# *IN-VIVO* ANTI-PLASMODIAL ACTIVITY AND IN-VITRO ANTIOXIDANT PROPERTIES OF METHANOLIC LEAF EXTRACT OF *AZADIRACHTA INDICA* AND ITS POSITIVE EFFECT ON HEMATOLOGICAL AND LIPID PARAMETERS IN SWISS ALBINO MICE INFECTED WITH *PLASMODIUM BERGHEI NK 65.*

***Momoh. J.***
***Longe. A.O***
Department of Science Laboratory Technology (Biochemistry Unit),
School of Technology, Lagos State Polytechnic, Ikorodu, Lagos, Nigeria
***Aina. O.O.***
***Ajibaye.O.***
Department of Biochemistry, Nigerian Institute of Medical Research (NIMR),
Yaba, Lagos, Nigeria

**Abstract**

The study was conducted to determine the effect of in vivo anti-plasmodial and in vitro antioxidant properties of methanolic leaf extract of *Azadirachta indica* and its positive effect on hematological and lipid parameters in Swiss albino mice infected with *Plasmodium berghei NK65.* Swiss albino mice were inoculated intraperitoneally with *Plasmodium berghei NK65*. The mice were grouped into six groups, five per group. Group I were not infected with *P.berghei*, Group II and III served as both the negative and positive control while Group IV, V, and VI were treated with 200, 400, and 800 mg/kg body weight of methanolic leaf extract of *A. indica*. The secondary metabolites in the extract include tannin, flavonoids, glycosides and saponin etc. The extract does not have any toxic effect that can lead to the death of the animals. The median lethal dose $LD_{50}$ was estimated to be >5000mg/Kg body weight. The extract caused 47.80%, 50.96% and 52.30% suppression in parasitaemia at 200, 400 and 800mg/kg body weight respectively while Chloroquine exerted 100% suppression at 5mg/kg body weight. The curative test shows that the different concentration of the extract exert a growth inhibition of 50.1%, 74.57% and 73.68% at 200, 400, 800mg/kg body weight respectively while Chloroquine cleared the parasites by 94.07% at 5mg/kg body weight. The Hematological parameters showed that the extract is not hematotoxic since it significantly increase ($P<0.05$) RBC, HGB, and HCT values while their WBC count reduced significantly when compared to the infected untreated mice. There is a significant decrease ($P<0.05$) in plasma TC, TG and LDL-C in the treated groups and their HDL-C significantly increase when compared to infected untreated group. This study shows that *A.indica* extract has hypolipidemic effect. In the in-vitro antioxidant assay, the extract significantly increase ($P<0.05$) the level of SOD, CAT and GSH in the liver homogenate induced with oxidative stress using $H_2O_2$ while the MDA values reduced significantly with the administration of the extract of *A.indica.*

**Keywords:** *Azadirachta indica,* Anti-plasmodial activity, hematological and lipid parameters, in-vitro antioxidant properties and *Plasmodium berghei NK 65* infected Swiss mice

## Introduction

Malaria is a disease caused by Plasmodium species, is one of the oldest and greatest health challenges affecting 40% of the world's population (Greenwood, 2002). About 300-500 million clinical cases is observed and 1.2–2.8 million deaths occur each year due to malaria. (Linares and Rodriguez , 2007 and Sahu *et al* 2008). Severe malaria is a complex multisystem disorder. Complications such as cerebral malaria, anemia, acidosis, jaundice, coagulation anomalies, respiratory distress, renal insufficiency, and hyperparasitemia can occur in severe malaria (Penet *et al* 2005).Some of the problems associated with malaria are the control of mosquitoes which transmits malaria which is made difficult by their resistance to a wide range of insecticides. Secondly, is the production of fake anti malarial drugs. Thirdly, most of the Plasmodial parasites are resistant to most widely available and affordable drugs like Chloroquine and Fansidar (Kisame,2005). Lastly, most countries in Africa lack the necessary infrastructure and resources to manage and control malaria (WHO, 1994).

