THE ANTIPLASMODIAL ACTIVITYAND SUBCHRONIC EFFECTS OF EXTRACTS OF BALANITES AEGYPTIACA AND TRICHILIA EMETICA

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M.TECH/SSSE/2008/1887

DEPARTMENT OF BIOCHEMISTRY

FEDERAL UNIVERSITY OF TECHNOLOGY,

MINNA

MAY, 2012

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THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, NIGERIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF TECHNOLOGY (M.TECH) IN BIOCHEMISTRY

MAY, 2012.

DECLARATION

I hereby declare that this thesis titled: **the antiplasmodial activity and subchronic effects of extracts of *Balanites aegyptiaca* and *Trichilia emetica*** are a collection of my original research work and it has not been presented for any other qualification anywhere. Information from other sources (published or unpublished) has been duly acknowledged.

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Rukayyah..... SIGNATURE/DATE

CERTIFICATION

This thesis titled **the antiplasmodial activity and subchronic effects of extracts of *Balanites aegyptiaca* and *Trichilia emetica*** by: SULAIMAN, Rukayyah (M.Tech/SSSE/2008/1887) meets the regulations governing the award of the degree of Master of Technology in Biochemistry, Federal University of Technology, Minna and it is approved for its contribution to scientific knowledge and literary presentation.

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MAJOR SUPERVISOR — Signature & Date 13.08.12

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DEDICATION

This work is dedicated to Allah (S.W.T) the Creator of the worlds and all that are contained therein.

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All praise is due to Allah, Lord of the worlds. May His endless peace and blessing be upon our noble prophet (s. a.w), his chosen companions, beloved wives and progeny as well as all those who follow the pristine path till the last day.

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ABSTRACT

The antiplasmodial effects of different fractions of the stem of *Balanites aegyptiaca* and leaf of *Trichilia emetica* were evaluated in mice. *Plasmodium berghei* (NK 65 Chloroquine sensitive strain) was inoculated into eighteen mice assigned to 3 groups of 6 animals in each case. Group I was treated with the respective plant extracts, group II, was treated with 5mg/Kgbw chloroquine (Standard) while group III was administered 20ml/Kgbw normal saline (control). The results showed that the three fractions of *Balanites aegyptiaca* have no antiplasmodial effects. However hexane and methanolic fractions of *Trichilia emetica* significantly suppressed parasitaemia by 79.17% and 95.83%. The effects of medium term administration of crude *Balanites aegyptiaca* stem and *Trichilia emetica* leaf extracts on some biochemical parameters also were investigated in mice. Sixty mice were divided into three groups of twenty animals each. Animals in groups I and II were gavaged with the extracts of *B. aegyptiaca* and *T. emetica* at 300mg/kg/body weight for five weeks respectively. Group III received normal saline (0.09%w/v NaCI) and served as controls. Whole body weight, fresh organ weights, packed cell volumes (PCV) and some serum biochemical parameters were analyzed using standard methods. Results showed minimum variation in whole body weights and packed cell volumes of animals given the extracts. Also values for some organ weights, triacyglycerides (116.20 ±3.05 mg/dl, 100.05 ± 2.88mg/dl), and Alkaline Phosphatase (155.00±4.44 µ/l, 185.20 ± 4.25 µ/l,), glucose (77.30± 2.22mg/dl, 95.65± 2.05mg/dl) total proteins (5.30 ±1.04mg/dl), Asparate aminotransferase (89.00 ± 5.08µ/L, 23.00± 1.15), and Alanine aminotransferase (6.00 ± 0.19µL), were significantly ($p<0.05$) different between test and control animals in the final week. However, fresh organ weight of *B. aegyptiaca* treatment compared to controls indicated that long term consumption of *B. aegyptiaca* could predispose to adverse tissue effects as shown in histopathological findings.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Medicinal Plants

Medicinal plants are various plants used in herbalism and thought by some to have medicinal properties. Over the past decade, herbal medicine has become a topic of global importance, making an impact on both world health and international trade. 80% of the world populations rely solely upon medicinal plant as the source of remedies for treatment of diseases (Khalid *et al.*, 2005). Medicinal plants continue to play a central role in the healthcare system of large proportions of the world's population. This is particularly true in developing countries, where herbal medicine has long and uninterrupted history of use. Recognition and development of the medicinal and economic benefits of these plants are on the increase in both developing and industrialized countries (Koduru *et al.*, 2007).

Medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industries. Plant derived medicines have been the first line of defense in maintaining health and combating diseases (Samy *et al.*, 2008). Due to poor condition of modern health care facilities and poverty, people depend on local medicinal plants. These medicinal plants gain further importance in region where modern medicinal health facilities are either not available or not accessible and some are due to cultural and spiritual reasons (Rout *et al.*, 2009).

Detailed research on chemistry and pharmacology of product of plant origin are much essential and this may eventually lead to the discovery of medicine that can be used in the

treatment of diseases such as malaria, typhoid, and tuberculosis e.t.c. Over commercial exploitation of these plant products frequently lead to degradation of natural resources that are major threat to medicinal plants (Samy *et al.*, 2008).

Before onset of synthetic era, man was completely dependent on medicinal herbs for prevention and treatment of diseases. In those days man was not properly aware about the health hazards associated with irrational therapy (Obajuluwa *et al.*, 2010).

With scientific research, we are able to understand about toxic principles present in green flora. Scientists isolated active constituents of the medicinal plants and after testing some were found to be therapeutically active. Aconitive, Atisine, Nicotine, Strychnine, Digoxin, Morphine are some common examples (Venkatachalam *et al.*, 2001).

In 2001, researchers identified 122 compounds used in mainstream medicine which were derived from "Ethnomedical" plants sources, 80% of these compounds were used in the same or related manner as the traditional ethnomedical use (Fabricant and Farnsworth, 2001).

Plants have evolved the ability to synthesize chemical compounds that help them defend against attack from a wide variety of predators such as insects, fungi and herbivorous mammals. By chance, some of these compounds whilst being toxic to plant predator, turn out to have beneficial effects when used to treat human diseases. Such secondary metabolites are highly varied in structure many are aromatic substances, most of which are phenol or their oxygen substituted derivatives. Chemical compounds in plants mediate their effects on human by binding to the receptor molecules present in the body. Such processes are identical to those already well understood for conventional drugs and as such herbal medicines do not differ greatly from conventional drugs in terms of

mechanism of action. These enable herbal medicines to be, in principle, just as effective as conventional medicine but also give them the same potential to cause harmful side effects (Lai and Roy, 2004). Furthermore adulteration, in appropriate formulation, or lack of understanding of plant and drug interactions have led to adverse reaction that are sometime life threatening or lethal (Huffman, 2003).

Malaria is mosquito-borne infectious disease caused by a eukaryotic protist of the genus plasmodium [phylum apleomplethal] (Muller *et. al.*, 2007). It is wide spread in tropical and sub-tropical regions, including parts of the America (22 countries), Asia and Africa. Each year, there are approximately 350-500 million cases of malaria, killing between one and three million people, the majority of whom are young children in sub-saharan Africa (Snow *et al.*, 2005).

Five species of the Plasmodium parasite can infect humans, the most wide spread and dangerous form of the disease are caused by *Plasmodium falciparum* and is responsible for about 80% of all malaria cases, and also responsible for about 90% of the death from malaria (Mendis *et al.*, 2001, Jigam *et al.*, 2009 and Singh *et al.*, 2004). Malaria caused by *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae* causes milder disease in humans that is not generally fatal. A fifth species, *Plasmodium knowlesi*, is a zoonosis that causes malaria in macagues but can also in humans (Singh *et al.*, 2004). Parasitic plasmodium species also infect birds, reptiles, monkeys, chimpanzees and rodents (Escalante and Ayala, 1994).

Malaria parasites contain apicoplasts, an organelle usually found in plants, which compete with their own functioning genomes. These apicoplasts are thought to have originated through endosymbiosis of algae (Kohler *et al.*, 1997) and play a crucial role in

various aspects of parasite metabolism e.g fatty acid biosynthesis (Gardner *et al.*, 1998). To date, 466 proteins have been found to be produced by apicoplast and these are now being looked at as possible targets for novel anti-malaria drugs (Foth *et al.*, 2003).

The parasite's primary hosts and transmission vectors are female mosquitoes of the anopheles genus, while humans and other vertebrates are secondary hosts (Talman *et al.*, 2004).

Young mosquitoes first ingest the malaria parasite by feeding on an infected human carrier and the infected anopheles mosquitoes carry plasmodium sporozoites in their salivary glands. Once ingested the parasite gametocytes taken up in the blood will further differentiate into male or female gametes and then fuse in the mosquitoes gut. This produces an ookinete that penetrates the gut lining and produces an oocyst in the gut wall. When the oocyst ruptures it releases sporozoites that migrate through the mosquito's body to the salivary glands where they are to infect a new host. The sporozoites are injected into the skin, alongside saliva when the mosquito takes a subsequent blood meal. This type of transmission is occasionally referred to as anterior station transfer (Talman *et al.*, 2004). The female of the Anopheles genus of mosquito prefer to feed at night. Only female mosquitoes feed on blood, thus males do not transmit the disease. Malaria parasites can also be transmitted by blood transfusion, although this is rare (Marcucci *et al.*, 2004).

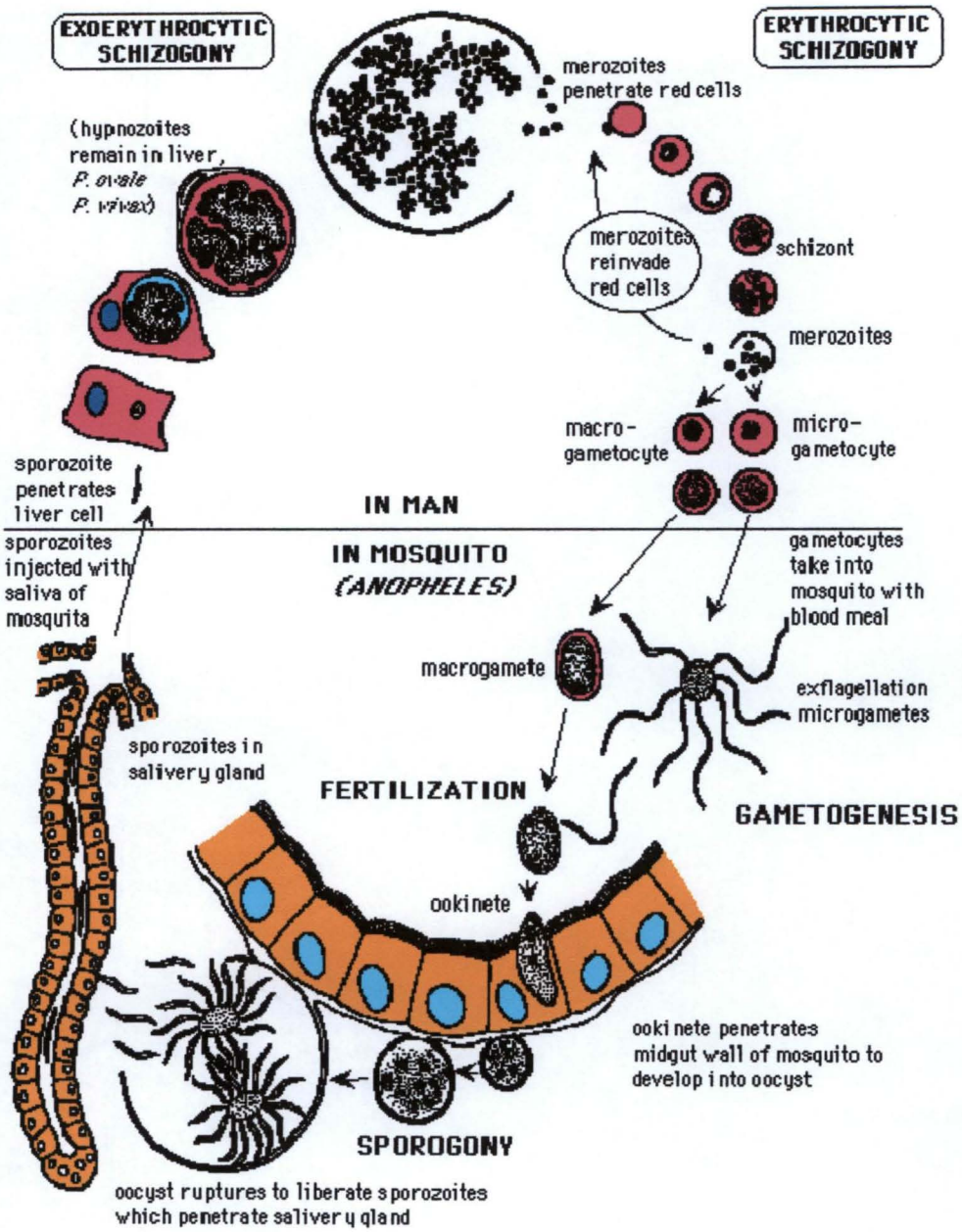

Figure1.1. The life-cycle of *Plasmodium vivax* in man and the mosquito. (Vickerman and Cox, 1967)

1.2 Pathogenesis

In humans the first sporozoites enter the blood stream and migrate to the liver. They infect liver cells, where they multiply asexually and asymptomatically into merozoites, rupture the liver cells (exoerythrocytic phase) and escape back into the blood stream. The merozoites infect red blood cells, where they develop into ring form, trophozoites and schizonts which in turn produce further merozoites (Bledsoe, 2005).

Some *P. vivax* and *P. ovale* sporozoites do not immediately develop into exoerythrocytic-phase merozoites instead produce hypozoites that remain dormant for long (6months-3years) and are responsible for long incubation and late relelapses in these two species of malaria (Cogswell, 1992).

The parasite is relatively invisible to immune surveillance because it resides in the liver and blood cells for its human life cycle. To prevent circulating infected blood cells from destruction in the spleen, P. falciparum displays adhesive protein on the surface of the infected blood cells, causing the blood cells to stick to the walls of small blood vessels (Chen *et al.*, 2000). This "stickness" is the main factor that causes hemorrhagic complication of malaria. Mass attachment of infected cells can cause blockage of high endothelial venules. In cerebral malaria the sequestrated red blood cells can breach the blood brain barrier possibly leading to coma (Adams *et al.*, 2002).

Although the red blood cell surface adhesive protein (called PFEMP1, for *Plasmodium falciparum* erythrocyte membrane protein 1) are exposed to the immune system, but do not serve as immune targets, because of their extreme diversity, at least 60 variations of protein within a single parasite (Adams *et al.*, 2002).

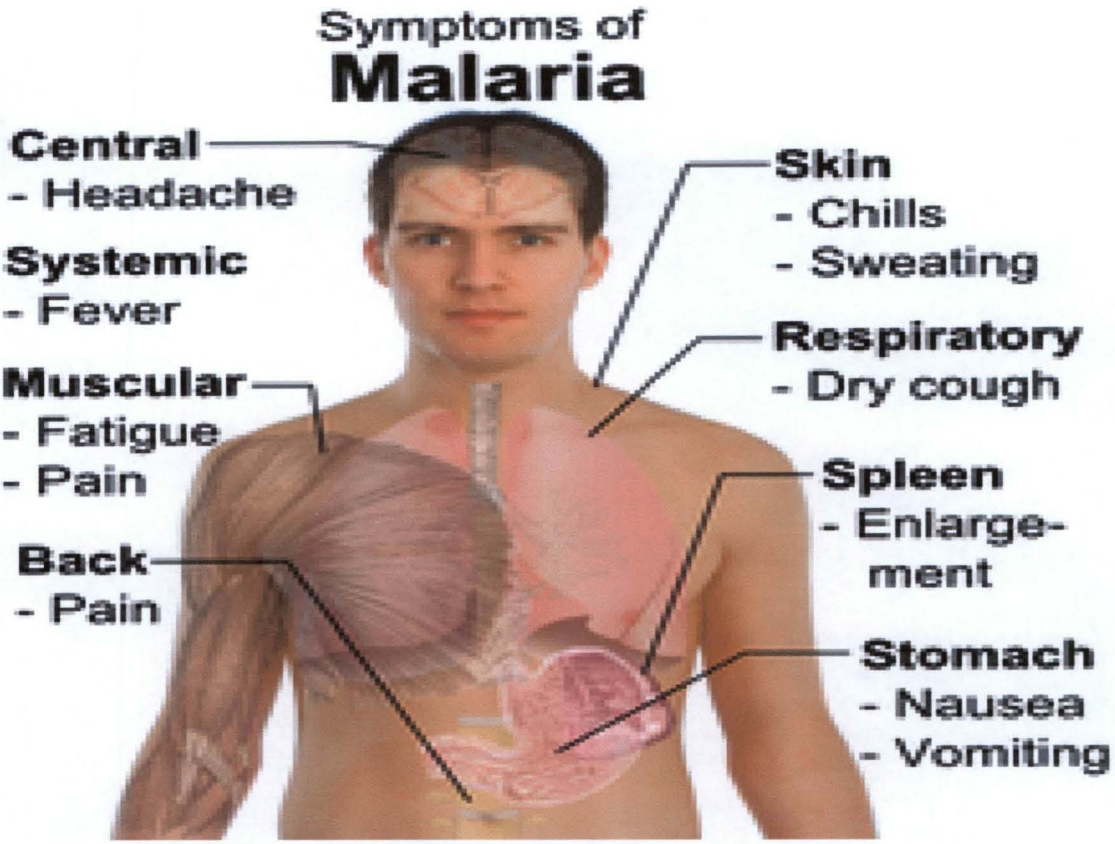

Figure 1.2 Main symptoms of malaria (Kilama and Ntoumi, 2009).

