

Antioxidative Stress and Hepatoprotective Activities of Leaf Extract and Fractions of *Setaria megaphylla* in *Plasmodium berghei* Infected Mice

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Abstract

Setaria megaphylla (Steud) Dur & Schinz (Poaceae), a perennial grass used traditionally in the treatment of various diseases such as malaria was, investigated for antioxidative stress activity in *Plasmodium berghei*-infected mice. The leaf extract (200-600 mg/kg) and fractions (hexane, dichloromethane, ethyl acetate and methanol; 400 mg/kg) of *S. megaphylla* were investigated for antioxidative stress and hepatoprotective activities in *Plasmodium berghei*-infected mice using a modified suppressive test model. Antioxidative stress and hepatoprotective potentials were assessed by determining oxidative stress markers levels, liver function indices and histopathology of liver. The extract/fractions progressively reduced parasitaemia induced by chloroquine-sensitive *P. berghei* infection with the methanol fraction exerting the highest activity. The leaf extract and fractions caused significant ($p < 0.05 - 0.001$) increases in the levels of oxidative stress markers enzymes and molecules (SOD, CAT, GPx, GSH) and also reduced MDA level significantly ($p < 0.05$) in the livers of the treated-infected mice. The extract/fractions treatment caused reduction in liver enzymes (ALT, AST and ALP), total and conjugated bilirubin. Histology of livers revealed absence or significant reductions in pathological features in the treated infected mice compared to untreated infected mice. The leaf of *S. megaphylla* may possess antioxidative stress and hepatoprotective effects which may in part be mediated through the chemical constituents of the plant.

Keywords: Antioxidative stress; *Setaria megaphylla*; antimalarial; antioxidative stress; malaria; *Plasmodium berghei*.

INTRODUCTION

Global malaria cases in 2019 were estimated to be about 229 million occurring in 87 malaria endemic countries (WHO, 2020). Contrary to significant reduction in malaria related mortality, Nigeria recorded the highest mortality rate of all malaria deaths globally in 2019. This portrays a serious threat to life in most countries of Africa especially Nigeria. Oxidative stress associated with malaria infection has been implicated in the pathogenesis and development of systemic complications caused by malaria (Guha *et al.*, 2006; Ojezele *et al.*, 2017). Malaria complications such as anemia, jaundice and pre-eclampsia have been linked to oxidative stress damage caused by the parasite (Fabbri *et al.*, 2013; Sarr *et al.*, 2017). Medicinal plants, therefore, serve as a great reservoir for antimalarial remedies having the advantage of being safer and providing many therapeutic effects.

Setaria megaphylla (Steud) Dur & Schinz (Poaceae), a perennial grass found in tropical and subtropical areas of World (Van Oudtshoorn, 1999), is traditionally used for the treatment of malaria and diabetes among others (Okokon *et al.*, 2007). Preliminary reports of

antiplasmodial activity on the leaves have been published (Okokon *et al.*, 2007; Okokon *et al.*, 2017). The leaf extract also possesses antidiabetic and hypoglycaemic (Okokon and Antia, 2007; Okokon *et al.*, 2022), anti-inflammatory, analgesic (Okokon *et al.*, 2006), cytotoxic, immunomodulatory and antileishmanial (Okokon *et al.*, 2013), antidepressant (Okokon *et al.*, 2016), inhibitory effect on α -amylase and α -glucosidase (Okokon *et al.*, 2021) activities. Phytochemical analysis of the leaf extract shows that it contains phytochemical compounds such as flavonoid, carbohydrate, terpenes, saponins, tannins, anthraquinones, cardiac glycosides (Z,Z,Z)-8,11,14-eicosatrienoic acid, phthalic acid, diisooctyl ester, vitamin E, γ -elemene, urs-12-ene, bicyclogermacrene, α -muurolene, germacrene-A, and guaiol have been reported (Okokon *et al.*, 2006; Okokon *et al.*, 2013). 1-triacontanal, 1-triacontanol, 1-dotriacontanol, 1-triacontyl cerotate, and stigmaterol have also been isolated from the plant leaves (Okokon *et al.*, 2022). We report in this study the antioxidative stress and hepatoprotective potentials of leaf extract and fractions of *Setaria megaphylla* in *Plasmodium berghei*-infected mice.

