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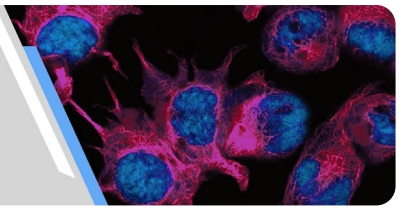

Pharmacological Efficacy of Ethanol Leaf Extract of *Justicia secunda* in Swiss Albino Mice Experimentally Infected with *Plasmodium berghei*

Nasiru Ademola ADEYEMI[1], Sonnie Joshua ONIYE[2], Peter Ibrahim REKWOT[4], Abdullateef YUSUF[3], Yunusa Adamu WADA[1], Muhammad HUSSAINI[3]

Abstract

Plasmodium parasite that causes malaria has developed resistance to most antimalarial medications and this has prompted research on natural products. The plant, *Justicia secunda*, is domesticated in the tropical region of Africa, and is used for the treatment of anaemia and other debilities. We evaluated the antiplasmodial and immunomodulatory activity of ethanol leaf extract of *J. secunda* in mice experimentally infected with *Plasmodium berghei*. Ethanol leaf extract of *Justicia secunda* contains steroids, tannins, flavonoids, terpenoids, phenols, glycosides and carbohydrate as well as thirty (30) compounds with numerous pharmacological activities. The LD_{50} was above 5,000mg/kg with no mortality. A dose-dependent percentage suppression of *P. berghei* was observed in the curative test for the three doses respectively (53.13%, 60.71%, 71.21%). The suppressive test for ethanol leaf extract of *J. secunda* also showed a similar trend (52.2%, 62.16%, 75.18%). The ethanol leaf extract of *J. secunda* effectively prevented anaemia, reduced the level of ALT enzyme and also increased the level of TNF-α and IL-10 in the plasma of *P. berghei*-infected mice. It also significantly reduced the oxidative stress and vascular congestion in the liver of mice infected with *P. berghei*. This study revealed that ethanol leaf extract of *J. secunda* could be utilized to treat infection caused by *Plasmodium* parasite and also ameliorate the pathogenesis of the disease.

Keywords: Antiplasmodial, Phytochemicals, Immunomodulatory, Histopathology.

Introduction

Malaria is more prevalent in African nations than it is worldwide. According to the World Malaria Report 2020; Nigeria (31.3%), the Democratic Republic of Congo (12.6%), the United Republic of Tanzania (4.1%) and Niger (3.9%) account for more than half of all malaria death [1]. Although, sociopolitical issues are connected to the causes and consequences of malaria disease [2]; explicitly, the major contributing factor to the prevalence of malaria is the lack of mosquito protection and appropriate malaria treatment [3].

Malaria is an infectious disease affecting humans and animals [4]. The etiologic agent of the disease is the parasitic protozoans of the genus *Plasmodium*, and the sole biological vector is the female *Anopheles* mosquitoes [5]. The asymptomatic human is an unwitting parasite reservoir, allowing continuous transmission [6]. The initial clinical symptom of this illness leads to headaches, fatigue, soreness in the joints and muscles, vomiting, and anorexia. Acidemia, hypoglycemia, anaemia, pulmonary oedema, coma and renal failure are common signs of acute malaria [7].

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Antimalarial drug resistance occurs in the malaria parasite due to ineffective chemoprophylaxis [8], fake and irregular pharmaceutical administration leading to genetic mutation [9]. Therefore, searching for a new anti-malarial compound is a continuous process, and numerous studies on African medicinal plants with anti-malarial potentials have been conducted over time. However, there is a dearth of practical information regarding the pharmacological efficacy of *J. secunda* as an antimalarial agent.

Justicia secunda is taxonomically classified in the family Acanthaceae and is notably utilized for treating anaemia in tropical regions of Africa [10]. It is commonly known as 'Christ blood' and 'Blood herb' in Nigeria. The herbaceous plant has green leaves and pink inflorescence (Figure 1) [11]. According to Irinmwinuwa and Afonne [12], the ethanol leaf extract of *J. secunda* contains saponin (9.2%), tannins (9.0), flavonoids (7.0%) and alkaloid (2.4%). It has also been established that *J. secunda* has hematinic effect in rats with anaemia induced by phenylhydrazine [13]. Likewise, it exhibited anti-inflammatory and antioxidant activity in an *in vitro* study using heat-induced bovine serum albumin (BSA) denaturation and erythrocyte membrane stabilization assay [14].

This study evaluates the phytochemical constituent and lethal dose of the ethanol leaf extract of *J. secunda,* as well as the effect of the extract on the parasitaemia level, hematological profile, pro-inflammatory (TNF-α) and anti-inflammatory (IL-10) cytokines, liver biochemicals, and histopathology of the liver in *Plasmodium berghei* NK65-infected mice.

Figure 1: Aerial part of *Justicia secunda*

Material and Methods

Collection of Plant Sample

Matured leaves were harvested from stands of *J. secunda* plants from three different sites in Lusada, Ado Odo Ota Local Government Area (LGA) Ogun State, Nigeria.

Preparation of Plant Extract

Cold maceration technique was adopted for the extraction by simply soaking five hundred grams (500g) of the powdered leaves in five (5) litres of eighty percent (80%) ethanol for three days, and the bottle containing the mixtures was agitated at intervals. The liquid extract was filtered into a clean container using a piece of chiffon material after 72 hours. The filtrate was poured into an evaporating dish and placed on a water bath to obtain a solid and more concentrated form of the extract [15].

