TITLE PAGE

EFFECTS OF AQUEOUS LEAF EXTRACTS OF *LORANTHUS MICRANTHUS* LINN. (LORANTHACEAE) ON BIOCHEMICAL AND HAEMATOLOGICAL PROFILES OF ALBINO RATS INFECTED WITH *TRYPANOSOMA BRUCEI BRUCEI*

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A PROJECT SUBMITTED IN PARTIAL FULFILMENT FOR THE AWARD OF MASTERS DEGREE IN ANIMAL PHYSIOLOGY IN THE DEPARTMENT OF ZOOLOGY AND ENVIRONMENTAL BIOLOGY, FACULTY OF BIOLOGICAL SCIENCES, UNIVERSITY OF NIGERIA, NSUKKA.

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OCTOBER, 2014

APPROVAL PAGE

I approve that this project was carried out under my supervision by Egbuji, Jude Victor Ifeanyi, in the Department of Zoology, University of Nigeria, Nsukka in partial fulfillment of the requirement leading to the award of Masters Degree in Animal Physiology

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DEDICATION

This work is dedicated to God Almighty whose love and grace lifted me up to this level

ACKNOWLEDGEMENT

My profound gratitude goes to God through whom all things were made possible, for the good health, strength, grace and wisdom he bestowed on me throughout this period of study. A special thank goes to my insightful and resourceful project supervisor Dr. V.C. Ejere, for his selfless service, intimidating humility, assistance and advice towards the progress of the work. The Head of Zoology Department, Dr. N. Ivoke and the P.G coordinator, Prof. B.O. Mgbenka should not be left unmentioned, your moral support are ever appreciated. To my mentor, my friend and my adviser in the name of Dr. Nwaigwe, who showed fatherly advice, counseling and academic support to this work, your efforts is ever remembered. My appreciation goes to my beloved parents who always stood by my side in good and in bad times, their moral, financial and social support and encouragement to me cannot be overemphasized. Also worthy of my thanks are Miss Ogochukwu Okeke and my good friends Godwin Ugwu, Chinagorom Okanya, Asogwa Chinyere Obiageli, Asogwa Norman, Chiemeka Ezechukwu and all the PG students in the Department who in one way or the other has helped in actualizing this work. I thank you all. God bless you

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Abstract

The effects of oral administration of aqueous crude seed extracts of *Loranthus micranthus* on the biochemical and haematological parameters of albino rats infected with *Trypanosoma brucei brucei* were investigated for 28days. A total of seventy-two adult-male albino rats were divided into six groups: 3 treatment groups (400 mg/kg and 800 mg/kg and 1200mg/kg), infected and untreated control, one standard drug group and one normal control groups of twelve rats each. Each group was further replicated three times with four rats per replicate. The treatment groups were administered 400 mg/kg and 800 mg/kg and 1200mg/kg of aqueous leaf extracts of *L. micranthus* according to their body weights. Level of parasitaemia was ascertained in rats that were inoculated with *T. brucei brucei* at Day zero and subsequently every two days until the end of treatment. Blood samples were collected on weekly basis for various biochemical and haematological parameters using standard methods and assay kits. Two-way ANOVA was used to determine interactive effects of treatment and time while One-way ANOVA was used to test the effect of treatment. The phytochemical screening revealed high concentrations of tannins and flavonoids; moderate concentrations of alkaloids, saponins and steroids; and trace concentrations of glycosides and terpenes to be present in the aqueous crude extracts of *L. micranthus* leaves. The LD_{50} of the crude leaf extracts of *L. micranthus* showed no mortality at dose levels of 5000 mg/kg after twenty four hours. A significant decrease ($P < 0.05$) was observed in the mean values of body weights of the infected and treated animals throughout the duration of the experiment. Data from the study revealed that the level of parasitaemia in all the infected animals together with the infected and untreated control group was significantly high ($P < 0.05$) in comparison with those of the standard drug and normal control groups throughout the duration of the experiment. Liver marker enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) of the infected and treated rats as well as those of the infected and untreated control group showed marked significant increases ($P < 0.05$) in comparison with the standard drug and normal control groups. Similarly, data obtained show that serum levels of bilirubin, urea, creatinine and cholesterol were significantly high ($P < 0.05$) in the infected animals and in the infected and untreated control group. Serum albumin was significantly lower ($P > 0.05$) in the treatment groups with minimal increases in the group administered 800mg/kg of the aqueous leaf extract. The result obtained from the haematological analysis revealed that the haemoglobin (HB), packed cell volume (PCV), red blood cell (RBC) and its indices (mean cell haemoglobin, MCH, mean cell volume, MCV and mean cell haemoglobin, MCHC) of the infected rats significantly decreased ($P < 0.05$) at various dose levels of the aqueous leaf extract of *L. micranthus* when compared with the standard drug and normal control groups. The white blood cells (WBC) of the infected and treated rats and those of the infected and untreated control group showed significant increases ($P < 0.05$) in WBC counts when compared with the normal control group in all the weeks. WBC differentials revealed that neutrophils were significantly higher ($P < 0.05$) in the infected and treated animals in comparison to the standard drug control group in week 3, however lymphocytes, eosinophils, basophils were not significantly different ($P > 0.05$) from the standard drug control group in week 3 of extract administration. Furthermore, minimal increases in the WBC differentials were observed in the group administered 800mg/kg of the aqueous leaf extract of *L. micranthus.* Data generated in the present study showed that all infected and treated rats as well as the infected and untreated control group died from the resultant overwhelming parasitaemia unlike the case of those administered the standard drug. This is an indication that the extract lacks any anti-tryopanocidal activity. Thus, the aqueous leaf extract of *Loranthus micranthus* inhabiting the host plant *Kola acuminata* is an inadequate anti-trypanosomal agent.

CHAPTER ONE

1.1 Introduction

The incidence of trypanosomiasis remains a source of concern in the tropics and other parts of the world. Trypanosomiasis is a lethal disease which affects both man and animals; and caused by a parasitic protozoa of the Genus *Trypanosoma* (Adeiza *et al.*, 2010). This parasite is transmitted by a vector, tsetse fly, most of which belong to the species *Glossina palpalis*. The distribution of trypanosomiasis corresponds roughly with that of tse-tse flies. Trypanosomiasis, which is also known as sleeping sickness is caused by *Trypanosoma brucei gambiense* and or *Trypanosoma brucei rhodesiense* following an infective bite from tse-tse fly (Welburn *et al.*,2001; Haydon *et al.*, 2002; WHO, 2006;).

Transmission of this parasite is mostly through the bite of an infected tse-tse fly; however there are other ways in which people can be infected. These include ï mother to child transmission through the placenta, mechanical transmission through other blood sucking insects (though it is difficult to assess the epidemiological impact of this mode of transmission), accidental transmissions/infection due to pricks from contaminated needles in the laboratory (Seed, 1998; WHO, 2006; Kennedy, 2006).

Trypanosomiasis and its vectors occur in vast areas of the sub-Saharan Africa with devastating impact on livestock productivity as well as posing a serious threat to the lives and livelihood of entire communities (Doua and Yapo, 1993; Rates, 2001; Engels and Savioli, 2006). Trypanosomiasis infestation constitutes the greatest single constraint to livestock and crop production thereby directly contributing to hunger, poverty, protein malnutrition and suffering of entire communities in Africa. This is as its name suggest, infected people tend to sleep a lot, leading to loss of man hours that could have been applied to productive farm work (Murray, 1994; Aroke *et al.*,1998 Jodi *et al.*, 2011).

Treatment of trypanosomiasis infection is dependent on the stage of infection (that is whether it is chronic and or acute infection). Normally the drugs used in the initial stage of the disease infection are of lower toxicity and easier to administer (Burri *et al.*, 2001; Chappius *et al.,* 2005). The earlier the disease is identified the better the prospect of a cure. Treatment success in the second stage of infection (that is the advanced stage) depends on a drug that can cross the bloodbrain barrier to reach the parasite. Some of the drugs used include; Pentamidine, Suramin, and Eflornithine (Burri *et al.,* 2001; Chappius *et al.,* 2005; WHO, 2008).

Despite the efficacious nature of these drugs, researchers have directed their energies into research projects designed to screen local medicinal plants as potential trypanocides. Although some herbal formula are already in circulation (such as Jubi herbal formula, African herbal formula among others) efforts are made to develop effective drugs from medicinal plants for both the management and treatment of trypanosomiasis (Okochi, *et al.,* 2003; Erah, *et al*., 2003).

The African mistletoe, *Loranthus micranthus* Linn is an ubiquitous hemiparasitic plant that thrives well in tropical climates (Obatomi *et al.*, 1996). It depends on its host for mineral salts and water but can photosynthesize its own carbohydrates. Mistletoe grows on a wide range of evergreen and deciduous trees. Host plants of mistletoe include *Persea americana, Kola accuminata, Baphia nitida, Treculia africana* etc (Nzekwe *et al.,* 2009). Mistletoe has a long history of traditional use for a wide range of diseases such as diabetes, diarrhea, epilepsy etc. Due to its medicinal value/uses and pharmacological activities, mistletoe have been revealed to have a great potential for its use in various systemic and non-systemic infections due to bacteria and fungi (Osadebe and Ukwueze, 2004).

This study is therefore designed to investigate the trypanocidal effects of aqueous leaf extracts of African mistletoe, *Loranthus micranthus* on the biochemical and haematological profile of albino rats infected with *Trypanosoma brucei.*

1.2 Aims and Objective of the Study

i. To determine the effect of the aqueous leaf extract on the body weight of the infected rats.
ii. To ascertain the level of parasitaemia in albino rats infected with trypanosome.
iii. To determine the effect of the aqueous leaf extract of mistletoe on the serum biochemistry of albino rats infected with trypanosome.
iv. To ascertain the effect of the aqueous leaf extract of mistletoe on the haematological profile of albino rats infected with trypanosome.

1.3 Literature Review

1.3.1 Epidemiology

Trypanosomiasis or sleeping sickness is endemic in some regions of sub-Saharan Africa, covering about 37 countries and about 60 million people. It is estimated that about 50,000 to 70,000 people are currently infected, the number having declined somewhat in recent years (Donelson *et al.*, 1998).

Although the two protozoan parasites transmitted by tse-tse fly have identical morphologic appearance, clinical infections differ in presentation, prognosis, and subspecies involved. At present there is no overlap in their geographical distribution (Adeiza *et al*., 2008).

T. brucei gambiense (causative agent of West African Sleeping Sickness) is predominantly found in Central Africa and in limited areas of West Africa. Most of the

sleeping sickness disease in Africa is caused by this form of the parasite. Epidemic outbreaks in the past have been a significant public health problem. However, the disease has been reasonably controlled and well managed at present with about estimated 10,000 cases reported in recent years (Truc, 2003). About 90%-95% of the cases and of human infection are found in Democratic Republic of Congo, Angola, Sudan, Central African Republic, Chad and Northern Uganda. Humans constitute an important reservoir of infection, although the parasite can sometimes be found in domestic animals such as cattle, goats, pigs etc (Truc, 2003).

T. brucei rhodesiense (the causative agent of East African Sleeping sickness) is found in focal areas of Eastern and Southeastern Africa, with a few hundred cases each year as reported by World Health Organization (W.H.O.). Over 95% of the cases of human infection occur in Uganda, Tanzania, Malawi and Zambia (WHO, 2006). In this region animals are the primary reservoir of infection. Cattle have been implicated in the spread of the disease to new areas and in local outbreaks within this region. This animal reservoir is thought to be responsible for the sporadic transmission of the disease to hunters and visitors to game parks as well as travelers/tourists who are on safari in East Africa (WHO, 2008).

Another form of trypanosomiasis occurs mainly in 21 Latin American countries. It is known as American trypanosomiasis or Chagas disease. This is transmitted by the organism called the assassin bug which acts as a vector for *Trypanosoma cruzi* (Hunter *et al.,* 1992; Murray, 1994).

The vector, tse-tse fly, inhabit rural areas, living in the woodlands that dot the East African Savannah. In Central and West Africa they are found in the forests and vegetations along streams. Tse-tse flies bite during the daylight hours. Both male and female flies can transmit the infection (Alafiatayo *et al*., 2003). Although the vast majority of infections are

transmitted by the tse-tse fly, other modes of transmission are possible. In theory, the infection can also be transmitted by blood transfusion or sexual contact but such cases have rarely been documented (Adewummi *et al*., 2001).

1.3.2 Disease Burden of Trypanosomiasis

Trypanosomiasis is restricted to sub-Saharan Africa in the range of the tse-tse fly vector. The distributions of tse-tse fly (possibly more than 30 species) and of the parasites within the vector range, constitute a public health problem where the vector, the parasite (and its reservoir hosts) and humans co-exist (WHO, 2003). It is estimated that about 50,000 to 70,000 people are currently infected, the number having declined somewhat in recent years. It is believed that many cases go unreported, the number of reported cases in 2008 was below 10,000; the first time in 50 years (WHO, 2008).

Four major epidemics have occurred in recent history in Africa

i. One from 1896 ï 1906 primarily in Uganda and the Congo basin.
ii. Two epidemics in 1920 and 1970 in several African countries
iii. A recent epidemic in 2008 in Uganda. (Truc *etal.*, 2002).

The transmission of trypanosomiasis occurs primarily in rural areas that are at the furthest extremities of the formal health system, creating particular problems for patients to access healthcare, for control campaigns to have effective outreach, and importantly, in the assessment of the burden of infections, handling efforts to collect data on how many people are at risk, how many people are infected, and most importantly, the impact of the disease on the social environment (Truc, 2003).

1.3.3 Life-Cycle of *Trypanosoma* Parasite

Human African trypanosomes are transmitted by several species of tse-tse flies of the genus *Glossina*. It is generally accepted that *T. brucei* var. *rhodesiense* is transmitted by tsetse flies (of the *G. morsitans* group). Development of the parasite in the tse-tse fly commences when an uninfected fly bites an infected vertebrate, ingesting trypomastigote forms of *T. brucei* (Seed, 1998). The trypanosomes in the vertebrateôs blood migrate into the vectorôs midgut, where the short stumpy (SS) forms complete the development of their mitochondrion and change their surface coat to differentiate into the long, slender procyclic stages. As the procyclic stages have a fully developed mitochondrion and polysomes highly loaded with mRNA, they exhibit significantly higher levels of metabolic activity and protein synthesis than do the bloodstream stages (Brecht and Parsons 1998). These stages have Krebôs cycle enzymes and actively respire using an electron transport system. This mitochondrial development also brings about a change in the positional relationship between the nucleus and the kinetoplast-mitochondrial complex, as well as in flagellar motion and hence trypanosomal motility (Braakman *et al.*, 2006). The procyclic forms develop further, undergoing more morphological changes and, after numerous cycles of multiplication, migrate into the vectorôs salivary glands and differentiate into epimastigotes which attach to the cells of the gland and continue multiplying. Eventually, some epimastigotes undergo a final transformation stage into non-dividing metacyclic trypomastigotes which are short, stumpy and highly motile. They lack free flagella, and have a terminally located kinetoplast. Mature metacyclic trypomastigotes detach from the salivary gland cells, synthesize a surface coat and are then able to infect a vertebrate bitten by the vector. This completion of development of the vector stages in the salivary glands (anterior station), and subsequent inoculative transmission to the mammalian host (Seed,1998).

After metacyclic trypomastigotes have been transmitted from the tsetse fly to the vertebrate host, they transform into long slender (LS) forms (20-40 x 0.1 um). The LS forms lack cytochromes and several Krebs cycle enzymes, and generate ATP solely by glycolysis (Braakman *et al.*, 2006). These forms multiply by binary fission until a threshold population is reached, whereupon a switch occurs, resulting in LS forms transforming first into intermediate forms and then into SS forms (15-25 x3.5 um). This transition involves cell cycle arrest and a decrease in protein synthesis, mediated by a reduction in ribosome loading (Brecht and Parsons 1998). The SS forms are morphologically and functionally very different. They do not divide and have no free flagellum. Considerable changes are also seen in their kinetoplast-mitochondrial complex ï the kinetoplast is posteriorly located, and the first stages of functional mitochondrial development are seen. It is likely that the SS form is the infective for the tse-tse, and the switch from a predominance of LS forms to SS forms is therefore essential for the cycle to continue (Seed, 1998).

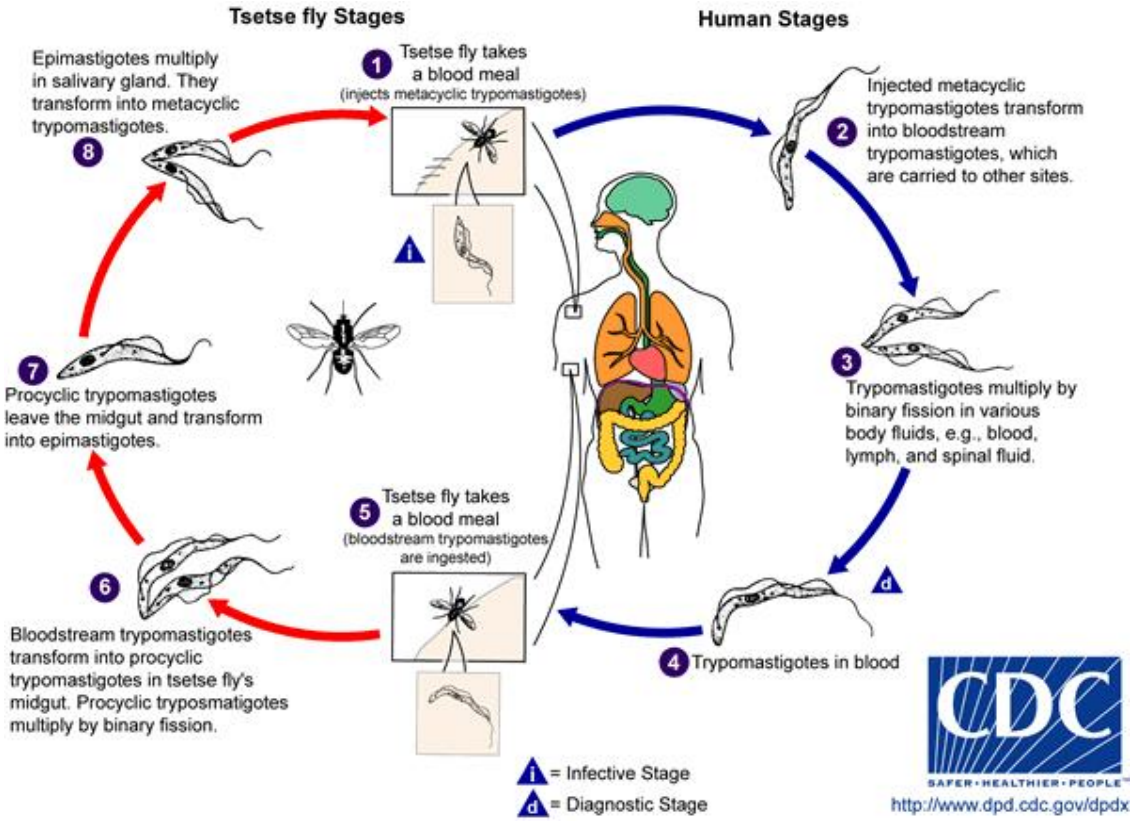

Fig.1: Life cycle of *Trypanosoma* parasite Source: WHO (2006).

