

Evaluation of Antimalarial and Toxicity Potentials of Methanolic Fraction of *Cocos Nucifera* (West African Tall Variety) Husk Fibre Extract in Animal Models

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Abstract

The antimalarial and toxicity potentials of the methanolic fraction of *Cocos nucifera* (West African tall variety) husk fibre extract were investigated using animal models. For the 4-day suppressive antimalarial test, thirty *Plasmodium berghei* NK65-infected mice were randomly divided into six groups (A-F) with five mice each. Mice in group A (control) received orally appropriate volume of distilled water while those in group B were orally administered chloroquine (20 mg/kg body weight) for three days post-inoculation. Mice in groups C-F were orally administered 62.5, 125, 250 and 500 mg/kg body weight of the extract fraction for three days post-inoculation. For toxicological studies, twenty albino rats were randomly divided into four groups (G-J) with five rats each. Rats in group G (control) were orally administered appropriate volume of distilled water while those in groups H-J were orally administered 25, 50 and 100 mg/kg body weight of the extract fraction respectively for fourteen days. At the end of the experimental period, venous blood was collected and selected tissues isolated and homogenised. The full blood count and activities of alkaline phosphatase, aspartate and alanine aminotransferase in the tissues were determined. The results revealed that the methanolic fraction of *C. nucifera* (West African Tall variety) husk fibre extract had no antimalarial activity. The extract, at all doses administered, had no significant effect ($P > 0.05$) on the red blood cell indices, white blood cell indices and the activities of all the enzymes in the liver, kidney, heart and brain compared to controls. The results thus suggested that the methanolic fraction of the husk fibre extract was not responsible for the acclaimed antimalarial action of *C. nucifera* (West African Tall variety) husk fibre.

Keywords: *Cocos nucifera*, *Plasmodium berghei*, antiplasmodial, haematology, biochemical assays, animal model.

Introduction

Malaria is one of the most widely prevalent diseases in the world and is known to be endemic in the tropics [1, 2]. Annually, about 300 - 500 million people get infected with the disease worldwide; of these, 1-3 million people die, especially in the under-developed and developing countries [3]. Nigeria is known for a high prevalence of malaria [4, 5] and is a leading cause of morbidity and

mortality in the country. Malaria is caused by *Plasmodium* spp. which has its life cycle alternately in humans and female *Anopheles* mosquitoes [6]. Of the various species that infect humans, *P. falciparum* is the most important in most parts of the tropics and is responsible for most severe illnesses and death.

Despite significant progress in the treatment of malaria, the disease has staged a huge comeback in many parts of the world due to the development of

drug resistant parasites [7, 1]. Moreover, many of the potent drugs are too expensive for the rural dwellers. Hence, many people in the rural and even urban places have adopted alternative or complementary therapies including medicinal herbs. Thus, the increased level of drug resistance and difficulties for households to afford and access effective antimalarial drugs call for extensive research and development of cheap, effective, readily available and safe antimalarials, especially from medicinal plants which have served as sustainable source of malaria treatment amidst the indigenous people. One of such plants is *Cocos nucifera* (Linn). The decoction of its husk fibre is popularly used among the indigenous people of Nigeria for the treatment of malaria. Moreover, the decoction of the white flesh of the fruit is used for the treatment of malaria or fever by the rural population in Malaysia. In recent studies, these acclaimed uses have been authenticated [8].

The present study investigated the antimalarial and toxicity potentials of the methanolic fraction of *Cocos nucifera* husk fibre extract in animal models to ascertain the reported *in vitro* antiplasmodial activity and its safety for consumption.

Methodology

Reagents

Absolute n-Hexane, Ethyl acetate, Methanol and Ethanol were obtained from Sigma-Aldrich Laborchemikalien GmbH, Germany. Giemsa stain was obtained from Anosantec Laboratory, UK. Sodium Chloride was obtained from BDH Chemical Limited, Poole, England. Immersion oil was obtained from Panzonal Laboratory Supplies, Button road, Canada. Enzyme assay kits for alkaline phosphatase (ALP), aspartate and alanine aminotransferases (AST and ALT respectively) were obtained from Randox laboratories Ltd, UK.

