MALARIA is one of the essential protozoal parasitic diseases globally. The present study investigated the antimalarial effect of Dracocephalum kotschyi compared to chloroquine due to the possible side effects of chemical drugs used to treat malaria and the increase in parasite resistance to these drugs. Thirty mice were divided into five groups of six infected with the Plasmodium berghei parasite. The first group represented the negative control group, the second group received chloroquine, and the third to fifth groups were treated with 100, 150, and 250 mg/kg of plant extract. After that, blood samples were taken on days 4, 7, and 14 to determine the parasitemia rate, body temperature, and survival rate. The results indicated that the lowest amount of parasitemia was observed in the group receiving 250 mg/kg (P <0.05). On the fourteenth day, the lowest parasitemia was in the groups receiving 100 and 250 mg/kg (P <0.05). On the fourteenth day, the group receiving a 250 mg/kg treatment had the highest mean temperature (P<.05). Also, the groups receiving chloroquine and D. kotschyi extract were increased until day 17. Based on the results, it can be stated that the effect of D. kotschyi on Plasmodium parasites is remarkable. The segregation and reinforcement of the active antimalarial ingredient of this plant can be considered a promising candidate in the treatment of malaria.

Keywords: Malaria, Antimalarial agent, Zarrin-Giah.

Introduction

Human malaria, also known as paludism, tropical, nubia, intermittent, and forest fever, is a blood-borne infection caused by a unicellular parasite of the genus Plasmodium transmitted by Anopheles mosquitoes. This disease is one of the essential protozoan parasitic diseases globally; based on estimates by the World Health Organization in 2015, about 214 million people were infected with malaria, of which about 400 thousand people have lost their lives due to the disease. Therefore, it is the most important parasitic disease globally in terms of prevalence and mortality [1].

Among the five species of human Plasmodium, P. falciparum disease is the most severe and causes cerebral malaria, coma, and eventual death by binding to cerebral vascular endothelial cells [2]. This protozoan causes significant side effects such as trembling, fever, sweating, tissue oxygen deprivation, anemia, and renal hypoxia in humans [1,3]. Chemical drugs like chloroquine, quinine, pyrimethamine, and sulfadoxine treat malaria. Still, the parasites have developed resistance to these drugs, which is one of the main reasons for the failure to control, prevent, and treat this disease in some areas of the world [4,5].

On the other hand, these drugs may cause hazardous side effects, especially in children and pregnant women, due to restricted use [4-6]. Therefore, based on these findings, the search for drugs with fewer side effects capable of treating drug-resistant strains of malaria seems attractive, necessary, and critical [7,8].

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Since medicinal plants have traditionally been used in malaria-prone areas to treat malaria since ancient times, their therapeutic properties and scientifically examining their effects are prioritized. *D. kotschyi* is a plant that belongs to the Labiatae family, a valuable and exclusive medicinal plant of our country, Iran. Several medicinal properties were reported, including antioxidant, antibacterial, antinociceptive, and immunomodulatory\[9-11\].

So far, no studies have been performed on the anti-malarial activity of *D. kotschyi* in the form of an extract. Therefore, according to those mentioned above and the growing consumption of medicinal plants globally, this study aims to evaluate the anti-malarial effect of the *D. kotschyi* extract.

**Material and Methods**

**Animals and test conditions**

In this experimental study, thirty female albino mice aged about 4 to 6 weeks (25± 5 g) were divided into five groups of six in separate cages. Mice were kept in the animal house of the Faculty of Veterinary Medicine of Shahrekord Azad University in conditions of 12 hours of darkness and 12 hours of light and at a temperature of 22–25 °C.

**Preparation of plant extracts**

First, *D. kotschyi* was collected and purchased from Semirom City and approved by the Herbarium Center. After transferring the plant to the laboratory, it was air-dried and powdered under normal heat and shade. Extraction was performed by the maceration method. The 1000 g of crushed *D. kotschyi* was carefully weighed and soaked in ethanol-water. The resulting extract was filtered and concentrated during the maceration by a rotary apparatus, and its solvent was recycled and added to the vegetable pulp. Some of the total extract obtained from this step was kept as a whole hydroalcoholic extract. The required amounts of the extract were weighed using a sensitive scale of up to 0.001 mg to obtain the necessary concentration of the extract [12].