MATERIALS AND METHODS

Plants Collection

The leaves of *Setaria megaphylla* were collected from farms in the Uruan area of Akwa Ibom State, Nigeria. The plant was identified by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. A voucher specimen (UUPH. 221 d) of the plant was deposited in the Department of Pharmacognosy and Natural Medicine herbarium at the University of Uyo.

Extraction

The leaves were washed and shade dried for two weeks. The dried leaves were cut into smaller pieces and pulverized to powder. The powdered leaves was divided into two parts. One part was macerated in ethanol for 72 hours, while the remaining part was successively and gradually macerated for 72 hours in each of, n-hexane, dichloromethane, ethyl acetate and methanol respectively, which is along their polarities to give the corresponding gradient fraction for each solvent. The liquid filtrate of each extract and fraction were concentrated and evaporated to dryness in *vacuo* 40°C using a rotary evaporator. The various yields were calculated and the extract and fractions were stored in a refrigerator at -4°C, until used for the proposed experiments.

Microorganism (parasite)

Chloroquine-sensitive strain of *Plasmodium berghei* ANKA strain was obtained from the National Institute of Medical Research (NIMR), Yaba Lagos, Nigeria and maintained by subpassage of blood from infected to healthy mouse once every 7-8 days.

Parasite inoculation

Each mouse used in the experiment was inoculated intraperitoneally with 0.2 mL of infected blood containing about $1 \times 10^7 P. berghei$ parasitized erythrocytes collected from an infected mouse with 20-30% parasitaemia. The inoculum consisted of $5 \times 10^7 P. berghei$ infected erythrocytes per milliliter prepared by determining both the percentage parasitemia and the erythrocytes count of the donor mouse and diluting the blood with isotonic saline in proportions indicated by both determinations (Atanu *et al.*, 2021). Parasitemia was monitored by standard methods; thin blood smears were made on glass slides, fixed using methanol, and stained using Giemsa stain parasitemia was counted using a microscope and was calculated as a percentage of infected red blood cells (RBCs) relative to the total number of cells in a microscopic field at $\times 100$ magnification according to the formula of Peters and Robinson (1992) as given below:

$$\text{Parasitemia (\%)} = \frac{\text{Total number of parasitised RBCs}}{\text{Total number of RBCs}} \times 100$$

Experimental animals

Male and female Swiss albino mice, each weighing 21-32 g, were obtained from the University of Uyo's animal house. They were kept in standard cages and acclimatized for a period of 10 days before use in the experiments. The mice were fed on standard pelleted diet and water *ad libitum*. All animals were kept at room temperature in cross ventilated rooms. The care and use of animals was conducted in accordance with the National Institute of Health Guide for the Care and Use of laboratory Animals (NIH Publication, 1996). Approval for the study was obtained from the University of Uyo's Animal Ethics Committee.

Drug administration

The extract, fractions, chloroquine and pyrimethamine that were used in the antimalarial study, were administered orally with the aid of a stainless metallic feeding cannula.

Evaluation of antioxidative and liver protective activities of the leaf extract and fractions of *Saccharum officinarum* using 4-day test

A modified early infection test model was used to assess the antioxidative stress and hepatoprotective potentials of the leaf extract and fractions as well as chloroquine in *P. berghei* infected mice. This was done using the method described by Knight and Peters (1980). Forty-five mice were randomly divided into nine groups of five (5) mice each based on body weights on the first day (D₀). They were further infected with the parasite and treated with the leaf extract, fractions, chloroquine and distilled water. Based on previously determined median lethal dose (LD₅₀) of 2.4 ± 0.5 g/kg, (Okokon *et al.*, 2006), the mice in groups 1-3 were given 200 mg/kg, 400 mg/kg and 600 mg/kg of crude extract respectively, while groups 4, 5, 6, 7 were administered 400 mg/kg of n-hexane, dichloromethane, ethyl acetate, and methanol fractions respectively, group 8 was given 5 mg/kg of chloroquine (positive control) and group 9 was given 10 mL/kg of distilled water (negative control) for four consecutive days (D₀-D₃) between 8am to 9am. On the fifth day (D₄), thin films were made from the tail blood. The films were stained with Giemsa stain to reveal parasitized erythrocytes out of 500 in a random field of the microscope. On the tenth day, the mice from various group were sacrificed under diethyl ether vapour. Blood samples were collected into EDTA bottles and plain centrifuge tubes. The blood samples in the EDTA bottles were used for hematological analysis, while those in centrifuge tubes were centrifuged immediately at 2500 rpm for 15 mins to separate the serum at room temperature to avoid haemolysis and used for biochemical assays such as determination of liver function indices. Liver from each mouse was surgically removed, weighed and divided into two parts. One part was fixed in 10% formaldehyde for histological process