Parasite Strain

A chloroquine-sensitive strain of *Plasmodium berghei* (NK-65) was obtained from the Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Ibadan, Nigeria.

Experimental Animals

Thirty adult Swiss albino mice (*Mus musculus*) with an average weight of 18 ± 2 g were obtained

from the Animal Breeding Unit of the Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria, while twenty adult male albino rats (*Rattus norvegicus*) with an average weight of 112 ± 4 g were obtained from the small Animal Holding Unit of the Department of Biochemistry, University of Ilorin. The animals were housed in standard plastic cages and acclimatised for a period of 2 weeks. They were maintained under standard conditions (12h light and 12h dark cycle) and had access to chow (Bendel Feeds, Ewu, Delta State) and clean tap water *ad libitum*.

Plant Material

Coconut husk fibres of the West African Tall variety dried at room temperature under shade were obtained from Nigeria Institute for Oil Palm Research (NIFOR), Badagry, Lagos State, Nigeria, in October, 2010. It was botanically authenticated at the Institute.

Preparation of Extract

The extract was prepared according to the method of [9]. The husk fibres were allowed to dry under shade at room temperature and then pulverised into powder. Four hundred and fifty grams (450 g) of the powder was percolated in 5 L of n-Hexane for 3 days in a tightly stoppered glass container. After this, it was filtered with Whatmann filter paper No. 1. The residue was again percolated in 5 L ethylacetate for another 3 days and the filtrate obtained. Lastly, the remaining residue was percolated in 5 L absolute methanol for another 3 days and filtrate was obtained, which was evaporated to dryness. The amount of the methanolic fraction of the extract obtained was 3.54g, with a percentage yield of 0.79%.

Qualitative phytochemical screening

Qualitative phytochemical screenings were performed using standard procedures described by [10]. Phytochemicals assayed for include tannins, anthraquinones, alkaloids, triterpenes, saponins, phlobatannins, glycosides, flavonoids and phenolics.

Antimalarial tests

Suppressive test *in vivo* was used. Briefly, the adult Swiss outbred mice were inoculated by the intraperitoneal route with $\sim 10^5$ red blood cells infected with *P. berghei* NK65 strain, a chloroquine-sensitive parasite. The infected animals were divided randomly into six groups (A-F) of 5 mice

each. Mice in group A (control) received orally appropriate volume of distilled water while those in group B were orally administered chloroquine (20 mg/Kg body weight) for three days post-inoculation. Mice in groups C-F were orally administered 62.5, 125, 250 and 500 mg/kg body weight of the extract fraction for three days post-inoculation. All extract solutions were prepared on the day of treatment and each mouse received 200 μ l. Every other day starting from day 4 post-inoculation, blood smears were prepared from the mouse tail, methanol-fixed, stained with Giemsa, and examined microscopically. Parasitemia was evaluated in coded slides, and up to 6,000 erythrocytes were examined, in the case of negative smears. Inhibition of parasite growth was calculated in relation to the untreated control group. Overall mortality was monitored daily until the 30th day post-infection.

Toxicological studies

Animal grouping and extract administration

Twenty adult male albino rats were randomly divided into four groups (G-J) of five rats each. Rats in group G (control) were orally administered appropriate volume of distilled water while those in groups H-J were orally administered 25, 50 and 100 mg/Kg body weight of the extract fraction respectively for fourteen days. All extract solutions were prepared on the day of treatment and each mouse received 200 μ l.

Sample collection and preparation

At the end of the 14-day experimental period, the rats were sacrificed by slight diethyl ether anesthesia and venous blood was collected into EDTA bottle to prevent clotting. The animals were then quickly dissected and the liver, kidney, heart and brain were isolated, cleaned of blood, weighed and homogenised in ice-cold 0.25M sucrose solution (1:5 w/v). The homogenates were then stored frozen overnight for maximum release of the enzymes.

