SCREENING OF EXTRACTS OF SOME NIGERIAN MEDICINAL PLANTS FOR TRYPANOCIDAL ACTIVITY

BY

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DEDICATION

This work is dedicated to my aged parents Alhaji Raimi Orimadegun Akinsunbo and Alhaja Nin'motallahi Arike Orimadegun Akinsunbo.

CERTIFICATION

This thesis entitled "Screening of Extracts of Some Nigerian Medicinal Plants for Trypanocidal Activity" was carried out by Akinsunbo Orimadegun AbdulGaniy under my supervision and has been examined, read and found to meet the regulations governing the award of the Degree of Masters of Technology in Biochemistry of the Federal University of Technology Minna, and is approved for its contribution to knowledge and literary presentation.

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DECLARATION

I hereby declare that this Thesis is my original work and has never to the best of my knowledge been submitted before for the award of any degree.All literature used in the course of this study has been duly cited in references.

AKINSUNBO ORIMADEGUN ABDULGANIY _____ Date

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ABSTRACT

Extracts from various parts of *Annona senegalensis* plant , stem bark of *Enantia chlorantha* and *Okoubaka aubrevellei and* leaf of *Calotropis procera* and *mitracapus scabra* were screened for *trypanocidal* activity in *albino mice* and white rats infected with *Trypanosoma brucei brucei.* Both the crude and the partially purified forms of aqeous extract of *Annona senegalenisis* leaf satisfactorily cleared *trypanosomes* from the systems of the experimentally infected animals at the dose of 200mgkg^{-1} body weight. There was also no sign of the presence of parasites in the *cerebrospinal* fluid of the treated mice. The treated animals survived for more than five months post treatment. Acute toxicity study showed that the extract is non-toxic. Phytochemical screening revealed the presence of *Tannin, Saponin* and *phlobatannin.* The aqeous and alcoholic extracts of the root bark and the stem bark showed no appreciable trypanocidal activity even at a high dose of 350mg kg^{-1} body weight. *Enantia chlorantha* at a dose of 100mg kg^{-1} body weight brought a very high level of parasitemia load down considerably. However, the parasitemia later rose up again and killed the animals two weeks post treatment. The remaining plants screened showed no trypanocidal activities at the doses used.

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CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

State of health and ill health (or disease) are parts of what man, animal and plant pass through in their life time. The World Health Organization (WHO) defines health as a state of complete physical, mental and social well being. But from a strictly biochemical view point, health may be considered as that situation in which all of many thousands of intra and extracellular reactions that occur in the body are proceeding at a rate commensurate with its maximal survival in the physiological state (Robert, 2000).

Conversely, any continuing disturbance in the structure and function of a bodily organ that causes damage is a disease (Stenesh, 1975).

Parasitic protozoa are responsible for some of the most devastating and prevalent diseases of human and domestic animals *(Borst and Ovellette, 1995).* Malaria (*plasmodium species*), the various forms of *(muco)* cutaneous and visceral, Leishamiasis *(Leishmania species),* African sleeping sickness *(Trypanosoma brucei gambiense, Trypanosoma brucei Rhodesiense),* south-America's charged disease *(Trypanosoma cruzi),* amoebic dysentary *(entamoeba species)* and *toxoplasmosis (Toxoplasma species)* are serious diseases that threaten the lives of nearly one quarter of the human population world wide *(Borst and Ovellette, 1995)* . Protozoan parasites also result in enormous losses of life and productivity of domesticated animals; both mammals and fowl. Economic and social conditions such as in adequate sanitation, Ignorance and poverty may encourage the spread of parasitic diseases *(Togboto and Townson, 2001)*

Trypanosomiasis is a parasitic disease prevailing in both human and his domestic animals. It is caused by a single-cell parasite, *trypanosoma species*, and is mainly transmitted by insect vectors *(tsetse fly in African trypanosomiasis and heamatophagous insects of the genus Triatoma in American trypanosomiasis).* Other modes of transmission of *trypanosomiasis* include blood transfusion, coitus (Molyneux and Ashford,1993), congenital infection, organ transplantation, accidental infection and or a transmission during breast feeding (Maria, 1992). Over 50 million people and 25 million live stock in Africa and several other people in South America are at risk of acquiring the infection with pathogenic trypanosome (Yoshisada et al, 1998).

Until recently effective chemotherapeutic drugs for treatment against human trypanosome infection have been *pentamidine, suramin* and *melarsoprol* while recently *diminazene aceturate, isometamedion chloride* and *difluoromethyl ornithine* (DFMO *eflornithine*) are available for treating trypanosomiasis in domestic animals these drug are beset with different kinds of problems ranging from drug resistance, toxicity and adverse side effects. Moreover, there is no available trypanocidal drug that can be used routinely for the prevention of the disease during blood transfusion (Andrew et al, 1993). These inherent problems associated with current *trypanocides* make the sourcing for better *trypanocides* mandatory.

Plant materials have been in use for the treatment and prevention of diseases and it is from plant materials that most of today's orthodox medicine emanated. Example of such drugs are *quinine* and *artemisine* for the treatment of malaria.

Annona senegalensis root was reported to have *trypanocidal* activity (Igweh and Onabanjo, 1989) and it was in corroboration of the claim of trypanocidal potential. of the plant made by the traditional people. No person however has reported the

chemotherapeutic potential of *Annona senegalensis* leaf extract in the treatment of *trypanosomiasis.*

Enantia chlorantha on the other hand is a well known plant in different parts of Africa where is has been used to cure many diseases. (Onabanjo and Agbaje 1991) reported the chemotherapeutic effect of *Enantia chlorantha* in the treatment of malaria.

Calotropis procera was reported to contain *calotropain* that have proteolytic and antimicrobial activities against *Escherichia* coli, *neisaria gonorheoa* etc.

Mitrocarpus scabra is popularly known *to* have been used *in the* treatment *of* skin *infection while Okoubaka aubrevillei is a* herbicide that kills any *tree* near it. It is however not reported to have chemotherapeutic properly.

1.2 LITERATURE REVIEW

1.2.1 THE PARASITE (*TRYPANOSOME*)

Trypanosomes are parasitic protozoa that cause several serious diseases of humans and domestic animals in tropical Africa and South America (Fairlamb, 1982). The first pathogenic trypanosome (*trypanosoma evansi*) was discovered in camels in India in 1880 (Borst, 1997), Not all trypanosomes are pathogenic, example of non pathogenic *trypanosomes* is the crocodile *trypanosome T. gray* (Molyneux and Ashford, 1983).

Trypanosomes are very variable in size from 10µm to over 1200µm depending on the species and subgenus.

1.2.2 TAXONOMY AND NOMENCLATURE

The protozoans that cause sleeping sickness belong to the genus *Trypanosoma*; subgenus *Trypanozoon*. They are classified in the phylum *sacomastig phora*, the order *kinetoplastida*, and the family *trypanosomatidae* (WHO, 1998A).

Depending on the mode of transmission by the insect vector, the genus *Trypanosomes* (order *kinetoplasida*) is generally subdivided into two main groups: the *STECORARIA* and *SALIVARIA*. The salivaria is the group whose mode of transimission to the vertebrate hosts is by the bite of a vector while the transmission of the stercoraria is by the contermination of the skin or mucous membrane by faecal material containing infective metacyclic trypanosomes (Molyneux and Ashford, 1983).

Salivarian trypanosomes cause African sleeping sickness in human*(trypanosoma rhodesciense and Trypanosoma gambiense) nagana* in cattle *(trypanosoma brucei, trypanosoma vivax and Trypanosoma congolense) surra* in horses and camel *(Trypanosoma evansi) and dourine* in horses *(Trypanosoma equipardum* (Fairlamb, 1982).

Example of *stercoraria* parasite is *trapanosoma cruzi,* which causes *chaga* disease in man and animal (Molyneux and Ashford, 1983).

1.2.3 MORPHOLOGY

Trypanosomes are very variable in size 10μm to over 1200μm, but generally these spindle shaped organism are 15 - 100μm depending on species and subgenus. The characteristics morphological forms are *trypomastigotes* - typically found in the blood and tissue of vertebrate hosts and the *epimastigote* forms which are found in the invertebrate vectors. Other configuration of morphological forms are found; such

as *amastigote* and more rarely *promastigotes*. The infective forms which are produced in the vector are known as the metacyclic and they are *trypomastigote* in configuration (see figure 1.1).

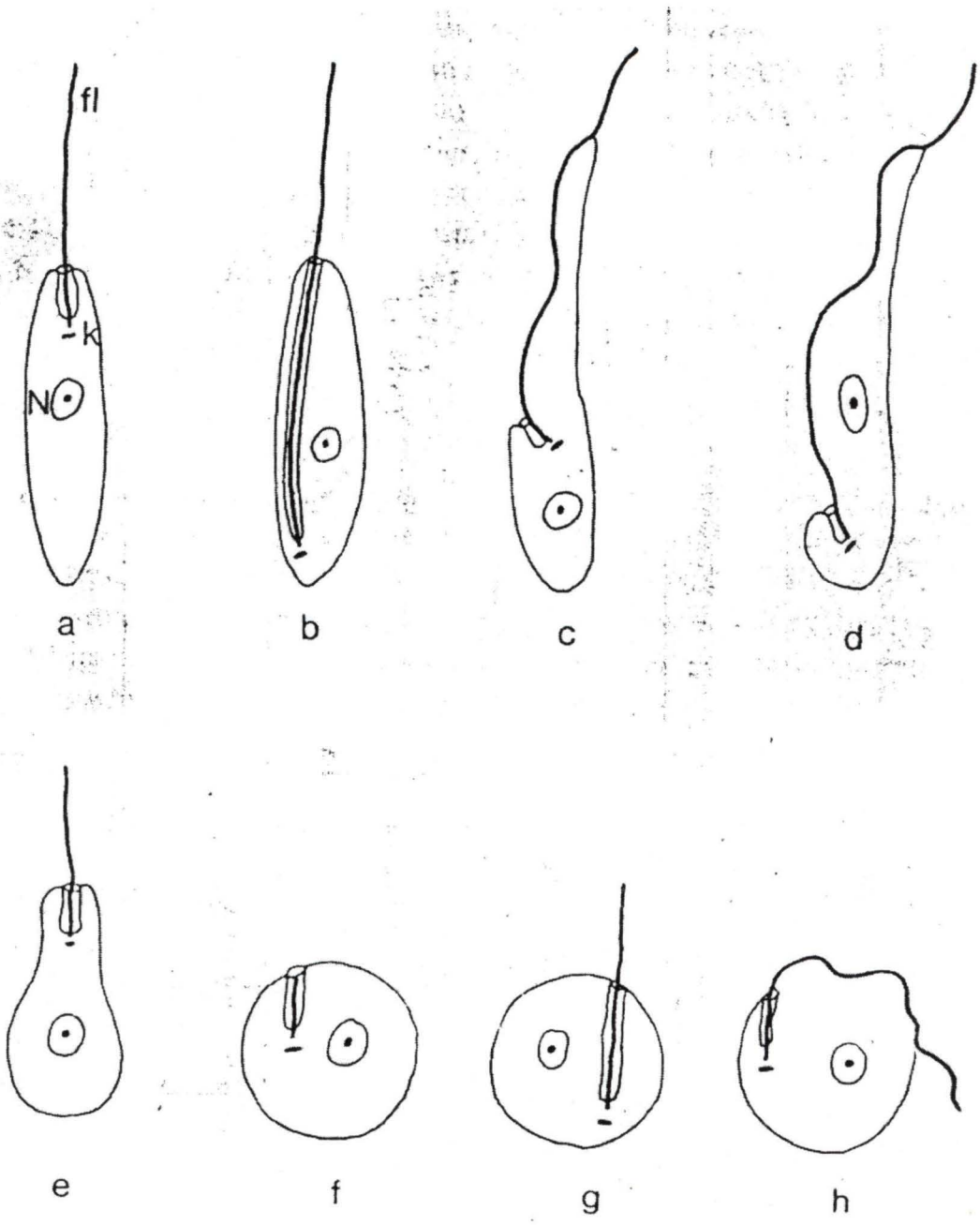


Fig 1.1 Diagramatic configuration of morphological types of Trypanomastidae

a. Promastigote
b. Opistomastigote
c. Epimastigote
d. Trypomastigote
e. Choanomastigote
f. Amastigote
g. Paramastigote
h. Sphaeromastigote

Abbreviations: fl = flagellum; N = nucleus; k = kinetoplast

Taken from Molyneux and Ashford (1983)

Trypanosomes are characterized by five main structural features: a single flagellum, a single often highly branched mitochondrium, which contains a dense network of mitrochondrial DNA (kinetoplast), a complex skeletal array of sub pellicular microtubules and highly unusual micro body - like organelles (*glycosomes*) which contains the first nine enzymes of glycolysis (Fairlamb, 1982) (see figure 1.2)

Trypanosoma b. rhodesiense

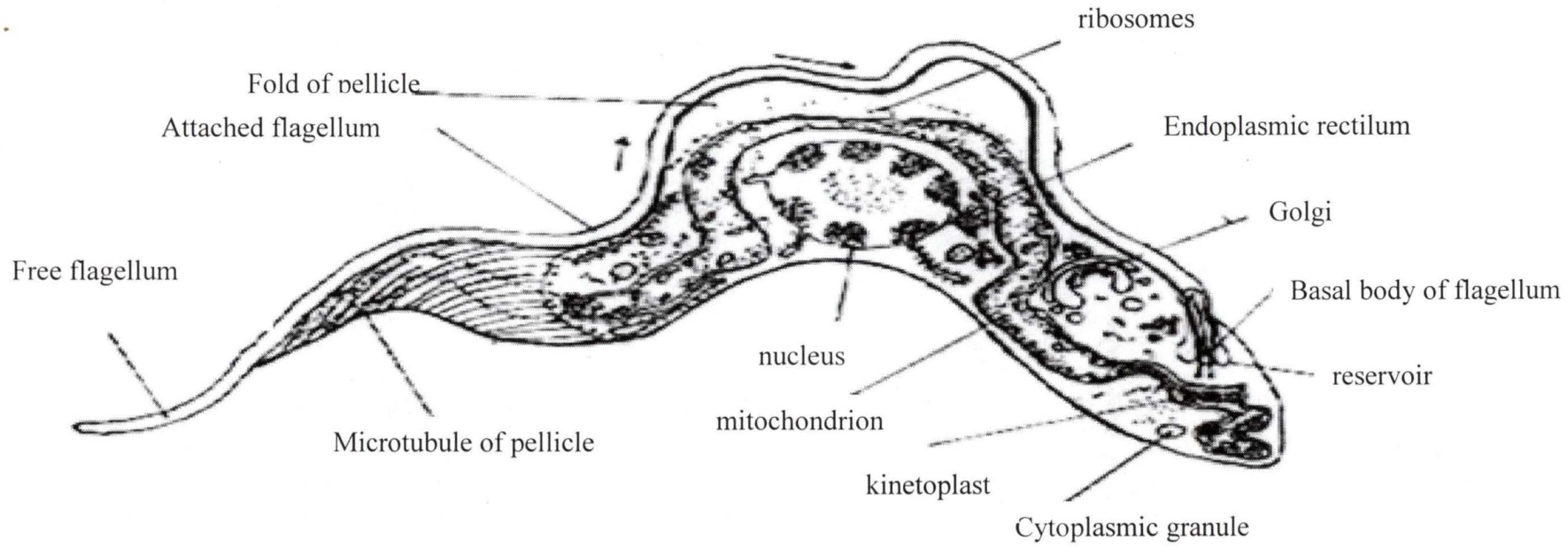


Fig 1.2 Diagram of trypanomastigote intermediate bloodstream form of *Trypanosoma b. rhodensiense* as seen at the level of the electron microscope. The arrows along the flagellum indicate the direction of travel of flagellar waves.
Courtesy of George Allen & Unwin, Col. H.W. Mulligan and Prof K. Vickerman

1.2.4 LIFE CYCLE

T. brucei is cyclically transmitted from mammal to mammal by the insect vector tsetse fly (figure1.3). Different developmental stages of the life cycle have markedly different morphology and biochemical activities.

When an infected tsetse fly bites an uninfected human or animal, metacyclic forms are infected with the fly's salivary secretion. These forms develop into trypomastegotes forms and migrate to the blood stream. Blood stream trypomastigotes show considerable variation in morphology (pleomorphism) ranging from slender to stumpy forms. Stumpy forms are non-dividing and are thought to be a pre adaption necessary for survival in the insect mid gut. After a blood meal from an infected animal, short stumpy forms develop into procyclic trypomastigotes which ultimately migrate to the fly's salivary gland to complete the cycle (see figure1. 4A).

1.2.5 BIOCHEMISTRY AND MOLECULAR BIOLOGY.

Since conventional approaches to the control of trypanosomiasis have largely met with failure, there has been a renewed interest in identifying novel aspects of biology, biochemistry and molecular biology of trypanosomes that might be exploited to develop new targets for vaccines or chemotherapy

For a thorough in-depth research on the biochemistry of trypanosomiasis, it is pertinent one knows much on trypanosome's kinetoplast DNA, Electron transport, intermediary metabolism and life cycle of the parasite.

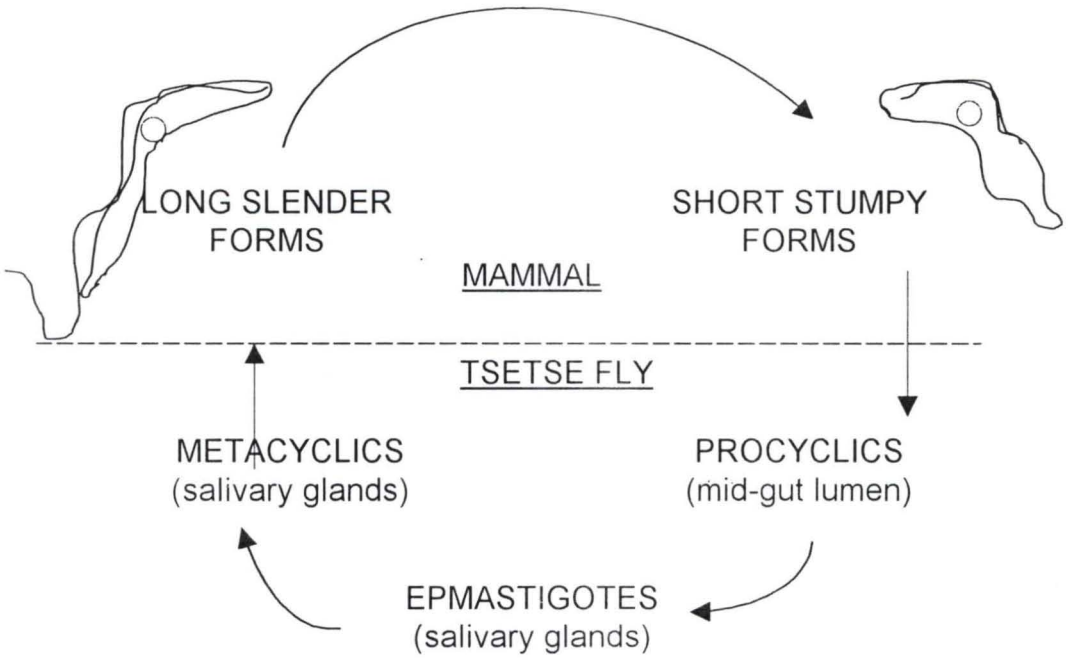


Fig.1.3 life cycle of the Salivarian Trypanosome, (T. brucei)

Courtesy of Fairlamb, (1982)

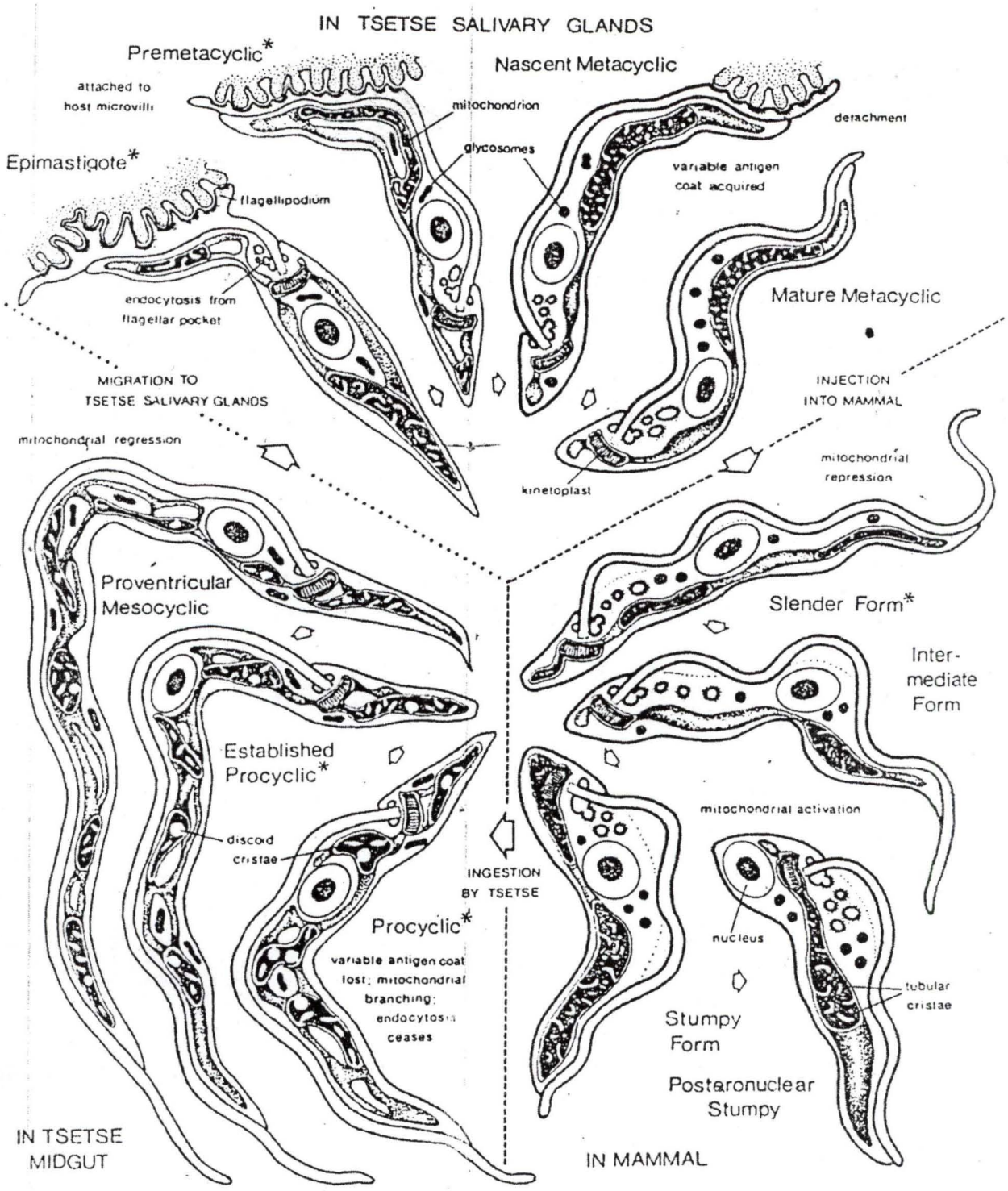


Fig 1.4a Schematic diagram of *Trypanosoma brucei* developmental cycle in mammal and tse-tse fly, showing changes in cell surface, mitochondrion, *glycosomes* and receptor mediated *endocytosis*, also in relative size of different stages. Stages possessing the variable antigen coat lie to the right, uncoated stages to the left. The mitochondrion is depicted partly in section to show changes in its cistae. The posteronuclear stumpy (bottom right) is included as an example of a form produced by some stocks but not playing an essential part in the cycle.