1.3.4 Pathology/Pathogenesis of the Disease

Early involvement of the cardiovascular and lymphatic systems results in perivascular cellular infiltration, haemorrhage and oedema (Pentreath, 1995). Widespread meningeal inflammation with injury to the choroid plexus soon follows, resulting in choroid plexus breakdown, allowing parasites to enter into the cerebrospinal fluid (CSF) and infiltrate periventricular areas and a relatively thin blood-brain barrier. Although parasite entry into the CSF is achieved at this early state, trypanosomes usually remain undetectable in the CSF until considerably later (weeks in the case of *T. brucei* var. *rhodesiense* and months in *T. brucei* var. *gambiense* infection). This is probably because the CSF is a relatively hostile environment for trypanosomal survival. However, permeation into the CSF allows trypanosomes of the subarachnoid space, which penetrate deep into cerebral tissue (Hunter and Kennedy, 1992). This generates an intense inflammatory reaction, resulting in generalized perivascular cuffing with T helper and B lymphocytes and morular cells (plasma cells producing IgM), particularly in the cerebellum and brainstem, and marks the transition from meningitis to encephalitis. There is also evidence of astrocyte and microglial hyperplasia and microglial activation (Chianella et al., 1999). The cellular infiltrates described suggest that the immune response against trypanosomal invasion of the central nervous system is primarily a T-dependent B cell response(Alafiatayo *et al.*, 2003).

As disease progresses, vasogenic oedemas are seen, with characteristic changes in brain tissue density and electrolyte levels. The blood-brain barriers progressively more damaged. Trypanosomes infiltrate areas of cerebral parenchyma with relatively little blood-brain barrier (e.g pineal gland, median eminence) and spinal sensory ganglia (Poltera, 1985). Except in terminal disease, trypanosomal invasion of brain tissue is rare. Expression of major histocompatibility complex (MHC) Class I molecules is upregulated in parasite-infiltrated

parenchyma; this is accompanied by a cellular influx of $CD8^+$ T lymphocytes and macrophages, and may be mediated largely by interferon-gamma (IFNγ) (Hertz *et al.*, 1998).

The biochemical disturbances produced by trypanosomal invasion of the CNS also extend to chemicals not directly related to the immune response. Several monoamine neurotransmitters have been shown to have increased turnover (Stibbs and Curtis, 1987), and there is substantial overproduction of lipid mediators, such as prostaglandins D_2 and $F_{2\alpha,}$ the release of which is the result of synergistic activity between endotoxin and various trypanosomal products (Pentreath *et al.,* 1990). Endotoxaemia is a common occurrence in both the blood and CSF of patients with late-stage trypanosomiasis (Alafiatayo *et al.,* 2003). The source of the endotoxin is unclear, but non-specific endotoxin-like substances may be released by trypanosomes, and intestinal and hepatic damage could contribute by producing increased bacterial translocation and reduced clearance, respectively (Chechet *et al.,* 2010).

1.3.5 Immunology

The immune response to African trypanosome infection is complex and remains poorly elucidated (Donelson *et al*., 1998). The immune response is challenged by a series of rapidly changing variant surface glycoproteins (VSGs), together with thousands of invariant antigens, as if the host were being invaded by a series of organisms that were closely related but not identical. The inaccessibility of the invariant antigens in the living trypanosome prevents effective clearing of the parasite. The response is characterized by two major phenomena:

Massive polyclonal activation of B cells, resulting in the release of large amounts of IgM (Donelson *et al.,* 1998). This response is stimulated partly by the changing VSG epitopes, but the antibodies produced are heterospecific and bind other trypanosomal antigens as well as host proteins and nucleic acids. The elevated levels of IgM result in the formation

of large numbers of immune complexes, which lead to reticuloendothelial hyperplasia. Although the precise stimuli are unknown, it is likely that a trypanosomal antigen acts as the mitogen driving this hyper-responsiveness (Hunter *et al.,* 1991).

Generalized suppression of both T and B cell function, resulting in a failure of humoral and cellular responses. This leaves the host with increased susceptibility to opportunistic infection, and also prevents maturation of the initial IgM response to trypanosomal infection. Hence, the usual secondary response manifested by the appearance of IgG and other immunoglobulin classes is absent (Hunter *et al.*,1991).

Immune suppression is produced in two ways. Early on, there is a rapid but transient suppression resulting from the production of large amounts of nitric oxide in the spleen. The protracted immune suppression of chronic infection is the result of the parasiteôs ability to induce production of very high titres of IFNγ by $CD8^+$ cells, by secreting a protein called T lymphocyte triggering factor (TLTF) (Olsson *et al*., 1992). IFNγ stimulates macrophages to produce large quantities of tumor necrosis factor (TNF) and to adopt an immunosuppressive phenotype, ultimately leading to downregulation of the production of IL-2 and its receptor. Despite the immune suppression mediated by IFNγ, there is evidence that host resistance is associated with an intense Th l response (Hertz et al., 1998), the mainstay of host defense against *T. brucei being* opsonic phagocytosis by liver macrophages (Dempsey and Mansfield, 1983).

1.3.6 Immunopathology

The immune response generated in response to trypanosomal invasion of the CNS contributes significantly to the neurological damage inflicted by a number of mechanisms (Hunter and Kennedy, 1992).

Autoantibody production is a feature of trypanosomiasis, autoantibodies being produced against antigens both outside and within the CNS. Autoantibodies have been demonstrated against muscle, single-stranded deoxyribonucleic acid (DNA), erythrocytes, brain myelin proteins and galactocerebrosides. Good correlation has been shown between the levels of antibody to myelin basic protein, galactocerebrosides and gangliosides and damage sustained by the CNS. However, it should be the result of extensive tissue damage resulting in the release of auto antigens, and could therefore be the result of neuropathology, rather than its cause (Hunter *et al.,* 1992).

The neuroglial cells contribute significantly to the course and outcome of African trypanosomiasis. Astrocytes respond to trypanosomal invasion by reactive gliosis, a response marked by increases in several enzyme activities and increased production of numerous cytokines (Eddleston and Mucke, 1993). Astrocyte activation has been shown to precede the development of significant brain lesions, and is marked by upregulation of MHC Class I and Class II, ILlα, IL-6 IFNγ, TNF and prostaglandins D_2 and E_2 (Hunter *et al.,* 1991, 1992). Hence, the microglia could act as accessory immune cells, playing a role in both cytokine production and antigen presentation, to modulate the cytokine network and mediate intracerebral inflammatory processes.

Trypanosomes directly modulate the production of some cytokines ï *T. brucei var. brucei* stimulates IFNγ production by $CD8^+$ T lymphocytes by releasing a triggering factor (trypanosome lymphocyte triggering factor, TLTF) (Stibbs and Curtis, 1987). IFNγ, in addition to its immunomodulatory effects, stimulates trypanosmal TNFα production by macrophages. Both IFNγ and TNFα could act as pro-inflammatory mediators within the CNS. Studies using knockout mice have demonstrated that TNFα is a key mediator involved in both control of parasitaemia and infection-associated pathology (Magez *et al.,* 1999).

1.3.7 Signs and Symptoms:

African trypanosomiasis symptoms occurs in two stages. The first stage is known as the haemolymphatic phase. This phase is characterized by fever, headaches, painful joints, and itching. Invasion of the circulatory and lymphatic system by the parasites is associated with severe swelling of the lymphnodes often to tremendous sizes. *Winterbottom's Sign* the tell ï tale swollen lymph nodes along the back of the neck may appear and if left untreated the disease overcomes the hostõs defenses and can cause more extensive damage broadening symptoms to include anaemia, endocrine, cardiac, and kidney dysfunctions (Donelson *et al.*, 1998; Fevre *et al.*, 2001).

The second stage known as the neurological phase begins when the parasite invades the central nervous system by passing through the blood-brain barrier. The term sleeping sickness comes from the symptoms of the neurological phase. The symptoms, include confusion, reduced coordination, and disruption of the sleep cycle with bouts of fatigue punctuated by manic periods leading to daytime slumber and nighttime insomnia. Without treatment, the disease is invariably fatal, with progressive mental deterioration leading to coma and subsequently death. Damage caused in the neurological phase is irreversible (Frieburghaus *et al*., 1996; Engels and Savioli, 2006).

1.3.8 Diagnosis

Trypanosomes can be detected from patient samples using two different preparations. A wet preparation can be used to look for the motile trypanosomes. Alternatively a fixed (dried) smear can be stained with Geimsa and examined. Often, the parasite is in relatively low abundance in the sample, so techniques to concentrate the parasites can be used prior to microscopic examination. For the blood sample centrifuged, this is followed by examination of the buffy coat; mini anion-exchange/centrifugation; and the Quantitative Buffy Coat

(QBC) technique. For other samples such as spinal fluid, concentration techniques include centrifugation followed by examination of the sediment (Olsson *et al.,* 1992; Sam-Wobo *et al*., 2010).

The gold standard for diagnosis is identification of trypanosomes in a patient sample by microscopic examination. Patientôs samples that can be used for diagnosis include Chancre fluid, lymph node aspirates, blood, bone marrow; and during neurological stage, cerebrospinal fluid. Detection of trypanosome ï specific antibodies can be used for diagnosis, but the sensitivity and specificity of these methods are too variable to be used alone for clinical purposes (Priotto *et al.*, 2009). Other diagnostic methods for detection of the parasite are:

The first uses dried blood while the other two use whole blood samples. Recent studies show that the whole blood-card agglutination test for trypanosomiasis (wb-CATT) to be the most efficient for diagnosis of trypanosomiasis while the whole blood latex agglutination test for trypanosomiasis (wb-LATEX) is a better of exam for situations where greater sensitivity is required (Truc *et al*., 2002).

1.3.9 Treatment of Trypanosomiasis

In 1901 a devastating epidemic of trypanosomiasis erupted in Uganda, killing more than 250,000 people about two-thirds of the population which reside in the lake-shore areas. The causative agent and vector for the disease were identified in 1903 by David Bruce, and the differentiation between the subspecies of the protozoa made in 1910. The first affective treatment, *Atoxyl,* an arsenic ï based drug developed by Paul Ehrlich and Kiyoshi Shiga, was introduced in 1910 but blindness was a serious side effect. Numerous drugs designed to treat the disease have been introduced since then (Robays *et al.,* 2008).

The type of treatment depends on the stage/phase of the disease. The drugs used in the first stage/phase of the disease are of lower toxicity and easier to administer. The earlier the disease is identified, the better the prospect of a cure. Treatment success in the second stage/phase depends on the drugs used, some of which are toxic and complicated to administer. Four drugs are registered for the treatment of sleeping sickness and provided free of charge to endemic countries (Engels and Savioli, 2006).

1.3.9.1 First Stage/Phase Treatment:

The current standard treatment for first stage/phase (haemolymphatic phase) of the infection is:

Intravenous or intramuscular Pentamidine (For *T. brucei gambiense)*; a highly effective drug for the first stage of the disease, it has been in use since 1939. During the fifties, it was widely used as a prophylactic agent in West Africa leading to a sharp decline in infection rates (Fevre *et al*., 2001). Despite its non-negligible and undesirable effects, it is generally well tolerated by patients.

Intravenous Suramin (*T. brucei rhodesiense)*, introduced in 1920 to treat first stage of trypanosomiasis. By 1922 Suramin was generally combined with Tryparsamide (another pentavalent organo-arsenic drug) in the treatment of the second stage of the *T. brucei gambiense* infection. It was used during the grand epidemic in West and Central Africa and it was the mainstay of therapy until 1969. However it provokes certain undesirable effects in the urinary tract and induces allergic reactions (Robays *et al*., 2008).

1.3.9.2 Second Stage/Phase Treatment:

The current standard treatment for the second stage/phase (neurological phase) of the disease is:

Intravenous Melarsoprol; developed in the 1940ôs. It is used in both forms of infection, but it is more effective for patients with second stage sleeping sickness. It is derived from arsenic and has many undesirable side effects. The most dramatic side effects is reactive encephalopathy (Convulsions, progressive coma or psychotic reactions) which can be fatal in 3-10% injected; it can cause brain damage in those who survive the encephalopathy. However due to its effectiveness, Melarsoprol is still used today. Resistance to Melarsoprol is increasing, but combination therapy with Nifurtimox is currently under research (Rates, 2001; Welburn *et al.*, 2001).

Intravenous Eflornithine (difluoromethylornithine DFMO); the most modern treatment was developed in the 1970ôs by Albert Sjoerdsmanot and underwent clinical trials in the 1980ôs. The drug was registered in 1990 by Aventis, the company responsible for its manufacture. In 2001 Aventis in association with Medecins San Frontieres and the World Health Organization (WHO) signed a long term agreement to manufacture and donate the drug (WHO, 2008).

A combination treatment of Nifurtimox and Eflornithine has been recently introduced in 2009. It simplifies the use of eflornithine in monotherapy but unfortunately it is not effective for *T. brucei rhodesiense.* Nitfurtimox is registered for the treatment of the American trypanosomiasis (Chagas disease) but not for (human) African trypanosomiasis. Nevertheless, after safety and efficacy data provided by clinical trials, its use in combination with eflornithine has been accepted and included in the WHO list of essential medicine and it is provided free of charge for this purpose by WHO (Priotto, *et al.*, 2009).

1.3.10 Resistance to Drugs

The genome of the parasite has been sequenced and several proteins have been identified as potential targets for drug treatment. Analysis of the genome also revealed the reason why generating a vaccine for this disease has been difficult. *T. brucei* has over 800 genes that make proteins the parasite ñmixes and matchesò to evade immune system detection (Donelson *et al.,* 1998).

In 2006, a team of medical doctors at Johns Hopkins investigated the pathway by which Trypanosomes make *myristate,* a 14-carbon length fatty acid. Myristate is a component of the variant surface glycoprotein (VSG), the molecule that make up the trypanosomes outer layer. This outer layer surface coat of VSG is vital to the trypanosomes ability to avoid destruction by the hostôs immune system. These doctors discovered that trypanosomes use a novel fatty acid synthesis pathway involving fatty acid elongases to make myristate and other fatty acids (Sternberg, 2004).

Two independent variants of the APOL-1 gene found in African halotypes carrying signatures of natural selection have been shown to confer protection against the acute version of sleeping sickness caused by *T. brucei rhodesiense,* while at the same time increasing risk of kidney disease when inherited from both parents (Braakman *et al.*, 2006).

1.3.11 Herbal Medicine

Chemotherapy and chemoprophylaxis, which form the most important and major aspect of the control and eradication of trypanosomiasis in African countries is beset with problems. These include limited repertoire of compounds, high level of drug toxicity, development of resistance to drugs by the parasite, high cost of the drug, undesirable route(s) of administration, and exhibition of antigenic variation which hampers vaccine production.

These factors emphasize the need for research into a more comprehensive, formidable and cheaper sources of trypanocides using herbs (Okochi *et al*., 2003).

According to the World Health Organization (2006), more than 80% of the world population relies wholly or partially on traditional/herbal medicines. This is more so evident in most of the Third world countries where modern way of treatment of diseases is grossly inadequate. Most of the rural populace in these countries relies on medicinal plants for both human and animal treatment because of high level of poverty and unavailability of synthetic drugs (Adeyemi *et al*., 2009).

Plants have provided the basis for traditional treatment for different diseases and still offer an enormous potential source of new chemotherapeutic agents. Plants present a broad spectrum of biological compounds with activities against virus, cancer, and parasites. These plants contain compounds mainly secondary metabolites such as alkaloids, glycosides, flavonoids, terpenes and coumarins. Plants have been studied as trypanocides by many researchers (Jodi *et al*., 2011)

According to the works of Okochi *et al.* (2003) on the effect of African Herbal Formula (AHF) on the haematological profile of rats infected with *T. brucei,* they observed that there was an increased red cell count, haemoglobin and white cell count. Furthermore, there was a rise in percentage of lymphocyte levels in the uninfected animals which were treated with AHF. This study suggests that it may implicate immunological pathways. This particular study also showed that the parasite load in the bloodstream of the infected and treated rats when compared to that of the untreated rats reduced, suggesting that AHF may positively influence the defense capacity of the treated animals. AHF was also observed to increase the weight of the rats infected and treated compared to their untreated counterparts.

Wurochekke and Anyanwu (2012) investigated the trypanocidal activity of the plant, *Anogeissus leiocarpus* on rats infected with trypanosome. In this work, methanolic and aqueous extracts of the leaf, bark, and roots of the plants were used. Extracts of the different parts of the plant were found to posssess *in vitro* anti-trypanosomal activity against *Trypanosoma brucei brucei.* However this anti-trypanocidal activity varies among the different parts of the plant used. Methanolic and aqueous extracts of the stem bark of the plant had the highest in vitro activity against the parasite. This suggests that the bark of the plant may have a higher concentration of the active component responsible for the activity observed.

The work of Adeiza *et al.* (2010) investigated the trypanocidal activity of the plant *Khaya senegalensis.* They observed that the extracts from the plant *K. senegalensis* reduced the levels of parasitaemia and the severity of the manifested clinical signs. This suggests it possible usefulness in the treatment of trypanosomiasis. The result further suggests that the stem-bark extract was generally more efficacious against *Trypanosoma evansi* than the other two parts of the plant used.

