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Original Article

Anti-Malarial Activity of Nano Tannic Acid MgO Extract Alone and Combined with Chloroquine against *Plasmodium berghei*

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Abstract

Background: The global rise in malaria parasite resistance to antimalarial drugs necessitates new therapeutic strategies. Medicinal plant extracts, long used in traditional malaria treatment, have shown antiplasmodial potential in recent studies. This study investigated the effects of nano tannic acid MgO (NTA MgO) and chloroquine (CQ), both individually and in combination, on a chloroquine-sensitive *Plasmodium berghei* strain.

Methods: BALB/c mice infected with *P. berghei* were divided into 11 groups. Groups were treated with NTA MgO (12.5, 25, 50, 100 mg/kg), CQ (1, 3, 10, 20 mg/kg), pure tannic acid (100 mg/kg), or assigned as controls. Peter's method determined the fifty percent effective dose (ED50) for NTA MgO and CQ. Drug interactions were assessed using the fixed-ratio method (ratios: 100/0, 90/10, 70/30, 50/50, 30/70, 10/90, 0/100). Parasitemia and inhibition percentages were calculated and analysed using SPSS software.

Results: The ED_{50} values for CQ and NTA MgO were found to be 1.1 mg/kg and 25 mg/kg, respectively. A synergistic effect was observed when a combination of 30% CQ and 70% NTA MgO was used, which significantly reduced parasitemia compared to the control group ($P< 0.05$, Kruskal-Wallis test). Additionally, NTA MgO administered alone at a dosage of 25 mg/kg effectively reduced the parasite load.

Conclusion: NTA MgO showed strong antiplasmodial activity both alone and with chloroquine (CQ). The 30% CQ and 70% NTA MgO combination exhibited a significant synergistic effect, highlighting its potential as a new treatment for chloroquine-sensitive malaria and the promise of plant-based nanoparticles against drug-resistant malaria.

Keywords: Nano tannic acid; Malaria; Drug discovery; Combination therapy; Mice

Introduction

Malaria is the most important parasitic disease found mainly in tropical and sub-tropical countries around the world, caused by the parasitic protozoa of the *Plasmodium* genus. The parasite is transmitted to humans through the bite of certain species of female *Anopheles* mosquitoes (1). There are five species of *Plasmodium*, including *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi*, that cause the disease in humans, and *P. knowlesi* is considered a zoonotic parasite (2).

According to the World Health Organization's (WHO) report, 263 million malaria-infected cases with 597000 deaths were recorded globally in 2023. Most deaths were among children younger than 5 years old in African

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countries (3).

Emerging malaria resistance to some antimalarial drugs, limited access to effective drugs, drug expenses, safety concerns, and chemical side effects, especially in children and pregnant women, limit the utilization of many antimalarial drugs (4).

Combination therapy is a highly recommended method to delay the development of drug resistance and to enhance the efficacy of antimalarial drugs against *Plasmodium* strains. This subject is very important when extracts of herbal medicines are combined with synthetic antimalarial compounds (5).

There are approximately 500,000 plant species in the world, of which only 1% are being studied in the field of pharmaceutical research (6). According to some literature, about 75% of patients in tropical regions use traditional medicines for malaria (7). Because of geographical conditions, a great variety of medicinal plant species can be found in Iran. Thus, this study was conducted in this region (8).

Extracts of useful plants such as licorice (*Glycyrrhiza glabra*), artemisia (*Artemisia* spp.), and barberry (*Berberis* spp.) have been used for the treatment of malaria since ancient times, and in recent years their anti-plasmodial effects have been investigated (9). Hence, the World Health Organization has encouraged studies in the field of traditional herbal extracts in malaria-endemic areas (10).

Tannic acid is a natural tannin from a group of phenolic acids. Tannic acid is a high-molecular-weight plant polyphenol belonging to the tannin family, commonly found in coffee, tea, and fruits such as grapes and bananas (11). Tannic acid is a decagalloyl residue with a center glucose molecule esterified at all its hydroxyl groups with 10 gallic acid units (12). It has powerful antioxidant properties (13).

Due to the unique properties of Tannic acid, several studies have been conducted on the use of tannic acid as an additive to biopolymer materials. The antibacterial and antiviral activities of this compound make it a promising tool for application in the medical field. For example, it increases cell proliferation, tissue regeneration, and wound healing processes. It also acts as an antioxidant and homeostatic agent. In addition, tannic acid can neutralize free radicals that cause various diseases such as allergies, diabetes, Parkinson's disease, Alzheimer's disease, and cardiovascular disease, among others. Tannic acid also has anti-cancer properties (14).