and the other part stored in ice cold normal saline. The average suppression of parasitemia was calculated according to the formula of Peters and Robinson (1992) as follows: (average % parasitemia positive control – average % parasitemia negative control) / (average % parasitemia negative control).

Effect of the leaf extract and fractions on biochemical parameters and histology of livers of *P. berghei* infected mice

Serum was separated from the blood of each mouse sacrificed and these sera were stored at -20°C until used for biochemical determinations such as total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), conjugated and total bilirubin to assess the liver functions. The determinations were done spectrophotometrically using Randox analytical kits according to standard procedures of manufacturer's protocols (Tietz, 1976).

The livers of the sacrificed animals were surgically removed, weighed and a part of each fixed in 10% formaldehyde for histological processes, while the other part was washed with ice cold 0.9% NaCl and homogenates were made in a ratio of 1 g of wet tissue to 9 ml of 1.25% KCl by using motor driven Teflon-pestle. The homogenates were centrifuged at 7000 rpm for 10 min at 4°C and the supernatants were used for the assays of superoxide dismutase (SOD) (Marklund and Marklund, 1974), catalase (CAT) (Sinha, 1972), glutathione peroxidase (GPx) (Lawrence and Burk, 1976), and reduced glutathione (GSH) (Ellman, 1959). Malondialdehyde (MDA) and glutathione-S-transferase (GST) were determined using commercial diagnostic kits (Aldrich-Sigma, USA) using standard procedures of the manufacturer's protocol.

Statistical analysis

Data collected were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post-test (Graph pad prism software Inc. La Jolla, CA, USA). Values were expressed as mean ± SEM and significance relative to control were considered at $p < 0.05$.

RESULTS AND DISCUSSION

Effect of leaf extract and fractions on parasitaemia

The extract exerted a dose-dependent and significant ($p < 0.001$) reduction in parasitaemia levels of the treated mice at the different doses employed when compared to the control group in the study. The leaf fractions (n-hexane, DCM, ethyl acetate and methanol; 400 mg/kg), exerted prominent reductions in parasitaemia levels of the treated mice with chemosuppression of 11.12, 23.96, 20.81 and 41.00% for n-hexane, dichloromethane, ethyl

acetate and methanol respectively. However, methanol fraction exerted the highest activity (Figure 1).

Effect of leaf extract and fractions on liver function indices of *P. berghei*-infected mice.

The levels of liver function indices (AST, ALT, ALP, total protein, albumin, total and conjugated bilirubin) were found to be elevated in untreated *P. berghei* -infected mice. However, treatment of *P. berghei* infected mice with leaf extract and fractions of *S. megaphylla* caused non dose-dependent and significant ($p < 0.01-0.001$) reductions in the activities of AST and ALT especially at higher doses (400 and 600 mg/kg) with methanol fraction followed by ethyl acetate fraction treated group having the most significant ($p < 0.001$) reduction when compared to control though not comparable to that of chloroquine (Table 1). ALP levels of the treated groups of infected mice were non dose dependently and significantly ($p < 0.01-0.001$) reduced when compared to control untreated mice with the methanol fraction followed by n-hexane fraction treated group having the highest and most significant ($p < 0.001$) reductions. The total protein levels of the treated mice groups were similarly reduced non dose-dependently and significantly by the extract/ fractions treatments with the n-hexane fraction treated group having the highest significant effect ($p < 0.01$). Treatment of infected mice further produced non dose-dependent and significant ($p < 0.05-0.01$) reductions in albumin levels of the treated mice when compared to control. Although the fractions-treated groups had reduced albumin levels, these were not significant ($p > 0.05$) when compared to control. The extract/fractions treatment caused non dose-dependent reduction of total bilirubin level of the treated infected mice though not significant ($p > 0.05$) when compared to control. However, the methanol fraction significantly ($p < 0.05$) reduced the total bilirubin level of the mice when compared to control (Table 5). The leaf extract and fractions of *S. megaphylla* also caused non dose dependent reductions in the levels of conjugated bilirubin of the treated mice which was only significant ($p < 0.05$) at the middle dose (400 mg/kg) when compared to control. Methanol fraction also produced significant ($p < 0.05$) reduction of conjugated bilirubin when compared to control (Table 1).