Evaluation of Phytochemical Constituent of Plant Extract

A standard method [16] was adopted in the phytochemical screening of the extract. The Gas Chromatography-Mass Spectrometry (GC-MS) analysis was conducted using a 7890B GC System (Agilent Technologies, USA) coupled with a 5977A Mass Selective Detector (Agilent Technologies, USA). The leaf extract of *J. secunda* was diluted in methanol, and 2µL of the mixture was injected into the GC-MS machine using a micro-syringe. The experimental parameters for the GC-MS system were: initial oven temperature: 70°C, Equilibration Time: 1 min, Max Temperature: 325 ℃, Slow Fan: Disabled, Oven Program: 5°C/min On 250°C for 1 min, #1 then 30°C/min to 300°C for 0 min, Post Run: 50 °C. Cryo: Off, Front SS Inlet He – Mode: Split. Heater: On 250°C, Pressure: On 11.089psi, Total Flow on 19.204mL/min, Septum Purge Flow: On 3 mL/min, Gas Saver: off, Purge flow to Split vent: on 15mL/min at 0.75min. Holdup time: 1.2386min, Flowrate: 1.0mL/minute.

Maintenance of Experimental Animals

Seventy-five (75) male Swiss albino mice with body weight between 20 and 30g were obtained for this study. Chicken grower's marsh and water was provided at all time for the experimental mice [17]. The overall maintenance of the experimental animals was in accordance with the procedures approved by the ethical committee of the institution where the experiment was conducted.

Determination of Lethal Dose of Plant Extract

The median lethal dose of the ethanol leaf extract of *J. secunda* was determined per the Up and Down method described by the Organization for Economic Co-orporation and Development [18].

Evaluation of Antiplasmodial Activity of Plant Extract

The infected experimental mice were inoculated

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intraperitoneally with 0.2mL of blood from donor mouse infected with *P. berghei* NK65 (30% parasitaemia) and normal saline (1:9) containing 10^5 *P. berghei* parasitized erythrocytes. The seven-day curative test was evaluated seventy-two (72) hours after inoculation and confirmation of parasitemia in thirty (30) mice, and they were randomly assigned into six (6) groups. The suppressive test was conducted, and the inception was precisely three (3) hours after the infection of mice for a four-day duration using thirty (30) mice. These tests were performed using the method of Onyegeme-Okerentaa *et al*. [19], with a slight modification. Groups 1–3 were the control groups treated with 1 mL of normal saline (the negative control), 25 mg/kg of chloroquine (the positive control), and the uninfected and untreated groups (the normal control), respectively. Groups 4-6 were given 500 mg/kg, 1000 mg/kg, and 1,500 mg/kg of the crude extract based on the LD_{50} for the ethanol leaf extract of *J. secunda* determined in this study. The oral treatment was done once a day.

Collection of Blood and Liver Samples

The animals were sacrificed after inhaling vapour from ketamine-damped cotton wool at the end of the experiment. The blood of experimental animals was collected in EDTA bottles for haematological and biochemical analysis through cardiac aspiration. A portion of the liver was placed in cold phosphate buffer for antioxidant and MDA analysis. A small portion of liver of mice was preserved in phosphate-buffered formalin.

Determination of Parasitaemia and Percentage Chemosuppression

The number of parasitized erythrocytes in ten (10) slides prepared with thin blood smears was counted, and the average was computed to give the parasitemia of each mouse [20].

Percentage parasitaemia and chemosuppression was calculated as:

Parasitaemia (%) = (Number of parasitized RBC/Total number of RBC) x 100

$$\% \text{ Chemosuppression} = \left\{ \frac{A - B}{} \right\} \times 100$$

A= mean % parasitaemia in negative control

B= mean % parasitaemia in treated group

Assessment of Haematological Parameters

The Red Blood Cell (RBC), White Blood Cell (WBC), Packed Cell Volume (PCV) and Haemoglobin (HGB) concentration were analyzed using a Biobase Bk6100 haematology analyzer [21] in blood samples collected in Ethylene Diamine Tetracetic Acid (EDTA) bottles.

Assessment of Plasma and Liver Biochemicals

The liver homogenate was used to evaluate the levels of Malondialdehyde (MDA), Superoxide dismutase (SOD), Catalase (CAT) and reduced Glutathione (GSH) using a Sigma Aldrich Assay kit. Blood in EDTA bottles was used to check the concentrations of Alanine aminotransferase (Randox kit), Interleukin-10 and Tumour Necrosis Factor-α using ELISA kits by FineTest.

Liver Histopathological Studies

The liver of mice preserved in phosphate-buffered formalin was prepared for histological study using standard histotechnique and viewed with a microscope at 400 × magnification. The photomicrographs were taken using a digital camera [22].

Statistical Analyses

One-way Analysis of Variance (ANOVA) was used to determine statistical differences in the antiplasmodial activity, haematological and biochemical parameters in the *in vivo* suppressive and curative test. Tukey HSD test was used to rank the difference between significant parameters at $p<0.05$. Data were represented in tables and summarized in form of mean ± standard error. Statistical analyses were done using 'R' statistical package (version 4.1.2) for windows.

