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Original Article

In Vivo Anti-malarial Activity and Toxicity of *Allium ursinum* (Wild Garlic) Hydroalcoholic Extract

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Abstract

Introduction: Malaria is a protozoan disease that is caused by different types of *Plasmodium* in humans and animals. Resistance to the main drugs in the treatment of malaria infections has led to studying alternative drugs. Therefore, in the present study, the effect of hydroalcoholic extract of wild garlic was studied on *Plasmodium berghei* malaria-infected mice.

Materials and Methods: This experimental study was conducted on 45 male mice infected with *Plasmodium berghei.* The treatment with hydroalcoholic extract of wild garlic was performed using Peter's proposed method. Statistical analysis of data was conducted using SPSS v.18 software.

Results: Findings showed that the wild garlic hydroalcoholic extract had the highest effect at the treatment dose of 800 mg/kg with 92.4% prevention of parasite growth compared to the control group ($p<0.05$). No significant difference was observed in the mean weight of the mice or the morphology of the liver and kidney in the group receiving wild garlic extract compared to the negative control group.

Conclusions: The anti-malarial effects of wild garlic plant observed in the present study, elicits the necessity for further research, evaluation and comparison of different extraction methods such as aqueous and chloroform as well as higher therapeutic dosages.

Keywords: Malaria, *Plasmodium berghei,* Garlic, Extract
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Introduction

Malaria is a protozoan disease that is caused by different types of *Plasmodium* in humans and animals. *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium knowlesi* are the species that are known to infect humans.[1] According to the latest report by the World Health Organization (WHO), malaria was asserted as the cause of the death of 409 thousand people worldwide in 2020.[2]

The agent of disease in mice is *Plasmodium berghei* which is highly lethal to them. Yet, this parasitic *Plasmodium* is invaluable for *in vivo* malaria research due to specificity to the host, i.e., human *Plasmodium* cannot induce disease in mice and vice versa.[3] Hence, *in vivo* studies of malaria are usually carried out in mice using *P. berghei*. Drugs such as artemisinin, primaquine, chloroquine, mefloquine and lumefantrine are used to treat malaria.[4] In recent years, the development of resistance to anti-malarial drugs such as chloroquine in *Plasmodium* has become one of the major health problems in developing countries.[5] Resistance to the major drugs in the treatment of parasitic infections has led to the formation of large research teams around the world and also the development of alternative drugs.

Side effects of the above drugs as well as the development of drug resistance in the parasite have caused major hindrances in the use of these drugs in malaria treatment. Therefore, obtaining effective anti-parasitic compounds from other sources such as herbs can be a big step forward. One approach suggested by WHO is the investigation of herbal medicines in the treatment of various diseases.[6-8]

Northern Iran, due to its favorable geographical and climatic conditions, is the habitat of many wild plant species, including wild garlic with the binomial name "*Allium ursinum*". The most important wild garlic chemical constituents with pharmacological activity are undoubtedly sulfur compounds;

among the most common are glutamyl peptides and sulfoxides, which are at the same time the most widely studied chemicals in this plant species. Allium species can have a strong smell due to the content of S-alk(en)yl-L-cysteine-sulfoxides and volatile compounds such as thiosulfinates and polysulfides. The quantitative profile of cysteine-sulfoxides which could be found in the extract is highly variable depending on plant organ and harvest time. The most abundant sulfoxides are: methiin [(+)-S-methyl-L-cysteine-sulfoxide], aliin [(+)-S-2-propenyl-L-cysteine-sulfoxide], isoaliin [(+)-S-(1-propenyl)-L-cysteine-sulfoxide], propiin [(+)-S-propyl-L-cysteine-sulfoxide], as well as ethiin (S-ethyl-cysteine-sulfoxide).[9]

Due to its high content of cysteine sulfoxides and sulfoxide amino acids, wild garlic is known to have anti-bacterial and antioxidant properties.[9-10] Effective substances in wild garlic can also reduce the production of lipid peroxidation products such as malondialdehyde and hydroperoxide, playing an important role in the prevention of vascular complications in various diseases as well as improving serum triglyceride and cholesterol levels in long-term administrations.[11] Since the anti-parasitic properties of this plant were not widely studied until 2021, in this *in vivo* study, thee anti-malarial and toxicity properties of the hydroalcoholic extract of wild garlic was investigated for the first time in *P. berghei* infected mice.

Materials and Methods

Hydro-alcoholic Extract of Wild Garlic

Plant Collection

Plant samples for this study were collected during spring in Alborz province, Chaloos road (at an altitude of 200 to 800 meters). Proper maintenance included drying in normal airflow and temperature, away from direct sunlight, and then grinding.

Extract Preparation

The percolation method was used to prepare a hydroalcoholic extract from the ground plants.[12] Wild garlic was fully grounded and weighed. The powder was mixed with Methanol -water (ratio of 4 to 1) in an Erlenmeyer flask with its top closed by an aluminum foil. Using an electric shaker, materials were mixed for 30 min. The Erlenmeyer flask containing the mixture was stored in darkness for 24 h. Then, sufficient solvent was again added and mixed as before. The resulting mixture was filtered. In the next step, the extract was then fully condensed using a vacuum apparatus. For removing the remaining solvent and further drying the powder, the extract was freeze-dried. To prevent any unwanted changes in the final extract and its active components content, it was stored in an airtight container inside a refrigerator (4 °C). The stock standard solution was prepared by dissolution of a proper proportion of powder in normal saline and Dimethyl Sulfoxide (DMSO).