Adeiza *et al.* (2008) in their work, Comparative haematological changes in experimentally infected Savannah brown goats with *Trypanosoma brucei* and *Trypanosoma vivax*, observed that there was a decline in the total plasma protein of the infected animals. All the goats challenged with *T. brucei and T. vivax* developed parasitaemia. Their investigations revealed that there was an increase in the population of the parasites with a corresponding rise in the rectal temperature, rapid weight loss, packed cell volume decrease and decrease in the total plasma protein, all in the infected goats. This decline was more significant in the *T. brucei*-infected goats when compared to the *T. vivax*-infected goats. This decline is similar to the pathological and biochemical changes associated with trypanosome infection. The onset of anaemia and the extent to which the packed cell volume falls correlate

closely with the appearance, height, and duration of parasitaemia. Their findings showed that the severity of the disease was more manifest in *T. brucei*-infected goats.

Adeyemi *et al.* (2009) reported that there was a considerable extension of lifespan of infected animals compared with the control, when infected animals were treated with ethanolic extract of *Psidium guajava.* The ethanolic extract produced an appreciable decline in the level of parasitaemia for the animals treated with the ethanolic extract of the plant especially for the prophylactic treatment in which parasitaemia was kept extremely low. This confirmed that herbal extracts from *P. guajava* have many pharmacological uses in the treatment of aneamia due to trypanosomiasis infection.

Jodi *et al.* (2011) studied the comparative effects of animals treated with ethanolic root extract of *Gardenia sokotensis* and diminazen aceturate (Berenil) on the biochemical and heamatological parameters of the infected rabbits. They observed there was a chronic and fluctuating parasitaemia which is typical of trypanosome infections. The level of parasitaemia decreased rapidly in rabbits treated with diminazen aceturate, but there was only a slight decrease in the parasitaemia level in rabbits treated with the ethanolic extract of *Gardenia sokotensis.* This findings suggest that the plant *G. sokotensis* has some medicinal potentials for treating trypanosomiasis. Treatment of the rabbits with *G. sokotensis* resulted in significant reduction in the level of parasite-induced hyperproteinaemia and hyperglobulinaemia as well as significant amelioration of infection associated hypoalbuminaemia.

The reports of Jodi *et al*. (2011) demonstrated the potential of the anti-trypanosomal property of *G. sokotensis* root extract, although there was no complete eliminination of the parasites as it was the case with diminazen aceturate (Berenil).

Mann *et al.* (2009), they investigated the efficacy of the plant *Dissotis rotundifolia* on *Trypanosoma brucei brucei* infection in rats. They confirmed that the crude extract of *D.*

rotundifolia was active against *T. brucei*. Oral and intraperitoneal administration showed 66.7 and 78.4% inhibition of parasitaemia respectively. The intraperitoneal route is more effective which can be expected because the active constituents may reach the bloodstream faster and to a larger extent. The lower activity after oral administration may be due to enzymatic inactivation of active compounds in the gut or reduced absorption from the gut or a combination of both. In cases of massive parasitaemia it is possible that early treatments with lower concentrations of the crude extract may have favoured treatment failure. It may be that blood parasitaemia could be completely eliminated if the treatment is initiated at high dose of the crude extract of *D rotundifolia*.

The African mistletoe plant (Eastern Nigeria version), *Loranthus micranthus* Linn is an ubiquitous hemiparasitic plant that thrives well in tropical and subtropical climates (Osadebe and Ukwueze, 2004). Mistletoe belongs to the family *Loranthaceae,* and it has a long history of traditional use for a wide range of diseases; such as epilepsy, diarrhea, diabetes, convulsion, dizziness etc. Mistletoe depends on its host for mineral salts and water only, but photosynthesizes its own carbohydrate by means of its green leaves (Griggs, 1991). Mistletoe grows on a host of evergreen and deciduous trees all year round, around the branches of the tree. Nzekwe *et al.* (2009) and Osadebe and Ukwueze (2004), reported *Pentacletthra macrophylla, Persea americana, Irvingia gabonensis, Kola accuminata, Baphia nitida* and *Azardirichta indica* as hosts of mistletoe. Several other mistletoe plants are well known worldwide. These include the European mistletoe (*Viscum album Linn);* Korean or Japanese mistletoe (*Viscum album coloratum);* Australian/Argentina mistletoe (*Ligaria cuneifolia);* American mistletoe (*Phoradendron flavescens)* etc (Osadebe and Ukwueze, 2004). A number of chemicals have been isolated from mistletoe such as lectins, alkaloids, viscotoxins etc and the plant have been studied in terms of pharmacological activity (Obatomi *et al*., 1996).

In Nigeria and some other parts of Africa, it is believed that the aqueous extract of mistletoe (Loranthus species), consumed over a long time will bid farewell to the cause of hypertension, diabetes, and other metabolic diseases (Obatomi *et al.,*1994). The mistletoe plant has been described as ónan all purpose herbô because of its rich folkloric uses. However, the use of the European mistletoe (*Viscum album)* as an anti cancer and immune system stimulating agent is well documented (Kuttan, 1990; Fischer *et al.,* 1997). Earlier studies have shown that the composition activities of mistletoe are host- tree, species and harvesting period- dependent (Obatomi *et al*., 1994). They showed that infusions of mistletoe parasitic on lemon and guava trees significantly decreased serum glucose levels; whereas preparations from mistletoe parasitic on *Jatropha curcas* did not (Obatomi *et al.,* 1996).

Osadebe and Ukwueze (2004) investigated the Phytochemical and anti-microbial properties of mistletoe. They observed that the sensitivity of the different extracts of *L. micranthus* leaves gave interesting profiles. The test organisms showed varying degrees of sensitivity to the extracts. Extracts from *K. accuminata, P. Americana* and to a lesser extent *I. gabonensis* showed a marked broad-spectrum activity against bacteria and fungi. When compared with standard antibiotics (amoxicillin and ketoconazole) as controls, some of the extracts were found to be significantly more active than the standard antibiotics used as control.

In view of this, there is a strong incentive for further research into the pharmacological activities of *Loranthus micranthus* against common diseases. Considering the fact that the plant is readily available in the tropics and within reach of the local populace, this experiment/research work is designed to assess the trypanocidal activity of mistletoe (*Loranthus micranthus)* leaf extract.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Experimental Plants

4kg of fresh leaves of mistletoe plant were procured from the forests around Obukpa, a community in Nsukka from the host tree *Kola accuminata*. The plant was identified by a plant biologist in the Department of Plant Biology and Biotechnology, University of Nigeria, Nsukka. The leaves were collected, weighed and shade dried for two (2) weeks. The weight of the dried leaves was taken again using a Mettler electronic weighing balance PC 2000. After shade drying, the leaves were pulverized into fine powder using a laboratory milling machine (Honda: model 622, China).

2.2 Preparation of Aqueous Plant Extract

About 500 grams of the powdered materials was soaked in 1000ml of distilled water and allowed to stand for twenty four hours (24hrs) at room temperature, with occasional shaking to increase the extraction capacity. The decotion was filtered using a muslin cloth (40-mesh sieve), and then concentrated to drynes. The residue or yield obtained was used to determine the concentration of the mistletoe extract to be administered to the different groups of the experimental animals. Weighed samples of the extract was re-dissolved in normal saline and used to prepare the stock solution for oral administration to the animals according to their body weights.

2.3 Phytochemical Screening of the Plant Material

The phytochemical analysis of the extract was done using the standard methods. The fractions screened were alkaloids, saponins, tannins, flavonoids, resins, terpenes, glycosides and steroids.

2.3.1 Determination of Alkaloids (General Tests)

About 2ml of 5% tetraoxosulphate (iv) acid in 50% ethanol was added to 5ml of the methanol and water extracts, respectively. The different mixtures were brought to heat on a boiling water bath for 10 minutes. They were cooled and filtered. To 2ml of each filtrate was added fewdrops of: Mayerôs Reagent (Potassium mercuric iodide solution) Dragendorffôs Reagent (Bismuth potassium iodide solution), Wagnerôs Reagent (Iodine in potassium iodide solution) Picric acid solution (1%).

The remaining filtrates were placed separately in 100ml separator funnels and made alkaline with dilute ammonia solution. The aqueous alkaline solution, of each extract, was separated and extracted with two 5ml portions of dilute sulphuric acid. Both extracts were tested with a few drops of Mayerôs, Wagnerôs, and Dragendorffôs reagents. Alkaloids showed up as milky precipitate with one drop of Mayerôs reagent and a reddish brown precipitate with one drop of Wagnerôs reagent.

2.3.2 Determination of Saponins (Fehling's Method)

About 20ml of water was poured into 0.25g of both extracts in a 100ml beaker and boiled gently on a hot water bath for 2 minutes. Both mixtures were respectively

filtered out and allowed to cool. The filtrates were used for the Fehlingôs test. A reddish precipitate indicated the presence of Saponins.

2.3.3 Determination of Tannins (Ferric Chloride Method)

One gram of both extracts was boiled respectively with 50ml of distilled water. Each was filtered and the filtrate used for the test. To about 3 ml of the respective filtrate, few drops of ferric chloride solution were added. A greenish black precipitate indicated the presence of tannins.

2.3.4 Determination of Flavonoids (Ammonium Test Method)

About 10ml of ethylacetate were added to 0.2g of both extracts. Both mixtures were treated on a water bath for 3 minutes. Each mixture was cooled, filtered and the filtrate used for the ammonium test. Ammonium Test: A quantity, 4ml of each of the filtrates was shaken with 1ml of ammonia solution. The layers were allowed to separate and the yellow colour in the ammoniacal layer indicated the presence of flavonoids.

2.3.5 Determination of Resins (Precipitation Test)

About 0.2g of both extracts was washed with about 15ml of 95% ethanol and the mixture poured into 20ml distilled water in a beaker. The formation of a precipitate indicated the presence of resins.

2.3.6 Determination of Steroids and Terpenoids

About 9ml of ethanol was poured into 1g of the extract. It was refluxed for a few minutes and then filtered. The filtrate was concentrated to 2.5ml on a boiling water bath and 5ml of hot water was added. The mixture was allowed to stand for 1 hour and the waxy matter filtered off. The filtrate was extracted with 2.5ml chloroform using a separating funnel. Later 1ml of concentrated sulphuric acid was poured into about 0.5ml of the chloroform extract in a test tube. The appearance of a reddish-brown interface showed the presence of steroids. Another 0.5ml of the chloroform extract was evaporated to dryness on a water bath and heated with 3ml of concentrated sulphuric acid for 10 minutes on a water bath. A grey colour indicated the presence of terpenoids.

2.4 Acute Toxicity Studies of the Plant Extract

This was carried out by the method described by Lorke (1983). In the initial phase, rats were divided into 3 groups (A, B and C) of three (3) rats each and were treated with the plant extract at doses of 200 mg, 400mg and 800 mg/kg body weight orally. The animals were observed for 24 hours for signs of toxicity including death. Based on the results of

phase one, three fresh rats were divided into 3 groups of two rats each and were treated with 1,000, 3,000 and 5,000 mg/kg body weight and signs of toxicity were also recorded. The lethal dose was calculated as the arithmetic mean of the dose that killed the least number of animals and the one next lower dose that did not kill any animal.

2.5 Procurement and Management of Experimental Animals

Animals used were seventy-two (72) adult male albino rats. They were purchased from the Genetic and Animal breeding laboratory of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. The rats had no history of drug consumption (i.e. those that have not been used for any investigation). The rats were kept in stainless wire rat cages equipped with drinkers and fecal collecting trays, in a clean and fly proof experimental animal house. The rats were fed commercial growers chick mash (18 % crude protein) made by Vital Feeds Nigeria Limited and clean drinking water, and allowed to get acclimatized for 14 days before the start of the experiment. They were allowed free access to food and water. The fecal droppings in the tray were removed daily.

However, the rats were weighed using an electronic weighing balance (Mettler, PC 2000) before treatment and on weekly basis during sampling. They were randomly assigned into five groups of 12 rats per group with each group comprising 3 replicates of 4 rats each. The individual rats in a group were differentially marked for proper identification during treatment.

2.6 Experimental Design

Table 1: Design That Was Used for the Experiment

Group	TREATMENTS (mg/kg)	WEEK 1	WEEK 2	WEEK 3	WEEK 4	TOTAL
A	400	3	3	3	3	12
B	800	3	3	3	3	12
C	1200	3	3	3	3	12
D	Infected and untreated	3	3	3	3	12
E	Standard drug	3	3	3	3	12
F	Normal	3	3	3	3	12
Total number of rats		**18**	**18**	**18**	**18**	**72**

The rats were divided into six groups (A, B, C, D, E and F) of 12 rats per group. Groups A, B, and C served as the treatment groups while groups D and E were the control groups. Three different concentrations of the aqueous extract were administered to the different treatment groups according to their body weight. Group A were given 400 mg/kg body weight of the leaf extract while groups B and C were administered 800 mg/kg and 1200 mg/kg respectively. Group D, (the infected and untreated) were infected with trypanosomes but were not administered the aqueous leaf extract. The standard drug control (group E) were inoculated, after which they were treated with a standard drug (dimenazene aceturate) also known as Berenil, according to their body weight. Normal control groups (Group F, experimental control) were used to monitor all the treated groups. All the doses were administered once daily orally for 28 days (four weeks) for all the groups using oral gavage.

2.7 Determination of Level of Parasitaemia in Rats Infected with *Trypanosoma brucei brucei*

The level of parasitaemia was determined by counting the number of trypanosomes per view under the light microscope at a final magnification of x100 from thin blood smear obtained from the tip of the tail of the infected rats. The level of parasitaemia was ascertained before the start of treatment (Day 0) and subsequently every two days until the end of treatment.

2.8 Collection of Blood Samples

Blood samples were collected before the start of the experiment and at weekly intervals during treatment. On a weekly basis, three rats each (i.e. one rat from each replicate) was sampled from the control and treatment groups. Blood samples from the selected rats were collected for the various haematological profile tests as well as the biochemical estimations.

Procedure

The rats were anaesthesized before the blood collection. The tip of a capillary tube was inserted into the corner of the eye socket underneath the eye ball of the anaesthetized rat at an angle of 45^0C. The tube will be rotated between fingers and should not be moved side to side or front and back. Then, little pressure will be applied gently downward until the ocular vein is cut. The blood sample was collected with a sterilized sample container through the capillary tube (Hoff, 2000).

2.9 Biochemical Analysis

2.9.1 Determination of Alanine Transaminase (ALT)

The alanine transaminase content was determined using the method described by Reitman and Frankel (1957). Two test tubes were labeled blank and sample respectively for

each of the samples. A total volume of 0.1 ml distilled water was pippeted into the blank test tubes, and 0.1 ml of serum was added to the sample test tubes. Similarly, 0.5 ml of the Buffer was added into all the test tubes, mixed thoroughly and incubated for 30 minutes at 37^{o}C. Thereafter, 0.5 ml of 2, 4- dinitrophenylhydrazine was added to all the test tubes. The contents were mixed again and allowed to stand for 20 minutes at 25^{o}C. At the expiration of this time, a total volume of 5 ml of NaOH was added into all the tubes, and the content was mixed and allowed to stand for another 5 minutes. The absorbance of the samples against the sample blank was read using spectrophotometer (Spectrumlab 752S, B) at 550 nm.

2.9.2 Determination of Aspartate Transaminase (AST)

The aspartate transaminase content was also determined using the method described by Reitman and Frankel (1957). Two test tubes were labeled blank and sample respectively for each of the samples. A total volume of 0.1 ml distilled water was pippeted into the blank test tubes, and 0.1 ml of serum was be added to the sample test tubes. Similarly, 0.5 ml of R1 was added into all the test tubes mixed thoroughly and incubated for 30 minutes at 37^{o}C. Thereafter, 0.5 ml of R2 was added to all the test tubes. The contents were mixed again and allowed to stand for 20 minutes at 25^{o}C. At the expiration of this time, a total volume of 5 ml of NaOH was added into all the tubes, and the content was mixed and allowed to stand for another 5 minutes. The absorbance of the samples against the sample blank was read using spectrophotometer (Spectrumlab 752S, B) at 546 nm.

2.9.3 Determination of Alkaline Phosphatase (ALP)

Assay followed the method used by Fishman and Lerner (1953). Approximately 0.02ml of the sample and 1.0ml of the reagent was added into a test tube and then thoroughly mixed. The absorbance of the content was measured at a wavelength of 405nm using a spectrophotometer (Spectrumlab 752S, B).

2.9.4 Serum Albumin

This assay follow the method of Cheesbrough (2005). The method requires 0.02ml of non-lipaemic, non-icteric serum. The blood must be collected with the minimum of venous stasis and haemolysis should be avoided. Take four or more tubes (depending on the number of tests) and label as follows:

B ï Reagent blank

S ï Standard, 30 g/l

C ï Control serum

P ï Patientsô tests

Pipette 4ml of BCG (Bromocresol green) reagent into each tube. Add to each tube as follows:

Tube

B ï 20µl (0.02ml) distilled water

S ï 20µl standard, 30g/l

C ï 20µl control serum

P ï 20µl patientôs serum

Mix well but avoid frothing of the solutions. If air bubbles are present the absorbance readings will be incorrect. Within 5 minutes read the absorbances of the solutions in a colorimeter using an orange filter 590nm or in a spectrophotometer set at 632nm. Zero the instrument with the reagent blank solution in tube B. calculates the concentration of albumin in the control and patientsô samples by:

- Using the formula:

$$\text{Albumin g/l} = \frac{\square\square}{\square\square} \times 30$$

Where: AT = Absorbance of test(s) or control

AS = Absorbance of 30g/l standard

2.9.5 Total bilirubin

This assay followed the method of Cheesebrough (2005). The method requires 0.2 ml (200µl) of patientôs serum or plasma from EDTA or heparin anti-coagulated blood. The specimen should be as fresh as possible (not more than 24 hours hold).

Take six or more tubes (depending on the number of tests) and label as follows:

S ï Standard

SB ï Standard blank

C ï Control serum

CB ï Control blank

PT ï Patientsô tests

PB ï Patientsô blanks

Pipette 1ml of caffeine-benzoate reagent into each tube. Add to each tube as follows:

S, SB ï 0.1ml standard serum (170-340µmol/l)

C, CB ï 0.1ml control serum

PT, PB ï 0.1ml patientôs serum/plasma.

