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Evaluation of the Effectiveness of Ethanolic Extract of Solanum Surattense against Plasmodium Berghei in Comparison with Chloroquine in Sourian Mice Using in Vivo Tests

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Abstract

Objective: Owing to the importance of employing native and traditional medicinal plants with good efficacy against malaria parasites, an ethanolic extract of Solanum surattense was tested on Plasmodium berghei in sourian mice. Moreover, the results were compared with that of the effect of chloroquine on the same parasite.

Materials and Methods: In this study, 80 sourian mice were divided into 8 groups, each consisting of 10 animals. The first 7 groups were infected with P. berghei and the last group was used as control. The first 7 groups were given chloroquine, solanum surattense at four different concentrations (20, 100, 300, and 450 mg/kg), and placebo, respectively, and the seventh group did not receive any treatment. The evaluation was done by Rane test. In each group, the level of parasitaemia was determined on days 4 and 7, and compared with values from day 0 (just before treatment) in order to record the decline in parasitaemia in treated groups. Results were analyzed using SPSS software and one-way analysis of variance (ANOVA).

Results: The results indicated that although all four concentrations of Solanum surattense extract significantly reduced parasitaemia in the infected subjects, the 450 mg/kg solution showed optimal effectiveness on the parasites in comparison with other concentrations and the no-treatment option.

Conclusion: We conclude that although the ethanolic extract of Solanum surattense is not as effective as chloroquine in reducing parasitaemia, it can nonetheless cause a significant decrease when compared to control and placebo groups.

Keywords: Chloroquine, Mice, Plasmodium Berghei, Solanum Surattense, Treatment

Introduction

Malaria is the most important parasitic disease and one of the world's major health problems in some countries, especially tropical countries. Malaria is a protozoan disease, and regarding its spreading prevalence, it is the most important parasitic disease causing mortality in the world. Every year, nearly 500 million people worldwide are infected with malaria and 1.5-2.7 million people die of malaria every year. The cause of the disease in humans is the protozoa of plasmodium genus (Plasmodium vivax, P. falciparum, P. Malariae, and P. ovale) (1,2).

Malaria has existed since ancient times in many parts of Iran and Iranian physicians, such as Bou Ali Sina and Abu Reyhan Biruni, had treated patients with compound drugs that were prepared from several species of plants and fruits. Herbal medicines were gradually replaced by new synthetic drugs and the most widely used and important of them was chloroquine of the 4-aminoquinoline family of drugs.

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After chloroquine, other drugs were produced and used in the treatment of malaria. In recent years, incidence and spread of resistance to chloroquine and other anti-malarial drugs in plasmodium and falciparum has caused issues for the treatment and controlling of falciparum malaria in many parts of the world, and recently, in vivax malaria treatment in some parts of the world. On the other hand, the side effects of anti-malarial chemical drugs, especially among children and pregnant women, have limited the use of these drugs. Research on new drugs that can be effective in the treatment of resistant strains and have fewer side-effects seems necessary and important (3-5). The use of medicinal plants for the treatment of malaria has been common since ancient times, and some types of these plants, such as Artemisinin, have been identified as effective drugs (6-9). Therefore, research on native plants in malaria-prone areas, which are locally used as antipyretics, may have a significant role in the treatment and control of malaria. Given the importance of this issue, it was decided that the antimalarial effects of plant extracts of Solanum surattense be investigated in this study. Solanum surattense is boiled in some southern parts of the country and used as antipyretic, and its antimalarial effects have not been scientifically studied until now. This review was the first study with in vivo condition using the mentioned plant in the experimental treatment of malaria. The present study was conducted in the malaria research laboratory in the protozoology unit, Department of Parasitology, Faculty of Veterinary Medicine of Islamic Azad University, Tabriz Branch, Iran.

Materials and Methods

In the present study, 80 mice that were similar in terms of gender, age, and weight (23 ± 2 g weight) were divided into 8 groups of 10. Then, 7 groups were injected intradermally with plasmodium bargei parasite (106 infected erythrocytes suspension in saline = final volume of 0.2 ml). After viewing parasites in peripheral blood, the treatment began. Groups 1 to 4 were treated with four different concentrations of plant extracts of Solanum surattense; 450, 300, 100, and 20 mg/kg.

For the solubility and dilution of the extract of Solanum surattense, tween 80 and physiological serum were used. Group 5 with chloroquine 20 mg/kg, group 6 with placebo injection (2.5% tween 80 in physiological serum), group 7 without medication, and group 8 in order to control the randomized mortality of mice were kept in animal shelter without injection of parasite and medication. Treatment was performed using the method proposed by Ryley and Peters (10). This method was based on beginning the treatment after viewing parasitaemia in the peripheral blood. Treatment was continued subcutaneous and up to 4 days. Blood samples were taken daily from the tip of the tail of the mice. After a thin smear of blood and staining with Giemsa, parasitemia rates were determined. The most effective drug concentration was the concentration that reduced the parasitemia rate to its lowest level compared with the other concentrations, and had no toxic effects on the mice. The mice were examined for parasitemia until day 21 and for mortality until day 35. Results were gathered using SPSS for Windows (version 16, SPSS Inc., Chicago, IL, USA).software and analyzed by Student's t-test. To ensure the lack of toxicity of ethanol extract, 10 healthy mice were selected and placed in a group. For two weeks, the mice were injected continuously with the highest concentration of plant medication. At the end, the follow-up period was continued for 50 days, and during this period the mice were examined regarding diarrhea, weight loss, blurred and clarity of vision, injection site necrosis, and mortality.

