

EFFECTIVENESS OF USING SPIRULINA IN CONJUNCTION WITH ARTEMISININ COMBINATION THERAPY AS AN ANTIMALARIAL TREATMENT

NAJMA ASSEGAFF¹, HENDRI ASTUTY^{2*}

¹Undergraduate Program, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia. ²Department of Parasitology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia. Email: astutiek@yahoo.com

Received 30 September 2018, Revised 30 September 2018 and Accepted 13 February 2019

ABSTRACT

Objective: This study investigated whether the herbal plant Spirulina could be used in crude form as an alternative therapy to artemisinin combination therapy (ACT) for the treatment of malaria caused by Plasmodium falciparum.

Methods: Mice infected by Plasmodium berghei were treated orally with a single dose of Spirulina (200 or 400 mg/kg body weight [BW]), dihydroartemisinin-piperaquine as a type of ACT, or a combination of the two. The level of parasitemia was then compared between the groups during the 4-day post-infection period.

Results: There was a significant difference in the change in the level of parasitemia from day 0 to day 4 between groups (Kruskal-Wallis test, $P < 0.05$). Mice that were treated with both doses of Spirulina alone had a significantly higher parasitemia density than those treated with ACT alone. However, the combination of Spirulina and ACT had a synergistic effect, with 200 mg/kg BW Spirulina + ACT giving significantly better results than ACT alone.

Conclusion: These findings indicate that treatment with Spirulina alone cannot be used as antimalarial medication, but its combination with ACT can lead to enhanced antimalarial activity.

Keywords: Spirulina, Artemisinin combination therapy, Parasitemia density, Antimalarial activity, Plasmodium berghei.

© 2019 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ijap.2019.v11s1.013>

INTRODUCTION

Malaria is a disease that is transmitted by female Anopheles mosquitoes carrying the Plasmodium parasite [1]. According to the World Health Organization (WHO), there were 214 million cases of malaria and 438,000 malaria deaths worldwide in 2015, with Africa having the greatest number of cases [2]. In Indonesia, malaria remains one of the major health problems, with a prevalence rate of 6% and an incidence of 1.9% according to Indonesian Basic Health Research in 2013.

Artemisinin combination therapy (ACT) is currently recommended by the WHO for the treatment of malaria caused by Plasmodium falciparum [3], as artemisinin can rapidly reduce the level of parasites in the blood. However, resistance to this drug was detected in a number of countries in 2015, and there is concern that this resistance will spread to other countries, which would pose a major health problem because no other antimalarials with an equivalent efficacy have been found to date [3].

Medicinal plants offer a safe and cheap alternative treatment for malaria with few side effects. Consequently, many studies have investigated whether the compounds they contain can be combined with standard drugs to resolve the current resistance problem [4]. Spirulina algae contain proteins, carbohydrates, fats, vitamins, and some types of pigments [5] and have been shown to have not only anti-inflammatory, antiviral, antioxidant, and anticancer effects [6-9] but also antimalarial activity through neutrophil blockage and pro-inflammatory cytokines [7]. Furthermore, Spirulina is classified as safe for use due to its minimal side effects even after long-term use [6].

One of the most studied pigments in Spirulina is phycocyanin, which can act as an anti-inflammatory, antimicrobial, and antimalarial [5,6,10,11] and is known to inhibit nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which plays a role in the development of oxidative stress [5]. Consequently, phycocyanin is considered to be of biomedical

interest, and the effect of low concentrations of phycocyanin on parasitemia levels in sensitive or chloroquine-resistant parasites has previously been investigated. In addition, phycocyanin is safe to use in combination with other antimalarials [10]. The beta-carotene content of Spirulina also plays an important role as an antioxidant and anti-inflammatory [11], inhibiting the pro-inflammatory cytokines produced by macrophages in a way similar to phycocyanin [6].

No studies to date have used Spirulina in combination with ACT for the treatment of malaria. Therefore, this study investigated the effect of treating mice infected with Plasmodium berghei with a combination of Spirulina and dihydroartemisinin-piperaquine (DHP), which is a type of ACT that has been shown to have high antimalarial efficacy [2].

METHODS

Study animals and treatment groups

This study used 3–4-month-old male Swiss mice that weighed 20–25 mg. The mice were kept in a laboratory at the Research and Development Center of Indonesian Ministry of Health (Salemba, Indonesia) between April and December 2016, where they were provided with standard food and clean drinking water each day. They were housed before the experiment for acclimation to the surrounding environment.

