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Original Article:

Evaluation of the Efficacy of Nano Spirulina Alcoholic Extract in Conjunction with Chloroquine Against Plasmodium berghei in BALB/c Mice

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Abstract

Introduction: The recent emergence of drug resistance among malaria parasites has led to increased interest in the use of medicinal plants for the treatment of malaria, particularly because these plants tend to have fewer side effects compared to conventional chemical medications. This study aimed to evaluate the effects of nano Spirulina algae extract in conjunction with chloroquine on laboratory mice.

Materials and Methods: An alcoholic extract of Spirulina was prepared and subsequently processed and characterized through Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and Fourier Transform Infrared Spectroscopy (FTIR). The antimalarial activity of these components was assessed in two distinct phases using white mice as the model organism. In the initial phase, the optimal concentration and the effective dose (ED50) of both Spirulina and nano-Spirulina against *Plasmodium berghei* were determined. Following this, the efficacy of the extract was examined in combination with chloroquine at a fixed ratio. The data were statistically analyzed using a one-way ANOVA test.

Results: The optimal concentration of the extract was established at 100 mg/kg, which resulted in 85% and 47% inhibition of parasite growth on days 4 and 7, respectively ($p < 0.05$). The effective doses (ED50) for nano Spirulina and chloroquine were determined to be 100 mg/kg and 1.2 mg/kg, respectively. Additionally, the findings from the combined treatment with chloroquine and nano Spirulina extract suggested an antagonistic interaction. Consequently, it is recommended to avoid the concurrent use of these two medications for therapeutic purposes.

Conclusion: The application of nano-extracts derived from Spirulina algae has demonstrated the ability to inhibit the proliferation and growth of parasites, indicating that this extract may significantly reduce the presence of *Plasmodium berghei* in murine models. However, when administered in conjunction with chloroquine, an antagonistic interaction occurs, leading to an enhancement in parasite growth.

Keywords: Spirulina algae, Plasmodium berghei, Combination therapy, Chloroquine

1. Introduction

Malaria continues to be one of the most significant and widespread parasitic diseases affecting humans worldwide, with a notably high mortality rate among pregnant women and children under the age of five. This disease is caused by Plasmodium parasites, which are prevalent in regions endemic to malaria. According to the 2022 report published by the World Health Organization (WHO), approximately 247 million cases of malaria were reported in 2021, affecting 84 endemic countries. Furthermore, the report indicates that the number of reported cases in 2021 was two million higher than in 2020, underscoring the neglect of malaria during the COVID-19 pandemic[1]. The World Health Organization recommends Artemisinin combination therapy for the treatment of malaria caused by Plasmodium falciparum due to the drug's significant efficacy in reducing parasitic load in the bloodstream. However, there have been reports of drug resistance in several countries which could be regarded as a global concern. In response to this challenge, the RTS, S vaccine has been administered experimentally in selected countries, including Kenya, Ghana, and Malawi, as endorsed by the WHO. Nevertheless, the treatment of malaria remains a critical priority until effective solutions are achieved [1, 2]. Algae constitute a diverse and extensive group of primitive photosynthetic organisms, which are commonly categorized into two primary classifications: microalgae and macroalgae. Microalgae encompass various taxa, including cyanobacteria (commonly known as blue-green algae), dinoflagellates, and diatoms (Bacillariophyta), while macroalgae, or seaweeds, include green, brown, and red algae. The phyla of microalgae have been recognized for their potential to yield novel chemical and pharmacological compounds. Furthermore, microalgae are acknowledged as the primary producers of several highly bioactive compounds found within marine ecosystems [3, 4]. Spirulina is a microscopic filamentous cyanobacterium, and its name is derived from its characteristic spiral shape and filamentous structure. The term Spirulina platensis and Spirulina maxima being the most significant species. These algae typically range in length from 3 to 33 micrometers, rendering them difficult to observe with the naked eye [5]. Numerous studies on Spirulina algae have identified its various properties, including high-quality proteins that contain essential amino acids, pigments such as carotenoids and phycocyanin, as well as vitamins and minerals like calcium and iron. Additionally, Spirulina exhibits antioxidant and antibacterial properties.[4, 6-8].

