IN VIVO ANTI-MALARIAL ACTIVITY OF THE HYDROALCOHOLIC

EXTRACTS OF *ASPARAGUS AFRICANUS, WITHANIA SOMNIFERA*

AND *LAGANARIA VULGARIS* IN MICE

A THESIS SUBMITTED TO THE RESEARCH AND GRADUATE PROGRAM OFFICE OF ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE IN PHARMACOLOGY

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LIST OF ACRONYMS AND ABBREVIATIONS USED

ACT	artemisinin-based combination therapy
AL	artemether-lumefantrine)
ANOVA	analysis of variance
AS	artesunate
CT	combination therapy
DDR	Department of Drug Research
DHFR	dihydrofolate reductase
EHNRI	Ethiopian Health and Nutrition Research Institute
ITNs	insecticide-treated bed nets
MDR	multidrug resistance
MOH	Ministry of Health
NRBM	National Roll Back Malaria
PABA	Para-aminobenzoic acid
P.C.V.	packed- cell volume
RBCs	red blood cells
SPSS	Statistical Package for Social Sciences
TDR	Tropical Diseases Research
WHO	World Health Organization

ABSTRACT

Malaria is a major public health problem in the world in general and developing world in particular. It is known to cause 1-2 million deaths per year, with an annual incidence of 300-500 million clinically manifested cases and with more than 2 billion people at risk of infection. It is becoming more difficult to prevent and to treat malaria due to the increasing resistance of the transmitting mosquito and of the malaria parasite to the insecticides and drugs that have been commonly used. The importance of malaria as a major public health and development problem has been reviewed on a number of occasions in Ethiopia. This study aims at investigating the *in vivo* antiplasmodial activity of the known traditionally used herbal drugs. A rodent malaria parasite*, Plasmodium berghei,* maintained in EHNRI laboratory, was inoculated into young male albino mice. Male mice were infected with $1x10^7$ parasites intraperitonially. The extracts were administered by intra gastric tube daily for four consecutive days starting from the day of parasite inoculation. Control groups received the same amount of solvent (vehicle) used to suspend each dose of the herbal drug and Chloroquine was used as a standard drug given by the same route. The results showed that *Asparagus africanus* Lam. (Liliaceae) root and areal part and *Withania somnifera* (L) Dunal (Solanaceae) leaf and root bark are effective in *P.bergei* malaria, which is inconformity to the claim that they have therapeutic values in human malaria in traditional medicine. This study could partly confirm the claim, facilitate in initiating further in-depth investigation using different experient model.

Key words: *Asparagus africana* Lam., *Withania somnifera* (L) Dunal, *Plasomodium berghei*, acute toxicity, *in vivo*, antimalarial activity,

1. INTRODUCTION

1.1. MALARIA PARASITE LIFE CYCLE AND PATHOGENESIS

Malaria is an infectious disease caused by the parasite plasmodia. There are four identified species of this parasite causing human malaria, namely, *Plasmodium vivax, Plasmodium falciparum, Plasmodium ovale & Plasmodium malariae.* The female anopheles mosquito transmits malaria (Hardman and Limbird, 2001).

To understand the ethiology of malaria, it is necessary to understand the life cycle of the parasites). Taking human blood that contains parasites in the sexual form infects the anopheles mosquito. The sporozoites that develop in the mosquito commence with the inoculation into the human host, migrate to the liver where they invade the hepathocytes and undergo the first stage of development in humans, the exoerythrocytic stage (Fig. 1) (Katzung, 1995). The sporozoites multiply in the liver to form schizonts. Later, the parasites escape from the liver into the bloodstream as merozoites to initiate the erythrocytic stage. In this stage they invade red blood cells, multiply in them to form blood schizonts, and finally rupture the cells, releasing a new crop of merozoites. Meanwhile, the gametocytes (sexual-stage parasites) that are infectious for mosquitoes are released into the circulation, where they would be taken by another mosquito (David, 2004; Katzung, 1995; Louis et al, 1994). The gametes ingested by female anopheles mosquito through blood meal are fertilized in the gut to form zygotes, which elongate and become motile ookinetes, invade the mosquito mid gut wall and develop into oocysts, in which the sporozoites develop. These sporozoites migrate to mosquito’s, salivary glands, upon

inoculation into a new human host perpetuating the malaria cycle (Hardman and Limbird, 2001; Louis et al, 1994).

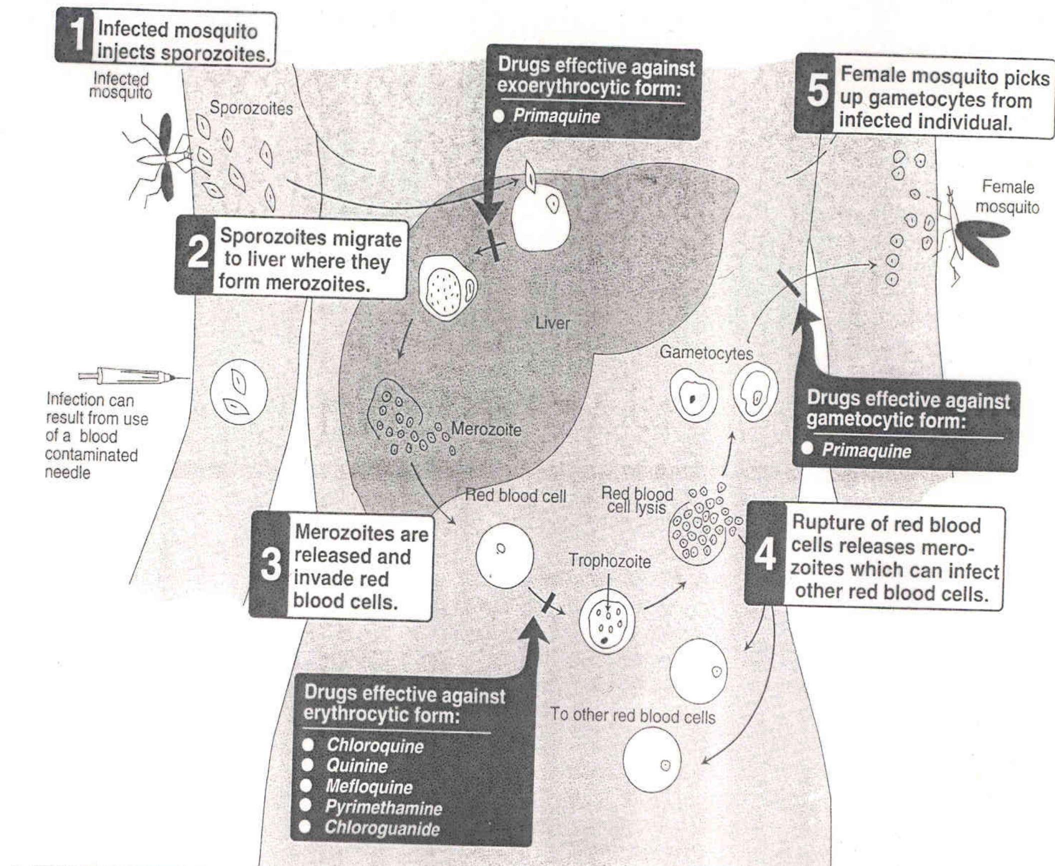

Fig. 1: Life cycle of the malaria parasite showing the sites of action of antimalarial drugs

(Reuters Health information, 2004)

Malaria is caused by repeated cycles of growth of the parasite, *P.falciparum,* in the erythrocyte cycle.Various cellular and molecular strategies allow the parasite to evade human immune response for many cycles of parasite multiplication, manifesting itself in the form of clinical symptoms, including flu like illnesses, fever with chills/shivering, diarrhea and abdominal pain (Miller et al., 1994). The most severe form of the disease, *P.falciparum* malaria, causes severe anemia, complications like cerebral malaria in which impaired consciousness is common, delirium, metabolic acidosis, weakness and jaundice, acute renal failure, nephritic syndrome, pulmonary edema, comma and death (David, 2004; Crlark and Schofield, 2000). The expression of disease is influenced by both parasite and host factors that are responsible for causing pathology, factors related to prophylaxis, contact with

mosquitoes, emergence of drug resistant parasites, need of chemotherapy etc (Louis et al, 1994; Miller et al., 1994)

The pathogenic process occurs only during the erythrocytic cycle. During this stage, there is a huge, periodic amplification of the size of parasite populations that may enhance the probability of differentiation to gametocytes, the stage infectious to mosquitoes. A peculiarity of *P.falciparum* is its ability to adhere to venular endothelium (cytoadherence) of erythrocytes infected with maturing parasites. The parasitized erythrocytes remain attacked until merozoites are formed that are released to invade other erythrocytes. Thus, the predominant form seen in the peripheral circulation is the ring –infected erythrocyte, the young form of the parasite (Louis et al., 1994; Peter et al., 1998)

Effects of Parasite invasion on pathogenesis of malaria

Invasion of all erythrocytes or only a subpopulation (for example, reticulocytes), and redundancy in invasion pathways is possible. *Plasmodium vivax* invades only reticulocytes, and *P. falciparum* invades erythrocytes of all ages (Louis et al., 1994). The *P. falciparum* ligand binds specifically to glycophorin A, binding that is dependent on both sialic acid and the peptide backbone of glycophorin A. In case of *P. falciparum*, however, there are a number of invasion pathways independent of glycophorin A. Consequently, erythrocytes missing glycophorine A can be invaded, although, at a lower frequency. An alternative path way invades glycophorine B and may explain the high frequency of glycophorin B-negative erythrocytes in a pygmy population (Louis et al., 1994; Williams et al., 2004).

Importance of host susceptibility to a parasite in disease pathogenesis

Individuals living in endemic areas although clinically immune, often remain persistently parasitemic, asexual parasites being continuously switching to gametocytes that are infectious to mosquitoes. In children, this parasite strategy for survival puts the children at continuous risk of disease until the development of clinical immunity. Transfer of purified IgG from sera of immune adults results in a rapid drop in parasitemia. It is often argued that, because natural immunity takes so long to develop, other mechanisms should be thought. However, natural immunity, when it does develop, is very effective, at least under permanent exposures that are freed to invade other parasites. These nonspecific signs of malaria are believed to be caused by release of a malaria toxin that induces macrophages to secrete TNF-@ and interleukin-1 (Wernsdorfer, 1991), common mediators induced by plasmodium species. In non immune patients, many of the severe complications, such as cerebral malaria, anemia, hypoglycemia, renal failure, and non cardiac pulmonary edema-occur in combination or as isolated complications (Louis et al., 1994; Williams et al., 2004).

Although four species of malaria parasites can infect humans and cause illness, only malaria caused by *Plasmodium falciparum* is potentially life threatening. Malaria caused by *Plasmodium falciparum* can cause kidney or liver failure, coma, and death. The trophozoites upon rupture of red blood cells (RBCs) release merozoites, which can infect other RBCs. The lysis of RBCs also results in the release of gametocytes, which get picked up by female mosquito to continue the cycle (Louis et al., 1994; Williams et al., 2004). Infection with *P.falciparum* is a medical emergency. About 2% of persons infected with falciparum malaria die, usually because of delayed treatment (Peter et al., 1998).

