

# EFFECT OF *Telfairia occidentalis* STEM EXTRACT AND FRACTIONS ON PARASITAEMIA, OXIDATIVE STRESS MARKERS, LIPID PROFILE, HEMATOLOGICAL PARAMETERS, LIVER FUNCTION INDICES AND LIVER HISTOLOGY IN *Plasmodium berghei* INFECTED MICE

## ABSTRACT

*Telfairia occidentalis* Hook (*Cucurbitaceae* family) is a fluted pumpkin of the widely used nutritionally and in Ibibio ethnomedicine for the treatment of various diseases such as malaria. The stem extract and fractions of *Telfairia occidentalis* were investigated for effect on parasitaemia, hematological parameters, oxidative stress markers, lipid profile, liver function indices and histopathology of the liver in *Plasmodium berghei*-infected mice using curative test model. The stem extract (200-600 mg/kg, p.o.) exerted dose-dependent significant ( $p < 0.05$ ) antimalarial activity against *P. berghei* infection in curative test with n-hexane fraction demonstrating the highest activity. The extract/fractions treatment did not affect any blood parameters except elevation of WBC counts and neutrophil percentages in the group treated with extract (400 and 600 mg/kg). The extract/fractions treatment did not alter significantly ( $p < 0.05$ ) the lipid profile indices, liver enzymes (ALT, AST and ALP), total and conjugated bilirubin, total protein and albumin levels of the treated infected mice relative to control. The stem extract and fractions also did not affect the levels of liver oxidative stress marker enzymes and molecules (CAT, GPx, GST, SOD, GSH, MDA) of the treated infected mice significantly ( $p > 0.05$ ) although there were observable changes. Histology of liver sections revealed moderate reductions in pathological features in infected mice treated with extract (200, 400 and 600 mg/kg), and hexane fraction compared to untreated infected mice. These results suggest that the stem extract/fractions of *Telfairia occidentalis* possess moderate antioxidative stress and liver protective potentials due to the activities of its phytochemical constituents which could be beneficial in malaria therapy.

**Keywords:** Malaria, *Telfairia occidentalis*, *Plasmodium berghei*, antioxidative stress, hepatoprotective

## 1. INTRODUCTION

Malaria has continued to ravage the world especially the developing countries despite relentless efforts to contain malaria (WHO, 2022; WHO, 2023). Eighty-four countries of the world are still endemic to the disease inspite of concerted efforts at eradicating

the disease (WHO, 2022; Center for Disease Control and Prevention, 2023). The recent reports documented that cases and deaths from malaria are escalating from year to year: 2019; 227 million cases, 568, 000 deaths (WHO, 2020), 2020; 241 million cases, 625,000 deaths (WHO, 2021) 2021; 247 million cases, 619, 000 deaths (WHO, 2022), and 2022; 249 million cases, 608,000 deaths (WHO, 2023), each consecutive year, responsible for more than half a million deaths. Worst still, about 95% of malaria cases and 96% of deaths occurred in sub-Saharan Africa. Every minute, malaria kills a child under 5 years (WHO, 2022; WHO, 2023; UNICEF, 2024). Besides, pregnant women living in sub-Saharan Africa are vulnerable to the danger of malaria and the disease affected about 32% of pregnancies (out of 42 million) in this region.(WHO, 2023). Oxidative stress associated with malaria infection contributes to development of systemic complications which leads to organ dysfunctions (Ojezele *et al.*, 2017). Medicinal plants, therefore serve as an alternative reservoir for antimalarial remedies being beneficial in providing many therapeutic effects and safe.

*Telfairia occidentalis* Hook is a fluted pumpkin of the *Cucurbitaceae* family widely consumed as food in Nigeria (Okokon *et al.*, 2009). It is a popular vegetable all over Nigeria, especially in the Niger-Delta region and the Eastern part of the country; varieties of meals are prepared from the leaves, stem and seeds of the plant (Usunomena *et al.*, 2023). The various parts of the plant (seeds, leaves and stem) are used traditionally in the treatment of various ailments and diseases. Antiplasmodial activities of the seed, leaves and roots of the plant have been previously reported (Okokon *et al.*, 2007; Okokon *et al.*, 2009). Enin *et al.*, (2023) had reported on antioxidant activity and in vivo inhibitory effect on alpha amylase and alpha glucosidase of the stem extract. Polyunsaturated fatty acids such as

hexadecanoic acid, methyl ester, 9,12-octadecadienoic acid methyl ester (linoleic acid), 9,12,15-octadecatrienoic acid, methyl ester (linoleic acid), and 9-octadecenoic acid have been found in the various fractions, as well as alkaloid, terpenes, saponin, flavonoid and tannin in the crude extract (Enin *et al.*, 2023). The present study was designed to evaluate the effect of stem extract and fractions of *T. occidentalis* on oxidative stress markers, liver function parameters and liver histology in mice infected with *Plasmodium berghei*.

## **2. MATERIALS AND METHODS**

### **2.1 Plant materials**

Fresh stems of *Telfairia occidentalis* were collected from farms in Uyo metropolis in Uyo LGA, Akwa Ibom State, Nigeria. The leaves were identified and authenticated as *Telfairia occidentalis* by a taxonomist in the Department of Botany and Ecological studies, University of Uyo, Uyo, Nigeria and a voucher specimen was prepared and deposited at the herbarium of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo.