Taken from Vickerman (1985)

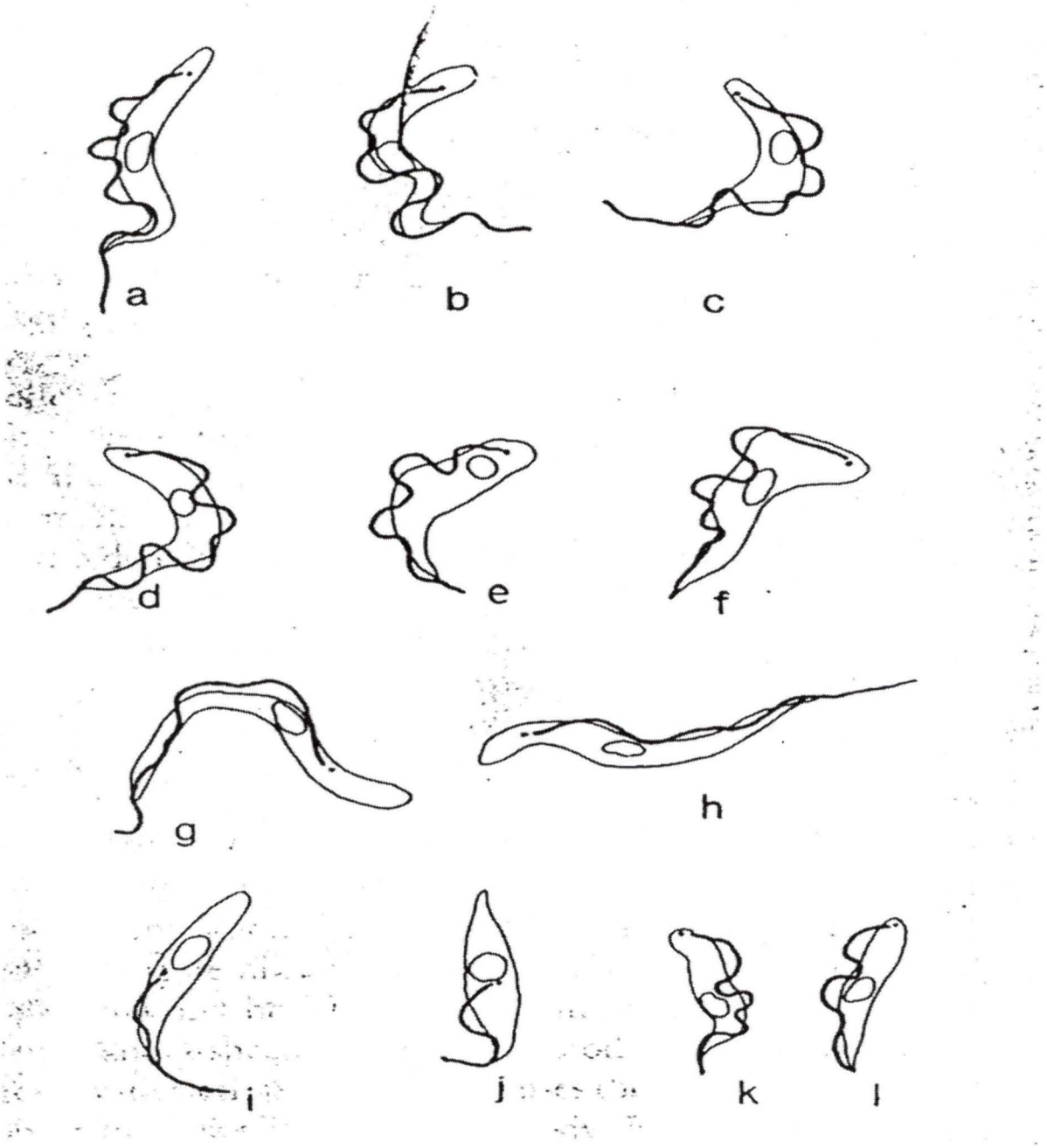


Fig 1.4b (Simplified form of Fig 1.4a) Morphological forms of *T. brucei* and other *Trypanozoon* species

a-c = Long slender forms of subgenus *Trypanozoon* e.g. *T.b. brucei, T.b. gambiense* and *T.b. Rhodesiense*

d-e = *Intermediate forms of* T. brucei

f = stumpy form of *T. brucei*

g-h = *Trypomastigote procyclic* form from the midgut of *Glossina*

i-j =*Epimastigote* from the salivary gland

Taken from Molyneux and Ashford (1983)

1.2.5.1 KINETOPLAST DNA (K DNA).

A characteristic feature of all members of the order, *kinetoplasidae* (of which the trypanosomes belong) is the presence of a single mitochondrion which contains large quantities of DNA, kinetoplast DNA (K DNA), which is usually localized in the part of the mitichondrion adjacent to the base of the flagellum (Hajduk et al, 1992).

Unlike any other DNA in nature, KDNA is organized into a network containing several thousand topologically interlocked DNA circles{ Shapiro and Englund, 1995). The major component of the K DNA network, representing more than 95% of the mass of the network is the minicircle. There are approximately 5,000-10,000 mini circle per K DNA network. As theirs name implies, minicircles are small, covalently closed circular DNA molecules which range in size from about 0.7kb in T. vivax to 2.5 kb in crithidia fasciculata {C .fasciculata) . The size of the minicircles within a given network, however, is constant. Minicircles are generally heterogenous in DNA sequence with the K DNA of T. brucei having the highest degree of heterogeneity with up to 300 different sequence classes per network (Hajduk et al,1992). This indicates that the genetic complexity of the minicircle genome couid be as high as 3×10^5 base pairs.

The other component of the K DNA network is termed the maxicircle. Maxi circles are present at about 50 copies per net work and range in size from about 20 kb in T. brucei to 40 kb in C. fasciculata. The maxicircles are structurally and functionally analogous to the mitochondria DNA of most other eukayotes. Maxi circles encode the mitochondrial ribosomal RNA'S subunits on NADH dehydrogenase (subunits 1, 4, 5 & 7), cytochrome b, sub unit of cytochrome oxidase (Subunits; I, II, and III), a subunit of mitochondrial ATPase (sub unit 6) and several other unidentified

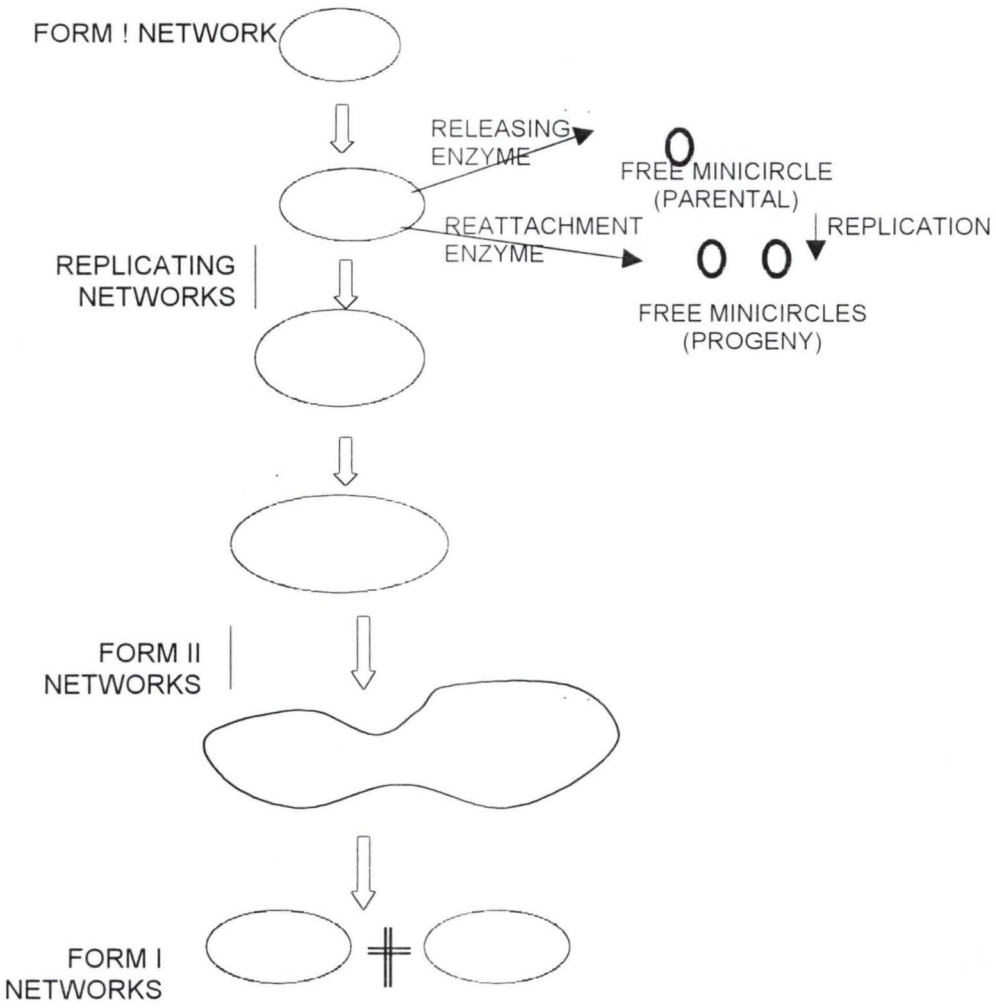


Fig 1.5 Model for replication of kinetoplast DNA network. The stippled areas represent nicked minicircles.

Fairlamb, (1992).

transcripts (Hajduk et al ,1992) However, the expression of maxicircle protein coding genes is unexpectedly complex. Their transcripts undergo RNA editing, a process in which uridine residues are added to or deleted from precise positions in the transcript, to create the open reading frame.

In contrast, the genetic function of minicircles is that they encode small guide RNAs which control the specificity of RNA editing (Sturm and Simpson, 1990).

1.2.5.2 KINETOPLAST REPLICATION

In contrast to replication of mitochondria DNA in mammalian cells which occurs throughout the entire cell cycle (Clayton ,1991), K DNA replication occurs during a distinct S phase approximately coincident with that of the nucleus {Clayton,1991} . During S phase, there is replication of each individual minicircle and maxicircle. The progeny circles are ultimately distributed into two daughter cells at the time of cell division (see fig.1.5).

1.2.5.3 MINICIRCLE REPLICATION

Replication begins soon after the covalently closed minicircle is decantenated from the network and is thought to occur in one of the two complexes of replication protein. Minicircle replication occurs via Q-intermediates and is unidirectional (Englund, 1979). Leading strand synthesis initiate complimentary to the universal mini circle sequence GGGGTTGGTGTA, a 12 mer found in a conserved region of mini circles from nearly all *trypanosomatids* that have been examined. The leading strand has one or more ribonucleotides at its 5` end, indicating that DNA synthesis is initiated by a primase (Ntambi et al, 1986). A second sequence, A C G CCC which is less well conserved, is located 70 - 90 nucleotides down stream from the 12 - mer; the first Okazaki fragment initiated complimentary to this 6-mer. In both *C. fasciculata* and *T. equiperdum,* the leading strand is synthesized continuously around the molecule and the lagging strand *Okazaki fragment* are roughly 100 *nucleotides* in size (Ryan and Englund, 1989) .

1.2.5.4 MAXICIRCLE REPLICATION

The current electron microscopy studies indicate that the maxicircle replication initiates in the variable region, a non-coding segment of the molecules which contains many representative sequences. This region in T. brucei contains low copies of the GGGTTGGTGT sequence found at the minicircle replication origin. If similar sequence exists in this region of C. fasciculata maxicircles and if they serve as replication origin, then the mechanism of maxicircle replication might be very similar to that of minicircles.

Minicircles and maxicircles replicate simultaneously and both duplication unidirectionaly as Q-structure. If they initiate at similar sequences, the factor which

controls initiation would probably be the same. The major difference is that mini circles replicate free of the network whereas maxicircle replicate while still linked to it (Hajduk et al ,1984).

1.2.6 MITOCHONDRIAL RNA PROCESSING. (RNA EDITING)

RNA editing is the term used to describe a variety of RNA modifications in which the coding information of mRNAs is changed from that encoded by the gene. The first examples of RNA editing were described in the mitochondrial mRNAs of T. brucei (Benne et. al, 1986). The editing of trypanosome mRNAs involves the addition and deletion of uracil residues in at least seven T. brucei mitochondrial mRNA. These addition and deletions are always uridines and are functionally significant in that they lead to the correction of frame shifts coding sequences of genes, formation of initiation codons for translation, and in several extreme cases, the formation of entire mRNA reading frames.

MODELS FOR RNA EDITING

Several models have been proposed for RNA editing in trypanosome mitochondria. The first is the enzymatic cascade in which the mRNA and gRNA form a complex that is recognized and cleaved by an editing site endo ribonuclease. A single U is either inserted or deleted by the action of a terminal uridyl transferase(TuTase). Addition or deletion is directed by the base pairing of the gRNA with the mRNA and the cleaved mRNA is joined by the action of an RNA ligase. *This model is supported by the identification of an editing site specific endonuclease,an RNA* ligase, *and a* TuTase *in T. brucei mitochondria (Hajduk et al,1992).*

The second model proposed for the insertion and deletion of uridines involves a series of paired trans esterification. This uridines could be donated to the editing site by a poly U tail either from the gRNA or other mitochondria transcripts. Conversely, deletion of uridines could be accomplished with the gRNA acting as an acceptor for the deleted uridine. This proposed model would not require an *endonuclease, TuTase* or ligase for editing. It does requires the interaction of gRNA with pre-edited uridine {Hajduk et al, 1992). The wide distribution of editing in the pathogenic *kinetoplastids* and the apparently unique nature of the editing process in *trypanosomes* make it an appealing target for the development of specifically targeted drugs.

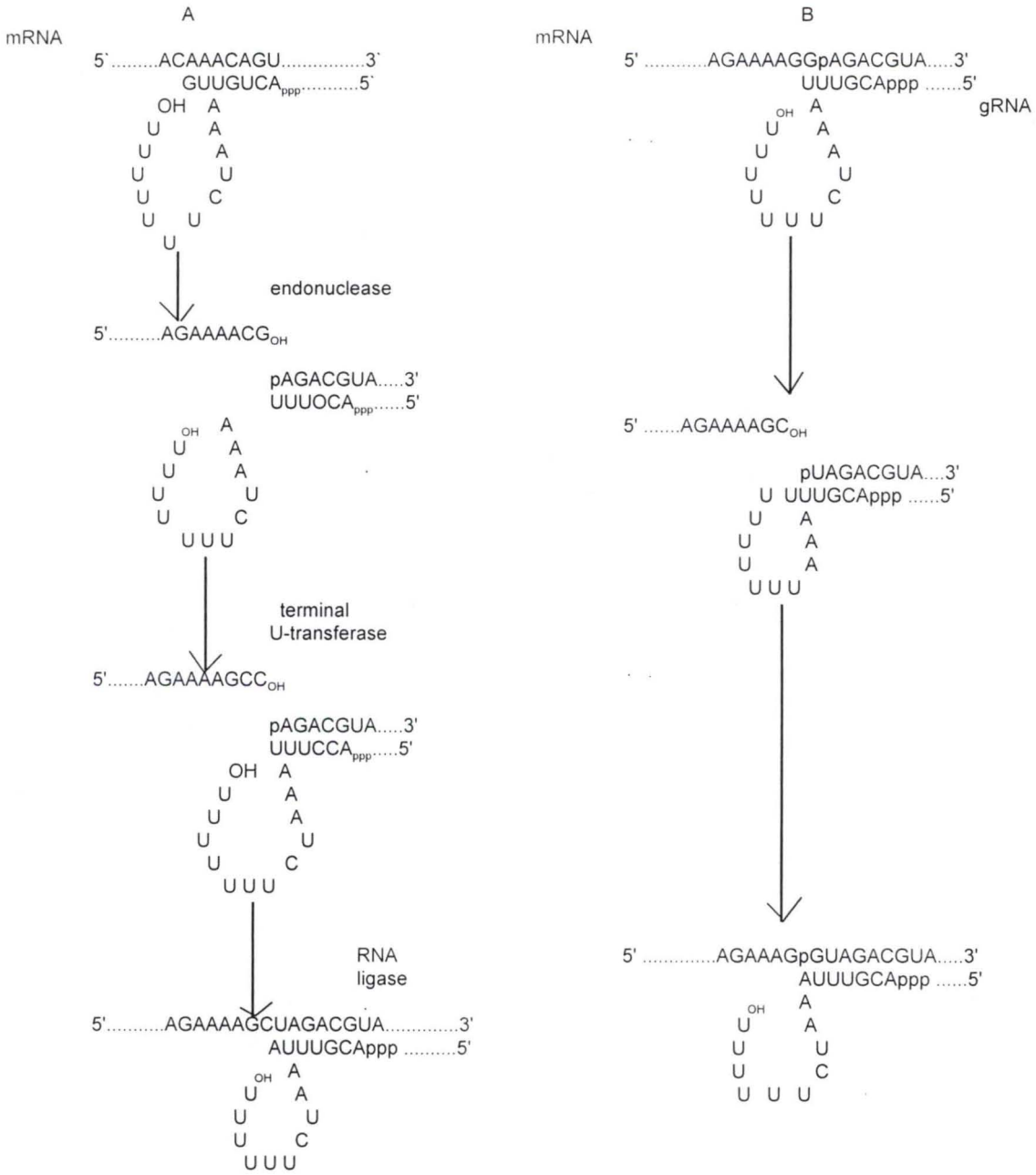


figure1.6: Two models for RNA editing in *trypanosome* mitochondira.

(A) Enzymatic cascade model proposes that the sequential action of a site specific *endoribonuclease*, terminal *uridyl transferase Tutase*), and RNA ligase leads to the addition or deletion of uridines.

(B) The transesterification model proposes paired reactions, the first mediated by the 3'-OH of the gRNA poly-U tail and the editing site on the pre-edited mRNA Hajduk et al, (1992)

to form a chimeric gRNA/mRNA molecule; the second mediated by the 3'-OH of the 5' cleavage product rejoining the two parts of the mRNA with a single uridine added at the editing site.

TRANS-SPLICING OF NUCLEAR PRE-mRNA.

Trans-splicing is an intermolecular RNA processing reaction in which exons from two separate RNA molecules are joined together to form a mature species. In one form of trans-splicing, the partners of this reaction are the pre-mRNA and small RNA, the spliced lender (SL) RNA. The SL sequence which represents the 5` end of the SL RNA is joined to the 3 spliced site located just at the boundary of the mature mRNA within the polycistronic pre mRNA. The joining of two exons located on the same pre -mRNA can be considered a cls -splicing reaction (Boothryd and Cross,1982). African *trypanosome* and other members of the order *kintoplastidae* do not appear to have intervening sequences within coding regions of genes and, thus do not cis-splice their mRNAs. However, every T. brucei mRNA is composed of two exons; they are encoded by separate genes (Hajduk et al, 1992). The first exon is identical for all mRNAs containing 39 nucleotides of non coding leader sequence called the spliced leader (SL) or the mini exon. In T. brucei, the mini exon is transcribed from a tandem array of approximately 200, 1.35 kb SL genes to form a 140 nucleotide mini exon derived RNA (med-RNA) or spliced leader RNA (SL - RNA). The exon makes up the 39 nucleotides of the SL-RNA. The SL is joined to the pre-mRNA by an intermolecular or trans-splicing mechanism.