Symptoms of malaria include fever, shivering, arthralgia (joint pain), vomiting, anaemia (caused by hemolysis), hemoglobinuria, retinal damage and convulsions (Beare *et al.*, 2006). Malaria has been found to cause cognitive impairments and also direct brain damage especially in children. It causes widespread anaemia during a period of rapid brain development and also direct brain damage. This neurological damage results from cerebral malarial to which children are more vulnerable (Boivin, 2002, and Holding and Snow, 2001). Cerebral malaria is associated with retinal whitening (Maude *et al.*, 2009), which may be a useful clinical sign in distinguishing malaria from other causes of fever (Beare *et al.*, 2006).

1.3 Diagnosis of Malaria

1.3.1 Microscopic Examination

Since Charles Laveran first visualized the malaria parasite in blood in 1880, the mainstay of malaria diagnosis has been the microscopic examination of blood. Although blood is the sample most frequently used to make diagnosis, both saliva and urine have been investigated as alternative less invasive specimens (Sutherland and Hallett, 2009) Microscopic examination of blood is most economic, preferred and reliable method of malaria diagnosis because each of the four major parasite species has distinguishing characteristics. Two sorts of blood films are traditionally used. Thin films are similar to usual blood films and allow species identification because the parasite's appearance is best preserved in this preparation. Thick films allow the microscopist to screen a larger volume of blood and are about eleven times more sensitive than the thin film, but the appearance of the parasite is much more distorted and therefore distinguishing between the different species can be much more difficult (Warhurst and Williams, 1996).

Where microscopy is not available or where laboratory staff are not experienced at malaria diagnosis, there are commercial antigen detection tests that require only a drop of blood. Immunochromatographic tests (also called: malaria rapid diagnostic tests, antigen-capture assay or dipticks") use finger-stick venous blood, the complete test takes a total of 15-20minutes, results are read visually as the presence or absence of coloured stripes on the dipslick. Threshold of detection by this method is in the range of 100 parasite/μl of blood (0.002% to 0.1% parasitemia). This method is qualitative but not quantitative (Pattanasin *et al.*, 2003) the first rapid diagnostic (*P. falciparum* glucose dehydrogenase

PGluDH) as antigen. PGluDH has been replaced by *P. falciparum* lactate dehydrogenase (PLDH). PLDH does not persist in the blood but clears about the same time as the parasites following successful treatment. Depending on which monoclonal antibodies are used, this type of assay can distinguish between all five different species of human malaria parasites, because of antigenic difference between their PLDH Isoenzymes (Ling *et al.*, 1986).

1.3.2 Molecular Methods

Molecular methods are available in some clinical laboratories and rapid- time assay (for example QT-NASBA based on polymerase chain reaction is being developed with the hope of being able to deploy them in endemic areas (Mens *et al.*, 2006). Molecular methods are more accurate than microscopy. However, it is expensive, and requires a specialized laboratory (Redd *et al.*, 2006).

1.4 PREVENTION

1.4.1 Vector Control

i. Eradication of malaria by eliminating mosquito has been successful in areas where draining of wet land and breeding grounds and better sanitation were adequate (Barat, 2006).

ii. Sterile insect techniques are emerging as a potential mosquito control. Researchers at Imperial College London created the world's first transgenic malaria mosquito, with the first plasmodium resistance species (Ito *et al.*, 2002), such transposable element allow for non-Mendelian inheritance of the gene of interest.

1.4.2 Prophylactic drugs

Several drugs, most of which are also used for treatment of malaria, can be taken preventively. Modern drugs used include mefloquine (Lariam), doxycycline, and combination of atovaquone and proguanil hydrochloride (Malarone). (Jacqurioz and Croft, 2009). The choice of drug used depends on which drugs the parasites in the area are resistant to, as well as side effects and other considerations. The drugs are taken 2weeks before visiting malaria endemic area and must continue for another 4weeks (except for atovaquone and proguanol that only need to be started to 2days before and continued for 7days afterwards). The prophylactic drugs are usually restricted to short-term visitors to malaria regions; due to it negative effect from long-term use and high cost (Roestenberg *et al.*, 2009).

1.4.3 Indoor residual spraying (IRS)

This is the practice of spraying insecticides on the interior walls of homes in malaria affected area. After feeding, many mosquito species rest on a nearby surface while digesting the bloodmeal, so if the walls of dwelling have been coated with insecticides, the resting mosquitoes will be killed before they can bite another victim, transferring the malaria parasite. The World Health Organization (WHO) currently advises the use of 12 different insecticides in IRS operations. These include DDT and a series of alternative (such as Pyrethroids, Premethrin and deltamethrin), to combat malaria in areas where mosquitoes are DDT-resistant and to slow down the evolution (WHO, 2006).

1.4.4 Mosquito nets

Help keep mosquitoes away from people and greatly reduce the infection and transmission of malaria. The nets are not a perfect barrier and they are often treated with an insecticide designed to kill the mosquito before it has time to search for a way past the net. Insecticide treated nets (ITNs) are estimated to be twice as effective as untreated nets and offer greater than 70% protection compared to no net (Bachou *et al.*, 2006).

1.4.5 Malaria Vaccination

Immunity does occur naturally, but only in response to repeated infection with multiple strain of malaria. After the mouse vaccination study in 1967, it was hypothesized that the injected sporozoites themselves were being recognized by the immune system, which in turn create antibodies against the parasites. The immune system was creating antibodies against the circumsporozoite protein (CSP) which coated the sporozoite. Antibodies against CSP prevent the sporozoite from invading hepatocytes (Farnert *et al.*, 2009). Pre-erythrocytic vaccines (Vaccine that target the parasite before it reaches the blood) based on CSP; make the largest group of research for the malaria vaccine. Others include vaccines that seek to avoid severe pathologies of malaria by preventing adherence of the parasite to the blood venules and placenta, and those that induce immunity to the blood stages of the infection and transmission blocking vaccine that stop development of the parasite in the mosquito right after the mosquito has taken a blood meal from an infected person. It is hoped that the knowledge of the *P. falciparum* genome the sequencing of which was completed in 2002, will provide target for new drugs and vaccines (Gardener *et al.*, 2002; Matuschewski, 2006).

1.4.6 Education

Recognizing the symptoms of malaria has reduced the number of malaria cases in developing world by 20%. Recognizing the disease in the early stages can also stop the disease from becoming a killer. Informing people to cover areas of stagnant water, cuts down the risk of transmission between people (Aguas *et al.*, 2008)

1.4.7 Other interventions

For the control of malaria include mass drug administration and intermittent preventive therapy. A proposed alternative to mosquito nets is mosquito laser, or photonic fence, which identities female mosquitoes and shoots them using a medium powered lasar (Aguas *et al.*, 2008).

1.5.0 Treatment

The treatment of malaria depends on the severity of the disease. Uncomplicated malaria is treated with oral drugs, depending on the assessment and the experience of the clinician. Severe malaria requires the parenteral administration of anti-malarial drugs. The traditional treatment for severe malaria has been quinine but there is evidence that the artemisinin are also superior for the treatment of severe malaria. With the exception of artemisinin derivatives, resistance has emerged to every antimalaria drug. There is a cross resistance in both *Plasmodium malaria* and *P. vivax* following the introduction of pyrimethamine in many parts of tropics. Mefloquine resistance was reported even before the drug had been routinely used in some parts southern Asia. Halofantrine resistance has emerged concomitantly. The use of atovaquone was curtailed in preliminary clinical trials. Chloroquine and quinine are no longer effective in the treatment of *falciparum*

malaria. *P. vivax* has developed resistance in some parts of Oceania. Quinine, too, is threatened in some parts of the world (Jigam *et al.*, 2010).

Chemotherapy therefore remains a vital component of malarial control because viable and relevant vaccines are unavailable for the disease. The scientific evaluation of relevant medicinal plants used in traditional health care offers some hope in the identification of novel antimalaria pharmacophores (Jigam *et al.*, 2010).

1.6.0 Genetic Resistance to Malaria

1.6.1 Sickle Cell Anaemia

In sickle cell disease, there is a mutation in the HBB gene which encodes the beta-globin subunit of haemoglobin. The normal allele encodes a glutamate at position six of the β-globin protein, whereas the sickle cell allele encodes a valine. This change from a hydrophilic to a hydrophobic amino acid encourages binding between haemoglobin molecules, with polymerization of haemoglobin deforming red blood cells into a "sickle" shape. Such deformed cells are cleared rapidly from the blood, mainly in the spleen, for destruction and recycling (Champe and Harvey, 1994). The frequency of sickle gene is as high as 40% in certain parts of Africa. Until recently, most homozygotes have died before adulthood, and so there must have been strong selective pressure to maintain the high incidence of the gene. James Neel proposed that the heterozygote enjoys advantages not shared either the normal homozygote or the sickle cell homozygote. Sickle cell trait confers a small but highly significant degree of protection against the most lethal form of malaria, perhaps by accelerating the destruction of infected erythrocytes. In a malaria

infected region, the reproductive fitness of a person with sickle cell trait is about 15% higher than that of someone with normal haemoglobin (Stryer, 1999).

1.6.2 Thalassaemias

Another well-documented set of mutations found in the human genome associated with malaria are those involved in causing blood disorders known as thalassaemias. Thalassaemias is characterized by defective synthesis of one or more haemoglobin chains. The geographic distribution of β- thalassaemias genes parallels that of malaria, which suggests that the heterozygote benefits from the presence of the gene (Stryer, 1999).

1.6.3 Duffy Antigens

The Duffy antigens are antigens expressed on red blood cells and other cells in the body acting as a chemokine receptor. The expression of Duffy antigens on blood cells is encoded by Fy genes (Fya, Fyb, Fyc e.t.c). *Plasmodium vivax* malaria uses the Duffy antigen to enter blood cells. However, it is possible to express no Duffy antigen on red cells (fy-/fy-). This genotype confers complete resistance to *P. vivax* infection. The genotype is very rare in European, Asian, and American population, but is found in almost all of the indigenous population of West and Central Africa. This is thought to be due to very high exposure to *P. vivax* in Africa in the last few thousand years (Kwaitkowski, 2005).

1.6.4 Glucose-6-phosphate dehydrogenase deficiency

Glucose-6-phosphates dehydrogenase (G6PD) deficiency is known to provide a partial protection against malaria, by providing defective environment in the affected red blood cells. Glucose-6-phosphates dehydrogenase is an enzyme in the Hexose Monophosphate Shunt.The shunt generate reduced glutathione that protect sulfhydryl groups of haemoglobin and red blood cell membrane from oxidation by oxygen radicals. Defects in this shunt leads to inadequate protection against oxidation, resulting in oxidation sulfhydryl groups and precipitation of haemoglobin as Heinz bodies and in the lyses of the red blood cell membrane. Individuals with the disease may exhibit nonimmune haemolytic anaemia in response to a number of causes, most commonly infections or exposure to certain medications or chemicals. This continuous destruction of the red blood cells by the spleen of the susceptible individuals makes it impossible for the malaria parasite to survive the erythrocytic phase (Champe and Harvey, 1994; Stryer, 1999).

1.7.0 *Plasmodium berghei*

Plasmodium berghei is one of the four malaria species that infect murine rodent from forests of central Africa, where its natural cyclic hosts are the thicket rat (Grammomys surdaster) and the mosquito (*Anopheles dureni*) (Janse *et al.*, 2006).

Plasmodium berghei is used in research for study aim at new drug or vaccine development against malaria (Janse *et al.*, 2006). In the laboratory the natural hosts have been replaced by a number of available mouse strain, and the mosquito *Anopheles*

stephensi, which is comparatively easily reared and maintained under defined laboratory condition (Janse *et al.*, 2006).

Rodent parasites are recognized as valuable model organisms for the investigation of human malaria because they are similar in most essential aspects of morphology, physiology, biochemistry and life cycle. The manipulation of the complete life cycle of these parasites, including mosquito infections, is simple and safe (Amino *et al.*, 2006).

Like all malaria parasites of mammals, including the four human malaria parasites *P. berghei* is transmitted by Anopheles mosquitoes and it infects the liver after being injected into the blood stream by a bite of an infected female mosquito. After a short period (a few days) of development and multiplication, these parasites in the blood causes the pathology such as anaemia and damage of essential organs of the host such as lungs, liver, spleen. *P. berghei* infection may also affect the brain and can be cause cerebral complication in laboratory nice. These symptoms are to a certain degree comparable to symptoms of cerebral malaria in patient infected with the human malaria parasite *Plasmodium falciparum* (Franke- fayard *et al.*, 2006).

The use of this model malaria parasite has provided researcher with more insight into: the interaction of malaria parasites with the immune system, the process of infection of the liver by the parasites, the cause of severe pathology, such as cerebral complication in malaria patients, the infection of the mosquitoes and transmission of the malaria (Franke-fayard *et al.*, 2006).

1.7.0 Importance of the Biochemical Parameters assayed

Each of the biochemical parameter assayed have normal range, above or below which is pointing to a pathologic condition

1.7.1 Blood Glucose

Blood glucose level or blood sugar concentration is the amount of glucose present in the blood of an animal. In mammals, the normal range is 4.4 – 6.1mM (mmol/L) or 82 – 110mg/dl. The body naturally tightly regulates blood glucose level as a part of metabolic homeostasis (Champe and Harvey, 1994).

Glucose is the primary source of energy for the body's cells and blood lipids are primarily a compact energy store. Glucose is transported from the intestine or liver to body cells via the blood stream and made available for cell absorption via the hormone insulin, produced by the pancreas (Stryer, 1999).

Blood sugar level outside the normal range is an indicator of a medical condition. Persistently high level is referred to as hyperglycemia; low levels are referred to as hypoglycemia. Diabetes mellitus is characterized by persistent hyperglycemia from any of the several causes and is prominent disease related to failure of blood sugar regulation. Temporary elevation of blood sugar may be due to severe stress, such as trauma, stroke, myocardial infarction, surgery or illness. Intake of alcohol causes an initial surge in blood sugar and later tends to cause levels to fall. Also, certain drugs can increase or decrease glucose levels (Champe and Harvey, 1994).

1.7.2 Total Protein

Total protein is a biochemical test measuring the total amount of protein in blood. It is also called serum total protein or plasma total protein. It was formally called albumin/globulin ratio ($^{A}/_{G}$ ratio) (Tricot, 2008). Total proteins reflect nutritional status and may be used to screen and help diagnose kidney disease, liver disease and cause of oedema (Klein, 2007).

There are two types of protein in the plasma: albumin and globulin (α1, α2, β and globulins). Albumin is made in the liver. It helps keep the blood from leaking out of blood vessels. Albumin also helps carry some medicines and other substances through the blood and is important for tissue growth and healing (Tricot, 2008).

Some globulins are made by the liver, while others are made by the immune system. Certain globulins are part of haemoglobin, other globulin transport metal such as iron in the blood and fight infection. Protein electrophoresis can be used to quantify these fractions of proteins (Champe and Harvey, 1994).

Total protein is measured using biruet reagent, kjeldahl method; dye-binding and refractometry method (Tricot, 2008). The normal range of total protein is 6.0 – 8.5gm/dl. Abnormal total protein can be lower or higher than the normal. Low total protein levels can suggest a liver disorder, a kidney disorder, or disorder in which protein is not digested or absorbed properly. Low levels may be seen in severe malnutrition and malabsorption such as celiac disease or inflammatory bowel disease (Bazari, 2007).

High total protein levels may be seen with chronic inflammation or infection such as viral hepatitis or HIV. They may be caused by bone marrow disorder such as multiple

myeloma. A low $^{A}/_{G}$ ratio may reflect overproduction of globulins such as seen in multiple myeloma or autoimmune diseases or underproduction of albumin such as in cirrhosis or selective loss of albumin from the circulation as occur with kidney disease (nephrotic syndrome). A high $^{A}/_{G}$ ratio suggests underproduction of immune-globulins like in some genetic deficiencies and leukemia. More specific tests such as albumin, liver enzyme tests and serum protein electrophoresis must be performed to make an accurate diagnosis (Tricot, 2008).

Total protein level may be high during pregnancy. Some drugs are found to increase total protein examples anabolic steroids, androgens, corticosteroids, dextran, growth hormone, insulin, phenazopyridine and progesterone. Hepatotoxic drugs, oral contraceptives, estrogen and ammonium ions are known to decrease total protein (Tricot, 2008).

1.7.3 Triglycerides

Triglycerides or triacylglycerol is an ester derived from glycerol and three fatty acids. It is the main constituent of vegetable oil and animal fats.

The three fatty acids RCOOH are usually different, depending on the type of triglycerides. The chain lengths of the fatty acids in naturally occurring triglycerides vary, but most contain 16, 18 or 20 carbon atoms. Naturally fatty acids found in plants and animals are typically composed of only even numbers of carbon atoms reflecting the path way for their biosynthesis from the two carbon building block acetyl CoA. Bacteria however, possess the ability to synthesize odd and branched chain fatty acids. As a result, ruminant animal fat contains odd number fatty acids such as 15, due to the action of bacteria in the rumen. Many fatty acids are unsaturated, some are polyunsaturated e.g.

those derived from linoleic acid. Triglycerides cannot be absorbed by the duodenum, so it is broken down by the pancreatic lipase, act at the ester bond, hydrolyzing the bond and releasing the fatty acids and monoglycerides (Stryer, 1999).

