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The Modulatory Activity of *Justicia carnea* in Plasmodium Infected Mice

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Abstract The study investigated the antiplasmodial potency of *Justicia carnea* in plasmodium infected mice. The aqueous leaf extract of *Justicia carnea* was subjected to phytochemical screening using GC-FID. The suppressive, prophylactic and curative activity of the studied plant was accessed after oral administration of standard drugs (5mgkg^{-1} choroquine and 4mgkg^{-1} artesunate) and 400-800mgkg^{-1} of the extract. The effect of the aqueous leaf extract of the studied plant was also explored in the activities of liver enzymes, oxidative stress markers and hematological indices of plasmodium infected mice using spectrophotomertic methods and a hematology auto analyzer (BC 5300 Mindray England) respectively. The aqueous leaf extract of *Justicia carnea* showed considerable dose dependent antiplasmodial activity in the suppressive, prophylactic and curative test conducted. The suppressive activity of (92%) of the aqueous leaf extract of the studied plant at 800mgkg^{-1} was comparable to the standard drugs used while the mean survival time of plasmodium infected mice at 800mgkg^{-1} was beyond the mean survival time of the infected untreated group. The aqueous leaf extract of the studied plant also exhibited significant improvements in the activities of liver enzymes, oxidative stress markers and some hematological parameters at 800mgkg^{-1} of the extract when compared with the infected and untreated group. The results of the study exhibited the antiplasmodial activity of the studied plant, nonetheless, the crude extracts of the studied plant can be further purified for synergistic use with other potent antimalarial drugs to combat and achieve total clearance of plasmodium resistant infections.

Keywords: *Antimalaria, Justicia carnea, Plasmodium berghei, Poly phenolic compounds, Aqueous leaf extract*

1. Introduction

Malaria in recent times has become more prevalent and difficult to contain using affordable and widely accessible anti-malarial therapies of Western origin probably as a consequence of the constant evolution of the parasite and mosquitoes that transmit these malaria parasites are now resistant to insecticides [1,2]. This trend is worrisome with a profound effect as malaria remains the world most devastating human parasitic infection with an estimated mortality of over a million people per year [1]. Environmental factors such as rainfall, warm temperature and stagnant waters provide the ideal habitats for the development and increase in mosquitoes where children, the aged and pregnant women are most vulnerable to the incidence of this disease with devastating outcome in Sub-Saharan Africa [2,3]. Currently, in developing countries such as Nigeria, traditional medicine remains the predominant means of healthcare adopted by the rural dwellers in Nigeria [4,5,6]. The increasing resistance of malaria parasites to anti-malarial medicines and orthodox treatments aggravate deep concerns that has resulted in the urgent need to evaluate, explore and utilize naturally endowed medicinal plants that could offer useful and optimal therapeutic effects against malaria parasite. And thus, ultimately enhance the synthesis and isolation of

bioactive compounds. Medicinal plants are of great importance in the development of modern drugs [7,8]. *Justicia carnea* is an ornamental plant that belongs to the *Acanthaceae* family with about 250 genera and 2500 species [9]. *Justicia carnea*, commonly referred to as flamingo flower, jacobinia and Brazilian plume flow are found in Nigeria with a folkloric origin and are widely deployed by traditional medical practitioners in the treatment of various conditions such as gastrointestinal diseases, respiratory tract infection, inflammation, mental disorders, epilepsy and heart diseases [10,11]. Consequently, the study investigated the anti-malarial potency of the aqueous leaves extract of *Justicia carnea hooker*.

2. Materials and methods

2.1 Collection of plant material

Fresh leaves of *Justicia carnea* Hooker were obtained from Choba community, Rivers State, Nigeria. The plant sample was identified and authenticated at the Department of Plant Science and Biotechnology, University of Port Harcourt, Choba, Rivers State.

