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Research article

Phytochemical analysis of Aqueous Methanolic Extract of *Acanthospermum hispidium* and its effect on Biochemical and Hematological indices in *Plasmodium falciparum* infected Rats

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ABSTRACT

Haematological (Red blood cell 'RBC', packed cell volume 'PCV', white blood cell 'WBC' and platelet 'PLA') and biochemical (total protein, serum albumin and creatinine) parameters of rats treated with aqueous-methanolic extract of *A. hispidum* leaves on blood serum of rats infected with *P. falciparum* was investigated. The chemical composition was determined using gas chromatography and mass spectrometry (GC-MS). Biochemical and haematological parameters were examined using standard methods. The aqueous-methanolic extract of *A. hispidum* consist of Allyldimethyl-Formamide, Erythritol, Glycerin, Benzoylmethyl-3-hydroxy-5-nitro-2-indolinone, Silanol and Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester with composition of 13.578, 2.796, 56.72, 5.486, 1.415 and 10.005%, respectively. The increase in RBC, PCV, PLA and albumin with reduction in WBC and creatinine level compared to normal control when treated with aqueous – methanolic extract of *A. hispidum* leaves were obtained with reduced parasitaemia. Aqueous – methanolic *A. hispidum* leaves, at 400mg/kg for 72 hours proved to be the best for treatment of malaria parasite based on haematological and biochemical parameters assessed.

Keywords: *Acanthospermum hispidum , Plasmodium falciparum, GC- MS, haematological and Biochemical parameters*

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INTRODUCTION

One of the most threatening communicable diseases to human health and economic development in most tropical and subtropical regions is malaria (Al-Omar *et al*, 2010). This is due to the fact that rainfall, warm temperatures, and stagnant waters provide habitats ideal for mosquito larvae (Adetola, Aishat, & Olusola, 2014). It has been reported that about 40% of the people living in this world live in malaria transmitting areas and more than two millions out of 500 million people die of malaria every year (Al-Omar *et al*, 2010; Gardner *et al*., 2002; Shandilya *et al*, 2013). Investigation revealed that sub-Saharan Africa has the highest mortality rate, where less than 5-year old children account for more than 80% deaths caused by malaria (Gardner *et al*., 2002).

Anopheles mosquitoes are the prime transmitters of parasites of the genus Plasmodium that cause malaria to humans by biting the human skin (Barat, 2006). *Plasmodium falciparum*, one of the species of genus Plasmodium called protozoan parasite, is quite lethal and causes more than 80% of malaria through infection into the red blood cell (Tilley *et al*, 2011). Other Plasmodium species such as Plasmodium ovale, Plasmodium malariae and Plasmodium vivax are less dangerous. After successful entry into the human body without invitation, the malaria parasite P. falciparum interacts with red blood cells (Tuteja, 2007; Vignali *et al*, 2008; Tilley *et al*, 2011), thereby, infected the individual with malaria. Ifoue *et al* (2009) reported *P. falciparum* to induce oxidative stress through sequestration of infected red blood cells (IRBCs) in intervillous space and increase in lipid peroxidase which raised reactive oxygen species production by the immune system of the body, as well as synchronised release of oxygen during haemoglobin degradation. This weaken the immune system.

P. falciparum is seen and tagged as a parasite resistant to quite cheaply and readily available medications such as chloroquine or sulfadoxine-pyrimethamine (Bell & Winstanley, 2004; Van-Schalkwyk *et al*., 2013). Artemisinin-based combination therapies (ACTs) have replaced those inactive medications so as to fight against the strong parasite. ACTs are short lived and are known to aid in the cure of malaria by the rapid reduction of the parasite biomass (Ojurongbe, Akindele, Adedokun, & Thomas, 2016). Some other medications have been produced while others are still

being produced to kill the parasite, hence treating malaria. Some of these medications are costly and have adverse effect whereas the Plasmodium species are resistant to others (Fegas *et al*, 2010). Researches are currently focuses on better natural solutions for the treatment of malaria. Our natural herbs or plants used as alternative medicine have been underestimated in one way or the other and could be a lasting solution to eradicate some diseases including malaria in sub-Saharan Africa. Reasons for underestimation of alternative medicine include unknown bioactive compounds derived from medicinal plants, dosage related medicinal plants and toxicological assessment are imperative for clinically efficient means (Alferah, 2012). It is of great importance that we carry out investigations on medicinal plants which are gift free in our local communities for management or treatment of diseases.

Acanthospermum hispidum , commonly called Bristly starbur belong to the family of Asteraceae and found in Tropical America, Europe, Asia and Africa as a weed with medicinal potential with antifungal, antiviral and antimicrobial activities (Adu, Gbedema, Akanwariwiak, Annan, & Boamah, 2011; Chakraborty, Gaikwad, & Singh, 2012). The extract of *A. hispidum* is used as a traditional medicine for the treatment of jaundice, malaria, stomach ache, constipation, fever and viral infections (Adu *et al*., 2011; Araújo *et al*., 2008; Asase, Akwetey, & Achel, 2010; Chakraborty *et al*., 2012). Changes in blood cell parameters are already a well-known feature of malarial infections (Kotepui, Piwkham, Phunphuech, & Phiwklam, 2015). Yet, there is paucity of information on the constituents of *Acanthospermum hispidum* plant extract and therapeutic effect on malaria. In this study, we investigated the constituents, haematological and biochemical indices of induced P. falciparum experimental rats treated with aqueous – methanolic extract of *A. hispidum*.

MATERIALS AND METHODS

Animals: Experimental rats was obtained from the small Animal Holding Unit, Department of Biochemistry, University of Nigeria, Nsukka, Nigeria. A total number of twenty-four male albino rats (170 - 225g) were acclimatized in a well-ventilated aluminum and iron net cage for three (3) weeks and fed with standard commercial pelleted feed and distilled water. After acclimatization, the rats were randomly shared into six (6) groups, induced and treated as presented in Table 1. *Plasmodium falciparum* for inoculation was obtained from Department of Biotechnology, Federal University of Technology, Owerri, Imo State, Nigeria.

Reagents: All chemicals used in this work were of analytical grade obtained from Chemisciences (Nig) Ltd, Owerri, Imo State, Nigeria.

Extraction: The leaves of *Acanthospermum hispidum*, collected from its natural habitat in the School of Health Technology, Federal University of Technology, Owerri, Imo State, Nigeria were washed and air dried at 25ºC for 28 days. After weighing and grinding into smooth fine powder (using an electric milling machine (Philips Blender, UK), 450g of powdered leaves were macerated in extracted medium (methanol: water in the ratio of 4:6) for 7 days in a clean transparent bottle. The extract was filtered using Whitman number 42 paper. The clean aqueous - methanolic extract of *A. hispidum* was concentrated using standard soxhlet apparatus after which a dark paste was obtained and weighed.