In the next step, different concentrations in mice’s mg/kg body weight were prepared from this extract. Also, DMSO was used in physiological serum to obtain the desired solubility and dilution of the extract.

**Preparation and culture of parasites**

Tehran University of Medical Sciences donated the *Plasmodium berghei* parasite of the SPH-TUMS strain. BALB/c mice infected with *P. berghei* with a 20–30% parasitemia were selected as donor mice. Blood samples were taken from these mice using the retro-orbital blood sampling method and heparin-impregnated Glass Pasteur Pipets. The collected blood was stored in K2EDTA tubes before injection into an experimental group of mice. This blood was diluted with a sterile physiological serum to achieve approximately 107 infected red blood cells and 107.5 infected red blood cells per milliliter of blood. To infect the mice in the experimental groups, 30 healthy female BALB/c mice aged 6 to 7 weeks and weighing 20–27 g body weight were selected (6 mice for each group).

Each mouse received 0.2 mL of blood infected with the parasite by intraperitoneal injection. Twenty-four hours later, the first ring shapes appeared, and their number increased 48 hours later.

**Experiment grouping**

After infection with the parasite, mice were randomly divided into five groups of six:

1- The first negative control group consisted of *Plasmodium*-infected mice that received DMSO10% for treatment.

2- The second negative control group consisted of *Plasmodium*-infected mice that received 25 mg/kg of chloroquine for treatment.

3- Intraperitoneal injection treated the third treatment group with the *D. kotschyi* extract at 100 mg/kg.

4- Intraperitoneal injection treated the fourth treatment group with the *D. kotschyi* extract at 150 mg/kg.

5- Intraperitoneal injection treated the fifth treatment group with the *D. kotschyi* extract at 250 mg/kg.

The experiment using the Peters treatment method on rats started two hours after the injection of *Plasmodium*-infected erythrocytes. Chloroquine extract and drugs were injected intraperitoneally once a day for 24 hours and continued for four days [13].

**Evaluation of parasitemia and mortality**

Following the treatment period’s completion, blood spread was prepared from the animals on
the fourth, seventh, and fourteenth days after treatment. To prepare a thin spread of malaria-infected blood, the animals’ tails were disinfected with alcohol, then the end of the tail was cut with sharp scissors, and a drop of blood was placed on the slide. The slide was placed between the thumb and middle finger, and a thin expansion was prepared using another 45-degree angle slide. The mouse tail was exposed to hot metal to prevent further bleeding. After the spread dried, it was fixed with absolute methanol. After 5 to 10 minutes, a 10% Giemsa solution (1 ml of Giemsa dye and 9 ml of distilled water) was used for staining. A few drops of the dye were poured on the blood spread, and staining was performed for 20 to 30 minutes.

The slides were washed with water and dried at room temperature. Then, using immersion oil on the slides, they were placed under a microscope, and 20 random fields and 200 erythrocytes in each field were counted to determine parasitemia. This operation was performed twice. The amounts of parasitemia (number of infected red blood cells in 20 fields/4000 healthy red blood cells × 100) and growth inhibition percentage (parasitic difference between the control group and drug/parasitemia of the control group × 100) were calculated.

Rectal temperature measurement

A digital contact thermometer was utilized to measure body temperature on days 4, 7, and 14, and rectal temperature was recorded. Also, from the first day of infection, i.e., day zero to the seventeenth day after infection of mice, the number of deaths in the treatment and positive and negative control groups was recorded.

Measuring the mean survival time

From the first day of infection, i.e., day zero, to the 30th day after infection of mice, namely day 29, the number of mortality days and the number of deaths in the treatment and positive and negative control groups were recorded. The mean survival time in each group (total survival time of mice in each group divided by the total number of mice) was calculated[14].

Statistical analysis

The data were analyzed using SPSS software version 22. Tukey, independent tests, and one-way variance analysis were used to compare groups, and P < 0.05 was considered the significance level.

Results

Measuring the amount of parasitemia in a 4-day test: As can be seen from the study results of parasitemia in different groups, shown in Table 1, on the fourth day, the highest percentage of parasitemia was in the control group, which was significantly higher than in other groups (P < 0.05). At this time, the lowest level of parasitemia was observed in the group receiving a 250 mg/kg concentration, which was significantly lower than the others (P < 0.05).

