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Ndem JI

Department of Biochemistry, Faculty of Basic Medical Sciences, University of Uyo, Uyo, Akwa Ibom State, Nigeria

Uwah AF

Department of Biochemistry, Faculty of Basic Medical Sciences, University of Uyo, Uyo, Akwa Ibom State, Nigeria

Effiong BO

Department of Biochemistry, Faculty of Basic Medical Sciences, University of Uyo, Uyo, Akwa Ibom State, Nigeria

Bassey UE

Department of Biochemistry, Faculty of Natural and Applied Sciences, Obong University, Etim Ekpo, Akwa Ibom State, Nigeria

Umanah BM

Department of Biochemistry, Faculty of Basic Medical Sciences, University of Uyo, Uyo, Akwa Ibom State, Nigeria

Chukwudike CP

Department of Biochemistry, Faculty of Basic Medical Sciences, University of Uyo, Uyo, Akwa Ibom State, Nigeria

Correspondence:

Ndem JI

Department of Biochemistry, Faculty of Basic Medical Sciences, University of Uyo, Uyo, Akwa Ibom State, Nigeria
Email: jessndem@yahoo.com

Ethanol leaf extract of *Jatropha tanjorensis* ameliorates hepatorenal toxicity of *Plasmodium berghi-berghi* infected mice treated with *Hippocratea africana* root bark extract

Ndem JI*, Uwah AF, Effiong BO, Bassey UE, Umanah BM, Chukwudike CP

ABSTRACT

The effect of ethanol leaf extract of *Jatropha tanjorensis* on hepatorenal function of *Plasmodium berghi-berghi* infected mice treated with root bark extract of *Hippocratea africana* was evaluated. Twenty-One (21) male mice weighing between 27 – 33 g used for the study were divided into seven groups. Group 1 served as normal control while Groups 2 – 7 were parasitized with *Plasmodium berghi-berghi* and Group 2 was the test control group (parasitized without treatment). Group 3 was administered 8 mg/kg bw of artemether-lumefantrine for 3 days. Group 4 and 5 received daily, 200 mg/kg bw and 300 mg/kg bw of *Hippocratea africana* and *Jatropha tanjorensis* respectively for 4 days. Group 6 received 8mg/kg bw of artemether-lumefantrine for 3 days followed with 300 mg/kg bw of *Jatropha tanjorensis* for 4 days. Group 7 was treated with 200 mg/kg bw of *Hippocratea africana* for 4 days followed by 300 mg/kg bw of *Jatropha tanjorensis* for 4 days. The concentration of urea, creatinine and the activities of the liver enzymes were observed to increase significantly following induction of malaria when compared to normal control. Treatment with artemether-lumefantrine and root bark extract of *Hippocratea africana* showed drug induced hepatorenal toxicity which was ameliorated with the administration of ethanol leaf extract of *Jatropha tanjorensis*. The study showed that *Jatropha tanjorensis* leaf extract had hepatorenal protective function against *Plasmodium berghi-berghi* infection and malaria treatment induced toxicity, that may be due to its rich phytochemicals with antioxidant activity.

Keywords: *Jatropha tanjorensis*, *Hippocratea africana*, Malaria, Artemether-Lumefantrine, Hepatorenal Function.

INTRODUCTION

Malaria is an infectious disease with enormous public health implication and economic consequences especially in Africa and Asia. Nigeria has been reported to account for 25% of all malaria cases in Africa with an all year transmission cycle in the southern and seasonal transmission cycle in the northern part of the country [1, 2]. The parasitic infection is responsible for significant morbidity and mortality among children and pregnant women with an estimated yearly mortality rate of 2 million [3].

The treatment of malaria has metamorphosed from the use of monotherapies to combination therapies and presently to the World Health Organisation (WHO) recommended artemisinin-based combination therapies (ACTs). However, malaria therapy has faced several challenges due to development of resistance by the plasmodium parasite [4], availability, affordability and efficacy of antimalarial drugs [5]. Cytotoxicity and undesirable side effects of antimalarial drugs also contribute to these challenges in the treatment of malaria [6].

In combating with the outlined challenges militating against malaria chemotherapy, herbal therapy has been widely utilized as alternative means of treating malaria [7]. There is a growing interest and dependence on herbal malaria therapy especially in the rural communities of Africa and Southern Asia where the disease is predominant. Current trend has also shown that herbal therapy for malaria are also being taken after completion of a chemotherapeutic regimen for malaria. This is a deliberate practice in the rural area because of dependence on herbal therapy or due to persistence of symptoms of malaria after a chemotherapeutic regimen has failed.

