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Effect of *Vernonia amygdalina and Ocimum gratissimum* on Antioxidant Status and Lipid Profile of Wistar Rats Exposed to Long-Term Administration of Artemisinin-based Combination Therapies

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Abstract **Original Research Article**

In this study the effect of *Vernonia amygdalina and Ocimum gratissimum* on antioxidant status and lipid profile of Wistar rats exposed to long-term administration of artemisinin-based combination therapies were investigated. Forty-two albino rats were divided into seven groups. The rats were given two different types of drugs, artesunate amodiaquine (AA) and artemether lumefantrine (AL) base on their body weight. Group 1: Control, received distilled water, group 2, received of 2.86mg/7.7mg AA, group 3, received of 1.14mg/6.86mg AL, group 4 received of 2.86mg/7.7mg AA + 200mg VA, group 5 received of 1.14mg/6.86mg AL + 200mg VA, group 6 received of 2.86mg/7.7mg AA + 200mg OG and group 7 received of 1.14mg/6.86mg AL + 200mg OG. The animals were sacrificed under chloroform anaesthesia and blood samples obtained through cardiac puncture for biochemical investigations. Artesunate-amodiaquine and arthemeter-lumefantrine and plant extracts were administered once daily for 21 days after which the total cholesterol, triacylglycerol, high density and low-density lipoprotein, superoxide dismutase (SOD), catalase (CAT) and gluthatione (GSH) and malondialdehyde (MDA) were determined. Co-administration of artesunate/amodiaquine and arthemeter/lumefantrine significantly increased ($P< 0.05$) total cholesterol, triacylglycerol and low-density lipoprotein. The SOD and CAT activities and GSH concentration significantly decreased ($P< 0.05$) with an increase in the MDA concentration in treated groups when compared with the normal control. The results obtained suggest that long term co-administration of artesunate/amodiaquine and arthemeter/lumefantrine could result to coronary heart disease and depletion of antioxidant capacity and should be given with caution.

Keywords: Malaria, antioxidant, lipid, Artemisinin, *Vernonia amygdalina, Ocimum gratissimum*.

INTRODUCTION

Malaria is the most common parasitic infection in the world, and it remains a major public health challenge in the tropics, particularly in Sub-Saharan Africa. According to recent WHO reports, fifteen countries in Sub-Saharan Africa and India account for 80% of the global malaria burden (Ayinbuomwan *et al.*, 2022). Malaria remains a major public health concern in low- and middle-income countries (LMICs). Malaria accounts for at least 600,000 deaths annually (WHO, 2021). Malaria is caused by *Plasmodium* parasites, which are spread to people through the bites of infected female *Anopheles* mosquitoes. There are several species of *Plasmodium*; however, *P. falciparum* is the most dominant in sub-Saharan Africa (WHO, 2021).

The emergence of antimalarial drug resistance has significantly contributed to the global resurgence of malaria in the last 30 years (Marsh, 1998, WHO, 2001). As a result of this development, the World Health Organization (WHO) advocated in 2001 for artemisinins to become an essential component of antimalarial treatment in countries experiencing antimalarial drug resistance. Since 2005, artemisinins have been the main-stay of malaria treatment. For uncomplicated malaria, they are administered as Artemisinin-based Combination Therapy (ACT) and as injectable monotherapy for severe malaria. Nigeria

adopted Artemether-Lumefantrine (AL) as her preferred ACT for uncomplicated malaria in 2005, and injection Artesunate as her drug of choice for severe cases (FMOH, 2014).

There is serious concern of their misuse as ACTs and other antimalarial drugs are frequently bought over-the-counter without proper prescription by an expert (Mankwe *et al.,* 2016; Agbafor, *et al.,* 2017) and unfortunately, ACT-resistant *Plasmodium falciparum* has emerged, and its prevalence is expanding (Imwong *et al.,* 2017; Aly *et al.,* 2023). Also wrong diagnosis of other fever related cases for malaria in very rampart and those people often uses antimalarial drugs in these regions. The implications of this are overdose and prolong administration of artemisinin-base combination therapies which raises the concern of drug toxicity (Mankwe *et al.,* 2016).

Prolonged and persistent malaria infections without adequate treatment can result to oxidative stress. Oxidative stress is brought on by the imbalance oxidant and antioxidant (Narsaria *et al.,* 2012). In normal cells, there is an appropriate pro-oxidant/antioxidant balance. Shifting this equilibrium in favour of the pro-oxidant side causes oxidative stress, which is characterised by increased numbers of free radicals and increased lipid peroxidation of cell membranes, which causes devastating cell damage (Ibrahim *et al.,* 2023).