Nanotechnology refers to the manipulation and utilization of matter at the nanometer scale. The dimensions of materials are critically significant in nanotechnology, as the physicochemical and biological properties of atoms and molecules differ markedly from those of bulk materials at this scale. The distinctive biological properties of nanomaterials arise from their unique characteristics at the nanoscale; consequently, by altering the shape and size of nanomaterials, novel properties and potential applications can be developed [9]. The initial effect of reducing particle size is the enhancement of the surface-to-mass ratio. As this ratio increases, the influence of surface atoms becomes more pronounced compared to that of the atoms located within the particle's interior. Consequently, this alteration significantly impacts the physical properties of the particles, leading to a marked increase in their reactivity. An increase in surface area results in modifications to various characteristics, including viscosity, surface pressure, surface properties, and magnetic properties of the particles. These changes subsequently affect the interatomic distances within the particles, reduce the increase in ionization potential, enhance the strength of electrical bonds, and ultimately alter the chemical interactions of the material. Nanoparticles are composed of both organic and inorganic chemical substances.[10,11]. This study explored the interaction between Spirulina algae nano extract and chloroquine in their combined effects against the Plasmodium berghei parasite in BALB/c mice.

2. Materials and Methods

Animals

In this study, mice weighing approximately 19 to 23 grams were approached, having been procured from the Pasteur Institute. The mice were housed in standard cages and provided with access to nutritious food and drinking water, maintained at the standard temperature of the animal house at Tehran University of Medical Sciences. Furthermore, it is important to note that the treatment of the mice involved in this study adhered to the ethical guidelines and regulations established by the Declaration of Helsinki.

Parasites

In the current study, *Plasmodium berghei*, which was propagated at the National Malaria Laboratory of Tehran University of Medical Sciences, was utilized. The *Plasmodium berghei* parasite was retrieved from liquid nitrogen storage in accordance with established

safety protocols, allowed to acclimatize to ambient temperature, and subsequently injected intraperitoneally into the designated mouse to facilitate the introduction of the parasite into the animal's body.

Herbal extract and drug

The dry powder of Spirulina algae was obtained from the Iranian National Algae Culture Collection, amounting to approximately 96 grams. Following this, an extraction process was carried out in collaboration with the Faculty of Pharmacy at Tehran University of Medical Sciences, applying the maceration method as detailed below:

Ninety six grams of Spirulina algae powder was thoroughly immersed in 300 ml of 80% ethanol and subjected to maceration for a duration of 24 hours. The resulting supernatant solvent was then filtered using a paper filter, after which an additional 200 ml of 80% ethanol was introduced to the remaining sediment. This filtration process was repeated after another 24 hours, and the supernatant was subsequently separated. This procedure was conducted for an additional four days. The collected supernatant, which contains the bioactive compounds of Spirulina algae, was concentrated using a vacuum distillation apparatus at a temperature of 40 degrees Celsius until the desired extract was achieved, yielding approximately 46 grams. Concentrations of 100, 50, 25, and 12.5 mg/kg were prepared from the Spirulina algae extract. Following the extraction process, which was conducted in collaboration with the Clinical Biochemistry Department of Iran University of Medical Sciences, the resultant extract was converted into a nano extract through the incorporation of magnesium oxide, as detailed in the subsequent methodology.

An approximate quantity of 40 grams of algae extract was measured, and its alcoholic aqueous extract was prepared utilizing the Soxhlet extraction system. The extract was subsequently filtered to eliminate impurities. In the following step, a specific amount of magnesium salt was introduced to the algae extract. Through the application of heat and stirring on a heating stirrer, magnesium oxide (MgO) nanoparticles were synthesized, which exhibited phenolic compounds derived from the algae extract on their surface. Ultimately, Nano Spirulina was processed and characterized using Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and Fourier Transform Infrared Spectroscopy (FTIR). Concentrations of the resulting substance were prepared at 100, 50, 25, and 12.5 mg/kg.

