

Original Research Article

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STUDIES ON ANTIMALARIAL ACTIVITY AND LIVER HISTOPATHOLOGICAL CHANGES OF *ARTOCARPUS ALTILIS* ON *PLASMODIUM BERGHEI*-INFECTED MICE

M. I. Udonkang^{1*}, M. A. Eluwa², B. K. Enun², P. C. Inyang-Etoh¹, I. J. Inyang¹

1. Department of Medical Laboratory Science, Faculty of Allied Medical Sciences, College of Medical Sciences, University of Calabar, Calabar, Nigeria.

2. Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, University of Calabar, Calabar, Nigeria.

ABSTRACT: *Artocarpus altilis* (breadfruit) leaf is used in the treatment of various ailments including malaria. This study was to investigate the acute toxicity (LD₅₀), antiplasmodial and liver histopathological effects of aqueous extract of breadfruit leaves. Mice infected with NK65 strain of *Plasmodium berghei* were administered 500mg, 1000mg, and 1500mg/kg of extract for 4 days after acute toxicity testing. Giemsa-stained blood films, paraffin wax-embedded haematoxylin and eosin-stained and Periodic acid schiff-stained liver sections were evaluated. The mean lethal dose was 1414.2mg/kg. Percentage suppression of parasite was 76%, 80%, 96%, and 82.5% for 500mg, 1000mg, 1500mg/kg and 5mg chloroquine respectively. This was statistically significant (p=0.001). The 500/1000mg groups had mild sinusoidal dilatation and congestion while 1500mg group had moderate sinusoidal dilatation. Pale glycogen staining with Periodic acid schiff stain was observed around areas congested with merozoites. Thus, aqueous extract of breadfruit leaves has antiplasmodial properties and is safer at lower doses.

KEYWORDS: *Artocarpus altilis*, antiplasmodial, *Plasmodium berghei*, histopathology, liver, glycogen

***Corresponding Author: M. I. Udonkang**

Department of Medical Laboratory Science, Faculty of Allied Medical Sciences, College of Medical Sciences, University of Calabar, Calabar, Nigeria.

*Email Address: – mfonisoudonkang@gmail.com

1. INTRODUCTION

Artocarpus altilis is also called *Artocarpus communis* and *Artocarpus incises* which is commonly known as breadfruit [1]. It is an edible tropical plant that belongs to the family Moraceae which is rich in carbohydrates [2]. The genus *Artocarpus* has more than 50 species and the extracts from their leaf, stem, fruit and bark contain numerous biologically active compounds [1]. These active compounds are rich in prenylated flavones, isoprenylated flavonoids, and flavonone. These compounds have been reported for various biological activities such as antiplasmodial, antibacterial, antitubercular, antiviral, antifungal, antiplatelet, antiarthritic, and antihypertensive effects; tyrosinase inhibitory and cytotoxicity [3]; [4]. Among over 130 compounds isolated from breadfruit, artocarpanone, artoindonesianin F and cycloheterophyllin have been reported for their antiplasmodial activities in mice [5]. This research on plasmodium species is bore out of the need to find more locally available, effective and affordable medicinal plants for the treatment of malaria in Nigeria. Malaria is highly endemic in Nigeria [6]. It is mostly transmitted by *Plasmodium falciparum* which is the major cause of malaria and the leading cause of morbidity and mortality in Nigeria [6]; [7]. Falciparum malaria is still responsible for about 60 per cent outpatient visits to hospitals in Nigeria. In addition, children under five years of age and pregnant women are the most at risk group of infection [6]. This high endemicity is largely due to her climatic and environmental factors that favour the breeding of the mosquito vector [7]. As such effective treatment remains the best option for proper management of malaria. Thus, this work is carried out to ascertain the antimalarial properties and determine the histopathological effect on the liver from aqueous extract of *Artocarpus altilis* leaves.

2. MATERIALS AND METHODS

Plant material

Fresh leaves of *Artocarpus altilis* were collected from the University of Calabar campus and were authenticated in the Department of Botany, University of Calabar with Voucher No. 385.

