ANTITRYPANOSOMAL ACTIVITIES OF *Tridax procumbens* and *Morinda lucida*" by ABUBAKAR, Abdulkadir (Ph.D/SSSE/2001/067) meets the regulations governing the award of the degree of Doctor of Philosophy in Biochemistry of Federal University of Technology, Minna, and is approved for its contribution to scientific knowledge and literary presentation.

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ABSTRACT

The therapeutic potential of twenty medicinal plant extracts were screened for antitrypanosomal properties in mice, out of which *Tridax procumbens* and *Morinda lucida* gave the highest prolongation of life and were further investigated. These were followed by *Securidaca longipenduculata* and *Cucumis metuliferus* which prolonged the life of the treated animals by 4 days. The stem bark and leaves of *M. lucida* and whole *T. procumbens* were sequentially extracted with hexane, ethyl acetate, methanol and water and the extracts obtained were tested for antitrypanosomal activity. Mice infected with *Trypanosoma brucei brucei* were administered with the extracts intraperitoneally at doses of 100, 200, 300 and 400mg/kg body weight respectively for 14 consecutive days. The methanol extract of *M. lucida* stem bark and leaves gave significant mean survival of 7.0 ± 3.3 and 9.7 ± 3.7 days respectively when compared to the untreated control ($P<0.05$). The ethyl acetate and methanol extracts of *T. procumbens* gave a mean survival of 11.7 ± 5.4 and 14.3 ± 10.2 days respectively ($P<0.05$). Phytochemical screening revealed the presence of steroids, saponins, tannins, alkaloids, flavonoids, phenols and carbohydrate in the crude methanol extract and phenols, flavonoids and steroids in the crude ethyl acetate extract of *T. procumbens*.The bioassay-guided fractionation of the crude ethyl acetate and methanol extracts of *T. procumbens* gave 12 and 11 fractions respectively. Fraction 11 of ethyl acetate exhibited higher antitrypanosomal activities than fraction 7 of methanol which was significantly different from that of the crude extract and the standard drug treated and untreated controls ($P\leq 0.05$). The thin layer chromatographic profile of the active ethyl acetate fraction 11 showed 3 spots; with PTLC band 2 producing highest antitrypanosomal effects. The TLC profile showed the presence of phenolic compounds. The spectrum of ^{13}C and ^{1}H NMR indicates the presence of sugars, fatty acids and phenolic related compounds like *cathechin, 3, 7 dihydroxyflavone, 3 – hydroxyflavone and quercetin. Although the* LD_{50} value is 2100 mg/kg body weight, all the survived animals in acute toxicity studies gained body weight and % organ / body weight ratio as compared to the untreated control ($P<0.05$). However, during the short term toxicity studies, there was a significant decrease in glucose levels ($P<0.05$) and significant increase in ALT and decrease in AST activities with 800mg/kg producing highest effect ($P<0.05$). ALP activity was not affected significantly ($P>0.05$). However, the urea and electrolyte levels increased ($P>0.05$), while the total protein was not affected at all the dose levels. All the animals also gained body weight and % organ / body weight ratio. The crude ethyl acetate extract increased the leucocyte count ($P>0.05$). The ethyl acetate active fraction demonstrated antibacterial activity against *Klebsiella pneumoniae, Salmonella typhi* and *Escherichia coli* while the crude methanol and ethyl acetate extracts were active only against *E. coli*. The result of histopathological studies showed the ethyl acetate extract to have endothelial toxicity at high dose level destroying the blood vessels leading to haemorrhage. Nevertheless, the current study suggests the *in vivo* antitrypanosomal activities of phenolic compounds which include flavonoids and encourages the use of medicinal chemistry approach to obtain more potent derivatives as lead compounds from plants.

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CHAPTER ONE

1.0 INTRODUCTION

In tropical Africa, protozoan parasites cause several diseases of social and economic importance. One of the most devastating, trypanosomiasis is caused by infection with trypanosomes, which are transmitted by tsetse flies to people, domestic livestock and wildlife. The disease constrains agricultural development on over a third of the African continent by causing livestock production losses due to poor weight gains, stunted growth, poor milk production, reproductive failure and finally death (ILRAD, 1990).

Human African trypanosomiasis (HAT), also known as sleeping sickness is caused by haemoflagellates of the genus *Trypanosoma*, subgenus Trypanozoon, which classically includes three subspecies: *T. brucei brucei, T. b. gambiense* and *T. b. rhodesiense* (Smith *et al.*, 1995). The three subspecies are morphologically identical but differ in their ability to infect various hosts. *Trypanosoma b. brucei* is essentially a parasite of domestic animals (Bovidae, Suidae and Canidae) and game animals (antelopes) and is not pathogenic to humans because it is lysed by a haptoglobin-like molecule (Smith *et al.*, 1995). Only *T. b. rhodesiense* and *T. b. gambiense* are known to be human pathogens. Two clinical variants of HAT are encountered: an acute syndrome, attributed to *T. b. rhodesiense* (Rhodesian trypanosomiasis) and a chronic syndrome caused by *T. b. gambiense* (Gambian trypanosomiasis). *Trypanosoma brucei gambiense* is seen in West and Central Africa and in parts of East Africa and *T. b. rhodesiense* only in East and Southern Africa (Masiga and Barrett, 2000).

The most important species of trypanosomes that cause disease in livestock are *Trypanosoma vivax, T. congolense* and *T. b. brucei* (Ikede, 1981). These trypanosome species are widely distributed in all the five agro-ecological zones of Nigeria. In Pigs, *T. simiae* is the most virulent (Agu, 1984); while *T. evansi* infects mainly camels and is

non-cyclically transmitted by some biting flies other than *Glossina* spp. (Scot, 1973). In all instances, the disease results from complex interactions between vertebrate hosts (human and animals), the parasite and its tsetse fly vector (*Glossina* spp.).

According to World Health Organization (1998), there remain within the tsetse belt more than 200 active foci of African Trypanosomiasis, located between 15°North and 15° south. Within these geographical areas, approximately 60 million individuals living in 36 countries are exposed to the infection. Less than 4 millions benefit from an adequate surveillance and case finding programme or from vector control activities. All endemic countries are characterised by shortages of the financial and human resources necessary to implement or sustain a comprehensive control programme.

It is difficult to estimate the overall burden of African Trypanosomiasis because of underdiagnosis in the most heavily infected countries, but it was suggested to be in the vicinity of 100,000 new cases per year, with between 1/3 and ½ of cases remaining undetected and untreated (Pepin and Meda, 2001).

African trypanosomes have evolved several mechanisms which enable them to escape being eliminated from the mammalian host; namely, antigenic variation, destruction of compliment and the ability to survive in elevated levels of interferon (Donelson *et al.*, 1998).

Anaemia is regarded as the main pathological feature in human and animal trypanosomiasis (Losos and Ikede, 1972). Opinion is divided on the pathogenesis of the anaemia but it is generally agreed that three broad factors; increased red cell destruction, inadequate bone marrow response and a haemodilution (or hydraemia), contribute to the anaemia (Fiennes, 1970).

The development of anaemia becomes obvious after the first peak of parasitaemia when, as a result of antibody responses, a major trypanolytic crisis, of which there subsequently several occurs. These crises lead to formation of antigen-antibody

complexes (Murray, 1974; Lambert and Houba, 1974) and probably to the release of a whole range of biologically- active factors known to present in trypanosomes (Tizard *et al.*, 1978). Some of these factors include free fatty acid (Tizard *et al.*, 1977) and hydrogen peroxide (Vray *et al* 1991).There is also evidence that living trypanosomes may lead to red cell damage due to the release of these biologically active factors (Esievo, 1983) and therefore may play key role in the induction of anaemia.

Currently, there are three principal control strategies for tsetse-transmitted trypanosomiasis: trypanocidal drugs (Chemotherapy and Chemoprophylaxis), trypanotolerant cattle and tsetse control/eradication (insecticidal spraying, insecticidal targets, traps and the sterile insect technique) (Freidman and Hendrichs, 2001). These control measures can be applied singly or in combination. However, it is increasingly becoming apparent that sustainable control of trypanosomiasis is more likely to be achieved by integrating the different control strategies. Furthermore, a proper understanding of the metabolic differences between parasite and host could represent good targets for chemotherapy.

There are rather limited numbers of safe and effective antiparasitic drugs licensed for human use (WHO, 1990). Furthermore, increasing drug resistance has limited the usefulness of some existing compounds. Parasitic diseases occur mostly in poorer countries, where the technical expertise and financial resources necessary for drug development are scarce. Despite efforts made by organizations like the World Health Organization (WHO) to find new treatments, there have been few new drugs in recent times. In view of this, there is a need for more effective, less toxic and readily available source of drugs, hence the extension of search to medicinal plants. It is estimated that about 400,000 plants grace the earth and less than 0.5% have been studied exhaustively for their chemical composition and medicinal value (Hoareau and Dasilva, 1999).

Traditional medical remedies for several diseases abound in most endemic regions and it is estimated that some 20,000 species of higher plants are used medicinally throughout the world (Phillipson, 1994). Many of these preparations have been used extensively and knowledge about them has been accrued by several generations of practitioners from experience, trial and error. The main advantages of using medicinal or even toxic plants include local availability, environmental friendliness of cultivation and processing as well as renewable source of raw materials (Wambebe, 1995). Unlike synthetic products, medicinal plants do not contaminate the environment and in most cases are very safe to handle.

Various well-known drugs listed in modern pharmacopoeia have their origins in nature. These include digitalis and related cardiac glycosides isolated from the leaves of foxglove plants (*Digitalis lantana* and *D. purpurea*), ephedrine is derived from ephedra plant (*Ephedria sinica, E. equisentina; E. gerardiana*), Morphine from the opium poppy (*Papaver somniferum*) etc. It is for these reasons that this work was intended to evaluate, the antitrypanosomal activities and toxity of *Tridax procumbens* and *Morinda lucida* extracts. These plants have been exploited for a long time as a source of traditional drugs (Burkill, 1985). The plants are used traditionally for fever, cough, skin infections and wounds (Ali and Earla, 2006). It is also shown to possess significant anti-inflammatory, hepatoprotective, wound healing and antimicrobial properties (Perumal *et al.*, 1999, and Taddei and Rosas, 2000). Preliminary studies on antitrypanosomal effect of some medicinal plants showed encouraging result (Abubakar *et al.*, 2008).

1.1 Justification

1.1.1 The existing trypanocides are not readily available and when available, the cost is so high that the rural populace who are mostly affected cannot afford to buy them (Adewumi *et al.*, 2001).

1.1.2 The development of new trypanocides has been very slow due to the fact that big pharmaceutical companies are no longer interested in the development of drugs with no promise of financial returns (Fairlamb, 1990).

1.1.3 Most of the existing trypanocides are toxic to the host (Schillinger, 1985; Onyekwelu, 1999).

1.1.4 Trypanosomes have also developed resistance to almost all the existing trypanocides (Schillinger, 1985; Rottcher and Schillinger, 1985).

1.1.5 The development of a conventional drug typically takes at least ten years and costs US$ 300 million to $500 million in research and development expenditure for every new product that reaches the market (Swerdlow, 2000).

1.1.6 The extension of the search for trypanocides to medicinal plants is justified since the use of plants in folkloric medicine dates back to the 3rd century.

1.1.7 Such a scientific evaluation would not promote phytomedical practice but also contribute to the search for ethnopharmaceuticals against trypanosomiasis. This will also help alleviate the current crisis in the manufacturing of synthetic drugs.

1.2 Aim and Objectives

1.2.1 Aim

The main aim of this research work is to obtain medicinal plant which is capable of treating African trypanosomiasis and to obtain lead compound(s) that will form the basis for the chemical synthesis of modern pharmaceuticals.

1.2.2 *Objectives*

1.2.2.1 Screening of a wide range of indigenous medicinal plants for anti-trypanosomal activities.

1.2.2.2 To identify the structure of the active antitrypanosomal chemical compound(s) in the plant extracts.

1.2.2.3 To determine the antimicrobial activities of the plant extracts

1.2.2.4 To ascertain whether the extracts have any serious deleterious side effects.

odour attractants) and cattle is an efficient and sufficiently specific method to suppress tsetse target populations in most situations (Brandl, 1988; Bauer *et al.*, 1995). Success largely depends on the density and placement of the impregnated attractive devices in the fly habitat (Vale, 1998); the availability of attractants for the target tsetse species (Torr *et al.*, 1995, 1997); the size of the control area; reinvasion pressure and the population dynamics of tsetse populations in adjacent areas (Hargrove *et al.*, 2000; Hargrove, 2000); tsetse host preference (Clausen *et al.*, 1998); and pastoralist practices, i.e. the time and location of grazing and peaks of tsetse activity. Infested areas may be sprayed with low levels of modern insecticides to kill the flies, but application of such compounds over large areas is discouraged because of the pollution this causes the environment. Habitat hospitable to tsetse may be destroyed to rid areas of flies, but the felling of trees and clearance of bush is an unacceptable waste of diminishing natural resources. This approach is also ineffective in poorest areas, as the vegetation quickly grows again.

The most effective of vector control is the elimination of fly population by the application of sterile insect technique (SIT) on a larger scale. The sterile insect technique involves sustained, systemic releases of sterile insects among the indigenous target population. When female flies are mated by sterile male flies, the female become infertile for the remainder of their life spans. The insects to be released are propagated at special large-scale rearing facilities. Males are sterilized by radiation at the appropriate stage and then taken to the selected area and released. Distribution of the sterile insets can be optimized by aerial release. By continually releasing sterile males in quantities and over a time span that is sufficient to cover several generations of the target population, its reproductive capacity and, hence, the fertile population are progressively reduced. Eventually, so few fertile insects remain that fertile mating do no occur and the population is eliminated. This method is effective solely for the control of savannah

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Classification of Trypanosomes

Trypanosomes are unicellular parasites responsible for a number of diseases of man and domestic animals. Various species of these organisms belonging to the genus *Trypanosoma* are responsible for sleeping sickness in humans in tropical Africa and a very similar disease called Nagana in cattle. A systematic position of *Trypanosoma* in the phylum protozoa and the revised classification are shown in table 2.1 below:

Table 2.1: Classification of Trypanosomes

Phylum: Protozoa

Subphylum: Sarcomastigophora

Superclass: Mastigophora

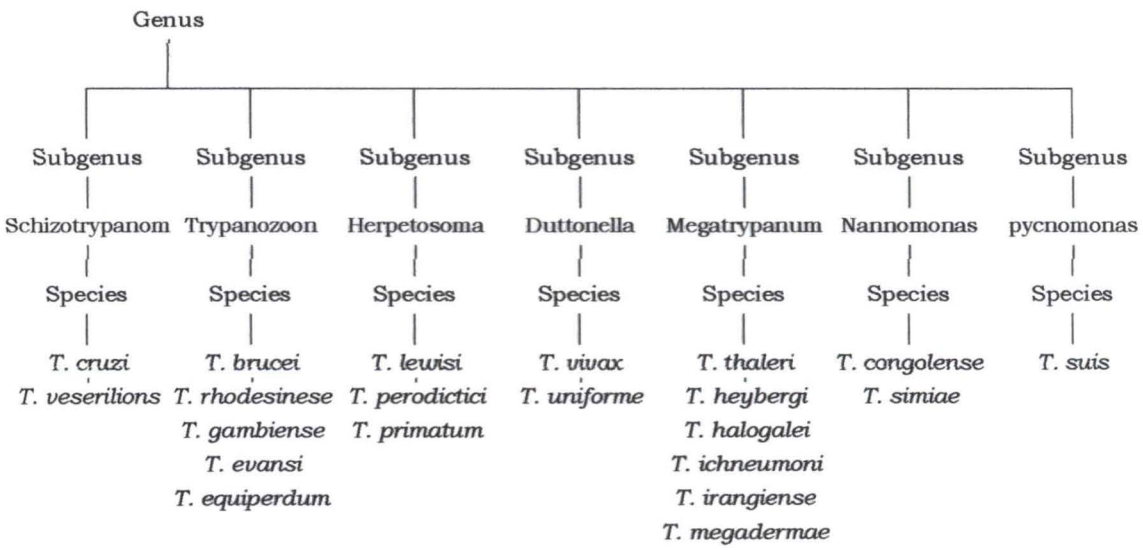
Class: Zoomastigophora

Order: Kinetoplastida

Suborder: Trypanosomatina

Family: Trypanosomatidae

Genus: Trypanosoma



In African Trypanosomiasis (Mulligan, 1970)

2.2 Medical and Economic Importance of African Trypanosomiasis

Pathogenic trypanosomes are divided into two groups, salivaria and stercoraria, according to their development in the vector and transmission by either the saliva or by faecal contamination of the wound caused by bite of the vector. The pathogenic salivarian species include *Trypanosoma vivax, T. congolense, T. simiae, T. brucei, T. b. rhodesiense, T. b. gambiense, T. evansi, T. equiperdum* and *T. suis* while the stercorarian species is *T. cruzi*. The more important trypanosome species affecting man, domestic and experimental animals have been sub-divided into two groups —the haematinic group (*Trypanosoma congolense, T. vivax*) which remains in the plasma and the tissue-invading group (*T. brucei, T. gambiense, T. rhodesiense* and *T. equiperdum*) which is found extravascularly and intravascularly (Losos and Ikede, 1972). Because of their presence in the blood, they produce numerous changes in its cellular and biochemical constituents (Anosa, 1988).

In humans, salivarian trypanosomes (*T. rhodesiense* and *T. gambiense*) cause the fatal disease sleeping sickness. Some 35million Africans are at risk of contracting sleeping sickness and about 10,000 new cases are reported each year (De Raadt, 1976). However, difficulties in monitoring the incidence of the disease in rural areas of Africa suggest that this represents a gross underestimate. Moreover, when medical surveillance and methods to control break down, the disease can reach epidemic proportions, such as occurred during the political upheavals in Uganda (Gashumba, 1981). The duration of the disease ranges from weeks to months in *T. rhodesiense* infection and from months to years in *T. gambiense* infections.

In animals, salivarian trypanosomes cause a variety of diseases including Nagana in cattle (*T. brucei, T. vivax, T. congolense*), Surra in horses and camels (*T. evansi*), and dourine, a venereal disease of horses and donkeys (*T. equiperdum*). *T. simiae*, which causes a severe disease in pigs, is related to *T. congolense* (Fairlamb, 1982). Animal

trypanosomiasis is a serious obstacle to human welfare, due to the severe nutritional and economic problems it causes. Over 3 million cattle die each year and the rearing of high-meat-and milk-producing cattle, sheep, and goats is impossible in 10 million km^2 of Africa (WHO, 1979).

2.3 Morphology of African Trypanosomes

Trypanosomes that are of interest in Africa are almost all belonging to the Salivarian group (Figure 2.1). These parasites all develop in the gut and/or mouth parts of the insect vector, tsetse fly and are transmitted to the mammalian host by injection into the bloodstream during insect feeding. Such trypanosomes include *Trypanosoma vivax*, *T. congolense*, *T. evansi*, *T. equiperdum* and trypanosomes of the "brucei" group (Hoare, 1972).

T. vivax is typically rounded at the posterior end and tapering at the anterior end. The undulating membrane is only slightly developed with a distinct free flagellum. It is pathogenic to various domestic animals even though infectivity in laboratory rodents (rats and rabbits) is low (Desowitz and Watson, 1953).

T. congolense infect both domestic and practically all laboratory animals. In the strains found in Nigeria, the mean lengths are correlated with pathogenicity of the parasite. Accordingly, the small congolense type is characterized by low infectivity, parasitaemia and virulence. The long type is highly infective and virulent with a high level of parasitaemia while the intermediate type shows high infectivity and virulence but low parasitaemia.

Among the salivarian trypanosomes, the subgenus Trypanozoon represents the most homogenous group since it contains a number of species which are indistinguishable by morphological criteria but differ importantly in the kinds of disease they cause. Extensive developments in biochemistry and molecular biology have enabled a variety of parasite identification techniques to be developed.

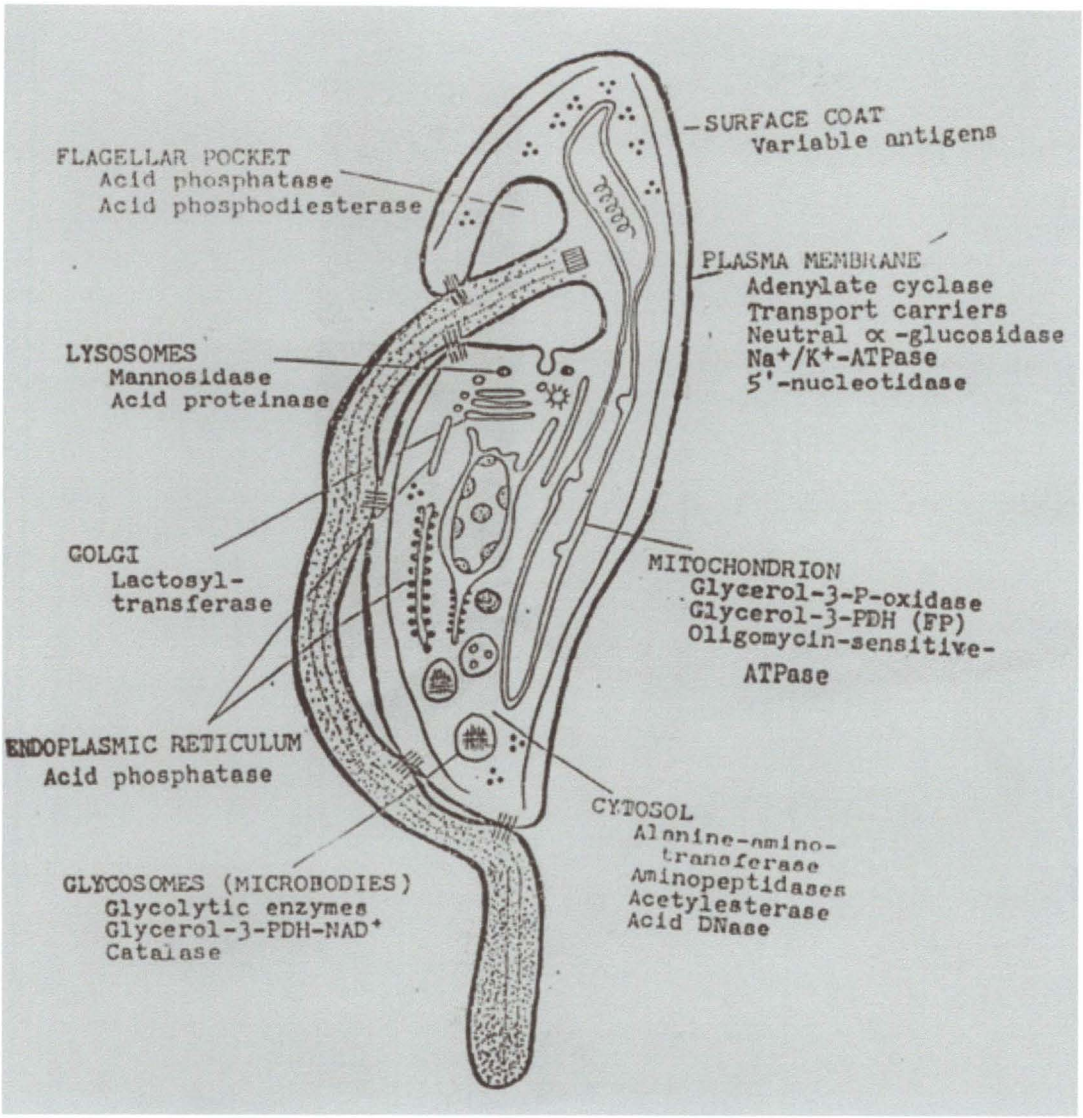


Figure 2.1: Components of the bloodstream form of *Trypanosoma brucei*. Possible locations of some enzymes are given (Vickerman, 1980).

In the past, a major constraint on many aspects of the epidemiological research has been the difficulty of distinguishing between members of the "brucei" group i.e. *T. brucei brucei* which does not infect man, *T. b. rhodesiense* which causes acute sleeping sickness and *T. b. gambiense* which causes chronic sleeping sickness (Cross, 1990). The three members can occur in animals as can the similar parasites *T. evansi* and *T. equiperdum*. The bloodstream form of Trypanozoon is polymorphic and typically comprise;

a) Stumpy trypanosomes, usually without a free flagellum but having a well developed undulating membrane, a rounded nucleus and the kinetoplast.

b) long slender trypanosomes possessing a long free flagellum, a well developed undulating membrane, elongated nucleus and a kinetoplast near the blunt posterior end

c) Intermediate forms which have a shorter flagellum and the kinetoplast nearer the blunter posterior end than the long slender forms (Vickerman, 1985).

2.4 Structural Functions of some Cellular Components of African Trypanosomes

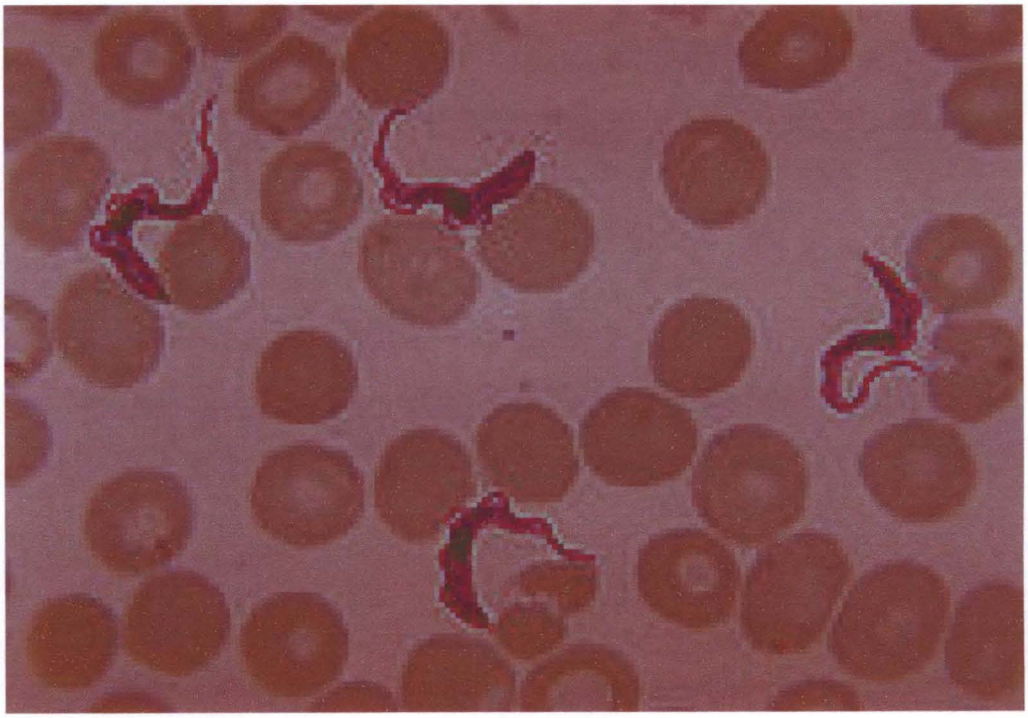


Figure 2.2: Trypanosome as seen in the blood smear.

Source: (CDC:http://www.dpd.cdc.gov/dpdx)

The details of the structure of trypanosomes have been described mainly by electron micrographs (Vickerman, 1974, 1980; Vickerman and Preston, 1976).

The plasma membrane which is fortified with sub-pellicular microbubules (Figure 2.2) is covered by a surface coat made up of variable antigens manifested through the sequential expression of variant surface glycoproteins (VSGs). The surface coat which accounts for about 10% for the total protein of the bloodstream forms of *T. brucei* but absent in the insect and culture forms (Tetley *et al.*, 1987; Vickerman *et al.*, 1988) covers the entire surface of the trypanosome including the flagellum. The base of the flagellum where it is inserted into the body forms a circular pocket distinct from the rest of the cytosol (Figures 2.1 and 2.2). The plasma membrane is continuous with the membrane of the flagella pocket which presumably is involved in exo-and endo-cytic pathways (Coppens *et al.*, 1987). As the flagellum beats from the tip downwards, areas of contiguous plasma membrane and cytoplasm become drawn out into a series of crests, forming the undulating membrane (Vickerman and Preston, 1976).

The plasma membrane of the salivarian trypanosome is of particular interest to the biochemist because it provides the attachment sites for the antigenic surface coat in bloodstream forms and the assembly template for a complex array of microtubules. A 60 kDa cytoskeletal protein from *T. b. brucei* has been reported to interact with plasma membrane and with microbubules (Seebeck *et al.*, 1988). Periera *et al.*, (1978) reported the presence of Mg^{2+}-dependent ATPase activity that was not sensitive to Na^{+}, K^{+} and Ca^{2+} ions. Another report (Bababunmi, 1987) also indicated the presence of Mg^{2+}-dependent ATPase in both the bloodstream and the procyclic forms of *T. brucei*. In addition, the parasite demonstrated Mg^{2+}-dependent and Mg^{2+}-stimulated ATPase (Bababunmi, 1987). The presence of Na^{+}/K^{+} ATPase and adenylate cyclase activities on the plasma membrane of *T. brucei* have also been reported (Voorheis *et al.*, 1978).

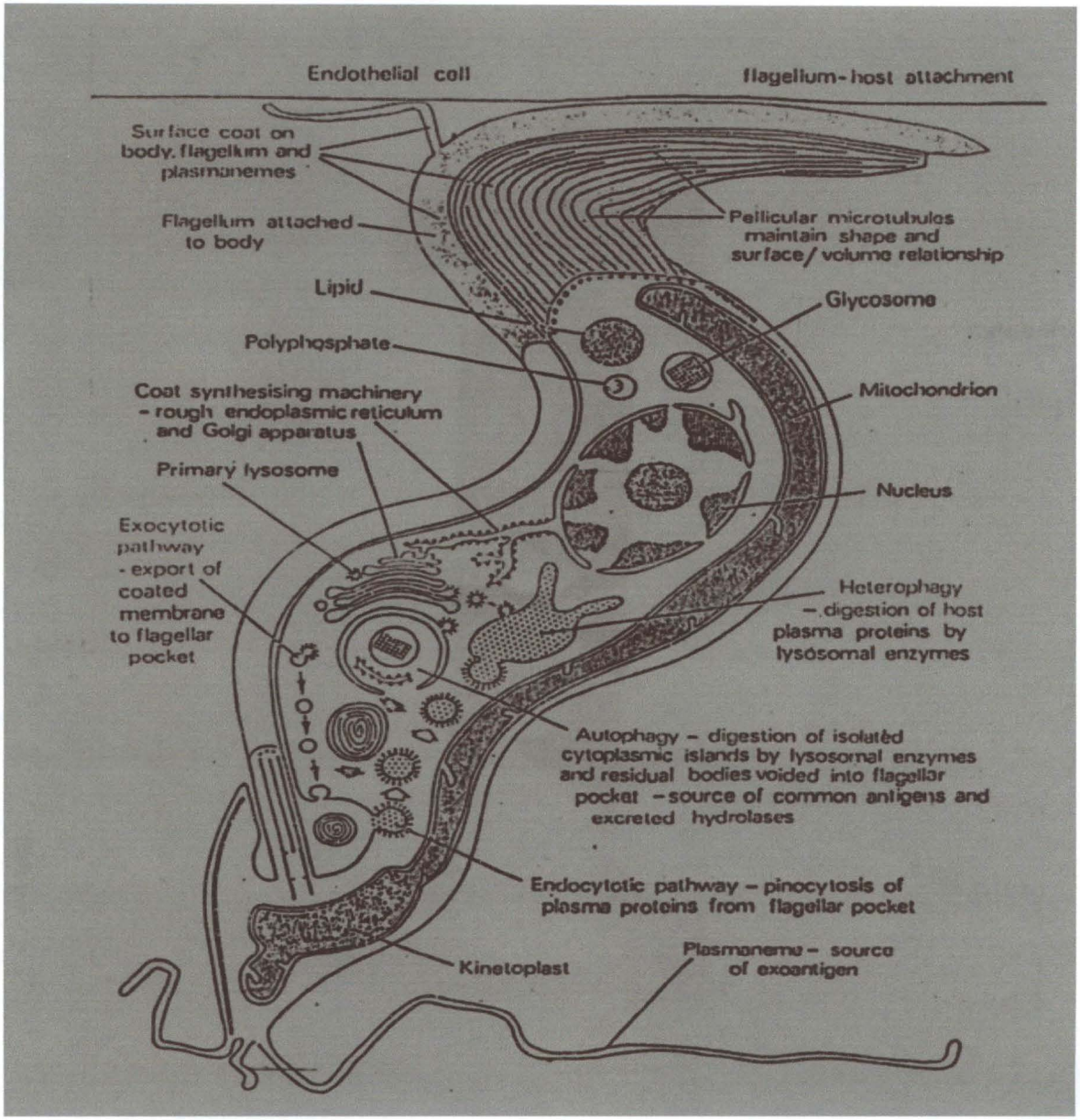


Figure 2.3: Principal structures of an African Salivarian Trypanosome. The function of some of them is given (Vickerman, 1980).

One mitochondrion of varying complexity and function is present in trypanosomes. In the *T. brucei* group, it has been suggested that pleomorphism has functional significance since only the short stumpy forms can transform further into insect midgut trypomastigotes and epimastigotes (Vickerman, 1962). Pleomorphism is accompanied by parallel changes in mitochondrial organization and function. In the long slender forms cristae are poorly developed or completely absent in the mitochondrion. Cytochromes are also not detectable and respiration is not inhibited by cyanide (Grant *et al.*, 1961; Flynn and Bowman, 1973). The Krebs cycle is also non-functional, the energy requirement being fully met by glycolysis with pyruvate as the end product (Flynn and Bowman, 1973; Bowman and Flynn, 1976). However, the stumpy trypomastigote has full mitochondrial competence in preparation for life in the tse-tse fly. Thus the transformation of slender bloodstream trypomastigotes of *T. brucei* group to the insect vector forms involves a switch from a mitochondrial respiration dependent on glycerol phosphate oxidase and no cytochromes to a mitochondrial respiration displaying Krebs cycle enzymes and cytochromes (Opperdoes, 1985). The mitochondrion of the trypanosome contains DNA packaged into a microscopically identifiable structure called kinetoplast (Simpson, 1987).

In African trypanosomes, most enzymes of the glycolytic pathway are found in a micro-body like organelle called the glycosome (Opperdoes, 1985).

2.5 Life Cycle of Trypanosomes

Trypanosoma brucei and other salivarian trypanosomes are transmitted from animal to animal by the tsetse fly. When an infected fly bites an uninfected animal to obtain a blood meal, it injects metacyclic forms along with its salivary secretions into the skin. Metacyclics multiply into trypomastigote forms at the site of the bite forming a chancre. Subsequently, parasites migrate via the bloodstream. Here, they multiply in large numbers as well as invade the intercellular spaces of other tissues.

In the mammalian bloodstream, slender forms of *T. brucei* are actively dividing cells in which the mitochondrial functions are repressed. These cells cannot engage in oxidative phosphorylation. Instead they metabolize glucose to pyruvate via a glycolytic pathway which is partially compartmentalized within specialized organelles termed glycosomes. During this stage, the trypanosomes are covered by a uniform coat of a variant surface glycoprotein (VSG). A continuous variation of this antigen allows the parasite to escape the defences of the host. As the immune system eliminates the trypanosomes covered by the previous VSG, individual parasites expressing a new VSG increase in numbers, creating the observed pattern of parasitaemic waves.

As the infection progresses, long slender forms are replaced by nondividing short stumpy forms preadapted to transmission in the fly. Once taken up by the fly, these cells quickly differentiate into actively dividing procyclic trypomastigole forms. The procyclic forms develop a fully active mitochondrion, respire on proline and can use the Krebs cycle as a source of energy. At this stage, the parasite has lost the VSG and is covered instead by a totally different glycoprotein termed procyclin or procyclic acidic repetititve protein (PARP). Procyclic forms of the parasite migrate from the midgut to the proventriculus and then to the salivary glands, where they stope dividing. This nondividing form, called the metacyclic form, reacquires a VSG coat in a preadaptation to its future life in the bloodstream of the mammal (Figure 2.4), (Vanhamme and Pays, 1995).

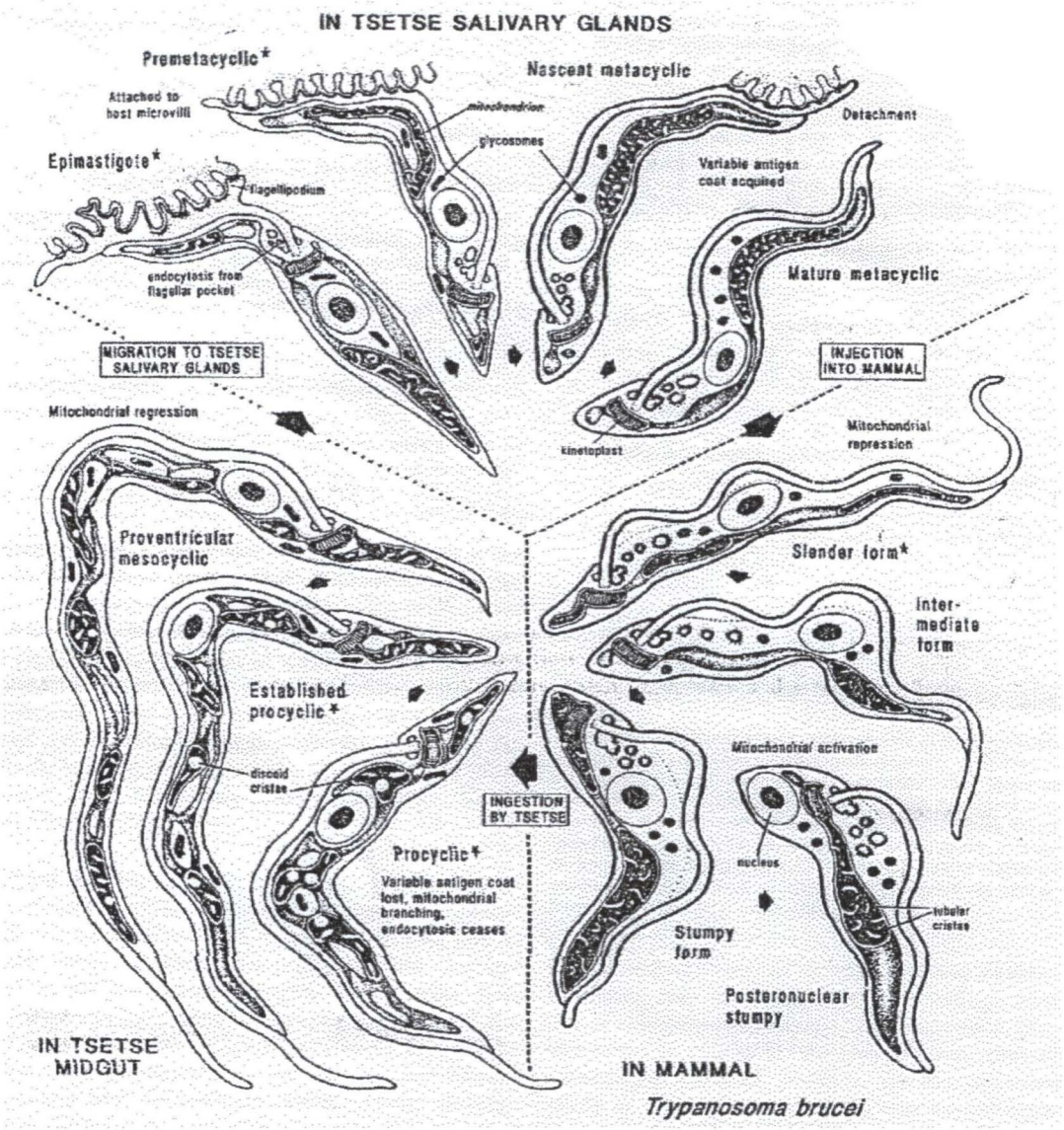


Figure 2.4: Life Cycle of *T. brucei*. In the insect host, the surface coat is lost but re-acquired at the metacyclic stage (Vickerman, 1985).

2.6 Control Strategies for African Trypanosomiasis

Different methods are used to control trypanosomiasis; parasite control through: the use of trypanocidal drugs and the promotion of trypanotolerant livestock, vector control or eradication through: traps and insecticide-treated targets, in some cases baited with attractant odours, insecticide-treated animals and the sterile insect technique (SIT). Each of these methods is useful, indeed, without them, many livestock farmers would be out of business. None, however, is ideal and no single method or combination of methods is sufficiently efficacious and cost-effective to sustain small scale livestock raising in much of the tsetse belt, which effectively seal off one third of the continent otherwise suitable for grazing and mixed livestock/crop farming (ILRAD, 1991).

2.6.1 Drug Treatment

The use of trypanocidal drugs is the most widely accepted means of controlling the disease. However, the drugs available are effective but also relatively expensive. The widespread, unsupervised and underdosed use of the few compounds developed for use against the trypanosomes that cause the disease has led to increasing resistance on the part of the parasite (Afewerk *et al.*, 2000), which retains its resistance after cyclical transmission by tsetse (Gray and Roberts, 1971). Furthermore, the high cost of developing new drugs, with little hope of a reasonable financial return for their investment in research and development, are a serious disincentive for most pharmaceutical companies. The drugs are also faced with the problem of toxicity.

Table 2.2: Summary of drugs available for treatment of human African trypanosomiasis

Drug	Marketed	Spectrum of Activity	Stage of disease
Suramin (Germanin)	1922	*T. b. rhodeasiense*	Stage 1
Pentamidine (Pentacarinat)	1937	*T. b. garnbiense*	Stage 1
Melasoprol	1949	*T. b. gambiense*	Stage I & 2
Arsobal		*T. b. rhodensiense*	Stage I & 2
Eflornithine (Orindyl)	*1981*	*T. b. gambense*	

Chemotherapy of early and late stages of sleeping sickness has been restricted to the use of suramin and pentamidine but the more toxic aromatic arsenicals or nitrofurazone are used when there is central nervous system (CNS) involvement, as the former drugs do not cross the "blood-brain barrier' (Fairlamb, 1982). Melorsoprol has been effective drug available for the treatment of the late stage sleeping sickness. Eflornithine (also known as difluoromethylornithine) is also effective against late stage disease caused by *T. b. gambiense* but is ineffective against *T. b. rhodesiense* (Masiga and Barett, 2000). Treatment of animal trypanosomiasis has been with diminazene aceturate (berenil) and isometamidium chloride (Samorin) which replaced the highly toxic and resistant antrycide and ethidium (Greenwood, 1979; Newton, 1974; Williamson et al, 1982). Of the few drugs available, samorin is the only drug which is used for prophylaxis. Its remarkable economic value in reducing livestock mortality and increasing productivity has been well demonstrated (Murray *et al.*, 1987). Suramin was

also used in animals against *brucei*-like infections in horses, donkeys and camels (Bennett, 1933).

2.6.2 Disease-Resistant Livestock

This method of control involved keeping cattle of a few ancient African breeds, such as the West African N'Dama (*Bos Taurus*), which are innately able to tolerate infection with trypanosomes. The genetic ability to resist the pathogenic effects of infection is called 'trypanotolerance' (Mulla and Rickman, 1988). When exposed to trypanosomiases, trypanotolerant cattle breeds generally show slightly lower mortalities that are shown by trpanosuceptible breeds and lower reductions in calving rate (FAO, 2000). Such trypanotolerant breeds are being promoted in several parts of Africa (FAO, 1988) but this method also has its drawbacks. First, the degree of disease resistance in an animal is not absolute, levels of trypanotolerance are reduced in animals under stress, particularly those with poor nutrition, a common condition of livestock raised in Africa's marginal farming areas. Secondly, although meat production of trypanotolerant livestock compares favourably with that of other breeds, the amount of milk trypanotolearnt cattle produce is relatively low. Furthermore, trypanotolerant animals raised in traditional farming system are trypically small and are therefore not ideal for drought work. Finally, and most importantly, few such animals are available. In spite of their importance in tsetse infested areas where other livestock cannot survive, trypanotolerant cattle constitute only 5% of the cattle raised in countries where tsetse flies occur (ILRAD, 1991).

2.6.3 Vector Control

Tsetse control involves the killing of large population of flies to allow man and his domestic animals to live in an area with limited risk of trypanosomiasis (Jordan, 1978). This can be achieved through the use of special insecticide formulations applied to artificial attractive devices (insecticide-impregnated targets with or without available

tsetse of the *G. morsitans* group, which are vectors for Rhodesian and animal trypanosomiasis.

2.7 Pathogenesis of Trypanosomiasis

In the absence of treatment, trypanosomiasis is often fatal in man. Trypanosomiasis with their antigenic variation has topological preferences within the host and within the particular organs (Poltera, 1985). The lymphatic system is an important site for multiplication as it provides a way of escaping host defences and trypanocides. The histological and haematological changes observed in trypanosome infections are determined by several factors including virulence of the parasite, the susceptibility of the host and, among others, the period of the infection during which samples are taken (Joshua, 1989). Metabolic alterations at the circulating blood constituents further contribute to tissue damage. A significant feature of the pathological state that has long been established (Fienne, 1954) is the anaemia. Some other consequences of trypanosome infection in mammals include thrombocytopenia (Davis, 1982) and malfunctions of the reproductive organs (Ikede *et al.*, 1988) and the liver (Arowolo *et al.*, 1988). Early studies described maningoencephalitis in sleeping sickness (Thomas and Brinl, 1905; Mott, 1906). The neuropathologies of untreated and insufficiently treated patients as well as myocarditis are documented (Calwell, 1973; Hawking and Greenfield, 1941; Pantreath, 1989, 1990). Indications are that all organs are invaded by the trypanosomes with the central nervous system involvement ultimately leading to coma and death (Anosa, 1988).

Despite much investigation, the mechanism by which *T. brucei* and other trypanosomes induce pathogenicity including anaemia is not known. The diverse natures of the consequences of trypanosome infection imply that several factors from the trypanosome could be involved. It is also possible that some of the factors are originating from the host having been induced by the presence of the trypanosome. As a result of

different combinations of these host parasite and perhaps other factors, the pattern of pathogenesis is quite variable. Thus acute, chronic and recovery or post-crisis phases have been described (Anosa, 1988; Zwart, 1989). The acute phase or phase 1 begins with the first appearance of trypanosomes in blood after the incubation period. The parasitaemia is high, although fluctuating. This phase appears to be a period when the inadequately organised defence mechanisms of the infected animal are shocked by a vicious foe which amongst its weapons possesses the ability to circumvent the alerted defence mechanisms of the host by manifesting antigenic variation. Unless the virulence of the parasite is not intense, the host particularly rodents often succumbs and dies within one week (Losos *et al.*, 1973).

Animals destined to enter the chronic phase show less parasitaemia and pathology in the acute phase. The chronic phase or phase II is characterised by low frequency and intensity of parasitatemia. It appears to be a period when the infected animal has fully mobilised its defence mechanism to a level that is adequate to depress parasite multiplication but is not yet adequate to completely abort the infection or reverse the pathology that developed during phase I (Anosa and Isoun, 1974, 1980). With the tissue invading trypanosomes, this is the period when the parasites 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 cannot mobilise commensurate immunologic response to non-trypanosme infections (Schmidt and Sayer, 1982). The recovery or post-crisis phase (phase II) is characterised by a parasitaemia. At this stage the pathological changes are slowly reversed (Anosa, 1988).

2.8 The Erythrocyte Ghost Membrane

It is well established that the erythrocyte membrane, like all biological membranes is made up of proteins, lipids and carbohydrates. The lipid globular protein,

mosaic (LGPM) model of biological membranes proposed by Singer and Nicolson, (1972) is defined as two-dimensional solution of oriented globular proteins and lipids. The model which is dynamic and applicable to functional membranes proposes a lipid bilayer as the fluid matrix in which the proteins are embedded (i.e. intrinsic or integral) or bound to the exposed surface of the integral proteins and the polar head-groups other lipid bilayer (i.e. extrinsic or peripheral). The LGPM model shown schematically in Figure 2.5 is consistent with all thermodynamic considerations (Singer and Nicolson, 1972). In this model the integral proteins of membranes and sections of phospholipid bilayer alternate to form a mosaic in the plane of the membrane. The phospholipids that are present (Figure 2.6) include phosphatidylserine (PS), phosphatidylcholine (PC), phosphatidylethano-lamine (PE) and phosphatidylinositol (PI). Cholesterol and sphingomyelin are also present (Marchesi *et al.*, 1976).

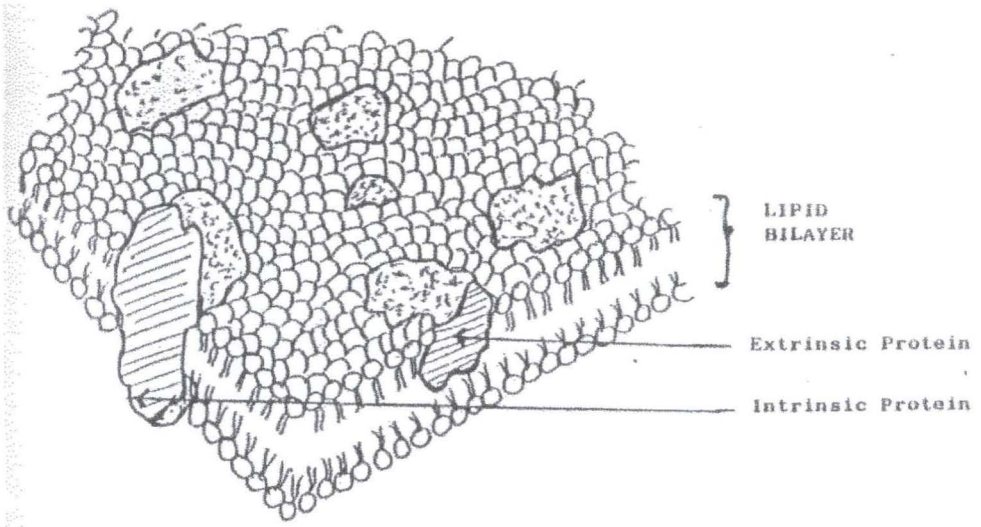


Figure 2.5: Schematic three-dimensional and cross-sectional view of the lipid-globular, protein fluid mosaic model with a lipid 'matrix (Singer and Nicholson, 1912).

One idea which has developed almost entirely from the study of the red cell membrane is that phospholipid types are distributed asymmetrically in specific halves of the lipid bilayer (Rothman and Lenard, 1977). The results have been obtained from several different approaches including the use of specific phospholipases (Colley *et al.*, 1973; Zwaal *et al.*, 1973) and labeling experiments (Marinetti, 1977). Conclusions from these experiments are that PC and sphingomyelin are the principal phospholipid components in the outer half. PE and PS which are abundant in the inner leaflet show non-random distribution. They are cross-linked as PE-PE, PE-PS and PS-PS in different amounts. Findings also showed that cholesterol is distributed throughout both halves of the bilayer (Smith and Oldfield, 1984).

The red cell, an end-stage non-nucleated component of the blood is typically biconcave in nature and it consists essentially of haemoglobin encapsulated by the phospholipid bilayer into which proteins are either embedded or are attached thus providing an enclosed environment for the cell and hence maintaining an internal milleau which is different from the plasma outside (Marchesi *et al.*, 1976). A pointer to the molecular basis of stability and maintenance of the shape of the red blood cell was provided by Guidotti, (1972) when he suggested that spectrin polymers bore some striking resemblances to the large molecular weight polypeptides of muscle myosin and further hypothesized that they interacted with actin or an actin-like protein to form a contractile apparatus for the membrane. Singer *et al.*, (1975) confirmed this idea and further proposed a model in which the spectrin polymers may be attached to the inner surface of red cell membrane with internal segments of trans-membrane proteins forming a new order of integral membrane proteins. The gross mechanical properties of the cell e.g. shape, is considerably influenced by the arrangement of spectrin, actin and ankyrin proteins with one or more intra-membraneous proteins which span the lipid bilayer e.g. glycophorin A.

Carbohydrates are present in the erythrocyte membrane in form of glycoproteins and glycolipids (Tanner and Boxer, 1972; Tomita and Marchesi, 1975). Glycophorin A is an example. It represents approximately 75% of total sialoglycopeptides of the erythrocyte ghost membrane and is a single polypeptide chain composed of 131 amino acids and 16 oligosacharide chains which comprise 60% of the total mass of the molecule (Tomita and Marchesi, 1975). Although ca^{2+}-ATPase is not known to contain carbohydrate moiety (Carafoli and Zurini, 1982), Na^{+}/K^{+}-ATPase is however a glycopeptide. Sialic acid, the acylated neuraminic acid occurs in appreciable quantities on the erythrocyte surface in several animal species (Esievo *et al.*, 1986).

The membrane-bound Na^{+}/K^{+}-ATPase is the ion-motive sodium pump that controls many essential cellular functions such as cell volume, heat production, intra cellular pH and membrane potential. The ouabain inhibitable enzyme couples the free energy from ATP hydrolysis to the translocation of Na^{+} and K^{+} across the plasma membrane. The sodium pump consists of an alpha beta heterodimer which constitutes the minimal functional unit able to hydrolyze ATP. Whether a higher degree of oligomerization is required for cation transport reactions is yet to be established (Sweadner and Goldin, 1980; Rossier *et al.*, 1987).

The membrane-bound Ca^{2+}-ATPase, together with its activator calmodulin is the major regulator of intracellular calcium ion concentration in the red cell. The ion-motive enzyme of 138kd probably contains 18 different types of amino acids with asparagine, glutamine and leucine in high proportion. Ca^{2+}-ATPase couples the tree energy from ATP hydrolysis to the export of excess calcium ions across the cell membrane against a concentration gradient (Carafoli and Zurini, 1982; Pedersen and Carafoli, 1987).

2.9 Anaemia in African Trypanosomiasis

The development of anaemia is a well recognised and inevitable consequence of trypanosome infection in domestic animals in general and cattle in particular (Hornby,

1921; Murray, 1974; Morrison *et al.*, 1982). It is also the most outstanding clinical and laboratory feature of trypanosomiasis in man (Suliman and Feldman, 1989). It has been established that the measurement of anaemia gives a reliable indication of the disease status (Murray, 1979) and productive performance (ILCA, 1986a,b) of trypanosome infected cattle.

The severity of the anaemia which follows infection is affected by several factors. These include differences in virulence that exist among the different species of trypanosome and among the large number o strains belonging to each species. At the same time, host factors such as age, nutritional status and breed are important (Murray *et al.*, 1982). The course of infection acute or chronic may also affect the severity (Cox, 1979; Wery *et al.*, 1982). The observed variations in severity may be summarised as due to the ability of the trypanosomes to vary antigens on their surface coat or to host efforts to rid themselves of the infection.

2.9.1 Mechanisms

The exact mechanism(s) responsible for the development of anaemia is still debatable. Mechanisms such as haemolysis (Amole *et al.*, 1982; Anosa and Kaneko, 1983) haemodilution (Holmes, 1976; Whitelaw *et al.*, 1980) and dyshaemopoiesis (Dargie *et al.*, 1979; Dodd *et al.*, 1978) have been proposed. The basis of anaemia is the increased rate of destruction of red blood cells. In general, the onset of anaemia and the extent to which packed cell volume (PCV) value fall correlate closely with the appearance, height and duration of parasitaemia. The parasitaemic crisis is usually accompanied by a severe drop in the PCV and red blood cell (RBC) count (Anosa, 1980; Akol *et al.*, 1986; Shoyinka and Uzoukwu, 1986). Studies on haemolytic activity in plasma of cattle with *T. vivax* showed that red cell destruction followed waves of parasitaemia and accompanied the progressive anaemia (Murray and Dexter, 1988). Furthermore, anaemia occurred in rats subjected to irradiation and infected with *T.*

brucei. It was also possible to demonstrate haemolytic activity in plasma of these animals (Figure 2.6), indicating that immunological competence is not essential for the development of anaemic (Murray and Dexter, 1988). Based on the presence or absence of trypanosomes, the anaemia in trypanosome-infected mammals can be divided into two distinct but overlapping phases as schematically represented in Figure 2.7. As the infection progresses in the trypanosome infected animal, the kinetics of anaemia change and with them almost certainly the underlying mechanisms (Murray and Dexter, 1988).

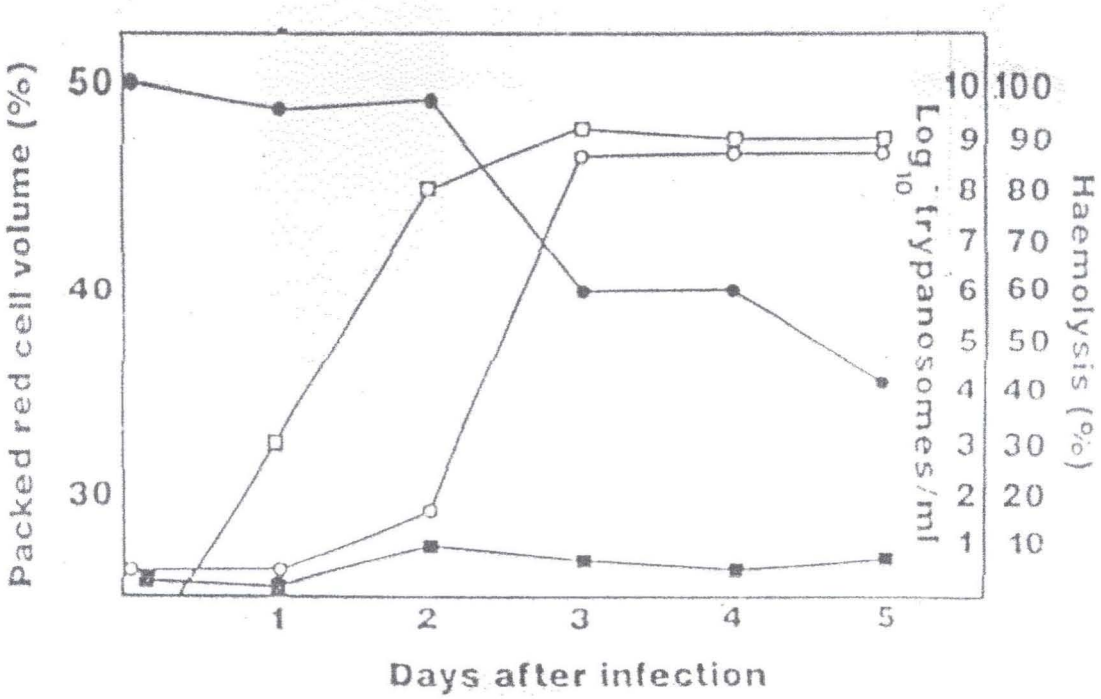


Figure 2.6: Development of anaemia (●) in relation to parasitaemia (□) and plasma haemolytic activity (○) in irradiated rats infected with *Trypanosoma brucei*. Haemolytic activity in control rat plasma is also shown (■) (Murray and Dexter, 1988).

2.9.1.1 Phase I

The first phase is an acute phase characterised by the onset of anaemia and the extent to which PCV values fall would appear to correlate closely with the appearance, intensity and duration of parasitaemia. The initial fall in PCV is associated with the first wave of parasitaemia while the rate of development and severity of anaemic usually reflect the intensity and duration of parasitaemia. Progressive decrease in PCV takes place in trypanosome infected cattle over a period of 4 to 12 weeks after infection by which time values of around 20% are reached. At this stage, PCV values may continue to fall until the animal dies (PCV = 15% or less). The presence of the parasite in the blood is the basis of the progress of the anaemia during phase I (Murray and Dexter, 1988). In certain animals, the elimination of the parasite or self-cure heralds recovery. If infected animals are treated with trypanocidal drug in the phase I, there is a return to normal haematological values (Holmes and Jennings, 1976). It would appear that phase one of the anaemia of African trypanosomiasis depends not only on the presence of the trypanosome but also on a number of other induced factors possibly involving trypanosome-generated enzymes, immunological mechanisms, complement activation through trypanosomes and/or antigen-antibody reactions, microangiopathic damage, fever and en expanded and active mononuclear phagocytic system (MPS). Although each factor may function independently, it is much more likely that they interact with trypanosome-derived factors possibly playing the key role in the inductive phase of red cell damage (Murray and Dexter, 1988).

2.9.1.2 Phase II

Hosts that survive phase one progress into phase two or the chronic phase of the disease syndrome that can end in death, spontaneous recovery or survival with persisting low grade anaemia (Murray and Dexter, 1988). This phase of the disease is characterised by low transient parasitaemia or the complete absence of detectable parasites in the blood

while PCV values stabilize at 20 to 25% in infected cattle for a variable period of time. Some animals, mainly the trypanotolerant breeds like N'Dama and West African Short-horned cattle make a complete recovery 2 to 4 months after infection (Murray, 1979). In some more susceptible breeds of cattle such as the Zebu or Boran, the rate of recovery is slower or partial. Other animals despite the absence of detectable parasite maintain low PCV values and make no clinical improvement (Murray *et al.*, 1979a). Blood and tissues from such animals fail to infect mice and rat. While some animals suffering from chronic trypanosomiasis die, many remain alive but in poor health characterized by stunting, wasting and infertility despite adequate feeding.

In contrast to phase one, the response to trypanocidal drug treatment during phase two is poor or sometimes non-existent (Murray *et al.*, 1979a, b).

Anaemia in trypanosomiasis is associated with significant abnormalities of iron metabolism, bone marrow activity, serum and plasma proteins together with thrombocytopaenia and hepatosplenomegaly (Anosa, 1988).