Testing method

Initially, *Plasmodium berghei* parasites were suspended in normal saline to prepare a suspension. A selection of mice was designated as parasite donors, and the parasites were injected intraperitoneally into the donor mice to induce infection. Blood samples were collected from the donor mice by performing a cardiac puncture, and the blood was subsequently diluted in normal saline. A total of 0.2 ml containing 10^6 parasitized red blood cells was intraperitoneally injected into a cohort of 45 male mice, which were divided into nine groups. Seven of these groups were administered varying concentrations of Spirulina and nano Spirulina, while the eighth group received chloroquine at a concentration lower than the standard therapeutic dose, in order to determine the effective dose (ED50) for this medication. The final group was administered parasites without any therapeutic intervention.

Different dosages of pure extract and nanoform of Spirulina algae, along with chloroquine, were administered to infected mice. The injections were administered once daily for a duration of four days.

Mice were subjected to daily examinations. The assessment of parasitemia in the mice continued for a duration of 7 days, while the monitoring of mortality extended until day 21. On the fourth and seventh days, blood samples were collected from the tails of the mice, and smears were prepared and stained with 10% Giemsa to quantify the percentage of parasitic presence and the inhibition rate. The results obtained from the microscopic counting of the prepared slides were recorded using SPSS statistical analysis software, and statistical analyses were conducted employing specialized tests. A One-Way ANOVA parametric test was utilized to compare the means of the measured variables across different groups.

The study was conducted over a period of 21 days, during which the survival times of the mice in each experimental group were recorded and compared to those of the control group. At the conclusion of the initial phase, the effective doses (ED50) for both the extract and chloroquine were determined. In the subsequent phase of the study, the two components were combined in fixed ratios of 100:0, 90:10, 70:30, 50:50, 30:70, 10:90, and 0:100, representing chloroquine and nano Spirulina algae, respectively, for the pooled treatment. These combination mixtures were administered intraperitoneally to the infected mice and continued until the fourth day to assess the effects of these components on the parasites when combined. Based on the results obtained, a FIX RATIO diagram was constructed for the various

compounds, and the connections formed on the diagram were analyzed to determine the presence of synergistic or antagonistic effects.

3. Results

Investigating the structure and size of Magnesium Oxide/Spirulina extract nanoparticles using Scanning Electron Microscopy (SEM)

Nanoparticles were analyzed utilizing an electron microscope. The microscopic images obtained from a scanning electron microscope (SEM) demonstrate the successful synthesis of magnesium oxide (MgO) nanoparticles and the formation of a Spirulina extract nanoparticle complex. The synthesized MgO nanoparticles are characterized as spherical in shape, with an approximate diameter of 36 nm. These findings indicate the successful synthesis of nanoparticles employing Spirulina algae extract and the preparation of an MgO extract nanocomplex [Figure 1].

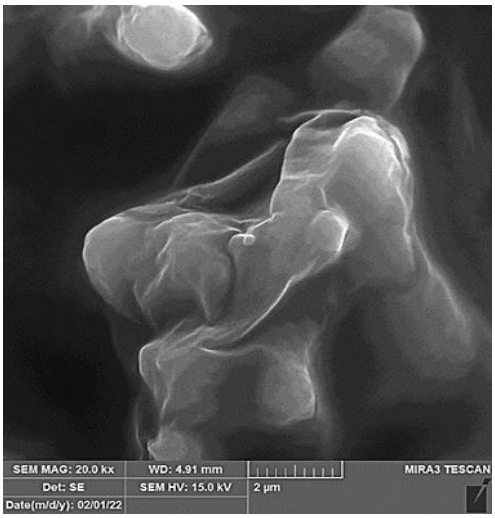

Figure1. Scanning Electron Microscope (SEM) image of magnesium oxide nanoparticles/Spirulina extract

Investigating the structure and size of Magnesium Oxide/Spirulina extract nanoparticles using Transmission Electron Microscopy (TEM)

The results obtained from transmission electron microscopy (TEM) images demonstrate a relatively uniform distribution of nanoparticles. Furthermore, the spherical morphology of the nanoparticles is distinctly observable in the TEM images, indicating the successful synthesis of spherical nanoparticles utilizing Spirulina algae extract [Figure 2].