One of the most important applications of nanotechnology is drug delivery to targeted points. Drugs developed and manufactured using nanotechnology have great potential to improve their properties, such as efficacy and safety. The advantage of these methods is the optimization of drug delivery (15).

Nanoparticles are finely dispersed particles or solid particles with a size of 10–100 nm that are produced by various methods. Nowadays, the use of nanoparticles in the field of delivery of various drugs has attracted the attention of researchers because of their extended shelf life and targeting properties. They can increase the water solubility of drugs. Since their small size, nanoparticles can pass through membrane barriers to target the specific organs (16).

Besides the advantages of using in vitro tests in plasmodia parasite studies, employing in vivo tests in rodent malaria parasites is also valuable for studying malaria. The rodent malaria parasite investigations owe their basic biological similarities to the human malaria parasites, due to their more or less stable genome, the genetic makeup of the parasites, and mostly the same biochemical processes. *Plasmodium berghei*, in different aspects of molecular concepts, is a valuable model for simple in vivo studies (17, 18).

This study is the first to investigate the effect of nano tannic acid MgO (NTA MgO) alone or in combination with chloroquine (CQ) on *P. berghei* in vivo.

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Materials and Methods

Experimental animals

A total of 95 outbred male BALB/c mice with an approximate weight of 20±2 grams were used for the study. These mice were purchased from Razi Institute of Tehran and kept in the animal house of the School of Public Health, Tehran University of Medical Sciences. In the first stage, 55 mice were distributed into 11 groups, with 5 mice in each group. The groups were categorized as: four groups for tannic acid MgO extract, plus one positive control group (This group was infected with parasites without receiving any treatment), four groups for chloroquine administrating as standard treatment, plus one relevant positive control group, and one group without any injection as negative control. The mice were maintained under conditions of 12 h of darkness and 12 h of light at a temperature range of 22 to 25 degrees Celsius. Water and food were freely provided to them. At the injecting or sampling time, the subject point of the mouse was anesthetized with 2% lidocaine hydrochloride applied on a cotton ball.

An in vivo test of Peter's method was conducted to determine the fifty percent effective dose (ED_{50}) of chloroquine and NTA MgO. The interaction between NTA MgO and chloroquine was also evaluated based on the fixed ratio method (5).

Parasites

In this study, we utilized a chloroquine-sensitive *P. berghei* strain (NICD originally obtained from the Hufkin Institute, India) that had been preserved in liquid nitrogen. Two weeks before testing, the cryopreserved parasites were thawed at room temperature for approximately ten minutes and then maintained through serial blood passages in BALB/c mice until the time of testing. This study was conducted in the National Malaria Laboratory, Department of Parasitology and Mycology, Faculty of Health, Tehran University of Medical Sciences.

Herbal extract and drug

Crude tannic acid and NTA MgO extracts were prepared in collaboration with the biochemistry department of Iran University of Medical Sciences. For tannin extraction, 500 mg of tannin powder was added to 100 mL of the ethanol/water (1:1) solution and was vigorously stirred for 90 minutes at 70 °C. The extract solution was then filtered and stored at 4 °C. To obtain the nano size of the extract as magnesium oxide (MgO) nanoparticles, some amounts of magnesium salt were added to the extract and then stirred on a heater stirrer for 12 hours.

Chloroquine drug in the form of diphosphate salt (COP26 C1N3.2H3Po4) was purchased from Sigma (Sigma Chemical Co.) and dissolved in distilled water up to concentrations of 1, 3, 10, and 20 mg/ml.

Investigating the size, surface charge, and shape of the synthesized products

Dynamic light scattering (DLS), Scanning Electron Microscope (SEM), and Transmission Electron Microscope (TEM) were used to determine the shape, size, and size distribution of the particles. A Fourier transform infrared spectroscopy (FTIR) method was used to scan the test samples to observe chemical properties and functional groups.

Infecting mice with *Plasmodium berghei* and preparation of blood slides

Red blood cells infected with *Plasmodium berghei* were injected intraperitoneally into a few mice as sources. Some amounts of the infected blood were collected via cardiac puncture and then diluted with sterile physiological serum. Each 0.2 ml of the prepared suspension was enriched with 10^6 parasitized erythrocytes. The suspension was injected subcutaneously into the subjected mice. Five days after the injection, a thin blood smear was prepared from the tail of each mouse. The slide was then stained with 5% Giemsa stain (diluted in distilled water) for 20 minutes.

