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Evaluation of the use of *Cocos nucifera* as antimalarial remedy in Malaysian folk medicine

Abdulelah H. Al-Adhroey[a,*], Zurainee M. Nor[a], Hesham M. Al-Mekhlafi[a], Adel A. Amran[b], Rohela Mahmud[a]

[a] Department of Parasitology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia
[b] Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia

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ABSTRACT

Ethnopharmacological relevance: White flesh extract of *Cocos nucifera* (coconut) was studied to ascertain the ethnopharmacological standing of its antimalarial usage in Malaysian folk medicine.
Materials and methods: The crude methanol extract was investigated for phytochemical constituents and acute oral toxicity. Antimalarial activity of different extract doses of 50, 100, 200 and 400 mg/kg were investigated *in vivo* against *Plasmodium berghei* (NK65) infections in mice during early, established and residual infections. Chloroquine (20 mg/kg) and pyrimethamine (1.2 mg/kg) were used as reference drugs.
Results: The results revealed that the extract contained some phytochemical constituents and is toxicologically safe by oral administration. The extract significantly reduced the parasitaemia by the 200 and 400 mg/kg doses in the all three *in vivo* assessment assays. However, the extract did not significantly increase the survival time of the infected mice.
Conclusions: The observed pharmacological activities suggest that the Malaysian folkloric medicinal application of *Cocos nucifera* has a pharmacological basis.

1. Introduction

The coconut *(Cocos nucifera* Linn) is an important member of the family Arecaceae (palm family). It is an ornamental tree grown in villages and towns in Malaysia. The coconut is native to the littoral zone of Southeast Asia (Malaysia, Indonesia, Philippines) and Melanesia (Chan and Elevitch, 2006). Ethnobotanically, a decoction of the white flesh of the fruit is used by the rural population in Malaysia to treat malaria or fever (Al-Adhroey et al., 2010). The husk fibre of the coconut fruit showed a number of activities against helminths (Oliveira et al., 2009), *Leishmania species* (Mendonça-Filho et al., 2004) and microbes (Esquenazi et al., 2002). However, studies on the phytochemical screening, acute oral toxicity of the white flesh and its activity against malaria are nonexistent in the literature. Hence, this study aims to investigate and find out the phytochemical constituents, acute oral toxicity and antiplasmodial activity of the methanol white flesh extract of *Cocos nucifera*.

2. Materials and methods

2.1. Plant materials

The plant part was collected in May 2009 and identified by a taxonomist in the Department of Botany, University of Malaya. A voucher specimen (KLU 047212) of the plant was deposited at the herbarium of University of Malaya, Malaysia.

2.2. Extraction

The white flesh was dried for 2 weeks. The powdered material was macerated in absolute methanol for 72 h to give the crude methanol extract. The filtrate was concentrated and evaporated to dryness in vacuum 40 °C using a rotary evaporator, which was freeze-dried. The extractive value (%, w/w) of the dry extracts was 4.19%. The dry extract was stored in a refrigerator at 4 °C until used.

2.3. Phytochemical screening

Phytochemical screening of the white flesh extract was carried out according to the qualitative phytochemical screening tests described by Hymete (1986), Trease and Evans (1989) and Sofowora (1993) to expose the presence of its chemical constituents.

* Corresponding author. Tel.: +60 37967 3789; fax: +60 37967 4754.
E-mail address: husssien75@yahoo.com (A.H. Al-Adhroey).

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2.4. Animals

The females (mean weight + SD; 22 ± 2 g) and males (27 ± 2 g) ICR mice were obtained from the Faculty of Medicine Laboratory Animal Centre, University of Malaya. The mice were caged in standard conditions and were maintained on a standard pelleted feed and water *ad libitum*. Permission and approval for animal studies were obtained from the Faculty of Medicine, Animal Ethics committee, University of Malaya.

2.5. Parasite

A chloroquine tolerant strain of *Plasmodium berghei* (NK65) was obtained from the National University of Malaysia, School of Bioscience and Biotechnology and was maintained by subpassage in mice.

