BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 13, Number 2, October 2024 | Pages: 351-359 | DOI: 10.14421/biomedich.2024.132.351-359 ISSN 2540-9328 (online)

Effect of *Justicia insularis* Leaf Extract and Fractions on Oxidative Stress Markers, Liver Function Parameters and Liver Histology of *Plasmodium berghei* -Infected Mice

Veronica James Enyiekere[1], Martin Osita Anagboso[2], Uduak Peter Ise[3], Grace Emmanuel Essien[1], Jude Efiom Okokon[1], Nwakaego Omonigho Ebong[4,*]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria
[2]Department of Microbiology, Madonna University Nigeria, Elele campus, Rivers State, Nigeria.
[3]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Bingham University, Karu, Nigeria
[4]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Madonna University Nigeria, Elele campus, Rivers State, Nigeria.

Corresponding author*
nwakaebong@gmail.com

Manuscript received: 06 June, 2024. Revision accepted: 08 August, 2024. Published: 01 October, 2024.

Abstract

Justicia insularis (Family-Acanthaceae) is used in Ibibio ethnomedicine to treat malaria. The leaf extract and fractions of *J. insularis* were investigated for antioxidative stress and hepatoprotective activities in *Plasmodium berghei*-infected mice. The leaf extract (100-300 mg/kg, p.o.) exerted significant ($p<0.05$) antimalarial activity against *P. berghei* infection in curative test with ethyl acetate fraction demonstrating the highest activity. The extract/fractions treatment caused significant ($p<0.05$) reductions in liver enzymes (ALT, AST and ALP), total and conjugated bilirubin of the treated infected mice and also decreased significantly ($p<0.05$) total protein and albumin levels of the treated mice relative to control. The leaf extract and fractions further improved significantly ($p<0.05$) the levels of oxidative stress markers enzymes and molecules (CAT, GPx, GST, SOD) of the treated infected mice with no significant ($p>0.05$) effect on GSH. The MDA levels in the livers of the treated infected mice were significantly ($p<0.05$) reduced relative to control. Histology of liver sections revealed absence or significant reductions in pathological features in infected mice treated with leaf extract (100 mg/kg), DCM and ethyl acetate fractions compared to untreated infected mice. These results suggest that the leaf extract/fractions of *Justicia insularis* possess antioxidative stress and hepatoprotective potentials, which is an added advantage to its antimalarial property.

Keywords: antioxidative stress; hepatoprotective; *Justicia insularis*; malaria; *Plasmodium berghei*.

INTRODUCTION

Malaria remains one of the deadliest diseases globally despite being able to be prevented and treated. According to the World Health Organization (WHO), the majority of malaria cases reported in 2023 were from the WHO African region, with about 94% cases and 95% deaths and Nigeria is one of the four African countries responsible for more than half of all malaria deaths worldwide (WHO, 2024). Malaria is known to be caused by the genus Plasmodium, which consists of five significant species, including *Plasmodium falciparum, Plasmodium vivax, Plasmodium malaria, Plasmodium ovale,* and *Plasmodium knowlesi*. In humans, malaria transmission is usually via the bite of the female Anopheles mosquito infected with the parasites, while in rodents, malaria is primarily caused by *P. berghei* (Banyal *et al*., 2016). The growth of the parasite Plasmodium majorly occurs in the host cell such as the liver cells and red blood cells, resulting in anaemia (Intan, 2017). Infection caused by *P. berghei* can cause damage to major organs such as the lungs, liver, spleen, and brain (Prasiwi *et al*., 2018). The liver, the target organ of Plasmodium, plays a crucial role in its life cycle and is also the site of parasitic activity and immune response (Vanderberg & Undra, 2004). The liver also serves as a site for detoxification of toxins and drugs. Thus, it is crucial to protect the liver from the damaging effects of infectious agents and toxic chemicals (Wahyuningsih *et al*., 2002).

Liver injury associated with malaria, which is one of the causes of death in severe malaria, contributes to 50% mortality in hospitalized patients (Whitten *et al*., 2011). The mechanism of liver injury during malaria infection is uncertain. However, it has been proposed to involve the inflammatory response, adhesion of infected red blood cells (iRBC) and oxidative stress induced liver toxicity (Viriyavejakul *et al*., 2014). Inflammatory cytokines associated with the pathogenesis of severe malaria have also been reported (Mbengue *et al*., 2015). During the asexual phase of the malaria parasite, there is also an increase in the consumption of haemoglobin by the

parasite and induction of oxidative stress due to oxidation of lipoprotein and liver damage (Rifkind *et al*., 2015).