Triglycerides are major components of a very low density lipoprotein (VLDL) and chylomicrons, playing important role in metabolism as energy sources and transporters of dietary fat. They contain more than twice energy (9kcal) as carbohydrate and proteins. In the intestine TAG are split into monoacylglycerol and free fatty acids in a process called lipolysis, with the secretion of lipases and bile, which are subsequently moved to absorptive enterocytes, cells lining the intestines.TAG are rebuilt in the enterocytes from their fragments and packaged together with cholesterol and protein to form chylomicrons. These are excreted from the cells and collected by the lymph system and transported to large vessels near the heart before being mixed into the blood. Various tissues can capture chylomicrons and use the triglycerides as source of energy. Fat and liver cells can synthesize and store triglycerides. When the body requires fatty acids as energy source, the hormone glucagon signals the breakdown of triglycerides by hormone sensitive lipase to release free fatty acid. Fatty acid can than go into β-oxidation to generate energy. The brain cannot utilize fatty acids as energy source (unless converted to a ketone), the glycerol component of triglycerides can be utilize as glucose, via glycolysis by conversion into dihydroxyacetone phosphates and the into glyceraldehyde-3 phosphate, fat cell also breakdown triglycerides when there is energy deficit in the body (Champe and Harvey, 1994).

Triglycerides cannot pass through cell membrane freely, special enzymes on the wall of blood vessels called lipoprotein lipase breakdown triglycerides into free fatty acids and

glycerol. Fatty acid can then be taken the cells via the fatty acid transporter FAT, (Champe and Harvey, 1994).

High levels of triglycerides in the blood stream levels been linked to atherosclerosis (hardening of the arteries) and by extension the risk of heart disease and stroke. However, the relative negative impact of raised levels of triglyceride compared to that of LDL-HDL ratios is as yet unknown. The risk can be partly accounts for by a strong inverse relationship between triglyceride and HDL-cholesterol level (Stryer, 1999).

Heavy use of alcohol can elevate triglyceride level. High consumption of carbohydrate increases glycemic index which in turn causes insulin over production and increase triglyceride levels, and are of stronger risk factors for the heart disease in women then in men. Exercise, carnitine and fibrates have been known to reduce the level of triglycerides (Stryer, 1999).

1.7.4 Alkaline Phosphatase

Alkaline phosphatase (ALP or orthophosphoric monoester phosphohydrolase) is a hydrolase. It has enzyme commission number E.C 3.1.3.1 and hydrolyses colourless p-nitrophenyl phosphate (pNPP) producing phosphate and p-nitrophenol which is yellow at alkaline pH.

Diethanolamine (DEA) regulates the reaction's pH and acts as acceptor of the phosphate release by phosphatase (Transphosphorylation), resulting in activation of the reaction. DEA has the best condition terms of activation and buffering when p-NPP is used as

substrate. Alkaline phosphatase is also found to hydrolyze phosphate from proteins and alkaloids apart from nucleotides (Kim and Wyckoff, 1991).

An alkaline phosphatase is found concentrated in the liver, bile duct, kidney, bone and the placenta. The normal range is 20 – 140IU/l. ALP levels are normally high in children and pregnant women (Kim and Wyckoff, 1991).

ALP test help detect liver diseases or bone disorders. In conditions affecting the liver damaged, liver cells release large amount ALP into the blood. It is often used to detect blocked bile ducts because ALP is especially high in the edges of cells that join to form bile duct. If one or more of them are obstructed, for example by a tumor, then blood level of ALP will often be high. An ALP test may be used to detect cancers that spread to the bone or to help diagnose Paget's disease or other bone condition such as vitamin D deficiency (Lange *et al.*, 1982).

If ALP level is high and it is not known if it is due to liver or bone disease, then tests for ALP isoenzymes are done to know the cause. A GGT test or test for 5' nucleotidase is carryout and if the level of GGT and 5'nucleotidase are increased then it is liver diseases, if not is a bone disorder. Conditions that result in abnormal high level of ALP are liver congestion or cholestasis (oral contraceptive, obstructive pancreatitis, hepatitis, heart failure, parasites and cancer), Osteoblastic or bone related (Paget's disease, Herpes zoster's, hyperthyroidism, over activities of parathyroid gland, Osteomalacia, vitamin D deficiency, healing fractures, Osteoporosis treatment and Adrenal cortical hyper function) and non-bone or non-liver condition (as normal part of late pregnancy, Amyloidosis, granulations tissues, gastrointestinal inflammation, systemic infection (sepsis), cancer

and acute tissue damage in the heart or lungs (myocardial or pulmonary infarctions) (Schiele *et al.*, 1998).

A decrease ALP may be due to Zn deficiency, hypothyroidism, Vitamin C deficiency, folic acid deficiency, excess vitamin D intake, hypophosphatasia, celiac disease, insufficient parathyroid gland function or of vitamin B_6 and pernicious anaemia (Hann and Smith, 2006).

1.7.5 Alanine Aminotransferase

Alanine aminotransferase (ALT) formally known as Glutamate Pyruvate Transaminase (GPT) or Serum Glutamate Pyruvate Transaminase (SGPT) is a transferase and has E.C 2.6.1.2. ALT catalyses the transfer of amino group of alanine to ketoglutarate, resulting is the formation of pyruvate and glutamate

ALT reaction is a readily reversible; however, during amino acid catabolism, this enzyme (like most other aminotransferases) functions in the direction of glutamate synthesis. Thus, glutamate acts as a "collector" of nitrogen from alanine (Champe and Harvey, 1994).

ALT is found primarily in the liver. High levels of ALT (more than 10times highest normal level) are usually due to acute hepatitis, often due to virus infection. In acute hepatitis, ALT levels usually stay high for about 1-2months but can take as long as 3-6 months to return to normal. Exposure to drugs or toxic substance as well as conditions that cause decreases blood flow (ischemia) to the liver (Hann and Smith, 2006).

In chronic hepatitis, ALT is about 4times higher than the highest normal value. Other causes of moderates increase in ALT include obstruction of bile ducts, cirrhosis, and tumors in the liver. In most liver diseases ALT lever is higher than AST expect in alcoholic hepatitis, cirrhosis and muscle injury (Stryer, 1999).

1.7.6 Aspartate Aminotransferase

Aspartate Aminotransferase or Aspartate transaminase (AST) formally known as Glutamate Oxaloacetate Transaminase (GOT) has EC 2.6.1.1. Aspartate aminotransferase is an exception to the rule that aminotransferase transfer amino groups to α-ketoglutarate to form glutamate. Since during amino acid catabolism, AST transfers amino groups from glutamate to oxoacetate to form α-ketoglutarate and aspartate is then used as a source of nitrogen in the urea cycle (Kirsch *et al.*, 1984).

Both ALT and AST have requirement for pyridoxal phosphate (PLP). PLP is covalent linked to the E – amino group of a specific lysine residue at the active site of the enzyme. AST act by transferring the amino group of an amino acid to the pyridoxal part of the coenzyme to generate pyridoxamine phosphate (Kirsch *et al.*, 1984).

Aspartate aminotransferase is found in the liver, Red blood cell, cardiac muscle, and skeletal muscle. Any disease in these organs raises the AST level significantly. Others less important sources of AST that raise the AST level significantly in the blood are kidney, brain pancreas, lungs leukocytes and erythrocytes in decreases order of concentration (Stryer, 1999).

1.7.7 Packed Cell Volume (PCV)

Packed Cell Volume (PCV) or erythrocyte volume fraction (EVF) is the proportion of blood volume that is occupied by the red blood cells. It is normally about 48% for man and 38% for women. It is considered an integral part of a person's complete blood count results, along with hemoglobin concentration, white blood cell count and platelet count. In mammals, PCV is independent of body size (Purves *et al.*, 2004).

Hematocrit (Hct) and PCV are used interchangeably. Hematocrit is the corresponding calculated value, calculated by multiplying a red blood cell count (RBC Count) with the mean erythrocyte volume (MCV). PCV is slightly higher than the more accurate HCT due to plasma trapping (between the packed cells in a centrifuged capillary) (Purves *et al.*, 2004).

There may be elevated cases of PCV due to

i. Dengue fever, a high hematocrit is a danger sign of an increased risk of dengue shock syndrome.
ii. Polycythemia vera (PV), a myeloproliferative disorder in which the bone marrow produces excessive numbers of red blood cells.
iii. Chronic obstructive pulmonary diseases (COPD) and other pulmonary conditions associated with hypoxia may elicit an increased production of red blood cells. This increase is mediated by the increased levels of erythropoietin by the kidney in response to hypoxia.
iv. Professional athletes hematocrit levels are measured as part of tests of blood doping or Erythropoietin (EPO) use; the level of hematocrit in a blood sample is

compared with the long term level for that athlete (to allow for individual variations in hematocrit level) and against an absolute permitted maximum (which is based on maximum expected levels within the population) and the hematocrit level that causes increased risk of blood clots resulting in strokes or heart attacks.

v. Anabolic Androgenic Steroid (AAS) use can also increase the amount of RBC and therefore, impact the hematocrit, in particular the compounds boldenone and oxymetholone.

vi. Dehydration: If a patient is dehydrated, the hematocrit may be elevated (Behrman, 2000 and Purves *et al.*, 2004).

Low hematocrit can imply significant haemorrhage. The mean corpuscular volume (MCV) and the red cell distribution width (RDW) can be quite useful in evaluating a low hematocrit, because it determines whether blood loss is chronic or acute. The MCV is the size of the red cells and RDW is a relative measure of the variation in the size of the red cell population. A low hematocrit with a low MCV with a high RDW suggests a chronic iron-deficient erythropoiesis, but a normal RDW suggest a blood loss that is more accurate, such as hemorrhage. PCV may be low due to:

i. Inadequate intake of iron in infants and children going through a rapid growth spurt.

ii. Chronic kidney disease which affects the synthesis of erythropoietin by the kidney (erythropoietin is the hormone that stimulate synthesis of red blood cells).

iii. Diseases of the bone marrow that causes a decrease in the synthesis of red blood cells.

iv. Menstruation: women of child bearing age also have abnormal low PCV due to menstrual cycle. (Johnson, 1993 and Purves *et al.*, 2004).

Due to the continuous problem of resistance development by malaria parasites there is a need to explore more medicinal plants that could be use in the treatment of malaria. The aim of this work is to see the effects of *Balanites aegyptiaca* and *Trichilia emetica* on malaria parasite, some vital organs, some essential biochemical enzymes and parameters. Also to determine the safe dose, detect and quantify some phytochemicals present in them.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. *Balanites aegyptiaca* (L) Delile.

2.1.1. Taxonomy and Nomenclature

Balanites aegyptiaca is a species of tree that belongs to the plantea kingdom, division of magnoliophyta class of magnoliophyta and order of zygophyllates. It is classified either as a family member of zygophyllaceae or Balanitaceae; with sub-family Tribuloideae and genus Balanites (Schmidt and Joker, 2000). *B. aegyptiacae is* commonly known as desert date or soapberry tree and as *Aduwa* in Hausa language (Yves and Dechass, 2009).

Natural distribution is obscured by cultivation and naturalization. It is believed that it is indigenous to all dry land south of the Sahara (Sahel), extending southwards to Malawi in the Rift valley, and to the Arabian Peninsula, Middle East and south Asia (Ndoye, 2004).

It has wide ecological distribution (i.e it can be found in many kinds of habitat) tolerating a wide variety soil types from sandy to heavy clay). It is a lowland species growing between 1000-1600m altitudes in areas with mean annual rainfall of 200-1200mm (Fernandes, 2003).

This is a multibranched spriny shrub tree that reaches 10m (33ft) in height (Shmidt and Joker, 2000) with a stem diameter of around 30cm. The canopy may be deciduous for part of the dry season. Tree coppice well (Fernandes, 2003).Trunk short and often branching from near the base. Branched armed with shout yellow or green thorns up to 8cm long. Leaves with two separate leaflets, leaflets obovate, asymmetric, 2.5-6cm long,

bright green, leathery, with fine hair when young. Flowers in fascicles in the leaf axils, fragrant, yellowish-green (Schmidt and Joker, 2000).

The fruit is narrow drupe, 2.5-7cm long, 1.5-4cm in diameter. Young fruits green and tormentose, turning yellow and glabrous when mature. Pulp is bitter-sweet and edible. The seed is pyrene (stone), 1.5-3cm long; light brown, fibrous and extremely hard. It makes up 50-60% of the fruit. There are 500-1500 dry, clean seed per kg (Schmidt and Joker,2000).

2.1.2 Flowering and Fruit habit

Flowers are small, inconspicuous, hermnproditic and pollinated by insects like halictid bees, *Rhinia apicalis* and *Chrysomia chloropiza* (Ndoye, 2004). Carpenter and *Camponotus sericeus* feed on the nectar and larva of cabbage tree emperior moth *Bunaea alcinoe* causes defoliation of the tree. In areas with pronounced seasonal climates (northern and southern part of the distribution range) fruit mature just before the rainy season (Schmidt and Joker, 2000). In most of the Sahelian region the main flowering season is between October and March, the main fruiting season between December and April. Flowering in Nigeria varies between November and April with ripe fruit in December and January and occasionally later, from March to July (Schimdt and Joker, 2000). Seed are dispersed by ingestion by birds (e.g hornbills) and larger animals (baboon and ruminants).The tree begins to flower and fruits at 5-7years old.

Fruits are harvested when they turn yellow and flesh becomes soft and sweet. Seed storage is orthodox .and moisture content for storage should be 6-10%. Cleaned, dried and insects free seeds remain viable for 1year at ambient temperature, 2years at cool

temperatures and several years in hermetic storage at 3^0C. The seed should be sown vertically with the stalk end down. Germination occurs in 1-4week and the seedlings are kept in the nursery for about 12weeks (Schmidt and Joker, 2000).

2.1.3 Uses of *Balanites aegyptiaca*

Many part of the plant are used as famine food in Africa .The leaves are eaten raw or cooked ,the oily seed is boiled to make it less bitter and eaten mixed with sorghum, the flowers can be eaten. The fruit can be fermented for alcoholic beverage. The seed contains 30-40% seed oil and contains the sapogenois, diosgein and yamogenin (Fernandes, 2003). Diosgenin can be used to produce hormone such as those in combined oral contraceptive pills and corticoids (Ndoye, 2004). The oil is used as cooking oil (Ndoye, 2004). The seed cake remaining after the oil is extracted is commonly used as animal fodder in Africa .Young foliage and fruit and eaten by goats, sheeps and camel (Fernandes, 2003). The plant parts are used as soap substitutes because of high saponin contents, resilient thorny branches are used for fencing. The pale brownish wood is used to make furniture and durable items such as tools, and it is a low-smoke firewood and good charcoal. The bark yields fibers, the natural gum from the branches are used as glue and seeds have been used to make jewelry and beads (Fernandes, 2003).

Various parts of B. a*egyptiaca* have it own traditional medicinal properties. The fruit is mixed into porridge and eaten by nursing mothers, and the oil consumed for headache, and to improve lactation. Bark extracts and the fruit repel snails and copepods,organism

that host the pariotes schiotosomes and guinea worm respectively (Hamidou *et al.*, 2002). This plant has been reported to be an antihelminthic, a purgative, vermifuge, febrifuge, emetic and can also cure other types of ailments like skin boils, lancoderma, malaria, wound, cold, syphills, liver and sleeping disorder and aches (Hamid *et al.*, 2001)The seeds have molluscicide effect on *Biomphaleria pfeiffer* (Hamidou *et al.*, 2002). The boiled root can be used as soup against stomach pain, anthrax, and the infusion of root also acts as an antidote to snake bite (Ojo *et al.*, 2006)The infusion of root bark has been used in diarrhea, in heamorrhold and also act as fish poison (Bukar *et al.*, 2004). Kernel oil helps in curing skin diseases (Yadav *et al.*, 2010). The fruit can cure mouth ulcer, whooping cough, sleeping sickness and skin diseases. Fruit kernel has been found as a mild laxative, an antidote to arrow poison and also acts as a vermifuge (Ojo *et al.*, 2006). The seeds are useful as ointments to cure cough, colic pain and also have magic-religious properties (Bukar *et al.*, 2004 and Ojo *et al.*, 2006). The paste of shoot has been used for dressing of wounds and as tooth brushes when frayed .The thorns are used in the treatment of leprosy. Plant leaves are used in curing anthrax for their antihelminthic activities and to clean malignant wound (Hamid *et al.*, 2001).

2.1.4 Phytochemistry

The phytochemistry of its root, stem, bark, leaves, fruit pulp, seed kernel and mesocarp has been studied by different researchers. *B. aegyptiaca* is rich source of saponins. Saponins are glycosides consisting of sugar residues (one or more unit of glucose, galactose, e.t.c) linked through oxygen with complex multring compounds usually containing 27-31carbon atoms. The aglycone (non-sugar) part, which is also called sapogenin, is either a steroid [C27] or a triterpene [C30] (Hostettmann and Marston,

1995). Saponin containing plants are used in folk medicine, especially in Asia and are intensively used in food, veterinary and medical industries. *B. aegyptiaca* contains different types of saponins, namely, balaniten-1, 2, 3, 4, 5, 6 and 7 (Yadav and Panghal, 2010).

The phytochemical identified from roots of *B. aegyptiaca* contains Balanitin-1, 2, and 3, alkaloid and diosgenin have been isolated from the east African specimen (Gaur *et al.*, 2005 and Yadav and Panghal, 2010). Diosgenin is a steroidal sapogenin (5-spiostan-3-ol) compound which is very useful in pharmaceutical industries as a natural source of steroidal hormones (Liu *et al.*, 2005).