*Plasmodium berghei* have been used in studying the activity of potential anti-malarials in mice (Pedronic,*et al*.2006 ) and in rats (English, *et al* 1996 ). It produces diseases similar to those of human plasmodium infection (Kumar *at al*, 2006, Peter and Anatoli 1998 )

*Azadirachta indica,* commonly known as Neem, is found in Nigeria and in most of the tropical and subtropical countries and is widely distributed in the world. The taxonomic classification of *Azadirachta indica* is as follows: Kingdom *Plantae*, Order: *Rutales*, Suborder: *Rutinae*, Family: *Meliaceae*, Subfamily : *Melioideae*, Genus : *Azadirachta*, Species: *indica* (Girish *et al*, 2008). All parts of the plant are useful and have been used in treatment of diseases ranging from teeth decay, swollen liver, ulcers, dysentery, diarrhea, malaria and other bacterial infections. (Allameh et al., 2002 and Mossini et al., 2004). Studies have shown that the generation of reactive oxygen and nitrogen species (ROS and RNS) associated with oxidative stress, plays a crucial role in the development of systemic complication caused by malaria. Atamma *et al* 1993 observed that erythrocytes infected with *Plasmodium falciparum* produced $OH^-$ radical and $H_2O_2$ about twice as much compared to normal erythrocytes. Malaria infection induces the production of hydroxyl radicals ($HO^-$) in the liver, which may be the reason for the induction of oxidative stress and apoptosis (Guha, 2006).

## Materials and methods

### Collection and identification of Plant extract

The leaves of *Azadirachta indica* were obtained from Ikorodu in Lagos State, Nigeria. The plant was authenticated by Miss Shokefun a botanist from Science Laboratory Technology Department, Environmental biology Unit, Lagos State Polytechnic, Ikorodu.

### Preparation of methanolic leaf extract of Azadirachta indica

The leaves of *Azadirachta indica* were washed, air dried under shade in the Biochemistry Laboratory, pulverised to coarse power using blender. Extraction was carried out by dispersing 200g of the grounded *A. indica* plant material in 1L of 80% ethanol and shaking was done with GFL shaker for 72 hours. This was followed by vacuum filtration and concentrated by rotary evaporator at a temperature not exceeding $40^oC$. The concentrated extract was dried to complete dryness in an aerated oven at $40^oC$ for 48 hours. The extract was latter stored in a refrigerator at $4^oC$.

### Phytochemical analysis

Phytochemical analysis for phytochemical constituents were carried out on the methanolic extract of *Azadirachta indica* using standard phytochemical procedures (Sofowora (1993), Harborne (1973), Trease and Evans (1985).

**Sources of Swiss albino mice**

Eight (8) weeks old Swiss albino mice weighing 24- 35g were obtained from Nigeria Institute of Medical Research (NIMR), Lagos, Nigeria. These animals were maintained under laboratory conditions of temperature (22 to 24°C), humidity (40 to 60%) and 12 h light/12 h dark regime at NIMR animal house. They were acclimatized for three days, housed in plastic cages with saw dust as beddings; they were exposed to both food and water *ad libitum* for the entire duration of the study. The mice were used in accordance with NIH Guide for the care and use of laboratory animals; NIH Publication revised (1985) NIPRD Standard Operation Procedures (SOPs).

**Acute toxicity test**

The acute toxicity test of nethanolic leaf extract of *Azadirachta indica* was carried out using modified Lorkes method (1993). Eighteen Swiss albino mice weighing 24 to 35 grams were randomized into three groups of six mice each and were given 1600, 2900 and 5000mg/Kg body weight of the extract orally. They were observed for signs of toxicity and mortality for the first critical four hours and thereafter daily for 7 days. The oral median dose $LD_{50}$ was calculated

**Grouping of animals for infection and treatment**

The parasite *Plasmodium berghei NK 65* was obtained from Nigeria Institute of Medical Research (NIMR), Lagos, Nigeria from Dr Aina, O.O. The parasites were kept alive by continuous intraperitoneal inoculation of known amount of the parasite into Swiss mice. Iml of blood was taken from donor mice and diluted with 5ml phosphate buffer; such that 0.1ml contained standard inoculum of $1^{7}$ infected red blood cells (Maegraith *et al*, 1952). Thirty acclimatized Swiss albino mice were randomly selected and twenty five Swiss mice were inoculated intraperitoneally from the same source to avoid variability in parasitemia. The mice were randomly distributed into six groups of five per group as shown below:

GROUP I (Normal control) Healthy uninfected Swiss mice

GROUP II (Negative control) mice infected with *P.berghei NK65* without treatment

GROUP III. (Positive control) = *P.berghei* +5mg/kg b,wt of Chloroquine (Standard drug)

GROUP IV= *P. berghei* + 200mg/kg b.wt of *Azadirachta indica* extract.