Gas Chromatography-Mass Spectrometry (GC–MS) Analysis

The phytochemical investigation of hydroalcoholic extract of wild garlic was performed using GC-MS equipment. In the gas chromatography device, the temperature program was set to 40 °C for 5 min, then raised at 5 °C/min to 200 °C and kept the temperature for 5 min, and again raised at 10 °C /min to 280 °C and stayed at this final temperature for 10 min. The carrier gas was pure helium with a constant flow rate of 1 ml/min. High ionization energy of 69.9 eV was adopted with a fragment scan range of 40 to 550 m/z.

Anti-plasmodial Activity of Wild Garlic

Experimental Mice

This experimental study was conducted on 45 male mice weighing 24 ± 2 grams which were similar in age. The population of mice in each cage was five where they were kept under 12 h dark/12 h light cycle and 22-25 °C temperature.

Infecting the Mice with Plasmodium berghei

P. berghei was used to infect the mice. In this study, the infection dosage was 10^6 parasitized erythrocytes that was prepared in a 0.2 ml volume of suspension form in physiologic serum before intraperitoneal inoculation.

Drug Testing in Mice

The treatment was performed using Peter's proposed method.[13] Mice were divided into nine groups (n = 5), including three control and six experimental groups. Three control groups included negative control (normal saline receiving infected mice), positive control (chloroquine receiving infected mice), and mortality control (healthy mice without infection nor treatment). Experimental *Plasmodium*-infected mice were subdivided into six groups receiving 25, 50, 100, 200, 400, and 800 mg/kg of wild garlic extract for treatment in volumes of 0.2 ml. to calculate the growth inhibition of the test plants. After four days of treatment, the growth inhibition of the wild garlic extract was measured by microscopy test.

Microscopic Test

The thin smears were prepared from the blood of mice and stained with 5% Giemsa. For more accuracy in parasitemia calculation, all infected Red Blood Ccells (pRBC) containing asexual stages of the parasites were counted at least in 500 scopes and the mean percentages of all observed scopes were measured. Finally, the ED_{50} was obtained from nonlinear regression analysis of dose-effect curves by the GraphPad Prism 9 program.

Toxicity Test of Wild Garlic

Twenty mice were divided into four groups for toxicity tests.[4] The first group received PBS (negative control), the second group received chloroquine (positive control) while the third and fourth groups received the treatment doses of

400 and 800 mg/kg of wild garlic extract, respectively.

Mice Weight, RBC Morphology and Biochemical Evaluation

The weights of the mice were measured on the first and fourth days of treatment. At the end of the experiment, after anesthesia, cardiac puncture sampling was performed to evaluate the RBC morphology as well as assessing ALT, AST, and ALP enzymes.

Histopathological and Histomorphometric Study

The abdominal region was then cut and liver, kidney, and spleen tissues were removed. After washing in physiological serum, samples were fixated in 10% formalin buffer solution. After 72 h of fixation, the samples were dehydrated (with ascending percentages of alcohol, respectively: 50%, 70%, 80%, 90%, 99/6%, and 99/6%) and submerged into xylol followed by embedding in paraffin (using tissue processor machine). Blocks of paraffin embedded samples were then sent for section cutting (microtomy) to produce sections of 5-7 um and stained using Hematoxylin and Eosin (H&E) for further microscopic appraisal. Images were captured by Dino-Late camera and recorded by Dino capture v.2 software.

Statistical Analysis

Statistical analysis of data was conducted using SPSS v.18 software. A one-way ANOVA followed by Tukey's posttest was employed to compare the parameters between the control and extract-treated groups of the study. All results were expressed by the mean ± Standard Error of the Mean (SEM), and statistical significance was considered if $p<0.05$ at the 95% confidence interval. Finally, GraphPad Prism version 9.0 was used for obtaining ED50 in this study.

Results

GC–MS Analysis of Wild Garlic

Based on the GC-MS dispersion spectrum, 39 major and primary compositions were identified in the hydro-alcoholic extract of wild garlic, with the most important organic compounds being Propenyl disulfide, Methyl-2-propenyl disulfide, Vinyl -1,3-dithiane, Propionic acid and Furfural (Table 1).

Table 1. The Most Phytochemical Constituents Identified in the Hydro-alcoholic Extract of Wild Garlic Using Gas Chromatography-Mass Spectrometry (In % of Total Ion Current)

No.	Compounds	% Of Total
1	Propenyl disulfide	3.2
2	Methyl-2-propenyl disulfide	9.7
3	Vinyl-1,3-dithiane	5.9
4	Heneicosane	1.6
5	di-2-Propenyl trisulfide	2.8
6	Propionic acid	1.8
7	Decanal	0.6
8	Heptacosane	0.2
9	Tricosane	0.1
10	Furfural	0.3

In Vivo Anti-malaria Activity of Wild Garlic

The Average Life Span of Treated Mice

The mean survival time for mice in the negative control and positive control groups were 9.8 ± 0.4 and 29.2 ± 0.3 days, respectively. The highest survival span in mice treated with different doses of the wild garlic extracts was related to receiving a dose of 800 mg/kg of plant extract that survived for 26.2 ± 0.3 days (Table 2).