Federer's formula showed that the minimum number of experimental animals that needed to be used was four per treatment group. Therefore, five mice were included in each group in anticipation that some mice would drop out of the experiment. The mice ($n=30$) were divided into six treatment groups: A positive control group that was given DHP, a negative control group that received no treatment, two Spirulina treatment groups (200 and 400 mg/kg body weight [BW]), and two combined treatment groups (200 and 400 mg/kg BW Spirulina + DHP).

Table 1: Effects of Spirulina and ACT on the levels of parasitemia and inhibition of parasite growth in mice infected with Plasmodium berghei from 0 to 4 days after infection (H0-H4)

Treatment group	n	H0		H1		H2		H3		H4		P H4-H0
		P	GI	P	GI	P	GI	P	GI	P	GI	
Negative control	5	0.8	0	10.6	0	28.96	0	48.44	0	55.26	0	54.46
Positive control	5	0.52	35	1.72	83.77	0.96	96.69	0.48	99.01	0.62	98.88	0.1
Sp 1	5	0.78	2.50	6.32	40.38	18.2	37.15	43.52	10.16	56.1	-1.52	55.32
Sp 2	5	0.78	2.50	6.64	37.36	26.94	6.98	47.02	2.93	60.42	-9.34	59.64
Combination 1	5	0.98	-22.50	0.56	95.09	0.64	97.79	0.52	99.01	0.6	98.91	-0.38
Combination 2	5	0.72	10.00	1.76	83.40	0.54	98.14	0.46	99.05	0.5	99.10	-0.22

Negative control, no treatment; positive control, ACT; Sp 1, 200 mg/kg BW Spirulina; Sp 2, 400 mg/kg BW Spirulina; combination 1, 200 mg/kg BW Spirulina+ACT; combination 2, 400 mg/kg BW Spirulina+ACT; P - Parasitemia level; GI - Growth inhibition ACT; Artemisinin combination therapy, BW: Body weight

Table 2: Differences in the parasitemia density in mice infected with Plasmodium berghei that had been administered Spirulina and/or ACT

Treatment group	n	Brinkman index	p value
Positive control	5	0.00 (-0.10-0.40)	< 0.000
Negative control	5	51.80 (49.00-69.00)	
Sp 1	5	55.00 (40.80-68.50)	
Sp 2	5	58.80 (53.60-69.30)	
Combination 1	5	-0.30 (-0.70--0.10)	
Combination 2	5	0.00 (-1.00-0.00)	

Kruskal-Wallis test. See Table 3 for the results of the post hoc. Positive control, ACT; Sp 1, 200 mg/kg BW Spirulina; Sp 2, 400 mg/kg BW Spirulina; combination 1, 200 mg/kg BW Spirulina+ACT; combination 2, 400 mg/kg BW Spirulina+ACT; negative control, no treatment. ACT: Artemisinin combination therapy

Table 3: Post hoc analysis of the differences in parasitemia density among treatment groups

Treatment group	Asymp. Sig. (two-tailed)	Significance
Positive control	Sp 1	0.009 *
	Sp 2	0.009 *
	Combination 1	0.012 *
	Combination 2	0.180 NS
Sp 1	Negative control	0.009 *
	Sp 2	0.465 NS
	Combination 1	0.009 *
	Combination 2	0.008 *
Sp 2	Negative control	0.917 NS
	Combination 1	0.009 *
	Combination 2	0.008 *
Combination 1	Negative control	0.76 NS
	Combination 2	0.138 NS
Combination 2	Negative control	0.009 *
	Negative control	0.008 *

*p<0.05, Mann-Whitney U-test. Positive control, ACT; Sp 1, 200 mg/kg BW Spirulina; Sp 2, 400 mg/kg BW Spirulina; combination 1, 200 mg/kg BW Spirulina+ACT; combination 2, 400 mg/kg BW Spirulina+ACT; negative control, no treatment

Experimental procedure

Parasitemia was induced in donor mice, following which blood suspensions from these donors were diluted to 2% parasitemia concentration. Each experimental mouse was injected with up to 1 ml of the diluted infected blood intraperitoneally and left for 24 h for the parasites to breed and has an effect on the mice.

After 24 h, the mice in each group were administered their respective treatment orally using a sonde that was carefully inserted into the stomach of the mouse. DHP was administered at a dose of 49.3 mg/kg BW, which was calculated by converting the dose of 4 mg/kg BW dihydroartemisinin that is recommended in the

Malaria Case Management Manual in Indonesia [12] using a surface-based calculation with a constant factor for each species. Spirulina was administered at 200 and 400 mg/kg BW, according to Siswanto [13].