Mix well. Add 0.5ml of diazo reagent to tubes S, C, PT and mix well. Add 0.5ml of sulphanilic acid, reagent to tubes SB, CB, PB and mix well. Leave at room temperature (30-28^{0}C) for 5 minutes. Add 1ml of alkaline tartrate reagent to each tube and mix well. With the addition of this reagent any turbidity in the solutions will clear. Read immediately the absorbances of the solutions (blanks first) in a colorimeter using an orange filter 590 nm or in a spectrophotometer set at wavelength 600nm. Zero the instrument with distilled water. Calculate the concentration of total bilirubin in the control and patientsô samples by:

- Reading the values from the calibration graph providing the reading of the standard agrees with the calibration, or if not by:

- Using the formula:

  Concentration of total bilirubin in µmol/l

  $\frac{\square\square}{\square\square} \times$ concentration of standard

  Where: AT = Absorbance of test(s) or control

  AS = Absorbance of standard

2.9.6 Total Serum Cholesterol

This assay will follow the method of Roeschlau *et al.* (1974). A total volume of 10ml of distilled water was pipette out into the test tube labeled as blank, 10ml of the cholesterol standard was pipette out into the tube labeled standard, and another 10ml of the serum sample was placed into the tube labeled as test sample. A total of 1000uL of the reagent solution was added to all the test tubes and the contents thoroughly mixed. The experimental tubes were then be incubated for about 5 minutes in a water bath set at 37^{o}C. The absorbance was measured at a wavelength of 500nm using a spectrophotometer (Spectrumlab 752S, B).

2.9.7 Blood Urea

The urea content will be determined using the method described by Weatherburn (1967). Three test tubes were labelled blank, sample and standard respectively for each of the samples. A total volume of 10 µl distilled water was pippeted into the blank test tubes, 10 µl of serum was added to the sample test tubes, and 10 µl of the standard to the test tubes labeled standard. Similarly, 100 µl of R1 was added into all the test tubes, mixed thoroughly and incubated for 10 minutes at 37^{o}C. Thereafter, 2.5 ml of R2 followed by 2.5 ml of R3 was added to all the test tubes. The content was mixed immediately and incubated again for 15 minutes at 37^{o}C. The absorbance of the samples (A_{sample}) and Standard ($A_{standard}$) was read

against the blank using spectrophotometer (Spectrumlab 752S, B) at 546 nm. The concentration of Urea in the serum will be calculated using the formula given below:

$$\text{Urea concentration in serum (mg/dl)} = \frac{\text{[illegible]}}{\text{[illegible]}} \times \text{[illegible]}$$

Where: A_{sample} = Absorbance of the sample and $A_{standard}$= Absorbance of the standard

2.9.8 Serum Creatinine

The creatinine content was determined using the method described by Bartels and Bohmer (1972). Three test tubes were labeled blank, sample and standard respectively for each of the samples. A total volume of 100 µl distilled water was pippeted into the blank test tubes, 100 µl of serum was added to the sample test tubes, and 100 µl of the standard to the test tubes labeled standard. Similarly, 1000 µl of working reagent (R1a and R2b) was added into all the test tubes and mixed. After 30 seconds, the absorbance (A1) of the standard and sample was read at 492 nm. Exactly 2 minutes later, the absorbance (A2) of the standard and sample was determined again using the same wavelength. The concentration of creatinine in the serum was calculated using the following formula:

A2 ï △A1 = $A\triangle_{Sample}$ or $A_{Standard}$

$$\text{Conc. of creatinine in serum (mg/dl)} = \frac{\text{[illegible]}}{\text{[illegible]}} \times \text{[illegible]}$$

Where:

A1 = Absorbance of sample or standard after 30 seconds

A2 = Absorbance of sample or standard after 2 minutes

$\triangle A_{sample}$ = change in absorbance of the sample

$\triangle A_{standard}$ = change in absorbance of the standard

A_{sample} = Absorbance of the sample

$A_{standard}$ = Absorbance of the standard

2.10 Hematological Analysis

2.10.1 Haemoglobin Estimation

Haemoglobin concentration was determined using the method described by Sood (2006). The graduated tube was filled to the mark 2 on the red graduations with N/10 HCL using a dropper. Blood sample was sucked into the capillary pipette up to 20cm mark, the end of the pipette was wiped and blown into the acid in the mixing tube. The pipette was then rinsed by sucking up the acid and blowing it out back into the tube 3 ï 4 times and then allowed to stand for 10 minutes for formation of acid haematin. Similarly, the acid haematin was diluted with distilled water in drop wise manner, mixed and the colour was compared with the standard by holding the comparator towards light. This was repeated until the colour of the diluted fluid matches with that of the standards in the comparator. From the level of the fluid in the mixing tube, the haemoglobin, Hb, was read out in gram percent.

2.10.2 Haematocrit (Packed Cell Volume) estimation

This was determined by the microhaematocrit method as described by Coles (1986). A heparinized capillary tube was filled to approximately three fourth (3/4) of its length with well-mixed anticoagulated blood. The coloured end of the capillary tube was sealed with plasticin. The capillary tube was placed into a microhaematocrit centrifuge set at 10,000 revolutions per minute (rpm) for 5 minutes. The height of the packed cell as well as the total height in millimeter was measured using the haematocrit reader. The packed cell volume was calculated using the formula:

$$PCV\ (\%) = \frac{\square\square\square\square\square\square\square\ \square\square\square\ \square\square\square\square(\square\ \square)}{\square\square\square\square\square\square\square\square\square\square\square\square(\square\ \square)} \times 100$$

2.10.3 Red Blood Cell Count (RBC)

The method of red blood cell count was according to Sood (2006). The blood was sucked slowly and carefully up to 0.5 mark in the red diluting pipette. Immediately, the pipette was plunged into the diluting fluid and sucked up to the 101 mark. Then, the ends of the pipette was gripped between the finger and the thumb and shaken thoroughly for 3 minutes. The diluted blood sample was loaded on a Neubauer counting chamber and all red blood cells in the five groups of 16 small squares in the central area of the Neubauer chamber was counted using a light microscope at a high magnification of x 100. The number of cells counted for each sample was calculated using the formula:

$$\text{RBC } (\text{cells/mm}^3) = \frac{\text{Number of cells counted} \times \text{Dilution factor}}{\text{Number of squares counted} \times \text{Volume counted}}$$

Where: dilution factor = 1: 200 and Volume counted = 0.02 mm^3

Then, RBC (mm^3) = $\frac{\text{Average number of cells counted} \times \text{Dilution factor}}{\text{Volume counted}}$

RBC (mm^3) = Average number of cells counted $\times$ 10^4

2.10.4 Red Blood Cell Indices

Red cell indices were calculated from the red blood cell count (RBC), haemoglobin (Hb) content and packed cell volume (PCV) according to Baker *et al*., (2001).

2.10.4.1 Mean Cell Volume (MCV)

The mean cell Volume (MCV) is the mean volume of the red cells. It was determined using the method as described by Baker *et al*. (2001). It was derived by calculating from packed cell volume (PCV) and Red blood cell (RBC) count as follows:

$$\text{MCV } (\text{fl}) = \frac{\text{PCV (\%)} \times 10}{\text{RBC (millions/mm}^3)}$$

2.10.4.2 Mean Cell Haemoglobin (MCH)

This refers to the amount of haemoglobin present in the average erythrocyte. It was determined using the method as described by Baker *et al*. (2001). It was derived by calculating from the ratio of haemoglobin (Hb) and Red blood cell (RBC) count according to the following formula:

$$\text{MCH (pg)} = \frac{\text{Hb g/100 ml} \times 10}{\text{RBC x } 10^{12}/\text{l}}$$

2.10.4.3. Mean Cell Haemoglobin Concentration (MCHC)

The MCHC refers to the amount of haemoglobin in 100 ml of packed cell volume (PCV), as opposed to the amount of haemoglobin in whole blood. It was determined using the method as described by Baker *et al*. (2001). It was derived by calculating from the ratio of haemoglobin (Hb) and packed cell volume (PCV) count according to the following formula:

$$\text{MCHC [\%(g/100 ml)]} = \frac{\text{Hb g/100 ml} \times 100}{\text{PCV}}$$

2.10.5 White Blood Cell Count (WBC)

The White blood cell (WBC) was determined using the method described by Sood (2006). The blood was sucked slowly and carefully up to 0.5 mark in the red diluting pipette. Immediately, the pipette was plunged into the diluting fluid and sucked up to 11 marks. Then, the ends of the pipette were gripped between the finger and the thumb and shaken thoroughly for 3 minutes. The diluted blood sample was loaded on a Neubauer counting chamber and all the cells in the four corner squares of the Neubauer chamber was counted using a light microscope at a high magnification of x 100. The number of cells counted for each sample was calculated using the formula:

$$\text{WBC x } 10^{9}/\text{l} = \frac{\text{number of cells counted} \times \text{dilution factor}}{\text{number of squares counted} \times 0.1}$$

Where: dilution factor = 1: 20, and Number of large squares counted = 4

$$\text{WBC/mm}^3 = \frac{\text{Total number of cells counted} \times \text{Dilution factor}}{\text{Area counted} \times \text{Depth of chamber} \times \text{Number of large squares counted}}$$

$$\text{WBC/mm}^3 = \text{Average number of cells counted} \times 50$$

2.10.5.1 Differential white cell count

The differential white cells count provides information on the different white cells present in the circulating blood i.e. neutrophils, lympholytes, monocytes, eosinophils, basophils (Cheesebrough, 2005). Providing the total white blood cell (WBC) count is known, the absolute number of each white cell type i.e. number of each cell per litre of blood can be calculated and an assessment made of whether the number of a particular cell type is increased or decreased (Cheesebrough, 2005).

Procedure

Make a thin blood film by

i. Placing a drop of blood on a clean slide
ii. Using a clean smooth edged spreader, spread the drop of blood to make a film
iii. And dry the film by waving the slide back and forth.
iv. Fix in absolute methanol
v. Stain with leishman stain for 10mins.
vi. Allow to dry and view under the microscope at x100 magnification.
vii. Record the numbers counted in up to 100 fields.

2.11 Statistical Analysis

Two-way ANOVA was used to determine interactive effects of treatment and time while One-way ANOVA was used to test the effect of treatment. One-way ANOVA was used to determine the level of parasitaemia in the infected animals. F-LSD (Fisherôs least significant difference) was used to establish if there were significant differences among the treatment groups. Means of the treatment groups were separated using DUNCAN multiple range test (DMRT). All results were expressed as Mean±S.E and the significant level was set at 0.05.

CHAPTER THREE

RESULTS

3.1 Qualitative Phytochemical Composition of the Crude Extract of *Loranthus micranthus* Leaves

Table 2 shows the qualitative phytochemicals composition of the aqueous extract of the *Loranthus micranthus* leaves. From the result, it was observed that tannins and flavonoids were present in high concentrations; alkaloids, resins, steroids and saponins were present in moderate quantities while terpenes and glycosides were present in trace amounts.

Table 2: Phytochemical Analysis of the Crude Extract of *Loranthus micranthus*

Parameter	
Resins	++
Flavonoids	+++
Tannins	+++
Terpenes	+
Alkaloids	++
Saponins	++
Glycosides	+
Steroids	++

+++ High concentration

++ = Moderate concentration

+ = Trace concentration

3.2 Acute Toxicity Studies of Extracts of *L. micranthus*

There was no mortality at different doses (200, 400 and 800 mg/kg) of the aqueous extract of *L micranthus* after 24 hours (Table 3). Also, at dose levels of 1,000 mg/kg, 3,000 mg/kg and 5,000 mg/kg, there was no death recorded although, behaviours such as shivering, bulging of eyes and dullness were observed in the animals.

Table 3: Acute Toxicity of Rats treated with *L. micranthus* leaf extract

DOSES (mg/kg)	MORTALITY
200	0
400	0
800	0
1000	0
3000	0
5000	0

3.3 Weekly effects of aqueous leaf extracts of *L. micranthus* on the Body weights of albino rats.

Table 4 shows the results of the weekly effects of the aqueous leaf extract of *L. micranthus* on the body weights of albino rats infected with *T. brucei brucei.* It was observed that there was no dose-dependent significant difference ($P>0.05$) in the body weights of the treated animals when compared with the control groups. Comparatively, however, the body weights of the animals administered 800mg/kg and 1200mg/kg of the aqueous *L. micranthus* leaf extract was not significantly different ($P>0.05$) in comparison with the control groups in weeks 1 and 2, whereas the animals administered the 400mg/mg of the aqueous leaf extract was significantly different ($P<0.05$) from those of the control groups. Similarly, it was observed that in week 3, the body weights of the animals treated with 800mg/kg and 1200mg/kg of the aqueous extract did not vary significantly ($P>0.05$) from the infected and untreated control group but varied significantly ($P<0.05$) from those of the standard drug and normal control groups respectively. It is also noteworthy that the animals that were administered 400mg/kg of the aqueous extract recorded mortality in week 3, while the animals treated with 800mg/kg and 1200mg/kg of the aqueous leaf extract alongside the infected and untreated control group all died in week 4.

Table 4: Effects of Different Treatments of the Aqueous Leaf Extract of *L. micranthus* on the Body Weights, BW (g) of albino rats on Weekly Basis

Treatment (mg/kg)	Duration				
	Week 0	Week 1	Week 2	Week 3	Week 4
(400mg/kg)	139.5±6.92^{1b}	135.0±16.21^{2b}	136.7±14.2^{2b}	0.00	
(800mg/kg)	149.2±1.59^{1a}	147.3±9.66^{1a}	142.0±12.36^{2a}	137.7±8.88^{3b}	0.00
(1200mg/kg)	141.4. ±6.31^{1b}	140.9±12.9^{12a}	138.5±13.30^{2ab}	136.0±9.20^{2b}	0.00
(Infected and untreated)	147.7±9.80^{1a}	144.0±8.33^{2a}	140.1±12.06^{2a}	137.4±4.02^{2b}	0.00
(Standard drug)	144.2±9.80^{1ab}	143.7±1.99^{1a}	141.3±8.11^{1a}	142.3±5.87^{1a}	140.5±8.63^{1a}
(Normal control)	138.1±4.12^{1b}	137.4±4.61^{1b}	138.8±11.54^{1ab}	140.5±6.32^{1a}	139.3±2.95^{1a}

Mean values in a column with different letters as superscript are significantly different at P<0.05.

Mean values in a row with different numbers as superscript are significantly different at P<0.05

3.4 Effects of Aqueous Extracts of *L. micranthus* on the Level of Parasitaemia

Table 5 shows the level of parasitaemia in rats fed with aqueous extracts of *L. micranthus.* The initial signs of parasitemia were intermittent pyrexia, lethargy, isolation, reduced feed intake, rapid weight loss, and rough hair coat.

There was a progressive and significant increase in the level of parasitaemia throughout the period of the research. In overall, the level of parasitaemia in all the infected and treated animals significantly increased ($P < 0.05$) throughout the duration of the experiment. Comparatively, there was no significant difference ($P>0.05$) in the level of parasitaemia of the infected and treated animals and that of the infected and untreated control groups through out the duration of the study. However, it was further observed that the level of parasitaemia in the treated rats was not significantly different ($P>0.05$) from that of the standard drug control group in week 1, but was significantly different ($P<0.05$) in weeks 2-4. Moreover, the level of parasitaemia in the infected and treated animals and that of the infected and untreated control group was significantly high ($P<0.05$) in comparison to the normal control group in all weeks.

Table 5: Level of Parasitaemia in infected albino rats (Trypanosomes/per field)

Days (P.I)	400mg/kg	800mg/kg	1200mg/kg	Infected and untreated	Standard drug	Normal control
1	0	0	0	0	0	0
3	1×10^3	1×10^3	1×10^3	1×10^3	1×10^3	0
5	4×10^4	3×10^4	3×10^4	7×10^5	5×10^5	0
7	5×10^5	3×10^4	4×10^4	6×10^5	4×10^4	0
9	7×10^5	5×10^5	7×10^5	10×10^5	5×10^5	0
11	9×10^5	9×10^5	11×10^5	13×10^5	3×10^2	0
13	11×10^5	13×10^5	11×10^5	13×10^5	2×10^2	0
15	19×10^5	17×10^5	23×10^5	31×10^5	2×10^2	0
17	17×10^5	17×10^5	21×10^5	33×10^5	1×10^2	0
19	20×10^5	21×10^5	20×10^5	33×10^5	1×10	0
21	00	25×10^5	27×10^5	37×10^5	0	0
23	00	30×10^5	35×10^5	00	0	0
25	00	00	00	00	0	0
27	00	00	00	00	0	0

- Each value is the average number of trypanosomes per microscopic field in each group.
- Figures in parentheses represent the corresponding number of trypanosomes per mili litre of blood.
- P.I = post infection.

3.5 Effects of Different Treatments of the Aqueous Extracts of *L. micranthus* Leaves on Biochemical Parameters of Albino Rats

3.51. Weekly effects of aqueous leaf extracts of *L. micranthus* on the serum aspartate transaminase (AST) levels of albino rats.

The results of the weekly effects of aqueous leaf extracts of *L. micranthus* on the serum AST levels of albino rats are shown in Table 6. The increasing dosage of the *L. micranthus* aqueous leaf extracts produced an overall dose- independent significant increase ($P < 0.05$) in the total serum levels of AST of albino rats infected with *T. brucei brucei*. Comparatively, however, the effect of the increasing dosage of the leaf extract on the serum AST levels of the treated animals was not significantly different ($P < 0.05$) from that of the infected and untreated control group in weeks 1 and 3, but was significantly different ($P < 0.05$) from those of the standard drug and normal control groups. However, it was observed that in week 2, the serum AST levels of rats administered the 800mg/kg and 1200mg/kg was not significantly different ($P<0.05$) from that of the infected and untreated control group but was significantly different ($P<0.05$) from those of the standard drug and normal control groups.

There was an overall time-dependent significant increase ($P<0.05$) in the serum AST levels of albino rats infected with *T. brucei brucei*. It was observed that in week 2, the serum AST levels in all the treated animals was not significantly different ($P>0.05$) from the infected and untreated control group but was significantly different ($P<0.05$) from those of the standard drug and normal control groups. Mortality occurred in the animals that were treated with 400mg/kg of the aqueous extract in week 3, whereas in week 4 the rats in the treatment groups administered 800mg/kg and 1200mg/kg of the aqueous leaf extract, as well as the infected and untreated control group all died.

