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Original Research Article

Hematinic, anti-plasmodial potential and toxicity of aqueous leaf extract of *Justicia secunda* in albino mice

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Abstract

Purpose: *To investigate the hematinic, anti-plasmodial potentials, and toxic effects of an aqueous extract of the leaf of Justicia secunda in mice (Mus musculus) made anemic by Plasmodium beighei.*
Methods: *The LD_{50} of J. secunda was determined using Lorke's method. Male albino mice ages 4 – 5 months, totaling forty-eight (48), were assigned at random to six groups (1 – 6). Groups 1, 2, and 3 mice were P. berghei-infected and were given extract doses of 200, 400, and 600 mg/kg of body weight, respectively. Group 4 (positive control) was infected and treated with 0.3 mL of vitamin B12 (standard). Group 5 (negative control) was infected and untreated, while group 6 (normal control) was uninfected and untreated. Blood samples were taken on days 7, 12, and 16 post-infections to measure haemoglobin (Hb), red blood cells (RBC), packed cell volume (PCV), alanine transaminase, aspartate transaminase, alkaline phosphatase, creatinine, and urea using standard methods.*
Results: *Lower concentrations (200 and 400 mg) of the extract reduced P. berghei parasitemia. In comparison to vitamin B12, there was a negligible drop in Hb, PCV, and RBC levels in the infected groups. Both J. secunda extract and vitamin B12 did not improve the hematinic status of the mice infected with P. berghei. The hematinic condition of the mice with P. berghei infection was not improved by J. secunda extract or vitamin B12. Biochemical analysis showed no toxicity due to extract intake.*
Conclusion: *Justicia secunda leaf extract has anti-plasmodial efficacy in P. berghei-infected mice. Studies are required to validate these findings in humans.*

Keywords: *Justicia secunda, Vitamin B12, Anemia, Antimalarial, Toxicity*

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INTRODUCTION

Anemia is a decline in the overall quantity of erythrocytes with a decreased concentration of haemoglobin per erythrocyte which leads to a decrease in the blood's capacity to transport oxygen [1]. Anemia is a significant global public health issue linked to an increased risk of all-cause and cardiovascular disease in both developed and developing nations of the world. In anemic conditions in humans, haemoglobin concentration decreases to below the normal ranges of 13 to 12 g/dL for males and non-

pregnant females over the age of 15; compared to 11 g/dL for pregnant women and children under the age of five [2]. Anemia is most prevalent in children below five years of age with about 67.6 % of global cases among children of African descent. Anemia is most commonly caused by nutritional deficiencies, hemoglobinopathies, and diseases like malaria and other parasitic infections, tuberculosis, and HIV-AIDS among others [3].

Malaria, being a major cause of anemia is known to be the commonest cause of death in children under 5 yr. and in pregnant women [4]. Anemia contributes significantly to the high death rate in these malarious individuals. The malaria pathogens, plasmodia, which are obligate intracellular organisms destroy the erythrocytes of their hosts thus popularizing malaria infection as a primary cause of severe anemia globally and particularly in sub-Saharan West Africa where sickle cell anemia alongside parasitic infections are very common [3]. In malaria control, anemia has been recommended by WHO and Roll Back Malaria (RBM) partnerships as an extra pointer to the status of malaria burden at the community level [5].

Different iron-rich medicinal plants have been extensively documented as being efficacious in the management of anemia and other ailments [6]. Anti-plasmodial properties have been documented for some other medicinal plants. These medicinal plants have been identified as having bioactive substances (phytochemicals and secondary metabolites) that are protective against various diseases. However, some medicinal plants are like double-edged swords which may cure and as well inflict a level of injury on the consumer [7]. This potential to inflict harm on the user should not be neglected while considering the medicinal properties.

Justicia secunda is a well-known medicinal plant and one of several species of the genus of flowering plants in the family Acanthaceae. It is a shrub located in tropical areas of the world, although it is native to the north of South America and Venezuela, where it is commonly referred to as Bloodroot and Sanguinaria, respectively. It is largely consumed for its anti-sickling, hematinic, antimicrobial, and anti-hypertensive potentials [8]. There have not been any reports on the haematopoietic potential of *J. secunda* in anemic malarious individuals.

