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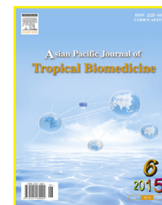

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In vivo antiplasmodial and *in vitro* antioxidant properties of stem bark extracts of *Haematostaphis barteri*

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ABSTRACT

Objective: To evaluate the antimalarial and antioxidant properties of stem bark extracts of *Haematostaphis barteri* (*H. barteri*).

Methods: The prophylactic activity of the plant was performed by dosing mice with sulfadoxine-pyrimethamine (1.2 mg/kg), aqueous extract (30, 100, 300 mg/kg) and dichloromethane/methanol (D/M) (30, 100, 300 mg/kg) extracts of *H. barteri* for 3 days. On the 4th day, the mice were inoculated with *Plasmodium berghei*. The parasite density was estimated for each mouse 72 h post-parasite inoculation. The curative activity of the plant was also performed by inoculating mice with *Plasmodium berghei*. Three days later, they were treated with artemether-lumefantrine (4 mg/kg), aqueous and D/M extracts of *H. barteri* stem bark for 5 days. The *in vitro* antioxidant property of the aqueous extract was determined by using the reducing power, nitric oxide and total antioxidant capacity assays.

Results: The aqueous extract exerted significant ($P < 0.05$) curative and prophylactic antimalarial activities. The D/M extract exhibited significant curative ($P < 0.05$) but not prophylactic antiplasmodial effect. The aqueous extract exhibited *in vitro* antioxidant property with IC_{50}'s of (0.930 ± 0.021) mg/mL, (0.800 ± 0.001) mg/mL and (0.22 ± 0.05) mg/mL in the total antioxidant capacity, reducing power and nitric oxide assays. Histological assessment of the liver of aqueous and D/M treated animals did not reveal any sign of toxicity.

Conclusions: *H. barteri* is not toxic which exerted significant curative antiplasmodial effects but the prophylactic property was however fraction dependent. The mechanism of the antiplasmodial activity of *H. barteri* may partly be mediated by its antioxidant property.

1. Introduction

Malaria is still regarded as a major global infectious disease in the 21st century, with a high pediatric mortality toll in the developing world [1]. In Africa, malaria is one of the diseases causing the most morbidity and mortality. The number of Africans who die as a result of malaria each year is estimated at 800 000 people of which a greater proportion is children aged below 5 years [2]. This is coupled with the emergence and spread of parasite resistance to well-established antimalarial drugs and mosquito vectors resistant to insecticides. It is therefore evident that newer agents with improved efficacy and toxicity should be developed. Medicinal plants presently constitute a popular source of potential antimalarial agents. About 30% of drugs for the management of diseases are obtained from nature [2]. In this regard, *Haematostaphis barteri* (*H. barteri*), commonly known as ''blood plum'', from the Anacardiaceae family was studied for its antimalarial activity. *H. barteri* is found in the northern region of Ghana. Different ethnic groups use it for a number of reasons. Notable among its uses include the leaves and bark infusion employed in the treatment of malaria, hepatitis and sleeping sickness [3].

2. Materials and methods

2.1. Collection and identification of plant material

The stem bark of *H. barteri* was collected from Wechiua in the northern region of Ghana in the months of November to

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December, 2013. It was identified and authenticated by a botanist at the University of Cape Coast Herbarium and a voucher specimen was deposited at the herbarium.

2.2. Preparation of plant extract

Preparation of the aqueous stem bark of *H. barteri* was done by washing thoroughly the stem bark with tap water and sun-dried. The dried stem bark was pulverized into fine powder and 30.98 g of the powder was weighed and soaked in boiled water for 3 days. The mixture was filtered and freeze dried. To obtain the dichloromethane/methanol (D/M) fraction, the plant sample was extracted with D/M (1:1) solvent mixture. The extract was concentrated under reduced pressure and dried on a water bath. The dried material was then weighed, dissolved in water and used for this study.

2.3. Screening for secondary metabolites

The aqueous and D/M extracts were screened for the presence of phytochemicals using standard procedures described elsewhere [4–6].

2.4. Animals and husbandry

Institute for Cancer Research mice bred in the Animal House of the School of Biological Sciences, University of Cape Coast weighing between 20 and 30 g were used for the antimalarial studies. The animals were housed in stainless steel cages (34 cm × 47 cm × 18 cm) with soft wood shavings as bedding, fed with normal commercial pellet diet (Agricare Ltd, Kumasi, Ghana), given water *ad libitum* and maintained under ambient laboratory conditions. All procedures and techniques used in these studies were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (Department of Health and Human Services publication no. 85-23, 1985, revised 1996). All ethical protocols used for the study were approved by the Departmental Ethics Committee.

