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• Research Article

***In vivo* antimalarial activity and toxicological effects of methanolic extract of *Cocos nucifera* (Dwarf red variety) husk fibre**

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OBJECTIVE: Phytochemical constituents as well as antimalarial and toxicity potentials of the methanolic extract of the husk fibre of Dwarf Red variety of *Cocos nucifera* were evaluated in this study.

METHODS: The dried powdered husk fibre was exhaustively extracted with hexane, ethyl acetate and methanol successively and the methanolic extract was screened for flavonoids, phenolics, tannins, alkaloids, steroids, triterpenes, phlobatannins, anthraquinones and glycosides. A 4-day suppressive antimalarial test was carried out using *Plasmodium berghei* NK65-infected mice, to which the extract was administered at doses of 31.25, 62.5, 125, 250 and 500 mg/kg body weight (BW). Toxicity of the extract was evaluated in rats using selected hematological parameters and organ function indices after orally administering doses of 25, 50 and 100 mg/kg BW for 14 d.

RESULTS: Phytochemical analysis revealed the presence of alkaloids, tannins, phenolics, saponins, glycosides, steroids and anthraquinones in the extract. Moreover, the extract reduced parasitemia by 39.2% and 45.8% at doses of 250 and 500 mg/kg BW respectively on day 8 post-inoculation. Various hematological parameters evaluated were not significantly altered ($P>0.05$) at all doses of the extract, except red blood cell count which was significantly elevated ($P<0.05$) at 100 mg/kg BW. The extract significantly increased ($P<0.05$) urea, creatinine, cholesterol, high-density lipoprotein-cholesterol and bilirubin concentrations in the serum as well as atherogenic index, while it reduced albumin concentration significantly ($P<0.05$) at higher doses compared to the controls. Alanine aminotransferase activity was reduced in the liver and heart significantly ($P<0.05$) but was increased in the serum significantly ($P<0.05$) at higher doses of the extract compared to the controls.

CONCLUSION: The results suggest that methanolic extract of the Dwarf red variety has partial antimalarial activity at higher doses, but is capable of impairing normal kidney and liver function as well as predisposing subjects to cardiovascular diseases.

KEYWORDS: herbal drugs; *Cocos*; antimalarials; toxicity; *Plasmodium berghei*; rats; mice

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1 Introduction

Malaria is an infectious disease caused by *Plasmodium* species. Malaria is a prominent cause of human morbidity and mortality. About 1 million deaths occur per year worldwide as a result of *Plasmodium* infections^[1], especially among children under 5 years of age in sub-Saharan African countries. The disease has been eradicated in most temperate zones but it continues to be endemic in the tropics and subtropics, with 40% of the world's population at risk^[2].

Though significant progress has been made in malaria treatment, the disease has staged a huge comeback due to the development of drug resistant parasites^[3,4]. Also, many of the potent drugs (the artemisinin-based combination therapies) are too expensive for the poor populations typically dwelling in rural areas. Thus, many people in both rural and urban areas have adopted alternative therapies including herbs, due to their lower cost and cultural familiarity with their use. One of such plants is *Cocos nucifera* Linn, commonly referred to as coconut in English. The decoction of the husk fibre of *C. nucifera* is indigenously used for the treatment of malaria, and its efficacy has been scientifically authenticated, with the ethyl acetate extract of the most common variety (West African Tall variety) showing a high degree of antimalarial activity^[5,6].

In recent years, there has been increasing interest in cost-effective natural products that have potent antimalarial properties. In this manner, our group has been screening some medicinal plants for antimalarial properties. We have evaluated the husk fibre of various varieties of *C. nucifera* for their antimalarial activity *in vitro*, of which only the West African Tall variety was found to be active^[5,6]. However, our experience with *Clerodendrum violaceum* leaf extract, which was inactive *in vitro* but active *in vivo* (unpublished data), prompted the investigation of the antimalarial activity of other inactive varieties of *C. nucifera* *in vivo*. Thus, this study investigated the antimalarial activity and toxicity of the methanolic extract of the husk fibre of Dwarf red variety of *C. nucifera* *in vivo*.

2 Materials and methods

2.1 Materials

2.1.1 Reagents

Disodium hydrogen phosphate and potassium dihydrogen phosphate were obtained from Tianjin Kermel Chemical Reagent Co., Ltd., Tianjin, China. Immersion oil was purchased from Panzoner Laboratory Supplies, Canada. Chloroquine diphosphate salt and Giemsa stain were obtained from Sigma Chemical Company, St. Louis, MO, USA. Assay kits for the determination of serum creatinine, urea, bilirubin, albumin, total cholesterol and high-density lipoprotein

(HDL)-cholesterol concentrations were products of Randox Laboratories Ltd., UK. All other chemicals used were of analytical grade.

2.1.2 Parasite strain

Plasmodium berghei (NK65), a chloroquine-sensitive strain, was obtained from the Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Ibadan, Nigeria.

