

ISSN 2249-8524

Original Article

## ANTIMALARIAL EFFECT OF NEEM LEAF AND NEEM STEM BARK EXTRACTS ON PLASMODIUM BERGHEI INFECTED IN THE PATHOLOGY AND TREATMENT OF MALARIA.

<sup>†</sup>Akin-Osanaiye BC, <sup>\*</sup>Nok AJ, <sup>\*</sup>Ibrahim S, <sup>\*</sup>Inuwa HM, <sup>\*</sup>Onyike E, <sup>\*</sup>Amlabu E and <sup>\*</sup>E. Haruna

<sup>†</sup>Department of Chemistry, Faculty of Science, University of Abuja, Abuja, Nigeria.

<sup>\*</sup>Department of Biochemistry, Faculty of Science, Ahmadu Bello University, Zaria, Nigeria.

Received 23 January 2013; accepted 15 March 2013

### Abstract

Malaria is a major public health problem in the world in general and developing countries in particular, and it is becoming more difficult to manage particularly in areas of multi-drug resistance. Hence, the study was designed to carry out the antimalarial screening of neem extracts using *Plasmodium berghei* infected albino mice. Albino mice free from infection were experimentally infected with *Plasmodium berghei*. The animals showed detectable parasitemia on day 4 post-infection with about 30 % of parasitemia before death of animal was recorded. Different parts of neem (leaf, stem bark and seed) were extracted with methanol and their efficacy tested on *Plasmodium berghei* infected albino mice using the 4-day suppressive test and secondary biological assessment procedures. The lethal median dose (LD<sub>50</sub>) recorded for neem leaf and stem bark extracts were 31.62 and 489.90 mg/kg body weight respectively. Neem leaf and stem bark extracts reduced the level of parasitemia in infected mice by about 51 - 80% and 56 - 87% respectively.

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**Key words:** *Plasmodium berghei*, antimalarial screening, neem extracts, parasitemia, LD<sub>50</sub>

### INTRODUCTION

Malaria in man is caused by a protozoan parasite transmitted by *Anopheline* mosquitoes (Daily, 2006). There are four *Plasmodium* species that infect humans. They are *P. falciparum*, *P. ovale*, *P. malariae* and *P. Vivax* and they can be distinguished by morphology (Mendis, *et al.*, 2001; Wisner, 2009). *P. vivax* and *P. falciparum* are the most prevalent species worldwide, *P. malariae* are rare and not lethal but commonly found in parts of Africa and Papua New Guinea. *P. falciparum* is uniquely responsible for mortality and severe disease (Daily, 2006). Thirty-seven *Anopheles* species have been documented in the country as malaria vectors and the main parasite specie in Nigeria is *P. falciparum*. The transmission of malaria occurs in the entire country, but this transmission is all year round in a small part of the South of the country. In the remaining parts of the country the duration of the transmission is 3-10 months, from February to December (WHO/AFRO, 2002). If not treated promptly with effective medicines, malaria can cause severe illness that is often fatal (WHO, 2007). Although effective anti-malarial drugs are in the market, the disease remains a threat to people living in endemic areas who have no proper and prompt access to effective

drugs. Access to pharmacies and health facilities, as well as drug costs, are major obstacles.

Medicinal plants are part and parcel of human society to combat diseases, from the dawn of civilization (Biswas *et al.*, 2002). Plants have invariably been a rich source for new drugs and some antimalarial drugs in use today (quinine and artemisinin) were either obtained from plants or developed using their chemical structures as templates (Gessler *et al.*, 1994; Daily, 2006; Robert *et al.*, 2001; Wang *et al.*, 2001).

The Neem tree, *Azadirachta indica* (Meliaceae) is native to Southeast Asia and grows in many countries throughout the world (Ascher, 1993; Schmutterer, 1990). *Azadirachta indica* A. Juss (syn. *Melia azadirachta*) is well known in India and its neighbouring countries for more than 2000 years as one of the most versatile medicinal plants having a wide spectrum of biological activity. Every part of the tree has been used as traditional medicine for household remedy against various human ailments (Chatterjee and Pakachi, 1994; Chopra *et al.*, 1956; Chopra *et al.*, 1958; Kirtriker and Basu, 1975; Koul *et al.*, 1990). The tree is still regarded as 'village dispensary' in India. The importance of the neem tree has been recognized by the US National

Academy of Sciences, which published a report in 1992 entitled 'Neem – a tree for solving global problems' (Biswas *et al.*, 2002).