The present study evaluated the hematinic and anti-plasmodial potential of *J. secunda* leaf extract and its safety profile. This study aimed to obtain insights into the efficacy of *J. secunda* as a hematinic agent, as claimed in traditional settings, as well as its effect on the biochemical profile of the mice. Its anti-plasmodial potential in the mice which had *P. berghei* infection was, as well, studied. These were not previously investigated.

EXPERIMENTAL

Study design

Forty-eight (48) male albino mice, weighing between 20 and 26 g, were used in the study. The Albino mice were distributed at random into six (6) experimental groups comprising 8 mice per group. Groups 1, 2, and 3 of mice were all infected with the parasite *P. berghei* at 1 x 10^7 parasitized red blood cells and were given extract doses of 200, 400, and 600 mg/kg body weight, respectively. The positive control was made up of mice in Group 4 (infected with the parasite and treated with 0.3 ml of vitamin B12), while those in Group 5 served as the negative control (infected with the parasite and received 0.3 mL of distilled water). Group 6 mice served as the normal control (uninfected and untreated). Seven days following the injection of the mice with *P. berghei*, blood samples were collected to measure percentage parasitemia and hematological and biochemical parameters. This was followed by a three-day course of treatment with *J. secunda* extract. Subsequent blood analyses were carried out on day 12 (5^{th} day after treatment) and on the 16^{th} day (9^{th} day after treatment).

Collection and preparation of *Justicia secunda* extract

Fresh *J. secunda* leaves were collected from the Zoology and Environmental Biology Department (Zoological Garden), University of Nigeria, Nsukka. The leaves were oven-dried at 36^{o}C for 48 h and pulverized using Model 4 of Thomas Wiley Mill. The amount of *J. secunda* powder that was produced after pulverization was approximately 262.88 g.

The extract was soaked in 2.5 L of water and stirred until a homogenous mixture was obtained. The setup was left to stand on the laboratory bench undisturbed for 48 h. It was then filtered using muslin cloth arranged in 4 layers. Shallow volumes of the liquid extract were air-dried in Petri dishes under an industrial fan within 48 h and then stored away in a screw-capped bottle in a refrigerator until needed. A stock extract concentration of 0.1 mg/mL was produced from which the mice in different groups were dosed.

Procurement of animals and parasites

Forty-eight (48) male albino mice weighing between 20 and 26 g were acquired from the laboratory Animal House of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, for this study. The University of Nigeria's best practices for the handling and use of laboratory animals were followed throughout the investigation. The Ethics Committee on Animals, Veterinary Medicine Faculty, University of Nigeria, Nsukka, approved the experimental procedure (dated: Feb 9, 202 The Faculty of Pharmaceutical Sciences Research Ethics Committee, University of Nigeria, Nsukka, approved (Ethical number FPSRE/UNN/20/0011) the experimental procedure. All procedures on the animals were carried out by following the Guide for the Care and Use of Laboratory Animals [9]. The mice were fed *ad libitum* on a regular pellet diet, with water, and kept in a normal laboratory environment with a cycle of 12-hour light and 12-hour darkness.

Procurement of the *Plasmodium berghei*

The National Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria, provided samples of *P. berghei* (NK-65) that are sensitive to chloroquine for the investigation.

Inoculum preparation

A stock of parasitized red cells with minimal surface parasitemia of 20 % was collected from the infected mice, through the retrobulbar plexus of the medial canthus. Blood was collected into EDTA bottles. By comparing the total red blood cells to the parasitized ones, the percentage of parasitemia was calculated. The stock's cell concentration was established, and physiological saline was added to dilute it so that 0.2 mL of the final inoculum contained 1 x 10^7 parasitized red blood cells. This served as the standard inoculum used to parasitize a single mouse [10]. Seven days after the mice were inoculated with the parasite, the percentage of parasitemia was determined, and a treatment regimen that lasted for three consecutive days was started.

Toxicity studies

Acute toxicity evaluation was done following Lorke's two-step procedure for lethal dose determination [11].