Effect of leaf extract and fraction on liver antioxidant enzymes of *P. berghei*-infected mice.

The levels of enzymatic and non-enzymatic endogenous antioxidants (GST, SOD, CAT, GPX and GSH) in the infected mice were found to be lowered by malaria parasite infection, while higher levels of MDA were also observed (Table 2). Treatment of *Plasmodium berghei*-infected mice with leaf extract and fractions of *S. megaphylla* caused significant ($p < 0.05-0.001$) dose-dependent elevation in the levels of SOD with the DCM fraction treated group having the most significant level ($p < 0.001$) followed by ethyl acetate and methanol

fractions treated groups. Treatment of infected mice with leaf extract/fractions of *S. megaphylla* also caused dose-dependent and significant ($p < 0.001$) increases in the activity of GST especially at the higher doses of the extract (400 and 600 mg/kg) when compared with the control untreated infected mice. Methanol fraction followed by *n*-hexane and DCM fractions produced the most significant effect ($p < 0.001$) when compared to control. CAT activity was increased non dose-dependently but significant ($p < 0.05-0.001$) by the leaf extract/fractions treatment of the infected mice when compared to control with the *n*-hexane fraction treated group having the highest activity. Leaf extract/fractions treatment caused non dose-dependent elevation of GPx activity in the treated infected mice which was only significant ($p < 0.001$) in the group treated with *n*-hexane fraction when compared to control. Similarly, GSH levels in the treated infected mice were significantly ($p < 0.001$) and non dose-dependently elevated following treatment with the leaf extract and fractions when compared to control. *n*-hexane fraction followed by ethyl acetate and methanol fractions exerted the highest significant ($p < 0.001$) activity when compared to control. However, there were significant ($p < 0.01-0.001$) and non dose-dependent reductions in the levels of MDA of the treated mice with DCM and ethyl acetate fractions having the most significant effects ($p < 0.01-0.001$) when compared to control (Table 2).

Effect of extract and fractions on the histology of liver of *P. berghei*-infected mice.

Histologic sections of livers of untreated infected mice showing distorted livers with congested central vein, hepatocytes, sinusoids containing inflammatory cells, necrotic tissues. Histologic sections of livers of infected mice treated with 200 mg/kg of leaf extract revealed distorted livers with hepatocytes characterised by fatty degenerations, steatosis (microvascular, macrovascular, steatosis). Livers of infected mice treated with 400 mg/kg of extract had liver sections revealing normal/borderline liver hepatocytes with mild steatosis, patent central vein and sinusoids. Histologic sections of livers of *P. berghei*-infected mice treated with 600 mg/kg showed distorted liver with congested central vein, hepatocytes, necrotic tissues, and sinusoids.

n-hexane fraction treated infected mice showed liver sections with mildly distorted livers/border line liver with hepatocytes, sinusoids and necrotic tissues. Infected mice treated with DCM fraction had livers histologic sections showing distorted liver with hepatocytes, sinusoids containing kupffer cells, generalised distribution of necrotic tissue and patent central vein (Figure 2). Group of infected mice treated with ethyl acetate fraction showed livers histologic sections with normal liver with intact hepatocytes, sinusoids and patent central vein (Figure 2). Also, infected mice treated with methanol fraction had normal livers sections with intact hepatocytes, sinusoids containing kupffer cells and patent central vein (Figure 2). *P. berghei*-infected mice treated with chloroquine had histologic sections of livers showing normal liver with intact hepatocytes, sinusoids containing kupfers cells and patent central vein (Figure 2).

