

ANTIOXIDANT EFFECT OF AQUEOUS SEED EXTRACT OF *AFRAMOMUM SCEPTRUM* (KSCHUM) ON BRAIN AND KIDNEY OF MALARIA INFESTED MICE

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ABSTRACT

The present study investigated the antioxidant effect of aqueous seed extract of *Aframomum sceptrum* against oxidative stress induced in *Plasmodium berghei* infected mice. Adult-albino mice (male), weighing 15-25 g, were inoculated intraperitoneally with 0.1 ml parasitized blood suspension. *A. sceptrum* extract was orally administered at different dosages (250 and 350 mg/kgb.wt), daily to both normal and malaria induced mice for a period of 4 days. There was increase in uric acid level and decrease in albumin, respectively, in brain and kidney in malaria mice compared to normal control. After treating with *A. sceptrum*, a significant ($p < 0.05$) decrease and increase in uric acid and albumin levels, respectively, in the parasitized mice's brain and kidney were observed compared to the parasitized control. The results suggest that *A. sceptrum* extract may contribute to the prevention of oxidative damage caused by malaria.

Key words: Malaria, oxidative stress, *Aframomum sceptrum*, antioxidants, brain, kidney.

INTRODUCTION

In the tropics and subtropical regions of the world, the endemic nature of malaria as well as the mortality associated with the infection particularly among children under the ages of five years has been reported (WHO, 2000). It is also documented that malaria parasite inside erythrocytes exerts oxidative stress within the parasitized red blood cell (Hunt and Stocker, 1990). The formation of reactive oxygen species (ROS) by malaria parasites if not checked by the host cytoprotective enzymes and antioxidants could lead to oxidative damage, and there are increasing evidences that injuries contribute to pathophysiology of many diseases (Gora et al., 2006; Janse et al., 2006; Shiff, 2002).

The potential toxicity of free radical generation by malaria parasites is counteracted by a large number of cytoprotective enzymes and antioxidants. Some of these antioxidants include ascorbic acid, uric acid and albumin (Halliwell, 1994; Aviran, 2000; Hayden et al., 2004). Excessive or inappropriate release of ROS may contribute to tissue injury (Jackson and Cochrane, 1988). Thus, activated phagocytes that attach to brain endothelial cell in murine cerebral malaria (Niel and Hunt,

1992) might cause oxidative damage to the endothelium and compromise the blood-brain barrier.

Administration of an antioxidant can prevent the development of cerebral malaria in *Plasmodium berghei* infected mice (Thumwood et al., 1989). Thus, ROS may be involved in the pathogenesis of cerebral malaria, especially since the brain is particularly vulnerable to oxidative damage (Floyd and Carney, 1992). Similarly, the kidney is subjected to oxidative damage upon exposure to reactive oxygen species which may lead to its malfunction (Iyawe and Onigbide, 2009).

Aframomum sceptrum belongs to the Zingiberaceae family and it is called Altiako by the Itsekiris and Urioma by the Urhobos of Delta State, Nigeria. The seeds are commonly used as spices in flavouring food. The present study investigates the effect of *A. sceptrum* aqueous seed extract on the brain and kidney uric acid and albumin in malaria infected mice.

MATERIALS AND METHODS

Experimental animals

Adult albino male mice of eight weeks weighing between 15 – 25 g were obtained from

Anigboro the animal house, Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria. They were fed on growers mash obtained from Top –Feeds Sapele, Delta State, and were given water *ad libitum*.

The animals were housed in cages constructed of stainless steel and plastic and were under controlled condition of 12h light/12 dark cycle.

Chemicals

Albumin and Uric acid test kits were supplied by RANDOX Laboratories Limited, United Kingdom and used according to the manufacturer's instruction guide.

Spices

Aframomum Sceptrum seeds (a spice) were purchased from the local market in Abraka, Delta State, Nigeria and identified at the Department of Botany, Delta State University, Abraka, Nigeria.

Preparation of extract

The aqueous extract of *A. sceptrum* was obtained using the hot water extraction technique as described by Akinyole and Olerede (2000).

The spices were sun-dried to constant weight for two weeks. This was followed by grinding to fine powder using Waren blender. 100 g of the grinded spice was soaked in 400 ml of distilled water and boiled for 5 min. This was shaken for 10 min and allowed to cool then filtered. The extract was then concentrated using Rotary evaporator at 40 – 50^oC under reduced pressure. The extracts were stored frozen in a deep freezer until use.

Inoculation of experimental animals

The parasite (*P. 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 (3-4 drops) was diluted in 0.9 ml phosphate buffer. The mice were inoculated intraperitoneally with 0.1 ml parasitized suspension. Parasitemia was assessed by thin blood films made by collecting blood from the cut tip of the tail and stained with Geimsa stain (WHO, 2000). The

Nigerian Journal of Science and Environment, Vol. 14 (1) (2016) administration of the extract was carried out using an intragastric tube for a period of four (4) days. On the last day, mice were tested overnight, sacrificed by cervical dislocation and the blood and organ (kidney and brain) were carefully removed for biochemical analysis.