Determination of hematological profiles

Whole blood samples were collected from the mice via the retrobulbar plexus of the medial canthus for this purpose. The blood samples were measured for packed cell volume (PCV), haemoglobin (Hb) levels, red blood cell (RBC) count, and percentage parasitemia before the treatment (day 7). These blood parameters were also determined on days 12 and 16 after treatment to evaluate the effect of the various treatments on these parameters. The hematological profile was determined in accordance with standard protocols. The PCV and Hb were determined using the microhematocrit and cyanomethamoglobin methods respectively. With an upgraded Neubauer chamber, the hemocytometer method [12] was used to determine the RBC number.

Biochemical determinations

Samples of blood were collected and kept at laboratory ambient temperature for 45 min to clot and then centrifuged at 3000 rpm for 10 min to separate the clear serum from the clot. The serum samples obtained from the separation were proximately used for biochemical evaluation. The serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured with Reitman-Frankel colorimetric methods, whereas the phenolphthalein monophosphate method was used to measure ALP. The modified Jaffe technique was used to measure plasma/serum creatinine whereas the Berthelot-Searcy modified approach was employed for the *in vitro* determination of serum [13].

Data analysis

The Statistical Package for the Social Sciences (version 23.0, IBM Corporation, Armonk, USA), was used to analyze the data. A generalized linear model (GLM) was used to estimate the effect of extract concentrations and duration of exposure on the hematological and biochemical status of the mice. Parasitemia was normally distributed and was analyzed using GLM. Post-hoc analyses were conducted using least square difference (LSD). The level of significance was set at 95 % probability (i.e., $p < 0.05$).

RESULTS

Yield of *J. secunda* extract and acute toxicity profile

The weight-to-weight yield (w/w) of the crude aqueous extract of *J. secunda* was 35.10 %.

Acute toxicity of *Justicia secunda* leaf extracts

The extract, administered orally, had no toxicity-indicating behaviour at 5000 mg/kg weight of the mice used.

Effect of *Justicia secunda* leaf extract on malaria parasitemia

On day 7 following parasite inoculation and establishment of infection, and just before the commencement of treatment with the extracts, parasitemia in the infected mice was 40 % on average. On day 10 and day 16 post-treatments with various doses of the extract, parasitemia was significantly reduced with respect to concentration (18 %) unlike the positive control (58 %). Lower concentrations of the test extracts had better anti-malarial activity; doses of 200 and 400 mg/kg of mice weight reduced parasitemia significantly on days 10 and 16 compared to 600 mg/kg of the extract and those administered vitamin B12 ($p < 0.05$; Figure 1). The anti-malarial effect of the standard drug and *J. secunda* dose of 600 mg/kg of mice weight waned on day 16 compared to day 12. However, none of the concentrations of the extracts completely eliminated the parasite during the test time frame.

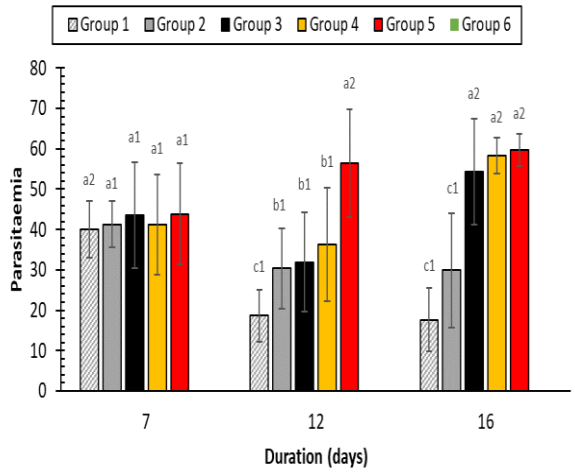

Figure 1: Effect of *J. secunda* extract on *P. berghei* malaria parasitemia levels in mice. Values labeled differently alphabetically were significantly different on a given day, while values labeled differently numerically were considerably different ($p < 0.05$) by duration in each group. Group 1 (infected and treated with 200 mg of *J. secunda* per kg weight of the mice; Group 2 (infected and treated with 400 mg of *J. secunda* per kg weight of the mice; group 3 (infected and treated with 600 mg of *J. secunda* per kg weight of the mice; Group 4 (infected and treated with standard drug, Vitamin B12); Group 5 (infected and untreated); Group 6 (uninfected and untreated)