### **2.2 Extraction**

Fresh stems of *Telfairia occidentalis* were washed, cut into smaller pieces and dried under shade for two weeks. The stems were further pulverized to powder using electric grinder. The powdered leaf material (2 kg) was soaked in 50% ethanol (7.5 L) at room temperature ( $28 \pm 2$  °C) for 72 hours. It was thereafter filtered and the liquid filtrate was concentrated and evaporated to dryness in *vacuo* 40 °C using a rotary evaporator (BuchiLab Switzerland). The crude extract (50.0 g) was dissolved in water (200 mL) and partitioned using n-hexane, dichloromethane, ethyl acetate and

methanol (4 x 500 mL each) to give the corresponding fractions of these solvents. The extract and fractions were weighed and stored in a refrigerator at -4 °C, until used for the proposed experiments.

### **2.3 Experimental animals**

Swiss albino mice (male and female) that were used in the study were obtained from the University of Uyo's Animal house. They were kept in standard plastic cages in a well ventilated room and left to acclimatized for a period of 10 days before the experiments. The mice were fed on standard pelleted diet and water *ad libitum*. 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.

### **2.4 Drug administration**

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

### **2.5 Determination of median lethal dose (LD<sub>50</sub>)**

The median lethal dose (LD<sub>50</sub>) of the extract was estimated using albino mice by intraperitoneal (i.p) route using the method of Lorke (1983). This involved oral administration of different doses of the extract (100 -1000 mg/kg) to groups of three mice each. The animals were observed for manifestation of physical signs of toxicity such as writhing, decreased motor activity, decreased body/limb tone, decreased respiration and death. The number of deaths in each group within 24 hours was

recorded. The LD<sub>50</sub> was calculated as geometrical means of the maximum dose producing 0% (a) and the minimum dose producing 100% mortality (b).

$$LD_{50} = \sqrt{ab}.$$

## 2.6 Micro-organism (parasite)

The chloroquine-sensitive strain of *Plasmodium berghei* was obtained from the National Institute of Medical Research (NIMR), Yaba Lagos, Nigeria and maintained by subpassage in mice.

## 2.7 Parasite inoculation

Each mouse that was 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 mice 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 (Okokon et al., 2019; Atanu et al., 2021; Okokon et al., 2022a). Parasitemia was monitored by standard methods; thin blood smears were made on glass slides, fixed using methanol, and stained using Giemsa stain, and parasitemia was counted using a microscope and 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 Enyiekere *et al* (2024) as given below:

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

Total number of RBCs

## 2.8 Evaluation of the *in vivo* antimalarial activities of stem extract and fractions of *Telfairia occidentalis* on established infection

This investigation was carried out using the curative test method earlier described by Okokon *et al.* (2017), to assess the antimalarial activity of the extract, fractions and chloroquine in established plasmodiasis. *P. berghei* parasites were injected intraperitoneally into ninety (90) mice on the first day (D<sub>0</sub>). The mice were divided into nine groups of ten mice per group after 72 hours (D<sub>2</sub>). The extract, at doses of 200, 400, and 600 mg/kg were respectively administered to groups 1-3 mice, while 400 mg/kg each of *n*-hexane, ethyl acetate, dichloromethane, and methanol fractions were given to groups 4 -7 respectively, group 8 was given 5 mg/kg of chloroquine (positive control) and group 9 was given 10 mL/kg distilled water (negative control). The crude extract, fractions and chloroquine was administered once daily for 5 days. Giemsa stained thin smears were prepared from tail blood samples collected on each day of treatment to monitor the parasitemia level. The mean survival time (MST) of the mice in each group were determined over a period of 29 days (D<sub>0</sub>-D<sub>28</sub>). On the sixth day, five mice were sacrificed from each group under diethyl ether vapour. Blood samples were collected by cardiac puncture into EDTA bottles and plain centrifuge tubes. Hematological analyses were carried out on blood samples in the EDTA bottles, while blood samples in plain tubes were centrifuged immediately at 2500 rpm for 15 mins to separate the serum at room temperature. These sera samples were stored at -20°C until used for biochemical determinations such as liver function test and lipid profile. 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 Enyiekere *et al.* (2024) as follows: (average % parasitemia positive control – average % parasitemia negative control) / (average % parasitemia negative control).

### **2.9 Effect of the stem extract and fractions of *T. occidentalis* on haematological parameters of *P. berghei* infected mice**

Blood samples that were collected from each mouse into ethylene diamine tetra-acetic acid (EDTA) – coated sample bottles were used to determine the effect of the extract/fractions on hematological parameters such as Red blood cell count (RBC), hemoglobin, (Hb), packed cell volume (PCV), platelet concentration (PLC) and total and differential white blood cell count (WBC). These parameters were analyzed using automated hematological system (Sysmex Hematology – Coagulation system, Model MO-1000 I, Trans Asia, Japan) at the University of Uyo Teaching Hospital.

### **2.10 Effect of the stem extract and fractions of *T. occidentalis* on liver function parameters of *P. berghei* infected mice**

Liver function parameters such as total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), conjugated and total bilirubin were determined in the stored sera samples collected from the sacrificed mice spectrophotometrically using Randox analytical kits according to standard procedures of manufacturer's protocols (Tietz, 1990).

### **2.11 Effect of the stem extract and fractions of *T. occidentalis* on liver oxidative stress markers of *P. berghei* infected mice**

The excised livers were stored and washed with ice cold 0.9% NaCl. 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), MDA (Esterbauer and Cheeseman,1990).

### **2.12 Effect of the stem extract and fractions of *T. occidentalis* on lipid profile of *P. berghei* infected mice**

Serum cholesterol, triglyceride and high-density lipoprotein (HDL) levels of the treated rats were measured using standard colorimetric methods. These determinations were done spectrophotometrically using Fortress Diagnostic Kits® according to standard procedures of manufacturer's protocols. Low and very low-density lipoprotein (LDL and VLDL) were estimated from the formula of Friedwald *et al.* (1972).