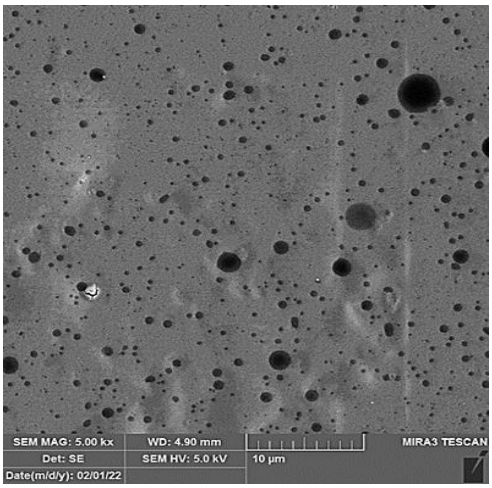

Figure 2. Transmission Electron Microscopy (TEM) image of magnesium oxide nanoparticles/spirulina extract

The results of the chemical configuration of samples analyzed through Fourier Transform Infrared Spectroscopy (FTIR)

The chemical configuration of Spirulina algae extract was analyzed using Fourier Transform Infrared (FTIR) spectroscopy. The infrared (IR) spectrum of Spirulina algae under optimal conditions is presented in Figure 4. The O-H stretching vibration observed at 3388 cm^{-1}, along with the peak at 2730 cm^{-1}, indicates the presence of phenolic compounds and alcohols, respectively. The C-H stretching observed at 2857 cm^{-1} suggests the presence of alkanes, while the strong C-H stretching at 12728 cm^{-1} is indicative of alkynes. The peak at 1728 cm^{-1} represents a carboxylic acid functional group, characterized by a strong and extensive O-H stretch. The C-H bending vibrations observed at 1452 cm^{-1} and 1375 cm^{-1} are robust, suggesting the presence of propane. Additionally, the C-O-C stretching observed at 1115 cm^{-1} indicates the presence of ester functional groups. The C-H stretching and bending vibrations between 1695 cm^{-1} and 1635 cm^{-1} further confirm the presence of alkanes.

The stretching vibrations observed in the range of 11590 cm^{-1} to 1780 cm^{-1} are indicative of C-H bending, which can be attributed to the presence of aromatic compounds. Furthermore, in the synthesized nanoparticle sample, a peak located at approximately 1555 cm^{-1} suggests the presence of the metal ion MgO in conjunction with the synthesized nanoparticles. Fourier Transform Infrared (FTIR) spectroscopy revealed a broad peak at 13400 cm^{-1} and a distinct peak at 1610 cm^{-1}, which correspond to the bending and stretching vibrations of hydroxyl (OH) groups resulting from absorbed water molecules and surface hydroxyl groups associated with hydrogen bonding within the structure. The peak at 1555 cm^{-1} further corroborates the presence of the metal ion MgO and its stretching vibrations. Additionally, the absorption band observed in the range of 3200-3500 cm^{-1} in the spectrum of MgO nanoparticles confirms a significant amount of hydrogen bonding involving water molecules in relation to the crystallite surface. Moreover, the peak in the 1520 cm^{-1} region is associated with the carbonyl (CO) group present in the surface structure of the nanoparticles [figure 3].

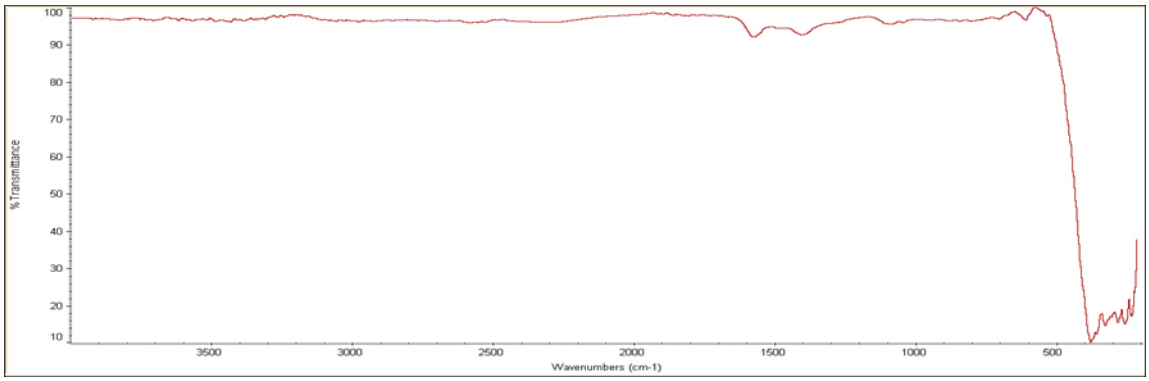

Figure 3. Fourier transform infrared spectrum of magnesium oxide nanoparticles synthesized by green method