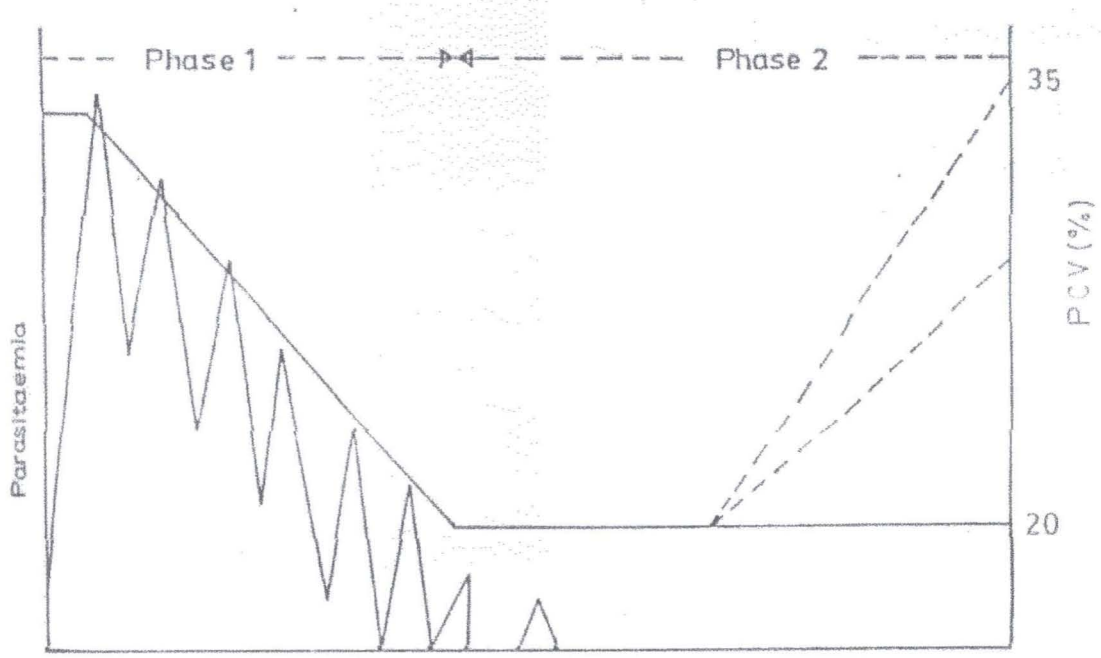


Figure 2.7: Anaemia of African trypanosomiasis (% PCV) in relation to parasitaemic waves, time and phases (Murray and Dexter, 1988).

2.10 Some Biochemical Peculiarities of African Trypanosomes

Trypanosomes are of interest to scientists not only because of their medical and veterinary importance, but also because of several unique features of their biochemistry and molecular biology. Such features include the mitochondrial DNA network (Kinetoplast) and its role in the life cycle (Englund *et al.*, 1982; Barker, 1980), the variant surface glycoprotein and its role in evading the immune response of the host (Cross, 1990), glycosome (Michels, 1988) and protection against oxidative stress (Murgelo *et al.*, 1989).

2.10.1 Carbohydrate Metabolism

All morphological bloodstream forms respire actively on glucose, fructose, mannose or glycerol (Ryley, 1956, 1962). In addition, short-stumpy trypomastigotes will respire with α-osoglutarate at about half the rate found with glucose (Flynn and Bowman, 1973). Neither form is able to respire using amino acids or fatty acids (Bowman and Flynn, 1976) and do no not possess any energy reserves as their ATP level are rapidly depleted in the absence of an external source of carbohydrate (Opperdoes *et al.*, 1976). Thus, in the mammal host, long-slender and short-stumpy trypomastigotes are dependent on a continuous supply of glucose from plasma or other extracellular fluids. Pyruvate is the major end product of aerobic glucose metabolism and is not further metabolized by long-slender forms as they are completely lacking in lactate deydrogenase (Dixon, 1966) and pyruvate decarboxylase (Flynn and Bowman, 1973). Short-stumpy forms also differ biochemically from long-slender types in their ability to use α-oxoglutarate as an energy source. In the absence of other exogenous carbohydrate, α-oxoglutarate preferentially confers survival to stumpy forms. Slender forms rapidly lose motility and disintegrate, whereas stumpy forms remain motile for at least 3hrs. *in vitro* (Vickerman, 1965). The NADH produced in glycolysis is reoxidized by a unique mitrochodrial glycerophosphate oxidase system consisting of glycerol-3-phosphate

dehydrogenase and glycerol-3-phosphate oxidase (Fairlamb and Bowman, 1977; Opperdoes *et al.*, 1977a, b). This terminal respiratory system is unique in that respiration is insensitive to inhibition by cyanide and does not contain cytochromes (Grant and Sargent, 1960; Flynn and Bowman, 1973; Fairlamb and Bowman, 1977a). In the presence of dihydroxyacetone phosphate the NAD- dependent glycerol-3- phosphate deydrogenase oxidizes NADH to glycerol-3-phosphate and NAD. Glycerol-3-phosphate diffuse from the glycosome to the mitochondrion, where it is reoxidized to dihydroxyacetone phosphate by the mitochondrial glycerol-3-phosphate oxidase (Fig. 8). Inhibitors of the mitochondrial dehydrogenase component include thiol-reactive compounds (P-hydroxymercuribenzoate and melarsen oxide), heavy metal ions (Cu^{2+}, Zn^{2+}, Hg^{2+}), certain chelating compounds (O-phenanthroline) and the trypanocidal drug suramin (Fairlamb and Bowman, 1977b). Inhibitors of the terminal oxidase components include; aromatic hydroxamic acids, 8-hydroxyquinoline, diphenylamine and hydrogen peroxide (Clarkson and Brohn, 1976).

In the absence of oxygen, or when glycerophosphate oxidase is inhibited by hydroxamic acids such as salicylhydroxamic acid (SHAM), long-slender bloodstream forms continue to utilize glucose at about the same rate as found under aerobic conditions (Opperdoes *et al.*, 1976). Because the glycerophosphate oxidase is inoperative, glucose is metabolized into equimolar amounts of pyruvate and glycerol (Ryley, 1956; Opperdoes *et al.*, 1976). Glycerol is not used under anaerobic conditions and, as first noted by Ryley (1962), millimolar concentrations of glycerol will completely inhibit utilization of glucose. No studies of anaerobic metabolism in shot-stumpy trypomastigotes have been reported.

Glycolysis in *T. brucei* is probably not regulated by any mechanism other than a rate-limiting first step in glycolysis. This could be either glucose transport into the

trypanosome as suggested by Gruenber *et al.*, (1978) or the phosphorylation of glucose itself.

2.10.1.1 Glycosomes

Glycosomes are specialized microbodylike organelles found in trypanosomatids. The organelles are likened to the peroxizomes or microbodies of other eukaryotic cells (Opperdoes *et al.*, 1984). The microbody-like organelles contain the first seven enzymes of glycolysis, the last two enzymes of the pyrimidine biosynthetic pathway (Hammond *et al.*, 1981), the first enzymes of the ether-lipid biosyntheitic pathway (Opperdoes, 1984) as well as (in *T. brucei*) enzymes of glycerol metabolism, adenylate kinase and enzymes of CO_2 fixation (Opperdoes and Borst, 1977; Opperdoes *et al.*, 1981; Broman *et al.*, 1983). Such compartmentation of the glycolytic pathway inside a sub-cellular organelle has never been found so far in any other eukaryote. Other eukaryotic organisms carry out glycolysis in the cytosol.

In intact bloodstream forms of *T. brucei*, glycosomes are spherical or elipsoid, bounded by a single membrane. The glycosomes of *T. brucei* are homogenous in size, with a mean diameter of $0.27 \pm 0.03\mu m$, and it has been calculated from morphometric studies that a single *T. brucei* would contain on average 230 glycosomes, representing 4.3% of the total cell volume and about 8% of the total cell protein (Opperdoes *et al.*, 1984). Since glycosomes contain neither DNA nor ribosomes (Opperdoes *et al.*, 1984) enzymic proteins must be synthesized elsewhere in the cytoplasm.

Studies on the organelles and their enzymes have revealed that even within the organelle, the enzymes are associated with each other constituting a multienzyme complex (Misset *et al.*, 1986).

The analysis of the structural and functional properties of the glycosomal enzymes has shown that the individual enzymes possess specific physical properties which are absent or quite different from those of their mammalian host or other

glycolytic pathways of other organisms where glycolysis is not compartmentalized within an organelle (Misset *et al.*, 1986; Michels, 1988). The unique location of the glycolytic enzymes, their specific structural features, their crucial role in glycolytic energy supply to the parasite and the extra-glycosomal synthesis of glycosomal proteins make the glycosome and its constituting proteins ideal targets for specific drugs.

2.10.1.2 Mitochondrial System

The African trypanosome belonging to the '*brucei*' sub-group are characterised by an unusual flexibility in the biogenes of their single large mitochondrion. In the insect stage, the mitochondrion is fully developed and respiratory chain phosphorylation provides the main source of energy. In the bloodstream and tissue fluids of the vertebrate host, however, mitochondrial biogenesis can be completely repressed. The mitochondrion regresses into a promitochondrion which lacks a functional respiratory chain and the parasite depends entirely on glycolysis for energy production (Bowman and Glynn, 1976).

Instead of using lactate dehydrogenase to convert pyruvate into lactate for the reoxidation of glycolytically reduced nicotinamide adenine dinucleotide (NADH) as is the case in anaerobic glycolysis in mammalian cells, *T. brucei* uses a dihydroxyacetone phosphate: glycerol-3-phosphate shuttle (Figure 8) in combination with a terminal glycerol-3-phosphate oxidase for the reoxidation of its NADH (Goant and Sargent, 1960). This oxidase has been localized in the mitochondrion (Opperdoes *et al.*, 1977) and it reacts with oxygen without the intervention of pyridine nucleotide coenzymes or cytochromes. There is no evidence that the oxidase is coupled to phosphorylation of ADP. The oxidase is insensitive to inhibitors or mammalian respiratory chain such as cyanide, azide and antimycin A (Bowman and Flynn, 1976). Its high activity and specificity for glycerol-3-phosphate is sufficient to account for the high rate of respiration of bloodstream trypanosomes. The aromatic hydroxamic acids m-

chlorobenzhydroxamic inhibitors of alternative cyanide insensitive, terminal oxidases of mitochondria of plants and some fungi are also potent inhibitors of the trypanosomal glycerol-3-phosphate oxidase (Hill, 1976). Inhibition of respiration, however, does not harm the bloodstream form of *T. brucei*, since the organism has the capacity of switching from an aerobic to an anaerobic type of glycolysis.

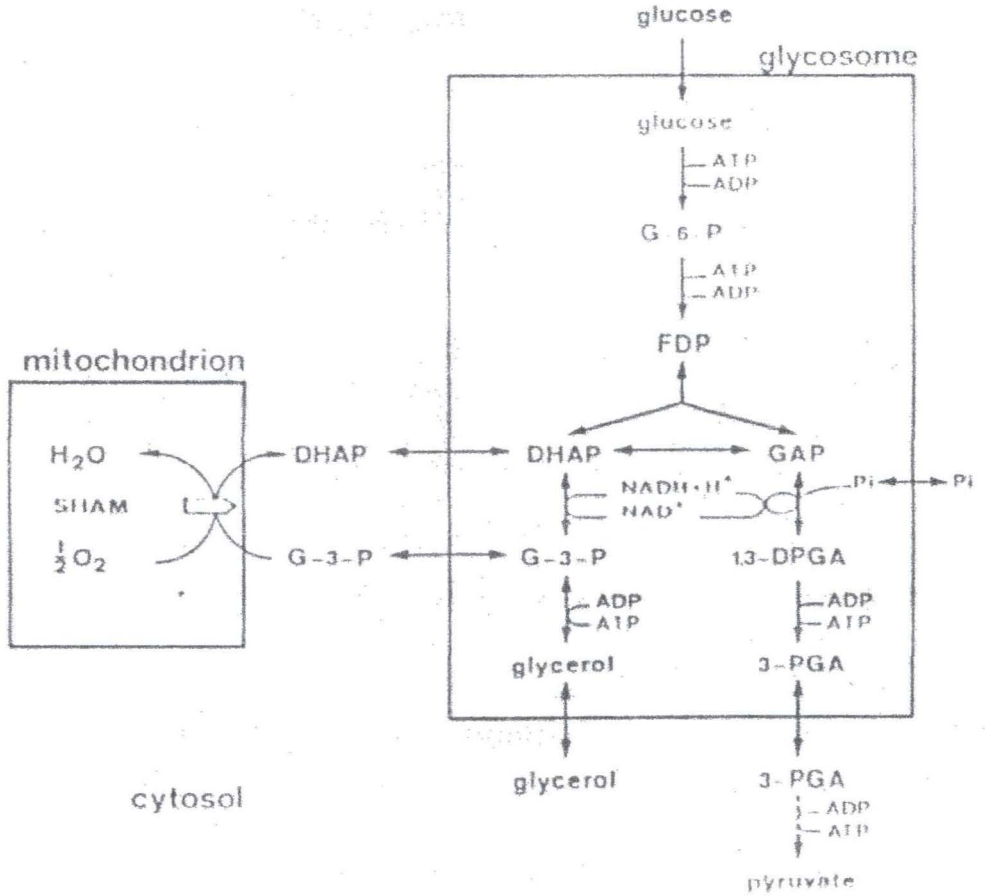


Figure 2.8: Compartmentation of glycolysis in *T. brucei*. Abbreviations: G-6-P, glucose 6-phosphate; FDP, fructose 1,6-diphosphate; DHAP, dihydroxy-acetone phosphate; GAP, glyceraldehyde phosphate; G-3-P, glycerol 3-phosphate; 1,3-DPGA, 1,3-diphosphoglyceric acid; Pi, inorganic phosphate; 3-PGA, 3-phosphoglycerate; SHAM, salicyl-hydroxamic acid (Opperdoes, 1982).

Upon transformation from bloodstream form to insect stage, first new dehydrogenases appear in the mitochondrion, allowing for the transfer of reducing equivalents from succinate, proline and NADH to oxygen via the alternative oxidase already present. In the second phase, a complete cytochrome chain appears and the synthesis of the alternative oxidase is partly, but not completely, shut off and respiration

becomes partly cyanide-sensitive (Bowman and Flynn, 1976). Based on inhibitor studies, it has been concluded that some of the glycerol-3-phosphate oxidase remains active in the insect stage and that at the position of cytochrome b, the trypanosomal respiratory chain is branched allowing for the flow of electrons from cytochrome b to oxygen, possibly via an O-type cytochrome. The presence of two terminal oxidases, cytochrome aa_3 and cytochrome o together, a situation usually found in prokaryotic cells, is highly unusual in eukaryotes (Stoppani *et al.*, 1980).

2.10.2 Lipid Metabolism

Lipids constitute 15 – 20% of the dry weight of African trypanosomes (Venkatesan and Ormerod, 1976), with the total lipid content of the stumpy forms being substantially higher than that of the slender forms. Trypanosomes contain the usual range of lipids found in eukaryotes including triacylglycerols, phospholipids, plasmalogens, sterols and isoperenoids (Dixon *et al.*, 1971, 1972; Carroll and McCrorie, 1986).

Although the fatty acid composition of bloodstream trypanosomes is similar to that of lipids found in the plasma of their mammalian hosts, some essential differences suggest that trypanosomes can regulate their fatty acid compostion (Mellors and Samad, 1989). *T. b. rhodesiense* possesses a higher proportion of long chain polyunsaturated fatty acids (22:5, 22:6) and linoleate (18:2), and lower levels of oleate (18:1) and C 16 fatty acids of the human host. The fatty acids of trypanosomes are mainly esterified as phosphoglycerides or cholesteryl esters, though they also exist as free fatty acids (Dixon *et al.*, 1972). Triacylglycerols constitute a minor part of tryapanosomal lipid in both slender and stumpy forms (Vankatesan and Ormerod, 1976). Myristate, a saturated fatty acid containing 14 carbon atoms plays an important role in the bloodstream stage of *T. brucei*. This molecule is a component of the glycosyl phosphatidylinositol (GPI) anchor of the trypanosome variant surface glycoprotein (VSG) (Ferguson, 1988). The two myristates, esterified to the glycerol of the GPI, inset into the lipid bi-layer of the

parasite's plasma membrane, thereby anchoring the VSG to the cell surface (Kimberly *et al.*, 2001).

African trypanosomes have very limited ability to synthesize fatty acids de novo and therefore dependent on external supplies and uptake. The stecorarian parasite, *T. lewisi*, can synthesize fatty acid from glucose, glycerol and acetate, though less glucose is used for fatty acid synthesis when exogenous fatty acid is available (Dixon *et al.*, 1971).

In eukaryotes, pyruvate dehydrogenase occurs in the mitochondria and the acetyl-CoA produced is used for fatty acid synthesis in the cytoplasm. *T. lewisi*, which has functional mitochondria, is able to oxidize pyruvate to acetyl-CoA and thereby synthesize fatty acids from glucose, glycerol and acetate. In contrast, salivarian bloodstream forms such as *T. b. brucei* and other trypanosomes readily incorporate exogenous fatty acids but with some selectivity so that, for example, linoleate is preferred over myristate or palmitate (Samad *et al.*, 1988). Specificity is also seen in the esterification of fatty acids. Linoleate and stearate are major components of the FFA fraction and are less common in cholesterol esters.

Phospholipids are essential components of the plasma membranes of all life forms and in trypanosomes they constitute 80% of the trypanosomal lipid (Dixon *et al.*, 1971). The major classes of phospholipids in trypanosomes are the same as those of found in the mammalian hosts namely; phosphatidylcholine, phosphatidylethano-lamine, sphingomyelin, phosphatidylinositol and phosphatidylserine. Plasmalogens have beenh found in the stumpy forms of *T. b. brucei* but not in the slender forms (Vankatesan and Ormerod, 1976). Phosphatidylcholine is the most abundant trypanosomal phospholipids (Carroll and McCrorie, 1986; Samad *et al.*, 1988). *T. b. brucei* does not metabolise exogenous phosphatidylcholine or phosphatidylethano-lamine, even when these are present in mixed phospholipids micelles. However, the organisms readily take up

exogenous lyso-phosphatidylcholine and lyso-phosphatidylethanolamine (Mellors and Samad, 1989). Trypanosomal phospolipase A_1 can hydrolyse exogenous lysophospholipids to yield glycerophospholipid and free fatty acid, and in the presence of amphiphiles it can cleave phosphatidylcholine to produce a 2-acylglycerophosphocholine (2-acyl-lyso-PC) and a fatty acid (Tizard *et al.,* 1977). The 2-acyl-lyso-PC undergoes a rearrangement to form 1-acyl-lyso-PC and can serve as a substrate for a second hydrolysis by the phospholipase A_1.

The bloodstream trypanosomes also contain acyltransferase activity which in the presence of exogenous acyl-coenzyme A and lysophospholipid can form phospholipids (PC) which are incorporated into their membranes (Mellors and Samad, 1989). Thus, the combination of the action of the trypanosomal phospholipase A_1 and the acyltransferase may lead to the depletion of exogenous lyso-PC levels observed in the plasma of infected ruminants (Roberts, 1975). The possible routes for the utilization of exogenous lysophosphatidylcholine and related lipids by *T. b. brucel* is summarised in Figure 2.9.

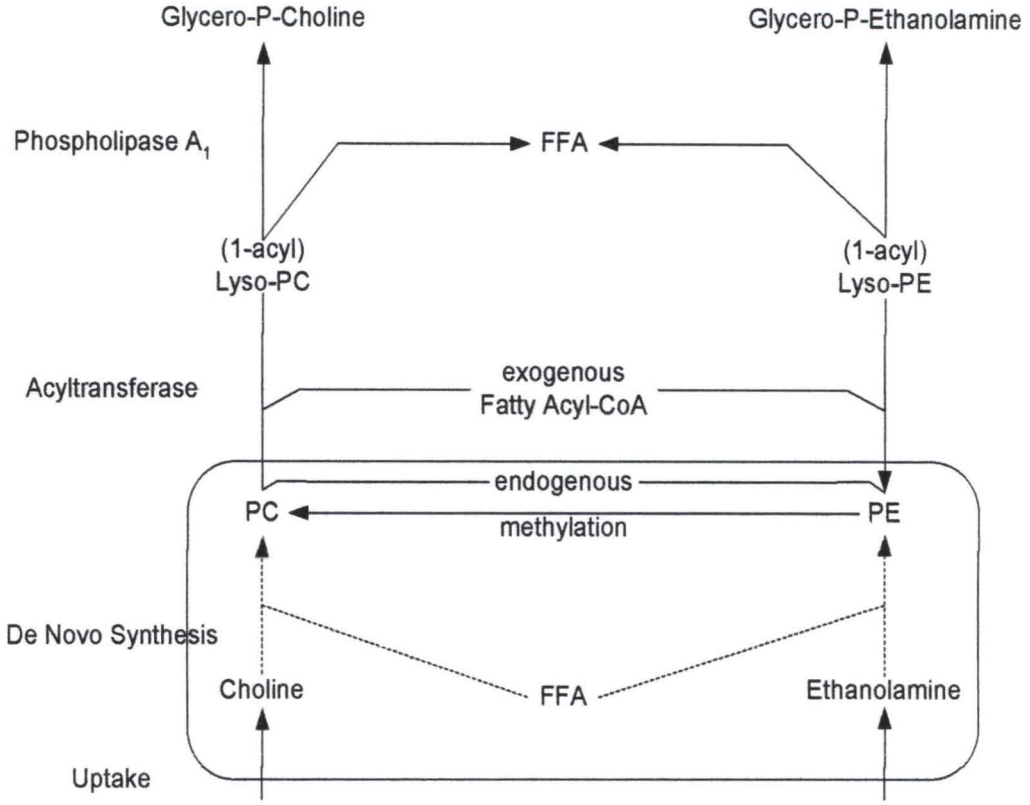


Figure 2.9: Metabolism of exogenous lysophosphatidylcholine (1-acyl-lyso-PC) and lysophosphatidylethanolamine (1-acyl-lyso-PE) by *T. b. brucei* (Mellors and Samad, 1989).

No phospholipase A_2 activity has been detected in trypanosomes (Mellors and Samad, 1989). Mammalian cells do not use exogenous CoA, hence competitive analogues of CoA may exert an inhibitory effect on trypanosomes but not highly unusual in eukaryotes (Stoppani *et al.*, 1980).

2.10.3 Amino Acids Metabolism

Exogenous amino acids are known to enter trypanosome by both diffusion and specific transport system (Voorheis et al., 1979), and there is evidence that blood forms of *T. brucei* are capable of ingesting and digesting proteins (Langreth and Balber, 1975). De Raadt and seed in 1977 suggested an important factor in determining when, during an

infection trypanosomes begin to develop in the cerebrospinal fluid (CSF). This factor is the availability of ingestible protein, amino acids and other nutrients. It was discovered that the parasite may have access to the CSF throughout an infection but be unable to grow until it has been enriched by proteins and other nutrients from degenerating tissues (Peruzzi, 1928). Goodwin and Guy, (1973) reported an increased levels of alnine and praline at certain stages of *T. brucei* infection of rabbits, whereas in the insect stages of *T. brucei*, the main energy sources are amino acids, especially proline, which is present in high concentrations in tsetse haemolymph. Proline is metabolized by proline oxidase and TCA cycle enzymes to aspartate, alanine and carbondioxide (Bowman and Flynn, 1976).

There is evidence that accumulation of end-products of amino acid metabolism contributes to the characteristic pathology of African trypanosomiasis (Newton, 1978). Transamination of tryrosine yields P-hydroxyphenylphyruvate, high concentration of which has been found in the urine but not in the blood of infected animals (Stibbs and Seed, 1975a). This metabolite is closely related to phenylpyruvate (formed by transamination of phenylalanine) which is a known inhibitor of adrenaline synthesis. Accumulation of either of these deamination products in the brain could contribute to the pathological picture of Gambian sleeping sickness (Newton, 1978).

Deamination of tryptophan yields pharmacologically active compounds. Indole pyruvate, the immediate product of tryptophan transamination is further metabolized to indole lactate, indole acetate and indole-3-ethanol (tryptophol) (Stibbs and Seed, 1975b; Sabella *et al.*, 1969). There is evidence that tryptophol can cause sleep, convulsion and death by respiratory depression when injected into mice and rats (Sabella *et al.*, 1969) and it has been suggested that trypanosomes in extravascular sites of the central nervous system produce similar effects. It is thought that tryptophol acts on cell membranes, perhaps by combining with the outer lipid bilayer (Tizard *et al.*, 1978) and in support of

this, Seed *et al.,* (1978) have shown that tryptophol rapidly lyse red blood cells. Similar action on synaptic membrane may cause changes in the transmission of impulses giving rise to behavioural changes and induce a steep-like state (coma). Seed and Hall, (1977) have estimated from *in vitro* measurements that a minimum of 3.2mg tryptophol/kg body weight could be formed in an infected mouse.

2.10.4 Purine and Pyrimidine Metabolism

In general, living organisms have two routes for purine nucleotide synthesis: the de novo pathway (Buchanam, 1960) involving the synthesis of purine and the salvage pathway (Kornberg, 1957) where preformed purine bases and nucleosides are taken up from the surrounding medium and utilized for the nucleotide synthesis. Parasitic protozoa, including the African trypanosome, generally lack the de novo pathway and instead rely on the preformed bases and nucleosides from the hosts (Gutteridge and Gaborak, 1979). In addition, the levels of phosphoribosyltransferase enzyme activities are higher than those of the nucleoside kinases (Davies *et al.,* 1983) suggesting that the trypanosomatidae preferentially salvage purine bases rather than nucleosides. These purines are acted upon by the salvage enzymes of the parasites and are trapped intracellularly as nucleotide to be incorporated into nuclei acids. The essential feature which distinguishes the purine metabolism in these parasites is an unusual substrate specificity of the hypoxanthine guaninephosphoribosyltransferease (Marr and Berens, 1983). The enzyme which is associated with the glycosome of the trypanosomes (Davies *et al.,* 1983) recognises the purine analogue allopurinol as the substrate and convert it to the corresponding ribonucleotide which then is accumulated in large quantities, become aminated, turned into triphosphate and finally incorporated into the RNA of the parasite Such incorporation is unique to trypanosomes (Marr and Berens, 1983).

Contrary to the situation with purine metabolism, the trypanosomes can synthesize their own pyrimidine bases (Hammond et al., 1981). The enzymes

carbamoylphosphate synthetase, aspartate carbamoyul trypanosomes and they are all soluble enzymes as in other cells (Hammond and Gutteridge, 1983). The fourth enzyme in this sequence in this sequence, which in mammalian cells is a dehydro-orotate dehydrogenase located in the mitochondria where it is ultimately linked to the respiratory chain, has been shown in trypanosomes to be cystosolic insofunctional dihydro-orotate oxidase which uses molecular oxygen (Pascal *et al.*, 1983). The last two enzymes of the pathway, orotate phosphoribosyl transferease and orodidine 5'-phosphate decarboxylase are soluble enzymes in mammals whereas in the trypanosomes both enzymes are tightly associated with glycosomes (Hammond and Gutteridge, 1983).

2.10.5 Peroxide Metabolism

Glutathione (GSH) is usually the major thiol species in both prokaryotic and eukaryotic cells (Meister and Anderson, 1983). The polyamines (Putrescine, spermidine and spermine) are also ubiquitous among living organisms and are also usually present in very high concentrations (Tabor and Tabor, 1984). Ameh, (1984) reported that the susceptibility to oxidative hemolysis increased in *T. gambiense* infected rats and Igbokwe *et al.*, (1994) showed an increased susceptibility of erythrocytes to *in vitro* peroxidation in *T. brucei* infected mice. Enormous amounts or hydrogen peroxide was produced by *T. brucei* organisms (Meshnick *et al.*, 1977) and macrophages activated by *T. brucei* also produced superoxide anions and hydrogen peroxide after respiratory burst (Vray *et al.*, 1991).

Glutathione has an important protective function in that together with glutathione peroxidase, it removes peroxides formed as toxic by-products of aerobic metabolism. GSH also acts as a free radical scavenger particularly for activated oxygen species resulting in the formation of oxidized glutathione (GSSG). This again can be cyclically reduced to reform GSH through the action of glutathione reductase. Examination of the GSH-metabolism in trypanosomes led to the discovery that they do not enzymatically

reduce glutathione, instead glutathione is reduced through a spermidine-containing glutathione based peptide, trypanothione that is apparently unique to kinetoplastida (Failamb *et al.*, 1985; Murgolo *et al.*, 1989). Key enzymes in the function of the trypanothione are trypanothione reductase and trypanothione peroxidase.

2.11 Some Biochemical Changes in African Trypanosomiasis

2.11.1 Changes in Serum and Plasma Proteins

Changes in the serum proteins have received considerable attention. The most striking abnormality of serum protein sin trypanosoma infections is decreased in albumin and elevation in total globulin concentration in infected animals (Anosa, 1988) resulting in a reduced albumin: globulin ratio. The decrease in plasma albumin concentrations could be due to plasma expansion (Anosa, 1988), proteinuria (Bruijn, 1987) or hepatocellular damage (Anosa and Isoun, 1983; Saror, 1980). The total plasma protein concentration was reported to be decreased (Kalu *et al.*, 1989), unaltered (Anosa, 1988) or increased (Anosa, 1988) in trypanosome infections of animals. It was also shown that total plasma concentration did not increase in trypanosome infected animals with little resistance, but increased in those with more resistance (Anosa, 1988).

Hypergammaglobulinaemia was consistently reported in several infections (Anosa and Isoun, 1976, Anosa and Kaneko; 1983; Singh *et al.*, 1982; Verma and Gautam, 1983) while alpha and beta-globulin were either normal (Anosa and Isoun, 1976) or depressed (Wellde *et al.*, 1974). These observations showed that the most consistent changes in serum protein levels are depression of albumin levels and elevation of globulin levels due to hypegammaglobulinaemia. Factionation of the gamma-globulins showed that increases in immunoglobulin M (IgM) levels are more commonly reported and pronounced than increases in IgG levels. The increases in these immunoglobulin levels are associated with polyclonal stimulation of β-lymphocytes (Wery *et al.*, 1982; Mansfield, 1978).

African sleeping sickness is associated with marked alterations in the composition and levels of host serum lipoproteins. Several studies have shown that lipoproteins play a key role in *T. brucei* parasites. *Trypanosome b. brucei* are able to obtain cholesterol by internalizing and degrading human low density lipoproteins (Gillett and Owen, 1987). Coppens *et al.*, (1987) reported that the host low density lipoprotein (LDL) are taken up by receptor mediated endocytosis through the flagella pocket and the trypanosome LDL receptor was subsequently isolated and analysed (Coppen *et al.*, 1988).

Humans are resistant to infection by *T. b. brucei* owing to the existence of a trypanosomicidal activity associated with the high density lipoprotein (HDL) fraction (Rifkin, 1991a, b). The human serum HDL fraction is rich in apolipoprotein A (Owen and Gillette, 1987). It is also known to contain in addition to the common apolipoproteins A-I, A-II, C-I, C-II and C-III, at least three unique proteins (Hajduk *et al.*, 1989). The analysis of plasma lipoproteins by polyacrylamide gel electrophoresis in rabbit infected with *T. b. brucei* in comparison with control rabbit showed that the very low density lipoprotein band was intensified. That of the low density lipoprotein band was to a lesser extent (Rouzer and Cerami, 1980).

2.11.2 Changes in Serum and Plasma Lipids

On autolysis, *T. congolense* and *T. brucei* have been shown to generate an active phospholipase A_1 (Mellors, 1985). Large quantities of phospholipase A_1 probably of trypanosome origin have also been found *in vivo* in tissue fluids of rabbits infected with *T. brucei* (Hambey *et al.*, 1980, 1981, 1984). This enzyme, absent in the fluid of normal rabbit was also demonstrated in the plasma but at a considerably lower level (Hambrey *et al.*, 1980; Mellors and Samad, 1989).

During mammalian experimental trypanosomiasis modification of serum triglyceride and cholesterol levels occur. An increase in triglycerides was found in rats

infected with *T. rhodesiense* (Dixon, 1967) and in rabbits infected with *T. b. brucei* (Rouzer and Cerami, 1980). Different variations in the cholesterol levels were observed. A rise was described in rabbits inoculated with *T. brucei* by Goodwin and Guy, (1973) while Dixon, (1967) reported a slight decrease in rats infected with *T. congolense* and *T. vivax*. In the same vein Traore-Leroux *et al.*, (1987) reported a large decrease in the HDL-cholesterol level in cattle infected with *T. congolense*. These are in contrast to the earlier report that serum lipid and cholesterol values increased three to four-fold at the terminal stages of the infection in rabbits (Goodwin and Guy, 1973; Diehl and Risby, 1974). A report by Huet *et al.*, (1990) however described a significant increase in cholesterol concentration and phospholipids concentration that did not appear significantly altered. In contrast to this report and that of Robert, (1975), Valli and Mills, (1980) reported significantly increased phospholipids levels.

Lipoprotein analysis (Huet *et al.*, 1990) revealed a significant rise in apolipoprotein B concentration, a significant decrease in apolipoprotein A-1, increase in low density lipoproteins and decrease in high density lipoprotein.

Serum-free fatty acid (FFA) concentration in trypanosomiasis has been reported (Akanji, 1985; Akanji and Kalu, 1986) to rise continuously with increase in level of parasitaemia. Other reports however, indicate that serum FFA bind to plasma albumin (Tizard *et al.*, 1977; 1978a, b, c). It is suggested that where heavy parasitaemia are produced, the amount of FFA may be sufficient to exceed the binding capacity of serum albumin (Tizard *et al.*, 1977).

2.11.3 Changes in Serum Enzymes

Serum Aspartate transaminase (AST) activity increased markedly in *T. rhodesiense* infection of mice (Moon *et al.*, 1968), man (Barret-Connor *et al.*, 1973) and monkeys (Sadun *et al.*, 1973); while decreased value was recorded in *T. congolense* infection of cattle. Serum Alanine transaminase (ALT) levels markedly increased in *T.*

rhodesiense infection of mice (Moon *et al.*, 1968) and monkeys (Sadun *et al.*, 1973), rose sharply in *T. vivax* infections of sheep and cattle (Stephen and Gray, 1960; Gray, 1963) and increased mildly in *T. evansi* infection of buffalo calves (Singh and Gaur, 1983). ALT and AST markedly increased in *T. congolense* infection of mice (Whitelaw *et al.*, 1980). The causes of the increases in transaminase activities could be specific organ damage and/or release of parasite metabolic products (Singh and Gaur, 1983; Whitelaw *et al.*, 1980). The observation that very marked increases in transaminases occurred in mice infected with *T. rhodesiense* for 5days (Moon *et al.*, 1968) or *T. congolense* for 8days (Whitelaw *et al.*, 1980) which are both too short for any significant organ damage to occur suggests that most of the transaminases are derived from parasites (Anosa, 1988). Lactic dehydrogenase levels increased markedly in acute *T. congolense* infection of mice (Whitelaw *et al.*, 1980) and in *T. rhodesiense* infection in man (Barret-Connor *et al.*, 1973) but decreased in *T. vivax* infection of cattle (Wellde *et al.*, 1983) and was normal in *T. gambiense* infection of rat despite the occurrence of necrotic lesions (Diehl and Risby, 1974). In an assessment of the blood sera of rabbits infected with *T. brucei*, Arowolo *et al.*, (1988) showed that the infected rabbits had high levels of alkaline phosphatase (ALP), bilirubin and cholesterol accompanied by low level of cholinesterase. Mercado, (1969) also reported that the activity of succinate dehydrogenase and cytochrome oxidase reduced significantly in the liver of heavily infected mice. The effect was attributed largely to marked liver necrosis which accompanied the infection and the consequent loss of mitochondria, the sites of these oxidative enzymes.

2.11.4 Electrolyte Changes in Serum and Plasma

Most investigations in blood chemistry alterations resulting from trypanosome infections in animals and man have given limited attention to inorganic elements of the blood and scarcely has the hydrogen ion concentration been considered.

Linton, (1930) described no variation in the blood chlorides of rats infected with *T. equiperdum.* Fiennes *et al.,* (1946) however demonstrated that in adult cattle and calves, blood chloride value may reach high levels and remain high in adult cattle but gradually return to normal in calves while Kalu *et al.,* (1989) described increased levels that were not significantly different from normal as the disease progressed. Hyponatraemia was reported in human *T. rhodesiense* infection (Barret-Connor *et al.,* 1973) and in *T. evansi* infection of camels (Raisinghani *et al.,* 1981) while blood sodium levels were normal in acute *T. rhodesiense* infection of mice (Moon *et al.,* 1968). Serum and plasma levels of potassium are reported to have been elevated in *T. brucei* and *T. equiperdum* infections in rats (Zwemer and Culbertson, 1939; Ikejiani, 1946), but decreased in *T. evansi* infection of camels (Raisinghani *et al.,* 1981) and normal in *T. rhodesiense* infection of mice (Moon *et al.,* 1968). The increases in postassium were correlated with decreases in RBC values and were attributed to release of potassium from RBC and damaged tissue coupled with the effects of kidney damage (Ikejiani, 1946). Calcium level was described as normal in the serum of mice infected with *T. rhodesiense* (Moon *et al.,* 1968) reduced in *T. evansi* infections of camels (Raisinghani *et al.,* 1981) and during relapses in *T. congolense* infection of cattle (Fiennes *et al.,* 1946). Phosphate levels decreased in *T. evansi* infection of camels (Raisinghani *et al.,* 1981) and *T. congolense* infection of cattle (Fiennes *et al.,* 1946) but was normal in *T. rhodesiense* infection of mice (Moon *et al.,* 1968). Depressions of calcium and phosphate levels were thought to be due to severe damage to the thyroid gland (Fiennes *et al.,* 1946). Serum magnesium levels decreased during relapses but returned to normal thereafter in cattle infected with *T. congolense* (Fiennes *et al.,* 1946). Plasma copper levels fluctuated in cattle infected with *T. vivax* or *T. congolense* but remained within normal limits (Saror, 1976).

In trypanosomes iron plays a pivotal role for DNA synthesis (Dormeyer *et al.*, 1997), energy generation (Fairlamb and Bowman, 1977; Clarkson *et al.*, 1989) and oxidative stress (Le Trant *et al* 1983). Compared with mammalian cells, bloodstream *T. brucei* has low iron content (Schell *et al.*, 1991), mainly due to the lack of cytochromes. Iron-chelation could thus be an attractive strategy for the development of new antiparasitic drug. So far, four iron-dependent enzymes have been identified in bloodstream forms of *T. brucei:* aconitase (Overath *et al.*, 1986), alternative oxidase (Fairlamb and Bowman, 1977; Clarkson *et al.*, 1989), ribonucleotide reductase (Dormeyer *et al.*, 1997; Hofer *et al.*, 1997), and superoxide dismutase (Le Trant *et al.*, 1983). Low serum iron was reported in animals with acute trypanosomiasis (Valli *et al.*, 1978) as well as in more chronic infections (Tartour and Idris, 1973).

2.12 Host and Parasite-Derived Factors

To view in a microscope field, a blood sample taken at peak parasitaemia of trypanosome infection evokes the intuitive realization that the entire body system of the patient will be affected. The damage caused to the host, the immune responses and the alterations in physiology and biochemistry in different organs and in the blood have been described in a vast amount of literature but an understanding of the mechanisms which bring about the changes has been slow to emerge (Pentreath, 1991). The problem consists of identifying substances or cell types which are produced or modified directly by the parasite and separating these from other effects. The primary biochemical or cellular lesions which could altered the production of an immuno-regulatory substance or I a chemical substance released by the parasite are in turn followed by the myriad lines of subsequent pathological events. The diverse nature of the consequences ranging from thrombocytopaenia, myocarditis, meningoencephalitis, anaemia, malfunctions of the reproductive organs and the liver to headache and somnolence among others (Davis, 1982; Ikede *et al.*, 1988; Murray and Dexter, 1988; Zwart, 1989) in trypanosomiasis

imply that, several factors from the trypanosome could be involved. It is also possible that some of these factors are originating from the host, induced by the presence of the trypanosomes.

2.12.1 Immune Response Modifiers

The search for keys to the pathology of African trypanosomiasis has revealed alterations in pharmacologically active substances that could bring about widespread malfunctions. The levels of several monoamine transmitters and their metabolites are significantly altered in localized brain areas in infected animals (Stibbs and Curtis, 1987). The pathogenesis is ultimately linked to the inability of the immune system to get rid of the parasite. The damage caused by the active amines and peptides are in a real sense subsequent to the altered immune response and immune complexes especially in the chronic disease caused by *T. b. gambiense* (Pentreath, 1991). The complex ways in which the parasite interacts with the immune system, upsetting the balance of cytokines and often mediator substances and thus producing the immunopathology are becoming clearer. The measurements of cytokines and immune mediators in the cerebrospinal fluid of late stage patients infected with *T. gambiense* showed selective and huge increases in prostaglandin D_2 in the fluid (Pentreath *et al.*, 1990). Prostaglandin D_2 is one of the ultimate sleep regulating substances (Kreuger, 1990). Others may include interleukin-1 (IL-1) and prostaglandin E_2 (Kreuger, 1990). The advanced stages of the trypanosome infection are characterized by marked immuno-suppression and continuous daytime sleep (Apted, 1970; Askonas and Bancroft, 1984). It seems likely that the elevated prostaglandin D_2 may be associated with several of the neurological manifestations such as headache and somnolence. The fact that prostaglandins are involved in multi-functional and feedback processes makes their roles in the initiation of pathology rather difficult.

2.12.2 Haemolytic Factors

While Davis *et al.*, (1974) reported thrombocytopaenia in trypanosomiasis extra cellular fractions derived from trypanosomes have been shown to elicit platelet aggregation (Nwagwu *et al.*, 1989). Landsteiner and Raubitsahek, (1907) found that degenerating trypanosomes, probably *T. brucei*, generated a lipid soluble factor that could lyse red cells. Subsequently, Fiennes (1954) demonstrated the intermittent presence in cattle infected with *T. congolense* of plasma factor that was capable of lysing normal bovine red cells. Studies on mice infected with *T.* congolense, *T. vivax* and *T. brucei* (Murray *et al.*, 1979a) and in rats infected with *T. brucei* (Murray, 1979) also indicated the presence of haemolysins in plasma within 2 to 3 days of infection.

Haemolytic activity has been demonstrated in lysates of *T. brucei* (Huan *et al.*., 1975) as well as in *T. congolense* and *T. vivax* (Murray *at al.*, 1979a). Although the nature of the haemolytic factors particularly those responsible for haemolytic activity in the plasma are yet to be extensively investigated, a range of enzymes that could playa role in host cell, or erythrocyte damage has been identified. These include proteases (Lonsdale-Eccles and Grab, 1986; Knowles *et al.*, 1989; Boutignon *et al.*, 1990), phospholipases (Mellors, 1985; Samad *et al.*, 1988) and neuraminidases (Esievo, 1983; Esievo *et al.*, 1990). Huan *et al.*, (1975) also identified a heat-stable, trypsin-sensitive substance with a molecular weight of 12 kDa which was present in the serum from day 2 after the onset of *T. brucei* infection of mice. Although there could be more factors yet unknown, only the proteolytic and perhaps membrane fraction phospholipase activities have been demonstrated as originating from trypanosomes by using extracellular fractions derived from trypanosomes (Lonsdale-Eccles and Grab, 1986; Nwagwu *et al.*, 1987; Boutignon *et al.*, 1990).

2.12.3 Trypanosomal Proteases

Many studies have been undertaken on parasitic proteinases in order to define their specific function in the life of parasites (Barxrett *et al.,* 1988). Several studies on trypanosomes have shown that they contain multiple proteases, including both cysteine and serine types, and have provided some information on their sub-cellular location and substrate specificity (North *et al.,* 1983; Lonsdale-Eccles and Mpimbaza, 1986; Lonsdale-Eccles and Grab, 1987; Pamer *et al.,* 1989; Sakanari *et al.,* 1989; North *et al.,* 1990; Robertson *et al.,* 1990). *T. b. brucei* lysates have shown five proteolytic forms at M_r 28,000, 42,000, 60,000, 90,000 and 105,000 (Lonsdale-Eccles and Mpimbaza, 1986) and Lonsdale-Eccles and Grab, (1987) reported that the thiol-dependent proteolytic activity in *T. b. brucei* is found in lysosome-like organelles while alkaline serine proteinase activity belong to the soluble portion. The lysosomal-like activity was predominantly cathepsin-L-like and had a M_r 27,000 when analyzed on fibrinogen-containing sodium dodecyl sulphate polyacrylamide gel (Lonsdale-Eccles and Grab, 1987). Reports have shown that *T. b. brucei* enzymes could be presented in the plasma of infected it hosts and hence could be involved in pathogenesis (Boid *et al.,* 1980; Hambrey *et al.,* 1980; Knowles *et al.,* 1987, 1989). In the plasma of mice infected with *T. b. brucei*, Knowles *et al.,* (1987) found a trypanosome equivalent of peptidase showing a molecular weight of 40,000. A similar parasite peptidase was found in the plasma of heifers infected with *T. congolense* (Knowles *et al.,* 1989). Although trypanosomes contain several proteases (Lonsdale-Eccles and Mpimbaza, 1986), Nwagwu *et al.,* (1987) detected only a protease of M_r 12,000 in trypanosome-derived extracellular fractions. Boutignon *et al.,* (1990) detected M_r 30,000 and 70,000 in similar preparations. It is possible that some of the proteases are never released.

2.12.4 Trypanosomal Phospholipase

African trypanosomes rapidly degrade their phospholipids when autolysed at room temperature and this degradation has been shown to be due to a very active phospholipase A_1 (Tizard *et al.*, 1978a). It has been suggested that this enzyme contributes to the pathology of trypanosome infections, damaging cells directly or through free fatty acids and lysophosphatidylcholine produced from host phospholipids (Tizard *et al.*, 1978b; Samad *et al.*, 1988). In contrast to lysophospholipids, exogenous phospholipids are not metabolized by *T. brucei*.

Phospholipase A_1 similar to that found in *T. brucei* appeared in the tissue fluid about seven days after infection with this parasite, increasing with the parasite burden (Hambrey *et al.*, 1980). Phospholipase A_1 was also detectable in blood plasma from the' infected rabbits but at a level considerably lower than in the tissue fluid (Hambrey *et al.*, 1980).

In *T. brucei*, the active phospholipase A_1 is present in high concentrations and it can generate lysophospholipids including both 1-acyl and 2-acyl sn-glycero-3-phosphocholine (Hambrey *et al.*, 1981, 1984). Living trypanosomes do not metabolize exogenous micellar phosphatidylcholine which in the absence of a detergent is a poor substrate for trypanosomal phospholipase A_1 (Samad *et al.*, 1988). The detergent-activated phospholipase activity is a membrane-bound trypanosomal enzyme (Hambrey *et al.*, 1981; Samad *et al.*, 1988; Mellors and Samad, 1989). In the absence of a detergent or exogenous fatty acyl-CoA, the product of lysophosphatidylcholine metabolism is principally glycero-3-phosphocholine which results from phospholipase A_1 action (Sage *et al.*, 1981). In the presence of exogenous oleoyl-CoA, the major metabolite of lysophosphatidylcholine by trypanosomes is phosphatidylcholine. The phosphatidylcholine product was found to be associated with trypanosomes whereas the water soluble glycero-3-phosphocholine was found exclusively in the suspending

medium. This suggests that phospholipase A_1 action is extracellular (Samad *et al.*, 1988). Although phospholipase A_2 has not been detected in trypanosomes, phospholipase A_1 activity appears to be part of a membrane-bound enzyme sequence also containing acyl-CoA ligase and lysophospholipid acyltransferase (Mellors and Samad, 1989).

2.12.5 Trypanosomal Neuraminidase

There is evidence that sialic acids may play a role in the pathogenesis of red cell destruction in African trypanosomiasis. There are reports that *T. vivax* produces neuraminidase (Esievo, 1981, 1983), that *T. congolense* attaches to sialic acids on red cells (Banks, 1979) and that sialic acids are constituents of the carbohydrate moieties of the variable surface glycoproteins of *T. congolense* (Rautenberg *et al.*, 1981).

It has been found that unlysed *T. vivax* exhibit neuraminidase activity *in vitro*. The degree of sialidase activity was linear with the number of trypanosomes and was inhibited by influenza serum (Esievo, 1983). Based on these findings it might be that the early anaemia which occurs in infected animals could be attributed to the activity of trypanosome sialidase which might cleave surface sialic acid (Durocher *et al.*, 1975), rendering erythrocytes more prone to phagocytosis, directly (Durocher *et al.*, 1975) by immunoglobulin (Jancik *et al.*, 1978) or by activation of the classical or alternate pathway of complement (Broman *et al.*, 1983). Furthermore, cleavage of sialic acid from red cell membranes would expose new epitopes on the surface of the affected cells (Pirofsky, 1969), an effect that could theoretically lead to antibody production against these exposed epitopes, and increased erythrophagocytosis.

It should also be noted that proteolytic enzymes which, as already discussed, may be released into the circulation from trypanosomes can remove variable fractions of sialic acid from red cell membranes in the form of glycopeptides (Cook *et al.*, 1960)

The possibility that parasitic neuraminidase might play a role in red cell damage *in vivo* was further supported by the finding that the onset of anaemia in *T. vivax* infected

cattle was preceded by a significant decrease in mean red cell surface sialic acid concentrations, starting 4 days after infection with the biggest drop occurring between 6 and 14 days, followed by a return to near-normality by day 15 (Esievo *et al.*, 1982). What was also significant was that these changes reflected changes in parasitaemia. The free serum sialic acid also showed an increase, an observation also made by Magaji, (1975) in cattle infected with *T. vivax* and *T. brucei*.

Another aspect of the possible role of sialic acid in the pathogenesis of anaemia is the observation that *T. congolense* binds *in vitro* to bovine red cells through neuraminic acid receptors (Banks, 1979). Banks, (1980) showed that damage to red cells occurred only when the organisms had attached to red cells, and antibody or complement had bound to the parasite. It was concluded that red cell damage was mediated through binding of anti-trypanosomal antibody and complement activation leading to increased erythrophagocytosis.

T. cruzi, the causative organism of Chagas disease has been found to contain a neuraminidase and live parasites have been shown, to have the capacity to remove sialic acid not only from red cells (Pereira, 1983) but also from the surface of endothelial and myocardial cells (Libby *et al.*, 1986).

2.12.6 Trypanocidal Factors

There is much evidence to support that in *T. congolense*, *T. vivax* and *T. brucei* infections in cattle, the height of parasitaemia can determine the severity of anaemia (Murray and Dexter, 1988), thrombocytopaenia (Davis, 1982) and leukopaenia (Ellis *et al.*, 1987). The host can and does play an important role in controlling parasite growth. Several host factors have now been shown to have a major effect on the severity of African trypanosomiasis. Of this, the most significant are age, nutritional status and breed.

It has long been recognized that certain breeds of cattle are able to survive in tse-tse infested areas and resist the effects of trypanosomiasis when other breeds rapidly succumb (Pierre, 1906; ILCA, 1979). This trait is termed 'trypanotolerance' and is generally attributed to the indigenous taurine breeds of cattle in West and Central Africa namely the N'Dama and West African Short-Horn. Major comparative investigations on the question of trypanotolerance have been carried out on cattle in Nigeria (Roberts and Gray, 1973), the Gambia (Murray *et al.,* 1982), Senegal (Traore *et al.,* 1978) and Kenya (Ismael *et al.,* 1985; Njoku *et al.,* 1985). The main breeds studied were the trypanotolerant N'Dama and trypanosusceptible Zebu (Boran). Irrespective of the route of infection, the outcome of each study consistently confirmed the superior resistance of the N'Dama (and the West African Short-Horn) and showed that the basis of the trait lay in the capacity of these animals to develop less severe anaemia. Furthermore, their resistance to anaemia appeared to be correlated with the ability to limit the intensity, prevalence and duration of parasitaemia to a significantly greater extent than the trypanosusceptible Zebu or European breeds (Murray *et al.,* 1982).

Humans are resistant to *T. b. brucei* infection. This has been associated with the high density lipoprotein (HDL) of the human serum (Rifkin, 1978, 1991a). The human serum HDL fraction is rich in apolipoprotein A and also contains minor sub-classes of apolipoprotein A-I, A-II, C-I, C-II and C-III (Owen and Gillette, 1987, Hajduk *et al.,* 1989). In contrast, humans infected with *T. b. gambiense* had low levels of HDL and apolipoprotein A-I but had increased levels of LDL and apolipoprotein B (Huet *et al.,* 1990).

The extent to which virulent African trypanosomes can multiply in different mammalian hosts may in part be regulated by toxic properties associated with the host HDL (Seed *et al.,* 1990; Rifkin, 1991b). HDL from hosts resistant (human, baboon) or susceptible (rat, rabbit) to *T. brucei* infection were isolated and their trypanocidal activity

was determined *in vitro* in cell lysis assays. Rabbit and rat HDL were not cytotoxic while baboon and human HDL rapidly lysed trypanosomes within 2hr. at 37°C. Analysis of the phospholipid composition of the HDL preparations suggested correlation between trypanocidal activity and phosphatidylinositol content. Phospholipase digestion of HDL led to loss of trypanocidal activity indicating the importance of native phospholipids in maintaining trypanocidal activity of HDL. Although the mechanism by which HDL lyses trypanosomes remains to be elucidated, these results suggest an important role for phospholipids in determining the specificity of this cytotoxic property of HDL.

The chemical nature of the trypanocidal factor in human serum has been investigated (Seed and Sechelski, 1989; Rifkin, 1991a). Results indicate that although trypanocidal factor is contained within the HDL fraction of the human serum, it is apparently not one of the HDL complex such as apolipoprotein A-I, A-II or apolipoprotein B. The factor, as suggested, appears to be a minor component of the HDL fraction whose chemical nature is still uncertain. In contrast to this, Barth (1989) reports that the trypanocidal factor from human serum is neither a component of the HDL nor the LDL. The factor isolated from, normal human serum (NHS) showed lytic activity against *T. b. brucei* strains as did normal human serum whereas NHS-resistant trypanosomes of the *T. b. rhodesiense* sub-species remained unaffected. The factor described by Barth, (1989) is a protein complex of high molecular mass (above 1000 kDa) and comprises of four peptides. No HDL proteins were present in the active fraction and neither HDL nor Low density lipoprotein (LDL) showed any trypanocidal activity.

Extracellular killing of trypanosome amastigotes by human eosinophils has been reported (Villalta *et al.*, 1987). Granules released from human eosinophils upon interaction with the amastigote *in vitro* were seen attached to the surface of non-internalized parasites by electron microscopy. Amastigote damage was preceded by the

binding of eosinophil granule material to its membrane and eosinophil major basic protein (MBP) bound to the parasite surface was readily detectable. Eosinophils known to be capable of destroying phagocytosed amastigotes could also contribute to the clearance of these parasites through extracellular killing (Villalta *et al.*, 1987).

An acid-lipase, capable of solubilising glycophosphatidylinositol-anchored proteins has been detected in the normal human serum (de Almeida *et al.*, 1988). The lipase can by its activity convert the membrane form of the variant surface glycoprotein of *T. brucei* to a water soluble form. The serum lipase is a glycoprotein and is optimally active at pH 5.4 but inhibited by chelating agents.

2.13 Drug Targets in African Trypanosome

A diverse range of drug targets has been identified and validated in trypanosomes. These include several organelles (glycosomes, acidocakisosomes kinetoplast) that are not represented in the mammalian host and biochemical pathways that differ significantly from host counterparts (carbohydrate metabolism, protein and lipid modification, response to oxidative stress, cell cycle), (Naula and Burchmore, 2003). However, there has been little progress in developing novel drugs. Pharmaceutical companies are unwilling to invest in the development of drugs for market that comprises some of the world's poorest people.

2.13.1 Carbohydrate Metabolism

Bloodstream form trypanosomes depend upon glycolysis for energy production and cannot survive even transiently in a glucose-deplete environment (Seyfang and Duszenko, 1991). Moreover, most of the trypanosome glycolytic pathway enzymes, unlike those of mammalian cells, are sequestered in microbodies called glycosomes. These features make glucose metabolism an attractive target for antitrypanosomal drug design but glucose metabolism is equally critical for the mammalian host and inhibitors must therefore act by targeting unique features of the trypanosome glycolytic pathway.

Trypanosome glucose uptake systems are necessarily exposed at the surface of the parasite and display a number of functional differences from mammalian counterparts (Barrett *et al.*, 1998). Modelling indicates that glucose uptake as an excellent drug target (Baker *et al.*, 1999).

The compartmentation of glycolysis appears to be essential for metabolic control in trypanosome (Blattner *et al.*, 1998) and recent data indicates that perturbation of glycosomes biogenesis is a promising target (Furuya *et al.*, 2002). Outside the glycosomes, targets that are vital for energy production have been validated by the use of inhibitors. Pyruvate kinase is a cytosolic enzyme that mediates net ATP production through glycolysis and the pyruvate is excreted via a plasma membrane pyruvate transporter (Wiemer *et al.*, 1992), inhibition of which rapidly kills the parasite (Wiemer *et al.*, 1995). The trypanosome alternative oxidase, responsible for oxidising glyceraldehydes-3-phosphate and critical for maintaining the redox balance of the glycosomes, is a plant-like enzyme that has no counterpart in the host (Chaudhuri *et al.*, 1998). A second pathway for glucose metabolism, the pentose phosphate pathway, is unusual in trypanososmes in that many of the constituent enzymes have plant-like ancestry (Hannert *et al.*, 2003) and thus present good drug targets.

Carbohydrate anabolism also represents an important target in trypanosomes because expression of cell surface glycoproteins is critical to survival. Galactose metabolism has been shown to be essential by disruption of the trypanosome UDP-Glc-4'-epimerase (Roper *et al.*, 2002). Many of the enzymatic steps that are involved in the synthesis of complex carbohydrates and glycoproteins may also prove to be useful drug targets.

2.13.2 Kinetoplast DNA

The mitochondrial DNA of trypanosomes forms an unusual network of several thousand catenated circles. Replication of this structure is mediated by multiple DNA

polymerases (Klingbeil *et al.*, 2002) and a battery of proteins that include a topoisomerase (Klingbeil *et al.*, 2002; Morris *et al.*, 2001). The kinetoplast is an attractive drug target because mammalian cells do not possess such an organelle and procyclic (Vector stage) trypanosomes die when expression of the kinetoplast topoisomerase is blocked by RNA interference (Wang and Englund, 2001). Topoisomerase inhibitors are effective against a number of parasites (Nenortas *et al.*, 1998) but it was not clear that perturbation of kinetoplast replication would be lethal because bloodstream trypanosomes of other species can exist without a kinetoplast (Schnaufer *et al.*, 2002).

2.13.3 Reactive Oxygen Intermediates

Trypanosomes encounter reactive oxygen species in the mammalian bloodstream. Enzymes that confer protection against oxidant stress are considered to be excellent drug targets and studies on trypanosomes and related parasites have revealed a number of unusual features in these pathways. Low molecular weight thiols are of critical importance for all cells in defence against reactive oxygen species but trypanosomes express a unique thiol called trypanothione that subsumes the role of glutathione in other cells. Trypanothione is maintained in a reduced state by the activity of trypanothione reductase, an enzyme that is unique to and essential for trypanosomes and related parasites (Dumas *et al.*, 1997). The primary source of reducing intermediates for the maintenance of a reduced pool of low molecular weight thiols is the pentose phosphate pathway (Barrett, 1997) and specific inhibitors of trypanothione reductse and of the synthesis of trypanothione, are reportedly under development (Schmidt and Krauth-Siegel, 2002). However, despite convincing studies that validate trypanothione reductase as a target, no inhibitor has been developed to date (Naula and Burchmore, 2003).

Nitrofurans, such as nifurtimox and aminoimidazoles, such as megazol, are believed to interfere with thiol groups and the generation of free radicals (Bouteilla *et al.*,

1995; Moreno, 1988; Maya *et al.*, 1997). Nifurtimox has been used to treat HAT without licence (Pepin *et al.*, 1992). Megazol has failed to progress to human trial because it is mutagenic in Ames test (Ferreira and Ferreira, 1986). Nevertheless, positive results with these compounds indicate that their supposed targets are valid and that nitrofurans and aminoimidazoles may represent useful lead compounds.

2.13.4 Lipid Metabolism

Lipid metabolism has not been well studied in trypanosomes but the phospholipid composition of the insect stage parasite is unusually high in ettherlipids (Croft *et al.*, 1996). Despite this, etherlipid analogs that have potent activity against the closely related parasite *Leishmania* spp. are much less active against *T. brucei* (Croft *et al.*, 1996); this may be due to a lower level of etherlipids in the bloodstream from trypanosome. Trypanosomes can synthesize fatty acids *de novo* via a plant-like Type II fatty acid synthase (Morita *et al.*, 2000) but this pathway operates alongside an efficient fatty acid scavenging system (Morita and Englund, 2001) and may therefore not be a valid drug target.

Farnesyl pyrophosphate is both the precursor of farnesylated and geranylgeranylated proteins and dolichol, and sterol biosynthesis. Screening of bisphosphonates against tryapanosomes has proven that bisphosphontes can inhibit sterol synthesis in these parasites. Some of the compounds tested have a low toxicity to host cells and have potential as lead compounds for the synthesis of novel parasite specific sterol synthesis inhibitors (Martin *et al.*, 2001).

2.13.5 Acidocalcisome

Acidocalcisome are acidic calcium-storage organelles that have been described in a number of protozoa, including *T. brucei*. These organelles contain large amounts of Ca^{2+}, Mg^{2+}, polyphosphates and other elements (Naula and Burchmore, 2003). Functions of the acidocalcisome may include a role in the storage of polyphophates, a potential

adaptation to environmental stresses as well as an involvement in Ca^{2+} storage and signalling. There is no evidence for the presence of acidocalcisomes in mammalian cells, making these organelles a promising target for the development of new broad spectrum drugs against various parasitic diseases (Docampo and Moreno, 2001). The membrane of acidocalcisomes contains a number of pumps and exchangers for the uptake and release of the various elements within the organelle. One of these pumps, the vacuolar H^+ pyrophosphatase (V-H^+-PPase) is of particular interest for the development of novel drugs as it is only found in plants and photosynthetic bacteria, as well as in the acidocalcisomes of parasitic protozoa. It has no counterpart in the animal kingdom (Naula and Burchmore, 2003). This pump translocates protons across the membrane by using potential energy released by hydrolysis of polyphosphates (McIntosh and Vaidya, 2002).