Table 1
Different Groups of induced albino rats and the various treatments

Groups	Infected Albino Rats
1	No parasitaemia, food and water given *ad libitum*
2	Induced and untreated
3	Plasmodium induced + Chloroquine treatment
4	Induced and treated with aqueous - methanolic leaf extract of *Acanthospermum hispidum* at a dosage of 100mg/kg
5	Induced and treated with aqueous methanolic leaf extract of *Acanthospermum hispidum* at a dosage of 200mg/kg
6	Induced and treated with aqueous methanolic leaf extract of *Acanthospermum hispidum* at a dosage of 400mg/kg

Phytochemical analysis of *A. hispidum*: The method described by Rajeswari *et al* (2012) was used to analyze phytochemical constituents of aqueous – methanolic extract of *A. hispidum* extracts using a Perkin-elmer GC clarus 500 systes and gas chromatograph interfaced to a mass spectrometer (GC-MS) and equipped with elite-1, fused silica capillary column with agilent 19091S-433 HP-5MS (30mm x 250µmm x 0.25µm, composed of 5% phenyl methyl silox). For GC-MS detection and electron ionization system with ionizing energy of 70eV was used. Helium gas (99.99%) was used as the carrier gas at constant flow rate of 1.5ml/min and an injection volume of 1µl was employed (split ratio of 50:1), injector temperature 250 ^{0}C, ion-source temperature 300 ^{0}C. The oven temperature was programmed from 35 ^{0}C (isothermal for 5 minutes), with an increase of 4 ^{0}C /min to 150 ^{0}C, then 20 ^{0}C /min to 250 ^{0}C, ending with a 5 minutes isothermal at 300 ^{0}C. Mass spectra were taken at 70eV; a scan interval of 0.25 minutes and fragments from 45 to 450Da. Total GC running was 45.75 minutes. The relative percentage of each component was calculated by comparing its average peak area to the total areas. Interpretation on mass spectrum GC-MS was done using the database of National Institute of Standard and Technology (NIST). The spectrum of the unknown compound was compared with the spectrum of the known components stored in NIST library installed with GC-MS.

Inoculation and treatment of experimental rats: Rats were inoculated using an intraperitoneal injection of standard inoculum (0.2ml containing 1×107/mL *Plasmodium falciparum* of the infected erythrocytes) extracted from a single donor mouse previously infected with *Plasmodium falciparum* (29% Parasitaemia) which was originally obtained from the Department of BTC, Federal University of Technology, Owerri, Imo State, Nigeria. The inoculum contained 1×10^7 *Plasmodium falciparum* parasitized erythrocytes per ml. On day zero, the individual mouse was inoculated with 0.2ml of infected blood containing 1×10^7 *Plasmodium falciparum* parasitized erythrocytes.

Collection of Blood sample: Blood samples were collected by ocular puncture and mixed with 2µL of prepared EDTA at room temperature of 36^0C and aliquots of serum were stored in plain plastic tubes at -20^0C after separation. Blood samples were analysed for haematological, biochemical indices and parasitaemia count before and after innoculation with *Plasmodium falciparum* as well as oral administration of the chloroquinine and A. hispidum extract.

Haematological analysis

The Red Blood Cell (RBC), White blood cell (WBC) and Platelet (PLA) counts were determined using the haemocytometer as described by Bigoniya *et al* (2015), Al-Omar *et al* (2010) and Obimba & Eziuzor (2015). Packed cell volume (PCV) using haematocrit reader.

Biochemical analysis

Total protein was determined using Biuret method as described by Obimba & Eziuzor (2015). Biuret reagent (containing sodium hydroxide 100mmol/l, Na- tartarate 16mmol/l, potassium iodide 15mmol/l and cupric sulphate 6mmol/l) was diluted with 400ml of double distilled water. Blank reagent (containing sodium hydroxide, 100mmol/l and Na-K tartarate 16mmol/l) was also diluted as above. 0.02ml DDH20 and 1.0ml of the biuret reagent were added to a cuvette and incubated for 10 minutes at 37^0C. Measurement of the absorbance of the standard (Astandard) and absorbance of sample (Asample) against the reagent blank was made after 10 minutes of incubation at a wavelength of 546nm, pathlength of 1cm, and temperature of 37^0C. The total protein concentration was calculated using the equation below:

$$\text{Total protein conc.} = \frac{A_{sample}}{A_{standard}} \text{ X standard concentration}$$

Serum albumin was determined the photometric test method was used to determine the serum albumin using the BCG (Bromocresol Green) albumin assay kit (Sigma, St. Louis, USA).

Serum creatinine was determined using the Jaffe's kinetic method. 100µl of sample or standard was added to 1ml of the Buffer/substrate reagent. It was stirred and left to stand for 15 minutes at room temperature to eliminate endogenous ammonia. The absorbance (A_1) at 340nm was measured vs. water. 20µl of creatinine iminohydrolase was added and stirred and allowed to stand for at least 15 minutes at room temperature. The absorbance (A_2) was then measured again. The change in absorbance (A_1-A_2) was determined and the value obtained for the sample was compared with that for the standard. The speed of absorbance change is proportional to the creatinine concentration.

Confirmation of Parasitaemia

The Geimsa's stain technique as described by Kotepui *et al* (2015) was used to determine the percentage parasitemia in the blood. A thick film was produced by depositing and spreading drops of the blood sample on a glass slide. The film was allowed to dry thoroughly without any contamination. Then, the slide was immersed in a mixture of 1 drop of Geimsa's stain to each ml of distilled water; washed off and air dried. Finally, the sample was examined under the microscope using the oil immersion objective (x100). A detergent, Triton-x-100 was added to the Geisma's stain in the amount of 0.1% for thick blood films. The parasitized red blood cells present were counted and recorded. The level of parasitemia was expressed as percentage (%) of erythrocytes infected with malaria parasites. In this study, one thousand erythrocytes were examined and the numbers of infected-RBC among these were noted. Percent parasitemia was determined using equation below:

$$\text{Percentage parasitaemia} = \frac{A_{sample}}{A_{standard}} \text{ X standard conc.}$$

Analysis of the Data

The data generated from this unique study was coded and analysed appropriately using bivariate statistics of Statistical Package for Social Science (SPSS) software package (version 21.0) and 95% confidence interval on data to obtain spearman's correlation coefficient and p-value with two tails. The p-value inferior to 0.05 was statistically significant.

RESULTS AND DISCUSSION

Fig. 1 shows the GC - MS spectra of aqueous – methanolic extract of *A. hispidum* leaves. The chemical compositions obtained from the spectra are presented in Figure 1. Peak areas represent the percentage compositions of metabolites. Six (6) chemical constituents were found in the extract of A. hispidum with composition of N – methyl thio monbactams (13.578%), Erythritol (2.796%), Glycerin (56.72%), Benzoylmethyl-3-hydroxy-5-nitro-2-indolinone (5.486), Silanol (1.415%) and Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester (10.005%) as presented in Table 2.