2.2 Sample preparation and extraction

The fresh leaves of *Justicia carnea* Hooker was air dried under room temperature and pulverized to coarse powder using a laboratory mill (Model 4 Arthur Thomas, USA). Precisely 400g of the ground leaves of the sample was macerated in 3 liters of de-ionized water for 24 hours. The solution was filtered with Whatman no.4 filter paper and the filtrate was concentrated to a semi-solid residue using rotary evaporator at 45- 60 ℃. The extract was stored in a refrigerator at 4 ℃ for subsequent usage.

2.3 Phytochemical screening

The quantitative phytochemical analysis of the sample was conducted using an auto system buck 530 chromatographer in gas phase equipped with an on - column automatic injector, flame ionization detector, and with Hp88 capillary column (100m x 0.25mm) with the following chromatographic conditions; injector temperature 220 ℃, detector temperature 250 ℃, oven temperature to 180 ℃, injection volume 1ml sample, hydrogen was used as a carrier gas (24 pound per square inch). The concentration of each active component was determined based on the ratio between the area and mass of internal standard and area under the peaks of the phytochemicals identified.

2.4 Acute toxicity test of the extract

The method of Lorke [12] with some modifications was adopted to determine the LD50 of the aqueous leaf extract of *Justicia carnea* Hooker. The study was conducted in two phases, male and female mice were fasted overnight. Three groups of 3 mice per cage in the first phase received intraperitoneally 10, 100 and 1000mgkg^{-1} of the aqueous extract. The experimental mice for the first 6 hours and 24 hours were observed for signs of toxicity and mortality. For the second phase, an additional 3 groups of 3 mice per cage received intraperitoneally 1600, 3200 and 5000mgkg^{-1} of the leaf extract. The mice were further observed for signs of toxicity and mortality at regular intervals for 24 hours, 48 hours and 72 hours respectively.

2.5 Experimental animals

Male and female Wistar mice weighing (20-24g) and consisting of 5 rats in each group were used for the study; they were bred and housed in the Animal house, Department of Biochemistry, University of Port Harcourt. The animals were reserved under naturally illuminated surroundings (twelve hours light-dark cycle) in cages and were allowed free access to standard rat pellet and water. Standard protocols for animal handling and treatment was adopted all through the duration of study [13]. Ethical clearance for the study was sought and received from the Animal Welfare Research Ethics Committee, University of Port Harcourt.

2.5.1 Malaria parasite and inoculation of mice

Plasmodium berghei (NK65) that served as the parasite source was obtained from National Institute for Medical Research Yaba, Lagos state, Nigeria. The donor mouse was intraperitoneally administered with a standard inoculum of *P. berghei* on the first day, and blood was withdrawn from the donor mouse via cardiac puncture after 5 days when the parasite had stabilized in the host mouse, and then diluted with isotonic saline. Precisely 0.1ml of acid citrate dextrose and normal saline were drawn into syringe to prepare the inoculums, the experimental mice were inoculated with 0.2 ml of parasite specimen containing about 1×10^7 parasitized cells. Malaria infection was ascertained after 5 days by viewing the Giemsa strained thin blood films prepared with drops of blood from the tail of the infected mice and studied under the microscope (H&E ×100) [14]

2.5.2 Suppressive test

The method explored by [15] was adopted with some modifications to determine the suppressive activity of the aqueous leaf extract of *Justicia carnea*. Thirty wistar mice were intraperitoneally administered 0.2ml of the infected red blood cells on the first day (D0). The experimental mice were separated into six groups of 5 mice in each group and were administered via oral route different concentrations of the extract and standard drugs after 15 minutes. Group 1 was left untreated [negative control] and received daily, $10mgkg^{-1}$ of distilled water, group 2 served as the positive control and was treated with $5mgkg^{-1}$ of chloroquine while group 3 received $4mgkg^{-1}$ of artesunate. Group 4, 5 and 6 were respectively administered $400mgkg^{-1}$, $600mgkg^{-1}$ and $800mgkg^{-1}$ of the extract. The infected mice were treated for a period of 4 days (D0-D3) and parasitic growth was assessed on the fifth day via Giemsa stained thin blood smear under light microscope (H&E ×100) using the formula

$$\%\text{Parasitemia} = \frac{No \text{ of parasitized RBC}}{No \text{ of total RBC}} \times 100$$

$$\text{Av. \% suppression} = \frac{\text{Av. \% parasitemia in the negative control - Av. \% parasitemia in test group}}{\text{Av. \% parasitemia in the negative control}}$$