2.1.3 Animals

Thirty-five adult Swiss albino mice 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, Osun State, Nigeria, while twenty adult male albino rats of Wistar strain with average weight of (112±2) g were obtained from the Small Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Kwara State, Nigeria.

2.1.4 Plant material

Husk fibres of *C. nucifera* (Dwarf red variety) dried in the shade at room temperature were obtained from Nigeria Institute for Oil Palm Research (NIFOR), Badagry, Lagos State, Nigeria, in March, 2010. It was botanically authenticated at NIFOR by Mr. Igbene Collins.

2.2 Methods

2.2.1 Preparation of extracts

The extract was prepared as described in a previous study^[7]. The dried husk fibers of the *C. nucifera* (Dwarf red variety) were pulverized to powder. The powder (450 g) was successively extracted with 4 L n-hexane, 4 L ethyl acetate and 4 L absolute methanol for 72 h per solvent. The extracts were filtered using Whatman filter paper No. 1 and then concentrated. The concentrates were then exposed to air and residual solvent was allowed to evaporate at room temperature to obtain the dry extract. The percentage yield for the methanolic extract (which was used for the present study) was 0.84%.

2.2.2 Qualitative phytochemical screening

The extract was screened for flavonoids, phenolics, tannins, alkaloids, steroids, triterpenes, phlobatannins, anthraquinones and glycosides using methods described by Odebiyi and Sofowora^[8].

2.2.3 Animal handling

The animals were housed in standard plastic cages and were maintained under standard conditions (12 h light-dark cycle). They had access to feed (Bendel Feeds, Ewu, Delta State, Nigeria) and clean tap water *ad libitum*.

2.2.4 Antimalarial studies

A 4-day suppressive test^[9] was used with some modifications^[10]. Blood was obtained from the tail of a donor mouse (infected with *P. berghei* NK65) with known parasitemia (>15%) into a sample bottle containing 2 mL citrate/glucose solution. The number of infected red blood cells (RBCs) was counted using a hemocytometer. The



blood sample was then diluted appropriately with more citrate/glucose solution to obtain an inoculum size of 1×10^5 infected RBCs in 200 μ L, which was used to inoculate intraperitoneally each of the 35 adult outbred Swiss mice on day 0.

The infected mice were randomly divided into 7 groups (A–G), of 5 mice each on day 1 post-inoculation. Group A mice (control) were orally administered sterile distilled water; group B mice were orally administered 20 mg/kg body weight (BW) of chloroquine, while mice in groups C, D, E, F and G were orally administered the extract at doses of 31.25, 62.5, 125, 250 and 500 mg/kg BW respectively for three consecutive days starting on day 1 post-inoculation. Blood was obtained from the tail of each mouse every other day, starting from day 4 post-inoculation, and was used to prepare blood smears, which were then fixed with methanol, stained with Giemsa, and microscopically examined (Mag. $\times 1\,000$) by counting parasites in 1 000 up to 6 000 erythrocytes. Mortality was monitored daily for 30 d post-inoculation and the mean survival time (MST) for each group was calculated. Parasitemia and inhibition of parasite growth in the extract-treated groups in relation to the non-treated control group were calculated by following formulae:

$$\text{Parasitemia (\%)} = \frac{\text{Total number of infected RBC}}{\text{Total number of RBC}} \times 100$$

$$\text{Inhibition (\%)} = \frac{\text{Parasitemia}_{\text{control}} - \text{Parasitemia}_{\text{treat}}}{\text{Parasitemia}_{\text{control}}} \times 100$$

2.2.5 Toxicological studies

2.2.5.1 Animal grouping and extract administration

Twenty albino rats of Wistar strain were randomly divided into four groups (A, B, C and D), of five rats each. The extract was orally administered to groups B, C and D rats at doses of 25, 50 and 100 mg/kg BW respectively, while group A (control) rats were administered sterile distilled water for 14 d.

2.2.5.2 Preparation of tissue homogenate and serum

After 14 d of extract administration, the rats were slightly anesthetized with diethyl ether and blood was collected from the jugular vein of each rat into the ethylene diamine tetraacetic acid sample bottles (for hematological analysis) and plain sample bottles (containing no anticoagulant). The clotted blood was centrifuged at $503 \times g$ for 5 min and a Pasteur pipette was used to collect the supernatant (*i.e.*, the serum) which was stored frozen at -20°C until needed for analysis. The rats were also quickly dissected in order to isolate tissues of interest (liver, heart and kidney). The isolated tissues were cleansed of blood, weighed, immediately homogenized in ice-cold 0.25 mol/L sucrose solution (1:5 w/v) and stored frozen overnight for maximum

release of the tissue enzymes.

2.2.5.3 Hematological analysis

The hemoglobin (Hb) concentration, packed cell volume (PCV), RBC count, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), white blood cell (WBC) count, and platelet count were estimated using an automated hematological analyzer, SYSMEX-KX21 (SYSMEX Corporation, Japan).