Results of the parasitological tests:

The efficacy of parasite inhibition in treated mice was assessed following administration of various concentrations of the extract during the initial phase of the study. As presented in Table 1, all concentrations of the herbal extract utilized in this investigation demonstrated the ability to inhibit parasite growth. Notably, the 100 mg/kg concentration of nano Spirulina exhibited the highest efficacy, achieving 85% and 47% inhibition of parasite growth on days D4 and D7, respectively ($p < 0.05$). In contrast, the pure extract of Spirulina at the same concentration resulted in inhibition rates of approximately 68% and 30% on these respective days.

The nano extract of Spirulina algae, at a concentration of 100 mg/kg, demonstrated a 47% inhibition of parasites by the seventh day and was selected as the effective dose (ED50) for nano Spirulina to be combined with chloroquine in the second stage of the experiment. Additionally, a concentration of 1.2 mg/kg of chloroquine was found to decrease the parasite rate by 53% and was designated as the ED50 for chloroquine [Table 2].

In the second stage, as presented in Table 3

, the ratios of 90:10 and 70:30 nano Spirulina to chloroquine exhibited inhibitory effects of 50% and 41%, respectively. The remaining combinations demonstrated lower inhibitory effects than those observed with pure Spirulina and chloroquine. This suggests that the combination of these two components may not be effective for malaria treatment, and it is possible that these substances exhibit an antagonistic reaction when utilized in combination therapy. [Figure 4].

Table 1. The results of the percentage of inhibiting the growth of Plasmodium berghei in mice treated with different concentrations of spirulina and nano spirulina on the fourth and seventh days

Groups	Dose(mg/kg)	Parasitemia Inhibition (%) D4	Parasitemia Inhibition (%) D7
Untreated	---------	----------	----------
Chloroquine	1.2	%90±1.4	%53±5
Nano Spirulina	100	%85±2	%47±3.3
Nano Spirulina	50	%77±1	%36±4.5
Nano Spirulina	25	%78±6	%29±3.7
Nano Spirulina	12.5	%79±0.4	%20±6.6
Pure Spirulina	100	%68±4	%30±3.3
Pure Spirulina	50	%64±3	%21±2
Pure Spirulina	25	%60±3.6	%17±5.4

Table 2. The table of results from the calculation of ED50 (mg/kg) for the medicinal groups of Spirulina nano extract and chloroquine in rats under test

Drug			
Nano Spirulina		Chloroquine	
Dose of ED50 (mg/kg)	Parasitemia Inhibition (%)	Dose of ED50 (mg/kg)	Parasitemia Inhibition (%)
100	47	1.2	53

Table 3. The results of parasitemia and the percentage of growth inhibition observed on the fourth day of combination therapy

Fixed Ratio (D4) CQ/SL	Parasitemia (%) (Mean± SD)	Parasitemia Inhibition % (Mean± SD)
Control (untreated)	% 1.5	----------
CQ 100/0 SL	%0.72±0.06	%52±4
CQ 90/10 SL	%0.75±0.01	%50±0.6
CQ 70/30 SL	%0.79±0.05	%47.3±3.3
CQ 50/50 SL	%0.8±0.05	%46.7±3.3
CQ 30/70 SL	%1±0.03	%44.4±2
CQ 10/90 SL	%1.2±0.02	%20±1.3
CQ 0/100 SL	%0.685±0.07	%56.6±4.6

