

Full Length Research Paper

A study of the hepato-protective effect of *Aframomum sceptrum* in mice infected with *Plasmodium berghei*

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One of the effects of *Plasmodium* infection which causes malaria is the invasion of the hepatocyte which affects liver and blood transaminases activities. This study was design to examine the hepato-protective effect of *Aframomum sceptrum* in mice infected with *Plasmodium berghei*. Thirty-six abino male mice infected (test) and non infected (control) of 8 weeks old were used for this research. The mice were divided into six groups of six mice per group. Biochemical parameters measured in serum and liver samples include, alanine aminotransferase (ALT), aspartate amniotransferase (AST) and alkaline phosphatase (ALP) activities. Serum and liver ALT, AST and ALP activities were significantly higher in parasitized control mice as compared with all other groups. Parasitized mice receiving 250 and 350 mg/kg body weight of *A. sceptrum* have comparable ($p>0.05$) serum and liver ALT activities with the normal control mice. However, normal mice receiving both doses of *A. sceptrum* did not show any increase of serum ALT, AST and ALP activities with their respective controls. This result is supported by the histological examination of liver section of parasitized mice treated with 350 mg/kg b. wt of *A. sceptrum* showing moderately brought central vein, hepatic cell with preserved cytoplasm and prominent nucleus. It can therefore be inferred from the study that the administration of *A. sceptrum* to malaria infected mice at 250 and 350 mg/kg b. wt did not damage hepatocyte as expressed by the parasitized mice and also treatment with extract could protect hepatocyte integrity of *Plasmodium* infected mice.

Key words: *Aframomum sceptrum*, malaria, liver enzymes, *Plasmodium berghei*.

INTRODUCTION

Malaria remains a devastating global health problem (Trampuz et al., 2003). It is known as the world's most important tropical parasitic infectious disease to human (Donovan et al., 2007). About 300 to 500 millions of cases and a mean death of 2 million are reported every year (Kochar et al., 2003).

Worldwide, the control of malaria has witnessed a serious deterioration (Fernex, 1985). Severe malaria is almost exclusively caused by *Plasmodium falciparum* in

humans, but other forms of *Plasmodium* include *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*. When the infected anopheline mosquito takes a blood meal, sporozoites are inoculated into the blood stream. Within an hour, sporozoites enter hepatocytes and begin to divide into exoerythrocytic merozoites (tissue schizogony) (Trampuz et al., 2003). Once merozoites leaves the liver, they invade erythrocytes. In effect malaria affects the liver. Alanine and aspartate transminase (ALT and AST) activities are used as indicators of hepatocytes damage (Asagba et al., 2004; Coppo et al., 2002; Dede et al., 2002; Whitehead et al., 1999). Akanji et al. (1993) reported that alkaline phosphatase (ALP) is a marker enzyme for the plasma membrane and endoplasmic reticulum.

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Aframomum sceptrum (Family- Zingiberaceae, Local name: Urioma/Alaiko) is a local spice commonly used to enhance flavour, aroma and palatability of cooking particularly by the Urhobos, Itsekiris and Ijaws of Delta State (George et al., 2010). Duker-Eshun et al. (2002) reported the anti-plasmodia activity of *A. sceptrum* while, George et al. (2010) reported the normalization of liver enzymes activities (AST and ALT) of diabetic rats treated with 200 mg/kg b. wt of *A. sceptrum*. However, there is dearth of information on the changes of the liver enzymes activities in mice infected with *Plasmodium berghei* treated with *A. sceptrum*. Liver damage caused by malaria infection may be prevented or reduced by treatment with this spice that forms part of our diet, hence, this study aim to examine the hepato-protective effect of *A. sceptrum* in mice infected with *P. berghei*.

MATERIALS AND METHODS

Experimental animals

Thirty-six albino male mice of 8 weeks old obtained from the animal house, Faculty of Basic Medical Sciences, Delta State University Abraka, Delta State, Nigeria were used for this study. They were fed on growers mash obtained from Top-Feeds, Sapele, Delta State, and were given water *ad libitum*. The animals were housed in metal cages under controlled conditions of 12 h light/ 12 h 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.

Inoculation of animals

The mice were infected with parasites (*P. berghei*) by obtaining parasitized blood from the cut-tip of the tail of an infected blood (3 to 4 drops) and diluted in 0.9 ml phosphate buffer, pH 7.4. The mice were inoculated intraperitoneally with 0.1 ml parasitized suspension. Parasitaemia was assessed by thin blood film made by collecting blood from the cut tip of the tail and this was stained with Geimisia stain (WHO, 2000). Inoculation was carried out in the Biochemistry Laboratory of the Nigerian Institute of Medical Research, Yaba Lagos.