GROUP V= *P. berghei* + 400mg/kg b.wt of *Azadirachta indica* extract.

GROUP VI=*P. berghei* + 800mg/kg b.wt of *Azadirachta indica* extract.

**Anti-plasmodium studies**

**Suppressive test**

The Peter's 4-day suppressive test against *P. berghei NK65* infection in Swiss mice was used (Peters, 1965). Adult Swiss mice weighing between 24 to 35gram were inoculated by intraperitonial injection with standard inoculum of *Plasmodium berghei NK65* with $1^{7}$ infected red blood cells. The mice were divided into six groups as shown above and treated for 4 consecutive days with 5mg/kg.b.wt of Chloroquine, 200, 400, and 800mg/kg body weight of *Azadirachta indica* extract orally daily. On day 5 of the experiment, blood was collected from the tail of each mouse and smeared onto microscope slide to make a film. The blood films were fixed with methanol, stained with Geimsa at pH 7.2 for 10 minutes and examined under the microscope for the presence of parasites. The parasite density was calculated for each group by comparing the parasitaemia in infected group (Group II) with those of control and treated groups..

**Curative test**

The Curative test of methanolic leaf extract of *Azadirachta indica* on another fresh infected Swiss albino mice were carried out according to the method described by Ryley and Peters, 1970. The Swiss mice were injected intraperitoneally with standard inoculums of $1\times10^7$ *Plasmodium berghei NK 65* infected erythrocytes on the first day (day 0). Seventy two hours later, thirty mice were divided into six groups of five mice per group as shown above. The treated groups were orally treated with 5mg/kg b.wt of chloroquine, 200,400 and 800mg/kg b.wt of *Azadirachta indica* respectively. The treatment was carried out once daily for 5 days, on each day of the treatment, blood was collected from the mice tail and smeared onto microscope slide to make thin and thick films. The blood films were fixed with methanol, stained with 10% Giemsa at pH 7.2 for 10 minutes and examined microscopically to monitor the parasitaemia level. The parasite density was calculated for each group over a period of six days.

**Hematological analysis**

The Swiss albino mice in the curative assay groups were sacrificed and their bloods were collected in EDTA bottles by ocular puncturing. The bloods in the EDTA bottles were assayed for hematological parameters using BC -3200 Auto Hematology Analyzer in Lagos University Teaching Hospital in Lagos- Nigeria.

**Lipid analysis**

The Swiss mice used for Curative test were sacrificed and their blood were collected by ocular puncturing in an heparinized bottles, the blood were centrifuged for 10 minutes and the plasma removed for lipid assay. Total cholesterol (TC), Triglyceride (TG) and High Density lipoprotein-Cholesterol (HDL-C) were assayed in the plasma using Randox Kits (Trinder,1969, Tietze, 1990 and NIHCDCS, 1992). Low density Lipoprotein-Cholesterol (LDL-C) were all calculated using formulae (Friedewald *et al*, 1972).

***In-vitro* antioxidant assay**

Two new healthy albino rats wistar strain with average weighing of 220 gram were scarified and their liver were removed and used for the assay. One gram of albino rat liver slice was taken in 4ml sterile PBS in flat bottom flask. $H_2O_2$ at 0.5M concentration was used as an oxidant for the induction of oxidative stress in the liver slices. 100µl of the methanolic leaf extract of *Azadirachta indica* were added and later incubated for 1 hour at $37^oC$

Group A: 1g of untreated liver slice (negative control)
B: 1g of liver slice + $H_2O_2$ (positive control)
C: 1g liver slice + 200mg of *Azadirachta indica* extract
D: 1g liver slice + $H_2O_2$ + 200mg of *Azadirachta indica* extract
E: 1g liver slice + $H_2O_2$ + 400mg of *Azadirachta indica* extract
F: 1g liver slice + $H_2O_2$ + 800mg of *Azadirachta indica* extract

After incubation for 1 hour, an homogenate was prepared from the slices using 10% (W/V) PBS buffer. The homogenate was centrifuged at 1500rpm for 20 minutes to clarify the debris and the supernatant was used for the in-vitro antioxidant assay.