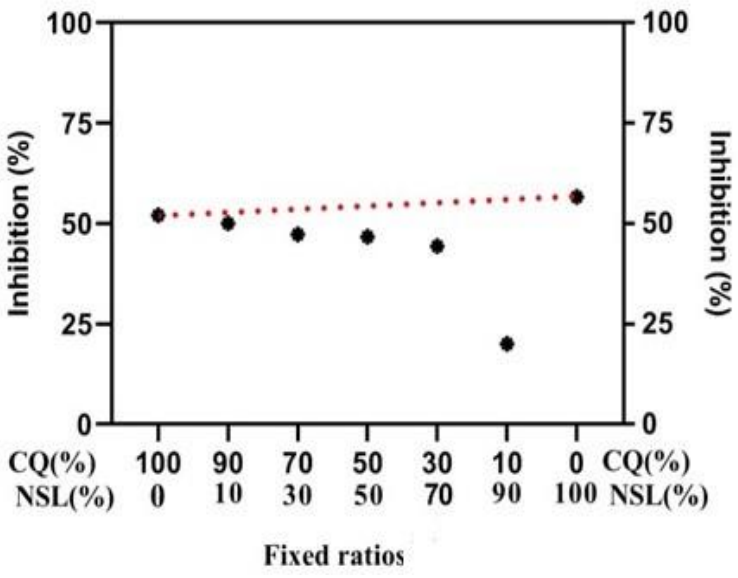

Figure 4. The percentage of growth inhibition for various ratios of chloroquine/Spirulina on the fourth day of cell treatment.

The average lifespan of mice in relation to the administered concentrations of injections.

The survival rates of the mice subjected to combination therapy with specified drug groups were analyzed. As illustrated in the accompanying figure, the highest survival rate was observed in the cohort treated with chloroquine, which exhibited a survival duration of 20 days. Conversely, the lowest survival rate was recorded in the control group that did not receive any pharmacological intervention, with a survival duration of only 7 days. Additionally, it is noteworthy that a survival duration of 15 days was documented in the group administered pure nano Spirulina. These findings are derived from the count of surviving mice in each cage, which is influenced by various factors, including the weight of the mice, their genetic background, and the strength of each individual mouse's immune system [Figure5].

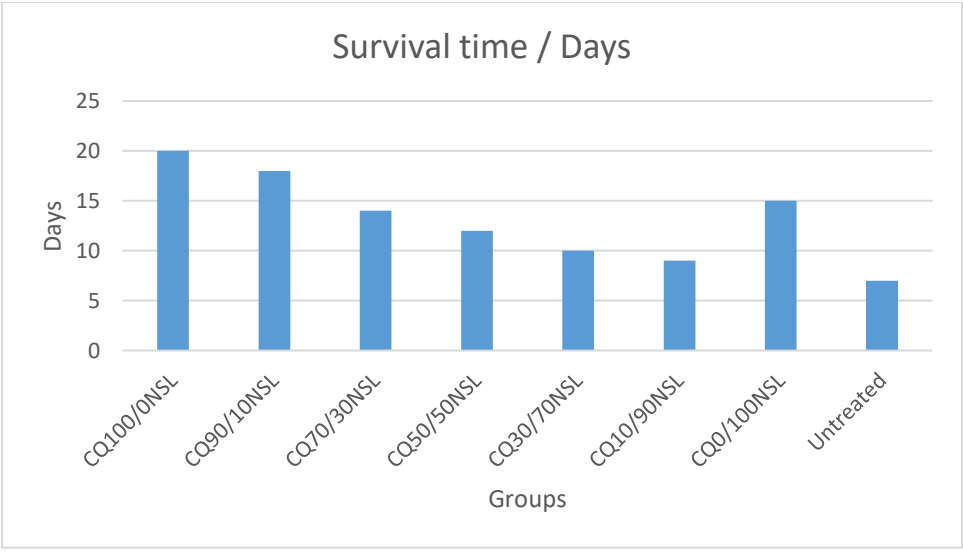

Figure 5. The survival rates of the studied mice following combination therapy with specified drug groups