2.13.6 Cell Cycle

Cyclin-dependent kinases (CDKs), a family of well-conserved serine/threonine kinases, have crucial roles in cell cycle progession, transcriptional control, differentiation and other cellular mechanisms. Their activity is regulated by the binding of a cyclin and by its state of phosphorylation (Nurse, 2000).

A deregulation of CDK function in many cancers has prompted an intensive search for pharmacological compounds that inhibit CDKs. Most of the inhibitors characterized to date bind in the active site, the ATP-binding pocket of the kinase. Some of the compounds found have proven to be potent antiproliferative agents (Knockaert *et al.*, 2002).

In *T. brucei*, six CDKs have been indentified and partially characterized (Hammarton *et al.*, 2002). Most of these enzymes are essential for the survival of the parasite (Naula and Burchmore, 2003). The parasite kinases share 40 – 60% identity with their mammalian counterparts (Mottram and Smith, 1995). However, the structural

differences between the host and parasite kinases might result in different affinities for inhibitory molecules that might be exploited. Alsterpaullone, a inhibitor of CDKs 1, 2 and 5, has been found to irreversibly inhibit growth of procyclic and bloodstream form trypanosomes at a concentration of 1 (Naula and Burchmore, 2003).

2.14 Screening of Commonly Used Medicinal Plants as Remedies

The quality and uniformity of the chemical constituents and their concentrations in natural products may be affected by factors such as subspecies or age of plant, geographical and seasonal variations, time, method of collection, storage and other environmental variables (Makinde *et al.,* 1993; Laughlin, 1994). There may also be major difficulties in isolating pure compounds from complex mixtures of substances often found in extracts submitted to screening.

No screening system is perfect and a properly controlled screen *in vitro* to measure the intrinsic activity of the substance is generally considered to be the first step in drug discorvery. Alternatively, where sophisticated incubation, tissue-culture facilities and reliable power supply are not available, it may be more practical to evaluate products/compounds directly in small animal model (Tagboto and Townson, 2001)

Literature surveys and field studies have shown that plants are used in traditional medicine in Africa to treat trypanosomiasis in Humans and Animals (Freiburghaus *et al.*, 1996; Youan *et al.*, 1997). This was one of the bases for the scientific evaluation of antitrypanosomal activity of selected Nigerian medicinal plants.

Medicinal plants, since times immemorial, have been used in virtually all cultures as a source of medicine (Hoareau and Dasilva, 1999). Traditional plants play an important role in medical system in Nigeria and plant materials remain an important resource to combat serious diseases in the world. Pharmacognostic investigations of plants are carried out to find novel drugs or templates for the development of new therapeutic agents. Since many drugs, e.g quinine and artemisinin were isolated from

plants and because of the increased resistance of many pathogens, e.g trypanosome parasites, towards established drugs, investigation of the chemical compounds within traditional plants is necessary, (Phillipson, 1991).

The most commonly used sources of drugs are herbs and plant extracts, seeds, leaves and barks of certain trees, tubers and roots. Others include wood ash, kaolin and potassium, local soap and spent engine oil (Adewumi, 2004). Noteworthy in this regard is the use of a single remedy or plant extract in treating more than one disease, as well as combinations of various plant extracts for broad-spectrum therapy. For example, extracts or ingredients from the mahogany tree (*Khaya senegalensis*) are used to treat anthrax, diarrhoea, dysentery, foot rot, helminth infections and ringworm. Extracts from the mahogany tress are also used to improve appetite and fertility as well as to relieve animals in cases of gastric/emetic problems, poisoning and as a laxative.

The anti-infective activities of some plant extracts have been documented. For instance, a 100ml extract obtained from 1.17g of *Vernonia amygdaline* leaves pounded and soaked in water significantly reduced worm burden (*Bonustumum*, *Dichrocelium* and *Fasciola* spp.) in calves (Alawa *et al.*, 2000). The antihelmintic properties of *V. amygdalina* and *Annona senegalensis* also have been demonstrated in cattle (Chiezey *et al.*, 2002; Jisaka *et al.*, 1992).

In addition to the effectiveness of *A. senegalensis* against worms, the extracts from this plant is also antitrypanosomal (Freiburghaus *et al.*, 1996). Asuzu and Chineme, (1990), Asuzu and Anaga, (1991) and Nok *et al.*, (1993) reported on the trypanocidal properties of leaf and bark extracts from *Morinda lucida*, *Histonia boonei* and *azadirachta indica* respectively. Similarly *Acacia nilotica* collected from Tanzania gave IC_{50} values between 4.4 ± 1.0 to 9.3 ± 0.3 µg/ml against *Trypanosoma brucei rhodesiense* and other eleven African plant species were also reported to have trypanosidal activity (Freiburghaus *et al.*, 1996). In an earlier study, the ethanolic

extracts of the leaves and fruits of *Alchornia cordifolia* were found to inhibit the growth of *T. brucei in vivo* (Agbe and Oguntimein, 1987).

In other studies carried out by Atawodi *et al.,* (2002), coccidiosis and worm infestation in poultry were controlled with extracts from various plants that included *K. senegalensis, Solanum nodiflorum, Bozwellia dalzieli, Momordica balsamina, Vitex doniana, Striga* spp. and *Butyrospermum paradoxum.* Abdu and Faya, (2000) also reported the use of *Solanum incanum* fruits to treat coccidiosis in poultry and of *M. balsamina* to treat fowlpox and *Capsicum frutenscens* for the treatment of Newcastle disease.

Plants have provided the basis for ethnomedicinal treatment for different types of animal and human diseases and still offer an enormous potential source of new chemotherapeutic agents.

2.15 Natural Products with Antiprotozoal Properties

Diseases caused by protozoa are responsible for considerable mortality and morbidity throughout the world. There are an estimated 20 million people infected with *Leishmania* species (Goto *et al.,* 1998) and nearly the same number infected with *Trypanosoma cruzi* (Burckner *et al.,* 1998). There are 50million cases of amoebiasis with up to 100,000 deaths each year (Huston and Petri, 1998). An estimated 60 million people are at risk of infection with African trypanosomiasis, with about 300,000 new cases each year (WHO, 1998). There are 2.8 billion people living in malaria endemic areas, and every year there are 300 – 500million clinical cases of malaria with over 1million deaths due to the disease (Trigg and Wernsdorfer, 1999). It has been predicted that global warming will cause the spread of many tropical diseases (Sharp, 1996).

There are many problems with the currently available drugs for treating protozoal diseases. Many of these drugs are poorly tolerated because of side effects. There are inadequate drug therapies for the treatment of some of these illnesses such as chronic

stage of chagas disease. Other problems include limited availability, prohibitive cost and increasing drug resistance (Tagboto and Townson, 2001). As a consequence of this, new, cheap, safe and effective drugs are urgently needed.

Established human antiprotozoal drugs from natural sources include quinine from *Chinchona* species, artemisinin from *Artemisia annua* (used in treating malaria) and emetine from *Psychotria* (*Cephaelis*) ipecacuanha (used in treating amoebiasis).

Some plant products that have undergone scientific evaluation for antitrypanosomal activity are listed below:

Table 2.3: Medicinal Plant with Activity against *Trypanosoma*

Species (Family)	Origin	Part (route)	Active Ingredients	Indications	References
Abrus precatorius (Leguminosae)	NC	Seed (na)	Plant Protein, arbrin	*T. rhodesiense,* inhibits ribosome translocation	Cenini *et al.,* (1988)
Abuta pahni A. rutescens (Menispermaceae)	Bolivia Bolivia	Stem (na) Stem (na)	Ehanolic extract Alkaloidal extract	*T. cruzi, in vitro* *T. cruzi, in vitro*	Fournet *et al.,* (1994) Fournet *et al.,* (1994)
Acaoypha gautemalensis (Euphorbiaceae)	Gautemala	Leaf (P.O)	Ethanol extract	*T. cruzi,* in mice	Caceres *et al.,* (1998)
Albizia gumjifera (Luguminosae)	E. Africa	N.C (na)	Lipophilic extract	*T. rhodesiense in vitro*	Freiburghaus *et al.,* (1996)
Allium sativum (Liliaceae)	Nigeria	Cloves (na)	Diallyldisulfide	*T. brucei in vitro* *T. brucei,* mice	Nok *et al.,* (1996)
Alstonia boonei (Apocynaceae)	Nigeria	Bark (ip)	Aq. Extract	*T. brucei,* mice	Asuzu and Anaga, (1991)

Annona senegalensis (Annonaceae)	Nigeria	Root (PO/im)	Aq. Extract	*T. brucei*, mice	Igweh and Onabanjo, (1989)
Ampelocera edentula (Ulmaceae)	Bolivia	Stem, root (top)	Hydroxytetralone	*T. cruzi, in vitro*	Fournet *et al.*, (1994)
Aspidosperma nigricans	Brazil	NC (ip/iv)	Alkaloid, Olivacine	Inactive in mice	Leon *et al.*, (1978)
Bocconia integrifolia *B. pearcei* (papaveraceae)	Bolivia	Leaf, latex, bark (top)	Benzophenathridine alkaloid	*T. cruzi, in vitro*	Fournet *et al.*, (1994)
Byrsonima crassifolia (Malpighiaceae)	Guatemala	Leaf (PO)	Ethanol extract	*T. cruzi, in vitro* *T. cruzi, mice*	Berger *et al.*, (1998)
Bussea occidentalis (leguminosae)	E. Africa	NC (na)	Lipophilic extract	*T. rhodesiense, in vitro*	Freiburghaus *et al* (1996)
Cardiopetalum calophyllum (Annonaceae)	Bolivia	Leaf, stem (na)	Alkaloidal extract	*T. cruzi, in vitro*	Fournet *et al.*, (1994)
Cinchona officinalis (Rubiaceae)	S. American a	Bark (na)	Alkaloidal extract	*T. cruzi, in vitro*	Cavin *et al.*, (1987)
Dianthus caryophyllus (caryophyllaceae)	NC	Leaf (na)	Plant proteins, dianthin 30 and 32	*T. rhodesiense*, inhibits ribosome translocation	Cenini *et al.*, (1988)
Ehretia amoena (Boraginaceae)	E. Africa	NC (na)	Lipophilic extract	*T. rhodesiense*,	Freiburghans *et al.*, (1996)

				in vitro	
Entada abyssinica (Leguminosae)	E. Africa	NC (na)	Lipophilic extract	*T. rhodesiense, in vitro*	Freiburghans *et al.*, (1996)
Gossipium hirsutum (Malvaceae)	NC	Cotton seed oil (na)	Gossypol	*T. cruzi, in vitro*	Montamat *et al.*, (1982) Turrens, (1986)
Lyceum chinense (Solanoceae)	China	Root (na)	Polyamine derivative kukoamine	Inhibitor of trypanothione reductase	Ponasik *et al.*, (1995)
Tridax procumbens (Compositae)	Gautemala	Leaf (na)	Ethanol extract Dichloromethane extract	*T. cruzi, in vitro*	Berger *et al.*, 1998 Caceres *et al.*, 1998
Annona senegalensis	Nigeria	Leaf (ip)	Aqueous	*T. b. brucei* in mice	Ogbadoyi *et al* 2007

Source: Tagboto and Townson, (2001)

Key:

NC = not cited

na = not applicable

PO = per os (orally)

ip = intraperitoneally

im = intramuscularly

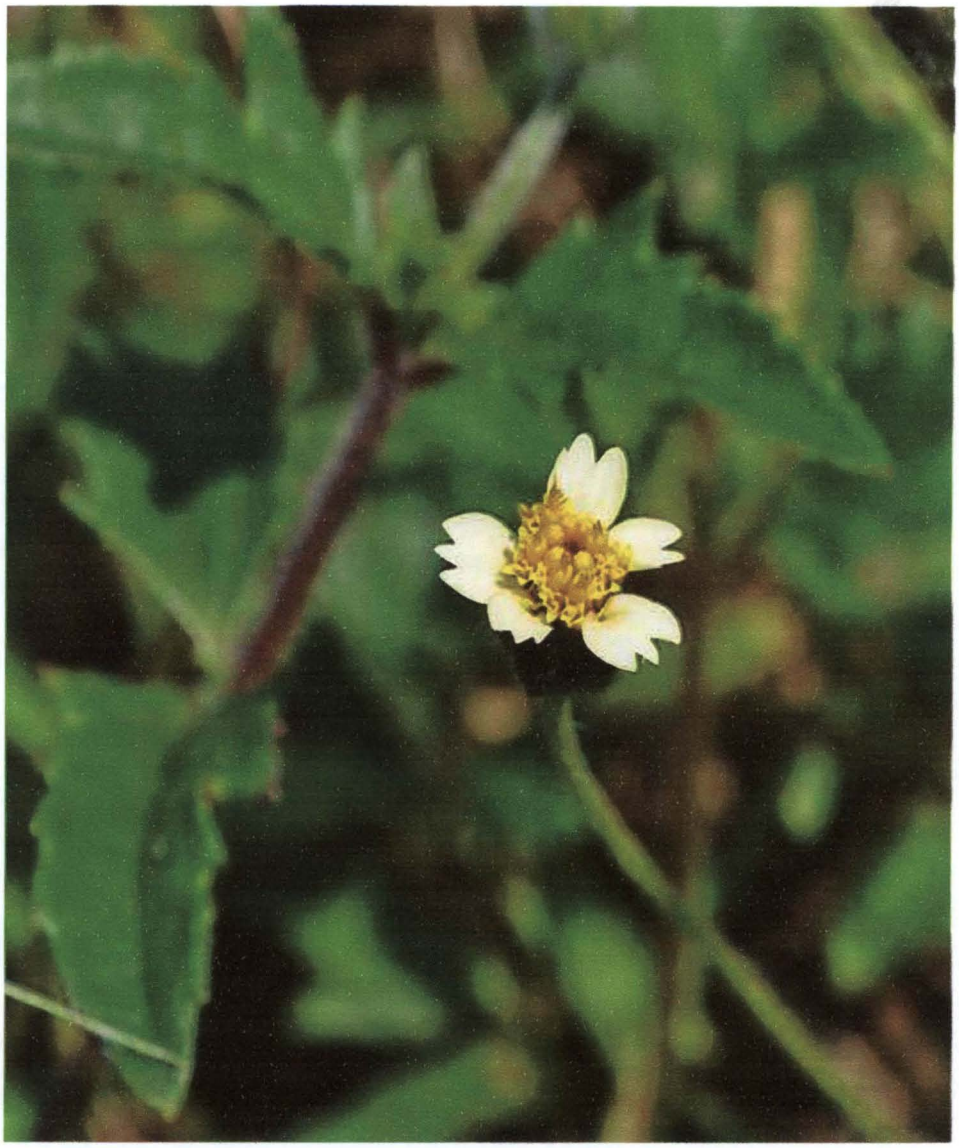


Figure 2.10: *Tridax procumbens* (Voucher number NIPRD/H/6155)

2.16 *Tridax procumbens* linn

2.16.1 Classification

Family:	Asteraceae / Compositae
Genus:	*Tridax*
Species:	*procumbens*
Common Names:	Coat buttons
	Tridax daisy

Local Names:	Ayara-utime-nse: —	Ibibio
	Harantama : ---	Hausa
	Sabaruma/Kodeleyiri:---	Yoruba

2.16.2 Description

Tridax procumbens is a species of flowering plant in the daisy family. It is best known as a widespread weed and pest plant. It is native to the tropical Americas but it has been introduced to tropical, subtropical, and mild temperate regions worldwide. It is listed as a noxious weed in the United States and has pest status in nine states. The plant bears daisylike yellow-centered white or yellow flowers with three-toothed ray florets. The leaves are toothed and generally arrowhead shaped.

2.16.3 Medicinal Action and Uses

Tridax procumbens is known for several potential therapeutic activities like antiviral, antibiotic efficacies, wound healing activity, insecticidal and anti-inflammatory activity (Suseela *et al* 2002). Some reports from tribal areas in India state that the leaf juice can be used to cure fresh wounds, to stop bleeding. The plants has been extensively used in Ayurvadic system of medicine for various ailments and is shown to possess significant antiinflamatory, hepatoprotective, wound healing and antimicrobial properties (Diwan *et al.,* 1989; Pathak *et al.,* 1991; Saraf *et al.,* 1991; Udupa *et al.,* 1991; Perumal *et al.,* 1999; and Taddei and Rosas 2000). The entire plant is used by indigenous people in Gautemala for the treatment of protozoal infections (malaria, leishmaniasis, vaginitis, dysentry) and gastrointestinal disorders (colic/stomach pains, gastritis/enterocolitis) (Caceres *et al.,* 1998; Berger *et al.,* 1998). Ethnobotanically, in Gautemala the whole plant of *Tridax procumbens* is used by the population for topical applications to treat chronic ulcers caused by leishmaniasis (Caceres *et al.,* 1998).

Figure 2.11: *Morinda lucida* (Voucher number NIPRD/H/6289)

2.17 *Morinda lucida*

2.17.1 Classification

Family:	Rubiaceae
Genus:	*Morinda*
Species:	*lucida*
Common Names:	Brimstone
Local Names: Yoruba;	Oruwo

2.17.2 Description

Morinda lucida belongs to the family of RUBIACEAE. It's common names in English is Brimstone tree (Nigeria, Oliver). Its tree is about 9-18m high, or 25m in coastal areas of Ivory Coast, hole crooked, or straight, sometimes shortly 20-30cm diameter, bearing a dense down of slender, crooked branches of various sorts of forest, and particularly in secondary Jungle and sometime the leaves are dark green and have a

leafy shape-like that of a Pumli leaves. The stem bark is brownish in colour and the plant has fruit and flower. The medicinal plants are mostly found in the Southern part of Nigeria; but this particular plant that was used in this research work was gotten from Osogbo. Osun State.

2.17.3 Medicinal Action and Uses

Different parts of *Morinda lucida* have been reported to possess medicinal properties. Many people in Southern Nigeria treat malaria by drinking aqueous leaf extract of *Morinda lucida.* The leaf extract of the plant was reported to possess trypanocidal (Asuzu and Chineme, 1990), antimalarial activities (Tona *et al.,* 1999 and Makinde and Obih, 1985) and aortic vasorelaxant effect (Ettarh and Emeka, 2004). Oliver-Bever (1986) documented the use of a weak decoction of the stem bark to treat severe jaundice. *Morinda lucida* leaf extract has also been reported to have a strong oral hypoglycemic property (Olajide *et al., 1999* and Adeneye and Agbaje, 2008) ; Adeneye and Agbaje (2008) attributed this property to increased peripheral utilization of glucose. The leaf extract of *Morinda lucida* has also been documented to possess reversible antispermatogenic activities in rats (Raji et al., 2005).

Bacteria	EF	MF	EC	MC	AMPICLOX

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Experimental Animals

Matured albino mice weighing between 22 and 35g were purchased from Small Laboratoray Unit of National Veterinary Research Institute, Vom, Plateau State, Nigeria. The albino rats (Wistar strain), weighing between 40 and 80g, used for short term and acute toxicity studies, were purchased from Small Animal House Unit of Nigeria Institute for Trypanosomiasis Research, Vom, Jos, Plateau State, Nigeria. The animals were fed with growers mash and water *ad libitum*. They were acclimatized for one week before the commencement of the experiment.

All experiments involving the animals were conducted in compliance with the internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care (CCAC, 1997) guidelines on animal use protocol review (1997).

3.1.2 Parasites (*Trypanosoma brucei brucei)*

A stabilate of *Trypanosoma brucei brucei*, a parasite originally isolated from cattle in Lafia, Nasarawa State and kept in liquid nitrogen at the Nigerian Institute for Trypanosomiasis Research Vom, Plateau State was used. It was maintained in rats by serial passaging.

3.1.3 Plant Materials

The plants were collected in Kaduna Vom of Plateau state; Bida and Minna of Niger state and Osogbo in Osun state of Nigeria. They are mainly selected because of claims that they possess antiprotozoal activity, and can alleviate one or many of the clinical symptoms such as intermittent fever, anaemia, jaundice, hepatomegaly commonly associated with trypanosomiasis. Consequently the following plants were selected after

their scientific identification; *Aloe vera, Cucumis metuliferus, Securidaca longipenduculata (root and Root bark), Enantia chlorantha, Khaya senegalenses, Dissortis rotundifolia triana, Azadirachta indica, Nauclea latifolia, Nelsonia compestris, Erythrina senegalensis, Tridax procumbens* and *Morinda lucida (*stem, root and leaves*)*. The information on these plants is obtained from traditional healers before collection. All these plants were screened for antitrypanosomal activities and *Tridax procumbens* and *Morinda lucida* gave the best result in terms of prolongation of life and were subjected to further screening.

The aerial part of *Tridax procumbens* were collected in the months of May and June within and around Kaduna Vom, Plateau state and FUT Minna, Bosso campus, Niger State Nigeria. Similarly, the leaves, flowers as well as the local names of *Morinda lucida* were collected in March at Oba Ile, Olorunda Local Government Area, Osogbo, Osun State, Nigeria. The two plants were all identified and deposited at the herbarium of the National Institute for Pharmaceutical Research and Development, Idu, Abuja, Nigeria.

3.1.4 Identification of Plants Materials

All the plant materials were identified at the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja. The Plant, *Tridax procumbens* with voucher number NIPRD/H/6155 and *Morinda lucida* with voucher number NIPRD/H/6289 were deposited at the herbarium of NIPRD, Idu, Abuja.

3.2 Methods

3.2.1 Preparation of Plant Materials

About 1 kg each of the *Tridax procumbens* and *Morinda lucida (*stem, root and leaves*)* were freshly obtained, washed with running tap water and dried at room temperature to a constant weight. The dried plant samples were grinded into powder form using mortar and pestle, and sometimes an electric blending machine where

necessary. The powdered samples were stored in clean polythene bags until required for use.

3.2.2 Preparation of Crude Extracts

The extracts were prepared and screening carried out using the method described by Ogbadoyi *et al.*, (2007). In this method, fifty grams (50g) of the dried powdered samples of each of the leaves, stem of the *Morinda lucida* and whole plant of *Tridax procumbens* were extracted sequentially under reflux with 400ml of hexane, ethyl acetate, methanol and water in that order for 2 hrs in each case. Extracts were filtered hot using muslin cloth and solvent was removed using rotatory evaporator for organic solvents and freeze-drier for water extracts. The extracts, are finally dried in steam bath and transferred into sterile sample bottles for storage stored at refrigerated temperature untill when required for use. The residue was dried after each extraction for the next extraction process.

3.2.3 Weight of extracts

When 100g of the crude *M. lucida* stem bark was extracted, hexane gave 0.37g; ethyl acetate = 0.42g, methanol = 1.19g and aqueous = 5.14g extracts. Similarly, 100g of crude *M. lucida* leaf extraction gave 1.27g with hexane, 5.12g for Ethyl acetate, Absolute Methanol 3.92g and Aqueous =10.62g extracts.

The weight of *T. procumbens* extracts using 100g of the crude is as follows: Ethyl acetate = 3.31g; Methanol = 4.5g and Aqueous = 7.12g.

3.2.4 Infection of Animals

Blood from heavily infected donor mouse was obtained by cardiac puncture and collected with EDTA coated syringe to avoid clotting. The blood was immediately diluted with physiological saline to give 1.0×10^7 parasites per ml to obtain inoculums. Healthy mice were then infected intraperitoneally with 0.1ml of the inoculum containing about 10^6 trypanosomes.

with the extract at 100, 200, 300 and 400 mg/kg body weight respectively for 14 consecutive days respectively. Mice in Group E were treated once intraperitoneally with berenil at 3.5 mg/kg body weight while Groups F was untreated and Group G was neither infected nor treated and served as control.

3.2.7 Confirmatory Screening with Effective Doses of *Tridax procumbens* and *Morinda lucida*

In order to ascertain the efficacy and reproducibility of the doses that demonstrated appreciable antitrypanosomal activities in the initial screening, eight groups of mice, (A - H) each containing three mice, were set up. Group A, B and C were administered with methanolic leaves, methanolic stem bark and ethyl acetate extracts of *Morinda lucida* at 400, 200 and 100mg/kg body weight respectively respectively. Groups D, E and F were administered with ethyl acetate, methanol and aqueous extracts of *Tridax procumbens* at 200, 300 and 300mg/kg body weight respectively. All the administration was through intraperitoneal route for 14 consecutive days. Groups G was infected untreated while Group H was neither infected nor treated and served as controls. Parasitemia was monitored three times weekly.

3.2.8 Combination Therapy

The possibility of synergistic action of different extracts in varying combinations was investigated using a modified method of Gerardo *et al.*, (2007). To establish this, different extracts that gave highest antitrypanosomal activity in the initial screening were combined in varying ratios and was screened against *T. b. brucei* infected mice. Thus, *Tridax procumbens* ethyl acetate extract at 200mg/kg body weight and *Morinda lucida* methanolic leaves extract at 400mg/kg body weight were combined in ratios 1:1, 2:1 and 1:2. Each ratio was used treat 3 groups of *T. b. brucei* infected mice comprising of 3 animals each at 200, 300 and 400 mg/kg body weight respectively. In all cases,

parasitaemia was monitored daily and means (±SD), maximum / minimum survival was calculated.

3.2.9 Screening for Prophylactic Activity of Extracts

Two extracts, namely methanol extract of *Tridax procumbens* at 300mg/kg body weight and ethyl acetate at 200mg/kg body weight that had demonstrated activity against *T. b. brucei* infected mice were screened for prophylactic activity. Three groups of three mice each were set up. Groups A and B were administered with methanol and ethyl acetate extracts respectively for 14 consecutive days. Group C was administered with 0.1ml of physiological saline. On the 15^{th} day, animals in the three groups were inoculated with 0.1ml of diluted parasite-infected blood (about 10^6 Trypanosomes/ml) intraperitoneally. Parasitemia was thereafter monitored twice weekly and mean survival (maximum and minimum) beyond the infected untreated control was determined.

3.2.10 Bioassay- Guided Fractionation of *Tridax procumbens* Extracts

3.2.10.1 Principle of Column Chromatography

Column chromatography is a separation technique in which the stationary bed is within a tube. The particles of the solid stationary phase or the support coated with a liquid stationary phase may fill the whole inside volume of the tube (packed column) or be concentrated on or along the inside tube wall leaving an open, unrestricted path for the mobile phase in the middle part of the tube (open tubular column). Differences in rates of movement through the medium are calculated to different retention times of the sample (Still *et al.,* 1978). Column chromatography is categorized into two categories, depending on how the solvent flows down the column. If the solvent is allowed to flow down the column by gravity, or percolation, it is called open column chromatography (gravity). If the solvent is forced down the column by positive air pressure, it is called flash chromatography.

3.2.10.2 Column Chromatography of Crude Ethyl Acetate and Methanol Extract

In column chromatography, the stationary phase, a solid adsorbent (Silica gel-60-120 mesh) soaked in hexane, was placed in a vertical glass column and the mobile phase, hexane, was added to the top and allowed to flow down through the column by gravity. Both the methanol and ethyl acetate extracts of *Tridax procumbens* were separately subjected to column chromatography to obtain various fractions from which their anti-trypanosomal activities were compared.

3.2.10.2.1 Procedure

About 30 g portion of the crude ethyl acetate and methanol extracts of *Tridax procumbens* were separately redissolved in their respective extracting solvent and mixed thoroughly with 120g of silica gel (60- 120 Mesh). The mixture was then allowed to evaporate to dryness. The dried extract/silica gel mixture was added carefully to the top of the column already packed with silica gel in hexane. First, the tap was opened to allow the solvent (hexane) already in the column to drain so that it will level with the top of the packing material. The dried extract/silica gel mixture was then added carefully to the top of the column. The tap was again opened to allow for complete absorption of the coloured extract into the top of the column.

Next, the solvent mixture, made up of hexane, ethyl acetate and methanol in increasing ratios of polarity was added to the top of the column, trying to disturb the packing material as little as possible. Then the tap was opened so that the solvent can flow down through the column, collecting it in a beaker or flask at the bottom. As the solvent ran through, fresh solvent was added to the top so that the column never dried out. Fractions were then collected sequentially in bottles, concentrated on a rotary evaporator and evaporated on steam bath at between 60- 80^0 C. The residues were kept in stoppered bottles until required for screening.

3.2.10.2.2 The Adsorbent

Silica gel (SiO_2) (mesh 60-120) was used in the fractionation of the methanol and ethyl acetate extracts of *Tridax procumbens*. The mesh number refers to the mesh of the sieve used to size the silica, specifically, the number of holes in the mesh or sieve through which the crude silica particle mixture is passed in the manufacturing process. If there are more holes per unit area, those holes are smaller, thus allowing only smaller silica particles go through the sieve. The relationship is: the larger the mesh size, the smaller the adsorbent particles.

Adsorbent particle size affects how the solvent flows through the column. Smaller particles (higher mesh values) are used for flash chromatography; larger particles (lower mesh values) are used for gravity chromatography.

3.2.10.2.3 The Solvent System

In this fractionation process, a series of increasingly polar solvent systems were used to elute the column. A non-polar solvent, hexane, was first used to elute less-polar compounds, and subsequently the polarity was gradually increased by varying the ratios of hexane, ethyl acetate, and methanol in the solvent mixture. The last elution was done with 100% ethyl acetate and methanol for ethyl acetate and methanol extracts respectively as shown in the Table below:

Table 3.1: Solvent System for Ethyl Acetate Extract Fractionation

HEXANE	ETHYL ACETATE	METHANOL	VOLUME (ML)
100	-	-	500
95	5	-	200
90	10	-	200
80	20	-	200
70	30	-	200
60	40	-	200
50	50	-	200
40	60	-	200
20	80	-	200
-	100	-	200
-	90	10	200
-	80	20	200
-	100	-	300

Table 3.2: Solvent System for Methanol Extract Fractionation

HEXANE	ETHYL ACETATE	METHANOL	VOLUME (ML)
100	-	-	200
90	10	-	300
80	20	-	200
70	30	-	200
60	40	-	200
50	50	-	200
40	60	-	200
30	70	-	200
20	80	-	200
10	90	-	200
-	100	-	200
-	90	10	200
-	80	20	200
-	70	30	200
-	60	40	200
-	40	60	200
-	-	100	200

3.2.11 Collection and Analysis of Column Eluents

Small fractions of the eluents were collected sequentially in labelled beakers and the composition of each fraction was separated by thin layer chromatography. Twenty nine (29) for ethyl acetate and thirty four (34) for methanol fractions obtained at the end of the column chromatography were subjected to thin layer chromatography in order to

pool together fractions with similar Rf values. Small fractions of the eluents were collected sequentially in labelled bottles and the fractions with similar Rf values were pooled together using thin layer chromatography.

3.2.12 Thin Layer Chromatography

3.2.12.1 Principle

Thin layer chromatography (TLC) is a chromatography technique used to separate mixtures. It involves a stationary phase consisting of a thin layer of adsorbent material, usually silica gel, aluminium oxide, or cellulose immobilized onto a flat, inert carrier sheet. A liquid phase consisting of the solution to be separated is then dissolved in an appropriate solvent and is drawn up the plate via capillary action, separating the experimental solution based on the polarity of the components of the compound in question.

3.2.12.2 Plate Preparation and Solvent System

TLC plates were made by mixing the *adsorbent*, 60 g silica gel, with a small amount of inert binder, calcium sulphate (gypsum) and 180ml water. This mixture was spread as thick slurry on an unreactive carrier sheet, glass, the resultant plate was dried and activated by heating in an oven for thirty minutes at 110 °C. The thickness of the adsorbent layer was typically around 0.1–0.25 mm for analytical purposes and around 1–2 mm for preparative TLC. Every type of chromatography contains a mobile phase and a stationary phase. In Ethyl acetate and methanol fractionation, the mobile phase for fractions 1-21 was made up of hexane, ethyl acetate (3 : 2), while that of fractions 22-30 was hexane, ethyl acetate and methanol (3 : 2 : 1). The spot were visualized using iodine in iodine tank and spraying with vanillin in sulphuric acid.

3.2.12.3 Sample Application

A small spot of the eluents were applied to the analytical plate using capillary tube, about one centimetre from the base. The plate was then dipped into the mobile

phase placed in a sealed tank. The solvent mixtures moved up the plate by capillary action and interacted with the fractions and were carried up the plate by the solvent. Different fractions moved at different rates due to the differences in their attraction to the stationary phase, and because of differences in solubility in the solvent.

3.2.12.4 Combining Together of Fractions

Using the RF values of the fractions after visualization of spots with iodine vapour, fractions with same values were pooled together as single fractions. Consequently, 12 fractions are obtained for ethyl acetate extract and 11 fractions are for methanol extract.

3.2.13 Screening for Antitrypanosomal Activity of Fractions

The pooled fractions obtained from column chromatography were screened for antitrypanosomal activity using the dose that gave the highest activity. Consequently, the ethyl acetate fractions were administered at 200mg/kg body weight while the methanol fractions were administered at 300mg/kg. Each fraction was screened for antitrypanosomal activity using three infected mice and parasitemia was monitored daily.

3.2.14 Thin Layer Chromatography of Methanol Fractions 7 and Ethyl Acetate Fraction 11

Using the method described above (3.19), the two fractions that gave highest survival beyond the untreated control (methanol fraction 7 and ethyl acetate fraction 11) were further subjected to analytical thin layer chromatography in order to ascertain the number of components in the two fractions as compared to their crude counterpart. The fractions were developed in 2 ml each of chloroform (100%), chloroform and methanol (9: 1) and chloroform and methanol (4: 1). The plates are removed from the tank when the solvent front is ¾ to the end of the plate. They were air dried and the components were then observed visually, spray with sulphuric acid and exposed to ultra violet light at 366nm.

3.2.15 Preparative Thin Layer Chromatography (PTLC)

The ethyl acetate fraction 11 was spread onto the prepared thin layer plates as earlier described. Several plates were spread and were later developed in chloroform: methanol (5:1). The plates were removed from the tank when the solvent front was ¾ to the end. The bands obtained were scrabed and eluted in Chloroform: Methanol (3:1) in a separate conical flask. They were separately filtered and concentrated to dryness at room temperature

3.2.16 Screening the Band for Antitrypanosomal Activity

The three band obtained from 3.2.15 above were separately screened for the antitrypanosomal activity. Five groups (A – E) of three mice each were inoculated with *T.b.brucei* and groups A – C were separately treated with bands 1 – 3 respectively. Group D was treated with fraction 11 while group E and F served as infected untreated and uninfected untreated respectively. All the animals were treated at 20 mg/kg body weight intraperitoneally for 3 consecutive days. The parasitaemia was monitored daily and mean survival and prolongation of life were determined.

3.2.17 Tests for Antibacterial Activity of the Extracts

Four extracts, namely, crude methanol and ethyl acetate extracts, ethyl acetate and methanol fractions with highest means survival were tested for antibacterial activities using the agar well diffusion method as described by Apak and Olila (2006).

3.2.17.1 Principle

When an antibiotic-impregnated disk is placed on agar previously inoculated with the test bacterium, the disk picks up moisture and the antibiotic diffuses radially outward through the agar, producing an antibiotic concentration gradient. The antibiotic is present at high concentrations near the disk and affects even minimally susceptible microorganisms (resistant organisms will grow up to the disk). A clear zone or ring is present around antibiotic disk after incubation if the agent inhibits bacterial growth.

3.2.17.2 Procedure

A sterilized cork borer (7mm in size) was used to drill holes in Nutrient Agar plates. An inoculating loop was used to inoculate the test organisms on the plates and incubated for a few hours at 35°C until it became slightly turbid and was diluted to march a turbidity standard. Using sterilized forceps, the extracts were dispensed into the holes on the plates. The plates were immediately placed in an incubator at 35°C. After 24 hours of incubation, the diameters of the zones of inhibition were measured to the nearest mm. The test organisms used were: *Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, and salmonella typhi* while a broad spectrum ampiclox was used as a standard.

3.2.18 Phytochemical Analysis of the Crude Methanol and Ethylacetate Extracts

The crude methanol and ethyl acetate extracts of *Tridax procumbens* was subjected to phytochemical analysis using standard analytical methods described by Sofowora (1979).

a. Test for flavonoids: About 3g of the crude extract was transferred into a clean test tube and 5ml of water was added. The mixture was boiled for 3 minutes and then filtered cool. Few drops of magnesium chips were added to the filtrate in a clean test tube and allowed to dissolve on addition of concentrated HCl. A green colour change indicates the presence of flavonoids.

b. Test for Tannins: A small amount of the extract was transferred into a test tube and 3ml of distilled water was added. To this mixture, a few drops of 10% ferric chloride were added. A deep brown coloration indicates the presence of tannins.

c. Test for Saponins: To 3g of the extract, 5ml of distilled was added. The tube was covered with the thumb and shaken vigorously for 30 minutes. The presence of saponins is confirmed by the persistence of frothing on warming.

d. Test for Terpenes: To 5ml of chloroform in a clean test tube, 3grams of the extract was added and mixed. The mixture was filtered and the filtrate was transferred into a clean test tube. Acetic anhydride was added to the filtrate followed by concentrated sulphuric acid. The formation of a ring at the interphase between the two immiscible liquids is a preliminary evidence for the presence of terpenes.

e. Test for steroids: 1ml of the extract was dissolved in 2ml of chloroform, and then concentrated H_2SO_4 was added to form a lower layer. The presence of a reddish violet ring at the interphase and a green chloroform upper layer indicates the presence of steroids.

f. Test for Anthraquinones: 1ml of the extract was dissolved in 10ml of benzene in a test tube. The mixture was filtered and to the filtrate, 5ml of 10% NH_3 was added and shaken. The presence of anthraquinones is indicated by the appearance of a pink red/violet colour in the ammonia phase.

g. Test for Alkaloids: 1ml of the extract was stirred with 5ml of 1% aqueous HCl in a steam bath. The mixture was filtered and 1ml of the filtrate was treated with few drops of Wagner's reagent.

h. Test for fatty acids: 1ml of the extract was made alkaline with 5ml of 25% NH_3. The mixture was exhaustively extracted with ether, and then acidified with concentrated HCl. The acidic aqueous solution was then shaken with ethyl ether in a separating funnel and subsequently the ether was evaporated to dryness. The presence of higher fatty acids is indicated by the oily nature of the residue.

3.2.19 Acute Toxicity Studies of Ethyl acetate Crude Extract

The acute toxicity of the ethyl acetate extract of *Tridax procumbens* was evaluated using the method described by Lorke (1983) with slight modification. In the first phase, nine mice randomly divided into three groups of three mice per group and

each were given 10, 100 and 1000 mg extract/kg body weight intraperitoneally respectively. The mice were observed for signs of adverse effects and death for the first critical 4 h, subsequently for 72 h and thereafter daily for 14 days. In the second phase of the study, the procedure was repeated using another set of nine mice randomly divided into three groups of three mice each, given 1600, 2900 and 5000 mg extract/kg body weight, respectively. Another fresh set of three mice were given normal saline to serve as the control and all the mice were kept under same conditions and observed for the first critical 4h, subsequently for 72h and thereafter for 14 days. The signs of toxicity such as physical activity and general appearance, paw-licking, salivation, stretching, Gastrointestinal Signs: Dropping (Faeces), rubbing of nose on the floor and wall of cage, sedation, coma, convulsion and death. The number of deaths in each group within 24 h was recorded and the final median lethal dose (LD_{50}) was calculated as the geometric mean of the highest non-lethal dose (with no deaths) and the lowest lethal dose (where deaths occurred).

3.2.20 Short-term Toxicity Study of *T. procumbens*

The short-term toxicity study of the extract was evaluated according to the format described by Wilson *et al.*, (2001). Six groups, (A – F), consisting of five Wistar rats each, were set up. Groups A – E animals were administered intraperitoneally with 50, 100, 200, 400 and 800mg/kg body weight per day repeatedly for 14 consecutive days. Group F rats which served as control were administered physiological saline for 14 consecutive days. All the groups were further monitored for another two weeks after the last administration.

The data that are obtained from short-term repeated dose studies include:

- Body / organ weight response
- Physical observations
- Clinical pathology (Clinical chemistry and haematology)

- Histopathology
- Gross necropsy
- Palatability of test material

3.2.20.1 Percentage Body / Organ Weight Ratio

All the animals are weighed weekly and at the end of the study the rats are terminated. Organs such as liver, lungs, kidneys, heart and spleen are collected and weighed. The weights obtained are divided by the last life weight of the rats multiplied by 100 to give percentage (%) organ body weight ratio.

3.2.20.2 Serum Clinical Chemistry

Albino rats used for short term toxicity studies were sacrificed at the end of study period and blood was withdrawn from the punctured heart of each rat into a clean, sterile centrifuge tubes. They were allowed to clot for 1 hour at room temperature. The serum obtained after centrifugation at 3000xg for 5 minutes was used for the determination of Alanine Amino Transferase, Aspartate Amino Transferase, Alkaline Phosphatase, Glucose, Urea nitrogen, Creatinine, Total protein, Sodium and Potassium.

3.2.20.2.1 Determination of Alanine Amino Transferase (ALT) Activity

a) Principle:

The assay method used for the determination of SGPT activity was based on the one recommended by the IFCC (1980). Kinetic determination of ALT is according to the following reaction.

$$\text{L – Alanine} + \alpha\text{-ketoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate} + \text{L-Glutamate}$$

$$\text{Pyruvate} + \text{NADH} + H^{+} \xrightarrow{\text{LDH}} \text{L-Lactate} + NAD^{+}$$

Where LDH is Lactate dehydrogenase

b) Preparation of Working Reagent and Procedure

The working reagent was reconstituted by mixing Reagent 2 (R2) comprising of LDH, NADH and α ketoglutarate with the volume of Reagent 1 (R1) comprising of Tris

buffer (pH 7.5) and L-Alanine as indicated on the vial label. To 0.1ml of the serum sample, 1.0ml of the working reagent was added. The mixture was incubated at 37°C for 1 minute, after which the change in absorbance per minute (OD/min.) at 340nm was measured during the next 3 minutes.

c) Calculation:

SGPT activity (U/L) = (Δ OD/min) × 1768

3.2.20.2.2 Determination of Aspartate Aminotransferase (AST) or Serum Glutamate – Oxaloacetate Transaminase (SGOT) Activity

a) Principle:

The assay method used for the determination of SGPT activity was based on the one recommended by the International Federation of Clinical Chemistry (1980). Kinetic determination of AST is according to the following reaction.

$$\text{L – Aspartate} + \alpha\text{-ketoglutarate} \xrightarrow{\text{AST}} \text{Oxaloacetate} + \text{L-Glutamate}$$

$$\text{Oxaloacetate} + \text{NADH} + H^{+} \xrightarrow{\text{MDH}} \text{L-Lactate} + NAD^{+}$$

Where MDH is Malate dehydrogenase

b) Preparation of Working Reagent and Procedure

Similarly, the working reagent was reconstituted by mixing Reagent 2 (R2) comprising of MDH, LDH, NADH and α ketoglutarate with the volume of Reagent 1 (R1) comprising of Tris buffer and L Aspartate as indicated on the vial label. The reconstituted reagent is stable for 30 days at 2-8°C. To 0.1ml of the serum sample, 1.0ml of the working reagent was added. The mixture was incubated at 37°C for 1 minute, after which the change in absorbance per minute (ΔOD/min.) at 340nm was measured during the next 3 minutes.

c) Calculation:

SGOT activity (IU/L) = (Δ OD/min. × 1768)

3.2.20.2.3 Determination of Serum Alkaline Phosphatase

a) Principle

p-nitrophenylphosphate + H_2O $\xrightarrow{\text{ALP}}$ phosphate + p-nitrophenol

b) Preparation of Reagents

The working reagent was prepared by reconstituting one vial of substrate with the appropriate volume of the buffer. To 0.02 ml of serum sample, 1.0ml of working reagent was added. After mixing samples and reagent, the initial absorbance was taken at 405 nm which was repeated after 1, 2 and 3 minutes.

c) Calculation

ALP activity (U/l) = 2760 X Δ Absorbance

3.2.20.2.4 Determination of Total Protein

`a) Principle:

Colorimetric determination of total protein based on the principle of the Biuret reaction (copper salt in an alkaline medium). Protein in plasma or serum sample forms a blue coloured complex when treated with cupric ions in alkaline solution. The intensity of the blue colour is proportional to the protein concentration.

b) Preparation of Working Reagent and Procedure

Potassium iodide 6 mmol/L, potassium sodium tartrate 21 mmol/L, copper sulphate 6 mmol/L and sodium hydroxide 58 mmol/L were combined together to form total protein reagent. To 1.0 ml of the protein reagent each, 0.02 ml of the protein standard and sample were mixed and incubate for 10 minutes at 37°C. The Asorbance reading of standard and sample was taken against reagent blank at 546 nm.

c) Calculation

$$\text{Total Protein Concentration (gm/dl)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times 6$$

3.2.20.2.5 Determination of Glucose

a) Principle:

Enzymatic colorimetric determination of glucose according to the following reaction

$$\text{Glucose} + O_2 \xrightarrow{\text{Glucose Oxidase}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Red quinone} + 4H_2O$$

b) Preparation of Working Reagent and Procedure

The working reagent was reconstituted by dissolving Reagent 2 comprising of Glucose oxidase, peroxidase and 4-aminoantipyrine with the volume of Reagent 1 (phosphate buffer, pH 7.4) as indicated on the label. To 0.1 ml of the sample, 1.0ml of working reagent was added. The mixture was incubated for 10 minutes at 37°C after which the change in absorbance at 505nm was measured.

b) Calculation

$$\text{Glucose Concentration (mg/ml)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times 100$$

3.2.20.2.6 Determination of Some Serum Kidneys Function Parameters

The serum obtained above was also used to determine the following parameters:

- Urea level
- Potassium (K^+) level
- Sodium (Na^+) level

3.2.20.3 Determination of Haematological Parameters

The collected blood samples into the EDTA containers are analysed for Packed Cell Volume (PCV) by the microhaematocrit method, haemoglobin concentration by cyanomethaemoglobin method, red blood cell (RBC) count, Total and differential leucocyte (WBC) count by the haemocytometer methods (Schalm *et al.*, 1975).

3.2.21 Gross Necropsy and Histopathology

After obtaining the weight of all the organs as in 3.2.20.1 above, they are quickly transfered into 10% buffered formalin and examined grossly. Thereafter, representative organs such as liver, kidney, spleen, heart and lungs from each of the treated groups and control are processed for histopathological studies.

3.2.21.1 Preparation of Tissues for Histopathological Studies

The tissues were prepared according to the method described by Wallace, (2001). All the animals that were exposed to short-term doses of the ethyl acetate extract of *T. procumbens* were subjected to histopathological studies. Animals were sacrificed and their livers, spleen, lungs, heart and kidneys were carefully removed. These organs were processed, sectioned and stained according to standard laboratory methods. They were fixed in 10% formalin- saline and 3-4mm thick tissue was cut from each organ for processing. The cut tissues were transferred to the automatic tissue processor where the tissues were further fixed in 10% buffered formol- saline for two hours and dehydrated for two hours in each ascending grades of alcohol (70%, 90%, and 100% v/v). The dehydrated tissues were then cleared in xylene for two hours and the tissues impregnated in molten paraffin wax for another two hours and left to cool. The sections were then trimmed and sectioned on the microtome at 5 microns (μ). The sections were floated out in a warm water bath, then attached to slides, and dried on a hot plate and stained. The slides were viewed on a microscope with the assistance of a Pathologist to enable interpretation of morphological changes in the tissues.

3.2.22 Determination of Chemical Composition of Active Fractions

The band obtained from 3.2.15 above of ethyl acetate Fractions 11 was subjected to Nuclear Magnetic Resonance (NMR) analysis in order to elucidate the chemical composition of each band of the active fraction. In addition, the TLC profiles of the 3 bands that constitute the fraction 11 were also determined using normal and reverse phase thin layer chromatography. The plates were developed in dichloromethane and methanol (4:1) for the normal phase and 50% methanol for reverse phase. The plates were also visualize under UV light at 366 and 254nm and sprayed with 10% vanillin in concentrated sulphuric acid and ferric chloride solution.

3.2.23 Statistical Analysis of Experimental Data

All data obtained in this work were statistically analyzed using analysis of variance (ANOVA) and Students T - test. Data obtained were subjected to a one-way analysis of variance to derive mean values of parasitemia which were compared with least significant difference. Mean values among the treated groups were considered to be different if the level of probability was < 0.05.

CHAPTER FOUR

4.0 RESULTS

4.1 Antitrypanosomal Activities of Selected Medicinal Plants Screened

The results of the first group of medicinal plants screened were presented below in table 6. These plants were screened because of their claimed antiprotozoal effect and have ability to alleviate one or two clinical symptoms associated with trypanosomiasis. They were obtained from local traditional healers. *Cucumis metuliferus and Securidaca longipenduculata* gave highest prolongation of life by 4 days while *Nelsonia compestris* did not even extent the life of the treated beyond the control.

Table 4.1: Effect of Some Medicinal Plants Extract on *T. b. brucei* Infected Rats

Scientific name / part Used	Local name	Family	Dosage (mg/kg)	Route	Extraction solvent	Survival Range	Survival beyond control (Days)
Enantia Chlorantha (SB)	Awo-Opa (Y)	Annonaceae	100 20	Oral SC	Aq. Ethanol	13-16 13-15	2 1
Khaya senegalensis (SB)	Madacii (H)	Meliaceae	60	Oral	Aq.	18-25	1
Dissortis rotundifolia triana	Edigibata (N)		400	Oral	Aq. Ethanol	9-10	1 0
Azadirachta indica (SB)	Dogon yaro (H)	Meliaceae	60	Oral	Aq.	13-17	2
Aloe vera			1ml/kg	Oral	Pulp	8-10	1
Cucumis metuliferus (F)	Burar Zaki (H)	Cucubitaceae	150	Oral	Pulp	10-13	4
Securidaca longipenduculata (R)	Uwar magunguna (H)	Polygalaceae	100	IP	Aq.	8-12	3
Securidaca longipenduculata (RB)	Uwar magunguna (H)	Polygalaceae	100	Oral	Aq.	9-13	4
Nauclea latifolia	Igiyaa (H)	Rubiaceae	1500	Oral	Aq.	17-18	2
Nelsonia compestris			1500	Oral	Aq.	14-15	-1
Erythrina senegalensis	Idon Zakaraa (H)		500	Oral	Aq.	17-20	2
Herbal mixture (*M. Lucida, E. chlorantha,Nettle, lemon grass)*			3.6ml/kg	Oral	Aq.	10-15	2
Tridax procumbens	Harantama (H)	Asteraceae	300kg	I.P	Methanol	7 - 30	14
Morinda lucida	Oruwo (Y)	Rubiaceae	400	I.P	Methanol	10 – 19	14

Aq = Aqueous; SB = Stem bark; H = Hausa; N = Nupe; Y = Yoruba SC = Sub – cutaneous; IP = Intraperitoreal; F = Fruit; R = Root; RB = Root bark

The result for the screening of leaf extract of *Morinda lucida* is presented in figures 4.1-4.4. The parasitaemia was lowered with methanol extract (figure 4.3) having a means prolongation of life by 9.7 days at 400mg/kg body weight (table 4.2). Other solvent extracts also extend the life of treated groups and the least was the hexane extract.

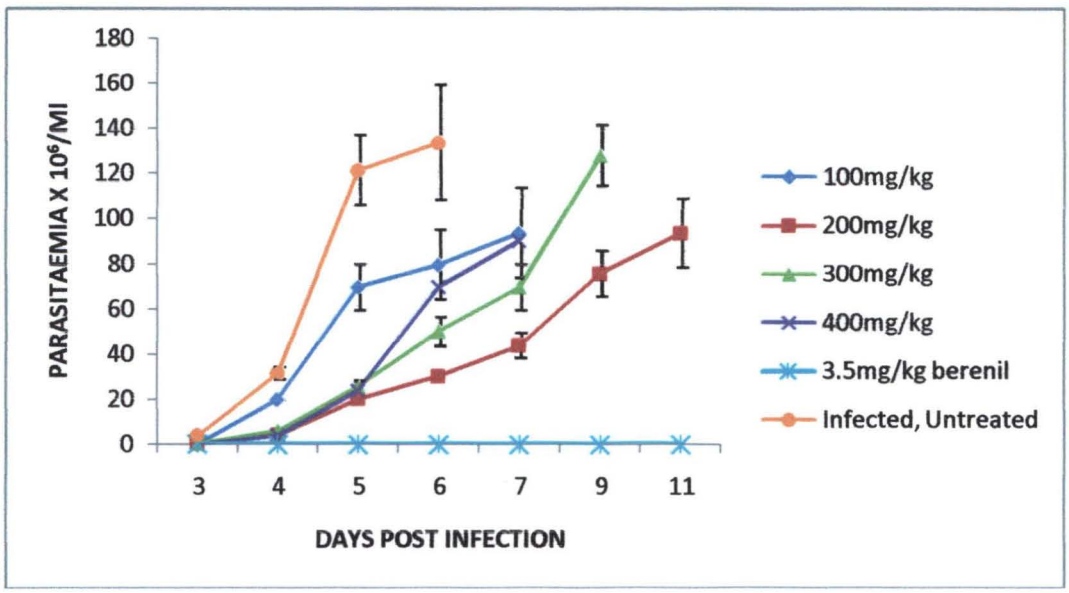


Figure 4.1: Effect of Different Doses of *Morinda lucida* Hexane Leaves Extract on *T. b. brucei* Infected Mice

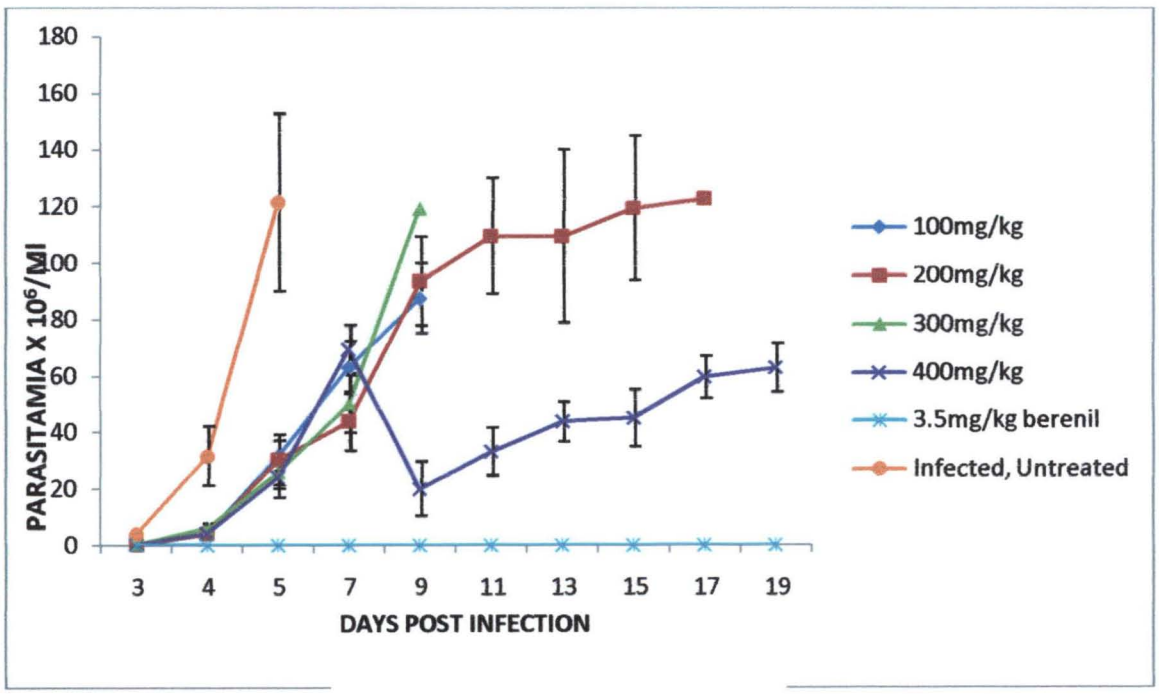


Figure 4.2: Effect of Different Doses of *Morinda lucida* Ethyl acetate Leaves Extract on *T. b. brucei* Infected Mice

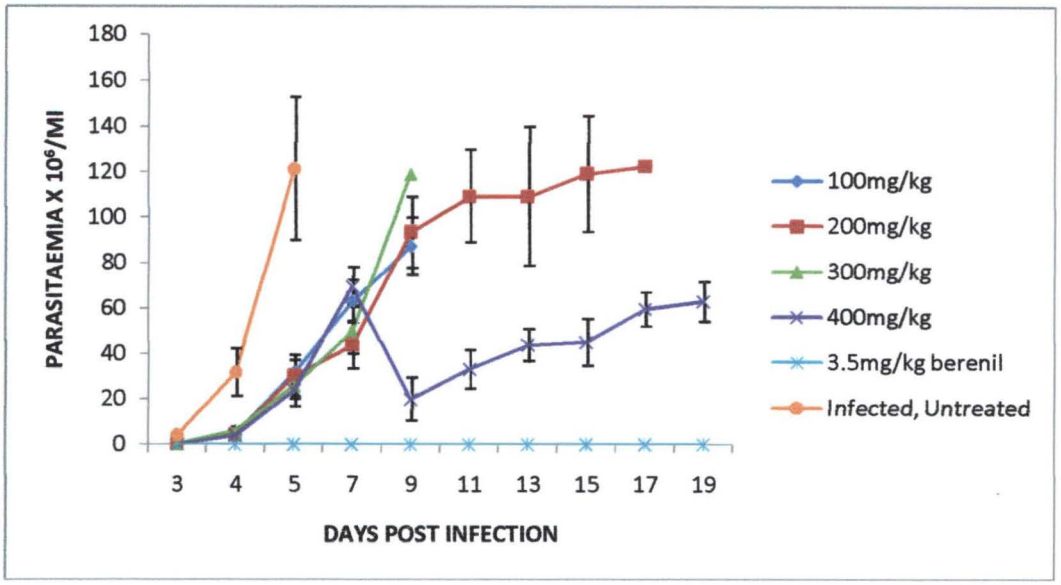


Figure 4.3: Effect of Different Doses of *Morinda lucida* Methanolic Leaves Extract on *T. b. brucei* Infected Mice

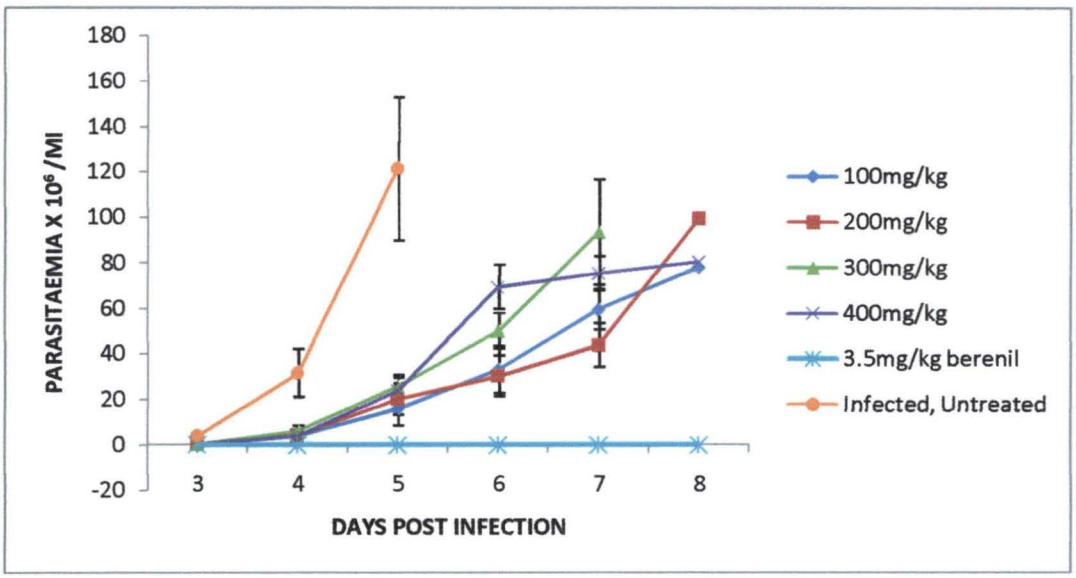


Figure 4.4: Effect of Different Doses of *Morinda lucida* Aqueous Leaves Extract on *T. b. brucei* Infected Mice

Table 4.2: Summary of Screening *Morinda lucida* Leaves Extract

Extraction Solvent	Dose (mg/Kg)	Survival Range	Survival beyond Control (Days). Min	Max	Means Survival (±SD)
Hexane	100	6-8	0	2	1.0±0.8
	200	9-11	3	5	4.0±0.8
	300	7-9	1	3	2.0±0.8
	400	7-8	1	2	1.3±0.5
Ethyl acetate	100	9-16	3		6.7±2.9
	200	8	10		2.0±0.0
	300	6-9	2	2	1.3±1.2
	400	7-11	0	3	3.0±1.6
	Infected, Untreated	6	1 -	5 -	-
Methanol	100	6-10	2	5	4.3±0.5
	200	9-17	5	12	7.6±3.3
	300	6-7	2	2	1.5±0.5
	400	10-19	6	14	9.7±3.7
Aqueous	100	7-8	3	3	2.3±0.5
	200	6-8	2	3	2.0±0.8
	300	6-7	2	2	1.6±0.5
	400	6-8	2	3	2.0±0.8
	Infected, Untreated	4-5	-	-	-

The result for screening stem extract of *Morinda lucida* was presented on figures 4.5-4.8. The methanol extract at a dose of 200 mg/kg recorded the longest mean prolongation of life by 7.0 days beyond the infected untreated control. The least was that of ethyl acetate extract with 0 day mean prolongation of life (Table 4.3). In all, the parasitaemia kept fluctuating until the animals died of acute infection.