The effectiveness of this aqueous - methanolic of A. hispidum leaves may be due to synergistic effect of the bioactive compounds in the leaves. N – methyl thio substituents have demonstrated intrinsic and potentiate activity and may lead to a new class of medicinal agents, even without its normal acidic sulfonate group at the nitrogen (Majewski *et al*., 2016). N-thiolation have been reported to generate bioactive compounds with anti-cancer and fungal activity, and excellent bacteriostatic activity against Staphylococcus aureus and Methicillin-Resistant Staphylococcus aureus (MRSA) by *in - vivo* transfer of the N-thiol group to coenzyme A which ultimately disrupting essential fatty acid biosynthesis (Hussein, 2016; Majewski *et al*., 2016). Erythritol, sugar alcohol had been considered as a safe, effective and prophylaxis drugs used for the treatment of cerebral malaria because it kills or impede the growth of P. falciparum (Chen, 2014; Huthmacher *et al*., 2010; Lutgen, 2016). Glycerin have been reported to be used as a laxative agent for children and suppositories of Artemether and Artemisinin for malaria treatment (Jannin *et al*, 2014).

Benzoylmethyl-3-hydroxy-5-nitro-2-indolinone had been reported to possess anti-inflammatory activity (Kadhim, 2016). Seth *et al*. (2007) reported that amorphous silica is safe for feed additive of poultry chicken as well as human consumption. The basic mechanism of action of these nanosilica molecules is mediated by the physical absorption of very – low – density lipoprotein (VLDL), serum triglycerides

and other serum cholesterol components in the lipophilic nanopores of nanosilica which reduced the supply of the host derived cholesterol, thus limiting the growth of the malarial parasite in vivo (Seth *et al*., 2007). The enhanced efficiency of alkaloids activity as a typical reversed mobile phase absorption in the treatment of malaria had been reported by Fegas *et al*. (2010). Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester, a saturated fatty acid ester possesses anti-inflammatory, antioxidant, hypocholesterolemic, antiandrogenic, hemolytic, Alpha reductase inhibitor activity (Ali *et al*., 2016; Chen, 2014; Fatemeh, 2011; Hussein, 2016; Markkas & Govindharajalu, 2015). This indicated that Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester may inhibit and limiting the growth of P. falciparum, malaria parasites.

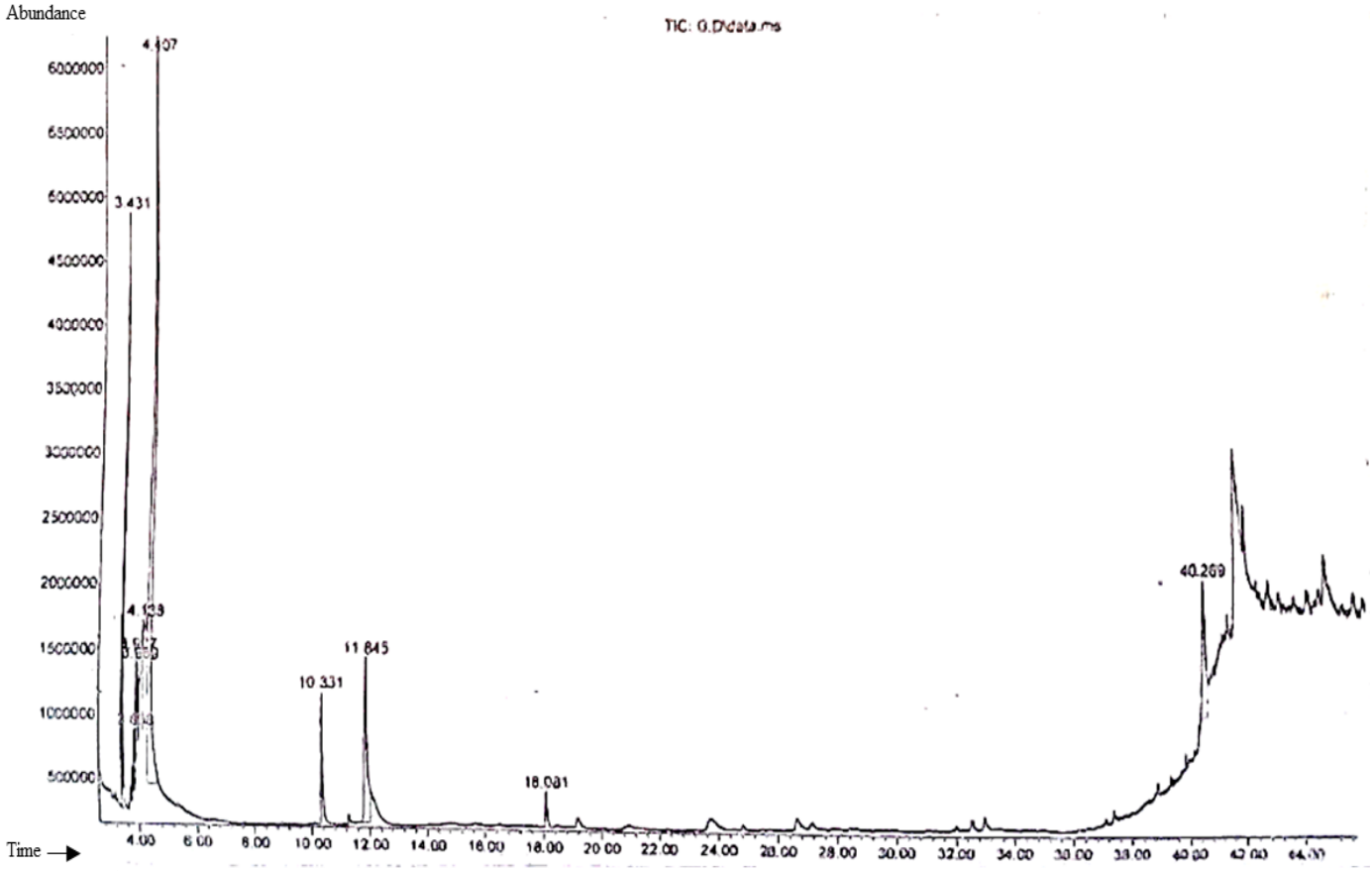


Fig. 1:
GC- MS spectra of aqueous – methanolic extract of *A. hispidum*

Table 2:
Chemical constituents of aqueous – methanolic extract of A. hispidum leaves using GC-MS detector

S/N	Retention time (min)	Chemical compound	Nature of the compound	Peak (%)
1	3.431	N – methylthio	Alcohol	13.578
2	3.838 and 11.845	Erythritol	Sugar alcohol	2.796
3	3.907 - 4.407	Glycerin	Fats & oils; Laxatives	56.720
4	10.331	Benzoylmethyl-3-hydroxy-5-nitro-2-indolinone	Terminal metabolites	5.486
5	18.081	Silanol	Polymer	1.415
6	40.269	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	Palmitic acid	10.005