The level of parasitemia in each group was assessed using the 4-day suppressive tests introduced by Trager and Jensen [14]. Before treatment and each day for 4 days after treatment, a blood sample was collected from the lateral vein in the tail of each mouse at 1-2 mm from the tip using sterile scissors. A blood smear was made on a glass slide and left to dry in the air, and was then fixed with 90% methanol and stained using Giemsa stain diluted with distilled water. The level of parasitemia in the peripheral blood smear was then calculated in 1000 red blood cells at $\times 1000$ magnification under a microscope. On day 8, the mice were terminated by cervical dislocation, following one of the termination guidelines produced by the Institutional Animal Care and Use Committee [12].

Statistical analysis

Since the data consisted of >2 unpaired groups and >50 samples, the Shapiro-Wilk test was used to examine the normality of the data set. This showed that the data were not normally distributed. Therefore, the Kruskal-Wallis test was used to compare the levels of parasitemia among groups and the Mann-Whitney U-test was used for post hoc analysis. All analyses were performed in SPSS version 20 with a significance level of $p < 0.05$.

RESULTS

The level of parasitemia increased each day in the negative control group and 200 and 400 mg/kg BW Spirulina treatment groups but generally decreased in the positive control group and Spirulina + ACT treatment groups (Table 1). At 4-day post-infection (H4), the highest rate of parasitemia was observed in 400 mg/kg BW Spirulina group (60.42%), while the lowest rate was observed in 400 mg/kg BW Spirulina + ACT group (0.5%).

Both of Spirulina + ACT treatment groups experienced decreased parasitemia levels between the day of infection with *P. berghei* (H0) and H4, though with slight increases on H2 and H4 (Table 1). They also had lower mean parasitemia rates than the positive control group, with this difference being significant for 200 mg/kg BW + ACT treatment group (Tables 2 and 3). In addition, 200 mg/kg BW Spirulina treatment group exhibited slightly greater reductions in parasitemia levels than 400 mg/kg BW Spirulina treatment group (Sp 200 mg/kg WB = -0.38% and Sp 400 mg/kg WB = -0.22%). By contrast, the negative control group and two Spirulina treatment groups had significantly higher parasitemia levels than the positive control group (Tables 2 and 3).

The positive control group and the two combined treatment groups had the highest rates of parasite growth inhibition (98.88-99.10%), while 400 mg/kg BW Spirulina treatment group had the lowest (-9.34%) (Table 1). However, there was little difference between Spirulina alone and combined treatment groups.

DISCUSSION

This study investigated the effects of using two different doses of Spirulina alone and in combination with ACT to treat mice infected with *P. berghei*. Neither of the Spirulina treatment groups exhibited any antimalarial effects, with the parasitemia level increasing each day in a way similar to the negative control group. However, a combination of Spirulina and ACT exhibited enhanced antimalarial activity, with a lower parasitemia level than the positive control group that was treated with ACT therapy.

Parasitic infections can induce the production of hydrogen peroxide and the formation of hydroxyl radicals (OH[•]), which cause apoptosis and oxidative stress [15], and can also lead to an increase in lipid peroxidation and a decrease in antioxidant capacity in the host's body [16]. The body responds to the presence of the infection by increasing macrophage and neutrophil phagocytosis activities, which leads to an imbalance between the antioxidant activity and free radicals in the body [17].

Spirulina has a potent antioxidant capacity through its ability to prevent lipid peroxidation and DNA damage and to increase the production of antioxidant enzymes [18]. The antioxidant and anti-inflammatory effects of this plant result from the phycocyanin, vitamins, and other substances it contains. Phycocyanin can inhibit the activity of NADPH oxidase, which is involved in the development of oxidative stress, and the vitamins in Spirulina, such as Vitamins E and C, also enhance the plant's antioxidant and anti-inflammatory effects [5]. In addition, phycocyanin has an antimalarial effect through the destruction of hemozoin polymerization [10]. Consequently, the consumption of Spirulina can improve the workings of the immune system, and it has been proposed that phycocyanin and beta-carotene pigments could act synergistically with ACT [5]. Therefore, the synergistic antimalarial effect of Spirulina when used in combination with ACT likely resulted from the antioxidant activities that are associated with the beta-carotene and phycocyanin pigments it contains.

Given these activities of the components of Spirulina, it was somewhat surprising that Spirulina alone did not exhibit antimalarial activity. It has previously been shown that phycocyanin obtained from *Nostoc* spp. has antimalarial activity and is a powerful antioxidant [10], whereas phycocyanin isolated from *Spirulina platensis* has a lower inhibitory effect on parasitic growth than artemisinin and is a weak antioxidant [19]. These different findings result from the different origins of the phycocyanin and the different extraction processes that were used, which will have affected the level of phycocyanin purity [19]. The present study used crude Spirulina that was sold commercially, the specific composition of which was unknown. Therefore, it is suspected that this contained some substance that acted as an antagonist of the antimalarial activity possessed by phycocyanin.

