Original Article

Preventive (myoglobin, transferrin) and scavenging (superoxide dismutase, glutathione peroxidase) anti-oxidative properties of raw liquid extract of *Morinda lucida* leaf in the traditional treatment of *Plasmodium* infection

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Abstract

Background: Liquid extract of *Morinda lucida* leaf has been demonstrated to have antiplasmodial activities. Some phytochemicals act as preventive and or scavenging antioxidants. This study aimed to investigate the preventative and scavenging properties of the raw liquid extract of *M. lucida* leaf using plasma myoglobin, transferrin, superoxide dismutase (SOD), and glutathione (GSH) peroxidase. **Materials and Methods:** Forty-eight *Plasmodium*-infected patients aged 29-47 years that have not been treated with any antimalaria medication but have decided to be treated traditionally using *M. lucida* leaf extract were recruited from 15 traditional homes in ATISBO, Saki-East, and Saki-West local government areas of Oke-Ogun — the Northern part of Oyo State-Nigeria. Identification of *Plasmodium* in the blood of the test and normal control subjects were carried out by Giemsha thick film technique. Packed cell volume, total bile acids, blood glucose, blood pressure, plasma myoglobin, transferrin, SOD, and GSH peroxidase (GPx) were evaluated in the normal control subjects and in the *Plasmodium*-infected patients before and after the treatment with raw liquid extract of *M. lucida* leaf. **Results:** A significant ($P < 0.05$) biochemical alterations were observed in the plasma values of transferrin, SOD, and GPx in the *Plasmodium*-infected patients when compared with the normal control subjects and after treatment with the raw liquid extract of *M. lucida* leaf. **Conclusion:** Our study supports the possible preventative and scavenging antioxidative effect of the raw liquid extract of *M. lucida* leaf in the traditional treatment of *Plasmodium* infection.

Key words: *Morinda lucida*, *Plasmodium* infection, preventive antioxidants, scavenging antioxidants

INTRODUCTION

Antioxidants are intimately involved in the prevention of cellular damage, which is the common pathway for cancer, aging, and a variety of diseases.[1] Antioxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (lipophilic). In general, water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma, while lipid-soluble antioxidants protect cell membranes from lipid peroxidation.[2]

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Free radicals have been implicated in the progression of numerous conditions including cancer, diabetes, cardiovascular disease, ageing, and neurological disorders. Hence, antioxidants have been investigated for the prevention of these diseases.[3,4]

Decrease in antioxidants level including tissue and cellular damage in plasmodia (falciparum, ovale, vivax, and malariae) infection are suggestive of oxidative stress in the infected individual. The role of oxidative stress during malarial infection needs clear illumination, as some have demonstrated a protective role while other suggested relation with malarial pathophysiology and complication. However, recent reports suggest that generation of reactive oxygen species (ROS) and associated oxidative stress play a crucial role in the development of systemic complications in malaria.[5-7] Malarial infection decreases the levels of antioxidant enzymes and other antioxidants such as catalase, glutathione (GSH) peroxidase, superoxide dismutase (SOD), albumin, GSH, ascorbate, and plasma tocopherol. *Plasmodium* infection in mice increases the activity of xanthine oxidase (XOD) and lipid peroxide content in liver, indicating the development of hepatic oxidative stress in malaria. However, there is no clear idea on the identity of the reactive species of ROS family involved in the development of oxidative stress.[5-7]

Phytochemical screening of *Morinda lucida* leaf was found to include: Alkaloids, saponnins, tannin, anthraquinones, glycosides, anthraquinols, flavonoids, and steroids[8-11] and two known triterpenic acids (ursolic and oleanolic acids) isolated from the leaves.[12] Leaf extracts showed *in vitro* antimalarial activity against *Plasmodium falciparum* while in several other tests antidiabetic properties were confirmed. Inhibiting effects on cancer tumors in mice have also been reported.[12]