Collection of *Aframomum sceptrum*

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

Preparation of extract

The spice was sun-dried to a constant weight for two weeks. This was followed by grinding to fine powder using warren blender. 100 g of the ground spice material was then soaked in 400 ml of distilled water and boiled for 5 min, followed by mechanical shaking for 10 min, cooled and filtered. The filtrate was then concentrated using rotary evaporator at 40 to 50°C under reduced pressure. The extract was stored frozen in a deep freezer until required for the experiment (Abukakar et al., 2008).

Experimental design

After the confirmation of parasitemia, the mice infected (parasitized) and non infected (normal) were divided into 6 groups of 6 mice per group treated as follows:

- Group 1: Normal control
- Group 2: Parasitized control
- Group 3: Normal mice + *A. sceptrum* (250 mg/kg b. wt)
- Group 4: Normal mice + *A. sceptrum* (350 mg/kg b. wt)
- Group 5: Parasitized mice + *A. sceptrum* (250 mg/kg b. wt)
- Group 6: Parasitized mice + *A. sceptrum* (350 mg/kg b. wt)

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

Preparation of serum

Fasting blood was collected from each mice into a sterile, plain tube, and then it was centrifuged at 1,200 × g for 5 min at room temperature to obtain the serum sample, which was stored frozen at -20°C until analyzed.

Preparation of tissue homogenate

0.5 g of wet liver tissue was homogenized in 4.5 ml of freshly prepared normal saline. The supernatant obtained was used for this experiment.

Biochemical investigations

The biochemical investigations in serum and liver samples were carried out with the following methods, using commercially available kits as supplied by TECO Diagnostic, Anaheim, USA. Alanine aminotransferase (ALT) was determined by method of Reitman and Frankel (1957), Aspartate aminotransferase (AST) by the method of Reitman and Frankel (1957) and while alkaline phosphatase (ALP) activity was estimated by the method of Tietz et al. (1983).

Histology

Histological study on liver tissues obtained from experimental mice was carried out following the method described by Drury and Wallington (1973).

Statistics

The repeat measure analysis of variance (ANOVA) was used to compare similar mean values, and the group means were compared by Duncan's multiple range test (DMRT). The level of statistical significant was established at 5% probability level.

RESULTS

The results in Table 1 indicate that the mice infected with *P. berghei* (Group 2) expressed a significantly ($P < 0.05$) higher serum alanine aminotransferase (ALT) activity

Table 1. Changes in serum alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase activities in parasitized mice treated with *Aframomum sceptrum*.

Groups	ALT (U/l)	AST (U/l)	ALP (U/l)
1	20.69±0.66 ^a	25.34±0.84 ^a	42.61±1.86 ^a
2	23.83±1.19 ^b	28.14±0.46 ^b	51.95±1.09 ^b
3	21.44±3.75 ^a	25.56±0.80 ^a	43.37±2.75 ^a
4	20.29±1.10 ^a	26.52±0.87 ^c	44.07±2.34 ^a
5	21.21±1.40 ^a	23.19±0.61 ^d	51.39±1.08 ^b
6	19.94±0.88 ^a	22.46±0.50 ^u	50.63±1.02 ^b

Values are means ± SD with n = 6. Column values with different superscript letters are significantly different (P<0.05).

Table 2. Changes in liver alanine aminotransferase, aspartate, aminotransferase and alkaline phosphatase activities in parasitized mice treated with *Aframomum sceptrum*.

Groups	ALT (U/l)	AST (U/l)	ALP (U/l)
1	29.99±0.74 ^{ad}	34.20±0.86 ^a	66.18±6.58 ^{ad}
2	32.44±0.88 ^b	37.19±0.50 ^b	93.03±4.91 ^b
3	29.26±0.60 ^a	33.20±1.29 ^{ac}	67.78±7.63 ^a
4	28.29±0.94 ^c	32.33±1.22 ^{cd}	69.16±7.24 ^a
5	30.20±0.67 ^d	33.49±1.41 ^{ad}	62.10±4.55 ^{dc}
6	29.36±0.64 ^{au}	32.70±1.53 ^{cu}	55.29±2.53 ^c

Values are means ± SD with n = 6. Column values with different superscript letters are significantly different (P<0.05).

compared with all other groups of experimental animals. The ALT activity of the control mice (Group 1) is comparable (P>0.05) to the normal and parasitized mice treated with 250 and 350 mg/kg b. wt (that is, Groups 3, 4, 5 and 6) respectively. However, the Group 6 animals showed the lowest ALT activity.