Results of measuring parasitemia on the seventh day showed that, on the fourth day, the group receiving 250 mg/kg of the *D. kotschyi* had the lowest parasitemia percentage. In contrast, the control group had the highest (P < 0.05).

On the fourteenth day, the lowest level of parasitemia was in the groups receiving different extract concentrations (P < 0.05). However, no significant difference was observed between these three groups (P < 0.05). As in previous times, the highest rate of parasitemia occurred in the control group, which was significantly higher than the other groups (P < 0.05). There was no significant

| TABLE 1. Results from the study of parasitemia in different groups (mean ± standard deviation). |
|-------------------------------------------------|-----------------|-----------------|-----------------|
| Time Group | Day 4           | Day 7           | Day 14          |
| Control    | 59.2 ± 84.21a   | 83.1 ± 57.30a   | 59.3 ± 39.40a   |
| Chloroquine| 63.0 ± 25.14b   | 38.0 ± 56.10b   | 42.0 ± 09.8b    |
| 100 Concentration | 98.0 ± 20.14a | 82.0 ± 92.9b | 44.0 ± 76.6b |
| 150 Concentration | 67.0 ± 05.8c | 68.0 ± 74.6c | 70.0 ± 69.4c |
| 250 Concentration | 29.1 ± 22.5d | 84.0 ± 08.4d | 01.1 ± 71.4d |

* Mismatched letters in each column indicate a significant difference (P < 0.05).
difference between the groups receiving 100 mg/kg of extract and those receiving chloroquine (P <0.05).

Measuring the percentage of parasitic inhibition in a 4-day test

The results of parasite inhibitory activity by different concentrations of plant essence are presented in Table 2. According to this table, the highest percentage of parasite inhibitory activity was on the fourth and seventh days after treatment with 250 mg/kg (76.09 and 86.65%, respectively). On the fourteenth day, the highest percentage of parasitic inhibition was observed in the group receiving a 150 mg/kg plant extract concentration.

Body temperature

The results of measuring the mean body temperature are shown in Table 3. As it can be seen, the highest mean body temperature on the fourth day was observed in the group receiving 150 mg/kg, and the lowest mean temperature was observed in the chloroquine group. However, no significant difference was observed between the control and treatment groups in the present study (P <0.05).

The groups observed no significant differences between the seventh day (P <0.05). On day 14, the highest mean temperature was found in the control group (P>0.05), significantly higher than the other groups. However, no significant difference was observed (P<0.05).

Body weight

The mean body weight analysis, shown in Table 4, shows no significant difference between the studied groups on the fourth day. The group receiving 250 mg/kg of the extract had the highest mean weight on the seventh day, significantly higher than the other groups except the group receiving 150 mg/kg (P <0.05). By the fourteenth day, the mean body weight of the groups receiving different concentrations of plant extract was significantly higher than that of the control group (P <0.05). However, the difference between these groups at this point was not statistically significant.

TABLE 2. Results of investigating the percentage of parasitic inhibition in different groups.

<table>
<thead>
<tr>
<th>Time Group</th>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>75.34</td>
<td>45.65</td>
<td>97.79</td>
</tr>
<tr>
<td>100 Concentration</td>
<td>98.34</td>
<td>73.67</td>
<td>26.83</td>
</tr>
<tr>
<td>150 Concentration</td>
<td>14.63</td>
<td>95.77</td>
<td>38.88</td>
</tr>
<tr>
<td>250 Concentration</td>
<td>09.76</td>
<td>65.86</td>
<td>33.88</td>
</tr>
</tbody>
</table>

* Mismatched letters in each column indicate a significant difference (P<0.05).

TABLE 3. Results of the mean body temperature in different groups (mean ± standard deviation)

<table>
<thead>
<tr>
<th>Time Group</th>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>61.0 ± 76.36b</td>
<td>64.0 ± 15.37a</td>
<td>24/0 ± 65.37b</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>86.1 ± 80.34a</td>
<td>31.0 ± 58.37a</td>
<td>82.0 ± 47.36a</td>
</tr>
<tr>
<td>100 Concentration</td>
<td>71.1 ± 05.35a</td>
<td>26.0 ± 63.37a</td>
<td>86.0 ± 94.35a</td>
</tr>
<tr>
<td>150 Concentration</td>
<td>37.0 ± 53.37b</td>
<td>30.0 ± 13.37b</td>
<td>31.0 ± 48.36a</td>
</tr>
<tr>
<td>250 Concentration</td>
<td>71.0 ± 98.35ab</td>
<td>32.0 ± 50.37b</td>
<td>41.0 ± 48.36a</td>
</tr>
</tbody>
</table>

* Mismatched letters in each column indicate a significant difference (P 0.05).

