COMPARATIVE STUDY OF KIDNEY LIPID PROFILE, GLUTATHIONE AND MALONDIALDEHYDE LEVELS IN MALARIA INFECTED MICE TREATED WITH AFRAMOMUM SCEPTRUM

Akpovwehe A. Anigboro
Department of Biochemistry, Delta State University, Abraka, Delta State. Nigeria. E-mail: akposanigboro@yahoo.co.uk.com. Tel: 08034230386.

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The effect of aqueous extract of Aframomum sceptrum (spice) on the kidney of mice induced with malaria was assessed in this study. Six groups of six mice each were used: normal control, parasite control, normal + 250 mg of spice, parasite + 250 mg of spice, parasite+350 mg of spice and normal+350 mg of spice. After four days of treatment with aqueous extract of A. sceptrum, the mice were sacrificed and the kidneys collected to determine the levels of some biomarkers. The results revealed that the parasitized mice had a significant (p<0.05) increase in triglyceride (TG), malondialdehyde (MDA), low-density lipoprotein (LDL)-cholesterol and a significant (p<0.05) decrease in high-density lipoprotein (HDL)-cholesterol. These parameters were, however, significantly decreased when parasitized mice were treated with the aqueous extract of A. sceptrum. However, no significant (p>0.05) alteration was observed in kidney level of reduced glutathione (GSH). It can be inferred that the antioxidants and bioactive metabolites of A. sceptrum are responsible for the observed change. Thus, A. sceptrum can play a crucial function in the management of malaria.

Key words: Aframomum sceptrum, kidney, lipid profile, glutathione, malondialdehyde, malaria.

INTRODUCTION

World Health Organization estimated that there are 270 million clinical cases of malarial diseases annually and these diseases result in at least 1.5 – 2.7 million deaths a year (WHO, 2000). Malaria is a pyrexia illness that typically begins with chills, headache, and sweating. Further threatening complications such as anaemia, jaundice, acute renal failure (ARF) and hepatic disorder occur with malaria parasites such as Plasmodium falciparum, P. vivax and P. malariae (Ozen et al., 2006; Kumar et al., 2007). These malaria parasites pose a severe challenge on infected individuals by exerting oxidative stress on red blood cells resulting in the destruction of infected erythrocytes leading to the development of anaemia (Das and Nanda, 1999; Kremsner et al., 2000). The parasites are thought to produce reactive oxygen species (ROS) from which they are endangered (Potter et al., 2005) through one or more of the following pathways; electron transport chain (Deslauriers et al., 1987), haemoglobin and cytosolic protein degradation (Atamma and Ginsburg, 1997),and redox reaction of hemin (Har-El et al., 1993). Phagocyte generated ROS are non-specific modulator molecules in their mode of defense mechanism thus destroying both parasitized and non-parasitized erythrocytes (Kiukarni et al., 2003). However, if not monitored by the host cytoprotective enzymes and antioxidants, it may lead to oxidative damage thereby increasing the evidence that such injuries contribute to the patho-physiology of many diseases (Gora et al., 2006).

Gilles et al. (1963) and Pakasa et al. (1985) have reported the implication of renal in P. falciparum, P. malariae, and P. virax infections and these are linked with nephropathy mainly in malaria prevalent areas of Africa (Gilles et al., 1963; Pakasa et al., 1985). Acute renal failure (ARF) is a common and dangerous complication of falciparum malaria in non-immune adults and older children (Eiam-Ong et al., 1998; Barsoum, 2000; Eiam-Ong, 2003). Recent studies have reported frequent morbidity and mortality caused by malaria induced ARF among semi-immune African children (Wasiu and Kayode, 1994; Zewdu, 1994).
Spices have been acknowledged for their ability to make food more pleasant as well as possessing preservative and antioxidant properties (Shobana and Naidu, 2000). Spices have been used for medicinal purposes as well as food additives over centuries. New medical research is now unraveling the basic physiological and molecular mechanism of their action as well as providing some scientific proofs of their effectiveness (Emily and Barbara, 2007).

*Aframomum scepturn*, a well known local spice consumed in south-south part of Nigeria, has been analyzed for its chemical and antioxidant composition (Erukainure et al., 2010). The presence of bioactive metabolites such as flavonoids, phenols, tannins alkaloids and saponins in it are responsible for its antioxidant activity and this affirms the use of this spice in the treatment of various diseases. The aim of this study was to assess the kidney lipid profile, glutathione and malondialdhyde in malaria infected mice as well as the effect of different concentration of *A. scepturm* extract on these biochemical parameters.

**MATERIALS AND METHODS**

**Experimental animals**

Healthy adult albino mice that weighed 17- 25 g were used in the investigation. They were bred and taken care of in the central animal house, College of Health Sciences, Delta State University, Abraka. They were kept in cages built of stainless steel and plastic under maintained condition of 12 h light/12 dark cycle. They were fed with typical laboratory pellet diet and water.