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Determination of 50% effective dose (ED_{50}) of chloroquine and NTA MgO

Concentrations of 1, 3, 10, and 20 mg/kg of mouse body weight were prepared for chloroquine to determine the ED_{50} of the drug against *P. berghei*. To accomplish this, twenty mice were divided equally into four groups. Two hours after infection, the mice were treated with the aforementioned doses for 4 consecutive days, and the growth inhibition rates of parasites were calculated at the end of the fourth day. To obtain the ED_{50} for NTA MgO, the same method was conducted but with different concentrations of 12.5, 25, 50, and 100 mg/kg. Additionally, one more infected group was kept without any injection as a control group.

The parasitemia and inhibition percentage of parasite growth were calculated according to the following formulas (19):

$$\text{Parasitemia\%} = \frac{\text{Number of parasites in test}}{\text{Number of parasites in control}} \times 100$$

$$\text{Inhibition\%} = \frac{\text{Parasitemia in control} - \text{Parasitemia in test}}{\text{Number of parasitemia in control}} \times 100$$

Parasite Count

To determine parasitaemia rates, parasite numbers were counted against 10,000 red blood cells and converted to percentages. To count parasites, all extra-erythrocyte parasites and those infected red blood cells were counted. Each infected red blood cell containing one or more parasites was counted as one parasite.

Assessing the combined effects of two drugs using the fixed ratio method

Combination therapy was employed based on the fixed ratio method as described previously (5). Ratios of 0, 10, 30, 50, 70, 90, and 100% of each ED_{50} were obtained and combined inversely to give fixed ratios of 100/0, 10/90, 30/70, 50/50, 30/70, 10/90, and 0/100 of the CQ/NTA MgO percent, respectively. Each combination was injected intraperitoneally into one group of infected mice, including five mice in each group, except the positive control group, which was left without any treatment.

Statistical analysis

Percentage inhibition and survival time during the study were presented as mean ± standard deviation for all groups. The mean percentage parasitemia on days 4 and 7 and the mean survival time were analyzed statistically using the ANOVA test to identify the significant differences between test groups and the negative control group using P-value criteria (<0.05). Also, Tukey's HSD test was used for post-hoc pairwise comparisons.

Results

This study was conducted in two steps using the chloroquine-sensitive *P. berghei* strain. In the first step, Peter`s method was used to determine the ED_{50} of each drug. In the second stage, the combined effect of different drug ratios at the ED_{50} concentration of each agent was investigated using the fixed ratios method.

Approval of product synthesis

Fourier transform infrared spectroscopy spectrum of NTA MgO

In the spectrum of this extract (Fig. 1), two strong absorption peaks can be seen in the regions of 3400 cm^{-1} and 1205 cm^{-1}, for the OH and C-O stretching frequencies in the structure, which shows the strong absorption in the region of 3400 cm^{-1} for the hydroxyl group. Also, the strong and broad spectrum related to the carbonyl group at 1730–1750 cm^{-1} is the O=C carbonyl group. In the absorption of carbonyl ester, a spectrum is visible in the region of 1718 cm^{-1}, due to the strong resonance bond with the aromatic ring. Also, in the region of 1000–1300 cm^{-1}, the presence of the C-O ester functional group is observed.

Generally, the methyl functional group can be seen in the 1370–1450 regions, which appeared in the 1445 cm^{-1} absorption region in this spectrum. In addition, the cyclic (aromatic) groups can be seen in the 3000 cm^{-1} region, which is overlapped and not visible in this spectrum due to the presence of OH groups,

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but the absorption peak related to C-C stretching can be seen in the 1616 cm^{-1} region, which indicates the presence of the aromatic ring of the structures in the extract of NTA MgO. Also, the absorption in the 2950 cm^{-1} region is related to C-H bonds. In addition, the absorption of OH groups can be seen in the 1330 to 1430 cm^{-1} regions.

Fourier transform infrared spectroscopy spectra related to nanoparticles

The absorption peaks of metal nanoparticles in the FTIR spectra of the synthesized nanoparticles were observed around 400 cm^{-1}. This indicates that the structure and stretching bands of the metal nanoparticles were centralized in that range. On the other hand, the coordination vibrational spectrum associated with the interaction of tannin is found in the 3400 cm^{-1} region, and the peaks seen in the 1450–1230 cm^{-1} region are associated with the interaction of phenolic groups present in the nanoparticles (Fig. 2).