*Av = Average

The dosage of administration for chloroquine and artesunate was obtained from [7].

2.5.3 Prophylactic test

The prophylactic or repository test was conducted via the method described by [16]. Twenty five wistar mice [20-24g] were randomly separated into 5 groups of 5 mice per group and received $10mgkg^{-1}$ of distilled water (negative control), $4mgkg^{-1}$ of artesunate (positive control), $400mgkg^{-1}$, $600mgkg^{-1}$ and $800mgkg^{-1}$ of the extract via oral routes respectively. The mice were inoculated with 0.2 ml of parasite specimen containing about 1×10^7 parasitized cells and after 3 days, thin blood films were made with the blood droplets from the tail of each mouse stained with 10% Giemsa. The percentage parasitemia and average percentage inhibition or suppression was estimated using the aforementioned formulas.

2.5.4 Curative test

Thirty six wistar mice were intraperitoneally injected standard inocula of 1×10^7 *Plasmodium berghei berghei* on the first day (D0) and after 3 days, the mice were randomly sorted into six groups of 6 mice per cage. Group 1 served as the negative control (untreated) and were administered $10mlkg^{-1}$ of distilled water, group 2 represented (positive control) and they received $5mgkg^{-1}$ of chloroquine, group 3 were administered $4mgkg^{-1}$ of artesunate while the remaining groups were treated with different concentrations of the extract via oral route ($400\text{-}800mgkg^{-1}$ per day). The experimental mice were treated with the drugs and different concentrations of the extract for 7 days. Thin films prepared from the tail blood of each mouse were fixed with methanol and stained with Giemsa to observe the level of parasitemia. Then over a period of 28 days (D0-D27), the mean survival time was accessed using the formula

$$\text{Mean survival time} = \frac{\text{Number of days survived} \times 100}{\text{Total number of days}}$$

2.6 Biochemical assays

The experimental mice were anaestized using dietyl ether on the sixth day and blood was obtained via cardiac puncture for the assessment of the activities of hepato specific markers, oxidative stress enzymes, and hematological parameters. The activity of alanine amino transferase and aspartate amino transferase was measured using the methods of [17]. Concentration of alkaline phosphatase was determined via the method of [18] while the activities of glutathione peroxidase, superoxide dismutase, glutathione and malondialdehyde were also evaluated [19, 20, 21].

2.7 Haematological assay

Blood samples stored in ethylene diamine tetraacetic acid (EDTA) anti-coagulant bottles were assayed using BC 5300 Mindray Hematology Auto-Analyzer.

2.8 Statistical analysis

Obtained data was expressed as mean ± standard error and one way analysis of variance was adopted to determine the difference between the controls and the test groups.

3. Results

3.1 Phytochemistry

The outcome of the phytochemical assessment (Table 1) revealed the presence of alkaloids, tannins, sapogenin and polyphenolic compounds.

Table 1. Phytochemical composition of aqueous leaf extract of *Justicia carnea*

Component	Concentration (µg/ml)
Anthocyanin	0.62 ± 0.06
Oxalate	4.29 ± 0.51
Tannin	15.22 ± 0.04
Sapogenin	13.23 ± 0.06
Lunamarine	22.30 ± 0.05
Ribalinidine	4.85±0.09
Saponin	5.36 ± 0.04
Phytate	0.72 ± 0.05
Rutin	10.43 ± 0.03
Kaempferol	33.61 ± 0.07
Catechin	12.94 ± 0.02

Values are means of three determinations ± standard errors of mean (SEM)

3.2 Acute toxicity test

There was zero mortality in all the treatment groups that received various doses of the extract even at 5000mgkg^{-1} (Table 2).