1.2. MALARIA GLOBAL SITUATION

The global momentum gained in malaria research and control activities during the 1950s was almost completely lost during the 1970s (Martin et al, 2004). The first large scale multilateral initiative, the WHO Malaria Eradication Program (1955-1969), which aimed to eradicate malaria in every part of the world, did not succeed, rendering a general sense of

disappointment globally (Martin et al, 2004). The reported problems included, the growing resistance of parasites to drugs and mosquitoes to insecticides, weaknesses in health care infrastructures, reduced government spending on health care, worsening the situation, fatalism and resignation towards the disease (Sachs, 2002).

Although Roll Back Malaria, launched in 1998 by the WHO and Partners, aimed to cut the malaria burden in half by 2010 using strategies that included distribution of insecticide treated bed nets to all pregnant women and children in sub Saharan Africa, but progress is slow (Balter, 2000). Despite many disappointments, there seemed to be one positive development with initiatives of Training in Tropical Diseases Research (TDR), greatly influencing the global efforts to combat malaria (Morel, 2002). However, with recent resurgence, malaria is still one of the most important communicable diseases, with an annual incidence of 300-500 million clinically manifest cases and a death toll of 1-2 million (David, 2004; WHO, 2000).

Currently about 40% of the world's population live in areas where malaria is endemic, 130 countries in tropics and 90% of them in sub Saharan Africa and with more than 2 billion people at risk of infection (Fig 2) (Louis et al., 1994; Williams et al., 2004). It is re-emerging as the No.1 infectious killer & priority tropical disease, killing in 1 year what AIDS killed in 15 years. WHO forecasts a 16% growth in malaria cases annually. A child dies every 20 sec, in Africa (Louis et al., 1994; Williams et al 2004; Laxminarayan R, 2004; Wernsdorfer and Wernsdorfer, 2003).

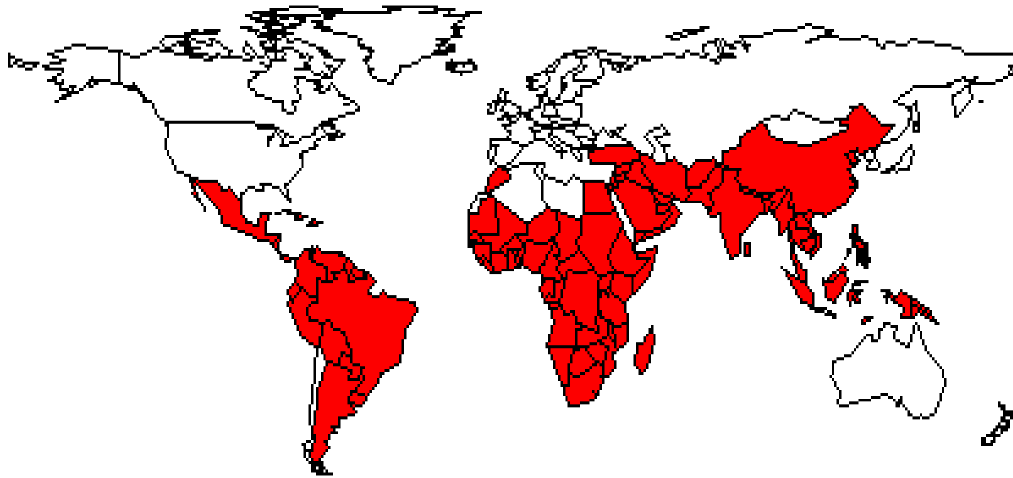

Fig. 2: Worldwide malaria distribution

(WHO, 2002)

Many countries have been experiencing resurgence in cases caused by *P.falciparum*, the most deadly of the four human malaria parasites. Chloroquine-(4-aminoquinololine) ,that had a world wide use beginning in the late 1940s, was reported to face resistant strains of *P.falciparum* in Africa, Asia, and the Americas (Laxminarayan R, 2004). The importance of malaria as a major public health and development problem is well recognized and the malaria situation has been reviewed on a number of occasions in Ethiopia (WHO, 1999; Teklehaimanot, 1986) also. It has also been reported that the species dominant are *P. falciparum* (60%) and *P.vivax* (40%) (Deresse et al., 2003). In Ethiopia, a large area of the country (75%) is malarious, and more than 60% of the population live in malaria endemic areas, and malaria is the top leading cause of out-patients morbidity, the fifth leading cause of hospitalization, and the most important cause of hospital deaths in Ethiopia (MOH, 2002). It has recently been reported that malaria in Ethiopia, is a top cause of out patient visits (15.5%), admission (20.4%) & death (27%), being the N0.1 killer followed by HIV/AIDS & Tuberculosis (MOH, 2004)

1.3. MALARIA TREATMENT

1. Chemotherapy: Malaria is a disease that can be treated in 48 hours, yet can cause fatal complications if the diagnosis and treatment are delayed (Louis et al., 1994). The common treatment for uncomplicated falciparum malaria (oral) was limited to Chloroquine in Chloroquine sensitive cases of malaria and to quinine sulfate or mefloquine, tetracycline or doxacycline, sulfadoxine /pyrimethamine in Chloroquine resistance cases. Treatment for complicated malaria, used to include quinine intravenious or oral, doxycycline and sulfadoxine /pyrimethamine. Drugs effective against erythrocytic form were Chloroquine, quinine, mefloquine, pyremethamine, chloroguanide while primaquine is effective for both exoerythrocytic and gametocytic forms (Williams et al., 2004; Laxminarayan, 2004). However, changes and developments have been evident following the development of resistance by malaria parasites against the conventional antimalarial drugs.

WHO's currently recommendations for malaria chemotherapy: Recent treatment protocols have been suggested by WHO giving emphasis to derivatives of the extracts of wormwood plant-*Artemisia annua* (Artemisininin, artemether, artheether, qinghaosu). The following combinations have been specifically recommended. 1) artemether/lumefantrine; 2) artesunate plus amodiaquine; 3) artesunate plus sulfadoxine /pyrimethamine (in areas where sulfadoxine /pyrimethamine efficacy remains high); 4) artesunate plus mefloquine (in areas with low to moderate transmission); and 5) amodiaquine plus sulfadoxine /pyrimethamine, in areas where efficacy of both amodiaquine and sulfadoxine /pyrimethamine remains high (mainly limited to countries in West Africa). This non artemisinin-based combination therapy is reserved as an interim option for countries, which, for whatever reason, are unable immediately to move to ACTs (WHO, 1999).

2.Vaccines: The status of malaria drug resistance is critical, leading to the appearance of virulent and deadly *P. falciparum* strains that are difficult to control. Number of areas where the mosquitoes are present, the cases increase and imported malaria is affecting the non immune host population of the world, making the need for vaccines so urgent (Millet, 1995). Efforts have been made to report success in development of safe and effective malaria vaccine, which of course, would be the most cost effective method. However, the candidate vaccines tested so far are not as effective as expected (Holder, 1999; Richie and Saul, 2002). Moreover that, development of new vaccines requires increased investments and formation of

public-private partnerships, because of the demands for due consideration of the disease burden, cost-effectiveness, financing, delivery systems, and approval by regulatory agencies (WHO, 1999). To ensure effective coordination and cooperation between the growing number of research and control coalitions forming in support of malaria activities, strong efforts are required globally and in regions most affected by malaria. Continued support for scientists and control workers globally, particularly in low-income malarious countries has been suggested (Breman et al., 2004)

3.Chemoprophylaxes: Malaria is becoming more and more difficult to prevent and to treat due to the increasing resistance of the transmitting mosquito and of the malaria parasite to the insecticides and drugs that have been commonly used. Attempts to reduce the prevalence of malaria by chemotherapy, environmental management, and insecticides have been hindered by the widespread emergence of resistant strains of both the parasite and the Anopheles vector (Ryall, 1987). Therefore, extracting lessons from the past, the researchers working for future malaria therapy directions have been suggesting the judicious use of existing antimalarials, preferably in combinations, in an attempt to delay the emergence of resistance and aggressive research effort aimed at identifying a new generation of antimalarial drugs (Schapira et al., 1993).

It has been suggested that the lessons over the past 20 years with the introduction of amodiaquine, pyrimethamine/dapsone and sulfadoxine /pyrimethamine, show how sensitive drugs for chemoprophylaxis are to side effects. Three levels of chemoprophylaxis are used: Chloroquine in areas with sensitive *P. falciparum*, Chloroquine plus proguanil in areas with low level Chloroquine resistance, and atovaquone/proguanil, doxycycline or mefloquine in areas with extensive resistance against Chloroquine and proguanil. Primaquine and the primaquine analog tafenoquine may be future alternatives but otherwise there are few new drugs for chemoprophylaxis (WHO, 1999; Petersen, 2004). However some may still ask, is drug resistance in malaria chemotherapy really a problem?

1.4. MALARIA DRUG RESISTANCE

Trends of Malaria Drug Resistance: Resistance of *P.falciparum* to CQ has emerged in the late 1950s and has now conquered the large majority of areas where this species is endemic (Hyde, 1989). Chloroquine resistant strains of *P.falciparum* were initially reported during the early 1960s around Thailand and Cambodia (Young and Moore, 1961) and thereafter spread in many areas of South America, with increasing prevalence and degree of resistance in all affected areas, representing a serious setback to antimalaria programs (Kevin et al. 1994). The World Health Organization (WHO) has developed global monitoring program, initially implemented in 1977 in the Southeast Asia Region Program (Wernsdorfer and Kouznetsov, 1980).

This situation lead the US Army to initiate a program to develop new antimalarials; in 1963, screening over 235 000 compounds, but very few were sufficiently active and safe for use in humans (Zhang, 1987). Refractoriness to many antimalarials has been steadily rising, starting with Chloroquine in 1960s, followed with sulfadoxine /pyrimethamine, then with quinine in 1980 and quinine and tetracycline in 1981, still followed finally with arteminsinine and mefloquine, in 1992 (Brasseure et al., 1992; WHO, 1987) and is now widespread to all parts of the world to the extent at which most of these drugs can no longer be recommended as a first line treatment for *P.falciparum* malaria and similar situation has been reported in all sub-Saharan African countries (WHO, 1999; Annon, 1999)

Chloroquine, a prominent antimalarial drug, has been the first line drug for the treatment of uncomplicated malaria, over the last forty years in Ethiopia ,but the first report on Chloroquine failure to clear asexual *P.falciparum* parasites from the blood of patients infected in various parts of Ethiopia appeared in1986 (Teklehaimanot, 1986). Studies of the situation in African countries and the East Africa have also been reviewed (Mengesha and Mekonen, 1999). There have been a number of studies conducted since then indicating the increasing levels of resistances of antimalarial drugs (Yeneneh et al, 1993; Elchalal et al., 1993; Fletcher, et al., 1993; Mengesha and Seboka, 1998)

With in the following ten years, in 1973, sulfadoxine /pyrimethamine cocktails replaced Chloroquine because of resistance, but by the year 1985, this too was ineffective. Although quinine remains effective, there was a 50% failure rate and that also lead to supplementing

therapy with tetracyclines or doxacycline, however, compliance with the 7-day regimen was poor. Between 1985 and 1990, the treatment with mefloquine, combined with sulfadoxine /pyrimethamine was started, but by the year 1990 the cure rate had fallen to 71% in adults and 50% in children and could no longer be used due to resistance (Zucker and Campbell, 1992; Terkuile et al., 1992).