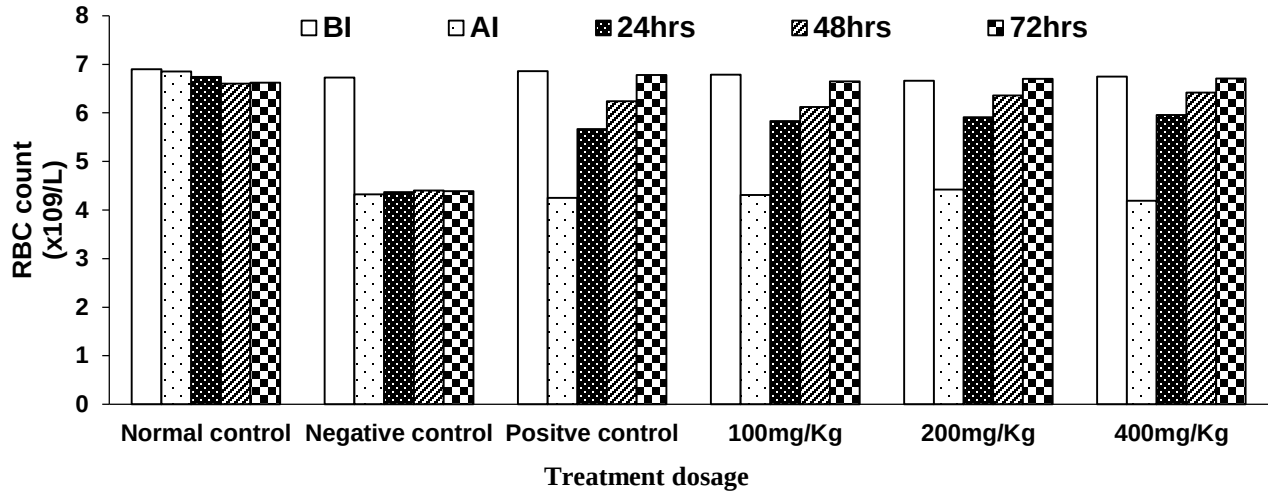


Figure 2
Effect of aqueous- methanol extract of *Acanthospermum hispidum* and chloroquine on RBC count before (BI) and after (AI) inoculation with *Plasmodium falciparum* in rats.

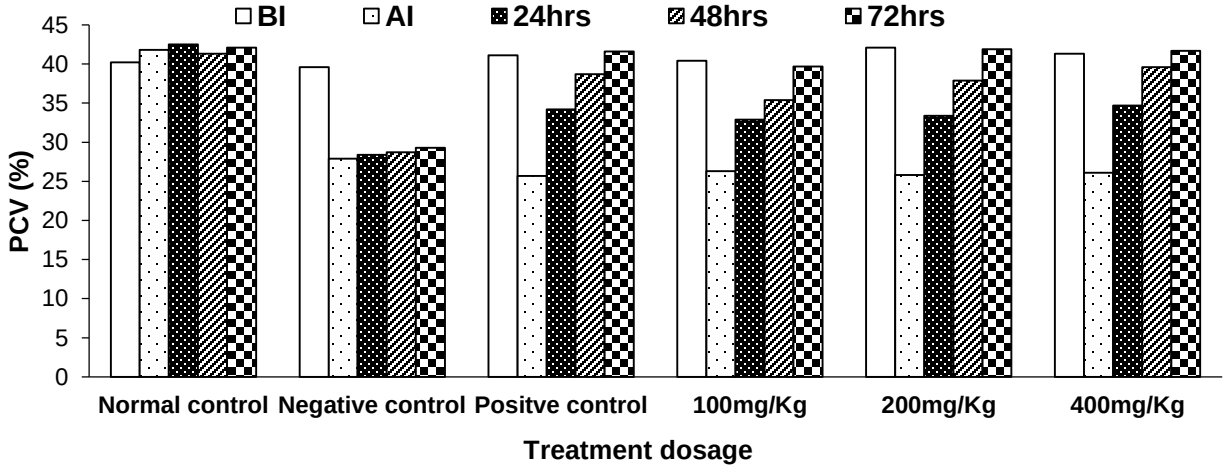


Figure 3
Effect of aqueous- methanol extract of *Acanthospermum hispidum* and chloroquine on RBC count before (BI) and after (AI) inoculation with *Plasmodium falciparum* in rats.

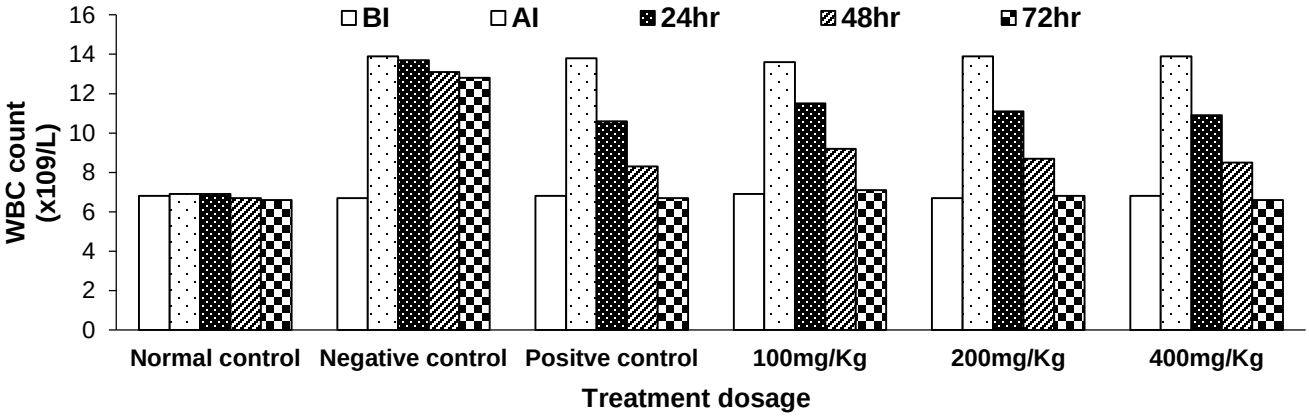


Figure 4
Effect of aqueous- methanol extract of *Acanthospermum hispidum* and chloroquine on WBC count before (BI) and after (AI) inoculation with *Plasmodium falciparum* in rats.

Figure 2 shows the effect of albino rats induced with P. falciparum and effect of chloroquine and aqueous – methanolic extract of A. hispidum leaves on RBC level. The average RBC level of normal albino rats of range 6.6 - 6.9 x 106/mm^3 was

obtained which is within the standard range of 6.76 - 9.75 x 106/mm^3 as reported by Chikezie & Okpara (2013) and Johnson-Delaney (1996) before induction but reduced by 37.03% of the normal albino rats with R2 value of 0.900 and p value of 0.037 (< 0.05). This indicated that the 0.2ml/kg P. falciparum significantly reduced the RBC of induced rats. Treatment of P. falciparum induced albino rats with dosage of chloroquine, 100mg, 200mg and 400mg of extract of A. hispidum leaves, respectively, increased the RBC of albino rats towards the normal range with R2 value of 0.900, 1.000, 1.000 and 0.900 with p values of less than 0.05 as shown in Figure 2. The increase in RBC level for all treatments was observed for period of 24, 48 and 72hours, respectively. Moreover, it can be observed that the higher volume of the aqueous extract yields more red blood cells which may be attributed to reduction in Plasmodium falciparum. This similar to the report of Bigoniya *et al* (2015).