Most phytochemicals have antioxidant activity and protect our cells against oxidative damage and reduce the risk of developing certain types of cancer. Saponins reduce bone loss and could act as antioxidant.[12,13] Saponins have many health benefits which include beneficial effects on blood cholesterol levels, cancer, bone health, and stimulation of the immune system. Saponins have antitumor and antimutagenic activities and can lower the risk of human cancers, by preventing cancer cells from growing and could boost immunity. Saponins found in beans interfere with the replication of cell DNA, thereby preventing the multiplication of cancer cells. Capsaicin, found in hot peppers, protects DNA from carcinogens.[13,14] The phytochemical allicin from garlic has antibacterial properties. Some phytochemicals bind physically to cell walls thereby preventing the adhesion of pathogens to human cell walls. Proanthocyanidins are responsible for the anti-adhesion properties of cranberry. Consumption of cranberries will reduce the risk of urinary tract infections and will improve dental health.[13]

This work was therefore designed to measure the plasma level of myoglobin, transferrin, SOD, and GSH peroxidase (GPx) to determine the preventative and scavenging anti-oxidative properties of *M. lucida* in *Plasmodia* infection. This was investigated in *Plasmodium*-infected patients aged 29-47 years that have not been treated with any malaria medication but have decided to be treated traditionally using *M. lucida* leaf extract were recruited from 15 traditional homes in ATISBO, Saki-East, and Saki-West local government areas of Oke-Ogun – the Northern part of Oyo State-Nigeria.

MATERIALS AND METHODS

Study area

The study was carried out in ATISBO, Saki-East, and Saki-West local government areas of Oke-Ogun — the Northern part of Oyo State-Nigeria. The three local governments constitute the former Ifedapo local government area of Oyo State and presently a Nigeria Federal Constituency. The three local governments share a border with Kwara State-Nigeria, Ogun State-Nigeria, and the Republic of Benin.

Study design

Experimental research design.

Study population

Forty-eight *Plasmodium*-infected patients aged 29-47 years that have not been treated with any antimalaria medication but have decided to be treated traditionally using *M. Lucida* leaf extract were recruited from 15 traditional homes in ATISBO, Saki-East, and Saki-West local government areas of Oke-Ogun — the Northern part of Oyo State-Nigeria. Apparently, healthy 50 age-matched male (n = 25) and female (n = 25) volunteers were studied as normal control.

Sample size

Forty-eight (48) out of the 57 *Plasmodium*-infected patients (female: n = 24; male: n = 24) that visited the traditional healers between August 2014 and March 2015 for treatment and volunteered themselves for this study were recruited based on the inclusion and the exclusion criteria.

Case selection procedure/s

Inclusion criteria

Anicteric *Plasmodium*-infected patients aged 29-47 years that have not been treated with any antimalarial medication but have volunteered to be treated with a raw liquid extract of the leaf of *M. lucida* in the traditional homes were

recruited. In addition, patients with normal blood pressure, blood glucose, total bile acids, packed cell volume, and nonpregnant and nonlactating females were included in the study.

Exclusion criteria

1. *Plasmodium*-infected patients that have been treated or being treated with antimalarial drugs were not recruited for the study.
2. Icteric subjects were not included in the study.
3. Pregnant and lactating mothers were not included.
4. Subjects with abnormal blood pressure, blood glucose, plasma total bile acids, and packed cell volume were excluded from the study.

Blood sample

Ten milliliters of blood was collected from each of the control and test subjects after an overnight fasting before and after the administration of the raw liquid of extract *M. lucida* (Oowo). The blood was divided into two parts. One part was preserved in lithium heparinized bottle. The blood sample was used for the estimation of total bile acids, myoglobin, transferrin, SOD, GPx, packed cell volume, and the identification of *Plasmodium* spp. in test and control subjects. The other part was preserved in the flouride-oxalate bottle for the estimation of blood glucose. The posttreatment blood sample was collected after 1 week of administration.

