Journal of Traditional and Complementary Medicine 6 (2016) 112–117

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Contents lists available at ScienceDirect

Journal of Traditional and Complementary Medicine

journal homepage: http://www.elsevier.com/locate/jtcme

Original article

Evaluation of *Senna singueana* leaf extract as an alternative or adjuvant therapy for malaria

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ARTICLE INFO

Article history:
Received 26 May 2014
Received in revised form
1 July 2014
Accepted 13 August 2014
Available online 22 January 2015

Keywords:
adjuvant
antimalarial
in vivo
Plasmodium berghei
Senna singueana

ABSTRACT

The emergence of malarial resistance to most antimalarial drugs is the main factor driving the continued effort to identify/discover new agents for combating the disease. Moreover, the unacceptably high mortality rate in severe malaria has led to the consideration of adjuvant therapies. *Senna singueana* leaves are traditionally used against malaria and fever. Extracts from the leaves of this plant demonstrated *in vitro* and *in vivo* antioxidant activities, which in turn could reduce the severity of malaria. Extracts from the root bark of this plant exhibited antiplasmodial activity; however, the leaves are the more sustainable resource. Thus, *S. singueana* leaf was selected for *in vivo* evaluation as a potential alternative or adjuvant therapy for malaria. Using malaria [*Plasmodium berghei* ANKA, chloroquine (CQ) sensitive]-infected Swiss albino mice of both sexes, 70% ethanol extract of *S. singueana* leaves (alone and in combination with CQ) was tested for antimalarial activity and adjuvancy potential. The 4-day suppressive test was used to evaluate antimalarial activity. The dose of *S. singueana* extract administered was safe to mice and exhibited some parasite suppression effect: extract doses of 200 mg/kg/d, 400 mg/kg/d, and 800 mg/kg/d caused 34.54%, 44.52%, and 47.32% parasite suppression, respectively. Concurrent administration of the extract with CQ phosphate at varied dose levels indicated that the percentage of parasite suppression of this combination was higher than administering CQ alone, but less than the sum of the effects of the extract and CQ acting separately. In conclusion, the study indicated that 70% ethanol extract of *S. singueana* leaf was safe to mice and possessed some parasite suppression effect. Coadministration of the extract with CQ appeared to boost the overall antimalarial effect, indicating that the combination may have a net health benefit if used as an adjuvant therapy.

1. Introduction

Human malaria is mainly caused by four plasmodium species (*Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale,* and *Plasmodium vivax).* Recently, a fifth human parasite (*Plasmodium knowlesi*) has been reported. Of these, *P. falciparum* is the most widespread, causes the most severe infections, and is responsible for nearly all malaria-related deaths.[1–4] Malaria remains a major cause of morbidity and mortality worldwide, with the vast majority of cases reported in African countries.[5] The global spread of multidrug-resistant malarial parasites has led to an urgent need to develop new chemotherapeutic agents.[6] Furthermore, a significant number of malarial patients progress to severe malaria with organ dysfunction, which is more common in immunologically naïve individuals, especially young children.[7] The current primary treatment for severe malaria is parenteral quinine or administration of artemisinin derivatives. Despite improved survival with artemisinin derivatives, mortality rate in severe malarial cases treated with either artemisinin derivatives or quinine remains unacceptably high, and therefore, adjuvant therapies (additional therapies that modify the pathophysiologic processes caused by malaria) are urgently needed.[5,7] Accordingly, numerous adjuvant therapies have been tested and some of those under clinical trials for severe

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Peer review under responsibility of The Center for Food and Biomolecules, National Taiwan University.

http://dx.doi.org/10.1016/j.jtcme.2014.11.014

malaria are immune response modulators, antioxidants, anticoagulants, and agents with antiseizure activity.[5]