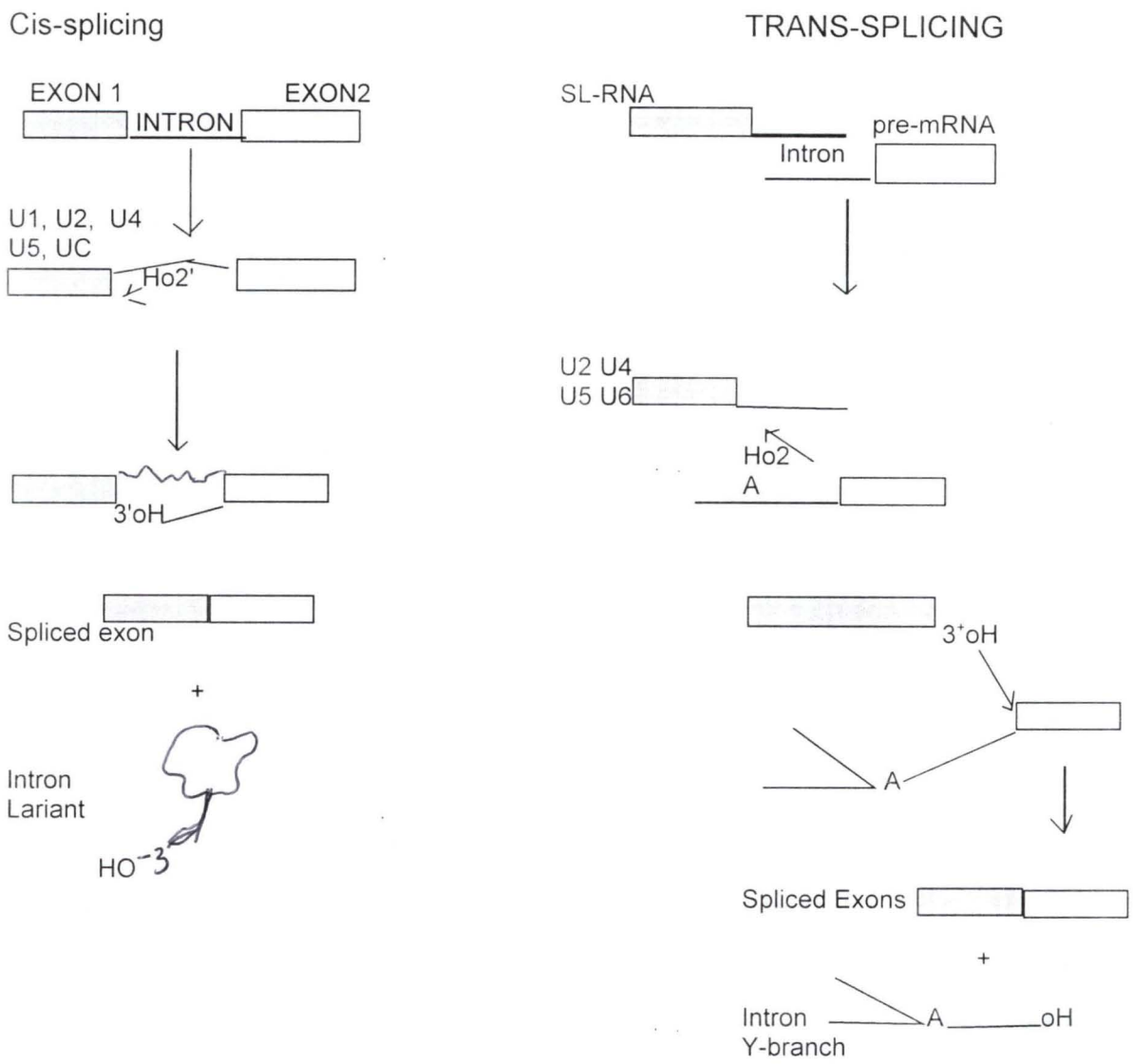


Figure1.7 Trans and cis - splicing are fundamentally similar processes with a slight difference. (Hajduk *et* al, 1992).

FUNCTIONS OF TRANS-SPLICING IN TRYPANOSOME

It has been suggested that the function of trans-splicing might be to add the capped SL to the 5` end of trypanosomes mRNA. The 5` cap is required for several important cellular processes including transport of mRNA from the nucleus to the cytoplasm, stability of mRNA and translation of mRNAs. Thus, trans-splicing of SL-RNA and pre-mRNA might be an indispensable event in trypanosomes. This offers the intriguing possibility of specifically inhibiting several essential pathways in

trypanosomes by either blocking trans-splicing or destroying the SL- RNA (Hajduk, et al 1992).

1.2.7. **ELECTRON TRANSPORT**

One striking feature of blood stream *T. brucei i*s that respiration is completely insensitive to inhibition by cyanide. These forms contain no cytochromes or haem. NADH generated during glycolyisis is reoxidize by means of the glycerophosphate shuttle involving the concerted action of glycosomes NAD^+ dependent glycerophosphate oxidase. Electron transport does not appear to be coupled to oxidase phosphorylation. The terminal oxidase component catalyses a four electron reduction of oxygen to water, and can be inhibited by a number of metal chelating compounds notably salicylhydroxamic acid (SHAM) but not cyanide (Fairlamb,1982). For the shuttle. (See Fig.1. 8)

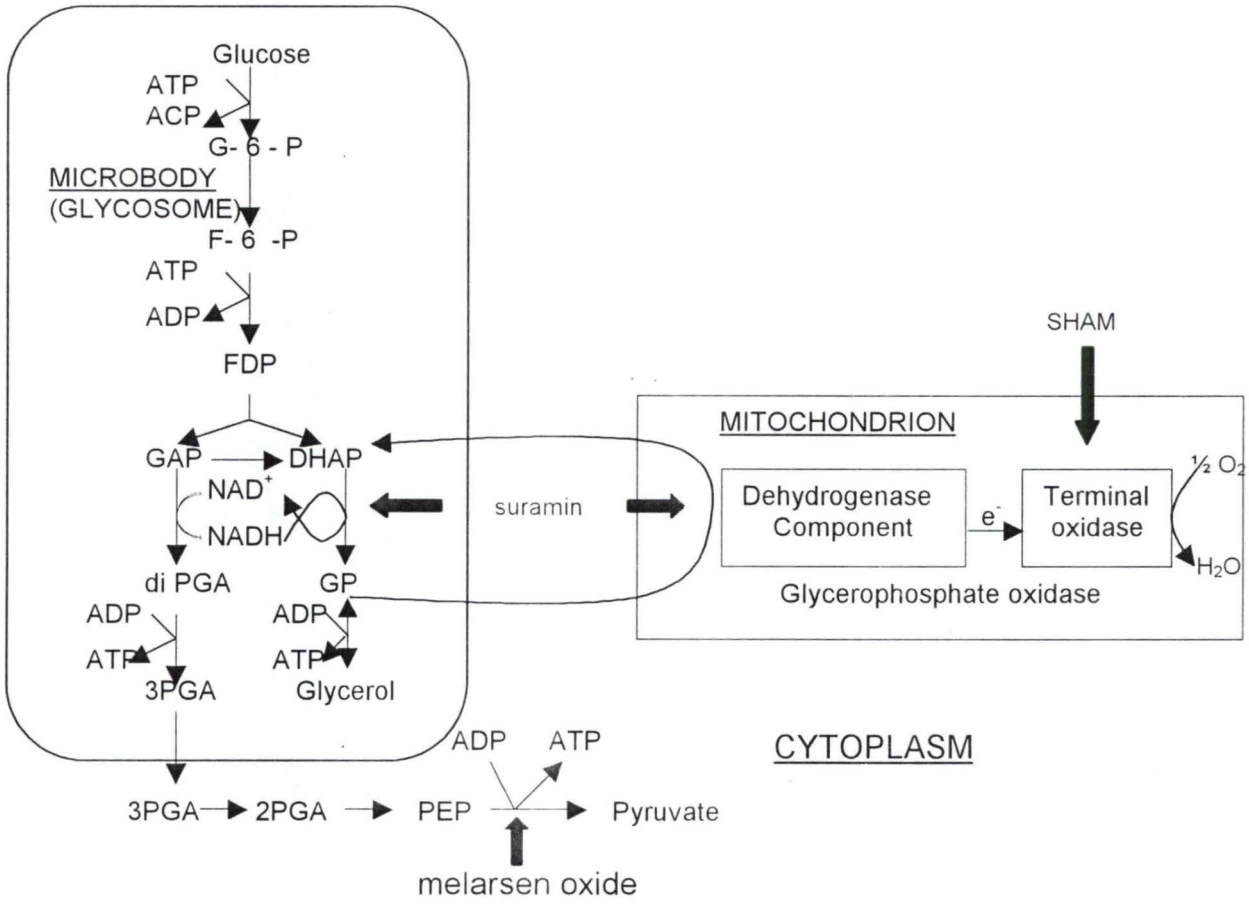


Figure 1.8 Glycolytic pathway in long-slender bloodstream forms of T. brucei. The principal sites of inhibition by trypanocidal drugs invivo indicated by bold arrows. Abbreviations: SHAM, salicylhydroxamic acid; G-6-P. glucose-6-phosphate; F-6-P, fructose-6-phosphate; FDP, fructose –1, 6-diphosphate; GAP, glyceraldehydes-3-phosphate; DHAP, dihydroxyacetone phosphate, GP glycerol-3-phosphat; diPGA, 1-3- diphosphoglycerate; 3PGA and 2PGA, 3- and 2-phosphoglycerate respectively, PEP, phosphoenol-pyruvate.
(Fairlamb, 1982).

1.2.8 **INTERMEDIARY METABOLISM**

In the mammals, blood stream forms of *T. brucei* are entirely dependent on glucose for energy supply. These forms do not have energy reserves and do not oxidize fatty acids or amino acids. The long- slender forms metabolize glucose aerobically exclusively to *pyruvate* since they lack both *lactate dehydrogenase* and tricarboxylic acid (TCA) cycle enzymes. Under anaerobic conditions or when the glycerophosphate oxidase is inhibited by SHAM, glucose is metabolized at the same rate as aerobically forming equimolar pyruvate and glycerol. For each mole of triose phosphate converted to pyruvate, a mole of NAD^+ is reduced to NADH which is in turn is re oxidized to a mole of DHAP to glycerophosphate. Glycerophosphate is then converted to glycerol under the catalysis of glycerol kinase. This pathway is unique to blood stream African trypanosomes and not found in other *trypanosomatids* (Fairlamb, 1982)

1.2.9s **ENERGY PRODUCTION**

Blood stream *trypanosoma brucei* possesses an extremely high rate of glycolysis and under aerobic conditions, blood stream *trypanosomes* should obtain a net gain of 2ATP/mole of glucose used. Classically, one would expect that under anaerobic condition, the net energy gain would be zero. However, another studies have shown that ATP can be produced anaerobically from glycerophosphate and ADP under the catalysis of the highly active glycosomal glyceral kinase. Thus the *glycosome* is capable of carrying out the following thermodynamically favourable sequence of reaction

Glucose + pi → 3PGA + glycerol ΔG^x = -31KJ/mol.

1.3 VARIABLE SURFACE GLYCOPROTEIN AND ANTIGENIC VARIATION

As parasites evolve, they can develop sophisticated means for pursuing their exploitative life styles and no where is this more elaborate than in antigenic variation system of African Trypanosomes.

The surfaces of all blood stream stages of African trypanosomes is completely covered by a, 15nm thick coat (Hajduk et al, 1992). This coat is known as variant antigen. This variant antigen has been shown to be a *glylcoprotein* and its location on the surface of the blood stream trypanosomes led to its description as the *variant surface glycoprotein* (V.S.G) or *variant specific surface antigen*, (Molyneux and Ashford, 1983). Functionally, this surface coat required by the parasite to evade the host's immune response also prevents complement mediated action of the blood stream trypanosomes. Biochemical analysis indicates that this coat is homogeneous and is composed of about 10^7 glycoprotein molecules. This size of the variable surface glycoprotein (VSG) varies from 50 to 65 kilodaltons (Cross ,1990). Each blood stream *trypanosome* synthesizes a single type of VSG, but this VSG is spontaneously replaced by a different one at a frequency of up to 10^{-2} per generation. the immune response against the VSG is sufficient to kill the majority of parasites after a few days. However, the emergence of new variants allows the propagation of infection through the development of new waves of parasitemia. Thus the continuos interplay between antigenic variation of the parasite and antibody response of the host maintain a relatively constant number of trypanosome for long period in the blood stream(pays et al,1994).

The constant replacement of one VSG by another is accomplished by selective expression of one out of approximately 1000genes, approximately one in every 10^6

trypanosomes undergoes antigenic variation during cell doubling time. This change of VSG composition occurs spontaneously without induction by host's immune system or other factors in the bloodstream on the Mammalia host

When the trypanosome enters the mid gut of the tsetse or cultured at 26^{0}c, the VSG coat is rapidly replaced by another abundant surface glycoprotein called either procylin or procyclic acidic repititive protein (PARP)(Roditi et. al1989) PARP is a single glycoprotein species of 15,000 Da which cover the surface of the procyclic trypanosome *and in also attached to the plasma membrane by a c-terminal GPI anchor. The function of PARP is completely unknown.*

1.3.1 CHROMOSOME STRUCTURE AND GENE ORGANIZATION

T. brucei nuclear genome is organized into at least eleven diploid megabase chromosome (1-6Mb), variable number of intermediate-sized chromosome of indeterminate ploidy (200-900kb) and 50-100 mini chromosome (25-100kb)(Melville and Masiga, 2001). The chromosome number does not change in the blood stream and the procyclic development stages of *T.brucei* and these cells are diploid for most genes. Multiplication is mainly asexual by simple binary fission, however, sexual reproduction occurs at an undefined stage in the insect vector (Hajduk et al,1992). The termini of both the large chromosomes and the minichromosomes have the same telomeric repeat sequence $(GGGTTA)_n$ Functionally, the large chromosomes contain most of the coding information of the genome while the minichromosomes have been found to be mainly composed of tandem array of 177bp repeat (> 90% of the sequence), GC rich direct repeats separated by a bent helical A -T rich region and a usually a VSG gene. Minichromosomes are only found in trypanosomes species which undergo antigenic variation, raising the possibility that minichromosome might

have a role in antigenic variation. While most of the minichromosomes have a VSG gene, these are not transcribed (Hajduk et al 1992). It appears that the function of the minichromosomal VSG genes is to serve as a source of VSG gene for translocation to expression sites at the teleomeres of the large chromosome.

Most megabase chromosomes differ in size from their homologues by up to 15%, but homologous chromosomes in different stocks vary more - considerably more than reported in other organism (Melville and Masiga, 2001).

With the exception of some variant glycoprotein genes (VSGs) most expressed genes are located in the megabase chromosomes. These are inherited in a Mendelian fashion (Turner et al, 1990). Many genes are expressed in polycistronic transcripts and some chromosomes ends carry VSG expression sites but only one is active at one time, resulting in a uniform surface protein coat. Gene- or promoter - swtsiching result in antigenic variation. The intermediate chromosome also carry expression site and most mini-chromosome contain non-transcribed and simple repeat sequence {Weiden et. al, 1991).

1.3.2 EXPRESSED SEQUENCE TAGS { E S T_S}

Rapid gene discovery was achieved in the early phase of the genome project by sequencing of randomly selected cDNA clones. There are 5133 *T. brucel* ESTs in the public data bases from four cloned stocks of *T. brucei* (Melville and Masiga,2001) most of EST sequence were generated from cDNA clones of blood stream from mRNA. In addition to discovery of many novel *T.brucel* genes, the ESTs have provided a rich sources of makers and acid sequence annotation (Melville et al, 1998)

1.3.3 GENE SURVEY SEQUENCES (GSSs)

Sequencing of random, short pieces of DNA(GSSs) of both ends of almost 2500 clones (almost 50,000 short sequence) provided a total of 29mb of the TREU927 genome. A proportion of these sequence derive from the ends of large genomic clones ands these contribute to the mapping and sequence of whole chromosome (excepting the minichromosome}{Melville et al,1998}

1.4 *TRYPANSOMIASIS*

Trypanosomiasis is a severe, often fatal disease that occurs in parts of Africa and south American. African trypanosomiasis is an ancient disease of man and his domesticated animals. The disease, also known as sleeping sickness is caused by African trypanosomes (protozoan parasites)

Two subspieces of *trypanosoma brucei* are the causative agents of the human disease, African sleeping sickness(Hajduk et. al,1990). They are *T.brucei gambiense* and *T.brucei rhodesience*. *T.b rhodesience* is mainly found in eastern African from Ethiopian and eastern Uganda to zambia and Zimbabwe. A number of wild animals hogs and antelopes can act as reservoirs for *T.brucei rhodesience*. Clinically, the "*Rhodesian*" sleeping sickness is more acute in nature and involvement of central nervous system occurs earlier than in the"Gambian" form of the disease caused by *T. brucei gambiense* (Hajduk et al,1990)

T.brucei gambianse occurs mainly in western and northern region of sub-saharan Africa from Senegal on the west coast through western African as far east as the Sudan and Uganda. Gambian sleeping sickness is generally chronic inform and the initial phase of the disease , involving the blood and lymphatic tissues, can

persist for the years prior to involvement of the central nervous system (Hajduk et. at ,1990). The reservoir for *T. brucei* Gambians is man and his domesticated pig.

It is estimated that 50million people in 36 sub-saharan countries are at risk to human sleeping sickness and there are approximately 50,000 new cases reported annually (WHO, 1998B).

1.4.1 ANIMAL TRYPANOSOMIASIS

Different form of animal trypanosomiasis occur over large areas of Africa, Latin American and the middle east. In tropical African, tsetse transmitted trypanosomiasis is formed over an areas of 10million square kilometers whereas non-tsetse transmitted trypanosomasis occurs in 12 south American countries. The disease is one of the major constrains to agricultural development in the endemic area (Melville et al, 1998).

Animals *trynasomiasis* is cased by different species of *trypanosome* parasites. They include *trypanosoma vivax* , *T. congolense*, *T. evansi*, *T. simiae*, T. equiperdum, T. go*dfreyi* among others. Different sub - species and strains exist and they cause different disease syndrome in different animal species.

1.4.2 AMERICAN TRYPANOSOMIASIS

American trypanosomiasis (Chagas' disease) is a zoonotic infection caused by trypanosoma cruzi .It causes acute, subacute and chronic parasitemia with dissemination to many organs such as the heart, brain, oesophagus and colon . The acute disease can occur at any age and is characterized by fever, *oedema*, *lymphadenopathy* and *spleenomegaly*, *myocarditis*, *meningo - encephalitis* (Maria, 1992).

1.4.3 CLINICAL FEATURES

The *trypanosoma chancre*: This is a swollen, inflamed and indurated lesion which develops at the site of the tsetse fly bite. *Chancres* are usually painful and tender. *Chancres* heal with overlying desquamation. They are substantially more common in *rhodesian trypanasomiasis* than in *gambian trypanosomiasis*

THE *HAEMOLYMPHATIC STAGE* (STAGE1) Follow haemolymphatic spread, period of *febrile illness* are accompanied by severe, progressive headache general *malaise*, *myalgia* and joint pains and progressive *lymphadenopathy*, especially in *T. brucei gambiense* infection (Maria, 1992)

Febrile symptoms may be mild, particularly in *T. brucei gambiense* infection. A transitory *erythmatous* rash is seen in light skinned patients. As the illness progresses, periods of fever become infrequent and in the late stages are unusual. A variety of organ lesions may develop during this phase, including : *myocarditis, spleenomegaly*, *hepatomegaly*, *anaemia*, *peripheral oedema*, *ascites*, *pericardial effusion* and *pulmonary oedema*.

***MENINGO - ENCEPHALITIS (STAGE II)*:**

During the course of infection, trypanosomes become established in the brain and spinal cord and cause a *meningo - encephalitis* which :(a) develops within the first month of infection in *Rhodesian trypanosomiasis* and is rapidly progressive,(b) it also develops slowly in *Gambian trypanosomiasis* and may not be apparent clinically for many months or even years.

Meningo-encephalitis affects the brain stem and diencephalon preferentially;cortical areas are affected only late in the disease process.In addition

to vasculitis and round cell infiltration, which is mainly prevascular,there is progressive neuronal destruction.

Headache is a feature of trypanosomiasis even in the early stage, and become severe and intractable. Sleep disorder appears, notably *diurnal somnolence* and *insomnia*. As the disease progresses, cerebral function and conscious level deteriorate ,and lead eventually to irreversible *coma* (Maria, 1992).

1.4.4 **PATHOGENESIS**

The *lymphoid* system, brain, heart and lungs are the most severally affected. Enlargement of the lymph nodes, spleen and liver is consistently observed in both forms of the disease (WHO, 1998A) but the most significant pathology in the initial stage is increase in the B *lymphocytes* which result in lymph node enlargement (Molyneux and Ashford, 1983). The *meninges* are also infiltrated with lymphocytes and there is a chronic progressive meninges with the invasion of the brain with plasma cells which form characteristic *morula cells* associated with the vessels of the brain, the infiltration of the brain later produces neuronal degradation.

Cerebral and *meningeal oedema* and *punctate haemorrhages* are also typical of second stage of the disease (WHO, 1998A).*Hyperplasia* of *microglia* and reactive *astrocytes* occurs and occasionally a diffuse pallor of the myelin has been described.

The severity of the pathological lesions that arise during the haematolymphatic stage (e.g *thrombocytopenia*, *disseminated intra vascular coagulation*, *anaemia*, *tissue lesions*, immunodepression, IgM and IgG, *hyperglobulinaemia* and immune complex disease)only partly correlates with the level of parasitemia.

High parasitemia leads to the host being exposed to high levels of toxic metabolites, lytic enzymes, immunosuppressive membrane components and other component of trypanosomes that induce unregulatory response. It is generally believed, however, that many cytotoxic and physiopathological processes are the result of indirect biochemical and immunological mechanism.

1.4.5 **DIAGNOSIS**

There is no single clinical diagnostic sign or symptom that can be regarded as satisfactory for sleeping sickness. For accurate diagnosis, therefore it is necessary to obtain laboratory confirmation (WHO, 1998A) .

DETECTION OF THE PARASITE

Conclusive proof of infection rests on finding characteristic *trypanosome* in blood or tissue fluid. In acute infection with *T. brucei rhodesiense, trypanosomes* are found in the peripheral blood and are seen readily in wet preparations or *giemsa* stained blood films.

During the initial phase of infection, trypanosomes may be found in fluid aspirated from *chancres* or enlarged peripheral *lymph nodes.* Aspiration of enlarged posterior cervical node particularly in *T brucei gambiense* infection, may establish the parasitic diagnosis (Maria, 1992). A number of concentration techniques have been developed to increase the frequency of establishing a parasitic diagnosis from blood. These include the *microhaematocrit centrifugation* technique where parasites are concentrated in the buffy coat, and the mini anion exchange column

INDIRECT DIAGNOSIS

Although demonstration of trypanosomes is desirable, their concentration in the patient's body fluid is often below limit of detection. The detection of antibodies, circulating antigens or trypanosomal DNA is suggestive of an infection in such cases.

There are two indirect field tests used for mass screening ; they are; the card agglutination test for trypanosomiasis CATT and the card indirect antigen test for trypanosomiasis CIATT. The former is a serological screening and uses a reagent composed of stained, freeze- dried trypanosomes of selected variable antigen type VATS (WHO, 1998B), while the latter is a rapid and simple to perform test which detects antigens unlike the CATT which detect antibodies. CIATT is sensitive and has a potential for assessing cure following treatment (WHO, 1998B).

LABORATORY TESTS

For practical and logistical reasons, some tests are better suited for use in the laboratory than in the field. These includes antibody tests, namely immuno fluorescence (IF), Indirect haemaglutination (IHA) and the enzyme - linked immunosorbent assay (ELISA) as well as DNA techniques based on antigen detection.