Preparation of the raw liquid extract of Morinda lucida (Oowo)

The leaves of the *M. lucida* were plucked and washed in water. The water was drained, and the leaves were crushed or squished for the extraction of the liquid content into a container. A plastic cup with a capacity of 70 ml was dedicated by the healers for the measurement of the extract to be administered to the patients. The liquid extract is freshly prepared on daily bases prior to administration. The raw liquid content of the leaf is extracted without the addition of water and also administered undiluted.

Methods

1. Based on the information obtained from the 15 traditional homes visited in the three local governments about 70 ml of the raw undiluted liquid extract of the *M. lucida* leaf is administered to the patients on daily bases for at least 1 week.
2. *Plasmodium* spp. was determined in the blood of the control and the test subjects using giemsha thick blood staining technique described by Monica.[15]
3. Glucose oxidase method was used to measure plasma glucose using reagent kit of Randox in test and control subjects.
4. Sphygmomanometer was used to measure the diastolic and systolic blood pressure in test and control subjects.
5. Microhaematocrit method was used to determine packed cell volume in test and control subjects as described by Monica.[15]

Estimation of plasma total bile acids was carried out on the plasma samples of the subjects using Randox reagent kit. The manufacturer's instruction was strictly followed.

Principle

Two reactions are combined in this kinetic enzyme cycling method. In the first reaction, bile acids are oxidized by 3-α hydroxysteroid dehydrogenase with the subsequent reduction of Thio-NAD to Thio-NADH. In the second reaction, the oxidized bile acids are reduced by the same enzyme with the subsequent oxidation of NADH to NAD. The rate of formation of Thio-NADH is determined by measuring the specific absorbance change at 405 nm.

Plasma transferrin was estimated by ELIZA technique in test and control subjects out using the reagent kit of Abcam.

Principle

Abcam's transferrin human *in vitro* competitive enzyme-linked immunosorbent assay (ELISA) kit is designed for the quantitative measurement of transferrin in plasma and serum samples.

A transferrin specific antibody has been precoated onto 96-well plates and blocked. Standards or test samples are added to the wells, and subsequently, biotinylated transferrin is added and then followed by washing with wash buffer. Streptavidin-peroxidase complex is added, and unbound conjugates are washed away with wash buffer. Tetramethyl benzidine (TMB) is then used to visualize streptavidin-peroxidase enzymatic reaction. TMB is catalyzed by streptavidin-peroxidase to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow coloration is inversely proportional to the amount of transferrin captured in plate.

Plasma SOD was estimated in test and control subjects out using the reagent kit of Randox.

Principle

The role of SOD is to accelerate the dismutation of the toxic superoxide radical (O_2), produced during oxidative energy processes, to hydrogen peroxide and molecular oxygen. This method employs xanthine and XOD to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. The SOD activity is then measured by the degree of inhibition of this reaction. One unit of SOD is that which causes a

50% inhibition of the rate of reduction of INT under the conditions of the assay.

Plasma myoglobin was estimated by ELIZA technique in test and control subjects using Abcam's myoglobin *in vitro* ELISA kit is designed for the accurate quantitative measurement of myoglobin in human serum.

Principle

A 96-well plate has been precoated with anti-myoglobin antibodies. Samples and standards are added to the wells, where myoglobin in the sample and standards binds to the precoated antibody. Added anti-myoglobin horseradish peroxidase (HRP) conjugate binds to this antibody-myoglobin complex. After incubation, the wells are washed to remove unbound material, and TMB substrate is then added which is catalyzed by HRP to produce blue coloration. The reaction is terminated by addition of stop solution which stops the color development and produces a color change from blue to yellow. The intensity of the signal is directly proportional to the amount of myoglobin in the sample, and the intensity is measured at 450 nm.

Plasma GPx was estimated by ELIZA technique in test and control subjects using Abcam's GPx assay kit.