Fig. 3 shows the PCV level in albino rats against chloroquine and variation in administration of A. hispidum leaves extract. The albino rats had its PCV level within the standard range of 37.6-50.6% prior to inoculation but reduced significantly after inoculation as shown in Figure 3 and conforms to report of Ogbodo *et al* (2010). The significant decrease in PCV may be as a result of increasing breakdown of RBCs by P. falciparum, thus resulting to anaemia as reported by Goselle and Onwuliri (2009). During the treatment, PCV level of the induced albino rats significantly increased with dosage and time with as compared with negative control. This is similar to the report of Adesina *et al* (2009). After 72hours treatment, the PCV value of P. falciparum induced experimental albino rats treated with chloroquine increased more than the samples treated with an extract dose of 100 mg/kg but lower than that of 200 with R2 value of 0.9 and p value of 0.037 (< 0.05) and 400mg/kg with R2 value of 1.000 and p value of 0.000 (< 0.05).

Effect of albino rats induced with P. falciparum and effect of chloroquine and aqueous – methanolic extract of A. hispidum leaves on WBC level are presented in Fig. 4. The WBC tested in all six samples before inoculation was within the standard range of 6.6 - 12.6 x 103/mm3 and increased significantly after inoculation with P. falciparum, but reduced after the first treatment. The increase in WBC is a prove of inflammatory response infection in the body, particularly from the parasitic infection (Obimba & Eziuzor, 2015; Sumbele *et al*, 2010; Mattson, 2011). Then, the WBC gradually returned to the normal range at subsequent administration of both the positive drug (Chloroquine) as well as the A. hispidum leaves extract for 100, 200 and 400mk/kg until the dosage was completed for 72hours as shown in Figure 4. The statistical analysis shows the significant reduction in WBC of P. falciparum induced albino rats when administered with chloroquine and A. hispidum leaves extract of 100 and 200mg/kg with R2 value of 0.900 and p value of 0.037 (< 0.05) as well as 400mg/kg with R2 value of 1.000 and p value of 0.000 (< 0.05) on. This is similar to the report of Bigoniya *et al* (2015). Significant reductions observed of the white blood cell may be attributed to localization of leukocytes away from the peripheral circulation and to the spleen and other marginal pools, rather than actual depletion or stasis (Obimba & Eziuzor, 2015)

Fig. 5 shows the PLA level in albino rats against chloroquine and variation in administration of A. hispidum leaves extract. Before inoculation, there was no significant difference in platelets of the albino rats. After inoculation, the PLA level reduced drastically and statistically significant ($p<0.005$). The decrease in platelet level with increase in parasitemia (P. falciparum) level investigated in this research has been reported in previous studies (Erhart *et al*, 2004; Saravu *et al*, 2011). The platelet level began to increase significantly towards the normal range in subsequent treatments with time for chloroquine and aqueous - methanolic extract of Acanthospermum hispidum. The statistical analysis shows the significant of the chloroquine and A. hispidum leaves extract of 100, 200 and 400mg/kg with R2 value of 0.900 and p value of 0.037 (< 0.05) on PLA of P. falciparum induced albino rats. This is similar to the report of Bigoniya *et al* (2015) and Al-Omar *et al* (2010).

The abnormalities in the haematological (RBC, WBC, PCV and Platelet) parameters are most pronounced, particularly in P. falciparum infected subjects. The improvement in haematological parameters may be attributed to effectiveness of A. hispidum leaves extracts. Fig. 6 shows the effect of chloroquine and A. hispidum leaves extract on the total proteins of induced P. falciparum albino rats.

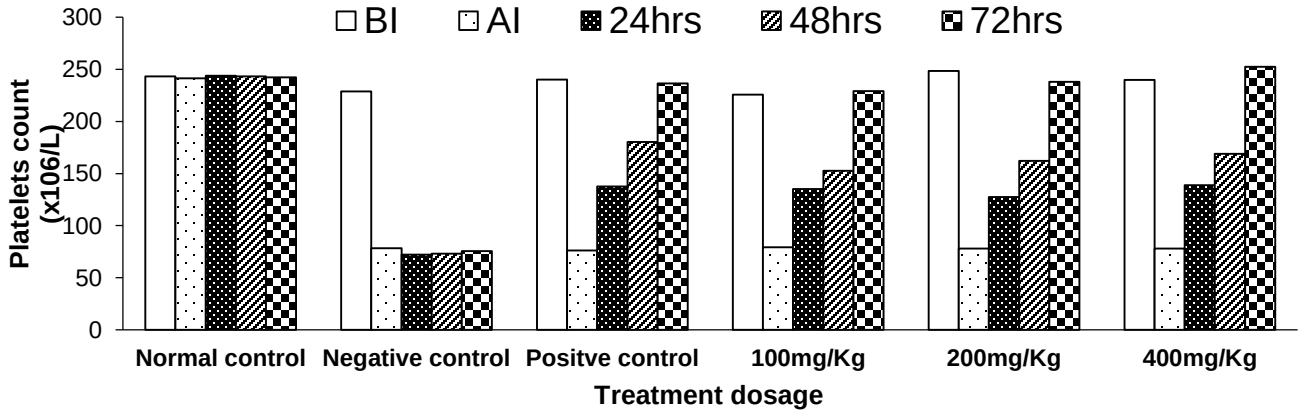


Figure 5
Effect of aqueous- methanol extract of *Acanthospermum hispidum* and chloroquine on platelet counts before (BI) and after (AI) inoculation with *Plasmodium falciparum* in rats

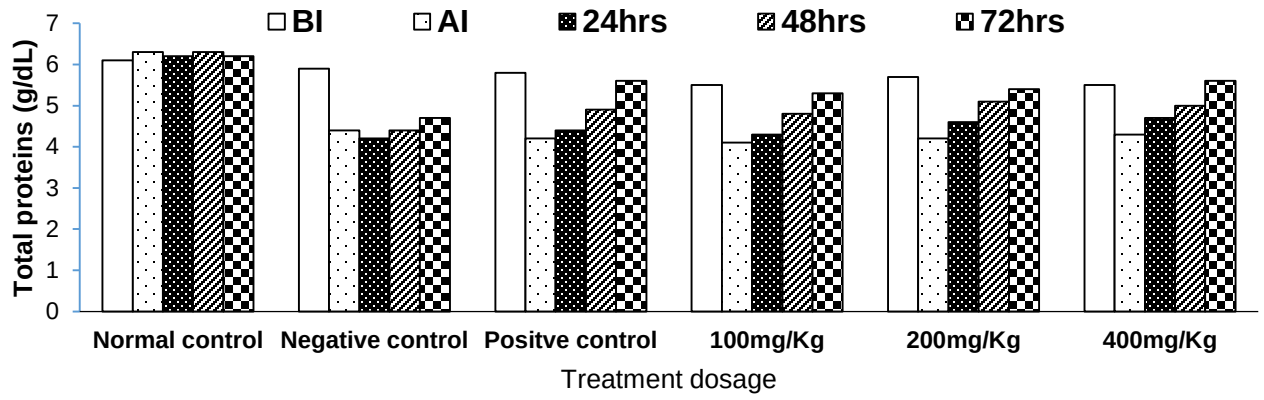


Figure 6
Effect of aqueous- methanol extract of *Acanthospermum hispidum* and chloroquine on total proteins before (BI) and after (AI) inoculation with *Plasmodium falciparum* in rats