Malaria is managed solely with use of chemically synthesized drugs. However, antimalarial resistance has led to research on more effective novel antimalarial medicines. Research on medicinal plants that are used locally by various tribes to treat malaria would be beneficial. Thus, extracts from plant materials may have a protective effect on liver injury during malaria infection. At this point, the extracts of medicinal plants are suitable targets for study.

Among such plants is *Justicia insularis* T. Anderson (Acanthaceae family), grown in home gardens in west and central African, especially in Guinea, Sierra Leone, Ghana, Togo, Benin, Nigeria, Cameroon and DR Congo (Burkill, 1985). In Southern part of Nigeria, the leaves are used in cooking soup and are called Isepe-akera by the Akwa Ibom community (Akuodor *et al*., 2020). The vegetable is used for both nutritional and medicinal purposes as a digestive, weaning agent and laxative (Telefo *et al*., 2004; Ajibesin *et al*., 2008; Telefo *et al*., 2011; Adeyemi & Babatunde, 2014) as well as a local malaria remedy in Nigeria and across Africa. Aqueous extracts of *J. insularis* leaves have been shown to produce estradiol in vitro (Telefo *et al*., 2004), promote ovarian folliculogenesis and fertility in female rats (Telefo *et al*., 2011), possess anti-oxidant activity (Adeyemi & Babatunde, 2014), and to benefit the treatment of anaemia (Wood *et al*., 2020). Phytochemical compounds such as saponins, alkaloids, tannins, flavonoids, anthraquinones, cardiac glycosides (Telefo *et al*., 2004; Oyomah *et al*., 2019) and clerodane diterpenoids; 16(α/β)-hydroxy-cleroda-3,13 (14)Z-dien-15,16-olide and 2, 16-oxo-cleroda-3,13(14)E-dien-15-oic acid have been isolated and characterised from the leaf extract (Fadayomi *et al*., 2021). We report the leaf extract's antioxidative stress and hepatoprotective potentials and fractions of *Justicia insularis* in *Plasmodium berghei*-infected mice.

MATERIALS AND METHODS

Collection and identification of plant material

Justicia insularis leaves were collected from the Medicinal Plants Farm of the University of Uyo, Uyo, Akwa Ibom State, Nigeria. A taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria identificatied and authenticatied the plant. the plant's voucher specimen (FPH 83b) was conveyed to the herbarium of the Department of Pharmacognosy and Natural Medicine, University of Uyo.

Extraction

The leaves were washed and shadeed, and after two weeks, they were sliced into smaller pieces and pulverized to powder. The powdered leaves were divided into two pieces. One of them was macerated in ethanol for 72 hours, while the other was successively and gradient macerated for 72 hours in each of n-hexane, dichloromethane, ethyl acetate and methanol respectively, which is along their polarity to give the corresponding gradient extract for each solvent. The liquid filtrate of each extract and fraction was concentrated and evaporated to dryness in *vacuo* 40^0C using a rotary evaporator. The different yields were determined, and the extract and fractions were stored in a refrigerator at -4^{o}C until they used for the proposed experiments.

Microorganisms (parasites)

The National Institute of Medical Research (NIMER), Yaba Lagos, Nigeria supplied Chloroquine-sensitive strains of *Plasmodium berghei* ANKA which were maintained by sub-passaging blood from infected mouse to a 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 x 10^7*P. berghei* parasitized erythrocytes were collected from infected mice with 20-30% parasitaemia. The inoculum consisted of 5 x 10^7 *P. berghei* infected erythrocytes per millilitre 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 (Odetola & Basir, 1980; Atanu *et al*., 2021). Standard methods monitored parasitemia; thin blood smears were made on glass slides, fixed using methanol, and stained using Giemsa stain, and 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 ×100 magnification according to the formula of Peters and Robinson (1992) as given below:

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

Experimental animals

Swiss albino mice (18-25 g), male and female, 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 acclimatise for a period of 10 days before the experiments. The mice were fed on a standard pelleted diet and water *ad libtum* according to the National Institute of Health Guide conducted for the care and use of animals 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 and chloroquine used in the study were administered orally through a stainless metallic feeding cannula.