One of the plants often utilized by the locals in the South Eastern part of Nigeria for the treatment of malaria is *Hippocratea africana* and there are several documented reports establishing its antimalarial efficacy [8, 9]. *Hippocratea africana* is a green forest perennial climber. The root of the plant is traditionally used in the treatment of various ailments such as fever, malaria, body pains, diabetes and diarrhea [9]. The root bark extract of *Hippocratea africana* has been reported to be rich in phytochemicals such as alkaloids, cardiac glycosides, tannins, anthraquinones and flavonoids [7] conferring on it, the reported medicinal potentials.

Infection with plasmodium parasite and malaria treatments are associated with altered biochemical parameters and have effects on several organs in the body. Malaria and its treatments have also been known to affect blood parameters necessitating the use of hematopoietic agents [5]. Herbal extract is widely taken after malaria treatment amongst the locals. one of such herbs is *J. tanjorensis* commonly called "Hospital too far" in the South Eastern part of Nigeria. It is consumed as vegetable and the leaf extract have been reported to have hematopoietic potential [8]. Many edible vegetables have been reported to have good antioxidant and cyto-protective properties [10].

The aim of this study was to evaluate the effect of ethanol leaf extract of *Jatropha tanjorensis* on hepatorenal function of *Plasmodium berghei-berghi* infected mice treated with root bark extract of *Hippocratea africana*.

MATERIALS AND METHODS

Plant Material

The root of *Hippocratea africana* was collected from Afaha Etok forest in Ibesikpo-Asutan Local Government Area, Akwa Ibom State. It was identified and authenticated by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo. Uyo. The voucher number (UUH3394) was assigned. The roots were gently washed to get rid of debris and processed to obtain crude extract according to the method described by Ndem and Bassey, [11]. The bark was peeled from the root, cut into small pieces and pulverized. The pulverized sample was macerated in 80% ethanol (Sigma Aldrich) for 72 hours. After 72 hours, the clear orange colour supernatant was carefully and concentrated in a Waterbath at 40°C to obtain the crude extract.

Fresh leaves of *Jatropha tanjorensis* were collected from Uyo metropolis, Akwa Ibom State, Nigeria. The leaves were washed and reduced to pieces using a manual grinder. It was then macerated in water for 6 hours, filtered and the filtrate concentrated to dryness in Waterbath at 40°C to obtain brownish crude extract. Both extracts were preserved in a refrigerated at 4°C and used for this study.

Synthetic Drugs

Artemether-Lumefantrine [Coartem®, Novartis] was obtained from Uchris Pharmacy in Uyo Metropolis in Akwa Ibom State, Nigeria.

Experimental Animals and Design

Twenty-One (21) male mice weighing between 27 – 33 g were used for the study. They were obtained from the Animal House, Department of Pharmacology, Faculty of Pharmacy, University of Uyo, Uyo, Akwa Ibom State, Nigeria. The animals were maintained under standard laboratory conditions and fed with normal rat chow and clean drinking water *ad libitum*. The mice were divided into 7 groups with 3 animals in each group.

Group 1 served as normal control while Groups 2 – 7 were parasitized with *Plasmodium berghei-berghi* and Group 2 was the test control group (parasitized without treatment). Group 3 was administered 8 mg/kg bw of artemether-lumefantrine for 3 days. Group 4 and 5 received daily, 200 mg/kg bw and 300 mg/kg bw of *Hippocratea africana* and *Jatropha tanjorensis* respectively for 4 days. Group 6 received 8mg/kg bw of artemether-lumefantrine for 3 days followed with 300 mg/kg bw of *Jatropha tanjorensis* for 4 days. Group 7 was treated with 200 mg/kg

bw of *Hippocratea africana* for 4 days followed by 300 mg/kg bw of *Jatropha tanjorensis* for 4 days.

Malaria Parasite and Inoculation

Malaria parasite, *Plasmodium berghei-berghi* was obtained from the Department of Pharmacology, University of Uyo, Uyo, Nigeria through a donor mouse. The experimental animals in the present study were induced with malaria according to the method described by Ndem and Bassey, [11]. The parasite was obtained from the donor mouse through cardiac puncture after being anaesthetized with chloroform. The blood with a parasitaemia of 10^7 was diluted with normal saline and 0.3 ml of the infected blood was passage intraperitoneally into each of the mouse. The parasites were inoculated for 7 days then the animals were confirmed to be infected with malaria through microscopic examination of blood films from the tail of each mouse.

Collection of Blood Sample

The animals were fasted overnight after the administration of the last dosage of the extracts and drugs but were still allowed water *ad libitum*. They were anaesthetized with chloroform and blood samples were obtained by cardiac puncture using sterile needles and syringes into plain sample tubes. The blood was allowed to clot and centrifuged at 3,000 rpm for 15 minutes to obtain the serum which were used for assay of some parameters of liver and kidney function.

Assay of Biochemical Parameters

Biochemical parameters of liver function (serum liver enzyme – ALT, AST and ALP activities) and some parameters of renal function (urea and creatinine concentrations) were assayed using the protocol described by assay kit manufacturer (Randox Diagnostic, UK) in the reagent leaflet.

