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Original Article

Antimalarial activity of *Neopetrosia exigua* extract in mice

Syamsudin Abdillah [a], *Rahmatul Wahida Ahmad* [b], *Farid Kamal Muzaki* [c], *Noriah Mohd Noor* [b,*]

[a] *Department of Pharmacology, Faculty of Pharmacy, Pancasila University, Jakarta, Indonesia*
[b] *Department of Basic Medical Sciences, Kulliyyah of Pharmacy, International Islamic University Malaysia, Kuantan Campus, Malaysia*
[c] *Laboratorium of Ecology of Marine, Department of Biology, Institute of Technology Surabaya, Indonesia*

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ABSTRACT

Objective: To evaluate the antimalarial activity of ethanolic extract of *Neopetrosia exigua* in ICR mice. The safety of the extracts was also ICR mice by the acute oral toxicity test.
Methods: The crude ethanol extract of *Neopetrosia exigua* (50, 100, 200 and 400 mg/kg) was investigated for its antimalarial activity against *Plasmodium berghei* during early infection. The acute toxicity of the extract was also investigated. At the end of 14 days, mice were sacrificed for histopathology study.
Results: The extract of *Neopetrosia exigua* demonstrated significant ($p < 0.05$) schizonticidal activity. The acute toxicity test showed that the extract of *Neopetrosia exigua* is toxicological safe by oral administration. Histopathological study revealed normal architecture of kidney and liver of mice.
Conclusion: The extract of sponge *Neopetrosia exigua* has anti-malarial activity *in vivo*.

1. Introduction

Malaria during pregnancy is a major public health problem in tropical and subtropical regions throughout the world.[1] Malaria causes serious illness and death amongst children and pregnant women. There are between 300 and 500 million malaria infections and 1 million malaria-attributed deaths worldwide each year.[2] As malaria vaccines remain problematic, chemotherapy still is the most important weapon in the fight against the disease.[3] The antimalarial drugs including chloroquine, quinine, mefloquine, pyrimethamine, and artemisinin are currently used in malaria treatment. Part of the reason for the failure to control malaria is the spread of resistance to first-line antimalarial drugs, cross-resistance between the limited number of drug families available, and some multidrug resistance.[4]

Marine sponges have a potential to provide future drugs against important diseases, such as malaria, cancer and a range of viral diseases.[5] Of 10,000 marine sponges, 11 genera are known to produce bioactive compounds, and only three genera (Haliclona, Petrosia and Discodermia) are known to produce anti-malarial, anticancer and anti-inflammatory

* *Corresponding author*. Tel.: +60 9 5704873.
E-mail address: noriahmn@iium.edu.my (N. Mohd Noor).
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compounds.[6] Sponge from the genus of Petrosia commonly found in Situbondo waters, East Java, Indonesia is *Neopetrosia* sp. Marine sponge, *Neopetrosia* sp. is a newly revived genus name, but in the past, it might have been described as Xestospongiasp.[7] They produced many potential bioactive metabolites including cytotoxicity: Renieramycin J, Araguspongine B, D, M, and three 5α,8α-epidioxy sterol,[7,8] antileishmanial: Renieramycin A from the Satsunan island, Japan[9] and antimicrobial substance: N-ethylene methyl ketone derivative of renierone, 1,6-dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione, renierone and mimosamycin.[10] The study aims at finding out antimalarial effect *in* vivo the *Plasmodium berghei* infected mice and its safety profile in acute toxicity assay in mice when given orally.

2. Materials and methods

2.1. Animal materials

A sponge of the *Neopetrosia exigua* (order Hadromerida, family Suberitidae) was collected by scuba diving at 8 m depth at Tanjung Pecaron Bay, near Situbondo (Indonesia). A voucher specimen, Voucher No.A24354, is deposited at Department of Biology, Faculty of Sciences, Institute Technology of Surabaya. The strain of *P. berghei* was kindly provided by Dr. Hashida Mohd Sidek, Centre of Bioscience and Biotechnology, Faculty of Sciences and Technology, National University of Malaysia.