Serum aspartate aminotransferase activity is significantly lower (P<0.05) in the parasitized mice (22.46±0.50 U/l) administered with 350 mg/kg b. wt of *A. sceptrum* compared to all other groups except parasitized mice (Group 5) treated with 250 mg/kg b. wt of *A. sceptrum*. Serum AST activity of the control parasitized mice (Group 2) is statistically higher (p<0.05) than the AST activity of all the other groups.

The table also revealed that serum alkaline phosphatase activity significantly (P<0.05) increased in the serum of parasitized control mice (that is, Group 2) as compared with Groups 1, 2 and 3 respectively, but comparable (P>0.05) with parasitized mice administered with 250 mg/kg b. wt (51.39±1.08) and 350 mg/kg b. wt (50.63±1.02).

The liver marker enzymes (ALT, AST and ALP) activities according to Table 2 were significantly increased (P<0.05) in the liver of the parasitized mice as compared with all other groups. Alanine aminotransferase activities of parasitized mice in Groups 1 and 2 were statistically comparable (P>0.05) to ALT activity of the control mice (Group 1). According to Table 2 also, no significant difference was observed between aspartate aminotransferase activity of parasitized mice in Groups 5,

6 and control mice (Group 4) receiving 350 mg/kg b. wt of *A. sceptrum*.

Table 2 also reveal that a statistical (P<0.05) reduced alkaline phosphatase activity was observed between parasitized mice receiving 250 and 350 mg/kg b. wt of *A. sceptrum* (Groups 5 and 6) compared with the corresponding normal control mice receiving an equivalent dose of the spice material (Groups 3 and 4). The experimental mice in Group 1 showed a lower level or alkaline phosphatase activity as compared with the control mice in Groups 3 and 4. However, the observed increase was not significant. The histopathological section of mouse liver is presented in Figures 1 to 6.

DISCUSSION

Liver destruction can affect the metabolic processes in the body due to the role of liver in general metabolism of the organism. Enzymes are necessary for normal cellular metabolism including that of the liver (Rajamanickam and Muthuswamy, 2008). Within an hour of which an infected anopheline mosquito takes a blood meal, sporozites enter the hepatocytes and divide into exoerythrocytic merozoites. The degenerative changes in the hepatocytes due to the infection of *Plasmodium* may alter the activities its enzymes. This study investigated the possible effect of *A. sceptrum* amelioration on this cellular damage. Alanine aminotransferase (ALT), aspartate aminotranferase (AST) and alkaline phosphatase (ALP)

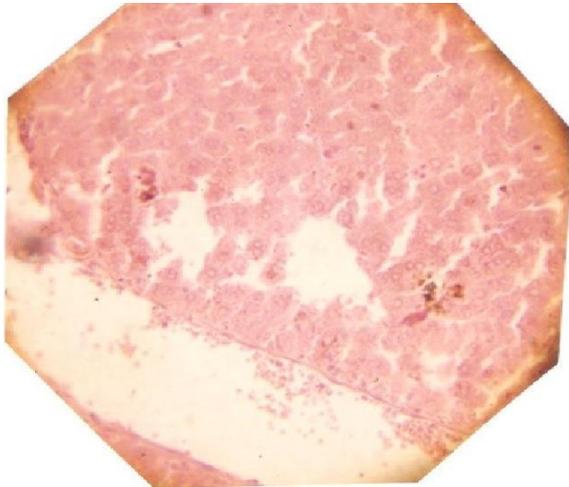


Figure 1. Photomicrograph of Group 1 mouse liver section showing well brought central vein and hepatic cells.

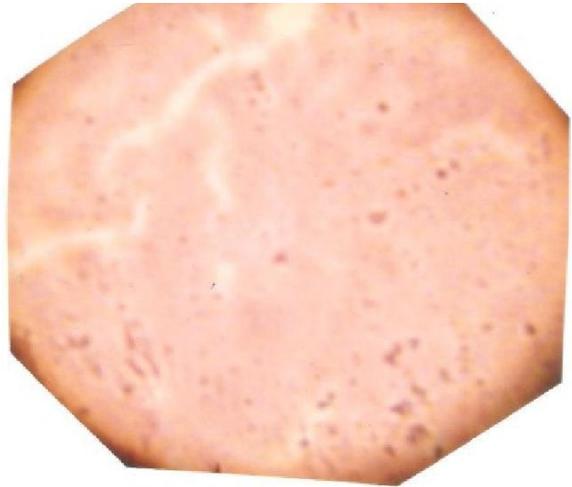


Figure 3. Photomicrograph of mouse liver section of Group 3 showing moderately ballooning degeneration.