The indirect immunofluorescence test using parasites of subgenus *trypanozoon* as antigen on slides is widely used; blood can be obtained by finger prick and dried on filter paper. The filter paper disc with a phosphate buffered saline (PBS) solution or serum itself are incubated on the slides, which are then washed with PBS and incubated with fluorescein labeled anti -human serum for 30 minutes. Examination is then carried out under a fluorescent microscope and a brilliant fluorescence is considered positive (Molyneux and Ashford, 1983)

Indirect haemaglutination test (IHA) is a test carried out in capillary tube using antigen-sensitized human erythrocytes. This capillary haemaglutination test is developed for human trypanosomiasis and it allows on the spot diagnosis.

Enzyme - linked immunosorbent assay (ELISA) method may be used to measure specific 1gG antibodies levels especially at the chronic stage of the disease. Here, the putative antigen attached to a solid phase (e.g cross - linked dextran or polyacrylamide beads, filter paper (cellulose) e.t.c, any specific antibody molecules bind to the antigen and all other material is washed away. . Exposure of the complex to enzyme- labeled anti- Immunoglobulin antibody results in binding to any specific antibody molecules adsorbed from the original serum. The complex is washed and the substrate for the enzyme added, resulting in activity proportional to the amount of specific antibody in the serum (Sofowora,1986)

1.4.6 EPIDEMIOLOGY

TRANSMISION DYNAMICS :- There are three major ways by which African trypanosomiasis are transmitted to man; these are identified as human - fly- human transmission cycle, fly - human transmission and Animal - fly- man transmission (Molyneux and Ashford, 1983)

The role of the animal reservoir in the transmission of African trypanosomiasis to human is believed to be more important in *T. b. rhodesiense* diseases than in T. b gambiense diseases (WHO, 1998). In *T. b gambiense* disease, the human-fly-human transmission cycle seems to be predominant involving mainly *Glossina palpalis* palpalis (G.p) gambience, *G. Fuscipes fuscipes* and *G. technoides*. Humans are important reservoir, since in the early stage of the disease, infected people can continue their normal activities while the parasite circulate in their blood and remains

available to the vector. Transmission depends on the site, intensity and frequency of contact between the Glossina spp and human.

Endemic T. b Rhodesiense

T. b rhodesiense diseases is generally believed to be zoonotic in nature. *In the endemic situation sporadic cases are found among those members of the population whose activities bring them into contact with the glossina morsitans group, which normally feed on game animals.* Those patients are usually adult males indulging in hunting, honey and bees wax gathering and fishing or who become infected during migration or local travel or who farm on the edge of game and fly - infested bush. This means that the mode of transmission of this type of disease is through wild animal - fly human transmission (Molyneux and Ashford, 1983)

Epidemic T. b. Rodesiense

T. brucei rhodesiense may be transmitted by river rine tsetse flies and under these circumstances, may become epidemic. This has occurred in the area around lake Victoria in Uganda and Kenya where transmission is effected by *G. fuscipes*. An epidemic was reported between 1975 and 1987 when up to 5000 individuals were infected per year (Maria, 1992). The epidemic occurred in settled rural communities and affects all age groups.

1.4.7 FACTORS INFLUENCING TRANSMISSION RATE

Several factors influence the transmission of trypanosomes to human. At the endemic level, those factors are related to the vectors and hosts in the transmission cycle (Glossina, human and animal reservoir).

For Glossina, the factors are vectorial capacity, degree of *anthropophily*, longevity dispersal and density of the fly population (for *T. b rhodesciense*). The other

factors are the place of residence, behavior, and agricultural and water related activities of humans and the nature and importance of the animal reservoir.

At the epidemic level, factors influencing transmission are:

i. Sudden modifications to the environment(such as deforestation)

ii. Ethnic diversity (leading to variation in human behaviour

iii. Mobility and displacements of population including massive population movements; and

iv. Degradation of the economic environment (resulting in changes in human behaviour.

1.5 CONTROL

Trypanosomiasis control has a long history. It started at the turn of 20th century as the infections agents and their vectors were identified in Africa and Latin America (Schofield and Maudlin, 2001) .There are two major ways of controlling trypanosomiasis; they include clinical treatment of the infection and by halting the transmission of disease by controlling the insect vectors - tsetse fly in Africa and domestic *triatominae* in Latin American

1.5.1 VECTOR CONTROL

Since the eradication of tsetse population is not considered realistic, the objective of vector control campaigns in an endemic focus is to reduce rapidly and drastically the vector population to a level at which disease transmission is significantly reduced or interrupted. A lot of techniques have been tried in order to bring tsetse population under control. These control measures include:

i. Destruction of wild host and bush clearing.

ii. Selective spraying of fly resting places in riverine fringing vegetation (Jodan, 1986)

iii. The use of bait technology in which odour baits are used to attract flies over relatively large distance to visual target treated with insecticide (Vale, 1974).

iv. Another form of bait technology used is the live bait technology in which cattle are treated with insecticide (usually synthetic pyrethroids applied as a dip or a pour - on {Thompson, 1987) . Apart from the aforementioned methods that could be referred to as the low tech methods there are still other methods called high tech methods. They include:

v. Aerial spraying and a sterile insect technique using sterile male tsetse to reduce the likelihood of naturally fertilized female flies.

Indeed, a cohesive technical package could be readily devised for most situations using low - tech approaches to reduce fly densities over large areas followed by a sterile insect technique to mop-up residual fly population.

1.5.2 **PROPHYLAXIS**

Pentamidine has been used in the past as a prophylactic to prevent infection in persons at risk, for example, tin miners in Nigeria (Molyneux and Ashford, 1983). The effect of *pentamidine* is said to last for six months, meaning that more frequent inoculation is desirable. There are also reports of resistance to pentamidine. A serious danger is that *pentamidine* may suppress an existing infection below potency, without cure permitting invasion of the central nervous system. Because of the danger of drug resistance and the possibility of masking a second stage infection prophylaxis is no longer recommended (WHO, 1998A).

1.5.3 **CLINICAL TREATMENT**

Trypanocides have played key roles in the control of human and animal trypanosomiasis the most popular and effective chemotherapeutic drugs for treatment against human trypanosome infection have been pentamidine, *suramin* and *melarsoprol* (Yoshisada et. al, 1998) while the three trypanocides available for controlling tsetse transmitted trypanosomiasis in domestic ruminants are *isometamedium*, *homidium* and *diminazene* aceturate(Stanny et. al, 2001).

Treatment recommendations vary according to the stage of the disease. During the first stage, treatment with suramin sodium or pentamidine is usually useful, in the second stage those drug are considered ineffective, presumably because the blood - brain barrier prevents the drugs from reaching trypanocidal levels in the cerebrospinal fluid (WHO, 1998A). *Melarsoprol* and *eflornithine* are commonly used during this stage.

.TREATMENT OF THE FIRST STAGE

The most commonly used drug for *T, b gambiense* infection is *pentamidine isotionate* which replace *pentamidine mesilate* in the early 1990s. *Suramin sodium* is also effective, but it is more often used in the treatment of *T.b rhodesiense* , the recommended dosage for pentamidine is a single intramuscular injection of 4mg of pentamidine isotionate per kilogram of body weight once daily for 7 - days (WHO, 1998A)

For suramin sodium the recommended therapy consists of a test dose of 5mg per kg of body weight on the first day followed by 20mg/kg body weight (up to a maximum of 1g on days 3, 10, 17, 24 and 31.

TREATMENT OF THE SECOND STAGE

Melarsoprol is a *melaminophenyl* based organic arsenical that was introduced as an anti-trypanosomiasis reagent in 1949 (Barrett and Barrette, 2000). Melarsoprol is effective both in the first and the second stage of the disease (WHO, 1998A)

Melarsoprol treatment is usually preceded by one or two injections of a first stage drug to eliminate the parasites in the blood and lymph .Melarsoprol is administered as three or four series of daily injection for 3 or 4 days with intervening periods of 7 - 10 days.

EFLONITHINE: The currently recommended treatment regimen for T. b gambiense disease in adults is 400mg/kg body weight daily in four divided doses for 14 days while that of children is $4g/m^2$.(the dosage for children is based on body surface area)

NIFURTIMOX;- The dosage in current use vary from one health center to another ranging from 15 to 20mg/kg body weight per day in three divided doses. The duration of treatment is between 30 and 60 days (WHO, 1998A).

1.6 PROBLEMS ASSOCIATED WITH THE ORTHODOX TREATMENT OF TRYPANOSOMIASIS

A lot of short comings have been attributed to the existing ways of trypanosomiasis treatment. These shortcomings have made (or are marking) the victims of trypanosomiasis hostages of some sorts. serious as the threat posed by trypanosomiasis to the health of both man and animals, there has been no satisfactory treatment for the disease in the case of chagas disease, the south American form of trypanosomiasis, caused by *trypanosoma cruzi*, there has been no completely suitable drugs for prevention or treatment (Togbato 2000). The chemotherapy of

trypanosomiasis is beset with problems including a limited repertoire of compounds, resistance to drugs, toxicity and protracted treatment protocol (Andrew et al,1992).until recently effective chemotherapeutic drug for treatment against human trypanosome infection has been *pentamidine*, *suramin* and *melarsoprol*. All these drugs have adverse side effects (Yabu et al, 1998) Melarsoprol for example, causes reactive *encephalopathy* in 5 - 10% of patient treated with a fatal outcome in 1 -5% (Kuzoe, 1993).

Resistance to *pentamidine* of *Trypanosoma brucei gambiense* and to *melarsoprol* of both *T. b. gambiense and T.b rhodesiense occurs . Eflornithine (DFMO) is effective against T. b .gambiense* infection but not for sickness caused by *T. b rhodensiense* (Bales et al, 1989). Moreover, *diminazene aceturate* and isometamidium chloride currently available for treating *trypanosomiasis* in domestic animals, also are challenged by the appearance of drug resistant trypanosomes (Codija et al, 1993).

Non accessibility and poverty are the other reasons for the present non-impressive chemotherapeutic treatment against typanosomiasis. For instance, DFMO that is relatively safe is very expensive especially for the poor African victims of trypanosomiasis (Motoves et al, 2001)

1.7 THE WAY FORWARD

In view of all these mentioned shortcomings against the present drugs used for the treatment of trypanosomiasis ; new drugs effective against trypanosomiasis infection that will be non toxic, cheaper, accessible, well absorbed orally are eagerly awaited.

According to the world health organization, more than 80% of the world population still rely on herbal medicines in their primary source of health care. Herbal treatment has indeed a long tradition in African Acacia nilotica Collected from Tanzania gave IC_{50} value against *T. brucei rhodesiense* and other countless African Plants Species were also reported to have trypanocidal activity (Freibughous et. al 1991)

Plants have provided the basis for traditional treatment for different type of diseases and still have enormous potential as a source of new chemotherapeutic agents. It is in due consideration of the foregoing that this present work was initiated with the interest of exploring the trypanocidal potentials of the plants under investigation.

The plants under investigation in this research are *Annona senegalensis, Enantia chlorantha, Calatropis pocera, Mitrocarpus scarber and Okoubaka aubrevillei.*

Annona senegalensis known as "Abo" in yoruba, guadan- daji in Hausa, dukunbe ladde in Fulani, anyan in Tiv, Uburu - ocha in Igbo (Keay, 1986) and Ikpokpo in idoma language belongs to the genus *Annona*. They are shrub or small tree with branchlets pubescent, leaves glaucous entire ovate to elliptic ovate (Shahina , 1989)". Base rounded or subcadate, upper surface hairy, the lower densely hairy flower ; pale yellow to orange. This plant is widely spread in the drier parts of savanna and near stream. Annona senegalensis is one of the plants claimed and used by the traditional health practitioners. The root of this plant is used in northern Nigeria to treat sleeping sickness . It is also used in the folk loric medicine for the treatment of cancer (Gbile, 1985)

Enantia chlorantha belongs to the family known as Annonaceae and to the genus called *Enantia* (Sofowora, 1986). This is a very small genus confined especially to west Africa with distinctive fruits composed of many stalks of the carpels in umbel - like heads. Stalks of the carpels is about 0.5 inches long. This fair - sized trees, usually growing in dense shade may be recognized by the bright yellow slash and conspicuous black fruit (keay, 1989). In English, the plant is known as African yellow wood and in french it is called "Moambe" Jaune ; the Edos in Nigeria call it "erembaubogo" , the Yoruba call it "Awopa" Osopupa" or "Iyani"(Sofowora, 1986). In western Cameroon, the Duale people refer to it as "bomuke" or "Nje", the Kpe call it "Woyoyo" implying bitterness. The plant is found in southern Nigeria, it is also widely distributed in Western Cameroon, Fernando and also extends to Zaire, Garbon and Angola (Sofowora,1986)

Enantia chlorantha grows in the middle storey of low land fair forest. The tree is tall (about 100ft high), the bark is grey - brown, shallady tissues, slash thin and blackish on the surface, thick and bright sulphur-yellow bellow.

For curative purpose, *Enantia chlorantha* is used traditionally among the Yoruba in the south western Nigeria to treat malaria, jaundice and leprosy spot. Oliver (1960). Claimed that therapy with decoction of it is known to be effective against many infections and it has striking antipyretic action.

The bark of *Enantia chlorantha* is used as healing powder on wound in Nigeria and congo (Sofowora,1986), and as scrapings on ulcers in Gabon, in congo, a bark decoction is taken for tuberculosis and blood vomit. It is used to bath in case of fatigue, it is also used by mouth and vapour baths for rheumatism, intercostal pain and to promote conception. Bark sap is taken in decoction in Gabon for excess of bile and

by injection for diarrhoea. The bark may also offer other medicinal use as uterus stimulant and antibiotic (Oliver, 1960).

Calotropis species are milky weed like shrubs or small tree with large glaucous leaves grown in the American tropics and wide spread in west Africa (Sofowora, 1986). *Calotropis* procera was reported to contain *Calotropain* which is a mixture of five proteins. *Calotropain* showed proteolytic activity. The leaves, flowers and root bark oil showed antimicrobial activity against *E scherichia coli, Neisaria, gonorrheoa, shigella, dysentaries, salmonella typhosy* and *treponema pallidum* (Sofowara 1986). *Mitracarpus scabra* is an annual weed about 30cm tall or much smaller (Omotoye, 1984) . The flowers are white crowded at the nodes within the divided stipple. The leaves are elliptic to obovate with whitish veins. *Mitracarpus scabra* is called "Emile" in Yoruba and "Gugamasu" or "Harwatsi" in Hausa.

Okoubaka aubrevellei; this plant together with *octoknema* belong to a family *actoknemacea* (keay, 1989). This plant is confined to tropical Africa and composed of trees and shrubs. *Okoubaka* is a large tree, well known in Benin where it is believed to kill any tree near it, a clear pointer that this plant is a great herbicide. *Okoubaka* may be recognized by its hard yellow fruits and the leaves are occasionally opposite.

This plant is known as "Iginla" amongst yorubas, the Edos call it "Akoebosi" and it is known among Igbo as "akoebilesi". *Okoubaka* extends from ivory coast to cameroon and zaire (Keay, 1989).

CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 *TRYPANOSOMES*:-

A stabilate of pleomorphic *Trypanosoma* brucei brucei (*T. brucei*) strain 8/18 was obtained from the Department of Parasitology, Nigeria Institute for Trypanosomiasis Research (NITR) Vom, near Jos, Nigeria and maintained in our biology laboratory by serial passage in mice.

2.1.2 **ANIMALS**:-

Sixty albino mice and six white rats free from infection {i.e apparently healthy} were purchased from the Department of Veterinary Parasitology, Ahmadu Bello University, Zaria, Nigeria. Subsequent animals used were bred. The mice and rats were on the average of about 30g and 130g each respectively when they were ready for use.

2.1.3 **PLANT MATERIALS**:-

Annona Senegalensis (A. Senegalensis) (leaf, root and stem bark) was collected between April and August at the farm of the Headquarters of the River Basin Authority Minna, Niger State, Nigeria. The plant was identified at the crop Production Department of the Federal University of Technology, Minna. *Enantia Chlorantha* (E. chlorantha) stem bark was obtained in April at Akufo forest, a village in Ibadan, south-western Nigeria,while *Calotropis procera, Mitracarpus scabra and Okoubaka aubrevillei* were bought at minna market in Niger State, Nigeria. The plants were identified at the Herbarium Department of National Institute for pharmaceutical research and Development (NIPRD) Idu, Abuja Nigeria.

All the plant parts used were thoroughly washed and sun dried to constant weight.

2.2 CHEMICALS AND REAGENTS

PHYSIOLOGICAL SALINE:- This was prepared by dissolving 9g pure grade of sodium chloride (NaCl) first in a small quantity of distilled water and then later made it up to 1000ml with distilled water (i.e 0.9% NaCl). This sodium chloride solution was stored in a washed stoppered bottle and kept in refrigerator at 4°C until when required for use.

ANTICOAGULANT:- The anticoagulant used is Ethylene diamine tetraacetic acid (EDTA). It was used as described by WHO (1998A}i.e 1mg of EDTA per ml of blood.

SOLVENTS:- Apart from distilled water that was prepared in our biochemistry laboratory ; ethanol, acetone, methanol, ethyl acetate and hexane were obtained from B D H company London. All chemicals and reagents used were of analytical grade.

2.3 METHODS

2.3.1 PREPARATION OF EXTRACTS:-

Parts of A. Senegalensis (the root, stem bark and leaf), *E. chlorantha* (stem bark), *Calotropis procera* (leaf), *Mitrocapus scabra* (leaf) and *Okoubaka aubrevillei* (stem bark) were properly washed and sun - dried to constant weight. 50g each of dried root and stem bark of *A. senegalensis,* 70g of dried leaves of *A. senegalensis,* 50g of dried stem bark of *E .chlorantha,* 50g of dried stem bark of *Okoubaka aubrevillei,* 60g each of dried leaves of *Calotropis procera and Mitracarpus scabra*

were separately cut into pieces and later made into powder using mortar and pestle and stored separately in covered bottles until when they were required for use. Each of these powdered samples was then extracted in 400ml of hexane, ethylacetate, methanol, ethanol and acetone for the duration of four hours under reflux. The extracts were filtered hot using muslin cloth, cooled, kept in stoppered flask and stored in the refrigerator at 4°c until when required for use.

2.3.2 INFECTION OF ANIMALS

Blood was collected by cardiac puncture with an E D T A coated syringe (Hen kesass 23G x 1" G M B H London) from a heavily infected mouse and instantly diluted to about twice the original volume with physiological saline (0.9% Nacl). Parasites in the diluted blood was used as Inoculum. Each clean, disease - free animal (mouse or rat) was there after inoculated intraperitoneally with 0.02ml of the diluted blood containing about 1 x 10^3 trypanosomes Parasitemia was monitored daily through out the infection by counting the number of parasites per microscope field.

2.3.3 ADMINISTRATION OF PLANT EXTRACTS

The prepared plant extracts were administered between 24 and 48 hours post infection when parasites usually began to come up. The extracts were administered to five groups of mice intraperitoneally. One animal was used per group for preliminary studies.Five groups of mice were each administered doses of 0, 100, 150, 200 and 250 mg/kg body weight respectively. The infected but not treated mouse was used as control.

2.3.4 SCREENING OF EXTRACTS FOR TRYPANOCIDAL ACTIVITY.

This was done by monitoring parasitemia on a daily basis and where necessary, the cerebrospinal fluid (CSF) was examined for parasites, where parasites was neither detected in the blood nor CSF, blood and CSF samples were withdrawn and used for inoculation of clean animals to confirm complete clearance of parasites from the infected animals.

2.3.4 INOCULATION OF HEALTHY MICE WITH BLOOD FROM CURED MICE

One of the surviving mice treated with aqueous extract of *A. senegalensis leaves* was sacrificed eight weeks post treatment and 0.02ml of the blood obtained from the sacrificed mouse was used to inoculate each of the two clean, disease -free mice. Presence of parasites was monitored daily on the microscope for two months.

2.3.5 INOCULATION OF HEALTHY MICE WITH CSF OF CURED MICE:-

This was done as described by Andrew et. al (1993); Briefly, the fur (hair) on the back of one of the surviving mice treated with aqueous leaf extract of *A. senegalensis* was shaven. The animal was positioned to make its head touch its limbs, a position that made the vertebrae conspicuous. In this position, *lumber* punjure was done by inserting the clean needle into the *lumber*, The clean, clear and transparent fluid gushed in to the needle. A syringe was then fixed to the needle and the CSF withdrawn and 0.02ml of the CSF was used to inoculate each of the two mice used for this test. Presence of parasites was monitored in the blood of the mice daily on the microscope for two months.

2.4 PROPHYLACTIC ACTIVITY TEST

The curative dose of the extract was used for testing the prophylactic activity. Two separate groups of four clean and disease free mice were used. One group was administered 200mg/kg body weight each of the aqueous extracts of *Annona senegalensis* leaf and the other group was administered 100mg/kg body weight each of the aqueous extract of *Enantia chlorantha* stem bark. These extracts were administered daily for four days before being inoculated with trypanosomes. The animals were then monitored for establishment of infection.

2.5 COLUMN CHROMATOGRAPHY

A slurry was prepared by dissolving 100g of silica gel (- 70 + 230 mesh powder S .A.) In 200ml (90% water and 10% methanol) and packed with 2g of the sample of the extract of *Annona senegalensis leaves* that had been previously mixed with 2g of the silica gel and distilled water.The column was eluted with water/methanol (1 :1). Six different fractions were separately collected under pressure by mechanical pump. The fractions were stored at 10°c. The fractions were each evaporated to dryness to obtain 0.53g, 0.68g, 0.45g, 0.09g, 0.01g and 0.002g of extract in fractions I - VI respectively

2.6 PHYTOCHEMICAL ANALYSIS

Chemical tests were performed to detect certain substances such as *alkaloid, saponin, glycosides, reducing sugars, anthraquinones, tannins and phlobatannin,* that may be present in the extract used. These tests were carried out on the aqueous leaf

extract of *Annona senegalensis* and aqueous extract of *Enantia chlorantha* stem bark since these were the only ones that showed significant anti trypanosome activities.

2.6.1 *TEST FOR TANNIN*

5g of aqueous leaf extract of *Annona senegalensis* was stirred with 10ml of distilled water, filtered and ferric chloride reagent added to the filtrate. A blue - black colour was taken as an evidence for the presence of *tannin* (Sofowora, 1982)

2.6.2 *TEST FOR SAPONIN*

0.5g of each extract was shaken with water in the test tube. Frothing which persists on warming was taken as preliminary evidence for the presence of *saponin*. In order to remove false positive results the blood haemolysis test was performed on those extracts that frothed in water.