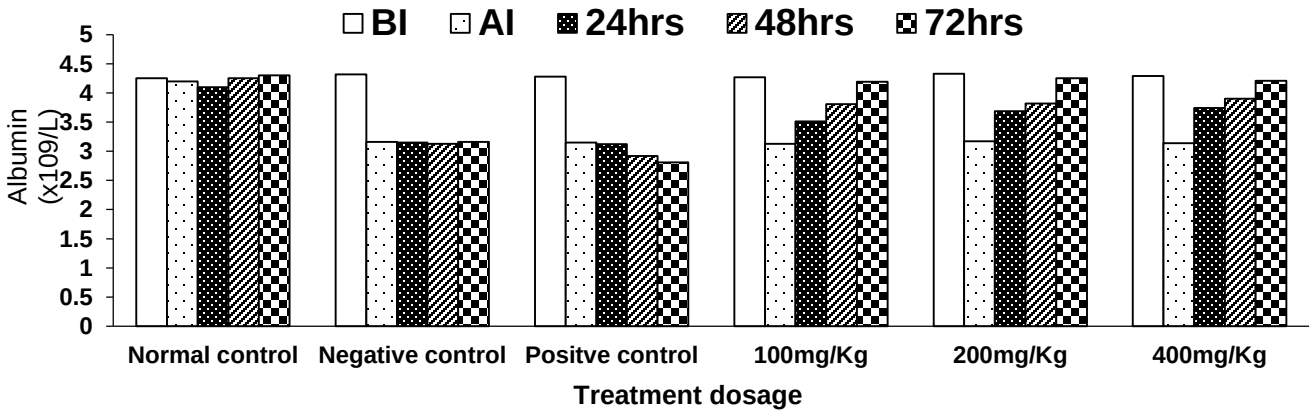


Figure 7
Effect of aqueous- methanol extract of *Acanthospermum hispidum* and chloroquine on serum albumin before (BI) and after (AI) inoculation with *Plasmodium falciparum* in rats

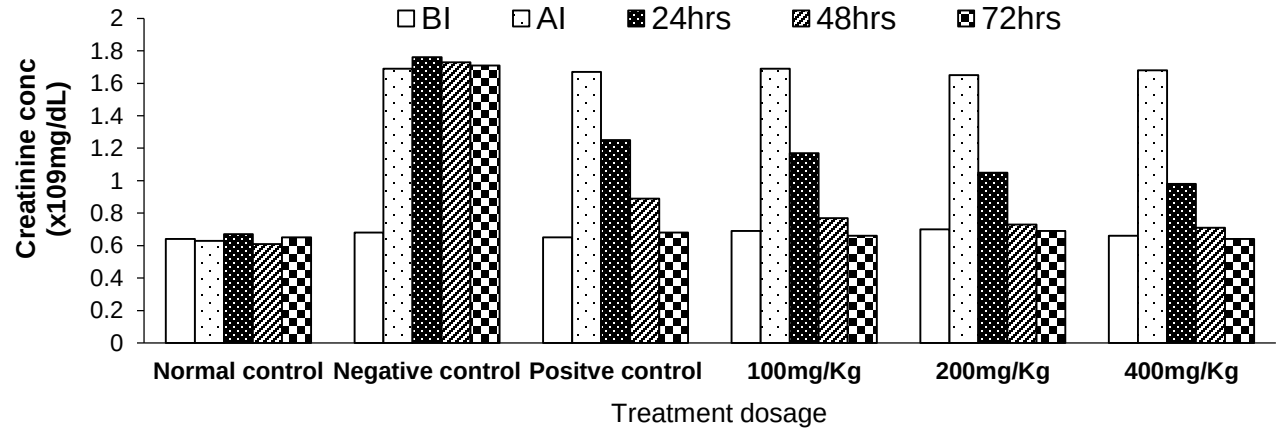
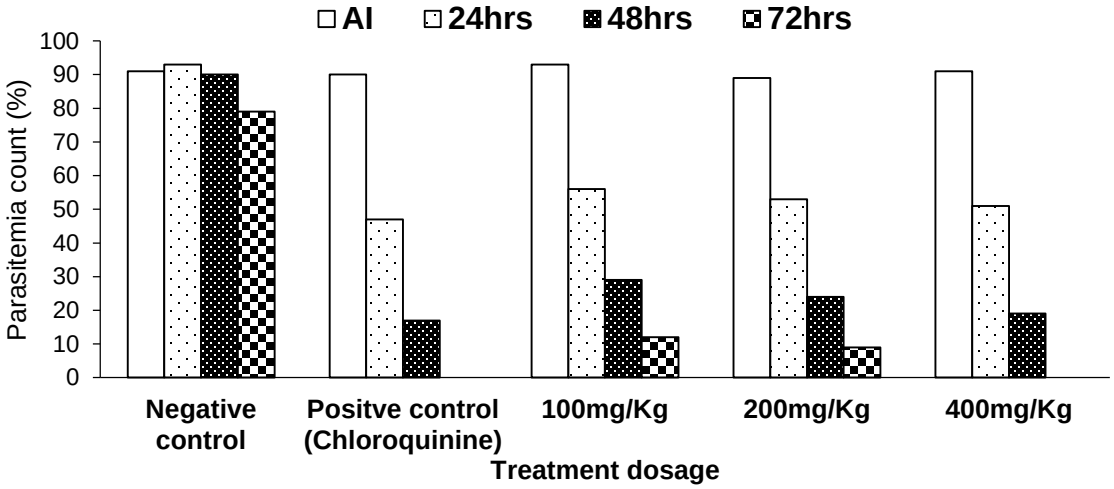


Figure 8
Effect of aqueous- methanol extract of *Acanthospermum hispidum* and chloroquine on serum creatinine levels before (BI) and after (AI) inoculation with *Plasmodium falciparum* in rats

There is no significant difference between total proteins of the experimental albino rats before induction for the period of 72hours. After induction with plasmodium falciparum, the significant decrease in total protein count of 0.037 ($p < 0.05$) was observed for the whole of 72 hours. Aqueous methanolic extract of A. hispidum leaves gradually increased the total proteins with increase in treatment time as well as that of chloroquine treatment. The maximum total proteins of 5.6g/dL was observed for albino rats administered with chloroquine and 400mk/kg of extract of A. hispidum leaves, respectively. There was slight increase in total protein in the chloroquine treated albino rats (Omotosho, Adebiyi, & Oyeyemi, 2014).