**Estimation of Lipid peroxidative (LPO) indices**

Lipid peroxidation as evidenced by the formation of thiobarbituric acid (TBARS) and malondialdehyde (MDA) . These parameters were measured in the liver homogenate by the method of Niechaus and Sameulsson 1968 and Jiang *et al*, 1992.

**Estimation of superoxide dismutase (SOD)**

The homogenate was assayed for the presence of SOD by utilizing the technique of Magwere *et al* 1997 with slight modification.

**Estimation of catalase (CAT)**

The liver homogenate was assayed for catalase colorimetrically at 620mm and expressed as μmoles of H2O2 consumed/min/mg protein as described by sinha,1972.

**Estimation of Reduced glutathione (GSH)**

Reduced glutathione (GSH) was determined in the liver homogenate using the method of Ellman, 1959.

**Data analysis**

Data analysis was done using the Graph Pad prism computer software. Student's'-test and one-way analysis of variance (ANOVA) were used for comparison. A *P*-value < 0.05 was considered significant.

**Results**

The results obtained from the phytochemicals analysis of *Azadirachta indica* extract showed the presence of some secondary metabolite like tannins, saponins, flavonoids, protein, glycoside, reducing sugar and fats and oil. (Table I). The presence of these secondary metabolites in this extract may be responsible for the anti-plasmodial activity of the extract.

Table 1: The phytochemical constituents of the methanolic leaf extract of *Azadirachta indica*.

| **Phytochemical components** | **Qualitative abundance** |
|---|---|
| Tannins<br>Ferric Chloride test | ++ |
| Saponins<br>Frothing test | ++ |
| Alkaloids<br>Wagners test | +++ |
| Steroids<br>Salkowiski test | - |
| Terpenoids | + |
| Flavonoids<br>Ammonium test | + |
| Test for protein<br>Millions test<br>Biuret test | <br>+<br>+ |
| Test for fat and oil | ++ |
| Test for reducing sugar | ++ |
| Glycoside | + |

+ (Present in low concentration), ++ (present in moderate concentration) and -(absent)

**Azadirachta indica toxicity test**

The mice were monitored for four hours, but no signs of toxicity were observed. The behavioural and physical observations revealed no involuntary urination, muscle weakness, and convulsion. The animals were physically active for the first four hours.

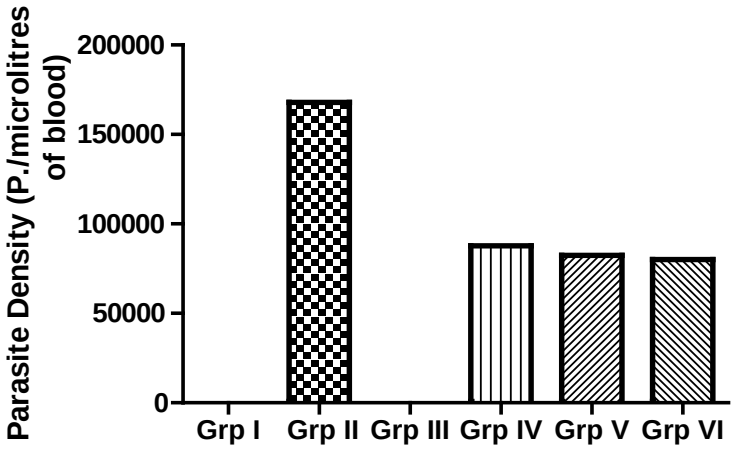


**Figure I.Suppresive test showing the effect of methanolic leaf extract of *A,indca* on Swiss mice infected with *Plasmodium berghei NK65*.**