2.2.5.4 Determination of concentrations of selected serum biomolecules

Protein concentration was determined by the method of Gornall *et al*^[11]. Creatinine and urea concentrations in the serum were determined by the methods of Bartels *et al*^[12] and Veniamin *et al*^[13] respectively. Serum bilirubin concentration was determined by the method of Malloy *et al*^[14]. Serum albumin concentration was determined using the method described by Doumas *et al*^[15]. The method of Frederickson *et al*^[16] was used for determining total cholesterol concentration in the serum while serum HDL-cholesterol concentration was determined by the method described by Albers *et al*^[17]. Assay kits were used for all these determinations. The atherogenic index (total cholesterol concentration/HDL-cholesterol concentration) was also calculated.

2.2.6 Enzyme assays

2.2.6.1 Determination of alkaline phosphatase activity

Alkaline phosphatase (ALP) activity was assayed by the method reported by Wright *et al*^[18]. Briefly, appropriately diluted sample (0.2 mL) was added to a mixture of 2.2 mL of carbonate buffer (0.1 mol/L, pH 10.1) and 0.1 mL of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.1 mol/L). This was mixed and incubated at 37°C for 10 min. Afterwards, 0.5 mL of *p*-nitrophenyl phosphate (10 mmol/L) was added and the resulting mixture was mixed and incubated at 37°C for 30 min. Thereafter, 2.0 mL of NaOH (1 mol/L) solution was added to the mixture. Absorbance of the mixture was read at 400 nm in a spectrophotometer against a blank (prepared in a similar manner, except that 0.2 mL of distilled water was used instead of the 0.2 mL of sample).

2.2.6.2 Determination of acid phosphatase activity

Assay for acid phosphatase (ACP) activity was carried out according to the method of Wright *et al*^[19]. Briefly, 0.2 mL diluted sample was added to 2.2 mL of sodium acetate buffer (0.1 mol/L, pH 4.5). This was mixed and incubated at 37°C for 10 min. The remaining assay procedure is as stipulated for ALP, beginning with the addition of *p*-nitrophenyl phosphate (10 mmol/L).

2.2.6.3 Determination of aminotransferase activity

The method of Reitman and Frankel^[20] was used to determine aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity. Briefly, 0.1 mL diluted homogenate/serum (0.1 mL) was added to 0.5 mL AST-

or ALT-buffered substrate solution (0.1 mol/L, pH 7.4). The mixture was mixed and incubated at 37 °C in water bath for 60 min (for AST) or 30 min (for ALT), after which, 0.5 mL of 2,4-dinitrophenylhydrazine solution (1 mmol/L) was added. The mixture was mixed and allowed to stand for 20 min at 25 °C. Five milliliter of 0.4 mol/L NaOH solution was then added and reaction mixture was allowed to stand for 5 min, after which its absorbance was read in a spectrophotometer against blank at 546 nm.

2.3 Statistical analysis

Data were subjected to statistical analysis using one-way analysis of variance and Duncan Multiple Range test (SPSS 16.0 version statistical package program, SPSS Inc., Chicago, IL). Differences in means were considered statistically significant at $P < 0.05$.

3 Results

3.1 Phytochemicals of the extract

The phytochemicals present in the methanolic extract of *C. nucifera* (Dwarf red variety) husk fibre include alkaloids, tannins, phenolics, glycosides, phlobatannins,

saponins and steroids.

3.2 Antimalarial activity of the extract

The extract was partially active against *P. berghei* NK65 *in vivo* at 250 and 500 mg/kg BW, causing between 30% and 50% reduction in parasitemia on days 4, 6, and 8 post-inoculation (Table 1). The results showed that the extract (at higher doses) increased the MST of the infected mice compared to the untreated control (Table 2).

3.3 Hematological parameters

No significant changes ($P > 0.05$) in Hb, PCV, MCH, MCHC, WBC, neutrophil, lymphocyte and platelet counts were observed at all doses of the administered extract compared to the controls (Table 3). However, the extract at 100 mg/kg BW increased RBC significantly ($P < 0.05$) compared with the control (Table 3).