Table 2. The Average Life Span (Days) of Mice Receiving Different Doses of Wild Garlic Extract *(Allium ursinum)* vs. Control Groups

Drug	Dose (mg/ml)	Life Span (Mean ± SEM)	*p value
Allium ursinum	25	10 ± 0.447	0.988
	50	10 ± 0.316	0.957
	100	11.8 ± 0.583	0.052
	200	14.4 ± 0.244	0.031
	400	18.6 ± 0.400	0.006
	800	26.2 ± 0.374	<0.001
Positive control		29.2 ± 0.348	<0.001
Negative control		9.8 ± 0.448	

*The differences between experimental groups and negative control ($p\leq0.05$ indicate significant statistical differences).

Anti-plasmodial Activity

In the present study, parasitemia in the negative control group (normal saline recipient) was increased while in the positive control group (chloroquine recipient) parasitemia reached ~1%. The results showed that the wild garlic hydroalcoholic extract had the highest effect at the treatment dose of 800 mg/kg with 92.4% prevention of parasite growth compared to the negative control group ($p<0.05$). The minimum effect was observed in dosages of 25 mg/kg where reduction in parasitemia was not significant compared to the control group ($p>0.05$). In concentrations of 200 and 400 mg/kg, the wild garlic extract showed 43.4% and 68.4% inhibition in the parasite growth, respectively (Figure 1). The results of parasite count in stained samples and nonlinear regression showed that 50% of maximum response (ED_{50}) can be achieved at 245 mg/kg dosage (Figure 2).

The maximum survival time was attributed to the mice treated with 800 mg/kg concentration with 26.2 ± 0.3 days, which was not significantly different from the positive control group with the mean survival time of 29.2 ± 0.3 days ($p>0.05$).

Toxicity Study of Wild Garlic

All the treated mice at the doses of 400 and 800 mg/kg of wild

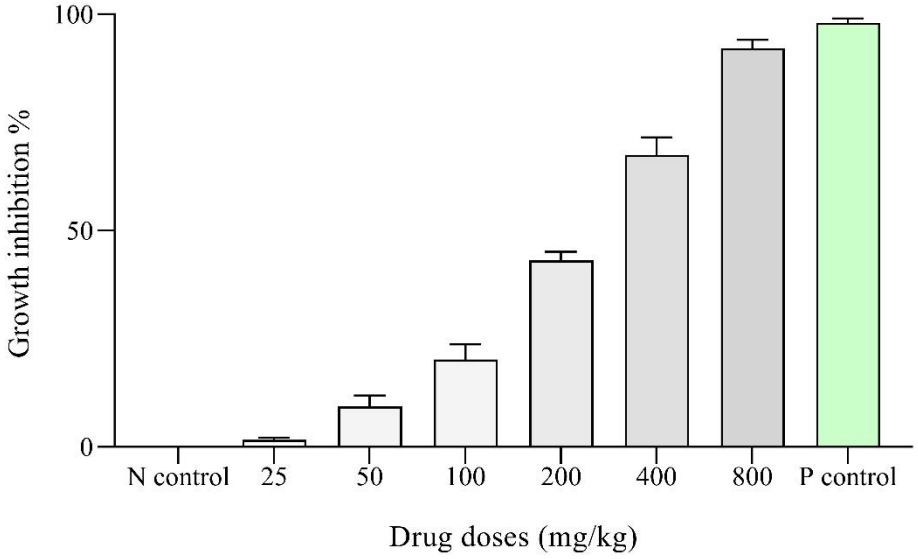

Figure 1. *Plasmodium* Growth Inhibition for Different Experimental Dosages of Wild Garlic Extract.

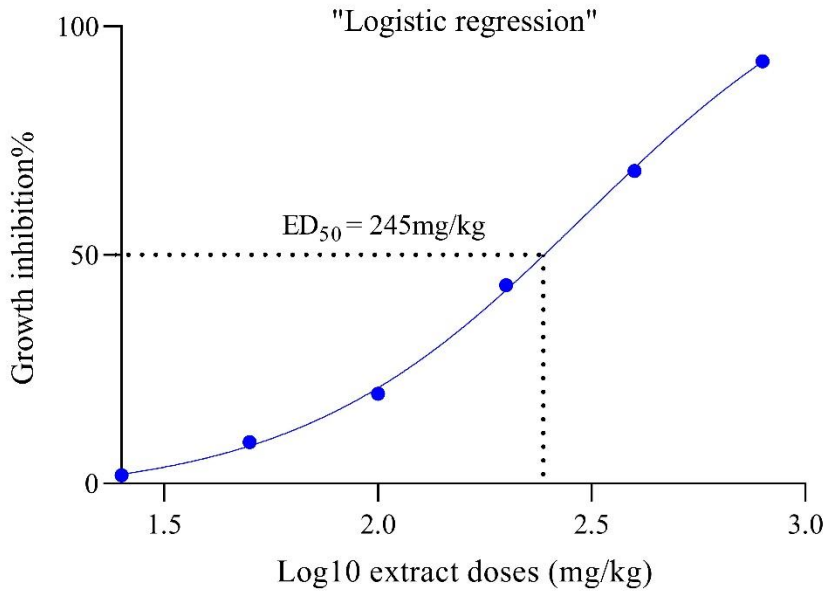

Figure 2. *In vivo* ED_{50} of Wild Garlic Extract.

garlic extract survived during the test period. No observable toxicity signs were noticed in the extract-treated mice compared to the negative control.

Mice Weight and RBC Morphology

No significant difference was seen in the mean weight of the mice and the morphology of the red blood cells in the group receiving wild garlic extract compared to the negative control group (Table 3).

Biochemical Findings

Changes in the levels of ALT, AST, and ALP in the control group and recipients of different doses of wild garlic extract have been presented in Table 4. Analysis of data obtained from Table 4 showed that in the experimental group receiving 800 mg/kg of wild garlic extract, ALT, AST, and ALP concentrations were not significantly higher than the negative control group ($p>0.05$), while they were still significantly lower than the positive control group ($p<0.05$).