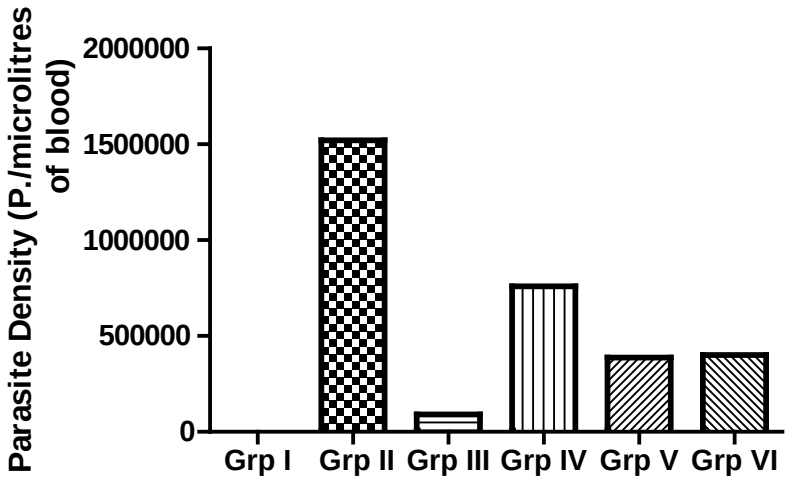


**Figure 2.Curative test showing the effect of methanolic leaf extract of *A.indica* on Swiss mice infected with *Plasmodium berghei NK65*.**

The methanolic leaf extract of *A.indica* caused 47.80%, 50.96% and 52.30% suppression in parasitaemia of *P.berghei NK65* infected mice at 200, 400 and 800mg/kg body weight respectively (Figure 1), while Chloroquine, a standard anti-malarial drug used exerted 100% suppression at 5mg/kg body weight. The curative test shows that the different concentration of the extract of *A. indica* exert a growth inhibition of 50.1%, 74.57% and 73.68% at 200, 400, 800mg/kg body weight of the extract respectively while Chloroquine, the standard drug, cleared the parasites by 94.07% at 5mg/kg body weight (Figure 2).

**Table 2**: Curative test showing the effect of methanolic leaf extract of *Azadirachta indica* and chloroquine on hematological parameters of Swiss albino mice infected with *P.berghei NK65*.

| **Hematological Parameters** | **GROUP I** | **GROUP II** | **GROUP III** | **GROUP IV** | **GROUP V** | **GROUP VI** |
|---|---|---|---|---|---|---|
| WBC (X $10^9$/L) | *15.9±4.50 | 69.7±10.40 | *39.20±7.1 | *49.6 ±6.20 | *45.5±3.60 | *36.50 ±5.2 |
| PCT (%) | 0.4640 ± 0.090 | 0.345 ±0.058 | 0.258 ±0.042 | 0.312 ±0.062 | 0.282 ±0.060 | 0.305 ±0.072 |
| MPV (fL) | 6.10 ± 0.50 | 8.1 ± 0.30 | 7.6 ± 0.30 | 7.7 ± 0.30 | 7.9 ± 0.40 | 7.1 ± 0.30 |
| PDW | 14.10±0.20 | 15.80±0.70 | 15.10±0 .30 | 15.2±0.40 | 15.40±0.60 | 15.0±0.10 |
| HGB (g/dl) | *12.9±0.10 | 4.50 ±0.90 | *9.30±0.30 | *6.80±0.30 | *7.70±0.50 | *7.10±0.60 |
| RBC (X $10^{12}$/L) | *8.20±0.03 | *2.65 ± 0.30 | 5.40±0.30 | *5.33±1.20 | *5.00±1.42 | *4.48±0.20 |
| HCT(%) | *42.5±2.20 | 17.2±1.00 | *32.50±1.2 | *25.4±0.3 | *26.1±0.7 | *23.1±0.8 |

| | | | | | | |
|---|---|---|---|---|---|---|
| MCV (fL) | 55.7±4.10 | 68.0±0.30 | 63.50±0.20 | 73.20 ± 0.20 | 75.20±0.20 | 70.3±3.60 |
| MCH (Pg) | 15.5±0.40 | 18.4±0.40 | 16.8±0.10 | 18.50±0.40 | 20.30±1.10 | 18.10±0.10 |
| MCHC (g/dL) | 31.9±0.80 | 25.2±0.23 | 25.4±0.21 | 25.3±0.30 | 28.2±0.50 | 26.9±0.30 |
| RDW-CV (%) | 18.10±0.20 | 20.50±0.70 | 18.00±0.30 | 19.82±0.40 | 21.90±1.40 | 19.40±0.50 |
| RDW-SD (fL) | 30.50±1.60 | 46.60±1.40 | 37.80±0.70 | 47.50±5.30 | 51.1±7.70 | 47.7±0.90 |

Values are expressed in mean ± SD of five mice in each group. * indicate Significant difference when compared to negative control (P <0.05). WBC: White blood cell; PCT: Mean platelet volume; MPV: Mean platelet volume; PDW: Platelet Distribution Width; HGB: Hemoglobin Concentration; RBC: Red Blood Cell; HCT: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean Cell hemoglobin; MCHC: Mean Cell hemoglobin concentration; RDW–CV: RBC distribution width–coefficient of variation; RDW–SD: RBC distribution width–standard deviation.