3.4 Serum parameters and lipid profile

There was a significant increase ($P < 0.05$) in the concentrations of serum creatinine, conjugated bilirubin, and HDL-cholesterol, with the atherogenic index of treated rats at 50 and 100 mg/kg BW of extract compared with the controls (Tables 4 and 5). Also, serum total cholesterol and urea concentrations of treated rats were increased

Table 1 Effects of methanolic extract of *Cocos nucifera* (Dwarf red variety) husk fibre on parasitemia in *Plasmodium berghei* NK65-infected mice

Doses	n	Parasitemia (%)		
		4 d	6 d	8 d
Control	5	1.20	1.82	4.21
31.25 mg/kg BW (extract)	5	1.16 (3.33)	1.73 (4.94)	4.11 (2.38)
62.5 mg/kg BW (extract)	5	1.03 (14.17)	1.68 (7.69)	3.39 (19.48)
125 mg/kg BW (extract)	5	0.86 (28.33)	1.46 (19.78)	3.16 (24.94)
250 mg/kg BW (extract)	5	0.82 (31.67)	1.44 (20.88)	2.56 (39.19)
500 mg/kg BW (extract)	5	0.97 (40.83)	1.13 (37.91)	2.28 (45.80)
20 mg/kg BW (Chloroquine)	5	0.12 (90.00)	0.16 (91.21)	0.17 (95.96)

d: days post-inoculation. Values outside the parentheses represent parasitemia in percentage while values in parentheses are percentage reduction in parasitemia compared to control. BW: body weight.

Table 2 Effects of methanolic extract of *Cocos nucifera* (Dwarf Red variety) husk fibre on mean survival time of *Plasmodium berghei* NK65-infected mice

Doses	n	Mean survival time (d)
Control	5	14±1
31.25 mg/kg BW (extract)	5	16±2
62.5 mg/kg BW (extract)	5	18±2
125 mg/kg BW (extract)	5	18±1
250 mg/kg BW (extract)	5	20±3
500 mg/kg BW (extract)	5	22±2
20 mg/kg BW (Chloroquine)	5	24±2

BW: body weight. Values are presented as mean ± standard error of mean.

significantly ($P < 0.05$) at all doses of the extract administered compared with the controls (Tables 4 and 5). However, serum albumin concentration in treated rats was reduced significantly ($P < 0.05$) at all doses of extract administered compared with the control (Table 4). The extract increased serum total bilirubin concentration significantly ($P < 0.05$) at the dose of 100 mg/kg BW compared to the control (Table 4).

3.5 Enzyme activities

No significant alteration ($P > 0.05$) in ALP activity was observed in all the tissues studied, at all doses of the extract administered compared with the controls (Table 6). AST activities in the liver, kidney and serum of the treated animals were increased significantly ($P < 0.05$) at higher doses of the extract compared to the controls (Table 7).

Table 3 Effects of methanolic extract of *Cocos nucifera* (Dwarf red variety) husk fibre on hematological indices in rats

Group	n	RBC ($\times 10^{12}/L$)	Hb (g/dL)	PCV (%)	MCV (fl)	MCH (pg)
Control	5	6.59 $\pm$ 0.24	12.60 $\pm$ 0.36	48.73 $\pm$ 1.51	78.93 $\pm$ 0.94	19.10 $\pm$ 0.19
25 mg/kg BW (extract)	5	6.83 $\pm$ 0.16	13.25 $\pm$ 0.13	52.83 $\pm$ 0.69	75.17 $\pm$ 1.58	19.35 $\pm$ 0.17
50 mg/kg BW (extract)	5	7.03 $\pm$ 0.11	13.35 $\pm$ 0.29	52.65 $\pm$ 1.68	73.15 $\pm$ 1.80	18.55 $\pm$ 0.18
100 mg/kg BW (extract)	5	7.19 $\pm$ 0.13*	12.80 $\pm$ 0.71	49.13 $\pm$ 1.09	72.03 $\pm$ 2.05	18.78 $\pm$ 0.39

Group	n	MCHC (g/dL)	WBC ($\times 10^9/L$)	Lymphocyte (%)	Neutrophil (%)	Platelet ($\times 10^9/L$)
Control	5	25.80 $\pm$ 2.94	10.13 $\pm$ 0.63	90.15 $\pm$ 1.81	14.19 $\pm$ 2.33	492.50 $\pm$ 21.47
25 mg/kg BW (extract)	5	25.10 $\pm$ 1.47	12.88 $\pm$ 1.30	87.28 $\pm$ 1.00	14.33 $\pm$ 2.17	549.25 $\pm$ 66.66
50 mg/kg BW (extract)	5	25.40 $\pm$ 0.56	9.70 $\pm$ 0.88	87.55 $\pm$ 4.07	15.25 $\pm$ 1.65	552.50 $\pm$ 58.17
100 mg/kg BW (extract)	5	26.05 $\pm$ 0.48	11.43 $\pm$ 1.14	89.78 $\pm$ 1.46	14.98 $\pm$ 1.81	574.00 $\pm$ 33.01

Values are presented as mean $\pm$ standard deviation. * P <0.05, vs control group. RBC: red blood cell; Hb: hemoglobin; PCV: packed cell volume; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; WBC: white blood cell; BW: body weight.