Histopathological and Histomorphometric Findings

Histopathologic studies of the liver in different groups were performed in regard to parameters such as apoptosis, necrosis, local inflammation, hyperemia, and histomorphometric such as hepatocyte nucleus diameter, hepatocyte cytoplasm volume, hepatic sinusoid volume, and Kupffer cell proliferation parameters. No significant difference was observed between the negative control group (normal saline recipient) and experimental groups

Table 3. The Average Weight of Mice in Experimental and Control Groups

Drug	Dose (mg/ml)	Weight (kg) (Mean ± SEM)	*p value
Allium ursinum	25	26.6 ± 0.374	0.598
	50	25.8 ± 0.509	0.995
	100	27.8 ± 0.679	0.983
	200	25.6 ± 0.316	0.816
	400	26 ± 0.632	0.951
	800	28.4 ± 0.244	0.201
Positive control		26.6 ± 0.489	0.373
Negative control		27.4 ± 0.374	

*No statistically significant differences were observed between experimental groups and negative control (p>0.05).

Table 4. ALT, AST, and ALP Levels (IU/L) in Plasma of Mice

Drug	Dose (mg/ml)	Serum Enzyme (Mean ± SEM)		
		ALT(U/L)	AST(U/L)	ALP(U/L)
Allium ursinum	25	75.2 ± 1.428^{a}	92 ± 1.581^{6}	128.8 ± 1.356$^{a''}$
	50	74.8 ± 1.655^{a}	98.8 ±0.969^{6}	135.6 ± 1.860$^{a''}$
	100	76.4 ± 1.503^{a}	90.2 ± 1.280^{6}	136 ± 1.643$^{a''}$
	200	79.6 ± 1.208^{a}	102.6 ± 1.878^{6}	147.4 ± 1.720$^{a''}$
	400	82.2 ± 1.655^{a}	98.8 ± 1.059^{6}	152.2 ± 2.108$^{a''}$
	800	84.4 ± 1.469^{a}	108.4 ± 2.238^{6}	161.2 ± 2.489$^{a''}$
Positive control		102.4 ± 1.157^{b}	174.2 ± 2.445^{b}	177.6 ± 1.177$^{b''}$
Negative control		75.2 ± 2.315^{a}	94 ± 1.612^{6}	133.2 ± 2.563$^{a''}$

Dissimilar letters in the table indicate a significant difference between groups (p≤0.05).
Similar letters indicate there wasn't a significant difference between groups (p>0.05).

groups in histopathologic and histomorphometric parameters (p>0.05). The parameters that were examined in the kidney included the condition of histopathologic parameters (apoptosis, necrosis, local inflammation, hyperemia) and histomorphometric parameters (renal corpuscle, urinary space, mesangial cells, proximal and distal convoluted tubules). Appraisal of renal tissue revealed no significant differences between control and experimental groups (p>0.05) as they were intact and similar to the negative control group (Figure 3).

Discussion

In this study, chloroquine as a standard drug reduced parasitemia in the positive control group to near zero (1%) and increased the average survival time in mice. In the infected group where no treatment was carried out, parasitemia increased significantly compared to the chloroquine recipient group (p<0.05).

Different studies have been conducted around the world in search of an anti-malarial alternative medicine. Elmi et al., showed that ginger extract can reduce parasitemia by 62% in doses of 250 mg/kg in mice.[3] In this study, wild garlic extract (800 mg/kg) could inhibit the growth of parasites in mice by 92.4%, implying that the wild garlic extract had a better therapeutic effect in malaria-infected mice when compared to ginger extract. This could be attributed to the exaggerated presence of cysteine sulfoxides and sulfoxide amino acids in the wild garlic composition. The anti-malarial effect of ethanolic and dichloromethane extracts of Iranian propolis was studied by Afrouzan et al.[14] The curative effect against established infection showed that both extracts at all doses (50, 100, and 200 mg/kg) produced anti-plasmodial activity against the parasite.

In the Habte et al. study, crude fruit extract of *C. frutescens* showed a significant dose-dependent inhibition of parasitemia in mice infected with *P. berghei* compared to the vehicle-treated group.[15] This was comparable with similar studies carried out on *Croton macrostachyus* and *Withania somnifera*.[16,17] However, the chemoprevention efficacy of the plant was superior to another report on *Schinus molle*.[18]

The results of Joseph et al., revealed a significant reduction in the parasitemia of the mice after being treated with varying doses (400 mg/kg, 600 mg/kg, and 800 mg/kg) of *A. occidentale* relative to the control groups.[19] This revealed that the tested doses of the plant extract produced various curative effects. *A. occidentale* exhibited high anti-malarial properties of 80.66% and 80.69% effectiveness's at 600 mg/kg and 800 mg/kg doses, respectively. However, low anti-malarial effectiveness (54.20%) was observed at 400 mg/kg treatment. Phytoconstituents such as alkaloids, abundantly localized in the plant extract, could be responsible for the anti-plasmodial activity as plant -derived alkaloids such as quinine are evidenced to possess a potent anti-malarial activity. This plant also contains flavonoids. These bioactive principles have been reported to possess a range of therapeutic activities including anti-malarial activity in the literature.[20,21] The effect exerted could be via indirect boosting of the immune system or inhibition of other target pathways which are not fully realized. The flavonoids observed in our plant have been proven to possess potent anti-inflammatory and antioxidant activities. Furthermore, the plant constituents may target the previously discovered targets in the pathogenesis and life cycle of the malaria parasite but with a unique or similar mechanism of action.[22-24]