Medicinal plants have been used to prevent and treat various health problems for many ages and despite the great advancement in modern medicine, phytotherapy is still commonly used (Veiga *et al.,* 2005). One of such promising plant is *Vernonia amygdalina*, especially its aerial parts (the leaves). *V. amygdalina* is documented to demonstrate diverse therapeutic activities, including anti-cancer properties, that have been attributed to its presence of coumarins, flavonoids, sesquiterpene lactones and edotides (Ijeh and Ejike, 2011; Ugwu *et al.,* 2019; Nowak *et al.,* 2022; Satria *et al.,* 2023).

Ocimum gratissimum commonly called scent leaf is a plant that is frequently grown for both culinary and medicinal purposes in West Africa, typically in and around village huts and gardens (Ugwu *et al.,* 2018; Edo *et al.,* 2023). The leaves, which have a potent scent, are frequently used to season meat and to flavour soup. The leaves are widely used as an essential seasoning in soups, especially "pepper soup," and other similar dishes in the southeast of Nigeria and beyond. Additionally, over the past 10 years, a number of researchers have been interested in medicinal plants and their bioactive chemicals due to their potential for managing and preventing both life-threatening and chronic disorders such as arthritis, diabetes, cancer, and stroke (Ugwu *et al.,* 2011; Ugwu *et al.,* 2013; Silveira *et al.,* 2020; Ugbogu *et al.,* 2021; Ghaleb-Dailah, 2022; Ademiluyi *et al.,* 2023). This research is vital to evaluate the long-term effect of ACTs and the protective effect of *Vernonia amygdalina and Ocimum gratissimum* leaves on antioxidant status and lipid profile as these drugs are commonly misused in Nigeria.

MATERIALS AND METHOD

Materials

Drugs

The study was conducted using ACTs; Artesunate/Amodiaquine (AA) 100/270mg (Sanofi Aventis), manufactured at Maphar laboratories 20250 Casablanca, Morocco and Arthemeter/Lumefantrine (AL) 20/120mg (Coartem Novartis) which was purchase from Odonah Pharmacy Okuku, Yala, Cross River State, manufactured by Novartis Pharmaceuticals Corporation Suffern, New York, U.S.A. Drugs were dissolved and reconstituted in distilled water and administered to the animals orally for 21 days based on their body weight.

Plant Materials

Fresh leaves of *Vernonia amygdalina and Ocimum gratissimum* were harvested from a garden in Okuku in Yala Local Government of Cross River State, South-South, Nigeria. The plants were identified at the herbarium unit of the Department of Biological Sciences, University of Calabar. Their fresh leaves were washed with clean water and dried under the shade for six days. Their dried leaves were milled using pestle and mortar to get a powder that was used for extraction.

Preparation of Extract

The powdered samples of *Vernonia amygdalina and Ocimum gratissimum* 100 g were soaked separately into 100 ml of distilled water, they filtered after 48 hours and filtrates were concentrated in water bath. The solutions were diluted with corn oil, to produce a solution 100 mg/ml. The administration of extract was totally by gavage. Proper concentrations were administered by the use of oropharyngeal canula and calibrated hypodermic syringe.

Experimental Animals

Forty-two **(**42) healthy adult albino rats of average weight (50-100g) were obtained from Animal House, Department of Medical Biochemistry, Cross River State University of Technology, Okuku Campus. The rats were weighed, marked and grouped into seven groups with six rats in each group. They were housed in clean well-ventilated cages and fed with vital feed and given water *ad libitum* for two weeks to acclimatize them to laboratory condition. The study lasted for three weeks. The principles governing the care of laboratory animals as laid out by the Department of Medical Biochemistry, Cross River State University of Technology, were duly observed.

Methods

Preparation of Drugs

One tablet (100/270) mg of Artesunate/Amodiaquine (AA) was dissolved in 35ml of distilled water and this was vigorously shaken for proper dissolution. 20/120mg Arthermether/lumefanthrine (AL) was dissolved in 17.5ml of distilled water with vigorous shaking for proper dissolution. Volumes corresponding to dose calculated for each rat was taken out of this stock and administered to the rats orally.

Experimental Design

The rats were given two different types of drugs, artesunate amodiaquine (AA) and artemether lumefantrine (AL) base on their body weight. Group 1: Control, received distilled water, group 2, received of 2.86mg/7.7mg AA, group 3, received of 1.14mg6.86mg AL, group 4 received of 2.86mg/7.7mg AA + 200mg VA, group 5 received of 1.14mg6.86mg AL + 200mg VA, group 6 received of 2.86mg/7.7mg AA + 200mg OG and group 7 received of 1.14mg6.86mg AL + 200mg OG.