Plant Extraction

The fresh leaves were air-dried, blended and powdered leaves (413g) were extracted with distilled water for 48 hours at room temperature. After evaporation and concentration, 11.4g of the crude extract was obtained.

Experimental animals

Swiss albino mice weighing 17-24g were used for the research. Five mice infected with NK65 strain of chloroquine sensitive *Plasmodium berghei* were gotten from the Nigerian Institute of Medical Research, Yaba, Lagos. Apparently healthy mice were gotten from the Department of Pharmacology, University of Calabar, Calabar. The animals were housed in the Animal house, College of Medicine, University of Calabar under standard conditions and fed with animal chow

and water *ad libitum*. All animals were treated under laid down procedures given by the Animals Ethical Committee of the College of Medicine, University of Calabar.

Acute toxicity testing

The LD50 of the aqueous crude leaf extract of *Artocarpus altilis* was carried out using the Lorke's method [8]. The mice were observed for signs of toxicity.

Animal inoculation

The infected mice were allowed to acclimatize. Serial passage of the parasite was done by taking blood through cardiac puncture after chloroform inhalation of a donor mouse into 25 recipients' mice. Inoculation was done according to method of Okokon et al. [9]. A standard inoculum of 1×10^7 was used by diluting 0.5ml of the blood in 5ml normal saline after which 0.2ml of the diluted blood was passage intraperitoneal into the recipients' mice. The mice were allowed for three days to establish peripheral parasitaemia before the administration of extract. This was done by staining the thick and thin blood films of tail blood stained with 10% Giemsa stain for 15 minutes.

Study design/Administration of extract

The study design was the 4-day curative or Rane test by Ryley and Peter [10] to check the schizontocidal effect of the extract on the parasite. On day 4 after passage of *P. berghei*, the mice were weighed and randomly divided into five groups of five animals each. Group A-C were given 500mg, 1000mg, and 1500mg/kg body weight respectively; once a day for 4 days. Group D was the positive control comprising infected mice treated with chloroquine (5 mg/kg) once a day for 4 days while group E was the negative control comprising infected mice given distilled water only. Thick and thin tail blood films were taken for each day (1-4). On day 5, the mice were sacrificed by chloroform inhalation method and the liver tissues were removed and fixed in 10% formal saline. The thick and thin blood films were stained in 10% Giemsa stain for 15 minutes and examined microscopically for parasites. Parasitaemia level was determined by counting the number of infected erythrocytes out of 1000 erythrocytes. Average percentage chemosuppression was calculated using $100(A-BA)$, where A is the average percentage parasitaemia in the negative control group and B, average percentage parasitaemia in the test group [9].

Histological analysis/Tissue processing and staining

The liver tissues were processed using the routine paraffin wax infiltration method. Embedded tissues were sectioned with a rotary microtome and stained with Cole's haematoxylin and eosin and Periodic acid schiff methods. Stained slides were viewed under a light microscope with x10 and x40 objectives. Photomicrographs were taken with a photomicroscope.

Data analysis

The results were expressed as percentages and data was analysed using Statistical Package for Social Sciences (SPSS) version 20 (Armonk, New York: IBM Corporation). Results were calculated using ANOVA and was statistically significant at probability level less than or equal to 0.05.

3. RESULTS AND DISCUSSION

Acute toxicity testing

The dose of 2000 mg/kg and above showed physical signs of toxicity. All mice treated with 3000 mg/kg and above all died immediately after extract administration. The oral LD50 of 1414g was obtained.

Curative test

The parasitaemia level in the negative control increased while that of the test groups and positive control decreased gradually from day 1-4. Parasitaemia in the negative control increased from 1.5%-10.9%. On day 4, percentage parasitaemia was 1.2%, 1.0%, 0.2% and 0.9% for 500, 1000, 1500mg/kg body weight and 5mg chloroquine respectively (Table 1). Table 2 shows the percentage suppression of 76%, 80%, 96% and 82.5% for 500, 1000, 1500mg/kg and 5mg chloroquine respectively. This was statistically significant ($p=0.001$).