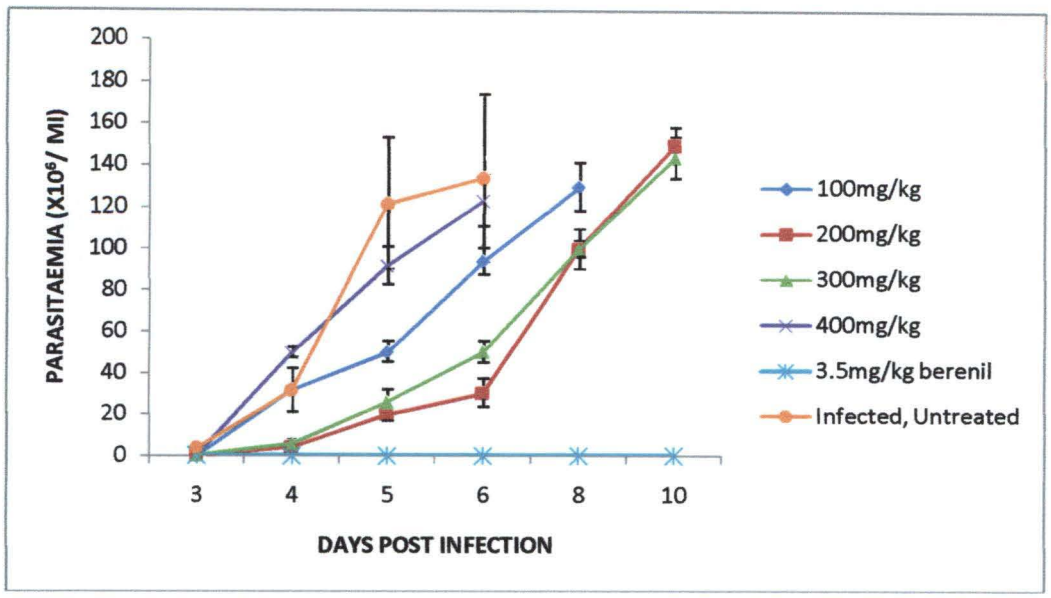


Figure 4.5: Effect of Different Doses of *Morinda lucida* Hexane Stem Bark Extract on *T. b. brucei* Infected Mice

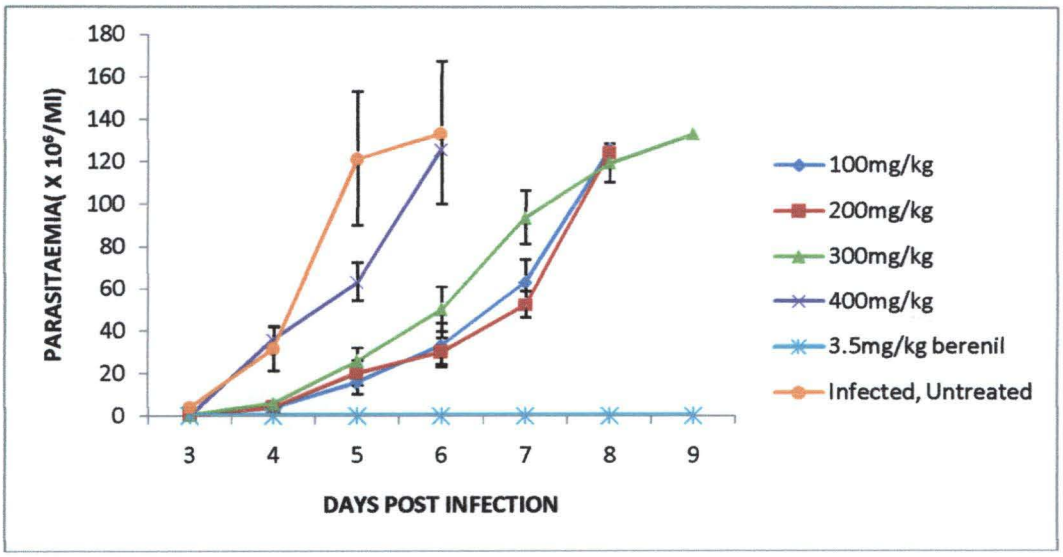


Figure 4.6: Effect of Different Doses of *Morinda lucida* Ethyl acetate Stem Bark Extract on *T. b. brucei* Infected Mice

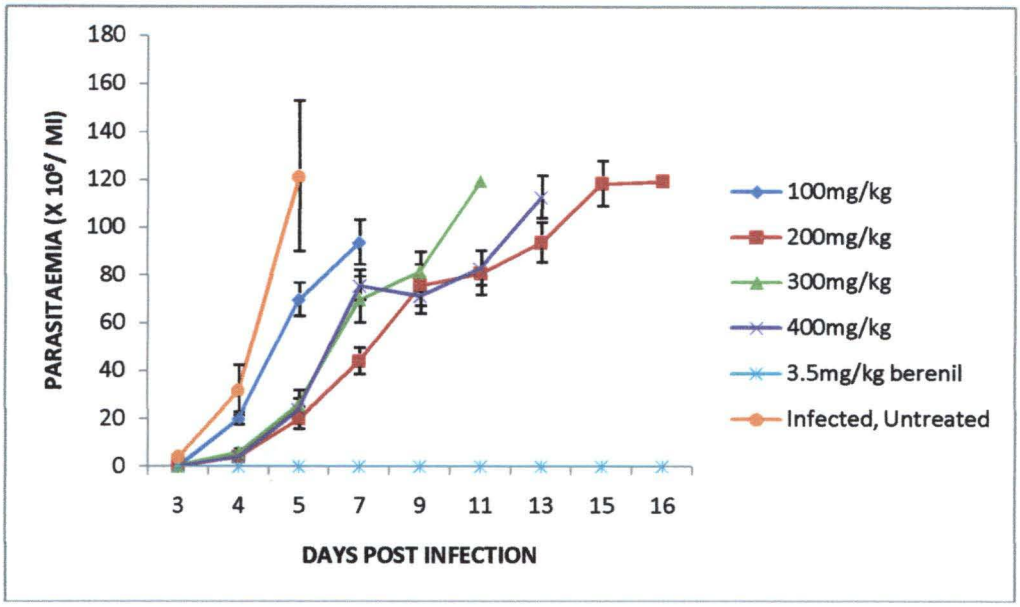


Figure 4.7: Effect of Different Doses of *Morinda lucida* Methanolic Stem Bark Extract on *T. b. brucei* Infected Mice

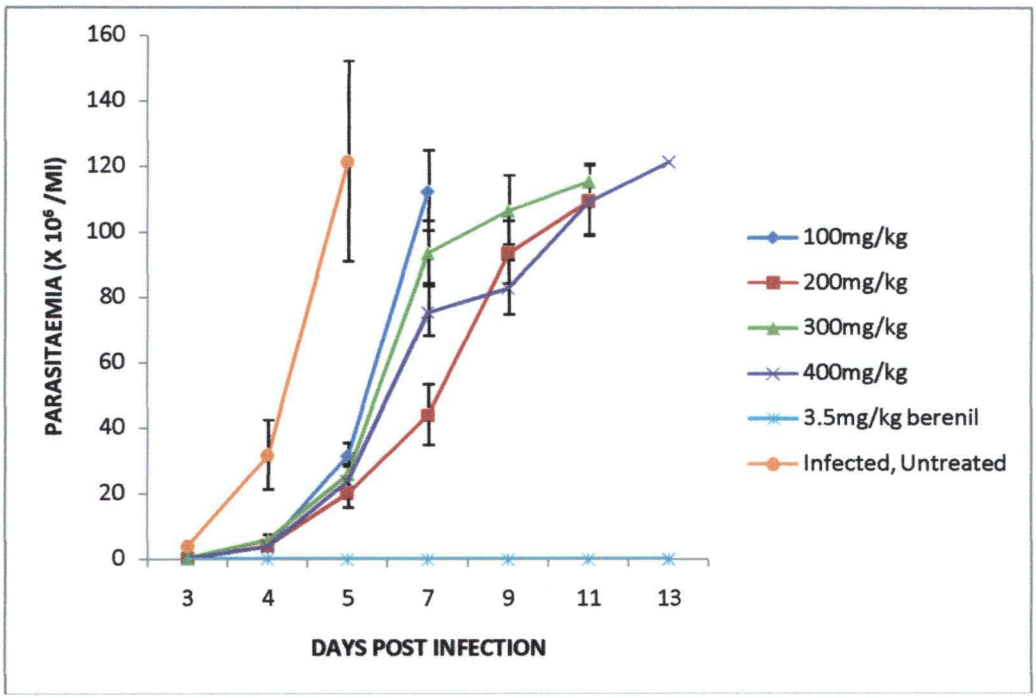


Figure 4.8: Effect of Different Doses of *Morinda lucida* Aqueous Stem Bark Extract on *T. b.brucei* Infected Mice

Table 4.3: Summary of Screening *Morinda lucida* Stem Bark Extract

Extraction Solvent	Dose (mg/Kg)	Survival Range	Survival beyond Control (Days). Min	Max	Means Survival (±SD)
Hexane	100	6-9	0	3	1.3±1.2
	200	6-10	0	4	2.3±1.7
	300	7-10	1	4	2.6±1.2
	400	6-7	0	1	0.6±0.5
Ethyl acetate	100	6-8	0	2	1.0±0.8
	200	6-8	0	2	1.0±0,8
	300	9	3	3	3.0±0.0
	400	4-6	-2	0	0.0±0.0
	Infected, Untreated	6	-		-
Methanol	100	6-8	2	3	2.0±0.8
	200	8-16	4	11	7.0±3.3
	300	7-12	3	7	4.3±2.1
	400	9-13	5	8	6.0±1.6
Aqueous	100	4-7	0	2	1.5±0.5
	200	5-12	1	7	4.0±2.9
	300	4-12	0	7	6.0±1.0
	400	9-13	5	8	6.0±1.6
	Infected Untreated	4-5	-	-	-

Treatment with the *Tridax procumbens* at all the dose levels resulted to lowering of the parasitaemia leading to prolongation of life (Figures 4.9 – 4.11). Ethyl acetate and methanol extract have the best trypanostatic effect resulting to significant means prolongation life by 11.7 and 14.3 days respectively ($P<0.05$). Aqueous extract has the least effect (Table 4.4).

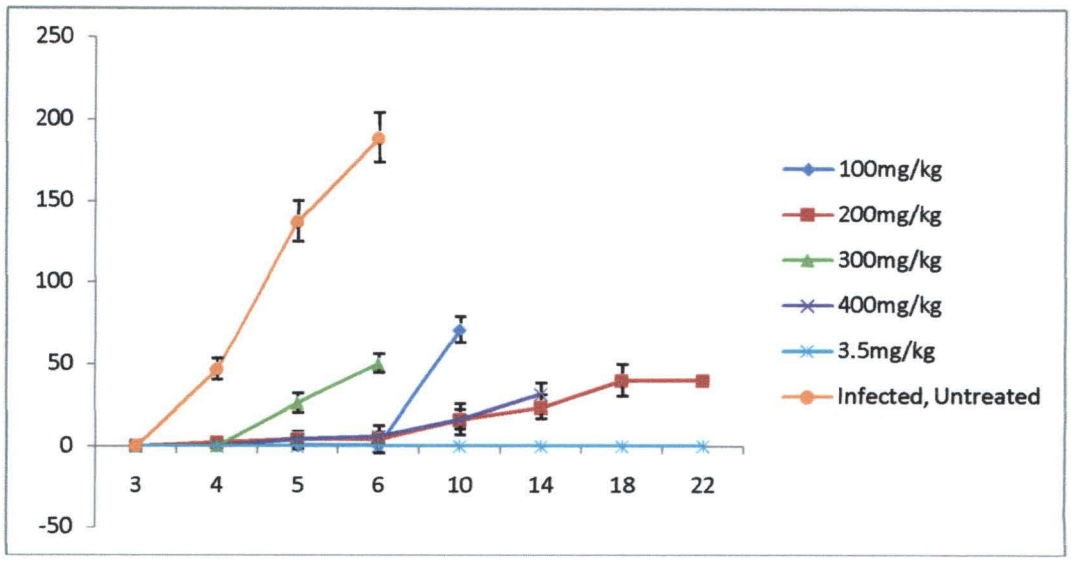


Figure 4.9: Effect of Different Doses of *Tridax procumbens* Ethyl acetate Extract on *T. b brucei* Infected Mice

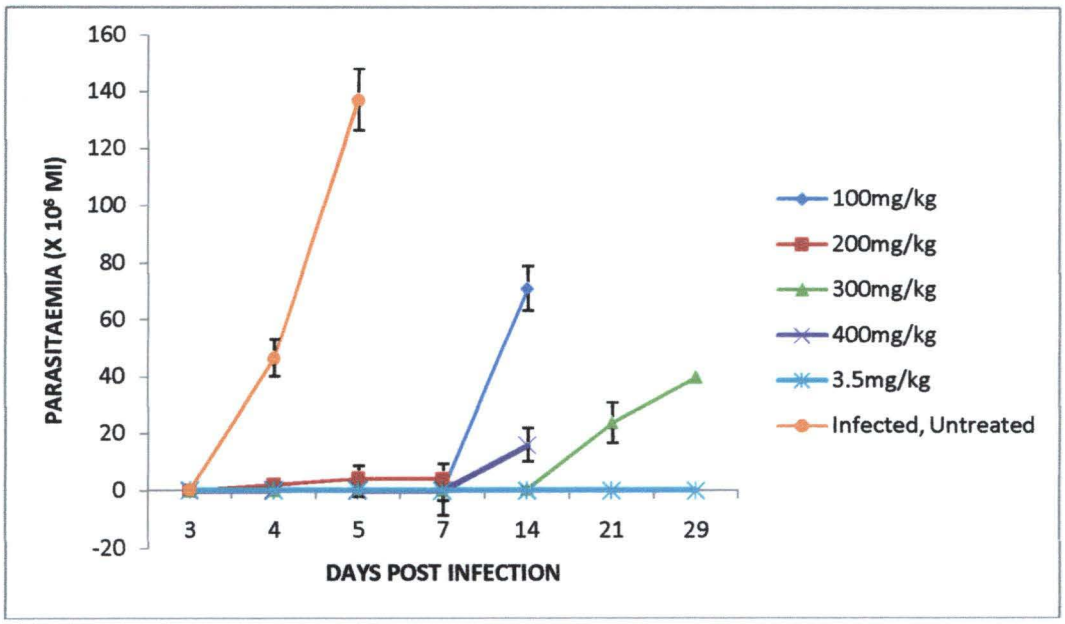


Figure 4.10: Effect of Different Doses of *Tridax procumbens* Methanolic Extract on *T. b. brucei* Infected Mice

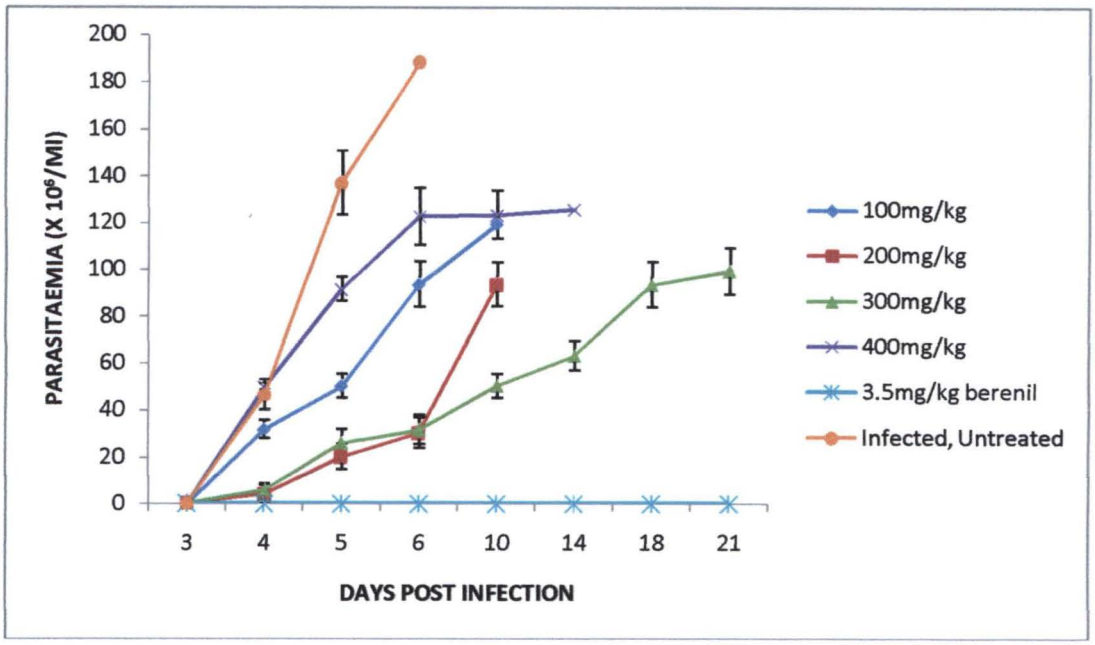


Figure 4.11: Effect of Different Doses of *Tridax procumbens* Aqueous Extract on *T. b brucei* Infected Mice

TABLE 4.4: Summary of Screening *Tridax procumbens* Extracts

Extraction Solvent	Dose (mg/Kg)	Survival Range	Survival beyond Control (Days). Min	Max	Means Survival (±SD)
Ethyl acetate	100	8-10	3	4	3.6±0.5
	200	9-22	4	16	11.7±5.4
	300	7-9	2	3	2.7±0.8
	400	6-16	1	10	6.3±3.8
Methanol	100	6-17	1	11	7.0±4.3
	200	6-10	1	4	2.6±1.2
	300	7-30	2	24	14.3±10.2
	400	5-15	0	10	5.6±4.2
Aqueous	100	8-10	3	4	3.7±0.5
	200	7-12	2	6	4.3±1.7
	300	7-21	2	15	8.0±5.4
	400	8-16	3	10	7.3±3.1
	Infected, Untreated	5-6	-	-	-

Confirmatory Test for the initial screening with the extracts at different doses and a subsequent repeated screening based on the results obtained from the initial screening showed that 400 mg/kg body weight of methanol extract of *M lucida* leaves and 200 mg/kg body weight of *T. procumbens* ethyl acetate extracts gave consistent antitrypanosomal activities. The means prolongation of life was almost the same with the result of the initial screening. However, the methanol stem bark extract of *M. Lucida* and methanol extract of *T. procumbens* at 200 mg/kg body weight could not reproduce the result of the initial screening as the value for means survival was lowered than the earlier one recorded. The control animals that were untreated died one week post infection.

Testing for the prophylactic activity of extracts showed that both the methanol leaves of *M.lucida* and the ethylacetate extracts of *T. procumbens* used to test for prophylaxis did not protect the animals from infection thus indicating no prophylactic activity against *T. b. brucei* infection. Parasites appeared in the blood almost at the same time with the control and death occurred at the same rate.

The combination of *Tridax procumbens* and *Morinda lucida* at 1:2 gave significant means survival of 10 days ($P<0.05$) at 200mg/kg body weight, while the least was when combined in ratio 1:1(Figures 4.12- 4.14).

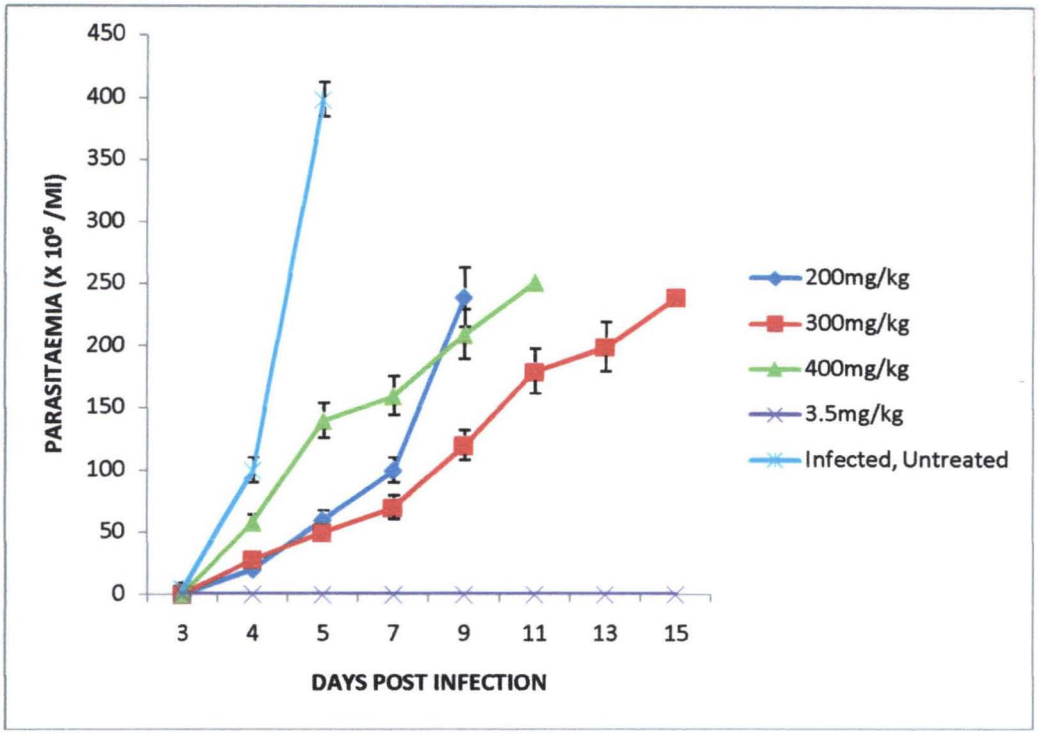


Figure 4.14: Effect of Different Doses of Combined *Tridax procumbens* and *Morinda lucida* Methanol Extracts (2:1) on *T. b. brucei* Infected Mice.

Table 4.5: Summary of Screening Combined *T. procumbens* and *M. lucida* Methanolic Extracts

Combination Type	Dosage (Mg/Kg)	Survival range	Survival beyond Control (Days) Min	Max	Means Survival (±SD)
TP:ML(1:1)	200	9-11	4	5	4.0±0.8
	300	13	-	7	7.0±0.0
	400	9-11	4	5	4.0±0.8
TP:ML (1:2)	200	13-19	8	13	10.0±2.2
	300	9-14	4	8	5.3±2.1
	400	9-11	4	5	4.0±0.8
TP:ML (2:1)	200	13-15	8	9	8.0±0.8
	300	12-17	7	11	8.3±2.1
	400	8-13	3	7	4.7±2.1
	Infected, Untreated	5-6	-	-	-

TP = *Tridax procumbens* ML = *Morinda lucida*

Table 4.6: Summary of Screening *T. procumbens* Ethyl acetate Fractions

Fraction Type	Survival Range (Days)	Survival Beyond Control(Days) Min.	Max.	Means Survival (±SD)
Fraction 1	8	-	3	3.0±0.0
Fraction 2	8	-	3	3.0±0.0
Fraction 3	8	-	3	3.0±0.0
Fraction 4	11	-	6	6.0±0.0
Fraction 5	11-15	7	10	8.2±1.2
Fraction 6	7-8	3	3	2.7±0.5
Fraction 7	9-14	5	9	7.0±1.6
Fraction 8	9-16	5	11	8.3±2.5
Fraction 9	8-9	4	4	3.7±0.5
Fraction 10	8-13	4	8	6.0±1.6
Fraction 11	18-21	14	16	15.0±0.8
Fraction 12	10-15	6	10	8.0±1.6
Crude	11-15	7	10	8.7±1.2

N.B. The highlighted are the fractions with the highest activity.

With the exception of fraction 2 which was negligible, all the other methanol fractions resulted to longer means survival of treated mice than the infected untreated control (Figures 4.17 – 4.18). The highest means survival was 12.3 days for fraction 7 while the least was 4.7 days for fraction 3 (Table 4.7).

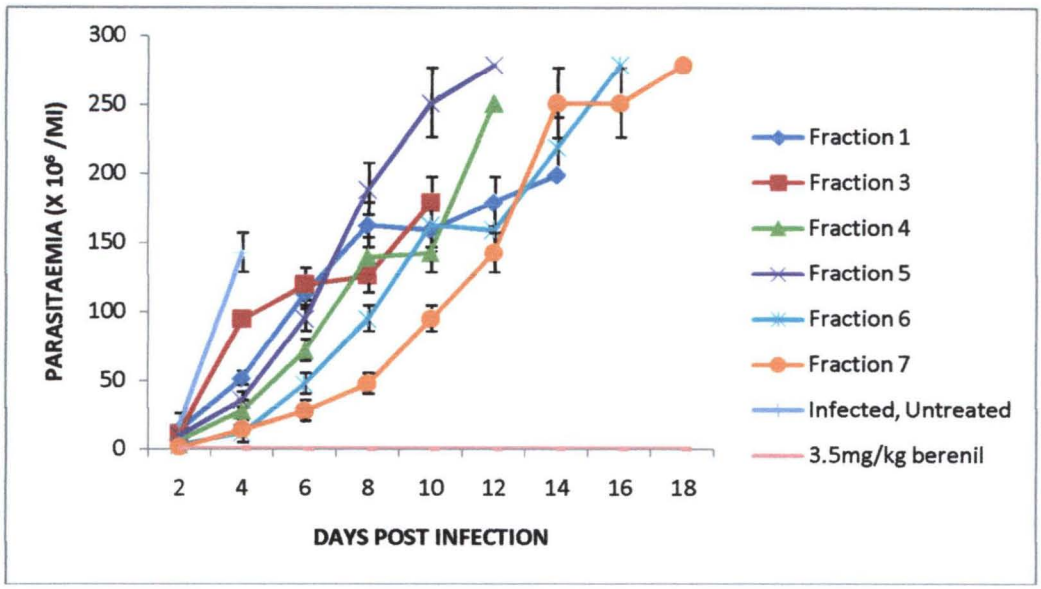


Figure 4.17: Effect of *Tridax procumbens* Different Methanol Fractions (1-7) on *T. b. brucei* Infected Mice.

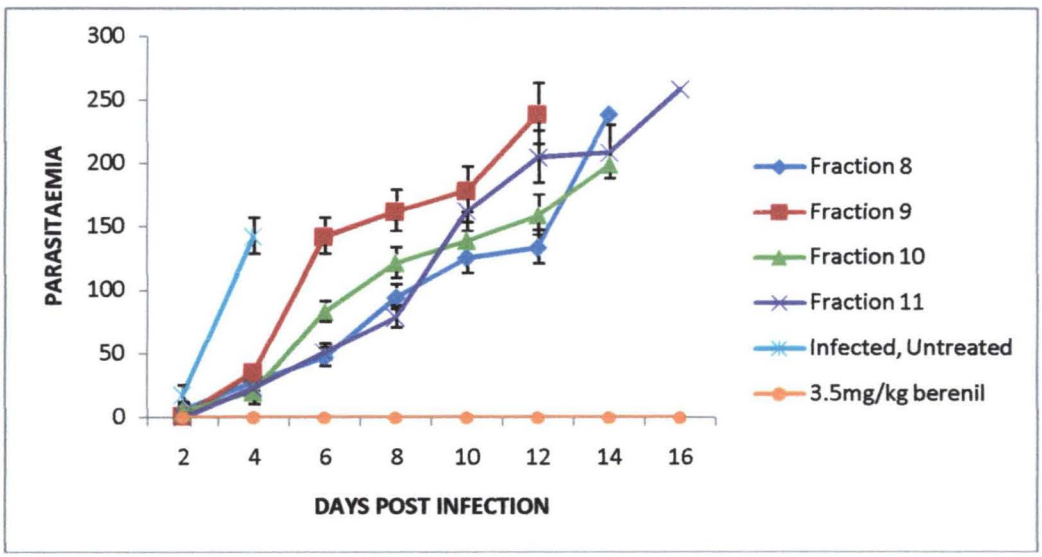


Figure 4.18: Effect of *Tridax procumbens* Different Methanol Fractions (8-11) on *T. b. brucei* Infected Mice.

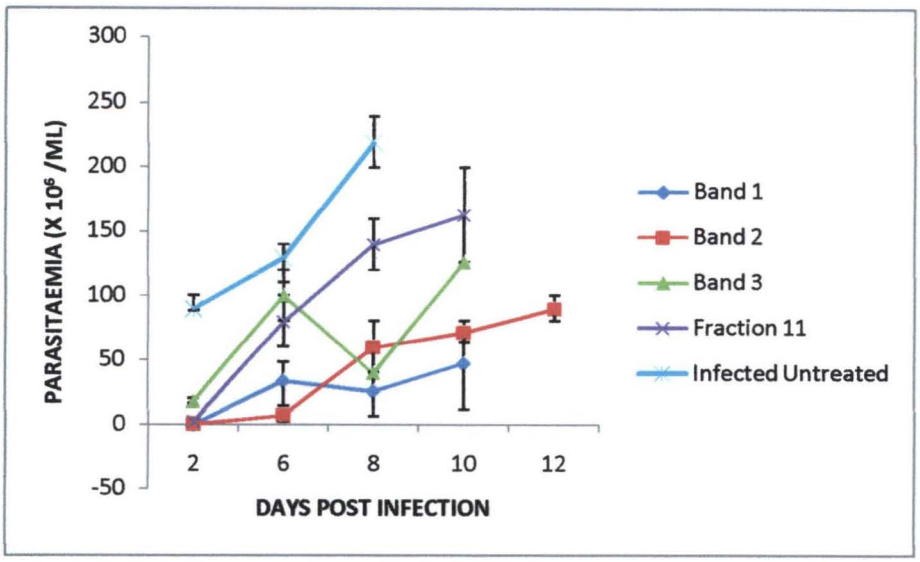


Figure 4.19: Effect of *Tridax procumbens* Different Bands (1-3) of Fraction 11 on *T. b. brucei* Infected Mice.

Table 4.8: Summary of Screening *T.procumbens* Ethyl acetate Bands and Fraction 11

Type	Survival Range (Days)	Survival Control (Days) Min.	Beyond Max.	Means Survival (±SD)
Band 1	10-11	2	2	2.0±0.0
Band 2	11-12	3	3	3.0±0.0
Band 3	8-10	0	1	0.5±0.5
Fraction 11	10-11	2	2	2.0±0.02
Infected, Untreated	8-9	-	-	-

4.2 Thin Layer Chromatography of Crudes and Active Fractions

The fractions with reproducible trypanostatic activities are fractions 11 and 7 respectively for ethyl acetate and methanol extracts. The respective thin layer chromatography of these fractions and their crude were then compared when developed in chloroform, chloroform: methanol (9:1) and chloroform: methanol (4:1). With the exception of the ethyl acetate fraction developed in chloroform: methanol (4:1), all the

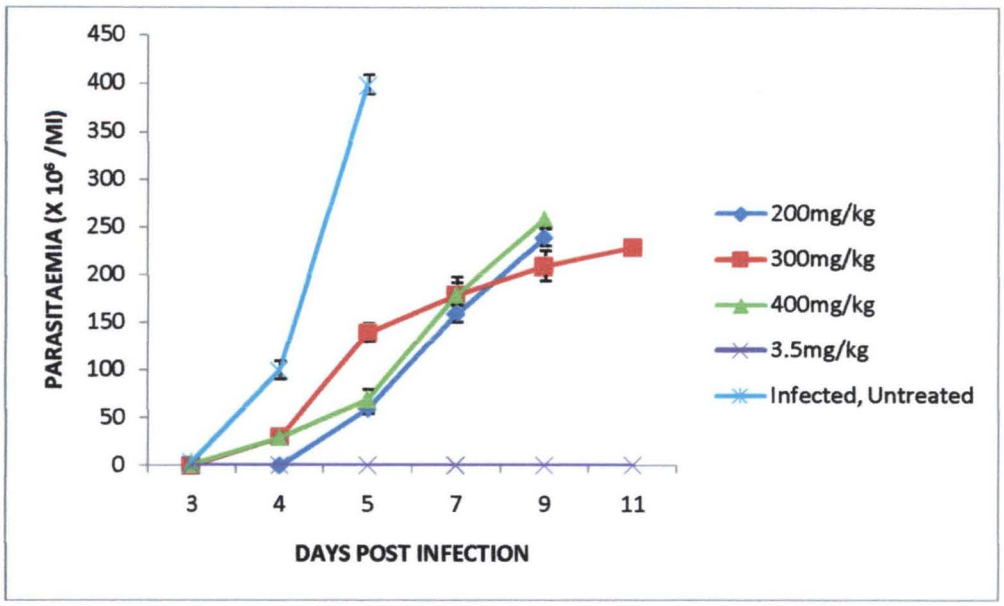


Figure 4.12: Effect of Different Doses of Combined *Tridax procumbens* and *Morinda lucida* Methanol Extract (1:1) on *T. b. brucei* Infected Mice.

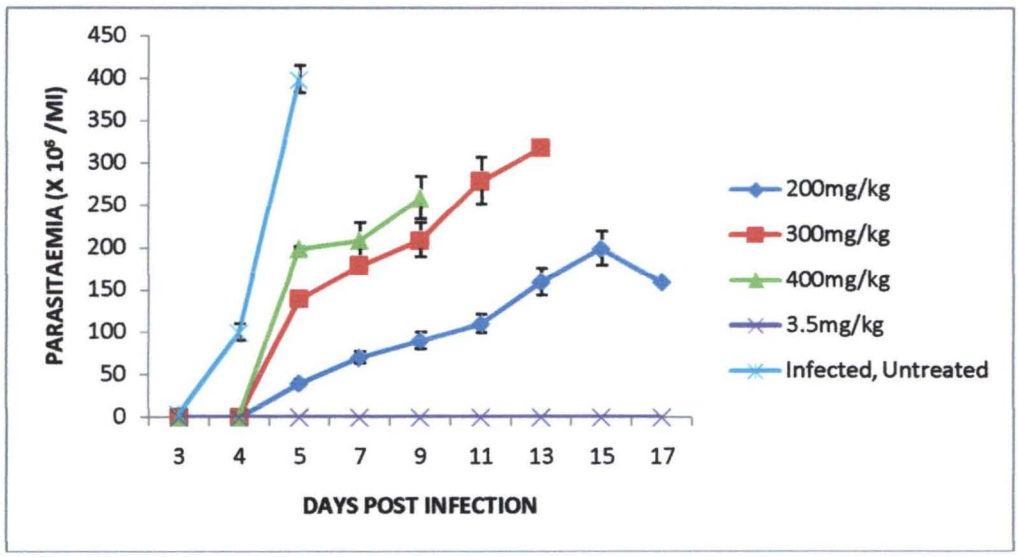


Figure 4.13: Effect of Different Doses of Combined *Tridax procumbens* and *Morinda lucida* Methanol Extract (1:2) on *T. b. brucei* Infected Mice.

Antitrypanosomal Effect of *T. procumbens* different ethyl acetate Fractions (Figures 4.15 -4.16) showed that fraction 11 gave the highest means survival of 15 days ($P<0.05$) while the least means survival was in fraction 6 which gave 2.7 days (Table 4.6).

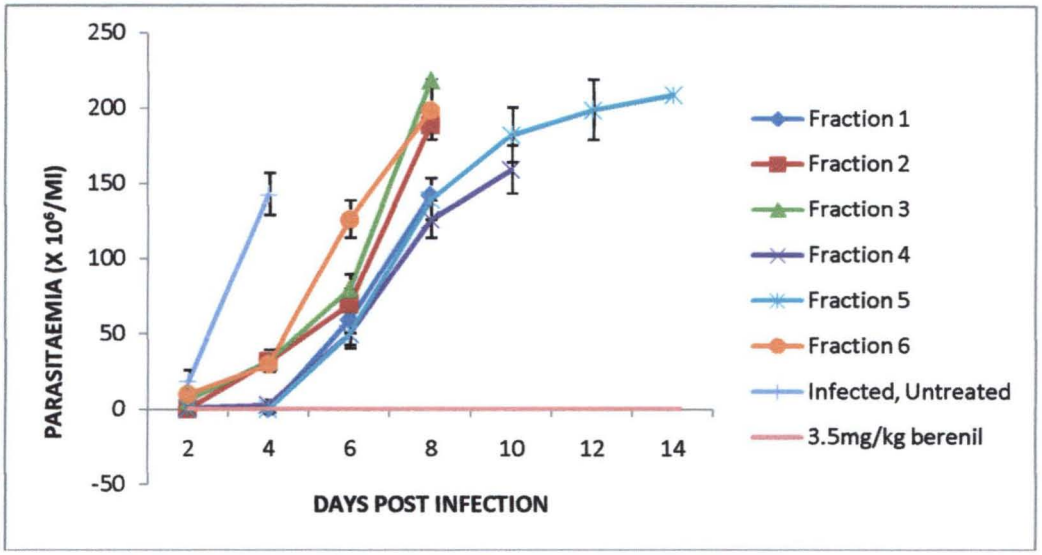


Figure 4.15: Effect of *Tridax procumbens* Different Ethylacetate Fractions (1-6) on *T. b. brucei* Infected Mice.

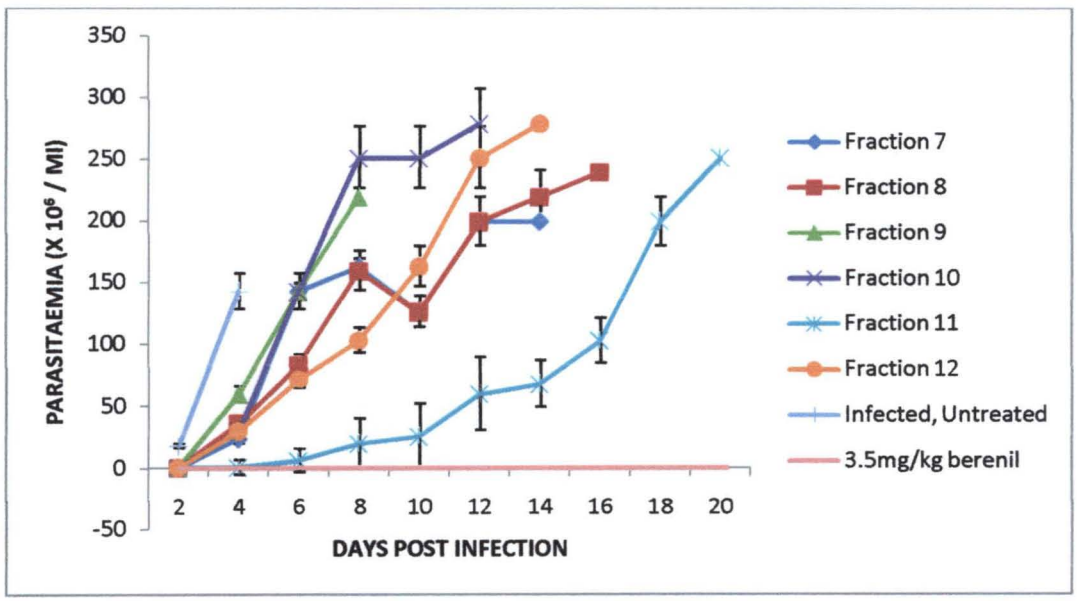
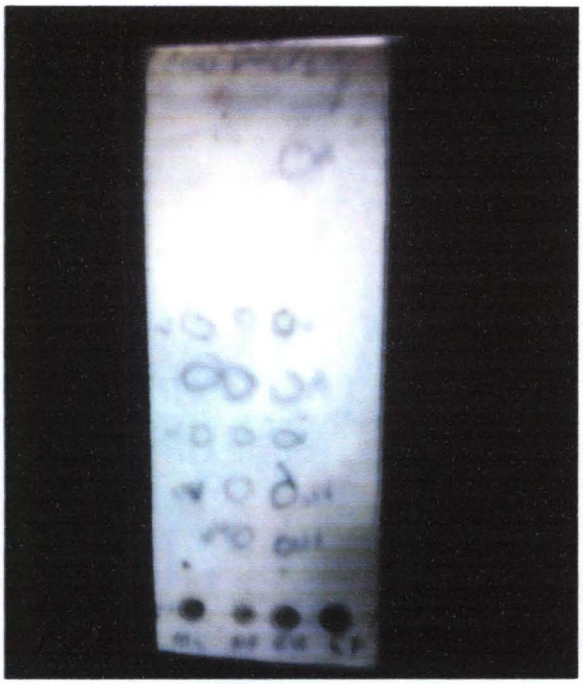


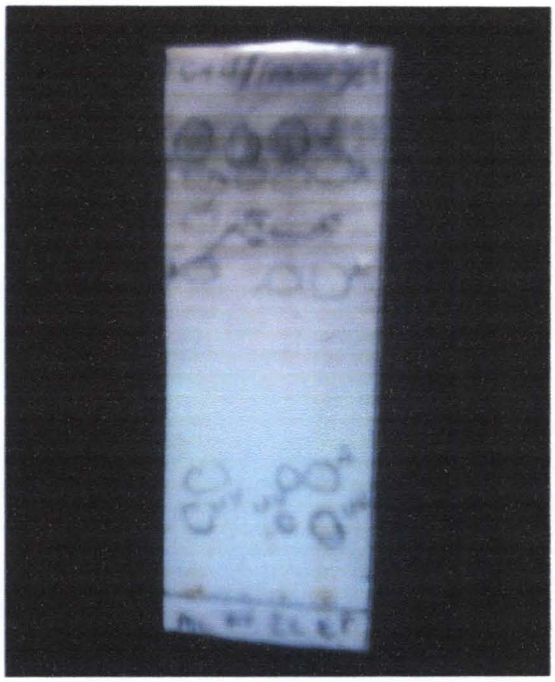
Figure 4.16: Effect of *Tridax procumbens* Different Ethyl acetate Fractions (7-12) on *T. b. brucei* Infected Mice.

others contained similar components based on their Rf values which were visible under long wave UV light at 366nm flourensced golden yellow colouration (Tables 4.1-4.3).



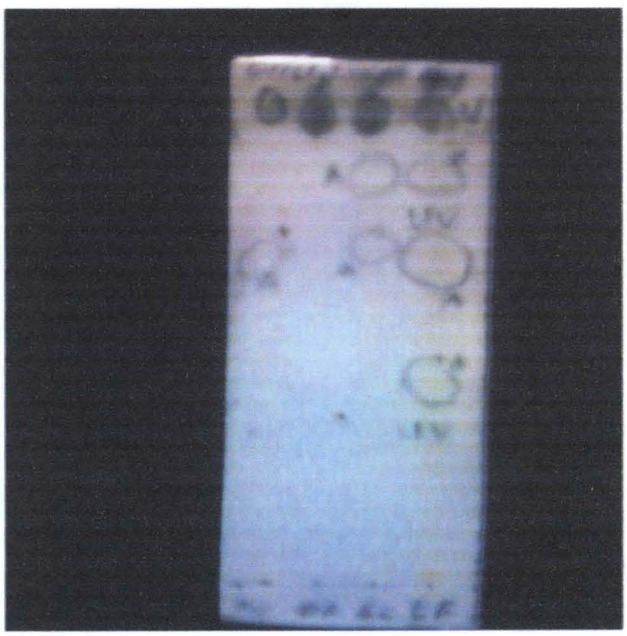
MC MF EC EF

Plate I: TLC plate of Different *T. procumbens* extract (Solvent =Chloroform)



MC MF EC EF

Plate II: TLC of plate Different *T. procumbens* extract (Solvent = Chloroform: Methanol, 9:1)



MC MF EC EF

Plate III: TLC plate of Different *T. procumbens* extract (Solvent = Chloroform: Methanol, 4:1)

Table 4.9: Rf Values of *Tridax procumbens* Crude and Fractions Using Choloroform as Mobile Phase.

Types of Extract	Rf Values	Visualization
Crude Methanol	0.25	Visible
	0.35	Visible
	0.46	Visible
	0.57	Visible
Methanol Fraction	0.16	UV Light
	0.25	Visible
	0.35	Visible
	0.46	Visible
	0.57	Visible
Crude Ethyl Acetate	0.16	UV Light
	0.25	UV Light
	0.35	Visible
	0.46	Acid Spray
	0.57	Visible
	0.86	Acid Spray
Ethyl Acetate Fraction	-	-

Table 4.10: Rf Values of *Tridax procumbens* Crude and Fractions Using Choloroform and Methanol (9: 1) as Mobile Phase.

Types of Extract	Rf Values	Visualization
Crude Methanol	0.16	UV Light
	0.25	Acid Spray
	0.67	Acid Spray
	0.75	Visible
	0.84	Acid Spray
	0.93	Visible
Methanol Fraction	0.75	Acid Spray
	0.84	Acid Spray
	0.93	Visible
Crude Ethyl Acetate	0.16	UV Light
	0.25	Acid Spray
	0.67	Acid Spray
	0.75	Acid Spray
	0.84	Acid Spray
	0.93	Visible
Ethyl acetate Fraction	0.16	UV Light
	0.25	Acid Spray
	0.67	Acid Spray
	0.84	Acid Spray
	0.93	Visible

Table 4.11: Rf Values of *Tridax procumbens* Crude and Fractions Using Choloroform and Methanol (4: 1) as Mobile Phase.

Types of Extract	Rf Values	Visualization
Crude Methanol	0.67	Visible
	0.93	Acid Spray
Methanol Fraction	0.93	Visible
Crude Ethyl Acetate	0.67	Acid Spray
	0.84	Acid Spray
	0.93	Visible
Ethyl Acetate Fraction	0.40	UV Light/Acid Spray
	0.67	UV Light/ Acid Spray
	0.84	Acid Spray
	0.93	Visible

4.3 NMR result and TLC profile of the Bands

The chemical compounds present were deduced from the standard NMR correlation charts. The nature of the active compound was clearly defined. From the spectra data obtained (Figures 4.20-4.28), the ^{1}H NMR of band 1 shows the methyl and polymethylene absorptions between 0.6 and 1.6 deltas (δ). The ^{13}C NMR shows the presence CH_3 at 14.354, CH and poly CH_2 at 29.595 ppm, quartenary carbon, unsaturation carbon absorption at 130.395 and 125.595 and weak C=O at about 160-170ppm indicating unsaturated long chain fatty acids.

Band 2 ^{1}H NMR shows olefinic hydrogens between 6.6 and 7.7 delta typical of aromatic hydrogen absorptions as shown by phenolic compounds including flavonoids. The spectrum also shows –CH-O- hydrogen absorptions typical of sugar fragments in the region 3.3 – 3.8 delta. Probable absorptions due to impurity from the Band 1 sample are

also present. ^{13}C NMR confirms olefinic / aromatic carbon absorptions at 130.03 and 115.67, -CH-O, CH_2-O carbon absorptions typical of sugar fragments between 102 and 61ppm and polymethylene group absorption at 29.581ppm. There are weak absorptions around 200, 190, 170, 158, 140 and 134ppm some of which are ascribable to aromatic carbonyl compounds including phenols and flavonoid compounds.

Band 3 ^{1}H NMR spectrum shows absorptions around 1.4 delta for polymethylene group, while the ^{13}C NMR shows -CH-O-, -CH_2-O- at 101-51 and 29.555ppm for polymethylene, -$(CH_2)_n$- group. The polymethylene absorptions shown in the three spectra may be due to fatty acid derivatives or the polar compounds themselves.

The result of normal phase TLC detected by vanillin in sulphuric acid indicates a prominnent yellow reacting spot at Rf 0.81 common to both the active fraction 11 as well as the PTLC band 2 (plate IV). Similarly, another normal phase TLC detected by ferric chloride solution indicate a normal blue-black reacting spot (phenol) at Rf 0.81 common to both the fraction 11 and PTLC band 2 (Plate V). The prominent colour was yellow in vanillin/ sulphuric acid sprayed while it is blue-black in ferric chloride sprayed. The Rf value is also the same in normal phase TLC plates but was however lower in reverse phase TLC plate (Table 4.12 – 4.14). The yellow reacting spot in vanillin/H_2SO_4 is the same as the black-blue reactions of spot in ferric chloride suggesting the spot to be phenolic. The compound present is related to flavonoids eg flavones, 7, 8 Dihydroxyflavone, 3 Hydroxyflavone, Genistein, Quercetin and catechin which all have phenolic groups. Their structures are hereby presented in Figures 4.29.