Principle

In Abcam's GPx assay kit, GPx reduces cumene hydroperoxide while oxidizing GSH to GSSG. The generated GSSG is reduced to GSH with consumption of NADPH by GR. The decrease of NADPH (easily measured at 340 nm) is proportional to GPx activity. The assay can be used to measure all of the GSH dependent peroxidases in plasma, erythrocyte lysates, tissue homogenates, and cell lysates with a detection sensitivity of ~0.5 mU/ml of GPx in samples.

Ethical consideration

The proposal was reviewed and approved by the Research and Ethical Committee of Baptist Medical Centre, Saki-Oyo State-Nigeria before the commencement of the work. This is to protect the interest of patients to ensure that the patients and the community are not harmed in any form by the procedure. Only *Plasmodium*-infected patients that volunteered themselves for the study were recruited.

Statistical analysis

The values of the biochemical parameters obtained in the patients before and after treatment with the raw liquid extract were subjected to statistical analysis to determine the mean values, standard deviation, and Student's *t*-test, for *t* value, *P* value, and level of significant at 0.01 (99%) using online Student *t*-test calculator for two independent means online at http://www.socscistatistics.com/tests/studentttest.

RESULTS

The result obtained in this study is as shown in Tables 1-3 and also represented in Figures 1-3 below. There was no significant difference in the mean value of plasma total

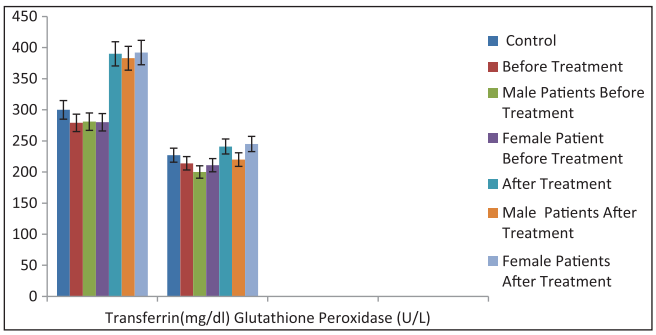

Figure 1: Results of plasma transferrin and glutathione peroxidase obtained in the control and *Plasmodium*-infected subjects before and after treatment with the liquid extract of *Morinda lucida*

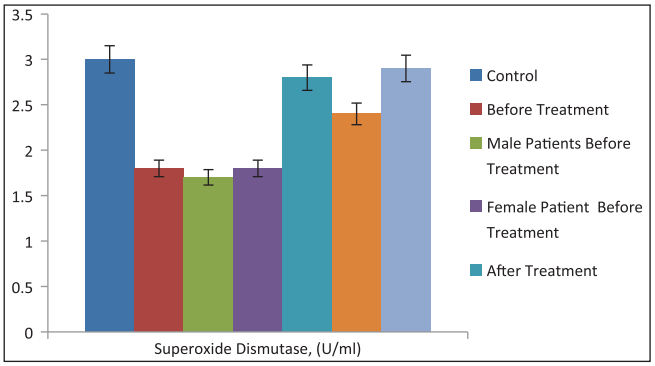

Figure 2: Results of plasma superoxide dismutase obtained in the control and *Plasmodium* infected subjects before and after treatment with the liquid extract of *Morinda lucida*

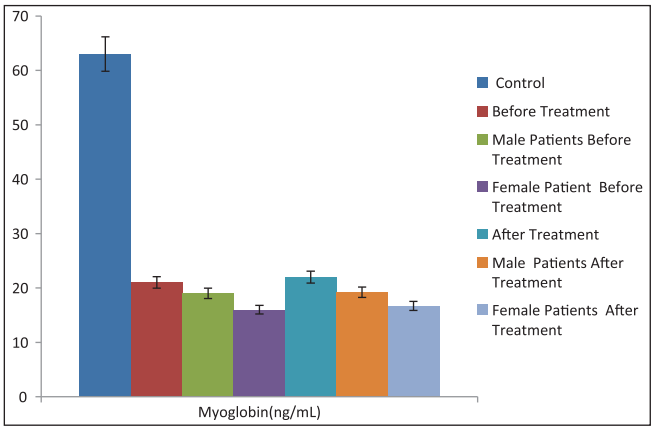

Figure 3: Results of plasma myoglobin obtained in the control and *Plasmodium* infected subjects before and after treatment with the liquid extract of *Morinda lucida*

bile acids, plasma glucose, packed cell volume, systolic, and diastolic blood pressure obtained in the *Plasmodium*-infected patients before and after treatment with the extract [Table 1].