### **2.13 Effect of the stem extract and fractions of *T. occidentalis* on liver histology of *P. berghei* infected mice**

The liver parts of mice that were fixed in buffered formalin were processed and stained with haematoxylin and eosin (H&E) for liver study according to standard procedures (Drury and Wallington, 1980) at Department of Chemical Pathology, University of Uyo Teaching Hospital, Uyo. Morphological changes observed and recorded in the excised organ of the sacrificed mice. Histologic pictures were taken as micrographs.

### **2.14 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  $\pm$  SEM and significance relative to control were considered at  $p < 0.05$ .

## **1. RESULTS**

### **3.1 Determination of Median lethal dose (LD<sub>50</sub>)**

Administration of stem extract of *T. occidentalis* (100 - 5000 mg/kg) orally did not cause any mortality to the animals groups administered. Moreover, no physical toxic signs of the extract was observed. The median lethal dose (LD<sub>50</sub>) of stem extract of *T. occidentalis* was therefore estimated to be =5000 mg/kg.

### **3.2 Antiplasmodial effect of ethanol stem extract and fractions of *Telfairia occidentalis* on established infection**

Administration of extract/fraction to *P. berghei* infected mice produced a dose-dependent reductions of parasitaemia in all the extract/fraction-treated groups progressively relative to control. These reductions were statistically significant relative to the control ( $p < 0.001$ ; Table 1) on day 7 with the highest dose (600 mg/kg) having the highest effect of 69.74% and m.s.t value of  $16.10 \pm 1.76$  d. The n-hexane fraction had the highest activity with chemosuppressive effect of 69.68 % and m.s.t value of  $15.60 \pm 2.06$  d) on day 7, this was lower compared with that of the standard, chloroquine, 92.77 %. The stem extract and fractions demonstrated considerable protective potentials on the infected mice as seen in the mean survival time of the animals. These were only significant ( $p < 0.05-0.001$ ) in the groups treated with the highest dose (600 mg/kg) and n-hexane fraction when compared to the control. The groups treated with n-hexane fraction had a longer mean survival time,  $15.60 \pm 2.06$  d followed by those of dichloromethane fraction treated mice,  $11.40 \pm 0.50$  d. These were less than that of the standard drug, chloroquine ( $29.83 \pm 0.16$  d; Table 1).

### **3.3 Effect of stem extract and fractions of *Telfairia occidentalis* on haematological indices *Plasmodium berghei*-infected mice.**

Administration of the stem extract and fractions of *T.occidentalis* to *P. berghei*-infected mice did not caused any significant ( $p > 0.05$ ) effect on RBC, Hb

concentration, PCV percentages, lymphocytes, monocytes, basophils and platelets counts when compared to the control group. The WBC count was significantly ( $p < 0.05$ ) elevated in groups treated with middle and high doses (400 and 600 mg/kg), hexane and methanol fractions as well as chloroquine, while neutrophils percentages were significantly ( $p < 0.05-0.001$ ) elevated in groups treated with low and middle doses (200 and 400 mg/kg) and methanol fraction (Table 2).

#### **3.4 Effect of stem extract and fractions of *Telfairia occidentalis* on liver function parameters of *Plasmodium berghei*-infected mice.**

Treatment of *P. berghei* infected mice with stem extract and fractions of *T. occidentalis* (200 - 600 mg/kg) did not cause any significant ( $p > 0.05$ ) alteration of liver function indices (AST, ALT, ALP, total protein, albumin, total and conjugated bilirubin) when compared to those of the control untreated *P. berghei* -infected mice (Table 3).

#### **3.5 Effect of stem extract and fraction of *Telfairia occidentalis* on liver oxidative stress markers of *Plasmodium berghei*-infected mice.**

Treatment of *Plasmodium berghei*-infected mice with stem extract and fractions of *T. occidentalis* (200 - 600 mg/kg) did not cause any significant ( $p > 0.05$ ) effect on the levels of GSH, SOD, GPX, CAT and MDA when compared to control. Although there were observable increases and/or decreases in the enzymes and molecules levels in the respective treatments, these changes were not significantly different ( $p > 0.05$ ) when compared with that of the control. (Table 4).

#### **3.6 Effect of stem extract and fraction of *Telfairia occidentalis* on lipid profile of *Plasmodium berghei*-infected mice.**

Treatment of *Plasmodium berghei*-infected mice with stem extract and fractions of *T.occidentalis* (200-600 mg/kg) did not cause any significant ( $p>0.05$ ) effect on the lipid profile parameters (total cholesterol, triglycerides, HDL, LDL, and VLDL) when compared to control. Although there were slight changes in the levels of these indices in the respective treatments, these changes were not significantly different ( $p>0.05$ ) when compared with that of the control (Table 5).

### **3.7 Effect of stem extract and fractions of *Telfairia occidentalis* on the histology of liver of *Plasmodium berghei*-infected mice.**