Experimental design

A total of 36 mice (18 surviving parasitized mice and 18 normal mice) were used for the study. The mice were divided into six (6) groups of 6 mice each as follows:

Group One: Normal Control (NC): This group was not infected with the parasite and spice extract was not administered.

Group Two: Parasitized control (PC): This group was infected with *Plasmodium berghei* and spice extract was not administered.

Group Three: Normal Spice 1 (NS1): Mice in this group were not infected with *Plasmodium berghei*, but 250 mg/kg/b.wt of *Aframomum Sceptrum* extract was given.

Group Four: Parasitized Spice 1 (PS1): This group was infected with *Plasmodium berghei* and 250 mg/kg/b.wt of *Aframomum Sceptrum* extract was administered.

Group Five: Parasitized Spice 2 (PS2): This group was infected with *Aframomum berghei* and 350 mg/kg/b.wt of *Aframomum Sceptrum* extract was administered.

Group Six: Normal Spice 2 (NS2): This group was not infected with *Aframomum berghei* but 350 mg/kg/b.wt of *Aframomum Sceptrum* extract was administered to them.

Collection of blood sample

Blood sample was collected from the mice at the end of the experimental period and put into heparinized tubes to prevent clotting. These tubes were then centrifuged at 1000 g for 10 min to obtain plasma as supernatant.

Preparation of organ homogenate

0.5 g of wet kidney and brain, respectively, were homogenized in 4.5 ml of normal saline. The kidney and brain homogenates were centrifuged at 1000 g for 15 min and the supernatant stored in a freezer until required.

Biochemical analysis

The levels of albumin and uric acid in plasma, brain and kidney were determined by

the method described by Doumus et al. (1971) and Caraway (1963), respectively using SPSS version 20.

Statistical analyses

The data for various biochemical parameters were analyzed using analysis of variance (ANOVA) and the group means were compared by Duncan’s Multiple Range Test

(DMRT) (Duncan, 1957).

RESULTS

The results in Figures 1a and 2a showed that mice in the parasitized control groups had a significantly higher ($p < 0.05$) brain and kidney uric acid when compared to the normal control. These, however, significantly ($p < 0.05$) decreased after treating with 250 and 350 mg/kg.

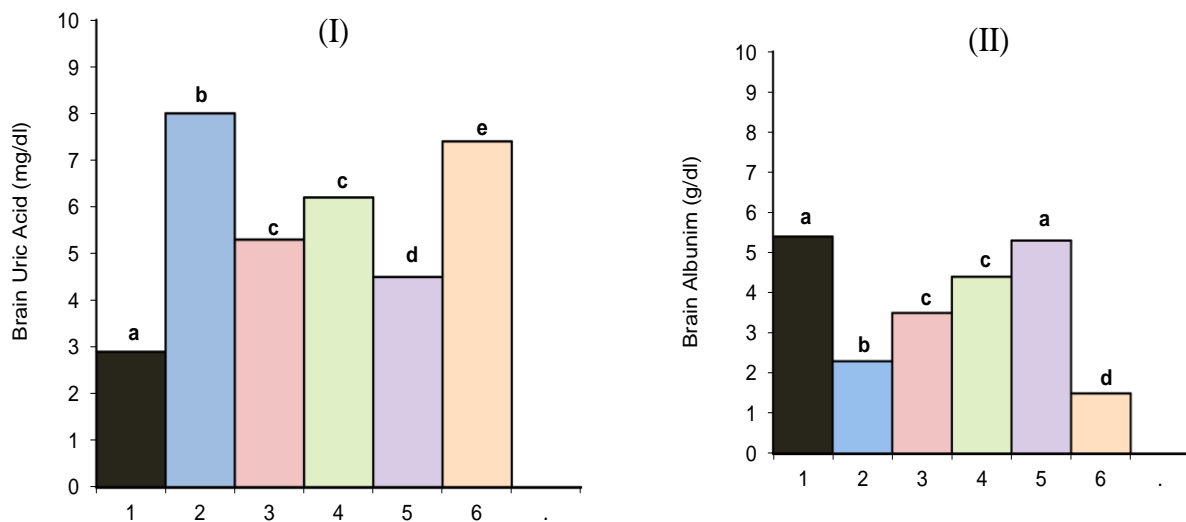


Figure 1. Levels of brain uric acid (I) and albumin (II) in malaria infected mice and effect of *Aframomum sceptrum* seed extract treatment. Values are given as mean ± S.D. n=6, Bar not sharing a common superscript letter differ significantly at $p < 0.05$.

b.wt spice extract compared to parasitized control. The normal control groups which were significantly ($p < 0.05$) lower in brain and kidney uric acid levels compared to the parasitized control increased significantly after treating with 250 and 350 mg/kg. b.wt spice extract.

The results in Figures 1b and 2b showed that mice in the parasitized control had a significantly lower ($p < 0.05$) brain and kidney albumin concentration compared to the mice in normal control group. These significantly increased after treating with the spice extract in dose-dependent manner. The normal control, which was significantly high compared to the parasitized control, increased significantly ($p < 0.05$) after treating with 250 and 350 mg/kg. b.wt of the spice extract.