Balanitin-1, 2 and 3 have been isolated from the stems African species of *B. aegyptiaca* (Yadav and Panghal, 2010). Dichloromethane extract has yielded two types of alkaloids, N-trans-fernloyl tramine and N-cis-feruloyltramine(figure 2.1)and other metabolites like vanillic acid, syringic acid and 3-hydroxy-1-(4-hydroxy-3 methanoxyphenyl)-1-propanone also from the stems(Sarker *et al.*, 2007).

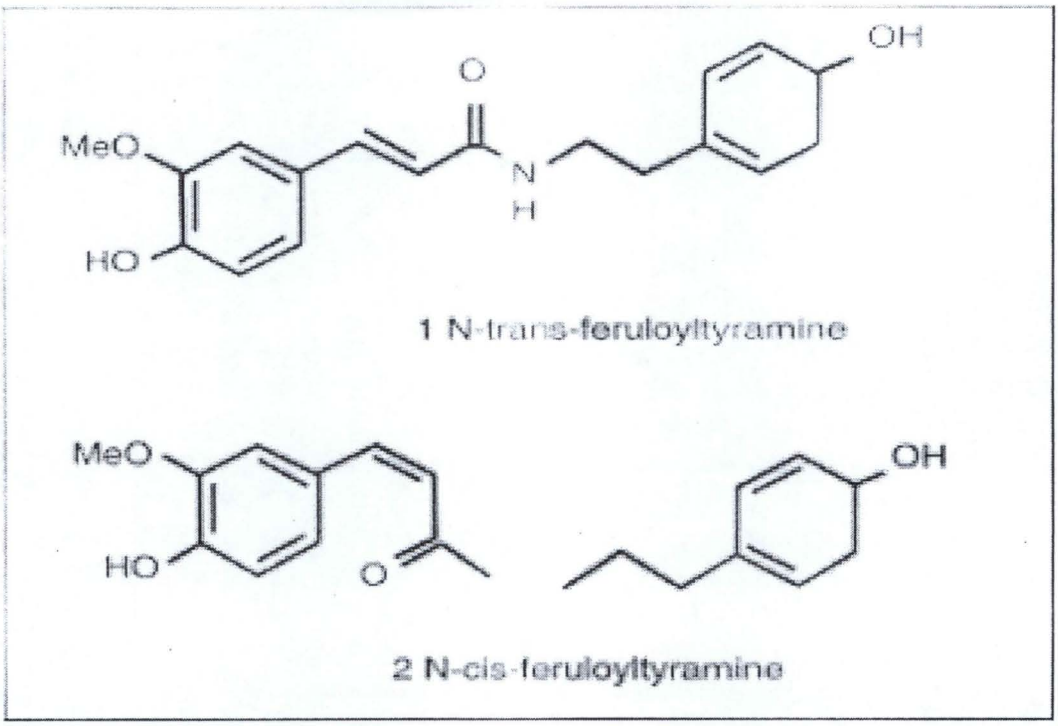

Figure 2.1: Structure of the two alkaloids isolated from stem bark of *B. aegyptiaca* (Yadav and Panghal, 2010).

The leaves contain six flavonoids, glycosides identified as quercetin-3-glucosides, quercetin3-rutinoside, 3-glucoside, 3-rutinoside, 3-7diglucoside and 3-rhamnogalactosides of isorhamnetin have been extracted and identified from the leaves and branches of Egyptian plant species (Markson and El-hadid, 1988).

The fruit of *B. aegyptiaca* consist s of an epicarp, a mesocarp,an endocarp and a kernel (Mohammed and Wolf, 2002). The total saponin content has been found to be 7.2% in the mesocarp and 6.7% in the kernel (Yadav and Panghal, 2010). Balanitin A, B, C, D, E and Balanitin F and G have been isolated from pulp and kernel respectively (Yadav and Panghal, 2010). The oil extracted from the kernel constituted 44-51%w/w and is composed of mainly triglycerides, phytosterols, sterol esters and tocopherols. Besides, a

known spirostenol glycoside, balanitin-3 and a new sapogenol, 6-methyldiosgenin, a new furostanol saponion, balanitoside and two pregnane glycosides have been isolated from fruits(mesocarp)of *B. aegyptiaca* (Kamel, 1998 and Kamel and Koskinen, 1995). Spectroscopic and chemical analysis suggested the structure of glycosides as 26-0-β-d-glucopyranosyl-3b, 22, 26- trihydroxy-furost-ene, 3-0-α-1-rhamnopyranosyl-(1-2)-β-d-glucopyranosyl(1-4)-β-d-glucopyranosides and the saponion present in the mesocarp of *B.aegyptica* fruit are a mixture of 22R and 22S epimers of 26-(0-β-d-glucopyranosyl)-3-β-[4-0-(-β-d-glucopyranosyl)-2-0-(α-1-rhamnopyranosy)-β-d-glucopyranosy}-22,26-dihydroxyfurost-5-ene (Staerk, 2006).

The seeds of *B. aegyptiaca* yielded four new cytostatic saponions, namely balanitins 4, 5, 6 and 7(Yadav and Panghal, 2010).The seed also contains deltonin and isodeltonin (Steroidal spirostanolglycosides) which are used as molluscicidal agents(Gnoula, 2007).

2.1.5 Pharmacological Properties.

The pharmacological activity, parts used, constituent compound responsible for the pharmacological properties and sources of information are discussed below:

2.1.5.1 Antidiabetic Activity

The bark extract of *B. aegyptiaca* has been shown to have a moderate effect on the activity of α-amylase which is responsible for the degradation of oligosaccharides (Funke *et al.*, 2005). *B. aegyptiaca* fruit extracts (1.5g/kg bw) reduced the blood glucose level by 24% and significantly decreased the liver glucose-6-phosphatase activity in diabetic rats (Mohammed *et al.*, 2006). The water and ethanolic extract of *B. aegyptiaca* fruit extract induced significant reduction in serum glucose, glucagon, total lipids, total cholesterol,

triglycerides level and transamimases[aspartate aminotransferase(AST) and alanine aminotransferase (ALT) and gamma aminotransferase (gGT)](Zaahkouk *et al.*, 2003). An aqueous extract of mesocarps of fruits of *B. aegyptiaca* exhibited a prominent antidiabetic activity on oral administration in streptozocin-induced diabetic mice. It is believed that the antidiabetic activity was due to the presence of steroidal saponins in the extract (Yadav and Panghal, 2010).

2.1.5.2 Anti-inflammatory Activity

The ethanolic extract of aerial part of *B. aegyptiaca*, when given orally as a suspension at 300mg/kg bw per day, reduced the paw volume by 55.03%, where in the case of administration of 600mg/kg bw per day it was 65.54%, indicating that the effect was dose dependent. The significant anti-inflammatory activity was evaluated in methanolic and ethanolic extracts of bark in two different animal models, the carrageenan-induced oedema in the rat, and acetic acid-induced writing test in mice (Speroni *et al.*, 2005). Ethanolic extract of fruit of *B. aegyptiaca* also exhibited a pro-inflammatory activity (Koko *et al.*, 2008).

The phytochemicals responsible for these activities were found to be flavonoids, saponins B1 and B2 isolated from bark and aerial parts of the plants (Speron *et al.*, 2005 and Gaur *et al.*, 2008)

2.1.5.3 Hepatoprotective Activities

The leaf, stem bark and root extracts of *B. aegyptiaca* were screened for hepatoprotective activity in Wistar albino rats. The stem bark extracts of the plant showed significant ($p < 0.05$) hepatoprotective effects as revealed by a decrease in the activity of the serum

transaminase and alkaline phosphatase enzymes as a compared to control rats (Ojo *et al.*, 2005). The effects of lysophilised extracts of *B. aegyptiaca* (1g/kg), and silymarin(0.1g/kg), a standard hepatoprotective agents, given for 5 consecutive days was tested on liver damage induced by paracetamol (0.6g/kg) in the mice. *B. aegyptiaca* had a relatively modest hepatoprotective activity (27%) while silymarin protected above 92% of the treated mice (Ali *et al.*, 2001). These results suggest that the extract could protect the paracetamol-induced liver damages perhaps by eliminating the deleterious effects of toxic metabolites from the drug (Yadav and Panghal, 2010).

2.1.5.4 Anticancerous and Antioxidants Activities

The mixture of balanitin-6 (28%) and balanitin-7 (72%) was evaluated in vitro for anticancer activity against six different human cancer cell lines, using the [3-(4, 5 dimethylthiozol–2yl) diplenyl tetrazolium bromide] colorimetric assay and in vivo in the murine L1210 leukemia model. The mixture demonstrated appreciable anticancer effects in human cancer cell lines in vitro as it displayed higher antiproliterative activity than etoposide and oxaliplatin but make lesser activity than taxol. The in vitro anticancer activities result at least partially from depletion of ATP leading in turn to major disorganization of actin cytoskeleton, ultimately resulting in the impairment of cancer cell proliferation and migration. In vivo, bal 6/7 increased the survival time of mice bearing murine L1210 leukemia graft to the same extent as that reported for vincristine. These preliminary in vivo date suggest that it may be possible to generate novel hemi-synthetic derivation of balanitins 6 and 7 with potentially improved in vitro and in vivo anticancer activity and reduced in vivo toxicity, thus markedly improving the therapeutic ratio (Gnoula *et al.*, 2008). Balanitin B1 and B2, isolated from methanol and butanol

extract of *B. aegyptiaca* bark have been evaluated in vitro and in vivo using a method based on Briggs-Rauscher (BR) oscillating reaction and this revealed the antioxidant activity (Speroni *et al.*, 2005).

2.1.5.5 Antihelminthic and Molluscicidal Activity

The root, bark, seed kernel fruit and whole plant extracts were found to be lethal to snails, miracidia and cerearіac of schistosome in various studies (Brimer *et al.*, 2007 and Yandav and Panghal, 2010). A mixture of deltonin and 25-isodeltonin extracted from seeds was found to be molluscicida against snail species *Biomphalaria glaybrata* (Brimer *et al.*, 2007). The antihelminthic properties of *B. aegyptiaca* were compared with those of albendazole and praziquantel (Koko *et al.*, 2000 and Koko *et al.*, 2005). The efficacy of *B. aegyptiaca* fruit mesocarp (200mg/kg) was compared with that of praziquantel (200mg/kg) in mice infected with Sudanese strain of *Schisosoma mansoni*. A significant reduction was observed in EPG (egg count per gram of faeces), egg burden in tissues and recovery of adult worm ($P<0.05$) for the extract and the drug-treated animal (Koko *et al.*, 2005).

2.1.5.6 Insecticidal Activity

B. aegyptiaca acts as a potential natural larvicidal agent against mosquito larvae due to the lavicidal activities present in the saponin rich extracts in the various tissues such a fruit kerned, root and leaf (Yandav and Panghal, 2010). The water extract of fruit kernel of *B. aegyptiaca* were found is be effective against the larvae of *Aedes arabiensis, Culex quinquefasciatus* and *Aedes aegypt*. The root extract was found to be most lethal, followed by the bark among the various parts tested (fruits) pulp, seed kernel, root bark

and leaves (Chapagain and Wiesman, 2005 and Wiesman and Chapagain, 2006). Saponin of *B. aegyptiaca* showed a great possibility for drastically reducing the *A. aegypti* population in the concerned areas (Chapagan *et al.*, 2008).

2.1.5.7 Antibacterial Activity

The leaf extracts of *B. aegyptiaca*, prepare in aqueous and organic solvents (acetone and ethanol), were tested for their antibacterial activity against *Salmonella typhi* by using the disc diffusion method. Ethanolic extracts demonstrated higher antibacterial activity (16mm zone of inhibition) while the aqueous extracts showed the least activity (4mm zone of inhibition) at 100mg/ml. The preliminary phytochemical analysis revealed the presence of saponins, tannins, phenols anthraquionones in the extract and these were considered for antibacterial activity (Doughari *et al.*, 2007). Methanolic and aqueous extract of whole plant extract showed 4mm inhibition zone in *Staphylococcus aureus* and 11mm zone of inhibition in the case of *Staphylococcus epidermidis* (Parekh and Chanda, 2007). *B. aegyptiaca* extract supplemented with a 60-100mg mineral (Kadosero) revealed 100% reduction in bacterial colony in untreated well water (Otieno *et al.*, 2007).

2.1.5.8 Antifungal Activity

Aqueous and methanolic (80%) extracts of root bark were screened for anticandidal activity by bioautography agar overlay method, using a standard strain of *Candida albicans* (ATCC 90028). The extract revealed strong anticandidal activity (Runyoro *et al.*, 2006). The stem bark extracts isolated in various solvent were screened for their antifungal effects against *C. albicans* and *Aspergillus niger*, and these extracts also showed high antifungal activity against C. albicans (Maregesi *et al.*, 2008). The fruit

mesocarp saponin rich extract has been tested against common phytopathogenic fungi (*Pythuim ultimum, Fusarium oxysporum, Alternaria solani, Colletotrichum coccodes and Verticillum dahliae*). The inhibitory effects of these extracts were measured in vitro and the concentrations that reduced the colony diameter of fungus to 50% of the control were determined. At 4% concentration, growth inhibition were reported against *P. ultimum*(81.1%) and *A. solani*(34.7%). The antifungal activity may be due to the presence of several triterpene saponins and steroidal saponins in *B. aegyptiaca* (Chapagain *et al.*, 2007 and Yadav and Panghal, 2010).

2.1.5.9 Antiparasitic Activity

The crude methanolic extract has been found to have a moderate biological activity on Leishmania major promastigotes in an in vitro study. With mean of growth inhibition of antileishmanial as 40.5 at a concentration of 1000µg/ml (Khalid *et al.*, 2005).

2.2.0 *Trichilia emetica*

2.2.1 Taxonomy and Nomenclature

Trichilia emetica belongs to the plantae kingdom, order of sapindals and family of Meliaceae. Genus Trichilia and species emetica (Pooley, 1993). *Trichilia emetica* is commonly known as Cape mahogany, Christmas bells, Natal mahogany, as *Jan saiwa* in Hausa and as *Ashapa* in Yoruba

2.2.2 Botanical Description

Trichilia emetica is an evergreen tree, usually up to 21m tall but occasionally reaching 30m, trunk swollen at the base, sometime fluted with age. Bark grey-brown or red-brown with fine, shallow striations and smallish scales. Branches erect, producing a pyramid shaped crown when young, oval to rounded and when matured with a diameter sometimes 15m. The tree has a non-aggressive root system. Leaves up to 50cm long, unevenly compound with 3-5pairs of leaflet plus a terminal one, dark green and glossy above, covered with short brownish hairs below, margins entire, vein prominent on lower surface. Flowers creamy to pale yellow-green, produced on short, congested axillary panicles, fragrant, with 5 thick petals, about 2cm. Fruits rounded, furry, red-brown capsules of 3cm across, split into 3 or 4 parts to reveal 3-6 shiny black seeds 14-18mm in length, each with a fleshy scarlet aril almost covering the seed. The named Trichilia is Greek' for in 3 parts, referring to the 3-lobed fruit and emetica means with emetic properties (Mbuya, 1994).

2.2.3 Ecology and Distribution

T. emetica is locally frequent in riparian forest and in some type of munga woodland, and can be found also in swamp forest, montane forest, savannah or alluvial lowland grass species. It grows naturally through sub-saharan Africa from Senegal to the red sea, throughout East and Central Africa to Congo and South Africa (Albrecht, 1993).

The plant shrives best at altitude of 0-2100m, Mean annual temperature 19-31^{0}C, Mean annual rainfall 600-2300mm, Soil type: Prefers well drained, rich alluvial or sandy soil and high water table (Mbuya, 1994).

Seeds are too heavy to be spread by wind and they fall to the ground, no animal or bird is known to disperse them. Flowering occurs from August to November and fruiting from December to April (Mbuya, 1994).

The species regenerates naturally by root suckers, coppice and seed. Capsules are collected when opened, dried in the shade and the seed shaked out pretreatment is by maceration in water to remove aril, and seed sown immediately. Sow fresh seed either directly or into nursery bags, germination within 10-20 days. The tree is also cultivated by means of cuttings. Make the cutting from layered branches or from 1 year old coppice shoots (Albrecht, 1993)

2.2.4 Functional Uses

A sweat, milky potable liquid is extracted from the arils. The skinned seeds are also edible and are eaten raw or soaked in water and ground, the resultant liquid mixed with spinach dishes. Domestic animals feed on its leaves. The tree is used for bee forage and firewood. Wood is soft .yet firm and works well. Good for furniture, shelving and carvings. Sunbirds visit the nectar-rich flower.

T. emetica oil produces a good finish on wood surfaces. Seed oil is used for candle making. The seed is extremely poisonous. The leaves used as antidote for the irritation caused by the buffalo bean and the bark in the treatment of skin complaints. Its oil is taken orally to relieve rheumatism. The kernels and husk of the nut produce a very good soap making oil, which is sometimes used as a cosmetic and also for preserving food stuffs (Grundy and Campbell, 1992).

Planting *T. emetica* greatly assists in soil conservation. A worthwhile tree to provide shade and protection for livestock and widely planted as windbreak. The presser seed cake left after oil has been extracted with an approximate protein content of 16%, is suitable as a fertilizer. *T. emetica* has been widely planted as a street or garden tree. Ideal for car parking areas as it never grows very high and has ever green, spreading crown (Mbuya, 1994).