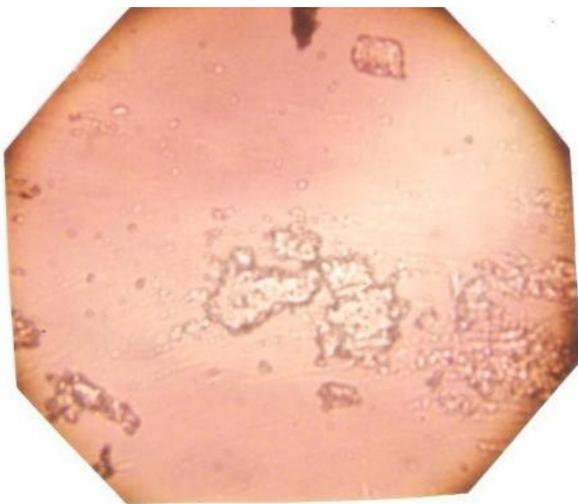


Figure 2. Photomicrograph of Group 2 liver section showing marked steatosis of the hepatocytes with ballooning degeneration, necrosis and mild periportal fibrosis.



Figure 4. Photomicrograph of mouse liver section of Group 4 showing degeneration brought central vein and hepatic cells.

are considered indicators of hepatocellular health (Yang and Chen, 2003; Vozarova et al., 2002).

A significant increase in the activities of ALT, AST and ALP in the blood and liver of control parasitized mice as compared with all the other groups was observed. The observed increase in enzyme activities may be as a result of liver injury and altered hepatocyte integrity caused by the *Plasmodium* infection and the consequent released of the enzymes into the blood stream. This is shown in the histopathological examination of the liver of the parasitized mouse which possesses a marked steatosis of the hepatocytes with ballooning degeneration, necrosis and mild periportal fibrosis (Figure 2) as

compared with the liver examination of the control mouse that showed a well brought central vein and hepatic cell (Figure 1).

The administration of *A. sceptrum* tends to normalize these enzymes (ALT, AST and ALP) activities which is in agreement with previously reported investigation by George et al. (2010). Although, the aforementioned liver enzymes normalization was more pronounced at a higher dose of 350 mg/kg b. wt of *A. sceptrum*. This spice is extensively used in cooking different types of soups and vegetables. It is very popular in cooking 'pepper soup' commonly prepared for individuals' suffering from malaria fever. The observation in the foregoing is supported by

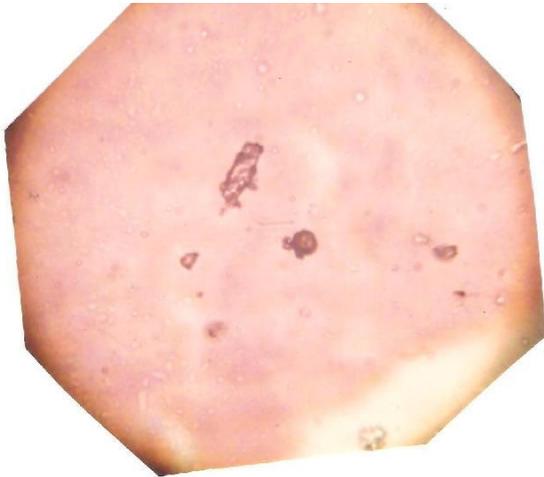


Figure 5. Photomicrograph of mouse liver section of Group 5 showing considerable reduction in necrosis moderately regeneration in hepatocellular architecture.

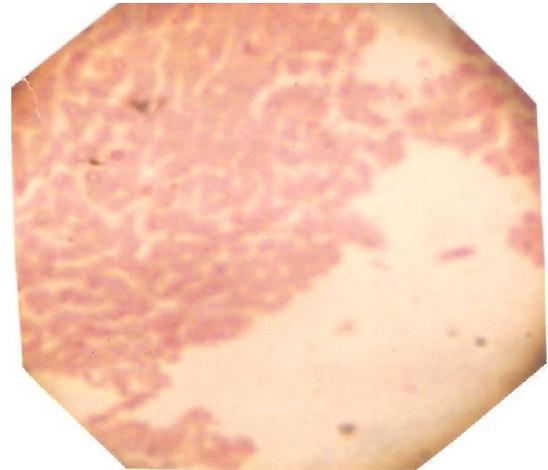


Figure 6. Photomicrograph of mouse liver section of Group 6 showing moderately brought central vein, hepatic cell with preserved cytoplasm and prominent nucleus.

the histological study as seen in Figure 6. Photomicrograph of liver section of parasitized mice treated with 350 mg/kg b. wt of *A. sceptrum* showing moderately brought central vein, hepatic cell with preserved cytoplasm and prominent nucleus.

It could therefore be concluded that the administration of *A. sceptrum* to parasitized mice at 250 and 350 mg/kg b. wt did not contribute to the hepatic injury expressed by the parasitized (Group 2) mice and may not be toxic. Treatment with extract from this study has shown to protect hepatocyte integrity of parasitized mice.

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