Table 1: Percentage parasitaemia among the groups

Day	Gp A-500mg (%)	Gp B-1000mg (%)	Gp C-1500mg (%)	Gp D-Chloroquine-5mg (%)
1	1.7	2.0	1.0	1.8
2	1.6	1.8	0.7	1.5
3	1.3	1.5	0.5	1.3
4	1.2	1.0	0.2	0.9
F=12.551	df=15	P=0.001		

Table 2: Percentage suppression of malaria parasite among the groups

Day	Gp A-500mg (%)	Gp B-1000mg (%)	Gp C-1500mg (%)	Gp D-Chloroquine-5mg (%)
1	66	60	80	60
2	68	64	86	80
3	74	70	90	85
4	76	80	96	86.5
F=12.551	df=15	P=0.001		

Histopathological studies

The H&E-stained liver sections in plate 1B and 1C were observed to have mild hepatic changes such as mild sinusoidal dilatation (D), hypochromatic staining of hepatocytes cytoplasm (N) and sinusoidal congestion with plasmodium merozoites (M) similar to the negative control in plate 1A. Plate 1D had moderate sinusoidal dilatation (D), dilatation of the central vein (CV) and sinusoidal congestion with merozoites (M).

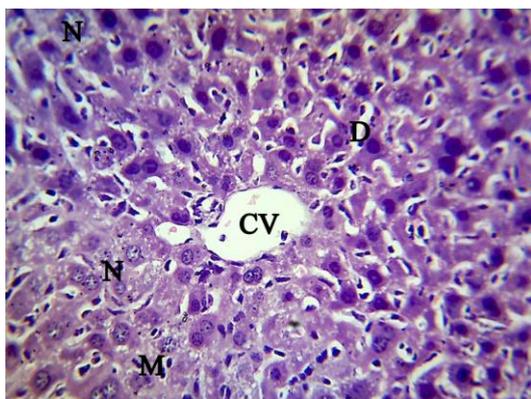


Plate 1A: Negative control

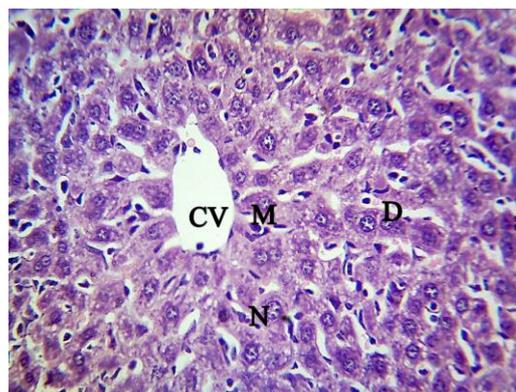


Plate 1B: 500mg/kg

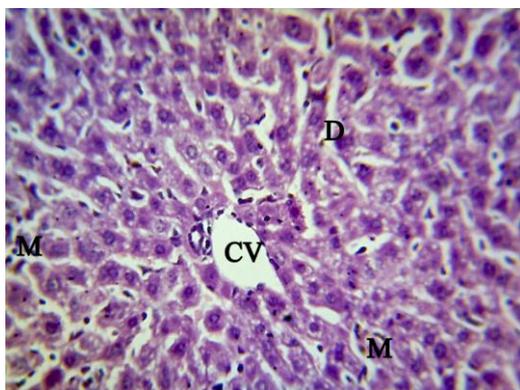


Plate 1C: 1000mg/kg

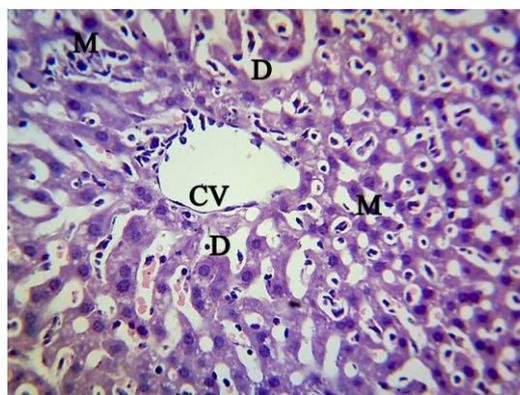


Plate 1D: 1500mg/kg

Plate 1: Photomicrographs of liver sections showing hepatic changes. Sinusoidal dilatation (D), hypochromatic staining (N) of nuclei and sinusoidal congestion with merozoites (M) are found in 1A-D. H&E x400 mag.