CONCLUSION

This study investigated the use of crude Spirulina as an antimalarial. It was found that Spirulina was not able to function as an antimalarial when used on its own. However, the use of 200 mg/kg BW Spirulina in combination with ACT led to enhanced antimalarial activity, indicating a synergistic interaction between the two treatments.

CONFLICTS OF INTEREST

All authors declare that they have no conflicts of interest.

REFERENCES

1. Global Health-Division of Parasitic Diseases and Malaria. Malaria Biology: Anopheles Mosquitoes. Centers for Disease Control and Prevention; 2016. Available from: <http://www.cdc.gov/malaria/about/biology/mosquitoes>. [Last cited on 2016 Apr 27].
2. WHO. World Malaria Report. Switzerland: WHO Press; 2015. Available from: <http://www.who.int/malaria/publications/world-malaria-report-2015/en>. [Last accessed on 2016 May 13].
3. WHO. Malaria: Q and A on Artemisinin Resistance. World Health Organization; 2015. Available from: http://www.who.int/malaria/media/artemisinin_resistance_qa/en. [Last accessed on 2016 May 17].
4. Muti'ah R, Enggar L, Winarsih S, Soemarmo S, Simamora D. Combination of talikuning stem and artemisinin as an antimalarial drug against *Plasmodium berghei*. J Kedokt Brawijaya 2010;26:8-13.
5. Ali SK, Saleh AM. Spirulina-an overview. Int J Pharm Pharm Sci 2012;4:9-15.
6. Chow TJ, Deng R. Hypolipidemic, antioxidant and antiinflammatory activities of microalgae spirulina. Cardiovasc Ther 2010;28:e33-45.
7. Rasool M, Sabina EP, Lavanya B. Anti-inflammatory effect of *Spirulina fusiformis* on adjuvant-induced arthritis in mice. Biol Pharm Bull 2006;29:2483-7.
8. Pugh ND, Edwall D, Lindmark L, Kousoulas KG, Iyer AV, Haron MH, et al. Oral administration of a spirulina extract enriched for braun-type lipoproteins protects mice against influenza A (H1N1) virus infection. Phytomedicine 2015;22:271-6.
9. Konickova R, Vankova K, Vanikova J, Vanova K, Muchova L, Subhanova I. Anti-cancer effects of blue-green alga *Spirulina platensis*, a natural source of bilirubin-like tetrapyrrolic compounds. Ann Hepatol 2014;13:273-83.
10. Pankaj PP, Seth RK, Mallick N, Biswas S. Isolation and purification of phycocyanin from *Nostoc muscorum* (cyanophyceae and cyanobacteria) exhibits antimalarial activity *in vitro*. J Adv Lab Res Biol 2010;1:87-91.
11. Kumar V, Tirumalai PS, Singh A, Bhatnagar AK, Shrivastava JN. Natural compounds from algae and *Spirulina platensis* and its antimicrobial activity. Indo Glob J Pharm Sci 2013;3:212-23.
12. Ministry of Health. Regulation of the Republic of Indonesia Health Minister Number 5 of 2013 Concerning Guidelines for the Management of Malaria. Jakarta: Ministry of Health; 2013.
13. Siswanto H, Hasugian AR, Avrina R, Risniati Y, Tjitra E. Efficacy and safety of dihydroartemisinin piperakuin (DHP) in patients with falciparum malaria without complication in Kalimantan and Sulawesi. Res Dev Health Media 2011;21:135-44.
14. Trager W, Jensen JB. Human malaria parasites in continuous culture. Science 1976;193:673-5.
15. Onyeneke EC, Okuda AO, Akunyele OR, Onumaegbu PU, Ukinebo OE, Chukudi JA, et al. Effects of antimalaria drugs on antioxidant status of malaria patients. Int J Pharm Med Sci 2016;6:20-9.
16. Euthanasia Guidelines. Research Animal Resources, University of Minnesota. Available from: <https://www.ahc.umn.edu/rar/euthanasia.html>. [Last cited on 2016 Apr 28].
17. Percario S, Moreira DR, Gomes BA, Michelli E, Sereira S, Goncalves AC, et al. Oxidative stress in malaria. Int J Mol Sci 2012;13:16346-72.
18. Abdelkhalek NK, Ghazy EW, Daim AM. Pharmacodynamic interaction of *Spirulina platensis* and deltamethrin in freshwater fish Nile tilapia, *Oreochromis niloticus*: Impact on lipid peroxidation and oxidative stress. Environ Sci Pollut Res Int 2015;22:3023-31.
19. Wulandari AF, Irwanti G. Arthrospira plantensis as alternative therapy in children with HIV (+). JIMKI 2010;1:7-10.