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Changes in Oxidative Indices in Plasmodium Berghei Infected Mice Treated with Aqueous Extract of Aframomum Sceptrum

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Abstract Malaria is associated with increased production of free radicals whose activities be reduced by antioxidants. This present study investigated the antioxidant ability of the aqueous extract of Aframomum sceptrum. Adult albino male mice, eight weeks old, weighing 15g-25g and divided into 6 groups of 6 mice per group were used for the experiment. Mice were inoculated intraperitoneally with 0.1ml parasitized blood suspension and parasitemia assessed by thin blood films stained with Geimsa stain. Aqueous extract of Aframomum sceptrum was orally administered at different doses (250mg/kg.wt and 350mg/kg b.wt daily) to both normal and malaria infected mice for a period of 4days. Blood reduced glutathione, hematocrit, liver reduced glutathione, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and lipid peroxidation levels were estimated. Significant ($p<0.05$) reduction in blood reduced glutathione, hematocrit and decrease activities of liver SOD, CAT, GPx and GSH was observed in parasitized control when compared with normal control mice. However oral administration of Aframomum sceptrum significantly ($p<0.05$) increased hematocrit and blood reduced glutathione content of parasitized mice. Enzymatic and non-enzymatic antioxidants (SOD, CAT, GSH and GPx) activities were also enhanced in a dose dependent manner of spice administration. Reduction in lipid peroxidation (malondialdehyde) level was also observed in the liver of parasitized mice receiving the spice treatment. These results suggest that aqueous extract of Aframomum sceptrum may contribute to the protection of malaria infected mice against oxidative damage by improving antioxidant status in a dose dependent manner.

Keywords Oxidative Stress, Plasmodium Berghei, Aframomum Sceptrum, Lipid Peroxidation

1. Introduction

Malaria is one of the most devastating diseases in the world, particularly in tropical countries[1]. Annually, about 300 – 500 million people get infected worldwide out of which 1-3 million die[2]. Malaria infection develops serious systemic complications such as hematological abnormalities[3, 4], splenomegaly, hepatitis and hepatic dysfunction[5]. The histopathological changes occurring in the liver of malaria patients include heapatocyte necrosis, cholestasis, bile stasis, granulomatous lesions and malaria nodules[6].

Malaria infection decreases the levels of antoxidant enzymes (catalase, glutathione peroxidase, superoxide dismutase) and other antioxidants: albumin, glutathione, ascorbate[7]. It is also documented that malaria parasites exert oxidative stress within the parasitized red blood cells[8,9]. 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.

Aframomum sceptrum (Family-zingiberaceae, local name: Urioma/Ataiko) is a native spice commonly use to enhance cooking flavour, aroma, and palatability especially in the southern part of Nigeria, particularly by the Urhobos, Itsekiri's and Ijaws of Delta State[10]. The antiplasmodial activity of Aframomum sceptrum has been reported[11,12]. However, studies on the effect of Aframomum sceptrum on oxidative indices of mice infected with Plasmodium berghei are scarce. This dearth of information prompted this study.

2. Materials and Methods

Experimental Animals:

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

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ad libitum. The animals were housed in cages constructed of stainless steel and plastic under control condition of 12h light/ 12h dark cycle. The animals used in this study were maintained in accordance with the guidelines approved by the Animal Ethical Committee, Delta State University, Abraka, Delta State, Nigeria.

Chemicals

Metaphosphoric acid and $5^1,5^1$-dithiobis-2-nitrobenzoic acid (DTNB) were obtained from Sigma-Aldrich, Germany. Disodium hydrogen orthophosphate, ethylendediamine tetraacetic acid (disodium salt) (EDTA), sodium citrate, sodium chloride, tricholoracetic acid were AnalaR Grade supplied by BDH Chemicals Limited, Poole, England. Sodium hydroxide pellets were packaged and delivered by Champroha, Netherlands. Di-potasium hydrogen phosphate was manufactuered and supplied by Griffin and George, Middlesex, England.

Spice

Aframomum Sceptrum was purchased from a local market in Abraka, Delta State. The spice was identified at the Department of Botany, Delta State University, Abraka, Delta State.

Preparation of Extract

The aqueous extract of Aframomum Sceptrum was obtained using hot water extraction technique as previously described by[13].

The spice was sun-dried to constant weight for two weeks. This was followed by grinding to fine powder using Warren blender. One hundred gramme (100g) of the ground spice was soaked in 400ml of distilled water and boiled for 5mins. This was shaken for 10min and allowed to cool, then filtered. The extract was then concentrated using Rotary Evaporator at 40 – 50℃ under reduced pressure. The extracts were stored at -8℃ until required.