Determination of Haematological Parameters

Haemoglobin concentration (Hb), packed cell volume (PCV), red blood cell count (RBC), white blood cell count (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin

concentration (MCHC), mean corpuscular haemoglobin (MCH), percentage neutrophil, percentage lymphocyte and platelet count were determined in the blood samples using the automated haematological analyzer SYSMEX KX21 (SYSMEX corporation, Japan) employing basically the method described by [11].

Biochemical assays

The protein concentrations in the tissues were determined using the Biuret method as described by [8]. Alkaline phosphatase activities in the serum and tissues were assayed by the method of [12]. Aspartate and alanine aminotransferase activities were determined by the method of [13].

Results

Phytochemical Screening

The phytochemicals present in the methanolic fraction of *Cocos nucifera* (West African tall) husk fibre extract were tannins, anthraquinones, alkaloids and triterpenes.

Antimalarial Test

Results of the 4-day suppressive test showed that the extract was not active at all doses administered compared to the non-treated control group (Figure 1). Drugs/extracts that have between 30 and 50% inhibition in parasite growth in animal models are generally considered to be partially active. The extract, at all doses administered did not cause any appreciable inhibition of parasite growth; hence, the extract fraction possessed no antimalarial activity.

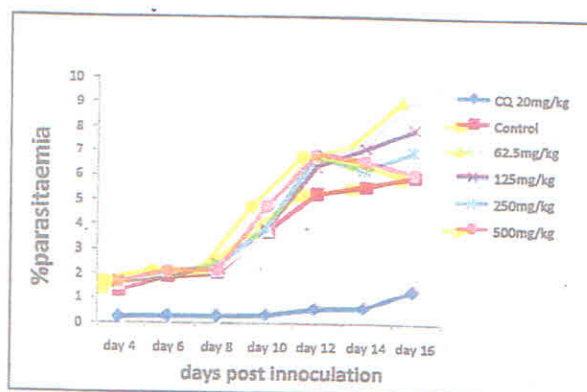


Fig.1: Parasitaemia in *P. berghei* NK65-infected mice treated with methanolic fraction of *Cocos nucifera* (West African tall variety) husk fibre extract.

The extract did not cause any significant alteration ($P>0.05$) to all the haematological parameters studied at all doses administered compared to the control (Tables 1 and 2).

Table 1: Effects of methanolic fraction of *Cocos nucifera* husk fibre extract on red blood cell indices in rats

GROUP	Hb (g/dl)	PCV (%)	RBC ($\times 10^{12}/L$)	MCV (fl)	MCH (pg)	MCHC (g/dl)
Ctrl	12.92 $\pm$ 0.48 ^a	44.00 $\pm$ 1.41 ^a	7.01 $\pm$ 0.19 ^a	62.40 $\pm$ 0.85 ^a	18.60 $\pm$ 0.55 ^a	29.60 $\pm$ 0.55 ^a
WAT 25	13.40 $\pm$ 0.70 ^a	48.00 $\pm$ 2.35 ^a	7.36 $\pm$ 0.71 ^a	65.60 $\pm$ 3.78 ^a	18.40 $\pm$ 1.14 ^a	28.40 $\pm$ 0.71 ^a
WAT 50	13.64 $\pm$ 0.57 ^a	49.75 $\pm$ 3.00 ^a	8.17 $\pm$ 0.51 ^a	64.40 $\pm$ 2.07 ^a	16.80 $\pm$ 0.84 ^a	26.80 $\pm$ 1.64 ^a
WAT 100	13.87 $\pm$ 0.89 ^a	50.67 $\pm$ 3.91 ^a	7.44 $\pm$ 0.51 ^a	65.40 $\pm$ 1.14 ^a	18.00 $\pm$ 0.00 ^a	27.60 $\pm$ 0.55 ^a

Results are mean $\pm$ S.D of 5 determinations. Means in each column with the same superscripts are not significantly different ($p>0.05$).