ANTI-MALARIAL EFFECT OF *Dracocephalum kotschyi* EXTRACT AGAINST…

### Survival rate

As shown in Fig.1, not all control mice survived until the seventh day. The survival rate of mice receiving chloroquine was increased by up to 17 days when *D. kotschyi* extract was administered at 100, 150, and 250 mg/kg concentrations.

### Discussion

As one of the world’s most important parasitic diseases, malaria is treated with various chemical drugs such as chloroquine, pyrimethamine, sulfadoxine, and quinine[15]. However, today the Plasmodium parasite has shown resistance to these drugs, one of the most important reasons for the failure to control and prevent this disease[16].

Therefore, finding a new drug with low side effects against this disease has become a focus of global attention. One of these options is products derived from medicinal plants, so in many countries, native plants are commonly used to treat most infectious diseases[17].

The *D. kotschyi*, with the scientific name *D. kotschyi* is a perennial and endemic herbaceous plant of Iran found in Central Asia and Europe. The current study is the first to investigate the effect of *D. kotschyi* extract in treating rat malaria, or *P. berghei*, compared to chloroquine. Based on the present study results, all three concentrations of the *D. kotschyi* extract could reduce the amount of parasitemia. This reduction effect was dose-dependent in that it was higher at a concentration of 250 mg/kg. The most critical factor in the effectiveness of a plant is the active compounds in that plant. Due to its active ingredient, various effects have been reported regarding *D. kotschyi*. This plant contains rosemary acid, which has antibacterial, anti-corruption, and antioxidant activities[18]. In addition, recent pharmacological studies have shown that methoxylated flavones in the vegetative body of the plant have anti-cancer properties[19].

A study of the methanolic extract of this plant against three gram-positive bacteria

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**TABLE 4.** Results of the mean body weight in different groups (mean ± standard deviation).

<table>
<thead>
<tr>
<th>Time Group</th>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>75.0 ± 62.24*</td>
<td>42.0 ± 69.23*</td>
<td>55.0 ± 78.22*</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>43.0 ± 81.24*</td>
<td>44.0 ± 78.23*</td>
<td>57.0 ± 92.22*</td>
</tr>
<tr>
<td>100 Concentration</td>
<td>67.0 ± 83.24*</td>
<td>29.0 ± 86.23*</td>
<td>35.0 ± 57.23*</td>
</tr>
<tr>
<td>150 Concentration</td>
<td>32.0 ± 04.25*</td>
<td>27.0 ± 08.24*</td>
<td>31.0 ± 51.23*</td>
</tr>
<tr>
<td>250 Concentration</td>
<td>41.0 ± 27.25*</td>
<td>45.0 ± 65.24*</td>
<td>27.0 ± 74.23*</td>
</tr>
</tbody>
</table>

*Mismatched letters in each column indicate a significant difference (P <0.05).*

---

*Fig. 1. Survival rate of mice receiving control, chloroquine and D. kotschyi extract.*
(Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes, and three gram-negative bacteria (Escherichia coli, Salmonella enterica, Enterica arugina) has shown strong antibacterial effects[20]. One of the essential properties of this plant that could explain its anti-malarial effects is its role in modulating the immune system, which has been well demonstrated in previous studies[21]. The immune system's immunomodulatory property helps clear the parasite from the host body.

Based on this, a drug derived from medicinal plants can be discovered that kills malaria parasites and strengthens the host immune system to repel them. Limonene is another component of the plant that acts as an inhibitor of angiotensin-converting enzymes and has anti-viral, anti-tumor, bactericidal, and cancer-preventing effects[22]. Geraniol, Xanthomicrol (Geraniol), and Spinal-Z are other compounds in this plant essence that inhibit polyamine production and growth in human cancer cells. Other main components of this plant include verbénol, alpha terpinol, perillyl alcohol, and caryophyllene[23]. Additionally, terpenoids and phytosterols have been isolated from the organs of this plant, which have been tested as an analgesic in mice[24].