Sample Collection

The rats were anaesthetized with chloroform and 5 ml of blood was collected from the rats through cardiac puncture and dispensed into a plane sample bottle. Sera were gotten from the blood by centrifugation and were used for biochemical investigations.

Estimation of Total Cholesterol Concentration

Total cholesterol concentration was estimated using Randox kit based on NCEP (2001).

Determination of Triacylglycerol Concentration

Triacylglycerol concentration was estimated using Randox kit as described by on Tietz (1995).

Determination of HDL-Cholesterol Concentration

High density lipoprotein (HDL-cholesterol) concentration was estimated using Randox kit based on NCEP (2001).

Estimation of VLDL-c and LDL-c by Calculations

Very low-density lipoprotein cholesterol (VLDL-c) and low density lipoprotein cholesterol (LDL-c) were calculated from relationships established by Burnstein and Sammaille, 1960; Friedewald *et al.*, (1972) respectively using triacylglycerol (TG), HDL-cholesterol and total cholesterol (Tc) levels.

Lipid Peroxidation and Antioxidant Determination

Malondialdehyde (MDA) was determined by using a method adapted from Khoschsorur *et al.*, (2000). The method of Rukkumani *et al.*, (2004) was followed in estimating the level of reduced glutathione (GSH). Superoxide dismutase (SOD) was determined by the method of Crosti *et al.*, (1987). Then catalase (CAT) activity was determined by the method of Aebi *et al.*, (1984).

Statistical Analysis

The experimental data were analysed for statistical significance by one-way analysis of variance and post hoc comparison using the SPSS version. All data were reported as mean ± SD and statistical significance was accepted at $P < 0.05$.

RESULTS

Results of the effect of daily oral administration of the Vernonia amygdalina and Ocimum gratissimum, artesunate-amodiaquine (AA) and arthemeter-lumefetrine on the Wistar albino rats are presented below

Table 1: Effect of oral administration of Artesunate amodiaquine (AA) and Arthemeter lumefetrine (AL) for 21 days on serum cholesterol, triacylglycerol, HDL, LDL and VLDL of Wister albino rat

GROUP	TCHOL (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
GROUP 1: CONTROL	105.39±4.99^{a}	88.61±3.70^{a}	29.98±1.74^{b}	35.14±3.93^{a}	40.29±1.65^{a}
GROUP 2: 1.43mg/3.86mg of AA + 100mg VA	117.36±2.63^{b}	93.02±3.59^{ab}	21.75±3.10^{a}	53.33±3.07^{bc}	42.28±1.63^{ab}
GROUP 3: 2.86mg/7.7mg of AA + 200mg VA	119.84±5.29^{b}	93.80±2.87^{bc}	22.75±3.79^{a}	54.45±5.84^{bc}	42.64±1.30^{bc}
GROUP 4: 5.72mg/15.42mg of AA	122.19±4.60^{b}	95.15±3.31^{bc}	22.52±4.09^{a}	56.43±3.23^{c}	43.25±1.51^{bc}
GROUP 5: 0.57mg/3.43mg of AL + 100mg VA	107.21±6.42^{a}	97.41±3.06^{bc}	23.10±4.67^{a}	39.84±6.84^{a}	44.28±1.39^{bc}
GROUP 6: 1.14mg/6.86mg of AL + 200mg VA	115.39±6.46^{b}	93.65±4.57^{bc}	23.27±3.87^{a}	49.55±5.20^{b}	42.57±2.08^{bc}
GROUP 7: 2.28mg/13.17mg of AL	121.34±4.33^{b}	98.18±2.62^{c}	22.01±1.98^{a}	54.71±4.04^{bc}	44.63±1.19^{c}

Values were expressed as Mean ± SD. Identical superscript (i.e. a) means there is no significant difference between the comparing group $P>0.05$. Non- identical superscripts (i.e. a, b, c) means there is significance between the comparing groups at $P<0.05$.

In the above table, total cholesterol, triacylglycerol, LDL and VLDL significantly (P<0.05) increased in all groups group when compared to the normal control while significant decrease (P<0.05) was observed in HDL in all groups when compared to the normal control.