**Inoculation of experimental animal**

The parasite (*Plasmodium berghei*) was obtained from Nigerian Institute of Medical Research, Yaba Lagos. The animals were infected with parasites by obtaining parasitized blood from the cut tip of the tail of an infected mouse. 0.1 mL of infected blood was diluted in 0.9 mL phosphate buffer. The mice were inoculated intraperitoneally with 0.1 mL parasitized suspension. The degree of parasitaemia was determined by staining thin blood films made by blood collected from the cut tip of the tail with Geimsa stain (WHO, 2000).

**Experimental procedure**

In this research, 36 mice were used and were divided into six groups, each containing six mice: Group 1, Normal control; Group 2, parasitized control; Group 3, normal + 250 mg spice extract / body weight; Group 4, parasitized + 250 mg spice extract / body weight; Group 5, parasitized + 350 mg spice extract / body weight; Group 6, normal + 350 mg spice extract / body weight.

**Feeding of experimental animal**

The *A. scepturm* extracts were administered once daily for 4 days using intragastric tube. After 4 days the mice were deprived of food overnight and sacrificed by cervical decapitation.

**Preparation of tissue homogenate**

Blood sample was collected from sacrificed mice (cervical decapitation) into heparinized containers. Plasma was separated from the blood by centrifugation at 1000 g for 15 min. 0.5 g of wet kidney tissue was homogenized in 4.5 mL of normal saline and the supernatant were stored in the freezer until required. The clear supernatants got from the process were filtered using whatman No. 1 filter paper and used for biochemical assay.

**Spice**

*A. scepturm* was bought from the local market in Abraka, Ethiope East Local Government Area, Delta State, Nigeria.

**Preparation of the spice extract**

The spice was dried to constant weight for two weeks by sunlight. This was ground to powder by using Warren blender. 100 g of the powder was soaked in 400 mL of distilled water and allowed to boil for 5 min. This was agitated for 10 min and allowed to cool, and filtered thereafter. Rotary evaporator at 40-50°C was used to concentrate the extract under reduced pressure. The concentrated extract was stored in a deep freezer until required.

**Lipid profile assays**

**Total cholesterol (TC)**

The total cholesterol content in the kidney
sample was determined using the enzymatic method described by Allan et al. (1979). 1.0 mL, 10.0 μL, 10.0 μL and 10 μL of reagent, distilled water, standard and specimen, respectively were added into tubes and left at room temperature and absorbance was taken at 530 nm after blanking the spectrometer.

**HDL -cholesterol**

HDL -cholesterol was determined according to the method described by Badimon et al. (1990). This reagent was first used for the treatment of the specimen before the determination of HDL -cholesterol with a reagent for total cholesterol. HDL -cholesterol obtained in supernatant after centrifugation was measured with total cholesterol. In this method, 1.0 mL, 25.0 μL, 25.0 μL and 25.0 μL of reagent, distilled water, standard and specimen, respectively were thoroughly mixed in various tubes. The content in each of these tubes was left for 5 min at 37°C at room temperature and the absorbance for each mixture was recorded at 500 nm against reagent blank.

**LDL - cholesterol**

LDL - cholesterol was quantified by the difference between the total cholesterol and HDL- cholesterol content of the supernatant obtained after the precipitation of the LDL fraction of polyvinyl sulphate (PVS) in the presence of polyethylene glycol mono methyl ether. This method requires the mixture of 0.1 mL (3 drops) of the precipitant solution and 0.2 mL of the samples in various tubes. The contents of the tubes were thoroughly mixed for 15 min at room temperature (20-25°C) and centrifuged at 2,000X g/5 min.

**Triglyceride (TG)**

The triglyceride was determined according to the method of Fossati and Prencipe (1983), associated with Trinder (1969). The present procedure which requires 1.0 mL, 10.0 μL, 1.0 mL and 10.0 μL of reagent, distilled water, standard and specimen involves the use of lipase to hydrolyze triglycerides. The concentration of glycerol was determined by enzymatic assay coupled with Trinder reaction that ends in the formation of a quinoneimine dye. The quantity of dye produced was determined spectrophotometrically at 540 nm and this was directly proportional to the concentration of triglycerides in the sample (Fossati, 1982).