Determination of median lethal dose (LD_{50})
The determination of median lethal dose (LD_{50}) of the extract was carried out in mice using oral (p.o) route by modified method of Lorke (1983). The animals in groups of three mice each were administered different doses of the extract (100– 5000 mg/kg). They were observed for manifestation of physical signs of toxicity such as writhing, decreased motor activity, decreased body/ limb tone, and decreased mobility and death. The mortality rate in each group within 24 hours was recorded. The LD_{50} value was calculated as geometrical means of the maximum dose producing 0% (a) and the minimum dose producing 100% mortality (b). $LD_{50} = \sqrt{ab}$

Evaluation of the *in vivo* antimalarial activities of leaf extract and fractions of *Justicia insularis* on established infection
This study used the curative test method described by Okokon *et al*. (2017) to determine 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_0). The mice were divided into nine groups of ten mice per group after 72 hours (D_2). The extract, at doses of 150, 300, and 450 mg/kg were respectively administered to groups 1-3 mice, while 300 mg/kg each of *n*-hexane, ethyl acetate, dichloromethane, and *n*-butanol 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 were administered once daily for 5 (five) days. Giemsa stained thin smears were prepared from tail blood samples collected on each day of treatment to monitor the parasitemia level. The mice's mean survival time (MST) in each group was determined over 29 days (D_0-D_{28}). Five mice were sacrificed from each group under diethyl ether vapour on the sixth day. Blood samples were collected into plain centrifuge tubes and centrifuged immediately at 2500 rpm for 15 mins to separate the serum at room temperature. These blood samples were stored at -20^{o}C until used for biochemical determinations. The Liver from each mouse was surgically removed, weighed and divided into two parts. One part was fixed in 10% formaldehyde for the histological process and the other part stored in ice-cold normal saline for antioxidative stress markers. 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 liver function parameters of *P. berghei* infected mice
Liver function markers like total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total cholesterol, total protein, albumin conjugated, and total bilirubin were determined in the stored sera samples collected from the sacrificed mice spectrophotometrically utilising Randox analytical kits following standard procedures of manufacturer's protocols (Tietz, 1990).

Effect of the leaf extract and fractions on liver antioxidative stress markers of *P. berghei* infected mice
The removed livers were kept 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. The supernatants were used for the assays of superoxide dismutase (SOD) (Marklund & Marklund, 1974), catalase (CAT) (Sinha,1972), glutathione peroxidase (GPx) (Lawrence & Burk,1976), and reduced glutathione (GSH) (Ellman,1959), MDA (Esterbauer & Cheeseman,1990).

Effect of the leaf extract and fractions on liver histology of *P. berghei* infected mice
The liver pieces from mice fixed in buffered formalin were prepared and stained with haematoxylin and eosin (H&E) for liver study following the standard procedures at the Department of Chemical Pathology, University of Uyo Teaching Hospital, Uyo. Changes in morphology from the excised organs of the sacrificed mice were observed and reported. Histologic pictures were taken as micrographs.

Gas Chromatography-Mass Spectrometry Analysis
Gas chromatography-mass spectrometry (GC-MS) data of the active fractions (ethyl acetate) were reported on an Agilent 7890A gas chromatograph linked with an Agilent MS model 5975C MSD detector (Agilent Technologies, USA). An HP5-MS column 5% phenyl-methylpolysiloxane, 30m × 0.25mm × 0.25µm was employed with a helium gas flow under a pressure of 10 psi. The injector temperature was set at 280°C. The oven temperature started at 150°C for 3 minutes increased to 300°C at 10°C/min, and was held for 5 minutes at 300°C. The mass spectrometer was operated using the electron ionization mode at 70eV (Aldulaimi *et al*., 2017). The phytochemicals were established by comparing the spectra using the NIST 2011 database.

Statistical analysis
Data was 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 presented as mean ± standard error

of the mean (SEM) and significance relative to the control was considered at $p < 0.05$.

RESULTS AND DISCUSSION

Determination of Median lethal dose (LD_{50})

Administration of leaf extract of *J. insularis* (100 - 5000 mg/kg) orally did not cause any mortality in the animal groups administered (Table 1). Moreover, no physical toxic signs of the extract were observed. The median lethal dose (LD_{50}) of leaf extract of *J. insularis* was therefore estimated to be =5000 mg/kg.

Table 1. Determination of oral LD_{50} (Lorke, 1983).