Table 6: Effects of Different Treatments of the Aqueous Leaf Extract of *L. micranthus* on the Aspartate Transaminase, AST (u/L) of albino rats on Weekly Basis

Treatment (mg/kg)	Duration				
	Week 0	Week 1	Week 2	\Week 3	Week 4
(400mg/kg)	110.00 ± 2.96^{2b}	171.33 ± 15.8^{1ab}	187.50 ± 3.17^{1b}	000	
(800mg/kg)	117.67 ± 3.06^{3ab}	168.67 ± 19.4^{2ab}	224.50 ± 3.17^{1a}	151.50 ± 0.50^{2b}	0.00
(1200mg/kg)	121.33 ± 3.09^{3a}	162.33 ± 8.3^{2ab}	215.00 ± 8.66^{1a}	162.00 ± 4.00^{2ab}	0.00
(Infected and untreated)	113.75 ± 2.15^{4ab}	180.00 ± 12.8^{2a}	215.00 ± 3.46^{1a}	155.50 ± 0.50^{3ab}	0.00
(Standard drug)	115.58 ± 2.81^{3ab}	133.07 ± 8.18^{2b}	154.33 ± 1.85^{1c}	90.00 ± 2.082^{4c}	97.33 ± 8.41^{4b}
(Normal control)	116.42 ± 2.43^{1ab}	86.72 ± 1.36^{2c}	87.191 ± 3.26^{2d}	81.124 ± 2.81^{2c}	80.416 ± 3.72^{2a}

Mean values in a column with different letters as superscript are significantly different at $P < 0.05$.

Mean values in a row with different numbers as superscript are significantly different at $P < 0.05$

3.5.2. Weekly effects of aqueous leaf extracts of *L. micranthus* on the serum alanine transaminase (ALT) levels of albino rats.

The results of the weekly effects of aqueous leaf extract of *L. micranthus* on the serum ALT levels of albino rats are shown in Table 7. In overall, there was a dose-independent significant increase ($P<0.05$) in the ALT of albino rats that were infected with aqueous leaf extracts of *L. micranthus.* The comparative effects of the increasing dosage of the aqueous leaf extract of *L. micranthus* on the serum ALT levels of the treated animals was not significantly different ($P>0.05$) from that of the infected and untreated control group in weeks 1 and 2, but was significantly different ($P<0.05$) from those of the standard drug and normal control groups. However, it was observed that in week 3, the serum ALT levels of rats administered the 800mg/kg of the extract was not significantly different ($P>0.05$) from that of infected and untreated control groups, whereas the serum ALT levels of the rats administered 1200mg/kg was significantly different ($P<0.05$) from the infected and untreated control group. Similarly, the serum ALT levels of rats administered 800mg/kg and 1200mg/kg were significantly different ($P<0.05$) from the standard drug and normal control groups in week 3.

There was an overall time-dependent significant increase ($P<0.05$) in the serum ALT levels of the treated rats from weeks 1-3. However, it was observed that the serum ALT levels of the animals administered the 400mg/kg of the aqueous leaf extract of *L. micranthus* varied significantly ($P<0.05$) from that of the infected and untreated and standard drug control groups in weeks 1 and 2. Secondly, there was no significant difference ($P>0.05$) recorded in the serum ALT levels of the treated animals in comparison with all the control groups in week 3. It is also noteworthy that all the animals that were administered 400mg/kg of the aqueous leaf extract died in week 3, while the animals treated with 800mg/kg and

1200mg/kg of the aqueous leaf extract alongside the infected and untreated control group all died in week 4.

Table 7: Effects of Different Treatments of the Aqueous Leaf Extract of *L. micranthus* on the Alanine Transaminase, ALT (u/L) of albino rats on Weekly Basis

Treatment (mg/kg)	Duration				
	Week 0	Week 1	Week 2	Week 3	Week 4
(400mg/kg)	38.50 ±1.18^{2a}	93.33±1.76^{1a}	95.500±.28^{1a}	00 ± 00^{3d}	
(800mg/kg)	39.25±1.54^{3a}	93.00±1.73^{2a}	98.500±28^{2a}	129.50±.500^{1a}	0.00
(1200mg/kg)	39.67±1.59^{3a}	97.67±5.50^{2a}	98.000±.00^{2a}	125.00±.000^{1b}	0.00
(Infected and untreated)	38.75±.78^{3a}	96.67±.88^{2a}	98.000±.00^{2a}	131.00±1.00^{1a}	0.00
(Standard drug)	38.83±.76^{2a}	25.00±1.52^{3b}	30.000±4.04^{3b}	56.00±1.00^{1c}	59.33 + 3.84^{1a}
(Normal control)	38.63±.75^{1a}	33.42±1.88^{1b}	32.63±2.13^{1b}	36.30±2.68^{1c}	35.12±2.87^{1b}

Mean values in a column with different letters as superscript are significantly different at $P < 0.05$.

Mean values in a row with different numbers as superscript are significantly different at $P < 0.05$

3.5.3 Weekly effects of aqueous leaf extracts of *L. micranthus* on the serum alkaline phosphatase (ALP) levels of albino rats.

Table 8 shows the results of the weekly effects of aqueous leaf extracts of *L. micranthus* on the serum ALP levels of albino rats. The increasing dose of *L. micranthus* aqueous leaf extracts produced an overall dose-independent significant increase ($P<0.05$) in the total serum levels of ALP of albino rats infected with *T. brucei brucei.* Comparatively, the effect of increasing dose of the aqueous leaf extract of *L. micranthus* on the serum ALP levels of the treated rats was not significantly different ($P>0.05$) from that of infected and untreated control group in weeks 1 and 2, but was significantly different ($P<0.05$) from those of standard drug and normal control groups. Similarly, the serum ALP levels of rats administered 800mg/kg and 1200mg/kg differed significantly ($P<0.05$) in comparison with all the control groups in week 3.

There was a time-dependent significant increase ($P<0.05$) in the serum ALP levels of the treated animals and the infected and untreated and the standard drug control group in week 2. The treated animals did not vary significantly ($P>0.05$) from the infected and untreated control groups in week 1 but varied significantly different ($P<0.05$) from that of the normal control group in weeks 1 and 3. However, the animals administered 400mg/kg of the extract recorded total mortality in week 3 of the study, whereas the animals administered 800mg/kg and 1200mg/kg of the aqueous leaf extract as well as the infected and untreated control group all died in week 4.

Table 8: Effects of Different Treatments of the Aqueous Leaf Extract of *L. micranthus* on the Alkaline Phosphatase, ALP (u/L) of Albino Rats on Weekly Basis

Treatment (mg/kg)	Duration				
	Week 0	Week 1	Week 2	Week 3	Week 4
(400mg/kg)	66.50 ± 2.67^{3ab}	152.67 ± 5.04^{2a}	185.500 ± 6.63^{1a}	000	
(800mg/kg)	63.08 ± 2.04^{4ab}	156.67 ± 4.05^{3a}	195.500 ± 3.17^{1a}	169.00 ± 1.00^{2a}	0.00
(1200mg/kg)	52.17 ± 3.59^{3b}	158.00 ± 7.21^{2a}	191.500 ± 3.17^{1a}	150.00 ± 2.00^{2b}	0.00
(Infected and untreated)	67.00 ± 2.24^{4a}	154.00 ± 6.24^{2a}	195.000 ± 2.88^{1a}	108.50 ± 0.50^{3c}	0.00
(Standard drug)	65.00 ± 3.10^{3ab}	72.33 ± 10.69^{3b}	152.333 ± 10.65^{1b}	90.33 ± 4.91^{2d}	$63.00\ \pm4.04^{3a}$
(Normal control)	62.03 ± 3.59^{1ab}	66.830 ± 3.74^{1b}	63.020 ± 6.43^{1c}	63.212 ± 1.75^{1d}	65.212 ± 3.04^{1a}

Mean values in a column with different letters as superscript are significantly different at $P < 0.05$.

Mean values in a row with different numbers as superscript are significantly different at $P < 0.05$

3.5.4 Weekly effects of aqueous leaf extracts of *L. micranthus* on the serum albumin levels of albino rats.

Table 9 show the results of the weekly effects of aqueous leaf extracts of *L. micranthus* on the serum albumin levels of albino rats infected with *T. brucei brucei* . There was no dose-dependent significant difference (P>0.05) in the total serum albumin levels of albino rats infected with *T. brucei brucei* when compared with the control groups. Comparatively, whereas the serum albumin levels of the rats administered 400mg/kg and 800mg/kg of the aqueous *L. micranthus* leaf extract was significantly different (P<0.05) from that of the standard drug control group in week 1, that of those administered 1200mg/kg was not significantly different (P>0.05) in weeks 1 and 3. Similarly, it was observed that the serum albumin levels of the treated animals were significantly different (P<0.05) from those of the infected and untreated; and normal control groups in week 1. However, there was no significant difference (P>0.05) in the serum albumin levels of all the treated animals in comparison with the control groups in week 2. In addition, it was observed that the serum albumin levels of the rats administered 800mg/kg of the aqueous leaf extract of *L. micranthus* was significantly different (P<0.05) from the infected and untreated control group in week 3, but was not significantly different (P>0.05) from that of standard drug and normal control groups.

There was no overall time dependent significant difference (P>0.05) in the serum albumin levels of the treated albino rats in comparison with the standard drug and the infected and untreated control groups in all the weeks. It was however observed that in week 2, the serum albumin levels of the treated rats were significantly different (P<0.05) from that of the normal control group. It is also noteworthy that the animals that were administered 400mg/kg of the aqueous extract recorded total mortality in week 3, while the animals treated with 800mg/kg and 1200mg/kg of the aqueous leaf extract alongside the infected and untreated control group all died in week 4.

Table 9: Effects of Different Treatments of the Aqueous Leaf Extract of *L. micranthus* on the Albumin (g/dl) of Albino Rats on Weekly Basis

Treatment (mg/kg)	Duration				
	Week 0	Week 1	Week 2	Week 3	Week 4
(400mg/kg)	37.67 ± 1.00^{2a}	43.67 ± 3.38^{1bc}	31.500 ± 3.75^{2a}	000	
(800mg/kg)	37.08 ± 0.82^{2a}	43.67 ± 1.76^{1bc}	38.500 ± 1.44^{2a}	43.50 ± 0.50^{1a}	000
(1200mg/kg)	37.00 ± 0.69^{2a}	47.33 ± 1.76^{1ab}	$36.000\pm.57^{2a}$	40.50 ± 4.50^{2ab}	000
(infected and untreated)	37.17 ± 0.83^{12a}	40.33 ± 1.33^{1c}	33.000 ± 1.73^{2a}	35.50 ± 0.50^{2b}	000
(Standard drug)	36.58 ± 0.80^{3a}	51.33 ± 0.33^{1a}	35.000 ± 1.73^{3a}	39.67 ± 0.33^{2ab}	41.33 ± 2.02^{2a}
(Standard drug)	36.58 ± 0.80^{3a}	51.33 ± 0.33^{1a}	35.000 ± 1.73^{3a}	39.67 ± 0.33^{2ab}	41.33 ± 2.02^{2a}
(Normal control)	37.19 ± 0.82^{1a}	38.53 ± 0.33^{1c}	37.28 ± 1.36^{1a}	37.21 ± 0.24^{1ab}	65.212 ± 3.04^{1a}

Mean values in a column with different letters as superscript are significantly different at P<0.05.

Mean values in a row with different numbers as superscript are significantly different at P<0.05

3.5.5 Weekly effects of aqueous leaf extracts of *L. micranthus* on the serum bilirubin levels of albino rats.

Table 10 shows the results of the weekly effects of the aqueous leaf extracts of *L. micranthus* on the serum bilirubin levels of albino rats. The increasing dose of the aqueous leaf extracts of *L. micranthus* produced a dose-independent significant increase ($P<0.05$) in the serum bilirubin levels of albino rats infected with *T. brucei brucei.* Comparatively, the effects of the increasing dose of the aqueous leaf extract of *L. micranthus* on the serum bilirubin levels of the treated animals was not significantly different ($P>0.05$) from those of the infected and untreated and standard drug control groups in week 1 but was significantly different ($P<0.05$) from that of the normal control group. However, it was observed in week 2, that the serum bilirubin levels of the rats administered 1200mg/kg was significantly different ($P<0.05$) from the normal and the infected and untreated control groups but was not significantly different ($P>0.05$) from that of the standard drug control group. Similarly, in week 3, the serum bilirubin levels of the rats administered 800mg/kg and 1200mg/kg was significantly different ($P<0.05$) from all the control groups.

There was an overall time independent significant increase ($P<0.05$) in the serum bilirubin levels of the treated animals above that of the baseline (week 0). The serum bilirubin levels of the treated animals were significantly different ($P<0.05$) from those of infected and untreated and normal control groups in weeks 2 and 3, whereas the treatment groups were not significantly different ($P>0.05$) from that of the standard drug control group. Total mortality occurred in the animals that were treated with 400mg/kg of the aqueous extract in week 3, whereas in week 4 the treatment groups that were administered 800mg/kg and 1200mg/kg of the aqueous leaf extract together with the infected and untreated control group all recorded total mortality in week 4.

Table 10: Effects of Different Treatments of the Aqueous Leaf Extract of *L. micranthus* on the Total Bilirubin (mg/dl) of Albino Rats on Weekly Basis

	Duration				
Treatment (mg/kg)	**Week 0**	**Week 1**	**Week 2**	**Week 3**	**Week 4**
(400mg/kg)	5.200 ± 0.31^{2ab}	17.033 ± 4.43^{1a}	5.3500 ± 0.83^{2cd}	0.00	
(800mg/kg)	7.4166 ± 0.94^{12a}	13.1000 ± 3.67^{1a}	4.2500 ± 1.06^{2d}	$13.500 \pm .10^{1a}$	0.00
(1200mg/kg)	5.066 ± 0.32^{3ab}	19.566 ± 5.33^{1a}	10.900 ± 1.61^{2b}	$11.800 \pm .80^{2ab}$	0.00
(Infected and untreated)	6.491 ± 0.85^{2ab}	17.166 ± 7.04^{1a}	16.000 ± 0.00^{1a}	$9.600 \pm .40^{12bc}$	0.00
(Standard drug)	5.525 ± 0.58^{3ab}	17.700 ± 0.49^{1a}	9.126 ± 2.30^{2bc}	8.333 ± 1.04^{2c}	$5.567 \pm .49^{3a}$
(Normal control)	5.500 ± 0.31^{1ab}	5.113 ± 0.22^{1b}	6.013 ± 0.00^{1c}	5.881 ± 1.10^{1c}	5.721 ± 4121^{1a}

Mean values in a column with different letters as superscript are significantly different at $P < 0.05$.

Mean values in a row with different numbers as superscript are significantly different at $P < 0.05$

3.5.6 Weekly effects of aqueous leaf extracts of *L. micranthus* on the serum urea of levels albino rats.

Table 11 shows the results of the weekly effects of aqueous leaf extracts of *L. micranthus* on the serum urea levels of albino rats. There was no overall dose-dependent significant difference ($P>0.05$) in the serum urea levels of the treated albino rats. The comparative effects of the increasing dose of the aqueous leaf extracts of *L. micranthus* on the serum urea levels of the treated rats was not significantly different ($P>0.05$) from those of the standard drug, and infected and untreated control groups in weeks 1 to 3 but was significantly different ($P<0.05$) from the normal control group in week 1.

There was a time-independent significant increase ($P<0.05$) in the serum urea levels of the albino rats treated with the aqueous leaf extract of *L. micranthus* in week 1. However, the animals administered the 400mg/kg and 1200mg/kg of the aqueous extract was significantly different ($P<0.05$) from the normal control group in week 2, whereas in week 3 the animals treated with the dose levels of 1200mg/kg was not significantly different ($P>0.05$) from the normal control group. However, the animals administered 400mg/kg of the extract recorded total mortality in week 3 of the study, whereas the animals administered 800mg/kg and 1200mg/kg of the aqueous leaf extract as well as the infected and untreated control group all died in week 4.

Table 11: Effects of Different Treatments of the Aqueous Leaf Extract of *L. micranthus* on the Urea (mg/dl) of Albino Rats on Weekly Basis

Treatment (mg/kg)	Duration				
	Week 0	**Week 1**	**Week 2**	**Week 3**	**Week 4**
(400mg/kg)	4.066 ± 0.27^{2a}	8.866 ± 0.61^{1a}	3.800 ± 0.05^{2a}	0.00	
(800mg/kg)	4.566 ± 0.91^{2a}	8.133 ± 1.63^{1a}	5.4500 ± 2.04^{12a}	3.850 ± 0.05^{2ab}	0.00
(1200mg/kg)	4.533 ± 0.31^{2a}	7.100 ± 1.01^{12a}	4.800 ± 1.38^{2a}	10.250 ± 5.75^{1a}	0.00
(Infected and untreated)	4.675 ± 0.40^{2a}	7.900 ± 1.41^{1a}	3.7500 ± 0.43^{2a}	5.500 ± 1.50^{2ab}	0.00
(Standard drug)	4.266 ± 0.21^{12a}	8.500 ± 4.65^{1a}	5.366 ± 0.47^{12a}	3.800 ± 0.15^{2ab}	$3.733 \pm .24^{2a}$
(Normal control)	$4.213 \pm .43^{1a}$	$4.439 \pm .21^{1b}$	$4.491 \pm .20^{1a}$	$4.334 \pm .13^{1ab}$	$4.310 \pm .11^{1a}$

Mean values in a column with different letters as superscript are significantly different at P<0.05.

Mean values in a row with different numbers as superscript are significantly different at P<0.05

3.5.7 Weekly effects of aqueous leaf extracts of *L. micranthus* on the serum creatinine levels of albino rats.

The results of the weekly effects of aqueous leaf extracts of *L. micranthus* on the serum creatinine levels of albino rats are shown in Table 12. Comparatively, the effect of the increasing dose of the aqueous leaf extracts of *L. micranthus* on the serum creatinine levels of the treated animals was not significantly different ($P>0.05$) from that of the infected and untreated control group in weeks 1 and 3, but was significantly different ($P<0.05$) in week 2. Similarly, it was observed that in weeks 2 and 3, the serum creatinine levels of rats administered the 800mg/kg and 1200mg/kg was not significantly different ($P>0.05$) from those of standard drug and normal control groups but was significantly different ($P<0.05$) in week 1.