**Reduced glutathione (GSH) assay**

The determination of kidney reduced glutathione was carried out by the method of Ellman (1959). It involves the reaction between the sulphhydryl group of reduced glutathione and (5,5 -dithio bis-2- nitrobenzoic acid (DTNB). The reaction produces a yellow colour, 5-thio-2-nitrobenzoic acid (TNB). The generated mixed disulfide, GSTNB (between GSH and TNB) is reduced by glutathione reductase to recycle the GSH and produce more TNB. The rate of TNB production is directly proportional to GSH level in the sample. To 0.5 ml of tissue homogenate was added 2 ml of 10% trichloroacetic acid (TCA), centrifuged and supernatant collected. To 1 ml of the supernatant was added 0.5 mL of Ellman’s reagent followed by the addition of 3 mL of phosphate buffer. The generated colour was read at 412 nm. A series of standards and blank were prepared in similar way along with a blank containing 3.5 ml of buffer. The concentration of reduced GSH in micromole (μmole of GSH per 0.5g of wet tissue) was intrapulated from the standard curve plotted.

**Malondialdehyde assay**

Beuge and Augustine (1978)’s method was used to determine malondialdehyde content (lipid peroxidation) in kidney tissue. To 0.2 mL of the tissue suspension was added 2 mL of glacial acetic acid followed by 2 mL of 1% thiobarbituric acid (TBA). The loosely stoppered tube was then immersed in boiling water for 15 min thereafter the tube was swirled slightly at intervals. The cooled mixture was centrifuged for 10 min at 5000 g. and absorbance was read at 532 nm after using the blank (made up of 2 mL glacial acetic acid, 2 mL TBA and 0.2 mL of distilled water) to standardize the instrument.

**Statistical analysis**

Statistical analysis was carried out using students’ T -test – one-way analysis of variance (ANOVA) and mean values were calculated using Turkey’s multiple comparison test, with the help of WINKS software. Values of p < 0.05 were considered significant. Values were expressed as mean ± SD.
RESULTS AND DISCUSSION

The overall focus of this study was to determine the lipid profile [triglyceride (TG), total cholesterol, HDL-cholesterol and LDL-cholesterol] as well as glutathione and malondialdehyde (MDA) levels in the kidney of malaria infected mice treated with *A. sceptrum.*

In Figure 1, there was a significant \((p<0.05)\) increase in triacylglyceride level in group 2 (parasitized control) when compared with that in groups 1 (Normal control), 3 (Normal mice + 250 mg spice extract / bwt) and 6 (Normal mice + 350 mg spice extract / bwt).

This may be due to the combined effect of lipid degradation and *de novo* lipid synthesis induced by the malaria parasite (whose genome contain gene similar to those for type II fatty acid synthesis) in an attempt to meet its lipid requirements for it growth and metabolism (Maegrith, 1981).

Furthermore, according to Maheshwari et al. (2004), increased kidney TG may be associated with increased proteinuria or albuminuria, commonly diagnosed in malaria induced acute renal failure. Therefore, the significant decrease of triacylglyceride in groups 4 and 5 compared to that in group 2 may probably be due to the antioxidant activity of the spice extract. There is a further reduction in TG level in group 5 when compared with that in group 4, and this may be attributed to the increased concentration of antioxidants activity in group 5 treated with 350 mg of the spice extract.

It has been reported previously (Nishida et al., 1999) that hypertriglyceridemia was a significant risk factor for progression of renal disease in chronic renal failure. Syrjänen et al. (2000) also acknowledged the presence of high triglyceride level at the time of biopsy in patients with progressive nephropathy than those with stable disease. Samuelsson et al. (1998) demonstrated that triglyceride apolipoprotein- B containing lipoproteins are associated with a frequent loss of kidney function in chronic renal insufficiency. All these studies explained the connection between lipid and kidney survival in diseased conditions.

There is a significant \((P<0.05)\) increase in total cholesterol level in groups 2, 4 and 5 when compared with that in groups 1, 3 and 6 respectively as shown in Figure 1. This may probably be attributed to the action of the parasite on the body which altered the cholesterol level in the kidney while there was no significant \((p>0.05)\) change between group 3 and 6 on one hand together with 4 and 5. This may probably be due to the insufficient antioxidant activities of the bioactive metabolites in the spice extract.

The kidney of malaria infected mice...
showed a significant increase in the level of total cholesterol in group 2. This may be as a result of a corresponding increase in triglycerides as confirmed by previous studies on biochemical status in malaria (Adekunle et al., 2007).

HDL-cholesterol in the tissue showed a significant (p<0.05) decrease in group 2 when compared with that of groups 1, 3, 4, 5, and 6 while there was no observable significant (p>0.05) change in groups 5 and 6 as shown in Figure 1. This indicates that the serum HDL-cholesterol will probably be high. This could be associated with the alteration in lipid metabolism induced by the parasite activity in the host. Further explanation to this could be attributed to the decrease in the activity of the enzymes involved in the synthesis of apolipoprotein A (apo-A), a major protein component of HDL. This may be due to hyperpyrexia, which provides an unsuitable condition for the synthesis of apolipoprotein A. Apo-A level may also be reduced, as protein may be lost via denaturation caused by ROS, proteinuria and anorexia which are clinical symptoms of malaria. However, the administration of A. sceptrum extract caused a significant increase in HDL-cholesterol in group 4 and 5 when compared with that in group 2. This change may be attributed to the effect of the bioactive constituents of the extract in the tissue which may enhance the activity of lecithin cholesterol acyl-transferase (LCAT) that catalyzes the esterification of cholesterol preparing it for storage as well as paroxane which plays an important antioxidant role.