2.2.5 Phytochemistry

2.2.5.1 Hepatoprotective and Antibacterial Activities

The hepatoprotective activity of the root extracts of *T. emetica* on CCl_4 induced damage in rat hepatocytes was examined, and their antibacterial activity against clinical isolated bacterial strain, which are commonly responsible for respiratory infections. A preliminary phytochemical analysis showed high polyphenolic content in aqueous extract and the presence of limonoids in the ethyl ether fraction. These latter compounds may be responsible for activity against the bacterial strains tested. *T. emetica* extracts exerted significant ($P<0.05$) heptoprotective effect at dose of 100µg/ml both on plasma membrane and mitochonelrial function compared to silymarin used as a positive control. Both the aqueous extract and its ethyl fraction did not show toxicity ($LC_{50}>1000\mu g/ml$) in the brine shrimp bioassay (Germao *et al.*, 2005).

2.2.5.2 Complement fixing ability of *T. emetica* Polysaccharides

The polysaccharides extracted from *T. emetica* and polymeric material was separated into neutral and acidic polymer by anion exchange chromatogramphy. The acidic fraction 4 of the 100^0c extract was most active. Structural studies showed that the most active fraction

was pectin of the rhamnogalacturonen type I with side chains of the arabinogalactan type II. Removal of terminal arabinofuranosides lead to drop in the activity indicating that this structural unit may be involved in the bioactive site of the molecule (Diallo *et al.*, 2003).

2.2.5.3 Anticancer Activity

A pioneering report of humulenes found in the genus of *T. emetica* has been reported. The aldehyde is a modest inhibitor of the slow-proliferating breast cancer cells MCF, (78μm) but a potent inhibitor of proliferation of S180 cancer cells (IC50 7.5μm) (Traore *et al.*, 2007).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Identification and Collection of Plant Materials

Fresh leaves of *Trichilia emetica* were collected in Bida Local Government Area of Niger State between July and September, after identification by herbal practitioner and authentitication at the Biology Department FUT Minna. These leaves were air dried at room temperature and pounded into powdered form using pestle and mortar. The powder was packaged in an air tight container, labelled and stored until analysed.

The stem bark of *Balanites aegyptiaca* was collected in the morning at Batagi in Lemu Local Government Area of Niger State, between July and September, after identification and authentification at the Biology Department FUT Minna. The debris on the plant was removed and air-dried at room temperature. It was pounded in to fine powder using mortar and pestle. The powder was packaged in an air-light container, labelled and store for analysis.

3.1.2 Animals

Healthy Swiss albino mice of either sex of about 7weeks old weighing between 20-30g were obtained from NITECO Road, Tunga, Minna, in Niger State, Nigeria were used for the experiments. The rodents were conveniently housed under standard environmental conditions, temperature 27 $\pm$ 2^0C, and 70% relative humidity, free access to commercial food pellets, water and natural 12hours day light/night cycles. The experiments were

conducted in strict compliance with internationally accepted principles for laboratory animals' use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review (Ernnest *et al*, 1993).

3.1.3 Parasites

Plasmodium berghei Nk65 chloroquine sensitive strain was obtained from National Emergency Maintenance Agency (NEMA) Lagos, Nigeria and maintained in the laboratory by serial passage in mice.

3.2 Methods

3.2.1 Preparation of Crude Extracts

Eighty grams of air dried sample *Trichilia emetica* and *Balanites aegyptiaca* were extracted exhaustively (48hours) in the cold sequentially with two liters each of n-hexane, ethylacetate and methanol (Sigma-Aldrich Europe) in that order separately. The marc was filtered with muslin cloth and solvent removed under reduced pressure in a rotary evaporator. The pastes were poured into beakers and placed on a water bath for complete evaporation of the organic solvent.

Each of the green and yellow paste from *Trichilia* emetica and *Balanites aegyptiaca* respectively, were weighed, and labelled prior to further analysis.

3.2.2 Phytochemical Screening

Phytochemical screening were carried out on aqueous extract and on the powdered specimen using standard procedures described by Edeoga *et al.* (2005) and Akinyemi (2005). The aqueous extract of each sample was prepared by percolating hundred grams

of dried powered samples with two hundred millilitre (ml) of distilled water in the cold for 12hours. The extracts were then filtered using Whatman filter paper No. 42 (125mm).

3.2.2.1 Test for Tannins

Zero point five grams of the dried powdered sample was boiled in 20ml of water in a test tube and then filtered. Few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration (Edeoga *et al.*, 2005).

3.2.2.2 Test for Phlobatannins

Five millilitre of aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid (HCl). Deposition of a red precipitate is taken as evidence for the presence of Phlobatannins (Edeoga *et al.*, 2005).

3.2.2.3 Test for Saponins

Two grams of powdered sample was boiled in 20ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for stable persistent froth. The froth was mixed with 3 drops of olive oil and shaken vigorously then observed for the formation of emulsion (Edeoga *et al.*, 2005).

3.2.2.4 Test for Flavonoids

A portion of the powdered plant sample was in each case heated with 10ml of ethyl acetate over a steam bath for 3minutes.The mixture was filtered and 4ml of the filtrate was shaken with 1ml of filtrate ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids (Edeoga *et al.*, 2005).

3.2.2.5 Test for Cardiac Glycosides (Keller-killani Test)

Five millilitre of each extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, whole in the acetic acid layer, a greenish ring may form just gradually throughout thin layer (Akinyemi *et al.*, 2005).

3.2.2.6 Test for Alkaloids

Zero point five gram of each extract was stirred with 5ml of 1% aqueous hydrochloric acid on a steam bath, 1ml of the filtrate was treated with a few drops of Mayer's reagent. Turbidity or precipitation with this reagent was taken as evidence for the presence of alkaloids (Akinyemi *et al.*, 2005).

3.2.3 Quantitative determination of some chemical constituents

The method of Bohm and Kocipai – Abyazan (1994) for flavonoids determination was used. Ten grams of each of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125mm). The filtrate was transferred into evaporating dish and placed on a water bath until a constant weight was maintained (Edeoga *et al.*, 2005).

Harbone's (1973) Method of alkaloid determination was used. Five grams of the samples were weighed into 250ml beaker and 200ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated

ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed (Edeoga *et al.*, 2005)

Obadani and Ochuka (2001) method was used in saponin determination. Twenty grams of each plant sample was weighed into a conical flask and 100cm^3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 hour with continuous stirring at 55^{0C}. The mixture was filtered and the residue re-extracted with another 200ml 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90^{0C}. The concentrate was transferred into a 250ml seperatory funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer recovered while the ether layer was discarded. The purification process was repeated. 50ml of n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated on a water bath. After evaporation the samples were dried in the oven to a constant weight, the saponin content was calculated as percentage.

Total phenol was determined using Edeoga *et al.*, 2005 method. Two grams of each plant sample were defatted with 100ml of diethyl ether using a soxhlet apparatus for 2 hours. The fat free sample was boiled with 50ml of ether for the extraction of the phenolic component for 15minutes. Five millilitre of the extract was pipette into a 50ml flask, then 10ml of distilled water was added. Two millilitre of ammonium hydroxide solution and 5ml of concentrated amylalcohol were also added. The samples were made up to mark and left to react for 30minutes for colour development. This was measured at 505nm.

3.2.4 Safe dose and acute toxicity (LD_{50})

Ten groups of four mice and were used and the animals were given extracts intraperitoneally (I.P) at doses of 200, 400, 800, 1600, and 3000mg/kg body weight (b/w) respectively. Extracts were dissolved in dimenthylsulphoxide (DMSO) (Sigma Chemical St Louis, MO, USA).

A control group was given normal saline (0.9% w/v NaCl) at 20ml/Kg bw. Mice were observed over 72hours for clinical signs and mortality was recorded. LD_{50} was obtained as the intercept of % mortality (y-axis) and dosages (x-axis).

3.2.5 Antiplasmodal Screening

Mice were pre-screened by microscopy of thin and thick tail tip blood smears. This was necessary to exclude the possibility of test animals harbouring rodent Plasmodium species.

3.2.6 Curative Test

This is a procedure whereby mice are infected and left for 72 hours before treatment with test and standard as in Rane test or established infection (Jigam, *et. al.*, 2000). Twenty albino mice were selected and divided into five groups. One group served as control, one as standard and the other as the test groups for hexane, ethylacetate and methanolic extracts. The two groups are infected with Plasmodium berghei (approximately 1 x 10^7 infected red cells) by the intraperitoneal routes. The animals were left for 72hours for the infection to be established. The control group was given 0.9w/v of normal saline. 300 $mgkg^{-1}$ bw day^{-1} dose was selected. On D3 i.e, after 72 hours of infection, the test drugs

were administered subcutaneously once daily for 4 days from D3 to D7, thick and thin blood smears from the tail blood were examined for parasite suppression. Chloroquine (Standard drug) 5mg/kg bw was ran for comparison. The equivalent CQ dose was determined from the rate of Chloroquine (standard) dose to dose of test drug giving identical average percentage suppression (Jigam *et al.*, 2000).

3.2.7 Determination of some Biochemical Parameters

Sixty mice were kept in three groups (A, B and C) of the twenty each. Group A was used as test for *B. aegyptiaca*, group B was used as test for *T. emetica* and group C as control. Group A and B were given 300mg/Kg bw of the extracts and group C given 20ml/Kg bw normal saline on alternating days. All animals were monitored for different biochemical parameters at weekly intervals for five weeks. Weight of mice were taken with Avery Balance (W and T) Avery Ltd, Birmingham, UK

3.2.7.1 Packed Cell Volume

Heparinized Capillary tube was filled to two-third with blood samples. One end was sealed with plastisin and placed into the microhematocrit centrifuge, with the plugged end away from the centre of the centrifuge. The spinning was done at 5000rpm for 10minutes. Those with hematocrit above 50% were spun for another 3minutes. The tube was placed in hematocrit reader and PCV determined.

3.2.7.2 Serum Glucose Estimation

Serum glucose was determined using Randox Glucose Kit. Glucose is oxidized in the presence of glucose oxidase to give glucoronic acid and hydrogen peroxide. The later,

under catalysis of peroxidase with phenol and 4 – aminophenalzone form a red – violet quinoneimine dye as indicator.

$$\text{Glucose} + O_2 + H_2O \longrightarrow \text{glucoronic acid} + H_2O_2.$$

$$2H_2O_2 + 4 - \text{amino phenazone} + \text{phenol} \longrightarrow \text{quinoneimine} + 4H_2O.$$

Three test tubes were used. To the first tube (standard), 0.0ml of the standard solution was pipette, and 1.0ml of reagent was added. To the second test tube (reagent blank), 1.0ml of reagent was pipette then 0.01ml of distilled water. To the third tube, 0.01ml of sample was pipette, and 1.0ml of reagent added.

The content of each testtube was shaked and mixed properly. These were incubated at 37^0C for 10 minutes. The absorbance of test was measured at 546nm against blank. The Glucose concentration was determined using.

$$\text{Glucose concentration (mg/dl)} = \frac{\text{A sample}}{\text{A standard}} \times \text{conc. of standard (mg/dl)}$$

3.2.7.3 Estimation of serum total proteins

The determination serum total protein was carryout with Total Protein Randox kit.

Cupric ions, in an alkaline medium, interact with protein peptides bonds resulting in the formation of a coloured complex compound which is proportional to the amount of protein present in the sample.

Three test tubes were used. To the first tube (blank) 0.02ml of distilled water was pipette and 1.0ml of reagent added. To the second test tube (standard) 0.02ml of standard solution was pipette and 1.0ml of reagent added. To the third test tube (test) 0.02ml of sample was pipette and 1.0ml of reagent 1 added. Each test tube was shaked properly.

These test-tube were incubated at 37^0C for 10 minutes. Absorbance of sample (A sample) and of the standard (A standard) were read against the reagent blank at 546nm. The total protein concentration was determined using:

$$\text{Total protein conc. (g/dl)} = \frac{\text{A sample}}{\text{A standard}} \times \text{conc. of standard (g/dl)}$$

3.2.7.4 Determination of Triglycerides

Estimation of Triglycerides was carried out using Randox Triglycerides kit. The triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminophonazone and 4-chlorophenol under the catalytic influence of peroxidase.

Triglyceride + water $\longrightarrow$ Glycerol + Fatty acids

Glycerol + ATP $\longrightarrow$ Glycerol – 3 – phosphate + ADP

Glycerol – 3 – phosphate + O_2 $\longrightarrow$ dihydroxuacetoxic + phosphate + H_2O_2

$2H_2O_2$ + 4 – amino phenazone + 4 – chlorophenol $\longrightarrow$ quinoneimine + HCl $4H_2O$

Three test tubes were used. To the first (reagent blank), 0.01ml of the distilled water was pipetted and 1.0ml of reagent added. To the second tube (standard) 0.01ml of the standard solution was pipetted, the 1.0ml of the reagent was added. To the third tube, 0.01ml serum was pipette, and then 1.0ml of the reagent added.

The content of each test-tube was mixed properly and incubated at 37^0C for 5minutes. The absorbance of sample (A sample) and standard (A standard) against blank was measured at 546nm.

Concentration of triglycerides was calculated using:

$$\text{Triglycerides Conc. (mg/dl)} = \frac{\text{A sample}}{\text{A standard}} \times \text{standard conc. (mg/dl)}$$

3.2.7.5 Determination of Aspartate Aminotransferase

Estimation of AST was done using AST Randox kit.

Oxogluturate + L – aspartate $\xrightarrow{\text{AST}}$ L – glutamate + Oxaloacetate

AST is measured by monitoring the concentration of oxoloacetate hydrazone formed with 2, 4 – dinitrophenylhydrazone.

Two test tubes were used. To the first tube (reagent blank), 0.5ml of solution R1, was pipetted, and then 0.1ml of distilled water added. To the second tube (sample), 0.5ml of solution R, was pipetted and 0.1ml of serum added. The content of each test tube was mixed properly and incubated at 37^0C for 30minutes. 0.5ml of R2 was added to each test tube. The content of each test tube was mixed properly and incubated at 37^0C for 10minutes. 5.0ml of sodium hydroxide was pipetted into each test tube. The content of the testtube were mixed and the absorbance of sample (A sample) was read against blank at 546nm after 5minutes.

The activity of AST was obtained from a standard calibration of AST activity (in u/l) against absorbance.

3.2.7.6 Determination of Alanine Aminotransferase (ALT) Activity

Estimation of Alanine Aminotransferase was done using Randox ALT kit. The activities of ALT is measured by measuring the concentration of the product of the reaction catalyze by ALT. ALT reaction is reversible, but adding a substance (2, 4-dinitro–phenylhydrazine) which will react with pyruvate (one of the products of ALT reaction) the reaction will be shuffled to the formation of pyruvate. 2, 4 –dintrophenyl hydrazine reacts with pyruvate to form the corresponding 2, 4-dinitrophenyl hydrazine derivative of pyruvate which is measured spectrophotomerically at 546nm.

Oxoglutarate + L – alanine $\xrightarrow{ALT}$ L – glutamate + Pyruvate

Two test tubes were used. To the first tube (reagent blank), 0.5ml of solution R, was pipetted, then 0.1ml of distilled water added. To the second tube (sample), 0.5ml of solution R, was pipetted and 0.1ml of serum added. The content of each test tube was mixed properly and incubated at 37^0C for 30minutes. 0.5ml of R2 was added to each test tube. The content of each test tube was mixed properly and incubated at 37^0C for 10minutes. 5.0ml of sodium hydroxide was pipetted into each test tube. The content of test tubes were shaked to mix properly and absorbance of sample (A sample) against blank was read at 546nm after 5 minutes. The activities of Alanine aminotransferase was read from standard calibration curve.

3.2.7.7 Estimation of Alkaline Phosphatase (ALP) Activity

The estimation ALP activity was done using Randox ALP kit. ALP hydrolyses colourfulness p-nitrophenyl phosphate (pNPP) producing phosphate and p-nitropenol at alkaline pH. The speed at which the p-nitrophenolate anion (yellow) appears is read colorimeterically at 405nm, is directly proportional to the enzymatic activity of the sample (Kim and Wyckoff, 1991).

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

Diethanolamine (DEA) regulates the reaction's pH and acts as acceptor of the phosphate release by phosphatase (Transphosphorylation), resulting in activation of the reaction. DEA has the best condition terms of activation and buffering when p-NPP is used as substrate.

Zero point zero one millilitre of the sample was measured into a test-tube and 0.5ml of ALP reagent was added. The content of the test-tube was mixed property and the absorbance was read at 405nm. The activity of ALP was calculated by multiplying the absorbance of sample by 2760, to give the activities in u/L.

3.2.8 Histopathological screening of tissues

At the end of the treatment period, mice were sacrificed and liver and kidney collected in sterile saline. Freshly dissected organs from each animal were cut rapidly and fixed in buffered neutral formalin (10%). The tissues were dehydrate in ascending grades ethanol (70%, 80%, 90%, 95% and 100%), cleared in 2 changes of Xylene, impregnated with 2 changes of molten paraffin and finally embedded in wax. Tissue sections of 4-5μm in thickness were cut with a microtome and stained with hematoxylin and eosin (Pearse, 1985).

3.2.9 Statistical Analysis

Results are expressed as mean ± standard error.of the mean, while student's t-test was used to test for differences between groups using Statistical package for social sciences (SPSS) version 16. A value of $P<0.05$ was accepted as significant

CHAPTER FOUR

4. 0 RESULTS AND DISCUSSIONS

4 .1 RESULTS

4.1.1 Extract Yields

The extract yields of *Balanites aegyptiaca* and *Trichilia emetica* obtained with different solvents are shown in Table 4.1. The values are in the order: methanol > hexane > ethyl acetate for the two plants analysed.