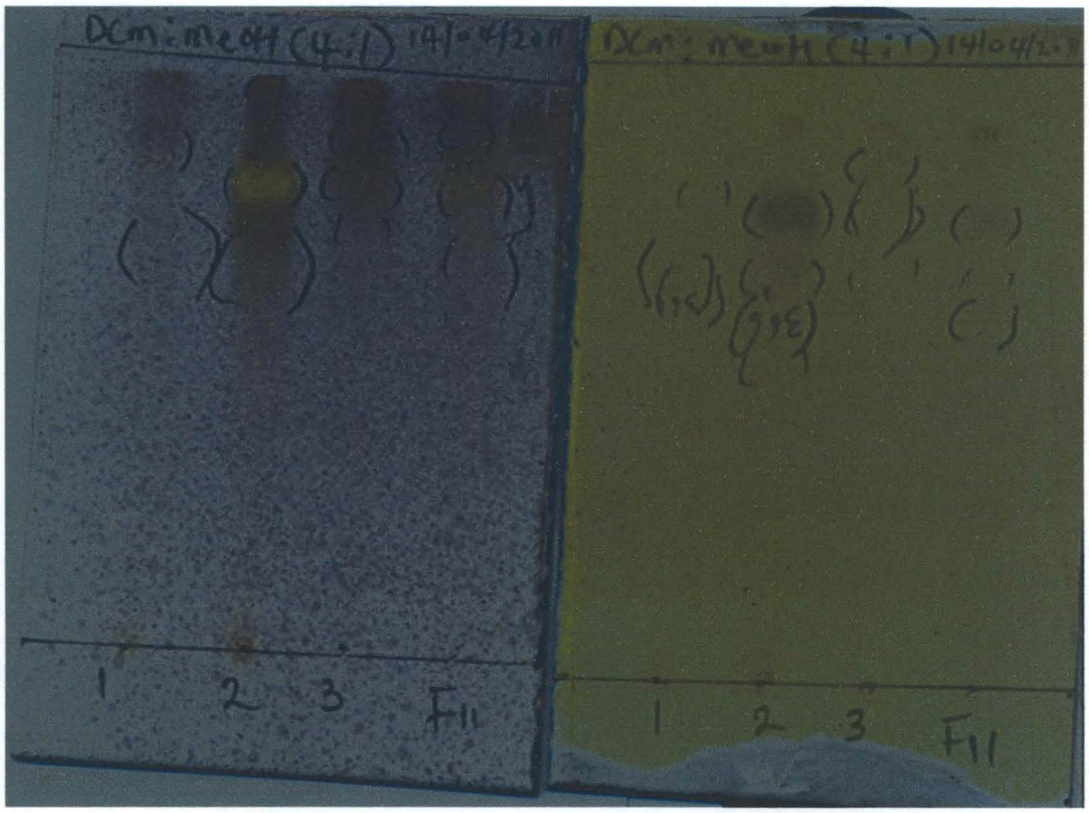


Plate IV: Normal Phase TLC Sprayed with Vanillin/Sulphuric Acid

Plate V: Normal Phase TLC Sprayed with Ferric Chloride Solution

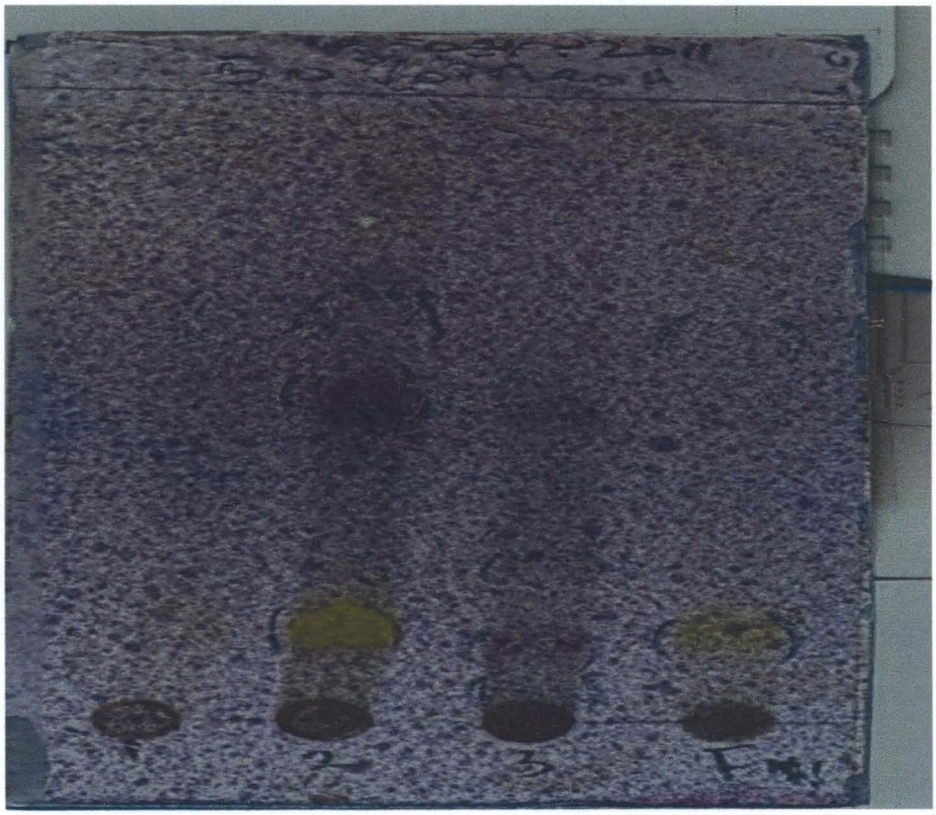


Plate VI: Reverse Phase TLC Sprayed with Vanillin/Sulphuric Acid

KEY: 1= BAND 1; 2= BAND 2; 3= BAND 3; F11= FRACTION 11

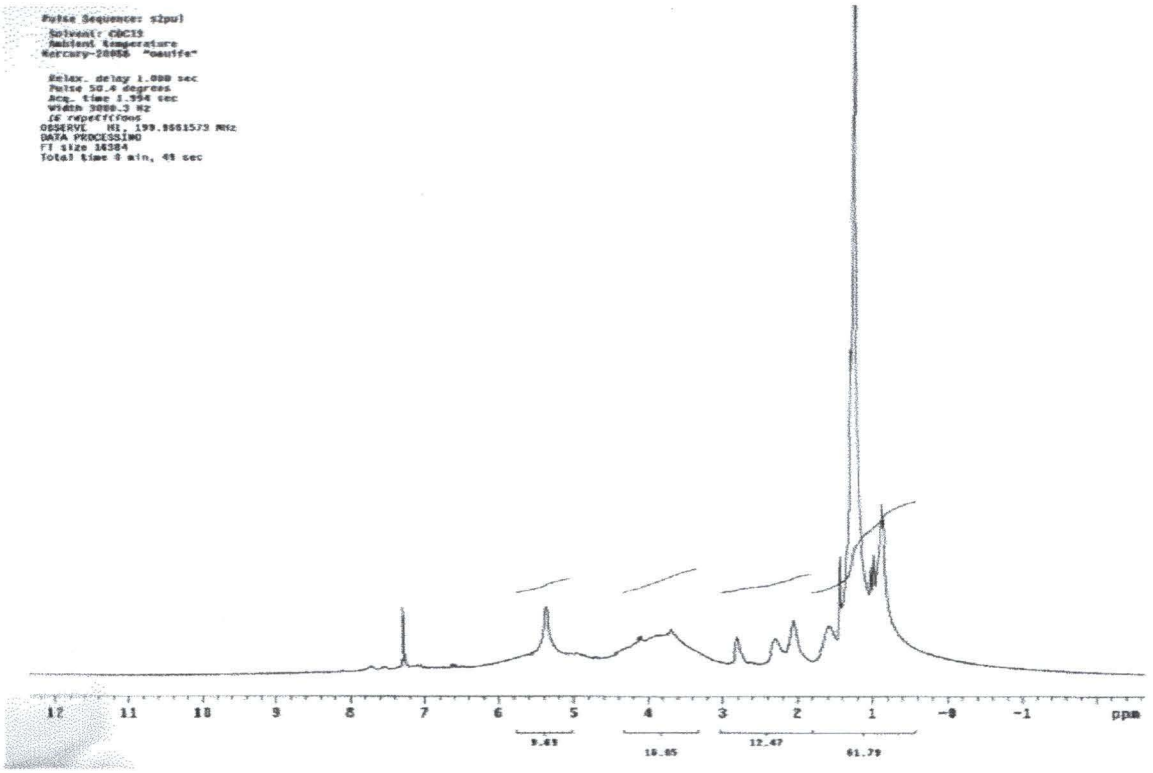


Figure 4.20: ^{1}H NMR Spectrum of Band 1 in Deuterated Methanol

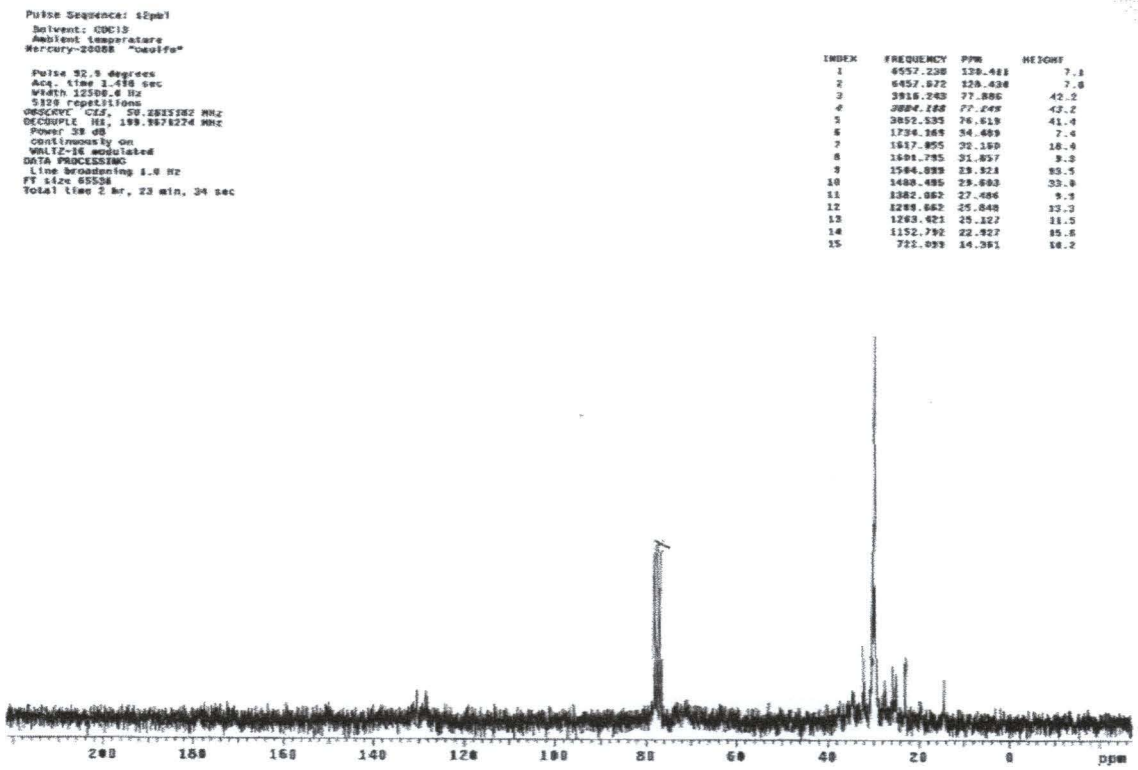


Figure 4.21: ^{13}C NMR Spectrum of Band 1 in Deuterated Methanol

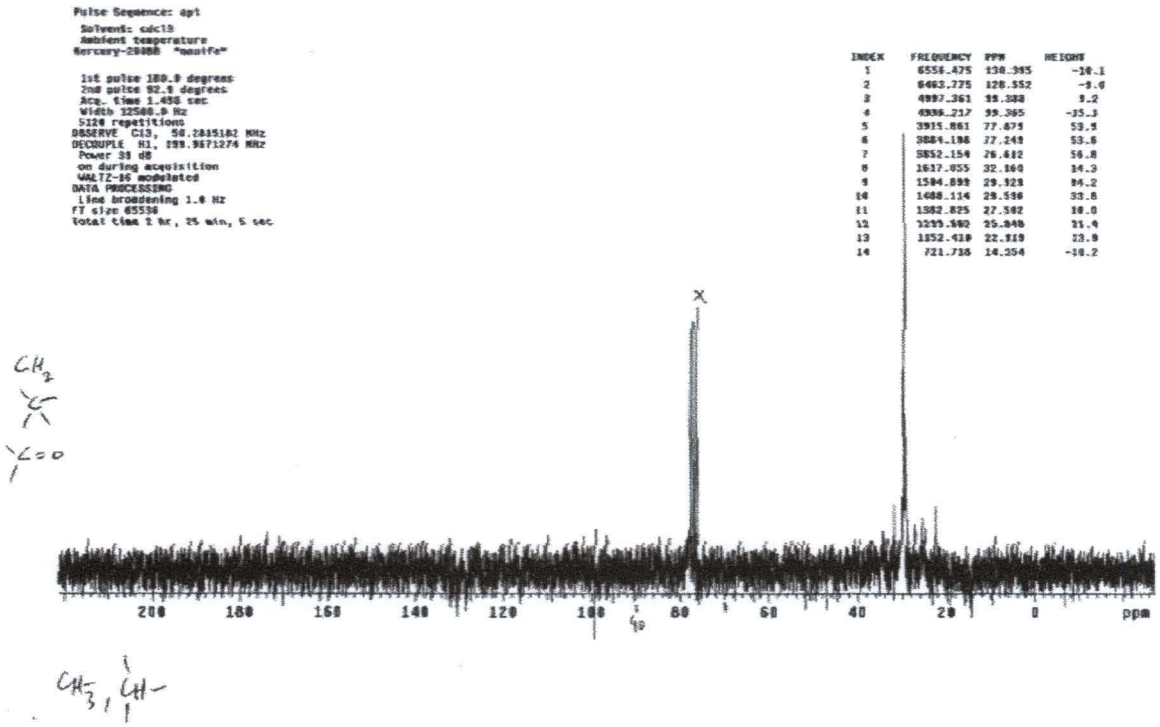


Figure 4.22: Attached Protein Test ^{13}C NMR of Band 1 in Deuterated Methanol

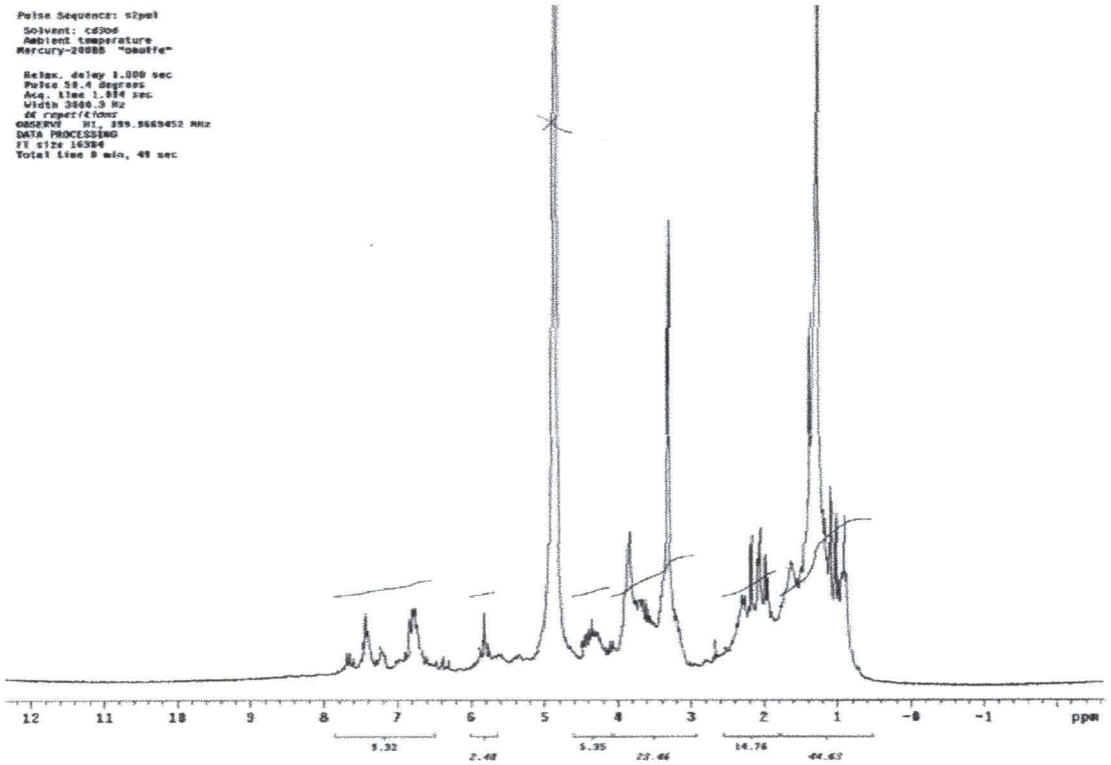


Figure 4.23: ^{1}H NMR Spectrum of Band 2 in Deuterated Methanol

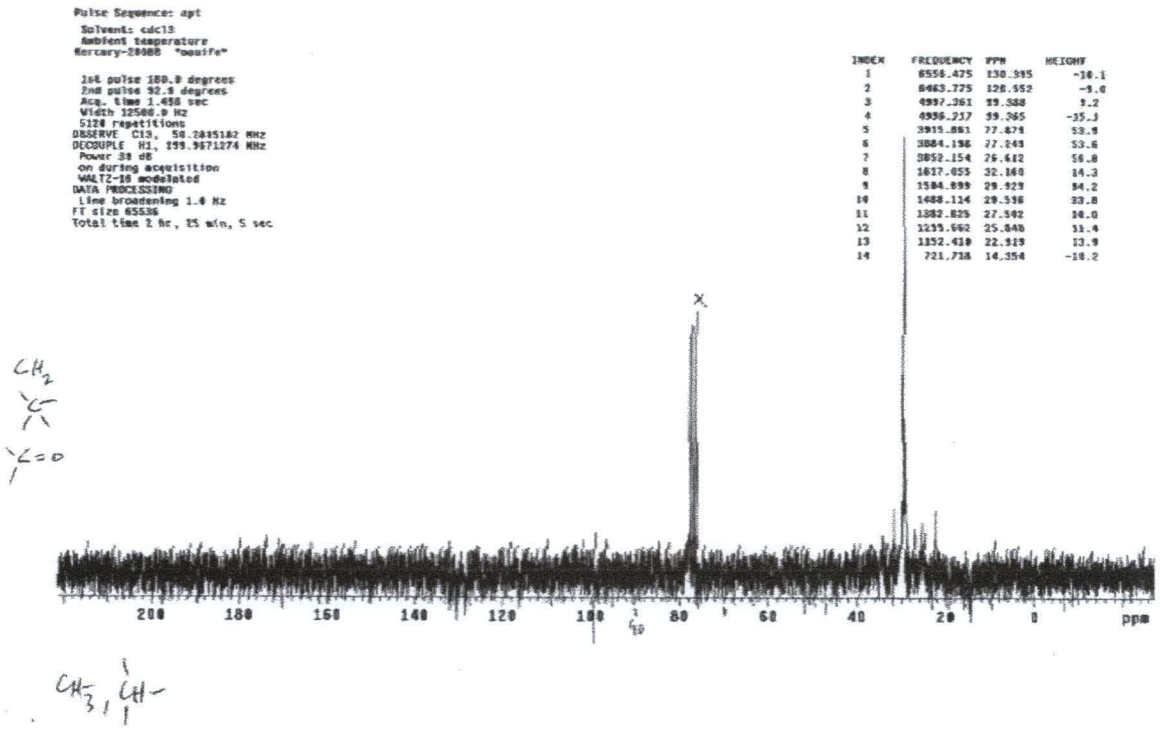


Figure 4.24: Attached Protein Test ^{13}C NMR of Band 1 in Deuterated Methanol

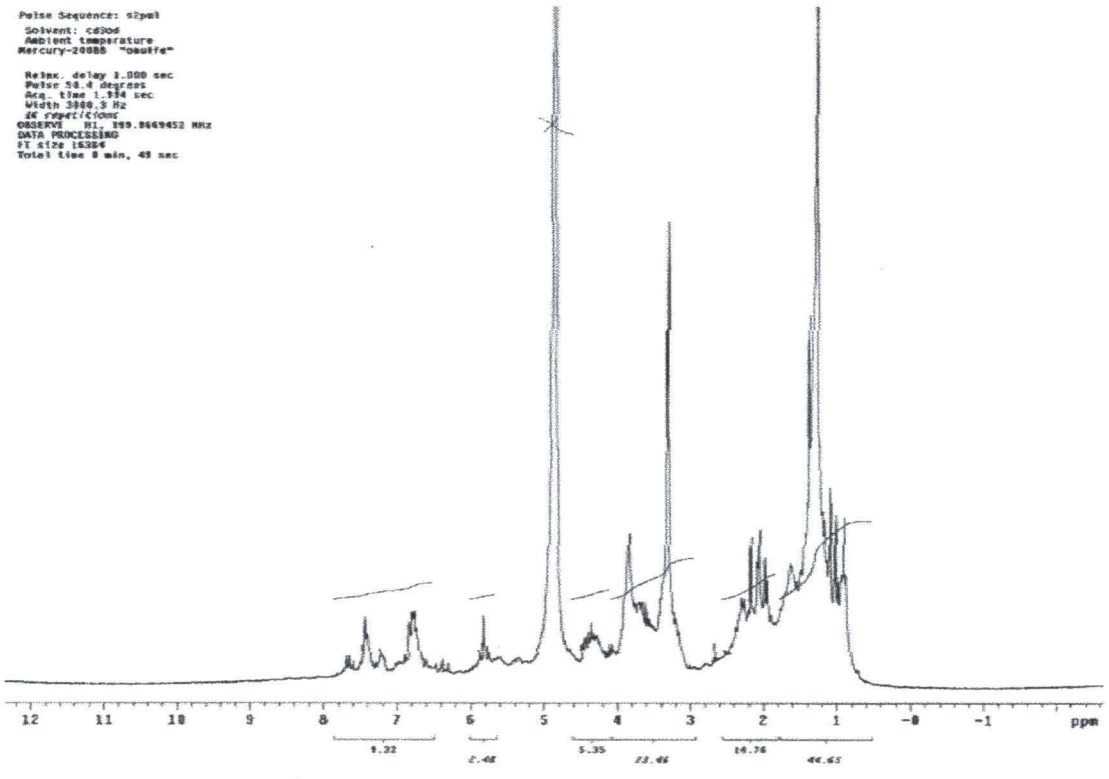


Figure 4.25: ^{1}H NMR Spectrum of Band 2 in Deuterated Methanol

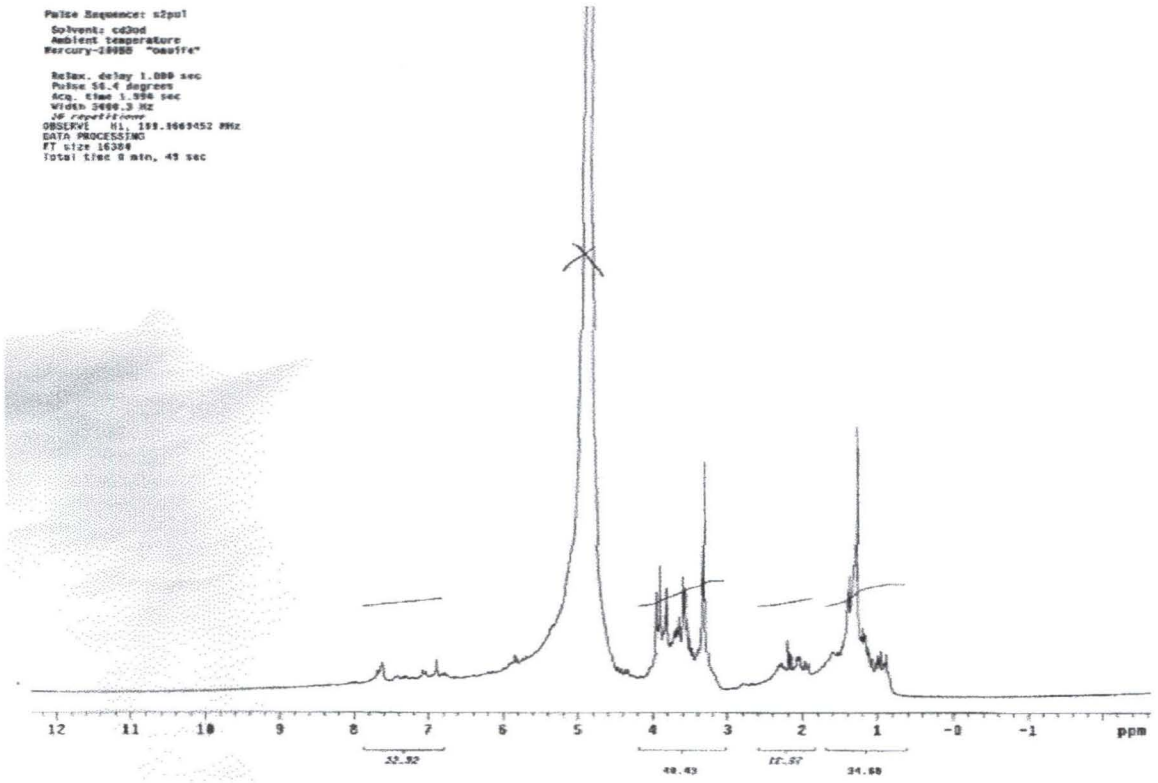


Figure 4.26: ^{1}H NMR Spectrum of Band 3 in Deuterated Methanol

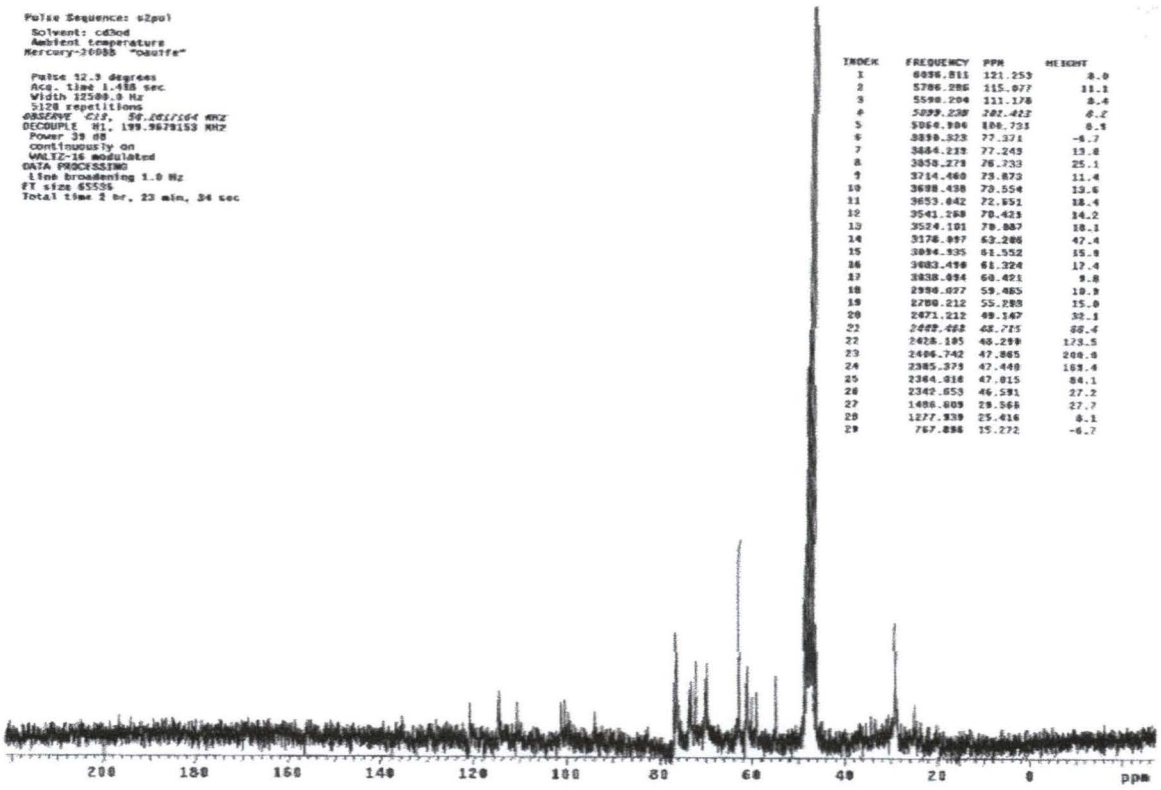


Figure 4.27: ^{13}C NMR Spectrum of Band 3 in Deuterated Methanol

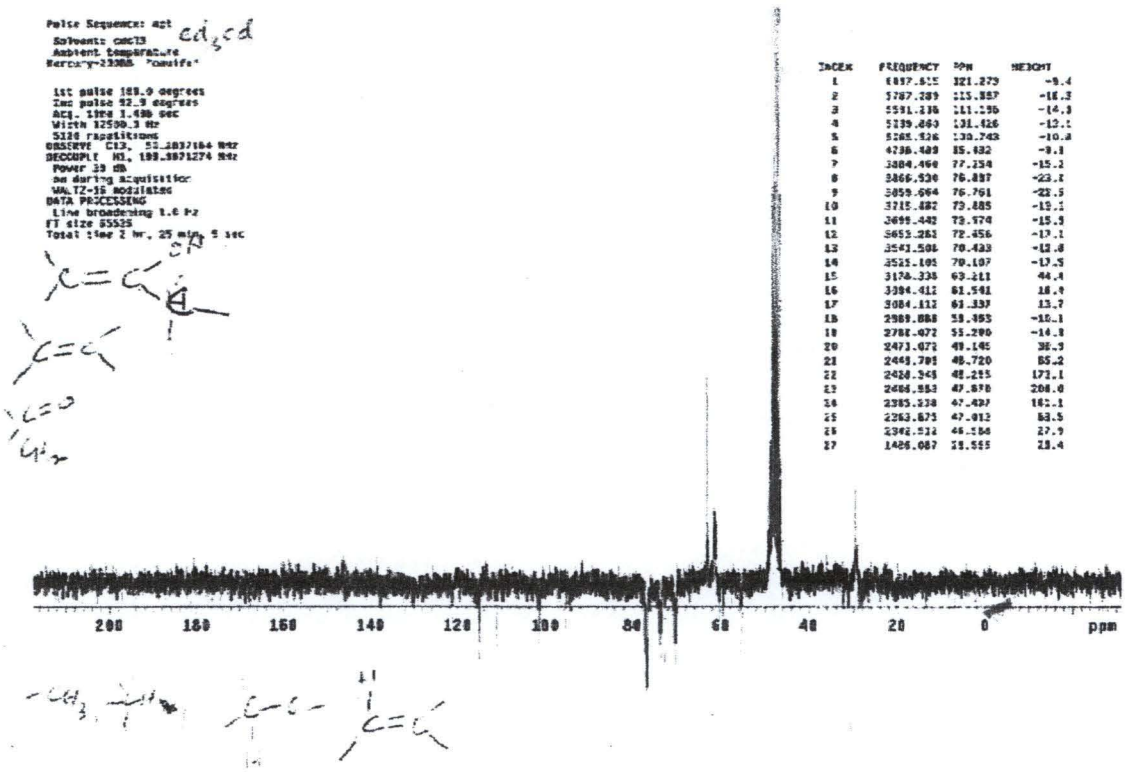
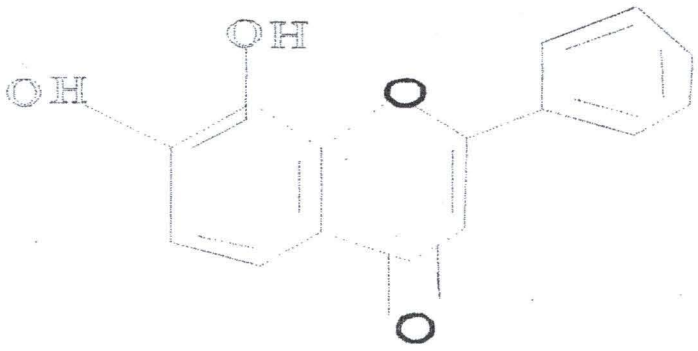
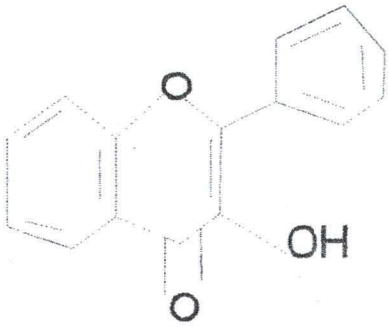


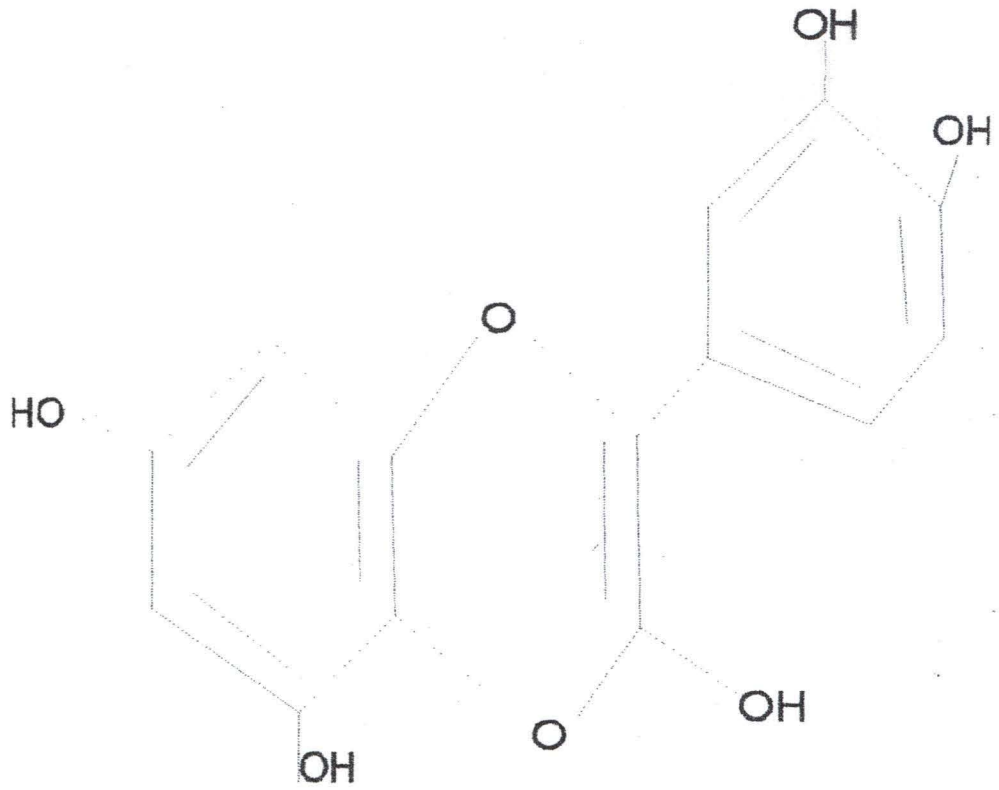
Figure 4.28: Attached Protein Test ^{13}C NMR of Band 3 in Deuterated Methanol



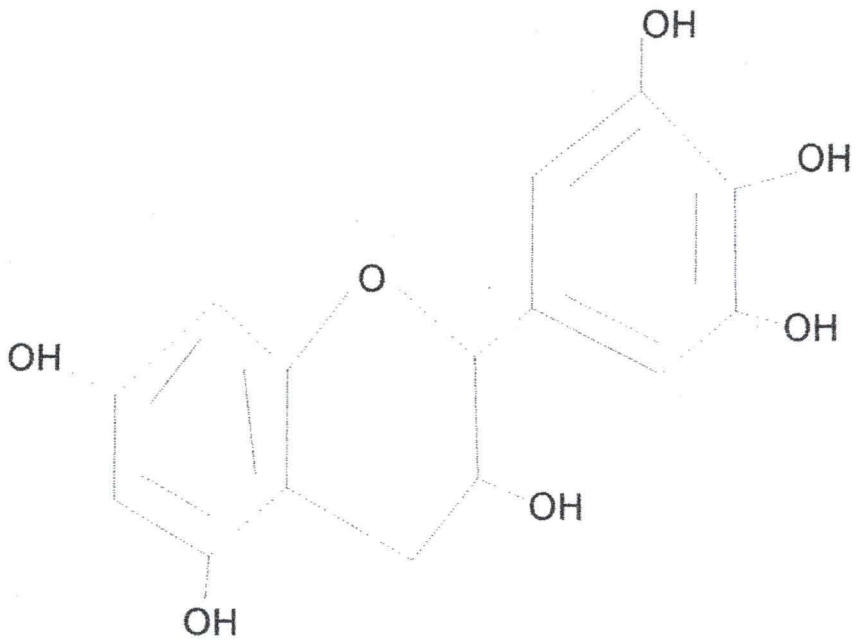
7, 8 - Dihydroxyflavone



3- Hydroxyflavone



Quercetin



Catechin

Figure 4.29: Possible Chemical Compounds in Band 2

Table 4.12: Rf Values of *T. procumbens* Different Bands and Active Fraction 11 Sprayed with Ferric Chloride Solution Using Dichloromethane and Methanol (4: 1) as Mobile Phase.

Types of Extract	Rf Values	Visualization
Band 1	*0.70*	*UV Light*
	0.76	UV Light
Band 2	0.55	UV Light
	0.60	UV Light
	0.70	UV Light
	0.76	$FeCl_3$Spray
Band 3	0.70	UV Light
	0.76	$FeCl_3$Spray
	0.81	$FeCl_3$Spray
Fraction 11	0.60	UV Light
	0.70	UV Light
	0.76	UV Light

Table 4.13: Rf Values of *T. procumbens* Different Bands and Active Fraction 11 Sprayed with Vanillin in Sulphuric Acid Using Dichloromethane and Methanol (4: 1) as Mobile Phase.

Types of Extract	Rf Values	Visualization
Band 1	*0.70*	*UV Light*
	0.76	UV / Vanillin/H_2SO_4 Spray
Band 2	0.70	UV Light
	0.77	Vanillin/H_2SO_4 Spray
Band 3	0.70	UV Light
	0.77	Vanillin/H_2SO_4 Spray
	0.83	Vanillin/H_2SO_4 Spray
Fraction 11	0.70	UV Light
	0.77	Vanillin/H_2SO_4 Spray
	0.83	Vanillin/H_2SO_4 Spray

Table 4.14: Rf Values of *T. procumbens* Different Bands and Active Fraction 11 Sprayed with Vanillin in Sulphuric Acid Using 50% Methanol as Mobile Phase.

Types of Extract	Rf Values	Visualization
Band 2	0.15	Vanillin/H_2SO_4 Spray
	0.83	UV / Vanillin/H_2SO_4Spray
Band 3	0.13	Vanillin/H_2SO_4 Spray
	0.50	UV / Vanillin/H_2SO_4 Spray
	0.55	UV / Vanillin/H_2SO_4Spray
Fraction 11	0.13	Vanillin/H_2SO_4 Spray
	0.55	UV/ Vanillin/H_2SO_4 Spray

4.4 *In vitro* Antibacterial Activity of *T. procumbens* Fractions and Crude Extracts

The antibacterial activities of the crude methanol and ethylacetate extracts and their active trypanostatic fractions 7 and 11 respectively of *T. procumbens* were determined against five bacterial strains using a uniform concentration of 0.2mg/ml. The fraction 11 of the ethyl acetate recorded highest activity against *E. Coli* producing a zone of inhibition of 24mm (Plate X) as compared to control having 33mm (Table 4.15). Similarly, only ethyl acetate fraction 11 was effective against *Salmonella typhi* and *Klebsiella pneumonia (*Plates IX and VII).

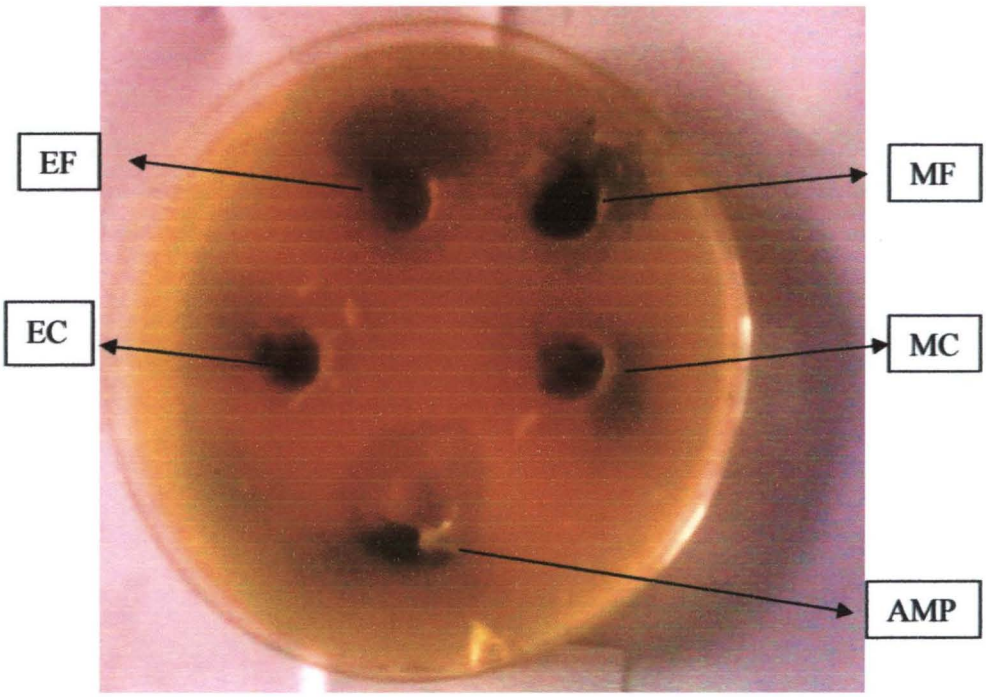


Plate VII: Antimicrobial Activity of Different *T. procumbens* Extracts on *Klebsiella pneumonia*

EF = Ethyl Acetate Fraction MF = Methanol Fraction

EC = Crude Ethyl Acetate MC = Crude Methanol

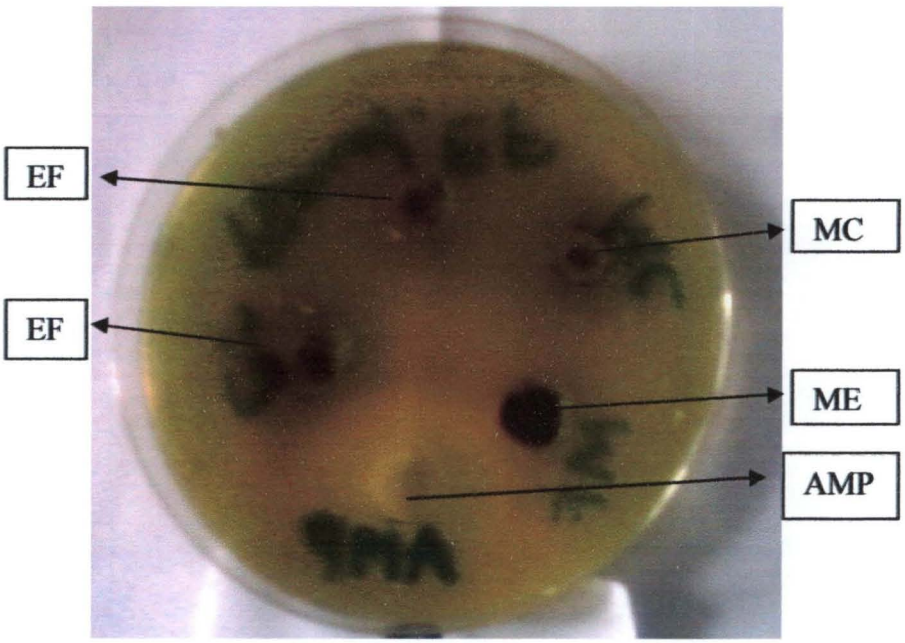


Plate VIII: Antimicrobial Activity of Different *T. procumbens* Extracts on *Pseudomonas aerogenosa*

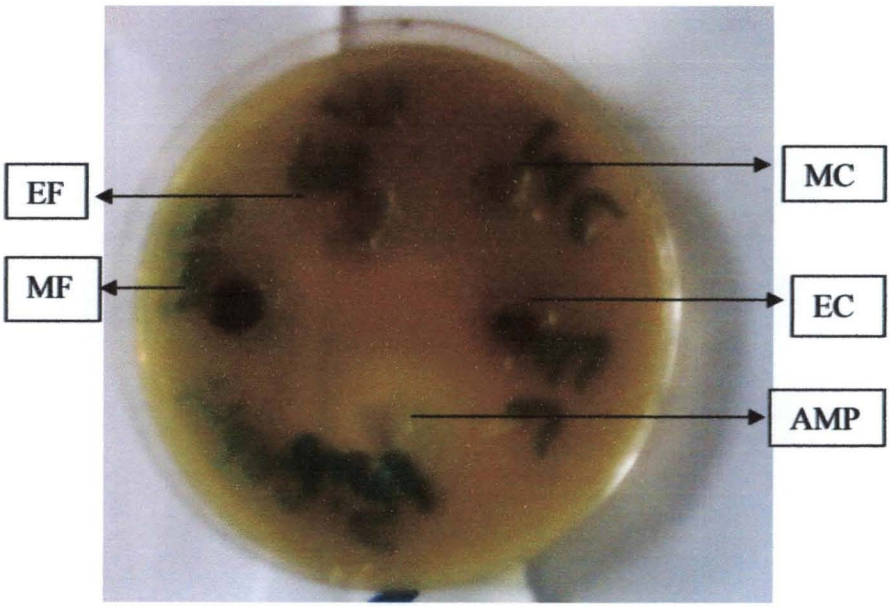


Plate IX: Antimicrobial Activity of Different *T. procumbens* Extracts on *Salmonela typhi*

EF = Ethyl Acetate Fraction MF = Methanol Fraction
EC = Crude Ethyl Acetate MC = Crude Methanol

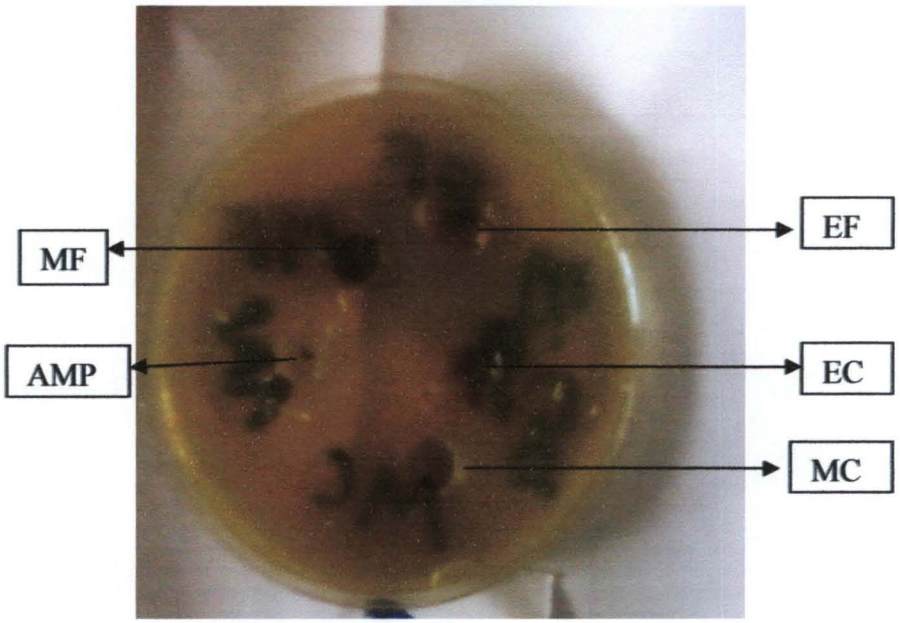


Plate X: Antimicrobial Activity of Different *T. procumbens* Extracts on *Escherichia coli*

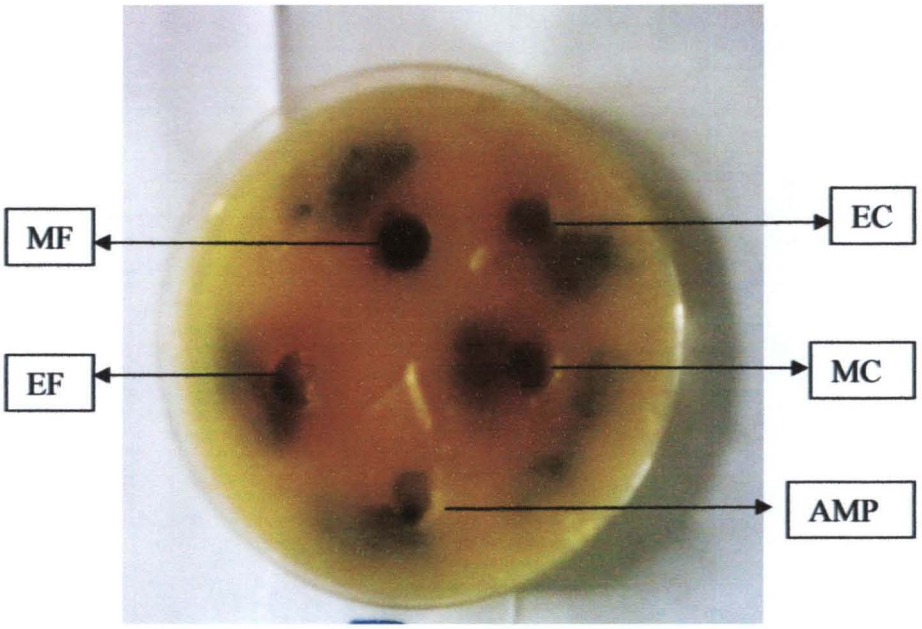


Plate XI: Antimicrobial Activity of Different *T. procumbens* Extracts on *Staphylococcus aureus*

EF = Ethyl Acetate Fraction MF = Methanol Fraction

EC = Crude Ethyl Acetate MC = Crude Methanol

Table 4.15: Effects of *Tridax procumbens* Extracts and Standard Antibiotics on some Test Bacteria

	Different Extract of *T. procumbens* and Standard Antibiotic				
	Zone of Inhibition (mm)				
Bacteria	EF	MF	EC	MC	AMPICLOX
Klebsiela pneumonia	11	-	-	-	16
Pseudomonas aeroginosa	-	-	-	6	34
Salmonela typhi	9	-	-	-	26
Escherichia coli	24	17	13	-	33
Staphylococcus aureus	-	-	-	-	-

EF = Ethyl Acetate Fraction MF = Methanol Fraction

EC = Crude Ethyl Acetate MC = Crude Methanol

4.5 Phytochemical Analysis of the crudes Extracts

The phytochemical analysis of the ethylacetate crude showed the presence of steroid and flavonoids which are also present in crude methanol extract in addition to the presence of other phytochemicals such as saponins, tannins, alkaloids and carbohydrate (Table 4.16). Resins and anthraquinones are conspicously absent in the two extracts.

Table 4.16: Phytochemical Constituents of *T. procumbens* Crude Methanol and Ethyl acetate Extracts

Phytochemical	Methanol	Ethyl acetate
Carbohydrates	+	-
Steroids	+	++
Saponins	+	-
Flavonoids	++	+++
Tannins	+	-
Alkaloids	+	-
Anthraquinones	-	-
Resins	-	-

Notations:

++ Highly present

\+ Fairly present

_ Absent

4.6 Acute Toxicity Studies

In the first phase of the acute toxicity studies, there were no remarkable signs of toxicity observed at 10, while at 100 and 1000 mg extract/kg body weight there was however salivation, rubbing at site of application, nose and mouth on the floor of the cage and restlessness. In the second phase of the study, the signs of toxicity observed were same and more severe than those observed at the phase one. However, at 2900 and 5000 mg/kg body weight all the animals died 2 hours after the extract administration all survived at 1600mg/kg body weighy. Therefore the median lethal (LD_{50}) dose was calculated to be 2100mg/kg body weight as geometric means on the doses for which there is zero mortality (0/3) and total mortality (3/3) ie between 1600 and 2900 mg/kg.

$$\text{i.e } \sqrt{1600} \times 2{,}900$$

$$\cong 2100\ mg/kg$$

Table 4.17: Acute Toxicity Assay Using Mice

Groups	Dose (mg/kg body weight)	No. of Dead/Survived mice
Control	0.4ml of DMSO	0/3
1	10	0/3
1	100	0/3
2	1000	0/3
4	1600	0/3
5	2900	3/3
6	5000	3/3

4.6.1 Changes in Total and Percent Organ / Body Weight Ratio

Throughout the period of observation, there was a significant gain in the total body weight with highest increase found in group that received lowest dose level of 50 mg/kg body weight and lowest increase is in 1600mg/kg when compared with their base line data ($P<0.05$). There was a significant increase in the body weight of the control compared to groups that were treated with the extract at 100, 1000 and 1600 mg/kg body weight (Figure 4.30).

Spleen recorded a general increase in the percent organ/body weight ratio with highest increase of 42% found in the 1000 mg/kg treated group. Both the liver and lungs recorded highest %organ/body weght ratio of 12.3% and 18.1% respectively when compare to the control at 100 mg/kg body weight. The heart decreased at all the dose levels while the kidney enlarged at 10 and 1600 mg/kg body weight.

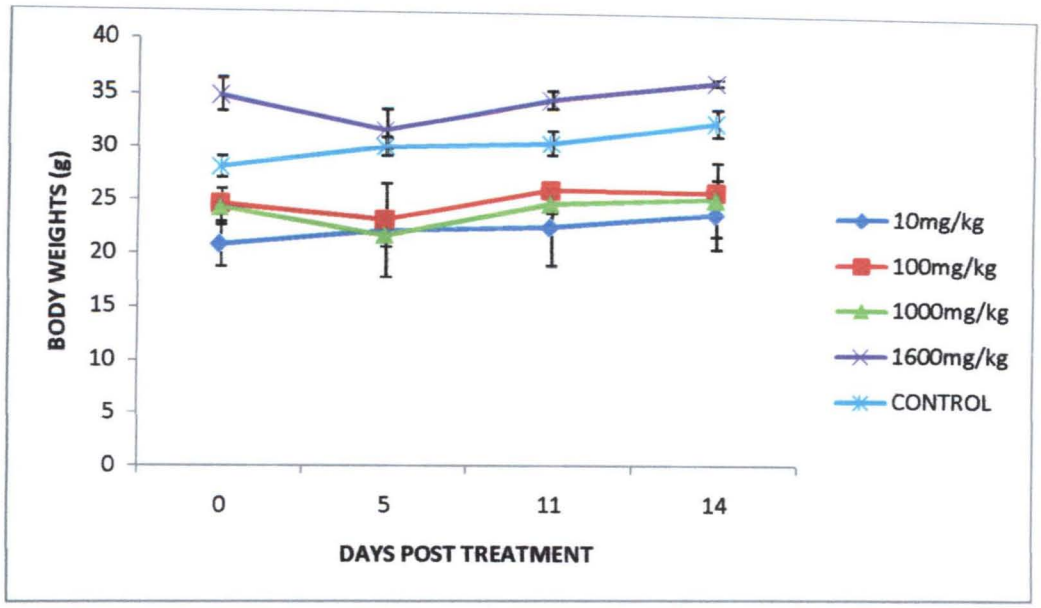


Figure 4.30: Changes in Body Weight of Mice Treated Acutely with *T. procumbens*

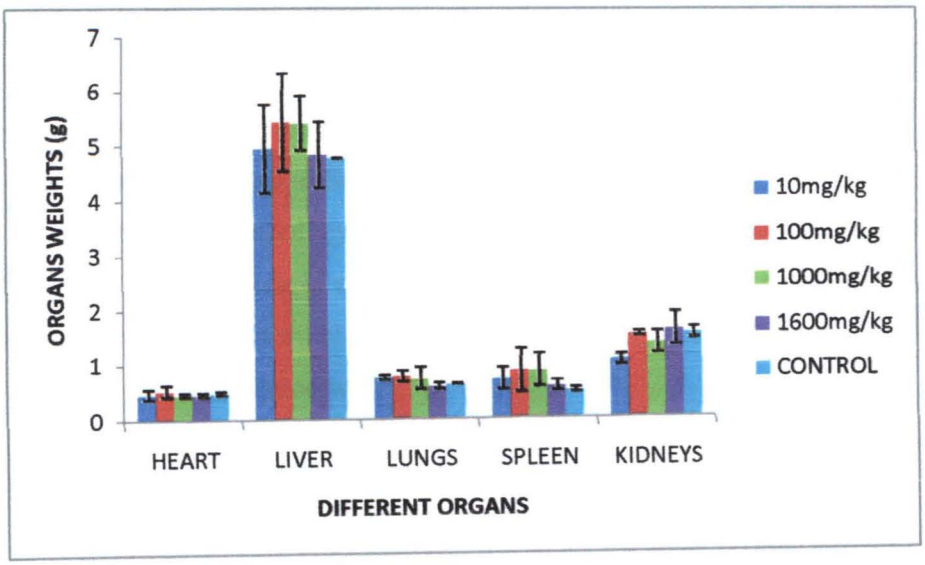


Figure 4.31: Changes in Organ Weight (g) of Mice Treated Acutely with *T. procumbens*

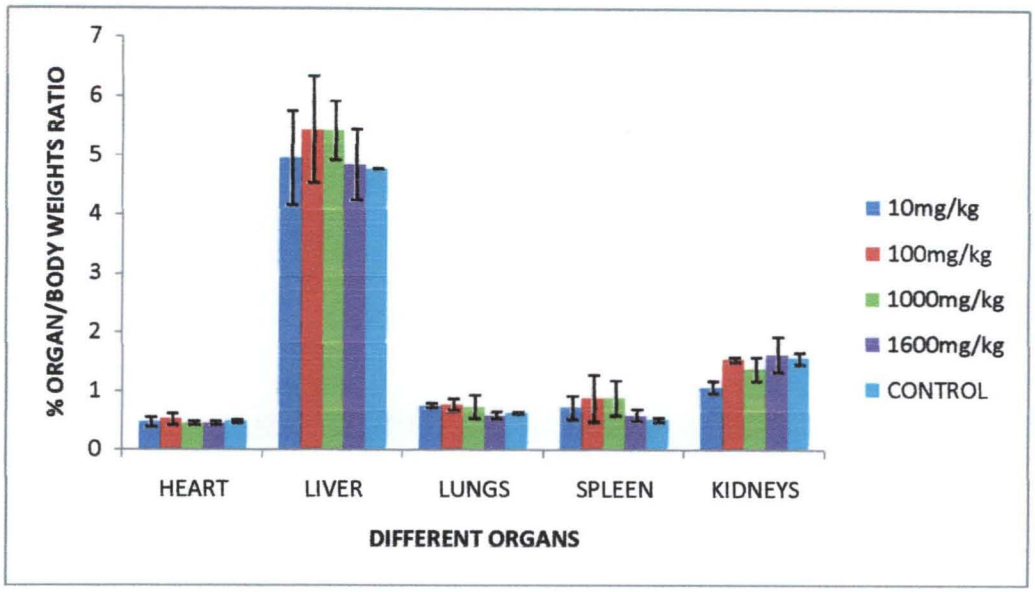


Figure 4.32: Changes in Percent Organ / Body Weight Ratio of Mice Treated Acutely with *T. procumbens*

4.7 Short Term Toxicity Studies

4.7.1 Gross pathology of Some Organs

The gross pathology of organs revealed a slight enlargement of the spleen and liver at all the dose levels with dark pathes seen on the liver at 800mg/kg. All the other organs are grossly normal and no mortality was recorded throughout the period of treatment and observation.w

4.7. 2 Changes in Total and Percent Organ / Body Weight Ratio

Administration of *T. procumbens* at all the dose levels resulted to a significant increase in the total body weight as compared to their baseline data ($P<0.05$). Among the treated groups, the highest weight increase was observed in 800mg/kg body weight treated group, where the body weight increased by 47.5% of the baseline.

Similarly, there was increase in percent organ/body weight ratio of liver and spleen in a dose dependant pattern, with highest liver increase found in group that receive lowest dose ($P<0.05$). However, the kidneys, heart and lungs are not significantly affected when compared to the untreated control group ($P>0.05$). At 50 mg/kg body weight, there was % organ / body weight ratio of liver recorded 17% while that of spleen was 61% as compared to the untreated control. These observations are the same with the changes in organ weight.

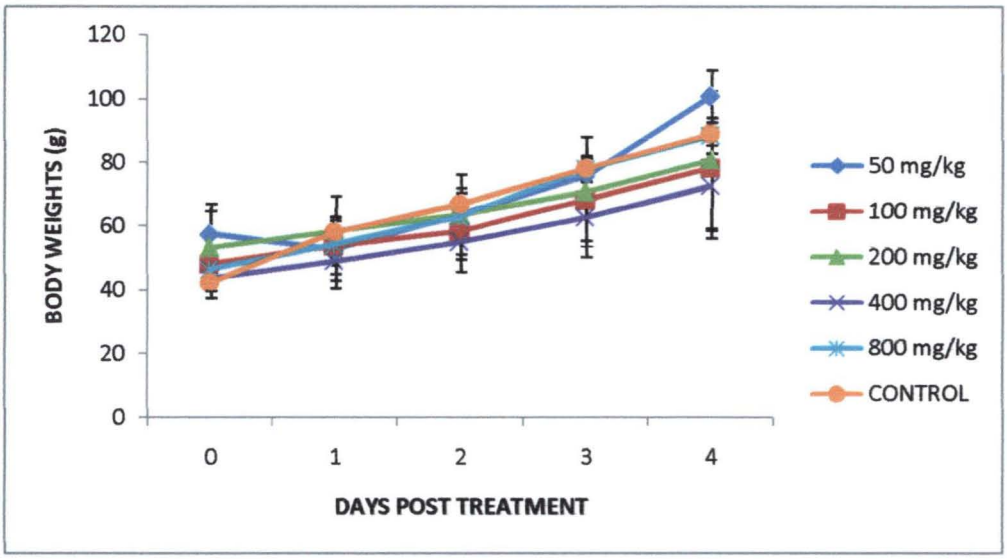


Figure 4.33: Changes in Body Weight of Rats Treated with *T. procumbens* Ethyl acetate extract at Short Term Toxicity Studies

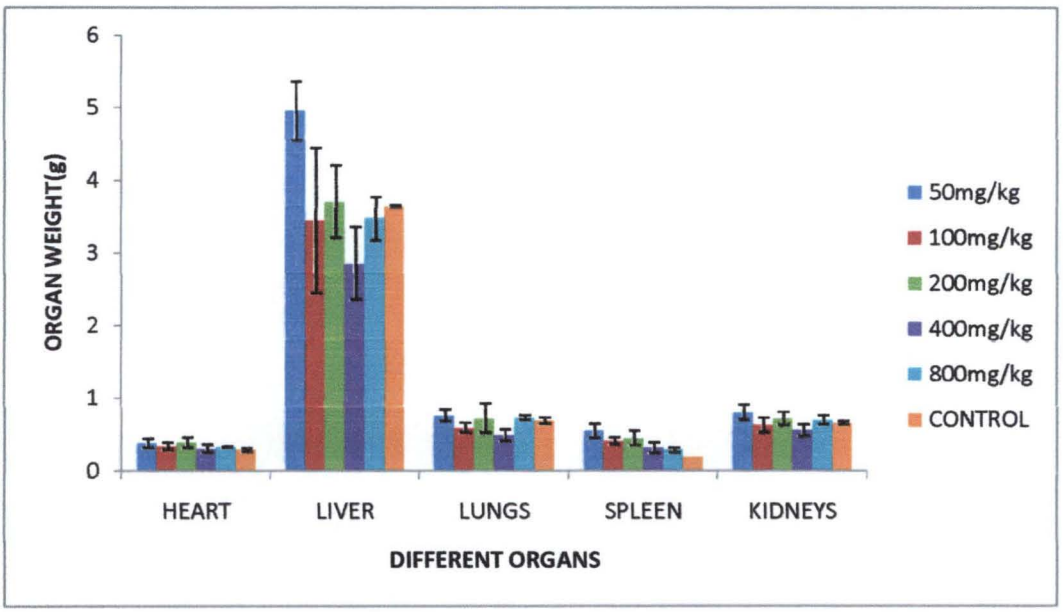


Figure 4.34: Changes in Organ Weight of Rats Treated with *T. procumbens* at Short Term Toxicity Studies

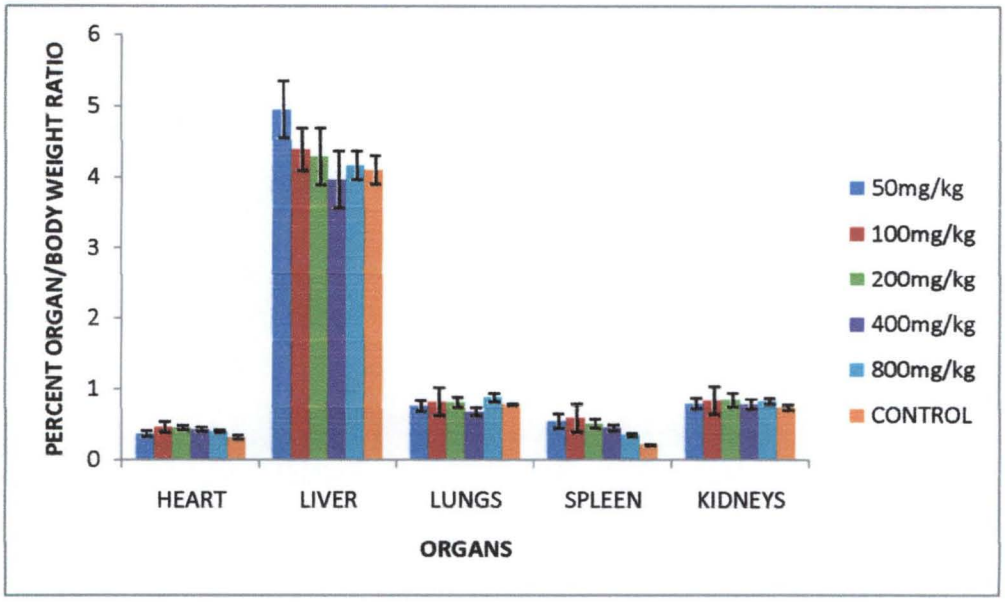


Figure 4.35: Percent Organ / Body Weight Ratio of Rats Treated with *T. procumbens* at Short Term Toxicity Studies

4.7.3 Haematological Changes in Rats

The packed cell volume (PCV), lymphocyte and RBC counts increased particularly with groups treated at 400 and 800 mg/kg body weight ($P>0.05$). However, the WBC counts and Neutrophils raised at all the dose levels though not statistically significant ($P>0.05$).

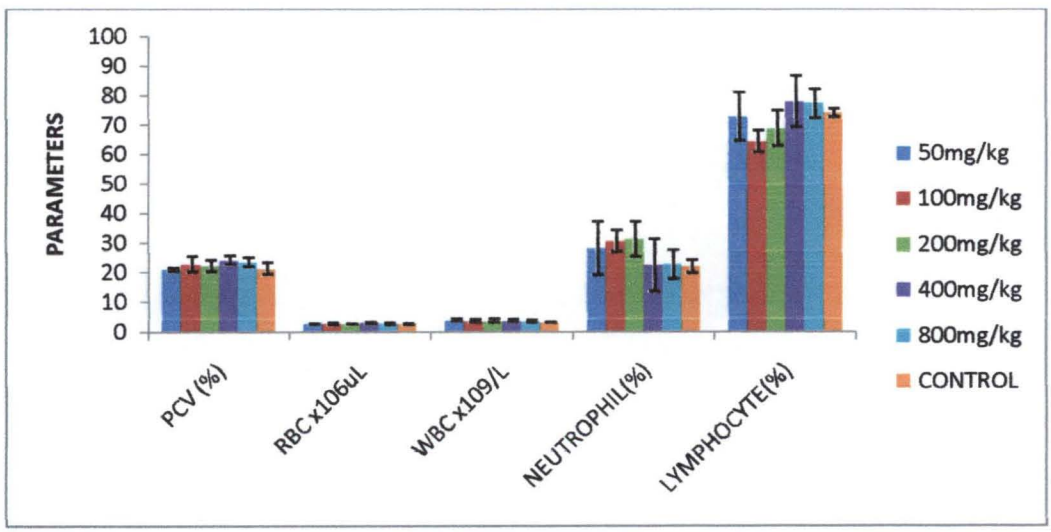


Figure 4.36: Haematological Changes of Rats Treated with *T. procumbens* at Short Term Toxicity Studies

4.7.4 Biochemical Changes in Rats

With the exception of the lowest dose administered, all the other doses resulted to a significant decrease in glucose levels ($P<0.05$). Similarly there is there is significant increase in ALT and decrease in AST with 800mg/kg producing highest effect ($P <0.05$). ALP was not affected significantly ($P>0.05$). However, the urea and electrolyte levels increase slightly ($P>0.05$), while the total protein was not affected at all the dose levels (Table 4.18).

Table 4.18: Effect of *Tridax procumbens* Ethyl acetate Extract on some Biochemical Parameters of Rat Liver and Kidneys

Parameters	50mg/kg	100mg/kg	200mg/kg	400mg/kg	800mg/kg	Control
AST (IU/L)	12.99±1.5	7.07±3.4	5.75±1.9	7.43±2.8	2.22±0.8	14.73±3
ALT (IU/L)	67.36±4.4	50.39±7.1	64.98±5.8	61.00±0.6	50.10±0.5	39.78±0.2
ALP (IU/L)	75.23±6.4	76.31±2.7	74.35±3.5	75.33±3.8	76.28±5.2	74.67±6.4
UREA (mmol/L)	8.72±0.6	8.85±0.8	7.41±0.9	7.70±0.6	8.80±1.2	6.73±1.0
TOTAL PROTEIN (g/L)	6.68±0.5	5.96±0.7	6.10±0.3	6.13±0.3	6.91±0.4	6.47±0.2
GLUCOSE (mmol/L)	7.56±1.1	5.26±2.7	4.84±3.1	3.73±1.3	2.81±0.2	8.17±1.6
Na^+ (mmol/L)	125.21±5.0	128.18±3.5	130.33±6.2	132.17±6.4	134.01±3.7	126.00±5.0
K^+ (mmol/L)	5.56±0.8	5.46±0.3	5.65±0.1	6.20±0.9	6.81±0.5	5.13±0.1

Values are means of three determinations (±SD)

4.7.5 Histopathological Studies of Rat Liver and Kidney

There is a general deposition of haemosiderin in the liver of treated animals which was dose dependant. The deposition of haemosiderin was highest in 800mg/kg body weight and lowest in 50 mg/kg body weight (Plate XIII). The section of the control shows an unremarkable normal liver tissue with a well preserved hepatic architecture (Plate XII). The liver of 50mg/kg treated rats showed a sporatic infilteration of the liver parenchyma cells and haemosiderin is absent. In addition the changes in liver tissue are irremakable (Plate XIII). At 100mg/kg body weight, there is mild infiltration of inflammatory cells (WBC) and haemosiderin deposition is insignificant. There are inflammation and infiltration of liver, pericortal inflammation, as evidenced by numerous polymorphonuclear leucocytes in the liver of rats treated at 200mg/kg body weight (Plate XIV). The haemosiderin deposition is also fewer (Plate XIV). Similarly, the liver section of rats treated at 400 and 800mg/kg body weight shows old haemorhage as indicated by haemosiderin deposit throughout the entire liver parenchyma.

The glomerular apparatus and tubules of the control rat kidney are visible (Plate XV). At 50 mg/kg body weight there is mild interstitial haemosiderin deposition and occasional intraglomeruli bleeding (Plate XVI). There is foci glomeruli haemorrhage in addition to interstitial haemosiderin deposition in kidney of rat treated at 100mg/kg body weight. At 200mg/kg body weight, the interstitial haemorrhage as evidenced by collection of haemosiderin laden macrophage was observed (Plate XVII). Similar interstitial haemosiderin deposition signifying interstitial haemorrhage with glomerulareExtravasation of RBC was also seen in other kidneys dosed at 400 and 800mg/kg treated rat.

Plate XII: Micrograph of normal liver tissue of extract untreated control group (X 100).

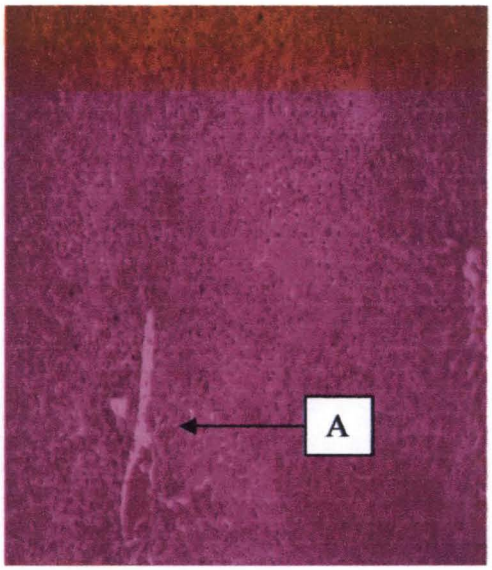


Plate XIII Micrograph of rat liver tissue Treated at 50mg/kg body weight (X 100).

Arrow A indicates shows polymorphonuclear leucocyte.