The resistance of the malarial parasites to chloroquine and other drugs prompts the search for chemotherapeutic agents with novel modes of action. Treatment of malaria with African Herbs and medicines has been in existence long before the arrival of western drugs (Abdul-Kareem, 2005). Many malaria victims worldwide, especially those living in developing countries, consult traditional healers, using medicinal plants to combat illness (Folb *et al.*, 2001). Medicinal plants researched to date were usually selected on the basis of their traditional use and reputation for efficacy in the treatment of malaria and other diseases. The few that are being studied currently and scientifically are Lemon grass in many parts of Nigeria, Neem leaves and barks in Northern Nigeria and the bark of Cherry mango in Southern Nigeria which have yielded positive results in reducing parasitemia (Abdul-Kareem, 2005).

## MATERIALS AND METHODS

### *Experimental Animals*

Mature Swiss albino mice (20-25g) of either sex free from infection, were obtained from the animal house facility from the department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria, Nigeria. They were stabilized for seven days in the laboratory before being used for experiments. The animals were maintained in a well ventilated room, with temperature of  $25 \pm 1^\circ\text{C}$ , fed on Excel feeds (Feed Masters Ilorin) and water ad libitum.

### *Parasite Strain*

Malaria parasite (*Plasmodium berghei*, Anka strain) was obtained from Prof. Jacob Golenser of Kuvin Medical Centre, Hebrew University of Jerusalem. The strain was maintained in the laboratory by serial blood passage from mouse to mouse.

### *Plant Collection*

Stem bark, seeds and leaves of *Azadirachta indica* (Neem) were collected from National Research Institute for Chemical Technology, Zaria and were identified at the Herbarium unit, Biological science Department, Ahmadu Bello University, Zaria, Nigeria.

### **Experimental procedures**

#### *Methanolic extract preparation*

The stem bark and leaf were cut into smaller pieces and dried in the laboratory and dried seeds collected, were processed and the kernel removed, then the samples were powdered and kept at room temperature in a well-closed amber coloured bottle until extracted. Hundred grammes of each sample were extracted by mixing with 500 ml methanol thoroughly shaken, and left for twenty four hours at room temperature. The extracts were then filtered and concentrated under vacuum in a rotatory evaporator which yielded about 18 % of gummy residue, as extracts of plant parts. The extracts were kept in a tightly closed bottle in a refrigerator until used for anti-malarial testing.

#### *Inoculation of Mice and parasite counting*

A donor mouse with a rising parasitemia of 20 % was sacrificed and its blood was collected in heparinized

syringe and diluted in phosphate buffered saline to  $10^8$  parasitized erythrocytes/ml. The infection of mice was initiated by needle passage of the *Plasmodium berghei* parasite preparation from the donor mouse to healthy test mice via an intraperitoneal route (Peter and Anatoli, 1998). Parasitemia was monitored by microscopic Giemsa-stained thin blood smears. The number of parasitized erythrocytes in each of the ten such fields was counted thrice and the average was computed to give the level of parasitemia of each mouse (Peter and Anatoli, 1998; Dawit *et al.*, 2006).

#### *Antimalarial Screening of Neem Extracts*

The antimalarial screening of neem extracts was carried out using the following assessment methods:

##### *Primary biological assessment of neem extracts efficacy:*

Thirty two mice were used and they were divided into eight groups (A, B, C, D, E, F, G and H) of four each. The mice were infected by method described by Peter and Anatoli (1998) and David, *et al* (2004), with 0.2ml of diluted ( $10^8$  parasitized erythrocytes/ml) infected blood each intraperitoneally (i.p) except those in group H that form normal control group. Treatment of mice in experimental groups A to F commenced 2 h after infection at a dose of 10mg/kg body weight and treatment was repeated daily with the same dose for 72 h. The Neem extracts were dissolved in dimethyl sulfoxide (DMSO) and 13% Ethanol.