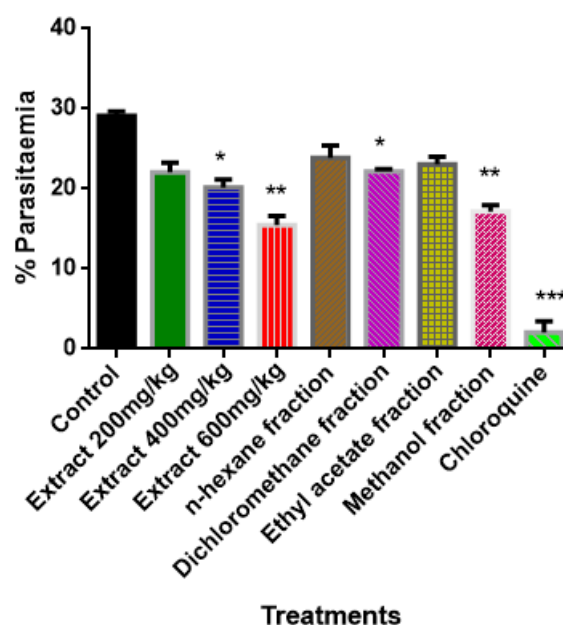


Figure 1. Suppressive activities of leaf extract and fractions of *S. megaphylla* during early *Plasmodium berghei* infection in mice. Values are expressed as mean \pm SEM. Significant relative to control. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. $n = 6$.