Table 2: Effect of oral administration of Artesunate-amodiaquine (AA) and arthemeter-lumefetrine (AL) for 21 days on serum SOD, CAT, GSH and MDA of Wistar albino rat

GROUP	SOD (mg protein)	CAT (mg protein)	GSH (mg/g)	MDA (mg protein)
GROUP 1: CONTROL	0.38 ± 0.06^{c}	151.45 ± 5.77^{d}	35.75 ± 1.95^{d}	4.05 ± 0.30^{a}
GROUP 2: 2.86mg/7.7mg AA	0.29 ± 0.09^{ab}	134.70 ± 4.97^{a}	28.76 ± 1.78^{a}	8.05 ± 0.46^{d}
GROUP 3: 1.14mg6.86mg AL	0.27 ± 0.05^{a}	135.31 ± 5.02^{a}	29.54 ± 1.68^{ab}	7.74 ± 0.45^{d}
GROUP 4: 2.86mg/7.7mg AA + 200mg VA	0.34 ± 0.04^{abc}	143.69 ± 2.63^{bc}	31.45 ± 0.95^{bc}	6.12 ± 0.23^{b}
GROUP 5: 1.14mg6.86mg AL + 200mg VA	0.35 ± 0.04^{bc}	145.94 ± 4.27^{c}	32.18 ± 1.48^{c}	5.83 ± 0.53^{b}
GROUP 6: 2.86mg/7.7mg AA + 200mg OG	0.32 ± 0.03^{abc}	139.06 ± 2.17^{ab}	31.78 ± 1.27^{c}	6.76 ± 0.41^{c}
GROUP 7: 1.14mg6.86mg AL + 200mg OG	0.33 ± 0.03^{abc}	140.09 ± 2.34^{ab}	32.31 ± 0.96^{c}	6.40 ± 0.70^{bc}

Values were expressed as Mean ± SD. Identical superscript (i.e. a) means there is no significant difference between the comparing group $P>0.05$. Non- identical superscripts (i.e. a, b, c, d) means there is significance between the comparing groups at $P<0.05$.

In the above table, SOD, CAT and GSH significantly ($P<0.05$) decreased in all groups group when compared to the normal control. There was a significant ($P<0.05$) increase in MDA in all groups when compared to the normal control.

DISCUSSION

The malaria problem has been greatest in Africa through recent times, but the imbalance between Africa and the rest of the world has been growing. Malaria has a huge disease burden globally with about 40% of world population at risk, though it occurs almost exclusively in the tropics and subtropics (Bhatt *et al.,* 2015; Badmos *et al.,* 2021; WHO, 2022; Bello *et al.,* 2023). In this study the effect of long-term oral administration of artemisininm based-combination therapy and protective effect on lipid profile and antioxidant status were investigated. As it has been established, total lipid profile of an individual is a contributory factor resulting from blood cholesterol along with its associated varieties of lipoproteins and triacylglycerol (Maruf *et al.,* 2006). Cholesterol is an amphipathic lipid and as such is an essential structural component of all cell membranes and of the outer layer of plasma lipoproteins. It is present in tissues and in plasma lipoprotein either as free cholesterol or, combined with a long-chain fatty acid, as cholesteryl ester (Bansal *et al.,* 2005).

In this present study we observed that long term administration of the drugs altered the metabolism of lipids resulting in a significant ($P<0.05$) elevation of total cholesterol. Also there was a significant ($P<0.05$) elevation of triacylglycerol, low density lipoprotein and very low density lipoprotein in all the treated groups while the levels of high density lipoprotein decreased significantly ($P<0.05$) in all the treated groups. Under normal physiological conditions, liver ensures homeostasis of lipid and lipoprotein metabolism (Jiang *et al.,* 2006). Most plasma apolipoproteins, endogenous lipids and lipoproteins have their origin from the liver (Tietge *et al.,* 1998; Mayes and Botham, 2003; Jiang *et al.,* 2006), which depends on cellular integrity and functionality of the hepatocytes. This altered lipid metabolism observed by the increase in the above parameters were ameliorated by administration of *Vernonia amygdalina and Ocimum gratissimum*.

Elevations of serum total cholesterol and low density lipoprotein (LDL) cholesterol have been implicated as primary risk factor for cardiovascular diseases (Edijala *et al.,* 2005; Dasofunjo *et al.,* 2013). Cholesterol is insoluble in the blood and needed the help carrier molecules called lipoproteins such as LDL, HDL and VLDL to transport it within the body. Disposition of blood pressure and coronary heart disease has been found to have strong correlation with lipid profile especially blood cholesterol level (Cotaran, 1999; Oluba *et al.,* 2012). Previous studies have shown that high cholesterol and triglycerides plasma level have been implicated to be risk factors for progression of coronary heart disease (Abrass, 2004).