DISCUSSION

The role of reactive oxygen species by malaria parasites if not checked by the host cytoprotective enzymes and antioxidants could lead to oxidative damage, and there are evidences that such injuries contribute to pathophysiology of many diseases (Gora et al., 2006). Cerebral malaria is the most severe neurological complication of infection with *Plasmodium berghei* and is a major cause of acute non-traumatic encephalopathy in tropical countries. During malaria infection, oxidative stress can lead to kidney damage (Iyawe and Onigbide, 2009).

There have been earlier studies on the evaluation and screening of anti-malarial activity aqueous extract of *Nigella sativa* seed in dose-dependent against *Plasmodium beghei* that infects mice (Abdulah and Zainal-Abidin, 2007). The

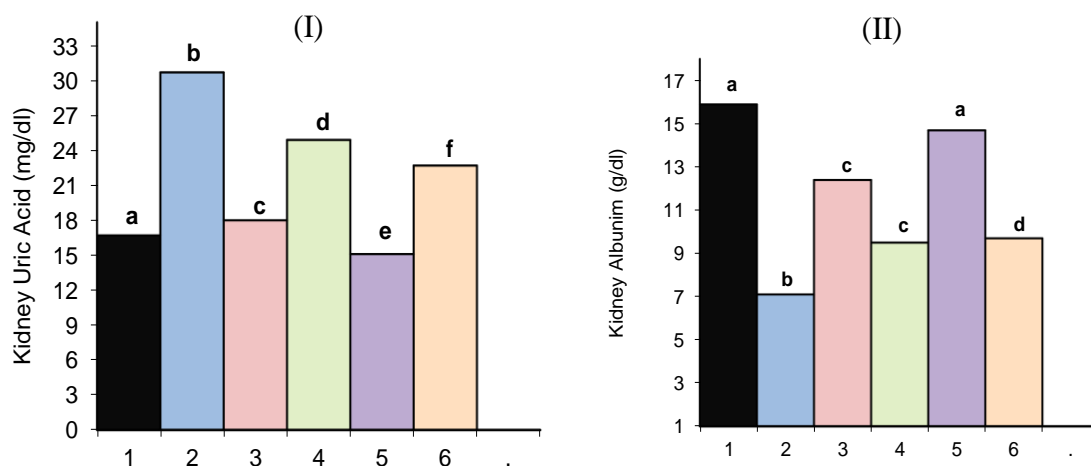


Figure 2. Levels of kidney uric acid (I) and albumin (II) in malaria infected mice and effect of *Aframomum sceptrum* seed extract treatment. Values are given as mean \pm S.D. n=6, Bar not sharing a common superscript letter differ significantly at $p < 0.05$.

The antimalarial activities exhibited by these extracts were perhaps due to the possible presence of active compounds and different classes of alkaloid and phenolics (Nergiz and Otless, 1993). These molecules are well known for their physiological properties, including among others, anticarcinogenic, anti-inflammation and anti-parasitic.

The observed increment in uric acid concentration in the brain and kidney of parasitized control compared to normal control indicates an increased rate of purine catabolism in the parasitized mice which probably led to production of uric acid, a general phenomenon during malaria infection (Nelson and Cox, 2005). In fact, the increased brain uric acid concentration may be due to increased xanthine dehydrogenase activity rather than xanthine oxidase activity (Nelson and Cox, 2005). Xanthine oxidase is formed in tissue from xanthine dehydrogenase and converts hypoxanthine to uric acid (Nelson and Cox, 2005).

According to Levander et al. (1995), dietary antioxidants reduce oxidative stress and also exhibit some antimalarial actions which prevent murine cerebral malaria. This is consistent with the result of this study.

The decrease in the brain albumin level observed in this study in the parasitized control mice compared to the normal control may be due to reactive oxygen species (ROS) which is

produced during infection. Molecular oxygen is the precursor of superoxide and other ROS (Roman, 1991). ROS interfered with the microglial cells that synthesized albumin in the brain which probably led to its decrease (Shamay, 2005).

In agreement with previous study, during malaria infection, there is a decrease in brain albumin compared to that of normal control. This implies that a dynamic equilibrium exists between the rates of production of this protein and a perturbation of balance can occur (Roman, 1991; Elesha et al., 1993). Inhibition of ROS with *Aframomum sceptrum* seed extract in different dosages may be due to the antioxidant and free radical scavenging activities (Adegoke and Skura, 1994). The decrease in kidney albumin level of parasitized mice compared to the normal control may be due to reduced absorption and utilization of amino acids. This is in agreement with the report of Iyawe and Onigbide (2009) that during malaria infection, oxidative stress leads to damage of the kidney. There was a decrease in kidney albumin in malaria infected mice when compared with mice not infected with malaria, and infected mice were restored close to normal after treating with ascorbic acid (Iyawe and Onigbide, 2009). In conclusion, the results showed that *Aframomum sceptrum* seed extract in a dose-dependent manner (250 and 350 mg/kg. b.wt) exhibits significant anti-malaria and antioxidant activity in malaria infected mice.

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