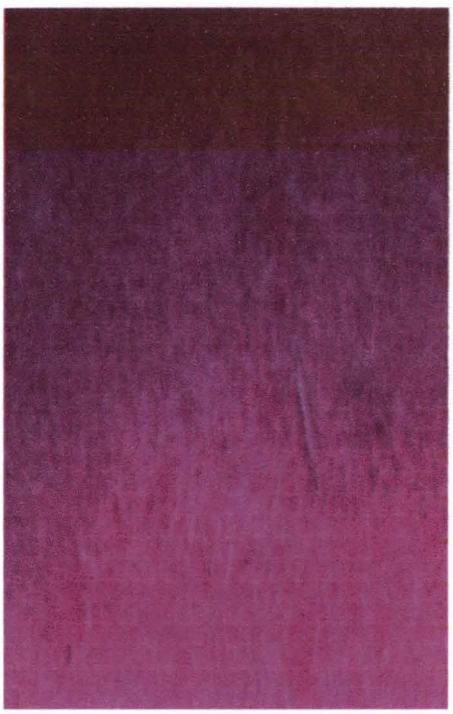


Plate XII: micrograph of well preserved hepatic Architecture of untreated liver tissue

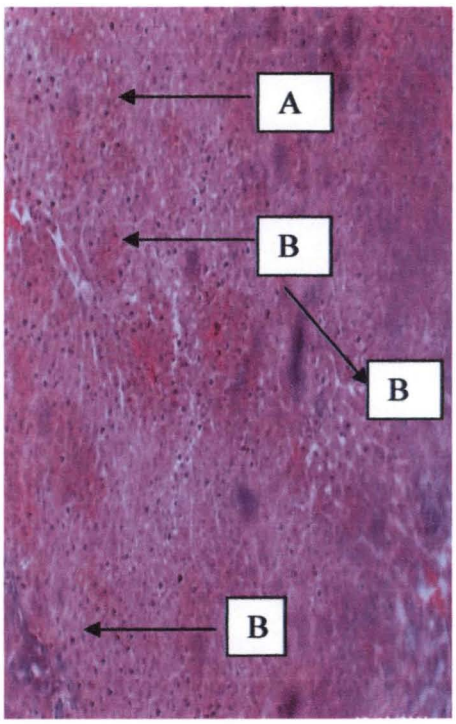


Plate XIV Micrograph of rat liver treated at 200mg/kg with numerous polymorphonuclear (B) leucocyte and fewer haemosiderin (A)

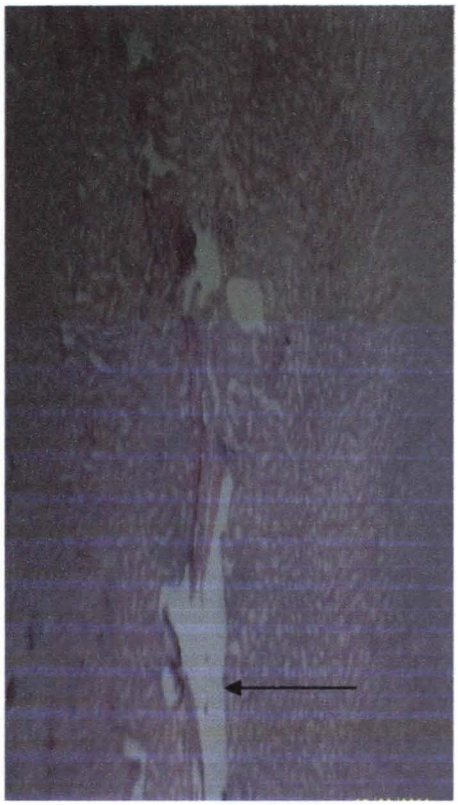


Plate XV: Micrograph of Rat kidney tissue of the control group (X 100). Normal glomerular apparatus and tubules are visible (arrows)

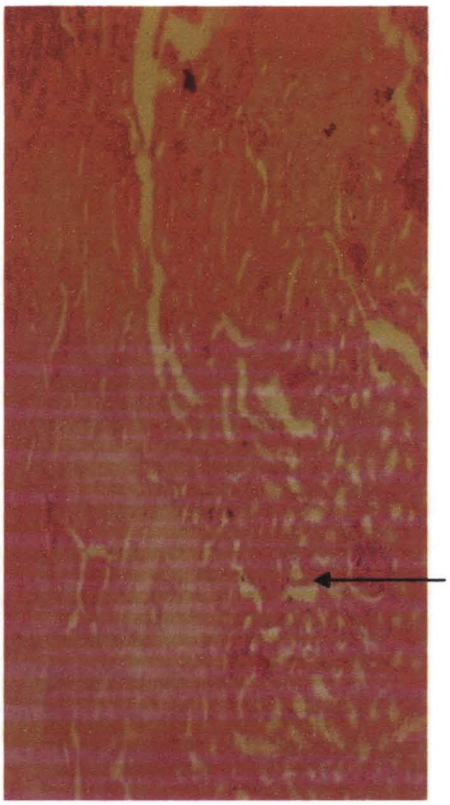


PLATE XVI: Micrograph of Rat kidney Tissue treated at 50mg/kg showing mild interstitial haemosiderin deposit (arrow)

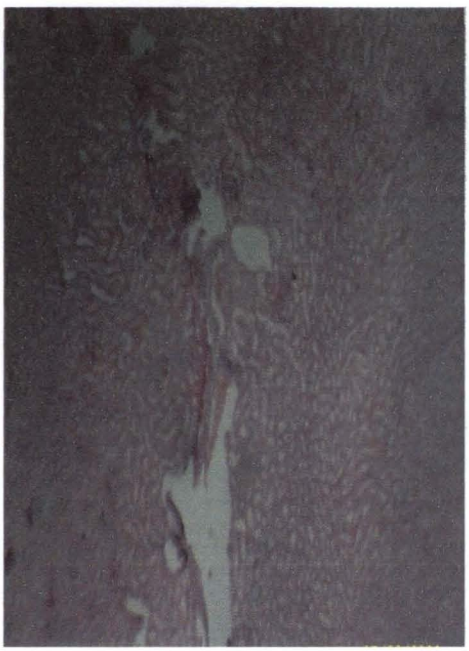


Plate XV: Micrograph of Rat kidney tissue of the control group (X 100). Normal glomerular apparatus and tubules are visible (arrows)

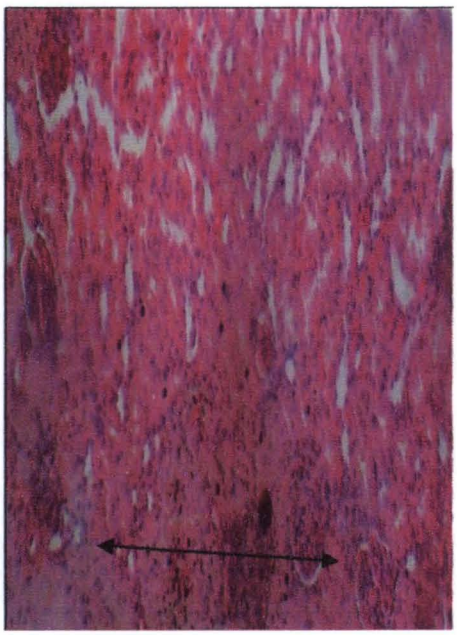


PLATE XVII: Micrograph of Rat kidney tissue treated at 200mg/kg showing mild interstitial haemorrhage with haemosiderin laden macrophage deposit (arrow)

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

The result of this study showed that these plants investigated have potential in the management of African Trypanosomiasis due to *T. b. brucei* tested at recommended doses by traditional healers (Table 4.1). *Cucumis metuliferus* (pulp) and *Securidaca longipenduculata* (Root bark) recorded highest potential due to reduction in parasitaemia and prolongation of life by 4 days. However, *Nelsonia compestris* recorded the list survival days.

Among the plants screened is the *Dissortis rotundifolia triana* which is a medicinal plant widely used in Nupe ethnomedicine (Mann *et al, 2003)* for the treatment of sleeping sickness. The treament of human trypanosomiasis by some healers using this plant is either orally, intraperitoneally or both. Earlier studies by Onyekwelu and Okwuasaba (2006) and Man *et al* (2009) showed the plant to only prolong the life of treated animals when treated intraperitoneally. Total clearance of the parasite was not observed. Oral treatment of both aqueous and ethanol extract of the plant did not significantly prolong the life of treated animals as compared to the infected, untreated control counterpart. In this studies, we observed no significant changes in parasitaemia of the treated when compared to the untreated control ($P>0.05$).

Enantia clorantha is another plant screened against *T.b.brucei.* The plant was selected to be screened because it has been established to have effect on *Plasmodium (*Agbaje and Onabanjo, 1991) and also has antipyretic effect (Adesokan *et al.,* 2008). Since trypanosomes are also protozoan parasite like plasmodium and produces pyrexia, its screening against trypanosomes is therefore justified. The aqueous extract of the plant prolonged the life of treated animals longer than the ethanol extract when compared to the infected untreated control.

Khaya senegalensis is plant of interest because it is commonly used by Fulani herdsmen for the management of animal trypanosomiasis. Our present and previous studies (Antia et al 2009; Frieburghaus *et al.*, 1996) did not record any outstanding *in vivo* activity of the extract. This observation may be attributed to degradation or metabolisation of the active principle through various metabolic processes in the host animal, or to the toxicity of high levels of the crude extract required for therapeutic efficacy. *Azadirachta indica* has a lot of beneficial uses. The biological activity of neem compound includes Anti-inflammatory; Antiarthritic; Antipyretic; Hypoglycaemic; Antigastric ulcer; Spermicidal; Antifungal; Antibacterial; Diuretic; Antimalarial; Antitumour; Immunomodulatory etc. (Biswas, *et al.*, 2002). However, no significant *in vivo* effect was observed in *T.b.brucei* infected rats but it's *in vitro* activity has been reported (Githua *et al.*, 2010).

Similarly, *Erythrina senegalensis* and *Nauclea latifolia* did not have significant effect on experimental trypanosomiasis ($P>0.05$). The *in vivo* antitrypanosomal activity of *Securidaca longipenduculata* and *Cucumis metuliferus* have shown their ability to reduce the parasitaemia of the infected animals resulting to prolongation of life by 4 days each beyond that of infected, untreated control . However, most of these plants screened have been reported to possess *in vitro* activity. The activity of the test material *in vivo* is influenced by a number of factors: The compound effective *in vitro* may not be effective *in vivo* due to their failure to reach the requisite site of action or they metabolise too quickly to a less active or inactive form. Or a compound can be more active because it metabolised to a more active form.

Morinda lucida is a medicinal plant that has been ascribed with several medicinal properties. This plant is therefore selected for the antitrypanosomal screening on the basis of their traditional reputation for efficacy in the treatment of trypanosomiasis and other diseases. The stem and leaves are widely used in the treatment of malaria,

jaundice, dysentry, abdominal colic and intestinal worm infestation (Bello *et al.,* 2009). Some of these symptoms are also commonly found in trypanosomiaisis. The stem and leaves of this plant are therefore screened for their possible antitrypanosomal properties against *T.b.brucei.*

As a medicinal plant, all the various solvent extract of the dried leaves, in order of increasing polarity, has shown some level of sporatic antitrypanosomal activity. The most promising activity was recorded with groups treated with more polar methanolic leaves extract. The highest dose level of 400 mg/kg body weight recorded highest means survival of 9.7 days with maximum life prolongation of 14 days. This was possible because there was suppression of parasitaemia which was more in group treated at 400 mg/kg body weight (Figure 4.3). The ethyl acetate treated group was the second best in terms of mean survival and parasitaemia. The ethyl acetate extract is more polar than hexane extract and less than methanol. The effect of hexane and aqueous extract on the parasitaemia and prolongation of life beyond the control are minimal ($P>0.05$).

The stem bark extract showed a similar activity with leaves extract although to a lesser extent. The methanol extract at 200 mg/kg recorded highest means survival of 7.0 days and tolerance of 11 days. Therefore, the antitrypanosomal property of leaves methanol extract supercede that of the stem bark methanol extract both in term of mean survival and prolongation of life beyond the untreated control.

The transient trypanocidal activity of methanol extracts of *Morinda lucida* leaves and stem bark is in agreement with the earlier studies by Asuzu and Chineme (1990). They reported the suppresion of the *T. b. brucei* infected animal when treated with 50% methanol dry leaves extract and 1000 mg/kg produced the highest suppression. In this study, the extracted leaves of *Morinda lucida* were in absolute methanol and the dose level that *gave* highest suppression of parasite was 400 mg/kg body weight. Furthermore, Asuzu and Chineme (1990) obtained the best trypanocidal activity when treatment with

M. lucida extract commenced simultaneously with trypanosome inoculation. In this study the best antitrypanosomal activity was observed when treatment commenced at 24 hours post parasite inoculation. Therefore the efficacy of treament is dependant on the number of parasite inoculated and time the treatment commenced. This studies and many other investigators (Asuzu and Chineme, 1990; Aguiyi *et al* 1999; Onyekwelu and Okwuasaba, 2006; Ogbadoyi *et al* 2011) all observed that the trypanocidal activity of the extracts from the various plants was not sustained and parasites were not cleared from the peripheral circulation.

Extracts of *Tridax procumbens* has shown antitrypanomal potential against *T. b. brucei* infected mice. Both the prolongation of life and suppression of parasitaemia in infected animals is possible because *T. procumbens* was earlier reported to have immunomodulatory activity which suggest it's therapeutic usefulness (Tiwari *et al.*, 2004). *T. procumbens* has been demonstrated to stimulate both humoral as well as cell mediated immune system vis-a-vis assists in genesis of improved antibody response against specific clinical antigen (Tiwari *et al.*, 2004). Since trypanosome infection causes immunosuppression, any herbal preparation that is immune boosting may have a significant effect on trypanosomes. Infection with trypanosomes has been known to impair the immune system of the host, cause anaemia, weight loss, reproductive disorders and death of animals if not treated (ILRAD 1991). Consequently in the presence of antigen (parasites) in the blood circulation, the administration of *Tridax procumbens* therefore may have activated the lymphocytes to increase the effectiveness of antigen clearance by phagocytosis or to secrete various immune effector molecules. This resulted to low parasitaemia and prolongation of life beyond the untreated control of all this plant extract investigated. The immunostimulative effect of *Tridax procumbens* may have occured in all the extract and methanolic extract recorded highest means survival followed by ethyl acetate, while aqueous extract was the least. The prophylactic

administration of all the extracts at the dose level tested has no effect on the prolongation of life and parasitaemia ($P>0.05$). This probably means that the extract could be immunostimulative only in the presence of the parasites. Furthermore, the presence of various phytochemicals particularly, flavonoid in this plant could be responsible for the observation recorded. The phytochemical analysis of the methanol and ethyl acetate extract of the plant revealed the presence of flavonoid in high amount in ethyl acetate than methanol extract. An important effect of flavonoids is the scavenging of oxygen-derived free radicals. *In vitro* experimental systems also showed that flavonoids possess antiinflammatory, antiallergic, antiviral, and anticarcinogenic properties (Middleton, 1998). Any anticarcinogenic plant could serve as a good source of trypanocide since trypanocide currently in use to treat sleeping sickness are known to have some level of anticancer activities (Barrett and Barrett, 2000).

All the screened extracts are polar and most likely the active compound could therefore be polar in nature. In addition to it's reported immunomodulatory effect, *Tridax procumbens* Linn (compositae) is also employed as indigenous medicine for a variety of ailments, including jaundice (Saraf et al., 1991). The plant has been extensively used in traditional medicine as anticoagulant, antifungal and insect repellent; in bronchial catarrh, diarrhoea and dysentery (Ali *et al.*, 2001). Moreover, it also possesses wound healing activity and promotes hair growth (Saraf *et al.*, 1991). *Tridax procumbens* is also dispensed as 'Bhringraj', which is well known Ayurvedic medicine for liver disorders (Pathak *et al* 1991). Antioxidant properties (Ravikumar *et al.*, 2005), have also been demonstrated.

In Africa, Nigeria in particular, most of herbal preparations used for the treatment of illnesses is usually a combination of two or more herbs. Combinations of medicinal herbs in medicinal prescriptions may not only affect a balance of active components, but

also undergo a mutual synergy which improves efficacy, safety, and minimizes side-effects.

A major problem bessetting the chemotherapy of African Trypanosomiasis is parasite resistance to the few available drugs (De Koning, 2001). One major benefit of combination therapies therefore is that they reduce development of drug resistance, since a pathogen is less likely to have resistance to multiple drugs simultaneously. Drugs that has different mode of action can be combined to achieve desirable effect. In these studies an attempt was made to explore the potentials of combination therapy using *T. procumbens* and *M. lucida* leaves extract in different combinations to treat *T.b.brucei*-infected mice. The only combination that has antitrypanosomal activity from the infected mice was the combination of the methanol extracts of the leaves of the two plants (fig.4.12-4.14). Though the mean survival rate was not very high as compared to when treated singly, but there was prolongation of life particularly at 200mg/kg. This also provided evidence that the combination of plants has some efficacy as practise in tradomedicine. Sometimes combination chemotherapy is used not to cure but to reduce severe symptoms and prolong life. However, a prolongation of life was observed which is an interesting development because this is the second reported screening for antitrypanosomal activity medicinal plants using combination of extracts from these two plants (Kabir, 2010).

The combination that gave highest mean survival was *T. procumbens* and *M. lucida* (1:2) treated at 200 mg/kg body weight. This was followed by the combination of 2:1 at 300 mg/kg. When used singly, *M. lucida* at 400 mg/kg produced highest effect while the effective dose in *T. procumbens* used singly or in combination did not change and still remains 300 mg/kg. This implies that *T. procumbens* could be responsible for the observed activity. Another advantage of this combination studies is that the dose level that gave highest activity was lower than those that gave activity singly. Dosage

reductions of each drug combined may reduce the overall toxicity while maintaining good efficacy (Gerardo *et al.*, 2007).

The methanol and ethyl acetate extracts of *T. procumbens*, exhibited good activity against *Trypanosoma brucei brucei*. The bioassay-guided fractionation of methanol extract gave eleven fractions while the ethyl acetate gave twelve fractions. When subjected to *in vivo* activity against *T. b. brucei* the fraction 11 of ethyl acetate gave a repeated highest activity which was statistically significant when compared to the untreated control ($P<0.05$). The antitrypanosomal activity was not high in the first few fractions collected where the polarity was low while the activities increased towards the last fractions where the polarity was high. This implies that the active compound must be a highly polar compound. A similar observation was made with methanol fraction but however, there was lost of activity with the methanol crude extract. Fraction 7 of methanol gave that gave the highest means antitrypanosomal activity of 11.3 days is not as polar as fraction 11 of ethyl acetate. It is possible therefore, that the remaining compound that was not completely extracted by ethyl acetate during the successive extraction was now removed by methanol hence this observation. The results obtained in these studies tend to lay credence to the fact that polar solvents as an extracting solvent, has the ability to extract phytochemicals that exhibit strong antitrypanosomal profile, thus making methanol and ethyl acetate a good extracting solvent for plants that are reported to have antitrypanosomal activity. This is because all the fractions of the 2 extracts showed antitrypanosomal activity and prolonged the survival of the treated animals.

The anti-trypanosomal activity exhibited by both the crude and fractions of *T. procumbens* was very obvious because mice in the control group that were infected but not treated presented massive parasitemia culminating in death within one week post infection.

Further attempt was carried out to determine the antitrypanosomal activity of each band obtained after subjecting the ethyl acetate fraction with highest activity to screening against *T. b. brucei* infected mice. Three bands were obtained and their antitrypanosomal activities were compared with the crude at the same dose level. On the basis of prolongation of life, band 2 gave the highest activity of three days while band one and crude gave 2 days each when the dose level of 200 mg/kg for the fraction was diluted 1:10. Consequently, there could be presence of certain compound in this band that has been responsible for the antitrypanosomal activity recorded throughout the screening process. Band 2 contain high amount of phenolic compound (flavonoid) when compared with both the crude and other bands. This could be reason for the observed highest activity.

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives (Geissman, 1963). Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total (Schultes, 1978). In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. Some, such as terpenoids, give plants their odors; others (quinones and tannins) are responsible for plant pigment. Many compounds are responsible for plant flavor (e.g., the terpenoid capsaicin from chili peppers), and some of the same herbs and spices used by humans to season food yield useful medicinal compounds.

Both the phytochemical analysis of the crude and TLC profile of the active fraction and 3 different bands obtained from PTLC suggested that the major component that is responsible for the higher biological activity is due to phenolic compounds which include flavonoids. The phenolic content is higher in band 2 relative to the crude fraction 11.

The crude extract of *T. procumbens* was earlier shown to exhibit antitrypanosomal activity and through bioassay guided fractionation the biological activity was more in fraction 11. Although further purification of the fraction 11 by preparative TLC do not yield a pure compound as shown by the result of both proton and ^{13}C- NMR., the spectra generated indicated the presence of phenols (aromatic compound) which include flavonoids. Phenols give a characteristic blue-black colouration with ferric chloride and yellow with vanillin/H_2SO_4 solution. Thus this therefore implies that the aromatic compound indicated by NMR suggest the presence of phenols.

Flavonoids are a large group of polyphenolic compounds possessing a basic flavan nucleus with two aromatic rings (the A and the B rings) interconnected by a three-carbon-atom

heterocyclic ring (the C ring). The most widespread flavonoids contain a double bond between C-2 and C-3 ($\Delta^{2,3}$) and a keto function at C-4 of ring C, which is attached to ring B at C-2

(flavone) or at C-3 (isoflavone). As a result of a number of further modifications on all three rings, particularly on ring C, flavonoids represent one of the largest and the most diverse class of plant secondary metabolites. Such modifications could remove the carbonyl group leaving the phenolic groups intact. Phenols and Flavonoids have been known for a long time to exert diverse biological effects and bioflavonoids in particular to act as antioxidants and preventive agents against cancer (Harborne and Williams, 2000). They are also common constituents of medicinal plants, and the therapeutic effects of many traditional medicines have been ascribed to these phytochemicals.

From the NMR and TLC profile results, the biological active band 2 has 4 peaks that correspond to 4 Rf values on TLC plate when sprayed with ferric chloride solution. The antitrypanosomal activity observed in this study could be as a result of high

concentration of the phenolic compound. 7, 8-dihydroxyflavone may be included in the band 2. This is because, when a six selected pure flavonoid compounds (3-hydroxyflavone, 7, 8-dihydroxyflavone, genistein, catechol, caffeic acid, and hydrocaffeic acid) were tested for their *in vivo* efficacies in mice infected with *T.brucei brucei,* only 7, 8-dihydroxyflavone significantly reduced the level of parasitemia; but the mice relapsed and showed a mean survival of 13 days, which represents an extension of the length of survival of 6 days for the untreated control group (Tasdemir *et al,* 2006). With the exception of genistein, all the other flavonoids tested did not prolong the life of treated as they died before the untreated control. In this study, the partially purified fraction recorded higher mean survival than the crude. Similarly, the purified band 2 from the PTLC recorded higher means survival than the crude and bands 1 and 3. Therefore, the prolongation of life observed could also be due the presence of these compounds in the band 2. However, since metabolism might transform one class of flavonoid into another (Nikolic and van Breemen, 2004) new pharmacological activity or the loss of previous activity can be observed. All these facts may underlie the absence of notable *in vivo* activity for the majority of the polyphenols investigated here. Quercetin (3,3′,4′,5,7-pentahydroxyflavone), a potent immunomodulating flavonoid, was shown to directly induce the death of *Trypanosoma brucei gambiense,* the causative agent of HAT, without affecting normal human cell viability (Mamani-Matsuda *et al* 2004). Quercetin was reported to directly promote *T. b. gambiense* death by apoptosis. In addition to microbicidal activity, quercetin induced dose-dependent decreases in the levels of TNF-α and nitric oxide produced by activated human macrophages (Mamani-Matsuda *et al* 2004). These results highlight the potential use of quercetin as an antimicrobial and anti-inflammatory agent for the treatment of African trypanomiasis. However, 7, 8-dihydroxyflavone and quercetin appeared to have some *in vivo* activity and therefore,

could serve as pharmacophore models for the rational design of synthetic analogs with higher *in vitro* and *in vivo* activities and more favourable chemical properties.

The antibacterial activity of the crude ethyl acetate, crude methanol extract, active fractions of methanol and ethylacetate of *T. procumbens* have been demonstrated in these studies. The compared activity has shown that the ethyl acetate fraction recorded broad antimicrobial activity spectrum against pathogenic *Klebsiella pneumoniae, Salmonella typhi* and *Escherichia coli* (Plates VII, IX, X). The antimicrobial activity to *Klebsiella pneumonia* and *Salmonella typhi* is lost in the crude ethylacetate which implies that the active antimicrobial compound is concentrated in it's fraction 11. It is noteworthy that the extract exhibited an appreciable measure of inhibitory activity against *Klebsiella pneumoniae,* an organism that causes pneumonia. Since the medicinal plants studied appear to have a broad antimicrobial activity spectrum, they could be useful in antiseptic and disinfectant formulations as well as in chemotherapy. Infection with trypanosomes usually results to immunosuppression which can cause onset of opportunistic diseases. Therefore, inaddition to it's antitrypanosomal property, ethylacetate fraction is a good source for managing infection due to bacteria.

The optimal effectiveness of a medicinal plant may not be due to one main active constituent, but to the combined action of different compounds originally in the plant (Gonzalez *et al.*, 1994). In literature, it has been indicated that the antibacterial activity is due to different chemical agents in the extract, including essential oils (especially thymol), flavonoids and triterpenoids and other compounds of phenolic nature or free hydroxyl group, which are classified as active antimicrobial compounds. A complete study conducted with the purpose of finding these chemicals is worthwhile. These findings can form the basis of further studies to isolate active flavonoid compound, elucidate them against wider range of bacterial strains with the goal to find new therapeutic principles. Under this experimental study the extract was active for

bactericidal action. The findings revealed that the extract's capability to penetrate the cell walls with hydrophobic environment (gram negative) and hydrophilic environment (gram positive) bacterias is responsible for the bactericidal action which can be isolated and identified by some analytical techniques (Kudi *et al.*, 1999). The results of the study supports to a certain degree, traditional medicinal uses of the plant evaluated both for human and animal diseases therapy and reinforce the concept that ethno - botanical approach to screening plants as potential sources of bioactive substances can be successful (Valsaraj *et al.*, 1997). Plants showing significant activity may be due to the presence of alkaloids, flavonoids, tannins and polyphenols. These results suggest the presence of either good antibacterial potency or high concentration of an active principle in the fraction. This antibacterial activity would support the folk therapy of infections whose symptoms might involve bacteria (Verpoorte *et al.*, 1982). Plant extracts and phytochemicals are becoming popular as potential sources of antibacterial and several reviews have been written (Rojas *et al.*, 1992). Results from this investigation show the rationale behind the use of *T. procumbens* in traditional medicine. *Tridax procumbens* are not only interesting sources for antimicrobial activities but also potential source of phenolic antioxidants.

Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases and natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action (Hamil *et al.*, 2003; Barbour *et al.*, 2004). In addition the number of multidrug resistant microbial strain and appearance of strains with reduced susceptibility to antibiotics are continously increasing. This increase has been attributed to indiscriminate use of broad spectrum antibiotics, immunosuppressive agent, catheters, organ transplantation and ongoing epidemics of human immunodefficient virus (H.I.V.) infections (Gonzalez *et al.*, 1994).

Furthermore, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases, but also often with adulteration and side effects (Aibinu *et al.*, 2004).

Investigation of the acute toxicity is the first step in the toxicological investigation of an unknown substance. The index of acute toxicity is the LD_{50}. However, LD_{50} should not be regarded as a biological constant, since differing results are obtained on repetition or when the determinations are carried out in different laboratories (Lorke, 1983), due to many variables such as animals' species and strain, age, gender, diet, bedding, ambient temperature, and time of the day. Hence, there are considerable uncertainties in extrapolating LD_{50} value obtained for species to other species, consequently, recognizing LD_{50} test as providing, at best, only a ballpark estimate of human lethality has been advocated (Zbinden and Flury-Roversi, 1981).

The result of acute toxicity (LD_{50}) of *Tridax procumbens* extract was found to be 2100 mg/kg body weight as all the animals died at 2900 mg/kg boody weight and survived at 1600 mg/kg body weight. This result is based on intraperitoneal administration where the compound in the extract is directly transported through the blood circulation to the target organ where they exert their toxic effect. However, if the administeration is oral, the LD_{50} may be much higher since the extract will undergo metabolism to a new product which could be less or not toxic. The current LD_{50} values based on acute oral toxicity recommended by the Globally Harmonized System of Classification and Labeling of Chemicals (Link/URL, 2000) are as follows: Category 1, $\leq$ 5mg/kg; category 2, > 5mg/kg$\leq$50mg/kg [they are both labeled as danger: fatal if swallowed]; category 3, >50mg/kg$\leq$300mg/kg [danger: toxic if swallowed]; category 4, >300mg/kg $\leq$ 2000mg/kg [warning: harmful if swallowed], category 5, >2000mg/kg$\leq$5000mg/kg [warning: may be harmful if swallowed] and LD_{50} >5000mg/kg [not classified: no specified label]. According to Lorke (1983), substances

more toxic than 1 mg/kg are so highly toxic that it is not so important to calculate the LD_{50} exactly while LD_{50} values greater than 5000 mg/kg are of no practical interest. Hence, the LD_{50} of 2100 mg/kg body weight is an indication that the extract may be harmful when administered intraperitoneally.

The liver is the principal organ that is capable of converting drugs into forms that can be readily eliminated from the body. A broad spectrum of adverse drug's effects on liver functions and structures has been documented. The reactions range from mild and transient changes in the results of liver function tests to complete liver failure with death of the host. Many drugs may affect the liver adversely in more than one way.

The measurement of the activities of marker or diagnostic enzymes in tissue plays a significant and well known role in diagnosis, disease investigation and in the assessment of drug or plant extract for safety/toxicity risk. The enzymes considered in this study are useful marker enzymes of liver cytolysis and damage of the liver cells (Schmidt and Schmidt, 1979).

Elevated liver enzymes indicate inflammation or damage to cells in the liver. Inflamed or injured liver cells may leak higher than normal amounts of certain chemicals, including liver enzymes, into the bloodstream, resulting in elevated liver enzymes on blood tests. The specific elevated liver enzymes most commonly found are: Alanine transaminase (ALT), Aspartate transaminase (AST) and alkaline phosphatase (ALP). In most cases, liver enzyme levels are elevated mildly and temporarily. Most of the time, elevated liver enzymes don't signal a chronic, serious liver problem (Green, 2002). ALP is a marker enzyme for the plasma membrane and endoplasmic reticulum of the tissue (Wright and Plummer, 1974). It is often employed to assess the integrity of plasma membrane, since it is localised predominantly in the microvilli of the bile canaliculi, located in the plasma membrane (Akanji, *et al.*,1993). The administration of the extract did not produce significant increase in the ALP activity ($P>0.05$). Since, ALP

hydrolyses phosphate monoesters, non increase of this enzyme may not constitute a threat to the life of the cells that are dependent on a variety of phosphate esters for their vital process, as increase in the activity of this enzyme may lead to indiscriminate hydrolysis of phosphate ester metabolite of the liver, an important biochemical symptom of cytolysis.

AST and ALT are normally localised within the cells of the liver, heart, kidney, muscles and other organs. The enzymes are of major importance in assessing and monitoring liver cytolysis (Wada and Snell, 1962). Their presence in the serum may give information on organ dysfunction (Wells *et al.,* 1986). ALT is very specific for hepatic tissue and is much more sensitive to hepatic damage and levels rise higher than those of the AST in most type of hepatocellular damage. Results from short term toxicity studies of the crude ethyl acetate extract of *T. procumbens* indicated that the liver of test animals were significantly affected at all the dose levels between the test and control. There was a significant increase in ALT and decrease in AST activities ($P<0.05$). The reduction in the serum AST activity following administration of the extract may be attributed to the reduced rate of synthesis in the liver. The rise in the ALT activity may implies that the administration of the extract have resulted to hepatocellular damage that lead to the leakage of these enzymes into circulation (Table 4.18).

Measurement of plasma Na^+, K^+, and HCO_3^- usually accompanied by plasma urea or creatinine and sometimes Cl^- concentrations together make up the most frequently requested group of test for kidney function (Whitby *et al.,* 1988). The principal univalent cations in the extracellular fluid (ECF) and intracellular fluid (ICF) are Na^+ and K^+ respectively (Whitby *et al.,* 1988). Although the increment in the serum levels of Na^+, K^+ and urea as compared to control is not significant, there is an indication that the kidneys was spared from any damage and so maintained its integrity (Table 4.18). The concentrations proteins may be altered both in diseases that primarily affect protein

metabolism and in diseases where there is dehydration or overhydration (Whitby *et al.*, 1988). Most diseases that alter plasma proteins affect the synthesis of proteins in the liver, or the distribution of or their rate of catabolism or rate excretion (Whitby *et al.*, 1988). Therefore, the intraperitoneal administration of *T. procumbens* to rats as compared to control animals did neither affect protein synthesis nor it's rate of excretion since values obtained are not significant between the test and control ($P>0.05$). The administration of crude extract of *T. procumbens* to experimental rats results to a significant reduction of glucose levels in a dose dependant manner with highest dose having highest effect ($P<0.05$). Glucose is one of the clinically important carbohydrates. Disorders of carbohydrate metabolism such as diabetes are evaluated in part by measurement of plasma glucose in either the fasting state or after suppression or stimulation. The significant decrease in glucose levels in the test group implies that the extract could serve as a good source for antidiabetic agent. In addition, any parasitic infection that largely dependant on glucose for survival like in trypanosomiasis can deprive be of this important nutrient and therefore a source of treatment for the host.

In the haematological analysis of the animals treated with *T.procumbens,* packed cell volume (PCV) and red blood cell (RBC) counts were determined. The measurement of PCV and RBC count cab be used as a simple screening test for anaemia. Anaemia is an absolute decrease in the total number of RBC per ml of blood, decrease in PCV due fewer RBC. With the exception of group treated at 50mg/kg body weight, where there is a decrease in the value, although not signinificant ($P>0.05$), there was an increase in the PCV and RBC count at all other dose levels. This means that administration of ethylacetate exratct of *T, procumbens* can beneficial in anaemia related disorder particularly in trypanosomiasis. Similarly, there is general increase in total white blood cell (leucocyte) and lymphocyce counts. The entire system of WBC focusses on host defence while the lymphocytes are essential to immune defense system as their primary

function is to repond to antigens by initiating the immune response (Odutola, 2000). The increases observed in WBC and lymphocyte is a further confirmation of earlier report that *T. procumbens* influenses both humoral as well as cell mediated immune system Vis a vis assists in genesisof imroved antibody response against specific clinical antigen (Tiwari *et al.,* 2004). Slight increase in neutrophils are also observed in the test group when compare with the control group ($P>0.05$). Neutrophilia is most frequently caused by systemic or severe local bacterial infection where their primary role is in immunity against bacterial and fungal infection by phagocytosis (Odutola, 2000). Neutrophils, also known as Granulocytes or segmented neutrophils, are the main defender of the body against infection and antigens. High levels may indicate an active infection; a low count may indicate a compromised immune system or depressed bone marrow (low neutrophil production). Therefore administration of this extract can be of help when there is presence of a clinical antigen like trypasomes.

It is recommended that body weight be measured atleast once a week during toxicity studies. Weekly measurement is recommended because body weight is one of the most sensitive indicators of the condition of an animal if it is monitored frequently and carefully during a study (Wilson *et al.,* 2001). Rapid and/or marked body weight loss is usually a harbinger of ill health or death. Rapid body weight loss can be due to either decreased feed or water consumption, disease or specific toxic effect (Wilson *et al.,* 2001). Collection of terminal body weight and organ weights for all animals during necropsy is normal practise in repeated dose toxicity studies (Wilson *et al.,* 2001).

The positive effect of the extract can be deduced from the body weight status of the animals at all the dose levels. The percent organ/body weight ratio during the acute toxicity studies are higher at low dosage than the groups treated with the extract at high dosage. This observation is also similar during the short term toxicity studies. Hence there is an enlargement of both the liver and kidneys when compared to the untreated

control. These indicated that animals administered with *T. procumbens* at low dosage are in in a better physical state to eat more than those in the higher dosage groups of 1000-1600 for acute toxicity and 200-800mg/kg body weight short term toxicity studies.

There is specific toxic effect of this extract particularly at high dose level, corroborating the result of histopathology where there is haemosiderin deposition. In histopathology, generally there is old haemorrhage indicated by haemosiderin deposition throughout the entire liver and kidney tissues. In kidney tissue, there is interstitial haemosiderin deposition signifying interstitial haemorrhage as evidence by collection of haemosiderin laden macrophages particularly at 100- 400 mg/kg body weight. It is observed that the amount of haemosiderin deposition depend on dose level with highest dose lsevel of 800mg/kg body weight prodcing highest haemosiderin deposition. Since the PCV value did not fall when compare with the control, implies that the destruction of red blood cells and endothelial of blood vessel might not be massive and hence anaemia could not have resulted because of administration.*T. procumbens* ethyacetate extract. The high amount of macophages observed from the kidneys means that the extract has ability to stimulate the immune reaction through the production of antibodies. Earlier study has shown that the *T. procumbens* influences both humoral as well as cell mediated immune system vis-à-vis assist in genesis of improve antibody response against specific clinical antigen (Tiwari *et al.,* 2004). Therefore the extract of this plant could be useful in infections where the immune system will be compromise.

5.2 Conclusion

The result of this study has shown that both the ethyl acetate and methanol extracts of *T. procumbens* exhibited antitrypanosomal activity which was due to the presence of flavonoids. Based on the TLC profile and NMR analysis, the fraction with highest antitrypanosomal effect contains flavonoid compounds which could include, 3 –

hydroxyflavonoid, 7,8 – dihydroxyflavonoid, catechin, quercetin and other phenolic compounds. With the exception of 7, 8 – dihydroxyflavonoid, all the other mentioned flavonoids did not have antitrypanosomal activity. The ethyl acetate fraction also showed broad spectrum antibacterial activity. The LD_{50} was 2100mg/kg body weight and administration of the extract for 14 days resulted into the impairment of liver function while the kidneys are not affected. It also has endothelial toxicity destroying the endothelial of blood vessels leading to haemorrhage into the tissues. Severity depends on dose level. Nevertheless, the current study therefore suggests the *in vivo* antitrypanosomal activities of phenolic compounds which include flavonoids and encourages the use of medicinal chemistry approach to obtain more potent derivatives from plants.

5.3 Recommendations

There is a need to screen for activity of the various *T. procumbens* and *M. lucida* extracts on *T. congolense.*

Attempt should be made to isolate all the phenolic compounds present in the *T. procumbens* and their antitrypanosomal activity assessed compared with the standards.

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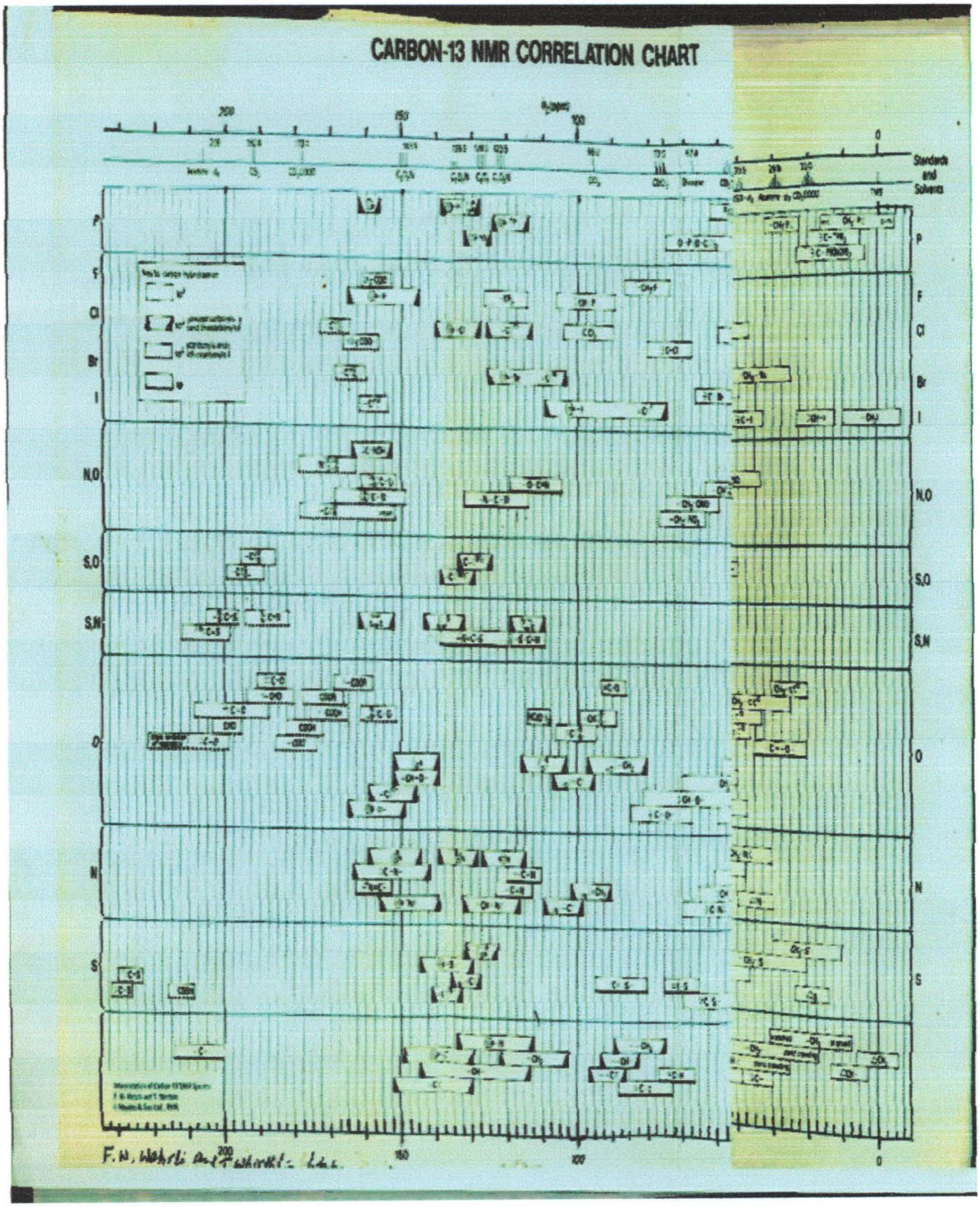
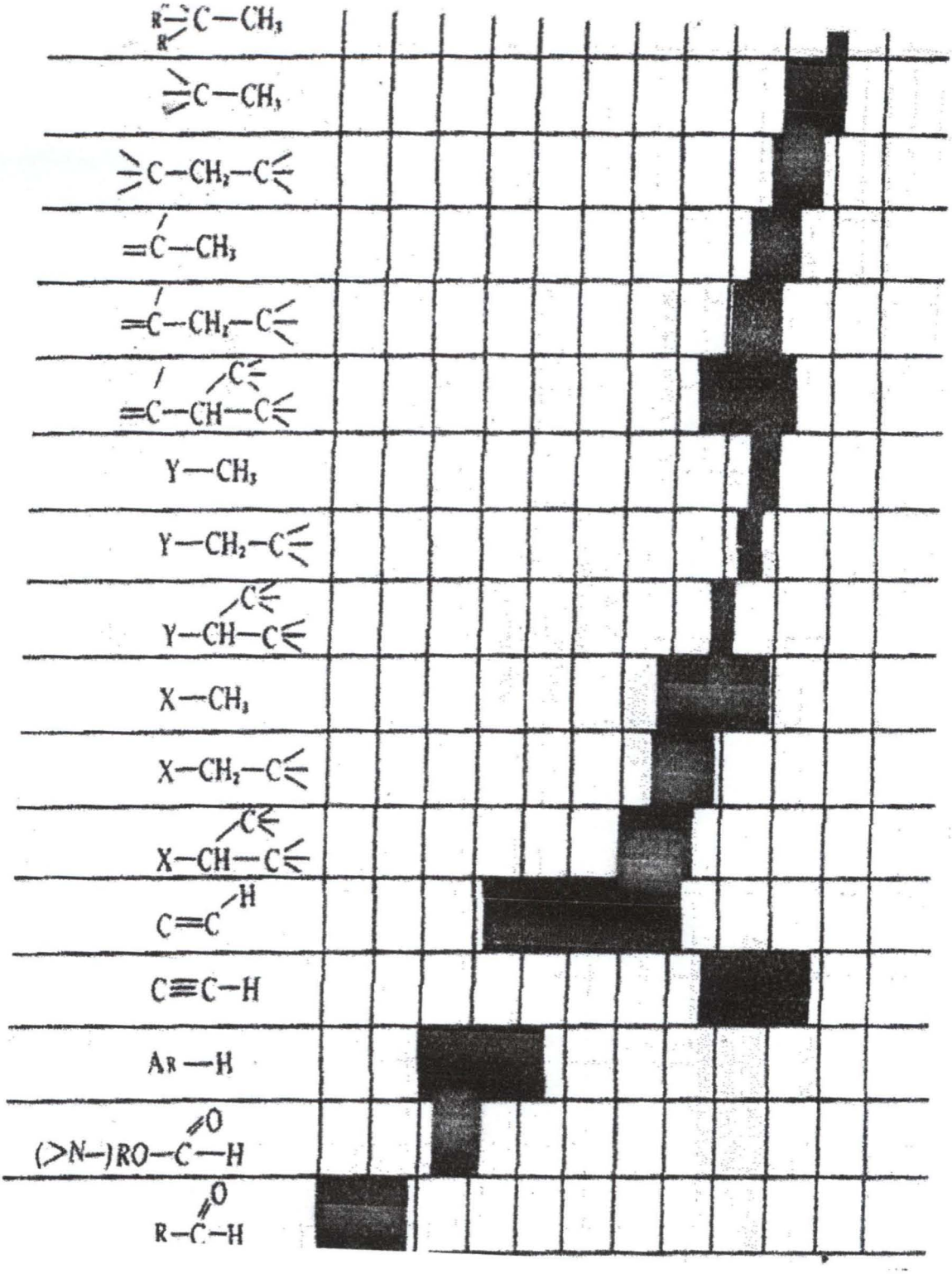


Appendix 1: Carbon [13] NMR Correlation Chart (Wehrli and Whirthlin, 1978)



Appendix II: Approximate Ranges of Proton Chemical Shift (R = H or alkyl, Y = SR, -NR_2; X = -OR, -NHCO.R, -O.CO.R, Halogen). Jacksman and Sternhell, 1969

Appendix III: Parasitaemia Profile With Methanolic Fractions.

Oneway

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Day 2	Fraction 1	3	13.9300	5.98501	3.45544	-.9376	28.7976	7.94	19.91
	Fraction 3	3	11.4933	3.85986	2.22849	1.9049	21.0818	7.94	15.60
	Fraction 4	3	5.9400	2.02030	1.16642	.9213	10.9587	3.90	7.94
	Fraction 5	3	9.9400	2.00000	1.15470	4.9717	14.9083	7.94	11.94
	Fraction 6	3	2.9867	.99500	.57447	.5149	5.4584	1.99	3.98
	Fraction 7	3	1.3867	.59518	.34362	-.0918	2.8652	.80	1.99
	Fraction 8	3	5.9000	1.88128	1.08616	1.2267	10.5733	3.98	7.74
	Fraction 9	3	1.3700	.61506	.35511	-.1579	2.8979	.76	1.99
	Fraction 10	3	4.9733	2.97504	1.71764	-2.4171	12.3637	1.99	7.94
	Fraction 11	3	.6000	.20000	.11547	.1032	1.0968	.40	.80
	Infected Untreated	3	18.4533	12.06092	6.96337	-11.5076	48.4143	7.94	31.62
	Total	33	6.9976	6.71075	1.16819	4.6180	9.3771	.40	31.62
Day 4	Fraction 1	3	51.2033	11.60026	6.69741	22.3867	80.0200	39.81	63.00
	Fraction 3	3	94.3333	31.50132	18.18730	16.0797	172.5870	63.00	126.00
	Fraction 4	3	31.0033	13.38537	7.72804	-2.2478	64.2544	15.60	39.81
	Fraction 5	3	*35.3367*	*4.15428*	*2.39847*	*25.0169*	*45.6565*	*31.60*	*39.81*
	Fraction 6	3	11.4800	3.86280	2.23019	1.8843	21.0757	7.94	15.60
	Fraction 7	3	13.9267	5.98001	3.45256	-.9285	28.7818	7.94	19.90
	Fraction 8	3	24.0033	13.69810	7.90860	-10.0246	58.0313	15.60	39.81
	Fraction 9	3	35.6700	4.10545	2.37028	25.4715	45.8685	31.60	39.81
	Fraction 10	3	23.6000	8.00000	4.61880	3.7269	43.4731	15.60	31.60
	Fraction 11	3	23.6000	8.00000	4.61880	3.7269	43.4731	15.60	31.60
	Infected Untreated	3	142.6000	16.60000	9.58401	101.3633	183.8367	126.00	159.20
	Total	33	44.2506	40.08055	6.97713	30.0387	58.4626	7.94	159.20

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Day 2	Between Groups	1004.255	10	100.425	5.058	.001
	Within Groups	436.838	22	19.856		
	Total	1441.092	32			
Day 4	Between Groups	47442.299	10	4744.230	26.329	.000
	Within Groups	*3964.120*	*22*	*180.187*		
	Total	51406.419	32			

Post Hoc Tests
Homogeneous Subsets

Day 2

Duncan

Methanolic Fractions	N	Subset for alpha = 0.05				
		1	2	3	4	5
Fraction 11	3	*.6000*				
Fraction 9	3	1.3700				
Fraction 7	3	1.3867				
Fraction 6	3	2.9867	2.9867			
Fraction 10	3	4.9733	4.9733	4.9733		
Fraction 8	3	5.9000	5.9000	5.9000	5.9000	
Fraction 4	3	5.9400	5.9400	5.9400	5.9400	
Fraction 5	3		9.9400	9.9400	9.9400	
Fraction 3	3			11.4933	11.4933	11.4933
Fraction 1	3				*13.9300*	*13.9300*
Infected Untreated	3					18.4533
Sig.		.212	.099	.121	.058	.083

Means for groups in homogeneous subsets are displayed.

Day 4

Duncan

Methanolic Fractions	N	Subset for alpha = 0.05			
		1	2	3	4
Fraction 6	3	11.4800			
Fraction 7	3	13.9267			
Fraction 10	3	23.6000			
Fraction 11	3	23.6000			
Fraction 8	3	24.0033			
Fraction 4	3	31.0033	31.0033		
Fraction 5	3	35.3367	35.3367		
Fraction 9	3	35.6700	35.6700		
Fraction 1	3		51.2033		
Fraction 3	3			94.3333	
Infected Untreated	3				142.6000
Sig.		.067	.104	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Day 6	Fraction 1	3	109.4200	14.44757	8.34131	73.5302	145.3098	99.53	126.00
	Fraction 3	3	106.4267	45.73944	26.40768	-7.1964	220.0497	79.62	159.24
	Fraction 4	3	71.5400	8.31954	4.80329	50.8731	92.2069	63.00	79.62
	Fraction 5	3	94.5000	31.50000	18.18653	16.2497	172.7503	63.00	126.00
	Fraction 6	3	46.9667	15.71061	9.07053	7.9393	85.9940	31.60	63.00
	Fraction 7	3	27.7033	12.10500	6.98883	-2.3672	57.7738	15.60	39.81
	Fraction 8	3	47.3000	15.70000	9.06440	8.2990	86.3010	31.60	63.00
	Fraction 9	3	142.6000	16.60000	9.58401	101.3633	183.8367	126.00	159.20
	Fraction 10	3	82.9067	43.09500	24.88091	-24.1472	189.9606	39.81	126.00
	Fraction 11	3	51.4067	11.59500	6.69438	22.6031	80.2102	39.81	63.00
	Total	30	78.0770	40.20874	7.34108	63.0628	93.0912	15.60	159.24
Day 8	Fraction 1	3	162.5267	36.52500	21.08772	71.7935	253.2598	126.00	199.05
	Fraction 3	2	126.0000	.00000	.00000	126.0000	126.0000	126.00	126.00
	Fraction 4	3	142.6200	16.62000	9.59556	101.3336	183.9064	126.00	159.24

	Fraction 5	3	188.5000	62.50000	36.08439	33.2414	343.7586	126.00	251.00
	Fraction 6	3	94.5000	31.50000	18.18653	16.2497	172.7503	63.00	126.00
	Fraction 7	3	94.5000	31.50000	18.18653	16.2497	172.7503	63.00	126.00
	Fraction 8	3	139.3000	19.90000	11.48927	89.8657	188.7343	119.40	159.20
	Fraction 9	2	183.8000	6.64680	4.70000	124.0808	243.5192	179.10	188.50
	Fraction 10	3	134.8667	7.67876	4.43333	115.7916	153.9418	126.00	139.30
	Fraction 11	3	162.5000	36.50000	21.07328	71.8290	253.1710	126.00	199.00
	Total	28	142.0550	40.98411	7.74527	126.1630	157.9470	63.00	251.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Day 6	Between Groups	34346.902	9	3816.322	6.087	.000
	Within Groups	12538.641	20	626.932		
	Total	46885.543	29			
Day 8	Between Groups	26731.104	9	2970.123	2.871	.027
	Within Groups	*18620.727*	*18*	*1034.485*		
	Total	45351.831	27			

Post Hoc Tests
Homogeneous Subsets

Day 6

Duncan

Methanolic Fractions	N	Subset for alpha = 0.05			
		1	2	3	4
Fraction 7	3	27.7033			
Fraction 6	3	46.9667	46.9667		
Fraction 8	3	47.3000	47.3000		
Fraction 11	3	51.4067	51.4067		
Fraction 4	3	71.5400	71.5400	71.5400	
Fraction 10	3		82.9067	82.9067	
Fraction 5	3		94.5000	94.5000	
Fraction 3	3			106.4267	106.4267
Fraction 1	3			109.4200	109.4200
Fraction 9	3				*142.6000*
Sig.		.067	.051	.110	.109

Means for groups in homogeneous subsets are displayed.

Day 8

Duncan

Methanolic Fractions	N	Subset for alpha = 0.05	
		1	2
Fraction 6	3	94.5000	
Fraction 7	3	94.5000	
Fraction 3	2	126.0000	126.0000
Fraction 10	3	134.8667	134.8667
Fraction 8	3	139.3000	139.3000
Fraction 4	3	142.6200	142.6200
Fraction 11	3		162.5000
Fraction 1	3		162.5267
Fraction 9	2		183.8000
Fraction 5	3		188.5000
Sig.		.137	.062

Means for groups in homogeneous subsets are displayed.

Warnings

Post hoc tests are not performed for Day 10 because at least one group has fewer than two cases.

Post hoc tests are not performed for Day 12 because at least one group has fewer than two cases.

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Day 10	Fraction 1	2	159.2400	.00000	.00000	159.2400	159.2400	159.24	159.24
	Fraction 3	2	179.1500	.00000	.00000	179.1500	179.1500	179.15	179.15
	Fraction 4	3	179.1467	19.90500	11.49216	129.6999	228.5934	159.24	199.05
	Fraction 5	2	251.0000	.00000	.00000	251.0000	251.0000	251.00	251.00
	Fraction 6	3	162.5267	36.52500	21.08772	71.7935	253.2598	126.00	199.05
	Fraction 7	3	94.5000	31.50000	18.18653	16.2497	172.7503	63.00	126.00
	Fraction 8	3	139.3000	19.90000	11.48927	89.8657	188.7343	119.40	159.20
	Fraction 9	2	179.1000	.00000	.00000	179.1000	179.1000	179.10	179.10
	Fraction 10	1	139.3000	.	.	.	.	139.30	139.30
	Fraction 11	3	162.5000	36.50000	21.07328	71.8290	253.1710	126.00	199.00

	Total	24	162.0917	43.00920	8.77922	143.9305	180.2529	63.00	251.00
Day 12	Fraction 1	1	199.0500	.	.	.	.	199.05	199.05
	Fraction 3	0	.	.	.	.	.	.	.
	Fraction 4	1	251.0000	.	.	.	.	251.00	251.00
	Fraction 5	1	278.6700	.	.	.	.	278.67	278.67
	Fraction 6	1	159.2400	.	.	.	.	159.24	159.24
	Fraction 7	3	142.6000	16.60000	9.58401	101.3633	183.8367	126.00	159.20
	Fraction 8	3	238.8000	39.80000	22.97854	139.9313	337.6687	199.00	278.60
	Fraction 9	1	238.0000	.	.	.	.	238.00	238.00
	Fraction 10	2	159.2000	.00000	.00000	159.2000	159.2000	159.20	159.20
	Fraction 11	*3*	*205.1000*	*45.90000*	*26.50038*	*91.0781*	*319.1219*	*159.20*	*251.00*
	Total	16	200.2412	50.09062	12.52265	173.5498	226.9327	126.00	278.67

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Day 10	Between Groups	33643.615	9	3738.179	5.879	.002
	Within Groups	8901.589	14	635.828		
	Total	42545.205	23			
Day 12	Between Groups	29703.228	8	3712.903	3.276	.068
	Within Groups	*7932.820*	*7*	*1133.260*		
	Total	37636.048	15			

Warnings

Post hoc tests are not performed for Day 14 because at least one group has fewer than two *cases.*

Post hoc tests are not performed for Day 16 because at least one group has fewer than two cases.

There are fewer than two groups for dependent variable Day 18. No statistics are computed.

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Day 14	Fraction 1	1	251.0000	.	.	.	.	251.00	251.00
	Fraction 6	1	218.9600	.	.	.	.	218.96	218.96
	Fraction 7	3	225.0000	26.00000	15.01111	160.4124	289.5876	199.00	251.00

	Fraction 8	1	238.8000	.	.	.	.	238.80	238.80
	Fraction 10	1	251.0000	.	.	.	.	251.00	251.00
	Fraction 11	1	251.0000	.	.	.	.	251.00	251.00
	Total	8	235.7200	19.59437	6.92765	219.3387	252.1013	199.00	251.00
Day 16	Fraction 1	0	.	.	.	.	.	.	.
	Fraction 6	1	251.0000	.	.	.	.	251.00	251.00
	Fraction 7	1	251.0000	.	.	.	.	251.00	251.00
	Fraction 8	0	.	.	.	.	.	.	.
	Fraction 10	0	.	.	.	.	.	.	.
	Fraction 11	1	298.5000	.	.	.	.	298.50	298.50
	Total	3	266.8333	27.42414	15.83333	198.7080	334.9587	251.00	298.50

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Day 14	*Between Groups*	*1335.574*	*5*	*267.115*	*.395*	*.826*
	Within Groups	1352.000	2	676.000		
	Total	2687.574	7			
Day 16	Between Groups	1504.167	2	752.083	.	.
	Within Groups	.000	0	.		
	Total	1504.167	2			

Appendix IV.Parasitaemia Profile On Different Days Of Treatment With Methanolic Fractions.

Oneway

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Methanol Fraction 1	Day 2	3	13.9300	5.98501	3.45544	-.9376	28.7976	7.94	19.91
	Day 4	3	51.2033	11.60026	6.69741	22.3867	80.0200	39.81	63.00
	Day 6	3	109.4200	14.44757	8.34131	73.5302	145.3098	99.53	126.00
	Day 8	3	162.5267	36.52500	21.08772	71.7935	253.2598	126.00	199.05
	Day 10	2	159.2400	.00000	.00000	159.2400	159.2400	159.24	159.24
	Total	14	94.9800	62.91311	16.81424	58.6550	131.3050	7.94	199.05

Methanol Fraction 3	Day 2	3	11.4933	3.85986	2.22849	1.9049	21.0818	7.94	15.60
	Day 4	3	94.3333	31.50132	18.18730	16.0797	172.5870	63.00	126.00
	Day 6	3	106.4267	45.73944	26.40768	-7.1964	220.0497	79.62	159.24
	Day 8	2	126.0000	.00000	.00000	126.0000	126.0000	126.00	126.00
	Day 10	2	179.1500	.00000	.00000	179.1500	179.1500	179.15	179.15
	Total	13	95.9277	60.26680	16.71500	59.5088	132.3466	7.94	179.15

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Methanol Fraction 1	Between Groups	48028.390	4	12007.097	31.539	.000
	Within Groups	3426.389	9	380.710		
	Total	51454.778	13			
Methanol Fraction 3	Between Groups	37386.387	4	9346.597	12.063	.002
	Within Groups	6198.656	8	774.832		
	Total	43585.043	12			

Post Hoc Tests
Homogeneous Subsets

Methanol Fraction 1

Duncan

		Subset for alpha = 0.05		
Days	N	1	2	3
Day 2	3	13.9300		
Day 4	3	51.2033		
Day 6	3		109.4200	
Day 10	2			159.2400
Day 8	3			162.5267
Sig.		.053	1.000	.848

Means for groups in homogeneous subsets are displayed.

Methanol Fraction 3

Duncan

		Subset for alpha = 0.05		
Days	N	1	2	3
Day 2	3	*11.4933*		
Day 4	3		94.3333	
Day 6	3		106.4267	

Day 8	2		126.0000	126.0000
Day 10	2			179.1500
Sig.		1.000	.257	.065

Means for groups in homogeneous subsets are displayed.

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Methanol Fraction 4	Day 2	3	5.9400	2.02030	1.16642	.9213	10.9587	3.90	7.94
	Day 4	3	31.0033	13.38537	7.72804	-2.2478	64.2544	15.60	39.81
	Day 6	3	71.5400	8.31954	4.80329	50.8731	92.2069	63.00	79.62
	Day 8	3	142.6200	16.62000	9.59556	101.3336	183.9064	126.00	159.24
	Day 10	3	179.1467	19.90500	11.49216	129.6999	228.5934	159.24	199.05
	Total	15	86.0500	68.88195	17.78524	47.9044	124.1956	3.90	199.05
Methanol Fraction 5	Day 2	3	9.9400	2.00000	1.15470	4.9717	14.9083	7.94	11.94
	Day 4	3	35.3367	4.15428	2.39847	25.0169	45.6565	31.60	39.81
	Day 6	3	94.5000	31.50000	18.18653	16.2497	172.7503	63.00	126.00
	Day 8	3	188.5000	62.50000	36.08439	33.2414	343.7586	126.00	251.00
	Day 10	2	251.0000	.00000	.00000	251.0000	251.0000	251.00	251.00
	Total	14	106.2021	94.20393	25.17706	51.8104	160.5939	7.94	251.00
Methanol Fraction 6	Day 2	3	2.9867	.99500	.57447	.5149	5.4584	1.99	3.98
	Day 4	3	11.4800	3.86280	2.23019	1.8843	21.0757	7.94	15.60
	Day 6	*3*	*46.9667*	*15.71061*	*9.07053*	*7.9393*	*85.9940*	*31.60*	*63.00*
	Day 8	3	94.5000	31.50000	18.18653	16.2497	172.7503	63.00	126.00
	Day 10	3	162.5267	36.52500	21.08772	71.7935	253.2598	126.00	199.05
	Total	15	63.6920	64.03308	16.53327	28.2317	99.1523	1.99	199.05

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Methanol Fraction 4	Between Groups	64576.326	4	16144.081	87.275	.00
	Within Groups	1849.796	10	184.980		
	Total	66426.122	14			
Methanol Fraction 5	Between Groups	105527.419	4	26381.855	24.131	.00
	Within Groups	9839.516	9	1093.280		
	Total	115366.935	13			

Methanol Fraction 6	Between Groups	52225.174	4	13056.294	25.214	.000
	Within Groups	5178.120	10	517.812		
	Total	57403.295	14			

Post Hoc Tests
Homogeneous Subsets

Methanol Fraction 4

Duncan

Days	N	Subset for alpha = 0.05				
		1	2	3	4	5
Day 2	3	5.9400				
Day 4	3		31.0033			
Day 6	3			71.5400		
Day 8	3				142.6200	
Day 10	3					179.1467
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Methanol Fraction 5

Duncan

Days	N	Subset for alpha = 0.05		
		1	2	3
Day 2	3	9.9400		
Day 4	3	35.3367	35.3367	
Day 6	3		94.5000	
Day 8	3			188.5000
Day 10	2			251.0000
Sig.		.393	.066	.055

Means for groups in homogeneous subsets are displayed.