2.6. Acute oral toxicity

The method used for conducting this study is the accepted standard described in the OECD guidelines for the testing of chemicals, acute oral toxicity, limit test – acute toxic class method sections 401, 423, (OECD, 1987, 2001). A total of 5 females and 5 males ICR mice were orally dosed with 5000 mg/kg as a single dose. The animals were fasted overnight prior to the administration and returned to feeding 3 h later. On the day of administration, all the males and females mice were observed for signs of toxicity and mortality at 1, 3 and 4 h following the administration and then they were observed twice a day for 14 days.

2.7. In vivo antimalarial assays

The three different methods of treating malaria infections, i.e., 4-day suppressive test, curative and prophylactic methods were applied according to Peters and Robinson (1992), Saidu et al. (2000) and Peters (1965), respectively. In the suppressive test, 2–4 h post-infection, the experimental groups were treated orally. All the treatments were repeated for the next three days (D1 to D3). On the fifth day (D4), blood smears were prepared from each mouse and stained with Giemsa's stain. However, 72 h post-infection, the experimental groups were treated and the treatments were continued daily until D7 in the curative test. The mean survival time (days) for each group was determined over a period of 30 days post-infection. In the prophylactic test, the mice were administered with the treatment, which was given for 3 consecutive days (D0–D2). On the fourth day (D3), all mice were infected with 1×10^6 *P. berghei* and kept for the next 3 days. On the seventh day, blood smears were prepared from the tail blood. The percentage of suppression of parasitaemia was then calculated.

The *in vivo* anti-malarial activities of the extract treated orally at 50, 100, 200 and 400 mg/kg doses were compared to control groups treated with distilled water (containing 10% DMSO, the solvent of the test extracts) and reference groups treated with standard drugs, chloroquine 20 mg/kg or pyrimethamine 1.2 mg/kg/day. Malaria infection was established in female ICR mice by the intraperitoneal (i.p.) administration of donor mouse blood containing about 1×10^6 parasites. The percentage of parasitaemia was determined by counting the parasitized red blood cells out of 9000 in random fields of the microscopic Giemsa's stained blood films.

$$\%\,\text{Parasitaemia} = \frac{\text{No. of parasitized RBC}}{\text{Total no. of RBC counted}} 100$$

Average percentage chemosuppression was calculated as: $[A–B/A] \times 100$where A is the mean percentage parasitaemia in the control group and B is the mean percentage parasitaemia in the test group.

2.8. Statistical analysis and data evaluation

Data obtained by this study were analyzed using SPSS (version 13, 2004). Student's *t*-test and ANOVA (one- or two-way) were used to test the difference between groups. Differences between means at 1% and 5% level ($P \leq 0.01$ and 0.05) were considered significant.

3. Results

3.1. Phytochemical screening

Phytochemical screening of the methanol extract of *Cocos nucifera* white flesh revealed the presence of terpenoids, tannins, steroids and glycosides.

3.2. Acute oral toxicity

No effects of toxicity or mortalities were recorded post dosing and during the 14-day observation period in any of the 10 animals. All animals gained body weight by D 7 and at the end of the 14-day observation period. Based on the above mentioned results, the acute oral LD_{50} in mice of the test extract was found to be in excess of 5000 mg/kg.

Table 1
The antiplasmodial activities of the methanol extract of coconut white flesh during early and established malaria infections.

Drug/extract	Dose	Parasitaemia	%Chemo-suppression	Mean survival time
Suppressive activity				
Control	0.2 ml	5.10 ± 0.33	00.00	
Coconut extract	50 mg/kg	2.82 ± 0.78	44.71	
	100 mg/kg	2.20 ± 0.49	56.86*	
	200 mg/kg	1.04 ± 0.75	79.61**	
	400 mg/kg	0.83 ± 0.47	83.73***	
Chloroquine	20 mg/kg	0	100	
Curative activity				
Control	0.2 ml	9.60 ± 0.93	00.00	13.60 ± 0.51
Coconut extract	50 mg/kg	5.90 ± 0.86	38.54	13.80 ± 1.69
	100 mg/kg	4.40 ± 0.97	54.17*	14.60 ± 1.21
	200 mg/kg	3.20 ± 0.51	66.67**	15.20 ± 0.66
	400 mg/kg	3.00 ± 0.51	68.57**	15.60 ± 1.36
Chloroquine	20 mg/kg	0	100	

Values are expressed as mean ± S.E.M. ($n = 5$).
* $P < 0.05$ as compared with control.
** $P < 0.01$ as compared with control.
*** $P < 0.001$ as compared with control.