Group A – Control (infected with no treatment)

Group B – Treated with chloroquine (CHQ) (10mg/kg)

Group C – Treated with Artheeter (ATM) (10mg/kg)

Group D – Treated with Quinine (QUI) (10mg/kg)

Group E – Treated with Neem leaf extract (NLE) (10mg/kg)

Group F – Treated with Neem stem bark extract (NBE) (10mg/kg)

Group G – Treated with Neem seed extract (NSE) (10mg/kg)

Group H – Not infected (normal control treated with DMSO)

Twenty four hours after the last treatment (96h post-infection) blood smears from all animals were prepared and stained with Giemsa stain. Parasitemia level was determined microscopically by counting 10 fields of approximately 100 erythrocytes per field. The difference between the mean values of the experimental group was calculated and expressed as percent Parasitemia inhibition, using the following equation:

$$\% \text{ Inhibition} = \frac{\text{Parasitized RBC in Negative control} - \text{parasitized RBC in study group}}{\text{Parasitized RBC in Negative control}} \times 100$$

*Acute toxicity studies:* Albino mice (20-25g) of either sex were used. The median lethal dose ( $\text{LD}_{50}$ ) was determined for each of the neem leaf and neem stem bark extracts using the method previously described by Lorke (1983). In the first phase, the mice were divided into three groups with three mice in each group and were administered with the methanol stem bark extract at doses of 10, 100 and 1000 mg/kg body weight respectively via the intraperitoneal route. The mice were then observed for signs of toxicity and death for 24h. In the second phase, groups of one mouse each were treated with more specific doses of the extract respectively depending on the result

obtained from the first phase and observed for signs of toxicity and death in 24h. The final LD<sub>50</sub> was calculated as the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived.

LD<sub>50</sub> =  $\sqrt{\text{mean of the lowest dose that caused death and the highest dose for which the animal survived.}}$

Matsumura (1975) and Corbett *et al.* (1984) classified chemicals based on their LD50 values as follows:

Extremely toxic	LD <sub>50</sub> ≤ 1 mg/kg
Highly toxic	LD <sub>50</sub> 1-50 mg/kg
Moderately toxic	LD <sub>50</sub> 50-500 mg/kg
Slightly toxic	LD <sub>50</sub> 500-5000 mg/kg
Practically non-toxic	LD <sub>50</sub> 5000-15000 mg/kg
Harmless	LD <sub>50</sub> > 15 g/kg

Deriving from the outcome of the acute toxicity studies, dosage ranging from 20 – 50 mg/kg body weight for both extract of neem leaf and stem bark were screened for secondary biological activities from which the doses that gave optimum and consistent results were selected. The graded doses used throughout the experiments reported in this study were 20 mg/kg for neem leaf and 50 mg/kg body weight for neem stem bark extract.

*Secondary biological assessment of Neem Extracts efficacy (David et al., 2004)*

The Neem extracts showing good in vivo activity in the primary 4-day suppressive test were evaluated further using secondary in vivo models. Mice maintenance, infection, drug preparation and application, and assessment of parasitemia were same as for the basic 4-day treatment protocol. These secondary in vivo methods involved dose ranging test, in which different dose of the neem extract was used for different group. Fifty two mice were used in this group and they were divided into thirteen groups of 4 per group.

Group A – Control (infected with no treatment)

Group B – Treated with chloroquine (10mg/kg)

Group C – Treated with Artheeter (10mg/kg)

Group D – Treated with Quinine (10mg/kg)

Group E1 – Treated with 20mg/kg Neem leaf extract

Group E2 – Treated with 30mg/kg Neem leaf extract

Group E3 – Treated with 40mg/kg Neem leaf extract

Group E4 – Treated with 50mg/kg Neem leaf extract

Group F1 – Treated with 20mg/kg Neem bark extract

Group F2 – Treated with 30mg/kg Neem bark extract

Group F3 – Treated with 40mg/kg Neem bark extract

Group F4 – Treated with 50mg/kg Neem bark extract

Group G – Normal Control (not infected)

Twenty four hours after the treatment, blood smears were made from tail-bleeding of treated and untreated mice stained with Giemsa. The parasite reduction was determined.