Low-density lipoproteins (LDLs) transport cholesterol from its site of synthesis in the liver to the various tissues and body cells, where it is separated from the lipoprotein and is used by the cell. Oxidation of LDL in the vascular endothelium is a precursor to plaque formation, thus leading to cardiovascular diseases. Cholesterol attached to LDLs primarily builds up in arteries of the blood vessels. LDL in the artery, react with oxygen free radical and get oxidized resulting in the formation of plaque and hardening of the arteries which can also lead to heart attack (Kunitomo, 2007).

The increase in serum LDL-cholesterol concentration, total cholesterol concentration and triacylglycerol caused by the administration of amodiaquine and its co-administration with artesunate suggest that they may predispose subjects to some

cardiovascular diseases. Assmann and Schulte (1992) reported that increase in triacylglycerol coinciding with high ratio of plasma LDL is a powerful additional coronary risk factor. High-density lipoproteins (HDLs) transport cholesterol from the tissues back to the liver, where it is broken down to bile acids and is then excreted, thus making HDL beneficial to health (Gordon *et al.*, 1977). A reduction in the HDL-cholesterol which is observed in this study could therefore be hazardous.

All these point to the fact that the long term co-administration of both drugs may increase the risk of cardiovascular diseases. This finding supports the earlier report by Ajani *et al.*, (2008) who indicated that amodiaquine may predispose patients to cardiovascular diseases. This finding supports the earlier report by Ajani *et al.*, (2008) who indicated that amodiaquine may predispose patients to cardiovascular diseases. The results obtained from this study suggest that co-administration of artesunate/amodiaquine and arthemeter/lumefantrine for a long time may increase the risk of coronary heart disease.

Oxidative stress contributes significantly in the pathogenesis of many diseases. The increase in lipid peroxides as an indicator of oxidative stress in the organism has been documented as an ultimate toxic effect of raised reactive oxygen species production by the immune system of the body, as well as a synchronised release of oxygen radicals during haemoglobin degradation by malaria parasites (Tiyong *et al.*, 2009; Atiku *et al.*, 2019; Ojongnkpot *et al.*, 2023). The mean concentration of MDA was significantly higher among the rats treated with ACTs compared to normal control and those treated with plant extracts. This is a consequence that the rats had significantly reduced antioxidant defence system such as the mean concentration of GSH, and the activities of CAT and SOD than normal control groups.

Oxidative stress is triggered by, respectively, the increase of reactive oxygen and/or nitrogen atoms that have unpaired electrons in their outer shell (Turrens, 2003). The oxidative stress marker (MDA) recorded in this study was higher in ACTs treated rats than in the normal control rats. This pathological development increases the oxidative stress index, as indicated in the results obtained, which is the ratio of total oxidative stress to total antioxidant activity and establishes the exact level of oxidative and antioxidant imbalance in ACTs treated rats (Ebrahim *et al.*, 2019).

Antioxidants are molecules which can combine with free radicals safely to stop the chain response before essential molecules are impaired. Excessive quantities of free radicals and oxidants can result in oxidative stress, a detrimental process that can dramatically affect wellbeing; and successively a variety of tissues, cellular elements, or components such as membrane lipids, proteins, lipoproteins, and deoxyribonucleic acid are all impacted (Lobo *et al.*, 2010). Antioxidants such as GSH, SOD, and CAT are known to play a major function in the preservation and control of ROS levels during malaria parasite infection and drug-drug interaction (Akanbi *et al.*, 2009). This may explain the variations in the level of oxidative stress in malaria patients using ACTs.

In this the long-term administration of artesunate amodiaquine and artemether lumefantrine seems to have altered the lipid metabolism and antioxidant status which were reversed by the treatment with *Vernonia amygdalina and Ocimum gratissimum*. The plants extracts helped in strengthening the endogenous antioxidant defense against oxidative stress. It then implies that co-administration of artesunate/amodiaquine and arthemeter/lumefantrine for malaria treatment in patients with heart related diseases should be with caution.

CONCLUSION

The findings from this study demonstrate that that long term co-administration of artesunate/amodiaquine and arthemeter/lumefantrine could lead to coronary heart disease and depletion of antioxidant capacity and should be given with caution. The findings further revealed 10 that consumption of *Vernonia amygdalina and Ocimum gratissimum* could help to reduce the induced oxidative damage and alteration in the lipid metabolism caused by the drugs.

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