Table 2. Suppressive activity of aqueous leaf extract of *Justicia carnea* in Plasmodium infected mice

Groups	Dose mgkg-1	Mean parasitemia	% Suppression
Distilled water	10ml	25.10±1.25	-
Positive control	5	0.80±0.01*	96
Artesunate	4	0.72±0.03*	97
Aqueous extract	400	7.25±0.11*	71
Aqueous extract	600	4.38±0.08*	82

Aqueous extract	800	2.00±0.03*	92

Values are expressed as Mean + SEM, *significantly different against the negative control *p< 0.05(n = 5)

3.3 Suppressive activity

The aqueous extract of *Justicia carnea* demonstrated antiplasmodial activity in a dose dependent manner (Table 3). The highest antiplasmodial activity (92%) of the extract was observed in rats treated with 800mgkg^{-1} of the extract and this outcome was comparable with the standard drugs used in the study chloroquine (96%) and artesunate (97%).

3.4 Prophylactic activity

The aqueous extract of the study plant showed statistically significant ($p<0.05$) dose dependent reduction in parasitemia as the concentration of the extract increased from 400mgkg^{-1} (69%) to 800mgkg^{-1} (86%) while the standard drug (artesunate) demonstrated 94% repository activity.

3.5 Curative test

Similar trends was observed in the curative test, however parasitemia rapidly increased in the untreated negative control as the days progressed. The highest curative activity was observed in the artesunate treated group (1.06±0.05), followed by choroquine treated group (1.12±0.01) and the group that received 800mgkg^{-1} of the extract (3.32±0.15). Also, there was a dose dependent increase in the mean survival time in the groups that received the aqueous extract of the study plant. Chloroquine and artesunate treated groups survived all through the 28 days duration and demonstrated the highest mean survival time (Table 4)

3.6 Biochemical Studies

The activities of liver enzymes were significantly ($p<0.05$) elevated when the negative control was compared with the uninfected and untreated group, however the activity of these enzymes dropped in a dose dependent manner as treatment progressed when the treated groups were compared against the negative control (Table 5). The packed cell volume and red blood cell concentration significantly ($p<0.05$) increased (Table 6) only in groups administered 800mgkg^{-1} of the extract (36.22±0.88 and 8.60±0.31), 5mgkg^{-1} of cholroquine (37.00±1.20 and 8.56±0.08) and 4mgkg^{-1} of artesunate (36.16±0.57 and 8.43±0.16) respectively, when compared with the negative control (30.33±1.45 and 6.23±0.21). The changes observed in the white blood cell count across all groups was not significant ($p>0.05$). The neutrophil count of the negative control was significantly ($p<0.05$) elevated when compared with the test groups while a significant ($p<0.05$) decrease was observed in the lymphocyte count of the negative control in comparison with the test groups. The malondialdehyde concentration of the negative control significant increased against the uninfected and untreated group. Nonetheless, the MDA concentration significantly ($p<0.05$) decreased across all treated groups when compared with the negative control (Table 7). In addition, glutathione peroxidase and superoxide dismutase activities significantly improved as treatment progressed in the positive control group (387.25±36.12 and 153±7.82), artesunate treated group (402.18±20.57 and 149±2.64) and group administered 800mgkg^{-1} of the extract (379.12±20.88 and 150.60±3.45) respectively, when compared with the negative control (249.16±17.40 and 130.32±3.62).