Results

Phytochemical Characterization and Acute Toxicity

The phytochemical screening detected eight different classes of phytochemicals (Table 1), and the GC-MS technique confirmed the presence of thirty different compounds, as shown in Table 2. Treatment of mice with limit dose of ethanol leaf extract of *J. secunda* (5000mg/kg) caused no mortality and signs of overtoxication. This suggests that the LD_{50} of the extract is greater than 5000mg/kg.

Table 1. Phytochemical screening of ethanol leaf extract of *Justicia secunda*

S/No	Phytoconstituent	Inference
1.	Alkaloids	+
2.	Cardiac glycosides	+
3.	Saponin	-
4.	Phenolic compounds	+
5.	Tannins	+
6.	Steroids	+
7.	Carbohydrates	+
8.	Flavonoids	+
9.	Terpenoids	+
10	Anthraquinones	-

Keys: (+) = Present, (-) = Absent

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Table 2: GC-MS profile of ethanol leaf extract of *Justicia secunda*

Peak	Retention Time (Minute)	Library I.D	Peak area (%)	Chemical Abstract Service Number
1	17.4652	17-Pentatriacontene	0.1691	006971-40-0
2	17.64	Oxalic acid, cyclobutyl heptadecyl ester	0.2012	1000309-70-7
3	20.0166	Hexadecanoic acid, methyl ester	2.6316	000112-39-0
4	21.4568	3-Chloropropionic acid, heptadecyl ester	0.2485	1000283-05-1
5	23.0506	9,12-Octadecadienoic acid, (linoleic) methyl ester, (E,E)-	1.1048	002566-97-4
6	23.2025	cis-13-Octadecenoic acid, methyl ester	6.3996	1000333-58-3
7	23.7984	Methyl stearate	0.8041	000112-61-8
8	25.1498	Carbonic acid, but-3-en-1-yl hexadecyl ester	0.1945	1000383-23-7
9	25.279	Aspidospermidin-17-ol, 1-acetyl-19,21-epoxy-15,16-dimethoxy-	0.2787	002122-26-1
10	28.5241	8-Hexadecenal, 14-methyl-, (Z)-	1.1543	060609-53-2
11	26.5546	Cyclododecane	1.5526	000294-62-2
12	26.7618	9,12-Octadecadienal	0.6438	026537-70-2
13	26.8969	p-Menth-8(10)-en-9-ol, cis-	0.1951	015714-13-3
14	26.9881	Oxirane, [(dodecyloxy)methyl]-	0.7356	002461-18-9
15	27.3173	Oleic Acid	2.1944	000112-80-1
16	27.7978	22-Stigmasten-3-one	3.3243	004736-95-2
17	28.2861	(S)(+)-Z-13-Methyl-11-pentadecen-1-ol acetate	3.9133	1000130-84-8
18	28.5241	8-Hexadecenal, 14-methyl-, (Z)-	1.2379	060609-53-2
19	28.9265	3-Cyclohexylthiolane,S,S-dioxide	1.317	071053-08-2
20	28.9874	cis-7, cis-11-Hexadecadien-1-yl acetate	1.3175	052207-99-5
21	29.3867	cis-9-Hexadecenal	0.7726	056219-04-6
22	29.8609	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	3.1331	000111-03-5
23	30.1094	Z-8-Methyl-9-tetradecenoic acid	3.1701	1000130-84-5
24	30.2394	1-Nonadecene	1.2528	018435-45-5
25	30.394	Cyclododecanol, 1-ethenyl-	5.5624	006244-49-1
26	30.9115	Cyclododecane, ethyl-	3.3424	028981-49-9
27	31.2385	2-Methyl-Z,Z-3,13-octadecadienol	3.9236	1000130-90-5
28	31.5878	E-9-Tetradecenal	0.9144	1000131-35-7
29	32.575	β-Sitosterol	19.7234	000083-46-5
30	34.706	Squalene	4.1521	000111-02-4

Antiplasmodial Activity of the Crude Extract

The crude extract was tested for curative and suppressive antiplasmodial activity as presented in Table 3 and the result was expressed in the form of percentage parasitaemia and percentage chemosuppression for the infected controls and the treatment groups. The extract significantly (p<0.05) reduced parasitaemia level in infected mice for both curative and suppressive tests compared with the negative control. A dose-dependent chemosuppression was observed for the experimental groups, which was lower than the standard drug with 100% chemosuppression.

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Statistical analysis performed amongst Negative control (C-), Positive control (C+), Normal control (NC), 500mg/kg(T1), 1000mg/kg(T2) and 1,500mg/kg(T3) dose of ethanol leaf extract of *J. secunda*, means with different superscript within a column are statistically significant at $p<0.05$

Haematological Profile of Experimental Mice

The haematological profile of mice measured at the end of the experiment shows that the extract significantly ($p<0.05$) prevented the reduction in Packed Cell Volume (PCV), Red Blood Cell (RBC) and Haemoglobin (HGB) for both curative and suppressive test as shown in Figure 2. The group of mice that received chloroquine (4.26±0.189) had a statistically ($p<0.05$) reduced White Blood Cell (WBC) than and the negative control (5.76±0.356) and experimental groups treated for seven days (4.8±0.362, 4.72±0.252, 4.76±0.218).