There was a time-independent significant increase ($P<0.05$) in the serum creatinine levels of the treated animals in all the weeks in comparison with the normal control group. However, there was a significant difference ($P<0.05$) in the serum creatinine levels of the treated animals in comparison to those of the standard drug and normal control groups in week 2, but no significant difference ($P>0.05$) was observed in the serum levels of creatinine of the animals administered the 1200mg/kg of the aqueous extract in comparison with the normal, and infected and untreated control groups. However, the animals administered 400mg/kg of the extract recorded total mortality in week 3 of the study, while the animals administered 800mg/kg and 1200mg/kg of the aqueous leaf extract as well as the infected and untreated control group all died in week 4.

Table 12: Effects of Different Treatments of the Aqueous Leaf Extract of *L. micranthus* on the Creatinine (mg/dl) of Albino Rats on Weekly Basis

Treatment (mg/kg)	Duration				
	Week 0	Week 1	Week 2	Week 3	Week 4
(400mg/kg)	65.750±4.49^{2a}	163.000±9.01^{1a}	67.00±10.97^{2c}	0.00	
(800mg/kg)	73.250±2.62^{2a}	150.333±24.91^{1ab}	84.00±37.52^{2ab}	88.50±.50^{2ab}	0.00
(1200mg/kg)	74.117±6.99^{2a}	111.000±14.17^{12ab}	99.00±32.33^{2a}	172.00±75.00^{1a}	0.00
(infected and untreated)	77.583±3.96^{23a}	165.000±20.21^{1a}	65.00±18.47^{3c}	103.50±0.50^{12ab}	0.00
(standard drug)	76.167±4.50^{2a}	43.300±20.21^{3c}	126.00±14.97^{1a}	67.00±8.08^{2ab}	86.67±8.96^{2a}
(Normal control)	73.504±6.21^{1a}	74.227±3.11^{1b}	76.215±5.32^{1ab}	74.100±4.757^{1ab}	75.313±3.72^{1a}

Mean values in a column with different letters as superscript are significantly different at P<0.05.

Mean values in a row with different numbers as superscript are significantly different at P<0.05

3.5.8 Weekly effects of aqueous leaf extracts of *L. micranthus* on the serum levels of total cholesterol of albino rats.

The results of the weekly effects of aqueous leaf extracts on the total serum cholesterol levels of albino rats are shown in Table 13. The rats administered with the aqueous leaf extracts of *L. micranthus* produced an overall dose-dependent significant increase ($P<0.05$) in the total serum cholesterol levels of rats infected with *T. burucei brucei*. The comparative effects of the increasing dose of the aqueous leaf extract of *L. micranthus* on the total serum cholesterol levels of the treated animals was not significantly different ($P>0.05$) from the standard drug control but differed significantly ($P<0.05$) from infected and untreated, and normal control groups in week 1, whereas it was observed in week 2, that the serum cholesterol levels of rats administered the 800mg/kg and 1200mg/kg did not significantly differ ($P>0.05$) from those of infected and untreated, and standard drug control groups but was significantly different ($P<0.05$) from the normal control group. However, there was no significant difference ($P>0.05$) in the total serum cholesterol levels of animals administered 800mg/kg and 1200mg/kg and all the control groups in week 3.

There was an overall time dependent significant increase ($P<0.05$) in the total serum cholesterol levels of the treated animals in comparison to that of standard drug control group. Moreover, the treated animals were not significantly different ($P>0.05$) from those of the infected and untreated and normal control groups in week 1. Similarly, week 2 recorded no significant difference ($P>0.05$) between the treated animals and all the control groups. However, the animals that were administered 800mg/kg and 1200mg/kg of the aqueous leaf extract of *L. micranthus* was significantly different ($P<0.05$) from that of the normal control group in week 3, but the infected and untreated control group did not vary significantly from them. Total mortality occurred in the animals that were treated with 400mg/kg of the aqueous extract in week 3, whereas the treatment groups that were administered 800mg/kg and

1200mg/kg of the aqueous leaf extract together with the infected and untreated control group all died off in week 4.

Table 13: Effects of Different Treatments of the Aqueous Leaf Extract of *L. micranthus* on the Total Cholesterol (mmol/L) ofAlbino Rats on Weekly Basis

Treatment (mg/kg)	Duration				
	Week 0	**Week 1**	**Week 2**	**Week 3**	**Week 4**
(400mg/kg)	$0.43667\pm.030^{2b}$	$1.566\pm.29^{1ab}$	$1.800\pm.05^{1b}$	0.00	
(800mg/kg)	$0.64667\pm.062^{4a}$	$1.110\pm.20^{3ab}$	$2.1500\pm.25^{1ab}$	$1.7500\pm.50^{2a}$	0.00
(1200mg/kg)	$0.63000\pm.08^{3ab}$	$1.166\pm.12^{12ab}$	$2.700\pm.00^{1a}$	$1.7500\pm.85^{2a}$	0.00
(Infected and untreated)	$0.751667\pm.07^{2a}$	$1.066\pm.08^{12b}$	$2.6500\pm.14^{1a}$	$0.9750\pm.025^{2ab}$	0.00
(Standard drug)	$0.595833\pm.05^{3ab}$	$1.866\pm.23^{2a}$	$2.800\pm.41^{1a}$	$0.8000\pm.057^{3ab}$	$0.767\pm.14^{3a}$
(Normal control)	$0.643149\pm.08^{1a}$	$0.7416\pm.29^{1c}$	$1.297\pm.04^{1b}$	$0.7984\pm.05^{1ab}$	$0.713\pm.50^{1a}$

Mean values in a column with different letters as superscript are significantly different at P<0.05.

Mean values in a row with different numbers as superscript are significantly different at P<0.05

3.6 Effects of the Aqueous Leaf Extract of *L. micranthus* on the Haemtological Indices of Albino Rats

3.6.1 Weekly effects of aqueous leaf extracts of *L. micranthus* on the haemogloblin levels of albino rats

Table 14 shows the results of the weekly effects of the aqueous *L. micranthus* leaf extracts of on the serum haemoglobin levels of the albino rats. The increasing dose of the aqueous leaf extract of *L. micranthus* produced an overall dose-independent significant decrease ($P<0.05$) in the haemoglobin levels of albino rats infected with *T. brucei brucei.* Comparatively, the effect of the increasing dose of the aqueous leaf extract of *L. micranthus* on the haemoglobin of the treated animals was not significantly different ($P>0.05$) from that of the infected and untreated control group in weeks 1 and 3, but was significantly different ($P<0.05$) from those of the standard drug and normal control groups. However, there was no significant difference ($P>0.05$) in the haemoglobin levels of rats that were administered the 400mg/kg and 1200mg/kg in comparison with the control groups in week 2.

There was an overall time-dependent significant decrease ($P<0.05$) in the haemoglobin of all the treatment groups from weeks 1-3. However, it was observed that whereas the haemoglobin levels of the treated rats was not significantly different ($P>0.05$) from that of the infected and untreated control group in all the weeks, but they were significantly lower ($P<0.05$) from those of the standard drug and normal control groups. It is also noteworthy that all the animals that were administered 400mg/kg of the aqueous leaf extract died in week 3, while the animals treated with 800mg/kg and 1200mg/kg of the aqueous leaf extract alongside the infected and untreated control group all died in week 4.

Table 14: Effects of Different Treatments of the Aqueous Leaf Extract of *L. micranthus* on the Haemoglobin, Hb (g/dl) of Albino Rats on Weekly Basis

Treatment (mg/kg)	Duration				
	Week 0	Week 1	Week 2	Week 3	Week 4
(400mg/kg)	13.083±0.25^{1a}	9.000±0.36^{2b}	9.050±2.95^{2ab}	000	
(800mg/kg)	12.967±0.25^{1a}	10.366±0.71^{2b}	7.200±0.50^{3b}	6.475±0.02^{3c}	000
(1200mg/kg)	13.00±0.24^{1a}	9.166±1.58^{2b}	11.000±0.00^{12ab}	10.00±1.00^{2b}	000
(Infected and untreated)	13.083±0.22^{1a}	9.933±0.63^{2b}	8.750±0.75^{2ab}	6.950±0.05^{3c}	000
(Standard drug)	12.817±0.27^{1a}	15.333±0.88^{1a}	14.900±2.15^{1a}	13.66±0.88^{1a}	14.67±0.66^{1a}
(Normal control)	13.762±0.25^{1a}	13.831±0.33^{1a}	13.583±0.64^{1a}	13.3021±.50^{1a}	13.511±0.51^{1a}

Mean values in a column with different letters as superscript are significantly different at P<0.05.

Mean values in a row with different numbers as superscript are significantly different at P<0.05

3.6.2 Weekly effects of aqueous leaf extracts of *L. micranthus* on the packed cell volume (PCV) of albino rats.

The results of the weekly effects of the aqueous leaf extract of *L. micranthus* on the packed cell volume (PCV) of albino rats are shown in Table 15. In overall, there was no dose-dependent significant difference ($P>0.05$) in the PCV levels of the albino rats infected with *T. brucei brucei.* The comparative effect of the increasing dose of the aqueous leaf extract of on the PCV of the treated rats was not significantly different ($P>0.05$) from those of the normal, and infected and untreated control groups, but was significantly different ($P<0.05$) from that of standard drug control group in week 1. Furthermore, it was observed that the PCV of the rats administered 400mg/kg and 1200mg/kg was not significantly different ($P<0.05$) in comparison with all the control groups in week 2. Similarly, the PCV of the animals that were administered the 800mg/kg and 1200mg/kg of the aqueous leaf extract of *L. micranthus* was not significantly different ($P>0.05$) from the infected and untreated control group but varied significantly ($P<0.05$) from that of the standard drug and normal control groups in week 3.

There was an overall duration dependent significant decrease ($P<0.05$) in the packed cell volume, PCV, of the treated rats in comparison with that of the PCV of the standard drug and normal control groups in all the weeks. However, the serum PCV levels of the infected and untreated control group was not significantly different ($P>0.05$) from that of the treated animals in all the weeks. In addition, it was observed that the animals administered 400mg/kg of the extract recorded total mortality in week 3 of the study, while the animals administered 800mg/kg and 1200mg/kg of the aqueous leaf extract as well as the infected and untreated control group all died in week 4.

Table 15: Effects of Different Treatments of the Aqueous Leaf Extract of *L. micranthus* on the Packed Cell Volume, PCV (%) of Albino Rats on Weekly Basis

Treatment (mg/kg)	Duration				
	Week 0	Week 1	Week 2	Week 3	Week 4
(400mg/kg)	38.17±0.62^{1a}	26.67±1.20^{2b}	27.00±9.00^{2ab}	.00±0.00	
(800mg/kg)	37.67±0.71^{1a}	30.67±2.18^{2b}	20.00±1.00^{3b}	20.50±0.50^{3b}	000
(1200mg/kg)	38.00±0.57^{1a}	27.00±4.61^{2b}	32.00±1.00^{12ab}	28.00±5.00^{2b}	000
(Infected and untreated)	38.42±0.55^{1a}	30.00+2.08^{2b}	26.00±2.00^{2ab}	20.50±0.50^{3b}	000
(Standard drug)	37.25±0.71^{2a}	46.33±2.02^{1a}	43.67±6.48^{12a}	39.67±2.02^{12a}	43.67±2.186^{12a}
(Normal control)	38.31±0.64^{1a}	38.69±2.41^{1b}	39.12±2.01^{1a}	39.28±1.00^{1a}	38.33±2.100^{1a}

Mean values in a column with different letters as superscript are significantly different at P<0.05.

Mean values in a row with different numbers as superscript are significantly different at P<0.05

3.6.3 Weekly effects of aqueous leaf extract of *L. micranthus* on the red blood cell, (RBC) of albino rats.

Table 16 shows the results of the weekly effects of the aqueous leaf extracts of *L. micranthus* on the red blood cells (RBC) of albino rats. The increasing dose of the aqueous leaf extracts of *L. micranthus* produced no dose-dependent significant difference ($P>0.05$) in the serum R.B.C levels/values of the albino rats infected with *T. brucei brucei.* The comparative effects of the increasing dose of the aqueous leaf extract of *L.micranthus* on the R.B.C levels of the infected and treated animals was not significantly different ($P>0.05$) from all the control groups in week 1, but was significantly different ($P<0.05$) from the standard drug control group in week 2. In addition, it was observed that in week 3, the serum RBC levels of rats administered 800mg/kg and 1200mg/kg were not significantly different ($P>0.05$) from that of the infected and untreated control group but was significantly different ($P<0.05$) from the normal control group.

In overall there was a time-dependent significant decrease ($P<0.05$) in the red blood cell levels of the treated animals in comparison with the normal control group in all the weeks. However, the red blood cell levels of the infected and untreated, and standard drug control groups were not significantly different ($P>0.05$) to those of the treated animals in weeks 1 and 2. It was further observed that the serum RBC levels of the animals administered 400mg/kg and 800mg/kg of the aqueous leaf extracts was not significantly different ($P>0.05$) from that of the standard drug control group in week 2, while those of the rats treated of 800mg/kg and 1200mg/kg doses of the aqueous leaf extracts of differed significantly ($P<0.05$) from those of the infected and untreated, and standard drug control groups in week 3. Similarly, whereas the animals administered 400mg/kg of the aqueous leaf extract recorded total mortality in week 3 of the study, the animals administered 800mg/kg and 1200mg/kg of the aqueous leaf extract as well as the infected and untreated control group all died in week 4.

Table 16: Effects of Different Treatments of the Aqueous Leaf Extract of *L. micranthus* on the Red Blood Cells, RBC ($x10^6/mm^3$) of Albino Rats on Weekly Basis

Treatment (mg/kg)	Duration				
	Week 0	Week 1	Week 2	Week 3	Week 4
(400mg/kg)	7.90 ± 0.29^{1a}	5.14 ± 1.49^{2a}	$4.07\pm.500^{3b}$	0.00	
(800mg/kg)	7.25 ± 0.81^{1a}	6.53 ± 1.13^{2a}	5.70 ± 1.00^{3a}	3.11 ± 0.51^{4c}	000
(1200mg/kg)	7.78 ± 0.13^{1a}	5.11 ± 0.55^{3a}	6.01 ± 1.19^{2a}	4.41 ± 0.24^{4bc}	000
(Infected and untreated)	6.62 ± 0.38^{1a}	5.78 ± 2.93^{2a}	5.81 ± 1.00^{2a}	3.20 ± 0.23^{3c}	000
(Standard drug)	8.11 ± 0.01^{1a}	6.83 ± 0.81^{2a}	4.22 ± 0.67^{3b}	5.58 ± 1.23^{3b}	6.70 ± 0.64^{2a}
(Normal control)	7.39 ± 1.14^{1a}	7.27 ± 0.56^{1a}	7.59 ± 0.72^{1a}	7.30 ± 1.18^{1a}	7.21 ± 2.07^{1a}

Mean values in a column with different letters as superscript are significantly different at $P < 0.05$.

Mean values in a row with different numbers as superscript are significantly different at $P < 0.05$

3.6.4 Weekly effects of aqueous leaf extracts of *L. micranthus* on the mean cell haemoglobin (MCH) of albino rats.

Table 17 shows the results of the weekly effects of aqueous leaf extract of *L. micranthus* on the mean cell haemoglobin (MCH) of albino rats infected with *T. brucei brucei.* There was a dose-independent significant difference (P>0.05) in the mean cell haemoglobin of the albino rats infected with *T. brucei brucei.* The comparative effects of the increasing dose of the aqueous leaf extract of *L. micranthus* on the mean cell haemoglobin of the treated animals was not significantly different (P>0.05) in comparison to the control groups in week 1. However, it was observed that the mean cell haemoglobin of the treated rats was significantly different (P<0.05) in comparison with the standard drug and the normal control groups in weeks 2 and 3 respectively.

There was no significant difference (P>0.05) observed in all the treatment groups from weeks 1-3. However, the MCH of the animals administered the 400mg/kg of the aqueous leaf extract of *L. micranthus* was significantly different (P<0.05) from the infected and untreated but not from standard drug and normal control groups in week 2, whereas no significant difference (P>0.05) was observed for all the groups in week 3. Mortality occurred in all the animals that were treated with 400mg/kg of the aqueous extract in week 3, whereas in week 4 the rats in the treatment groups administered 800mg/kg and 1200mg/kg of the aqueous leaf extract, as well as the infected and untreated control group all died.

Table 17: Effects of Different Treatments of the Aqueous Leaf Extract of *L. micranthus* on the MCH (Pg) of Albino Rats on Weekly Basis

Treatment (mg/kg)	Duration				
	Week 0	Week 1	Week 2	Week 3	Week 4
(400mg/kg)	18 ± 0.57^{2a}	18 ± 0.00^{2a}	22 ± 0.50^{1b}	0.00±.00	
(800mg/kg)	17 ± 0.14^{12a}	16 ± 0.66^{12b}	14 ± 1.0^{2c}	20 ± 0.00^{1a}	000
(1200mg/kg)	18 ± 0.57^{2a}	18 ± 0.10^{2a}	18 ± 0.33^{2b}	25 ± 0.10^{1a}	000
(Infected and untreated)	$21\pm.66^{1a}$	18 ± 0.00^{12a}	16 ± 1.0^{2b}	21 ± 0.721^{1a}	000
(Standard drug)	15 ± 1.0^{3a}	25 ± 0.00^{12a}	35 ± 1.0^{1a}	26 ± 0.00^{12a}	23 ± 0.33^{2a}
(Normal control)	18 ± 0.57^{1a}	18 ± 0.57^{1a}	18 ± 0.57^{1b}	18 ± 0.50^{1b}	18 ± 0.57^{1a}

Mean values in a column with different letters as superscript are significantly different at P<0.05.

Mean values in a row with different numbers as superscript are significantly different at P<0.05

3.6.5 Weekly effects of aqueous leaf extracts of *L. micranthus* on the mean cell volume (MCV) of albino rats.

The results of the weekly effects of aqueous leaf extracts of *L. micranthus* on the mean cell volume (MCV) of albino rats infected with *T. brucei brucei* are shown in Table 18. From observations made in the increasing doses of the aqueous leaf extracts of *L. micranthus*, there was no overall significant difference ($P>0.05$) in the mean cell volume of albino rats infected with *T. brucei brucei*. Comparatively also, the effects of the increasing dosage of the aqueous leaf extract on the mean cell volume of the treated animals was not significantly different ($P>0.05$) in comparison with the control groups in all the weeks.