Histological sections of livers of rats receiving various treatments at magnification (x100) stained with H&E method revealed that untreated infected mice in group 1(control) given distilled water (10 mL/kg) had liver tissue demonstrating a severely altered hepato-architecture with areas of degenerated hepatic cells, inter-hepatic fibrosis, increased degenerating and vacuolated hepatocytes, widespread micro-vesicular steatosis, and shrunk ductal cells within the hepatic portal area. Infected mice in group 2 treated with low dose of the extract (200 mg/kg) had liver tissue showing moderately altered hepato-architecture with increased degenerating and vacuolated hepatocytes, widespread micro-vesicular steatosis and organic deposits within the blood vessel and hepatic lobules. Group 3 infected mice treated with the middle dose of the extract (400 mg/kg) had liver tissue demonstrating a moderately altered hepato-architecture with increased degenerating and vacuolated hepatocytes and widespread micro-vesicular steatosis and organic deposits within the hepatic lobules. Mice in group 4 infected with *P. berghei* and treated with high dose extract (600 mg/kg) had liver tissue demonstrating a moderately altered hepato-architecture with increased degenerating and vacuolated hepatocytes, widespread micro-vesicular

steatosis and organic deposits within the hepatic lobules. Liver tissues of infected mice in group 5 treated with n-hexane fraction showed moderately altered hepato-architecture with areas of degenerated hepatic cells and organic deposits within the hepatic lobules. Group 6 infected mice treated with DCM fraction had liver tissue showing a severely altered hepato-architecture with areas of degenerated hepatic cells increased degenerating, vacuolated hepatocytes, widespread micro-vesicular steatosis, fibrosis of connective tissues and organic deposits within the hepatic lobules. Ethyl acetate fraction treated infected mice in group 7 had liver tissue demonstrating a severely altered hepato-architecture with areas of degenerated hepatic cells increased degenerating and vacuolated hepatocytes, widespread micro-vesicular steatosis, and organic deposits within the hepatic lobules. Methanol fraction treated mice had liver tissue demonstrating a severely altered hepato-architecture with areas of degenerated hepatic cells increased degenerating and vacuolated hepatocytes, widespread micro-vesicular steatosis, and organic deposits within the hepatic lobules. Chloroquine treated mice had liver tissue demonstrating a severely altered hepato-architecture with areas of degenerated hepatic cells increased degenerating and vacuolated hepatocytes, widespread micro-vesicular steatosis and organic deposits within the hepatic lobules. (Figure 1).

## **2. DISCUSSION**

The stem of *T. occidentalis* are used locally as vegetable and to treat various diseases such as malaria, diabetes, fever, inflammatory diseases and pain among others. This work was designed to investigate its effect on parasitaemia, hematological parameters, oxidative stress markers, lipid profile, liver function indices and histopathology of the liver in *Plasmodium berghei*-infected mice using curative test model to provide scientific basis for its usage in traditional medicine. The stem

extract and fractions of *T. occidentalis* were investigated for antimalarial potentials against rodent malaria parasite, *P. berghei* infection in mice using standard *in vivo* models. It was found that the stem extract and fractions significantly reduced the parasitaemia in the established *P. falciparum* infection model evaluated in a dose-dependent fashion with n-hexane fraction followed by dichloromethane fraction exerting significant *in vivo* activity confirming the antimalarial potential of this extract. The extract and fractions also prolonged the MST of the mice, but could only offer minimal degree of protection to the treated mice as most of the treated animals eventually died from infection. This activity could have resulted from plasmodicidal or plasmodistatic activity of the extract and fractions. These results corroborate previous reports on antimalarial activities of the leaf, seed and root extracts of *T. occidentalis* (Okokon *et al.*, 2007; Okokon *et al.*, 2009; Ebong *et al.*, 2020). These results validate the use of the stem extract decoctions as malarial remedy.

The observed curative activities of the extract/fractions on the progression of *Plasmodium berghei* parasitaemia as observed during *in vivo* study, suggests that the extract and fractions has effect on the erythrocytic stages of the parasite (Waako *et al.*, 2005). The inability of the extract/fractions to give complete protection to the infected animals in most cases could have resulted from low doses (200 -600 mg/kg) used, short half life/ duration of action of the extract/fractions due to rapid biotransformation processes and subsequent elimination (Waako *et al.*, 2005). Thus, resulting in further development and multiplication of the parasites as well as short MST of the treated mice as observed in this study.

The reported phytochemical screening and GCMS analyses of the n-hexane, DCM, ethyl acetate and n-butanol fractions revealed the present of some

pharmacologically active compounds such as tannins, flavonoids, alkaloids, terpenes (Enin *et al.*, 2024), and polyunsaturated fatty acids (PUFAs) among others. These compounds are likely to be responsible for the observed activities of the extract and fractions. Some secondary metabolites like alkaloids, flavonoids and triterpenoids have been shown to possess antiplasmodial properties (Kirby *et al.*, 1989; Philipson and Wright 1991; Christensen and Kharazmi 2001). Also, polyunsaturated fatty acids such as hexadecanoic acid, methyl ester, 9,12-octadecadienoic acid methyl ester (linoleic acid), 9,12,15-octadecatrienoic acid, methyl ester (linoleic acid), and 9-octadecenoic acid have been reported by Enin *et al.* (2024) to be present in the hexane fraction which showed the highest antiplasmodial potential. Antiplasmodial activities of these PUFAs have been reported and their activities have been reported to increase with the degree of unsaturation (Kumaratilake *et al.*, 1992; Krugliak *et al.*, 1995; Suksamrarn *et al.*, 2005; Attioua *et al.*, 2007; Melariri *et al.*, 2011, 2012). Flavonoids found to be present in the stem extract have been shown to possess significant antiplasmodial activity against chloroquine sensitive and resistant strains of *P. falciparum* (Attioua *et al.*, 2011; Ganesh *et al.*, 2012; Ezenyi *et al.*, 2014). Also, these PUFAs mentioned above are active antioxidant compounds (Kohno *et al.*, 1995; Ponnamma and Manjunath, 2012; Khan and Siddique, 2019) and maybe responsible for the observed antiplasmodial activities. Antiplasmodial potentials of flavonoids have been suggested to be due to its antioxidant activity (Cimanga *et al.*, 2009; Ganesh *et al.*, 2012), as elevated free radical levels are common features of malaria disease and are implicated in severe malaria complications. Scavenging of these free radicals could be one of the mechanisms of action of this extract as the stem extract and fractions have been reported to exert strong antioxidant activity (Enin *et al.*, 2024). Flavonoids could also exert antiplasmodial activity by chelation of nucleic acid