Various *P. falciparum* 'strains' have now attained resistance to all commonly used and generally available antimalarial drugs (Terkuile. et al., 1992). Although, this is the case, the problem of resistance to the common antimalarial drugs such as Chloroquine and pyrimethamine, and the decreasing effectiveness of quinine is mainly limited to *P.falciparum* infection; Chloroquine still remains the treatment of choice for *P.vivax* (David, 2004).

Pyrimethamine was first introduced as a prophylactic antimalarial drug. As early as the mid-1950s, parasite resistance to this compound had been reported from several areas, and it has become widespread on all continents where malaria is found, even in conjunction with sulphone or sulphonamide drugs. Today, Chloroquine and Pyrimethamine are completely ineffective for the treatment of *P.falciparum* malaria (Smithuis, et al., 2004). Pyrimethamine resistance is well known and a series of pyrimethamine-resistant mutants of *P. falciparum* have been produced (Louis, et al., 1994), as with sulfonides, resistance occurs in all stages of the lifecycle. Although, combination treatments have had good effects, resistance to these drug cocktails is now becoming apparent (Louis et al., 1994; Smithuis, et al., 2004).

Mefloquine, a drug developed during the Vietnam War, is now generally agreed to be the preferred antimalarial drug for travelers at high risk for chloroquine-resistant *P. falciparum* and quinine is still one of the drugs of choice for malaria. However, it was reported that even quinine resistance is increasing to serious levels, and the use of mefloquine - the most recently introduced antimalarial - is already facing problems (Louis et al., 1994; Hallett, et al., 2004).

Although Artemisnins are claimed to be the most effective of all antimalarial drugs, artemisnin resistant forms have already been demonstrated in laboratories. It was suggested that one strategy for delaying the development of resistance to effective drugs is the wide-scale deployment of artemisinin-based combination therapy (Coleman, et al., 2004; Hallett, et al., 2004). However, the cost of these combinations is higher than that of the currently used monotherapies or alternative non-artemisinin-based combinations. In addition, uncertainty

about the actual impact in real-life settings has made them a controversial choice for first-line treatment. The early use of artemisinin-based combination therapy (ACT) may delay development of resistance to these drugs and prevent the medical toll associated with use of ineffective drugs (Hallett, et al., 2004; Yeung, et al., 2004). Caution has also been suggested during the use of artemisnin, because of its indicated resistant forms that have already been demonstrated (Coleman, et al., 2004; Louis et al., 1994)

WHY AND HOW MALARIA DRUG RESISTANCE ARISES

Antimalarial drug resistance usually arises when spontaneously arising mutants are selected by antimalarial drug concentrations that provide differential inhibition to distinct genetic parasite types: i.e. the drug concentrations are sufficient to reduce the susceptible parasite population, but inhibit less or do not inhibit multiplication of the mutants (Peter, 1980). Several mechanisms of malaria drugs resistance have been studied and most of them account for changes in drug sensitivity in the malaria parasites. The following are some:

Mutation: Physiological adaptations due to non-genetic changes, selection of previously existing drug resistant cells from a mixed population under drug pressure, spontaneous mutation, mutation of extra nuclear genes, or the existence of plasmid-like factors. Point mutations in the respective genes encoding the target, in protein target genes are most common. e.g. pyrimethamine, cycloguanil, sulfonamide, atovoquone resistance (Arav-Boger and Shapiro, 2004; Nicholas, 1999).

Efflux mechanism: Mechanism responsible for resistance, where by the parasites expel drugs via a membrane p-glycoprotein pump similar to that described for multi drug resistance of cancer cells e.g. Chloroquine, amodaquine, quinine, mefloquine, halofantrine resistance (Cowman, 1991; Nicholas, 1999).

Drug pressure selection: Selection of mutants by the drugs themselves appears to be an important mechanism (Louis et al., 1994)

Subtherapeutic levels of the antimalarial drugs: In an environment where subtherapeutic levels of the antimalarial drugs are present, those parasites, which have resistance through their natural variation or through mutations, even though the resistant forms were initially in the minority, the continued drug mediated elimination of intraspecific competition from the

non resistant forms has allowed the resistant forms to attain numerical superiority. Where there is uncontrolled and irresponsible prophylaxis and treatment will kill the most drug sensitive forms of the parasite, but select the less sensitive ones- and spontaneous mutations in these forms tends to further reduce the sensitivity of the parasites to the drug (Wellems, 1991)

Metabolism of the parasites: Intra erythrocytic stages of malaria ingest haemoglobin into food vacuoles. Here exopeptidases and endopeptidases break down haemoglobin into haemozoin (malaria pigment), of which the cytotoxic ferriprotoporphyrin IX is a major component, potentially toxic for the parasite. A parasite synthesized binding protein, 'haembinder', seems to sequester the membrane-lytic ferriprotoporphyrin IX into the inert haemozoin, polymer complex to protect the Plasmodium membranes from damage. In non-resistant forms, most of the ferriprotoporphyrin IX is sequestered in haemozoin, but in the resistant forms, this toxic metabolite seems to become available to the host cell haemoxygenase system for elimination (Wernsdorfer, 1991). In Chloroquine sensitive malaria, the drug is taken up into food vacuoles, and here it competes with the haembinder for the ferriprotoporphyrin IX, to form a destructive compound (Fitch, 1983).

Defects in pigment formation: Many Chloroquine-resistant *Plasmodium* strains are not capable of pigment formation. The absence of pigment would protect the parasite against the toxic action of Chloroquine (Fitch, 1983).

Effect protein synthesis: Artemisinins are most effective of all antimalarial drugs, and seem to affect protein synthesis. Other mechanisms of drug resistance found in bacteria such as transferable resistance genes do not appear to be involved in antimalarial resistance (Nicholas, 1999).

DRUG RESISTANCE AND COMBINATION CHEMOTHERAPY

Although combination therapy is believed to be the key strategy being implemented to reduce the impact of resistance, its effect on the transmission of genetically resistant parasites from treated patients to mosquito vectors has not been measured directly (Hallett, et al., 2004). The

addition of artemisinin derivatives to sulfadoxine /pyrimethamine was also believed to delay the development of drug resistance, improve cure rates, and reduce transmission, but parasite clearance and gametocyte carriage were reduced significantly in both combination groups compared with SP alone (Mc Gready, et al., 2003).

Although, ACTs have been shown to improve treatment efficacy, the major challenges include: 1) the choice of drug combinations best suited for the different epidemiological situations 2) the cost of combination therapy 3) the timing of the introduction of combination therapy 4) the operational obstacles to implementation, especially compliance (Louis, et al., 1994; Peter and Anatoli, 1998; Breman, Alilio, Mills, 2004). Moreover, the combination of two or more drugs may also result in pharmacological interactions between the components leading to altered disposition, or toxicity (Nicholas, 1999). These drugs also have very short – lives, which necessitates their use in combination with long acting drugs, making the need for new drugs imperative (David, 2004).

Africa is facing a crisis, with its high birth rates, high incidence of communicable diseases, increasing prevalence of chronic diseases, climate related disasters such as droughts and floods, recurrent epidemics, number of refugees and displaced persons. Malaria is pushing the situation to the purse. Resistance of the most conventional chemotherapeutic agents is prevalent through out the continent, increasing morbidity and mortality is expected. Therefore, the urgent need for new antimalarials is unquestionable and we are still left with the sense of urgency to look for alternatives.

If we are to contain the ever increasing toll of malaria, it is imperative to rapidly put into action strategic plans for the discovery and development of new antimalarial compounds that are not encumbered by the pre-existing mechanisms of drug resistance (David, 2004). One of the rich areas for the unavoidable search for the newer drugs is the traditionally claimed antimalarial plants from the African flora.

1.5. TRADITIONAL REMEDIES IN MALARIA TREATMENT

Medicinal plants have been the focus of many drug studies and also considered alternative sources of animalarial agents since long ago (Attiso, 1983). The WHO organized an international meeting of the 1st type, in Tanzania in 1999, addressing the need for research and policy on the prophylactic and therapeutic effects of antimalarial medicinal plants, through formation of linkages between researchers working on traditional antimalaial methods (Bodeker and Willcox, 2000). It has also been well accepted that medicinal plants are part of the human life since time immemorial, and the history of drugs and numbers of plant products have extensive use in ethnomedicine, i.e., traditional systems of medicine (Whitefield, 1995).

Since a large number of drugs were first obtained from plants, medicinal plants are still and even today considered untapped reservoirs for new anti-malarials with novel modes of actions (Lee, 2002; Schuster, 2001). One of the most impressive developments in the history of medicine is the emergence of the Peruvian (Cinchona) bark (Rubiaceae) and also its pharmacologically active substance, quinine. Quinine is a classic antimalarial drug found in this bark, which is a tree native to South America. An alternative drug, Artemisinin from the leaves of Chinese herbal drug *Artemisa annua*, for instance, are plant products, which have been in use for centuries for the treatment of malaria, in traditional medicine as well as in standard drug- development paradigm (Kiseko et al., 2000; Peters, 1980). The standard antimalarials, Chloroquine and mefloquine, were also derived by completing from the basic chemical structure of quinine as a template. The development of plant based drugs that are used to treat this disease therefore should be given first priority.

The increasing prevalence and distribution of malaria mainly attributed to the emergence and spread of drug resistant parasites, have recently led to the study of medicinal plants with claimed antimalarial activities compared to those used empirically to treat other diseases (Rasoanaivo, et al., 1992; Clarkson, et al., 2004). Efforts have been directed towards the discovery and development of new chemically diverse antimalarial agents and a number of *in vitro* antimalarial tests have been conducted in many African countries. A remarkable number of investigations have also been conducted on medicinal plants traditionally used in African traditional medicine and other traditional health care systems like that of Vietnamese (Vonthron-Senecheau, et al., 2003; Banzouzi, et al., 2004; Tran, et al., 2003; Chan, et al., 2003)

It has been reported that about two hundred thirty-nine Madagascan medicinal plants have been identified as having antimalarial properties (Rasoanaivo, et al., 1992). An *in vitro* study of seven plants from the Democratic Republic of Congo has indicated antiplasmodial activities, a study of 134 plants from South Africa and that of 18 plant species from Venezuela have also reported the *in vitro* antiplasmodial activities (Clarkson, et al., 2004; Tona, et al., 2004; Caraballo, et al., 2004). Other recent *in vitro* studies from eight Ivorian medicinal plants and fifty-five extracts from eleven plants in malaria therapy in Kenya were reported to have antiplasmodal activities both on chloroquine sensitive and chloroquine resistant strains of *P. falciparum* (Vonthron-Senecheau, et al., 2003. Tran, et al., 2003).