DOSE (mg/kg)	MORTALITY
5000	0/3
4000	0/3
3000	0/3
1000	0/3
100	0/3
10	0/3

LD50= 5000 mg/kg

Antiplasmodial effect of ethanol leaf extract and fractions of *J. insularis* on established infection

There were 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; Figure 1). The ethyl acetate fraction had the highest activity with a chemosuppressive effect of 73.15 %, this was lower compared with that of the standard, chloroquine, 81.58 %. The leaf extract and fractions demonstrated significant (p<0.05-0.001) protective potentials in the mice as was seen in the mean survival time of the animals. The groups treated with ethyl acetate fraction had a longer mean survival time, 22.66 ± 0.80 d, followed by those of dichloromethane fraction treated mice 15.0 ± 0.57 d. These were less than the standard chloroquine drug (29.83 ± 0.16 d; Figure 2).

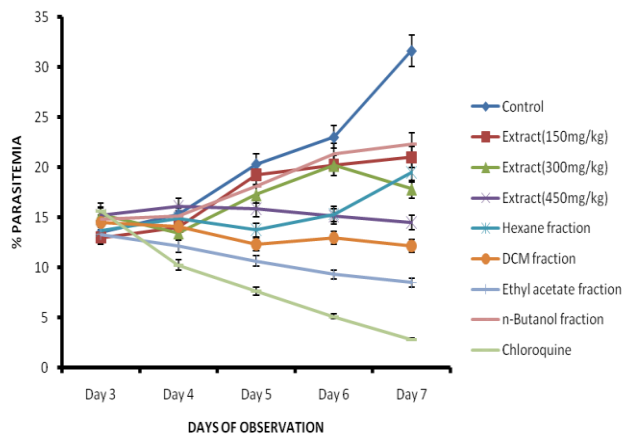

Figure 1. Effect of leaf extract and fractions of *Justicia insularis* on established *Plasmodium berghei* infection in mice.

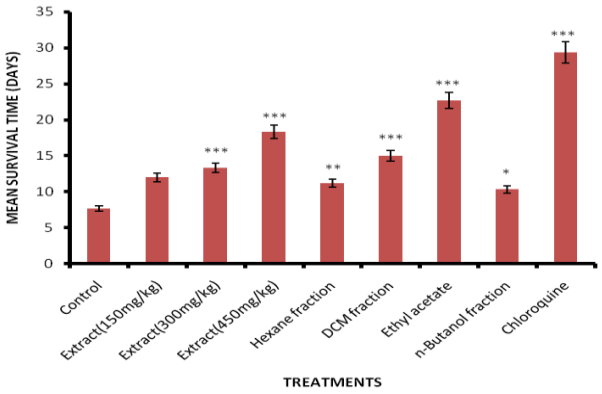

Figure 2. Effect of leaf extract and fractions of *Justicia insularis* on mean survival time (MST) of *Plasmodium berghei*-infected mice.

Values are expressed as mean ± SEM. Significant relative to control at $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$. n = 10.

Effect of leaf extract and fractions on liver function parameters of *Plasmodium berghei*-infected mice.

The liver function indices (AST, ALT, ALP, total cholesterol, total protein, albumin, total and conjugated bilirubin) were elevated in untreated *P. berghei* -infected mice. However, treatment of *P. berghei*-infected mice with leaf extract and fractions of *Justicia insularis* caused non dose-dependent and significant (p<0.05-0.001) reductions in the levels of AST, ALP, total cholesterol, and conjugated bilirubin with ethyl acetate fraction followed by DCM fraction treated groups having the most significant (p<0.05-0.001) reduction when compared to control. Similarly, dose-dependent and significant (p<0.05-0.001) reductions in ALT and total bilirubin levels were recorded in the extract treated groups, with ethyl acetate fraction exerting the highest effect. Some of the effects were better than those of the chloroquine-treated group (Table 2). The total protein and albumin levels of the treated infected mice were similarly reduced non-dose-dependently and significantly by the extract/fractions treatments with ethyl acetate fraction and DCM fraction-treated groups having the highest significant effect (p<0.001) (Table 2).

Effect of leaf extract and fraction on liver oxidative stress markers of *Plasmodium berghei*-infected mice.