base pairing of the parasite (Lui *et al.* 1992), modulation of host immunity to tackle disease and inhibition of plasmodial enoyl-ACP reductase (FAB I enzyme) – a key regulator of type II fatty synthases (FAS-II) in *P. falciparum* (Kirmizibekmez *et al.*, 2004; Teffo *et al.*, 2010) and binding to parasite's serinethreonine kinase with high affinity thereby affecting its development (Ferreira *et al.*, 2010). The stem extract/fractions may be acting through one of these mechanisms. The findings of this study suggest that stem extract and fractions of *T. occidentalis* possess antimalarial activity which is due to the activities of its phytochemical constituents. This validates its use as malarial remedy in folkloric medicine.

The stem extract and fractions of *T. occidentalis* were observed not to have any significant effect on the liver function parameters and oxidative stress markers (SOD, CAT, GPx, GST and GSH) compared to untreated control. when investigated for antioxidative stress and hepatoprotective activities in *P. berghei* infected mice using curative test. However, moderate pathological signs were observed in liver histologies of groups treated with extract (400 and 600 mg/kg) and hexane fraction, whereas other treatment groups had severe pathology similar to that of the control . This shows that the stem extract and fractions at the doses (200 - 600 mg/kg) used in this study could minimally protect the liver and improve the antioxidant enzymes and molecules (SOD, CAT, GPx, GST and GSH) to curb the generated oxidative stress. This could have been due to low doses administered in this study.

Obstruction of hepatic blood and blockade of sinusoids by parasitized erythrocytes in addition to destruction of the liver cells and membranes integrity by the activities of generated free radicals during malaria infection are known to cause injuries to the liver, thereby leading to leakages of cellular enzymes such as transaminases.

Reticuloendothelial blockage and disturbance of hepatocyte microvilli compromised the secretory capacity in the liver thereby resulting in hyperbilirubinemia (Onyesom and Onyemakonor, 2011). This could have been the case in this study which the extract/fractions were unable to ameliorate effectively.

Oxidative stress leads to complications observed during malaria infection such as anemia, jaundice and pre-eclampsia (Fabbri *et al.*, 2013; Sarr *et al.*, 2017) as large quantity of free radicals are generated by malaria infection which triggers body immune responses (Becker *et al.*, 2004; Percario *et al.*, 2012), leading to pathogenesis and development of systemic complications caused by malaria (Guha *et al.*, 2006; Ojezele *et al.*, 2017). Malarial infection results in reduced the levels of antioxidant enzymes and other non enzymatic anti-oxidants such as catalase (CAT), glutathione (GSH) peroxidase, superoxide dismutase (SOD), albumin, glutathione, ascorbate and plasma tocopherol (Asagba *et al.*, 2010). Increased lipid peroxidation and malondialdehyde levels have been correlated with malaria severity(Asagba *et al.*, 2010; Adil *et al.*, 2013). These indices have been used as indicators of malaria infection severity. In this study, the activities of the enzymes and MDA levels in the extract/fractions treated groups were found to be insignificantly different from that of the untreated infected mice. This suggest that the plant extract and fractions were unable to reduced parasitaemia significantly to curb the prevalent oxidative stress generated by the activities of the parasites perhaps due to the low doses used in this study.

Besides, malaria infection also induced oxidative modification of lipoproteins thereby contributing to oxidative stress, progression and complications of malaria infections (Nathawut *et al.*, 2004; Krishna *et al.*, 2009). The alteration in lipid metabolism has been attributed to acute phase response to the infection (Memon *et*

*al.*, 2000). However, treatment with *T.occidentalis* extract and fractions did not cause any significant ( $p>0.05$ ) effect on serum concentration of total cholesterol (TC), triglyceride (TG), LDL and very low density lipoprotein (VLDL) and HDL in the parasitized treated mice. The results suggest that the plant may not possess hypolipidemic potential at the doses administered in this study may be due to its weak antiplasmodial effect and free radical scavenging potentials.

Haematological alterations resulting in the decrease of RBC count, Hb level, PCV, and mean haemoglobin concentration levels are often observed in infected animals as conventional signs of anaemia (Surve *et al.*, 2017). During malaria infection, *Plasmodium* invades the host cells and shorten the lifespan of RBC through the digestion of Hb using glucose, oxygen and hemozoin formation and finally the bursting of the erythrocytes during the development of their asexual blood stage (Buffet *et al.*, 2010; Saganuwan *et al.*, 2011). The treatment of infected mice with the stem extract and fractions did not alter significantly the haematological parameters of the treated mice when compared to untreated control, except significant elevation of WBC count of the treated mice. This implies that the stem extract has no erythropoietic effect perhaps due to the low doses administered. The significant increase in WBC observed in infected mice as reported previously in malaria infection (Guyton, 2007), is attributable to immunogenic response to the parasite and malaria pigment (hemozoin) (Malaguarnera *et al.*, 2002). The extract/fractions treatment of infected mice may have stimulated the immune system thereby offering some degree of protection to the infected mice. This suggests the immunomodulatory activities of some phytochemical constituents of the stem extract and fractions (Bero and Quertin-Leclercg, 2009).