In line with the results obtained in the present study, the anti-malarial activity of some medicinal plants has been well demonstrated in a 4-day test. Karbalaei Pazoki et al. (2014) investigated the effect of an alcoholic extract of Turkish artichoke (Artemisia annua) compared to chloroquine in a 4-day test on P.berghei. They found that plant extract concentrations of 1100 mg/kg and 1300 mg/kg reduced parasitemia in infected mice more than other concentrations, but the 1100 mg/kg concentration was less toxic. However, the effect of chloroquine on the parasites under study was more significant than the different concentrations of alcoholic extract of Turkish artichoke[25].

Simlar to the present study, in a study by Khodadadi on the effect of alcoholic extract of Artemisia aucheri on P.berghei in a 4-day test comparing it with chloroquine in mice, there were dose-dependent antimalarial effects. It was observed that a concentration of 1000 mg/kg of plant extract is the best concentration for killing P.berghei[26]. In addition, in the present study, it was observed that not all control mice survived until day seven. In the groups receiving chloroquine, concentrations of 100, 150, and 250 mg/kg of D. kotschyi extract increased the survival rate of mice by up to 17 days.

Their results showed that the extract of this plant in doses of 12.5, 25, 50, and 100 micrograms per milliliter significantly increased the survival time of mice. In contrast, with an increase in concentration, this effect increases. Mediseh et al. (2016) compared the effects of alcoholic extract and saffron fractions with chloroquine on P. berghei in mice. In this study, after infection and parasite observation in peripheral blood at doses of 350, 700, and 1050 mg/kg of aqueous, alcoholic, and ethyl acetate extracts of saffron, a concentration of 20 mg/kg of chloroquine and 50 mg/kg of iron chelator was used. The results showed that the concentration of 700 mg/kg of ethyl acetate and 350 mg/kg of plant extract significantly increased the survival time of infected mice and reduced the parasitemia rate[27]. Another study was carried out to determine the effect of aqueous cinnamon extract on P.falciparum. Results showed that 50 IC for aqueous extract of cinnamon in culture (invitro) was equal to 1.25 mg/ml, and the use of this extract significantly increased the survival time compared to the control group[28].

Garedaghi et al. (2016) studied the effects of an alcoholic extract of chestnut rye seed at 20, 100, 300, and 450 mg/kg of P. berghei in mice. They compared it with the effect of chloroquine. According to the results, even though the parasitemia level was reduced significantly with all concentrations tested compared to the control and placebo groups, the 450 mg/kg concentration had the most significant effect. It has been stated that despite the effectiveness of plant extracts, chloroquine is still more effective at reducing parasitemia and increasing survival rates than plant extracts[29].

In the present study, the group receiving a 250 mg/kg concentration had the highest mean body temperature on the fourteenth day. Using the extract has prevented a drop in body temperature due to disease. In some previous studies, the effect of plant extracts on controlling temperature changes has been indicated.

In a study by Najm et al. (2019) to evaluate the effect of an alcoholic extract of aromatic Artemisia annua on P.berghei in comparison with chloroquine in laboratory mice, in groups treated
with a concentration of 75 mg of the extract, the amount of parasitemia on the fifth and eighth days was significantly reduced compared to the placebo group[30]. However, on the fifth day, the rate of parasitic reduction was higher in the group receiving 75 mg than in the group receiving 25 mg. Also, mice’s lifespan in the group treated with chloroquine and the concentration of 75 mg was statistically significantly different from other groups[30].

Conclusion

As a result of this study, it can be said that D. kotschyi has a significant effect on Plasmodium parasites and that the most effective concentration was 250 mg/kg. In addition, the survival rate of mice increased in all concentrations examined compared to the control and chloroquine-treated groups. Due to resistance to existing antimalarial drugs, the segregation and strengthening of this plant’s active antimalarial ingredient can be considered a promising candidate in treating malaria with the least resistance to the parasite and minor side effects for the patient.

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Conflicts of Interest

No potential conflict of interest was reported by the authors.

Authors’ Contributions

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors read and approved the final manuscript.

Data Availability

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Ethical Considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Funding statements

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