Some of the studies conducted so far have reported that the observed antiplasmodial activities have been linked to the range of compounds including terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthones, anthraquinones, berberine, limonoids, naphthquinones, sesquiterpenes, quassinoids, indole and quinoline alkaloids (Banzouzi, et al., 2004; Caraballo, et al., 2004).

A number of studies have also been conducted on the *in vitro* evaluation of the anti-malarial activity of Ethiopian traditional medicinal plants. It has been reported that extracts from plants such as *Hagenia abysinica* and *Berssama abysinica* (Kassa, et al., 1996), *Croton machrostachys*, *Calpurina aura*, *Dodnia angustifolia* (Solomon, 1992), *Withania somenifera*, *Vernonia amygdalina* (Bogale and Petros, 1996)*, Artemesia afra*, *Artemesia rehan* and *Ajuga remota* (Kassa et al., 1998), have significant antimalarial activity against *P.falciparum.* Although the *in vitro* studies conducted in African medicinal plants and elsewhere support the efforts of looking for plant based antimalarial agents and indicate their promising antiplasmodal activities (O'Neill, et al., 1985; WHO, 1993), no remarkable *in vivo* studies have been reported so far, to strengthen the preclinical study profile. Therefore, this study aims at investigating *in vivo* antiplasmodial activity of the traditionally used herbal remedies, as the main body of Ethiopian traditional medicine based on the use of ethnobotany (Teferi Gedif and Heinz-Jurgen, 2002).

Traditional medicine in Ethiopia is characterized by a great variation and has been shaped by a host of ecological, socio-cultural, and historical factors in Ethiopia. It has been influenced by the multiethnic characters of the population and the uniqueness of the socio-cultural environments within which it developed. The materia medica of the Ethiopian pharmacopoeia is based largely on derivatives from the natural world (Abebe, and Ayehu, 1993). As long as most of the population in Ethiopia remains rural, the traditional medicine, which is essentially based on the use of plant preparations, animal products and mineral substances, will no doubt continue to predominate.

The fact that the practices and remedies in this system of health care are based on repeated observations over centuries of use was considerable indication for their efficacy and innocuousness. The fact that infectious diseases of mostly bacterial, parasitic and fungal origin are the most common causes of morbidity and mortality in the country makes the field

more attractive to investigations. It is believed that many of the drugs in this system could have been exploited better had it been given due attention and would have been successfully streamlined with the conventional health care. It is believed that the health care of the majority of Ethiopian population may be improved by officially bringing the positive elements of traditional medicine into the public health process. In appreciation of these facts, and the consensus being formed within the health authorities and policy makers of the country in this endeavor, one should think of investigating into this natural resource.

Having this conviction, this preliminary investigation has been conducted into medicinal plants, which have been used for treatment of malaria in Ethiopia. The plants selected for this study have been commonly used, by the Ethiopian traditional healers, in central and Southern Ethiopia, to treat malaria (Teferi G. and Heinz J., 2002), and are described as follows.

The screening pilot study included the hydroalcoholic extracts of the root and areal parts of *Asparagus africanus*, leaf, root and root bark *Withania somnifera*, and the leaf of *Laganaria vulgaris*.

BRIEF DESCRIPTIONS ON THE INVESTIGATED PLANTS

1. *Asparagus africanus* Lam. (Liliaceae), "Seriti in Oromifa and Kestencha in Amharic", a perinial woody scrambler or climber with hooked spines; leaves needle shaped, corolla white; fruit berry, yellow- orange when ripe (Abebe et.al, 2003) (Fig.3). Two antiprotozoal compounds were isolated from the roots, a sapogenin, named muzanzagenin and a lignan (+) nyasol. Muzanzagenine showed *in vitro* activity against four different malaria shizont strains and moderately affected Leishmania major prostigotes while nyasol inhibited the growth of Leishmania major prostigotes and mederately inhibited *Plasmodium falciparum* schizontes. (Oketch-Rabaha et al., 2003).

Fig.3: *Asparagus africanus (*Kestencha, Amharic)

Three major saponins and glycosides have also been isolated from the root of Asparagus (Asfaw et al., 1999). Root and Arial parts were collected for this study. Indigenous use of Asparagus includes the use of fresh pulverized root against gouty arthritis, has ovarian and uterine tonic. Galic acid, tannic acid and vitamins have been isolated (Abebe et al, 2003) It has been used as antimalarial drug in southern Ethiopia (Teferi G. and Heinz J., 2002).

2. *Withania somnifera*, L. Dunal, (Solanaceae), “Hanzo in Oromifa and Gizawa in Amharic”, a brunched under shrub up to 2m high; leaves ecliptic, fruits a red globosely berry, enclosed by the inflated calyx (Fig.4). Root, root bark and leaves were collected. Withania bark powder is mixed with butter and applied as a remedy in swelling; root powder is used for rheumatoid arthritis in indigenous medical practice. Steroidal lactones, withaferine A and withanolide D, tropine, isopellertine and cusohygrine have been isolated from leaf and root and shown to be active against inflammation and microbial infections (Abebe et al, 2003). Indigenous use indicates the use of infusion of fresh leaf sprayed in residences during epidemic out breaks and grown in garden for protective purposes. Its traditional use as antimalarial agent, in south Ethiopia, has been reported (Teferi G. and Heinz J., 2002).

Fig. 4: *Withania somnifera (*Gizawa, Amharic)

3. ***Laganaria vulgaris,*** **(**Cucurbitaceae), "Kil in Amaharic", annual monoecious, trailing or climbing herb; leaves widely oviate; weakly cordate and usually palmately 5-lobed. Flowers sweetly scented, solitary; petals white; fruit variable in shape, globular, bottle or club-shaped and many seeded (Fig.5). Dried mature fruits are mainly used as containers (Abebe et al., 2003). Its leaf has been traditionally used as antimalarial drug in southern Ethiopia (Teferi G. and Heinz J., 2002).

Fig. 5: *Laganaria vulgaris (*Kil, Amharic)

2. OBJECTIVES

2.1. General Objective

To evaluate the three traditionally claimed anti-malarial herbal remedies (*Asparagus africanus, Withania somnifera* and *Laganaria vulgaris),* screen their major chemical constituents and perform acute toxicity studies *in vivo*, using mice

2.2. Specific Objectives

1. To determine changes in Parasitemia load after the administration of the extracts of *Asparagus africanus, Withania somnifera* and *Laganaria vulgaris*.

2. To determine rectal temperatures during the administration of the plant extracts

2. To perform phytochemical screening on the extracts used for antimicrobial study in

   order to identify the major class of compounds

4. To determine the acute toxicity including LD_{50} of the extracts, which show antimalarial activity

3. MATERIALS AND METHODS

3.1. PLANT COLLECTION AND SAMPLE PREPARATION

3.1.1. Plant Materials

The root and areal part of *Asparagus africanus,* leaf, root and root bark of *Withania somnifera* and leaf of *Laganaria vulgaris* were collected around Shashamane and Awassa (about 270 km South of Addis Ababa, 10 km North of Awassa), where they have been used in traditional treatment of malaria. A field trip consisting of a Taxonomist, a Phytochemist, a Plant Collector and the PI was organized and six plant parts were collected. A voucher specimen of *A. africanus,* Herb No, AA-2163, *Withania somnifera* Herb No, WS-2164 and *L.vulgaris* Herb No, LV-2165 were deposited at the Herbarium of Ethiopian Health and Nutrition Research Institute (EHNRI), Department of Drug Research (DDR). The collected plant parts were garbled and dried in the processing room, powdered and kept at room temperature in well-closed amber coloured battle until extracted.

3.1.2. Extract Preparation

Air-dried and powdered plant materials (100g) were extracted by maceration with 80% methanol for three consecutive days at room temperature. The extracts were then filtered and concentrated under vacuum in rotary evaporator to give (as percentage of powdered plant materials) 13%-28% of gummy residue, as extract of the the various plant parts. All the extracts were kept in tightly stoppered battle in a refrigerator until used for anti-malarial testing. The extracts were diluted with the vehicle (Double distilled water with 5 drops of ethanol. However, slightly soluble *A.africanus* root and aerial part were dissolved by adding 5 drops 10% EtOH in 10 ml double distilled water per dosage dilution.

3.1.3. Phytochemical Screening

The powdered plant materials and portion of the total extracts used for *in vivo* antimalarial screening were subjected for the identification of the major secondary metabolites by the methodology outlined by (Asfaw Debella, 2002). The phytochemical studies were conducted on the plant extracts tested, *A.africanus* root and aerial part and *W.somnifera* leaf and root bark. The tests employed to identify the presence of the following major chemical constituents (secondary metabolites), Alkaloids, Polyphenols, Saponins, Glycosides, quinones, Flavonoids, Cardiac Glycosides, Antranides, Phytosteroids and Withanoids, tannins and Phenolic glycosides.

Chemicals and reagents used

A lot of chemicals and standards, purity grade chemicals were used

a) 1% HCl, Dragendroffs, Mayer's and 0.5% Tannin Sol, were used to identify the presence of alkaloids
b) acetic anhydride, chloroform, and sulphuric acid were used to confirm the test for saponins.
c) 10% ammonia solution; benzo (napthoquinonee), 5% KOH solution and anthrone (positive control) were used to identify quinones.
d) 1% $FeCl_3$ and 1% $K_3Fe\ (CN)_6$ was used for phenols, tannins, flavonoids, phenolic acid
e) 2% NaCl and 1% gelatine, $K_3Fe\ (CN)_6$, Conc. Ammonia, 5% tannin solution (positive control) and methanol (negative control) were used to identify tannins.
f) Chloroform and conc. Sulphuric acid were used to identify Phytosteroids (Terpens). 1% $FeCl_3$ and 1% $K_3Fe\ (CN)_6$ were used to identify phenolic glycosides
g) Chloroform and sulphuric acid were used to test the presence of phytosteroids/Terpens, in pet ether extracts.

Water, 80% methanol, chloroform and acidic water were used as negative controls and saponin, berberine anthrone tanic acid and retinone were used as standard drugs.

3.2. *IN VIVO* ANTIMALARIAL TESTS

3.2.1. GENERAL EXPERIMENTAL PROCEDURES

3.2.1.1. Parasite

Plasmodium species that cause human malaria are unable to infect non-primate animal models. Therefore, the most widely employed rodent malaria parasite, original strain of *Plasmodium berghei* (chloroquine sensitive strain ANKA), was used to infect mice for a four- day suppressive test, as this runs acute course in random-bred Swiss albino mice (David, 2004; Peters et al, 1975). The *P. berghie* was subsequently maintained in our laboratory by serial blood passage from mouse to mouse. A donor mouse with rising parasitemia (% infected red blood cells) of 20% was determined by counting 20 parasitized cells of 100 erythrocytes and the animal was sacrificed by head blow, and blood collected in a slightly heparinated syringe from the auxiliary vessels (David, 2004; Peters et al., 1975). The blood is diluted with Trisodium Citrate medium so that each 0.2 ml contains approximately 10^7 infected red cells, each animal to receive inoculums of about 10 million parasites per gram body weight, which is expected to produce steadily rising consistent infection in the required intensity in mice.