Statistical analysis performed amongst negative control (C-), positive control (C+), normal control (NC), 500mg/kg(T1), 1000mg/kg(T2) and 1500mg/kg(T3) dose of ethanol leaf extract of *J. secunda* respectively, groups with different letters on bars are statistically significant at $p<0.05$, each point is the mean ± SEM (Standard error of mean) n=5.

Blood Plasma Biochemicals of Experimental Mice

The immunomodulatory and hepatoprotective potentials of the plant extract was assessed by analysing the biochemical components of plasma such as TNF-α, IL-10 and ALT. The outcome of this biochemical assay for the therapeutic and inhibitory test is presented in Figure 3. The infected mice treated with ethanol leaf extract of *J. secunda* had significantly higher ($p<0.05$) concentrations of TNF-α and IL-10 compared to negative control for both curative and suppressive test. Furthermore, the plasma concentration of ALT in negative control was significantly higher ($p<0.05$) than groups that received plant extract treatment.

Table 3. Antiplasmodial activity of ethanol leaf extract of *Justicia secunda* against *P. berghei* in mice

	Curative test		Suppressive test	
Experimental groups	**% Parasitaemia**	**% Chemo-suppression**	**% Parasitaemia**	**% Chemo-suppression**
C-	44.8 ± 1.61[a]	0.00	41.5 ± 2.40[a]	0.00
C+	0.00[c]	100	0.00[c]	100
NC	0.00	0.00	0.00	0.00
T1	21.0 ± 1.29[b]	53.13	19.8 ± 3.70[b]	52.2
T2	17.6 ± 0.959[b]	60.71	15.7 ± 1.46[b]	62.16
T3	13.9 ± 2.81[b]	71.21	10.3 ± 2.67[b]	75.18
p-value	0.00		0.00	

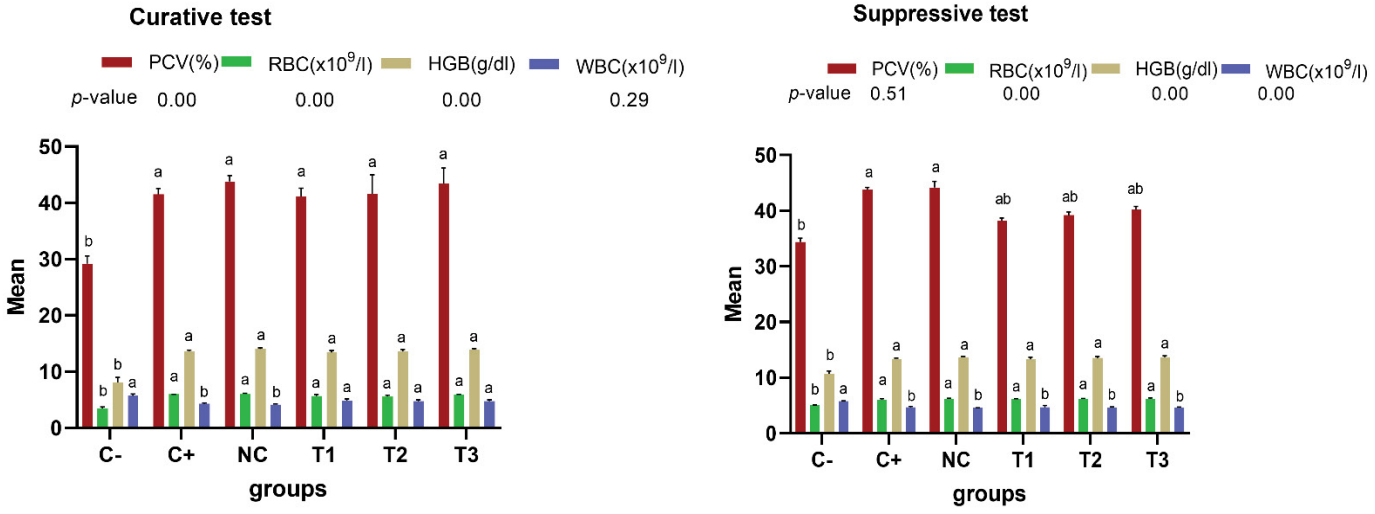

Figure 2: Haematological profile of experimental mice treated with ethanol leaf extract of *Justicia secunda*