The results obtained showed a significantly higher mean value of plasma myoglobin, transferrin, SOD, and GPx in normal control subjects than the *Plasmodium*-infected patients before the treatment with $P < 0.05$ [Tables 2 and 3].

There was a significantly lower mean value of plasma transferrin, SOD, and GPx in *Plasmodium*-infected patients before treatment than in *Plasmodium*-infected patients after treatment with $P < 0.05$. However, there was no significant difference in the value of myoglobin obtained in *Plasmodium*-infected patients before treatment and in the *Plasmodium*-infected patients after treatment with $P > 0.05$ [Tables 2 and 3].

The result obtained showed no significant difference in the mean value of plasma myoglobin, transferrin, SOD, and GPx in *Plasmodium*-infected male patients before treatment and *Plasmodium*-infected female patient before treatment ($P > 0.05$) as shown in Tables 2 and 3.

Table 1: Plasma TBA (µmol/L) plasma glucose (mg/dl) packed cell volume (%) systolic blood pressure (mmHg), and diastolic blood pressure (mmHg) in normal control subjects

Biochemical parameters	Normal control	Plasmodium infected patients before treatment	Plasmodium infected male patients before treatment	Plasmodium infected female patient before treatment	Plasmodium infected patients after treatment	Plasmodium infected male patients after treatment	Plasmodium infected female patients after treatment
n	50	48	24	24	48	24	24
TBA (µmol/L)	6.5±1.6	6.6±1.8	6.5±1.0	6.3±1.6	6.1±1.0	6.3±1.5	6.2±1.7
PCV (%)	40±6.1	39±5.1	42±2.1	37±3.0	40±4.1	41±3.1	38±2.5
Blood glucose (mg/dl)	90±15.0	88±14.0	89±15.1	91±14.0	86±13.0	88±14	89±14
Systolic blood pressure (mmHg)	110±10.0	117±2.1	110±9.0	118±2.0	115±5.0	117±2.0	117±3.0
Diastolic blood pressure (mmHg)	75±5.1	80±1.1	79±1.0	77±2.1	70±8.1	70±3.1	70±2.3

PCV: Packed cell volume, TBA: Total bile acids

Table 2: Results of plasma myoglobin, transferrin, SOD and GPx obtained in the control and *Plasmodium* infected subjects before and after treatment with the liquid extract of *Morinda lucida*

Biochemical parameters	Normal control	Plasmodium infected patients before treatment	Plasmodium infected male patients before treatment	Plasmodium infected female patient before treatment	Plasmodium infected patients after treatment	Plasmodium infected male patients after treatment	Plasmodium infected female patients after treatment
n	50	48	24	24	48	24	24
Myoglobin (ng/ml)	63±2.1	21±3.2	19±2.0	16±2.1	22±1.8	19.2±2.0	16.7±2.0
Transferrin (mg/dl)	300±1.1	279±2.0	281±3.0	280±4.0	390±2.5	383±6.0	392±2.0
SOD (U/ml)	3.01±0.2	1.8±0.3	1.7±0.1	1.8±0.2	2.8±0.1	2.4±0.1	2.9±0.3
GPx (U/L)	277±6.2	214±5.1	200±7.1	211±6.5	241±6.0	220±7.0	245±9.1

SOD: Superoxide dismutase, GPx: Glutathione peroxidase

Table 3: Comparative analysis of plasma myoglobin, transferrin, SOD and GPx obtained in the control and *Plasmodium*-infected subjects before and after treatment with the liquid extract of *Morinda lucida*