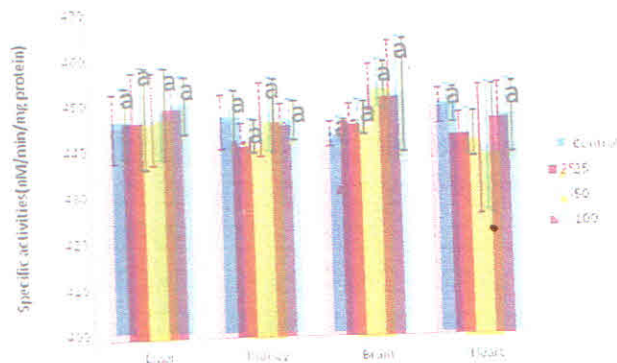
Table 2: Effect of methanolic fraction of *Cocos nucifera* husk fibre extract on white blood cell indices and platelet count in rats

GROUP	TWBC ($\times 10^9/L$)	NEUTROPHIL (%)	LYMPHOCYTE (%)	PLATELET ($\times 10^9/L$)
Ctrl	11.98 $\pm$ 5.48 ^a	14.67 $\pm$ 6.02 ^a	84.33 $\pm$ 6.02 ^a	411.80 $\pm$ 94.09 ^a
WAT 25	12.18 $\pm$ 3.10 ^a	8.60 $\pm$ 5.03 ^a	83.12 $\pm$ 5.03 ^a	571.67 $\pm$ 82.18 ^a
WAT 50	12.33 $\pm$ 3.59 ^a	16.80 $\pm$ 6.42 ^a	83.20 $\pm$ 6.42 ^a	597 $\pm$ 53.90 ^a
WAT 100	14.08 $\pm$ 4.69 ^a	20.40 $\pm$ 15.47 ^a	79.60 $\pm$ 15.47 ^a	611 $\pm$ 134.05 ^a

Results are mean $\pm$ S.D of 5 determinations. Means in each column with the same superscripts are not significantly different ($p>0.05$).

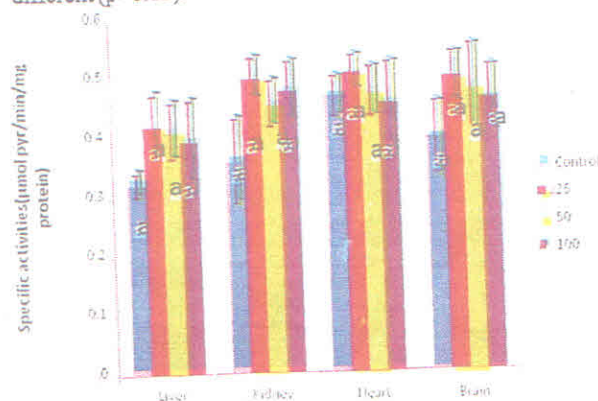
Enzymes

The extract at all doses administered had no significant effect ($p>0.05$) on ALP activities in all the tissues studied compared to controls (Figure 2). Similarly, ALT and AST activities in all the tissues studied were not significantly different ($p>0.05$) from those of controls at all doses administered (Figures 3 and 4 respectively).



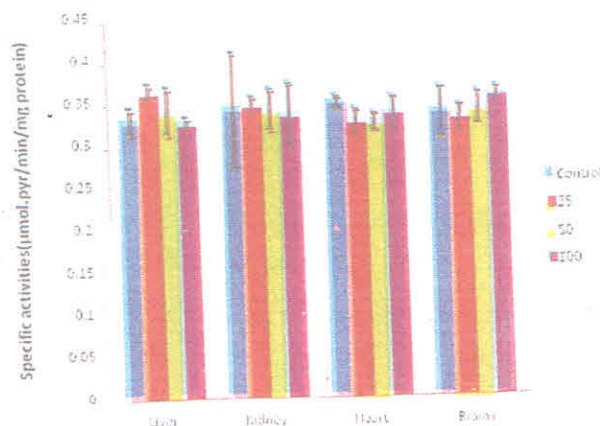
Tissues

Fig 2: Effects of methanolic fraction of *C. nucifera* (West African Tall variety) husk fibre extract on alkaline phosphatase activities in tissues of experimental animals. Values are means $\pm$ SD of 5 determinations. Values for each tissue with the same letter scripts are not significantly different ($p>0.05$).