4. Discussion

Although artemisinin and chloroquine are widely employed in the treatment of malaria caused by Plasmodium species, their associated toxicity raises significant concerns. Additionally, the emergence of resistance is attributed to genetic modifications that provide the parasites with protection against antimalarial agents [12]. Considering the known adverse and toxic effects of these medications, it is essential to investigate novel therapeutic alternatives and to revise the treatment regimens of current drugs. This endeavor should encompass the development of safer formulations that exhibit improved drug selectivity and sustained release properties [13, 14]. In this context, nanotechnology emerges as a powerful instrument capable of addressing these challenges and may promote the development of antimalarial therapies that are both less toxic and more effective. In particular, metal nanoparticles synthesized through environmentally friendly and green methodologies have demonstrated potential antimalarial activity, thereby representing a promising strategy for disease control [15, 16]. Rajakumar et al. synthesized nickel nanoparticles (Ni-NPs) utilizing leaf extract from Eclipta prostrata and examined their anti-plasmodial effects against Plasmodium berghei in Swiss albino mice. The study determined an inhibitory concentration (IC50) of 16.44 mg/kg body weight for the tested mice. Additionally, the nanoparticles exhibited sizes ranging from 18 to 64 nm, with an average size of 27 nm on the fourth day of treatment. The results indicated that the combined treatment effectively inhibited 78.13% of parasite growth [17]. The use of natural compounds in the treatment of malaria has a well-documented history. A study conducted by Nateghpour et al. examined the effects of the alcoholic extract of Otostegia persica on the Plasmodium berghei parasite. The findings indicated that a concentration of 450 mg of the plant extract significantly decreased the parasitemia rate in infected mice [18]. Govindan et al. conducted a study to evaluate the effectiveness of a combination of garlic oil and artichoke as an antimalarial treatment in mice. The results showed a 100% survival rate among the mice treated with this combination. This study is the first to demonstrate the potential of garlic oil as a complementary therapy to Artemether in treating malaria[19]. Kusmardi et al. conducted a study utilizing sambiloto plant extract and Spirulina algae to prevent anemia in laboratory mice infected with *Plasmodium berghei*. The findings indicated that the combination of sambiloto extract and Spirulina powder resulted in a statistically significant reduction in parasite load, as well as an increase in both the number of blood cells and hemoglobin levels by day 15, in comparison to the group that received only sambiloto extract [20]. Assegaf et al. concluded that Spirulina algae, when used alone, does not exhibit anti-malarial properties. However, their study demonstrated that administering 200 mg/kg body weight of Spirulina in conjunction with artemisinin-based combination therapy (ACT) resulted in an enhanced anti-malarial effect. This finding suggests a synergistic interaction between the two treatment modalities[21]. In the current study, nanoparticle Spirulina demonstrated significant efficacy in reducing the rate of parasitemia in infected mice. However, the interaction between the Spirulina algae nano extract and chloroquine exhibited an antagonistic effect on the *Plasmodium berghei* parasite.

5. Conclusion

The study demonstrated that the nano extract of Spirulina algae, when combined with chloroquine in varying proportions, exhibits an antagonistic effect on the Plasmodium berghei parasite. Consequently, the concurrent use of these two components for treatment is not recommended. Furthermore, it is noteworthy that the results obtained from the pure proportion of Spirulina algae nano extract indicated its efficacy in inhibiting the proliferation of the Plasmodium berghei parasite within the murine model.

Ethical Considerations

Compliance with ethical guidelines

The favorable ethical opinion (IR.TUMS.AEC.1401.177) can be accessed at https://ethics.research.ac.ir/IR.TUMS.AEC.1401.177. It is important to emphasize that the authors have diligently adhered to all ethical standards including, but not limited to, issues of plagiarism, misconduct, data falsification, and informed consent.

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Author's contributions

The authors equally contributed to preparing this article.

Conflict of interest

The authors have no conflicts of interest to declare.

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References

[1] World Health Organization. World malaria report 2023: World Health Organization; 2023. [LINK]

[2] Shibeshi W, Alemkere G, Mulu A, Engidawork E. Efficacy and safety of artemisinin-based combination therapies for the treatment of uncomplicated malaria in pediatrics: a systematic review and meta-analysis. BMC Infectious Diseases. 2021;21(1):1-12. [DOI:10.1186/s12879-021-06018-6] [PMID] [PMCID]

[3] El Gamal AA. Biological importance of marine algae. Saudi pharmaceutical journal. 2010;18(1):1-25.[DOI:10.1016/j.jsps.2009.12.001] [PMID] [PMCID]

[4] Fernández F, Reis A, Wijffels R, Barbosa M, Verdelho V, Llamas B. The role of microalgae in the bioeconomy. New Biotechnol 61: 99–107. 2021.[DOI:10.1016/j.nbt.2020.11.011] [PMID]