*Therapeutic Effects of Neem Extracts:* This was carried out following the method of David *et al* (2004) with some slight modifications. Twenty four mice were used for this experiment. Mice maintenance, infection, drug preparation and application were same as for the basic 4-day treatment protocol. Treatment commenced on day four when parasitemia was well observed in the infected mice and percentage parasitemia was assessed beginning from 24 h post treatment. Twenty milligram per kilogram body weight of NLE and 50 mg/kg body weight NBE were used for the treatment.

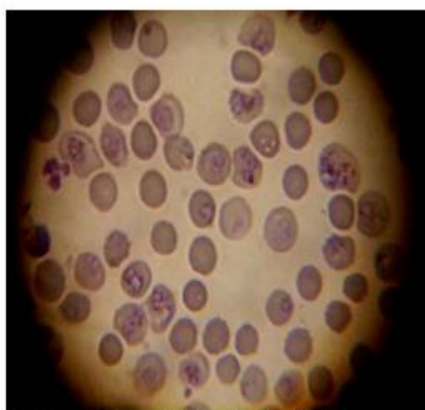
*In vitro treatments of parasitized red blood cell:* The infected blood was incubated with 10mg/kg concentration of each tested standard drugs (CHQ, ATM, and QUI), 20 mg/kg NLE and 50 mg/kg body weight NBE at 37°C for 30mins. This was injected intraperitoneally into each test mouse. The % parasitemia reduction was determined as earlier.

## RESULTS

### *Development of Parasitemia*

Albino mice were experimentally infected with about  $1 \times 10^7$  *Plasmodium berghei* intraperitoneally. They started showing noticeable parasitemia on day 3-post infection (p.i)

(Figure 1). About 30% parasitemia was recorded before the death of the animals. Microscopic views of Giemsa stained thin blood smears showed the *P. berghei* parasites as intracellular parasites, which caused enlargement of infected cell and produced stainable alteration on the red cell membrane (Plate 1). Clinical signs observed during the course of treatment ranged from diarrhea, anaemia, loss of appetite, loss of weight to cerebral hemorrhage (Plate 2) and splenomegaly (Plate 3).



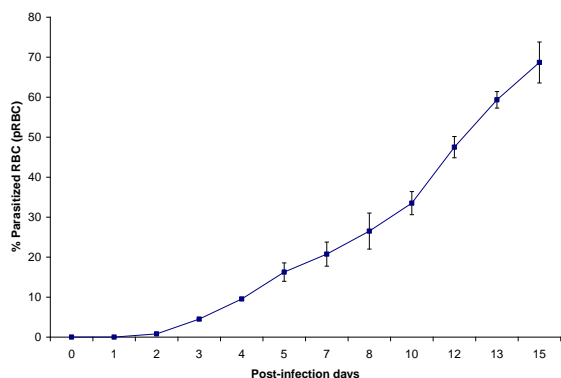
**Plate 1 Parasitized RBC of a donor mouse**



**Plate 2: Cerebral haemorrhage of mouse infected with *P.berghei***



**Plate 3: Micrograph showing splenomegaly**



**Figure 1:** Percentage Parasitized RBC (pRBC) during course of infection

#### Antimalarial Screening of Neem Extracts

At the initial trials of 4-day suppressive test, the animals treated with 10mg/kg/day (i.p) of the three different neem extracts (leaf, stem bark and seed kernel) showed lower parasitemia compared to untreated infected animals (Figure 2) indicating a significant antimalarial activity against *Plasmodium berghei*. The stem bark extract is significantly more effective than others. However, the neem seed extract was very toxic to the animals, and all animals in this group died within six days. Some of them showed signs of non-coordination and somersaulting before death. The neem stem bark and the neem leaf recorded about 55.83% and 50.74% parasitemia reduction respectively (Figure 2). The standard drugs recorded 85.49%, 73.39 and 29.85% for arthemeter (ATM), quinine (QUI) and chloroquine (CHQ) respectively. The value recorded for chloroquine showed that the parasite is chloroquine resistant specie. The antimalarial activities of the three neem extracts were not as good as that of some standard drugs (ATM and QUI) but better than that of chloroquine.