Dynamic light scattering spectrum

Dynamic light scattering (DLS) analysis was performed based on the impact of Takfam laser light on a sample of nanoparticles. The dispersed amount and average size of the synthesized nanoparticles were obtained as well. Based on the relevant graph and results, the nanoparticles have an average size of about 40 nm, and the particle size distribution is relatively uniform (Fig. 3).

Zeta potential

Results of the zeta potential analysis of nanoparticles (synthesized with NTA MgO extract) showed a zeta potential of 25.4, indicating the uniform colloidal nature of the particles and reasonable stability of the synthesized nanoparticles.

Scanning electron microscopy results

The results obtained from scanning electron microscopy (SEM) indicate that the size of the monodisperse nanoparticles is around 36–45 nm in these images, indicating the homogeneity of the nanoparticles synthesized, which is useful for the data in terms of DLS. These images also show that the synthesized nanoparticles are spherical (Fig. 4).

Transmission electron microscopy results

Results obtained from transmission electron microscopy (TEM) images show a relatively uniform distribution of nanoparticles. The spherical shape of the nanoparticles can also be seen in transmission electron microscope images (Fig. 5). This indicates the successful synthesis of spherical nanoparticles by a green synthesis method utilizing NTA MgO extracts.

Anti-plasmodia activity of the drugs in the ED_{50} assay

Administering 1 mg/kg of chloroquine resulted in 40% growth inhibition of the *P. berghei* at day 4 of assessment. Moreover, plotting results of the utilized concentrations on the semi-log chart illustrated 1.1 mg/kg as the ED_{50} of the drug against the parasite (Table 1). This table summarizes the efficacy of chloroquine in reducing parasitemia, expressed as percentage inhibition relative to untreated controls. Among the mentioned concentrations of NTA, MgO 25 mg/kg could inhibit 66.6% growth of the parasite (Table 2).

Combination assessments

Results from the combination assessments of Chloroquine (CQ) and NTA MgO indicated that a 30% CQ+70% NTA MgO combination inhibited 73.08% of parasite growth (Table 3 and Fig. 6), showing a statistically significant difference compared to the control group ($P< 0.05$). Results in Table 3 are based on the fixed-ratio method and show the combined efficacy in reducing parasitemia, expressed as percentage inhibition relative to untreated controls. Other ratios resulted in additive or antagonistic effects.

Survival time

The average lifespan in the first group was

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29 days; in the six groups after the first group (90/10, 70/30, 50/50, 30/70, 10/90, 0/100, CQ/ NTA MgO), the longest lifespan was 27 days (30/70 CQ/NTA MgO); in the untreated group, it was 27 days. After the first, the longest life span was 27 days, and in the untreated group, this average was 14 days (Fig. 7). The data were analyzed using an ANOVA statistical test.