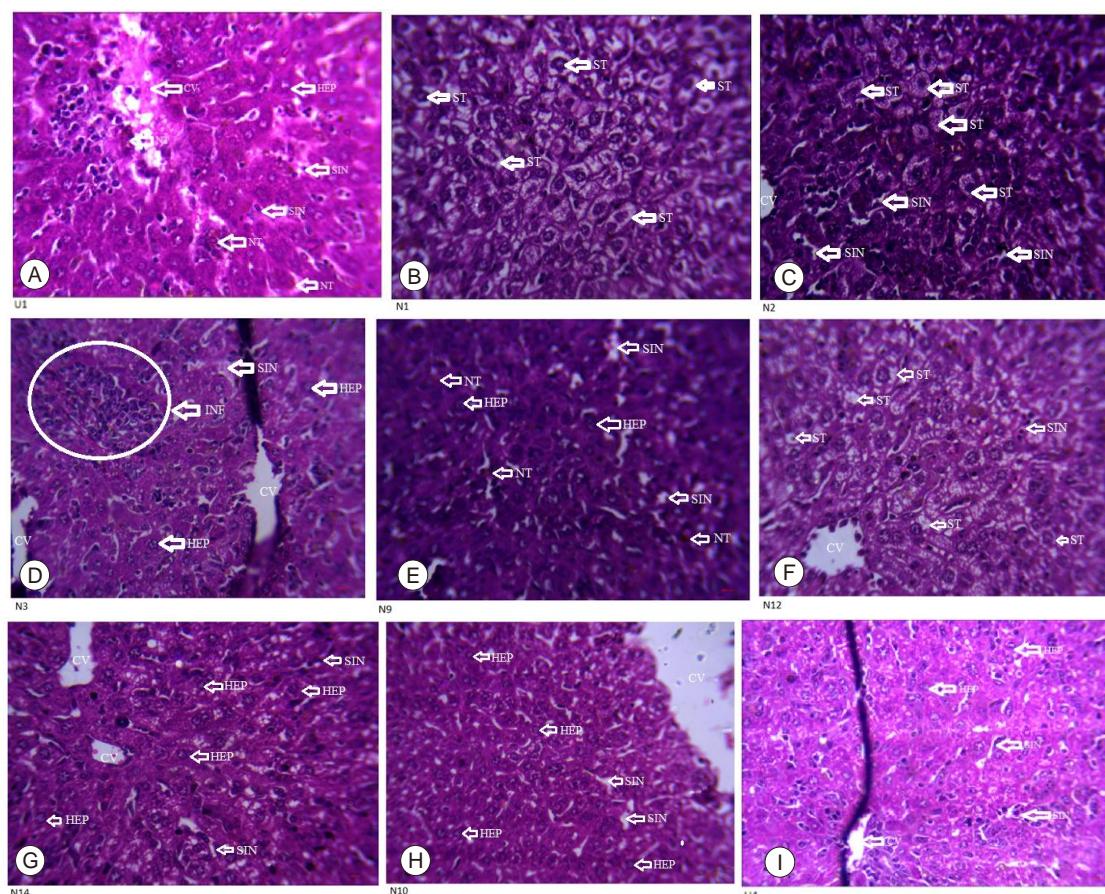


Figure 2. Histologic Liver sections of *Plasmodium berghei*-infected mice untreated (A), treated with leaf extract of *S. megaphylla*, 200 mg/kg (B), 400 mg/kg (C), 600 mg/kg (D), n-hexane fraction(E), DCM fraction (F), ethyl acetate fraction (G), methanol fraction (H) and chloroquine, 5 mg/kg (I) at magnification X400. Keys: Hepatocytes (HEP), Sinusoids (SIN), Necrotic tissues (NT), Steatosis (ST), Inflammatory cells (INF).

Table 1. Effect of leaf extract and fractions of *Setaria megaphylla* on liver function parameters of mice infected with *Plasmodium berghei* during established infection.

Treatment	Dose (mg/kg)	Liver Function Parameters						
		AST (IU/L)	ALT(IU/L)	ALP(IU/L)	Total Protein (g/L)	ALBUMIN (g/L)	Total bilirubin (μmol/mL)	Conjugate d bilirubin (μmol/mL)
Control	-	61.33±1.84	27.46 ±1.35	31.6 ±1.06	69.6 ±3.38	48.0 ±2.08	13.53±0.63	8.20±0.55
Extract	200	54.66±1.17	21.6±1.76	17.33±1.85 ^b	57.0±0.57 ^b	33.0±0.57 ^b	10.93±1.83	7.0±1.65
	400	40.0±3.21 ^c	14.66±1.79 ^c	17.16±1.20 ^b	59.0±2.64 ^b	37.3±1.85 ^a	8.16±1.33	4.66±0.79 ^a
	600	51.0±1.35 ^b	19.66±1.80 ^a	11.66±1.20 ^c	54.66±2.18 ^c	34.6±2.72 ^b	10.13±0.86	6.06±0.98
n-hexane	400	44.33±3.92 ^b	22.66 ±2.09	19.66±1.85 ^a	57.33±1.20 ^b	39.0 ±3.51	8.70±1.04	5.33±0.68
Dichloromethane	400	46.66±3.66 ^b	20.66± 2.17	24.33±2.72	58.0 ±0.57 ^b	38.0 ±1.52	9.16±1.67	5.10±1.20
Ethyl acetate	400	41.33±2.45 ^c	16.33± 1.36 ^c	26.0± 3.21	58.33 ±1.20 ^b	40.0 ±1.15	8.93±1.78	6.10±1.60
Methanol	400	37.0±3.21 ^c	16.33± 1.10 ^c	17.0± 2.51 ^b	58.66 ±2.33 ^b	39.0 ±2.64	7.43±0.66 ^a	3.80±0.17 ^a
Chloroquine	5	25.66 ±2.90 ^c	15.36 ±0.85 ^c	18.16 ±4.41 ^b	52.0±1.73 ^c	35.66±1.85 ^a	5.30±0.55 ^b	3.73±0.57 ^a

Values are expressed as mean ± SEM. Significant relative to control. ^ap<0.05; ^bp<0.01; ^cp<0.001. (n = 6).

Table 2. Effect of leaf extract and fractions of *Setaria megaphylla* on liver antioxidant enzymes of mice infected with *Plasmodium berghei* during established infection.