Medicinal plants are the most important sources of new antimalarial drugs as well as adjuvant therapies for severe malaria. Historically, plants have provided two major drugs for the treatment of malaria, namely, *quinine* from *Cinchona* species and *artemisinin* from *Artemisia annua*.[8,9] Major drugs that are available for infectious diseases, particularly for malaria, have been obtained from plants. In malaria-endemic regions, people use traditional medicinal plants to treat malaria and the fever or symptoms associated with this pathology.[9] One such medicinal plant is *Senna singueana* (Del.) Lock (Syn: *Cassia singueana*; Fabaceae), which has many medicinal uses throughout Africa,[10] including traditional antimalarial uses. Its leaf sap is drank to combat malaria in Tanzania[11] and a hot decoction of powdered leaves, taken orally, is indicated for malaria and fever in Kenya[12] and Burkina Faso.[11,13] Previous studies have indicated that methanolic extract of the root bark of the plant exhibited significant antiplasmodial activity against *Plasmodium berghei*.[14] The root bark is also reported to contain lupeol,[10] a triterpene that exhibits a wide spectrum of biological activity such as antimalarial effects against chloroquine (CQ)-resistant *P. falciparum*.[15] Although *S. singueana* has numerous medicinal uses, research into its pharmacology has been scarce and is restricted to the root bark. Because of its many medicinal uses, research into the properties of its leaves is warranted, as they are a more sustainable source of medicine than the root or stem bark.[10] Furthermore, scientific claims indicate that various extracts of the leaf possess *in vitro* and *in vivo* antioxidant activities[16–18]. Antioxidant therapy using the leaf extract of *S. singueana* is claimed to reduce malarial severity.[7] Taking these considerations into account, the aim of this work was to evaluate the effectiveness of *S. singueana* as an antimalarial agent or as an adjuvant therapy for severe malaria.

2. Materials and methods

2.1. Collection of plant material and preparation of extract

Sufficient amounts of *S. singueana* leaves were collected in March 2013 from the northwest and central zones of Tigray, Northern Ethiopia. The plant material was authenticated at the National Herbarium, Department of Biology, Addis Ababa University (Addis Ababa, Ethiopia). Photographs of *S. singueana* taken during collection at the collection site and collected leaves are shown in Fig. 1. The collected leaves were sorted, dried, powdered, and then defatted using petroleum ether (HiMedia Laboratories, Mumbai, India) for 48 hours. The marc was then allowed to dry and extracted three times by maceration using 70% ethanol with intermittent agitation. Each maceration was carried out for 72 hours. The extracts were filtered, collected, concentrated under reduced pressure using Rotavapor (BIBBY Sterlin Ltd, stuart®, UK), and dried in a vacuum oven at 35°C. The dried extracts were then transferred into vials and stored for further use.

2.2. Experimental animals and parasite

Swiss albino mice of both sexes (8–12 weeks of age) were obtained from the Pharmacology Animal House of the Department of Pharmacy, College of Health Sciences (Mekelle University, Mekelle, Ethiopia). The animals were housed in an air-conditioned room and were allowed to acclimatize for 1 week before the study. Before and during the experiment, the animals were provided with standard animal feed or pellets and clean water *ad libitum*. *P. berghei* ANKA strain (CQ sensitive)-infected donor mice were obtained from the Faculty of Science, Addis Ababa University (Addis Ababa, Ethiopia). The parasite was subsequently maintained in the Pharmacology Laboratory of the Department of Pharmacy, College of Health Sciences, Mekelle University by serial blood passage from one mouse to another.

2.3. Ethical considerations

The study was performed after obtaining ethical approval from the Institutional Review Committee of the College of Health Sciences, Mekelle University. All experiments were carried out in accordance with scientific procedures. Ethical issues, especially the handling of experimental animals, were respected.

2.4. Acute oral toxicity test

An acute oral toxicity study was performed according to the Organization for Economic Cooperation and Development guidelines 423,[19] but with a slight modification in the number of mice used. The study animals (n = 20 mice) were divided into four groups of five mice per cage. The animals were physically active and their consumption of food and water was normal. Before the administration of a single dose of the extract, the mice fasted for 2 hours. First, an acute oral toxicity study was performed on five female mice (weight, 30–32 g). The mice were given 2000 mg/kg of the extract dissolved in distilled water. The test was then performed on the remaining 15 mice (10 female and 5 male; weight, 23–25 g), which were divided into three groups of five each: The first group (5 female mice) was given distilled water, whereas the second (5 female mice) and third (5 male mice) groups were given the hydroalcoholic leaf extract of *S. singueana* (5000 mg/kg dissolved in distilled water). After the oral administration of 0.5 mL of the test

Fig. 1. Photographs of *Senna singueana* (Del.) Lock (Fabaceae) and the collected leaves.