The non-enzymatic and enzymatic endogenous antioxidants (GSH, SOD, CAT, GPx, and GST) were found to be reduced in the untreated *Plasmodium berghei*-infected mice. Treatment of *Plasmodium berghei*-infected mice with leaf extract and fractions *of Justicia insularis* caused non dose-dependent increases and non-significant (p>0.05) increases in the levels of GSH and GPx compared to control. The extract/fractions treatment further increased SOD level, which was only significant in the group treated with the low dose of the extract (150 mg/kg) when compared to the control. CAT levels of the treated infected mice were similarly

increased. However, these increases were only significant (0.05-0.001) in the groups treated with 150 and 300 mg/kg of the extract, DCM and *n*-butanol fractions, as well as chloroquine when compared to control. GST levels of the treated infected mice were increased also but the increases were significant (0.05-0.001) when compared to control in groups treated with extract (300 mg/kg) and all the fractions with *n*-butanol fraction treated group having the highest effect. The MDA level which was elevated in the untreated infected mice was decreased by extract/fractions treatment and the decrease was significant when compared to control (Table 3).

Table 2. Effect of leaf extract and fractions of *Justicia insularis* 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 Cholesterol	Total Protein (g/L)	Albumin (g/L)	Total Bilirubin (µmol/mL)	Conjugated bilirubin (µmol/mL)
Control	-	32.33±2.40	25.0±1.15	38.0±0.57	3.50±0.23	73.33±0.88	45.0 ±1.15	6.06±0.23	4.73±0.43
Extract	150	21.00±2.08^{b}	16.66±3.18^{b}	28.0±0.57^{c}	3.50±0.05	66.33±0.88	41.0±1.45	4.06±0.23^{b}	3.83±0.21
	300	20.66±1.76^{b}	17.00±0.57^{b}	21.0±0.57^{c}	2.80±0.17	61.33±2.88^{c}	42.60±1.45	4.53±0.20	2.56±0.14^{c}
	450	20.00±1.73^{b}	14.33±0.88^{c}	22.0±1.15^{c}	2.70±0.05	60.66±2.33^{c}	40.33±1.85	4.36±0.17^{a}	2.46±0.20^{c}
n-hexane	300	21.66±3.18^{b}	17.00±1.73^{b}	29.0±0.57^{c}	2.86±0.08	61.00±0.57^{c}	42.66 ±1.45	5.10±0.60	3.88±0.49
Dichloromethane	300	22.00±2.88^{b}	18.00±1.73^{a}	21.0±0.57^{c}	3.36±0.14	59.66±1.45^{c}	37.0±1.15^{b}	4.40±0.55^{a}	3.13±0.42^{b}
Ethyl acetate	300	20.00±0.57^{b}	15.33±0.88^{c}	19.0±0.57^{c}	2.56±0.12^{a}	64.00±0.57^{c}	34.66±1.20^{c}	4.50±0.17^{a}	2.70±0.11^{c}
n-butanol	300	25.66±0.33^{b}	17.64.0±0.57^{b}	31.0±0.57^{c}	2.70±0.11	65.66±1.20^{a}	42.0 ±0.57	5.16±0.16	4.00±0.11
Chloroquine	5	21.66±2.02^{b}	18.02±2.51^{a}	19.33±0.66^{c}	2.30±0.36^{b}	62.00±1.73^{c}	40.66±0.88	4.06±0.34^{b}	3.16±0.20^{a}

Values are expressed as mean ± SEM. Significant relative to control. $^{a}p<0.05$; $^{b}p<0.01$; $^{c}p<0.001$. n = 10.

Table 3. Effect of leaf extract and fractions of *Justicia insularis* on liver oxidative stress markers of mice infected with *Plasmodium berghei.*