Inoculation of Experimental Animals

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. Then, 0.1ml of infected blood (3-4 drops) was diluted in 0.9ml phosphate buffer, pH 7.2. The mice were inoculated intraperitoneally with 0.1ml parasitized suspension. Parasitemia was assessed by thin blood films made by collecting blood from the cut tip of the tail and this was stained with Giemsa stain[14].

Experimental Design:

A total of 36 mice (18 surviving parasitized mice and 18 normal mice) were used for the study. They were separated into 6 groups of 6 mice per group as follows:

Group 1: Normal Control: non-parasitized mice.

Group 2: Parasitized mice

Group 3: Parasitized mice given 250mg/kg b.wt of Aframomum sceptrum

Group 4: Parasitized mice given 350mg/kg b.wt of Aframomum sceptrum

Group 5: Non-parasitized mice given 250mg/kg b.wt of Aframomum sceptrum

Group 6: Non-parasitized mice given 350mg/kg b.wt of Aframomum Sceptrum

The administration of the extract was carried out using an intragastric tube for a period of four (4) days. On the last day, mice were fasted overnight, sacrificed by cervical decapitation and the blood and tissue (liver) were collected for various biochemical estimations.

Preparation of whole blood:

A portion of blood was collected in a heparinised tube and used for the estimation of blood reduced glutathione (Reduced GSH).

Preparation of Tissue Homogenate:

Half gramme (0.5g) of wet tissue was homogenized in 9.0ml of normal saline. The supernatant obtained was used for the experiment.

Biochemical Analysis:

The blood reduced glutathione level was estimated using the method of[15]. Liver reduced glutathione and glutathione peroxidase was determined using the method of[16]. The activities of superoxide dismutase (SOD) and catalase (CAT) was assayed using the method of[17] and[18], respectively. Lipid peroxidation was determined by using the method of[19]. The % packed cell volume (PCV) was measured according to the method of[20].

Statistical Analysis:

The data was analyzed using analysis of variance (ANOVA) and the group means were compared by Duncan's Multiple Range Test (DMRT). The SPSS-PC programme package (version 16.0) was used for statistical analysis.

3. Results

Table 1. Blood reduced glutathione and hematocrit value of control and parasitised mice treated with Aframomum sceptrum

Groups	Blood GSH mg% in blood	Hematocrit (%)
1	6.76 ± 0.14^a	41.83 ± 1.64^a
2	5.24 ± 0.01^c	29.67 ± 0.88^b
3	5.89 ± 0.08^b	36.33 ± 1.27^c
4	5.95 ± 0.07^b	39.83 ± 1.32^d
5	6.18 ± 0.17^a	39.83 ± 0.79^d
6	6.25 ± 0.03^a	40.00 ± 0.10^e

Values are given as means ±SD, n = 6. Values not sharing a common superscript letter down a column differ significantly at $p< 0.05$.

Table 1 indicates the blood reduced glutathione and hematocrite levels in parasitised and non-parasitised mice treated with Aframomum sceptrum. The blood reduced glutathione level of the parasitised control mice (ie group 2) is significantly lower than that of all other groups but comparable with the group 6 mice. Parasitised mice treated with 250mg/kg bwt A. sceptrum have comparable ($p>0.05$) blood GSH with the normal control mice. The table also revealed that no significant difference ($p>0.05$) exists between the normal mice receiving 250mg and 350mg/kg bwt A. sceptrum

The hematocrit value of the Group 2 Animals (i.e. parasitised control) was significantly (P< 0.05) lower than all other groups. No significant difference was observed in the hematocrit value of the Group 4 and 5 mice. Normal control mice had the highest hematrocrit value.

Values are given as means ±SD, n = 6. Values not sharing a common superscript letter down a column differ significantly at p< 0.05.

* Units/g of wet tissue.

Table 2 indicates changes in enzymatic and non enzymatic antioxidant activities in the liver of Plasmodium berghei infected mice and non infected mice treated with Aframomum sceptrum. The activity of superoxide dismutase (SOD) in normal control mice (Group 1) was significantly higher (p<0.05) than that of groups 2, 3, 4 and 6, but is comparable to the SOD activity of Group 5. Parasitized control mice expressed the lowest SOD activity at P< 0.05. The table also revealed comparable (P>0.05) SOD activities between the parasitized and non parasitized mice administered with 250mg/kg bwt A. sceptrum.

Infected mice receiving no treatment (i.e. parasitized control group) showed the lowest catalase activity (P< 0.05) compound with all other groups. The highest catalase activity was recorded for the normal control mice (i.e. group 1), while group 4 and 6 have comparable catalase activities.