Statistical Analysis

Statistical analysis was carried out using Widows SPSS Version 25. One-way analysis of variance (ANOVA) and Least Significant Difference Multiple Post Hoc test were employed for comparison to assess statistical significance. All the results are presented as Mean \pm Standard Deviation (SD). Probability level < 0.05 was considered significant.

RESULTS

Effect of *Jatropha tanjorensis* Leaf Extract on Liver and Kidney Function in *Plasmodium berghei-berghi* Infected Mice Treated with Root Bark Extract of *Hippocratea africana*

The results of the effect of ethanol leaf extract of *Jatropha tanjorensis* on hepatorenal function of *Plasmodium berghei-berghi* infected mice treated with root bark extract of *Hippocratea africana* are presented in Tables 1 and 2. Table 1 shows the effect of the treatments on the activities of the liver enzyme while Table 2 presents the urea and creatinine concentration following the treatments in the present study.

DISCUSSION

The prevalence of malaria continues to increase globally despite advances in the area of malaria chemotherapy. Malaria is a public health problem associated with poverty with deleterious effect on the economy of endemic countries. Resistance of the malaria parasite to

Table 1: Effect of *Jatropha tanjorensis* Leaf Extract on Liver Enzyme Activity of *Plasmodium berghi-berghi* Infected Mice Treated with Root Bark Extract of *Hippocratea africana*

Groups/Treatments	ALT Activity (IU/L)	AST Activity (IU/L)	ALP Activity (IU/L)
1 – Normal Control	25.33 ± 1.53	96.00 ± 2.65	47.00 ± 1.00
2 – Test Control	38.33 ± 8.51*	141.70 ± 17.50*	44.33 ± 3.51
3 – 8 mg/kg bw of artemether-lumefantrine for 3 days	54.33 ± 8.51*	161.70 ± 10.97*	59.33 ± 3.51*
4 – 200 mg/kg bw of <i>Hippocratea africana</i> for 4 days	36.33 ± 3.01*	126.30 ± 14.50*	34.67 ± 1.53*
5 – 300 mg/kg bw of <i>Jatropha tanjorensis</i> for 4 days	25.50 ± 6.36	99.00 ± 4.43	40.67 ± 6.01
6 – 8mg/kg bw of artemether-lumefantrine followed with 300 mg/kg bw of <i>Jatropha tanjorensis</i>	34.00 ± 3.00	136.00 ± 9.17*	42.00 ± 4.36
7 – 200mg/kg bw of <i>Hippocratea africana</i> followed with 300 mg/kg bw of <i>Jatropha tanjorensis</i>	20.00 ± 3.00	81.67 ± 6.51	33.50 ± 4.95

Data presented as Mean ± Standard Deviation (SD). Mean difference was considered significantly different at $p < 0.05$. * = significantly different when compared to Group 1 at $p < 0.05$.

Table 2: Effect of *Jatropha tanjorensis* Leaf Extract on Concentration of Urea and Creatinine in *Plasmodium berghi-berghi* Infected Mice Treated with Root Bark Extract of *Hippocratea africana*

Groups/Treatments	Urea Conc. (mmol/L)	Creatinine Conc. (µmol/L)
1 – Normal Control	7.00 ± 0.40	25.00 ± 2.00
2 – Test Control	10.23 ± 1.06*	45.00 ± 6.24*
3 – 8 mg/kg bw of artemether-lumefantrine for 3 days	15.18 ± 1.32*	60.74 ± 4.98*
4 – 200 mg/kg bw of <i>Hippocratea africana</i> for 4 days	9.50 ± 1.40	34.00 ± 2.00*
5 – 300 mg/kg bw of <i>Jatropha tanjorensis</i> for 4 days	6.60 ± 0.62	29.00 ± 1.00
6 – 8 mg/kg bw of artemether-lumefantrine followed with 300 mg/kg bw of <i>Jatropha tanjorensis</i>	7.20 ± 0.72	30.33 ± 4.50
7 – 200 mg/kg bw of <i>Hippocratea africana</i> followed with 300 mg/kg bw of <i>Jatropha tanjorensis</i>	6.73 ± 0.03	31.00 ± 3.60

Data presented as Mean ± Standard Deviation (SD). Mean difference was considered significantly different at $p < 0.05$. * = significantly different when compared to Group 1 at $p < 0.05$.

available drugs, unavailability and unaffordability of the drugs in some areas continue to pose serious challenges to the eradication of the disease [5]. These challenges necessitate the need for alternative treatment for malaria. Plant sources have been widely utilized as a safer alternative for malaria therapy. Antioxidants from plant sources have also received a lot of attention and are preferred to synthetic ones due to their health benefits, availability, affordability and safety profile [12]. Furthermore, the complications of malaria infection have been reported to include altered biochemical parameters in the host which reflects pathologies associated with certain organ dysfunction. Organs with altered biochemical function in malaria infection include the liver, kidney, blood, brain and many others. In addition, administration of antimalarial therapy has also been reported to worsen the deteriorating organ function associated with malaria infection. Consequently, antioxidants and haematopoietic agents are often recommended after successful malaria chemotherapy in clinical practice. In view of these underlining issues, the present study was designed to evaluate the effect of *Jatropha tanjorensis* leaf extract on some parameters of hepatorenal function in *Plasmodium berghi-berghi* infected mice treated with *Hippocratea africana* root bark extract.