Table 4 Effects of methanolic extract of *Cocos nucifera* (Dwarf red variety) husk fibre on concentrations of selected serum biomolecules in rats

Group	n	Albumin (g/L)	Total bilirubin (μ mol/L)	Conjugated bilirubin (μ mol/L)	Urea (mmol/L)	Creatinine (μ mol/L)
Control	5	32.30 $\pm$ 0.17	9.81 $\pm$ 0.13	3.06 $\pm$ 0.25	2.15 $\pm$ 0.02	34.77 $\pm$ 0.60
25 mg/kg	5	32.28 $\pm$ 0.29	9.80 $\pm$ 0.23	3.20 $\pm$ 0.17	2.25 $\pm$ 0.04*	36.49 $\pm$ 0.53
50 mg/kg	5	29.68 $\pm$ 0.19* Δ	9.47 $\pm$ 0.19	3.93 $\pm$ 0.17* Δ	2.26 $\pm$ 0.03*	36.99 $\pm$ 0.44*
100 mg/kg	5	28.67 $\pm$ 0.22* Δ	14.40 $\pm$ 0.14* Δ	4.42 $\pm$ 0.18* Δ	2.44 $\pm$ 0.02* Δ	39.82 $\pm$ 0.82* Δ

Values are presented as mean $\pm$ standard error of mean. * P <0.05, vs control group; Δ P <0.05, vs 25 mg/kg group; Δ P <0.05, vs 50 mg/kg group.

Table 5 Effects of methanolic extract of *Cocos nucifera* (Dwarf red variety) husk fibre on serum cholesterol concentration and atherogenic index in rats

Group	n	Serum total cholesterol (mmol/L)	Serum HDL-cholesterol (mmol/L)	Atherogenic index
Control	5	1.34 $\pm$ 0.02	0.86 $\pm$ 0.03	1.150 $\pm$ 0.054
25 mg/kg	5	1.70 $\pm$ 0.07*	0.88 $\pm$ 0.02	1.930 $\pm$ 0.078
50 mg/kg	5	1.62 $\pm$ 0.06*	1.18 $\pm$ 0.03* Δ	1.380 $\pm$ 0.047* Δ
100 mg/kg	5	2.85 $\pm$ 0.05* Δ	1.33 $\pm$ 0.01* Δ	2.140 $\pm$ 0.039* Δ

Values are presented as mean $\pm$ standard error of mean. * P <0.05, vs control group; Δ P <0.05, vs 25 mg/kg group; Δ P <0.05, vs 50 mg/kg group. HDL: high-density lipoprotein.

Table 6 Effects of methanolic extract of *Cocos nucifera* (Dwarf red variety) husk fibre on alkaline phosphatase activity in selected rat tissues

Group	n	Alkaline phosphatase activity (nmol/min/mg protein)				
		Liver	Kidney	Heart	Brain	Serum
Control	5	625.85 $\pm$ 1.65	542.34 $\pm$ 1.41	514.53 $\pm$ 0.93	464.48 $\pm$ 1.50	230.95 $\pm$ 0.53
25 mg/kg	5	625.65 $\pm$ 1.06	543.93 $\pm$ 1.51	516.73 $\pm$ 1.53	463.53 $\pm$ 1.37	231.66 $\pm$ 0.78
50 mg/kg	5	622.95 $\pm$ 1.97	544.33 $\pm$ 1.72	514.96 $\pm$ 1.33	462.96 $\pm$ 0.93	231.91 $\pm$ 0.85
100 mg/kg	5	622.43 $\pm$ 0.93	544.49 $\pm$ 1.26	514.53 $\pm$ 1.58	463.86 $\pm$ 1.19	232.12 $\pm$ 0.38

Values are presented as mean $\pm$ standard error of mean. Means in the same column are not significantly different (P >0.05).



However, no significant change ($P>0.05$) in heart AST activity was observed while a significant increase ($P<0.05$) in brain AST activity was observed at all doses of extract administered compared to the controls (Table 7). In the treated animals, liver ALT activity was significantly reduced ($P<0.05$), with a significant increase ($P<0.05$) in brain and serum ALT activities at higher doses of extract compared to the controls (Table 8). The extract significantly reduced ($P<0.05$) heart ALT activity with a significant increase ($P<0.05$) in kidney ALT activity at all doses administered compared to the controls (Table 8). No significant alteration ($P<0.05$) in acid phosphatase activities in the liver and serum of treated rats was observed at all doses of extract compared to the controls. However, the extract significantly increased ($P<0.05$) ACP activities in the kidney and brain

at higher doses compared to the controls. A significant decrease ($P<0.05$) in heart ACP activity was observed at all doses of the extract compared to the control (Table 9).