0.5g of each extract (*A. senegalensis* or *E .chlorantha*) was boiled briefly with 50ml phosphate buffer (pH 7.4) and then allowed to cool and filtered; 5ml of the filtrate was passed for three hours through an asbestos disc (1.5mm thick and about 7mm in diameter), which had been previously soaked with two drops of 1% cholesterol in ether and dried.

After filtration the disc was washed with 0.5ml of distilled water, dried and boiled in 20ml of oxylol for two hours. The disc was then washed in ether, dried and placed on a blood nutrient agar. Complete haemolysis of red blood cells around the disc after six hours was taken as further evidence of presence of *saponins* (Sofowora, 1982).

2.6.3 TEST FOR *ALKALOID*

0.5g of the extract (*E. Chlorantha*) was stirred with 5ml of 1% aqueous hydrochloric acid on a steam bath; 1ml of the filtrate was treated with two drops of mayer's reagent and a second 1ml portion was treated similarly with dragendorff's

reagent. Formation of precipitate in both reagents were taken as preliminary evidence for the presence of *alkaloids* in the extract.

For the confirmatory test of the alkaloid, 1g of the extract was treated with 40% calcium hydroxide solution until the extract was distinctly alkaline to litmus paper and then extracted twice with 10ml portions of chloroform. The chloroform extracts were combined and concentrated in vacuo to about 5ml and then spotted on thin - layer plates. The presence of alkaloids in the developed chromatograms was detected by spraying the chromatogram with freshly prepared dragenedorff's spray reagent. A positive reaction on the chromatogram (indicated by an orange colour spot against a pale yellow back ground) was confirmatory evidence that the plant extract contained an alkaloid. (Sofowora, 1986)

2.6.4 TEST FOR SIMPLE SUGAR

0.5g of the sample (*E. chlorantha* extract) was mixed with 5ml of benedict's solution in a beaker, the beaker was placed in a boiling water bath. For 5minites. The appearance of an orange - red precipitate was taken as evidence for the presence of simple sugar (Sarojini, 1996).

2.7 ACUTE TOXICITY STUDY

5000mg\kg body weight of the aqueous leaf extract of *Annona senegalensis* was administered intraperetonealy into each of the two mice used for the test. These mice were monitored for one month for any possible symptoms and signs of acute toxicity from the extracts

CHAPTER THREE

RESULTS

3.1 CHEMOTHERAPEUTIC EFFECTS OF THE EXTRACTS:

ANNONA SENEGALENSIS.

Of the three parts (i.e the leaf, stem bark and root bark) of *Annona senegalensis* plant screened for trypanocidal activity, it is only the aqueous leaf extract that was found to possess a tremendous potential. Trypanocidal activity was found in neither the crude aqueous extract nor in the alcoholic extract of both the stem and the root bark of the plant.

The crude aqueous extract of *Annona senegalensis* leaf was used to cure *T. brucei* infected rat and mice completely at 200mg/kg body weight. The treated animals survived for 12 and more than 60 days post treatment respectively.(figs3.10and3.11)

Partly purified form of the aqueous leaf extract was also found to possess anti - trypanosome activity. It was able to satisfactorily clear parasites from the systems of the infected mice(fig.3.13)The treated mice survived for more than 120 days post treatment.

As a form of a confirmatory test for the efficacy of the aqueous extract of *Annona senegalensis* leaf; the blood and cerebrospinal fluid (CSF) obtained from the treated mice were used to innoculate two groups of clean and disease - free animals. The animals were routinely monitored daily for two months by checking their blood for possible appearance of parasites. No parasite was found in the blood of any animal of the two groups through out the duration of the experiments(table 2B).

ENANTIA CHLORANTHA

In this work, the crude aqueous extract of *Enantia chlorantha* stem bark was found to suppress parasitemia for a while. The extract at 100mg/kg body weight was able to bring down the high parasitemia load in *T. b brucei* infected mice from the average of twenty five parasite to just one parasite per microscope field. The parasitemia gradually rose again and latter killed the animals. The maximum survival period of the treated animals was 17 days post treatment (fig.3.18)

This suppressive trypanocidal effect of the aqueous extract of *Enantia chlorantha* seemed to be *synergistic* for neither the defated extract(table17) nor the petroleum ether extract(i.e fat component)(table18) of the plant alone had any *antitrypanosome* activity in the infected mice.

CALOTROPIS PROCERA, MITRACARPUS SCABRA AND OKOUBAKA AUBREVILLIE:-

None of the aqueous or alcoholic extracts of these plants was found to possess trypanocidal activity even at high doses of between 250 - 300 mg/kg body weight(figs.3.22-3.24).

3.2 PROPHYLACTIC ACTIVITY.

Doses of 200mg/kg and 100mg/kg body weight of the crude aqueous extract of *Annona senegalensis* leaf and the crude aqueous extract of Enantia *chlorantha* stem bark respectively given intraperitoneally to two groups of mice did not protect them from infection. The animals were observed and found to come up with parasites at about 24 hours after infection(fig.3.25).

3.3 ACUTE TOXICITY STUDY

The Intraperitoneal administration of the aqueous leaf extract of *Annona senegalensis* into non-infected mice revealed that the extract is not acutely toxic. At a very high dose of 5000mg/kg body weight, no symptoms and signs of acute toxicity was noticed in the animals.

3.4 PHYTOCHEMICAL ANALYSIS

Phytochemical assay of the aqueous crude extract of *Annona senegalensis* leaf showed that it contained *Tannin*, *saponin* and *phlobatannin* while the aqueous crude extract of *Enantia chlorantha* stem bark revealed the presence of alkaloid, *Saponin* and simple sugar.

TABLE 3.1 SUMMARY OF RESULTS

PLANT	PART	EXTRACT	ACTIVITY	ACUTE TOXICITY	PROPHYLAXIS	PHYTOCHEMICALS PRESENT
Annona senegalensis	Leaf	Aqueous	Cured Infection	Not Toxic	None	Tannin, Saponin and phlobatannin
Annona senegalensis	Leaf	Ethyl acetate	In infected mice			
Annona senegalensis	Leaf	Ethanol	No Activity			
Annona senegalensis	Leaf	Methanol	No Activity			
Annona senegalensis	Root Bark	Aqueous	No Activity			
Annona senegalensis	Root Bark	Ethanol	No Activity			
Annona senegalensis	Root Bark	Methanol	No Activity			
Annona senegalensis	Stem Bark	Aqueous	No Activity			
Annona senegalensis	Stem Bark	Ethanol	No Activity			
Enantia chlorantha	Stem Bark	Methanol	No Activity		None	Alkaloids Saponin and simple Sugar
Enantia chlorantha	Stem Bark	Aqueous	No Activity			
Enantia chlorantha	Defated stem Bark	Ethanol	Suppressive			
Calotropis procera	Leaf	Methanol	No Activity			
Calotropis procera	Leaf	Aqueous	No Activity			
Calotropis procera	Leaf	Ethanol	No Activity			
Mitracarpus scabra	Leaf	Methanol	No Activity			
Mitracarpus scabra	Leaf	Aqueous	No Activity			
Mitracarpaus scabra	Leaf	Ethanol	No Activity			
Okoubaka aubrevillie	Stem Bark	Methanol	No Activity			
Okoubaka aubrevillie	Stem Bark	Aqueous	No Activity			
Okoubaka aubrevillie	Stem Bark	Ethanol	No Activity			
		Methanol	No Activity			

Fig 3.10 : Trypanocidal Activity of aqueous leaf extract of *Annona senegalensis* in *T. brucei* infected rats A and B.

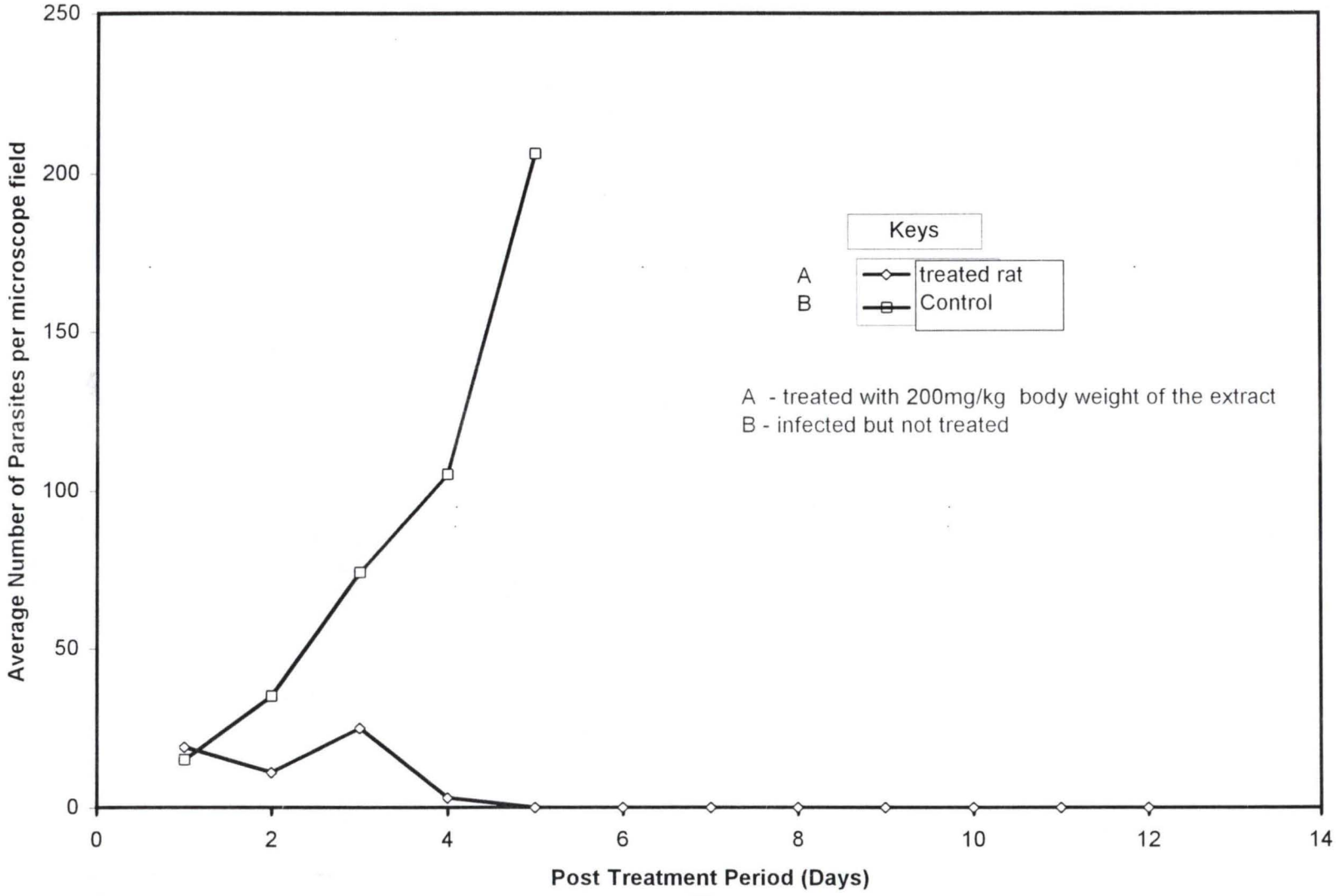


Fig 3.11 Trypanocidal Activity of aqueous leaf extract of *Annona senegalensis* in *T. brucei* infected mice (A-E)

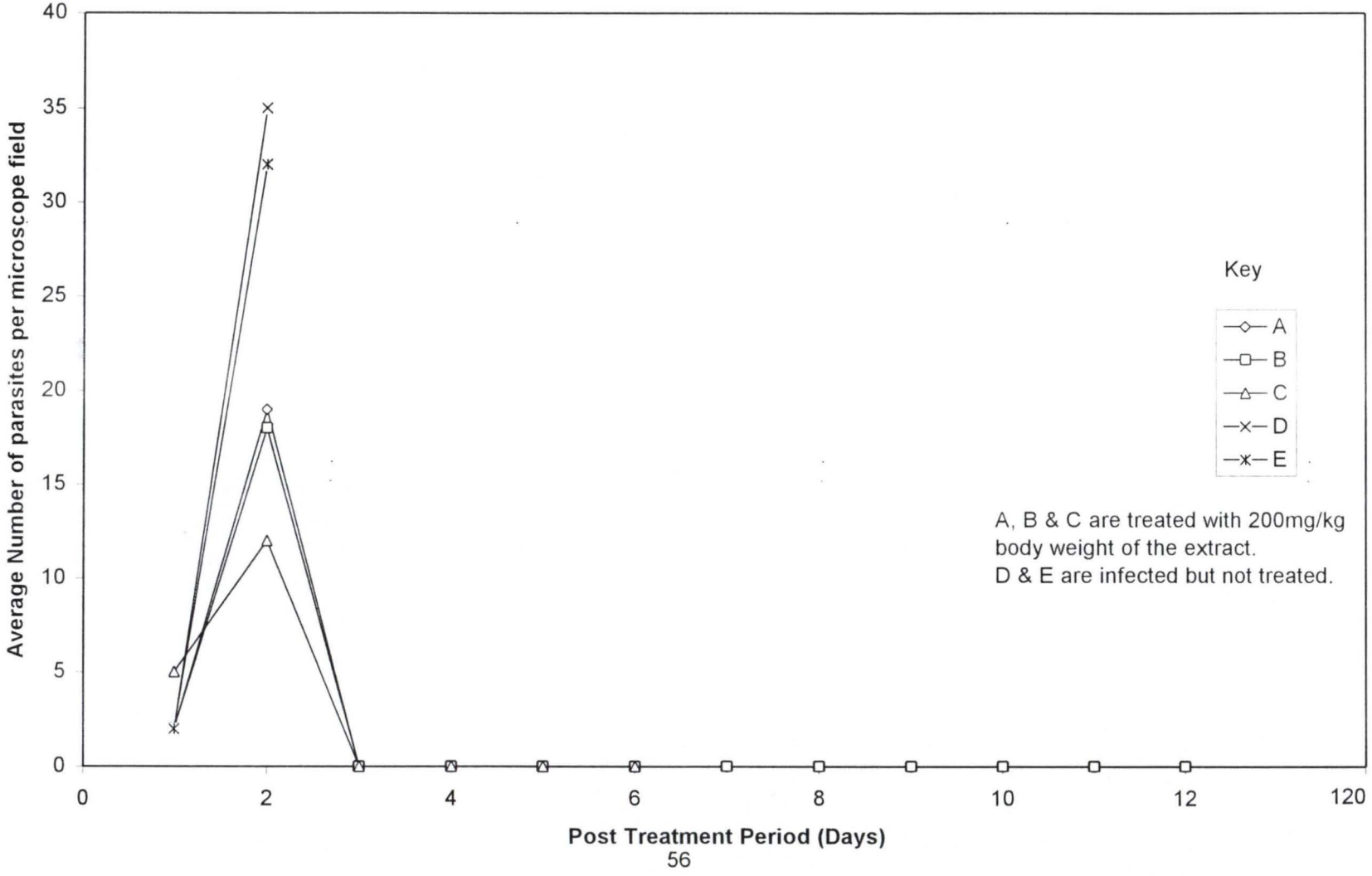


Fig 3.12 Trypanocidal activity of Ethyl Acetate, Hexane and Methanolic Leaf Extract of *Annona senegalensis* in Infeected Mice

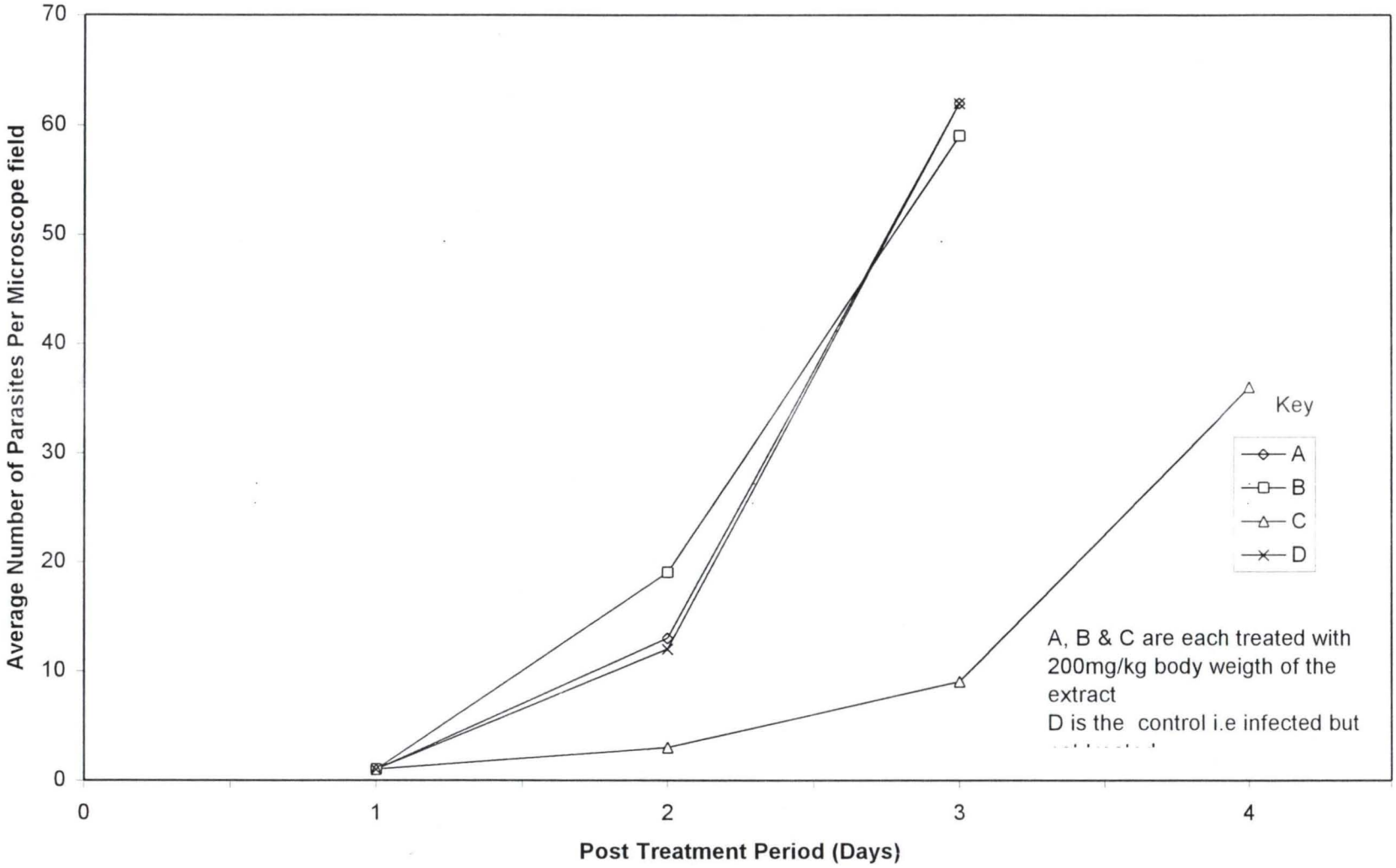


Fig 3.13 Trypanocidal Activity of Partially Purified Leaf Extract of *Annona Sengalensis*

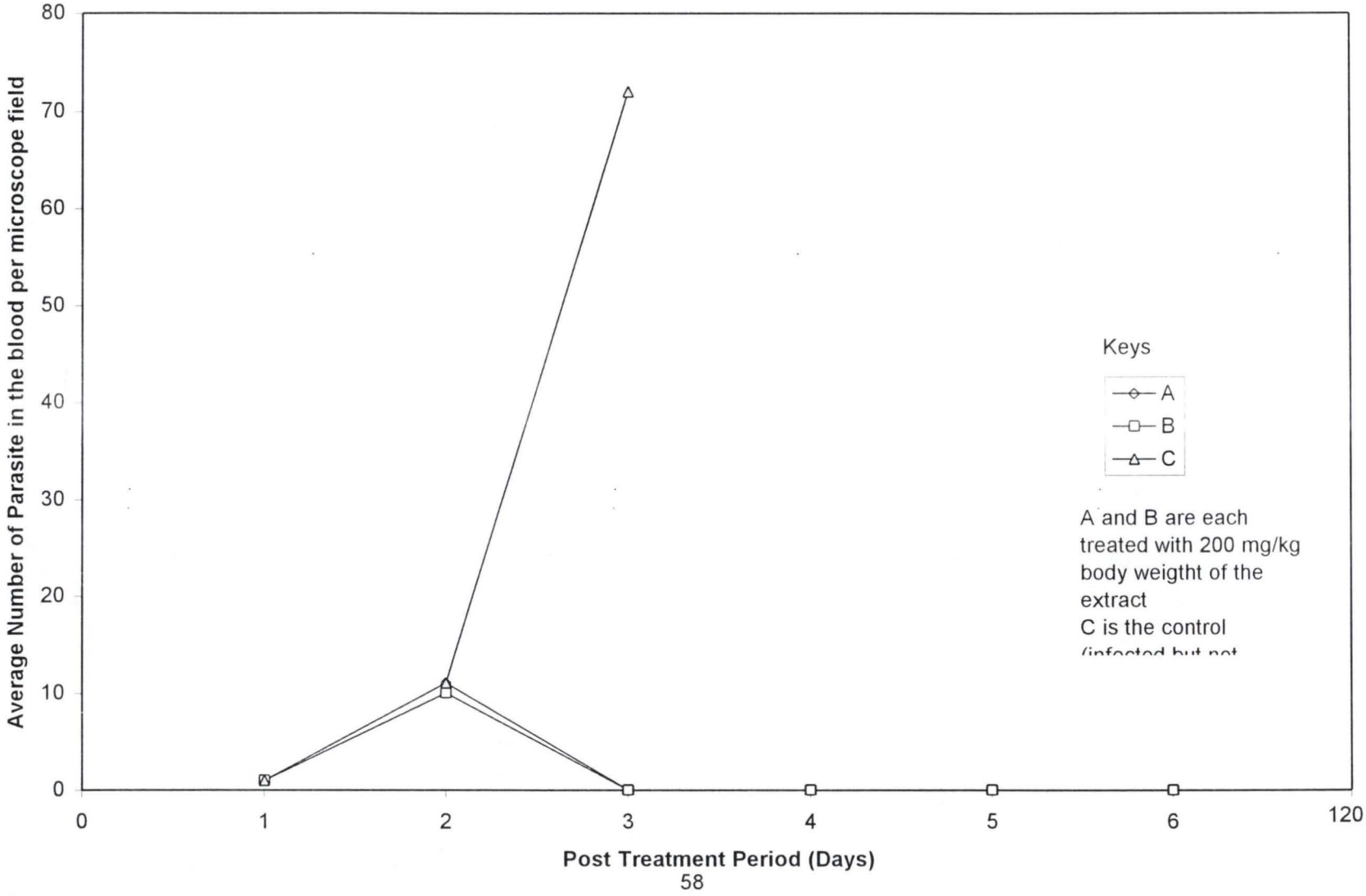


Fig 3.14 Anti Trypanosome Activity of aqueos extract of *Annona senegalensis* Stem Bark in *T. brucei* infected mice