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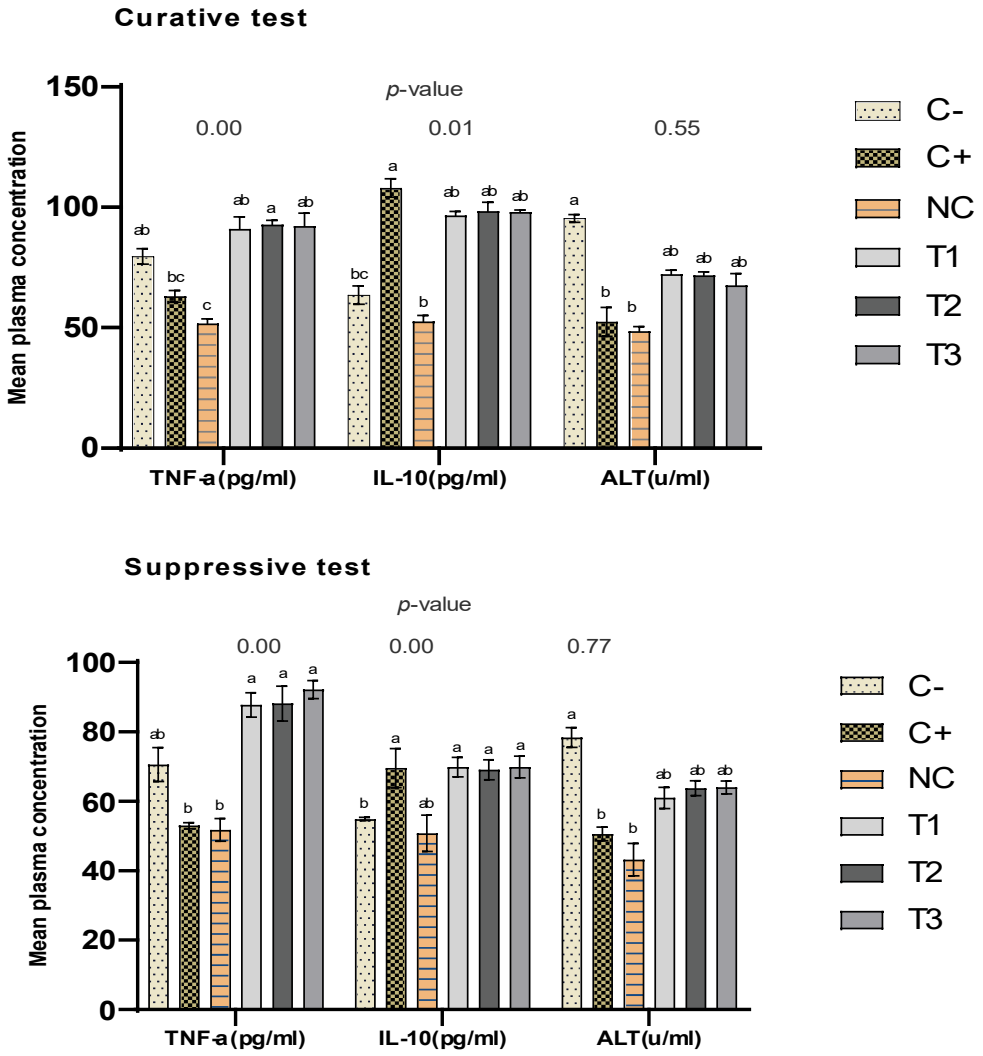

Figure 3: Plasma biochemical composition of mice treated with ethanol leaf extract of *Justicia secunda*

Statistical analysis performed amongst negative control (C-), positive control (C+), normal control (NC), 500mg/kg(T1), 1000mg/kg (T2), 1500mg/kg (T3) dose of ethanol leaf extract of *J. secunda*, groups with different letters on bars are statistically significant $p<0.05$, each point is the mean ± SEM (Standard error of mean) n=5.

Antioxidant and Lipid Peroxidation Status of the Liver

Figure 4 shows the liver's antioxidant and lipid peroxidation profile of experimental mice for the curative and suppressive test. The groups treated with plant extract exhibited a significant ($p<0.05$) increase in the bioactivity of antioxidants, including SOD, CAT, and GSH, compared to the negative control. The SOD concentration was, however, significantly ($p<0.05$) lower in the extract-treated group (3.32±0.447u/ml, 3.48±0.526u/ml, 3.47±0.579u/ml) and negative control (3.38±0.481u/ml) than in the normal control (7.26±0.462u/ml) and positive control (6.84±0.331u/ml). In addition, the hepatic malondialdehyde (MDA) concentrations were significantly ($p<0.05$) reduced in treatment groups compared to the negative control.

Liver Histology of Experimental Mice

The liver of negative control shows mass vascular congestion when compared to liver sections of other groups for the curative test (Figure 5). The positive control and normal control have normal architecture of a liver photomicrograph, while the 500mg/kg, 1000mg/kg and 1,500mg/kg have a moderate hepatic necrosis, moderate vacuolation and slight Kupffer cell hyperplasia respectively. Comparing the histological sections of the treatment groups to those of the negative control group for the suppressive test (Figure 6), the treatment groups showed slight vacuolation and necrosis (500 mg/kg), slight Kupffer cell hyperplasia (1,000mg/kg and 1,500 mg/kg) and the negative control had slight vascular congestion. Also, the liver photomicrographs of the positive and normal control exhibit normal features in their hepatic histology.