Methanol Fraction 6

Duncan

Days	N	Subset for alpha = 0.05			
		1	2	3	4
Day 2	3	2.9867			
Day 4	3	11.4800	11.4800		
Day 6	3		46.9667		
Day 8	3			94.5000	

Day 12	3			142.6000	
Day 14	3				225.0000
Sig.		.169	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Methanol Fraction 8	Day 2	3	5.9000	1.88128	1.08616	1.2267	10.5733	3.98	7.74
	Day 4	3	24.0033	13.69810	7.90860	-10.0246	58.0313	15.60	39.81
	Day 6	3	47.3000	15.70000	9.06440	8.2990	86.3010	31.60	63.00
	Day 8	3	139.3000	19.90000	11.48927	89.8657	188.7343	119.40	159.20
	Day 10	3	139.3000	19.90000	11.48927	89.8657	188.7343	119.40	159.20
	Day 12	3	238.8000	39.80000	22.97854	139.9313	337.6687	199.00	278.60
	Total	18	99.1006	85.71462	20.20313	56.4757	141.7254	3.98	278.60
Methanol Fraction 11	Day 2	3	.6000	.20000	.11547	.1032	1.0968	.40	.80
	Day 4	3	23.6000	8.00000	4.61880	3.7269	43.4731	15.60	31.60
	Day 6	3	51.4067	11.59500	6.69438	22.6031	80.2102	39.81	63.00
	Day 8	3	162.5000	36.50000	21.07328	71.8290	253.1710	126.00	199.00
	Day 10	3	162.5000	36.50000	21.07328	71.8290	253.1710	126.00	199.00
	Day 12	3	205.1000	45.90000	26.50038	91.0781	319.1219	159.20	251.00
	Total	18	100.9511	84.27362	19.86348	59.0428	142.8594	.40	251.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Methanol Fraction 8	Between Groups	119271.478	5	23854.296	50.867	.0
	Within Groups	5627.454	12	468.955		
	Total	124898.932	17			
Methanol Fraction 11	Between Groups	110795.143	5	22159.029	26.752	.0
	Within Groups	9939.588	12	828.299		
	Total	120734.731	17			

Post Hoc Tests

Homogeneous Subsets

Methanol Fraction 8

Duncan

Between Groups	73505.713	4	18376.428	233.438	.000
Within Groups	629.766	8	78.721		
Total	74135.479	12			

Post Hoc Tests
Homogeneous Subsets

Methanol Fraction 9

Duncan

Days	N	Subset for alpha = 0.05			
		1	2	3	4
Day 2	3	1.3700			
Day 4	3		35.6700		
Day 6	3			142.6000	
Day 10	2				179.1000
Day 8	2				183.8000
Sig.		1.000	1.000	1.000	.570

Means for groups in homogeneous subsets are displayed.

Descriptives

Methanol Fraction 10

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Day 2	3	4.9733	2.97504	1.71764	-2.4171	12.3637	1.99	7.94
Day 4	3	23.6000	8.00000	4.61880	3.7269	43.4731	15.60	31.60
Day 6	3	82.9067	43.09500	24.88091	-24.1472	189.9606	39.81	126.00
Day 8	3	134.8667	7.67876	4.43333	115.7916	153.9418	126.00	139.30
Total	12	61.5867	56.72554	16.37525	25.5450	97.6284	1.99	139.30

ANOVA

Methanol Fraction 10

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	31417.671	3	10472.557	21.061	.000
Within Groups	3977.986	8	497.248		
Total	35395.658	11			

Post Hoc Tests
Homogeneous Subsets

Methanol Fraction 10

Duncan

Days	N	Subset for alpha = 0.05		
		1	2	3
Day 2	3	4.9733		
Day 4	3	23.6000		
Day 6	3		82.9067	
Day 8	3			134.8667
Sig.		.336	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Warnings

Post hoc tests are not performed for Infected Untraeted because there are fewer than three groups.

Descriptives

Infected Untraeted

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Day 2	3	18.4533	12.06092	6.96337	-11.5076	48.4143	7.94	31.62
Day 4	3	142.6000	16.60000	9.58401	101.3633	183.8367	126.00	159.20
Total	6	80.5267	69.22520	28.26107	7.8793	153.1741	7.94	159.20

ANOVA

Infected Untraeted

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	23118.592	1	23118.592	109.820	.000
Within Groups	842.051	4	210.513		
Total	23960.644	5			

Appendix V. Parasitaemia Profile With Ethyl Acetate Fractions

Oneway

Day 2

Descriptives

Day 2

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Fraction 1	3	.1903	.06050	.03493	.0400	.3406	.13	.25
Fraction 2	3	.3133	.18502	.10682	-.1463	.7730	.13	.50

Fraction 3	3	5.9267	1.98084	1.14364	1.0060	10.8473	3.98	7.94
Fraction 4	3	.7067	.54994	.31751	-.6595	2.0728	.29	1.33
Fraction 5	3	.1903	.06050	.03493	.0400	.3406	.13	.25
Fraction 6	3	9.5967	6.00129	3.46484	-5.3114	24.5047	3.98	15.92
Fraction 7	3	.5967	.19502	.11260	.1122	1.0811	.40	.79
Fraction 8	3	.2933	.10066	.05812	.0433	.5434	.20	.40
Fraction 9	3	1.3300	.60556	.34962	-.1743	2.8343	.80	1.99
Fraction 10	3	.6000	.20000	.11547	.1032	1.0968	.40	.80
Fraction 11	3	.3233	.07506	.04333	.1369	.5098	.25	.40
Control (Infec.Untreated)	3	18.4533	12.06092	6.96337	-11.5076	48.4143	7.94	31.62
Total	36	3.2101	6.35775	1.05963	1.0589	5.3612	.13	31.62

ANOVA

Day 2

	Sum of Squares	*df*	*Mean Square*	*F*	*Sig.*
Between Groups	1042.317	11	94.756	6.106	.000
Within Groups	372.419	24	15.517		
Total	1414.736	35			

Post Hoc Tests
Homogeneous Subsets

Day 2

Duncan

		Subset for alpha = 0.05		
Fractions (Ethyl acetate)	N	1	2	3
Fraction 1	3	.1903		
Fraction 5	3	.1903		
Fraction 8	3	.2933		
Fraction 2	3	.3133		
Fraction 11	3	.3233		
Fraction 7	3	.5967		
Fraction 10	3	.6000		
Fraction 4	3	.7067		
Fraction 9	3	1.3300		
Fraction 3	3	5.9267	5.9267	
Fraction 6	3		9.5967	

Control (Infec.Untreated)	3			18.4533
Sig.		.139	.265	1.000

Means for groups in homogeneous subsets are displayed.

Day 4

Descriptives

Day 4

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Fraction 1	3	.3067	.18556	.10713	-.1543	.7676	.13	.50
Fraction 2	3	31.7100	7.83000	4.52065	12.2592	51.1608	23.88	39.54
Fraction 3	3	31.0433	7.91468	4.56955	11.3822	50.7045	23.88	39.54
Fraction 4	3	2.8533	1.02080	.58936	.3175	5.3891	1.99	3.98
Fraction 5	3	.3833	.12583	.07265	.0708	.6959	.25	.50
Fraction 6	3	29.8567	9.95500	5.74752	5.1271	54.5863	19.90	39.81
Fraction 7	3	23.8400	15.93008	9.19724	-15.7325	63.4125	7.94	39.80
Fraction 8	3	35.8200	27.88000	16.09653	-33.4378	105.0778	7.94	63.70
Fraction 9	3	59.9000	19.90758	11.49365	10.4468	109.3532	39.81	79.62
Fraction 10	3	29.8600	9.95000	5.74464	5.1428	54.5772	19.91	39.81
Fraction 11	3	.6000	.20000	.11547	.1032	1.0968	.40	.80
Control (Infec.Untreated)	3	142.6000	16.60000	9.58401	101.3633	183.8367	126.00	159.20
Total	36	32.3978	39.59496	6.59916	19.0008	45.7948	.13	159.20

ANOVA

Day 4

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	50819.374	11	4619.943	27.362	.000
Within Groups	4052.243	24	168.843		
Total	54871.617	35			

Post Hoc Tests
Homogeneous Subsets

Day 4

Duncan

Fractions (Ethyl acetate)	N	Subset for alpha = 0.05			
		1	2	3	4

Fraction 1	3	.3067			
Fraction 5	3	.3833			
Fraction 11	3	.6000			
Fraction 4	3	2.8533			
Fraction 7	3	23.8400	23.8400		
Fraction 6	3		29.8567		
Fraction 10	3		29.8600		
Fraction 3	3		31.0433		
Fraction 2	3		31.7100		
Fraction 8	3		35.8200		
Fraction 9	3			59.9000	
Control (Infec.Untreated)	3				142.6000
Sig.		.056	.329	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Day 6

Descriptives

Day 6

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Fraction 1	3	59.9033	20.03832	11.56913	10.1254	109.6813	39.54	79.60
Fraction 2	3	69.6500	9.95000	5.74464	44.9328	94.3672	59.70	79.60
Fraction 3	3	79.6000	19.90000	11.48927	30.1657	129.0343	59.70	99.50
Fraction 4	3	50.0900	9.96191	5.75151	25.3432	74.8368	39.81	59.70
Fraction 5	3	49.7567	9.94500	5.74175	25.0519	74.4614	39.81	59.70
Fraction 6	2	126.0000	.00000	.00000	126.0000	126.0000	126.00	126.00
Fraction 7	3	142.6200	16.62000	9.59556	101.3336	183.9064	126.00	159.24
Fraction 8	3	82.9067	43.09500	24.88091	-24.1472	189.9606	39.81	126.00
Fraction 9	3	142.6200	16.62000	9.59556	101.3336	183.9064	126.00	159.24
Fraction 10	3	142.6200	16.62000	9.59556	101.3336	183.9064	126.00	159.24
Fraction 11	3	5.9600	1.98000	1.14315	1.0414	10.8786	3.98	7.94
Total	32	85.2869	47.39343	8.37805	68.1997	102.3740	3.98	159.24

ANOVA

Day 6

	Sum of Squares	Df	Mean Square	F	Sig.

Between Groups	62061.334	10	6206.133	17.219	.000
Within Groups	7568.924	21	360.425		
Total	69630.259	31			

Post Hoc Tests
Homogeneous Subsets

Day 6

Duncan

Fractions (Ethyl acetate)	N	*Subset for alpha = 0.05*		
		1	2	3
Fraction 11	3	5.9600		
Fraction 5	3		49.7567	
Fraction 4	3		50.0900	
Fraction 1	3		59.9033	
Fraction 2	3		69.6500	
Fraction 3	3		79.6000	
Fraction 8	3		82.9067	
Fraction 6	2			126.0000
Fraction 7	3			142.6200
Fraction 9	3			142.6200
Fraction 10	3			142.6200
Sig.		1.000	.077	.348

Means for groups in homogeneous subsets are displayed.

Day 8

Descriptives

Day 8

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Fraction 1	3	142.6000	16.60000	9.58401	101.3633	183.8367	126.00	159.20
Fraction 2	3	189.0767	9.97500	5.75907	164.2974	213.8559	179.10	199.05
Fraction 3	3	218.9267	19.87500	11.47484	169.5544	268.2989	199.05	238.80
Fraction 4	3	126.7333	1.27017	.73333	123.5781	129.8886	126.00	128.20
Fraction 5	3	139.3000	19.90000	11.48927	89.8657	188.7343	119.40	159.20
Fraction 6	2	199.0000	.00000	.00000	199.0000	199.0000	199.00	199.00
Fraction 7	3	162.5100	36.50000	21.07329	71.8390	253.1810	126.00	199.00

Fraction 8	3	159.2467	99.52500	57.46079	-87.9871	406.4805	59.72	258.77
Fraction 9	3	218.9567	19.90500	11.49216	169.5099	268.4034	199.05	238.86
Fraction 10	3	251.0000	.00000	.00000	251.0000	251.0000	251.00	251.00
Fraction 11	3	19.9100	.00000	.00000	19.9100	19.9100	19.91	19.91
Total	32	165.0869	67.31026	11.89889	140.8189	189.3548	19.91	258.77

ANOVA

Day 8

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	114848.028	10	11484.803	9.420	.000
Within Groups	25602.769	21	1219.179		
Total	140450.797	31			

Post Hoc Tests

Homogeneous Subsets

Day 8

Duncan

Fractions (Ethyl acetate)	N	Subset for alpha = 0.05				
		1	2	3	4	5
Fraction 11	3	19.9100				
Fraction 4	3		126.7333			
Fraction 5	3		139.3000	139.3000		
Fraction 1	3		142.6000	142.6000		
Fraction 8	3		159.2467	159.2467	159.2467	
Fraction 7	3		162.5100	162.5100	162.5100	
Fraction 2	3		189.0767	189.0767	189.0767	189.0767
Fraction 6	2			199.0000	199.0000	199.0000
Fraction 3	3				218.9267	218.9267
Fraction 9	3				218.9567	218.9567
Fraction 10	3					251.0000
Sig.		1.000	.071	.083	.083	.068

Means for groups in homogeneous subsets are displayed.

Day 10

Descriptives

Day 10

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Fraction 4	2	159.2000	.00000	.00000	159.2000	159.2000	159.20	159.20
Fraction 5	3	182.4000	56.40000	32.56256	42.2946	322.5054	126.00	238.80
Fraction 7	2	126.0000	.00000	.00000	126.0000	126.0000	126.00	126.00
Fraction 8	2	126.0000	.00000	.00000	126.0000	126.0000	126.00	126.00
Fraction 10	2	251.0000	.00000	.00000	251.0000	251.0000	251.00	251.00
Fraction 11	3	25.8767	13.93500	8.04538	-8.7398	60.4931	11.94	39.81
Total	14	139.2307	77.14670	20.61832	94.6875	183.7739	11.94	251.00

ANOVA

Day 10

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	70620.680	5	14124.136	16.739	.000
Within Groups	6750.288	8	843.786		
Total	77370.969	13			

Post Hoc Tests
Homogeneous Subsets

Day 10

Duncan

Fractions (Ethyl acetate)	N	Subset for alpha = 0.05		
		1	2	3
Fraction 11	3	25.8767		
Fraction 7	2		126.0000	
Fraction 8	2		126.0000	
Fraction 4	2		159.2000	
Fraction 5	3		182.4000	
Fraction 10	2			251.0000
Sig.		1.000	.089	1.000

Means for groups in homogeneous subsets are displayed.

Day 12

Descriptives

Day 12

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Fraction 5	3	199.0500	.00000	.00000	199.0500	199.0500	199.05	199.05
Fraction 7	2	208.9900	14.05728	9.94000	82.6903	335.2897	199.05	218.93
Fraction 8	*2*	*199.0400*	*.01414*	*.01000*	*198.9129*	*199.1671*	*199.03*	*199.05*
Fraction 10	2	278.6700	.00000	.00000	278.6700	278.6700	278.67	278.67
Fraction 11	3	59.7167	19.90500	11.49216	10.2699	109.1634	39.81	79.62
Total	12	179.1417	78.24876	22.58847	129.4248	228.8586	39.81	278.67

ANOVA

Day 12

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	66361.529	4	16590.382	117.303	.000
Within Groups	990.025	7	141.432		
Total	67351.554	11			

Post Hoc Tests

Homogeneous Subsets

Day 12

Duncan

Fractions (Ethyl acetate)	N	Subset for alpha = 0.05		
		1	2	3
Fraction 11	3	59.7167		
Fraction 8	2		199.0400	
Fraction 5	3		199.0500	
Fraction 7	2		208.9900	
Fraction 10	2			278.6700
Sig.		1.000	.415	1.000

Means for groups in homogeneous subsets are displayed.

Warnings

There are fewer than two groups for dependent variable Day 14. No statistics are computed.

There are fewer than two groups for dependent variable Day 16. No statistics are computed.

There are fewer than two groups for dependent variable Day 18. No statistics are computed.

There are fewer than two groups for dependent variable Day 20. No statistics are computed.

Appendix VI. Parasitaemia On Different Days (Ethyl acetate Fractionation)

Oneway

Fraction 1

Descriptives

Fraction 1 (Ethyl acetate)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Day 2	3	.1903	.06050	.03493	.0400	.3406	.13	.25
Day 4	3	.3067	.18556	.10713	-.1543	.7676	.13	.50
Day 6	3	59.9033	20.03832	11.56913	10.1254	109.6813	39.54	79.60
Day 8	3	142.6000	16.60000	9.58401	101.3633	183.8367	126.00	159.20
Total	12	50.7501	61.95109	17.88374	11.3882	90.1119	.13	159.20

ANOVA

Fraction 1 (Ethyl acetate)

	Sum of Squares	*Df*	*Mean Square*	*F*	*Sig.*
Between Groups	40863.047	3	13621.016	80.463	.000
Within Groups	1354.265	8	169.283		
Total	42217.312	11			

Post Hoc Tests
Homogeneous Subsets

Fraction 1 (Ethyl acetate)

Duncan

Days	N	*Subset for alpha = 0.05*		
		1	2	3
Day 2	3	.1903		
Day 4	3	.3067		
Day 6	3		59.9033	
Day 8	3			142.6000
Sig.		.992	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Fraction 2

Descriptives

Fraction 2 (Ethyl acetate)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Day 2	3	.3133	.18502	.10682	-.1463	.7730	.13	.50
Day 4	3	31.7100	7.83000	4.52065	12.2592	51.1608	23.88	39.54
Day 6	3	69.6500	9.95000	5.74464	44.9328	94.3672	59.70	79.60
Day 8	*3*	*189.0767*	*9.97500*	*5.75907*	*164.2974*	*213.8559*	*179.10*	*199.05*
Total	12	72.6875	75.03830	21.66169	25.0104	120.3646	.13	199.05

ANOVA

Fraction 2 (Ethyl acetate)

	Sum of Squares	*Df*	*Mean Square*	*F*	*Sig.*
Between Groups	61418.520	3	20472.840	315.153	.000
Within Groups	519.693	8	64.962		
Total	61938.213	11			

Post Hoc Tests
Homogeneous Subsets

Fraction 2 (Ethyl acetate)

Duncan

Days	N	*Subset for alpha = 0.05*			
		1	2	3	4
Day 2	3	.3133			
Day 4	3		31.7100		
Day 6	3			69.6500	
Day 8	3				189.0767
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Fraction 3

Descriptives

Fraction 3 (Ethyl acetate)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Day 2	3	5.9267	1.98084	1.14364	1.0060	10.8473	3.98	7.94
Day 4	3	31.0433	7.91468	4.56955	11.3822	50.7045	23.88	39.54

Day 6	3	79.6000	19.90000	11.48927	30.1657	129.0343	59.70	99.50
Day 8	3	218.9267	19.87500	11.47484	169.5544	268.2989	199.05	238.80
Total	12	83.8742	86.91074	25.08897	28.6537	139.0946	3.98	238.80

ANOVA

Fraction 3 (Ethyl acetate)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	81373.068	3	27124.356	126.514	.000
Within Groups	*1715.183*	*8*	*214.398*		
Total	83088.251	11			

Post Hoc Tests
Homogeneous Subsets

Fraction 3 (Ethyl acetate)

Duncan

		Subset for alpha = 0.05		
Days	N	1	2	3
Day 2	*3*	*5.9267*		
Day 4	3	31.0433		
Day 6	3		79.6000	
Day 8	3			218.9267
Sig.		.069	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Fraction 4

Descriptives

Fraction 4 (Ethyl acetate)

					95% Confidence Interval for Mean			
	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
Day 2	3	.7067	.54994	.31751	-.6595	2.0728	.29	1.33
Day 4	3	2.8533	1.02080	.58936	.3175	5.3891	1.99	3.98
Day 6	3	50.0900	9.96191	5.75151	25.3432	74.8368	39.81	59.70
Day 8	3	126.7333	1.27017	.73333	123.5781	129.8886	126.00	128.20
Day 10	2	159.2000	.00000	.00000	159.2000	159.2000	159.20	159.20
Total	14	61.3964	64.36515	17.20231	24.2331	98.5598	.29	159.20

ANOVA

Fraction 4 (Ethyl acetate)

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	53652.940	4	13413.235	590.617	.000
Within Groups	204.395	9	22.711		
Total	53857.335	13			

Post Hoc Tests
Homogeneous Subsets

Fraction 4 (Ethyl acetate)

Duncan

Days	N	Subset for alpha = 0.05			
		1	2	3	4
Day 2	3	.7067			
Day 4	3	2.8533			
Day 6	3		50.0900		
Day 8	3			126.7333	
Day 10	2				159.2000
Sig.		.612	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Fraction 5

Descriptives

Fraction 5 (Ethyl acetate)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Day 2	3	.1903	.06050	.03493	.0400	.3406	.13	.25
Day 4	3	.3833	.12583	.07265	.0708	.6959	.25	.50
Day 6	3	49.7567	9.94500	5.74175	25.0519	74.4614	39.81	59.70
Day 8	3	139.3000	19.90000	11.48927	89.8657	188.7343	119.40	159.20
Day 10	3	182.4000	56.40000	32.56256	42.2946	322.5054	126.00	238.80

Day 12	3	199.0500	.00000	.00000	199.0500	199.0500	199.05	199.05
Day 14	3	251.0000	.00000	.00000	251.0000	251.0000	251.00	251.00
Total	21	117.4400	97.73242	21.32696	72.9528	161.9273	.13	251.00

ANOVA

Fraction 5 (Ethyl acetate)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	183680.724	6	30613.454	58.297	.000
Within Groups	*7351.785*	*14*	*525.128*		
Total	191032.509	20			

Post Hoc Tests
Homogeneous Subsets

Fraction 5 (Ethyl acetate)

Duncan

Days	N	Subset for alpha = 0.05				
		1	2	3	4	5
Day 2	*3*	*.1903*				
Day 4	3	.3833				
Day 6	3		49.7567			
Day 8	3			139.3000		
Day 10	3				182.4000	
Day 12	3				199.0500	
Day 14	3					251.0000
Sig.		.992	1.000	1.000	.389	1.000

Means for groups in homogeneous subsets are displayed.

Fraction 6

Descriptives

Fraction 6 (Ethyl acetate)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Day 2	3	9.5967	6.00129	3.46484	-5.3114	24.5047	3.98	15.92
Day 4	3	29.8567	9.95500	5.74752	5.1271	54.5863	19.90	39.81
Day 6	2	126.0000	.00000	.00000	126.0000	126.0000	126.00	126.00

Day 8	2	199.0000	.00000	.00000	199.0000	199.0000	199.00	199.00
Total	10	76.8360	78.27095	24.75145	20.8443	132.8277	3.98	199.00

ANOVA

Fraction 6 (Ethyl acetate)

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	54866.841	3	18288.947	406.068	.000
Within Groups	*270.235*	*6*	*45.039*		
Total	55137.076	9			

Post Hoc Tests
Homogeneous Subsets

Fraction 6 (Ethyl acetate)

Duncan

		Subset for alpha = 0.05			
Days	N	1	2	3	4
Day 2	*3*	*9.5967*			
Day 4	3		29.8567		
Day 6	2			126.0000	
Day 8	2				199.0000
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Fraction 7

Descriptives

Fraction 7 (Ethyl acetate)

					95% Confidence Interval for Mean			
	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
Day 2	3	.5967	.19502	.11260	.1122	1.0811	.40	.79
Day 4	3	23.8400	15.93008	9.19724	-15.7325	63.4125	7.94	39.80
Day 6	3	142.6200	16.62000	9.59556	101.3336	183.9064	126.00	159.24
Day 8	3	162.5100	36.50000	21.07329	71.8390	253.1810	126.00	199.00
Day 10	2	126.0000	.00000	.00000	126.0000	126.0000	126.00	126.00
Day 12	2	208.9900	14.05728	9.94000	82.6903	335.2897	199.05	218.93

Descriptives

Fraction 7 (Ethyl acetate)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Day 2	3	.5967	.19502	.11260	.1122	1.0811	.40	.79
Day 4	3	23.8400	15.93008	9.19724	-15.7325	63.4125	7.94	39.80
Day 6	3	142.6200	16.62000	9.59556	101.3336	183.9064	126.00	159.24
Day 8	3	162.5100	36.50000	21.07329	71.8390	253.1810	126.00	199.00
Day 10	2	126.0000	.00000	.00000	126.0000	126.0000	126.00	126.00
Day 12	2	208.9900	14.05728	9.94000	82.6903	335.2897	199.05	218.93
Total	16	103.6675	78.75388	19.68847	61.7025	145.6325	.40	218.93

ANOVA

Fraction 7 (Ethyl acetate)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	89110.430	5	17822.086	45.439	.000
Within Groups	3922.168	10	392.217		
Total	93032.598	15			

Post Hoc Tests
Homogeneous Subsets

Fraction 7 (Ethyl acetate)

Duncan

Days	N	Subset for alpha = 0.05		
		1	2	3
Day 2	3	.5967		
Day 4	3	23.8400		
Day 10	2		126.0000	
Day 6	3		142.6200	
Day 8	3		162.5100	
Day 12	2			208.9900
Sig.		.213	.073	1.000

Means for groups in homogeneous subsets are displayed.

Fraction 8

Descriptives

Fraction 8 (Ethyl acetate)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Day 2	3	.2933	.10066	.05812	.0433	.5434	.20	.40
Day 4	3	35.8200	27.88000	16.09653	-33.4378	105.0778	7.94	63.70
Day 6	3	82.9067	43.09500	24.88091	-24.1472	189.9606	39.81	126.00
Day 8	3	159.2467	99.52500	57.46079	-87.9871	406.4805	59.72	258.77
Day 10	2	126.0000	.00000	.00000	126.0000	126.0000	126.00	126.00
Day 12	2	199.0400	.01414	.01000	198.9129	199.1671	199.03	199.05
Total	16	92.8050	81.16218	20.29055	49.5567	136.0533	.20	258.77

ANOVA

Fraction 8 (Ethyl acetate)

	Sum of Squares	*Df*	*Mean Square*	*F*	*Sig.*
Between Groups	73730.079	5	14746.016	5.880	.009
Within Groups	25079.419	10	2507.942		
Total	98809.497	15			

Post Hoc Tests
Homogeneous Subsets

Fraction 8 (Ethyl acetate)

Duncan

Days	N	*Subset for alpha = 0.05*			
		1	2	3	4
Day 2	3	.2933			
Day 4	3	35.8200	35.8200		
Day 6	3	82.9067	82.9067	82.9067	
Day 10	2		126.0000	126.0000	126.0000
Day 8	3			159.2467	159.2467
Day 12	2				199.0400
Sig.		.104	.079	.130	.145

Means for groups in homogeneous subsets are displayed.

Fraction 9

Descriptives

Fraction 9 (Ethyl acetate)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Day 2	3	1.3300	.60556	.34962	-.1743	2.8343	.80	1.99
Day 4	3	59.9000	19.90758	11.49365	10.4468	109.3532	39.81	79.62
Day 6	3	142.6200	16.62000	9.59556	101.3336	183.9064	126.00	159.24
Day 8	3	218.9567	19.90500	11.49216	169.5099	268.4034	199.05	238.86
Total	12	105.7017	87.22016	25.17829	50.2846	161.1187	.80	238.86

ANOVA

Fraction 9 (Ethyl acetate)

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	81542.688	3	27180.896	101.695	.000
Within Groups	*2138.224*	*8*	*267.278*		
Total	83680.911	11			

Post Hoc Tests
Homogeneous Subsets

Fraction 9 (Ethyl acetate)

Duncan

Days	N	Subset for alpha = 0.05			
		1	2	3	4
Day 2	*3*	*1.3300*			
Day 4	3		59.9000		
Day 6	3			142.6200	
Day 8	3				218.9567
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Fraction 10

Descriptives

Fraction 10 (Ethyl acetate)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	Minimum	Maximum

					Lower Bound	Upper Bound		
Day 2	3	.6000	.20000	.11547	.1032	1.0968	.40	.80
Day 4	3	29.8600	9.95000	5.74464	5.1428	54.5772	19.91	39.81
Day 6	3	142.6200	16.62000	9.59556	101.3336	183.9064	126.00	159.24
Day 8	3	251.0000	.00000	.00000	251.0000	251.0000	251.00	251.00
Day 10	2	251.0000	.00000	.00000	251.0000	251.0000	251.00	251.00
Day 12	2	278.6700	.00000	.00000	278.6700	278.6700	278.67	278.67
Total	16	145.7238	114.01761	28.50440	84.9681	206.4794	.40	278.67

ANOVA

Fraction 10 (Ethyl acetate)

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	194249.689	5	38849.938	517.631	.000
Within Groups	750.534	10	75.053		
Total	195000.223	15			

Post Hoc Tests
Homogeneous Subsets

Fraction 10 (Ethyl acetate)

Duncan

Days	N	Subset for alpha = 0.05				
		1	2	3	4	5
Day 2	3	.6000				
Day 4	3		29.8600			
Day 6	3			142.6200		
Day 8	3				251.0000	
Day 10	2				251.0000	
Day 12	2					278.6700
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Fraction 11

Descriptives

Fraction 11 (Ethyl acetate)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		

Day 2	3	.3233	.07506	.04333	.1369	.5098	.25	.40
Day 4	3	.6000	.20000	.11547	.1032	1.0968	.40	.80
Day 6	3	5.9600	1.98000	1.14315	1.0414	10.8786	3.98	7.94
Day 8	3	19.9100	.00000	.00000	19.9100	19.9100	19.91	19.91
Day 10	3	25.8767	13.93500	8.04538	-8.7398	60.4931	11.94	39.81
Day 12	3	59.7167	19.90500	11.49216	10.2699	109.1634	39.81	79.62
Day 14	*3*	*67.6800*	*3.98000*	*2.29785*	*57.7931*	*77.5669*	*63.70*	*71.66*
Day 16	3	102.5400	23.19471	13.39147	44.9211	160.1589	79.62	126.00
Day 18	3	199.0500	39.81000	22.98431	100.1565	297.9435	159.24	238.86
Day 20	2	251.0000	.00000	.00000	251.0000	251.0000	251.00	251.00
Total	29	67.1369	79.93448	14.84346	36.7314	97.5423	.25	251.00

ANOVA

Fraction 11 (Ethyl acetate)

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	173440.515	9	19271.168	66.986	.000
Within Groups	*5466.061*	*19*	*287.687*		
Total	178906.576	28			

Post Hoc Tests

Homogeneous Subsets

Fraction 11 (Ethyl acetate)

Duncan

		Subset for alpha = 0.05				
Days	N	1	2	3	4	5
Day 2	3	.3233				
Day 4	*3*	*.6000*				
Day 6	3	5.9600				
Day 8	3	19.9100				
Day 10	3	25.8767				
Day 12	3		59.7167			
Day 14	3		67.6800			
Day 16	3			102.5400		
Day 18	3				199.0500	
Day 20	2					251.0000
Sig.		*.121*	*.581*	*1.000*	*1.000*	*1.000*

Means for groups in homogeneous subsets are displayed.

Neg.Control (Infected Untreated)

Warnings

Post hoc tests are not performed for Neg.Control (Infected Untreated) because there are fewer than three groups.

Descriptives

Neg.Control (Infected Untreated)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Day 2	3	18.4533	12.06092	6.96337	-11.5076	48.4143	7.94	31.62
Day 4	3	142.6000	16.60000	9.58401	101.3633	183.8367	126.00	159.20
Total	6	80.5267	69.22520	28.26107	7.8793	153.1741	7.94	159.20

ANOVA

Neg.Control (Infected Untreated)

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	23118.592	1	23118.592	109.820	.000
Within Groups	842.051	4	210.513		
Total	23960.644	5			

Appendix VII.Parasitaemia Profile At Different Doses
(*M.lucida* & *T.procumbens, ratio 1:1*)

Oneway

Day 3 & 4

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Day 3	Between Groups	19.850	3	6.617	1.471	.294
	Within Groups	35.973	8	4.497		
	Total	55.823	11			
Day 4	Between Groups	16046.764	3	5348.921	36.007	.000
	Within Groups	*1188.428*	*8*	*148.554*		
	Total	17235.192	11			

Post Hoc Tests

Homogeneous Subsets

Day 3

Duncan

Doses (M.L.& T.P. 1:1)	N	Subset for alpha = 0.05

		1
200mg/kg b.w.	3	.0000
300mg/kg b.w.	3	.0000
400mg/kg b.w.	3	1.1933
Neg.Control (Infected Untreated)	3	3.1467
Sig.		*.126*

Means for groups in homogeneous subsets are displayed.

Day 4

Duncan

Doses (M.L.& T.P. 1:1)	N	Subset for alpha = 0.05		
		1	2	3
200mg/kg b.w.	3	.0000		
300mg/kg b.w.	3		29.8600	
400mg/kg b.w.	3		29.8600	
Neg.Control (Infected Untreated)	3			99.5267
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Day 5,7 & 9

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Day 5	Between Groups	11300.817	2	5650.408	19.016	.003
	Within Groups	1782.847	6	297.141		
	Total	13083.664	8			
Day 7	Between Groups	754.737	2	377.369	.198	.826
	Within Groups	11458.153	6	1909.692		
	Total	12212.890	8			
Day 9	Between Groups	3090.105	2	1545.053	31.181	.004
	Within Groups	198.204	4	49.551		
	Total	3288.310	6			

Post Hoc Tests
Homogeneous Subsets

Day 5

Duncan

Doses (M.L.& T.P.	N	Subset for alpha = 0.05

1:1)		1	2
200mg/kg b.w.	3	59.7167	
400mg/kg b.w.	3	69.6700	
300mg/kg b.w.	3		139.3667
Sig.		.506	1.000

Means for groups in homogeneous subsets are displayed.

Day 7

Duncan

Doses (M.L.& T.P. 1:1)	N	Subset for alpha = 0.05
		1
200mg/kg b.w.	3	159.2400
400mg/kg b.w.	3	178.1467
300mg/kg b.w.	3	179.1467
Sig.		.608

Means for groups in homogeneous subsets are displayed.

Day 9

Duncan

Doses (M.L.& T.P. 1:1)	N	*Subset for alpha = 0.05*		
		1	2	3
300mg/kg b.w.	3	209.0033		
200mg/kg b.w.	2		238.0000	
400mg/kg b.w.	2			258.7700
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Warnings

There are fewer than two groups for dependent variable Day 11. No statistics are computed.

There are fewer than two groups for dependent variable Day 13. No statistics are computed.

Appendix VIII.Parasitaemia Profile On Different Days
(*M.lucida* & *T.procumbens*, ratio 1:1)

Oneway

ANOVA

200mg/kg b.w.

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	108387.342	4	27096.836	61.551	.000
Within Groups	3962.090	9	440.232		
Total	112349.433	13			

Post Hoc Tests
Homogeneous Subsets

200mg/kg b.w.

Duncan

Days (M.L.& T.P. 1:1)	N	Subset for alpha = 0.05			
		1	2	3	4
Day 3	3	.0000			
Day 4	3	.0000			
Day 5	3		59.7167		
Day 7	3			159.2400	
Day 9	2				238.0000
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

ANOVA

300mg/kg b.w.(M.L.&T.P. ratio 1:1)

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	136347.830	5	27269.566	150.173	.000
Within Groups	2179.056	12	181.588		
Total	138526.886	17			

Post Hoc Tests
Homogeneous Subsets

300mg/kg b.w.(M.L.&T.P. ratio 1:1)

Duncan

Days (M.L.& T.P. 1:1)	N	Subset for alpha = 0.05				
		1	2	3	4	5
Day 3	3	.0000				

Day 4	3		29.8600			
Day 5	3			139.3667		
Day 7	3				179.1467	
Day 9	3					209.0033
Day 11	3					228.9100
Sig.		1.000	1.000	1.000	1.000	.096

Means for groups in homogeneous subsets are displayed.

ANOVA

400mg/kg b.w.(M.L.&T.P. ratio 1:1)

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	115393.229	4	28848.307	32.893	.000
Within Groups	7893.337	9	877.037		
Total	123286.566	13			

Post Hoc Tests
Homogeneous Subsets

400mg/kg b.w.(M.L.&T.P. ratio 1:1)

Duncan

Days (M.L.& T.P. 1:1)	N	Subset for alpha = 0.05			
		1	2	3	4
Day 3	3	1.1933			
Day 4	3	29.8600	29.8600		
Day 5	3		69.6700		
Day 7	3			178.1467	
Day 9	2				258.7700
Sig.		.288	.151	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Warnings

Post hoc tests are not performed for Neg.Control (Infected Untreated) because there are fewer than three groups.

ANOVA

Neg.Control (Infected Untreated)

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	13933.657	1	13933.657	67.383	.001

Within Groups	827.127	4	206.782		
Total	14760.784	5			

Appendix IX.Parasitaemia Profile At Different Doses
(*M.lucida* & *T.procumbens*,ratio 1:2)

Oneway

Day 3 & 4

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Day 3	Between Groups	22.278	3	7.426	1.712	.241
	Within Groups	34.709	8	4.339		
	Total	*56.987*	*11*			
Day 4	Between Groups	22265.131	3	7421.710	74.925	.000
	Within Groups	792.443	8	99.055		
	Total	23057.574	11			

Post Hoc Tests
Homogeneous Subsets

Day 3

Duncan

Dose (M.L.& T.P., 1:2)	N	Subset for alpha = 0.05 1
200mg/kg b.w.	3	.0000
300mg/kg b.w.	3	.0000
400mg/kg b.w.	3	.0000
Neg.Control (Infected Untreated)	3	3.1467
Sig.		.120

Means for groups in homogeneous subsets are displayed.

Day 4

Duncan

Dose (M.L.& T.P., 1:2)	N	Subset for alpha = 0.05	
		1	2
200mg/kg b.w.	3	.0000	
400mg/kg b.w.	3	.0500	

300mg/kg b.w.	3	.1000	
Neg.Control (Infected Untreated)	3		99.5267
Sig.		.991	1.000

Means for groups in homogeneous subsets are displayed.

Day 5 & 7

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Day 5	Between Groups	16579.670	2	8289.835	5.905	.038
	Within Groups	8423.409	6	1403.901		
	Total	25003.078	8			
Day 7	Between Groups	54091.589	2	27045.795	3.515	.098
	Within Groups	46164.224	6	7694.037		
	Total	100255.813	8			

Post Hoc Tests

Homogeneous Subsets

Day 5

Duncan

Dose (M.L.& T.P., 1:2)	N	Subset for alpha = 0.05	
		1	2
200mg/kg b.w.	3	39.8000	
400mg/kg b.w.	3	60.2567	
300mg/kg b.w.	3		139.3367
Sig.		.529	1.000

Means for groups in homogeneous subsets are displayed.

Day 7

Duncan

Dose (M.L.& T.P., 1:2)	N	*Subset for alpha = 0.05*	
		1	2
200mg/kg b.w.	3	69.6600	
300mg/kg b.w.	3	179.1467	179.1467
400mg/kg b.w.	3		258.7733
Sig.		.177	.309

Means for groups in homogeneous subsets are displayed.

Day 9,11 & 13

Warnings

Post hoc tests are not performed for Day 9 because there are fewer than three groups.
Post hoc tests are not performed for Day 11 because there are fewer than three groups.
There are fewer than two groups for dependent variable Day 13. No statistics are computed.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Day 9	Between Groups	14381.293	1	14381.293	217.674	.001
	Within Groups	198.204	3	66.068		
	Total	14579.497	4			
Day 11	Between Groups	52417.200	1	52417.200	794.180	.000
	Within Groups	198.005	3	66.002		
	Total	52615.205	4			

Appendix X.Parasitaemia Profile On Different Days
(*M.lucida* & *T.procumbens*, ratio 1:2)

Oneway

ANOVA

200mg/kg b.w.(M.L.& T.P., 1:2)

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	52673.439	6	8778.906	191.933	.000
Within Groups	594.612	13	45.739		
Total	53268.051	19			

Post Hoc Tests

Homogeneous Subsets

200mg/kg b.w.(M.L.& T.P., 1:2)

Duncan

		Subset for alpha = 0.05					
Day	N	1	2	3	4	5	6
Day 3	3	.0000					
Day 4	3	.0000					
Day 5	3		39.8000				
Day 7	3			69.6600			
Day 9	3				89.5767		
Day 11	3					109.4800	
Day 13	2						159.2400
Sig.		1.000	1.000	1.000	1.000	1.000	1.000

200mg/kg b.w.(M.L.& T.P., 1:2)

Duncan

Day	N	Subset for alpha = 0.05					
		1	2	3	4	5	6
Day 3	3	.0000					
Day 4	3	.0000					
Day 5	3		39.8000				
Day 7	3			69.6600			
Day 9	3				89.5767		
Day 11	3					109.4800	
Day 13	2						159.2400
Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

ANOVA

300mg/kg b.w.(M.L.& T.P., 1:2)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	188917.782	5	37783.556	26.490	.000
Within Groups	14263.545	10	1426.354		
Total	203181.327	15			

Post Hoc Tests

Homogeneous Subsets

300mg/kg b.w.(M.L.& T.P., 1:2)

Duncan

Day	N	Subset for alpha = 0.05		
		1	2	3
Day 3	3	.0000		
Day 4	3	.1000		
Day 5	3		139.3367	
Day 7	3		179.1467	
Day 9	2		199.0500	
Day 11	2			318.4800
Sig.		.998	.117	1.000

Means for groups in homogeneous subsets are displayed.

ANOVA

400mg/kg b.w.(M.L.& T.P., 1:2)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	135424.484	3	45141.495	9.000	.006
Within Groups	40125.709	8	5015.714		
Total	175550.194	11			

Post Hoc Tests
Homogeneous Subsets

400mg/kg b.w.(M.L.& T.P., 1:2)

Duncan

Day	N	Subset for alpha = 0.05	
		1	2
Day 3	3	*.0000*	
Day 4	3	.0500	
Day 5	3	60.2567	
Day 7	3		258.7733
Sig.		.347	1.000

Means for groups in homogeneous subsets are displayed.

Warnings

Post hoc tests are not performed for Neg.Control (Infected Untreated) because there are fewer than three groups.

ANOVA

Neg.Control (Infected Untreated)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	13933.657	1	13933.657	67.383	.001
Within Groups	*827.127*	4	*206.782*		
Total	14760.784	5			

Appendix XI.Parasitaemia Profile With Different Doses Of *M.lucida* & *T.procumbens*, ratio 2:1

Oneway

Day 3 & 4

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Day 3	Between Groups	21.424	3	7.141	1.642	.255
	Within Groups	34.789	8	4.349		
	Total	56.213	11			
Day 4	Between Groups	11759.509	3	3919.836	19.367	.001
	Within Groups	*1619.170*	*8*	*202.396*		
	Total	13378.679	11			

Post Hoc Tests
Homogeneous Subsets

Day 3

Duncan

Dose (ML&TP ratio 2:1)	N	Subset for alpha = 0.05
		1
200mg/kg b.w.	3	.0000
300mg/kg b.w.	3	.0000
400mg/kg b.w.	3	.2000
Neg.Control(Infec.Untreated)	3	3.1467
Sig.		.121

Means for groups in homogeneous subsets are displayed.

Day 4

Duncan

Dose (ML&TP ratio 2:1)	N	*Subset for alpha = 0.05*		
		1	2	3
200mg/kg b.w.	3	19.9100		
300mg/kg b.w.	3	27.8700		
400mg/kg b.w.	3		59.0500	
Neg.Control(Infec.Untreated)	3			99.5267
Sig.		.513	1.000	1.000

Day 3

Duncan

Dose (ML&TP ratio 2:1)	N	Subset for alpha = 0.05
		1
200mg/kg b.w.	3	.0000
300mg/kg b.w.	3	.0000
400mg/kg b.w.	3	.2000
Neg.Control(Infec.Untreated)	3	3.1467
Sig.		.121

Means for groups in homogeneous subsets are displayed.

Day 5 & 7

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Day 5	Between Groups	58528.048	2	29264.024	3.782	.087
	Within Groups	46422.590	6	7737.098		
	Total	104950.639	8			
Day 7	Between Groups	32288.680	2	16144.340	9.054	.015
	Within Groups	10698.241	6	1783.040		
	Total	42986.921	8			

Post Hoc Tests
Homogeneous Subsets

Day 5

Duncan

Dose (ML&TP ratio 2:1)	N	Subset for alpha = 0.05
		1
300mg/kg b.w.	3	49.7633
200mg/kg b.w.	3	59.7167
400mg/kg b.w.	3	225.5900
Sig.		.056

Day 5

Duncan

Dose (ML&TP ratio 2:1)	N	Subset for alpha = 0.05
		1
300mg/kg b.w.	3	49.7633
200mg/kg b.w.	3	59.7167
400mg/kg b.w.	3	225.5900
Sig.		.056

Means for groups in homogeneous subsets are displayed.

Day 7

Duncan

Dose (ML&TP ratio 2:1)	N	Subset for alpha = 0.05	
		1	2
300mg/kg b.w.	3	69.6700	
200mg/kg b.w.	3	99.5267	
400mg/kg b.w.	3		209.0000
Sig.		.420	1.000

Means for groups in homogeneous subsets are displayed.

Warnings

Post hoc tests are not performed for Day 9 because there are fewer than three groups.
There are fewer than two groups for dependent variable Day 11. No statistics are computed.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Day 9	Between Groups	17116.230	1	17116.230	16.200	.028
	Within Groups	3169.672	3	1056.557		
	Total	20285.902	4			

Appendix XII.Parasitaemia Profile On Different Days
(*M.lucida* & *T.procumbens*, ratio 2:1)

Oneway

ANOVA

200mg/kg b.w.(ML&TP ratio 2:1)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	82098.734	4	20524.684	116.556	.000
Within Groups	1584.836	9	176.093		
Total	83683.570	13			

Post Hoc Tests
Homogeneous Subsets

200mg/kg b.w.(ML&TP ratio 2:1)

Duncan

Days	N	Subset for alpha = 0.05			
		1	2	3	4
Day 3	3	.0000			
Day 4	3	19.9100			
Day 5	3		59.7167		
Day 7	3			99.5267	
Day 9	2				238.8600
Sig.		.114	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

ANOVA

300mg/kg b.w.(MLTP ratio 1:2)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	63994.068	5	12798.814	14.315	.000
Within Groups	10729.325	12	894.110		
Total	74723.393	17			

Post Hoc Tests
Homogeneous Subsets

300mg/kg b.w.(MLTP ratio 1:2)

Duncan

Days	N	Subset for alpha = 0.05			
		1	2	3	4
Day 3	3	.0000			
Day 4	3	27.8700	27.8700		

Day 5	3	49.7633	49.7633		
Day 7	3		69.6700	69.6700	
Day 9	3			119.4300	
Day 11	3				179.1567
Sig.		.076	.129	.064	1.000

Means for groups in homogeneous subsets are displayed.

ANOVA

400mg/kg b.w.(MLTP ratio 1:2)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	111267.913	3	37089.304	5.305	.026
Within Groups	55934.937	8	6991.867		
Total	167202.850	11			

Post Hoc Tests
Homogeneous Subsets

400mg/kg b.w.(MLTP ratio 1:2)

Duncan

Days	N	Subset for alpha = 0.05		
		1	2	3
Day 3	3	.2000		
Day 4	3	59.0500	59.0500	
Day 7	3		209.0000	209.0000
Day 5	3			225.5900
Sig.		.414	.059	.814

Means for groups in homogeneous subsets are displayed.

Warnings

Post hoc tests are not performed for Neg.Control (Infected Untreated) because there are *fewer than three groups.*

ANOVA

Neg.Control (Infected Untreated)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	13933.657	1	13933.657	67.383	.001
Within Groups	827.127	4	206.782		
Total	14760.784	5			

Appendix XIII. Trypanosome Parasitaemia Profile Of Bands

Oneway

Day 2

Descriptives

Day 2

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Band 1	3	.0667	.06506	.03756	-.0950	.2283	.00	.13
Band 2	3	.2967	.09504	.05487	.0606	.5328	.20	.39
Band 3	3	17.9100	1.99000	1.14893	12.9666	22.8534	15.92	19.90
Fraction 11	3	2.0900	1.89000	1.09119	-2.6050	6.7850	.20	3.98
Control (Infected Untreated)	3	89.5767	9.95500	5.74752	64.8471	114.3063	79.62	99.53
Total	15	21.9880	35.86566	9.26047	2.1263	41.8497	.00	99.53

ANOVA

Day 2

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	17795.541	4	4448.885	208.579	.000
Within Groups	213.295	10	21.329		
Total	18008.836	14			

Post Hoc Tests

Homogeneous Subsets

Day 2

Duncan

Bands	N	Subset for alpha = 0.05		
		1	2	3
Band 1	3	.0667		
Band 2	3	.2967		
Fraction 11	3	2.0900		
Band 3	3		17.9100	
Control (Infected Untreated)	3			89.5767
Sig.		.620	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Day 6

Descriptives

Day 6	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Band 1	3	33.8367	13.93500	8.04538	-.7798	68.4531	19.90	47.77
Band 2	3	6.9700	4.97000	2.86943	-5.3762	19.3162	2.00	11.94
Band 3	3	99.5267	19.90500	11.49216	50.0799	148.9734	79.62	119.43
Fraction 11	3	79.6267	19.90500	11.49216	30.1799	129.0734	59.72	99.53
Control (Infected *Untreated)*	3	129.3867	9.95500	5.74752	104.6571	154.1163	119.43	139.34
Total	15	69.8693	47.46132	12.25446	43.5861	96.1525	2.00	139.34

ANOVA

Day 6	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	29315.265	4	7328.816	33.001	.000
Within Groups	2220.810	10	222.081		
Total	31536.076	14			

Post Hoc Tests

Homogeneous Subsets

Day 6

Duncan

Bands	*N*	Subset for alpha = 0.05		
		1	*2*	*3*
Band 2	3	6.9700		
Band 1	3	33.8367		
Fraction 11	3		79.6267	
Band 3	3		99.5267	
Control (Infected Untreated)	3			129.3867
Sig.		.052	.133	1.000

Means for groups in homogeneous subsets are displayed.

Day 8

Descriptives

Day 8

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Band 1	3	25.8767	13.93500	8.04538	-8.7398	60.4931	11.94	39.81
Band 2	3	59.7167	19.90500	11.49216	10.2699	109.1634	39.81	79.62
Band 3	3	40.4767	40.01027	23.09994	-58.9144	139.8677	1.00	81.00
Fraction 11	3	139.3367	19.90500	11.49216	89.8899	188.7834	119.43	159.24
Control (Infected *Untreated*)	3	218.9567	19.90500	11.49216	169.5099	268.4034	199.05	238.86
Total	15	96.8727	77.87117	20.10625	53.7490	139.9963	1.00	238.86

ANOVA

Day 8

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	78927.611	4	19731.903	33.067	.000
Within Groups	5967.266	10	596.727		
Total	84894.876	14			

Post Hoc Tests

Homogeneous Subsets

Day 8

Duncan

Bands	*N*	Subset for alpha = 0.05		
		1	*2*	*3*
Band 1	3	25.8767		
Band 3	3	40.4767		
Band 2	3	59.7167		
Fraction 11	3		139.3367	
Control (Infected Untreated)	3			218.9567
Sig.		.136	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Day 10

Descriptives

Day 10

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Band 1	3	47.3600	15.74000	9.08749	8.2597	86.4603	31.62	63.10
Band 2	3	71.3600	8.26000	4.76891	50.8410	91.8790	63.10	79.62
Fraction 11	3	162.4767	36.57500	21.11659	71.6193	253.3340	125.90	199.05
Total	9	93.7322	56.38872	18.79624	50.3880	137.0764	31.62	199.05

ANOVA

Day 10

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	22130.094	2	11065.047	20.073	.002
Within Groups	3307.412	6	551.235		
Total	25437.506	8			

Post Hoc Tests

Homogeneous Subsets

Day 10

Duncan

Bands	N	Subset for alpha = 0.05	
		1	2
Band 1	3	47.3600	
Band 2	3	71.3600	
Fraction 11	3		162.4767
Sig.		.257	1.000

Means for groups in homogeneous subsets are displayed.

Day 12

Warnings

There are fewer than two groups for dependent variable Day 12. No statistics are computed.

Appendix XIV. Trypanosome Parasitaemia Profile On Different Days

Oneway

Band 1

Descriptives

Band 1

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Day 2	3	.0667	.06506	.03756	-.0950	.2283	.00	.13
Day 6	3	33.8367	13.93500	8.04538	-.7798	68.4531	19.90	47.77
Day 8	3	25.8767	13.93500	8.04538	-8.7398	60.4931	11.94	39.81
Day 10	*3*	*47.3600*	*15.74000*	*9.08749*	*8.2597*	*86.4603*	*31.62*	*63.10*
Total	12	26.7850	20.96641	6.05248	13.4636	40.1064	.00	63.10

ANOVA

Band 1

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	3563.253	3	1187.751	7.469	.010
Within Groups	1272.241	8	159.030		
Total	4835.494	11			

Post Hoc Tests

Homogeneous Subsets

Band 1

Duncan

Days	*N*	Subset for alpha = 0.05	
		1	*2*
Day 2	3	.0667	
Day 8	3		25.8767
Day 6	3		33.8367
Day 10	3		47.3600
Sig.		1.000	.080

Means for groups in homogeneous subsets are displayed.

Descriptives

Band 2

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Day 2	3	.2967	.09504	.05487	.0606	.5328	.20	.39
Day 6	3	6.9700	4.97000	2.86943	-5.3762	19.3162	2.00	11.94
Day 8	3	59.7167	19.90500	11.49216	10.2699	109.1634	39.81	79.62
Day 10	3	71.3600	8.26000	4.76891	50.8410	91.8790	63.10	79.62
Day 12	3	89.5767	9.95500	5.74752	64.8471	114.3063	79.62	99.53
Total	15	45.5840	37.98582	9.80790	24.5482	66.6198	.20	99.53

ANOVA

Band 2

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	19024.418	4	4756.105	40.426	.000
Within Groups	1176.497	10	117.650		
Total	20200.915	14			

Post Hoc Tests

Homogeneous Subsets

Band 2

Duncan

Days	N	Subset for alpha = 0.05		
		1	2	3
Day 2	3	.2967		
Day 6	3	6.9700		
Day 8	3		59.7167	
Day 10	3		71.3600	71.3600
Day 12	3			89.5767
Sig.		.469	.218	.067

Means for groups in homogeneous subsets are displayed.

Descriptives

Band 3

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Day 2	3	17.9100	1.99000	1.14893	12.9666	22.8534	15.92	19.90

Day 6	3	99.5267	19.90500	11.49216	50.0799	148.9734	79.62	119.43
Day 8	3	40.4767	40.01027	23.09994	-58.9144	139.8677	1.00	81.00
Total	9	52.6378	42.80686	14.26895	19.7335	85.5420	1.00	119.43

ANOVA

Band 3

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	10657.437	2	5328.719	7.989	.020
Within Groups	4001.982	6	666.997		
Total	14659.419	8			

Post Hoc Tests

Homogeneous Subsets

Band 3

Duncan

Days	N	Subset for alpha = 0.05	
		1	2
Day 2	3	17.9100	
Day 8	3	40.4767	
Day 6	3		99.5267
Sig.		.326	1.000

Means for groups in homogeneous subsets are displayed.

Fraction 11

Descriptives

Fraction 11

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Day 2	3	2.0900	1.89000	1.09119	-2.6050	6.7850	.20	3.98
Day 6	3	79.6267	19.90500	11.49216	30.1799	129.0734	59.72	99.53
Day 8	3	139.3367	19.90500	11.49216	89.8899	188.7834	119.43	159.24
Day 10	3	162.4767	36.57500	21.11659	71.6193	253.3340	125.90	199.05
Total	12	95.8825	67.70285	19.54413	52.8662	138.8988	.20	199.05

ANOVA

Fraction 11

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	46152.998	3	15384.333	28.840	.000

Within Groups	4267.442	8	533.430		
Total	50420.440	11			

Post Hoc Tests

Homogeneous Subsets

Fraction 11

Duncan

Days	N	*Subset for alpha = 0.05*		
		1	2	3
Day 2	3	2.0900		
Day 6	3		79.6267	
Day 8	3			139.3367
Day 10	3			162.4767
Sig.		1.000	1.000	.255

Means for groups in homogeneous subsets are displayed.