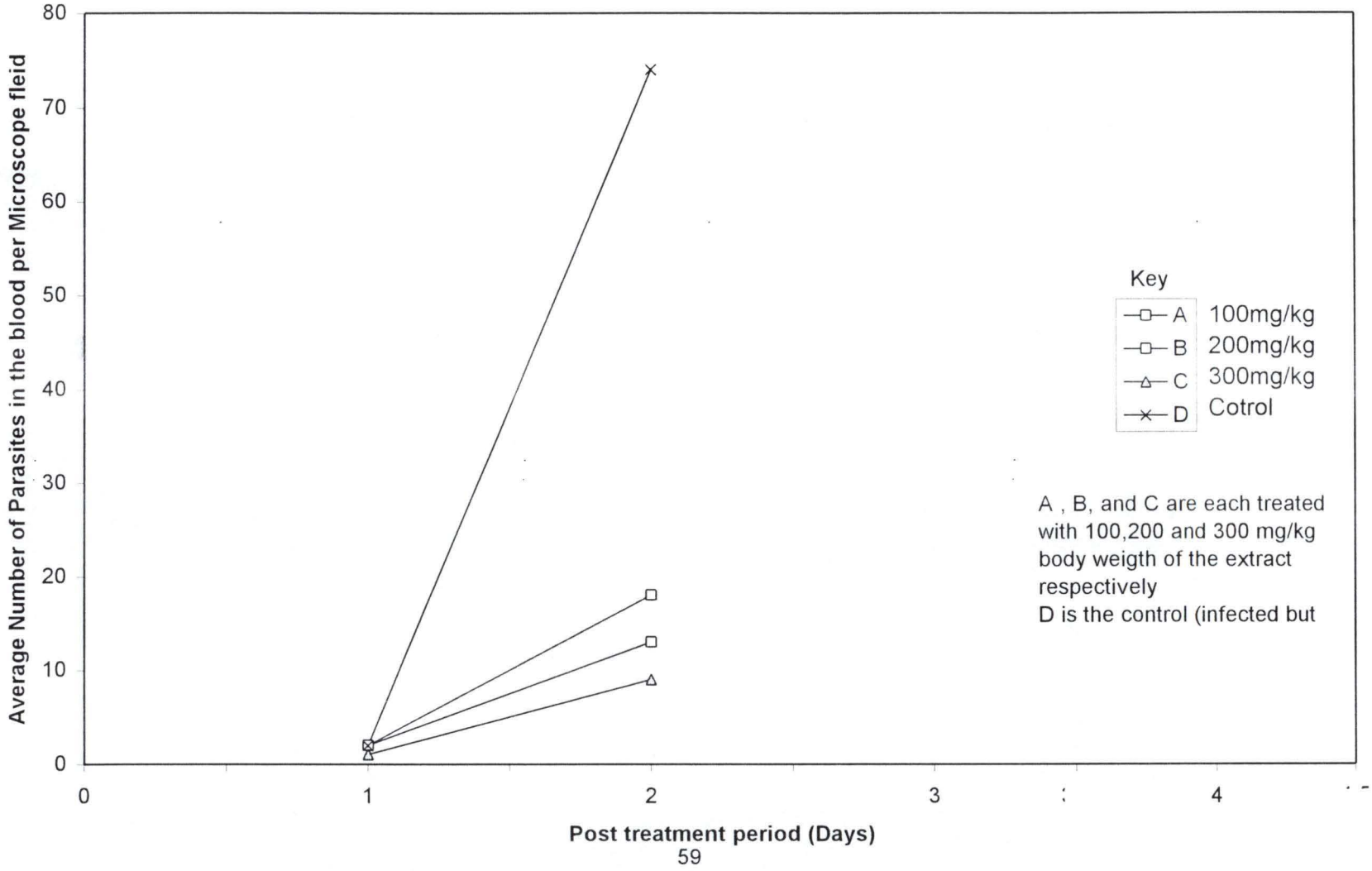


Fig 3.15 Anti-trpanosome Activity of Aqueous Extract of *Annona senegalensis* Root Bark in *T. brucei* Infected mice (A - D)

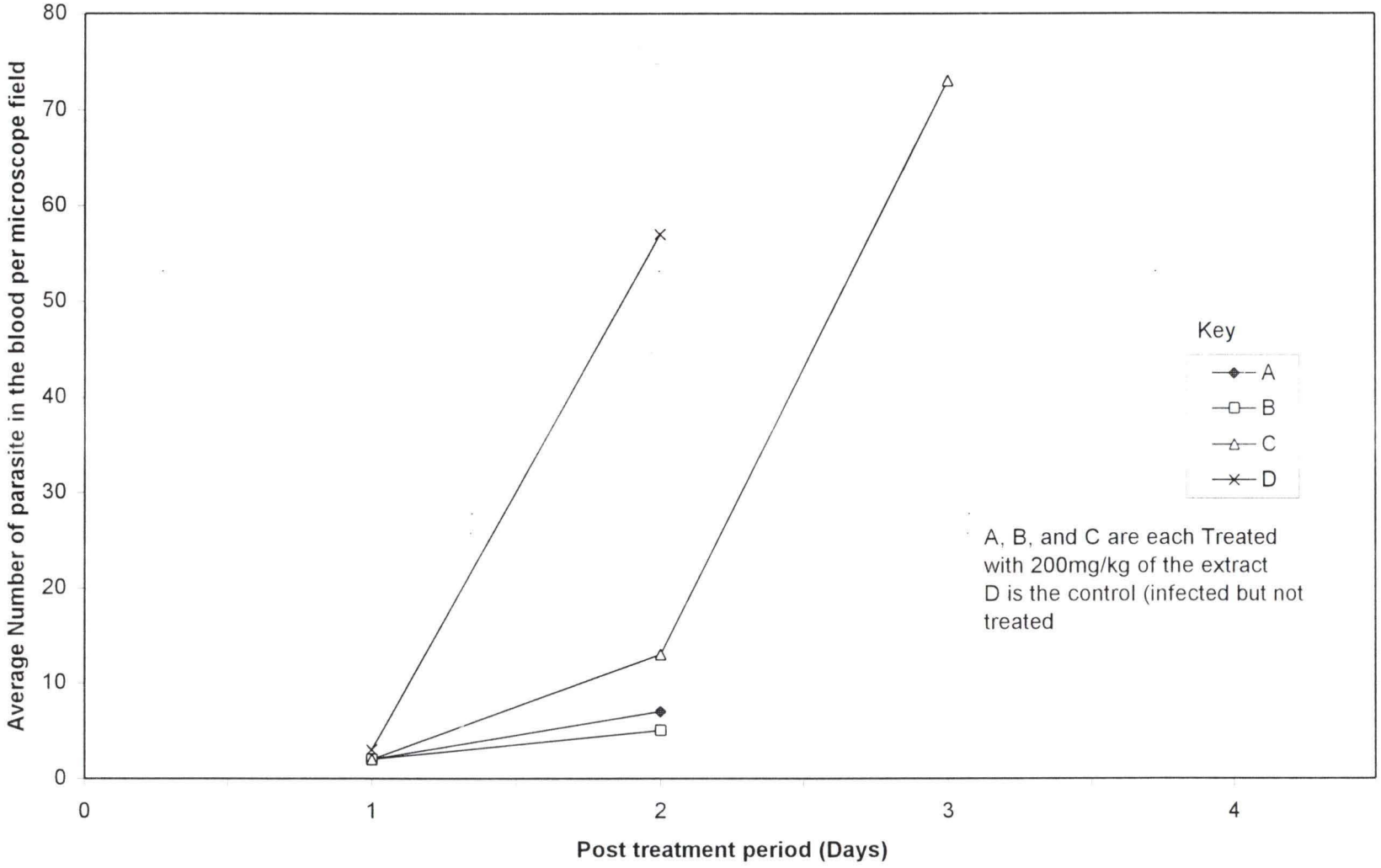


fig 3.19 Trypanocidal Activity of Aqueous Extract of *Enantia chlorantha* in *T. Brucei* Infected Mice (A - D)

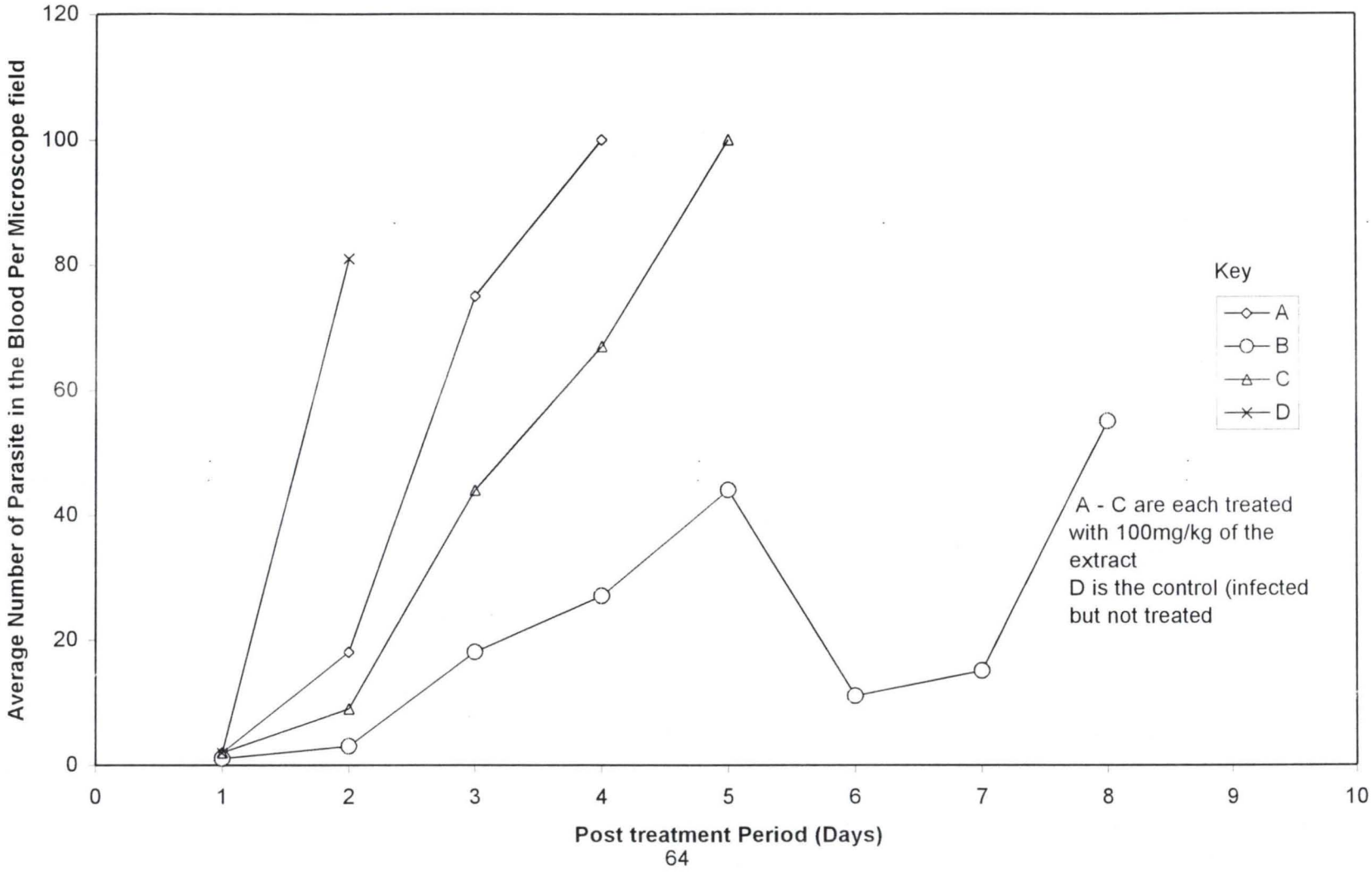


fig 3.20 Trypanocidal Activity of Defacted Extract of *Enantia chlorantha* Stem Bark in *T. brucei* Infected Mice (A - D)

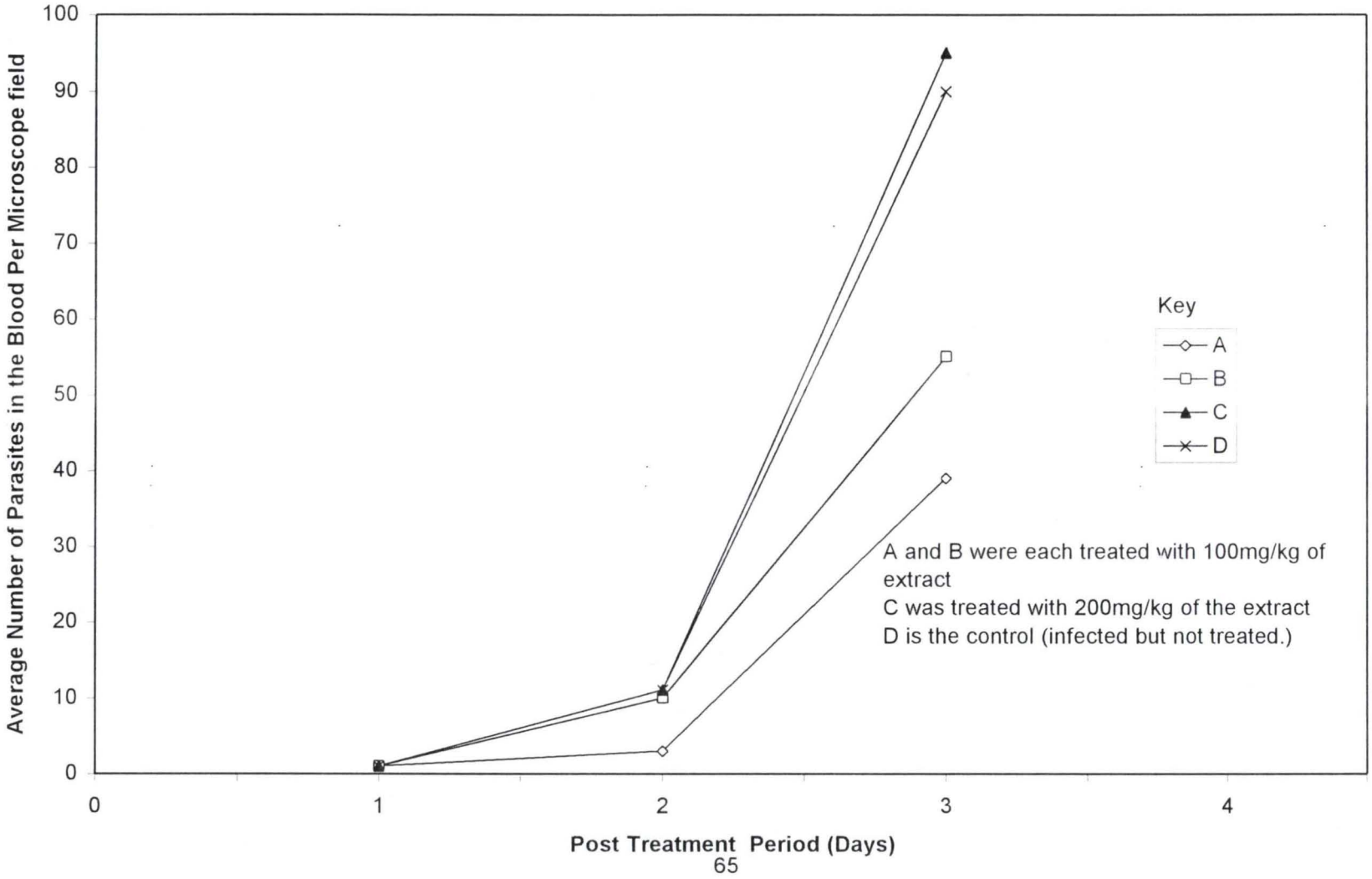


fig 3.21 Trypanocidal Activity of the Fat-soluble extract of *Enantia chlorantha* Stem Bark in three Infected Mice (A - C)

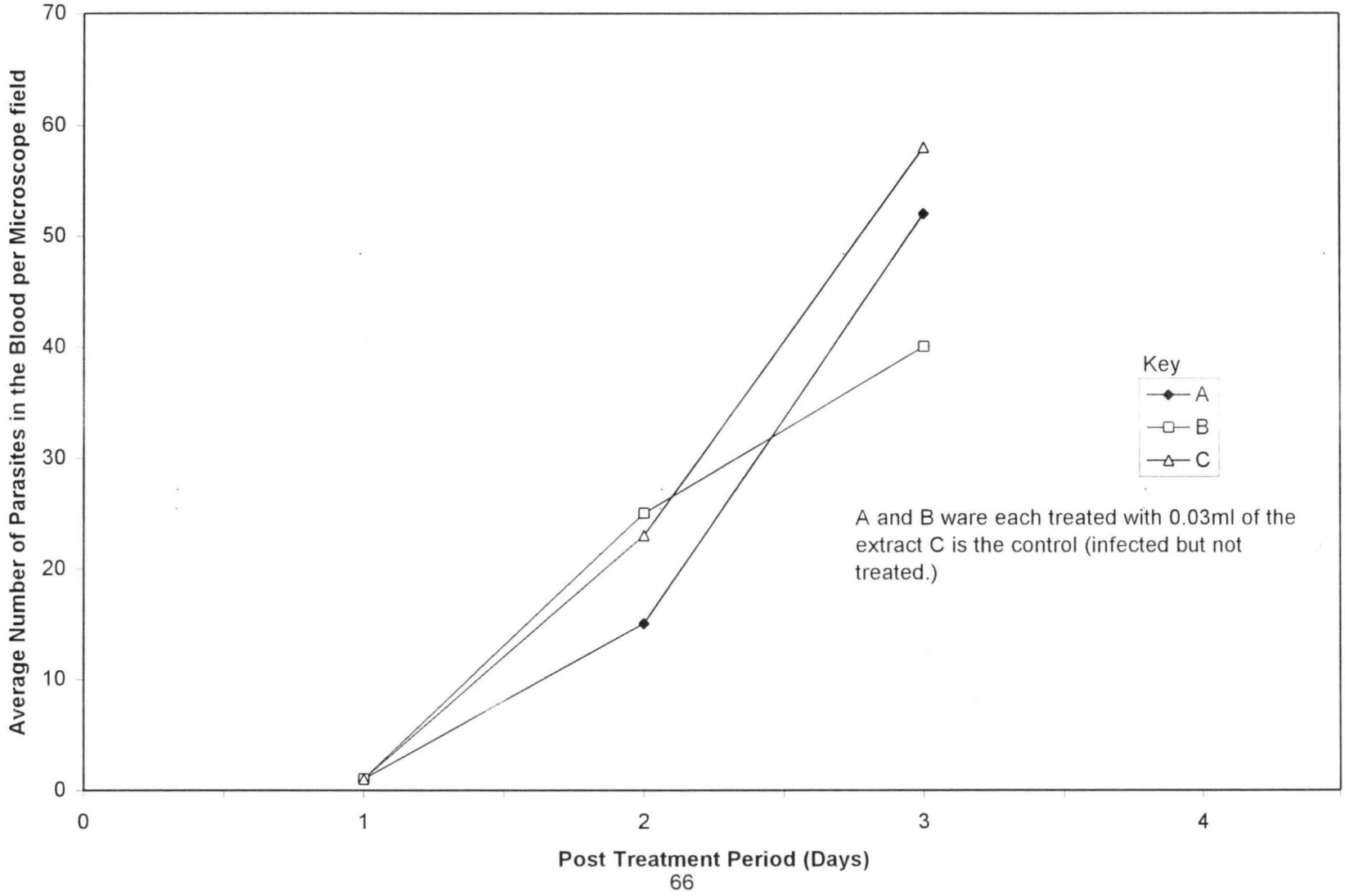


fig 3.22 Trypanocidal Activity of the Aquoeous Extract of *calotropis procera* in *T. brucei* Infected Mice (A - C)