The total WBC counts were significantly lowered ( P<0.05) by 28.12%, 33.72% and 48.63% following *A. indica* extract administration at different dosage. The treated groups all showed elevated WBC counts when compared to Group I (healthy mice). This is caused by the *Plasmodium berghei* infection present in the treated group. The mean HGB values are significantly increased by 34.12%, 41.68% and 36.16% on administration of the extract of *A. indica* at different dosage compared to the untreated infected mice. Treatment with 200, 400 and 800mg/kg body weight of the extract significantly (P<0.05) increase the RBC values by 50.80%, 48.10% and 41.12% respectively when compared to group II mice. The HCT values of the groups treated with *A. indica* were significantly increased by 33.03%, 34.94% and 26.07% respectively when compared to infected untreated group. (Table 2). There are no significant differences in the other hematological parameters of the infected untreated group compared to other group.

**Table 3:** The effect of Chloroquine and methanolic leaf extract of *Azadirachta indica* treatment on plasma Lipid profile of healthy and infected Swiss mice.

| **Lipid profile** | **GROUP I** | **GROUP II** | **GROUP III** | **GROUP IV** | **GROUP V** | **GROUP VI** |
|---|---|---|---|---|---|---|
| Total cholesterol (TC) mg/dl | *70.80 ± 4.40 | 87.33 ± 4.30 | *72.50 ± 4.20 | *71.52 ± 3.30 | *70. 20 ± 3.20 | *66.40 ± 2.80 |
| Triglyceride (TG) mg/dl | *64.20 ± 4.23 | 79.85 ± 3.23 | *66.30. ± 3.40 | *63.60 ± 2.40 | *61.30 ± 4.70 | *60.50 ± 3.60 |
| HDL –C mg/dl | *39.90 ± 2.60 | 27.42 ± 4.10 | *32.40 ± 2.15 | *37.30 ± 3.40 | *33.10 ± 3.40 | *39.40 ± 2.40 |
| LDL-C mg/dl | *18.33 ± 0.31 | 40.21 ± 2.10 | *25.40 ±2.21 | *22.25 ± 1.60 | *23.40 ± 2.60 | *17.40 ± 1.60 |

Values are expressed in Mean SD of five mice in each group. * indicate Significant difference when compared to negative control (P <0.05).

From Table 3, plasma TC is reduced by 18.93%, 19.83% and 24.03% respectively on administration of the different dosage of the extracts in the treated groups compared to the untreated infected group. The values of the plasma TG in group IV to VI were reduced by 20.35%, 23.63% and 24.01% respectively on administration of the extract. The different dosage of the extract significantly reduce the low density lipoprotein-Cholesterol (LDL-C) by 44.67%, 42.12% and 56.02% respectively after treatment compared to the untreated infected group. However, significant decreases (P < 0.05) in plasma TC, TG and LDL-C levels in infected mice treated with chloroquine were also observed. The HDL-C values were increased by 25.82%, 17.09% and 31.02% respectively on treatment with the extract compared to untreated infected group.

Table 4: In-vitro antioxidant activity of *Azadirachta indica* on $H_2O_2$ induced oxidative stress on liver tissue.

| **Oxidative stress Parameters** | **GROUP A** | **GROUP B** | **GROUP C** | **GROUP D** | **GROUP E** | **GROUP F** |
|---|---|---|---|---|---|---|
| **Lipid Peroxidation X $10^3$ mm MDA/mg protein** | *4.70±1.20 | 9.20±3.40 | *3.11±1.20 | *5.20±1.30 | *4.90±1.60 | *3.90±1.50 |
| **Catalase (CAT) unit*/ mg protein** | *41.20±4.10 | 22.20±4.20 | *47.10±3.40 | *30.20±4.2 | *31.50±3.5 | *38.20±3.1 |
| **Superoxide dismutase (SOD) unit*/ mg protein** | *11.30±0.80 | 4.60±0.50 | *11.80±1.20 | *8.60±0.70 | *7.40±1.60 | *8.50±1.30 |
| **Reduced glutathione (GSH) mg/mg protein** | *0.41±0.09 | 0.13±0.02 | *0.32±0.08 | *0.26±0.07 | *0.24±0.03 | *0.22±0.06 |

Values are expressed in Mean  SD of five mice in each group. * indicate Significant difference when compared to negative control (P <0.05).