Treatment	Dose (mg/kg)	Antioxidant Parameters						
		GSH ($\mu\text{g/mL}$)	SOD ($\mu\text{g/mL}$)	CAT ($\mu\text{g/mL}$)	GPX ($\mu\text{m/mL}$)	GST ($\mu\text{g/mL}$)	MDA ($\mu\text{mol/mL}$)	Liver weight (g)
Control	-	1.16 \pm 0.06	0.17 \pm 0.02	1.03 \pm 0.13	0.050 \pm 0.003	0.074 \pm 0.002	0.57 \pm 0.01	2.76 \pm 0.16
Extract	200	1.30 \pm 0.05 ^c	0.25 \pm 0.02	3.59 \pm 1.27 ^c	0.054 \pm 0.002	0.107 \pm 0.007	0.46 \pm 0.03	2.69 \pm 0.19
	400	1.56 \pm 0.03 ^c	0.34 \pm 0.04 ^b	1.78 \pm 0.50 ^b	0.049 \pm 0.004	0.177 \pm 0.004 ^c	0.36 \pm 0.02 ^c	2.28 \pm 0.16
	600	1.50 \pm 0.03 ^c	0.39 \pm 0.09 ^c	3.73 \pm 1.23 ^c	0.054 \pm 0.005	0.211 \pm 0.006 ^c	0.39 \pm 0.03 ^b	2.86 \pm 0.18
<i>n</i> -hexane	400	1.95 \pm 0.05 ^c	0.31 \pm 0.01 ^a	6.35 \pm 0.08 ^c	0.660 \pm 0.003 ^c	0.116 \pm 0.005 ^c	0.48 \pm 0.03	2.41 \pm 0.16
Dichloromethane	400	1.44 \pm 0.02 ^c	0.39 \pm 0.01 ^c	1.99 \pm 0.10 ^c	0.060 \pm 0.007	0.116 \pm 0.009 ^c	0.35 \pm 0.03 ^c	2.24 \pm 0.18
Ethyl acetate	400	1.70 \pm 0.03 ^c	0.33 \pm 0.01 ^b	1.67 \pm 0.12 ^b	0.058 \pm 0.006	0.100 \pm 0.005	0.41 \pm 0.02 ^b	2.39 \pm 0.13
Methanol	400	1.51 \pm 0.04 ^c	0.32 \pm 0.03 ^b	2.19 \pm 0.29 ^c	0.062 \pm 0.001	0.380 \pm 0.01 ^c	0.47 \pm 0.04	2.16 \pm 0.10
Chloroquine	5	1.18 \pm 0.04	0.20 \pm 0.05	2.73 \pm 0.39 ^c	0.057 \pm 0.008	0.155 \pm 0.01 ^c	0.56 \pm 0.04	2.22 \pm 0.18

Values are expressed as mean \pm SEM. Significant relative to control. ^a p <0.05; ^b p <0.01; ^c p <0.001. (n = 6).

Discussion

The leaves of *S. megaphylla* are used in Ibibio traditional medicine as malaria remedy and this work was designed to investigate its antioxidative stress and hepatoprotective potentials in *Plasmodium berghei*-infected mice. The leaf extract and fractions of *S. megaphylla* were investigated for antimalarial activity against rodent malaria parasite, *P. berghei* infection in mice using early infection test model. It was found that the extract and fractions significantly reduced the parasitaemia with methanol fraction exhibiting the highest suppressive activity, confirming the antimalarial potential of these extract and fractions. These activities could have resulted from plasmodicidal or plasmodistatic activity of the extract and fractions. The results of this study corroborate earlier reports of antimalarial and antiplasmodial activities of the leaf extract and fractions (Okokon *et al.*, 2007; 2017). This observed activity may have resulted from the activities of the phytochemical constituents of this plant earlier reported (Okokon *et al.*, 2006; 2013; 2021).

In the present study, higher levels of transaminases and hyperbilirubinemia were observed in untreated infected animals. Temporary hepatic dysfunction is associated with malaria infection characterized by the increase of relative liver weight and liver enzymes activities. The distortions in liver may result from alteration in blood flow through the organ as parasitized RBC adhere to endothelial cells, blocking the sinusoids and obstructing the intrahepatic blood flow. Similarly, liver damage could be also due to the leakage of some hepatic cells which were killed or injured by the immune response progress and/or by abnormal cell activation induced by the parasites (Guthrow *et al.*, 1979). Free radicals have been implicated in liver impairment which results in leakage of some enzymes. Hyperbilirubinemia results from the impairment of drainage capacity of the liver due to endothelial blockage and disturbance of hepatocytes (Onyesom and Onyemakonor, 2011). Administration of leaf extract and fractions of *S.*