Results

In this study, the mean parasitaemia of the studied groups were compared during different days from day 0 (one day before treatment) until day 28, and on fixed days, such as day 4 (24 hours after last treatment dose) and day 7 (3 days after cessation of treatment). In the chloroquine group (group 5), parasitemia rate gradually decreased, so that on day 4 this level was zero and remained zero until day 7. The amount of parasitaemia in groups 6 (placebo) and 7 (no treatment) on day 4 were 17.2 and 17.8 percent, respectively, and had a significant difference with group 5 ($P < 0.05$).

Increased levels of parasitaemia for groups 6 and 7 on day 4 compared to the day prior to treatment were, respectively, 10.2 and 9.1%. Moreover, the increase in parasitaemia for groups 6 and 7 on day 7 of the treatment compared to the day prior to treatment was 15.3 and 18.7%, respectively. The mean parasitaemia in the group 1 that was treated with a concentration of 20 mg/kg of Solanum surattense on day 4 was 18.6 percent. The increase in parasitaemia in this group on day 4 and day 7 compared to the day before the treatment was 7% and 15.3 percent, respectively.

The mean parasitaemia in the group 2, which was treated with 100 mg/kg Solanum surattense, was 18.6% on day 4. The amount of increase of parasitaemia on day 4 and day 7compared to the day before the treatment was 11.7 and 15.8 percent, respectively. The mean parasitaemia in group 3, which was treated with 300 mg/kg Solanum surattense, was 12.8% on day 4. The amount of increase of parasitaemia on day 4 and day 7 compared to the day before the treatment was 6.8 and 10.7 percent, respectively. The mean parasitaemia in group 4, which was treated with 450 mg/kg Solanum surattense, was 4.19% on day 4. The amount of increase of parasitaemia on day 4 and day 7 compared to the day before the treatment was 6 and 9 percent, respectively (Figure 1 and 2).

The comparison of lifespan of the mice was performed in the treatment group, placebo, control, and non-infected group. The comparison of death rates between groups of mice, showed that it was

lower in the group treated with chloroquine than other groups, their average of life was 29 days. Group 4 (treated with the extract at a concentration of 450 mg/kg), after chloroquine, had the highest life expectancy, and the mean age in this group was 2.20 days. Nevertheless, the life expectancy in the placebo group and control were, respectively, 10 and 9 days. These results show a clear difference.

Group 1, which was treated with a concentration of 20 mg/kg of the extract, had a mean survival of 10 days. Group 2, which was treated with a concentration of 100 mg/kg of the extract, had a mean survival of 12 days. In addition, group 3, which was treated with a concentration of 300 mg/kg of the extract, had a mean survival of 14 days.

It should be noted that at the end of the study (35 days) no death was observed in the uninfected group that was kept as the randomized mortality control group of the mice. In evaluating the potential toxicity of Solanum surattense on the mice, there was no negative or suspicion observation and the mice were weighted and seemed healthy.

Discussion

The aim of this study was to evaluate the effect of Solanum surattense extracts on Plasmodium berghi in sourian mice in comparison with chloroquine. Previous studies had been conducted on the effect of medicinal plants, such as espand and chicory, on Plasmodium berghi. In a study on the alcoholic extract of espand seed with a concentration of 100 mg/kg with minimal toxic effects, this extract was shown to be more effective in reducing parasitaemia in mice. Furthermore, in this study, the toxicity of the extract was detected at high concentrations (11).