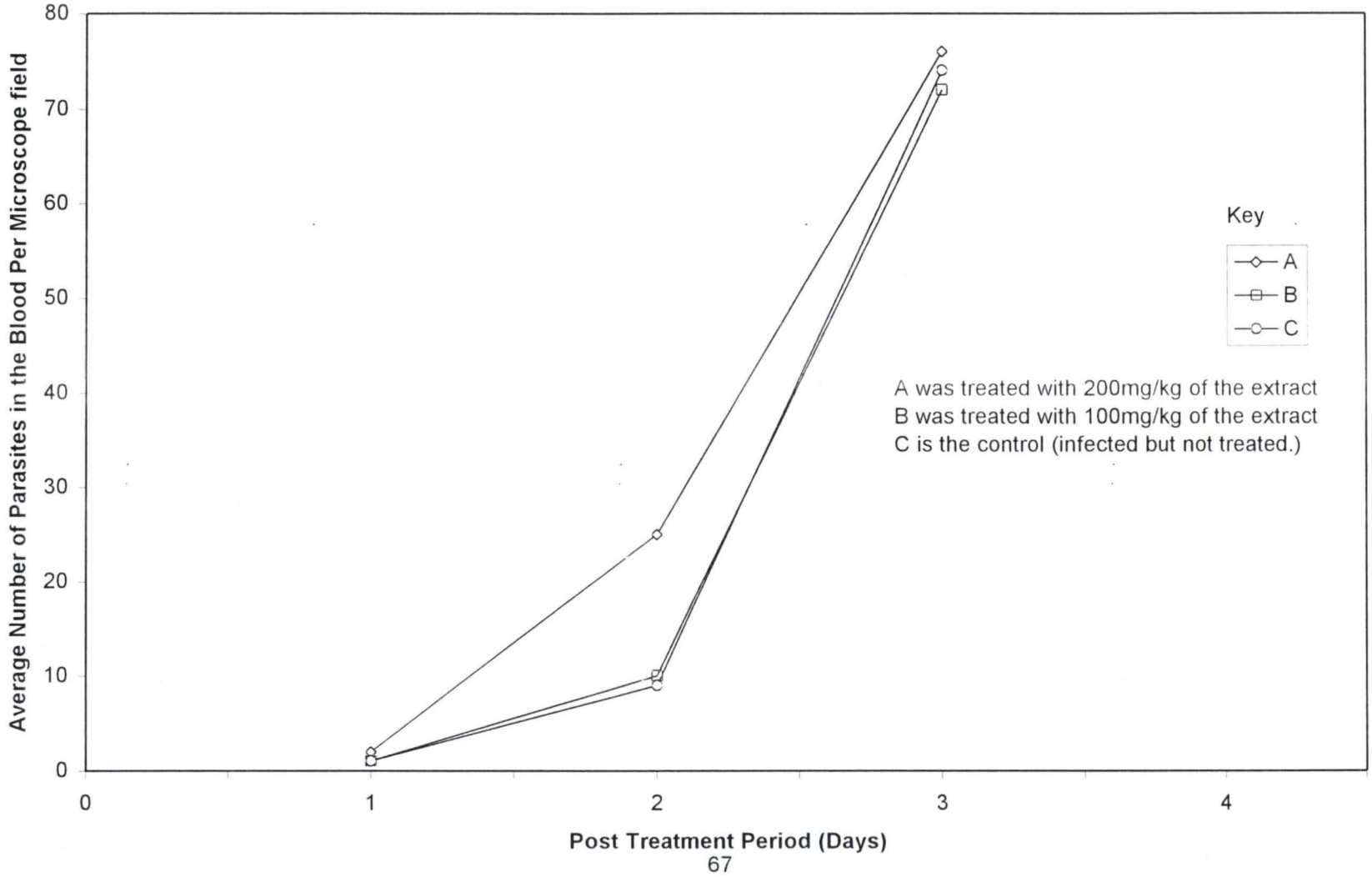


Fig 3.23 Trypanocidal Activity of the Aquoeus Extract of *Mitracarpus scabra* in *T. brucei* Infected Mice (A - C)

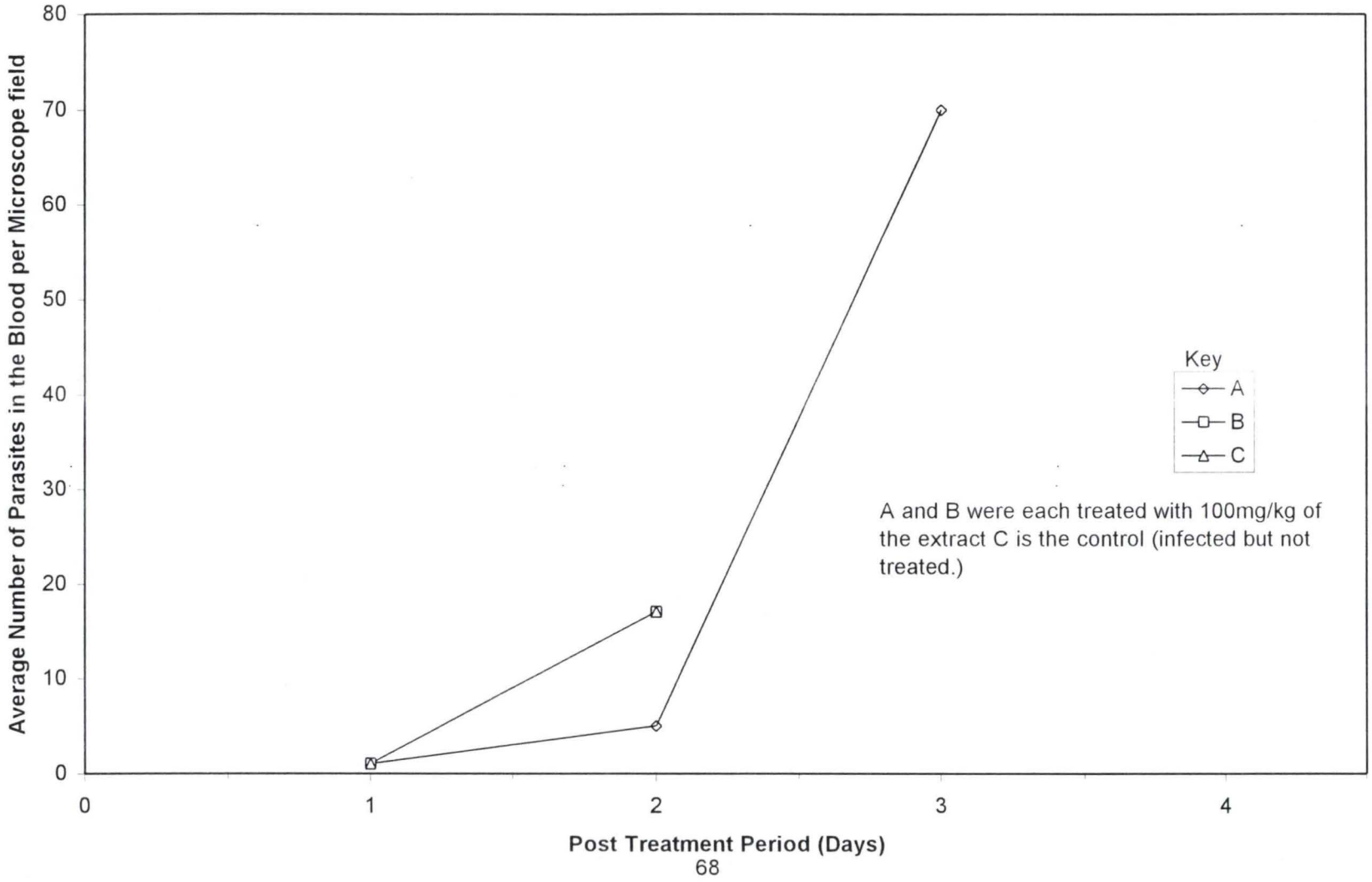


fig 3.24 Trypanocidal Activity of Aquoeus Extract of *Okoubaka abrevillei* in *T. brucei* Infected Mice (A -C)

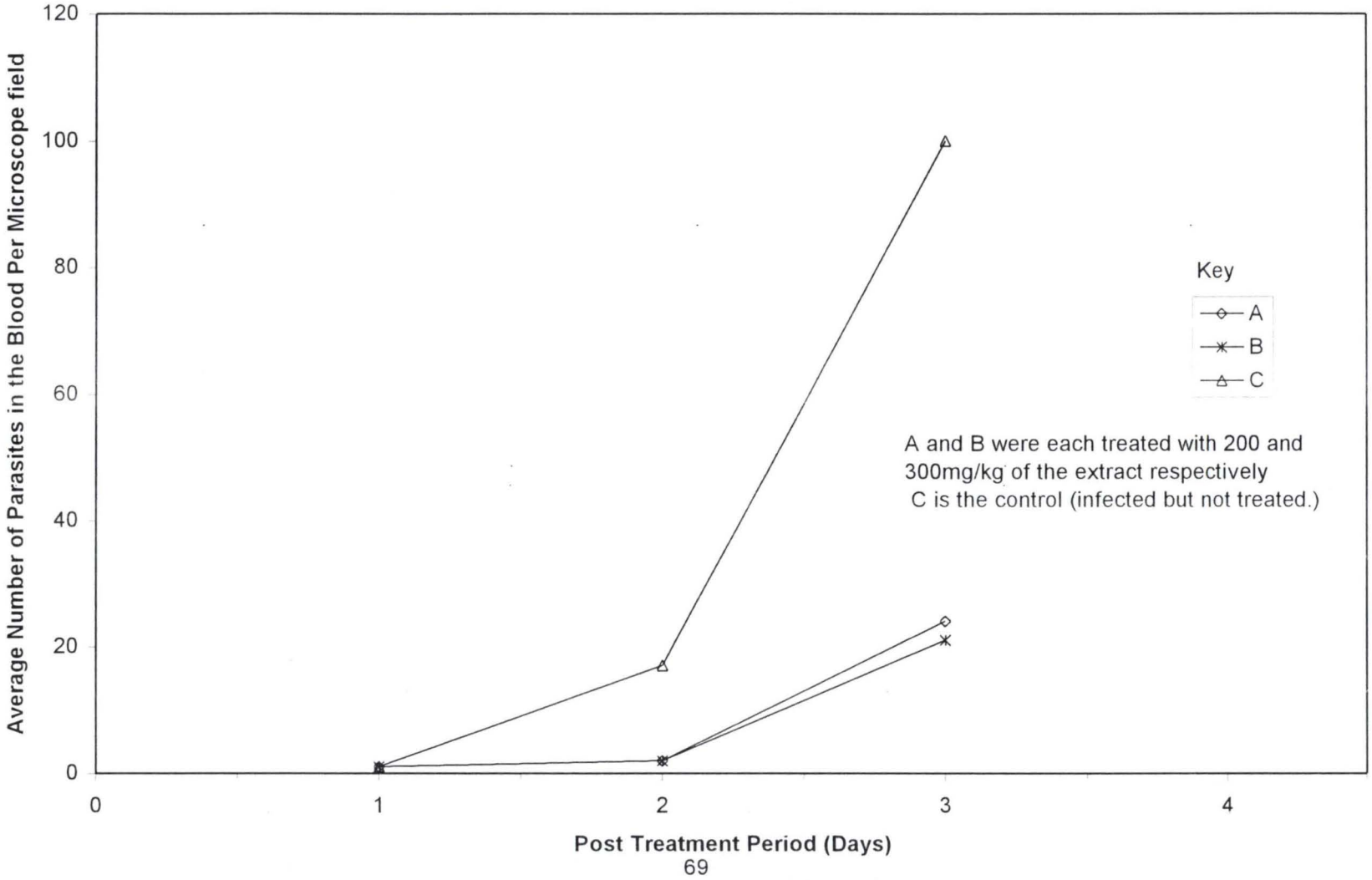
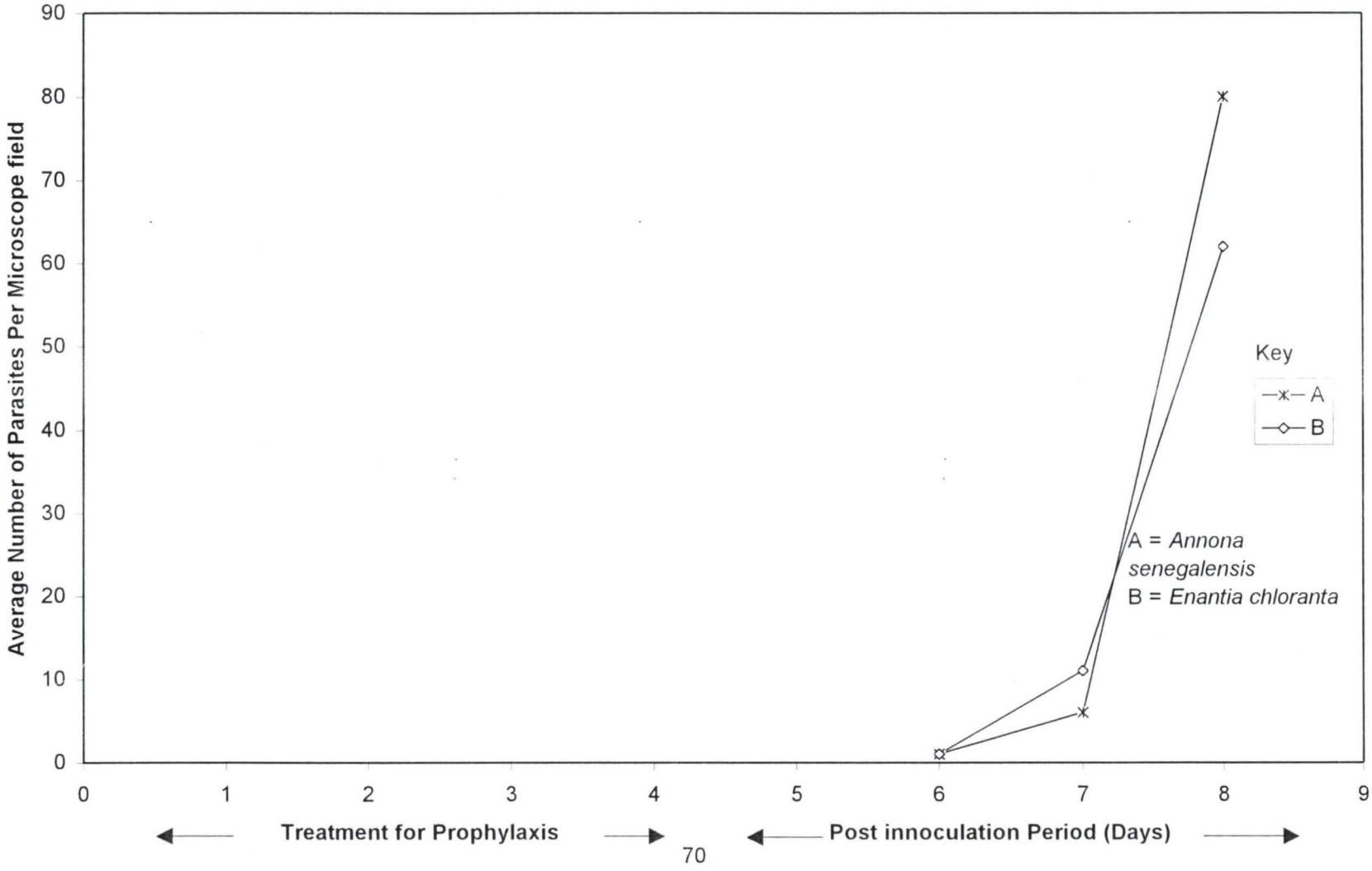


fig 3.25 Prophylactic Effect of Crude Aquoeus Extracts of *Annona senegalensis* Leaf and *Enantia chlorantha* Stem Bark in Trypanosomaisis Infection



CHAPTER FOUR

4.1 DISCUSSION

Annona senegalensis (*A. senegalensis*) leaf and *Enantia chlorantha* (*E. chlorantha*) stem bark, traditionally used in Nigeria for the treatment of trypanosomiasis and malaria respectively have been shown in this work to have activity against trypanosomes. Intraperitoneal administration of 200mg/kg body weight of the crude aqueous leaf extract of *A. senegalensis* was used in the treatment of *T. brucei* infection in a rat and mice (figs.3.10 and 3.11) The cured rat survived for twelve days post treatment. One of the treated mice survived for more than four months while the remaining two treated mice survived for seven and nine days respectively, This apparent difference might be due to differences in biochemical and physiological individuality of the animals.

There was a complete elimination of trypanosomes from the central nervous system (CNS) of the cured mice as evidenced by non-infectivity of their cerebrospinal fluid CSF(tabie2B). This is an encouraging result and it is a good indication of trypanocidal activity of the extract . Since the secondary stage of trypanosomiasis is linked with the presence of trypanosome in the CSF, the clearance of the parasites from the CNS by the extract is an indication that the drug can cross the blood- brain barrier.This is a rare potential, the like of which is lacked by most orthodox drugs currently used in the treatment of trypanosomiasis.This is at the moment, a major obstacle in the treatment of the late stage of the disease. Even if this drug has to be improved upon, there might be no need coupling its active ingredient with any carrier for the purpose of transporting it across the blood - brain barrier.

Bioassay directed fractionation indicates that only one fraction is active against trypanosomes(table 6). This is encouraging with regards to the extension of this work - identification and characterization of active constituents. It also suggests that synergistic effects of constituent of the crude extract might be an important factor in the trypanocidal effect of the extract.

Crude aqueous extract of *E. chlorantha* stem bark was found to possess some trypanocidal activity against *T. b. brucei*. The preliminary work showed how the parasitemia was fluctuating, the average number of parasites being about twenty five per microscope field on the fourth day post treatment but came down to just "one" on the ninth day post treatment and later rose up gradually till when the animal died seventeenth day post treatment. That the plant extract was the one responsible for this activity in the treated mice was obvious giving the fact that the untreated mouse had died on the forth day of infection. A subsequent experiment using the same plant extract is a replica of the previous result (fig.3.19) though the level of parasitemia rose gradually in mice A and C till the animals died on the 5^{th} and 6^{th} day post treatment respectively .If this is compared with the untreated (control) animal which died on the third day of infection in the the first and second experiment, one would come to conclussion that this fluctuation in the parasitemia which ultimatly lead to the extension of the time to death of the treated animal is definitely not unconnected with the activity of the extract.

The suppressive activity of *E. chlorantha* aqueous extract was further substantiated as the defated extract of the plant administered to the infected mice did not cure them of their infection(table17) here, both the treated and the untreated animals died around the same time when the parasitemia in them rose gradually at the same pace(table17). Also not effective against the infected mice was the

petroleum ether extract {fatty component} of *E.chlorantha* as evidenced by the death of the treated and control animals at around the same time (table18). These observations convincingly show that some anti-trypanosome activity possess by *E. chlorantha* is contained in the whole extract and this is a clear pointer to the synergistic trypanocidal action of the plant extract.

The outcome of these findings has shown that both the crude aqueous extract and partially purified form of *Annona senegalensis* leaf extract possess a convincing trypanocidal activity against *T. b brucei* in rats and mice. This is an encouraging finding since it corroborates the claims of the traditional health practitioners in Nigeria especially the Fulanis who use the leaves alone to treat their cattle suffering from trypanosomasis.

The aqueous and the alcoholic extract of the root bark and the stem bark of A.. *senegalensis* was not found to possess any activity against trypanosomes in the infected mice even though Igewh and Onabanjo {1989} reported the trypanocidal effect of the aqueous extract of the plant's root. The possible reasons for non-correlation between the outcome of this work and that of Igewh and Onabanjo might be due to the differences in the route of administration of the drug, season, in which the plant sample was collected and the age of the plant.

Onabanjo and Igewh administered the drug using both oral and intramuscular routes while intraperitoneal route of administration was employed in this work. The season in which the plant is collected affects its therapeutic action, giving the fact that the medicinal plants active ingredients are usually more concentrated during the dry season and somewhat less concentrated during the rainy season. Also the medicinal activity of some plants are only pronounced during the rainy season while some are pronounced during the dry season.

As for the age of the plant, the active ingredients required to cure the disease might not have been synthesized in the young plant or might have been negatively affected by the old age of the matured plant. In this present work, the root and the bark of *A. senegalensis* used were collected in the bush between April and June and they were from the matured plant. The age and season in which Igweh and Onabanjo collected their plant are not known. It is therefore suggested that subsequent work on these parts (i.e root and stem) of the plant should compare their potency in different seasons and at different ages (i.e young and old plants) so as to be sure of the season and age is the plant is more active against trypanosome. The intraperetoneal routes of administration of the plant's leaf extract was found to be useful in the treatment of the infected animals even though the drug was found to be ineffective prophylactically(fig.3.25)..

No acute toxic reaction (to the best of my knowledge) has been reported by the traditional healers when the drug was given orally to their patients (man and animals) to treat trypanosomiasis or other diseases. Similarly in this work, extracts of the plant given intraperitoneally to the infected animals did not produce any acute toxic reaction even at a very high dose of 5000mg/kg body weight. This finding is important because acute toxicity is one of the side effects of the current orthodox trypanocides.

When the parasitemia was allowed to be massive at the late stage of the disease i.e at the third to forth day of infection before the aqueous extract of the curative plant was administered the animals died a day or two after the commencement of treatment. The deduction that could possibly be made out of this is that at the advanced stages of the disease. serious damage to the host organs might have occurred (Jenning et, al, 1982) and anaemic conditions would have

developed. At this stage the host is exposed to high level of toxic metabolites,.lytic enzymes, immuno-suppressive membrane components and other components of trypanosomes that induce unregulated lymphocytes and destructive inflammatory responsse (WHO, 1998B). Also, high parasitemia elicits high levels of immunoglobulin M (IgM) antibodies and it has been suggested that raised IgM levels increase osmotic pressure, erythrocyte sedimentation rate and blood viscosity (WHO, 1998A).All these activities are already markedly occurring at the advanced stages of the infection, and at this time, the extract could presumable produce a negligible effect because of the combined patho physiological and lethal actions of these factors which finally led to the death of the host.

4.2 SUGGESTION AND CONCLUSION

In this present study the efficacy of intraperitoneally administered crude and partially purified form of aqueous leaf extract of *Annona senegalensis* was demonstrated as the chemotherapeutic agent for the treatment of African trypanosomiasis using infected mice. But for the practical use of *A. senegalensis* as a drug for African trypanosomiasis, oral treatment will certainly be an extremely important factor; apart from the fact that most of the available drugs for human and animal trypanosomiasis are being administered through intravenous and intramuscular routes ;also, a simple mode of administration is needed in the rural areas. For these reasons ,it is therefore suggested that oral route of administration of *A. senegalensis* leaf extract should further be investigated.

Despite the fact that there is no record of the therapeutic value or doses of *Annona senegalensis* leaf extract in the treatment of trypanosomiasis infection, it is evident from the result of this work of the anti-trypanosome activity of *A. senegalensis* which is an indication of the accuracy of its suggested effectiveness against human infection with *T. brucei* and *T. rhodesiense*. Therefore it is suggested that further purification of the plant extract be carried out, and the active factor isolated and evaluated so that we could know what exactly is responsible for the trypanocidal activity in the plant extract. May be in this form, the drug could be used to treat different stages of *T. brucei* infection and possibly be used for prophylaxis.

It is also not going to be out of place if the leaf is extracted in different solvents other than the ones used in this work and the extract obtained is administered in the infected animals using different other vehicles apart from the one used in this work, Comparison can then be made between the results obtained from such trials and that obtained in this work. Perhaps in doing so a new fact might emerge that would enrich our understanding on the anti trypanosome activity of the plant.

It also suggested that subsequent work on *A.senegalensis* should compare the efficacy of its different parts in both the young and old plant and also at different seasons.This is important so as to be sure of the part ,the age and the season the extract of the plant is more active against trypanosome

Enantia chlorantha failed to completely cure the infected mice of their infection but showed considerable anti- trypanosome activity.It is suggested that trypanocidal actions of this plant be investigated further.This is important because of the claimed anti malarial effect of this plant, which will make it more attractive to drug developers because of the obviously more profit that could be made.