Treatment	Dose (mg/kg)	Antioxidant Parameters						
		GSH (µg/mL)	SOD (µg/mL)	CAT (µg/mL)	GPX (µm/mL)	GST (µg/mL)	MDA (µmol/mL)	Liver weight (g)
Control	-	1.10±0.20	0.19±0.01	3.51±0.05	0.048±0.008	0.033 ±0.05	0.55±0.02	2.31±0.16
Extract	150	1.18±0.11	0.34±0.02^{a}	4.26±0.21^{a}	0.053±0.005	0.029±0.03	0.38±0.02^{c}	2.16±0.01
	300	1.29±0.18	0.25±0.04	2.67±0.20	0.057±0.008	0.18±0.01^{a}	0.42±0.03^{a}	2.24±0.20
	450	1.44±0.22	0.20±0.02	4.59±0.20^{c}	0.064±0.009	0.29±0.02^{c}	0.46±0.02	2.32±0.16
n-hexane	300	1.45±0.19	0.17±0.01	2.89±0.32	0.064±0.008	0.19 ±0.02^{a}	0.55±0.03	2.14±0.18
Dichloromethane	300	1.38±0.25	0.23±0.08	5.08±0.40^{c}	0.066±0.013	0.34±0.02^{c}	0.45±0.06	2.14±0.05
Ethyl acetate	300	1.34±0.24	0.21± 0.04	3.65± 0.32	0.062±0.012	0.24 ±0.02^{b}	0.42±0.06^{a}	2.29±0.13
n-butanol	300	1.29±0.21	0.25± 0.04	5.67± 0.21^{c}	0.059±0.010	0.41 ±0.03^{c}	0.49±0.04	2.21±0.22
Chloroquine	5	0.93 ±0.25	0.21±0.02	4.96±0.75^{c}	0.042±0.011	0.33±0.05^{c}	0.44±0.02^{a}	2.28±0.12

Values are expressed as mean ± SEM. Significant relative to control. $^{a}p<0.05$; $^{b}p<0.01$; $^{c}p<0.001$. n = 10.

Effect of Extract and Fractions on the Histology of liver of *Plasmodium berghei*-infected mice.

Histologic sections of untreated infected mice livers showed distorted liver with congested central vein, hepatocytes, sinosoids containing inflammatory cells, necrotic tissues. A similar pattern of distortion of liver architecture was observed in groups of infected mice treated with 300 and 450 mg/kg of the extract, *n*-hexane and *n*-butanol fractions. However, *P. berghei* infected mice groups treated with 150 mg/kg of the extract, DCM fraction, ethyl acetate fraction and chloroquine showed regular liver section with intact hepatocytes, patent central vein and sinusoids containing Kupffer cells without any pathological signs. (Figure 3).

GCMS analysis of ethyl acetate fraction

The results of GCMS analysis of ethyl acetate fractions show that the fraction contains various pharmacologically active compounds such as hexanoic acid, pentanoic acid, 3-methyl-, hexanoic acid, 1,1-dimethylethyl ester, hexadec-9-enoic acid, 7-tert-butyldimethylsilyloxy-, methyl ester, heneicosanoic acid, methyl ester, octa-2,4,6-triene, 1,3,6-heptatriene, 5-methyl-, (E)-, phytol, acetate, octadecanoic acid, 2-hydroxy-1,3-propanediyl ester, octadecanoic acid, docosyl ester and others (Table 4).