#### Acute Toxicity Study and Behavioural Effects of Neem Extracts

Acute toxicity test of the neem leaf extract at concentration of 100 and 1000 mg/kg produced mortality after 24 h of observation. The median lethal dosage (LD<sub>50</sub>) of this extract was 31.62 mg/kg. However, the animals were observed to experience slow movement within the first six hours of administration of the extracts. The neem bark did not show any mortality for the 100mg/kg dose and it recorded a higher lethal dosage of 489.90 mg/kg (Table 1). According to Matsumura (1975) and Corbett *et al.* (1984), the neem leaf is highly toxic while the stem bark is classified as moderately toxic. The neem seed extract was lethal at 10 mg/kg and therefore its use was discontinued.

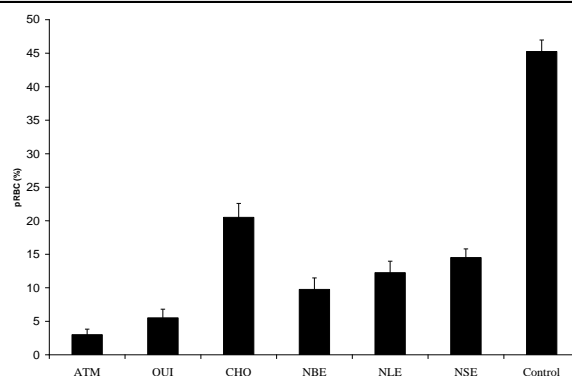
The treatment of animals with different dosages of methanolic neem extracts showed a different response to increase in dosage in line with protection against infection and survival. The optimal dosage recorded for neem leaf extract was 20mg/kg/day i.p as against higher dosages (Figure 3). This dosage conferred higher parasitemia inhibition for longer days up to twenty eight days by protecting the animals against earlier infection as compared to other dosages. The other dosages used were not as effective compared to chloroquine treated animals because they died earlier than even the chloroquine treated ones at

day 14 and 16 respectively with lower parasitized RBC. The death of the animals may be as a result of toxicity of the extract rather than parasitemia load. On the other hand, neem stem bark extract showed increase in parasitemia inhibition with increase in dose administered on the infected animals (Figure 4). It showed a dose dependent inhibition of parasitemia and even at the higher dosages the animals survived longer than chloroquine treated ones. The therapeutic effect of the leaf and stem bark extracts of neem on the infected animals showed that, the animals responded to the extracts within the first seven days of treatment with about 60% reduction (Figure 5) in parasitemia before the parasitized RBCs started to increase which later resulted in the deterioration of the health condition of the animals.

The results of the in vitro treated parasitized red blood cell in mice are shown in Figure 6. The in vitro treatment showed delayed of parasitemia and survived longer than those infected with unincubated infected blood. Some of the chloroquine treated mice survived beyond 30 days and the untreated blood survived for 28 days while arthemeter and quinine treated mice showed total parasite clearance at day 14 and 16 respectively with their parasitized RBCs not exceeding 3.66 and 4.20% respectively. The neem extracts also prolonged the survival of the mice with reduction of parasitized RBCs ranging from 32.09 to 35.22%. Two mice among the ones treated with neem leaf extract survived for about five months before they were sacrificed for further analysis. It was observed that the mice were at their early pregnancy stage when the treatment was administered, while those at the late or middle stage of their pregnancy died within forty-five days.