The table also showed that the glutathione peroxidase (GPx) activity of the group 1 mice is comparable to that of group 4, but significantly elevated with respect to all the other groups. The parasitized control mice (i.e. Group 2) have comparable GPx activity with group 6 mice.

The reduced glutathione levels in the liver of parasitized and non parasitized mice indicate that the Group 5mice have comparable (P> 0.05) liver GSH level with the group 6 mice, but is significantly lower than the other groups (i.e. 1,3,4 and 5). Groups 1 and 4 mice showed comparable GPx activities which is significantly (P< 0.05) higher than all other groups.

Values are given as means ±SD, n = 6.

Values no sharing acommon superscript letter down a column differ significantly at p< 0.05.

The results in Table 3 revealed that none of the experimental groups had comparable (P> 0.05) MDA level. MDA level of the parasitized control mice was significantly (P<0.05) higher compared with all the other five groups. Infected mice receiving 350mg/kg bwt of A. scepturm recorded the lowest lipid peroxidation value.

4. Discussion

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 is of great concern[14,21].

Reduced glutathione acts as primary line of defence to cope with the deleterious effect of reactive oxygen species [22]. Glutathione protect the cellular system against toxic effect of lipid peroxidation. Decreased level of reduced glutathione observed in the blood and liver of malaria mice could represent its increased utilization due to oxidative stress and the low hematocrit observed in the parasitized mice is an indication that the Plasmodium parasite destroys red blood cell.

Biological system protect itself against the damaging effects of activated species by the actions of free radical scavengers and chain terminator enzymes such as SOD, CAT and GPx system[23]. The result of this study showed a decreased hematocrit value, reduced antioxidant activity of SOD, CAT and GPx and a higher lipid peroxidation level in the parasitized control mice. Plasmodium invades the red blood cells leading to anaemic condition (indicated by the low hematocrit value). The invasion also exerts oxidative stress within the parasitized erythrocyte with concomitant decrease in antioxidant enzymes[7] which is expressed by high lipid peroxidation value (a maker of damaged lipid membrane).

Table 2. Changes in liver superoxide dismutase, catalase, glutathione peroxidase activities and reduced glutathione level in parasitised and non parasitised mice treated with Aframomum sceptrum

Group	Superoxide dismutase (SOD) *	Catalase *	Glutathione Peroxidase*	Reduced glutathione *
1	74.21 ± 1.36^{a}	67.02 ± 0.99^{a}	4.70 ± 0.15^{a}	0.92 ± 0.08^{a}
2	55.68 ± 1.29^{b}	42.50 ± 1.09^{b}	2.50 ± 0.55^{b}	0.66 ± 0.03^{b}
3	63.69 ± 1.44^{c}	47.07 ± 3.64^{c}	3.40 ± 0.43^{c}	0.71 ± 0.02^{c}
4	68.16 ± 2.52^{d}	55.86 ± 1.35^{d}	4.20 ± 0.60^{a}	0.93 ± 0.06^{a}
5	73.18 ± 0.66^{a}	64.45 ± 1.55^{a}	3.50 ± 0.46^{c}	0.82 ± 0.06^{d}
6	69.61 ± 1.57^{d}	52.06 ± 1.19^{d}	2.40 ± 0.37^{b}	0.61 ± 0.05^{b}

Table 3. Changes in the liver lipid peroxidation level of the control and parasitized mice treated with A. sceptrum

Groups	Malondialdehyde (MDA) (Units/g of wet tissue)
1	0.25 ± 0.02^{a}
2	0.47 ± 0.01^{b}
3	0.34 ± 0.01^{c}
4	0.24 ± 0.01^{d}
5	0.33 ± 0.01^{e}
6	0.41 ± 0.02^{f}

Administration of 250mg/350mg/kg bwt of A. sceptrum increase the blood glutathione and hematocrite level of parasitized mice in a dose-dependent manner. The enzymatic and non-enzymatic antioxidants in the liver of infected mice were also enhanced by the treatment with the spice extract. Reduction in the lipid membrane damage was also observed in the parasitized mice receiving the spice treatment. Comparatively, the 350mg/kg bwt treatment had more effect on the enhancement of the antioxidant enzyme and the hematocrit when compared with the 250mg/kg bwt treatment. Spice extract may have contributed to the biosynthesis of the antioxidant enzymes thereby reducing membrane lipid peroxidation and consequently elevating the hematocrit value in parasitized mice. Therefore, aqueous extract of Aframomum sceptrum could contribute to the protection against oxidative damage in malaria and improving antioxidant status in a dose dependent manner.

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