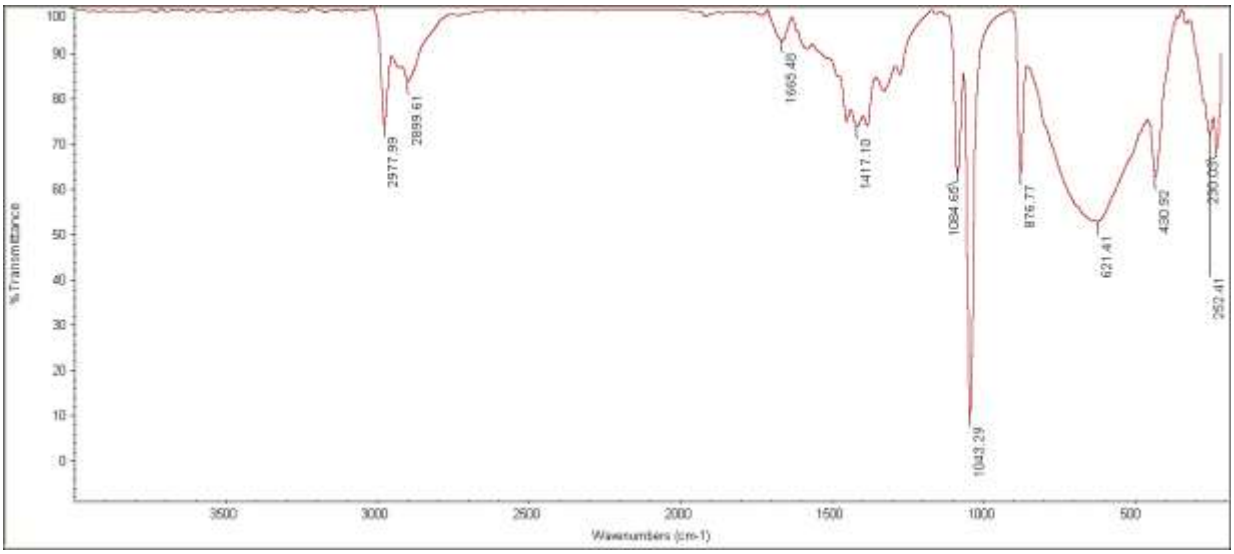

Fig. 1. Fourier transform infrared (FTIR) spectroscopy spectrum of nano tannic acid MgO (NTA MgO) nanoparticles. The spectrum shows characteristic absorption bands: a broad O–H stretch at 3400 cm^{-1}, C=O carbonyl vibrations at 1718–1750 cm^{-1}, and C–O ester groups in the 1000–1300 cm^{-1} region. The aromatic C=C stretch appears at 1616 cm^{-1}, while C–H bonds are observed near 2950 cm^{-1}. Additional peaks include: Methyl groups at 1445 cm^{-1} (1370–1450 cm^{-1} range). O–H bending vibrations at 1330–1430 cm^{-1}. C–O phenolic stretches at 1205 cm^{-1}. The aromatic C–H stretches (~3000 cm^{-1}) are obscured by the broad O–H band

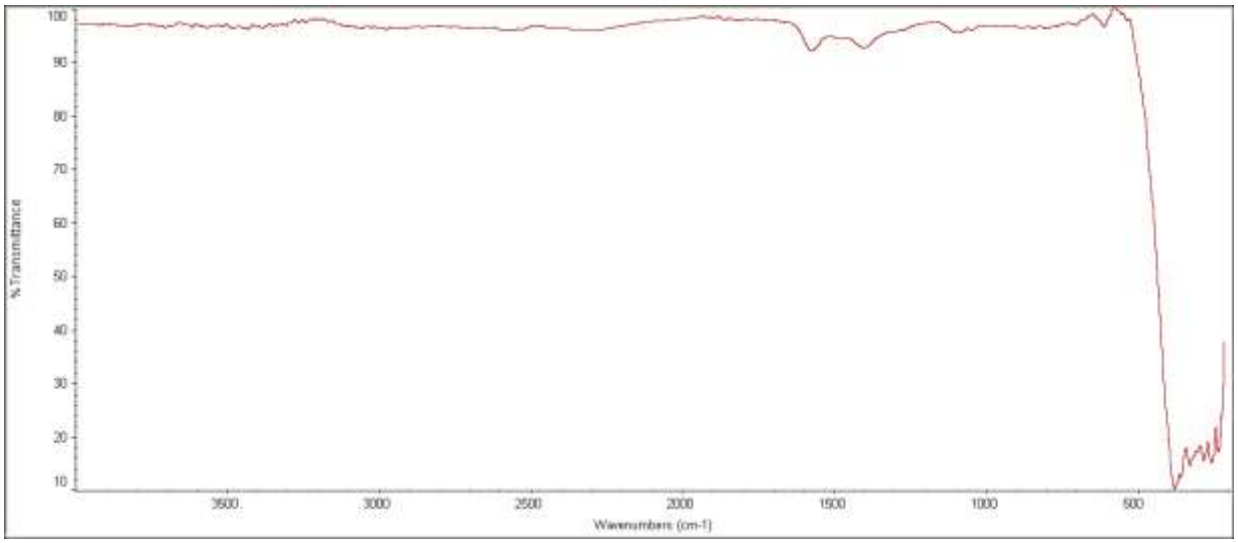

Fig. 2. Fourier transform infrared (FTIR) of the synthesized nano tannic acid MgO (NTA MgO) nanoparticles. The absorption peaks around 400 cm^{-1} correspond to metal nanoparticle structural vibrations, while the broad band near 3400 cm^{-1} indicates tannin coordination. Peaks in the 1230–1450 cm^{-1} region are attributed to phenolic group interactions in the nanoparticle

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Table 1. Inhibition pattern of chloroquine against *Plasmodium berghei* (Anka strain) in mice on the fourth day of treatment