Hematological indices of *Mus musculus* after treatment with *J. secunda* extract

The extract's impacts on the PCV, Hb, and RBC of *Mus musculus* are presented in Table 1. Effect of the extract on PCV was restricted in Group 3 on day 16. It decreased significantly in Group 3 relative to other groups ($p < 0.05$). The effect on Hb on day 7, after inoculation of the parasite and before treatment of parasitemia, was different among the groups. Haemoglobin was highest in Groups 2 and 3 on day 7 with a significant increase over the positive control (Group 5; $p < 0.05$). Hemoglobin in Groups 1 and 6 was also significantly higher on day 7 compared to Group 5. The *J. secunda* treatment appeared to be associated with a decline in Hb, as Hb in all groups administered the plant extract reduced to a similar level as the positive control (Group 5). The hemoglobin level in Group 3 dropped significantly in comparison to Group 5 ($p < 0.05$). Red blood cells in Group 3 also decreased considerably ($p < 0.05$) in comparison to the other groups in days 12 and 16 of treatment with the extract (Table 1). Different alphabets and numeric superscripts within columns and across rows respectively show that values are significant ($p < 0.05$). Values of parameters (variables) are presented as mean ± SEM (n = 6).

Serum biochemistry of *Mus musculus* after treatment with extract of *Justicia secunda*

Activities of ALT, AST, and ALP, varied with treatment of the parasitized mice with *J. secunda* leaf extract. Both ALT and ALP showed no significant changes across the groups before and after treatment with the extract (Table 2). However, with the exception of Group 3 ($p < 0.05$), Group 2's AST significantly rose on day 12 compared to the other groups. On day 16, AST became higher in Groups 1, 2, and 3 than the rest of the groups. These variations were substantial ($p < 0.05$; Table 2).

There was a general decrease in creatinine concentration during treatment with *J. secunda*, between day 7 and day 16 (Figure 2). The differences between day 7 creatinine levels in Group 2 and Groups 3, 4, 5, and 6 were significant ($p < 0.05$). Creatinine concentration showed a mixed trend on day 12. On day 16, creatinine level was similar in all groups (Figure 2).

Urea concentration decreased in Groups 4 and 5 on day 7 after inoculation of the parasite. The decrease was significant when compared to the levels of urea in Groups 1, 2, and 3, but on day

Table 1: Erythrocyte profiles of *P. berghei*-infected mice treated with *J. secunda* extract

Parameter	Group	Duration (days)		
		7	12	16
PCV (%)	1	50.50±3.04^{a2}	43.00±3.04^{a1}	40.17±7.78^{a1}
	2	51.67±2.47^{a2}	41.33±2.47^{a1}	47.17±1.76^{a1}
	3	50.50±2.18^{a2}	30.17±6.75^{a1}	24.33±8.08^{b1}
	4	45.00±4.44^{a1}	35.67±12.7^{a1}	42.67±2.52^{a1}
	5	45.67±1.89^{a1}	35.00±13.76^{a1}	38.00±5.22^{a1}
	6	47.17±2.60^{a1}	43.50±5.89^{a1}	43.67±1.26^{a1}
Hb (g/dL)	1	16.55±1.15^{ab1}	14.99±1.83^{a1}	12.98±3.27^{a1}
	2	18.34±0.62^{a1}	14.82±3.44^{a1}	15.17±1.37^{a1}
	3	18.23±0.82^{a2}	10.36±3.67^{a1}	7.84±3.23^{b1}
	4	15.33±1.40^{bc1}	11.39±4.64^{a1}	15.05±1.20^{a1}
	5	14.83±1.06^{c1}	12.48±5.89^{a1}	12.85±2.26^{a1}
	6	15.99±0.67^{b1}	15.14±2.94^{a1}	15.59±3.30^{a1}
RBC (x10^6/µL)	1	10.02±0.68^{a1}	8.47±0.76^{a1}	7.63±1.90^{a1}
	2	10.47±1.07^{a2}	8.09±1.99^{a1}	9.31±0.57^{a12}
	3	10.47±1.07^{a2}	5.77±1.09^{b1}	5.06±0.56^{b1}
	4	8.89±0.85^{a1}	6.90±2.54^{a1}	8.64±0.56^{a1}
	5	9.21±0.50^{a1}	6.94±2.65^{a1}	7.14±1.67^{a1}
	6	10.24±1.00^{a1}	8.64±1.40^{a1}	8.79±0.25^{a1}