### 3. CONCLUSION

The results of this study show that the stem extract and fractions of *T. occidentalis* possess considerable antimalarial potential with minimal antioxidative stress and liver protective potentials which maybe attributed to the activities of its phytochemical constituents.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE):** Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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Table 1: Curative activity and mean survival time of mice treated with stem extract and fractions of *Telfairia occidentalis* during established *Plasmodium berghei* infection

Treatment	Dose (mg/kg)	Parasitaemia					MST
		Day3	Day 4	Day 5	Day 6	Day 7	
Control	-	12.33±0.92	14.56±0.48	22.66±1.36	24.04±0.91	33.78±1.16	7.20±0.13
Extract	200	12.24±0.35	13.14±0.19	19.10±0.16	21.22±0.32	23.06±0.15 <sup>c</sup>	10.60±1.20
	400	13.35±0.49	14.55±1.12	16.31±0.68	18.39±0.28	20.20±0.55 <sup>c</sup>	11.20±0.80 <sup>c</sup>
	600	13.26±0.81	12.20±0.17	13.88±0.18 <sup>c</sup>	12.11±0.33	10.22±1.54 <sup>c</sup>	16.10±1.76 <sup>c</sup>
<i>n</i> -hexane	400	15.38±1.22	13.29±1.04	12.76±1.68	10.33±0.44	10.24±0.24 <sup>c</sup>	15.60±2.06 <sup>b</sup>
Dichloromethane	400	13.21±0.51	13.22±0.56	15.33±0.96	18.0±0.55	22.24±1.29 <sup>c</sup>	11.40±0.50 <sup>c</sup>
Ethyl acetate	400	13.25±1.44	15.15±1.53	18.66±0.20	25.28±1.14	31.28±1.02	7.40±0.24
Methanol	400	14.71±0.62	15.90±1.16	18.13±0.63	21.34±0.26	24.54±1.38 <sup>c</sup>	8.60±1.60
Chloroquine	5	14.62±0.43	11.44±0.23	7.66±0.18 <sup>c</sup>	5.12±0.22 <sup>c</sup>	2.44±0.28 <sup>c</sup>	29.83±0.16 <sup>c</sup>

Values are expressed as mean ± SEM. Significant relative to control. <sup>c</sup>p<0.001. n = 6.

**Table 2:** Effect of *Telfairia occidentalis* stem extract/fractions on haematological parameters of *P. berghei*-infected mice

Treatment	WBC (x10 <sup>9</sup> /L)	LYM (%)	NEUT (%)	MONO (%)	ESINO (%)	BASO (%)	RBC (x10 <sup>12</sup> /L)	HGB (g/dL)	PCV (%)	PLT (x10 <sup>9</sup> /L)
Control	7.72±1.36	39.36±8.61	32.26±1.22	2.46±0.83	1.26±0.76	1.30±1.06	6.83±0.37	11.30±0.83	36.40±2.93	133.0±11.71
Extract(200 mg/kg)	6.40±0.46	54.60±2.12	43.73±1.81 <sup>a</sup>	1.00±0.45	0.66±0.17	0.00±0.00	4.45±1.51	6.0±2.65	22.56±9.36	141.6±2.60
Extract(400mg /kg)	7.37±0.98	47.63±2.75	48.23±1.23 <sup>b</sup>	1.76±0.80	1.86±0.34	0.50±0.45	5.96±0.64	11.23±1.18	36.66±1.93	199.30±68.68
Extract (600 mg/kg)	12.34±4.12 <sup>a</sup>	59.46±8.43	39.23±1.53	1.43±0.62	1.33±0.39	0.03±0.03	4.62±1.12	7.06±1.75	24.86±4.80	129.0±42.72
Hexane fraction	18.55±1.14 <sup>a</sup>	59.90±3.61	37.83±3.34	5.70±2.32	1.16±0.96	0.03±0.03	4.23±1.00	8.13±3.13	25.83±8.53	96.66±23.69
Dichloromethane fraction	6.49±1.15	60.86±2.98	37.43±3.16	0.76±0.21	0.83±0.28	0.10±0.05	4.77±0.79	8.76±2.02	30.03±5.88	131.0±58.85
Ethyl acetate fraction	8.29±1.23	67.60±5.33	22.86±4.35	3.26±0.96	3.56±2.69	0.26±0.26	5.71±1.08	11.56±0.89	40.16±3.32	137.3±13.34
Methanol Fraction	12.19±2.00 <sup>a</sup>	12.63±2.86	82.16±0.73 <sup>c</sup>	1.73±0.21	1.73±1.03	0.20±0.15	4.62±0.05	11.96±0.66	41.46±0.84	196.0±26.40
Chloroquine	18.46±4.30 <sup>a</sup>	64.93±3.51	32.26±9.22	2.46±0.83	1.26±0.76	0.00±0.00	4.34±0.63	5.70±3.48	25.66±6.00	121.0±13.20

All values are presented as mean±S.E.M. for six rats in each group.compared with control group <sup>a</sup>p<0.05, <sup>b</sup> p<0.01, <sup>c</sup> p<0.001.

