INTRODUCTION

Malaria is one of the oldest and most life threatening parasitic diseases that human has faced till now. The infection is caused by plasmodia species that are transmitted by the female Anopheles mosquitoes. Five species of Plasmodium have been identified which cause disease in human with different clinical manifestations. However, out of the five species, Plasmodium falciparum is considered to be potentially fatal in delayed treatments. Despite continuous global attempts to fight parasitic infections, malaria still remains one of the major public health problems in tropic and subtropic areas. In 2015, there were estimated 214 million new cases of malaria, and approximately 438,000 deaths; the majority of them were children. In spite of launching malaria elimination programme (elimination phase started from 2010) in Iran, the infection is still, more or less, a health problem in the southeast of the country. According to the recent report of WHO, in total 1243 confirmed malaria cases were recorded during 2015 in Iran.

Antimalarial drugs such as chloroquine, quinine, artemisinine and primaquine are currently being used to prevent and treat human malaria. However in the past 40 years, malaria parasites have rapidly developed resistant to the most of the antimalarial drugs. Plasmodium falciparum became resistant to chloroquine (CQ) in Iran since 1983. Difficulty of producing efficient antimalaria vaccines and increasing drug-resistant strains, highlight the urgent need to search for a new alternative antimalaria drug. The aim of this study was to find a new agent against malaria parasite with maximum efficacy and minimum range of side-effects. For this, the antiplasmodial activity of commercial chitosan, a natural carbohydrate polymer, was evaluated on Plasmodium berghei via in vivo experiments. This is the first report that to highlight antimalarial effects of low molecular weight chitosan against P. berghei in vivo.

ABSTRACT

Background & objectives: Despite continuous global attempts to fight parasitic infections, malaria still remains one of the major human life threatening diseases. Difficulty of producing efficient antimalaria vaccines and increasing drug-resistant strains, highlight the urgent need to search for a new alternative antimalaria drug. The aim of this study was to find a new agent against malaria parasite with maximum efficacy and minimum range of side-effects. For this, the antiplasmodial activity of commercial chitosan, a natural carbohydrate polymer, was evaluated on Plasmodium berghei via in vivo experiments. This is the first report that to highlight antimalarial effects of low molecular weight chitosan against P. berghei in vivo.

Methods: Low molecular weight chitosan with 95% degree of deacetylation was melted in normal saline with 1% (w/v) acetic acid for preparing 10, 20, 40 and 80 mg/kg concentrations of chitosan, which were then examined for their antimalarial efficacy in P. berghei infected mice.

Results: The study showed that different concentrations of chitosan exhibited significant antimalarial effect (p=0.002) when compared with the control group. Also, analysis of mice survival time showed significant differences between 20 and 80 mg/kg concentrations of used chitosan in comparison to negative control group.

Interpretation & conclusion: The results of this study showed that the chitosan has potent antimalarial activity and could be suggested as an alternative antimalarial drug component.

Key words In vivo; Iran; low molecular weight chitosan; malaria; Plasmodium berghei
safe than other human malaria parasites.

Chitin is a long-chain carbohydrate polymer and commonly found in nature as structural element in exoskeleton of crustaceans and many other organisms, like arthropods and fungi. Commercial chitosan is produced by deacetylation of chitin which is characterized by its wide applications. In vitro and in vivo experiments have demonstrated considerable effectiveness of chitosan, against wide range of micro-organisms including bacteria, fungi, yeasts and parasites whithout justifiable effects on mammalian cells.

Necessity for developing a new agent with maximum antimalarial efficacy and minimum side-effects encouraged us to carry out this study with the aim of evaluating antiplasmodial activity of commercial chitosan on P. berghei in vivo. This is the first study reporting antimalarial effects of low molecular weight chitosan against P. berghei infection in mice.

MATERIAL & METHODS

Animals

Male white small saurian mice, weighing 20–25 g (supplied by the Animal Laboratory in Pasteur Institute of Iran) were used in this study. The mice were kept in normal temperature and light-dark cycle situation, with unlimited food and tap water supplies.

Ethical considerations

Animal experiments were done according to ethical standards formulated in the Helsinki Declaration, and approved by Ethical Committee of the Pasteur Institute of Iran for the use of laboratory animals.

Parasite culture

Chloroquine sensitive strain of P. berghei (NICD strain) that was stored in liquid nitrogen (originally obtained from Haffkine Institute, India) was maintained by blood passage in mice under laboratory conditions as described by Nateghpour et al.

Chitosan solution preparation

Low molecular weight chitosan (Mol. wt. 50,000–190,000 Da, Sigma-Aldrich, Cat No.: 448869–50G, St. Louis, MO, USA) with 95% degree of deacetylation was melted in normal saline with 1% (w/v) acetic acid to a final concentrations of 1, 2, 4 and 8 mg/ml. All concentrations were kept overnight at room temperature to reach complete dispersion. The pH of all tubes was then adjusted to 7 with 1 mol NaOH.