Group	Dose mg/kg	Inhibition % (Mean±SD)
1	1	40.4±7.1
2	3	100±0
3	10	100±0
4	20	100±0

Table 2. Inhibition pattern of nano tannic acid MgO (NTA MgO) against *Plasmodium berghei* in mice on the fourth day of treatment

Group	Dose mg/Kg	Inhibition %
1	12/5	33.3
2	25	66.6
3	50	6.6
4	100	20
5	100*	80

*Pure tannic acid

Table 3. Inhibitory effects of combined chloroquine (CQ) and nano tannic acid MgO (NTA MgO) nanoparticles against *Plasmodium berghei* in mice on the fourth day of treatment

Groups	CQ+NTA MgO %	Inhibition (Mean±SD) %
1	100+0	50.2±0.77
2	90+10	42.32±6.2
3	70+30	41.92±5.9
4	50+50	40.8±4.3
5	30+70	73.08±7.2
6	10+90	37.16±3.3
7	0+100	53.52±8.6

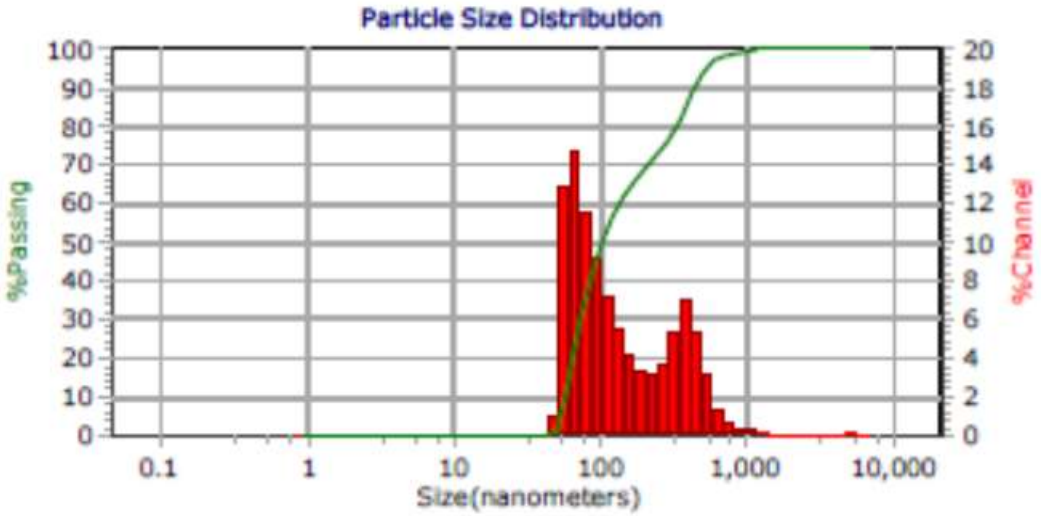

Fig. 3. Particle size distribution of synthesized nano tannic acid MgO (NTA MgO) nanoparticles measured by dynamic light scattering (DLS). Y-axis represents the intensity of scattered light, which correlates with the distribution of particle sizes in the sample. The X-axis represents the particle size

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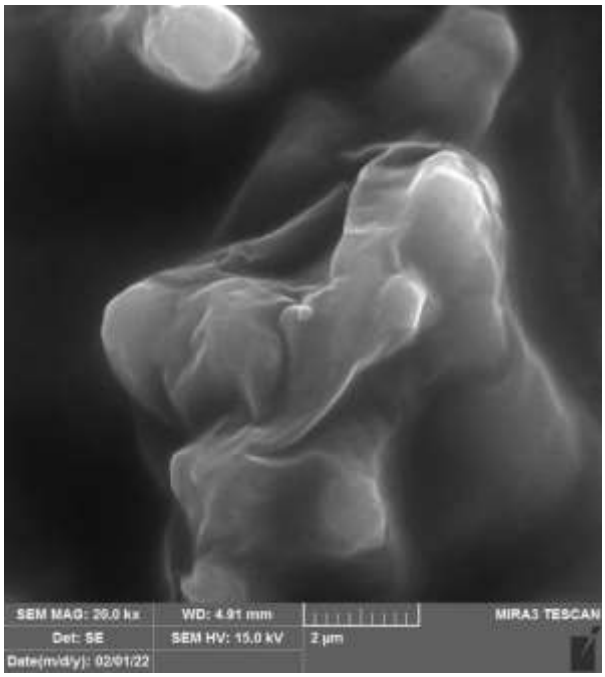