Table 3: Effect of stem extract and fractions of *Telfairia occidentalis* on liver function parameters of mice infected with *Plasmodium berghei*

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 ( $\mu\text{mol/mL}$ )	Conjugated bilirubin ( $\mu\text{mol/mL}$ )
Control	-	46.0 $\pm$ 0.57	44.66 $\pm$ 2.60	56.0 $\pm$ 1.73	78.66 $\pm$ 2.02	47.33 $\pm$ 1.20	8.76 $\pm$ 0.5	6.20 $\pm$ 0.17
Extract	200	51.33 $\pm$ 1.18	48.66 $\pm$ 0.10	54.66 $\pm$ 4.33	71.33 $\pm$ 1.76	44.66 $\pm$ 2.02	10.16 $\pm$ 0.63	7.50 $\pm$ 0.40
	400	48.0 $\pm$ 0.57	45.0 $\pm$ 1.15	63.66 $\pm$ 0.88	72.66 $\pm$ 1.45	43.0 $\pm$ 2.08	9.63 $\pm$ 0.08	6.43 $\pm$ 0.17
	600	46.33 $\pm$ 1.76	43.38 $\pm$ 0.88	61.33 $\pm$ 2.02	67.0 $\pm$ 5.50	41.0 $\pm$ 1.52	9.13 $\pm$ 0.23	6.60 $\pm$ 0.40
<i>n</i> -hexane	400	47.66 $\pm$ 3.18	55.0 $\pm$ 4.61	57.0 $\pm$ 1.15	72.33 $\pm$ 3.84	43.33 $\pm$ 2.72	9.46 $\pm$ 0.50	6.80 $\pm$ 0.51
Dichloromethane	400	47.0 $\pm$ 1.15	51.66 $\pm$ 2.60	65.66 $\pm$ 1.76	70.66 $\pm$ 2.33	43.0 $\pm$ 2.08	9.16 $\pm$ 0.08	6.50 $\pm$ 0.17
Ethyl acetate	400	45.0 $\pm$ 1.15	46.0 $\pm$ 0.57	57.33 $\pm$ 2.02	60.0 $\pm$ 2.30	35.33 $\pm$ 1.45 <sup>a</sup>	9.23 $\pm$ 0.14	6.53 $\pm$ 0.14
Methanol	400	42.0 $\pm$ 0.57	42.0 $\pm$ 1.73	60.00 $\pm$ 1.15	75.66 $\pm$ 1.85	46.33 $\pm$ 1.20	8.30 $\pm$ 0.11	5.63 $\pm$ 0.17
Chloroquine	5	44.38 $\pm$ 0.88	44.0 $\pm$ 0.57	59.0 $\pm$ 1.15	75.66 $\pm$ 1.85	48.0 $\pm$ 0.57	8.80 $\pm$ 0.20	6.06 $\pm$ 0.14

Values are expressed as mean  $\pm$  SEM. Significant relative to control. <sup>a</sup>p<0.05; <sup>b</sup>p<0.01; <sup>c</sup>p<0.001. n = 6.

Table 4: Effect of stem extract and fractions of *Telfairia occidentalis* on liver antioxidant enzymes of mice infected with *Plasmodium berghei* .

Treatment	Dose (mg/kg)	ANTIOXIDANT PARAMETERS					Liver weight (g)
		GSH( $\mu\text{g/mL}$ )	SOD( $\mu\text{g/mL}$ )	CAT( $\mu\text{g/mL}$ )	GPX ( $\mu\text{m/mL}$ )	MDA ( $\mu\text{mol/mL}$ )	
Control	-	1.89 $\pm$ 0.29	0.43 $\pm$ 0.02	3.65 $\pm$ 0.27	0.056 $\pm$ 0.008	0.35 $\pm$ 0.02	2.91 $\pm$ 0.12
Extract	200	2.02 $\pm$ 0.34	0.36 $\pm$ 0.07	5.61 $\pm$ 0.66	0.059 $\pm$ 0.009	0.42 $\pm$ 0.07	2.46 $\pm$ 0.11
	400	1.83 $\pm$ 0.35	0.36 $\pm$ 0.03	1.83 $\pm$ 0.48	0.054 $\pm$ 0.01	0.43 $\pm$ 0.03	2.33 $\pm$ 0.10
	600	2.20 $\pm$ 0.54	0.36 $\pm$ 0.05	3.89 $\pm$ 0.64	0.065 $\pm$ 0.01	0.44 $\pm$ 0.05	2.28 $\pm$ 0.13
<i>n</i> -hexane	400	1.55 $\pm$ 0.07	0.33 $\pm$ 0.07	3.58 $\pm$ 0.19	0.056 $\pm$ 0.01	0.46 $\pm$ 0.07	2.41 $\pm$ 0.14
Dichloromethane	400	2.35 $\pm$ 0.57	0.37 $\pm$ 0.01	4.44 $\pm$ 0.98	0.068 $\pm$ 0.01	0.44 $\pm$ 0.01	2.20 $\pm$ 0.15
Ethyl acetate	400	2.26 $\pm$ 0.19	0.40 $\pm$ 0.01	2.55 $\pm$ 0.23	0.067 $\pm$ 0.005	0.39 $\pm$ 0.01	2.40 $\pm$ 0.16
Methanol	400	2.46 $\pm$ 0.39	0.36 $\pm$ 0.03	3.50 $\pm$ 0.20	0.075 $\pm$ 0.01	0.60 $\pm$ 0.13	2.31 $\pm$ 0.14
Chloroquine	5	1.70 $\pm$ 0.07	0.32 $\pm$ 0.05	2.88 $\pm$ 0.81 <sup>c</sup>	0.049 $\pm$ 0.001	0.35 $\pm$ 0.02	2.21 $\pm$ 0.16

Values are expressed as mean  $\pm$  SEM. Significant relative to control. <sup>c</sup>p<0.001. n = 6.

Table 5: Effect of stem extract and fractions of *Telfairia occidentalis* on lipid profile of mice infected with *Plasmodium berghei* .