4 Discussion

The extract was partially active against *P. berghei* NK65 at 250 and 500 mg/kg BW and also increased MST of mice at these doses. Earlier reports have indicated the presence of polyphenols in *C. nucifera* husk fibre extract^[21]. Polyphenolic contents (such as flavonoids and tannins) of the coconut husk fibre are reported to be responsible for its antimicrobial and antileishmanial activities^[21,22]. Thus, the activities observed in this study may be due to the presence of such phytochemicals in

Table 7 Effects of methanolic extract of *Cocos nucifera* (Dwarf red variety) husk fibre on aspartate aminotransferase activity in selected rat tissues

Group	n	Aspartate aminotransferase activity ($\times 10^{-1}$ $\mu\text{mol/min/mg protein}$)				
		Liver	Kidney	Heart	Brain	Serum
Control	5	3.41 $\pm$ 0.19	2.61 $\pm$ 0.16	3.15 $\pm$ 0.22	2.34 $\pm$ 0.16	1.48 $\pm$ 0.17
25 mg/kg	5	3.43 $\pm$ 0.24	2.63 $\pm$ 0.12	3.16 $\pm$ 0.13	2.39 $\pm$ 0.15*	1.49 $\pm$ 0.16
50 mg/kg	5	3.45 $\pm$ 0.17	2.67 $\pm$ 0.15* Δ	3.15 $\pm$ 0.16	2.47 $\pm$ 0.13* Δ	1.54 $\pm$ 0.15*
100 mg/kg	5	3.51 $\pm$ 0.13* Δ	2.68 $\pm$ 0.14* Δ	3.20 $\pm$ 0.18	2.47 $\pm$ 0.17* Δ	1.62 $\pm$ 0.17* Δ Δ

Values are presented as mean $\pm$ standard error of mean. * $P<0.05$, vs control group; Δ $P<0.05$, vs 25 mg/kg group; Δ $P<0.05$, vs 50 mg/kg group.

Table 8 Effects of methanolic extract of *Cocos nucifera* (Dwarf red variety) husk fibre on alanine aminotransferase activity in selected rat tissues

Group	n	Alanine aminotransferase activity ($\times 10^{-1}$ $\mu\text{mol/min/mg protein}$)				
		Liver	Kidney	Heart	Brain	Serum
Control	5	4.69 $\pm$ 0.20	4.53 $\pm$ 0.18	5.51 $\pm$ 0.06	3.62 $\pm$ 0.16	1.62 $\pm$ 0.14
25 mg/kg	5	4.48 $\pm$ 0.22	4.63 $\pm$ 0.19*	5.39 $\pm$ 0.04*	3.64 $\pm$ 0.17	1.63 $\pm$ 0.16
50 mg/kg	5	4.36 $\pm$ 0.16*	4.65 $\pm$ 0.20*	5.28 $\pm$ 0.05* Δ	3.72 $\pm$ 0.14* Δ	1.65 $\pm$ 0.15
100 mg/kg	5	4.15 $\pm$ 1.89* Δ	4.71 $\pm$ 0.14* Δ Δ	5.30 $\pm$ 0.05* Δ	3.69 $\pm$ 0.17* Δ	1.71 $\pm$ 0.15* Δ Δ

Values are presented as mean $\pm$ standard error of mean. * $P<0.05$, vs control group; Δ $P<0.05$, vs 25 mg/kg group; Δ $P<0.05$, vs 50 mg/kg group.

Table 9 Effects of methanolic extract of *Cocos nucifera* (Dwarf red variety) husk fibre on acid phosphatase activity in selected rat tissues

Group	n	Acid phosphatase activity (nmol/min/mg protein)				
		Liver	Kidney	Heart	Brain	Serum
Control	5	486.01 $\pm$ 1.49	447.57 $\pm$ 0.91	354.73 $\pm$ 1.68	322.77 $\pm$ 0.98	216.74 $\pm$ 1.39
25 mg/kg	5	485.57 $\pm$ 1.32	446.70 $\pm$ 1.02	348.51 $\pm$ 1.06*	321.57 $\pm$ 1.09	219.86 $\pm$ 0.78
50 mg/kg	5	485.70 $\pm$ 1.09	457.09 $\pm$ 1.19* Δ	342.74 $\pm$ 0.88* Δ	324.71 $\pm$ 1.14	217.91 $\pm$ 1.42
100 mg/kg	5	485.25 $\pm$ 1.03	456.62 $\pm$ 1.75* Δ	343.29 $\pm$ 1.60* Δ	329.44 $\pm$ 1.11* Δ Δ	217.69 $\pm$ 1.02

Values are presented as mean $\pm$ standard error of mean. * $P<0.05$, vs control group; Δ $P<0.05$, vs 25 mg/kg group; Δ $P<0.05$, vs 50 mg/kg group.



the extract. Results of the present study also corroborate our earlier report on ethyl acetate extract of husk fibre of West African Tall variety of *C. nucifera*, which was found to be active even at the dose of 31.25 mg/kg^[6]. The reported antimalarial activity of ethyl acetate extract of the husk fibre of West African Tall variety of *C. nucifera* was recently found to be due to its polyphenolic content (Adebayo *et al*, unpublished data). These results suggest that the West African Tall variety is a better candidate for rational drug design and development than the Dwarf Red variety.