2.2. Preparation of extract

Freezed dried or wet samples were soaked twice in ethanol. Each soaking lasted 24 h. After filtration, solvents were evaporated under reduced pressure in a rotary evaporator and the extracts were combined.

2.3. Animals

ICR mice, male (29 ± 2 g) and female (25 ± 2 g), 7–8 weeks old were used in the experiment. The mice were kept in the stable and fed with standard pellet and water in libitum at Animal House. Department of Basic Medical Sciences, Kulliyyah of Pharmacy, International Islamic University Malaysia. The animals were housed under standard conditions of temperature (25 ± 10 °C) and relative humidity (60 ± 10%), 12/12 h light/dark cycle, and fed with standard pellet diet and tap water. Animals were fasted prior to dosing and the test substance was administered in a single dose by oral route.

2.4. Acute toxicity assays

Acute toxicity assay was conducted by using ICR strain of mice of both sexes with body weight range of 25–30 g. The extract of *Neopetrosia exigua* was given with varied dosages (5000, 2500, 1250, and 625 mg/kg). Every animal model was precisely observed and recorded for any toxicity effect that occurred within the first 24 h. The observation took 14 days. Every dead mouse was observed macroscopically and microscopically for crucial organs such as liver, kidney, lung, abdomen, intestine, and heart. LD_{50} value referred to the dosage that caused 50% of death in animal models. The value was determined from the number of dead mice within the first 24 h and for 14 days of observation after a single dosage administration.

2.5. Parasite inoculum

The blood of donor mice with 30–40% increase in parasitemia rate was taken through the heart, and then diluted with 0.9% of Nacl solution (1:1) up to the parasite density of 1×10^7. Inoculation was conducted in IP method by injecting 0.2 mL of inoculum. Inoculated mice were randomly taken into a stable that consisted of 5 mice and kept in Animal Room, Department of Basic Medical Sciences, Kulliyyah of Pharmacy, International Islamic University, in accordance with the internationally accepted principles for laboratory animal use and care.

2.6. In vivo antimalarial assays

In vivo assay was conducted upon ICR strain of *P. berghei* infected mice given with the extract of *Neopetrosia exigua* with the dosages of 50, 100, 200, and 400 mg/kg and compared with control group that was treated only with distilled water (containing DMSO 10% and solvent used to dilute the extract) as well as reference group that was treated with standard chloroquine with a dosage of 10 mg/kg. Percent of parasitemia was determined by using a microscope (Olympus, cover-015) from the infected red blood cells compared to 4000 RBC in random fields of the microscope.

2.6.1. Early malaria infection

Early malaria infection model was used based on the method applied by Peters.[11] Thirty mice of ICR strain were inoculated in IP using 0.2 mL and suspense that contained 1 × 106 of *P. berghei* in the first day (D0). Twenty four (24) hours after initiation of the infection, the mice were given the extract of *Neopetrosia exigua* with the dosages of 50, 100, 200, and 400 mg/kg/bwt in an oral way. Reference group was treated with 10 mg/kg of chloroquine and control group with 0.2 ml of distilled water. The treatment was repeated after 3 days (D1–D3). On the fourth day (D-4), thin blood smear was prepared using Giemsa stain for every mouse.

2.6.2. Established malaria infection

Established malaria infection model was used for 30 mice of ICR strain inoculated in IP of 0.2 ml and suspense that contained 1×10^6 of *P. berghei*. Seventy two hours after initiation of infection, the treatment group was orally given the extract of *Neopetrosia exigua* with the dosages of 50, 100, 200, and 400 mg/kg, the reference group with 10 mg/kg of chloroquine, and control group with 0.2 ml of distilled water every day for 6 days. On the seventh day, the blood was taken through the tail to prepare thin blood smear by using Giemsa stain. Observation was conducted up to 30 days after the initiation of infection to determine the survival of infected mice and the effect of the extract.

2.6.3. Residual malaria infection

Residual malaria infection model was used for 30 mice of ICR strain that had been randomly taken into every stable, which consisted of 5 mice. The treatment group was given the