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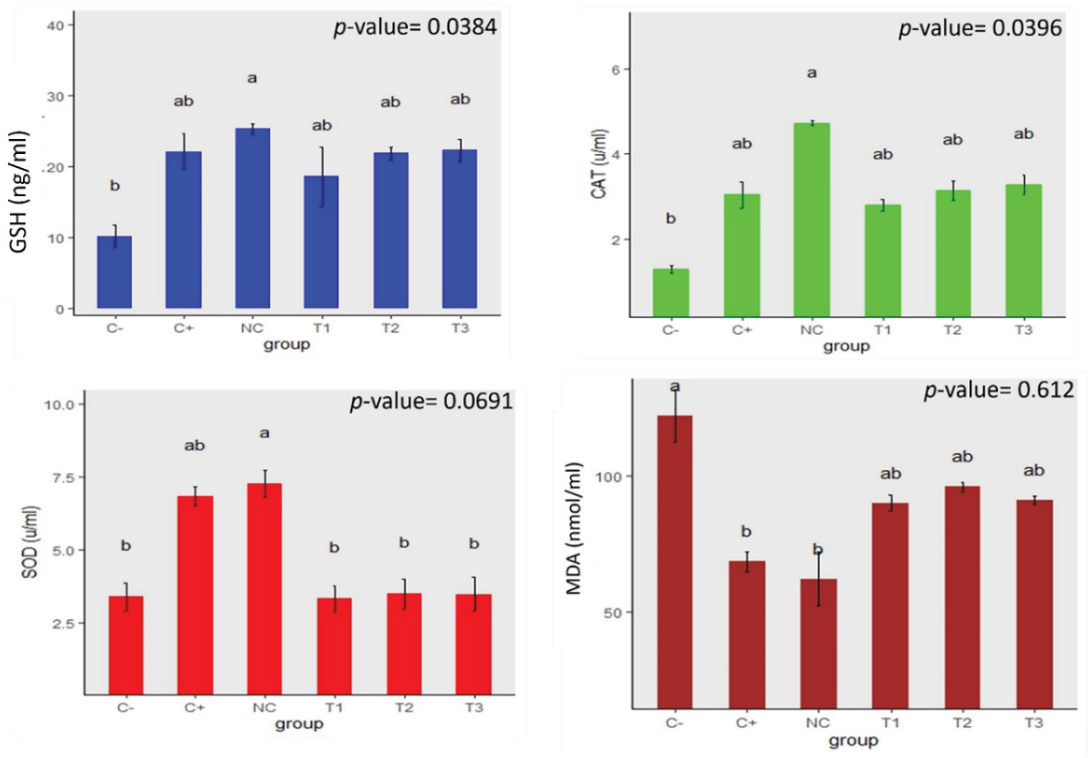
A. Curative test



B. Suppressive test

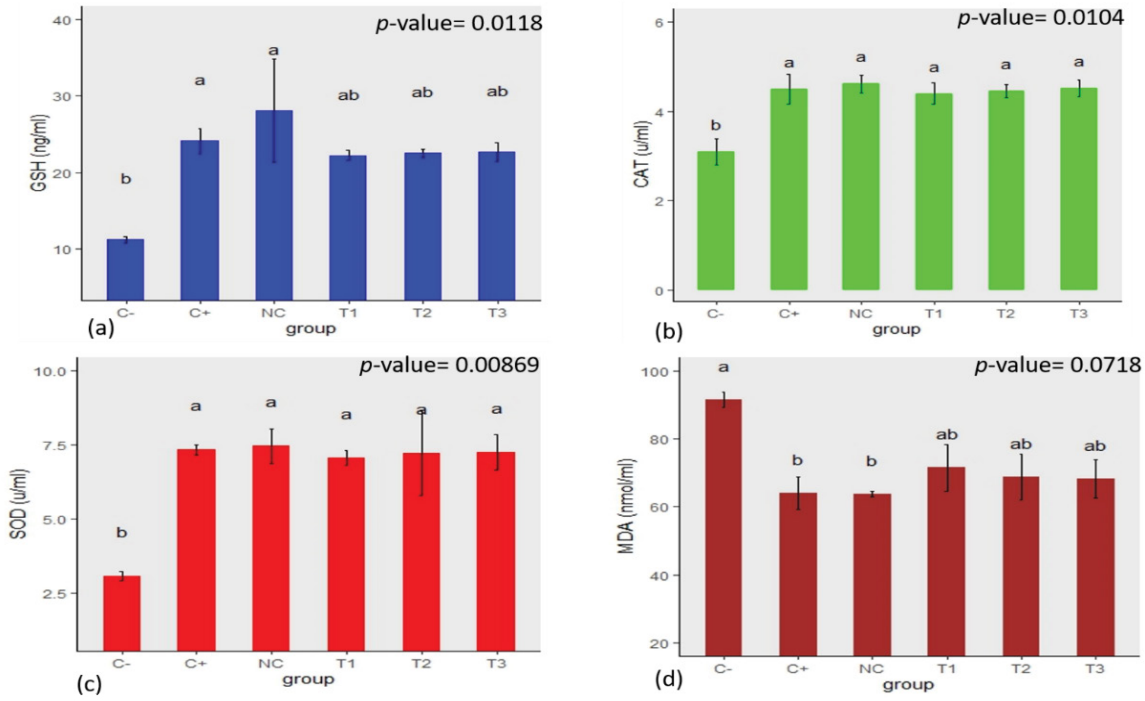


Figure 4: Antioxidant and lipid peroxidation status of the liver for mice treated with ethanol leaf extract of *Justicia secunda*

Statistical analysis performed amongst negative control (C-), positive control (C+), normal control (NC), 500mg/kg(T1), 1000mg/kg (T2), 1500mg/kg (T3) dose of ethanol leaf extract of *J. secunda*, groups with different letters on bars are statistically significant $p<0.05$, each point is the mean ± SEM (Standard error of mean) n=5.

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KEYS

Negative control (C-), positive control (C+), normal control (NC), 500mg/kg(T1), 1000mg/kg (T2), 1500mg/kg (T3) dose of ethanol leaf extract of *J. secunda*, MVC= Mass Vascular Congestion, NF= Normal Feature, VC= Vacuolation, MHN= Moderate Hepatic Necrosis, MVC= Mass Vascular Congestion, SKH= Slight Kupffer cell Hyperplasia.