[5] Nege AS, Masithah ED, Khotib J. Trends in the uses of spirulina microalga: a mini-review. Scientific Journal of Fisheries and Marine. 2020;12(1). [LINK]

[6] Miranda M, Cintra R, Barros SBdM, Mancini-Filho J. Antioxidant activity of the microalga Spirulina maxima. Brazilian Journal of Medical and biological research. 1998;31:1075-9. [DOI:10.1590/s0100-879x1998000800007] [PMID]

[7] Gupta RC, Lall R, Srivastava A. Nutraceuticals: efficacy, safety and toxicity: Academic Press; 2021.

[8] Pannindrya P, Safithri M, Tarman K. Antibacterial activity of Ethanol Extract of Spirulina platensis. Current Biochemistry. 2020 Dec 21;7(2):47-51. [LINK]

[9] Remington JP. Remington: the science and practice of pharmacy: Lippincott Williams & Wilkins; 2006. [LINK]

[10] Bayda S, Adeel M, Tuccinardi T, Cordani M, Rizzolio F. The history of nanoscience and nanotechnology: from chemical–physical applications to nanomedicine. Molecules. 2019;25(1):112. [DOI:10.3390/molecules25010112] [PMID] [PMCID]

[11] Khan I, Saeed K, Khan I. Nanoparticles: Properties, applications and toxicities. Arabian journal of chemistry. 2019;12(7):908-31. [LINK]

[12] HUANG F, XiaoNong Z. Roles of artemisinin in global malaria control program. Chinese Science Bulletin. 2016;61(15):1623-5.

[13] Plowe CV. Malaria chemoprevention and drug resistance: a review of the literature and policy implications. Malaria Journal. 2022;21(1):104. [DOI:10.1186/s12936-022-04115-8][PMID] [PMCID]

[14] Jadoun S, Arif R, Jangid NK, Meena RK. Green synthesis of nanoparticles using plant extracts: A review. Environmental Chemistry Letters. 2021;19:355-74. [LINK]

[15] Ojemaye MO, Okoh SO, Okoh AI. Silver nanoparticles (AgNPs) facilitated by plant parts of Crataegus ambigua Becker AK extracts and their antibacterial, antioxidant and antimalarial activities. Green Chemistry Letters and Reviews. 2021;14(1):51-61. [LINK]

[16] Santos-Magalhães NS, Mosqueira VCF. Nanotechnology applied to the treatment of malaria. Advanced drug delivery reviews. 2010;62(4-5):560-75. [PMID]

[17] Rajakumar G, Rahuman AA, Velayutham K, Ramyadevi J, Jeyasubramanian K, Marikani A, et al. Novel and simple approach using synthesized nickel nanoparticles to control blood-sucking parasites. Veterinary Parasitology. 2013;191(3):332-9. [DOI: 10.1016/j.vetpar.2012.08.028][PMID]

[18] Nateghpour M, Farivar L, Souri E, Hajjaran H, Mohebali M, Haghi AM. The effect of Otostegia persica in combination with chloroquine on chloroquine-sensitive and chloroquine-resistant strains of Plasmodium berghei using in-vivo fixed ratios method. Iranian Journal of Pharmaceutical Research: IJPR. 2012;11(2):583. [PMID] [PMCID]

[19] Govindan VP, Panduranga AN, Murthy PK. Assessment of in vivo antimalarial activity of arteether and garlic oil combination therapy. Biochemistry and biophysics reports. 2016;5:359-64. [DOI: 10.1016/j.bbrep.2016.01.015] [PMID] [PMCID]

[20] Kusmardi K, Elya B, Wahyuni T, Paramita RI. Combination of sambiloto (Andrographis paniculata (Burm. F.) nees) extract and spirulina (Arthrospira platensis gomont) to prevent anemia in mice infected with Plasmodium berghei anka. Asian Journal of Pharmaceutical and Clinical Research. 2017:269-73. [LINK]

[21] Assegaf N, Astuty H. Effectiveness of using spirulina in conjunction with artemisinin combination therapy as an antimalarial treatment. International Journal of Applied Pharmaceutics. 2019;11:265-7. [LINK]