Histochemical studies

The intensity of staining of glycogen in the hepatocytes with periodic acid schiff stain shows very pale stained areas (P) among the hepatocytes in areas heavily infiltrated with merozoites in the negative control group (plate 2A). But the hepatocytes in areas with few or no merozoites had an intense staining (I) with PAS method. Similar observation was found in plates 2B, 2C and 2D.

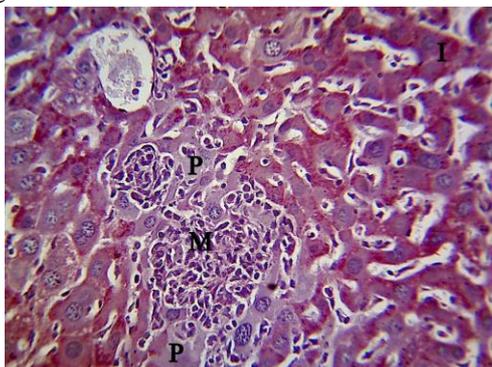


Plate 2A: Negative control

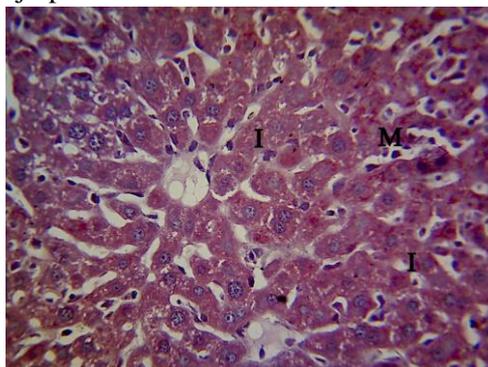


Plate 2B: 500mg/kg

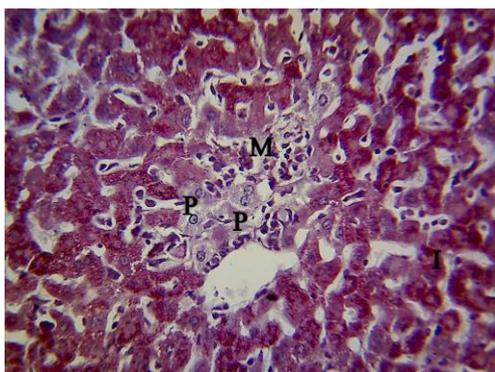


Plate 2C: 1000mg/kg

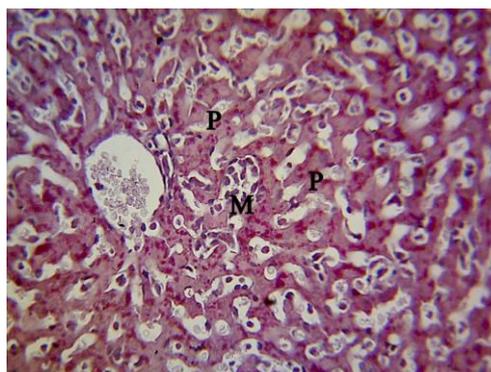


Plate 2D: 1500mg/kg

Plate 2: Photomicrographs of liver sections showing glycogen staining. Pale glycogen staining (P) of hepatocytes in areas with heavy sinusoidal congestion with merozoites (M) are found in 2A-D. Intense staining (I) in areas devoid or with few merozoites are seen. PAS 400x mag.