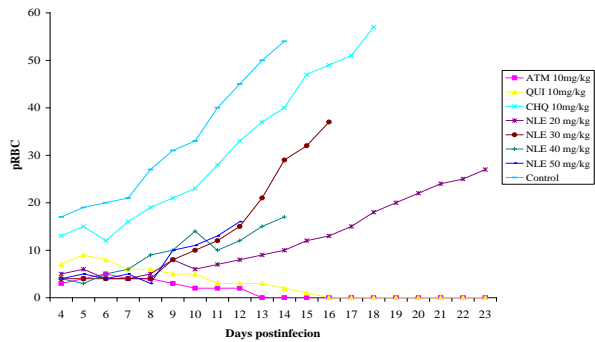
**Table 1:** Lethal dose (LD<sub>50</sub>) of the Methanol extracts of Neem leaf and Stem bark Extracts

Extract	LD <sub>50</sub> value (mg/kg)	Verdict
Neem leaf extract (NLE)	31.62	Slightly toxic
Neem Stem bark (NSE)	489.90	Moderately toxic



Key: NLE- Neem leaf extracts, NBE – Neem stem bark extract, NSE- Neem seed extract, ATM- Artemether, QUI- Quinine, and CHQ- Chloroquine

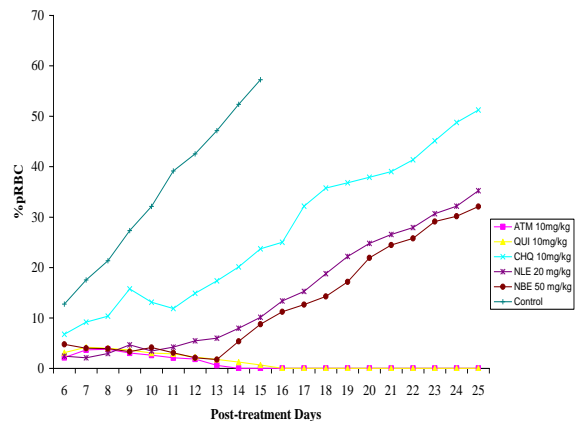
**Figure 2:** Antimalarial activity of Neem extracts and Standard drugs against *P. berghei* Infected Mice using Peter's four-day suppressive test



**Figure 3:** Effect of Different Doses of Neem Stem Bark Extract on Parasitized RBC of *P. berghei* infected Mice.

**Key:** NBE – Neem Leaf extract, ATM- Arteether, QUI- Quinine, and CHQ- Chloroquine

**Note:** Treatment was commenced 2 h after infection and observation was stopped at the death of the animals except in the case of ATM and QUI treatments when there was no detectable Parasitemia.