The result in Figure 1 shows that there was a high level of LDL – cholesterol in the parasitized group (group 2) when compared with that in normal control (group 1). This could probably be due to an increase in the hydrolysis of cholesterylesters by cholesterylester hydrolase and the activation of apo – B, thus increasing the level of cholesterol in the kidney. However, the administration of the spice extract resulted to a decrease in the LDL cholesterol level. The bioactive metabolite of the extract may probably act on ACAT (acyl CoA: cholesterol acyl transferase), thus enhancing its activity.

LDL cholesterol commonly referred to as “bad cholesterol” poses a risk of LDL invades the glomeruli and interstitial region of the nephrons it becomes oxidized by mesangial cells (Miyata and Takebayshi, 1987). The oxidized form is more retained by proteoglycan, I increase in the LDL- cholesterol which is associated with peripheral vascular disease, stroke and atherosclerosis (Crowell and Otvos, 2004).

The result in Figure 2 shows a significant (p<0.05) increase in lipid peroxidation in the untreated parasitized mice (group 2) which indicate that the malaria parasite inside the erythrocytes of the kidney tissue exerts oxidative stress which triggers non- specific defense mechanisms that cause damage on the surrounding cell membrane thus contributing to the patho-physiology of malaria. This result is in accordance with the work of Hunt and Stocker (1990) on the effect of malaria on oxidative stress and redox status. The result of this study demonstrated a significant (p<0.05) reduction in the level of malondialdehyde (MDA) in the parasitized mice treated with 250 and 350 mg spice extract / body weight, respectively, when compared with the MDA level in untreated parasitized mice. This may probably be the resulting effect of the bioactive metabolite and antioxidant activity of the extract which helps in reducing or scavenging the reactive oxygen species (ROS) generated by the immune response of the host. However, there was a further reduction of MDA level in group 5 when compared with that in group 4. This may also be attributed to the higher concentration of the antioxidant activity in group 5 treated with 350 mg of the spice extract. The striking increase in malondialdehyde level in group 6 as compared with that in groups 1 and 3 may probably be attributed to the presence of increased concentration of saponin content of the A. sceptrum which can destroy erythrocyte membrane as it has been known to have anti-nutritional factor (Gordon, 1999; Antwi et al., 2009).

The results displayed in Figure 2 showed a statistical significant (p<0.05) decrease in reduced glutathione level in group 2 (Parasitized control) when compared with that in group 1. This may indicate that the malaria parasite LDL-cholesterol in its oxidized form is toxic. cardiovascular disease. This is because the When reduces the level of reduced glutathione. This
effect may probably be as a result of the activity of reduced glutathione which functions as an oxidant that scavenges free radicals released by the parasite. The significant (p<0.05) increase in reduced glutathione level in group 5 (parasite + 350 mg of spice) when compared with group 4 (parasite + 250 mg of spice) may be attributed to the effect of increased concentration of antioxidants present in the spice extract which helps to boost the level of reduced glutathione under parasitized condition. However, when group 3 (normal + 250 mg of spice) was compared with group 6 (normal + 350 mg of spice), reduced glutathione level was observed to be higher in group 3 than that in group 6. This may probably be due to the antioxidant activity of the spice extract which replenishes the reduced glutathione, thus increasing its level beyond that in the parasitized state. The administration of increased concentration of spice extract under normal control may reduce glutathione level because of the anti-nutritional factors present, which may induce lipid peroxidation on cell membrane. This process decreases the reduced glutathione level, as reduced glutathione may be involved in the mechanism that counters lipid peroxidation of cell membrane.

CONCLUSION
This study on lipid profile show that, there was a significant (p < 0.05) increase in kidney triglyceride, total cholesterol, LDL-cholesterol, MDA levels as well as a significant (p<0.05) decrease in HDL-cholesterol and reduced glutathione levels in mice induced with malaria. However, upon treatment with *A. sceptrum*, a significant decrease was observed in the lipid profile with an exception in the HDL-cholesterol and reduced glutathione level which showed a significant increase. From the literature review, it is apparent that the total phenolic and flavonoid constituents of *A. sceptrum* are responsible for its antioxidant and reducing effects on the kidney. The potency of this spice is as effective as that observed in vitamin E (Gora et al., 2006). This present study has clearly revealed that *A. spectrum* has hypolipidemic and anti-atherogenic effect on malaria infected mice.

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