Biochemical parameters	Normal control and Plasmodium infected patients before treatment		Plasmodium infected patients before treatment and Plasmodium infected patients after treatment		Plasmodium infected male patients before treatment and Plasmodium infected female patient before treatment		Plasmodium infected male patients after treatment and Plasmodium infected female patient after treatment		Plasmodium infected male patients before and after treatment		Plasmodium infected female patients before and after treatment	
	t	*P*	*t*	*P*	*t*	*P*	*t*	*P*	*t*	*P*	*t*	*P*
Myoglobin (ng/ml)	15.0	0.02**	0.37	0.3*	0.2	0.4*	1.06	0.2*	0.2	0.4*	0.16	0.4*
Transferrin (mg/dl)	8.94	0.06**	3.59	0.03**	0.2	0.4*	1.42	0.14*	0.93	0.2*	2.67	0.05*
SOD (U/ml)	3.59	0.034**	3.5	0.03**	0.45	0.3*	2.04	0.08*	4.95	0.01**	3.05	0.04**
GPx (U/L)	8.06	0.07**	3.46	0.03**	1.20	0.1*	2.20	0.07*	2.02	0.09*	2.78	0.05*

*Not significant, **Significant. SOD: Superoxide dismutase, GPx: Glutathione peroxidase

The result obtained also showed no significant difference in the mean value of plasma myoglobin, transferrin, SOD, and GPx in *Plasmodium*-infected male patients after treatment and *Plasmodium*-infected female patient after treatment ($P > 0.05$) as shown in Tables 2 and 3.

The result obtained also showed no significant difference in the mean value of plasma myoglobin, transferrin, and GPx in *Plasmodium*-infected male patients before and after treatment with $P > 0.05$ [Tables 2 and 3].

There was a significantly lower mean value of plasma SOD in *Plasmodium*-infected male patients before than the result obtained after treatment with $P < 0.05$ [Tables 2 and 3].

The result obtained also showed no significant difference in the mean value of plasma myoglobin, transferrin, and GPx in *Plasmodium*-infected female patients before and after treatment with $P > 0.05$. However, there was a significantly lower mean value of plasma SOD in *Plasmodium*-infected female patients before than the result obtained after treatment with $P < 0.05$ [Tables 2 and 3].

DISCUSSION

There was no difference in the mean value of plasma total bile acids, plasma glucose, packed cell volume, systolic, and diastolic blood pressure obtained in the *Plasmodium*-infected patients before and after treatment with the extract. Plasma myoglobin, transferrin, SOD, and GPx were found to be significantly higher in normal control subjects than the *Plasmodium*-infected patients before the treatment. This is a result of *Plasmodium* infection, because malarial infection decreases the levels of antioxidant enzymes and other antioxidants such as catalase, GPx, SOD, albumin, GSH, ascorbate, and plasma tocopherol, which has also been demonstrated in mice to increase the activity of XOD and lipid peroxide content in liver, indicating development of hepatic oxidative stress in malaria.[5-7]

There was a significantly lower mean value of plasma transferrin, SOD, and GPx in *Plasmodium*-infected patients before treatment than in *Plasmodium*-infected patients after treatment. Transferrin is an antioxidant and a negative acute-phase protein that decreases in inflammation by providing an innate immunity preventing tissue and cell damage.[16] Oxidative damage is one of the most important pathological consequences of malarial infections. It affects vital organs of the body manifesting in changes such as splenomegaly, hepatomegaly, endothelial, and cognitive damages, therefore, low plasma transferrin, SOD, and GPx level is due to preventative (transferrin) and scavenging (SOD and GPx) effect of the these substances against the reactive oxygen or the oxidative stress.[17] The level of these antioxidants, therefore, increases after treatment affirming the liquid extract of *M. lucida* as an antiplasmodial agent.[18] Furthermore, phytochemicals found in *M. lucida* leaf such as alkaloids, saponnins, flavonoids, and have antioxidant activities.[8-14]

There was a significantly lower mean value of plasma SOD in *Plasmodium*-infected male patients before than the result obtained after treatment. This was also found in female patients. The above explanation also holds for this finding.