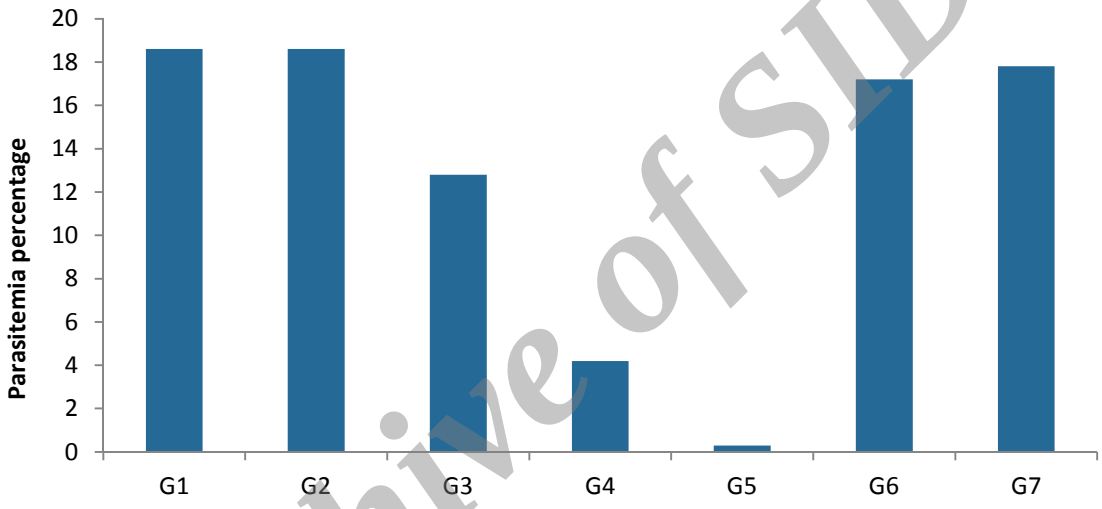

Figure 1. Comparison of Plasmodium parasitemia rate in the groups treated with Solanum surattense and chloroquine, and control group on days 0 to 4 of the treatment
G1: 20 mg/kg; G2: 100 mg/kg; G3: 300 mg/kg; G4: 450 mg/kg
G5: Chloroquine; G6: Placebo; G7: Without treatment

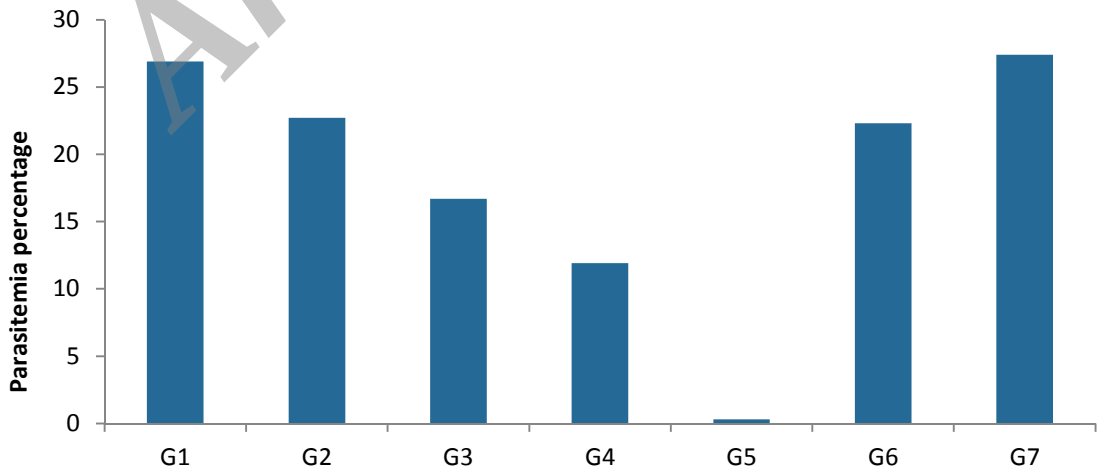

Figure 2. Comparison of Plasmodium parasitaemia in groups treated with the extract of Solanum surattense and chloroquine, and control group on days 4-7 after treatment
G1: 20 mg/kg; G2: 100 mg/kg; G3: 300 mg/kg; G4: 450 mg/kg
G5: Chloroquine; G6: Placebo; G7: Without treatment

In the present study, the concentration of 450 mg/kg Solanum surattense extract had the greatest impact in reducing parasitaemia in infected mice ($P < 0.05$). Because of the lack of toxicity and use of this drug as an antipyretic in natives of malaria-prone areas of Iran, the use of higher concentrations, compared to the ethanol extract of espand seeds, was possible. In another study on the chicory plant, concentrations of 0.1 mg/kg and 0.07 mg/kg had the greatest impact in reducing parasitaemia in the infected mice. The extract of this plant, like the alcoholic extract of espand seed, had toxic effects at higher concentrations (12). The difference between that study and the present research was the method of drug prescription; the chicory plant extract was used orally, but in this study on Solanum surattense, the extract was used by injection. Solanum surattense plants, due to their high concentration of antioxidant compounds, can have positive effects on the body (13,14).

Conclusion

Ethanolic extract of Solanum surattense, at a concentration of 450 mg/kg, had a considerable effect on reducing the plasmodium parasitaemia berghi parasite in mice being studied compared to the control group. The antiparasitic properties of the mentioned extract, compared to chloroquine, on the mentioned parasite were lower. However, if it was possible to increase its concentration with other solvents or to purify its active ingredient and apply it, perhaps better results, in terms of reducing parasitaemia and increasing the lifespan of mice, could be obtained.

Ethical issues

We have no ethical issues to declare.

Conflict of interests

We declare that we have no conflict of interests.

Acknowledgments

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