It may be wrong to quickly jump into conclusion that *Calotropis procera*, *Mitracarpus scabra* and *Okoubaka aubrevillei* do not have trypanocidal potential even though the results of the present work gave that indication. Perhaps something positive may come from these plants if further studies are done. It is therefore suggested that the plant be extracted in different other solvents and another vehicle be used when administering the drugs into the infected animals.

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APPENDIX I

AMOUNT OF EXTRACTS OBTAINED FROM POWDERED SAMPLE OF EACH PLANT

Plant	Part	Powder Weight(g)	solvent	Extract(g)
A. *senegalensis*	Leaf	70	Water	20
A. senegalensis	Leaf	70	Ethanol	25
A. senegalensis	Leaf	70	Methanol	23
A. senegalensis	Leaf	70	Acetone	22
A. senegalensis	Leaf	70	Hexane	3.74
A. senegalensis	Leaf	70	Ethylacetate	1.77
A. senegalensis	Leaf	70	Methanol	11.47
A. senegalensis	Root bark	50	Water	16.7
A. senegalensis	Root bark	50	Ethanol	20
A. *senegalensis*	Root bark	50	Methanol	18.33
A. *senegalensis*	Root bark	50	Acetone	21.6
A. *senegalensis*	Stem bark	50	Water	20
A. *senegalensis*	Stem bark	50	Ethanol	18.33
A. *senegalensis*	Stem bark	50	Methanol	20
A. *senegalensis*	Stem bark	50	Acetone	20
Calotropis procera	Leaf	60	Water	10
Calotropis procera	Leaf	60	Ethanol	14
Calotropis procera	Leaf	60	Methanol	17
Calotropis procera	Leaf	60	Acetone	8.4
Enantia *chlorantha*	Stem bark	50	Water	11.4

Enantia *chlorantha*	Stem bar	50	Ethanol	12.9
Enantia chlorantha	Stem bark	50	Methanol	12.29
Enantia chlorantha	Stem bark	50	Acetone	12.9
Mitracarpus scarbra	Leaf	60	Water	12
Mitracarpus scarbra	Leaf	60	Ethanol	16
Mitracarpus scarbra	Leaf	60	Methanol	18
Mitracarpus scarbra	Leaf	60	Acetone	18.4
Okoubaka aubrevillei	Stem bark	50	Water	20
Okoubaka aubrevillei	Stem bark	50	Ethanol	23.3
Okoubaka aubrevillei	Stem bark	50	Methanol	21.6
Okoubaka aubrevillei	Stem bark	50	Acetone	16.6

APPENDIX II

PREPARATION OF STOCK SOLUTION AND VARIOUS DOSAGES

STOCK SOLUTION

5ml of 100mg \ml solution was prepared by dissolving 0.5g of the sample in 5ml of physiological saline.

i.e 0.1g (100mg) ≡ 1ml physiological saline. 100mg of the sample in 1ml physiological saline hence 100mg /1ml.

i. Preparation of 200mg/kg body weight for the rat that weighed 125g.

200mg/kg means 200mg ≡ 1kg (1000g)

But the animal weighed 125g.

⇒ If 200 mg ≡ 1000g

xmg ≡ 125g

Xmg ≡ 25mg.

But the concentration of the stock solution is 100mg/1ml

⇒ If 100mg is dissolved in 1ml.

i.e 100mg ≡ 1ml

25mg ≡ yml

yml = 0.25ml

∴ 0.25ml of the extract was injected into 125g of the rat.

ii Preparation of 100mg/kg for the mouse that weighed 25g.

Dose 100mg/kg mean 100mg = 1 kg (1000g) but the animal weight 25g.

⇒ 100mg = 1000g

Xmg = 25g

Xmg = 2.5mg

But the concentration of the stock solution is 100mg/ml (100mg ≡ 1ml)

⇒ If 100mg ≡ 1ml

2.5mg ≡ yml

Yml = 0.025ml

∴ 0.025ml of the solution will be injected as 100mg/ kg of the extract into the animal

I.

APPENDIX III

Calculation of the dosage of crude extract injected in the mice for toxicity study

weight of the animals = 30g each

dose injected to the animals = 5000mgkg^{-1}body weight

Stock concentration = 100mg/ml.

⇒ 5000mgkg^{-1} body weight

= 5000mg ≡ 1kg (1000g)

But the animals weight 30g each.

⇒ 5000mg = 1000g

Xmg = 30g

⇒ xmg = 150mg

But the stock solution = 100mg/ml.

⇒ if 100mg = 1ml

150mg = xml

xml = 1.50ml

∴ 1.50ml was injected in to each of the two animals used for toxicity study.

APPENDIX IV

TABLE 1: TRYPANOCIDAL ACTIVITY OF AQUEOUS LEAF EXTRACT OF *ANNONA SENEGALENSIS* IN *T. brucei* INFECTED RATS A AND B MONITORED OVER A 12 - DAY PERIOD

POST TREATMENT PERIOD (DAYS)	NO OF PARASITES IN THE BLOOD PER MICROSCOPE FIELD	
	RAT A	RAT B
1	19	15
2	11	35
3	25	74
4	03	105
5	NIL	206
6	NIL	DEAD
7	NIL	
8	NIL	
9	NIL	
10	NIL	
11	NIL	
12	DEAD	

Rat A was treatment with 200mg/kg of the extract .

Rat B was the control (Infected but not treated).

APPENDIX V

TABLE 2A: TRYPANOCIDAL ACTIVITY OF AQUEOUS LEAF EXTRACT OF *ANNONA SENEGALENSIS* IN INFECTED MICE A - E MONITORED OVER A FOUR MONTH PERIOD

	NO OF PARASITES IN THE BLOOD PER MICROSCOPE FIELD				
POST TREATMENT PERIOD (DAYS)	A	B	C	D	E
1	02	02	05	02	02
2	19	18	12	35	32
3	NIL	NIL	NIL	DEAD	DEAD
4	NIL	NIL	NIL		
5	NIL	NIL	NIL		
6	NIL	NIL	DEAD		
7	NIL	NIL			
8	NIL	NIL			
9	NIL	NIL			
10	NIL	DEAD			
120	NIL				

Mice A, B and C were each treated with 200mg/kg body weight of the extract.

Mice D and E were the Control (Infected but not treated).

TABLE 2B: COURSE OF PARASITEMIA IN FOUR INFECTED MICE A - D MONITORED OVER A TWO MONTHS PERIOD

POST TREATMENT PERIOD (DAYS)	NO OF PARASITES IN THE BLOOD PER MICROSCOPE FIELD			
	A	B	C	D
1	NIL	NIL	NIL	NIL
2	NIL	NIL	NIL	NIL
3	NIL	NIL	NIL	NIL
60	NIL	NIL	NIL	NIL

Mice A and B were each innocolated with 0.02ml of CSF, while Mice C and D were each innoculated with 0.02ml of the blood obtained from one of the cured mice with the aqueous leaf extract of *Annona senegalensis*.

APPENDIX VI

TABLE 3: ANTI TRYPANOSOME ACTIVITY OF ETHYL ACETATE EXTRACT OF *ANNONA SENEGALENSIS* LEAF IN 3 MICE A, B AND C

POST TREATMENT PERIOD (DAYS)	NUMBER OF PARASITES IN THE BLOOD PER MICROSCOPE FIELD		
	A	B	C
1	01	01	01
2	13	13	12
3	62	60	62
4	DEAD	DEAD	DEAD

Mice A and B were each treated with 200mg/kg body weight in 0.02ml physiological saline.

Mouse C is the control.

APPENDIX VII

TABLE 4: TRYPANOCIDAL ACTIVITY OF HEXANE EXTRACT OF *ANNONA SENEGALENSIS* LEAF IN THREE INFECTED MICE A - C

POST TREATMENT PERIOD (DAYS)	NUMBER OF PARASITES IN THE BLOOD PER MICROSCOPE FIELD		
	A	B	C
1	01	01	01
2	19	09	12
3	59	54	62
4	DEAD	DEAD	DEAD

The table shows bioassay of the of the hexane extract of *Annona senegalensis* leaf mice.

A and B were each administered with 200mg/kg body weight of the extract.

C is the control.

APPENDIX VIII

TABLE 5: TRYPANOCIDAL ACTIVITY OF METHANOLIC EXTRACT OF *ANNONA SENEGALENSIS* LEAF IN THREE INFECTED MICE A - C

POST TREATMENT PERIOD (DAYS)	NUMBER OF PARASITES IN THE BLOOD PER MICROSCOPE FIELD		
	A	B	C
1	01	01	01
2	03	05	12
3	09	37	62
4	36	DEAD	DEAD
5	DEAD		

Bioassay of the methanolic extract of *Annona senegalensis* leaf.

Mice A and B were each treated with 200mg/kg body weight of the extract.

Mouse C is the control.

APPENDIX IX

TABLE 6: TRYPANOCIDAL ACTIVITY OF THE FRACTIONATED SAMPLES OF THE AQUEOUS EXTRACT OF *ANNONA SENEGALENSIS* LEAF IN SIX GROUPS OF INFECTED MICE MONITOR OVER A FOUR MONTH PERIOD

		NUMBER OF PARASITES IN THE BLOOD PER MICROSCOPE FIELD						
GROUP		POST TREATMENT PERIODS (DAYS)						
		1	2	3	4	5	6	120
A	i	01	07	41	DEAD			
	ii	01	06	40	DEAD			
B	i	01	11	39	DEAD			
	ii	01	09	69	DEAD			
C	i	01	08	50	DEAD			
	ii	01	13	53	DEAD			
D	i	01	11	49	DEAD			
	ii	01	06	45	DEAD			
E	i	01	11	NIL	NIL	NIL	NIL	NIL
	ii	01	10	NIL	NIL	NIL	NIL	NIL
F	i	01	07	44	DEAD			
	ii	01	10	39	DEAD			
G	i	01	11	72	DEAD			
	ii	01	10	70	DEAD			

Two animals (i and ii) per group of the infected mice were treated with 200mg/kg body weight of each sample from fractions 1 - 6 obtained from column chromatographic fractionation of the extract

Group G is the control

APPENDIX X

TABLE 7: TRYPANOCIDAL ACTIVITY OF THE AQUEOUS EXTRACT OF *ANNONA SENEGALENSIS* STEM BARK IN FIVE INFECTED MICE A- E

POST TREATMENT PERIOD (DAYS)	NUMBER OF PARASITES IN THE BLOOD PER MICROSCOPE FIELD				
	A	B	C	D	E
1	02	02	02	02	02
2	20	33	18	73	82
3	DEAD	DEAD	DEAD	DEAD	DEAD

Preliminary bioassay of the aqueous extract of *Annona senegalensis* stem bark.

Mice A, B, C and D were each treated with 100, 150, 200 and 250mg/kg body weight of the extract respectively.

Mouse E is the control (infected but not treated).

APPENDIX XI

TABLE 8: TRYPANOCIDAL ACTIVITY OF THE AQUEOUS EXTRACT OF ANNONA SENEGALENSIS STEM BARK IN 3 INFECTED MICE A - C

POST TREATMENT PERIOD (DAYS)	NUMBER OF PARASITES IN THE BLOOD PER MICROSCOPE FIELD		
	A	B	C
1	01	02	02
2	05	12	74
3	10	DEAD	DEAD
4	DEAD		

Bioassay of the aqueous extract of *Annona senegalensis* stem bark.

Mice A and B were each treated with 100 and 200mg/kg body weight of the extract respectively

Mouse C is the control.

APPENDIX XII

TABLE 9: TRYPANOCIDAL ACTIVITY OF AQUEOUS EXTRACT OF ANNONA SENEGALENSIS STEM BARK IN 3 INFECTED MICE A - C

POST TREATMENT PERIOD (DAYS)	NUMBER OF PARASITES IN THE BLOOD PER MICROSCOPE FIELD		
	A	B	C
1	04	01	01
2	29	09	12
3	DEAD	DEAD	DEAD

Bioassay of the aqueous extract of *Annona senegalensis* stem bark.

Mice A and B were treated with 300 and 350mg/kg body weight of the extract respectively.

Mouse C is the control.

APPENDIX XIII

TABLE 10: ANTI-TRYPANOSOME ACTIVITY OF THE AQUEOUS EXTRACT OF ROOT BARK OF *ANNONA SENEGALENSIS* IN SIX INFECTED MICE (A - F)

POST TREATMENT PERIOD (DAYS)	NUMBER OF PARASITES IN THE BLOOD PER MICROSCOPE FIELD					
	A	B	C	D	E	F
1	09	01	02	02	02	01
2	50	03	05	14	52	74
3	DEAD	25	22	85	DEAD	DEAD
4		DEAD	DEAD	DEAD		

Preliminary bioassay of the aqueous extract of *Annona senegalensis* root bark.

Mice A - D were treated with 100, 150, 200 and 250mg/kg body weight of the extract

Mice E and F were the control (Infected but not treated).

APPENDIX XIV

TABLE 11: ANTI - TRYPANOSOME ACTIVITY OF THE AQUEOUS EXTRACT OF *ANNONA SENEGALENSIS* ROOT BARK IN 4 INFECTED MICE A - D

POST TREATMENT PERIOD (DAYS)	NUMBER OF PARASITES IN THE BLOOD PER MICROSCOPE FIELD			
	A	B	C	D
1	02	02	02	02
2	07	05	13	57
3	DEAD	DEAD	73	DEAD
4			DEAD	

Bioassay of the aqueous extract of *Annona senegalensis* root bark.

Mice A - C were each treated with 200mg/kg body weight of the extract.

Mouse D is the control.

APPENDIX XV

TABLE 12: ANTI-TRYPANOSOME ACTIVITY OF THE AQUEOUS EXTRACT OF *ANNONA SENEGALENSIS* ROOT BARK IN 3 INFECTED MICE

POST TREATMENT PERIOD (DAYS)	NUMBER OF PARASITES IN THE BLOOD PER MICROSCOPE FIELD		
	A	B	C
1	01	01	01
2	17	02	05
3	DEAD	29	67
4		DEAD	DEAD

Bioassay of the aqueous extract of *Annona Senegalensis* root bark.

Mice A and B were each treated 200 and 250mg/kg body weight respectively.

Mouse C is the control.

APPENDIX XVI

TABLE 13: ANTI - TRYPANOSOME ACTIVITY OF THE ETHANOLIC EXTRACT OF *ANNONA SENEGALENSIS* ROOT BARK IN 3 INFECTED MICE A - C

POST TREATMENT PERIOD (DAYS)	NUMBER OF PARASITES IN THE BLOOD PER MICROSCOPE FIELD		
	A	B	C
1	01	01	01
2	05	11	12
3	52	35	60
4	DEAD	DEAD	DEAD

Bioassay of ethanolic extract of the root bark of *Annona senegalensis*.

Mice A and B were each treated with 200mg/kg body weight of the extract.

Mouse C is the control.

APPENDIX XVII

TABLE 14: ANTI TRYPANOSOME ACTIVITY OF METHANOLIC EXTRACT OF *ANNONA SENEGALENSIS* ROOT BARK IN 3 INFECTED MICE A - C

POST TREATMENT PERIOD (DAYS)	NUMBER OF PARASITES IN THE BLOOD PER MICROSCOPE FIELD		
	A	B	C
1	01	01	01
2	05	18	18
3	40	62	65
4	DEAD	DEAD	DEAD

Bioassay of the methanolic extract of the root bark of *Annona senegalensis*.

Mice A and B were each treated with 200mg/kg body weight of the extract.

Mouse C is the control.

APPENDIX XVIII

TABLE 15: TRYPANOCIDAL ACTIVITY OF THE AQUEOUS EXTRACT OF *ENANTIA CHLORANTHA* IN THREE INFECTED MICE A - C MONITORED OVER A 17-DAY PERIOD

POST TREATMENT PERIOD (DAYS)	NUMBER OF PARASITES IN THE BLOOD PER MICROSCOPE FIELD		
	A	B	C
1	01	01	01
2	12	19	68
3	32	33	DEAD
4	25	DEAD	
5	17		
6	14		
7	05		
8	02		
9	01		
10	03		
11	02		
12	08		
13	13		
14	56		
15	55		
16	113		
17	DEAD		

Preliminary bioassay of the aqueous extract of *Enantia Chlorantha* stem bark.

Mice A and B were each treated with 100 and 200mg/kg body weight of the extract.

Mouse C is the control (infected but not treated).

APPENDIX XIX

TABLE 16: TRYPANOCIDAL ACTIVITY OF THE AQUEOUS EXTRACT OF *ENANTIA CHLORANTHA* STEM BARK IN FOUR INFECTED MICE A-D

POST TREATMENT PERIOD (DAYS)	NUMBER OF PARASITES IN THE BLOOD PER MICROSCOPE FIELD			
	A	B	C	D
1	02	01	02	02
2	18	03	09	81
3	75	18	44	DEAD
4	100	27	67	
5	DEAD	44	100	
6		11	DEAD	
7		15		
8		55		
9		DEAD		

Bioassay of the aqueous extract of *Enantia Chlorantha* stem bark.

Mice A - C were each treated with 100mg/kg body weight of the extract.

Mouse D is the control.

APPENDIX XX

TABLE 17: TRYPANOCIDAL ACTIVITY OF DEFATED EXTRACT OF *ENANTIA CHLORANTHA* STEM BARK IN 4 INFECTED MICE A - D

POST TREATMENT PERIOD (DAYS)	NUMBER OF PARASITES IN THE BLOOD PER MICROSCOPE FIELD			
	A	B	C	D
1	01	01	01	01
2	03	10	11	11
3	39	55	95	90
4	DEAD	DEAD	DEAD	DEAD

Bioassay of defated extract of *Enantia Chlorantha* stem bark.

Mice A and B were each treated with 100mg/kg body weight of the extract.

Mouse C was treated with 200mg/kg body weight of the extract

Mouse D is the control.

APPENDIX XXI

TABLE 18: TRYPANOCIDAL ACTIVITY OF PETROLEUM ETHER EXTRACT (FATTY COMPONENT) OF *ENANTIA CHLORANTHA* STEM BARK IN THREE INFECTED MICE A - C

POST TREATMENT PERIOD (DAYS)	NUMBER OF PARASITES IN THE BLOOD PER MICROSCOPE FIELD		
	A	B	C
1	01	01	01
2	15	25	23
3	52	40	58
4	DEAD	DEAD	DEAD

Bioassay of the fat obtained from *Enantia Chlorantia* stem bark

Mice A and B were each treated with

3mol of the extract.

Mouse C is the control

APPENDIX XXVI

TABLE 23: ANTI-TRYPANOSOME ACTIVITY OF THE AQUEOUS EXTRACTS OF *OKOUBAKA AUBREVILLEI* IN THREE INFECTED MICE A - C

POST TREATMENT PERIOD (DAYS)	NUMBER OF PARASITES IN THE BLOOD PER MICROSCOPE FIELD		
	A	B	C
1	01	01	01
2	03	02	17
3	32	21	100
4	DEAD	DEAD	DEAD

Bioassay of the aqueous extract of *Okoubaka aubrivillei.*

Mice A and B were treated with 250 and 300mg/kg body weight of the extract.

Mouse C was the control.

APPENDIX XXVII

TABLE 24: PROPHYLACTIC ACTIVITY OF CRUDE EXTRACTS OF *ANNONA SENEGALENSIS* LEAF AND *ENANTIA CHLORANTHA* STEM BARK AGAINST TWO GROUPS OF *T. BRUCEI* INFECTED MICE (A AND B)

POST TREATMENT PERIOD (DAYS)	NUMBER OF PARASITES IN THE BLOOD PER MICROSCOPE FIELD	
	A	B
1	01	01
2	06	11
3	80	62
4	DEAD	DEAD

Prophylactic activity of crude extracts of *Annona Senegalensis* and *Enantia Chlorantha.*

Group A and B were treated with 200mg/kg and 100mg/kg body weight of the extract in 0.02ml physiological saline of *Annona Senegalensis* and *Enantia Chlorantha* respectively daily for 4 days before they are being innoculated with the parasite.

APPENDIX XXII

TABLE 19: ANTI-TRYPANOSOME ACTIVITY OF THE AQUEOUS EXTRACT OF *CALOTROPIS PROCERA* IN 3 INFECTED MICE

POST TREATMENT PERIOD (DAYS)	NUMBER OF PARASITES IN THE BLOOD PER MICROSCOPE FIELD A	B	C
1	02	01	01
2	25	10	09
3	76	72	74
4	DEAD	DEAD	DEAD

Bioassay of the aqueous extract of *calotropis procera*.

Mice A and B were each treated with 200 and 100mg/kg body weight in 0.02ml physiological saline respectively.

Mouse C is the control.

APPENDIX XXIII

TABLE 20: TRYPANOCIDAL ACTIVITY OF THE AQUEOUS EXTRACT OF *MITRACARPUS SCABRA* IN THREE INFECTED MICE (A - C)

POST TREATMENT PERIOD (DAYS)	NUMBER OF PARASITES IN THE BLOOD PER MICROSCOPE FIELD A	B	C
1	01	01	01
2	27	30	33
3	DEAD	DEAD	DEAD

Preliminary of the bioassay of the aqueous extract of *mitracapus scabra*.

Mice A and B were each treated with 100 and 200mg/kg body weight of the extract.

Mouse C was the control.

APPENDIX XXIV

TABLE 21: TRYPANOCIDAL ACTIVITY OF THE AQUEOUS EXTRACT OF *MITRACARPUS SCABRA* IN THREE INFECTED MICE (A - C)

POST TREATMENT PERIOD (DAYS)	NUMBER OF PARASITES IN THE BLOOD PER MICROSCOPE FIELD		
	A	B	C
1	01	01	01
2	05	17	17
3	70	DEAD	DEAD
4	DEAD		

Bioassay of the aqueous extract of *mitracarpus scabra.*

Mice A and B were each treated with 100mg/kg body weight of the extract

Mouse C is the control (infected but not treated).

APPENDIX XXV

TABLE 22: ANTI-TRYPANOSOME ACTIVITY AQUEOUS EXTRACT OF *OKOUBAKA ABREVILLEI* IN THREE INFECTED MICE A -C

POST TREATMENT PERIOD (DAYS)	NUMBER OF PARASITES IN THE BLOOD PER MICROSCOPE FIELD		
	A	B	C
1	02	01	02
2	10	02	90
3	DEAD	24	DEAD
4		DEAD	

Preliminary of the bioassay of aqueous extract of the stem bark of *Okoubaka Aubrevillei.*

Mice A and B were each treated with 100 and 200mg/kg body weight of the extract.

Mouse C was the control.