The ED_{50} value of wild garlic extract in the present study was calculated to be 245 mg/kg, which was in the general range of ED_{50} = 200 mg/kg for ginger extract calculated by

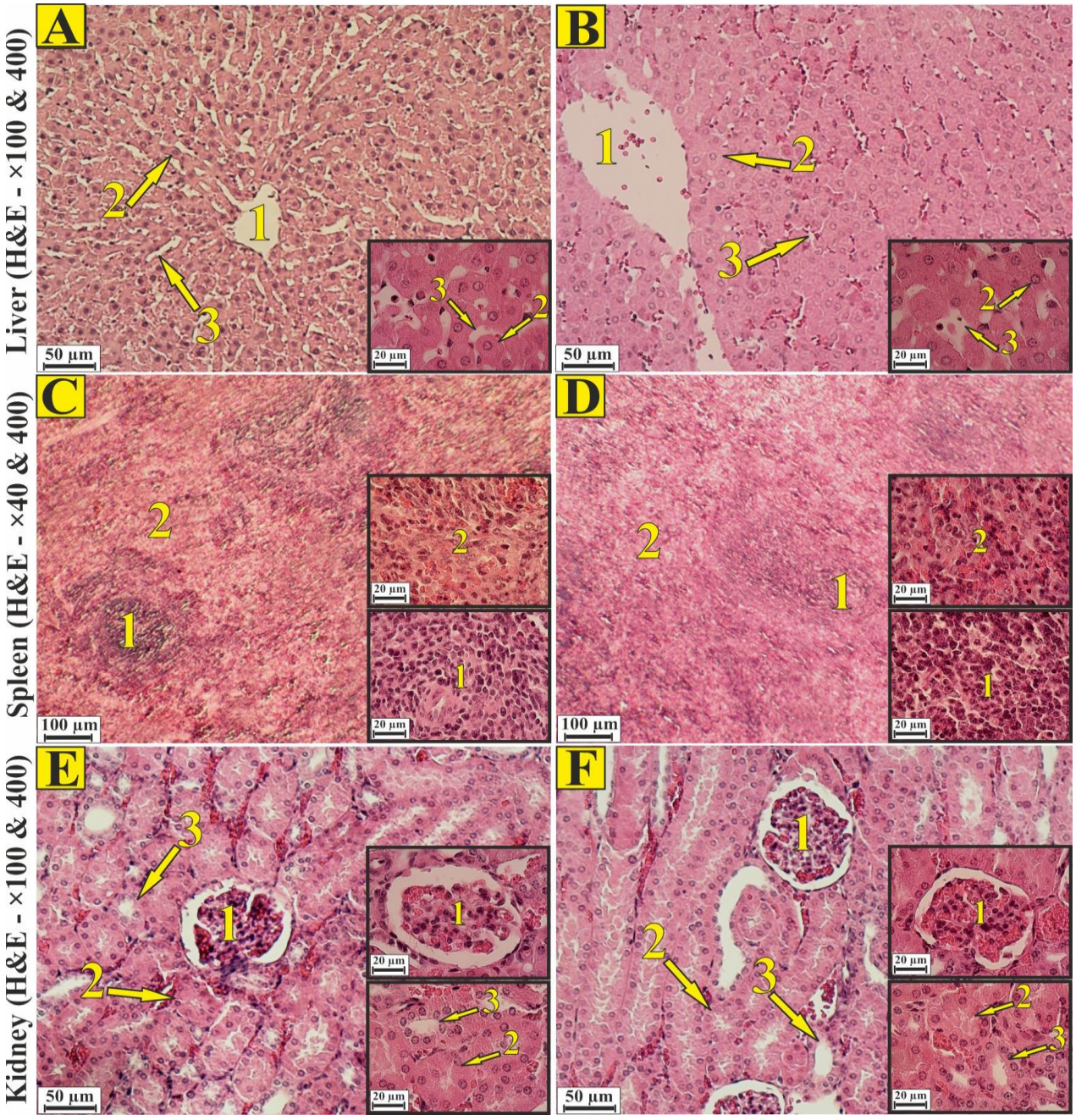

Figure 3. H & E Staining of Histological Sections of Experimental Group that Receiving 800 mg/kg of Wild Garlic (Left) and Negative Control (Right) Specimens. **A)** The histopathological structure of liver tissue in experimental group (800 mg/kg) was normal (based on the condition of central veins, hepatocytes and sinusoids); **B)** Central veins, hepatocytes and sinusoids were also normal in the liver tissue of negative group, ([1]central vein, [2]hepatocytes, [3]hepatic sinusoid); **C)** Study of the spleen tissues of the experimental group (800 mg/kg) showed no abnormalities in the structure of the organ in terms of status and volume of white and red pulps as well as the cellular structure in white pulps; **D)** We also observed normal tissue texture in negative group (regarding the status and volume of white and red pulps and white pulp cellular structure), ([1]white pulp, [2]red pulp); **E)** Histopathological study of kidney in the experimental group (800 mg/kg) was normal (in regard to the structure of renal corpuscle, distal and proximal convoluted tubules); **F)** Histological study of renal tissue structure in the negative group (structure of renal corpuscle, distal and proximal convoluted tubules) also showed a normal condition, ([1]renal corpuscle, [2]proximal convoluted tubule, [3]distal convoluted tubule).