There was no overall time-dependent significant difference ($P>0.05$) in the MCV levels of the treated groups. However, the animals administered the 400mg/kg of the aqueous leaf extract mean MCV levels which varied significantly ($P<0.05$) from that of the infected and untreated but not from those of the standard drug and normal control groups in week 2. Secondly, the animals administered 400mg/kg of the extract recorded total mortality in week 3 of the study, while those animals administered 800mg/kg and 1200mg/kg of the aqueous leaf extract as well as the infected and untreated control group all died in week 4.

Table 18: Effects of Different Treatments of the Aqueous Leaf Extract of *L. micranthus* on the Mean Cell Volume, MCV (u^3) of Albino Rats on Weekly Basis

Treatment (mg/kg)	Duration				
	Week 0	Week 1	Week 2	Week 3	Week 4
(400mg/kg)	5.4 ± 0.28^{2a}	5.2 ± 0.00^{2a}	6.7 ± 0.50^{1a}	0.00	
(800mg/kg)	5.2 ± 0.85^{12a}	5.0 ± 0.00^{12a}	4.0 ± 1.0^{2a}	$6.\ 6\pm0.66^{1a}$	000
(1200mg/kg)	5.4 ± 028^{2a}	5.4 ± 0.00^{2a}	5.3 ± 0.33^{2a}	7.0 ± 0.00^{1a}	000
(Infected and untreated)	6.3 ± 0.33^{1a}	6.0 ± 1.001^{1a}	5.2 ± 0.00^{2a}	6.6 ± 0.66^{1a}	000
(Standard drug)	4.6 ± 0.25^{2a}	7.6 ± 0.66^{1a}	7.5 ± 1.0^{1a}	7.8 ± 0.00^{1a}	7.1 ± 0.66^{1a}
(Normal control)	5.4 ± 0.28^{1a}	5.4 ± 0.28^{1a}	5.5 ± 0.71^{1a}	5.5 ± 0.71^{1a}	5.4 ± 0.28^{1a}

Mean values in a column with different letters as super script are significantly different at $P<0.05$.

Mean values in a row with different nun/ors as superscript are significantly different at $P<0.05$

3.6.6 Weekly effects of aqueous leaf extracts of *L. micranthus* on the mean cell haemoglobin concentration (MCHC) of albino rats.

The results of the weekly effects of the aqueous leaf extracts of *L. micranthus* on the mean cell haemoglobin concentration (MCHC) of albino rats are shown in Table 19. From the table, the mean cell haemoglobin concentration of animals administered the 400mg/kg was not significantly different ($P>0.05$) from the normal control group but was significantly different ($P<0.05$) from the standard drug and infected and untreated control groups

In week 1. Similarly, while the serum MCHC levels of those administered 800mg/kg and 1200mg/kg of the aqueous leaf extract was not significantly different ($P>0.05$) from the standard drug, and infected and untreated control groups, they were significantly different from that of the normal control group. However, while in weeks 2 and 3, it was observed that the mean cell haemoglobin concentration of rats administered the 800mg/kg and 1200mg/kg was not significantly different ($P>0.05$) from those of the standard drug and normal control groups, they were nonetheless significantly different ($P<0.05$) from that of the infected and untreated control group in week 2.

There was an overall time-independent significant increase ($P<0.05$) in the MCHC of the albino rats treated with the aqueous *L. micranthus* leaf extract when compared with the control groups. Nevertheless it was noticed that the mean serum MCHC levels of the treated animals did not significantly differ ($P>0.05$) from that of the infected and untreated control group in week 1, but was significantly different ($P<0.05$) from those of the standard drug and normal control groups. Secondly, the MCHC serum levels of the animals administered 800mg/kg of the extract was not significantly different ($P>0.05$) from that of the standard drug control group but was significantly different ($P<0.05$) from those of the infected and untreated and normal control groups in week 2. Furthermore, MCHC serum levels of the

animals administered the dose level of 1200mg/kg of the aqueous extract was significantly different ($P<0.05$) from that of the normal control group but was not significantly different ($P>0.05$) from those of the infected and untreated and standard drug control groups. It is also noteworthy that all the animals that were administered 400mg/kg of the aqueous leaf extract died in week 3, while the animals treated with 800mg/kg and 1200mg/kg of the aqueous leaf extract alongside the infected and untreated control group all died in week 4.

Table 19: Effects of Different Treatments of the Aqueous Leaf Extract of *L. micranthus* on the Mean Cell Haemoglobin Concentration, MCHC (g/dl) of Albino Rats on Weekly Basis

Treatment (mg/kg)	Duration				
	Week 0	Week 1	Week 2	Week 3	Week 4
(400mg/kg)	351 ± 0.35^{1a}	346 ± 0.15^{2a}	333 ± 0.33^{3b}	000±.00	
(800mg/kg)	308 ± 0.33^{4b}	333 ± 0.33^{2b}	360 ± 0.00^{1a}	320 ± 1.0^{3b}	000
(1200mg/kg)	342 ± 0.10^{2a}	337 ± 0.03^{3b}	343 ± 0.75^{2a}	357 ± 0.14^{1a}	000
(Infected and untreated)	342 ± 0.10^{1a}	333 ± 0.33^{2b}	334 ± 0.62^{2b}	345 ± 0.00^{1a}	000
(Standard drug)	345 ± 0.94^{12a}	332 ± 0.60^{3b}	346 ± 0.51^{1a}	348 ± 0.72^{1a}	339 ± 0.53^{2b}
(Normal control)	360 ± 0.52^{1a}	363 ± 0.15^{1a}	346 ± 0.15^{2a}	341 ± 0.02^{2a}	355 ± 0.26^{2a}

Mean values in a column with different letters as superscript are significantly different at P<0.05.

Mean values in a row with different numbers as superscript are significantly different at P<0.05

3.6.7 Weekly effects of aqueous leaf extracts of *L. micranthus* on the white blood cell (W.B.C) of albino rats.

The results of the weekly effects of the aqueous leaf extract of *L. micranthus* on the white blood cell (WBC) of albino rats are shown in Table 20. The increasing doses of the aqueous leaf extracts of *L. micranthus* produced a dose-dependent significant difference ($P>0.05$) in the WBC of the albino rats infected with *T. brucei brucei.* Comparatively, the effect of the increasing dose of the aqueous leaf extract of *L. micranthus* on the WBC of the treated rats was not significantly different ($P>0.05$) from those of the normal, and the infected and untreated control groups in weeks 1 and 3, but was significantly different ($P<0.05$) from the standard drug control group in week 1. However, it was observed in week 2, that the WBC of animals administered the 800mg/kg and 1200mg/kg of the aqueous leaf extract of *L. micranthus* was not significantly different ($P>0.05$) from that of the standard drug control group but was significantly different ($P<0.05$) from the normal, and infected and untreated control groups.

In overall, there was a time-dependent significant increase ($P<0.05$) in the white blood cell levels of the animals infected with *T. brucei brucei* and treated with the aqueous leaf extracts of *L. micranthus* in comparison to the normal control group in all the weeks. However, it was observed that in weeks 1 and 3, the white blood cells of the infected and untreated, and standard drug control groups were not significantly different ($P>0.05$) from that of the treated animals. Furthermore, the animals administered 400mg/kg of the extract recorded total mortality in week 3 of the study, whereas the animals administered 800mg/kg and 1200mg/kg of the aqueous leaf extract as well as the infected and untreated control group all died in week 4.

Table 20: Effects of Different Treatments of the Aqueous Leaf Extract of *L. micranthus* on the White Blood Cells, WBC ($x10^3/mm^3$) of Albino Rats on Weekly Basis

Treatment (mg/kg)	Duration				
	Week 0	Week 1	Week 2	Week 3	Week 4
(400mg/kg)	$4.958\pm.27^{1a}$	$5.066\pm.58^{1b}$	$4.100\pm.90^{1b}$	000±.00	
(800mg/kg)	$4.333\pm.27^{3a}$	7.600 ± 1.92^{2b}	6.800 ± 2.20^{23a}	$24.750\pm.25^{1a}$	000
(1200mg/kg)	$4.191\pm.39^{2a}$	$8.933\pm.52^{12b}$	8.900 ± 5.30^{12a}	10.00 ± 4.00^{1b}	000
(Infected and untreated)	$4.300\pm.26^{3a}$	$9.066\pm.58^{2b}$	4.500 ± 1.50^{3b}	$11.950\pm.05^{1b}$	000
(Standard drug)	$4.216\pm.26^{3a}$	14.400 ± 1.83^{1a}	8.866 ± 1.86^{2a}	6.067 ± 1.29^{23b}	8.33 ± 2.33^{2a}
(Normal control)	$4.432\pm.27^{1a}$	$4.398\pm.12^{1b}$	$4.414\pm.36^{1b}$	$4.224\pm.27^{1bc}$	$4.210\pm.38^{1b}$

Mean values in a column with different letters as superscript are significantly different at P<0.05.

Mean values in a row with different numbers as superscript are significantly different at P<0.05

3.6.8 Weekly effects of aqueous leaf extracts of *L. micranthus* on the neutrophil levels of albino rats.

The results of the weekly effects of the aqueous leaf extract of *L.micranthus* on the neutrophil count of albino rats are shown in Table 21. There was a dose-dependent significant difference (P>0.05) in the neutrophil of albino rats infected with *T. brucei brucei.* The comparative effect of the increasing dose of the aqueous leaf extract of *L. micranthus* of the treated animals was not significantly different (P>0.05) from that of the normal and infected and untreated control groups in weeks 1 and 3, but was significantly different (P<0.05) from the standard drug control group in week 1. However, there was no significant difference (P>0.05) in the neutrophils of the treated animals in comparison with the control groups in week 2. It was equally observed that the animals that were administered the 800mg/kg of the aqueous leaf extracts of *L. micranthus* varied with those of the control groups in week 3.

There was an overall, time independent significant difference (P<0.05) in the neutrophil counts of the treated animals in comparison to that of the normal control group. Similarly, there was a significant difference (P<0.05) in the neutrophils of the treated animals in comparison with those of the infected and untreated control group in weeks 1 and 2, and the standard drug control group in weeks 2 and 3 respectively. However, the animals administered 400mg/kg of the extract recorded total mortality in week 3 of the study, whereas the animals administered 800mg/kg and 1200mg/kg of the aqueous leaf extract as well as the infected and untreated control group all died in week 4.

Table 21: Effects of Different Treatments of the Aqueous Leaf Extract of *L. micranthus* on the Neutrophils (uL) of Albino Rats on Weekly Basis

Treatment (mg/kg)	Duration				
	Week 0	**Week 1**	**Week 2**	**Week 3**	**Week 4**
(400mg/kg)	2.600 ± 0.16^{1a}	2.6300 ± 0.34^{1b}	2.250 ± 0.25^{1a}	000	
(800mg/kg)	2.608 ± 0.16^{2a}	4.267 ± 1.09^{2b}	3.900 ± 0.90^{2a}	13.250 ± 0.25^{1a}	000
(1200mg/kg)	2.33 ± 0.21^{2a}	5.100 ± 0.37^{1b}	5.050 ± 2.95^{1a}	5.100 ± 2.10^{1b}	000
(Infected and untreated)	2.291 ± 0.16^{3a}	5.167 ± 0.55^{2b}	2.750 ± 0.75^{3a}	6.5950 ± 0.00^{1b}	000
(Standard drug)	2.291 ± 0.16^{3a}	8.00 ± 1.02^{1a}	5.000 ± 1.00^{2a}	3.333 ± 0.76^{23bc}	4.600 ± 1.53^{2a}
(Normal control)	2.323 ± 0.24^{1a}	2.668 ± 0.51^{1b}	2.515 ± 0.31^{1a}	2.633 ± 0.30^{1bc}	2.602 ± 0.12^{1a}

Mean values in a column with different letters as superscript are significantly different at P<0.05.

Mean values in a row with different numbers as superscript are significantly different at P<0.05

3.6.9 Weekly effects of aqueous leaf extracts of *L. micranthus* on the lymphocyte levels of albino rats.

Table 22 shows the results of the weekly effects of aqueous leaf extracts of *L. micranthus* on the lymphocyte counts of albino rats infected with *T. brucei brucei*. In overall, there was an overall no significant difference (P>0.05) in the lymphocyte levels of the albino rats infected with *T. brucei brucei*. Comparatively, the effect of the increasing dose of the aqueous leaf extract of *L. micranthus* on the lymphocyte of the treated animals was not significantly different (P>0.05) from those of the normal, and infected and untreated control groups in week 1, but was significantly different (P<0.05) from that of the standard drug control group. However, it was observed that in week 2, the lymphocyte levels of rats administered the 800mg/kg and 1200mg/kg was not significantly different (P>0.05) from those of the standard drug and normal control groups but was significantly different (P<0.05) from that of the infected and untreated control group. In week 3 also, rats administered the 800mg/kg of the aqueous leaf extract of *L. micranthus* varied in comparison with those of the control groups.

There was no overall time dependent significant difference (P>0.05) in the lymphocyte levels of the treated animals in comparison to the normal control group in all the weeks. However, the infected and untreated and standard drug control groups did not significantly differ (P>0.05) from the treated animals in weeks 1 and 3 but significantly varied (P<0.05) in weeks 1 and 3 respectively. Mortality occurred in all the animals that were treated with 400mg/kg of the aqueous extract in week 3, whereas in week 4 the rats in the treatment groups administered 800mg/kg and 1200mg/kg of the aqueous leaf extract, as well as the infected and untreated control group all died.

Table 22: Effects of Different Treatments of the Aqueous Leaf Extract of *L. micranthus* on the Lymphocyte (uL) of Albino Rats on Weekly Basis

Treatment (mg/kg)	Duration				
	Week 0	**Week 1**	**Week 2**	**Week 3**	**Week 4**
(400mg/kg)	2.075 ± 0.12^{1a}	2.066 ± 0.26^{1b}	1.750 ± 0.55^{1b}	000	
(800mg/kg)	1.616 ± 0.12^{3ab}	3.200 ± 0.80^{2b}	2.500 ± 1.30^{2a}	12.250 ± 0.25^{1a}	000
(1200mg/kg)	1.591 ± 0.14^{2b}	3.600 ± 0.40^{12b}	4.000 ± 2.80^{1a}	4.950 ± 0.05^{1b}	000
(Infected and untreated)	1.783 ± 0.11^{3ab}	3.466 ± 0.29^{2b}	1.500 ± 0.50^{3b}	4.950 ± 0.05^{1b}	000
(Standard drug)	1.691 ± 0.10^{3ab}	6.033 ± 0.53^{1a}	3.533 ± 1.03^{2a}	2.433 ± 0.61^{23bc}	3.600 ± 0.94^{2a}
(Normal control)	1.641 ± 0.12^{1ab}	1.881 ± 0.19^{1b}	2.133 ± 0.73^{1a}	1.891 ± 0.50^{1c}	1.811 ± 0.11^{1a}

Mean values in a column with different letters as superscript are significantly different at $P<0.05$.

Mean values in a row with different numbers as superscript are significantly different at $P<0.05$

3.6.10 Weekly effects of aqueous leaf extracts of *L. micranthus* on the eosinophil levels of albino rats.

Table 23 shows the results of the weekly effects of the aqueous leaf extract of *L. micranthus* on the eosinophil counts of albino rats infected with *T. brucei brucei*. There was no overall significant difference (P>0.05) in the eosinophil levels of albino rats infected with *T. brucei brucei.* The comparative effects of the increasing dose of the aqueous leaf extract of *L. micranthus* on the eosinophil levels of the animals that were administered the 400mg/kg was significantly different (P<0.05) from the control groups in week 1. However, there was no significant difference (P>0.05) in the eosinophil levels of the treated animals in week 2. There was no overall duration dependent significant difference (P>0.05) in the eosinophil on the duration of treatment of the treated animals in comparison with the control groups in all the weeks. However, the animals administered 400mg/kg of the extract recorded total mortality in week 3 of the study, whereas the animals administered 800mg/kg and 1200mg/kg of the aqueous leaf extract as well as the infected and untreated control group all died in week 4.

Table 23: Effects of Different Treatments of the Aqueous Leaf Extract of *L. micranthus* on the Eosinophil (uL) of Albino Rats on Weekly Basis

Treatment (mg/kg)	Duration				
	Week 0	Week 1	Week 2	Week 3	Week 4
(400mg/kg)	0.033 ± 0.0188^{1a}	0.067 ± 0.0333^{1a}	0.000 ± 0.000^{1a}	000	
(800mg/kg)	0.000 ± 0.000^{1a}	0.000 ± 0.000^{1b}	0.000 ± 0.000^{1a}	000	000
(1200mg/kg)	0.017 ± 0.0112^{1a}	0.000 ± 0.000^{1b}	0.000 ± 0.000^{1a}	000	000
(Infected and untreated)	0.008 ± 0.0083^{1a}	0.000 ± 0.000^{1b}	0.000 ± 0.000^{1a}	000	000
(Standard drug)	0.033 ± 0.0142^{1a}	0.000 ± 0.000^{1b}	0.067 ± 0.0333^{1a}	000	000
(Normal control)	0.030 ± 0.0127^{1a}	0.029 ± 0.0100^{1b}	0.031 ± 0.01413^{1a}	000	000

Mean values in a column with different letters as superscript are significantly different at $P<0.05$.

Mean values in a row with different numbers as superscript are significantly different at $P<0.05$

3.6.11 Weekly effects of aqueous leaf extracts of *L. micranthus* on the basophil levels of albino rats.

Table 24 shows the results of the weekly effects of aqueous leaf extract of *L. micranthus* on the basophil levels of albino rats infected with *T. brucei brucei* . There was no overall significant difference (P>0.05) in the basophil counts of the albino rats infected with *T. brucei brucei.* Comparatively, the effects of the increasing dose of the aqueous leaf extract of *L. micranthus* on the basophil of the treated rats was not significantly different (P>0.05) from all the control groups from weeks 1 to 3.

There was no overall significant difference (P>0.05) in the basophil counts of the treated animals when compared with the control groups in all the weeks. It is also noteworthy that all the animals that were administered 400mg/kg of the aqueous leaf extract died in week 3, while the animals treated with 800mg/kg and 1200mg/kg of the aqueous leaf extract alongside the infected and untreated control group all died in week 4.