DISCUSSION

The LD₅₀ of aqueous extract of *Artocarpus altilis* leaf was 1414.2mg/kg in this study, which shows it has low toxicity. But this is in contrast to report by [11] that the aqueous leaf extract had an LD₅₀ of 2000mg/kg. In this work, aqueous extract of *Artocarpus altilis* leaves possessed antiplasmodial activity against *Plasmodium berghei*-infected erythrocytes. This is similar to reports of antiplasmodial activity of its leaves [12], stem bark [13], and roots [4]. Its chemosuppression was statistically significant against standard drug chloroquine and negative control ($p=0.001$). The antimalarial phytochemicals of the leaves that have been reported are artocarpone, artoindonesianin F and cycloheterophyllin. These phytochemicals along with others have equally been isolated in *Artocarpus champeden* for their antiplasmodial activities [4]. The possible mode of action of these phytochemicals which are active prenylated flavones is by being highly lipophilic, thereby increasing their resorption through cell membranes [14]. In the liver, there was no observable antiplasmodial effect of the extract as the Plasmodium merozoites were still present in the stained liver sections in groups A-C. Thus, suggesting that its antiplasmodial effect is only on the erythrocytes in the peripheral blood circulation. The mild hepatic changes such as mild sinusoidal dilatation, hypochromatic staining of hepatocytes nuclei

and cytoplasm suggesting hepatic necrosis, and sinusoidal congestion with plasmodium merozoites where similar to the negative control. This suggests that these changes are attributed to the presence of the malaria merozoites and not as a result of administration of the extract. This is similar to reports by Viriyavejakul et al. [15] who reported that merozoites can cause hepatocellular changes such as sinusoidal congestion and dilatation, hepatic necrosis and Kupffer cell hyperplasia in *P. falciparum* infection. However, in the 1500mg group in Plate 1D, the moderate sinusoidal dilatation and dilatation of the central vein different from the control group points to the effect of the extract at higher doses. Thus, the toxicity of *Artocarpus altilis* leaf extract on the liver was mild in the lower doses which suggested that the extract is safe to the liver. This is similar to report by [11] who reported that no major histopathological changes in the liver were observed in Wistar rats. The pale glycogen staining of the hepatocytes with periodic acid schiff stain in areas heavily infiltrated with merozoites shows that the merozoites utilize glucose from glycogen metabolism as a source of energy. This is similar to reports by [16]; [17]; [18]. The observation was not attributed to the extract because areas without merozoites still maintained normal glycogen staining. This is in consonant with report that breadfruit leaves act as hypoglycaemic agent by increasing glycogenesis through glucose adsorption in the liver, facilitating glucose diffusion through cell membrane into cells and by inhibiting the activities of α -amylase, α -glycosidase and sucrase needed for carbohydrate metabolism into glucose [19]. This enzyme inhibitory action is attributed to the heavy presence of starch fibres and phytochemicals common among plants belonging to Moraceae family [20]. Finally, this antimalarial activity of *Artocarpus altilis* leaves is comparable to other medicinal plants like *Stachytarpheta cayennensis* leaves [9] and *Ageratum conyzoides* leaves [21] known for their antiplasmodial activity.

4. CONCLUSION

The aqueous leaf extract of *Artocarpus altilis* is a safe pharmaceutical agent against *Plasmodium berghei*. Its antimalarial activity is on the erythrocytic stages of Plasmodium and not on the liver stages. The extract is safe to the liver at low doses not beyond 1500mg/kg body weight. Thus, it is recommended as a good medicinal plant for the treatment of malaria especially in endemic areas like Nigeria. This will aid in reducing the morbidity and mortality associated with malaria in Nigeria.

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6. CONFLICT OF INTEREST

There is no financial interest or any conflict of interest among the authors.