Table 3. Prophylactic or repository activity of aqueous leaf extract of *Justicia carnea* in plasmodium infected mice

Groups	Dose mgkg^{-1}	Mean parasitemia	% Suppression
Distilled water	10ml	27.10±1.76	-
Positive control	4	0.46±0.05*	94
Aqueous extract	400	8.47±0.26*	69
Aqueous extract	600	6.05±0.03*	78
Aqueous extract	800	2.72±0.11*	86

Values are expressed as Mean + SEM, *significantly different against the negative control *p< 0.05(n = 5)

Table 4. Curative test and effect of aqueous leaf extract of *Justicia carnea* against Plasmodium in mice Mean parasitemia

Groups	Dose mgkg^{-1}	Day 3	Day 7	Mean survival time
Distilled water	10ml	26.22±0.33	37.33±0.76	9.45±0.08
Positive control	5	27.37±0.15	1.12±0.01*	28.00±0.00*
Artesunate	4	28.26±0.21	1.06±0.05*	28.00±0.00*
Aqueous extract	400	27.00±0.88	9.61±0.12*	16.23±0.45*
Aqueous extract	600	28.52±0.11	5.03±0.03*	19.47±0.32*
Aqueous extract	800	26.88±0.26	3.32±0.15*	21.74±0.55*

Values are expressed as Mean + SEM, *significantly different against the negative control *p< 0.05(n = 5)

Table 5. Effect of aqueous leaf extract of *Justicia carnea* on hepato specific markers in mice

Groups	Dose mgkg^{-1}	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Not inoculated (untreated)	10ml	37.00±1.64e	117.66±1.45e	111.00±3.06e
Negative control	10ml	53.66±3.76e*	149.33±3.28e*	155.62±2.48e*
Positive control	5	31.00±1.20*	125.33±1.18*	117.30±3.25*
Artesunate	4	34.16±0.57*	130.00±1.76*	121.00±1.40*
Aqueous extract	400	47.33±1.75	136.50±1.40	139.33±1.55
Aqueous extract	600	40.00±1.40*	138.32±2.08	128.34±1.88*
Aqueous extract	800	37.22±0.88*	128.20±1.52*	121.33±2.60*

Data are Mean ± SEM of five determinations. [e] significant difference when not inoculated are compared with the negative control ($p<0.05$). * Significant difference when negative control are compared against the test groups.

Table 6. Effect of aqueous leaf extract of *Justicia carnea* on hematological indices of mice

Groups	Conc.	PCV (%)	RBC (10^6/ul)	WBC (10^9/ul)	HGB(g/dl	NEU (%)	LYMP (%)
Uninfected	10ml	39.00±0.57e	9.80±0.11e	6.00±0.36	12.65±0.47e	25.21±1.56e	53.00±0.88*
N. control	10ml	30.33±1.45e*	6.23±0.21e*	4.87±0.21	8.34±0.36e*	78.00±3.00e*	28.25±2.64*
P. control	5	37.00±1.20*	8.56±0.08*	4.23±0.05	14.32±0.45*	40.32±2.46*	40.33±3.65*
Artesunate	4	36.16±0.57*	8.43±0.16*	4.06±0.40	12.00±0.27*	33.38±1.72*	47.62±2.00*
Extract	400	31.23±1.75	6.53±0.08	5.00±0.51	8.77±0.15	46.00±4.32*	36.22±1.25*
Extract	600	34.65±1.40	6.72±0.04	5.35±0.04	9.33±0.33	43.00±3.78*	39.00±1.45*
Extract	800	36.22±0.88*	8.60±0.31*	4.18±0.60	11.86±0.52*	44.33±1.45*	39.42±1.15*

Data are Mean ± SEM of five determinations. [e] significant difference when not inoculated are compared with the negative control ($p<0.05$). * Significant difference when negative control are compared against the test groups. WBC= White blood cell, HGB= hemoglobin, NEU= neutrophils, RBC= red blood cell, PCV= packed cell volume, LYMP= lymphocytes N. control= negative control, P. control= Positive control.