Elmi et al.[3] Comparison of the current study with the study of Lima et al. on *Andropogon leucostachyus* shows a better anti-parasitic effect of the latter plant *in vivo* with 70% parasite growth prevention at 250 mg/kg.[25] This different effectiveness between an Iranian native plant from Amaryllidaceae family and a Brazilian plant of Poaceae family could be attributed to their different phytochemical compounds which is seen in their distinct therapeutic properties.

In another study, Hajialiani et al.,[26] reported a clear inhibitory effect of the active fraction of Iranian *naja naja oxiana* snake venom on *P. berghei* at ED_{50} = 2.5 mg/kg which shows higher therapeutic efficiency compared to the current study.

However, the wild garlic extract showed no toxicity for higher therapeutic doses while the *naja naja oxiana* venom showed toxicity in mice liver tissues at high concentrations. Analysis of chemical composition of wild garlic extract using GC-MS revealed several effective compounds such as sulfur compounds that have proven to show anti-bacterial, anti-fungal and anti-parasite properties in several studies.[27-30] The result of Elmi et al.,[28] showed that the highest parasite growth inhibition at 88.71% compared to control group was seen in the mice infected with *P. berghei* when administered with 400 mg/kg hydroalcoholic extract of *Allium paradoxum*. Analysis of the *Allium paradoxum* extract showed the presence of sulfur compounds, such as allicin and ajoene, to which the biological activity of *Allium* has been attributed. On the other hand, the phytochemical analysis of *Allium ursinum* in the present study revealed one sulfur-containing compound, di-2-Propenyl trisulfide, which had been previously detected in onion extracts. The fact that garlic produces more sulfur compounds than onion could be an explanation to the stronger activity of garlic in our study. We assume that the ability of these extracts to kill parasites is mediated by sulfur compounds, which are produced in the alliinase pathway after the bulb tissue was damaged. Sulfur-containing compounds can probably establish disulfide bonds (-S-S-) with free thiol groups (-SH), and thus inhibit enzymes or other proteins, which are important for survival.

The propionic acid present in the wild garlic can inhibit the growth of microorganisms such as pathogenic fungi by changing pH and disrupting glucose synthesis. Also, non-toxicity of acetyl-L-serine in this plant has already been demonstrated by Mortel et al.[31,32]

Some medicinal plants, like many chemical drugs, have shown adverse effects on human cells and animal models. Karbalaei et al., studied the effect of the *Artemisia annua* plant on *P. berghei* and reported toxicity in liver tissue samples of mice for higher administration doses.[33] This is while in our study, histological studies of liver and kidney tissue revealed no visible changes or adverse effects in treatment groups receiving hydro-alcoholic extract of wild garlic plant when compared to the negative control group, although more investigations may be required for higher treatment dosage regimens. Furthermore, various studies have shown that extract preparation methods (aqueous, alcoholic, chloroform) can influence their efficacy. Elmi et al., showed that the chloroform extract of *Tanacetum parthenium* was more effective against *giardia* cysts in BALB/c mice when compared to its hydro-alcoholic extract.[34] Another study also reported higher effectivity of the alcoholic extract of *Asafetida* plant on *Giardia* parasites compared to its aqueous extract.[35] Similar studies in the past years have shown that various medicinal plants such as *Solanum Surattense*, *Ocimum basilicum*, *Crocus sativus*, *Artemisia annua*, *Peganum harmala*, and *nitida Parkia* are effective against *Plasmodium* and other parasite.[36-39] The results of the present study also showed the effect of wild garlic extract on *P. berghei* in mice. There is little pharmacological information about the therapeutic properties of wild garlic, although its antioxidant and anti-bacterial properties have already been proven. The present study shows the anti-plasmodial properties of wild garlic while further and complementary studies in this regard may provide more insights into its prospect as an anti-malarial drug.

Conclusion

Increasing consumption of chloroquine, primaquine, and other anti-malarial drugs has resulted in the resistance of *Plasmodium falciparum* to these drugs, which promotes the necessity of searching for new substitute drugs. Due to the anti-malarial effects of the wild garlic plant observed in the present study, further research is recommended to evaluate and compare different extraction methods such as aqueous and chloroform as well as higher therapeutic dosages. We also recommend further studies of other effective compounds in different plants using methods such as gas chromatography techniques, to distinguish and isolate a purified and more effective compound among them.

Authors' Contributions

FK and TE conceived, analyzed data, designed the study, performed experiments and wrote the paper. HT, MD, AKH, MA, and AB performed experiments, provided samples and wrote the paper.

Ethics Approval

The study protocol was approved by the ethical committee of the Iran University of Medical Sciences (IR.IUMS.REC. 1399.1052).

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Conflict of Interest Disclosures

The authors declare that they have no conflicts interest.