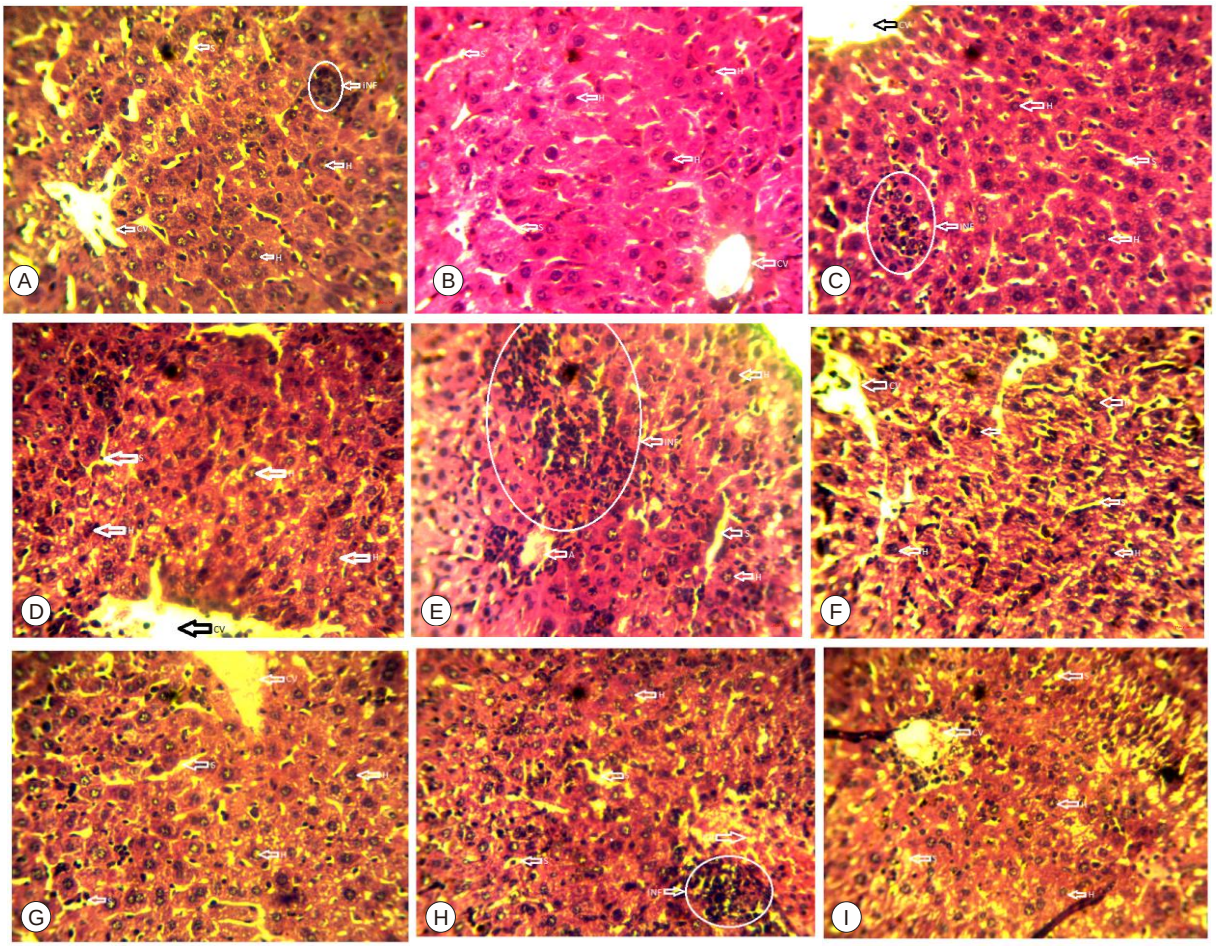

Figure 3. Histologic Liver sections of Plasmodium berghei-infected mice untreated with normal saline (A), leaf extract of *Justicia insularis*, 150 mg/kg (B), 300 mg/kg (C),450 mg/kg (D), n-hexane fraction(E), DCM fraction (F), ethyl acetate fraction (G), n-butanol fraction (H) and chloroquine, 5 mg/kg (I) at magnification X400. Keys: Congested Central vein (CV), Cords of normal Hepatocytes (H), Sinusoids (S) containing: Capillaries (C) and Kupffer cells (KC), Central vein (CV), Inflammatory cells (INF).

Discussion

The people of Ibibio traditionally use the leaves of *J. insularis* as a malaria remedy. This study was designed to evaluate the effect of the leaf extract and fractions on parasitemia, liver function parameters, liver oxidative stress markers and liver histology of *P. berghei* infected mice.

In this study, the extract and fractions significantly reduced the parasitemia in a dose-dependent pattern with the ethyl acetate fraction showing the highest schizonticidal activity, indicating this extract's has antimalarial potential. The phytochemical constituents of the extracts and fractions may be responsible for this effect, thus validating the use of the leaf extract decoctions locally in treating malaria.

Phytochemicals like secondary metabolites and other chemical compounds of plants contribute to the antimalarial properties of plants. Ethyl acetate, the most active fraction of the GCMS analysis shows the presence of hexanoic acid, pentanoic acid, 3-methyl-, hexanoic acid, 1,1-dimethylethyl ester, hexadec-9-enoic acid, 7-tert-butyldimethylsilyloxy-, methyl ester, heneicosanoic acid, methyl ester, octa-2,4,6-triene, 1,3,6-heptatriene, 5-methyl-, (E)-, phytol, acetate, octadecanoic acid, 2-hydroxy-1,3-propanediyl ester, octadecanoic acid, docosyl ester and others; which are known antimalarial compounds. Furthermore, chemical constituents of the leaf extract like alkaloids, saponins, tannins, anthraquinones, flavonoids and cardiac glycosides are under study (Telefo *et al*., 2004; Oyomah *et al*., 2019). So far, isolation and characterization of Clerodane diterpenoids; 2, 16-oxo-cleroda-3,13(14)E-dien-15-oic acid and 16(α/β)-hydroxy-cleroda-3,13 (14)Z-dien-15,16-olide from the leaf extract have been done (Fadayomi *et al*., 2021). This implies that these compounds are accountable for the notable activities of the extract and fractions particularly the terpenoids and polyunsaturated fatty acids (PUFAs) 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 which have been responsible for antiplasmodial activities of plants (Kirby *et al*., 1989; Philipson & Wright 1991; Kumaratilake *et al*., 1992; Krugliak *et al*., 1995;

Christensen & Kharazmi, 2001; Hakzakis *et al*., 2007, Suksamrarn *et al*., 2005; Attioua *et al*., 2007; Melariri *et al*., 2011, 2012).