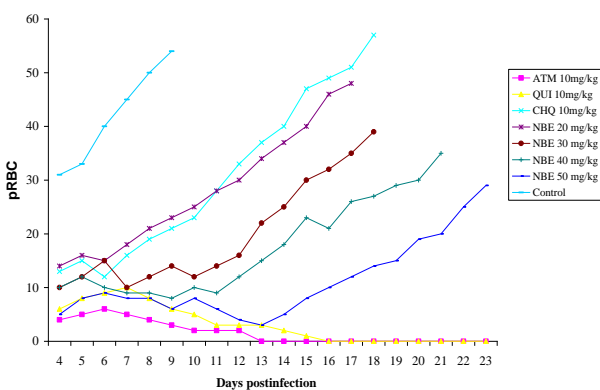


**Figure 6:** Effect of in vitro treated pRBC on the the level of parasitemia in *P. berghei* infected mice.

### DISCUSSIONS

The 4-day suppressive test commonly used for antimalarial screening and the determination of percent inhibition of parasitemia is the most reliable parameter (Dawitt *et al.*, 2006). A mean group parasitemia level of less than or equal to 90%, that of mock-treated control animals usually indicates that the test compound is active in standard screening (Peter and Anatoli, 1998). Therefore, it is clear from the results obtained (Fig. 1- 5) in this work that in *Plasmodium berghei* infected mice treated with extracts of neem leaf and stem bark, the percentage of parasitemia measured changed significantly ( $p \leq 0.05$ ) from those in the non-treated infected mice. This significant suppression of parasitemia by the extracts of neem leaf and neem stem bark is in agreement with the inhibition of parasitemia potential of neem extracts reported by earlier workers (Ekanem, 1978; Isah *et al.*, 2003; Odetola and Basir, 1986; Tella, 1977; Udeinya *et al.*, 2008; Ukaga *et al.*, 2006). Usha *et al* (2001), tested aqueous extracts of *Azadirachta indica* (bark), in vivo against *P. berghei* following Peter's 4-day test and recorded about 70% parasitemia inhibition. The antimalarial activity of neem leaf extract has also been reported by Udeinya *et al* (2008) to have both schizonticidal and gametocytocidal activities. They reported a crude acetone/water (50/50) extract of neem leaves activity against the asexual (trophozoites/schizonts) and the sexual (gametocytes) forms of the malarial parasite, *Plasmodium falciparum*, in vitro to reduce parasite numbers by less than 50% of the numbers in control cultures. The oil from the neem seed was also reported to possess larvicidal effects on the malarial vector *Anopheles gambiae* by inhibiting adult emergence by about 50% at a concentration of 6 ppm (Okumu *et al.*, 2007).

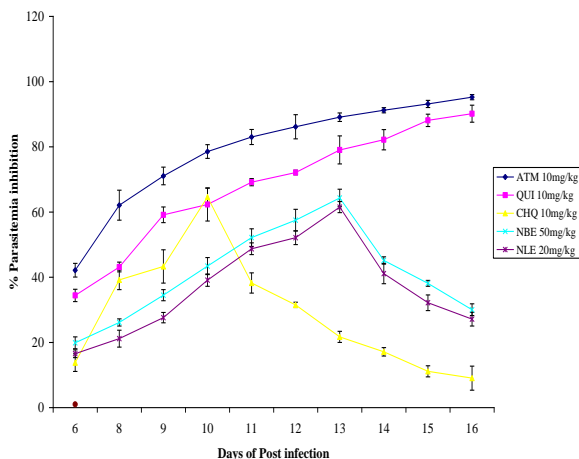
Also, other workers have reported earlier the antimalaria activity of different parts of neem tree (leaf, stem bark and seed) against *Plasmodium berghei* and *Plasmodium falciparum* responsible for causing mammalian malaria (Udeinya, 1993; Osujih, 1993; Udeinya *et al.*, 2006; Khalid *et al.*, 1986; Khalid *et al.*, 1989). It was reported that, components of alcoholic extracts of leaves and seeds are effective against both chloroquine-resistant and sensitive strains of malarial parasite (Badani *et al.*, 1987). Therefore, the results obtained from this research are in agreement



**Figure 4:** Effect of Different Doses of Neem Leaf Extract on Parasitized RBC of *P. berghei* infected Mice.

**Key:** NBE – Neem stem bark extract, ATM- Arteether, QUI- Quinine, and CHQ- Chloroquine

**Note:** Treatment was commenced 2 h after infection and observation was stopped at the death of the animals except in the case of ATM and QUI treatments when there was no detectable Parasitemia.



**Figure 5:** Therapeutic Effect of methanolic extracts of Neem leaf and stem bark on Albino mice infected with *P. berghei*

**Note:** treatment was started six days post infection.

with the findings of earlier documented results. It showed that, neem extracts possess antimalarial properties.

The neem stem bark and neem leaf recorded about 56-87% and 51-80% parasitemia inhibition respectively. The neem leaf extract parasitemia inhibition was highest at a treatment concentration of 20 mg/kg body weight of which higher concentration resulted in the earlier death of the animal than even the animals treated with chloroquine. On the other hand, the higher the doses of neem stem bark used for the treatment of the *Plasmodium berghei* infected mice, the higher the percent parasitemia inhibition. This is supported by the report of Usha *et al* (2001) who recorded about 60-80% parasitemia inhibition using 200mg/kg body weight of aqueous extracts of neem stem bark in the treatment of *P. berghei* infected mice through oral route. These results indicated that neem extracts can be used in the treatment of malaria. The *P. berghei* infected mice treated with neem seed extract died within six days as a result of toxicity of the extracts rather than as a result of the parasite load since the level of parasitemia in the animals was low. This was because the animals were observed to exhibit loss of coordination that resulted in convulsion, loss of appetite, paralysis, unconsciousness and death. Hence, this extract was not used for further experiments.