2.5. Drugs and chemicals

Artemether-lumefantrine (A-L) used for the curative antimalarial test was obtained from Ajanta Pharma Ltd. (Mumbai, India). Sulfadoxine/pyrimethamine (SP) was also obtained from Maxheal Laboratories Pvt Ltd. (Sachin, India). Ascorbic acid, ammonium molybdate, disodium hydrogen phosphate, sodium dihydrogen phosphate, ferric chloride, potassium ferricyanide, sodium bicarbonate, sodium carbonate and sulphuric acid were obtained from British Drug Houses, Poole, England.

2.6. Curative antiplasmodial effect of stem bark extracts of H. barteri

Mice were divided into eight groups and each inoculated with 1×10^6 *Plasmodium berghei* (*P. berghei*). About 72 h later, parasitaemia was determined in all the mice followed by the administration of the extracts. The various groups of mice were treated with aqueous (30, 100 and 300 mg/kg; *p.o.*) and D/M (30, 100 and 300 mg/kg; *p.o.*) and A-L (1.14:6.9 mg/kg, *p.o.* for 5 days. The negative control group was treated with normal saline. On Day 12, two animals from each group were randomly selected and sacrificed. Their livers were harvested, fixed in 4% phosphate-buffered paraformaldehyde, and embedded in paraffin. Sections were stained with hematoxylin and eosin, and fixed on glass slides for microscopic examination to estimate parasite density [7].

2.7. Prophylactic effect of stem bark extracts of H. barteri on P. berghei infection

The prophylactic antimalarial properties of the extracts were determined by treating mice with aqueous (30, 100 and 300 mg/kg), D/M (30, 100 and 300 mg/kg), SP (1.2 mg/kg) and normal saline. The mice were treated daily for three consecutive days. On Day 4, all the mice were infected with 1×10^6 *P. berghei*. About 72 h later, blood smears were prepared from the tail of the mice for the determination of parasite density [7].

2.8. In vitro antioxidant assays

2.8.1. Total antioxidant capacity

The antioxidant capacity or power of the aqueous extract was evaluated by the phosphomolybdenum reduction method with slight modifications [8]. The reagent solution prepared contained ammonium molybdate (4 mmol/L), sodium phosphate (28 mmol/L) and sulfuric acid (0.06 mol/L) mixed in 1:1:1 ratio respectively. Accurately 0.3 mL of the various concentrations of the ascorbic acid (20–200 μg/mL) and aqueous (20–200 μg/mL) were mixed with 3 mL of the reagent solution. The mixture was incubated for 90 min at 95 °C after which the absorbance of the green phosphomolybdenum complex formed was measured at 695 nm against a reagent blank (0.3 mL distilled water and 0.3 mL reagent solution). The results were expressed as ascorbic acid equivalents.

2.8.2. Reducing power capacity

The reducing power of aqueous was carried out using the potassium ferricyanide method by mixing 1 mL of aqueous and *n*-propyl gallate (20–200 μg/mL) with 2.5 mL phosphate buffer (0.2 mol/L, pH 6.6) and 2.5 mL potassium ferricyanide. The mixture was then incubated at 50 °C for 20 min. A volume of 2.5 mL of trichloroacetic acid was added to this mixture, which was then centrifuged at 3000 r/min for 30 min. Finally, 2.5 mL of the supernatant solution was collected and mixed with 2.5 mL of distilled water and 0.5 mL ferric chloride and absorbance was measured at 700 nm [9].

2.8.3. Nitric oxide radical scavenging activity

The nitric oxide radical scavenging activity of the extract was determined by mixing 2 mL of 10 mmol/L sodium nitroprusside prepared in 0.025 mol/L of phosphate buffer (pH 7.4) with 0.5 mL of ascorbic acid or aqueous at concentrations of 20–200 μg/mL [10]. The mixture was incubated at 25 °C for 150 min. A volume of 0.5 mL of the incubated solution was mixed with 0.5 mL of Griess reagent. The mixture was incubated at room temperature for 30 min. The absorbance of the mixture was measured at 540 nm against a phosphate buffer blank.

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