3.2.1.2. Host animals

Male albino mice belonging to strains maintained in the Ethiopian Health and Nutrition Research Institute laboratory were used. They were kept in cages in animal house with a 12 hour light-dark cycle and fed on pellets and tap water *ad libitum*. Mice were allowed to acclimatize to the laboratory environment under controlled temperature of 20^0C and optimum humidity at least for three days before being subjected to the experiments (Peters, 1975). All experiments were carried out in a quite laboratory setting with ambient illuminations and temperature close to those of the animal house. All experiments were performed on in house–bred male Swiss albino mice. They were divided into eight groups, each consisting of at least 3 mice for screening pilot study, and five groups of six each for the main investigation, all infected with malaria parasites. Six groups received extract treatments, one group received the vehicle (negative control) and the 8th group received chloroquine (standard antimalarial

drug), for the pilot screening study. The same batch of animals but 6 mice in each group was used for the study of the selected two plants (*A.africanus* and *W.somnifera*).

3.2.1.3. Infecting the mice

Infecting the recipient mice was initiated by needle passage of the above mentioned parasite preparation to a naïve test animals via intraperitonial route, using a few No of inoculums (David, 2004; Peter et al., 1975). Therefore, the *P. berghei*- infected red blood cells were intraperitonially injected into the mice from the blood diluted with TC medium so that each 0.2 ml contains approximately 10^{6}- 10^{7} infected red cells (parasite per kg body weight). Each mouse was infected with a single inoculum of 0.2 ml blood containing 10^{6}- 10^{7} infected red cells intraperitonially.

3.2.1.4. Administration of the plant extracts

Infected mice were weighed, randomly divided into treatment, vehicle and standard drug groups and the treatment groups were put on extracts starting 3 hours after infection. The plant extracts were administered by intra gastric route using the stomach tube to ensure the safe ingestion of the extracts and the vehicle. Three dose levels of the extracts, namely 200 mg per kilogram of body weight, medium dose of 400 mg per kilogram body weight and a high dose of 600 mg per kilogram of weight were selected from the pilot study carried out in mice based on the information obtained from the literature on traditional usage of these plants (Teferi G. and Heinz J., 2002). Extracts were made to suitable dose levels (200 mg/kg, 400 mg/kg and 600 mg/kg) in solution and suspension, the later requiring the addition of 5 drops of 10% ethanol in 10 ml double distilled water. The extracts of a) *A. africanus* Root and Arial parts (200 mg/kg, 400 mg/kg and 600 mg/kg); b) *W. somnifera* leaf and root bark (200 mg/kg, 400 mg/kg, 600 mg/kg) and the vehicle (5 drop 10% Ethanol and double distilled water), chloroquine 10 mg/kg (David, 2004), was used as a standard drug, and all were given for 4 consecutive days daily

starting 3 hours after infection, i.e. from day 0 ($D_{0)}$ to day (D_3), receiving a total of 4 intra gastric doses

3.2.1.5. Parasite counts

A mean group level of about 90% that of the control animals is believed to indicate the activities of the test compounds. Counting the number of parasitized erythrocytes in each of 30 fields of a microscope slide and taking the average, which was finally expressed as a percentage of the total, was determined as parasitemia of individual animal. Percentage of suppression was calculated by using:-

$$\textbf{\% Suppression} = \frac{\text{Parasitemia in negative control - Parasitemia in study group}}{\text{Parasitemia in negative control}}$$

(Munoz et al., 2000)

Thin smears of blood films were made from peripheral blood of the tail from each mouse on day four after infection [David, 2004; WHO, 1980]. The smears on microscopic slides were fixed with methanol and stained with Gimsa stain at pH 7.2 and parasitized red blood cells were examined under a 1/12 inch oil immersion objective. The microscope had an Ehrlich's eyepiece, which was adjusted so that the diaphragm exposed about 100 red blood cells per field. Counting the number of parasitized erythrocytes in each of 10-50 such fields and taking the average, which was finally expressed as a percentage of the total, determined Parasitemia of individual animal.

3.2.1.6. Body weight

The measurement of the body weight was to predict the effectiveness of the test extracts to prevent the expected body weight fall in infected mice with increasing parasitemia. that successful therapy would do (Jatamaad et al., 1998).The initial and final body weight measurements were done to indicate the protective effects of the extracts expected weight reduction.

3.2.1.7. Determination of Packed Cell Volume

The measurement of Packed Cell Volume (Erythrocyte volume fraction) (PCV) was done to predict the effectiveness of the test extracts [Jutamaad, 1998]. On day 4, a modified Wintrobe's method was employed using the Manual of basic Techniques for a health laboratory (WHO, 1980), with haematocrit tubes of thick-walled capillary glass, 1.0 mm internal diameter, 110 mm in length and open at both ends. Each was dipped in heparinein sodium edetate so that the inner wall was wetted with the anticoagulant. Blood from the tail of the animals was then drawn up to a 100 mm mark previously etched on the tube. The tube was next closed at both ends with an elastic band and centrifuged at 3,000 r.p.m. for 30 minutes. Duplicate and triplicate determinations were made, the relative volume of the blood occupied by erythrocytes, which is represented by

$$\text{Packed Cell Volume (PCV)} = \frac{\text{Volume of erythrocytes in a given volume of blood}}{\text{Total blood volume}}$$

Which is the measure of the proportion of red blood cells to plasma, used in estimating the mean erythrocyte hemoglobin concentration, was calculated as a simple decimal. The hemoglobin number is determined by calculation from this PCV value. This test was done from each mouth just before infection and on the 5th day of the infection.

3.2.1.8. Rectal Temperatures

The daily measurement of rectal temperature was done to predict the effectiveness of the test extracts [Jutamaad, 1998]. The study as well as control animals were checked for the rise in body temperature, by using rectal thermometers, daily for 6 consecutive days starting from the day prior to parasite inoculation.

3.2.2. SUPPRESSIVE AND ACUTE TOXICITY TESTS

3.2.2.1. Experiment 1

In vivo **antimalarial activity tests were performed in a 4-day suppressive test according to the methods of David, Thurston and Peters, to screen the 6 plant extracts, using a single arbitrary dose level. The extracts included: - a)** ***Asparagus africanus*** **Root and Arial parts (300mg/kg) b)** ***Withania somnifera*** **root, root bark and leaf (300mg/kg) c)** ***Laganaria vulgaris*** **leaf (300 mg/kg) for antimalarial activities. The 4-day procedures were started on Mondays and blood smears for parasitemia were collected on Fridays. From among plant extracts demonstrating the positive or potential efficacy, the most promising ones,** ***A. africanus*** **root and arial parts b)** ***W.somnifera*** **root bark and leaf were selected for detailed comprehensive investigation,** ***W.somnifera*** **root and** ***L.vulgaris*** **were kept for future consideration.**

3.2.2.2. Experiment 2

Asparagus africanus root and areal parts:

In vivo antimalarial activity tests were performed in a 4-day suppressive test according to experiment one above on two extracts of *A. africanus* root and aerial part. The 4-day procedures were started on Mondays, as usual, and blood smears for parasitemia and samples for PVC were collected by thin smears of blood films and hematocrit tubes from peripheral blood of the tail from each mouse (WHO, 1980). The smears on microscopic slides were fixed with methanol and stained with Gimsa at pH 7.2 stain on Fridays. *P. berghei* parasites were inoculated into 30 mice separating them into 5 groups, for each extract. Three of them (groups 1, 2 and 3) served as experimental groups, received extract of *A. africanus* root (200 mg/kg, 400 mg/kg and 600 mg/kg). Group 4, the vehicle (5 drops 10% Ethanol in double distilled water), and the fifth group, chloroquine 10 mg/kg, daily for 4 days, starting on the same day as that of the parasite inoculation. The same study was repeated on *A. africanus* aerial part (200 mg/kg, 400 mg/kg and 600 mg/kg). Stomach tube was used to ensure the safe ingestion of the extract and the vehicle. Parasitemia and PCV blood sample was extracted from the tail vein and was examined on day 4. Weight was measured on D0 and D4 and temperature was measured daily starting from the day prior to infection.

Withania somnifera leaf and root bark

The same *P. berghei* parasites were inoculated into another batch of 30 mice by the same method as that used in the above experiment. Mice were grouped into 5. Three of them (groups 1, 2 and 3) served as experimental groups, received extracts of *W. somnifera* leaf (200 mg/kg, 400 mg/kg and 600 mg/kg). Group 4, the vehicle, and the fifth group, chloroquine, daily for 4 days, starting on the same day as the of the parasite inoculation. The same procedure was repeated for

W. somnifera root (200 mg/kg, 400 mgkg and 600 mg/kg), with another 30 mice. Stomach tube was used to ensure the safe ingestion of the extract and the vehicle. Parasitemia, PCV, weight and temperature were determined as above

3.2.2.3. Experiment 3

This was done for acute and sub acute toxicity of *A. africanus* root and aerial part and *W. somnifera* leaf and root bark. Eight groups of mice, five male and five female for *A. africanus* root, five male and five female for *A. africanus* aerial part and the same groups for *W. somnifera* extracts were acclimatized and fasted over night. The weight of each mouse was taken and calculated dose of 5,000 mg/kg of respective extract was given on empty stomach. The toxicity signs expected read as death, changes in physical appearance, behavioral change, and organ damage were observed for 72 hours.

3.2.3. DATA ANALYSIS

The data of the parasitemia, body weight, packed cell volume and body temperature were analysed using SPSS version 8. The one-way ANOVA, Student's t-test and independent comparison tests were used to compare results among groups and with in groups for difference between initial and final results. All data were analysed at 95% confidence interval ($\alpha = 0.05$). The formulae for calculation of percentage suppression of parasitemia and percentage packed cell volume are used as indicated above.