Inoculation of malaria parasites

A number of mice were used as infected donors and as parasite reservoir. Mice infected via the intraperitoneal route were allowed to build parasitaemia level up to ~22% (till four days after the infection). The parasitaemia of the donor mice was first determined and infected blood was collected into the heparinized tubes directly from heart via the cardiac puncture using ether as anesthesia. The standard four-day suppressive method described in a study by Peters was used. The mice were randomly divided into six groups with six mice in each group. On the first day each mouse was inoculated by intraperitoneal injection with 0.2 ml of infected blood suspension diluted in physiological saline, and containing about 10⁶ P. berghei parasitized erythrocytes per ml. Treatment was initiated 2 h after the injection of the infected erythrocytes on Day 0 (D0, inoculation day) and continued daily until Day 3 (for four days). The negative control group was given 0.2 ml of normal saline with 1% (w/v) acetic acid. The positive control group was given 0.2 ml of chloroquine (20 mg/kg) subcutaneously, while groups 3–6 were administered single dose of 0.2 ml chitosan subcutaneously, with concentrations of 1, 2, 4 and 8 mg/ml per day which equaled to 10, 20, 40 and 80 mg/kg respectively. Thin blood films were made by collecting blood from the tail of each mouse on the D4, D7 and D14 days. Smears were fixed with methanol and stained with 3% Giemsa at pH 7.2 for 30 min. The percent parasitaemia was determined by counting the number of parasitized erythrocytes out of 10,000 blood cells. Then, average suppression percentage of parasitaemia was calculated for each concentration by comparing the control, using the modified method described by the Peters and Robinson formula:

\[
\text{Percent suppression} = 100 \left( \frac{(A - B)}{A} \right)
\]

Where, \(A\) is the mean percentage parasitaemia in negative control group; and \(B\) is the mean percentage parasitaemia in treated group.

Evaluation of mean survival time

Mean survival time (MST) is another factor that is usually used to calculate the efficacy of antimalarial activity of drugs. Mortality was checked daily and period of survival time was recorded for each mouse in all groups and the MST was assessed for each group by using the following formula:

\[
\text{MST} = \frac{\text{Sum of survival time of all mice in a group (days)}}{\text{Total number of mice in that group}}
\]
**Data analysis**

Descriptive results were expressed as mean ± standard deviation. The statistical differences was calculated by Mann-Whitney U-test and survival analysis using the statistical package for the social sciences (SPSS) software (SPSS IBM version 21, Chicago, IL, USA).

**RESULTS**

**Antiplasmodial studies**

As shown in Fig. 1, the antimalarial activity of the low molecular weight chitosan against *P. berghei* in white small mice was quite obvious due to considerable suppression of parasitemia on Days 4, 7 and 14. The different concentrations of chitosan showed significant antimalarial efficacy ($p = 0.002$) when compared with the control group treated with chloroquine (20 mg/kg/day) (Table 1).

**Survival analysis**

In the four-day suppressive test, the mean survival time in all groups treated with different concentrations of chitosan was longer than negative control group. Groups with concentration 20 mg/kg and 80 mg/kg, and with p-value 0.018 and 0.029 respectively showed significant differences in comparison with the negative control group treated with chloroquine (20 mg/kg/day).

![Fig. 1: Percentage chemosuppression of P. berghei induced malaria in mice.](image)

Table 1. Antimalarial effect of chitosan against *P. berghei* in concentrations of 10, 20, 40 and 80 mg/kg

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Percent parasitaemia (Mean ± SD)</th>
<th>Suppression (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 14</td>
<td>Day 7</td>
</tr>
<tr>
<td>10</td>
<td>7.17 ± 0.72</td>
<td>11.12 ± 0.93</td>
</tr>
<tr>
<td>20</td>
<td>5.62 ± 0.35</td>
<td>8.78 ± 0.98</td>
</tr>
<tr>
<td>40</td>
<td>8.50 ± 0.54</td>
<td>12.28 ± 0.42</td>
</tr>
<tr>
<td>80</td>
<td>7.70 ± 0.43</td>
<td>10.10 ± 0.58</td>
</tr>
<tr>
<td>Negative control</td>
<td>16.1 ± 0.52</td>
<td>18.6 ± 0.44</td>
</tr>
<tr>
<td>Positive control</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Malaria is one of the most perilous and important infectious diseases in the world. Since developing effective antimalarial vaccines is not an easy task and faced with several problems, chemotherapy still is the first-line action against the disease. The development of resistance against these chemotherapeutic agents necessitates further search for new antimalarials. In addition to the need of developing new antimalarial drugs, it is also important to prove the efficacy and safety of natural agents and traditional medicinal plants which are used against malaria.

It is well-known that natural agents and traditional medicine used against malaria and other diseases play important role in some endemic countries lacking access to essential medicines. For example, the herbal extract of Iranian Flora *Artemisia khorassanica* has been successfully tested in vivo for its antiplasmodial activity through artemisin composition, which is globally used as a standard malaria treatment. Similiar studies establish the efficacy of traditional remedies and natural agents.