4.1.2 Phytochemical Contents

The results of qualitative and quantitative phytochemical screening of *Balanites aegyptiaca* and *Trichilia emetica* are given in Table 4.2. Tannins were detected in *B. aegyptiaca* while pholobatannins were detected in *T. emetica.* Cardiac glycosides were only detected in *T. emetica.* Total phenols were most abundant in both plants. Other phytochemicals detected in both plants include saponins, flavonoids and alkaloids.

4.1.3 Safe Dose Determination (pre LD_{50}).

The result of safe dose determination of *B. aegyptiacae* is in Table 4.3. The extract was generally devoid of adverse symptoms up to 600mg/kg bw hence the dose chosen as safe. The result of safe dose determination of *T. emetica* is in Table 4.4.The extract was devoid of adverse symptoms up to 300 mg/kg bw hence the dose chosen as safe. The safe dose chosen was used for subsequent administration of the extracts to the experimental animals

4.1.4 Results of the preliminary antiplasmodial effects of *Balanites aegyptiaca* and *Trichilia emetica*

The activity of *Balanites aegyptiaca* fractions against parasites in mice are shown in Table 4.7 and the activity of *Trichilia emetica* fractions against parasites in mice are shown in Table 4.8. In the preliminary antiplasmodial screening of *B. aegyptiaca* fractions, hexane fraction shows greater efficacy than the methanolic and ethylacetate fractions and the control group all died while for the *T. emetica* all the three fractions show the same efficacy with parasites slightly present and the control group showed highest level of parasitaemia.

4.1.5 Results of Parasite suppression

The suppression of parasitaemia in mice treated with different fractions *B. aegyptiaca* of is in figure 4.3. None of the three fractions (Hexane, Ethylacetate and Methanolic extracts) of *B. aegyptiaca* was able to clear the parasite or decrease parasite level. In fact, the group of mice given methanolic extract of *B. aegyptiaca* died before the control group while both groups given ethylacetate and Hexane fractions died on the 14th day after infection.

The suppression of parasitaemia in mice treated with different fractions *T. emetica* of is in figure 4.4. Ethylacetate fraction of *T. emetica* is not effective in the treatment of malaria, because the mice in this particular group died before the control group. Hexane and methanolic fraction of *T. emetica*, shows a promising effect on malaria parasite, with the methanolic fraction having higher activity than the Hexane fraction as shown in figure 4.3. Since it is know that Hexane fraction contain non-polar constituents and the

methanolic fraction contain both polar and non-polar constituents, we can infer that, the substance responsible for the activity requires a polar medium to be more effective or there is a polar and non-polar constituents that are working synergistically to give better parasite suppression in the methanolic extract.

4.1.6 Results of weight variations

Weight variations in mice chronically dosed with *B. aegyptiaca* and *T. emetica* compared with the control is represented in figure 4.1. The figure shows that there was shape retardation in the weight of *T. emetica* treatment while there was a progressive increase in weight of the control as the weeks go by. There was weight reduction in *B. aegyptiaca* treatment compared to the control.

4.1.7 Results of Packed Cell Volume

Variations in packed cell volume in mice chronically dosed with *B. aegyptiaca, T. emetica* and the control is represented in figure 4.2. The Packed cell volume of both *B. aegyptiacae* and *T. emetica* were progressively decreasing while the PCV for the control was increasing in all the weeks.

4.1.8 Results of whole body and fresh organ weight

The results of whole body and fresh organ weight are presented in Tables 4.5 and 4.6 for *T. emetica* and *B. aegyptiacae* respectively. There was no significant difference in fresh organ weights (liver, kidney, intestine, and spleen) of mice dosed with *T. emetica* compared to the control expect in stomach weight which showed significant difference

($p< 0.05$), while for the *B. aegyptiacae* there was significant difference in the percentage fresh organ weight to whole body weight compared to the control.

4.1.9 Results of effects of *Balanites aegyptiaca* and *Trichilia emetica* on glucose, total proteins and triacylglycerides.

The results of effects of *Balanites aegyptiaca* and *Trichilia emetica* on glucose, total proteins and triacylglycerides in mice are presented in Tables 4.9 and 4.11. The results indicate that during the first week of *B. aegyptiaca* administration there was no significant difference in glucose and total proteins levels compared to the control expect in triacylglycerides level, but after week five there was significant difference in glucose, total proteins and triacylglycerides level. For *T. emetica* treatment there was significant difference ($p<0.05$) in glucose and triacylglycerides level in both the first week and fifth week of administration compared to the control values but there was no difference ($p< 0.05$) in the total proteins level in both first and fifth week.

4.1.10 Results of effects of *Balanites aegyptiaca* and *Trichilia emetica* on Aspartate aminotransferase, Alanine aminotransferase and Alkaline phosphatase.

The results of effect of *Balanites aegyptiaca* and *Trichilia emetica* on Aspartate aminotransferase, Alanine aminotransferase and Alkaline phosphatase in mice are presented in Tables 4.10 and 4.12. There was a significant ($p<0.05$) increase in AST and ALP activity but not in ALT levels compared to the control in the first week of *B. aegyptiaca* treatment. The AST, ALT and ALP activities of *Balanites aegyptiaca* treatment varied significantly compared to the control in week five. The ALT and ALP levels of *B. aegyptiaca* decreased compared to the control while AST of *B. aegyptiaca*

treatment was higher. For *T. emetica* treatment ALT and ALP levels were not different compared to the control at 95% confidence limit but AST varied significantly in the first week. In the fifth week ALT and ALP activities of *T. emetica* treatment decreased while AST activity increased compared the control group.

4.1.11 Results of *Balanites aegyptiaca* effect on liver and kidney

The histopathology result of the liver and kidney administered *Balanites aegyptiaca* on a medium term are in plate I and plate II respectively. The liver of the mice given *B. Aegyptiaca* lost the normal hepatocyte hexagonal archs and became disintegrated while the kidney nephrons become ghostlike in nature

Table 4.1 Extract yields of *B. aegyptiaca* and *T. emetica*

Solvents	*B. aegyptiaca* (g)	(%)	*T. emetica* (g)	(%)
Hexane	4	4.00	6	6.00
Ethylacetate	1.42	1.48	2.27	2.42
Methanol	5.73	6.23	9.33	10.48

Table 4.2: Phytochemical contents of *T. emetica* and *B. aegyptiaca* extracts

Phytochemicals	Test	T.emetica		*B. aegyptiaca*	
			%		%
Tannins	0.1% FeCl			+++	
	Bromine H_2O	-		-	
Pholobatannins	Lead acetate	-		-	
	1% HCl	+		-	
Saponins	Frothing	++	13.40	+++	16.10
	Emulsion	+		+++	
Flavonoids	Dilute NH_3/Conc H_2SO_4	-	32.20	+	12.50
	Lead Acetate	++		-	
	Dilute NaOH	-		-	
Cardiac glycosides	Keller-killeni	+		-	
Alkaloids	Mayer's test	++	19.40	+	4.00
Total phenol	Spectrophotometric test	-	64.71		32.35

+++= highly present ++= moderately present, += slightly present, - = absent

Table 4.3 Results of dose determination (Pre LD_{50}) for *Balanites aegyptiaca*

Dose (mg/kg bw i.p)	Observation	Mortality
200	No observable change	0/4
400	Apparently Normal	0/4
600	Appear Stable and Normal	0/4
800	Somnolence but Normal	0/4
1600	Somnolence Normal	0/4
3000	Laboured Breathing	0/4

Selected dose = 600 mg/kg bw,

Table 4.4 Results of dose determination (Pre LD_{50}) for *Trichilia emetica*

Dose (mg/kg bw. ip.)	Observations	Mortality
200	Animal appears normal	0/4
400	No observable Changes	0/4
800	Initial restlessness but normal	0/4
1600	Slow activity, laboured breathing	0/4
3000	Somnolence with a single mortality	1/4

Selected dose = 300mg/kg bw

Table 4.5 Results of fresh organ weights of mice dosed with *T. emetica* extract

Organs	Control	Test
Liver	4.55	4.69
Kidney	1.27	1.25
Intestine	11.32	11.30
Spleen	0.39	0.45
Stomach	2.34	2.68*

* means the sample result is significantly different at 5% ($p<0.05$) across the row.

Table 4.6 Results of fresh organ weights of mice dosed with *B. aegyptiaca* extract.

Organs	Control	Test
Liver	4.55	4.94*
Kidney	1.27	1.12*
Intestine	11.32	10.99*
Spleen	0.39	0.56*
Stomach	2.34	2.00*

* means the sample result is significantly different at 5% ($p<0.05$) across the row.

Table 4.7 Results of preliminary antiplasmodial screening of *B. aegyptiaca* fractions

Treatment	Dose (mg/kgbw)	Parasitaemia	
		Male mice	Female mice
ee	600	+ + +	+ + +
he	600	+ +	+ +
me	600	+ + +	*
chloroquine	5	+ +	+
Normal saline	20ml	*	*

ee:ethylacetate extract, he :hexane extract, me :methanolic extract, + : slightly present, ++ : moderately present, +++ : highly present, - : absent and*: mortality

Table 4.8 Results of preliminary antiplasmodial screening of *T. emetica* fractions

		Parasitaemia	
Treatment	Dose (mg/kg bw)	Male mice	Female mice
ee	300	+ +	+ +
he	300	+ +	+ +
me	300	+ +	+ +
chloroquine	5	+	-
Normal Saline	20ml	+ + +	+ + +

ee :ethylacetate extract, he :hexane extract, me :methanolic extract, ,+ : slightly present, ++ : moderately present, +++ : highly present, - : absent and*: mortality

Table 4.9: Effects of *Balanites aegyptiaca* extracts on some serum biochemical parameters in mice

		Parameters (mg/dl)	
Period	Glucose	Total proteins	Triacylglycerides
WK1 Ctrl	96.00 ± 1.84	7.00 ± 1.11	187.40 ± 5.13
Test	100.00 ± 3.00	9.00 ± 2.16	169.00 ± 4.58*
WK 5 Ctrl	109.00 ± 1.28	8.00 ± 2.16	223.00 ± 2.74
Test	77.30 ± 2.22*	5.30 ± 1.04*	116.20 ± 3.05*

The results are expressed as mean ± standard error of mean ($\bar{X}$ ± SEM) with ten sample size (n=10) and * mean the result is significantly different at 5% ($p<0.05$) across the row

Table 4.10: Effects of *Balanites aegyptiaca* extracts on some serum enzymes in mice

	Serum enzymes (μ/L)		
Period	AST	ALT	ALP
WK1 Ctrl	9.80 ± 1.40	12.00 ± 1.14	202.32 ± 8.18
Test	31.00 ± 1.05*	13.00 ± 0.99	173.60 ± 5.28*
WK 5 Ctrl	11.60 ± 1.11	12.60 ± 2.79	200.00 ± 3.89
Test	89.00 ± 5.08*	6.00 ± 0.19*	155.00 ± 4.44*

The results are expressed as mean ± standard error of mean ($\bar{X}$ ± SEM) with ten sample size (n=10) and * mean the result is significantly different at 5% ($p<0.05$) across the row

Table 4.11: Effects of *T.emetica* extracts on some serum biochemical parameters in mice.

	Parameters (mg/dl)		
Period	Glucose	Total proteins	Triacylglycerides
WR1 Ctrl	96.00 ± 1.84	7.00 ± 1.11	187.40 ± 5.13
Test	110.13 ± 2.6*	7.80 ± 0.34	144.87 ± 3.13*
WK 5 Ctrl	109.08 ± 1.28	8.00 ± 1.20	223.00 ± 2.94
Test	95.65 ± 2.05*	6.10 ± 0.22	100.05 ± 2.88*

The results are expressed as mean ± standard error of mean ($\bar{X}$ ± SEM) with ten sample size (n=10) and * mean the result is significantly different at 5% ($p<0.05$) across the row

Table 4.12: Effects of *T. emetica* extracts on some serum enzymes in mice.

	Serum enzymes (μ/L)		
Period	AST	ALT	ALP
WR1 Ctrl	9.80 ± 1.40	12.00 ± 1.14	202.32 ± 8.18
Test	60.00 ± 2.85*	12.00 ± 1.20	199.00 ± 4.02
WK 5 Ctrl	11.60 ± 1.11	12.60 ± 2.79	200.00 ± 3.89
Test	23.00 ± 1.15*	3.50 ± 0.76*	185.20 ± 4.25*

The results are expressed as mean ± standard error of mean ($\bar{X}$ ± SEM) with ten sample size (n=10) and * mean the result is significantly different at 5% ($p<0.05$) across the row

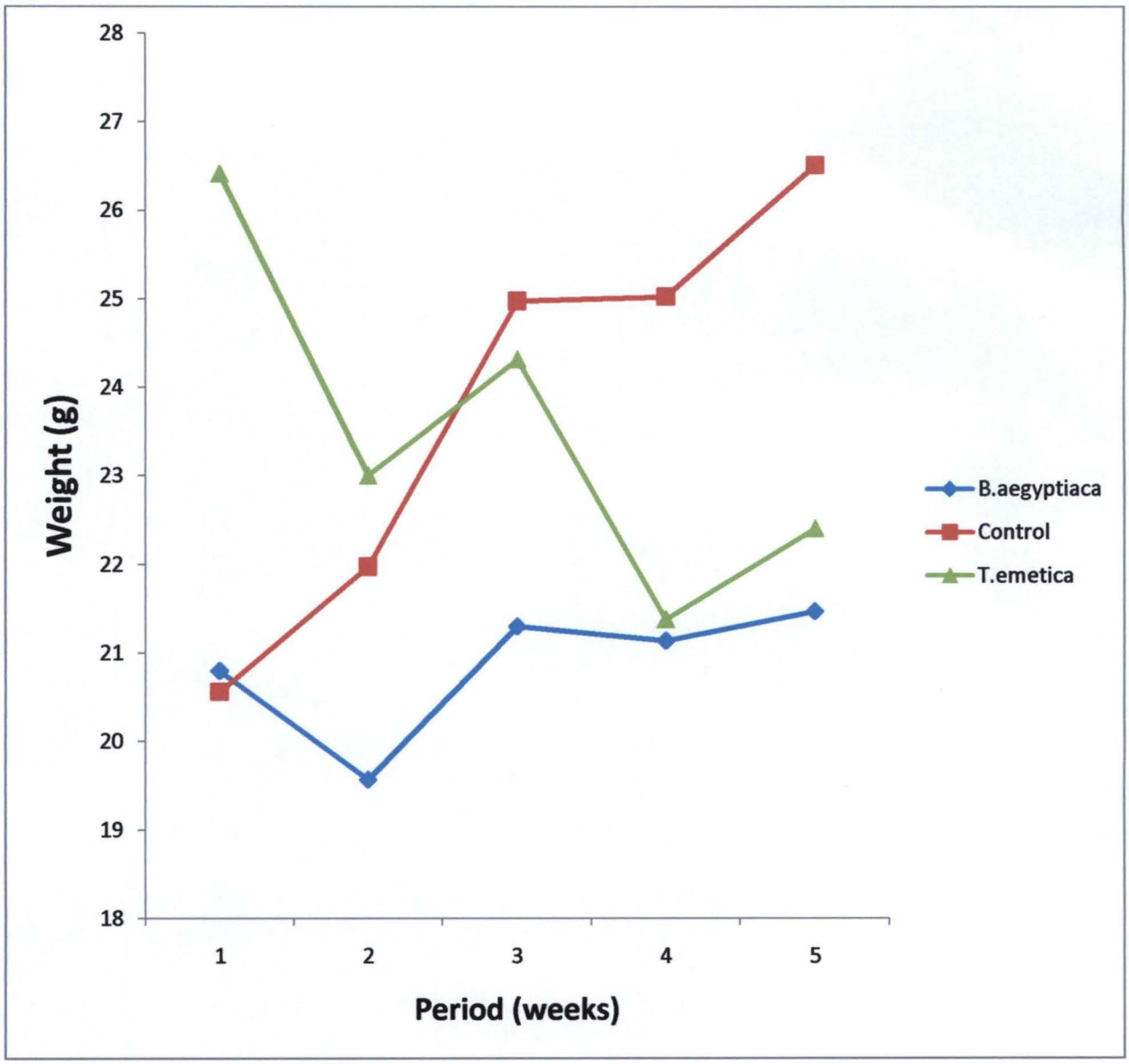

Figure. 4.1: Variations in weight (g) of mice chronically dosed with *B. aegyptiacae* and *T. emetica*.