REFERENCES

1. Sikarwar MS, Hui BJ, Subramaniam K, Valeisamy BD, Yean LK, Balaji K. A Review on *Artocarpus altilis* (Parkinson) Fosberg (breadfruit). *J App Pharm Sci*, 2014; 4 (08): 091-097.
2. Akanbi TO, Nazamid S, Adebowale AA. Functional and pasting properties of a tropical breadfruit (*Artocarpus altilis*) starch from Ile-Ife, Osun State, Nigeria. *Int Food Res J*. 2009; 1(6): 151.
3. Jagtap UB, Bapat VA. *Artocarpus*: A review of its traditional uses, phytochemistry and pharmacology. *J Ethnopharmacol*. 2010; 129(2): 142-166.
4. Boonphong S, Baramée A, Kittakoop P, Puangsombat P. Antitubercular and antiplasmodial prenylated flavones from the roots of *Artocarpus altilis*. *Chiang Mai J Sci*. 2007; 34(3): 339–344.
5. Solanki R, Nagori BP. New method for extracting phytoconstituents from plants. *Int J Biomed Adv Res*. 2012; 03(10): 770-773.
6. WHO: World Health Organization World Malaria Report 2014, Geneva: 2014; 1-242. http://www.who.int/malaria/publications/world_malaria_report_2014/en/
7. Njar GN, Akpama WA, Iwara AI, Ita EA, Lasisi JC. Mapping risk prone zones of malaria vector species in Cross River State, Nigeria. *J Med Sci*. 2013; 13: 76-85.
8. Lorke D. A new approach to practical acute toxicity test. *Arch Toxicol*. 1983;54:275–86.
9. Okokon J E, Etebong E, Antia BS. *In vivo* antimalarial activity of ethanolic leaf extract of *Stachytarpheta cayennensis*. *Indian J Pharmacol*. 2008; 40(3): 111–113.
10. Ryley JF, Peters W. The antimalarial activity of some quinone esters. *Ann Trop Med Parasitol*. 1970;84:209–22.
11. Sairam S, Urooj A. Safety Evaluation of *Artocarpus altilis* as Pharmaceutical Agent in Wistar Rats. *J Toxicol*. 2014;2014:980404.
12. Baba S, Chan HT, Kezuka M, Inoue T, Chan EWC. *Artocarpus altilis* and *Pandanus tectorius*: Two important fruits of Oceania with medicinal values. *Emir. J. Food Agric*. 2016; 28(8): 531-539.
13. Hafid AF, Septiani RP, Fabriana LH, Febrianty F, Ranggaditya D, Widyawaruyanti A. Antimalarial activity of crude extracts of *artocarpus heterophyllus*, *artocarpus altilis*, and *artocarpus camansi*. *Asian J Pharma Clin Res*. 2016; 9(1): 279-281.
14. Bero J, Fre´de´rich M, Quetin-Leclercq J. Antimalarial compounds isolated from plants used in traditional medicine. *J Pharm Pharmacol*. 2009; 61(11): 1401-1433.
15. Viriyavejakul P, Khachonsaksumet V, Punsawad C. Liver changes in severe *Plasmodium falciparum* malaria: histopathology, apoptosis and nuclear factor kappa B expression. *Malaria J*. 2014; 13: 106.

16. Kauser MW, Moeed K, Mohmand AQK. Correlation of haemoglobin versus liver function test in patients of falciparum malaria. *Isra Med J.* 2011; 3(1): 10-15.
17. Green K. Whole body modelling of glucose metabolism in malaria patients. M. S. thesis, Stellenbosch Univ., 2017; 1-158. [http://: scholar.sun.ac.za](http://scholar.sun.ac.za)
18. Kauser MW, Raza S, Mumtaz S, Abbasi I, Moeed K, Zafar S. Comparison of hepatic biochemical derangements induced by falciparum and vivax malaria. *Ann Pak Inst Med Sci.* 2010; 6(2): 80-84.
19. Sairam S, Urooj A. Effect of *Artocarpus altilis* on carbohydrate hydrolyzing enzymes and glucose uptake by yeast cells: An ex-vivo study. *J Herbs Spices Med Plants.* 2012; 18(2):140-151.
20. Sairam S and Urooj A: *Artocarpus altilis* –Mode of anti-hyperglycemic activity: Elucidation by suitable *in-vitro* and *ex-vivo* techniques. *Int J Pharm Sci Res.* 2013; 4(8): 3013-3019.
21. Ukwe VC, Epueke EA, Ekwunife OI, Okoye TC, Akudor GC, Ubaka CM. Antimalarial activity of aqueous extract and fractions of leaves of *Ageratum conyzoides* in mice infected with *Plasmodium berghei*. *Int J Pharmaceut Res.* 2010; 2(1): 33-38.