The present study was carried out to evaluate the antimalarial activity of chitosan, a natural carbohydrate polymer, in vivo on *P. berghei*. Chitin and chitosan have been...
explored as an antimicrobial agent against a wide range of organisms like algae, bacteria, yeasts and fungi in in vivo and in vitro experiments9–10. Although, the precise mechanism of the antimicrobial action of chitin, chitosan, and their derivatives is not fully understood; but different mechanisms have been proposed, the most passable being the interaction between negatively charged microbial cell surface and positively charged chitin/chitosan molecules19–22. Another posed mechanism is the binding of chitosan with microbial DNA via the penetration of chitosan into the nuclei of the microorganisms causing inhibition of mRNA and proteins synthesis23. The third mechanism is the chelation of metals, suppression of spore elements and binding to necessary nutrients required for microbial growth24. All of these properties, with the nontoxicity feature, make chitosan suitable for the pharmaceutical production for present and future uses. In many studies, the antimicrobial activity (antibacterial, antiviral and antifungal) of low molecular weight chitosan has been reported at pH values lower than 6. However, only a few studies have been performed to evaluate antiparasitic effect of chitosan23–24.

Recent studies have shown considerable antileishmanial activity of chitin/chitosan against Leishmania infantum LIPA 137, but not against L. infantum LIPA 155/1025. Results of other studies have shown that chitosan with 0.025% concentration inhibited the growth of fungi and bacteria including Helminth osporium, Fusarium oxysporum, Alternaria alternata and Escherichia coli22. Another study revealed that combination of chitosan and an antiparasitic drug prolonged the releasing time in gastrointestinal tract in Cryptosporidium infections26. Results of a comparative study among different types of fungal chitosan against protoscolices of hydatid cyst using in vitro experiments showed their scolicidal activity equivalent to commercial chitosan22.

Yarahmadi et al27, evaluated the effect of chitosan on the viability of Giardia lamblia cysts that resulted in 100% mortality rate in 180 min of exposure in concentration of 400 µg/ml of chitosan. Similiar studies showed that 1.250 µg/ml of chitosan after 360 min exposure time could completely inhibit the viability of Trichomonas gallinae28.

On the other hand, nanomedicine is expecting to present an exact delivery system and offers the ability of enhancing the quality of chloroquine by decreasing its toxicity, increasing bioavailability and potent of distribution. Tripathy et al29 conjugated chloroquine to chitosan–tripolyphosphate (CS–TPP) nanoparticles to prepare nanochloroquine (Nch) particles by inotropic gelation ranging from 150–300 nm. They showed that efficacy of Nch is more than chloroquine against P. berghei NK65 infection in Swiss mice. In addition they proved that Nch is able to keep tissues safe from oxidative damage, caused by P. berghei infection and capable to decrease free radical generation and also increases antioxidant enzymes activity. The results of their study showed that chitosan conjugated CQ delivery is more potent than only CQ in decreasing parasitemia and host pathology. This study established that CQ in 68.5 mg/kg body weight is not as effective as same amount of CQ conjugated with chitosan in attenuating the parasitaemia29–31. The present study revealed that chitosan alone can reduce parasitemia and its efficacy may be increased when used as a component in combination therapy.

Another parameter to evaluate antimalarial activity of plant extracts is mean survival time. The prolonged survival time of cured infected mice with chitosan in comparison to the non treated group (negative control) revealed considerable anti-plasmodial activities of the agent, but not as effective as chloroquine; in four-day suppressive test.

As mentioned previously, very few studies about antiparasitic effect of chitosan are available, and this work is the first report of using chitosan alone in treatment of murine malaria. The low molecular weight chitosan was successfully tested in vivo for its anti-malarial activity. The results indicated that the various concentrations of chitosan exhibited significant antimalarial effect (p=0.002) when compared with the negative control groups. Some natural substances, possess significant efficacy against the parasitic agents with low toxicity and can be used, more or less, as a safe treatment against murine malaria infection. Among the natural agents, chitosan has a reasonable efficacy to be supposed as a new therapeutic product. However, more studies are needed to analyse further aspects of chitosan and its exact effectiveness.

CONCLUSION

The results of this study showed that the low molecular weight chitosan has potent antimalarial activity and could be selected as an alternative natural antimalarial drug candidate. More investigations on different plasmodia species, animal hosts and combination between chloroquine and chitosan on chloroquine-sensitive and chloroquine-resistant strains of P. berghei are necessary to elucidate the antimalarial activity of chitosan and its natural components.

Conflict of interest

All the authors declare that they have no conflict of interests.
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REFERENCES

6. Nateghpour M, Farivar L, Souri E, Hajjaran H, Mohebali M, Mr. R. Eskandary for their useful technical support. The authors would like to thank Ms. M. Amirzadeh and Tehran University of Medical Sciences and Health Services.