Figure 7 shows the behaviour of aqueous - methanolic extract of A. hispidum and its effect on albumin after induction of albino rats with P. falciparum. Albumin is made in the liver which keep the blood from leaking out of blood vessels. Albumin transport some medicines and other substances through the blood that contributed to tissue growth and healing. The albumin level was found to be reduced in all P. falciparum induced rats compared to the normal control. The decrease in albumin level during malaria infection may be a consequence of parasite adhesion as well as exacerbated immune response against products of oxidative stress released (Akaninwor, Essien, Chikezie, & Okpara, 2013; Chikezie & Okpara, 2013; Muangpruan, Janruengsri, Chutoam, Klongthalay, & Somsak, 2015). The further decrease in albumin level was observed when treated with and chloroquine (positive control) and it may be attributed to hemolysis during blood stage infection accumulates high levels of toxic free heme that has ability to induce oxidative stress from production of hydroxyl radicals coupled with heme-derived oxidative stress as a major factor in the iron-induced lipid peroxidation resulting in liver damage (Muangpruan *et al*., 2015). Treatment with the extract at different dosages resulted to a significant ($p < 0.05$) increase in albumin level compared to non-treated (negative control) groups and control groups. The increase in albumin indicated that aqueous – methanolic extract of A. leaves enhanced the damage liver cells with no significant difference in normal control. This is similar to the report of Edewor *et al* (2007).

Figure 8 depicts the level of creatinine in P. falciparum induced albino rats. The significant ($p < 0.05$) increase in creatinine compared to the non-induced group (normal control) may when induced with P. falciparum damage the kidney cells and may be the consequence of parasite adhesion as well as exacerbated immune response against products of oxidative stress released during infection of albino rats as reported by researchers (Muangpruan *et al*., 2015; Obimba & Eziuzor, 2015).



The level of creatinine of the extract at different dosages and the standard drug (chloroquine) gradually reduced compared to the negative control after subsequent treatment. This may be due to protective effects of aqueous – methanolic extract of A. hispidum leaves on kidney against P. falciparum induced albino rats (Muangpruan *et al*., 2015). The level of creatinine in the 400mg/kg extract insignificantly ($P > 0.05$) reduced compared to the positive control group. The accumulation in kidney cells and elevation of this electrolyte may be due to intrinsic renal lesions, decreased perfusion of the kidney, or obstruction of the lower urinary tract as the tendency of chloroquine to cause nephrotoxicity, renal function or kidney impairment (Muangpruan *et al*., 2015; Omotosho *et al*., 2014). Although the finding of several authors suggest that chloroquine may affect kidney function when taken either during treatment or prophylaxis of malaria or administered acutely or chronically in rats (Muangpruan *et al*., 2015; Omotosho *et al*., 2014)

Figure 9 shows the parasitaemia level after induced albino rats. It can be deduced that there is increase in parasitaemia significantly ($P<0.05$) compared to the normal group. After several treatment with the extracts at different dosage and the chloroquine at different time intervals, the level of parasitemia significantly ($P<0.05$) reduce compared to the negative control. The rate at which the level of parasitemia decrease in positive control during the 24hours treatment was faster compared to the 400mg/kg extract. After, the 72 hours treatment, it was observed that both the positive control and 400mg/kg extract had equivalent level of significant ($P<0.05$) with disappearance of parasitemia

In conclusion, the results of the present study showed that *P. falciparum* reduced the RBC, PCV, Platelet, total protein and albumin but increased the WBC and creatinine level of the albino rats. These observations were reversed by aqueous – methanolic extract of A. hispidium leaves. Therefore, extract of *A. hispidum* may be an effective and cheaper alternative medicine against convectional drugs if well prepared, to treat malaria and serves as a great tool in the development of more substantial drugs to eradicate most diseases caused by genus plasmodium.

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REFERENCES

Adetola O. T., Aishat I. I. and Olusola O. (2014). Perception

and Treatment Practices of malaria among tertiary institution students in Oyo and Osun States , Nigeria. Journal of Natural Sciences Research, 4(5), 33–42.

Adesina K. T., Balogun O. R., Babatunde A. S., Sanni M. A., Fadeyi A. and Aderibigbe S. (2009). Impact of malaria parasitaemia on haematologic parameters in pregnant women at booking in Ilorin, Nigeria. Trends in Medical Research, 4, 84-90.

Adu F., Gbedema S. Y., Akanwariwiak W. G., Annan K. and Boamah, V. E. (2011). The Effects of Acanthospermum hispidum extract on the antibacterial activity of amoxicillin and ciprofloxacin. Hygeia.J.D.Med, 3(1), 58–63.

Akaninwor J. O., Essien E. B., Chikezie P. C. and Okpara R. T. (2013). Haematologic and Biochemical Indices of Plasmodiumfalciparum Infected Inhabitants of Owerri, Imo State, Nigeria. Global Journal of Medical Research Diseases, 13(4).

Alferah M. A. Z. (2012). Toxicity induced histological changes in selected organs of male (Wistar) Rats by Lawsonia inermis leaf extract. European Journal of Medicinal Plants, 2(2), 151–158.

Ali F. U., Ominyi M. C., Ogbanshi M. E., Nwankwo O. V. U., Ebenyi L. N., Ogah O. and Ezenwali, M. (2016). Comparative evaluation of phytoconstituents by GC-MS of four selected herbal drugs used in Abakaliki, Ebonyi State, Nigeria . IOSR Journal of Pharmacy and Biological Sciences, 11(3), 35–42. http://doi.org/10.9790/3008-1103043542

Al-Omar I. A., Eligail A. M., Al-Ashban R. M. and Shah A. H. (2010). Effect of falciparum malaria infection on blood cholesterol and platelets. Journal of Saudi Chemical Society, 14(1), 83–89. http://doi.org/10.1016/j.jscs.2009.12.013

Araújo E. de L., Randau K. P., Sena-Filho J. G., Pimentel R. M. M. and Xavier, H. S. (2008). Revisão Acanthospermum hispidum DC (Asteraceae): perspectives for a phytotherapeutic product. Brazilian Journal of Pharmacognosy, 18(November), 777–784.

Asase A., Akwetey G. A. and Achel D. G. (2010). Ethnopharmacological use of herbal remedies for the treatment of malaria in the Dangme West District of Ghana. Journal of Ethnopharmacology, 129(3), 367–376. http://doi.org/10.1016/j.jep.2010.04.001

Barat L. M. (2006). Four malaria success stories: how malaria burden was successfully reduced in Brazil, Eritrea, India, and Vietnam. American Journal of Tropical Medicine and Hygiene, 74(1), 12–16.

Bell D. and Winstanley P. (2004). Current issues in the treatment of uncomplicated malaria in Africa. British Medical Bulletin, 71, 29–43. http://doi.org/10.1093/bmb/ldh031

Bigoniya P., Sahu T. and Tiwari, V. (2015). Hematological and biochemical effects of Sub- chronic artesunate exposure in rats. Toxicology Reports, 83(2), 280–288. http://doi.org/10.1016/j.toxrep.2015.01.007

Chakraborty A. K., Gaikwad A. V. and Singh K. B. (2012). Phytopharmacological review on Acanthospermum Hispidum. Journal of Applied Pharmaceutical Science, 02(01), 144–148.