However, during the treatment, those animals infected with in vitro treated infected red blood cells had their parasitemia reduction greater than those treated with primary antimalarial screening (Peter's 4-days suppressive treatment) which also had higher parasitemia inhibition than the therapeutic treatment. The control in the group of in vitro test survived for about 30 days before they all died. The therapeutic test also proved that the animals responded to treatment using the neem extracts when compared to those treated with chloroquine and the non-treated infected mice. The observed antimalarial activity in this study is consistent with the traditional use of the plant as herbal medication against the disease in Nigeria.

When a standard antimalarial drug is used in mice infected with *P. berghei*, it suppresses parasitemia to non detectable levels (Kiseko *et al.*, 2000), which is in agreement with the effects of arthemeter and quinine used in this study. The protection of the infected animals from losing body weight by the extracts of the neem leaf and stem bark was observed during the course of this study when compared with the weight loss in the untreated infected mice. Artemeter is a semi-synthetic derivative of artemisinin, the active constituent of the plant, *Artemisia annua*. It is a fast acting, schizontocidal agent which attacks at the erythrocytic stage of malaria in blood (Yarnell and Abascal, 2004). The antimalarial effect of neem extracts seem to be closely related to the effect of quinine and artemisinin than chloroquine. The anti-plasmodial effect of neem components was also observed on parasites previously shown to be resistant to other anti-malarial drugs, like chloroquine and pyrimethamine suggesting a different mode of action (Dhar *et al.*, 1998). Chloroquine is said to operate against asexual forms of pathogenic malaria parasites called the "hemo-schizontocidal effect" (Raynes, 1999). Meanwhile, quinine acts by blocking the

polymerization of haem to haemozoin (malarial pigment) (Zhang *et al.*, 1999).

Acute toxicity test gives clues on the range of doses that could be toxic to the animal. It could also be used to estimate the therapeutic index ( $LD_{50}/ED_{50}$ ) of drugs and xenobiotics (Rang *et al.*, 2001). The intraperitoneal acute toxicity values ( $LD_{50}$ ) in mice for the extracts indicated that the neem leaf recorded median lethal dose ( $LD_{50}$ ) of 31.62 mg/kg body weight of which higher dose used in the treatment of the infected mice resulted in earlier death of the animals. This recorded  $LD_{50}$  is much lower than that reported by Pennington and Syles (1975). They reported that intravenously administered aqueous leaf extracts of neem at a dose greater than 40mg/kg body weight produces toxic manifestation leading to death in guinea pig while Kanugo (1996) reported the extract of neem leaf to exhibit oral toxicity in mice, showing signs of ill health and discomfort, gastrointestinal spasms, hypothermia and terminal convulsions, leading to death. These symptoms were observed in mice treated with 40-50mg/kg body weight. The difference in the  $LD_{50}$  values of the neem extracts may be accounted for different methods of extraction, and by the reason given by Lorke (1983), that acute toxicity test on the same extract or drug yielded different values from laboratory to laboratory. However, the  $LD_{50}$  value recorded for neem stem bark (425mg/kg body weight intraperitoneal) was closer to what was reported (390mg/kg body weight intraperitoneal) in mice by the United state patent 4515785 but lower than the one reported by other workers who claimed that neem stem bark has  $LD_{50} >1000$  mg/kg body weight through intraperitoneal. Kanugo (1996) reported the methanolic extract of neem stem bark demonstrated oral toxicity at  $LD_{50}$  of about 13g/kg on mice. The different reports in acute toxicity may be due to difference in the route of administration and the variation in the active components of the neem tree which differ from location to location. Also, it was suggested by (Jutamaad *et al.*, (1998), that oral administration is about 100 times less toxic than intraperitoneal. According to Matsumura (1975) and Corbett *et al.* (1984), the neem leaf extract can be classified as highly toxic while the neem stem bark as moderately toxic.

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Source of support: Nil; Conflict of interest: None declared