Medicinal plants or drugs can alter the normal range of hematological parameters^[23]. In this study, usage of the extracts were not associated with significant changes in RBC indices (Hb, PCV, MCV, MCH, and MCHC) in rats, except that RBC count was significantly increased compared to the controls (Table 3). This suggests that the extract may not induce hemolysis and may not adversely affect the oxygen-carrying capacity of the blood at the doses administered^[24]. Thus, the methanolic extract of *C. nucifera* (Dwarf red variety) husk fibre extract may not induce anemia. In this study, WBC indices were not significantly changed at all doses of the extract administered. This suggests that the extract has no modulatory effect on the immune system.

Liver function tests are designed to assess liver damage^[25]. The observed increase in serum total bilirubin concentration may not be a result of increased hemolysis, since the RBC count was not adversely affected by the extract. However, it may be caused by an increase in the conjugated bilirubin fraction, probably resulting from decreased secretion of the conjugated bilirubin into the bile duct or blockage of the bile duct. The decrease in serum albumin concentration observed at higher doses of the extract administered suggests that the extract may adversely affect the synthetic function of the liver, since albumin is synthesized by the liver. This may adversely affect the maintenance of oncotic pressure in the blood, a role mainly performed by albumin. The increased serum creatinine and urea concentrations observed in this study suggest a defect in the excretion of these biomolecules as a result of impaired renal function in the extract-treated rats^[26,27]. In this study, serum total cholesterol and HDL-cholesterol concentrations were increased in the extract-treated group with an increase in atherogenic index compared to controls, suggesting that the extract may predispose subjects to cardiovascular diseases at high doses.

Measurement of enzyme activities in tissues and body fluids plays a significant role in disease investigation and diagnosis^[28]. ALP, a membrane-bound enzyme, catalyzes the hydrolysis of a number of phosphate esters. It is often used as a marker enzyme for plasma membrane integrity. In this study, there was no significant change in ALP

activity in all the tissues and the serum of the extract-treated mice. This thus suggests that plasma membrane integrity in various tissues was not compromised and that there was no leakage of cellular components into extracellular fluids^[29]. AST and ALT are found in various tissues. They are released into the serum when these tissues are damaged. Activities of both enzymes in the serum are increased during liver damage; hence they are sensitive indicators of liver-cell injury^[30]. Since the membrane integrity in the various tissues was not compromised, increased AST and ALT activities in the tissues suggest activation of the enzymes in situ or induction of the synthesis of the enzymes. Reduction in liver and heart ALT activities suggests inhibition of the enzyme in situ. This thus suggests that the regulation of the enzyme activities is tissue-specific. ACP is a marker enzyme for the lysosome. Different isozymes of ACP are found in different organs and their serum levels are used to diagnose various types of diseases, *e.g.*, prostate cancer. No significant increase was recorded in activity of ACP in either the liver or the serum. This suggests that the extract may not predispose the cells to hydrolytic damage, since the integrity of the lysosomes was not compromised.

These results suggest that the methanolic extract of *C. nucifera* (Dwarf red variety) husk fibre may adversely affect the functions of the kidney and liver and may also predispose subjects to cardiovascular diseases. Thus, this extract is more toxic than the ethyl acetate extract of the husk fibre of West African Tall variety, which has been reported to be well tolerated^[6]. These findings suggest that the husk fibre of West African Tall variety may be a better candidate for drug development than the husk fibre of Dwarf red variety.

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6 Conflict of interests

There is no conflict of interests to declare.

REFERENCES

- 1 World Health Organization. World malaria report 2013. (2013) [2014-03-12]. http://www.who.int/malaria/publications/world_malaria_report_2013/report/en/.
- 2 Snow RW, Guerra CA, Noor AM, Myint HY, Hay SI. The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature*. 2005; 434(7030): 214–217.
- 3 Nájera JA. Malaria control: achievements, problems and strategies. *Parassitologia*. 2001; 43(1–2): 1–89.
- 4 Shift C. Integrated approach to malaria control. *Clin Microbiol*