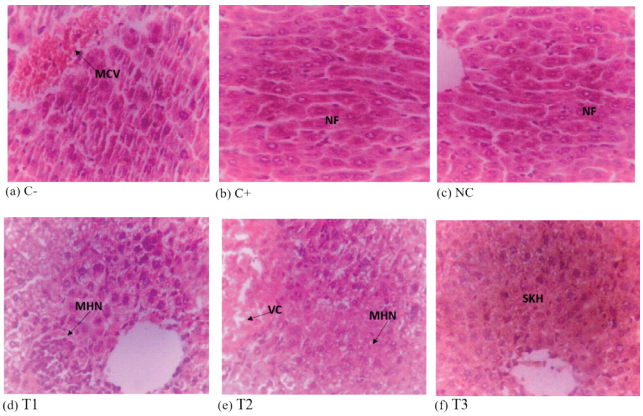

Figure 5: Liver Photomicrograph of mice treated with ethanol leaf extract of *Justicia secunda* (Curative test)- 400 × magnification.

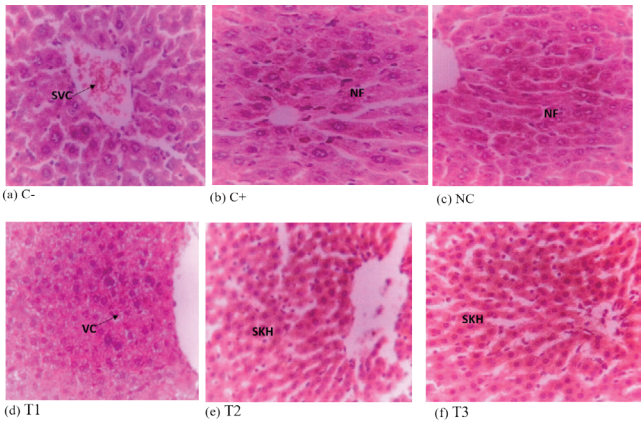

Figure 6: Liver Photomicrograph of mice treated with ethanol leaf extract of *Justicia secunda* (Suppressive test)- 400 × magnification.

KEYS

Negative control (C-), positive control (C+), normal control (NC), 500mg/kg(T1), 1000mg/kg (T2), 1500mg/kg (T3) dose of ethanol leaf extract of *J. secunda*, SVC= Slight Vascular Congestion, NF= Normal Feature, VC= Vacuolation, SKH= Slight Kupffer cell Hyperplasia.

Discussions

Eight (8) different secondary metabolites were present in the ethanol leaf extract of *J. secunda* namely: alkaloids, cardiac glycosides, phenolic compounds, tannins, steroids, carbohydrates, flavonoids and terpenoids. In addition, several phytochemical compounds with antioxidant, anti-inflammatory and anti-plasmodial activity were detected in the extract. For instance, β-sitosterol has anti-inflammatory properties [23], Squalene has antiparasitic and anti-inflammatory properties [24], 2-Methyl-Z,Z-3,13-octadecadienol has antimicrobial properties [25], 17-Pentatriacontene has anti-inflammatory properties [26], hexadacenoic acid methyl ester has anti-inflammatory properties [27], 9,12-Octadecanoic (linoleic) acid methyl ester has anti-plasmodial and anti-inflammatory [28], Methyl stearate has anti-inflammatory properties [24], cis-13-Octadecenoic acid, methyl ester has anti-inflammatory activity [29], 16-dimethoxy-aspidospermidin-17-ol has anti-plasmodial activity [30], 8-Hexadecenal 14-methyl-, (Z)- has antioxidant activity [31], Oleic acid has antioxidant activity [32], cis-9-Hexadecenal has anti-inflammatory properties [33] and cyclododecane, ethyl has antioxidant activity [34].

These myriads of phytochemicals may have conferred on this plant its popularity and wide use in African traditional medicine [35]. The phytochemical screening contrasts with the findings of a study conducted on a similar extract of *J. secunda* leaves collected from a farm in south-east Nigeria that is devoid of steroids and glycosides [36]; however, hexadecenoic acid methyl ester and octadecanoic acid methyl ester was also detected in extract of *J. secunda* [37]. The difference in phytochemical constituents in both studies could be attributed to ecological variation [38]. According to Hodge and Sterner's [39] toxicity scale, this plant extract is practically non-toxic. A study [40] that processed the leaves of *J. secunda* for three weeks and macerated them in ethanol for 24 hours reported an LD_{50} of 3,800 mg/kg body weight in rats due to the cyanide content of the extract. It is very likely that the selection of mature leaves and the longer processing duration of the extract for this present study may be reasons for the reduced toxicity observed [41]. One of the major foci of this study is to include *Justicia secunda* in the record of antimalarial study, and the crude extract has a percentage chemosuppression of 71.21% (1,500 mg/kg) and 75.18% (1,500 mg/kg) in the suppressive and curative tests, respectively. The result is less than the antimalarial activity of the ethanol leaf extract of *Justicia carnea* (800 mg/kg dose) with a percentage chemosuppression of 82% [42]. The efficacy of plant extracts in treating malaria disease depends on several factors, such as the *Plasmodium* species, the dosage, the duration of treatment, and the host immune response [43]. Experimental research on 16-dimethoxy-aspidospermidin-17-ol [31] and linoleic acid-methyl esters [29] suggest that these phytochemicals inhibit the growth of *Plasmodium* parasites by binding with the Fab-I enzyme responsible for fatty acid biosynthesis. This is to create awareness that *J. secunda* could be an alternative source of antimalarial compounds.