CONCLUSION

Oxidative stress in *Plasmodium* infection is prevalent due to a significant decrease in the plasma myoglobin, transferrin, SOD, and GPx. The preventative and scavenging antioxidative properties of the liquid extract of *M. lucida* leaf was evident due to alterations in the plasma level of transferrin, SOD, and GSH peroxidase in *Plasmodium*-infected patients.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Benzie IF. Evolution of dietary antioxidants. Comp Biochem Physiol A Mol Integr Physiol 2003;136:113-26.
2. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007;39:44-84.
3. Sies H. Oxidative stress: Oxidants and antioxidants. Exp Physiol 1997;82:291-5.
4. Baillie JK, Thompson AA, Irving JB, Bates MG, Sutherland AI, Macnee W, *et al.* Oral antioxidant supplementation does not prevent acute mountain sickness: Double blind, randomized placebo-controlled trial. QJM 2009;102:341-8.
5. Clark IA, Chaudhri G, Cowden WB. Some roles of free radicals in malaria. Free Radic Biol Med 1989;6:315-21.
6. Siddiqi NJ, Pandey VC. Studies on hepatic oxidative stress and antioxidant defence systems during arteether treatment of *Plasmodium yoelii* nigeriensis infected mice. Mol Cell Biochem 1999;196:169-73.
7. Pabón A, Carmona J, Burgos LC, Blair S. Oxidative stress in patients with non-complicated malaria. Clin Biochem 2003;36:71-8.
8. Nweze EI, Okafor JI, Njoku O. Antimicrobial activities of methanolic extracts of Trema guineensus (schunm and Thorn) *Morinda lucida* (Benth) used in Nigeria. Bioresearch 2004;2:39-46.
9. Akinyemi KO, Mendie VE, Smith ST, Oyefolu AO, Coker AO. Screening of some medicinal plants used in southwest Nigerian traditional medicine for anti-*Salmonella typhi* activity. J Herbal Pharmacother 2005;5:45-60.
10. Ajayeoba FO, Abiodun OO, Falade MO, Ogbole NO, Ashidi JS, Happi CT, *et al*. *In vitro* cytotoxicity studies of twenty plants used in Nigerian antimalarial ethnomedicine. Phytomedicine 2006;13:295-8.
11. Ebiloma GU, Omale J, Aminu RO. Suppressive, curative and prophylactic potentials of *Morinda lucida* (Benth) against erythrocytic

stage of mice infective chloroquine sensitive *Plasmodium* berghei NK-65. Br J Appl Sci Technol 2011;1:131-40, 201.
12. Odugbemi T. A Textbook of Medicinal Plants from Nigeria. Lagos: University of Lagos Press; 2008.
13. Kutalek R, Prinz A. African medicinal plants. In: Yaniv Z, Bachrach U, editors. Handbook of Medicinal Plants. New Delhi: CBS Publishers; 2007.
14. Prajapati ND, Purohit SS, Sharma AK, Kumar T. A Handbook of Medicinal Plants: A Complete Source Book. India: Agrobios Publishers; 2007.
15. Monica C. District Laboratory practice in tropical Countries Part 1. Low-price edition. Cambridge: Cambridge University Press; 2002.
16. Abbas A, Lichtman A, Pillai S. Basic Immunology Functions and Disorders of the Immune System. 4th ed. Philadelphia, PA: Saunders/Elsevier; 2012. p. 40.
17. Isah MB, Ibrahim MA. The role of antioxidants treatment on the pathogenesis of malarial infections: A review. Parasitol Res 2014;113:801-9.
18. Olaniyan MF, Babatunde EM. Evaluation of parasite density, plasma total bile acids, Alanine transaminase, Lactate dehydrogenase and CD4 in *Plasmodium*-infected patients treated with *Morinda Lucida* (Oowo). Am J Biochem 2014;4:52-8. Available from: http://www.journal.sapub.org/ajb