Fig. 4. Scanning electron microscopy (SEM) image of the synthesized nano tannic acid MgO (NTA MgO) nanoparticle. The image, captured using a MIRA3 TESCAN microscope, shows the morphology and size distribution of the nanoparticles

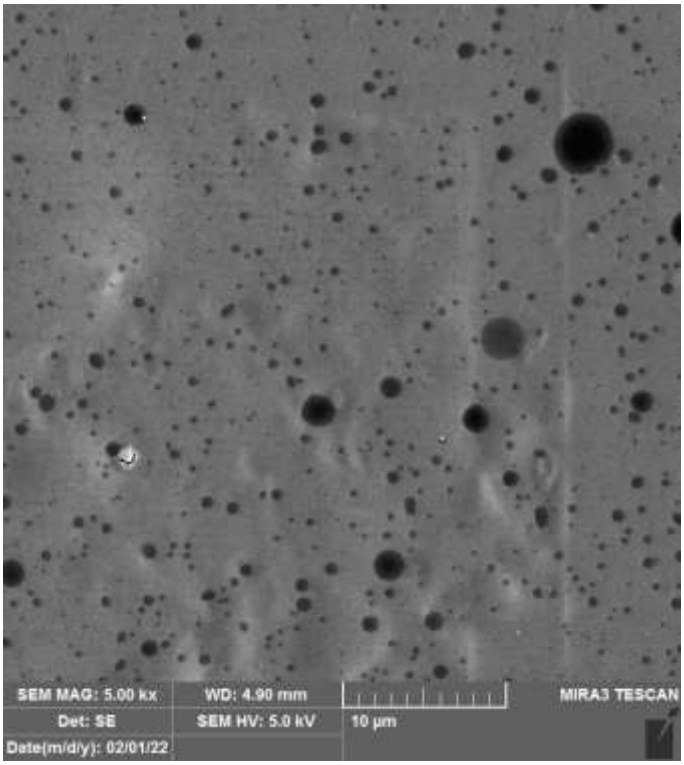

Fig. 5. Transmission electron microscopy (TEM) images of the synthesized nano tannic acid MgO (NTA MgO) nanoparticles. The image, captured using a MIRA3 TESCAN microscope, shows the morphology and size distribution of the nanoparticles

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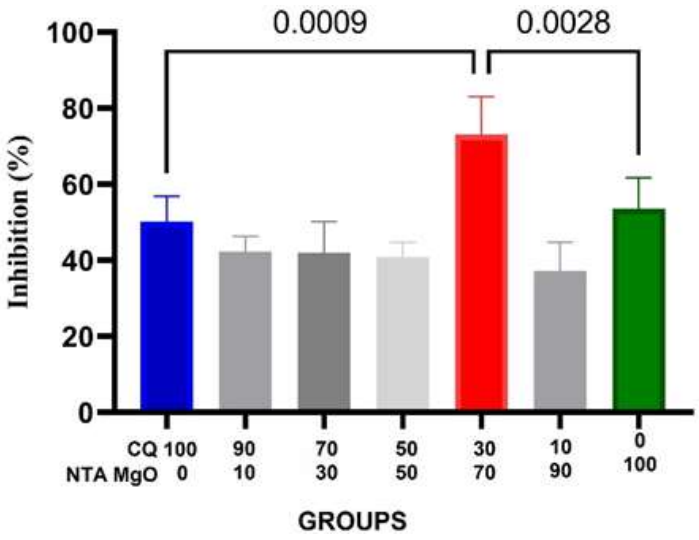

Fig. 6. Statistical analysis of the combined inhibitory effect of chloroquine (CQ) and nano tannic acid MgO (NTA MgO) nanoparticles against *Plasmodium berghei*. The analysis is based on fixed-ratio combinations of 100%, 90%, 70%, 50%, 30%, 10%, and 0%, showing the interaction between CQ and NTA MgO in reducing parasitemia expressed as percentage inhibition relative to untreated controls. A one-way ANOVA identified significant differences among groups ($P< 0.05$), and Tukey's HSD test was used for post-hoc pairwise comparisons