Chen L. Y. (2014). Does Plasmodium falciparum have an Achilles' heel? Malar Chemother Control Elimin, 3(1), 1–10. http://doi.org/10.4172/2090-2778.1000114

Chikezie P. C. and Okpara R. T. (2013). Haematologic and biochemical indices of Plasmodium falciparum infected inhabitants of Owerri, Imo State, Nigeria. Journal of Medical Laboratory and Diagnosis, 4(3), 38–44. http://doi.org/10.5897/JMLD2013.0068

Edewor T. I., Olajire A. A. and Olaniyan L. E. B. (2007). Effect of Oral Administration of Ethanolic leaf extract of Acanthospermum hispidum DC on Carbon Tetrachloride Induced Acute Liver Injury in Rats. Research Journal of Medical Sciences, 1(1), 39 – 41.

Erhart L. M., Yingyuen K., Chuanak N., Buathong N., Laoboonchai A, Miller R. S., Meshnick S. R., Gasser R. A. and Wongsrichanalai C. (2004). Hematologic and clinical indices of malaria in a semi-immune population of western Thailand. Americal Journal of Tropical Medicine and Hygiene, 70 (1), 8–14.

Fatemeh T. M. (2011). Review of the role of mannitol in the therapy of children. In 18th Expert Committee on the Selection and Use of Essential Medicines (pp. 1 –43).

Fegas R., Bensalem A., Bettache Z. and Righezza M. (2010). Simultaneous Separation of Quinine and Its Diastereoisomer Quinidine by RP-HPLC. Asian Journal of Chemistry, 22(2), 1587–1590.

Gardner M. J., Hall N., Fung E., White O., Berriman M., Hyman R. W., …and Barrell B. (2002). Genome sequence of the human malaria parasite Plasmodium falciparum. Nature, 419, 498 – 511.

Goselle O. N., Onwuliri C. O. E. and Onwuliri V. A. (2009). Malaria infection in HIV/AIDS patients and its correlation with Packed Cell Volume (PCV). Journal Vector Borne Diseases, 46, 205-211.

Hussein H. M. (2016). Determination of phytochemical composition and ten elements content (Cd, Ca, Cr, Co , Fe , Pb , Mg , Mn , Ni and Zn) of cardaria draba by GC-MS , FT-IR and AAS techniques. International Journal of Pharma and Bio Sciences, 7(3), 1009–1017.

Huthmacher C., Hoppe A., Bulik S. and Holzhutter H.-G. (2010). Antimalarial drug targets in Plasmodium falciparum predicted by stage-specific metabolic network analysis. BMC Systems Biology, 4, 120. http://doi.org/10.1186/1752-0509-4-120

Ifoue S. H. T., Mofor C. T., Gouado I., Teto G., Asonganyi T. and Zollo P. H. A. (2009). Evaluation of oxidative stress and antioxidant status of pregnant women suffering from malaria in Cameroon. Indian Journal of Clinical Biochemistry, 24(3), 288–293.

Jannin V., Lemagnen G., Gueroult P., Larrouture D. and Tuleu C. (2014). Rectal route in the 21st Century to treat children. Advanced Drug Delivery Reviews, 73, 34–49. http://doi.org/10.1016/j.addr.2014.05.012

Kadhim M. J. (2016). In Vitro antifungal potential of Acinetobacter baumannii and determination of its chemical composition by gas chromatography-mass spectrometry. Der Pharma Chemica, 8(19), 657–665.

Kotepui M., Piwkham D., Phunphuech B. and Phiwklam N. (2015). Effects of malaria parasite density on blood cell parameters. Plos, 10(3), 1–11. http://doi.org/10.1371/journal.pone.0121057

Lutgen, P. (2016). The strong prophylactic and antimalarial properties of polyunsaturated fatty acids . Malaria World, 1 – 10.

Majewski M. W., Watson K. D., Cho S., Miller P. A., Franzblau S. G., and Miller M. J. (2016). Syntheses and biological evaluations of highly functionalized hydroxamate containing and N-methylthio monobactams as anti-tuberculosis and β-lactamase inhibitory agents. Med. Chem. Commun., 7(1), 141–147. http://doi.org/10.1039/C5MD00340G

Markkas N. and Govindharajalu M. (2015). Determination of phytocomponents in the methanolic extract of mollugo cerviana by GC-MS analysis. International Journal of Research in Biological Sciences, 5(4), 26–29**.**

Mattson,P. C. (2011). White blood cell response, Essentials of Pathophysiology: Concepts of Altered Health States (3rd ed.), Philadelphia: Wolters Klower Health/Lippincott Williams & Wilkins, pp. 64–65.

Muangpruan P., Janruengsri T., Chutoam P., Klongthalay S. and Somsak V. (2015). Protective effects of Andrographis paniculata leaf extract on liver and renal\r\ndamage and hypoglycemia during Plasmodium berghei infection. Malaria Control & Elimination, 2015(2), 2–5. http://doi.org/10.4172/1000135

Obimba K. C., and Eziuzor C. S. (2015). Comparative biochemical and hematological analyses of malaria patients and normal human subjects of the Federal Medical Centre Owerri, Nigeria. International Journal of Medical Advances and Discovery, 2(1), 32–40.

Ogbodo S. O., Okeke A. O., Obu H. A., Shu E. N. and Chukwurah E. F. (2010). Nutritional status of parasitemic children from malaria endemic rural communities in Eastern Nigeria. Current Pediatric and Research, 14, 131-135.

Ojurongbe O., Akindele A. A., Adedokun S. A. and Thomas B. N. (2016). Malaria : Control, Elimination, and Eradication. Human Parasitic Disease, 8, 11–15. http://doi.org/10.4137/HPD.S16590

Omotosho O. O., Adebiyi M. A. and Oyeyemi M. O. (2014). Comparative study of the haematology and serum biochemistry of male wistar rats treated with chloroquine and artesunate. Journal of Physiology and Pharmacology Advances, 4(8), 413–419. http://doi.org/10.5455/jppa.20140827112119

Saravu K. Docherla M., Vasudev A. and Shastry B. A. (2011). Thrombocytopenia in vivax and falciparum malaria: an observational study of 131 patients in Karnataka, India. Annual Tropical Medical Parasitology, 105, 593–598.

Seth D., Debnath N., Rahman A., Mukhopadhyaya S., Mewis I., Ulrichs C., …and Goswami A. (2007). Control of poultry chicken malaria by surface functionalized amorphous nanosilica. Quantitative Biology - Biomolecules, 7(7), 1 – 10.

Sumbele I. U. N., Theresa N. A., Samje M., Ndzeize T., Ngwa E. M. and Titanji V. P. K. (2010). Hematological changes and recovery associated with untreated and treated plasmodium falciparum infection in children in the mount Cameroon region. Journal of Clinical Mediine and Research, 2, 143-151.

Tuteja R. (2007). Malaria - an overview. FEBS Journal, 274, 4670–4679. http://doi.org/10.1111/j.1742-4658.2007.05997.x

Van-Schalkwyk D. A., Burrow R., Henriques G., Gadalla N. B., Beshir K. B., Hasford C., …and Sutherland C. J. (2013). Culture-adapted Plasmodium falciparum isolates from UK travellers : in vitro drug sensitivity , clonality and drug resistance markers. Malaria Journal, 12(1), 1 – 15. http://doi.org/10.1186/1475-2875-12-320.