The hepatoprotective potentials were examined by evaluating the effect of extract and fractions on the liver function indices of the *P. berghei*-infected mice. Increased levels of transaminases and hyperbilirubinemia were detected in the untreated infected mice, suggesting liver injuries, usually associated with malaria infection which are likely due to hepatic blood flow obstruction and blockade of sinusoids by parasitized erythrocytes. Also, liver cell destruction and membrane integrity by free radicals following malaria infection, coupled with reticuloendothelial blockage and alterations in the hepatocyte microvilli could compromise the secretory capacity in the liver thus resulting in hyperbilirubinemia (Onyesom & Onyemakonor, 2011). After administration of leaf extract and fractions of *J. insularis* to *P. berghei*-infected mice, the elevated total protein, albumin, AST, ALT, ALP, total and conjugated bilirubin levels were reduced. These impacts suggest that the hepatoprotective properties of the leaf extract and fractions may be due to the antioxidant properties of the phytoconstituents. However, the histological examination of liver sections of untreated *P. berghei*-infected mice revealed severe pathologic signs like a distorted liver with congested central vein, hepatocytes with sinusoids containing inflammatory cells and necrotic tissues which are indicate inflammatory reaction in the tissue. These pathological effects significantly decreased mainly in *P. berghei* infected mice groups treated with 150 mg/kg of the extract, DCM fraction, ethyl acetate fraction and chloroquine exhibiting regular liver section with intact hepatocytes, patent central vein and sinusoids containing Kupffer cells without any pathological signs. Hence, the hepatoprotective activity of the leaf extract and fractions is probably due to the antioxidant properties of its phytochemical constituents as earlier reported (Adeyemi & Babatunde, 2014).

Oxidative stress is crusial in malaria complications such as anemia, jaundice and pre-eclampsia (Fabbri *et al*., 2013; Sarr *et al*., 2017). Hypoxic conditions due to malaria infection produce large amounts of free radicals triggering body immune responses (Becker *et al*., 2004; Percario *et al*., 2012), leading to the development of systemic complications associated with malaria (Guha *et al*., 2006; Ojezele *et al*., 2017). The Malarial infection has been observed to decrease enzymatic and other nonenzymatic endogenous anti-oxidants levels like catalase (CAT), glutathione (GSH) peroxidase, superoxide dismutase (SOD), albumin, ascorbate and plasma tocopherol. Increased lipid peroxidation and malondialdehyde levels have been associated with the severity of malaria (Asagba *et al*., 2010; Raza *et al*., 2013), hence they are used as biomarkers in determining the severity of malaria infection. In this study, the activities of SOD, CAT, GPx and GST levels which were found to reduce significantly in the untreated infected mice were elevated by the extract /fractions treatment, while GSH was not significantly affected by the treatment when compared to the untreated infected group and MDA level was significantly decreased especially in the groups treated with the extract (300 mg/kg), ethyl acetate fraction and chloroquine. The plant extract and fractions exerted antioxidative stress potentials by increasing the levels of some antioxidative stress markers. This activity can be explained as a results of the antioxidant activities of the phytochemical constituents as earlier reported (Adeyemi & Babatunde, 2014).

CONCLUSIONS

This study shows that the leaf extract and fractions of *Justicia insularis* possess antimalarial, antioxidative stress and liver protective potentials which may be due to the activities of its phytochemical constituents.

***Acknowledgements*:** The authors are grateful to Mr. Nsikan Malachy and other staff Animal House of Pharmacology and Toxicology Department, University of Uyo for providing technical assistance.

***Authors' Contributions*:** VJE, JEA and UPI conceived and designed this study. JEA, VJE and GEE carried out the experiments and drafted the manuscript. NOE and MOA performed the statistical analysis, edited and reviewed the manuscript, NOE, MOA and GEE read and approved the final manuscript.

***Competing Interests*:** The authors have not declared any conflict of interest.

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