4. RESULTS

4.1. *IN VIVO* ANTIMALARIAL TESTS

4.1.1. Screening Tests

The pilot *in vivo* antimalarial screening experiments on the six plant extracts showed considerable antimalarial activities against *P. bergei*. The hydro alcoholic extracts could produce the required antimalaial activities by significantly suppressing the parasitemia of *P. bergei*, at the dose level of 300 mg/kg. However, *A. africanus* roots and aerial parts (300 mg/kg), *W. somnifera* roots, root barks and leaves (300 mg/kg) showed better inhibitory effects on parasite growth than that of 300 mg/kg of *L. vulgaris*

The results are summarized in Table 1 for the study groups, negative and the positive control groups. The percentage inhibitions of the parasite counts were significantly higher for the study groups when compared to the negative control (p=0.000). The percent of inhibition of groups treated with the hydro alcoholic extract of *A. africanus* roots was (55.47 %), *A. africanus* aerial parts (22.12 %); *W. somnifera* leaves (54.49 %), *W. somnifera* roots (50.73 %) and *W. somnifera* root barks (50.80 %) and *L. vulgaris l*eaves (22.99 %)

Table. 1: **Pilot antimalarial activity screening tests of *A. africanus*, *W. somnifera* and *L. vulgaris* hydroalcoholic extracts against *P. berghei* in mice**

Test Substances	Wt	Dose/kg	% parasitemia	% Inhibition
A. africanus roots	28.83	300mg/kg	16.77	55.47
A. africanus aerial parts	26.10	300mg/kg	29.33	22.12
W. somnifera leaves	24.46	300mg/kg	17.14	54.49
W. somnifera root barks	26.27	300mg/kg	18.53	50.80
W. somnifera roots	27.85	300mg/kg	18.55	50.73
L. vulgaris leaves	27.2	300mg/kg	29	22.99
Vehicle (-)	28.42	0.00	37.66	0
Chloroquine (+)	27.6	10mg/kg	0.00	100

4.1.2. Parasite Counts

Asparagus africanus

***In vivo*, hydro alcoholic extracts of *A. africanus* displayed a very good activity against *P. berghei* malaria parasite. The comparison analysis indicated that the 13.62 % inhibition with the 200 mg/kg hydro alcoholic extract of *A. africanus* roots and 13.47 % inhibition of *A. africanus* aerial parts showed statistically significant difference on day 4 parasitemia level (p=0.000) respectively, compared to the negative control. The 27.84 % inhibition with the 400 mg/kg *A. africanus* roots extract and 22.67 % inhibition of *A. africanus* aerial parts also showed statistically significant difference on day 4 parasitemia level (p= 0.000), compared with the control groups.**

The high level of 46.12 % inhibition with the 600 mg/kg *A. africanus* roots and 40.73 % inhibition of *A. africanus* aerial parts is also remarkable and strong statistical significance was seen on day 4 parasitemia level (p=0.000), compared to the negative control. The highest level of inhibition (46.12 %) was seen with 600 mg/kg of *A. africanus* roots in this test (Table 2).

Table 2**: Parasitemia suppressive test of *A. africanus* roots and aerial parts hydro alcoholic extracts against *P. berghei* in mice**

Test substances	Dose mg/kg	% parasitemia	% inhibition
***A. africanus* roots**	**200**	35.27±0.14	13.62
	400	29.46±0.48	27.84
	600	22.00±0.11	46.12
Vehicle	**1 ml (-)**	40.83±0.29	0.00
Chloroquine	**10 (+)**	**0.00**	**100.00**
***A.africanus* aerial parts**	**200**	45.25±0.29	13.47
	400	40.45±0.21	22.67
	600	31.00±0.24	40.73
Vehicle	**1 ml (-)**	52.30±0.38	0.00
Chloroquine	**10 (+)**	**0.00**	**100.00**

- **Each result is a mean of 6 mice**
- **P=0.05 * ± controls**

Withania somnifera

***In vivo*, hydro alcoholic extracts of *W. somnifera* also displayed a very good activity against *P. berghei* malaia parasite. The multiple comparison analysis indicated that the 13.33 % inhibition with the 200 mg/kg hydro alcoholic extract of *W. somnifera* leaves and 17.17 % inhibition of *W. somnifera* root barks showed statistically significant difference on day 4 parasitemia level (p= 0.000) compared to the negative control. The 33.75% inhibition with the 400mg/kg of *W. somnifera* leaves and 23.04 % inhibition of *W. somnifera* root barks also showed statistically significant difference on day 4 parasitemia level (p=0.000) and the 50.43 % inhibition with the 600 mg/kg *W. somnifera* leaves and 29.13 % inhibition of *W. somnifera* root barks also showed statistically significant difference on day 4 parasitemia level (p=0.000) compared to the negative control. The maximum inhibition (50.43%) was seen with 600 mg/kg of *Withania* leaves in this test (Table 3).**

Table 3**: Parasitemia suppressive test of *W. somnifera* leaves and root barks hydroalcoholic extracts against *P. berghei* in mice**

Test substances	Dose mg/kg	% Parasitemia	% Inhibition

***W. somnifera* leaves**	**200**	34.67±0.42	13.33
	400	26.50±0.22	33.75
	600	19.83±0.48	50.43
Vehicle	**1 ml**	**40.00±0.63**	**0.00**
Chloroquine	**10**	**0.00**	**100.00**
***W. somnifera* roots**	**200**	44.83±0.66	17.17
	400	41.65±0.39	23.04
	600	38.35±0.88	29.13
Vehicle	**1 ml**	**54.12±0.83**	**0.00**
Chloroquine	**10**	**0.00**	**100.00**

- **Each result is a mean of 6 mice**
- **P<0.05**

4.1.3. Body Weight Measurement

Asparagus africanus

The over all ANOVA among groups of mice on D4 measurements of body weight, with *A. africanus* roots showed that there is a significant difference (p=0.000) among groups on body weight. The Student's paired t-test analysis indicated a significant difference (p=0.020) between the initial body weight (D-0) and the final body weight (D4), which is expected due to an increase because of drug effect or a decrease because of the parasitemia. The comparison analysis indicated that test extracts significantly prevented the expected body weight loss in infected mice with increasing parasitemia. There was no significant difference (p=0.100) between the initial weights of group one and group four, but there was a significant difference (p=0.000) on day 4. The increase 32.20 mg from the initial average mice weight of 30.26 g (the weight increment of 1.94 g), with the 200 mg/kg hydro alcoholic extract of *A. africanus* roots. This showed statistically significant difference, compared to the negative control.

The over all ANOVA among groups of mice on D4 measurements of body weight, with *A. africanus* aerial parts also showed that there is a significant difference (p=0.001) among groups on body weight. The Student's paired t-test result indicated a significant difference (p=0.020) between the initial body weight (D-0) and the final body weight (D4), which is expected due to an increase because of drug effect or a decrease because of the parasitemia. The comparison analysis indicated that test extract of *A. africanus* aerial parts (Table 4) significantly prevented the expected body weight loss in infected mice with increasing parasitemia. There was an increase of 1.10 g (from initial weight of 29.97 to 31.07), which showed statistically significant difference (p=0.001), compared to the negative control.

There was no significant difference (p=0.800) between groups two (400 mg/kg) and the control on D0, but there was significant difference (p=0.000) on D4, because of increase in weight in the treatment group.

The 33.20 g increase from 32.30 g with the 600 mg/kg *A. africanus* roots extract and 32.90 g increase from 32.58 g. *A. africanus* aerial parts also showed statistically significant difference on day 4 (p=0.001), compared with the control groups (which showed a weight reduction to 33.20g from 34.38 g on day 4). This indicates the protective effect, on weight reduction, of the hydro alcoholic test extracts of *A. africanus* of the infected mice (Table 4).

Table 4: Body weight measurement for *A. africanus* hydro alcoholic extracts against *P. berghei* in mice

Test substances	Dose mg/kg	Wt.D-0	Wt. D-4	% change
A.africanus roots	200	30.26±0.33	32.20±0.07	6.41
	400	27.30±0.28	28.50±0.26	4.40
	600	32.30±0.33	33.20±0.43	2.78
Vehicle	1ml	29.40±0.34	28.64±0.47	-2.59
Chloroquine	10	29.24±0.55	**29.84±0.48**	1.50
A.africanus aerial parts	200	29.97±1.30	**31.07±0.60**	3.67
	400	29.78±0.42	**30.55±0.66**	2.59
	600	32.58±0.48	**32.90±0.54**	0.98
Vehicle	1ml	34.38±0.61	**33.20±0.43**	-3.43
Chloroquine	10	**29.66±0.25**	**30.10±0.27**	1.48

Day 0 = day infection initiated **$* \ P<0.05$**

Day 4 = 5th day of infection * Each result is a mean of 6 mice

Withania somnifera

The ANOVA among groups of mice on D4 measurements of body weight, with *W. somnifera* leaves showed no significant difference (p=0.525) on body weight. However, the Student's paired t-test analysis indicated a significant difference (p=0.027) between the initial body weight (D-0) and the final body weight (D4), which is expected due to an increase because of drug effect or a decrease because of the parasitemia. The comparison analysis indicated that test extracts significantly prevented the expected body weight loss in infected mice. The 32.20 g increases from 30.26 g, with the 200 mg/kg hydro alcoholic extract of *W. somnifera* leaves showed statistically significant difference (p=0.001), compared to the negative control.

The over all ANOVA among groups of mice on D4 measurements of body weight, with *W. somnifera* root barks on the other hand showed a significant difference (p=0.000) among groups on body weight. Although the Student's paired t-test result indicated no significant difference (p=0.651) between the initial body weight (D-0) and the final body weight (D4). The comparison analysis also indicated that some doses of test extracts significantly prevented the expected body weight loss in infected mice. The 29.32 g increases from 26.57 g, with the 200 mg/kg hydro alcoholic extract of *W. somnifera* leaves showed statistically significant difference (p=0.001), compared to the negative control. The 28.55 g increase from 27.15 g, with the 600 mg/kg hydro alcoholic extract of *W. somnifera* leaves also showed statistically significant difference on day 4 parasitemia level (p=0.035), compared to the negative control.

The 32.65 g increases from 30.28 with the 600 mg/kg *W. somnifera* root barks showed statistically significant difference on day 4 (p= 0.003), compared with the control groups (which shows a weight reduction to 28.40 g from 32.10 g) (Table 5).

Table 5: **Body weight measurement for *W. somnifera* hydro alcoholic extracts against *P. berghei* in mice**

Test substances	Dose mg/kg	Wt.D-0	Wt. D-4	% change
W.somnifera leaves	200	26.57±0.23	29.32±0.66	10.35
	400	29.55±0.29	29.02±0.79	-1.79
	600	27.15±0.29	28.55±0.42	5.16
Vehicle	1ml	30.37±0.37	29.82±0.31	-1.81
Chloroquine	10	28.43±0.36	29.53±0.38	3.87
W.somnifera root barks	200	27.24±0.38	26.48±0.49	-2.79
	400	29.42±0.58	29.25±0.60	-0.58
	600	30.28±0.42	32.65±0.40	7.87
Vehicle	1ml	32.10±0.64	28.40±0.67	-11.53
Chloroquine	10	31.20±0.36	32.38±0.0.39	3.78

- Day 0 = day infection initiated

* Each result is a mean of 6 mice

- Day 4 = 5th day of infection * $p<0.05$

4.1.4. Determination of Packed Cell Volume

Asparagus africanus

The over all ANOVA among groups of mice on D4 measurements of the Packed Cell Volume (PCV), with roots of *A. africanus* tests showed that there is a significant difference (p=0.000) among groups on the PCV. The Student's paired t-test result also indicated a significant difference (p=0.000) between the initial body weight (D-0) and the final body weight (D4), which is expected due to an increase because of drug effect or a decrease because of the parasitemia. The comparison analysis of Packed Cell Volume (PCV), on day 4, determination indicated that the hydro alcoholic extract of both roots and aerial parts of *A. africanus* significantly prevented the reduction in PCV, at some dose levels. There was a significant preventive effect of *A. africanus* roots with 400 mg/kg (p=0.006) and 600 mg/kg (p=0.032) (Table 6).