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References

1. Elmi T, Ardestani MS, Hajialiani F, Motevalian M, Mohamadi M, Sadeghi S, et al. Novel chloroquine loaded curcumin based anionic linear globular dendrimer G2: A metabolomics study on *Plasmodium falciparum in vitro* using 1H NMR spectroscopy. Parasitology. 2020;147(7):

747-59. doi:10.1017/S0031182020000372
2. World Health Organization. World malaria report 2020: 20 years of global progress and challenges. Available from: https://www.who.int/publications/i/item/9789240015791
3. Elmi T, Hajialiani F, Asadi MR, Orujzadeh F, Kalantari Hesari A, Rahimi Esboei B, et al. A study on the effect of Zingiber Officinale hydroalcoholic extract on *Plasmodium berghei* in infected mice: an experimental study. J Rafsanjan Univ Med Sci. 2019;18(4):353-64.
4. Elmi T, Ardestani MS, Motevalian M, Hesari AK, Zamani Z, Tabatabaie F. Antiplasmodial effect of nano dendrimer G2 loaded with chloroquine in mice infected with *Plasmodium berghei*. Acta Parasit. 2021;67:298-308. doi:10.1007/s11686-021-00459-4
5. Myjak P, Nahorski W, Szostakowska B, Żarnowska-Prymek H, Pietkiewicz H. Detection of molecular markers for chloroquine and pyrimethamine/sulfadoxine resistance in imported cases of *Plasmodium falciparum* malaria in Poland. Acta Parasitol. 2007;52:286-91. doi:10.2478/s11686-007-0031-2
6. Elmi T, Rahimi Esboei B, Sadeghi F, Zamani Z, Didehdar M, Fakhar M, et al. In vitro antiprotozoal effects of nano-chitosan on *Plasmodium falciparum*, *Giardia lamblia* and *Trichomonas vaginalis*. Acta Parasitol. 2021;66:39-52. doi:10.1007/s11686-020-00255-6
7. Lu DY. Drug combinations. Personalized Cancer Chemotherapy. 1st ed. Woodhead Publishing. 2015. pp. 37-41. doi:10.1016/C2014-0-04049-X
8. Derda M, Hadaś E. The use of phytotherapy in diseases caused by parasitic protozoa. Acta Parasitol. 2014;60(1): 1-8. doi:10.1515/ap-2015-0001
9. Stanisavljević N, Soković Bajić S, Jovanović Ž, Matić I, Tolinački M, Popović D, et al. Antioxidant and antiproliferative activity of *Allium ursinum* and their associated microbiota during simulated *in vitro* digestion in the presence of food matrix. Front Microbiol. 2020;11:601616. doi:10.3389/fmicb.2020.601616
10. Krstin S, Sobeh M, Braun MS, Wink M. *Tulbaghia violacea* and *Allium ursinum* extracts exhibit anti-parasitic and antimicrobial activities. Molecules. 2018;23(2):313. doi:10.3390/molecules23020313
11. Roghani M, Baluchnejadmojarad T, Ogbi K. Survey the effect of feeding of Allium Latifolium on contractile reactivity of aorta of diabetic rats. J Guilan Univ Med Sci. 2008;17(65):1-6
12. Elmi T, Gholami S, Azadbakht M, Rahimi-Osboei B, Garayli Z. The effects of hydroalcoholic extract of leaves and onion of *Allium paradoxum* on *Giardia lamblia* in mice. J Shahrekord Univ Med Sci. 2014;16(5):13-22.
13. Knight DJ, Peters W. The antimalarial activity of N-benzyloxydihydrotriazines: I. The activity of clociguanil (BRL 50216) against rodent malaria, and studies on its mode of action. Ann Trop Med Parasitol. 1980;74(4): 393-404. doi:10.1080/00034983.1980.11687360
14. Afrouzan H, Zakeri S, Mehrizi AA, Molasalehi S, Tahghighi A, Shokrgozar MA, et al. Anti-plasmodial assessment of four different Iranian propolis extracts. Arch Iran Med. 2017;20(5):270-81.
15. Habte G, Assefa S. In Vivo Antimalarial Activity of Crude Fruit Extract of *Capsicum frutescens* Var. Minima (Solanaceae) against *Plasmodium berghei*-Infected Mice. BioMed Research International. 2020;2020:1320952. doi:10.1155/2020/1320952
16. Bantie L, Assefa S, Teklehaimanot T, Engidawork E. *In vivo* antimalarial activity of the crude leaf extract and solvent fractions of *Croton macrostachyus* Hocsht. (Euphorbiaceae) against *Plasmodium berghei* in mice. BMC Complement Altern Med. 2014;14(1):79. doi:10.1186/1472-6882-14-79
17. Dikasso D, Makonnen E, Debella A, Abebe D, Urga K, Makonnen W, et al. Anti-malarial activity of withania somnifera L. Dunal extracts in mice. Ethiop Med J. 2006;44(3):279-85.
18. Habte G, Nedi T, Assefa S. Antimalarial activity of aqueous and 80% methanol crude seed extracts and solvent fractions of *schinus molle* linnaeus (*anacardiaceae*) in *plasmodium berghei*-infected mice. J Trop Med. 2020;2020:9473250. doi:10.1155/2020/9473250
19. Joseph AO, Samson OT. Antiplasmodial Efficacy of *Anacardium occidentale* in Albino Mice Infected with *Plasmodium berghei*. J Fam Med Dis Prev. 2020;6(3):123. doi:10.23937/2469-5793/1510123
20. Kouassi CK, Koffi-Nevry R, Nanga ZY, Teixeira Da Silva JA, Yao K, Lathro JS, et al. Assessing the antibacterial activity and phytochemical screening of Capsicum varieties from Côte d'Ivoire. Food. 2010;4(1):27-32.
21. Olatunji TL, Afolayan AJ. Comparative quantitative study on phytochemical contents and antioxidant activities of *Capsicum annuum* L. and *Capsicum frutescens* L. Sci World J. 2019;2019:4705140. doi:10.1155/2019/4705140
22. Perozzo R, Kuo M, Valiyaveettil JT, Bittman R, Jacobs WR, Fidock DA, et al. Structural elucidation of the specificity of the antibacterial agent triclosan for malarial enoyl acyl carrier protein reductase. J Biol Chem. 2002; 277(15):13106-14. doi:10.1074/jbc.M112000200
23. Amoa Onguene P, Ntie-Kang F, Lifongo LL, Ndom JC, Sippl W, Mbaze LM. The potential of anti-malarial compounds derived from African medicinal plants. Part I: A pharmacological evaluation of alkaloids and terpenoids. Malar J. 2013;12(1):449. doi:10.1186/1475-2875-12-449
24. Ntie-Kang F, Onguene PA, Lifongo LL, Ndom JC, Sippl W, Mbaze LM. The potential of anti-malarial compounds derived from African medicinal plants, part II: a pharmacological evaluation of non-alkaloids and non-terpenoids. Malar J. 2014;13(1):81. doi:10.1186/1475-2875-13-81
25. Lima R, Rocha e Silva LF, Melo MR, Costa JS, Picanço NS, Lima ES, et al. *In vitro* and *in vivo* anti-malarial activity of plants from the Brazilian Amazon. Malar J. 2015;14:508. doi:10.1186/s12936-015-0999-2
26. Hajialiani F, Shahbazzadeh D, Maleki F, Elmi T, Tabatabaie F, Zamani Z. The Metabolomic Profiles of Sera of Mice Infected with *Plasmodium berghei* and Treated by Effective Fraction of *Naja naja oxiana* Using ^{1}H Nuclear Magnetic Resonance Spectroscopy. Acta Parasitol. 2021;66:1517-27. doi:10.1007/s11686-021-00456-7
27. Bhatwalkar SB, Mondal R, Krishna SB, Adam JK, Govender P, Anupam R. Antibacterial properties of organosulfur compounds of garlic (*Allium sativum*). Front Microbiol. 2021;12:613070. doi:10.3389/fmicb.2021.613077
28. Elmi T, Hajialiani F, Asadi MR, Sadeghi S, Namazi MJ, Tabatabaie F, Zamani Z. Antimalarial effects of the hydroalcoholic extract of *Allium paradoxum in vitro* and *in vivo*. J Parasit Dis. 2021;45(4):1055-64. doi:10.1007/s12639-021-01359-0
29. Fong J, Yuan M, Jakobsen TH, Mortensen KT, Delos Santos MM, Chua SL, et al. Disulfide bond-containing ajoene analogues as novel quorum sensing inhibitors of *Pseudomonas aeruginosa*. J Med Chem. 2017;12;60(1): 215-227. doi:10.1021/acs.jmedchem.6b01025
30. Jadoun J, Yazbak A, Rushrush S, Rudy A, Azaizeh H. Identification of a new antibacterial sulfur compound from *Raphanus sativus* seeds. Evid-based Complement Altern Med. 2016;2016:927185. doi:10.1155/2016/9271285