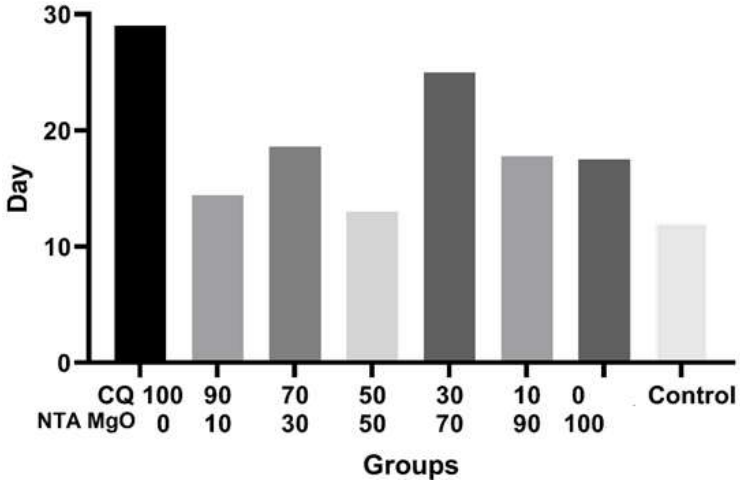

Fig. 7. The average lifespan of (day) mouse groups treated with combined chloroquine (CQ) and **nano tannic acid MgO (NTA MgO)** nanoparticles at different ratios. The graph illustrates the impact of CQ and NTA MgO combinations on survival rates. A one-way ANOVA identified significant differences among groups ($P< 0.05$), and Tukey's HSD test was used for post-hoc pairwise comparisons

Discussion

This study aimed to investigate the effect of the combination of NTA MgO extract and chloroquine against *P. berghei* in BALB/c mice. Implementing *in vivo* tests to assess the effectiveness of the prepared anti-malarial drug in this study to find a valuable opportunity to make the results close to those findings that are obtained from human malaria assessments, particularly in immune and therapeutic patterns (20).

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Results of some studies in the field of medicinal plant extracts and natural agents have been released during the past years for malaria therapeutic purposes, including tannin substances (21, 22).

Tannic acid inhibits processes binding of proteins of live micro-agents, such as protozoan parasites, to host cells and interferes with the interactions of receptors, which are necessary for metabolic activities of the parasites. Tannins implement their destructive activities using destroying cellular metabolism (23).

Nanoparticles such as MgO are now widely used in various medical and pharmaceutical industries due to their high efficacy in delivering desired drugs or herbal extracts under *in vivo/in vitro* conditions (24). Nanoparticles are smaller in size and ratio, so they have better and more pronounced catalytic properties and roles (25). As combination therapy has replaced monotherapy as a remedy for malaria, delivery carriers based on nanotechnology allow different drug components to be encapsulated in a single package (26).

The obtained ED_{50} value for the NTA MgO extract against *P. berghei* in this study was 25 mg/kg, but it was 250 mg/kg in tests on the *Xylopia amazonica* plant from the Brazilian Amazon. Comparing these results reveals that the antimalarial properties of NTA MgO are more effective than *X. amazonica* (27). Employing extracts of Saye, *N. dribala*, and *Azadirachta indica* by Yerbanga and colleagues showed that the extracts had 52%, 45.5%, and 45% preventive activity to reduce parasitemia of *Plasmodium berghei* in mice, respectively (28). In the current study, NTA MgO at a dose of 25 mg/kg inhibited the parasite by 66.6%, indicating that the NTA MgO extract had a higher inhibitory effect.

Somsak and colleagues exposed a chloroquine-sensitive strain of *P. berghei* to the extract of Kaempferol (3, 4′, 5, 7-tetrahydroxyflavone) alone and in combination with chloroquine in concentrations of 10 and 20 mg/kg in some mice. The therapeutic effect of Kaempferol was 52% in the best situation. Although the plant in combination with chloroquine had an increasing impact, statistically it was not significant (29), while using NAT MgO in this study resulted in 66.6% growth inhibition of *P. berghei* at a concentration of 25 mg/kg. In the combination form of NAT MgO with CQ, ED_{50}s, a considerable 73.08% growth inhibition was observed in the parasite.

Conclusion

This study showed that NTA MgO significantly inhibits the growth of *P. berghei* in BALB/c mice, both alone and in combination with chloroquine, without notable side effects at the concentrations used.

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Ethical consideration

This study was approved by the Research Ethical Committee of Tehran University of Medical Sciences with the code number of IR. TUMS.AEC. 1400.010.

Conflict of interest statement

The authors declare there is no conflict of interest.

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