The kidney maintains optimum chemical composition of the body fluids by removal of metabolic wastes such as urea, uric acid, electrolytes and creatinine. These parameters have been reported to increase significantly in serum in a state of renal dysfunction [13] hence are used as biomarkers of kidney function. The present study revealed that urea and creatinine concentrations were significantly increased compared to normal control following induction of *Plasmodium berghi berghi* parasites in the experimental animals. This further establish the occurrence of malaria induced nephrotoxicity. However, administration of *Hippocratea africana* root bark extract and *Jatropha tanjorensis* leaf extract singly, caused a significant decrease in the serum creatinine and urea concentrations when compared to the test control. These parameters are freely filtered by the glomeruli of the kidneys [14]. Creatinine is produced by the metabolism of creatinine

phosphate in the skeletal muscle but can also be obtained from dietary sources such as meat [15] while urea is a metabolic product of protein metabolism. The observed decrease in urea and creatinine concentration in the present study when the extract treated groups were compared to the test control group (Group 2) is an indication of nephroprotective potential of the plants. The administration of artemether-lumefantrine significantly increased the concentration of urea and creatinine, an indication of nephrotoxicity following the administration of the drugs. This corroborates earlier reports that antimalarial drugs exert deleterious effect on the kidney [16].

Significant changes in creatinine and urea concentrations were not observed following the administration of *Jatropha tanjorensis* leaf extract after treatment with artemether-lumefantrine or *Hippocratea africana* root bark extract when compared to the normal control, showing the nephroprotective potential of the *Jatropha tanjorensis* leaf extract which was used as hematopoietic agent in the present study. The nephroprotective potential of the extracts may be mediated by the antioxidant activity of the herbal extracts due to the presence of phytochemical such as flavonoid in the plant [17]. Flavonoids have been reported to possess antioxidant and free radical scavenging properties which confers on them nephron-protective activity [18]. The observed decrease in urea and creatinine implies that glomerular function of the renal tubules of the experimental animals were restored by the extracts. This corroborates report by Ndem et al., [5] that *Hippocratea africana* root bark extract possesses nephroprotective activity.

Investigation into the effects of plasmodium parasites on serum enzymes activities have gained recognition as an important area of research in the pathogenesis of malaria [19]. Malaria infection has been reported to be associated with liver damage which manifest as increase in liver enzyme activities in serum [20]. The result of the present study showed increased activity of AST and ALP following infection with *Plasmodium berghi berghi* and is in alignment with reports by

Enemchukwu et al., [20]. It has been reported that infective sporozoites invade and multiply in the hepatocytes and erythrocytes causing destruction to the tissues [21].

The present study also showed hepatoprotective effect of *Jatropha tanjorensis* leaf extract when administered alone to parasitized mice while *Hippocratea africana* root bark extract resulted in elevated activities of liver enzymes under investigation when compared to the control. Hepatotoxicity has been reported as a serious adverse effect associated with malaria therapy [22]. *Hippocratea africana* root bark extract has been previously reported to increase ALT and AST activities in albino Wistar rats and was attributed to first line defense of an organism in response to foreign substance(s) [23]. This corroborates the result of the present study. The administration of *Jatropha tanjorensis* leaf extract after treatment with *Hippocratea africana* root bark extract improved the liver function as the activities of the liver enzymes were decreased compared to the test control group. Furthermore, interaction of artemether-lumefantrine and *Jatropha tanjorensis* leaf extract decreased the activity of ALT, AST and ALP when compared to artemether-lumefantrine alone treated group. The observed effect may be due to the free radical scavenging properties of flavonoids that are richly present in *J. tanjorensis* [18].

CONCLUSION

It can be concluded that malaria infection induces hepatorenal toxicity in albino mice. Treatments with *Hippocratea africana* root bark extract and artemether-lumefantrine showed drug-induced elevation of the liver and kidney function parameters that were ameliorated with *J. tanjorensis* leaf extract treatment. The presence of bioactive principles such as flavonoids in *Jatropha tanjorensis* leaf extract may be responsible for the improved hepatorenal function in the plasmodium berghi berghi infected and treated mice.

Conflict of interest

The authors declare no conflict of interests

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