Values are significant ($p < 0.05$) where different alphabets and numeric superscripts are used within and across rows, respectively. Values of parameters (variables) are presented as mean ± SEM (n = 6). Group 1 (infected and treated with *J. secunda* dose of 200 mg/kg of mice weight; Group 2 (infected and treated with *J. secunda* dose of 400 mg/kg of mice weight; Group 3 (infected and treated with *J. secunda* dose of 600 mg/kg of mice weight; Group 4 (infected and treated with standard drug, Vitamin B12); Group 5 (infected and untreated); Group 6 (uninfected and untreated)

Table 2: Serum enzyme intensities in *P. berghei*-infected mice treated with *J. secunda* extract

Parameter	Group	Duration (day)		
		7	12	16
ALT (IU/L)	1	22.18±2.05^{a1}	36.46±16.15^{a1}	73.38±47.18^{a1}
	2	50.35±12.33^{a2}	47.89±17.26^{a2}	28.34±2.95^{a1}
	3	40.61±17.23^{a2}	73.09±22.30^{a2}	36.52±2.09^{a1}
	4	30.96±1.36^{a1}	64.14±12.44^{a2}	21.54±8.26^{a1}
	5	35.15±7.52^{a1}	41.96±19.99^{a2}	18.12±2.76^{a1}
	6	32.98±3.78^{a1}	46.32±22.59^{a1}	20.04±0.91^{a1}
AST (IU/L)	1	56.80±8.95^{b1}	67.01±11.06^{a1}	122.58±29.25^{a2}
	2	109.46±8.06^{a1}	94.24±18.34^{a1}	92.57±14.07^{a1}
	3	83.75±14.73^{ab1}	108.28±12.75^{a1}	107.38±8.65^{a1}
	4	72.53±5.51^{b1}	113.50±2.73^{a2}	66.46±10.49^{b1}
	5	70.20±10.69^{b1}	94.64±35.74^{a2}	68.97±4.19^{b1}
	6	73.31±5.15^{b1}	86.28±14.06^{a2}	72.37±12.44^{b1}
ALP (IU/L)	1	58.26±18.13^{a1}	33.60±14.06^{a1}	30.70±9.22^{a1}
	2	36.04±11.56^{a1}	32.68±9.69^{a1}	35.22±6.51^{a1}
	3	28.09±4.94^{a1}	66.91±54.23^{a2}	37.61±13.07^{a1}
	4	21.23±5.91^{a1}	34.49±3.65^{a1}	18.31±2.65^{a1}
	5	21.54±1.45^{a1}	42.61±10.53^{a1}	33.42±15.97^{a1}
	6	35.15±17.01^{a1}	37.83±6.12^{a1}	36.84±11.48^{a1}

Values are significant ($p < 0.05$) where different alphabets and numeric superscripts are used along columns and across rows, respectively. Values of parameters are presented as mean ± SEM (n = 6) Group 1 (infected and treated with *J. secunda* dose of 200 mg/kg of mice weight); Group 2 (infected and treated with *J. secunda* dose of 400 mg/kg of mice weight; Group 3 (infected and treated with *J. secunda* dose of 600 mg/kg of mice weight); Group 4 (infected and treated with standard drug, vitamin B12); Group 5 (infected and untreated); Group 6 (uninfected and untreated).

12 of treatment, the urea level did not increase appreciably ($p > 0.05$) in comparison to all other groups. On day 16, however, the level decreased appreciably ($p < 0.05$) in Groups 4 and 5 (Figure 3).

DISCUSSION

The crude aqueous extract of *J. secunda* yielded 35.10 % (w/w). A percentage yield of 10.7 % w/w dry matter, using methanol as the solvent of extraction, was reported by [14] while [15]

reported more yield using methanol (2.47 %) than using water (2.03 %).