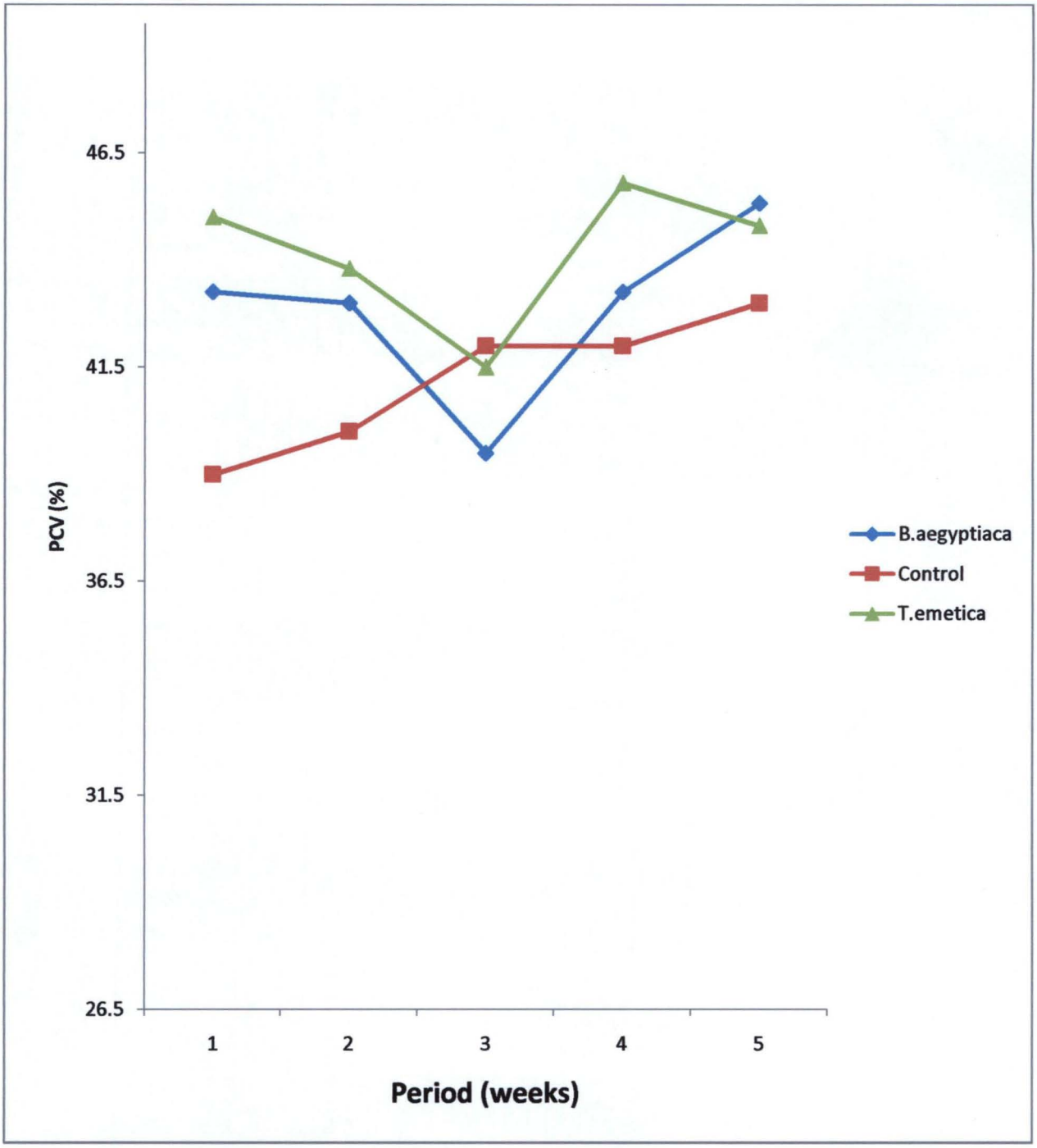

Figure. 4.2: Variations in packed cell volume in mice chronically dosed with *B. aegyptiacae* and *T. emetica.*

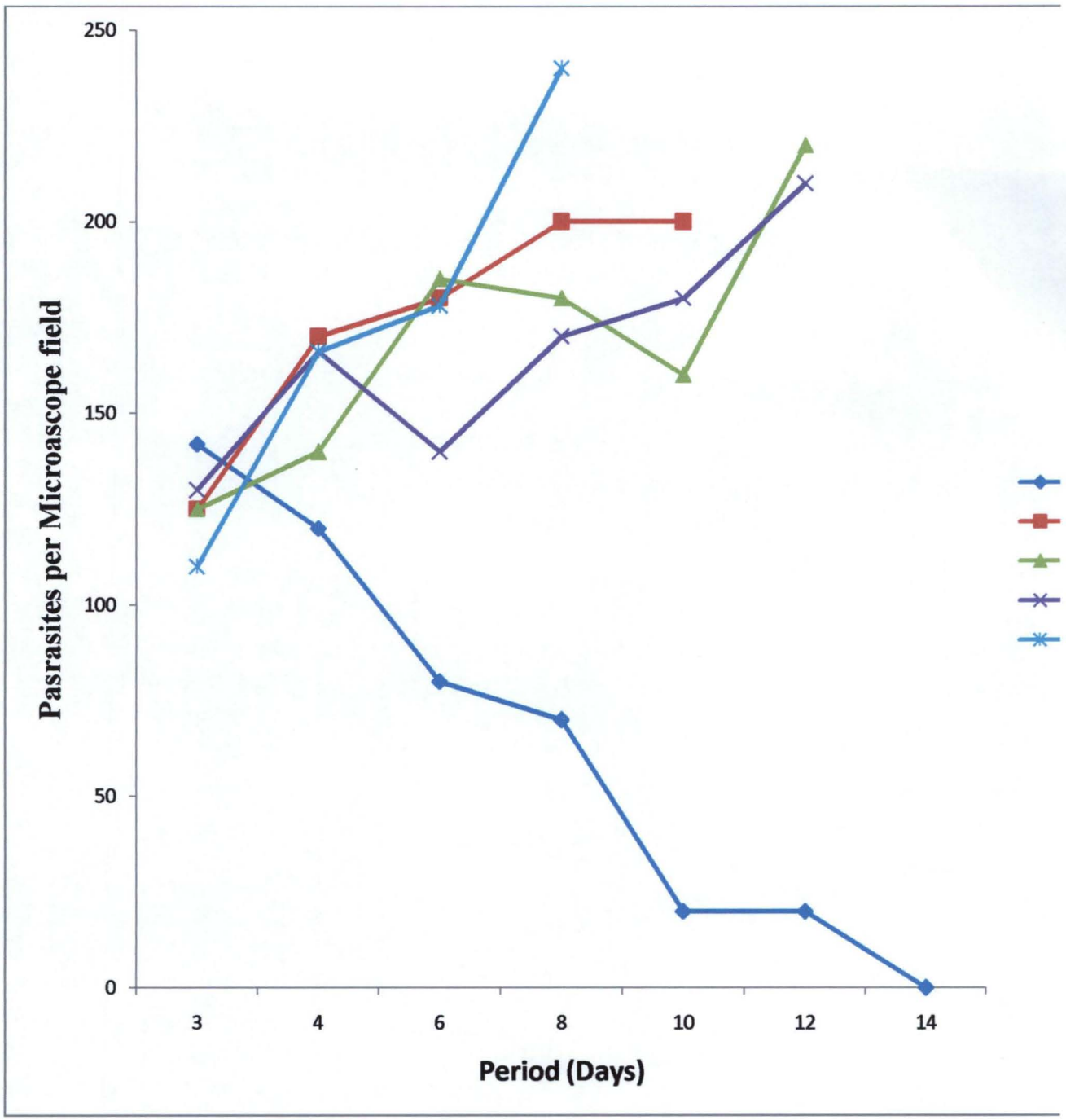

Figure. 4.3 Suppression of parasitaemia in mice treated with different fractions of *B. aegyptiaca*

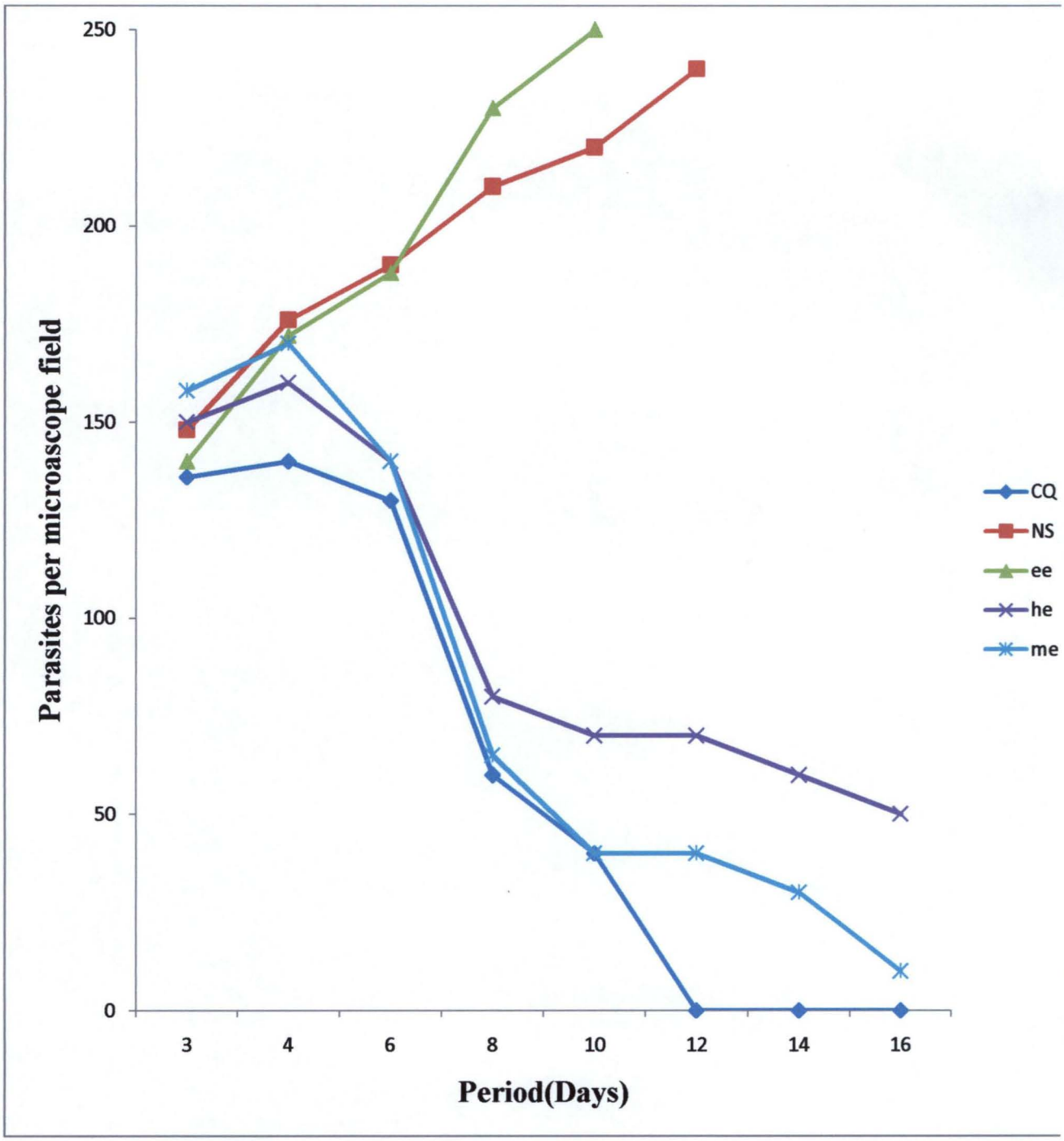

Figure. 4.4 Suppression of parasitaemia in mice treated with different fractions of *T. emetica*

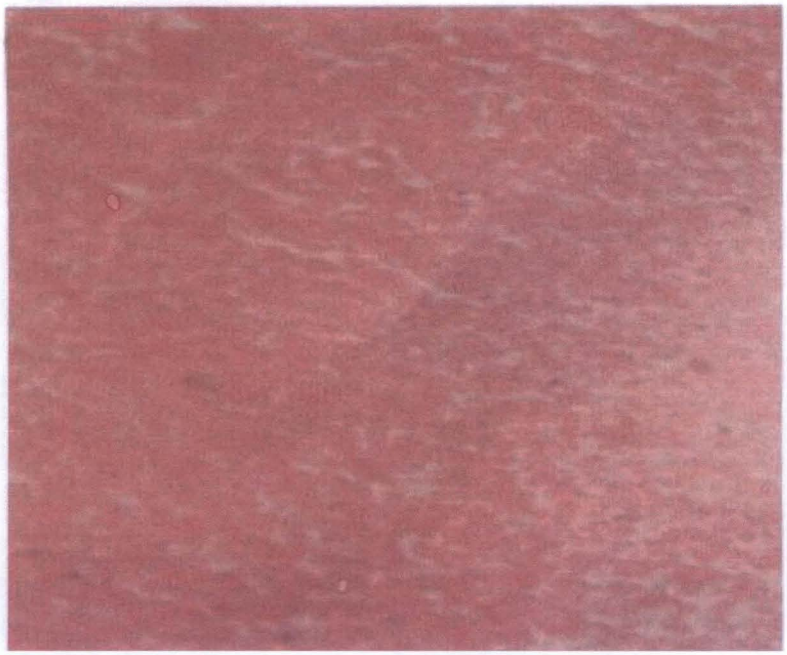

Plate Ia liver tissue (control) with normal hexagonal archs.

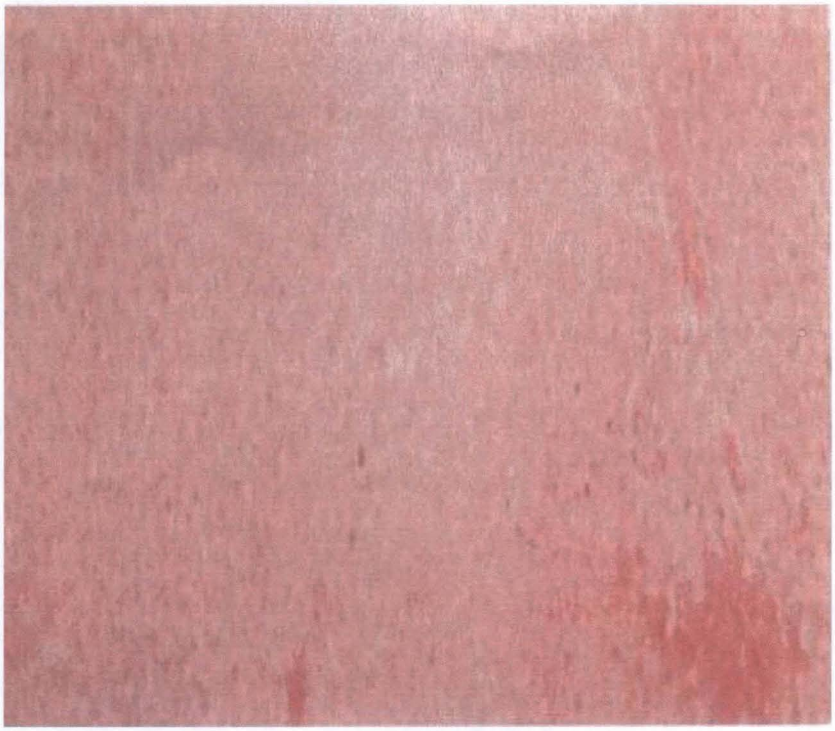

Plate Ib liver tissue (test) with feathery disintegration of hepato cytes

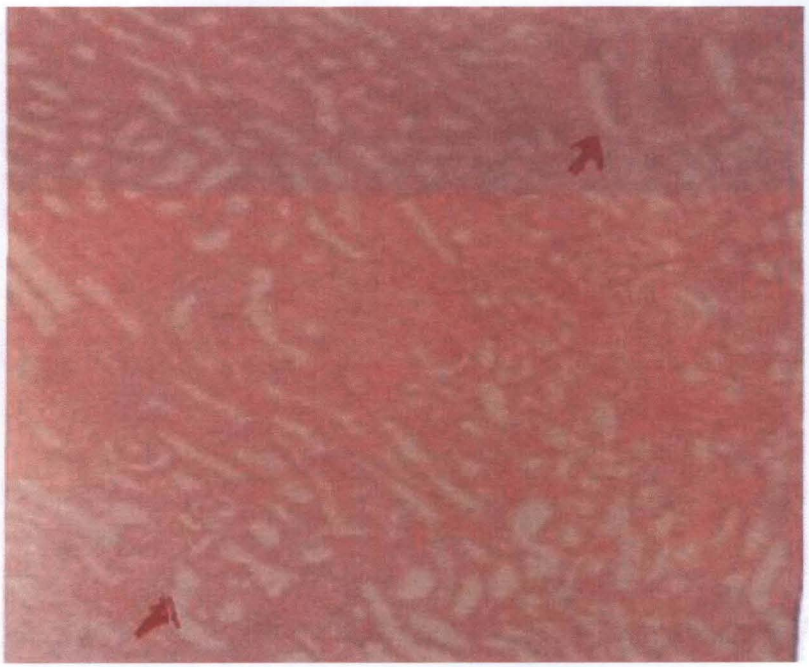

Plate IIa kidney tissue (control) with intact nephrons

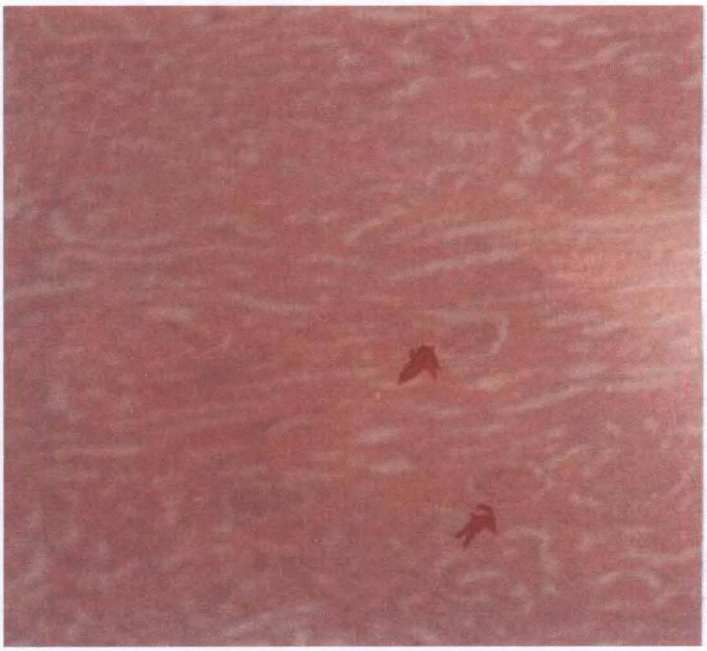

Plate IIa kidney tissue (test) with ghostlike nephrons

4.2 Discussion

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Properties of a good solvent in plant extractions includes, low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action, inability to cause the extract to complex or dissociate. The factors affecting the choice of solvent are quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process, potential health hazard of the extractants (Prashant, *et al.*, 2011). The choice of solvent is influenced by what is intended with the extract. Since the end product will contain traces of residual solvent, the solvent should be non-toxic and should not interfere with the bioassay. The choice will also depend on the targeted compounds to be extracted (Ncube *et al.*, 2008, Das *et al.*, 2010).