TREATMENT	DOSE mg/kg	TOTAL CHOLESTEROL (mMol/L)	TRIGLYCERIDE (mMol/L)	HDL-C (mMol/L)	LDL-C (mMol/L)	VLDL (mMol/L)
Control	10 mL/kg	4.46± 0.33	1.58± 0.20	1.68± 0.17	3.50± 0.26	0.71± 0.09
Crude extract	200	3.63±0.31	1.34± 0.10	1.39± 0.08	2.84± 0.32	0.61± 0.05
	400	4.40± 0.17	1.55± 0.09	1.88± 0.08	3.42± 0.12	0.70± 0.03
	600	4.13± 0.36	1.18± 0.05	1.21± 0.08	3.45± 0.36	0.53± 0.02
n-hexane	400	3.20± 0.47	1.32± 0.21	1.36± 0.16	2.43± 0.43	0.54± 0.09
Dichloromethane	400	4.43± 0.37	1.64± 0.10	1.75± 0.08	3.43± 0.34	0.74± 0.04
Ethyl acetate	400	3.83± 0.17	1.54± 0.11	1.58± 0.08	2.95± 0.22	0.70± 0.05
Methanol	400	4.13± 0.61	1.43± 0.05	1.49± 0.21	3.28± 0.54	0.65± 0.07
Chloroquine	5	4.36± 0.53	1.69± 0.11	1.70± 0.16	3.50± 0.26	0.71± 0.09

Data are expressed as MEAN ± SEM. (n=6).

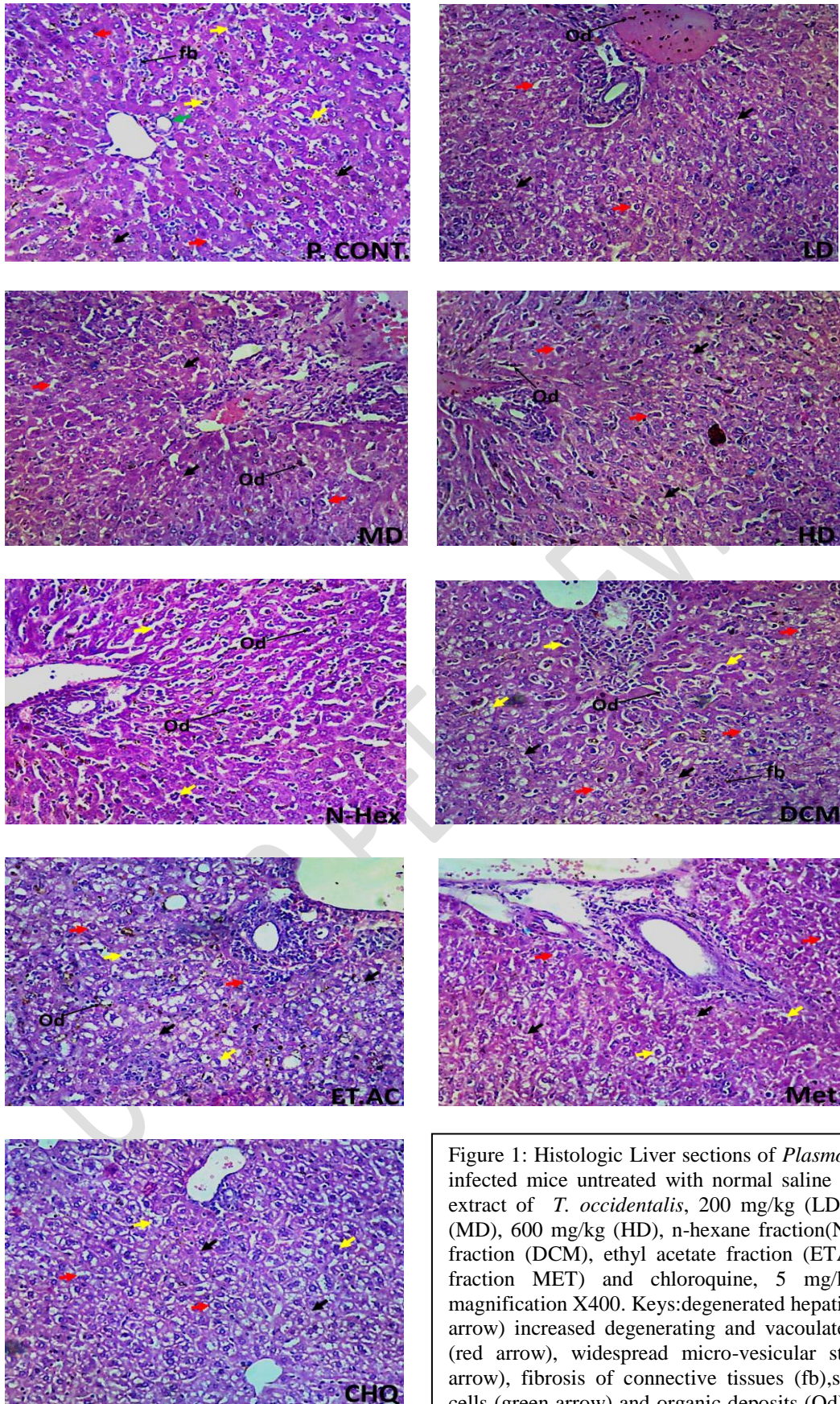


Figure 1: Histologic Liver sections of *Plasmodium berghei*-infected mice untreated with normal saline (CONT), stem extract of *T. occidentalis*, 200 mg/kg (LD), 400 mg/kg (MD), 600 mg/kg (HD), n-hexane fraction(N-HEX), DCM fraction (DCM), ethyl acetate fraction (ETAC), methanol fraction MET) and chloroquine, 5 mg/kg (CHQ) at magnification X400. Keys:degenerated hepatic cells (yellow arrow) increased degenerating and vacuolated hepatocytes (red arrow), widespread micro-vesicular steatosis (black arrow), fibrosis of connective tissues (fb),shrunken ductal cells (green arrow) and organic deposits (Od) inter-hepatic fibrosis (fb)

UNDER PEER REVIEW