The ethanol leaf extract of *J. secunda* prevented PCV, HGB, and RBC reduction in mice, but there was an increase in WBC in the curative test. A different study observed

similar trend for these haematological parameters with a normal WBC in curative and suppressive tests on the antioxidant and antimalarial activity of the ethanol stem bark extract of *Terminalia macroptera* in *P. berghei*-infected mice [44]. The haematinic properties of the extract in this study may be due to the presence of flavonoid compounds that have been reported to increase iron absorption and deposition in tissues while reducing iron excretion [45]. In addition, many antimalarial herbal preparations may exert their anti-infective activity not only by affecting the parasite directly but may also stimulate the defensive system of the host through many other mechanisms [46]. The assessment of modulatory and hepatoprotective potentials reveals that TNF-α and IL-10 increased and the concentrations of ALT reduced in treatment groups. This outcome corroborates the TNF and IL-10-increasing potentials observed in *P. berghei*-infected mice treated with the ethyl acetate leaf extract of *Sonchus arvensis* [47] and the hepatoprotective nature of *J. secunda* in reducing plasma ALT in rats administered carbon tetrachloride [48]. Infection and immunological reactions to the extract may be reasons for the increased level of TNF-α in treated mice. The anti-inflammatory activity of β-sitosterol was harnessed in reducing inflammation in Zebra fish [49] and the presence of this phytochemical in the extract utilized for this research may be the reason for the observed pharmacological effect. This is an indication that *J. secunda* may be utilized for treatment associated with pathogenesis of malaria infection.

The liver homogenate of the experimental groups sustained an increased bioactivity of antioxidants (SOD, GSH, and CAT). Yet, a reduced level of SOD was observed in mice treated days after infection. The lipid peroxidation biomarker (MDA) is equally reduced in mice treated with crude extract. Hepatic antioxidant and MDA concentrations follow the same pattern as those of experimental mice treated with an extract of *Croton membranaceus* but differ with an increased SOD level [50]. The infection also caused a reduction in hepatic SOD in mice infected with *P. berghei* and treated with a stem-bark extract of *Terminalia macroptera* [51]. *Justicia secunda* exhibited antioxidant properties in this study, which may be attributed to components of the extract with the relevant pharmacological activity [49, 52, 53]. The ethanol leaf extract of *J. secunda* reduced vascular congestion in the liver of mice, as the photomicrograph of the treatment groups only shows sections with Kupffer cell hyperplasia, vacuolation, and hepatic necrosis. Ibukunoluwa [54] also reported similar histopathology in the antiplasmodial activity of polyherbal mixtures. The slight changes seen in the liver when used to test the efficacy of the plant extract may be due to the induced infection and the activation of immune cells in the organs. Ayawa *et al.* [55] also advanced cellular damage by immunological reactions as a reason for histopathological changes in the liver of mice. Pure compounds should be isolated from *J. secunda* extract to conduct trials on their pharmacological activity in relation to malaria infection and this could potentially uncover another effective antimalarial compound.

Conclusion

In conclusion, the ethanol leaf extract of *J. secunda* contains phytochemicals with numerous pharmacological activities, and is non-toxic at 5,000mg/kg. It exhibits a dose-dependent percentage of chemosuppression of *P. berghei* at 500mg/kg, 1,000mg/kg and 1,500mg/kg. The extract prevented the reduction of the studied haematological parameters such as RBC, HGB and PCV. The concentrations of TNF-α and IL-10 increased and ALT concentrations reduced in the plasma of mice treated with extract. Evaluation of liver biochemicals revealed that MDA concentration was reduced with increased bioactivity of the studied antioxidants for the infected mice treated with the plant extract. Vascular congestion was also reduced in the liver histology of mice infected and treated with the plant extract but shows increased infiltration of immune cells and necrosis. This present study infers that ethanol leaf extract of *J. secunda* contains phytochemicals that have been tested individually to treat malaria and inflammation, which can be isolated for treating malarial pathogenesis.

Abbreviations

ALT	Alanine aminotransferase
MDA	Malondialdehyde
SOD	Superoxide dismutase
CAT	Catalase
GSH	Reduced Glutathione
IL-10	Interleukin-10
TNF-α	Tumour Necrosis Factor-alpha
HGB	Haemoglobin
PCV	Packed Cell Volume
RBC	Red Blood Cell
WBC	White Blood Cell

Declarations

Ethical approval

Maintenance and procedures performed involving animals in this study follow the ethical standards of Ahmadu Bello University, Zaria Committee on Animal Use and Care (ABUCAUC) with the approval number ABUCAUC/2023/055.

Consent for Publication: Not applicable

Availability of Data and Materials

All data generated or analyzed in this study are included in this article.

Competing Interest: None

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Authors' Contributions

N.A.A, S.J.O, P.I.R, A.Y., and Y.A.W conceptualized the study, conducted the investigation and formal analysis.

N.A.A and M.H. prepared figures and tables. All authors reviewed the manuscript.

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