Control (Infected Untreated)

Descriptives

Control (Infected Untreated)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Day 2	3	89.5767	9.95500	5.74752	64.8471	114.3063	79.62	99.53
Day 6	3	129.3867	9.95500	5.74752	104.6571	154.1163	119.43	139.34
Day 8	3	218.9567	19.90500	11.49216	169.5099	268.4034	199.05	238.86
Total	9	145.9733	58.66817	19.55606	100.8770	191.0697	79.62	238.86

ANOVA

Control (Infected Untreated)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	26346.805	2	13173.403	66.486	.000
Within Groups	1188.826	6	198.138		
Total	27535.632	8			

Post Hoc Tests

Homogeneous Subsets

Control (Infected Untreated)

Duncan

Days	N	Subset for alpha = 0.05

		1	2	3
Day 2	3	89.5767		
Day 6	3		129.3867	
Day 8	3			218.9567
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Appendix XV: Clinical Parameters: Glucose (mMol/L)

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	*Control (Glucose)*	*8.1667*	*3*	*1.96554*	*1.13480*
	50mg/kg b.w.	7.6000	3	1.51327	.87369
Pair 2	Control (Glucose)	8.1667	3	1.96554	1.13480
	100mg/kg b.w.	3.0000	3	1.73205	1.00000
Pair 3	Control (Glucose)	8.1667	3	1.96554	1.13480
	200mg/kg b.w.	3.9667	3	1.72434	.99555
Pair 4	Control (Glucose)	8.1667	3	1.96554	1.13480
	400mg/kg b.w.	1.8333	3	.49329	.28480
Pair 5	Control (Glucose)	8.1667	3	1.96554	1.13480
	800mg/kg b.w.	3.7333	3	2.39653	1.38363

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Control (Glucose) & 50mg/kg b.w.	3	.999	.035
Pair 2	Control (Glucose) & 100mg/kg b.w.	3	.984	.114
Pair 3	Control (Glucose) & 200mg/kg b.w.	3	-.861	.340
Pair 4	*Control (Glucose) & 400mg/kg b.w.*	3	.997	.049
Pair 5	Control (Glucose) & 800mg/kg b.w.	3	-.357	.767

Paired Samples Test

	Paired Differences	t	df	Sig. (2-

		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				tailed)
					Lower	Upper			
Pair 1	Control (Glucose) - 50mg/kg b.w.	.56667	.46188	.26667	-.58071	1.71404	2.125	2	.168
Pair 2	Control (Glucose) - 100mg/kg b.w.	5.16667	.40415	.23333	4.16271	6.17062	22.143	2	.002
Pair 3	Control (Glucose) - 200mg/kg b.w.	4.20000	3.55949	2.05508	-4.64227	13.04227	2.044	2	.178
Pair 4	Control (Glucose) - 400mg/kg b.w.	6.33333	1.47422	.85114	2.67116	9.99551	7.441	2	.018
Pair 5	Control (Glucose) - 800mg/kg b.w.	4.43333	3.60185	2.07953	-4.51416	13.38083	2.132	2	.167

Total Protein (g/L)
T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Control (T.Protein)	6.4733	3	.25007	.14438
	50mg/kg b.w.	6.8267	3	.67988	.39253
Pair 2	Control (T.Protein)	6.4733	3	.25007	.14438
	100mg/kg b.w.	5.9333	3	.69010	.39843
Pair 3	Control (T.Protein)	6.4733	3	.25007	.14438
	200mg/kg b.w.	6.2600	3	.10583	.06110
Pair 4	Control (T.Protein)	6.4733	3	.25007	.14438
	400mg/kg b.w.	6.0433	3	.26539	.15322
Pair 5	Control (T.Protein)	6.4733	3	.25007	.14438
	800mg/kg b.w.	7.0367	3	.38280	.22101

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Control (T.Protein) & 50mg/kg b.w.	3	.050	.968
Pair 2	Control (T.Protein) & 100mg/kg b.w.	3	.865	.334
Pair 3	Control (T.Protein) & 200mg/kg b.w.	3	-.741	.469
Pair 4	Control (T.Protein) & 400mg/kg b.w.	3	-.831	.375

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Control (T.Protein)	6.4733	3	.25007	.14438
	50mg/kg b.w.	6.8267	3	.67988	.39253
Pair 2	Control (T.Protein)	6.4733	3	.25007	.14438
	100mg/kg b.w.	5.9333	3	.69010	.39843
Pair 3	Control (T.Protein)	6.4733	3	.25007	.14438
	200mg/kg b.w.	6.2600	3	.10583	.06110
Pair 4	Control (T.Protein)	6.4733	3	.25007	.14438
	400mg/kg b.w.	6.0433	3	.26539	.15322
Pair 5	Control (T.Protein)	6.4733	3	.25007	.14438
Pair 5	Control (T.Protein) & 800mg/kg b.w.	3	-.927	.245	

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Control (T.Protein) - 50mg/kg b.w.	-.35333	.71248	.41135	-2.12324	1.41657	-.859	2	.481
Pair 2	Control (T.Protein) - 100mg/kg b.w.	.54000	.49000	.28290	-.67723	1.75723	1.909	2	.197
Pair 3	Control (T.Protein) - 200mg/kg b.w.	.21333	.33606	.19402	-.62147	1.04814	1.100	2	.386
Pair 4	Control (T.Protein) - 400mg/kg b.w.	.43000	.49325	.28478	-.79531	1.65531	1.510	2	.270
Pair 5	Control (T.Protein) - 800mg/kg b.w.	-.56333	.62172	.35895	-2.10777	.98110	-1.569	2	.257

Urea (mMol/L)
T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Control (Urea)	8.7333	3	1.23791	.71471
	50mg/kg b.w.	8.7167	3	.70465	.40683
Pair 2	Control (Urea)	8.7333	3	1.23791	.71471
	100mg/kg b.w.	5.8600	3	1.14092	.65871
Pair 3	Control (Urea)	8.7333	3	1.23791	.71471

	200mg/kg b.w.	6.4100	3	1.41905	.81929
Pair 4	Control (Urea)	8.7333	3	1.23791	.71471
	400mg/kg b.w.	7.9433	3	.63106	.36434
Pair 5	Control (Urea)	8.7333	3	1.23791	.71471
	800mg/kg b.w.	9.2000	3	1.50190	.86712

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Control (Urea) & 50mg/kg b.w.	3	.994	.067
Pair 2	Control (Urea) & 100mg/kg b.w.	3	.588	.600
Pair 3	Control (Urea) & 200mg/kg b.w.	3	-.969	.159
Pair 4	Control (Urea) & 400mg/kg b.w.	3	.609	.583
Pair 5	Control (Urea) & 800mg/kg b.w.	3	.918	.259

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Control (Urea) - 50mg/kg b.w.	.01667	.54225	.31307	-1.33035	1.36369	.053	2	.962
Pair 2	Control (Urea) - 100mg/kg b.w.	2.87333	1.08288	.62520	.18331	5.56336	4.596	2	.044
Pair 3	Control (Urea) - 200mg/kg b.w.	2.32333	2.63648	1.52217	-4.22605	8.87272	1.526	2	.266
Pair 4	Control (Urea) - 400mg/kg b.w.	.79000	.98975	.57143	-1.66867	3.24867	1.382	2	.301
Pair 5	Control (Urea) - 800mg/kg b.w.	-.46667	.61076	.35263	-1.98389	1.05056	-1.323	2	.317

S GOT (AST)

T-Test

Paired Samples Statistics

		Mean	*N*	*Std. Deviation*	*Std. Error Mean*
Pair 1	Control (S GOT)	14.7300	3	3.00000	1.73205
	50mg/kg b.w.	12.9900	3	1.50000	.86603
Pair 2	Control (S GOT)	14.7300	3	3.00000	1.73205
	100mg/kg b.w.	7.0700	3	3.40000	1.96299

Pair 3	Control (S GOT)	14.7300	3	3.00000	1.73205
	200mg/kg b.w.	5.7500	3	1.90000	1.09697
Pair 4	Control (S GOT)	14.7300	3	3.00000	1.73205
	400mg/kg b.w.	7.2967	3	3.00222	1.73333
Pair 5	Control (S GOT)	14.7300	3	3.00000	1.73205
	800mg/kg b.w.	2.2200	3	.80000	.46188

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Control (S GOT) & 50mg/kg b.w.	3	.500	.667
Pair 2	Control (S GOT) & 100mg/kg b.w.	3	.500	.667
Pair 3	Control (S GOT) & 200mg/kg b.w.	3	1.000	.000
Pair 4	Control (S GOT) & 400mg/kg b.w.	3	.533	.642
Pair 5	Control (S GOT) & 800mg/kg b.w.	3	1.000	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Control (S GOT) - 50mg/kg b.w.	*1.74000*	*2.59808*	*1.50000*	*-4.71398*	*8.19398*	*1.160*	2	.366
Pair 2	Control (S GOT) - 100mg/kg b.w.	7.66000	3.21870	1.85831	-.33568	15.65568	4.122	2	.054
Pair 3	Control (S GOT) - 200mg/kg b.w.	8.98000	1.10000	.63509	6.24745	11.71255	14.140	2	.005
Pair 4	Control (S GOT) - 400mg/kg b.w.	7.43333	2.90057	1.67465	.22791	14.63876	4.439	2	.047
Pair 5	Control (S GOT) - 800mg/kg b.w.	12.51000	2.20000	1.27017	7.04490	17.97510	9.849	2	.010

S GPT (ALP)

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Control (S GPT)	39.7800	3	.20000	.11547
	50mg/kg b.w.	67.3600	3	4.40000	2.54034
Pair 2	Control (S GPT)	39.7800	3	.20000	.11547
	100mg/kg b.w.	50.3900	3	7.10000	4.09919
Pair 3	Control (S GPT)	39.7800	3	.20000	.11547
	200mg/kg b.w.	64.9800	3	5.80000	3.34863
Pair 4	Control (S GPT)	39.7800	3	.20000	.11547
	400mg/kg b.w.	60.8200	3	.36497	.21071
Pair 5	Control (S GPT)	39.7800	3	.20000	.11547
	800mg/kg b.w.	50.1000	3	.05000	.02887

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Control (S GPT) & 50mg/kg b.w.	3	1.000	.000
Pair 2	Control (S GPT) & 100mg/kg b.w.	3	.500	.667
Pair 3	Control (S GPT) & 200mg/kg b.w.	3	-.500	.667
Pair 4	Control (S GPT) & 400mg/kg b.w.	3	-.822	.386
Pair 5	Control (S GPT) & 800mg/kg b.w.	3	.500	.667

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Control (S GPT) - 50mg/kg b.w.	-27.58000	4.20000	2.42487	-38.01338	-17.14662	-11.374	2	.008
Pair 2	Control (S GPT) - 100mg/kg b.w.	-10.61000	7.00214	4.04269	-28.00429	6.78429	-2.624	2	.120

Pair 3	Control (S GPT) - 200mg/kg b.w.	-25.20000	5.90254	3.40783	-39.86273	-10.53727	-7.395	2	.018
Pair 4	Control (S GPT) - 400mg/kg b.w.	-21.04000	.54148	.31262	-22.38511	-19.69489	-67.301	2	.000
Pair 5	Control (S GPT) - 800mg/kg b.w.	-10.32000	.18028	.10408	-10.76783	-9.87217	-99.151	2	.000

ALP

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Control (ALP)	74.6700	3	6.40000	3.69504
	50mg/kg b.w.	75.2300	3	6.40000	3.69504
Pair 2	Control (ALP)	74.6700	3	6.40000	3.69504
	100mg/kg b.w.	76.3100	3	2.70000	1.55885
Pair 3	Control (ALP)	74.6700	3	6.40000	3.69504
	200mg/kg b.w.	74.3500	3	3.50000	2.02073
Pair 4	Control (ALP)	74.6700	3	6.40000	3.69504
	400mg/kg b.w.	75.3300	3	3.80000	2.19393
Pair 5	Control (ALP)	74.6700	3	6.40000	3.69504
	800mg/kg b.w.	76.2800	3	5.20000	3.00222

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Control (ALP) & 50mg/kg b.w.	3	.500	.667
Pair 2	Control (ALP) & 100mg/kg b.w.	3	-.500	.667
Pair 3	Control (ALP) & 200mg/kg b.w.	3	1.000	.000
Pair 4	Control (ALP) & 400mg/kg b.w.	3	1.000	.000
Pair 5	Control (ALP) & 800mg/kg b.w.	3	-.500	.667

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			

Pair 1	Control (ALP) - 50mg/kg b.w.	-.56000	6.40000	3.69504	-16.45848	15.33848	-.152	2	.893
Pair 2	Control (ALP) - 100mg/kg b.w.	-1.64000	8.09506	4.67369	-21.74924	18.46924	-.351	2	.759
Pair 3	Control (ALP) - 200mg/kg b.w.	.32000	2.90000	1.67432	-6.88400	7.52400	.191	2	.866
Pair 4	Control (ALP) - 400mg/kg b.w.	-.66000	2.60000	1.50111	-7.11876	5.79876	-.440	2	.703
Pair 5	Control (ALP) - 800mg/kg b.w.	-1.61000	10.06380	5.81034	-26.60986	23.38986	-.277	2	.808

Sodium (Na+)
T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Control (Na+)	126.0000	3	5.00000	2.88675
	50mg/kg b.w.	125.2100	3	5.00000	2.88675
Pair 2	Control (Na+)	126.0000	3	5.00000	2.88675
	100mg/kg b.w.	128.1800	3	3.50000	2.02073
Pair 3	Control (Na+)	126.0000	3	5.00000	2.88675
	200mg/kg b.w.	130.3300	3	6.20000	3.57957
Pair 4	Control (Na+)	126.0000	3	5.00000	2.88675
	400mg/kg b.w.	132.1700	3	6.40000	3.69504
Pair 5	Control (Na+)	126.0000	3	5.00000	2.88675
	800mg/kg b.w.	134.0100	3	3.70000	2.13620

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Control (Na+) & 50mg/kg b.w.	3	-1.000	.000
Pair 2	Control (Na+) & 100mg/kg b.w.	3	-1.000	.000
Pair 3	Control (Na+) & 200mg/kg b.w.	3	-.500	.667
Pair 4	Control (Na+) & 400mg/kg b.w.	3	-.500	.667
Pair 5	Control (Na+) & 800mg/kg b.w.	3	1.000	.000

Paired Samples Test

	Paired Differences						
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference	t	df	Sig. (2-tailed)

				Lower	Upper			
Pair 1	Control (Na+) - 50mg/kg b.w.	.79000	10.00000	5.77350	-24.05138	25.63138	.137	2	.904
Pair 2	Control (Na+) - 100mg/kg b.w.	-2.18000	8.50000	4.90748	-23.29517	18.93517	-.444	2	.700
Pair 3	Control (Na+) - 200mg/kg b.w.	-4.33000	9.71802	5.61070	-28.47091	19.81091	-.772	2	.521
Pair 4	Control (Na+) - 400mg/kg b.w.	-6.17000	9.89747	5.71431	-30.75669	18.41669	-1.080	2	.393
Pair 5	Control (Na+) - 800mg/kg b.w.	-8.01000	1.30000	.75056	-11.23938	-4.78062	-10.672	2	.009

Potassium (K+)
T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Control (K+)	5.1300	3	.10000	.05774
	50mg/kg b.w.	5.5600	3	.80000	.46188
Pair 2	Control (K+)	5.1300	3	.10000	.05774
	100mg/kg b.w.	5.4600	3	.30000	.17321
Pair 3	Control (K+)	5.1300	3	.10000	.05774
	200mg/kg b.w.	5.6500	3	.10000	.05774
Pair 4	Control (K+)	5.1300	3	.10000	.05774
	400mg/kg b.w.	6.2000	3	.90000	.51962
Pair 5	Control (K+)	5.1300	3	.10000	.05774
	800mg/kg b.w.	6.8100	3	.50000	.28868

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Control (K+) & 50mg/kg b.w.	3	-1.000	.000
Pair 2	Control (K+) & 100mg/kg b.w.	3	-.500	.667
Pair 3	Control (K+) & 200mg/kg b.w.	3	-.500	.667
Pair 4	Control (K+) & 400mg/kg b.w.	3	-1.000	.000
Pair 5	Control (K+) & 800mg/kg b.w.	3	.500	.667

Paired Samples Test

	Paired Differences	t	df	Sig. (2-

		Std.	Std. Error	95% Confidence Interval of the Difference				tailed)
	Mean	Deviation	Mean	Lower	Upper			
Pair 1	Control (K+) - 50mg/kg b.w.	-.43000	.90000	.51962	-2.66572	1.80572	-.828	2	.495
Pair 2	Control (K+) - 100mg/kg b.w.	-.33000	.36056	.20817	-1.22567	.56567	-1.585	2	.254
Pair 3	Control (K+) - 200mg/kg b.w.	-.52000	.17321	.10000	-.95027	-.08973	-5.200	2	.035
Pair 4	Control (K+) - 400mg/kg b.w.	-1.07000	1.00000	.57735	-3.55414	1.41414	-1.853	2	.205
Pair 5	Control (K+) - 800mg/kg b.w.	-1.68000	.45826	.26458	-2.81837	-.54163	-6.350	2	.024

Appendix XVI. Haematological Parameters: PCV

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Control (PCV)	21.3333	3	2.30940	1.33333
	50mg/kg b.w.	21.0000	3	1.00000	.57735
Pair 2	Control (PCV)	21.3333	3	2.30940	1.33333
	100mg/kg b.w.	24.6667	3	2.08167	1.20185
Pair 3	Control (PCV)	21.3333	3	2.30940	1.33333
	200mg/kg b.w.	23.0000	3	2.64575	1.52753
Pair 4	Control (PCV)	21.3333	3	2.30940	1.33333
	400mg/kg b.w.	24.6667	3	1.52753	.88192
Pair 5	Control (PCV)	21.3333	3	2.30940	1.33333
	800mg/kg b.w.	24.0000	3	2.00000	1.15470

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Control (PCV) & 50mg/kg b.w.	3	.000	1.000
Pair 2	Control (PCV) & 100mg/kg b.w.	3	.971	.154
Pair 3	Control (PCV) & 200mg/kg b.w.	3	.655	.546
Pair 4	Control (PCV) & 400mg/kg b.w.	3	.756	.454
Pair 5	Control (PCV) & 800mg/kg b.w.	3	.866	.333

Paired Samples Test

	Paired Differences	t	df	Sig. (2-

		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				tailed)
					Lower	Upper			
Pair 1	Control (PCV) - 50mg/kg b.w.	.33333	2.51661	1.45297	-5.91828	6.58494	.229	2	.840
Pair 2	Control (PCV) - 100mg/kg b.w.	-3.33333	.57735	.33333	-4.76755	-1.89912	-10.000	2	.010
Pair 3	Control (PCV) - 200mg/kg b.w.	-1.66667	2.08167	1.20185	-6.83781	3.50448	-1.387	2	.300
Pair 4	Control (PCV) - 400mg/kg b.w.	-3.33333	1.52753	.88192	-7.12792	.46125	-3.780	2	.063
Pair 5	Control (PCV) - 800mg/kg b.w.	-2.66667	1.15470	.66667	-5.53510	.20177	-4.000	2	.057

T-Test

RBC (x 10^6/uL)

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	control (RBC)	2.6000	3	.30000	.17321
	50mg/kg b.w.	2.6000	3	.20000	.11547
Pair 2	control (RBC)	2.6000	3	.30000	.17321
	100mg/kg b.w.	2.9667	3	.20817	.12019
Pair 3	control (RBC)	2.6000	3	.30000	.17321
	200mg/kg b.w.	2.8000	3	.26458	.15275
Pair 4	control (RBC)	2.6000	3	.30000	.17321
	400mg/kg b.w.	3.0667	3	.23094	.13333
Pair 5	control (RBC)	2.6000	3	.30000	.17321
	800mg/kg b.w.	2.7333	3	.35119	.20276

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	control (RBC) & 50mg/kg b.w.	3	1.000	.000
Pair 2	control (RBC) & 100mg/kg b.w.	3	.721	.488
Pair 3	control (RBC) & 200mg/kg b.w.	3	.945	.212
Pair 4	control (RBC) & 400mg/kg b.w.	3	.000	1.000
Pair 5	control (RBC) & 800mg/kg b.w.	3	.569	.614

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	control (RBC) - 50mg/kg b.w.	.00000	.10000	.05774	-.24841	.24841	.000	2	1.000
Pair 2	control (RBC) - 100mg/kg b.w.	-.36667	.20817	.12019	-.88378	.15045	-3.051	2	.093
Pair 3	control (RBC) - 200mg/kg b.w.	-.20000	.10000	.05774	-.44841	.04841	-3.464	2	.074
Pair 4	control (RBC) - 400mg/kg b.w.	-.46667	.37859	.21858	-1.40715	.47381	-2.135	2	.166
Pair 5	control (RBC) - 800mg/kg b.w.	-.13333	.30551	.17638	-.89225	.62558	-.756	2	.529

T-Test

WBC (x 10^9/L)

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Control (WBC)	3.5333	3	.11547	.06667
	50mg/kg b.w.	4.2667	3	.25166	.14530
Pair 2	Control (WBC)	3.5333	3	.11547	.06667
	100mg/kg b.w.	3.8000	3	.43589	.25166
Pair 3	Control (WBC)	3.5333	3	.11547	.06667
	200mg/kg b.w.	3.7333	3	.61101	.35277
Pair 4	Control (WBC)	3.5333	3	.11547	.06667
	400mg/kg b.w.	3.5333	3	.41633	.24037
Pair 5	Control (WBC)	3.5333	3	.11547	.06667
	800mg/kg b.w.	3.7667	3	.20817	.12019

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Control (WBC) & 50mg/kg b.w.	3	-.115	.927
Pair 2	Control (WBC) & 100mg/kg b.w.	3	.993	.073
Pair 3	Control (WBC) & 200mg/kg b.w.	3	.756	.454

Pair 4	Control (WBC) & 400mg/kg b.w.	3	.277	.821
Pair 5	Control (WBC) & 800mg/kg b.w.	3	.693	.512

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Control (WBC) - 50mg/kg b.w.	-.73333	.28868	.16667	-1.45044	-.01622	-4.400	2	.048
Pair 2	Control (WBC) - 100mg/kg b.w.	-.26667	.32146	.18559	-1.06521	.53187	-1.437	2	.287
Pair 3	Control (WBC) - 200mg/kg b.w.	-.20000	.52915	.30551	-1.51448	1.11448	-.655	2	.580
Pair 4	Control (WBC) - 400mg/kg b.w.	.00000	.40000	.23094	-.99366	.99366	.000	2	1.000
Pair 5	Control (WBC) - 800mg/kg b.w.	-.23333	.15275	.08819	-.61279	.14612	-2.646	2	.118

T-Test

NEUTROPHILS (%)

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Control (NEUTROPHILS)	22.0000	3	2.64575	1.52753
	50mg/kg b.w.	26.6667	3	11.71893	6.76593
Pair 2	Control (NEUTROPHILS)	22.0000	3	2.64575	1.52753
	100mg/kg b.w.	29.6667	3	2.30940	1.33333
Pair 3	Control (NEUTROPHILS)	22.0000	3	2.64575	1.52753
	200mg/kg b.w.	31.0000	3	1.00000	.57735
Pair 4	Control (NEUTROPHILS)	22.0000	3	2.64575	1.52753
	400mg/kg b.w.	16.3333	3	6.02771	3.48010
Pair 5	Control (NEUTROPHILS)	22.0000	3	2.64575	1.52753
	800mg/kg b.w.	25.6667	3	4.72582	2.72845

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Control (NEUTROPHILS) & 50mg/kg b.w.	3	-.484	.679

Pair 2	Control (NEUTROPHILS) & 100mg/kg b.w.	3	-.982	.121
Pair 3	Control (NEUTROPHILS) & 200mg/kg b.w.	3	.189	.879
Pair 4	Control (NEUTROPHILS) & 400mg/kg b.w.	3	.690	.515
Pair 5	Control (NEUTROPHILS) & 800mg/kg b.w.	3	-.800	.410

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Control (NEUTROPHILS) - 50mg/kg b.w.	-4.66667	13.20353	7.62306	-37.46607	28.13273	-.612	2	.603
Pair 2	Control (NEUTROPHILS) - 100mg/kg b.w.	-7.66667	4.93288	2.84800	-19.92063	4.58729	-2.692	2	.115
Pair 3	Control (NEUTROPHILS) - 200mg/kg b.w.	-9.00000	2.64575	1.52753	-15.57241	-2.42759	-5.892	2	.028
Pair 4	Control (NEUTROPHILS) - 400mg/kg b.w.	5.66667	4.61880	2.66667	-5.80707	17.14041	2.125	2	.168
Pair 5	Control (NEUTROPHILS) - 800mg/kg b.w.	-3.66667	7.02377	4.05518	-21.11468	13.78134	-.904	2	.461

T-Test

LYMPHOCYTES (%)

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Control (LYMPHOCYTES)	74.0000	3	1.00000	.57735
	50mg/kg b.w.	73.3333	3	11.71893	6.76593
Pair 2	Control (LYMPHOCYTES)	74.0000	3	1.00000	.57735
	100mg/kg b.w.	70.3333	3	2.30940	1.33333
Pair 3	Control (LYMPHOCYTES)	74.0000	3	1.00000	.57735
	200mg/kg b.w.	69.0000	3	1.00000	.57735

Pair 4	Control (LYMPHOCYTES)	74.0000	3	1.00000	.57735
	400mg/kg b.w.	83.6667	3	6.02771	3.48010
Pair 5	Control (LYMPHOCYTES)	74.0000	3	1.00000	.57735
	800mg/kg b.w.	74.3333	3	4.72582	2.72845

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Control (LYMPHOCYTES) & 50mg/kg b.w.	3	-.939	.224
Pair 2	Control (LYMPHOCYTES) & 100mg/kg b.w.	3	-.866	.333
Pair 3	Control (LYMPHOCYTES) & 200mg/kg b.w.	3	-.500	.667
Pair 4	Control (LYMPHOCYTES) & 400mg/kg b.w.	3	.995	.061
Pair 5	Control (LYMPHOCYTES) & 800mg/kg b.w.	3	-.212	.864

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Control (LYMPHOCYTES) - 50mg/kg b.w.	.66667	12.66228	7.31057	-30.78818	32.12151	.091	2	.936
Pair 2	Control (LYMPHOCYTES) - 100mg/kg b.w.	3.66667	3.21455	1.85592	-4.31872	11.65205	1.976	2	.187
Pair 3	Control (LYMPHOCYTES) - 200mg/kg b.w.	5.00000	1.73205	1.00000	.69735	9.30265	5.000	2	.038
Pair 4	Control (LYMPHOCYTES) - 400mg/kg b.w.	-9.66667	5.03322	2.90593	-22.16989	2.83655	-3.327	2	.080
Pair 5	Control (LYMPHOCYTES) - 800mg/kg b.w.	-.33333	5.03322	2.90593	-12.83655	12.16989	-.115	2	.919

Appendix XI. Parasitaemia Profile With Different Doses Of *M.lucida* & *T.procumbens*, ratio 2:1

Oneway

Day 3 & 4

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Day 3	Between Groups	21.424	3	7.141	1.642	.255
	Within Groups	34.789	8	4.349		
	Total	56.213	11			
Day 4	Between Groups	11759.509	3	3919.836	19.367	.001
	Within Groups	1619.170	8	202.396		
	Total	13378.679	11			

Post Hoc Tests
Homogeneous Subsets

Day 3

Duncan

Dose (ML&TP ratio 2:1)	N	Subset for alpha = 0.05
		1
200mg/kg b.w.	3	.0000
300mg/kg b.w.	3	.0000
400mg/kg b.w.	3	.2000
Neg.Control(Infec.Untreated)	3	3.1467
Sig.		.121

Means for groups in homogeneous subsets are displayed.

Day 4

Duncan

Dose (ML&TP ratio 2:1)	N	Subset for alpha = 0.05		
		1	2	3
200mg/kg b.w.	3	19.9100		
300mg/kg b.w.	3	27.8700		
400mg/kg b.w.	3		59.0500	
Neg.Control(Infec.Untreated)	3			99.5267
Sig.		.513	1.000	1.000

Day 3

Duncan

Dose (ML&TP ratio 2:1)	N	Subset for alpha = 0.05
		1
200mg/kg b.w.	3	.0000
300mg/kg b.w.	3	.0000
400mg/kg b.w.	3	.2000
Neg.Control(Infec.Untreated)	3	3.1467
Sig.		.121

Means for groups in homogeneous subsets are displayed.

Day 5 & 7

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Day 5	Between Groups	58528.048	2	29264.024	3.782	.087
	Within Groups	46422.590	6	7737.098		
	Total	104950.639	8			
Day 7	Between Groups	32288.680	2	16144.340	9.054	.015
	Within Groups	10698.241	6	1783.040		
	Total	42986.921	8			

Post Hoc Tests
Homogeneous Subsets

Day 5

Duncan

Dose (ML&TP ratio 2:1)	N	Subset for alpha = 0.05
		1
300mg/kg b.w.	3	49.7633
200mg/kg b.w.	3	59.7167
400mg/kg b.w.	3	225.5900
Sig.		.056

Day 5

Duncan

Dose (ML&TP ratio 2:1)	N	Subset for alpha = 0.05
		1
300mg/kg b.w.	3	49.7633
200mg/kg b.w.	3	59.7167
400mg/kg b.w.	3	225.5900
Sig.		.056

Means for groups in homogeneous subsets are displayed.

Day 7

Duncan

Dose (ML&TP ratio 2:1)	N	Subset for alpha = 0.05	
		1	2
300mg/kg b.w.	3	69.6700	
200mg/kg b.w.	3	99.5267	
400mg/kg b.w.	3		209.0000
Sig.		.420	1.000

Means for groups in homogeneous subsets are displayed.

Warnings

Post hoc tests are not performed for Day 9 because there are fewer than three groups.
There are fewer than two groups for dependent variable Day 11. No statistics are computed.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Day 9	Between Groups	17116.230	1	17116.230	16.200	.028
	Within Groups	3169.672	3	1056.557		
	Total	20285.902	4			

Appendix XII. Parasitaemia Profile On Different Days
(*M.lucida* & *T.procumbens*, ratio 2:1)

Oneway

ANOVA

200mg/kg b.w.(ML&TP ratio 2:1)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	82098.734	4	20524.684	116.556	.000
Within Groups	1584.836	9	176.093		
Total	83683.570	13			

Post Hoc Tests
Homogeneous Subsets

200mg/kg b.w.(ML&TP ratio 2:1)

Duncan

Days	N	Subset for alpha = 0.05			
		1	2	3	4
Day 3	3	.0000			
Day 4	3	19.9100			
Day 5	3		59.7167		
Day 7	3			99.5267	
Day 9	2				238.8600
Sig.		.114	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

ANOVA

300mg/kg b.w.(MLTP ratio 1:2)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	63994.068	5	12798.814	14.315	.000
Within Groups	10729.325	12	894.110		
Total	74723.393	17			

Post Hoc Tests
Homogeneous Subsets

300mg/kg b.w.(MLTP ratio 1:2)

Duncan

Days	N	Subset for alpha = 0.05			
		1	2	3	4
Day 3	3	.0000			
Day 4	3	27.8700	27.8700		

Day 5	3	49.7633	49.7633		
Day 7	3		69.6700	69.6700	
Day 9	3			119.4300	
Day 11	3				179.1567
Sig.		.076	.129	.064	1.000

Means for groups in homogeneous subsets are displayed.

ANOVA

400mg/kg b.w.(MLTP ratio 1:2)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	111267.913	3	37089.304	5.305	.026
Within Groups	55934.937	8	6991.867		
Total	167202.850	11			

Post Hoc Tests

Homogeneous Subsets

400mg/kg b.w.(MLTP ratio 1:2)

Duncan

Days	N	Subset for alpha = 0.05		
		1	2	3
Day 3	3	.2000		
Day 4	3	59.0500	59.0500	
Day 7	3		209.0000	209.0000
Day 5	3			225.5900
Sig.		.414	.059	.814

Means for groups in homogeneous subsets are displayed.

Warnings

Post hoc tests are not performed for Neg.Control (Infected Untreated) because there are fewer than three groups.

ANOVA

Neg.Control (Infected Untreated)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	13933.657	1	13933.657	67.383	.001
Within Groups	827.127	4	206.782		
Total	14760.784	5			

Appendix XIII. Trypanosome Parasitaemia Profile Of Bands

Oneway

Day 2

Descriptives

Day 2

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Band 1	3	.0667	.06506	.03756	-.0950	.2283	.00	.13
Band 2	3	.2967	.09504	.05487	.0606	.5328	.20	.39
Band 3	3	17.9100	1.99000	1.14893	12.9666	22.8534	15.92	19.90
Fraction 11	3	2.0900	1.89000	1.09119	-2.6050	6.7850	.20	3.98
Control (Infected Untreated)	3	89.5767	9.95500	5.74752	64.8471	114.3063	79.62	99.53
Total	15	21.9880	35.86566	9.26047	2.1263	41.8497	.00	99.53

ANOVA

Day 2

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	17795.541	4	4448.885	208.579	.000
Within Groups	213.295	10	21.329		
Total	18008.836	14			

Post Hoc Tests

Homogeneous Subsets

Day 2

Duncan

Bands	N	Subset for alpha = 0.05		
		1	2	3
Band 1	3	.0667		
Band 2	3	.2967		
Fraction 11	3	2.0900		
Band 3	3		17.9100	
Control (Infected Untreated)	3			89.5767
Sig.		.620	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Day 6

Descriptives

Day 6

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Band 1	3	33.8367	13.93500	8.04538	-.7798	68.4531	19.90	47.77
Band 2	3	6.9700	4.97000	2.86943	-5.3762	19.3162	2.00	11.94
Band 3	3	99.5267	19.90500	11.49216	50.0799	148.9734	79.62	119.43
Fraction 11	3	79.6267	19.90500	11.49216	30.1799	129.0734	59.72	99.53
Control (Infected Untreated)	3	129.3867	9.95500	5.74752	104.6571	154.1163	119.43	139.34
Total	15	69.8693	47.46132	12.25446	43.5861	96.1525	2.00	139.34

ANOVA

Day 6

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	29315.265	4	7328.816	33.001	.000
Within Groups	2220.810	10	222.081		
Total	31536.076	14			

Post Hoc Tests

Homogeneous Subsets

Day 6

Duncan

Bands	N	Subset for alpha = 0.05		
		1	2	3
Band 2	3	6.9700		
Band 1	3	33.8367		
Fraction 11	3		79.6267	
Band 3	3		99.5267	
Control (Infected Untreated)	3			129.3867
Sig.		.052	.133	1.000

Means for groups in homogeneous subsets are displayed.

Day 8

Descriptives

Day 8

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Band 1	3	25.8767	13.93500	8.04538	-8.7398	60.4931	11.94	39.81
Band 2	3	59.7167	19.90500	11.49216	10.2699	109.1634	39.81	79.62
Band 3	3	40.4767	40.01027	23.09994	-58.9144	139.8677	1.00	81.00
Fraction 11	3	139.3367	19.90500	11.49216	89.8899	188.7834	119.43	159.24
Control (Infected Untreated)	3	218.9567	19.90500	11.49216	169.5099	268.4034	199.05	238.86
Total	15	96.8727	77.87117	20.10625	53.7490	139.9963	1.00	238.86

ANOVA

Day 8

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	78927.611	4	19731.903	33.067	.000
Within Groups	5967.266	10	596.727		
Total	84894.876	14			

Post Hoc Tests

Homogeneous Subsets

Day 8

Duncan

Bands	N	Subset for alpha = 0.05		
		1	2	3
Band 1	3	25.8767		
Band 3	3	40.4767		
Band 2	3	59.7167		
Fraction 11	3		139.3367	
Control (Infected Untreated)	3			218.9567
Sig.		.136	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Day 10

Descriptives

Day 10

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Band 1	3	47.3600	15.74000	9.08749	8.2597	86.4603	31.62	63.10
Band 2	3	71.3600	8.26000	4.76891	50.8410	91.8790	63.10	79.62
Fraction 11	3	162.4767	36.57500	21.11659	71.6193	253.3340	125.90	199.05
Total	9	93.7322	56.38872	18.79624	50.3880	137.0764	31.62	199.05

ANOVA

Day 10

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	22130.094	2	11065.047	20.073	.002
Within Groups	3307.412	6	551.235		
Total	25437.506	8			

Post Hoc Tests

Homogeneous Subsets

Day 10

Duncan

Bands	N	Subset for alpha = 0.05	
		1	2
Band 1	3	47.3600	
Band 2	3	71.3600	
Fraction 11	3		162.4767
Sig.		.257	1.000

Means for groups in homogeneous subsets are displayed.

Day 12

Warnings

There are fewer than two groups for dependent variable Day 12. No statistics are computed.

Appendix XIV. Trypanosome Parasitaemia Profile On Different Days

Oneway

Band 1

Descriptives

Band 1

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Day 2	3	.0667	.06506	.03756	-.0950	.2283	.00	.13
Day 6	3	33.8367	13.93500	8.04538	-.7798	68.4531	19.90	47.77
Day 8	3	25.8767	13.93500	8.04538	-8.7398	60.4931	11.94	39.81
Day 10	3	47.3600	15.74000	9.08749	8.2597	86.4603	31.62	63.10
Total	12	26.7850	20.96641	6.05248	13.4636	40.1064	.00	63.10

ANOVA

Band 1

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	3563.253	3	1187.751	7.469	.010
Within Groups	1272.241	8	159.030		
Total	4835.494	11			

Post Hoc Tests

Homogeneous Subsets

Band 1

Duncan

Days	N	Subset for alpha = 0.05	
		1	2
Day 2	3	.0667	
Day 8	3		25.8767
Day 6	3		33.8367
Day 10	3		47.3600
Sig.		1.000	.080

Means for groups in homogeneous subsets are displayed.

Descriptives

Band 2

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Day 2	3	.2967	.09504	.05487	.0606	.5328	.20	.39
Day 6	3	6.9700	4.97000	2.86943	-5.3762	19.3162	2.00	11.94
Day 8	3	59.7167	19.90500	11.49216	10.2699	109.1634	39.81	79.62
Day 10	3	71.3600	8.26000	4.76891	50.8410	91.8790	63.10	79.62
Day 12	3	89.5767	9.95500	5.74752	64.8471	114.3063	79.62	99.53
Total	15	45.5840	37.98582	9.80790	24.5482	66.6198	.20	99.53

ANOVA

Band 2

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	19024.418	4	4756.105	40.426	.000
Within Groups	1176.497	10	117.650		
Total	20200.915	14			

Post Hoc Tests

Homogeneous Subsets

Band 2

Duncan

Days	N	Subset for alpha = 0.05		
		1	2	3
Day 2	3	.2967		
Day 6	3	6.9700		
Day 8	3		59.7167	
Day 10	3		71.3600	71.3600
Day 12	3			89.5767
Sig.		.469	.218	.067

Means for groups in homogeneous subsets are displayed.

Descriptives

Band 3

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Day 2	3	17.9100	1.99000	1.14893	12.9666	22.8534	15.92	19.90

Day 6	3	99.5267	19.90500	11.49216	50.0799	148.9734	79.62	119.43
Day 8	3	40.4767	40.01027	23.09994	-58.9144	139.8677	1.00	81.00
Total	9	52.6378	42.80686	14.26895	19.7335	85.5420	1.00	119.43

ANOVA

Band 3

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	10657.437	2	5328.719	7.989	.020
Within Groups	4001.982	6	666.997		
Total	14659.419	8			

Post Hoc Tests

Homogeneous Subsets

Band 3

Duncan

Days	N	Subset for alpha = 0.05	
		1	2
Day 2	3	17.9100	
Day 8	3	40.4767	
Day 6	3		99.5267
Sig.		.326	1.000

Means for groups in homogeneous subsets are displayed.

Fraction 11

Descriptives

Fraction 11

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Day 2	3	2.0900	1.89000	1.09119	-2.6050	6.7850	.20	3.98
Day 6	3	79.6267	19.90500	11.49216	30.1799	129.0734	59.72	99.53
Day 8	3	139.3367	19.90500	11.49216	89.8899	188.7834	119.43	159.24
Day 10	3	162.4767	36.57500	21.11659	71.6193	253.3340	125.90	199.05
Total	12	95.8825	67.70285	19.54413	52.8662	138.8988	.20	199.05

ANOVA

Fraction 11

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	46152.998	3	15384.333	28.840	.000

Within Groups	4267.442	8	533.430		
Total	50420.440	11			

Post Hoc Tests

Homogeneous Subsets

Fraction 11

Duncan

Days	N	Subset for alpha = 0.05		
		1	2	3
Day 2	3	2.0900		
Day 6	3		79.6267	
Day 8	3			139.3367
Day 10	3			162.4767
Sig.		1.000	1.000	.255

Means for groups in homogeneous subsets are displayed.

Control (Infected Untreated)

Descriptives

Control (Infected Untreated)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Day 2	3	89.5767	9.95500	5.74752	64.8471	114.3063	79.62	99.53
Day 6	3	129.3867	9.95500	5.74752	104.6571	154.1163	119.43	139.34
Day 8	3	218.9567	19.90500	11.49216	169.5099	268.4034	199.05	238.86
Total	9	145.9733	58.66817	19.55606	100.8770	191.0697	79.62	238.86

ANOVA

Control (Infected Untreated)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	26346.805	2	13173.403	66.486	.000
Within Groups	1188.826	6	198.138		
Total	27535.632	8			

Post Hoc Tests

Homogeneous Subsets

Control (Infected Untreated)

Duncan

Days	N	Subset for alpha = 0.05

		1	2	3
Day 2	3	89.5767		
Day 6	3		129.3867	
Day 8	3			218.9567
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Appendix XV: Clinical Parameters: Glucose (mMol/L)

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Control (Glucose)	8.1667	3	1.96554	1.13480
	50mg/kg b.w.	7.6000	3	1.51327	.87369
Pair 2	Control (Glucose)	8.1667	3	1.96554	1.13480
	100mg/kg b.w.	3.0000	3	1.73205	1.00000
Pair 3	Control (Glucose)	8.1667	3	1.96554	1.13480
	200mg/kg b.w.	3.9667	3	1.72434	.99555
Pair 4	Control (Glucose)	8.1667	3	1.96554	1.13480
	400mg/kg b.w.	1.8333	3	.49329	.28480
Pair 5	Control (Glucose)	8.1667	3	1.96554	1.13480
	800mg/kg b.w.	3.7333	3	2.39653	1.38363

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Control (Glucose) & 50mg/kg b.w.	3	.999	.035
Pair 2	Control (Glucose) & 100mg/kg b.w.	3	.984	.114
Pair 3	Control (Glucose) & 200mg/kg b.w.	3	-.861	.340
Pair 4	Control (Glucose) & 400mg/kg b.w.	3	.997	.049
Pair 5	Control (Glucose) & 800mg/kg b.w.	3	-.357	.767

Paired Samples Test

	Paired Differences	t	df	Sig. (2-

		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				tailed)
					Lower	Upper			
Pair 1	Control (Glucose) - 50mg/kg b.w.	.56667	.46188	.26667	-.58071	1.71404	2.125	2	.168
Pair 2	Control (Glucose) - 100mg/kg b.w.	5.16667	.40415	.23333	4.16271	6.17062	22.143	2	.002
Pair 3	Control (Glucose) - 200mg/kg b.w.	4.20000	3.55949	2.05508	-4.64227	13.04227	2.044	2	.178
Pair 4	Control (Glucose) - 400mg/kg b.w.	6.33333	1.47422	.85114	2.67116	9.99551	7.441	2	.018
Pair 5	Control (Glucose) - 800mg/kg b.w.	4.43333	3.60185	2.07953	-4.51416	13.38083	2.132	2	.167

Total Protein (g/L)
T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Control (T.Protein)	6.4733	3	.25007	.14438
	50mg/kg b.w.	6.8267	3	.67988	.39253
Pair 2	Control (T.Protein)	6.4733	3	.25007	.14438
	100mg/kg b.w.	5.9333	3	.69010	.39843
Pair 3	Control (T.Protein)	6.4733	3	.25007	.14438
	200mg/kg b.w.	6.2600	3	.10583	.06110
Pair 4	Control (T.Protein)	6.4733	3	.25007	.14438
	400mg/kg b.w.	6.0433	3	.26539	.15322
Pair 5	Control (T.Protein)	6.4733	3	.25007	.14438
	800mg/kg b.w.	7.0367	3	.38280	.22101

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Control (T.Protein) & 50mg/kg b.w.	3	.050	.968
Pair 2	Control (T.Protein) & 100mg/kg b.w.	3	.865	.334
Pair 3	Control (T.Protein) & 200mg/kg b.w.	3	-.741	.469
Pair 4	Control (T.Protein) & 400mg/kg b.w.	3	-.831	.375

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Control (T.Protein)	6.4733	3	.25007	.14438
	50mg/kg b.w.	6.8267	3	.67988	.39253
Pair 2	Control (T.Protein)	6.4733	3	.25007	.14438
	100mg/kg b.w.	5.9333	3	.69010	.39843
Pair 3	Control (T.Protein)	6.4733	3	.25007	.14438
	200mg/kg b.w.	6.2600	3	.10583	.06110
Pair 4	Control (T.Protein)	6.4733	3	.25007	.14438
	400mg/kg b.w.	6.0433	3	.26539	.15322
Pair 5	Control (T.Protein)	6.4733	3	.25007	.14438
Pair 5	Control (T.Protein) & 800mg/kg b.w.		3	-.927	.245

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Control (T.Protein) - 50mg/kg b.w.	-.35333	.71248	.41135	-2.12324	1.41657	-.859	2	.481
Pair 2	Control (T.Protein) - 100mg/kg b.w.	.54000	.49000	.28290	-.67723	1.75723	1.909	2	.197
Pair 3	Control (T.Protein) - 200mg/kg b.w.	.21333	.33606	.19402	-.62147	1.04814	1.100	2	.386
Pair 4	Control (T.Protein) - 400mg/kg b.w.	.43000	.49325	.28478	-.79531	1.65531	1.510	2	.270
Pair 5	Control (T.Protein) - 800mg/kg b.w.	-.56333	.62172	.35895	-2.10777	.98110	-1.569	2	.257

Urea (mMol/L)

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Control (Urea)	8.7333	3	1.23791	.71471
	50mg/kg b.w.	8.7167	3	.70465	.40683
Pair 2	Control (Urea)	8.7333	3	1.23791	.71471
	100mg/kg b.w.	5.8600	3	1.14092	.65871
Pair 3	Control (Urea)	8.7333	3	1.23791	.71471

	200mg/kg b.w.	6.4100	3	1.41905	.81929
Pair 4	Control (Urea)	8.7333	3	1.23791	.71471
	400mg/kg b.w.	7.9433	3	.63106	.36434
Pair 5	Control (Urea)	8.7333	3	1.23791	.71471
	800mg/kg b.w.	9.2000	3	1.50190	.86712

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Control (Urea) & 50mg/kg b.w.	3	.994	.067
Pair 2	Control (Urea) & 100mg/kg b.w.	3	.588	.600
Pair 3	Control (Urea) & 200mg/kg b.w.	3	-.969	.159
Pair 4	Control (Urea) & 400mg/kg b.w.	3	.609	.583
Pair 5	Control (Urea) & 800mg/kg b.w.	3	.918	.259

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Control (Urea) - 50mg/kg b.w.	.01667	.54225	.31307	-1.33035	1.36369	.053	2	.962
Pair 2	Control (Urea) - 100mg/kg b.w.	2.87333	1.08288	.62520	.18331	5.56336	4.596	2	.044
Pair 3	Control (Urea) - 200mg/kg b.w.	2.32333	2.63648	1.52217	-4.22605	8.87272	1.526	2	.266
Pair 4	Control (Urea) - 400mg/kg b.w.	.79000	.98975	.57143	-1.66867	3.24867	1.382	2	.301
Pair 5	Control (Urea) - 800mg/kg b.w.	-.46667	.61076	.35263	-1.98389	1.05056	-1.323	2	.317

S GOT (AST)

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Control (S GOT)	14.7300	3	3.00000	1.73205
	50mg/kg b.w.	12.9900	3	1.50000	.86603
Pair 2	Control (S GOT)	14.7300	3	3.00000	1.73205
	100mg/kg b.w.	7.0700	3	3.40000	1.96299

Pair 3	Control (S GOT)	14.7300	3	3.00000	1.73205
	200mg/kg b.w.	5.7500	3	1.90000	1.09697
Pair 4	Control (S GOT)	14.7300	3	3.00000	1.73205
	400mg/kg b.w.	7.2967	3	3.00222	1.73333
Pair 5	Control (S GOT)	14.7300	3	3.00000	1.73205
	800mg/kg b.w.	2.2200	3	.80000	.46188

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Control (S GOT) & 50mg/kg b.w.	3	.500	.667
Pair 2	Control (S GOT) & 100mg/kg b.w.	3	.500	.667
Pair 3	Control (S GOT) & 200mg/kg b.w.	3	1.000	.000
Pair 4	Control (S GOT) & 400mg/kg b.w.	3	.533	.642
Pair 5	Control (S GOT) & 800mg/kg b.w.	3	1.000	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Control (S GOT) - 50mg/kg b.w.	1.74000	2.59808	1.50000	-4.71398	8.19398	1.160	2	.366
Pair 2	Control (S GOT) - 100mg/kg b.w.	7.66000	3.21870	1.85831	-.33568	15.65568	4.122	2	.054
Pair 3	Control (S GOT) - 200mg/kg b.w.	8.98000	1.10000	.63509	6.24745	11.71255	14.140	2	.005
Pair 4	Control (S GOT) - 400mg/kg b.w.	7.43333	2.90057	1.67465	.22791	14.63876	4.439	2	.047
Pair 5	Control (S GOT) - 800mg/kg b.w.	12.51000	2.20000	1.27017	7.04490	17.97510	9.849	2	.010

S GPT (ALP)

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Control (S GPT)	39.7800	3	.20000	.11547
	50mg/kg b.w.	67.3600	3	4.40000	2.54034
Pair 2	Control (S GPT)	39.7800	3	.20000	.11547
	100mg/kg b.w.	50.3900	3	7.10000	4.09919
Pair 3	Control (S GPT)	39.7800	3	.20000	.11547
	200mg/kg b.w.	64.9800	3	5.80000	3.34863
Pair 4	Control (S GPT)	39.7800	3	.20000	.11547
	400mg/kg b.w.	60.8200	3	.36497	.21071
Pair 5	Control (S GPT)	39.7800	3	.20000	.11547
	800mg/kg b.w.	50.1000	3	.05000	.02887

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Control (S GPT) & 50mg/kg b.w.	3	1.000	.000
Pair 2	Control (S GPT) & 100mg/kg b.w.	3	.500	.667
Pair 3	Control (S GPT) & 200mg/kg b.w.	3	-.500	.667
Pair 4	Control (S GPT) & 400mg/kg b.w.	3	-.822	.386
Pair 5	Control (S GPT) & 800mg/kg b.w.	3	.500	.667

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Control (S GPT) - 50mg/kg b.w.	-27.58000	4.20000	2.42487	-38.01338	-17.14662	-11.374	2	.008
Pair 2	Control (S GPT) - 100mg/kg b.w.	-10.61000	7.00214	4.04269	-28.00429	6.78429	-2.624	2	.120

Pair 3	Control (S GPT) - 200mg/kg b.w.	-25.20000	5.90254	3.40783	-39.86273	-10.53727	-7.395	2	.018
Pair 4	Control (S GPT) - 400mg/kg b.w.	-21.04000	.54148	.31262	-22.38511	-19.69489	-67.301	2	.000
Pair 5	Control (S GPT) - 800mg/kg b.w.	-10.32000	.18028	.10408	-10.76783	-9.87217	-99.151	2	.000

ALP

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Control (ALP)	74.6700	3	6.40000	3.69504
	50mg/kg b.w.	75.2300	3	6.40000	3.69504
Pair 2	Control (ALP)	74.6700	3	6.40000	3.69504
	100mg/kg b.w.	76.3100	3	2.70000	1.55885
Pair 3	Control (ALP)	74.6700	3	6.40000	3.69504
	200mg/kg b.w.	74.3500	3	3.50000	2.02073
Pair 4	Control (ALP)	74.6700	3	6.40000	3.69504
	400mg/kg b.w.	75.3300	3	3.80000	2.19393
Pair 5	Control (ALP)	74.6700	3	6.40000	3.69504
	800mg/kg b.w.	76.2800	3	5.20000	3.00222

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Control (ALP) & 50mg/kg b.w.	3	.500	.667
Pair 2	Control (ALP) & 100mg/kg b.w.	3	-.500	.667
Pair 3	Control (ALP) & 200mg/kg b.w.	3	1.000	.000
Pair 4	Control (ALP) & 400mg/kg b.w.	3	1.000	.000
Pair 5	Control (ALP) & 800mg/kg b.w.	3	-.500	.667

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			

Pair 1	Control (ALP) - 50mg/kg b.w.	-.56000	6.40000	3.69504	-16.45848	15.33848	-.152	2	.893
Pair 2	Control (ALP) - 100mg/kg b.w.	-1.64000	8.09506	4.67369	-21.74924	18.46924	-.351	2	.759
Pair 3	Control (ALP) - 200mg/kg b.w.	.32000	2.90000	1.67432	-6.88400	7.52400	.191	2	.866
Pair 4	Control (ALP) - 400mg/kg b.w.	-.66000	2.60000	1.50111	-7.11876	5.79876	-.440	2	.703
Pair 5	Control (ALP) - 800mg/kg b.w.	-1.61000	10.06380	5.81034	-26.60986	23.38986	-.277	2	.808

Sodium (Na+)
T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Control (Na+)	126.0000	3	5.00000	2.88675
	50mg/kg b.w.	125.2100	3	5.00000	2.88675
Pair 2	Control (Na+)	126.0000	3	5.00000	2.88675
	100mg/kg b.w.	128.1800	3	3.50000	2.02073
Pair 3	Control (Na+)	126.0000	3	5.00000	2.88675
	200mg/kg b.w.	130.3300	3	6.20000	3.57957
Pair 4	Control (Na+)	126.0000	3	5.00000	2.88675
	400mg/kg b.w.	132.1700	3	6.40000	3.69504
Pair 5	Control (Na+)	126.0000	3	5.00000	2.88675
	800mg/kg b.w.	134.0100	3	3.70000	2.13620

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Control (Na+) & 50mg/kg b.w.	3	-1.000	.000
Pair 2	Control (Na+) & 100mg/kg b.w.	3	-1.000	.000
Pair 3	Control (Na+) & 200mg/kg b.w.	3	-.500	.667
Pair 4	Control (Na+) & 400mg/kg b.w.	3	-.500	.667
Pair 5	Control (Na+) & 800mg/kg b.w.	3	1.000	.000

Paired Samples Test

	Paired Differences						
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference	t	df	Sig. (2-tailed)

				Lower	Upper			
Pair 1	Control (Na+) - 50mg/kg b.w.	.79000	10.00000	5.77350	-24.05138	25.63138	.137	2	.904
Pair 2	Control (Na+) - 100mg/kg b.w.	-2.18000	8.50000	4.90748	-23.29517	18.93517	-.444	2	.700
Pair 3	Control (Na+) - 200mg/kg b.w.	-4.33000	9.71802	5.61070	-28.47091	19.81091	-.772	2	.521
Pair 4	Control (Na+) - 400mg/kg b.w.	-6.17000	9.89747	5.71431	-30.75669	18.41669	-1.080	2	.393
Pair 5	Control (Na+) - 800mg/kg b.w.	-8.01000	1.30000	.75056	-11.23938	-4.78062	-10.672	2	.009

Potassium (K+)
T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Control (K+)	5.1300	3	.10000	.05774
	50mg/kg b.w.	5.5600	3	.80000	.46188
Pair 2	Control (K+)	5.1300	3	.10000	.05774
	100mg/kg b.w.	5.4600	3	.30000	.17321
Pair 3	Control (K+)	5.1300	3	.10000	.05774
	200mg/kg b.w.	5.6500	3	.10000	.05774
Pair 4	Control (K+)	5.1300	3	.10000	.05774
	400mg/kg b.w.	6.2000	3	.90000	.51962
Pair 5	Control (K+)	5.1300	3	.10000	.05774
	800mg/kg b.w.	6.8100	3	.50000	.28868

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Control (K+) & 50mg/kg b.w.	3	-1.000	.000
Pair 2	Control (K+) & 100mg/kg b.w.	3	-.500	.667
Pair 3	Control (K+) & 200mg/kg b.w.	3	-.500	.667
Pair 4	Control (K+) & 400mg/kg b.w.	3	-1.000	.000
Pair 5	Control (K+) & 800mg/kg b.w.	3	.500	.667

Paired Samples Test

	Paired Differences	t	df	Sig. (2-

		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				tailed)
					Lower	Upper			
Pair 1	Control (K+) - 50mg/kg b.w.	-.43000	.90000	.51962	-2.66572	1.80572	-.828	2	.495
Pair 2	Control (K+) - 100mg/kg b.w.	-.33000	.36056	.20817	-1.22567	.56567	-1.585	2	.254
Pair 3	Control (K+) - 200mg/kg b.w.	-.52000	.17321	.10000	-.95027	-.08973	-5.200	2	.035
Pair 4	Control (K+) - 400mg/kg b.w.	-1.07000	1.00000	.57735	-3.55414	1.41414	-1.853	2	.205
Pair 5	Control (K+) - 800mg/kg b.w.	-1.68000	.45826	.26458	-2.81837	-.54163	-6.350	2	.024

Appendix XVI. Haematological Parameters: PCV

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Control (PCV)	21.3333	3	2.30940	1.33333
	50mg/kg b.w.	21.0000	3	1.00000	.57735
Pair 2	Control (PCV)	21.3333	3	2.30940	1.33333
	100mg/kg b.w.	24.6667	3	2.08167	1.20185
Pair 3	Control (PCV)	21.3333	3	2.30940	1.33333
	200mg/kg b.w.	23.0000	3	2.64575	1.52753
Pair 4	Control (PCV)	21.3333	3	2.30940	1.33333
	400mg/kg b.w.	24.6667	3	1.52753	.88192
Pair 5	Control (PCV)	21.3333	3	2.30940	1.33333
	800mg/kg b.w.	24.0000	3	2.00000	1.15470

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Control (PCV) & 50mg/kg b.w.	3	.000	1.000
Pair 2	Control (PCV) & 100mg/kg b.w.	3	.971	.154
Pair 3	Control (PCV) & 200mg/kg b.w.	3	.655	.546
Pair 4	Control (PCV) & 400mg/kg b.w.	3	.756	.454
Pair 5	Control (PCV) & 800mg/kg b.w.	3	.866	.333

Paired Samples Test

	Paired Differences	t	df	Sig. (2-

	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				tailed)
				Lower	Upper			
Pair 1 Control (PCV) - 50mg/kg b.w.	.33333	2.51661	1.45297	-5.91828	6.58494	.229	2	.840
Pair 2 Control (PCV) - 100mg/kg b.w.	-3.33333	.57735	.33333	-4.76755	-1.89912	-10.000	2	.010
Pair 3 Control (PCV) - 200mg/kg b.w.	-1.66667	2.08167	1.20185	-6.83781	3.50448	-1.387	2	.300
Pair 4 Control (PCV) - 400mg/kg b.w.	-3.33333	1.52753	.88192	-7.12792	.46125	-3.780	2	.063
Pair 5 Control (PCV) - 800mg/kg b.w.	-2.66667	1.15470	.66667	-5.53510	.20177	-4.000	2	.057

T-Test

RBC (x 10^6/uL)

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	control (RBC)	2.6000	3	.30000	.17321
	50mg/kg b.w.	2.6000	3	.20000	.11547
Pair 2	control (RBC)	2.6000	3	.30000	.17321
	100mg/kg b.w.	2.9667	3	.20817	.12019
Pair 3	control (RBC)	2.6000	3	.30000	.17321
	200mg/kg b.w.	2.8000	3	.26458	.15275
Pair 4	control (RBC)	2.6000	3	.30000	.17321
	400mg/kg b.w.	3.0667	3	.23094	.13333
Pair 5	control (RBC)	2.6000	3	.30000	.17321
	800mg/kg b.w.	2.7333	3	.35119	.20276

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	control (RBC) & 50mg/kg b.w.	3	1.000	.000
Pair 2	control (RBC) & 100mg/kg b.w.	3	.721	.488
Pair 3	control (RBC) & 200mg/kg b.w.	3	.945	.212
Pair 4	control (RBC) & 400mg/kg b.w.	3	.000	1.000
Pair 5	control (RBC) & 800mg/kg b.w.	3	.569	.614

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	control (RBC) - 50mg/kg b.w.	.00000	.10000	.05774	-.24841	.24841	.000	2	1.000
Pair 2	control (RBC) - 100mg/kg b.w.	-.36667	.20817	.12019	-.88378	.15045	-3.051	2	.093
Pair 3	control (RBC) - 200mg/kg b.w.	-.20000	.10000	.05774	-.44841	.04841	-3.464	2	.074
Pair 4	control (RBC) - 400mg/kg b.w.	-.46667	.37859	.21858	-1.40715	.47381	-2.135	2	.166
Pair 5	control (RBC) - 800mg/kg b.w.	-.13333	.30551	.17638	-.89225	.62558	-.756	2	.529

T-Test

WBC (x 10^9/L)

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Control (WBC)	3.5333	3	.11547	.06667
	50mg/kg b.w.	4.2667	3	.25166	.14530
Pair 2	Control (WBC)	3.5333	3	.11547	.06667
	100mg/kg b.w.	3.8000	3	.43589	.25166
Pair 3	Control (WBC)	3.5333	3	.11547	.06667
	200mg/kg b.w.	3.7333	3	.61101	.35277
Pair 4	Control (WBC)	3.5333	3	.11547	.06667
	400mg/kg b.w.	3.5333	3	.41633	.24037
Pair 5	Control (WBC)	3.5333	3	.11547	.06667
	800mg/kg b.w.	3.7667	3	.20817	.12019

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Control (WBC) & 50mg/kg b.w.	3	-.115	.927
Pair 2	Control (WBC) & 100mg/kg b.w.	3	.993	.073
Pair 3	Control (WBC) & 200mg/kg b.w.	3	.756	.454

Pair 4	Control (WBC) & 400mg/kg b.w.	3	.277	.821
Pair 5	Control (WBC) & 800mg/kg b.w.	3	.693	.512

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Control (WBC) - 50mg/kg b.w.	-.73333	.28868	.16667	-1.45044	-.01622	-4.400	2	.048
Pair 2	Control (WBC) - 100mg/kg b.w.	-.26667	.32146	.18559	-1.06521	.53187	-1.437	2	.287
Pair 3	Control (WBC) - 200mg/kg b.w.	-.20000	.52915	.30551	-1.51448	1.11448	-.655	2	.580
Pair 4	Control (WBC) - 400mg/kg b.w.	.00000	.40000	.23094	-.99366	.99366	.000	2	1.000
Pair 5	Control (WBC) - 800mg/kg b.w.	-.23333	.15275	.08819	-.61279	.14612	-2.646	2	.118

T-Test

NEUTROPHILS (%)

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Control (NEUTROPHILS)	22.0000	3	2.64575	1.52753
	50mg/kg b.w.	26.6667	3	11.71893	6.76593
Pair 2	Control (NEUTROPHILS)	22.0000	3	2.64575	1.52753
	100mg/kg b.w.	29.6667	3	2.30940	1.33333
Pair 3	Control (NEUTROPHILS)	22.0000	3	2.64575	1.52753
	200mg/kg b.w.	31.0000	3	1.00000	.57735
Pair 4	Control (NEUTROPHILS)	22.0000	3	2.64575	1.52753
	400mg/kg b.w.	16.3333	3	6.02771	3.48010
Pair 5	Control (NEUTROPHILS)	22.0000	3	2.64575	1.52753
	800mg/kg b.w.	25.6667	3	4.72582	2.72845

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Control (NEUTROPHILS) & 50mg/kg b.w.	3	-.484	.679

Pair 2	Control (NEUTROPHILS) & 100mg/kg b.w.	3	-.982	.121
Pair 3	Control (NEUTROPHILS) & 200mg/kg b.w.	3	.189	.879
Pair 4	Control (NEUTROPHILS) & 400mg/kg b.w.	3	.690	.515
Pair 5	Control (NEUTROPHILS) & 800mg/kg b.w.	3	-.800	.410

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Control (NEUTROPHILS) - 50mg/kg b.w.	-4.66667	13.20353	7.62306	-37.46607	28.13273	-.612	2	.603
Pair 2	Control (NEUTROPHILS) - 100mg/kg b.w.	-7.66667	4.93288	2.84800	-19.92063	4.58729	-2.692	2	.115
Pair 3	Control (NEUTROPHILS) - 200mg/kg b.w.	-9.00000	2.64575	1.52753	-15.57241	-2.42759	-5.892	2	.028
Pair 4	Control (NEUTROPHILS) - 400mg/kg b.w.	5.66667	4.61880	2.66667	-5.80707	17.14041	2.125	2	.168
Pair 5	Control (NEUTROPHILS) - 800mg/kg b.w.	-3.66667	7.02377	4.05518	-21.11468	13.78134	-.904	2	.461

T-Test

LYMPHOCYTES (%)

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Control (LYMPHOCYTES)	74.0000	3	1.00000	.57735
	50mg/kg b.w.	73.3333	3	11.71893	6.76593
Pair 2	Control (LYMPHOCYTES)	74.0000	3	1.00000	.57735
	100mg/kg b.w.	70.3333	3	2.30940	1.33333
Pair 3	Control (LYMPHOCYTES)	74.0000	3	1.00000	.57735
	200mg/kg b.w.	69.0000	3	1.00000	.57735

Pair 4	Control (LYMPHOCYTES)	74.0000	3	1.00000	.57735
	400mg/kg b.w.	83.6667	3	6.02771	3.48010
Pair 5	Control (LYMPHOCYTES)	74.0000	3	1.00000	.57735
	800mg/kg b.w.	74.3333	3	4.72582	2.72845

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Control (LYMPHOCYTES) & 50mg/kg b.w.	3	-.939	.224
Pair 2	Control (LYMPHOCYTES) & 100mg/kg b.w.	3	-.866	.333
Pair 3	Control (LYMPHOCYTES) & 200mg/kg b.w.	3	-.500	.667
Pair 4	Control (LYMPHOCYTES) & 400mg/kg b.w.	3	.995	.061
Pair 5	Control (LYMPHOCYTES) & 800mg/kg b.w.	3	-.212	.864

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Control (LYMPHOCYTES) - 50mg/kg b.w.	.66667	12.66228	7.31057	-30.78818	32.12151	.091	2	.936
Pair 2	Control (LYMPHOCYTES) - 100mg/kg b.w.	3.66667	3.21455	1.85592	-4.31872	11.65205	1.976	2	.187
Pair 3	Control (LYMPHOCYTES) - 200mg/kg b.w.	5.00000	1.73205	1.00000	.69735	9.30265	5.000	2	.038
Pair 4	Control (LYMPHOCYTES) - 400mg/kg b.w.	-9.66667	5.03322	2.90593	-22.16989	2.83655	-3.327	2	.080
Pair 5	Control (LYMPHOCYTES) - 800mg/kg b.w.	-.33333	5.03322	2.90593	-12.83655	12.16989	-.115	2	.919