MDA is the major oxidation product of peroxidized poly-unsaturated fatty acids and the increased MDA content is an important indicator of lipid peroxidation. Table 4 shows that the group treated with *A.indica* extract dosage of 200, 400 and 800mg/kg body weight significantly reduce the MDA liver homogenate level by 43.82%, 45.98% and 57.28%, respectively compared to the untreated group B. Group C reduces the MDA level by 66.81% when compared to group B. Catalase is an enzymatic antioxidant widely distributed in all animal tissues. Catalase decomposes hydrogen peroxide and helps protect the tissues from highly reactive hydroxyl Radicals. The CAT liver homogenate was also increased by the administration of *A.indca* extract by 53.80%, 26.88%, 28.45% and 43.26% respectively (group C to F). SOD has been touted as one of the most important enzymes in the enzymatic anti-oxidant defense system. SOD level is increased by the administration of the different extract of *A.indica* by 61.82%, 47.062%, 38.54% and 46.63% respectively (group C to F). The GSH liver homogenate values increased by 59.38%, 50.20%, 45.58% and 40.64% respectively on the administration of different extract of *A.indica.*

## Discussion

The result of this study shows that methanolic leaf extract of *A. indica* contain some secondary metabolites like tannin, saponin, glycoside, reducing sugar and flavonoids (Table 1). The presence of these secondary metabolites in *A. indica* may be responsible for the plant anti-*plasmodium* activity. It is evident by these findings that *A. indica* possessed anti-plasmodium activity justifying its usage in the management of malaria. Milliken, 1997, Christensen and Kharazmi, 2001 showed clearly that anti-plasmodial screening of plant substances may be caused by terpenes, flavonoids and alkaloids. These compounds could be acting singly or in synergy with one another to exert the anti-plasmodial activity observed in this study.

No death occurred during toxicity test at all the dose level used which shows that the extract does not have any toxic effect that can lead to the death of the animals. The median lethal dose $LD_{50}$ was estimated to be >5000mg/Kg body weight. Behavioral signs of toxicity like salivation, paw licking, restlessness, reduced activities and stretching were observed.

The four day suppressive test is a standard test commonly used for anti-malarial screening, and the determination of percentage inhibition of parasitaemia is the most reliable parameter. The results obtained from this study showed significant decrease in parasitaemia of *P. berghei* after treatment with the extract of *A. indica.* The significant decrease in parasitaemia observed in this study was dose dependent. The methanolic leaf extract of *A. indica* caused 47.80%, 50.96% and 52.30% suppression in parasitaemia of *P.berghei* at 200, 400 and 800mg/kg body weight respectively while chloroquine, a standard anti-malarial drug

used exerted 100% suppression at 5mg/kg (Figure 1). When a standard anti-malarial drug is used in mice infected with *P. berghei*, it suppresses the parasiteamia to a non-dectable level (Kiseko,*et al* 2000). The curative test shows that only chloroquine cures the parasites from day 3 of treatment while the different concentration of the extract of *A. indica* exert a growth inhibition of 50.10%, 74.57% and 73.68% respectively. Studies showed that the *in vitro* anti-malarial activity of *A. indica*, previously reported by El-Tahir *et al* in 1999, indicate that an aqueous extract of Neem leaves showed an $IC_{50}$ value <5 μg/ml against *P. falciparum*. Similar results were obtained by Alshawsh and colleagues in 2007, where they reported that aqueous *A. indica* extracts inhibited the development of the ring stage of *P. falciparum*.