Table 6**: Packed Cell Volume tests of *A. africanus* roots and aerial parts hydro alcoholic extracts against *P.berghei* in mice**

Test substances	Dose mg/kg	D-0 PCV	D-4 PCV	% reduction
A.africanus roots	200	52.61±0.32	**43.24±0.42**	17.81
	400	51.24±0.30	**43.86±0.22**	14.40
	600	53.42±0.31	**43.73±0.41**	18.40
Vehicle	1ml	52.02±0.25	**42.34±0.37**	18.61
Chloroquine	10	51.38±0.34	**48.27±0.45**	6.05
A.africanus aerial parts	200	52.83±0.30	**43.37±0.31**	17.91
	400	51.80±0.44	**43.66±0.14**	15.71
	600	53.32±0.34	**43.08±0.70**	19.20
Vehicle	1ml	53.26±0.33	**42.97±0.63**	19.32
Chloroquine	10	52.68±0.55	**48.05±0.24**	8.79

- Day 0 = day infection initiated * Each result is a mean of 6 mice
- Day 4 = 5th day of infection * $p<0.05$

Withania somnifera

The ANOVA among groups of mice on D4 measurements of Packed Cell Volume (PCV), with *W. somnifera* leaves tests showed that there is a significant difference (p=0.000) among groups on the PCV. The Student's paired t-test result also indicated a significant difference (p=0.000) between the initial PCV (D-0) and the final PCV (D4), which is expected to decrease because of the parasitemia. The comparison analysis of Packed Cell Volume (PCV), on day 4, determination indicated that the hydro alcoholic extract of *W. somnifera* leaves significantly prevented the expected reduction in PCV, at some dose levels.

The variance analysis among groups of mice on D4 measurements of Packed Cell Volume (PCV), with *W. somnifera* root barks tests also indicated there is a significant difference (p=0.000) among groups on the PCV. The Student's paired t-test result also indicated a significant difference (p=0.000) between the initial PCV (D-0) and the final PCV (D4), which is expected to decrease because of the parasitemia. The comparison analysis of Packed Cell Volume (PCV), on day 4, determination indicated that the hydro alcoholic extract of *W. somnifera* root barks also significantly prevented the expected reduction in PCV, at some dose levels.

The day 4 determination indicated that the hydro alcoholic extract of *W. somnifera* leaves showed statistically significant difference in preventive effect, at dose level of 200 mg/kg (p=0.016) and 600 mg/kg (p=0.001) respectively, in comparison with the control group, and *W. somnifera* root barks also significantly prevented the drop in PCV at dose levels of 600 mg/kg in comparison with the negative control group of mice (p=0.004) (Table 7)

Table 7: **Packed Cell Volume tests of *W. somniferum* leaves and root barks hydro alcoholic extracts against *P. berghei* in mice**

Test substances	Dose mg/kg	D-0 PCV	D-4 PCV	% reduction
W.somnifera leaves	200	**50.08±1.36**	**40.26±0.69**	19.61
	400	**49.96±1.31**	**42.62±1.06**	14.69
	600	**50.89±1.15**	**46.28±0.76**	9.06
Vehicle	1ml	**51.07±1.37**	**40.80±1.11**	20.11
Chloroq.	10	**49.68±0.99**	**47.52±0.97**	4.35
W.somnifera root barks	200	**51.25±0.99**	**41.02±3.80**	19.96
	400	**50.62±1.06**	**43.03±2.27**	14.99
	600	**49.37±1.37**	**47.50±2.75**	3.79
Vehicle	1ml	**49.79±4.21**	**40.81±3.33**	18.04
Chloroq.	10	**48.54±0.70**	**48.03±0.61**	1.05

- Day 0 = day infection initiated
- Day 4 = 5th day of infection

* Each result is a mean of 6 mice

* $p<0.05$

4.1.5. Rectal Temperatures

Asparagus africanus

The ANOVA among groups of mice on D4 measurements of body temperature, with *A.africanus* roots test showed a significant difference (p=0.000) among groups on body temperature. The independent t-test result also indicated a significant difference (p=0.013) between the pre-treatment (normal) body temperature and the final body temperature (D4), because of parasitemia. The comparison analysis indicated that test extracts significantly prevented the expected body temperature variation in infected mice at some dose levels

The comparison analysis has shown statistically significant difference (p=0.001) between the study and the control groups with hydro alcoholic extract of roots of *A. africanus*, at the dose level of 600 mg/kg. Although the results indicated a fall from 38.2 ^{0}C pretreatment to 36.08 ^{0}C on day 4 with, 200 mg/kg, a fall from 38.18 ^{0}C pretreatment to 36.37 ^{0}C on day 4 with, 400 mg/kg, a fall from 37.62 ^{0}C pretreatment to 34.03 ^{0}C on day 4 with, 600 mg/kg in comparison to the fall from 38.10^{0}C to 34.50 ^{0}C on day 4 with control group, the comparison analysis showed significant difference only with 600 mg/kg dose level of the roots of *A. africanus*. There was no statistical significance with hydro alcoholic extract of *A. africanus* roots with the rest of the dose levels (Table 8)

Table 8: Rectal temperature test for *A. africanus* hydroalcoholic extracts against *P.berghei* in mice

Test Substanc	Dose mg/k	Pre-treatmen	D-0	D-1	D-2	D-3	D-4	% change
A.africanus	200	38.2±0.12	36.76±0.16	36.62±0.27	36.68±0.34	37.77±0.22	36.08±0.24	-3.62

roots	400	38.18±0.16	37.16±0.52	36.99±0.47	36.82±0.18	37.90±0.27	36.37±0.42	-0.38
	600	37.62±0.19	34.72±0.49	35.87±0.29	34.23±0.47	37..20±0.09	34.03±0.09	-6.41
Vehicle	1ml	38.10±0.16	37.12±0.33	37.23±0.44	37.60±0.22	37.45±0.25	34.5±0.24	-3.33
Chloroq.	10	38.40±0.16	38.20±0.27	37.38±0.23	37.67±0.17	38.28±0.15	38.38±0.13	-1.10
A.africanus Aerial parts	200	37.63±.0.15	37.42±0.16	37.98±0.09	37.65±0.25	38.05±0.20	37.21±0.58	0.12
	400	37.88±0.11	37.85±0.05	38.01±0.09	37.62±0.10	37.83±0.11	37.22±0.35	-0.43
	600	37.40±0.19	36.08±0.33	38.10±0.11	38.30±0.10	37.90±.0.24	37.32±0.37	-0.92
Vehicle	1ml	37.65±0.17	36.50±0.50	38.10±0.15	38.15±0.29	38.13±0.13	35.88±0.81	-0.78
Chloroq.	10	38.13±0.13	38.27±0.10	37.57±0.15	37.35±0.34	37.78±0.07	38.02±0.14	0-.80

- Day 0 = day infection initiated
- Day 4 = 5th day of infection

* Each result is a mean of 6 mice

* p<0.05

Withania somnifera

There is an over all significant difference (p=0.033) between the pretreatment (normal) temperature and the final temperature (D4), which is expected because of the parasitemia. The comparison analysis has shown statistically significant difference between the study and the control groups with hydro alcoholic extract of *W. somnifera* leaves, at the dose level of 200 mg/kg (p=0.024). The results indicated that a fall from 37.98^{0}C pretreatment to 36.47^{0}C on day 4 with, 200 mg/kg, a fall from 38.05 ^{0}C pretreatment to 35.70^{0}C on day 4 with, 400mg/kg, a fall from 38.87^{0}C pretreatment to 37.68^{0}C on day 4 with, 600 mg/kg in comparison to the fall from 38.72^{0}C to 37.27^{0}C on day 4 with control group. However, there was no statistical significance with hydro alcoholic extract of *W. somnifera* leaves for the rest of the dose levels (Table 9)

Table 9: Rectal Temperature tests for *W. somnifera* hydro alcoholic extracts against *P.berghei* in mice

Test Substanc	Dose mg/kg	Pre-treatmen	D-0	D-1	D-2	D-3	D-4	% change
W.somnifera leaves	200	37.57±0.13	36.32±0.58	37.88±0.18	37.56±0.09	37.64±0.16	36.48±0.56	-1.03
	400	37.83±0.13	37.53±0.31	37.98±0.19	37.92±0.11	37.53±0.33	34.17±0.10	-2.11
	600	37.38±0.22	35.10±0.40	37.43±0.20	36.73±0.42	37.08±0.24	36.32±0.54	-2.25
Vehicle	1ml	38.02±0.25	35.97±.0.20	36.08±0.34	36.58±0.22	36.38±0.30	37.22±0.32	-4.10
Chloroq.	10	38.33±0.09	38.08±0.11	37.26±0.10	37.35±0.19	38.20±0.11	38.26±0.07	-1..25
W.somnifera root barks	200	37.98±0.19	37.37±0.32	37.80±0.07	37.80±0.20	37.48±0.09	36.47±0.34	--1..50
	400	38.05±0.06	37.25±0.27	37.86±0.16	37.63±0.0.18	37.17±0.34	35.70±0.64	-2..52
	600	38.87±0.20	37.53±0.37	37.72±0.17	36.92±0.31	36.97±0.27	37.68±0.27	-3. 86

Vehicle	1ml	38.72±0.18	37.77±0.13	37.67±0.20	37.38±0.37	37.80±0.34	37.27±0.24	-2. 91
Chloroq.	10	38.43±0.19	38.07±0.19	37.82±0.08	37.60±0.18	38.24±0.20	38.13±0.01	-1..2.2

- Day 0 = day infection initiated
- Day 4 = 5th day of infection

* Each result is a mean of 6 mice

* $p<0.05$

4.2. TOXICITY TESTS

The hydro alcoholic extracts of *A. africanus* roots and aerial parts and *W. somnifera* leaves and root barks, showed no lethality at doses up to 5,000 mg/kg, when given through intragastric route (which is 25 MED, 200 mg/kg). The mice were followed for over a week but no sign of toxicity was seen. Gross behavioural and physical observations revealed no lacrimation, no urination, no muscle weakness, no sedation and no convulsion, that are commonly occurred in toxicity. They were physically active moving and feeding. Similarly, intraperitonial administration of the same extracts doses of up to 1000 mg/kg did not produce lethality.

4.3. PHYTOCHEMICAL STUDY

The phytochemical screening of the powdered plant materials and extracts used in this study revealed that there are several biologically active major secondary metabolites. polyphenols, saponins, phenolic glycosides, phytosteroids and terpens were identified in *A. africanus* roots and alkaloids, polyphenols, tannins, phenolic glycosides, and phytosterols and Terpens were revealed in *A. africanus* aerial parts.