The various solvents that used in the extraction procedures are water, alcohol, acetone, chloroform, ether, dichloromethanol. Water is universal solvent, used to extract plant products with antimicrobial activity. Though traditional healers use primarily water but plant extracts from organic solvent have been found to give more consistent antimicrobial activity compared to water extract. Also water soluble flavonoids (Mostly anthocyanins) have no antimicrobial significance and water soluble phenolics only important as antioxidant compound (Das *et al.*, 2010).

Acetone dissolves many hydrophilic and lipophilic components. It is miscible with water, is volatile and has a low toxicity to the bioassay used, it is a very useful extractant, especially for antimicrobial studies where more phenolic compounds are required to be extracted. A study reported that extraction of tannins and other phenolics was better in aqueous acetone than in aqueous methanol (Prashant *et al.*, 2011). Both acetone and methanol were found to extract saponins which have antimicrobial activity (Ncube *et al.*, 2008). The higher activity of the ethanolic extracts as compared to the aqueous extract can be attributed to the presence of higher amounts of polyphenols as compared to aqueous extracts. It means that they are more efficient in cell walls and seeds degradation which have unpolar character and cause polyphenols to be released from cells. More useful explanation for the decrease in activity of aqueous extract can be ascribed to the enzyme polyphenol oxidase, which degrade polyphenols in water extracts, whereas in methanol and ethanol they are inactive. Moreover, water is a better medium of the occurrence of the micro-organisms as compared to ethanol (Lapornik *et al.*, 2005). The higher concentrations of more bioactive flavonoids compounds were detected with ethanol 70% due to its higher polarity than pure ethanol. By addition water to the pure ethanol up to 30% for preparing ethanol 70% the polarity of solvent was increased (Bimakr, 2010). Additionally, ethanol was found easier to penetrate the cellular membrane to extract the intracellular ingredients form the plant material (Wang, 2010). Since nearly all of the identified components form plants active against microorganisms are aromatic or saturated organic compounds, they are most often obtained through initial ethanol or methanol extraction Cowan, 1999). Methanol is more polar than ethanol but

due to its cytotoxic nature, it is unsuitable for extraction in certain kind of studies as it may lead to incorrect results.

Terpenoid lactones have been obtained by successive extractions of dried barks with hexane, chloroform and methanol with activity concentrating in chloroform fraction. Occasionally tannins and terpeniods will be found in the aqueous phase, but they are more often obtained by treatment with less polar solvents. Ether is commonly used selectively for the extraction of coumarins and fatty acids while dichloromethanol is specially used for the selective extraction of only terpeniods (Prashant, *et al.*, 2011).

Solvent differ in polarity strength, they are three strength of polarity, and they are polar, medium polar and non polar. Polar solvent will extract polar chemical and the same is true for non-polar solvent. Polar solvent include methanol, ethanol and water, medium polar solvents examples are ethylacetate, acetone and dichloromethane and non-polar solvent include toluene, chloroform and hexane (Handa *et al.*, 2008).

The extract yield is in the order of methanol greater than hexane and hexane greater than ethylacetate. This shows that most of the phytochemical present in both *B. aegyptiaca* and *T. emetica* are polar compounds. The hexane and methanolic extracts of *T. emetica* show significant antiplasmodial activity but not ethylacetate extract, as a result of difference in strength of polarity. Alkaloids are usually complex structures with polar and non polar groups, so they dissolve more readily in somewhat polar organic solvents but not in water. Hexane is very non polar, so it would not dissolve most alkaloids (Handa *et al.*, 2008) from this it could be suggested that more than one phytochemical is responsible for the antiplasmodial effect of *T. emetica*. Or the concentration of the phytochemical responsible for the antiplasmodial effect in methanolic extract of *T.*

emetica is greater than hexane extract of *T. emetica.* Amusan *et al.*, (1996) isolated ursolic acid and its two derivatives tomentosolic acid from hexane and chloroform extracts of *Spathodea campanulata,* which suppressed malaria and prolonged the survival time of mice infected with *Plasmodium berghei.*

Phytochemical compounds such as terpenoids (sesquiterpene lactones and quassinoids) are commonly implicated in the antiprotozal and antiplasmodial activity of many plants. (Aarthi and Murgan, 2011, Tane *et al.*, 2005, Goffin *et al.*, 2002). An example of a common terpenoid is artemisinin (sesquiterpere lactone) the main active ingredient in the traditional Chinese antimalarial ginghaosu. (Aarthi and Murgan, 2011). It has a strong blood schizonticidal action and reduces gametocyte transmission (White, 2004).

A high level of tannins was detected in *B. aegyptiaca* extracts. Tannins are potential metal ion chelators, protein precipitating agents and biological antioxidant (Hagerman *et al.*, 1998). The administration of *B. aegyptiaca* extracts could predispose to any of the mentioned processes in vivo. Phlobatannins were detected in *T. emetica* leaves but absent in *B. aegyptiaca.* Tannins have been shown to bind and precipitate proteins and other organic compounds which in some case inhibits the absorption of nutrients, (Katie and Thorington, 2006). If injected in excess quantities, tannins inhibit the absorption of minerals such as iron, which may, if prolonged, lead to anemia (Brune *et al.*, 1989). This is because tannins are metal in chelators and tannin-chelated metal irons are not bioavailable. Tannins only reduce bioavailability of plant sources of iron, also known as non-heme. Animal sources or heme iron absorption are not affected by tannins (Brune *et al.*, 1989). Vitamin C or lemon juice help neutralize the tannin effect on iron absorption (Hurrell *et al.*, 1999). Despite the entire negative effects of tannins Banzouzi *et al.* (2002)

isolated ellagic acid (tannin) from *Alchornea cordifolia* as the active ingredient against *Plasmodium berghei* infected mice. This is to show tannins also have it own beneficial activity. Tannins were not detected in *T. emetica* but phlobatannins were detected. Phlobatannins are formed under action of acids or heating of condensed tannins. The term proanthocyanidin (condensed tannins) is derived from the acid catalyzed oxidation reaction that produces red anthocyanidins (phlobatannins) upon heating condensed tannins in acidic alcohol solution (Hagerman and Klucher, 1986). This positive phlobatannins test in *T. emetica* leaves shows that only condensed tannins are present in it and no hydrolysable tannins present.

Although both *T. emetica* and *B. aegyptiaca* contain alkaloids, the concentration was higher in *T. emetica*, Alkaloids exhibit important pharmacological properties, example Remijia species contain quinine which is an antimalarial agent. Most alkaloids show a good ability to quench singlet oxygen (Trease and Evans, 1999). The seed extracts of Tetracerpidium canophorum were found to contain alkaloids and were found to have antibacterial activity (Isaac and Chinwe, 2001). Alkaloids also have toxic effects especially the cyclic ester like retrorsine. This or any alkaloid administered in large doses have cytotoxic effect on vital organs and affect the central nervous system, pyrolizidine alkaloid during hepatic metabolism generate on active intermediates (pyrrole alkylation agent) which are toxic (Aiello and Susan, 1998). It is possible that the alkaloids present in *T. emetica* is responsible for it antimalaria property. Bioassay guided fractionation have shown that numerous alkaloids have antiplasmodial activities. Examples of such alkaloids are quinine, chloroquine, araliopdimerine A, indole alkaloids and alkaloid akuamine (Kapadia *et al.*, 1993, Tana *et al.*, 2005, Ngemenya *et al.*, 2005). Quinine

being the first alkaloid to been discovery as an antimalaria, acts as a blood schizonticidal and weak gametocide against *Plasmodium vivax* and *Plasmodium malariae*. As an alkaloid, it is accumulated in the food vacuoles of plasmodium species, especially plasmodium falciparum. It acts by inhibiting the hemozoin biocrystallization, thus facilitating an aggregation of cytotoxic heme (White, 2004). Quinine can cause hypoglycaemia through its action of stimulating insulin secretion (Seltzer, 1989). So the, hypoglycaemic effect of *B. aegyptiaca* and *T. emetica* could be as a result of presence of quinine alkaloid in the extracts.

Both *T. emetica* and *B. aegyptiaca* were found to contain saponins, with the latter having a high value than the former. Steroidal saponins have been shown to have antimicrobial properties (Isaac and Chinwe, 2001). Azebaze *et al.* (2006) discover the antimalaria efficacy of *Allanblackia monticola*, of which stigmasterol-3-o- beta-D-glucopyranosides saponin was detected as part of it phytochemical.

Saponins and flavonoids were the largest of phytochemical contents detected after quantitative analysis of *B. aegyptiaca* stem and *T. emetica* leaves respectively. Many flavonoids containing plants are diuretic or antiplasmodic in nature. Some flavonoids have fungicidal properties and protect the body against parasite. Flavonoids isolated from cition were found to decrease bleeding tendency in scurvy (Shafik *et al.*, 1976).

Flavonoids are the other form of the two plants phenolic structures detected in this study. Flavonoids are known to lower vein permeability and are hence used in the treatment of circulatory disorders. Different flavonoids and phenolic compounds react with free radicals to reduce the degeneration of membrane (Bruneton, 1995). They also possess

antibacterial properties (Hostettman *et al.*, 1995). Flavonoids can also be used as antioxidants and in vitro as enzyme inhibitors (Smith *et al.*, 1995). Flavonoids showed significant antiplasmodial activity against different strains of malaria parasites (Andrade – Neta *et al.*, 2004, Ngamga *et al.*, 2005), it could be possible that this phenolic group of phytochemicals is responsible for the antiplasmodial effect of *T. emetica*.

Cardiac glycoside was detected in *T. emetica* but not absent in *B. aegyptiaca*. Cardiac glycosides are used therapeutically mainly in the treatment of cardiac failure, due to their anti-arrhythmic effects. They increases cardiac output by increasing the force of contraction and prolonging the plateau phase of cardiac depolarization, thus slowing ventricular contraction and allowing more time for ventricular filling (Ruch *et al.*, 2003).

A preliminary phytochemistry revealed that *T. emetica* leaves are composed of mainly polyphenolic compounds up to 64.71% of the crude extract (Germao *et al* 2005). The polyphenolic constituent of *B. aegyptiaca* stems is just one third of the crude extract.

B. aegyptiaca has higher safe dose compared to *T. emetica*, meaning that at the same dose level *T. emetica* will be more toxic than *B. aegyptiaca*. The toxic nature of *T. emetica* might be due to the presence cardiac glycosides or alkaloids. Some cardiac glycosides possess negative effects on the cardiac output. Metabolism of some alkaloids yields toxic metabolite that affect vital organs like the liver, kidney and central nervous system.

None of the three fractions (Hexane, Ethylacetate and Methanolic extracts) of *B. aegyptiaca* was able to clear the parasite or decrease parasite level as shown in figure 3.0. In fact, the group of mice given methanolic extract of *B. aegyptiaca* died before the control group while both groups given ethylacetate and Hexane fractions died on the 14th

day after infection. The death of group given methanolic extracts before the control group might be due to toxic principal(s) or due to extraction solvent use. Methanol is polar, but due to its cytotoxic nature, it is unsuitable for extraction in certain kind of studies as it may lead to incorrect results (Prashant *et al.*, 2011).

Ethylacetate fraction of *T. emetica* is not effective in the treatment of malaria, because the mice in this particular group died before the control group. Hexane and methanolic fraction of *T. emetica*, shows a promising effect on malaria parasite, with the methanolic fraction having higher activity than the Hexane fraction as shown in figure 4.0. Since we know that Hexane fraction contain non-polar constituents and the methanolic fraction contain both polar and non-polar constituents, we can infer that, the substance responsible for the activity requires a polar medium to be more effective or there is a polar and non-polar constituents that are working synergistically to give better parasite suppression in the methanolic extract.

The decline in whole body weight and packed cell volume in mice chronically dosed with *B.* aegyptiaca and *T. emetica* are noteworthy. A weekly body weight measurement had been recommended for mice under chronic toxicological assessment. Body weight and feed utilization are often sensitive to xenobiotics and occasionally are the only significant toxicological findings with materials of low toxicity (Jigam *et al.*, 2011[b]). These can be attributed to antinutritive factors in the crude extract. There could be loss of appetite and poor feed utilization by such animals. Tannins inhibit growth by decreasing the digestion coefficient of most nutrients and the coagulation of proteins. Essential minerals such as calcium, iron, magnesium etc can be chelated and adversely affect vital on processes such as hematopoiesis (Sotohy *et al.*, 1997; Jigam *et al.*, 2011a). Stress, diarrhoea and

dehydration are other factors that influence weight change (Jigam *et al.*, 2011[b]). Some variations were obtained in the weights of kidneys, stomach, intestine liver and spleen of animals dosed with *B. aegyptiaca* could have impaired detoxification and haematopoiesis (Abdelgadir *et al.*, 2010).

The oral administration of methanolic extracts of *T. emetica* and *B. aegyptiaca* caused a reduction concentration of blood glucose the mice. This is similar to the report of Muhammad *et al.* (2006) that fruit extract *B. aegyptiaca* significantly decreased the liver glucose-6-phosphatase activity in diabetic rats. Based on the increasing number of reports on blood glucose, reduction in association with some saponins and alkaloids (Emeka *et al.*, 2010) isolate from other medicinal plants, it is likely that the active principles could be present in one or more of the chemical substances. These compounds stimulate influx of Na^+ and Ca^{2+}ions by inhibiting the $Na^+ - Ca^{2+}$ exchanger thereby stimulating the influx of Ca^{2+} into the inner membrane, hence there is membrane depolarization. This activates the synthesis of ATP through oxidative phosphorylation. This mechanism feed forward glucose, stimulated insulin secretion. The ATP produced mediates the secretion of insulin by cells of islets of langerhan's or its release form bound insulin by insulin secretogogues(Saidu *et al.*, 2008).Or it is likely that the extracts produce its hypoglycaemic effect by acting as an analogue of insulin and mimics some of the actions of insulin on glucose metabolism. Such as enhancing up- take of glucose into the cells, inhibition of glucose absorption in the intestine as well as acting as anti-metabolites that are capable of block the pathway of fatty acid oxidation, however the mechanism of action of these plant is yet unknown(Luka *et al.*, 2012).

There was also a decrease in total protein in the mice given *T. emetica* and *B. aegyptiaca* extract compared to control which could be due to the long-term complications of a disease or the significant decrease in serum total protein suggest a derangement in protein metabolism.

The decrease in triglyceride level of *T. emetica* and *B. aegyptiaca* is related to their promotion of utilization of glucose and inhibition of fatty acid oxidation (Emeka *et al.*, 2010). This result implies that the plant extracts may be helpful in reducing the complications which coexist quite often in diabetics.

AST, ALT and ALP were significantly ($p<0.05$) elevated in the *B. aegyptiaca* and *T. emetica* animals could indicate some tissue damage. Serum ALT level is of grater diagnostic specificity in respect to the liver than AST. AST exists in high levels in many tissues such as kidney, heart, liver, skeletal muscles etc (Atangwho *et al.*, 2007; Sodipo *et al.* 2011). Raised serum ALP is usually encountered in billiary disorders such as obstructive jaundice and cirrhosis, bone and intestinal disorders (Tietz, 1983). The decrease in total proteins at the end of week five could be due to early indications of liver damage, renal failure, malabsorption or nutritional deficiency (Sood 2006) reports exist of similar effects of some plant extracts in experimental animals (Sodipo *et al.*, 2011).The decrease in glucose, total proteins and triglycerides is supported by the fact that *B. aegyptiaca* and *T. emetica* could be used as antidiabetic agent (Mohammed *et al.*, 2006). The major problem associated with *B. aegyptiaca* been use as drug is the elevation of some serum enzymes due to long term consumption of the crude plant extract, which has been validated by histopathological finding involving kidney and liver damage. The hepatocytes became disintegrated and lost its normal hexagonal arches while the

nephrons lost their intact shape. It could be possible that the principal component responsible for a give pharmacological activity might not be the one responsible for the toxicity, after isolation and purification of the extract.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMEDATIONS

5.1 Conclusion

The medicinal properties of *Balanites aegyptiaca* have been widely documented. However, the potential toxicity of crude extracts especially with chronic intake cannot be ignored as validated by histopathological studies. Bioactive principle(s) could be further isolated to serve as lead compounds for the synthesis of more drugs with reduced toxicity

Trichilia emetica could be a source of an effective malarial medicament. However, this potential will be better harnessed by further purification in an attempt to isolate the bioactive principle (s) and minimize the toxicity inherent in the crude extracts.

5.2 Recommendations

People should stop the administration of crude extract of *Balanites aegyptiaca* in the treatment of diseases because of its toxic effect (i.e. only purify active components which have efficacy should be used). *Balanites aegyptiaca* has no antimalaria activity and therefore its use for such should be stopped.

Further studies on purification of hexane and methanolic extracts *Trichilia emetica* needs to be done for rational drug design against malaria parasites.

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