Tissues

Fig 3: Effects of methanolic fraction of *C. nucifera* (West African Tall variety) husk fibre extract on alanine aminotransferase activities in tissues of experimental animals. Values are means $\pm$ SD of 5 determinations. Values for each tissue with the same letter scripts are not significantly different ($p>0.05$).



Tissues

Fig 4: Effects of methanolic fraction of *C. nucifera* (West African Tall variety) husk fibre extract on aspartate aminotransferase activities in tissues of experimental animals. Values are means $\pm$ SD of 5 determinations. Values for each tissue with the same letter scripts are not significantly different ($p>0.05$).

Discussion

The popular medicinal uses of *Cocos nucifera* extracts for the treatment of diverse diseases and ailments have been reported, but scientific authentication of these potential uses and possible adverse effects are still very preliminary [14]. Only of recent was the edible white flesh of *Cocos nucifera* shown to possess antimalarial activity. However, people in the North Central region of Nigeria use the decoction of *Cocos nucifera* husk fibre as antimalarial remedy, which most of the time is considered a waste. The study's results have revealed the antiplasmodial activity of the husk fibre extract of West African Tall variety *in vitro* [8]. The results of the present study, which was aimed at confirming the *in vivo* antimalarial activity of one of the fractions of the extract, revealed that the methanolic fraction of the extract does not possess any antimalarial activity (Fig 1). The phytochemical screening of the extract revealed the presence of alkaloids, tannins, anthraquinones, and triterpenes which corroborated earlier findings that reported the presence of condensed tannins and polyphenols in *Cocos nucifera* husk fibre aqueous extract. Various compounds belonging to these classes of phytochemicals have been reported to inhibit the growth of several infectious protozoans, one of which is *P. falciparum* [15, 16, 17]. However, compounds belonging to these groups of phytochemicals present in the extract fraction did not exhibit such activity.

The extract caused no significant alterations in red blood cell and white blood cell indices at all doses administered. This suggested that the extract may not have a pronounced effect of the production of red blood cells nor aggravate the anaemia associated with malaria. Due to the fact that the extract lacked saponins, it thus suggested that it may not initiate RBC lysis. Moreover, since there was no alteration in the percentage neutrophil caused by the extract, the extract may not initiate inflammation in tissues.

The measurement of the activities of enzymes in tissues and body fluids play a significant role in disease investigation and diagnosis [18]. Tissue enzyme assay can also indicate tissue cellular damage long before structural damage can be picked by conventional histological techniques. Alkaline phosphatase is a marker enzyme for the plasma membrane and endoplasmic reticulum. It is

often used to assess the integrity of plasma membrane and endoplasmic reticulum. The results of this study suggested that the extract, at all doses administered, did not adversely affect cellular membrane integrity in all the tissues studied, since there was no alteration in ALP activities in all the tissues at the different doses administered compared to controls. AST and ALT are intracellular enzymes and there is always a leakage into extracellular fluid when there is cellular damage, most especially when the membrane integrity has been compromised. Lack of alterations in AST and ALT activities in the various tissues studied compared to controls suggested that the extract did not cause tissue damage and there was no leakage of the enzymes into the blood.

Based on the results obtained from this study, it may be concluded that the methanolic fraction of *C. nucifera* (West African Tall variety) husk fibre extract may not be responsible for the acclaimed antimalarial action of the husk fibre extract. However, the fraction may not aggravate the complications of the disease in the various tissues studied. Further studies are underway to evaluate the *in vivo* antimalarial activities of other extract fractions.

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