Tannins, alkaloids, polyphenols, flavonoids, phytosterols and phenolic glycosides, were identified in *W. somnifera* leaves and alkaloids, polyphenols, phenolic glycosides, phytosteroids and saponins were identified in the root barks. The Thin Layer Chromatography (TLC), using appropriate solvents, mobile phases and detection reagents for the indicated class of compounds, secondary metabolites, further resolved the major components of the two plants namely the alkaloids, and saponins. Three to five major alkaloids were detected for *W. somnifera* while 2-3 minor alkaloids were visualized for *A. africanus* roots. *A. africanus* roots and arerial parts showed 3 major spots of typical steroidal skeleton saponins using selective detecting reagent for this class of compounds

5. DISCUSSION

Although, it is clear that the primate models provide a clearer prediction of human efficacy than the rodent models, rodent models have also been validated through the identification of several conventional antimalarials, for example chloroquine, halofantrine, mefloquine and more recently artemisinin derivatives (David, 2004). The common malaria parasite, the *P.berghie*, is with proven use in the prediction of treatment outcomes and remains a standard part of the drug discovery pathway, hence, is the appropriate parasite for this study. Therefore, it was appropriate to start the 4-day suppressive study with the parasite, *P. berghei*, and chloroquine as a sensitive standard drug.

The *P.berghie*, is known to be infective to laboratory strains of rats and mice, being more infective to mice than rats, with increasing parasitemia indicating the drug response on the 4th day treatment and death of infected mice 7th-14th day after infection, in non treated (Hill,

1950). Hence, the use of mice instead of rats is rational and acceptable. The 4-day suppressive test is a standard test commonly used for antimalarial screening and the determination of percent inhibition of parasitemia is the most reliable parameter used. It has, therefore, been recommended that a mean group parasitemia level less or equal to 90% that of mock-treated control animals usually indicates that the test compound is active (Peters, 1975).

Although, the preliminary screening experiments on the six plant extracts, in this study, showed considerable suppressing of the parasitemia of *P. bergei,* all with parasitemia load less than 90% of the parasitemia load of the negative control group, *W. somnifera* roots and *L. vulgaris* leaves have not been selected for the subsequent studies because of the over all working facility capacity, and have been kept under deep freezer for future considerations.

In the *P. berghie* infected mice treated with the extracts of *A. africanus* roots and aerial parts and *W. somnifera* leaves and root barks*,* the percentage of parasitemia measured changed significantly from those of the control animals. This significant suppression of parasitemia by the hydroalcoholic roots extract of *A. africanus* on day 4 was in agreement with that shown for water extract against four different malaria shizont strains *in vitro* (Oketch-Rabaha et al., 2003). It is also consistent with the traditional claim that it is popularly used in the treatments of malaria, in traditional medicine, in Southern Ethiopia (Teferi Gedif and Heinz-Jurgen, 2002)

The change in parasitemia count with the extracts of the leaves and root barks of *W. somnifera* on infected mice, was also comparable to that observed by extracts of other plants (Kiseko et al., 2000, Obih and Makinde, 1985). The high (49.84 %) malaria suppression with 600 mg/kg of *W. somnifera* leaves was comparable to suppression reported with the leaf of *Solanum indicum* (31.9 %) (Abatana and Makinde, 1986), which is believed to be effective antimalaral agent. The appreciable efficacy that was indicated by this plant may also be attributed to its immune modulatory effect, which was reported in similar studies (Davis and Kuttan, 2000; Gandhi, et al. 1994). The inhibitory effect of parasite growth by the extracts increased with increase in dose administered, this was seen with all the extracts

When standard antimalarial drug is used against *P. bergei* infected mice parasitemia, it suppresses parasitemia to non-detectable level (Kiseko, *et al,* 2000), therefore the results of

the tests with chloroquine 10 mg/kg provided 100 % inhibition and are consistent with the previous findings. No parasite could be seen in the slides on day 4, and all the other parameters measured in mice had returned to normal.

The body weight measurement on the other hand has shown statistically significant difference between the study and the control groups. The hydro alcoholic extract of *A. africanus* roots significantly prevented the expected weight loss due to increasing parasitemia, which is inconformity with the previous studies (Jutamaad, et al., 1998)
The body weight increase with extracts is significantly higher with the extracts than with even the chloroquine treated groups. The possible explanation is that the extracts contain several nutrients and immunomodulatory substances (Abebe et al., 2003). Therefore, in addition to their parasitemia inhibitory activities their nutrient natural products could have contributed for the weight gain, which was not seen even with chloroquine treatment.

The purpose of the measurement of Packed Cell Volume was to predict the effectiveness of the test extracts, by protecting the massive blood cell distraction. In untreated mice, the parasite count increased and the hematocrit packed cell volume (PCV) decreased markedly from day to day until the death of the animal. One of the previous studies showed that PCV was 42.8% after 24 hours of infection and dropped to 10.8% after 5 days of infection (Ayodele, 1979). This study also indicated that the parasite caused massive destruction of red blood cells leading to reduction in packed cell volume. The PCV at some dose levels showed no statistically significant difference from the control group. The possible reason is that the time was too short to increase these vital values, which are expected to increase with significant decrease in parasitemia.

The purpose of the daily measurement of the rectal temperature was to predict the effectiveness of the test extracts, by normalizing the body temperature by suppressing infectious stage, erythrocytic malaria (Jutamaad, 1998). It was found that the temperature is reduced in infected mice with increasing parasitemia and the reduction was prevented to some extent by the plant extracts. However, the preventive effect in some treatment groups was not statistically significant compared to the control group. The comparison analysis showed no statistical significance with hydro alcoholic extract of *A. africanus* roots, while there was

statistically significant preventive effect with hydro alcoholic extract of *A. africanus* aerial parts.

The situation is similar with *W. somnifera*, the comparison analysis has shown statistically significant difference between the study and the control groups with hydro alcoholic extract of *W. somnifera* leaves, at the dose level of 200mg/kg (p=0.024). However, there was no statistical significance with hydro alcoholic extract of *W. somnifera* leaves with the rest of the dose levels. The possible explanation could be the variation in acclimatization that may interfere with normal body temperature that would directly affect the body physiology of the mice. It could also be because of the variation caused by repeatedly taking temperature when the existence of dormant parasite in the pre-erythrocyte stage avoiding the erythrocyte stages. The infectious stage, the maximum temperature hours would have been at night where it was difficult to record their temperature. Therefore, it might be wise to arrange a separate study unite, including the night shift observations, for such a delicate study.

In general, if the LD_{50} value of the test substance is 3 times more than the minimum effective dose (MED) the substance is considered a good candidate for further studies. It was also suggested that oral administration is about 100 times less toxic intraperitonealy (Jutamaad et al, 1998). However, the hydroalcoholic extracts on mice showed no lethality at 5,000 mg/kg, which is 25 times the MED and no gross behavioural and physical changes were revealed. Intraperitonial administration also did not produce lethality at the doses up to even 1000mg/kg and it was difficult to think of giving more doses because of the viscosity of extracts, being unable to pass through intraperitonial needle, even if higher doses were desirable to be tested.

The absence of toxicity is inconformity to the study report, which indicates that *W. somnifera* extracts are capable of suppressing toxicity and any inflammation induced as adverse effects of other chemicals. *W. somnifera* also has immune modulatory effects (Davis and Kuttan, 2000 Gandhi, et al. 1994), therefore one possible explanation of the high suppressive effect and lack of toxicity of the extracts might be due to the immune modulation of the plant. *Asparagus* also is traditionally employed for its anti-inflammatory and anti gouty arthritis and contains a number of protective vitamins like vitamine E (Abebe et al, 2003).

It is also in agreement with the fact that these plants have been used safely in traditional medicine, by the local people, in Southern Ethiopia for the treatment of malaria (Teferi and Heinz-Jurgen, 2002). The minimum effective dose was considered to be the lowest dose which produced a definitive decrease of parasitemia compared with untreated controls; that is to say, the dose that brought about 10% decrease on parasitemia compared to the controls (Jutamaad, 1998). From what WHO has been providing to developing world, it can be noted that the long experience acquired during decades of use constitutes a major advantage in

mastering the innocuousness of the remedies. Therefore, it can also be considered that there is the possibility of observing relative safety in traditional drugs because of their long use and observations in clinical practice. That is why WHO stresses that long experience in use can be considered as a guarantee of innocuousness for drugs from traditional health care system, belonging to category 2 and 3, according to its proposed guideline of use of herbal drugs (WHO, 1993). This can also explain that, the plants studied may be with relative safety and wider safety margin for the therapeutic dose. However, further study can consider more investigation with better-fractionated extracts

Plant extracts are by definition a composition of many different compounds. Mostly, the therapeutic utility of a natural herb or plant extract cannot be attributed to a single compound. It is the interaction of different compounds that often makes a plant extract therapeutically useful. The phytochemical studies of this study revealed several biologically active major secondary metabolites and this is in agreement with the findings of previous screening studies (Asfaw et al., 1999; Banzouzi, et al., 2004; Caraballo, et al., 2004). Further studies on the total extract that shows activity is required to isolate, test and characterize the compounds for standardization of the total extract.

6. CONCLUSION AND RECOMMENDATIONS

a) CONCLUSION

- The *in vivo* anti-malarial test using male Swiss albino mice against rodent malaria, *P. berghei,* revealed that the hydroalcoholic extracts of *A. africanus* roots and aerial parts and *W. somnifera* leaves and root barks showed significant parasite suppressive effect in comparison with the control groups.

- The highest level of inhibition was observed at a dose 600 mg/kg for *A. africanus* roots (% inhibition, 46.12) and *W. somnifera* leaves (% inhibition, 50.43 %), against *P. berghei* parasitemia

- The common malaria parasite, *P.berghie*, is with proven sensitivity to chloroquine, can be utilized for screening of antimalarial phytochemicals and chloroquine can be used still as a suitable standard drug.

- The results of the measurements of body weight showed that the test extracts of *A.africanus* and *W.somnifera* significantly prevented the expected body weight loss in infected mice due to increasing parasitemia

b) RECOMMENDATION

Based on what has been practically achieved from the field and laboratory experience and the results of this study the following recommendations may be made

- Activity guided isolation and characterization of *A. africanus* and *W. somnifera* hydroalcoholic extracts is necessary to facilitate standardization and determine possible new antimalarial compound

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- The principles of combination therapy can be tested with the plant extracts with positive antimalarial activities, for increased activity and relative safety

- Further investigation, phytochemical screening and pharmacological activity studies of other traditionally claimed antimalarial medicinal plants is strongly recommended

- Strong collaboration among researchers interested in screening phytochemicals with claimed antimalarial activities in order to strengthen research efforts and avoid duplication

- Sub acute and chronic toxicity studies on total, fractionated extracts through various route of administration to ensure safety is required

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