Hematological and biochemical indices have been reported to be a reliable parameter for assessment of the health status of animals (Sexena ,*et al* 2011 and Ohaeri, 2011). WBC count functions primarily in body defence against foreign bodies and this is often achieved through leucocytosis and antibody production (Marieb, 1995).There was a significant increase ($P < 0.05$) in the WBC count in group II mice compared to all other groups treated with *A.indica* extract (Table 2). Leukocytosis observed in group II may be due to luekemia, bone marrow tumors, tissue damage and inflammatory disease of the mice infected with *P.berghei NK65*. There were significant reduction ($P<0.05$) in HGB, RBC and HCT of the untreated infected mice compared to the group treated with chloroquine and *A.indica* extract . This is an indication of severe anemia in group II animals. The extract prevented a drastic reduction in HGB, RBC and HCT values, features signifying severe anemic conditions. This observation is supported by a report stating that anemia is characterized by decreased values of HGB, RBC and HCT (Aleksandro,2009). There were no significant change ($P<0.05$) in the MCV, MCH, MCHC, PCT, PDW, RDW-CV and RDW-SD values in the entire experimental groups. This study shows that the methanolic leaf of *A.indica* extract does possess hematopoietic activity and is not hematotoxic.

Several studies have shown that high plasma total cholesterol, triglyceride and LDL-cholesterol are the major cause of cardiovascular disease. There were significant increase ($P<0.05$) in plasma TC, TG and LDL-C in the untreated infected group compared to other groups. The extract significantly reduces ($P<0.05$) these lipid parameters (TC, TG and LDL-C) by an average of 20.93%, 22.66% and 47.60% respectively for the entire treated group (Table 3). This is an indication that *A. indica* may be used to prevent cardiovascular infections. This observation is consistent with report from earlier study by Onyeneke *et al*. 1997.They showed that there were increased in serum lipoprotein fractions in malarial patients compared with apparently healthy control subjects. The extract significantly increase ($P<0.05$) HDL-C in the treated groups by 25.82%, 17.09% and 31.02% respectively compared to the infected untreated animals. Njoku and colleque (2001) also reported significant increase in HDL- cholesterol levels of malaria patients treated with crude neem extracts relative to both malaria controls and chloroquine treated patients.

Liver is the major organ used for removing Xenobiotic substances from the body and as such it possesses a high metabolic rate and is subjected to many substances causing oxidative stress. The host system produces reactive oxygen and nitrogen species (ROS and RNS) in response to infection. The parasite is capable of producing free radicals, which interfere with the biochemistry of red blood cells and may facilitate the internalization of the parasite into hepatocytes and RBC. Malondialdehyde (MDA) concentration was used as an index for lipid peroxidation. The liver homogenate MDA value was significantly higher ($P<0.05$) in group B compared to other groups (Table 4). The result of lipid peroxidation in the liver homogenate of the group treated with the different doses of *A. indica* showed the least value. This is an indication that the extract reduces oxidative stress since increased in lipid peroxidation has been linked to cause oxidative stress. Evidence has shown that ROS like $H_2O_2$ are implicated in ethanol induced lipid paroxidation (Schlorff et al 1999). SOD is

one of the most important enzyme in the enzymatic anti-oxidant defense system. SOD catalyses the dismutation of superoxide anion ($0_2^-$) to form $H_2O_2$. CAT decomposes the $H_2O_2$ to form water and oxygen. SOD and CAT values in the liver homogenate were significantly lowered ( $P<0.05$) in group B compared to other group (group A, C, D, E and F). Group C has the highest values for SOD and CAT respectively. The reduced levels of SOD and CAT in group B liver homogenate clearly indicate hepatocytes necrosis due to production of reactive metabolities induced ROS production. Pegeolot *et al* 1990 observed that decreased in SOD and CAT could be due to feed back inhibition or oxidative inactivation of enzyme protein due to excess ROS generation. GSH acts as a free radical scavenger, a generator of α-tocopherol and plays an important role in the maintenance of protein sulfhydryl groups (Farnandez, and Videla, 1981). Group A, C, D, E and F showed significant higher GSH values ($P<0.05$) compared to group B. Low GSH value has been implicated with oxidative stress. Farnandez, and Videla, 1981 showed that acute ethanolic ingestion, depletes GSH levels which lead to oxidative stress. The above result showed clearly that *A.indica* possess antioxidant potentials.

**Conclusion**

The result obtained from this study reveals that 200, 400 and 800mg/kg body weight of methanolic leaf extract of *Azadirachta indica.* suppresses *Plasmodium berghei NK 65* and could be used in the management of malaria. The extract is not hematotoxic, it has hypolipidemic effect and possess antioxidant properties.

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