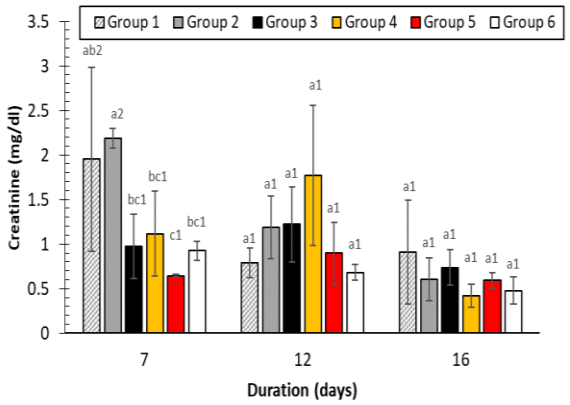

Figure 2: Serum creatinine concentrations of *P. berghei*-infected mice treated with *J. secunda* extract. Values with distinct numeric labels are statistically varied by duration for each group, while those with different alphabetic labels are significantly different on a specific day ($p < 0.05$). Group 1 (infected and treated with *J. secunda* dose of 200 mg/kg of mice weight); Group 2 (infected and treated *J. secunda* dose of 400 mg/kg of mice weight); Group 3 (infected and treated with *J. secunda* dose of 600 mg/kg of mice weight); Group 4 (infected and treated with standard drug, Vitamin B12); Group 5 (infected and untreated); Group 6 (uninfected and untreated)

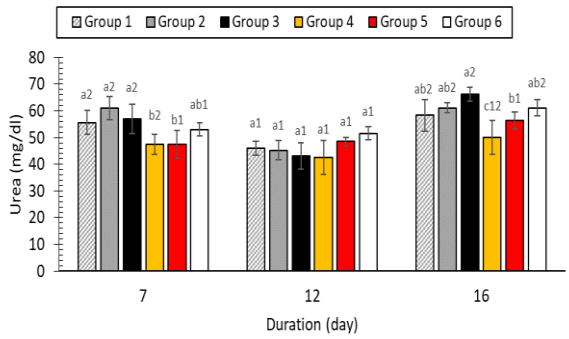

Figure 3: The serum urea concentrations of *P. berghei*-infected mice treated with *J. secunda* extract. Values with distinct numeric labels are statistically varied by duration for each group, while those with different alphabetic labels are significantly different on a specific day ($p < 0.05$). Group 1 (infected and treated with 200 mg/kg of *J. secunda*.); Group 2 (infected and treated with *J. secunda* dose of 400 mg/kg of mice weight); Group 3 (infected and treated with *J. secunda* dose of 400 mg/kg of mice weight); Group 4 (infected and treated with standard drug, vitamin B12); Group 5 (infected and untreated); Group 6 (uninfected and untreated)

The disparity between this report and previous ones could be attributed to the method of filtration adopted by users of the herbal product which was followed in this work. Plant source and geographical location could also be a factor. There were neither deaths nor signs of toxicity in all mice dosed with aqueous leaf extract of *J. secunda* at 5000 mg/kg of mice weight). The extract was, therefore, well tolerated by the mice. The LD_{50} of the aqueous extract was 5000 mg/kg. This is in disparity with the report of 2000 mg/kg body weight given by [14]. The variation in LD_{50} values could also be attributed to the type of solvent used during extraction.

The results of the malaria parasitemia evaluation in this study revealed a reduction in parasitemia levels with the administration of lower doses of *J. secunda* at 200 and 400 mg/kg of mice weight). Paradoxically, at a dose of 600 mg/kg, parasitemia levels continued to rise. This was also the case with the standard hematinic (Vit. B12) used. Even though parasitemia was not completely cleared at day 16 post-infection, the present study indicates that *J. secunda* had a dose-dependent antimalarial effect. Previous researchers have reported the presence of high concentrations of alkaloids in the plant and alkaloids have a spectrum of pharmacological effects, including antimalarial effects [16]. Although other plants in the genus, Justicia (such as the methanolic extract of *Justicia purpura*) have been reported to have antimalarial potential activity most research on *J. secunda* only reveal its anti-sickling, anti-hemolytic, and membrane-stabilizing effects. Results of the present study point to a need for further studies on the antimalarial activity of the aqueous leaf extract of *J. secunda* at concentrations below 400 mg/kg and also investigate the antimalarial activity noticed during these experiments. Other solvents may also be used to screen the extract for antimalarial effect. The results obtained in the antimalarial study are consistent with the outcome of the hematological studies, especially in mice dosed with 400 mg of the extract.

