Effect of Nelumbo nucifera Stamen Extract on Phagocytosis and Malaria Parasite Growth Against Plasmodium Berghei Infected Mice

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Abstract

Antimalarial drug resistant malaria parasites are causing not only the spread of malaria to new areas but also its re-emergence in areas where it had previously been eradicated. In addition, malaria-associated impairment of phagocytosis has been reported during malaria parasite infection. The present study has been carried out to investigate the effect of Nelumbo nucifera stamen extract on phagocytosis and malaria parasite growth against Plasmodium berghei infected mice. Groups of ICR mice were treated orally by gavage with N. nucifera stamen extract (500, 1,000 and 2,000 mg/kg) after infection with P. berghei ANKA. Parasitemia, percent phagocytosis and phagocytic index were determined. At these doses, N. nucifera stamen extract inhibited parasitemia in dose-dependent manner, with similar level of antimalarial activity to chloroquine (5 mg/kg). In addition, increasing of phagocytosis and phagocytic index has also been observed in dose-dependent in infected mice treated with the extracts. In particular, the highest activities of N. nucifera stamen extract were found at dose 2,000 mg/kg. These results indicated that aqueous crude extract of N. nucifera stamens have antimalarial and improve phagocytic activity against P. berghei ANKA infected mice.

Keywords: Nelumbo nucifera; Phagocytosis; Malaria; Plasmodium berghei

Introduction

Malaria remains one of the world’s largest burdens of disease. With an estimated 2.5 billion people at risk, it causes 300-500 million infections and 1-3 million deaths annually, the greatest part of the latter in children under five years of age [1]. Malaria is caused by protozoa parasite in genus Plasmodium that transmitted by female Anopheles mosquito. Five species of malaria parasites including P. falciparum, P. vivax, P. ovale, P. malariae, and P. knowlesi are responsible for human infection, although the majority of fatal cases are caused by P. falciparum [2,3]. Although an effective vaccine is the best long term control for malaria, current research on vaccine development is still in the laboratory. Therefore, the strategy for malaria mainly focuses on drug treatment. However, antimalarial drug resistant malaria parasites are causing not only the spread of malaria to new areas but also its re-emergence in areas where it had previously been eradicated [4,5]. In addition, malaria-associated impairment of phagocytosis has been reported during malaria parasite infection [6-9]. This has prompted research towards the discovery of new antimalarial drugs with phagocytosis recruitment properties. In this respect, plant extracts are potential targets for research and development of the alternative drugs.

Nelumbo nucifera Gaertn, commonly known as lotus, is a large aquatic plant and has long been used as a medicinal herb in China, Japan, India, Korea and Thailand. Nearly every part of N. nucifera including buds, flowers, anthers, stamens, fruits, leaves, stalks, rhizomes and roots have been used as medicinal plant for treatment of cancer, heart problems, hypertension and insomnia [10]. Catechin, quercetin, quercetin-3-O-glycoside, kaempferol-3-O-glycoside and myricetin-3-O-glucose have been reported as part of its major components [11,12]. Pharmacological and physiological activities including anti-diabetic, cytoprotective, hepatoprotective, anti-bacterial, antioxidant, anti-hypertensive, anti-hyperlipidemia, hematopoietic system, and anti-obesity effects have been described from N. nucifera leaves, seed and rhizome [13-16]. In particular, N. nucifera stamens are flavonoid-rich and have a variety properties including antioxidant, anti-inflammation, anti-microbial and anti-cancer [11,12,17,18]. Flavonoids are its important constituents that belong to a group of natural substances with variable phenolic structures found in fruits, vegetables, grains, flowers, tea and wine. Moreover, flavonoids have been described to increase phagocytic activity and exerted antimalarial effect in vitro against P. falciparum [19-21]. Although biological and therapeutic efficacies of N. nucifera have been reported to a certain extent, the studies on the antimalarial activity and phagocytosis during malaria infection have not yet been performed. Hence, in the present study, we investigated the effect of N. nucifera stamen extract on phagocytosis and malaria parasite growth in vivo using P. berghei infected mouse model.

Materials and Methods

Plant material

The stamens of lotus (N. nucifera) were obtained from Rangsit Science Centre for Education, and authenticated by Dr. Saowanee Buatone.

Preparation of crude extract

Aqueous crude extract of N. nucifera stamens was prepared as previously described [22]. In brief, the dried powdered sample was mixed with distilled water in a ratio of 1:5 (w:v), and heated using microwave at 360 W for 5 min. Incubation at room temperature for 30 min was subsequently performed with continuously stirring to complete.

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The standard 4 day test was employed in this study [26]. Randomly groups of naïve ICR mice (5 mice of each) were inoculated by intraperitoneal injection with 1×10⁷ infected erythrocytes of PbANKA, and treated for 4 consecutive days with 500, 1,000 and 2,000 mg/kg of *N. nucifera* stamen extracts orally by gavage twice a day (day 0-3). Three control groups were used; the healthy control was given either with distilled water or the extract (2,000 mg/kg); the untreated control was given distilled water; the drug treatment control was given 5.0 mg/kg of CQ. On day 4 of the experiment, tail blood was collected to determine parasitemia, percent phagocytosis and phagocytic index.

**Statistics**

Statistical analysis of the data was carried out using GraphPad Prism Software (GraphPad software, Inc., CA, USA). The one way ANOVA was used to analyze and compare the results at a 95% confidence level. Values of *p*<0.05 were considered significant. Results were expressed as mean + standard error of mean (SEM).