megaphylla to *P. berghei*-infected mice was found to decreased the elevated total protein, albumin, AST, ALT, ALP, conjugated and total bilirubin. The increases recorded in serum total protein, albumin, ALT, AST, ALP, conjugated and total bilirubin in the parasitized non-treated mice was suggested be due to response to hyper-parasitemia (Orhue *et al.*, 2005). Malaria parasite infections are accompanied by cellular mobilization of T-cells and its complements with a resultant synthesis and secretion of antibody molecules leading to elevated protein levels in parasitized non-treated mice (Orhue *et al.*, 2005). The increased activities of serum AST, ALT, and ALP in the liver and blood of *P. berghei* infected mice may be due to hepatic dysfunction (George *et al* 2011; Guthrow *et al.*, 2007) or hepatic damage as could be confirmed in the liver histology. The observed increases in activities of markers enzymes of hepatic damage is in agreement with the report of Uzuegbu and Emeka, (2011). However, administration of *S. megaphylla* reduced and restored normal levels of total protein, albumin, direct and total protein and the activities of AST, ALT and ALP in the serum of infected treated mice. The results are indicative of hepatoprotective activity of the leaf extract and fractions. Besides, the histological analyses of livers from malaria infected mice showed a significant pathological signs. This reticuloendothelial hyperplasia expressed by general architectural disorganization of liver with inflammatory sites, hepatocyte necrosis, vascular congestion, malarial pigment presence in the Kupffer cells and steatosis is suggestive of inflammatory reaction in the tissue. These were reduced or absent following extract/fractions treatments which further confirm the hepatoprotective activity of the leaf extract due to the antioxidant activities of its phytochemical constituents as previously reported (Okokon *et al.*, 2013, Okokon *et al.*, 2017; Okokon *et al.*, 2021).

Oxidative stress results from an imbalance between the generation of reactive oxygen species and

endogenous antioxidant systems (Chanda and Dave, 2009). The tissue hypoxia cause by malaria infection induces an activation of the natural host defence that generates large amounts of reactive oxygen species, causing an imbalance between the formation of oxidizing species and the activity of antioxidants which can lead to the death of the parasites (Becker *et al.*, 2004; Percario *et al.*, 2012). Studies suggest that the generation of reactive oxygen species and reactive nitrogen species (ROS and RNS) associated with oxidative stress is implicated in the pathogenesis and development of systemic complications caused by malaria (Guha *et al.*, 2006; Ojezele *et al.*, 2017). Malaria complications including anemia, jaundice and pre-eclampsia have been linked to oxidative stress damage caused by the parasite (Fabbri *et al.*, 2013; Sarr *et al.*, 2017). Malarial infection has been found to decrease the levels of antioxidant enzymes and other non-enzymatic anti-oxidants such as catalase (CAT), glutathione (GSH) peroxidase, super oxide dismutase (SOD), albumin, glutathione, ascorbate and plasma tocopherol. Malaria severity has also been found to be directly proportional to the level of lipid peroxidation - an indication of membrane damage which is associated with increased malondialdehyde levels (Asagba *et al.*, 2010; Adil *et al.*, 2013). The oxidative stress induction was also described as electrons produced during the oxidation of Fe²⁺ into Fe³⁺ following the Hb degradation by the parasites (Kumar and Bandyopadhyay, 2005). Moreover, antioxidant enzymes (catalase and SOD), and molecules such as proteins significantly decreased while lipid peroxidation (MDA) increased during malaria infection (Luersen *et al.*, 2000). These indices have been used to measure the severity of malaria infection. In this study, the activities of SOD, CAT, GPx and GSH level were found to decrease significantly, while MDA level was significantly increased in the untreated infected group. These findings corroborate earlier reports on the rise in MDA level as indicative of lipid peroxidation in the liver of *P. berghei*-infected mice and depletion in host's SOD and CAT activities being important features of malaria infections (Becker *et al.*, 2004; Gora *et al.*, 2006). The plant extract and fractions exerted protective effect by increasing the activity of antioxidant enzymes and molecules as well as reducing the level of MDA significantly, thus lipid peroxidation. This activity can be explained by the presence of phenols, flavonoids and tannins that are able to trap free radicals (Rice-Evans *et al.*, 1995). These activities may have resulted from the antioxidant activities of its phytochemical constituents as previously reported (Okokon *et al.*, 2013, Okokon *et al.*, 2017; Okokon *et al.*, 2021).

CONCLUSION

The results of this study show that the leaf extract and fractions of *Setaria megaphylla* possess antimalarial,

antioxidative stress and liver protective potentials which may be attributed to the activities of its phytochemical constituents.

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