31. Langfeld LQ, Du K, Bereswill S, Heimesaat MM. A review of the antimicrobial and immune-modulatory properties of the gut microbiota-derived short chain fatty acid propionate–What is new?. Eur J Microbiol Immunol. 2021;11(2):50-6. doi:10.1556/1886.2021.00005
32. Van de Mortel EL, Shen ZA, Barnett Jr JF, Krsmanovic L, Myhre A, Delaney BF. Toxicology studies with N-acetyl-L-serine. Food Chem Toxicol. 2010;48(8-9):2193-9. doi:10.1016/j.fct.2010.05.045
33. Karbalaei Pazoki Z, Nateghpour M, Maghsood AH, Souri E, Haghi AM, Farivar L, et al. Comparison between the effects of ethanolic extract of *Artemisia annua* and chloroquine on *Plasmodium berghei* in white mice. Sci J Kurd Univ Med Sci. 2014;19(2):9-20.
34. Elmi T, Gholami S, Azadbakht M, Ziaei H. Effect of Chloroformic Extract of *Tanacetum parthenium* in the treatment of *Giardia lamblia* infection in Balb/c Mice. J Mazandaran Univ Med Sci. 2014;23(1):157-65.
35. Rezaiemanesh M, Shirbazou S. *In vitro* giardicidal effect of aqueous and alcoholic extracts of *Asafoetida* on *Giardia lamblia* cyst. Birjand Univ Med Sci. 2012;19(1): 22-3.
36. Garedaghi Y, Khaki A. Evaluation of the effectiveness of ethanolic extract of *Solanum surattense* against *Plasmodium berghei* in comparison with chloroquine in Sourian Mice using *in vivo* tests. Crescent J Med Biol Sci. 2014;1(3):76-9.
37. Ntonga PA, Baldovini N, Mouray E, Mambu L, Belong P, Grellier P. Activity of *Ocimum basilicum*, *Ocimum canum*, and *Cymbopogon citratus* essential oils against *Plasmodium falciparum* and mature-stage larvae of *Anopheles funestus* ss. Parasite. 2014;21:33. doi:10.1051/parasite/2014033
38. Weathers PJ, Towler M, Hassanali A, Lutgen P, Engeu PO. Dried-leaf *Artemisia annua*: A practical malaria therapeutic for developing countries. World J Pharmacol. 2014;3(4):39-55. doi:10.5497/wjp. v3. i4.39
39. Hezarjaribi HZ, Elmi T, Dayer MS, Gholami S, Fakhar M, Akbariqomi M, et al. A systematic review of the effects of Iranian pharmaceutical plant extracts on *Giardia lamblia*. Asian Pac J Trop Dis. 2015;5(12):925-9. doi:10.1016/S2222-1808(15)60959-8