Generally, hematological parameters decreased during the test period, although not significantly in some groups. The results obtained at 200 and 400 mg of the extract per kg of mouse body weight did not significantly differ from those of the standard hematinic (Vit B12). However, there was a significant improvement in hematological parameters with the administration of standard hematinic (Vit B12) compared to 600 mg of the extract. The standard hematinic (Vit. B12) had no effect on the levels of parasitemia. Therefore, aqueous extract of *J. secunda* may have synergistic activity when used with potent antimalarials in malaria-induced anemia.

For the serum enzymes, while ALT and ALP showed no significant change across the groups, AST increased significantly after treatment of the

mice with the extract. The inappreciable changes in serum ALT and ALP show that the extract may not be harmful to the liver. Increases in ALT and ALP are more specific for obstructive liver damage or disease. The significant increase in serum AST as opposed to the negligible increases in ALT and ALP may be attributed to the peculiar intralobular distribution of AST which results in its increase in a situation of ischemia. It could also be attributed to partial hepatocellular damage which releases ALT and AST [17].

There was a mixed trend in creatinine on day 12 while serum urea remained clearly unaffected. In malaria, elevation in serum creatinine levels may be triggered on account of acute kidney and liver complications that arise from hemodynamic and liver dysfunctions. Plant extracts may also trigger changes in creatinine and urea levels by either increasing or reducing them. Serum creatinine has been severally reported as a glomerular filtration rate (GFR) estimate and cardiovascular disease (CVD danger signs [18] although there are reports that GFR is a different CVD risk factor [19]. The decrease in creatinine, when treated with *J. secunda,* showed that there was no kidney or liver damage. The serum urea was normal in the groups treated with the extract. The decrease in serum urea in the mice that did not receive the extract could have resulted from factors other than the extract.

CONCLUSION

Justicia secunda leaf extract at doses of 200 and 400 mg/kg of the mice exhibits anti-malarial and hematinic potential. However, the hematinic property of the extract is not significant. Biochemical analysis shows no significant toxicity potential of *Justicia secunda* leaf extract. More studies are required to determine whether there is synergism between anti-malarial drugs and *J. secunda* and whether it may serve as better hematinic in malaria patients.

DECLARATIONS

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Ethical approval

The Faculty of Pharmaceutical Sciences Research Ethics Committee, University of Nigeria, Nsukka, approved (Ethical number FPSRE/UNN/20/0011) the experimental procedure.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Conceptualization: Godwin I. Ngwu, Chinazom P. Agbo, Maria I. Ngwu, Wilfred I. Ugwuoke; Data curation: Edith U. Okagu, Ernest C. Onoyima; Formal analysis: Godwin I. Ngwu, Chinazom P. Agbo, Maria I. Ngwu, Wilfred I. Ugwuoke; Funding: Godwin I. Ngwu, Chinazom P. Agbo, Maria I. Ngwu, Wilfred I. Ugwuoke, Edith U. Okagua, Ernest C. Onoyima; Investigation: Godwin I. Ngwu, Chinazom P. Agbo, Maria I. Ngwu, Wilfred I. Ugwuoke, Ifeanyi O. Aguzie; Methodology: Godwin I Ngwu, Chinazom P Agbo, Wilfred I. Ugwuoke; Project administration: Godwin I. Ngwu, Wilfred I. Ugwuoke; Validation: Godwin I. Ngwu, Chinazom P. Agbo ; Writing – original draft: Godwin I Ngwu, Ifeanyi O. Aguzie, Edith U. Okagu, Ernest C. Onoyima; Writing – review & editing: Godwin I Ngwu, Chinazom P Agbo, Petromina C Ozioko.

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