Table 7. Effect of aqueous leaf extract of *Justicia carnea* on antioxidant enzymes and lipid peroxidation marker in mice

Groups	Dose mgkg^{-1}	Glutathione peroxidase (U/L)	Superoxide dismutase U/ml	Total Glutathione (ng/µL)	Malondialdehyde (µmol/L)
Not inoculated	10ml	485.85±19.62e	158.40±6.52e	1.32±0.01	1.42±0.02e
Negative control	10ml	249.16±17.40e*	130.32±3.62e*	0.93±0.26	3.90±0.13e*
Positive control	5	387.25±36.12*	153±7.82*	1.18±0.05	1.70±0.08*
Artesunate	4	402.18±20.57*	149±2.64*	1.26±0.40	1.64±0.05*
Aqueous extract	400	306.43±15.62	137.53±1.73	1.00±0.51	2.84±0.03
Aqueous extract	600	365.52±30.33*	144.00±4.64*	1.08±0.04	1.71±0.06*
Aqueous extract	800	379.12±20.88*	150.60±3.45*	1.13±0.60	162±0.22*

Data are Mean ± SEM of five determinations. [e] significant difference when not inoculated are compared with the negative control ($p<0.05$). * Significant difference when negative control are compared against the test groups

4. Discussion

Plants with optimal therapeutic benefits exert such attributes as a consequence of the bioactive constituents inherent in them, such as flavonoids, alkaloids, saponins, phenol, terpenoids and cyanogenic glycosides. The studied plant did not fell short of expectation in this regard as the quantitative phytochemical screening revealed the presence of most of these important bioactive constituents. Alkaloids, flavonoid, terpenoids, saponins and glycosides have all been implicated to posses antiplasmodial activity [22, 23] among other beneficial effects. Alkaloids are well recognized bioactive compounds with potent therapeutic activity, they are also, the parent compounds for synthetic products like chloroquine [7]. Alkaloids exert antimalrial effects by disrupting protein synthesis in the parasite while flavonoids chelate with nucleic acid base pairing of the parasite [24]. Sporozites, the infective stage of plasmodium require circumsporozite protein (CSP) and transmembrane proteins (TRAP) which covers the external surface of the sporozite and enhance its gliding motility to reach the liver [25]. On the other hand, proanthocyanidins (condensed tannins) form complexes with proteins that impair the gliding motility of sporozites, and also chelate zinc and iron which are crucial to the growth and replication of the parasite [25]. Compounds with oral LD_{50} of 5000mgkg^{-1} body weight or more are considered practically harmless. Nonetheless, extreme high doses might not be safe [12] and consequently, the LD_{50} of the aqueous extract of *Justicia carnea* leaves may be greater than 5000mgkg^{-1} body weight. Aqueous leaf extract of *Justicia carnea* demonstrated considerable anti-plasmodial activity against established and early infections in a dose dependent fashion. The results obtained from the 4 days suppression test showed a blood schizonticidal activity of 92% in group administered 800mgkg^{-1} of the extract and comparable to the 96% and 97% activities observed in groups treated with cholroquine and artesunate respectively. The mean survival time in group treated with 800mgkg^{-1} of the extract was impressive and more than doubled the mean survival time of the negative control. The studied plant also exhibited prophylactic activity, however, the highest chemo-suppression activity of 86% observed in the group treated with 800mgkg^{-1} of the extract was not comparable to the suppression activity of the standard drug used (aretsunate). In general, the aqueous extract of *Justicia carnea* exhibited considerable antiplasmodial activity possibly due to the bioactive compounds such as alkaloids, flavonoids and tannins present in the studied plant, though further purification of the extract is required to improve and enhance its therapeutic potentials against malaria parasites. The standard antimalarial drugs used in the study showed potent antimalarial activity in the entire conducted test. The plasmodicidal action of chloroquine and its exact mechanism is not clearly elucidated. Chloroquine like other quinoline derivatives is believed to posses lysomotropic characteristics via its ability to inhibit heme polymerase activity and thus, result in the accumulation of free heme in the food vacuole of the parasite thereby interfering with basic metabolism and ultimately result in the death of the parasite. Aretsunate, an artemisinin drug also portrays a similar mechanism of action with chloroquine through its ability to involve cleavage of the endoperoxide bond via reaction with haem, and this action generates free radicals that alkylate parasitic proteins [26]. Artesunate inhibits malaria proteins EXP1, a glutathione S-transferase which aids the parasite to break down cytotoxic hematin [26]. Alterations in the metabolic functions of the liver as a result of parasitic infections may disrupt the activities of liver enzymes considered as markers of hepatocellular health and are needed for optimal cellular function. Liver damage in sever malaria infection might manifest in portal tract inflammation, cholestasis, sequestration of parasitized erythrocytes and deposition of hemozoin pigment [27]. These changes may influence the consequent release of these enzymes into the blood stream. The total polyphenolic composition of the aqueous extract of the studied plant might have contributed to its antioxidant activity and the subsequent amelioration of the damage to the liver due to oxidative stress. This outcome was clearly evident in the activities of the liver enzymes in groups treated with the highest dose of the extract (800mgkg^{-1}) and the standard drugs in the present study. The observed significant decrease in packed cell volume and RBC count of the negative control against the uninfected group was not astonishing due to the lysing of infected blood cells which also affected erythropoiesis. This outcome corroborates the reports of [28, 29]. Nonetheless, the considerable increase in RBC, PCV, and HGB concentrations observed in groups treated with the standard drugs and the highest dose of the extract suggest enhancement of erythropoiesis in the experimental animals. The changes in WBC count across all groups were not significant while the neutrophil and lymphocyte counts exhibited considerable changes. Increased neutrophil count often portrays an infection while decreased lymphocyte counts suggest an invaded immune system [29, 30]. Hence, the increase in lymphocyte count and the concomitant decrease in neutrophil count observed in all treated groups suggest improvements in immune function. Malaria