Table 24: Effects of Different Treatments of the Aqueous Leaf Extract of *L. micranthus* on the Basophils (uL) of Albino Rats on Weekly Basis

Treatment (mg/kg)	Duration				
	Week 0	Week 1	Week 2	Week 3	Week 4
(400mg/kg)	0.075 ± 0.02^{1a}	0.067 ± 0.03^{1a}	0.050 ± 0.05^{1a}	0.000	
(800mg/kg)	0.017 ± 0.01^{2a}	0.000 ± 0.00^{2b}	0.100 ± 0.00^{1a}	0.1250 ± 0.02^{1a}	000
(1200mg/kg)	0.067 ± 0.02^{1a}	0.033 ± 0.03^{1a}	0.000 ± 0.00^{1a}	0.1000 ± 0.10^{1a}	000
(Infected and untreated)	0.033 ± 0.01^{1a}	0.067 ± 0.03^{1a}	0.050 ± 0.05^{1a}	0.000 ± 0.00^{1a}	000
(Standard drug)	0.067 ± 0.02^{1a}	0.067 ± 0.03^{1a}	0.100 ± 0.05^{1a}	0.0333 ± 0.03^{1a}	0.033 ± 0.03^{1a}
(Normal control)	0.067 ± 0.02^{1a}	0.067 ± 0.02^{1a}	0.050 ± 0.05^{1a}	0.0333 ± 0.01^{1a}	0.033 ± 0.03^{1a}

Mean values in a column with different letters as superscript are significantly different at $P<0.05$.

Mean values in a row with different numbers as superscript are significantly different at $P<0.05$

3.6.12 Weekly effects of aqueous leaf extracts of *L. micranthus* on the monocyte levels of albino rats.

The results of the weekly effects of the aqueous leaf extract of *L. micranthus* on the monocyte of albino rats infected with *T. brucei brucei* are shown in Table 25. The increasing dosage of the aqueous leaf extracts of *L. micranthus* showed that there was no dose-dependent significant difference ($P>0.05$) in the monocyte levels of albino rats infected with *T. brucei brucei.* Comparatively, the effects of increasing dose of the aqueous leaf extract of *L. micranthus* on the treated animals was not significantly different ($P>0.05$) in comparison with the control groups in weeks 1 and 2 respectively. However, it was observed that the monocyte level of the animals administered the 800mg/kg of the aqueous leaf extract of *L. micranthus* was significantly different ($P<0.05$) from all the control groups in week 3.

There was an overall time-dependent no significant difference ($P>0.05$) in the monocyte levels of treatment of the treated animals in comparison with the control group. It was however observed that whereas the monocyte counts of rats administered 800mg/kg varied from that of the control groups in weeks 1 and 2, those of the rats that received 400mg/kg varied significantly ($P<0.05$) in week 2 only. However, the animals administered 400mg/kg of the extract recorded total mortality in week 3 of the study, whereas the animals administered 800mg/kg and 1200mg/kg of the aqueous leaf extract as well as the infected and untreated control group all died in week 4.

Table 25: Effects of Different Treatments of the Aqueous Leaf Extract of *L. micranthus* on the Monocyte (uL) of Albino Rats on Weekly Basis

Treatment (mg/kg)	Duration				
	Week 0	Week 1	Week 2	Week 3	Week 4
(400mg/kg)	0.242 ± 0.02^{1a}	0.267 ± 0.06^{1a}	0.050 ± 0.05^{2a}	.000	
(800mg/kg)	0.100 ± 0.02^{3b}	0.200 ± 0.11^{23a}	0.350 ± 0.05^{2a}	1.375 ± 0.02^{1a}	000
(1200mg/kg)	0.183 ± 0.02^{2ab}	0.200 ± 0.15^{12a}	0.250 ± 0.05^{12a}	0.4500 ± 0.05^{1b}	000
(Infected and untreated)	0.183 ± 0.03^{1ab}	0.367 ± 0.12^{1a}	0.200 ± 0.20^{1a}	0.3750 ± 0.25^{1bc}	000
(Standard drug)	0.233 ± 0.03^{1a}	0.367 ± 0.23^{1a}	0.167 ± 0.08^{1a}	0.2667 ± 0.03^{1c}	0.433 ± 0.12^{1a}
(Normal control)	0.180 ± 0.02^{1ab}	0.183 ± 0.05^{1a}	0.181 ± 0.01^{1a}	0.181 ± 0.68^{1c}	0.181 ± 0.32^{1a}

Mean values in a column with different letters as superscript are significantly different at P<0.05.

Mean values in a row with different numbers as superscript are significantly different at P<0.05

CHAPTER FOUR

DISCUSSION AND CONCLUSION

4.1 Discussion

Plants all over the world are renowned for their medicinal value which lies in their bioactive phytochemical constituents (Veermuthu *et al*., 2006). Phytochemical screening of the aqueous leaf extract of the African mistletoe, *Loranthus micranthus* Linn., parasitic on *Kola acuminata* in Nsukka as reported in this study revealed a high concentration of flavonoids and tannins; moderate concentrations of alkaloids, saponins and steroids; and trace concentrations of glycosides and terpenes. This correlates with the observations of other researchers on the leaves of this same plant using different solvents such as methanol ethyl acetate (Uzochukwu and Osadebe, 2007); chloroform (Osadebe and Omeje, 2009); methanolic and aqueous (Eze-Steven and Njoku, 2010) and petroleum ether (Egbuonu and Nwankwo, 2012) respectively.

The observation made in the present study that the aqueous *L. micranthus* leaf extract showed no lethal effect on the experimental animals at the varying doses administered, positively corroborates past reports on this plant species. Eze-Steven and Njoku (2010) observed that the methanolic and aqueous leaf extracts of *L. micranthus* were not toxic even at a dose level of 5000mg/kg. This presupposes that the leaf extract of this plant species is non-toxic to the experimental animals irrespective of the solvent employed.

The observed steady decrease in the body weights of the infected and treated rats in comparison with the standard drug control group is very surprising. This presupposes that the observed decrease in body weight may be as a result of the severity of the *T. brucei brucei* infection consequent upon the inability of the leaf extract to checkmate the increase in the

population and activity of the parasite. This result may therefore suggest that the extract has no therapeutic effect on the *T. brucei brucei* infection. Jodi *et al*. (2011) observed a similar significant weight decrease and anaemia in rats infected with *T. brucei brucei* which they reported that trypanosomiasis was adduced as the plausible reason for the observed weight decrease. Mgbenka and Ufele (2004) and Ajakiye *et al*. (2013) reported a similar weight loss in the body weights of wistar rats infected with trypanosomiasis which they attributed to parasite-induced anorexia and increased parasitaemia of the circulating parasites.

The results of this present investigation revealed that the parasitaemia was high in all infected and treated animals as well as the infected and untreated control group. This is presumed to be as a result of the overwhelming number of circulating trypanosomes in the blood. Trypanosomes, upon infestation of any mammalian system proliferate rapidly to establish its population in the infected host (Olsson *et al.*, 1992). The antibodies produced by the host against the parasites are in some cases not effective because of the ability of the parasite to produce a large repertoire of antigens (Sternberg, 2004). One can therefore presume that the leaf extract of *L. micranthus* was not able to checkmate the circulating population of parasites in the blood and as such may not be a good therapeutic agent in the treatment of trypanosomiasis after total mortality was recorded for all the infected and treated animals. A similar work by Jodi *et al. (*2011) showed a chronic and fluctuating parasitaemia in rabbits that is suffering from trypanosome infections with complications arising from anaemia, thrombocytopaenia and leucopaenia. The inference is further supported by observations made by Sulaiman and Adeyemi (2010), who reported an increase in the level of parasitaemia of rats infected with *T. brucei brucei* and treated with Novidium and Berenil.

The serum biochemical parameters that were investigated in this research are useful indices for evaluating any plantôs toxicity in an animal. The results obtained in this work showed significant increase among the mean values of the biochemical parameters

investigated. Liver marker enzymes such as alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) are always released into the blood whenever the liver cells experienced damage as the data collected shows and this often results in the increased enzymatic activity. The observations made in this work therefore suggests that parasitic protozoa and viral infections may be among the most common causes of liver injury and this results in AST and ALT being elevated to a greater degree than ALP. The predominant rise in ALP, from this work, is suggestive of damage of the billiary tree which disrupts flow of bile out of the liver. This may imply that the extract might have no hepato-protective properties. Jodi *et al*. (2011) who did a similar work, reported elevated levels of serum ALT, ALP and AST in *T. brucei brucei* infected rats; a situation which implies that there was liver damage. A contrary result obtained by Edem and Usoh (2009), who ascertained significant decreases in the serum AST and ALT of normal male wistar rats administered aqueous extracts of the leaves of *L. micranthus*, but reported elevated levels of serum ALP which agrees with this present work.

Albumin, although, produced entirely in the liver is of great importance in regulating the flow of water between the plasma and tissue fluids, and any hepatic pathology could result in impairment of its production which could lead to increased protein loss through the kidney (Onyelili *et al*., 2005). The observed low serum albumin levels of the infected and treated animals may be linked to renal dysfunction as a result of excretion of albumin in the urine, chronic liver disease and viral infections. The low serum albumin levels obtained in this study followed a fluctuating pattern with minimal increases recorded for the group administered the 800mg/kg of the aqueous extract of *L. micranthus* in week 3. This result is contrary to the views of Edem and Usoh (2009), who reported significant increases in the serum albumin of wistar rats leading to protein loss through the kidney.

Several disorders may cause elevated levels of bilirubin in animals infected with *T. brucei brucei* due to a variety of reasons including reabsorption of large haematomas, ineffective erythropoiesis and liver damage (Halsted and Halsted, 1991; Mayne, 1994). The observations made in this work therefore suggest that persistently high levels of bilirubin in the infected animals may be due to reactions to toxins or the leaf extract, autoimmune hepatitis or chronic liver diseases. The present result further shows there was a dose independent increase in the serum bilirubin of the infected animals treated with aqueous leaf extract of *L. micranthus.* This result correlates with the observations of Igbokwe and Mohammed (1992), who reported elevated levels of bilirubin which they attributed to liver damage leading to clusters of bilirubin showing through the eyes first before manifesting through the skin as jaundice. However, the results from this present investigation differed from the works of Edem and Usoh (2009), who reported decreased levels of serum bilirubin an indication that there was no impairment of hepatic excretory function.

High blood urea is usually associated with excess breakdown of proteins in the blood, increased catabolism of tissue proteins and reduced urea excretion. From the data collected in this investigation, the high levels of serum urea have been suggested to mean that there is an acute impairment of the kidney. However, as the result on kidney markers show high serum urea, one can assume that the extract by itself does not have adverse effects on renal function in the infected animals. High serum urea levels have been linked to low blood flow to the kidneys caused by severe dehydration and in extreme cases heart failure (Nduka, 1999). In the weeks following extract administration, serum urea levels of all the rats infected with *T. brucei brucei* continued to show an undulating pattern of increase and decrease in serum urea levels with the group administered 1200mg/kg of the aqueous leaf extract showing the highest serum urea levels in week 3 of extract administration. Conversely, however,

Abubakar *et al*. (2005) reported lower serum urea levels of hypertensive rats treated with methanolic extract of African mistletoe.

Creatinine is a more specific kidney function marker than urea. It is an important indicator that reflects kidney functions and persistently high creatinine levels are most possibly caused by kidney impairment (Guyton and Hall, 2006). The results of this work show that there was an increase in the serum creatinine levels of the infected rats in comparison to those of the standard drug and normal control groups in week 2. The reported high creatinine levels in the infected rats, however, may not be as a result of extract administration. Furthermore, the increased levels of serum creatinine observed in this work may be linked to renal dysfunction, and or reduced blood flow in the kidneys causing poor clearance of serum creatinine from the blood. This work supports the findings of Jodi *et al.* (2011), who ascertained an elevated level of creatinine of albino rabbits induced with *T. brucei brucei* which they presumed to be as a result of reduced blood flow to the kidneys as a consequence renal dysfunction.

From the result obtained in this present investigation, one can presuppose that the hypercholesterlaemia in the infected and treated animals as well as that of the infected and untreated control group may be connected with kidney diseases as atheriosclerosis have been linked to damaging the renal arteries of the kidney. Elevated total cholesterol levels have been associated with increased risk of coronary heart disease, liver diseases, diabetes, and stroke (Linda, 2007). Thus, it is worthy of note here that the plant extract may not have the potential to lower cholesterol levels in a disease condition like trypanosomiasis. A similar work by Obatomi *et al.,* (1996), reported that there was an increase in total cholesterol of streptozotocin-induced rats administered aqueous extracts of the leaves of the African mistletoe plant.

Haematolgical parameters are important physiological indices used to evaluate the extent of deleterious effects of foreign agents on the blood constituents of both animals and humans. They could also be used to explain blood relating functions of chemical compounds of plant extracts (Yakubu *et al*., 2007; Ashafa *et al*., 2009). The significant reductions observed in the red blood cells (RBC), packed cell volume (PCV) and haemoglobin (HB), of the infected animals treated with *L. micranthus* leaf extracts is very instructional. This may be attributed to the enormous number of circulating parasites which could not be cleared by the aqueous leaf extract. Similar works by Sam-Wobo *et al.* (2010) and Yakubu *et al.* (2014), noted that RBC and its associated parameters (PCV and HB) of animals infected with *T. brucei brucei* were lower than that of non-infected animals, and attributed this to the enormous number of circulating trypanosomes causing haemolysis of the red blood cells. This inference further supports the work of Igbokwe and Mohammed (1992), who opined that the decreases observed in the RBC, PCV and haemoglobin levels causing severe anaemia is a cardinal feature associated with *T. brucei brucei* infection in comparison with other species of trypanosomes. Ekanem and Yusuf (2007) also ascribed the decrease in RBC, PCV, and HB as a measurement of acute anaemia in *T. brucei brucei* infection as an indicator of the severity of the proliferating parasites.

The calculated blood indices mean cell haemoglobin concentration (MCHC), mean cell volume (MCV) and mean cell haemoglobin concentration (MCH) evaluated in this study are of crucial importance in anaemia diagnosis in most animals (Coles, 1986). The non-significant effects of these indices relating to the RBC suggest that there was no effect on the average size of RBC and also in the haemoglobin. Similarly, the absence of observable significant effect of the extract on MCHC, MCV and MCH between the treatment groups and all the control groups throughout the period of experiment suggests that neither the incorporation of haemoglobin into the red blood cells nor the morphology and osmotic

fragility of the red blood cells were altered. This result therefore suggests that the aqueous leaf extract may not possess any potential for inducing anaemia following prolonged period of administration. This result corroborates the views of Yakubu *et al.,* (2007) which reported the non-significant effects of these indices as it relates to the average size of RBC microcytes of rats infected with *T. brucei brucei*.

Total white blood cells (WBC) contribute to the host defense mechanism by defending the body against infectious diseases and foreign invaders (Barry and Turner, 1992). The hostôs defense system consists of an intricate network of cytokines and progenitor cells which maintain basal myelopoiesis (formation of WBCs) and allow rapid adjustments in the rates of production of WBCs in response to acute and chronic infections (Stock and Hoffman, 2000). The results of this study showed significant elevations in the levels of WBCs of the trypanosome infected rats from the first week to the last week of the leaf extract administration. This observed significant increase in WBC counts of the treated rats is not surprising as such is indicative of a disease condition. This may also be as a result of normal response of the animalsô immune system to the plant extract. Furthermore, observations made in this present study further suggest that the infected rats experienced severe protozoan infection and myeloproliferative disorders, leading to the mass production of WBCs. This condition may also be linked to abnormal bleeding, high fever, anaemia and weight loss. It is also worthy of note here to suggest that the increased number of the circulating WBCs may also be as a result of the overwhelming number of parasites (trypanonsomes) circulating in the blood. This result corroborates the views of Yusuf *et al*. (2013), who reported increase in the WBCs following acute and chronic *T. brucei brucei* infection of bone marrow and peripheral blood cells of wistar rats. Sulaiman and Adeyemi (2010), and Adeyemi *et al.* (2009) noted that increased WBC count in animals affected by *T. brucei brucei* infection is attributed to the efforts of the animalsô defense system in eliminating the invading parasites.

White blood cell differentials determine the number of each type of WBC present in the blood and how each of these WBC provides immunity against infections. This present investigation revealed elevated levels of neutrophils, whereas lymphocytes, basophils, eosinophils and monocytes were lower in the differential counts. The elevated neutrophil count in the infected and treated rats may be indicative or suggestive of severe viral infections, tissue death or chronic myeloid leukemia (Sternberg, 2004). The lower lymphocytes, monocytes, basophils and eosinophil counts further suggests that the animals infected with *T. brucei brucei* may have experienced autoimmune disorders, hairy cell leukemia, acute viral infection and bone marrow damage. This inference is supported by the observations made by Abubakar *et al.* (2005) who reported that high neutrophil count in animals infected with trypanosomiasis is as a result of leucocytosis which has been implicated in the wax and wear syndrome on the animals immune system caused by the ever changing variable surface glycoprotein of the infecting trypanosome. A similar work by Anosa and Kaneko (1983), reported lower counts of basophils, lymphocytes and eosinophils which they narrowed down to lymphocytosis. In addition, Sulaiman and Adeyemi (2010) reported higher counts of neutrophils and lower counts of lymphocytes, monocytes and basophils on *T. brucei brucei* infected rats and suggested that these lower counts to infection may be as a result of induced immunosuppression.

4.2 Conclusion

Data presented in the present study have clearly demonstrated that the aqueous leaf extract of *L. micranthus* appeared to have no therapeutic properties in organisms infected with *T. brucei brucei*. This is more so as findings from this study showed that all infected and treated rats as well as the infected and untreated control group died from the resultant overwhelming parasitaemia unlike the case of those administered the standard drug. This is an indication that the extract lacks any anti-tryopanosomal activity.

The available documented evidence of African mistletoe, *L. micranthus,* being a good medicinal plant with good medicine potentials may probably not have been exhausted. Hence more studies are advocated into the molecular constituents of its bioactive ingredients to ascertain its suitability in the management of trypanosomiasis (Monthana *et al*., 2000; Monthana *et al*., 2003).

Conclusively, the aqueous leaf extract of *Loranthus micranthus* inhabiting the host plant *Kola acuminata* is an inadequate antitrypanosomal agent. It is therefore advocated that other methods of utilizing this important medicinal plant should be explored.

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