**Results**

**Acute toxicity test**

Behavioral signs of toxicity observed in mice given 3000 mg/kg of *N. nucifera* stamen extract include; paw licking, salivation, stretching and reduce activity. There was however no mortality at all doses used. Therefore, 500, 1000, and 2000 mg/kg were suitable doses for using in this study.

**Malaria-associated phagocytic suppression during PbANKA infection**

There was a progressive increase in level of parasitemia as the days progressed from day 2 to 12 in the PbANKA infected mice (Figure 1A), and survival time was 12 days (Figure 1B). Interestingly, determination of phagocytosis showed a progressive decrease in the response to the presence of the parasites, which reached significant values on 4 after infection (Figure 1C). Moreover, decreasing of phagocytic index was also observed in the correlation with decreasing of phagocytosis (Figure 1D).

**Antimalarial activity of *N. nucifera* stamen extract against PbANKA infected mice**

During early malaria infection, *N. nucifera* stamen extract produced a dose-dependent antimalarial effect against PbANKA. The extract caused a significant (*p*<0.05) antimalarial when compared to the untreated control, especially at dose of 2,000 mg/kg showed the highest activity (Figure 2). The standard drug, CQ caused chemosuppression, which was similar to those of the extract treated groups.

**Anti-phagocytic suppression of *N. nucifera* stamen extract during PbANKA infection**

As showed in Figure 3, significant (*p*<0.01) suppression of phagocytosis was observed in untreated group. Interestingly, *N. nucifera* stamen extract exerted dose-dependent anti-phagocytic suppression in the extract treated groups, especially at a dose of 2,000 mg/kg showed the highest activity. In addition, no effect on phagocytosis was observed in normal mice treated with this extract and CQ treated group.

**Discussion**

There was a progressive increase in level of parasitemia at the days progressed from day 2 to 12 in the PbANKA infected mice (Figure 1A). Moreover, decreasing of phagocytic index was also observed in the correlation with increasing of parasitemia (Figure 1D). The standard drug, CQ caused chemosuppression, which was similar to those of the extract treated groups.
1A), and survival time was 12 days (Figure 1B). This is in line with the view that parasitemia increases progressively after inoculation or infection until the point of death in the absence of suitable treatment. Interestingly, determination of phagocytosis showed a progressive decrease in the response to the presence of the parasites, which reached significant values on 4 after infection (Figure 1C). Moreover, decreasing of phagocytic index was also observed in the correlation with decreasing of phagocytosis (Figure 1D). This could be due in part to the fact that during malaria infection, infected erythrocytes and hemozoin (malarial pigment) non-enzymatically generated large amounts of hydroxyl fatty acids that inhibit monocyte function followed by decreasing phagocytosis [27]. Phagocytosis of hemozoin or hemozoin-containing trophozoites alters functionality of monocytes and macrophages. Monocyte ability to perform oxidative burst is compromised, bacterial killing abolished, antigen presentation altered and ability to differentiate to functional dendritic cells disturbed. Moreover, hemozoin-laden monocytes produce increased amounts of peroxidation products of polyunsaturated fatty acids and stimulate generation of several cytokines, such as TNF, IL-1beta, MIP-1alpha and MIP-1beta [28,29]. These appears to be causally related to decreasing phagocytosis and phagocytic index in our finding during PbANKA infection in mice.

During early malaria infection, the extract of N. nucifera stamens produced a dose-dependent antimalarial effect against PbANKA. The extract caused a significant (*p<0.05) antimalarial when compared to the untreated control, especially at dose of 2,000 mg/kg showed the highest activity (Figure 2). The standard drug, CQ caused chemosuppression, which was similar to those of the extract treated groups. It has been reported the antioxidant potential was related to antimalarial activity in several plant extracts. Moreover, flavonoids, quercetin and kaempferol have been reported to have potent antimalarial activity against P. berghei infected mice [19,30,31]. Hence, these compounds in N. nucifera stamen extract, and its potent antioxidant activity might play a central role to inhibit PbANKA growth in vivo. Moreover, oxidative damage in order to inhibit malaria parasite of artemisinin has also been described [32], and might related to antimalarial activity of N. nucifera stamen extract. However, the modes of action and other mechanisms should be searched for.

As showed in Figure 3, significant (*p<0.01) suppression of phagocytosis was observed in untreated group. Interestingly, N. nucifera stamen extract exerted dose-dependent anti-phagocytic suppression in the extract treated groups, especially at a dose of 2,000 mg/kg showed the highest activity. Several studies have been reported the activity of N. nucifera extract to activate innate immune response and phagocytic
activity. Knowledge of properties of flavonoids and polyphenolic compounds in order to protect white blood cells and macrophage from oxidative stress and activate phagocytic activity have been described [20]. Moreover, protective effect of flavonoid-rich extract on macrophage from oxidative stress-induced apoptosis has also been reported [33]. Hence, significant antioxidant potential and flavonoid-rich stamen extract might be properties on increasing of phagocytosis during malaria infection.

It is interesting to note that *N. nucifera* stamen extract was found the antimarialar and anti-phagocytic suppression against *P. berghei* infected mice. Although the bioactive components and mechanism are yet to be identified, the results of this study provide the basis for further studies.

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