parasites exert oxidative stress during the conversion of heme (ferroprotoporphyrin) to hematin within the host's parasitized red blood cells and also, via the activation of the immune system resulting in the release of reactive oxygen species [31]. In addition, phagocytosis entails the production of superoxide anion subsequent to the creation of the NADPH-oxidase where leucocytes are able to swallow up the infectious agent [32]. The formations of these ROS if not regulated by the host cytoprotective antioxidants and enzymes or by exogenous substances could lead to oxidative damage [33]. A decline that is significant ($p<0.05$) in the activities of the anti-oxidant enzymes such as SOD and glutathione peroxidase coupled with a higher lipid peroxidation value was observed in the negative control against the uninfected and untreated group. A considerable ($p<0.05$) decrease in the lipid peroxide values and increase in the level of antioxidant enzymes were seen in groups administered the standard drugs, $600mgkg^{-1}$and $800mgkg^{-1}$ of the extract. This outcome is in agreement with the earlier report of [33]. Superoxide dismutase, glutathione peroxidase and catalase, through their respective actions shield the body system from the deleterious effects of free radicals and activated species [33]. The decrease in the level of glutathione observed in the plasmodium infected untreated mice suggests possible increased utilization as a result of oxidative stress while the considerable increase in the activity of these antioxidant enzymes in groups administered the standard drugs and increased doses of the extract points to positive effects of the treatment which led to possible reversal and improvements in the radical scavenging capabilities of these antioxidant enzymes.

Conclusion

The study has shown the antimalarial potentials of the aqueous leaf extract of *Justicia carnea* which validates its continuous use in folkloric medicine, this beneficial effect can be harnessed in combination therapy with other antimalarial drugs through the isolation and characterization of the bioactive molecules present in the studied plant in order to achieve total clearance of malaria parasite.

Conflict of interest

They authors declare no conflict of interest.

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