- Rev. 2002; 15(2): 278–293.
- 5 Adebayo JO, Santana AE, Krettli AU. Evaluation of the antiplasmodial and cytotoxicity potentials of husk fiber extracts from *Cocos nucifera*, a medicinal plant used in Nigeria to treat human malaria. *Hum Exp Toxicol*. 2012; 31(3): 244–249.
 - 6 Adebayo JO, Balogun EA, Malomo SO, Soladoye AO, Olatunji LA, Kolawole OM, Oguntoye OS, Babatunde AS, Akinola OB, Aguiar AC, Andrade IM, Souza NB, Krettli AU. Antimalarial activity of *Cocos nucifera* husk fibre: further studies. *Evid Based Complement Alternat Med*. 2013; 2013: 742476.
 - 7 Adebayo JO, Yakubu MT, Egwim EC, Owoyele VB, Enaibe BU. Effect of ethanolic extract of *Khaya senegalensis* on some biochemical parameters of rat kidney. *J Ethnopharmacol*. 2003; 88(1): 69–72.
 - 8 Odebiyi OO, Sofowora EA. Phytochemical screening of Nigeria medicinal plants. Part II. *Llyodia*. 1978; 41(3): 234–246.
 - 9 Peters W. Drug resistance in *Plasmodium berghei* Vincke and Lips, 1948. I. Chloroquine resistance. *Exp Parasitol*. 1965; 17(1): 80–89.
 - 10 Carvalho LH, Brandão MG, Santos-Filho D, Lopes JL, Krettli AU. Antimalarial activity of crude extracts from Brazilian plants studied *in vivo* in *Plasmodium berghei*-infected mice and *in vitro* against *Plasmodium falciparum* in culture. *Braz J Med Biol Res*. 1991; 24(11): 1113–1123.
 - 11 Gornall AG, Bardawill CJ, David MM. Determination of serum proteins by means of the biuret reaction. *J Biol Chem*. 1949; 177(2): 751–766.
 - 12 Bartels H, Böhmmer M, Heierli C. Serum creatinine determination without protein precipitation. *Clin Chim Acta*. 1972; 37: 193–197. German.
 - 13 Veniamin MP, Vakirtzi-Lemonias C. Chemical basis of the carbamidodiacyl micromethod for estimation of urea, citrulline, and carbamyl derivatives. *Clin Chem*. 1970; 16(1): 3–6.
 - 14 Malloy HT, Evelyn KA. The determination of bilirubin with photoelectric colorimeter. *J Biol Chem*. 1937; 119: 481–485.
 - 15 Dumas BT, Watson WA, Biggs HGG. Albumin standards and the measurement of serum albumin with bromocresol green. *Clin Chim Acta*. 1971; 31(1): 87–92.
 - 16 Fredrickson DS, Levy RI, Lees RS. Fat transport in lipoproteins — an integrated approach to mechanisms and disorders. *N Engl J Med*. 1967; 276(3): 148–156.
 - 17 Albers JJ, Warnick GR, Chenng MC. Quantitation of high density lipoproteins. *Lipids*. 1978; 13(12): 926–932.
 - 18 Wright PJ, Leathwood PD, Plummer DT. Enzymes in rat urine: alkaline phosphatase. *Enzymologia*. 1972; 42(4): 317–327.
 - 19 Wright PJ, Leathwood PD, Plummer DT. Enzymes in rat urine: acid-phosphatase. *Enzymologia*. 1972; 42(6): 459–462.
 - 20 Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am J Clin Pathol*. 1957; 28(1): 56–63.
 - 21 Mendonça-Filho RR, Rodrigues IA, Alviano DS, Santos AL, Soares RM, Alviano CS, Lopes AH, Rosa Mdo S. Leishmanicidal activity of polyphenolic-rich extract from husk fiber of *Cocos nucifera* Linn. (Palmae). *Res Microbiol*. 2004; 155(3): 136–143.
 - 22 Esquenazi D, Wigg MD, Miranda MM, Rodrigues HM, Tostes JB, Rozental S, da Silva AJ, Alviano CS. Antimicrobial and antiviral activities of polyphenolics from *Cocos nucifera* Linn. (Palmae) husk fiber extract. *Res Microbiol*. 2002; 153(10): 647–652.
 - 23 Ajagbonna OP, Onifade KI, Suleiman U. Haematological and biochemical changes in rats given extract of *Calotropis procera*. *Sokoto J Veterin Sci*. 1999; 1: 36–42.
 - 24 Suchantabud A, Talubmook C, Chomko S, Narkkong N. Some hematological values and ultrastructure of blood cells in *Piper sarmentosum* Roxb. and *Tinospora crispa* Miers ex Hook. F & Thoms. treated diabetic rats. *J Microsc Soc Thailand*. 2008; 22: 65–70.
 - 25 Sheila S, Dooley J. Diseases of the liver and biliary system. 11th ed. Oxford, UK; Malden MA: Blackwell Science. 2002.
 - 26 Sitprija V, Indraprasit S, Pochanugool C, Benyajati C, Piyaatn P. Renal failure in malaria. *Lancet*. 1967; 1(7483): 185–188.
 - 27 Ganong WF. Review of medical physiology. 20th ed. London: McGraw Hill Medical Publishing Division. 2001: 414–417.
 - 28 Malomo SO. Toxicological implication of cerftriaxone administration in rats. *Niger J Biochem Mol Biol*. 2000; (1): 33–38.
 - 29 White NJ, Ho M. The pathophysiology of malaria. *Adv Parasitol*. 1992; 31: 83–173.
 - 30 Pratt DS, Kaplan MM. Evaluation of abnormal liver-enzyme results in asymptomatic patients. *N Engl J Med*. 2000; 342(17): 1266–1271.



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