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Original Article

Evaluation of Effectiveness of Ethanolic Extract of *Artemisia aucheri*, Individually and in Combination with Chloroquine, on Chloroquine - Sensitive Strain of *Plasmodium berghei* in Sourian Mice

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Abstract

Background: Drug resistance in malaria parasites is extending in the world particularly in chemical synthesized drugs such as 4- aminoquinolines and aminoalcoholes. Employing herbal extracts is encouraged by WHO in the malarious areas. In this study, the effectiveness of ethanolic extract of *Artemisia aucheri* individually and in combination with chloroquine, has been considered against chloroquine - sensitive strain of *Plasmodium berghei*.

Methods: At the first stage, ED50 of *A. aucheri* and chloroquine on *P. berghei* was calculated using in vivo test. Then based on the ED50s combination of *A. aucheri* and chloroquine with ratios of 0/100,10/90,20/80,30/70,40/60,50/50,60/40,70/30,80/20,90/10 and100/0 were tested against the parasite. For evaluating the adverse effect of *A. aucheri* on the mice, for two weeks 1000mg/kg of the extract was daily employed and the mice were followed up for fifty days

Results: ED50s for chloroquine and *A. aucheri* were 1.6mg/kg and 1000mg/kg respectively. The outcome of two drugs combination on the mice showed antagonistic effects on the chloroquine – sensitive strain of parasite. Two weeks daily administration of *A. aucheri* had no toxic effect on the mice.

Conclusion: *A. aucheri* individually can be effective in reducing the parasite while in combination with chloroquine loses its property.

Keywords: *Artemisia aucheri*, *Plasmodium berghei*, Combination therapy, Chloroquine

Introduction

Malaria is one of the most important and widespread human parasitic diseases in the world with high mortality rate particularly among children and pregnant women. The disease is caused by plasmodium parasites in malarious areas (1). Prompt and accurate treatment of malaria play a crucial role in control of the disease, but drug resistance in malaria parasites especially in *Plasmodium falciparum* is an obstacle in the way of combating the infection. Emergence of multidrug – resistant strains in *P. falciparum* and chloroquine –resistant strains of *P. vivax* in malaria endemic areas emphasize on preparing new, effective and affordable antimalarial medications. Some species of artemisinin and its derivatives are very effective, safe and available medicines which can prevent

the development of resistance particularly if to be used in combination form. Artemisinin combination therapy is recommended by WHO for successful treatment of uncomplicated malaria (2-5). *Artemisia annua* is the most famous species of artemisinin plants due to its long time using in China and the first official and pharmaceutical herbal remedy administrated against *falciparum* malaria in where multidrug –resistant strains of *P. falciparum* were distributed (6, 7). Indeed, for more information about *Artemisia* besides *A. annua* other species are needed to be considered due to their effective antimalarial efficacy. There are some Iranian flora of Artemisinin growing in different parts of Iran and are utilized as traditional herbal remedy. *Artemisia aucheri* is classified among the Asteraceae family derived from tribe of Anthemideae. The plant is about 20-25 cm in highness with flat elliptical leaves and green yellowish in color. *A. aucheri* is used as anti-parasitic, anti-inflammatory and decreasing blood sugar herbal remedy in decoction or extract forms in some parts of Iran (8-10).

This study was proposed to investigate the effectiveness of *A. aucheri* individually and in combination with chloroquine, against chloroquine – sensitive strain of *P. berghei*

Materials and Methods

Animals

Saurian male mice with 20-25 g weight (supplied from Pasteur Institute of Iran) were used in this study. The animals were kept in standard plastic cages at room temperature under daytime light in Animal House of the School of Public Health, Tehran University of Medical Sciences (TUMS), with access to standard food and tap water. Dealing with the mice was done according to the ethical regulation of Helsinki.

Parasites

Chloroquine - sensitive *Plasmodium berghei* NICD strain (originally from Haffkine Institute, India) stored in nitrogen liquid was employed in this study. Two weeks previous to the tests the parasites were rethawed and maintained by blood passage in saurian mice.

Herbal extract and drug

Artemisia aucheri was collected from Golestan Province located at the northeast of Iran. Two-hundred grams of aerial parts of the plant were powdered and macerated in 96° ethanol for 24 h, and was allowed to settle. The supernatant was filtered and evaporated. Finally, the concentrations of 50, 100, 200, 300, 400, 500, 750, 1000, 1500, 2000 and 2500 mg/kg of the extract were produced via dissolving in 2.5% Tween 20(diluted in normal saline) under a sonicating process (Ultrasonic system, Tecna 6). Chloroquine diphosphate (Sigma Chemical Co.) was dissolved in distilled water and provided concentrations of 1, 3, 10, 20 and 30 mg/kg using the same solvent.

Testing method

Plasmodium berghei parasites were suspended in normal saline to prepare 0.2 ml suspension. Some mice were selected as donor and infected with the parasites intraperitoneally. Parasitaemia was measured every day and at the point of 10% Parasitaemia, blood of the donor mice was collected via cardiac puncture and diluted in normal saline. Ratio of 10^6 parasitized erythrocytes in 0.2 ml dilution was injected intraperitoneally into the test mice. Five mice were appropriated for each concentration of the extract and chloroquine. Moreover, two groups of control containing five uninfected and five infected but untreated mice were prepared besides the tests. Two hours after injection of parasites, that was named day zero, the prepared concentrations of the extract and chloroquine were injected into the relevant infected mice and repeated once daily until day 3. On day 4 a thin blood smear via puncturing the tail of each of the mice was prepared and stained with10% Giemsa stain in distilled water. Percentage of Parasitaemia was determined by counting parasites against 200erythrocytes. $ED_{50\ s}$ (fifty percent of effective dose) of the drug and extract were calculated based on semi-log papers. Combination therapy was conducted according to

fixed ratios method which was described previously (11). This method relies on the use of D_{50} values for each employed substance in the combination. Briefly, some amounts of ED_{50} concentration of chloroquine and *A. aucheri* extract with ratios of 100/0, 90/10, 80/20, 70/30, 60/40, 50/50, 40/60, 30/70, 20/80,10/90 and 0/100 percent respectively were combined together. Each combination was injected intraperitoneally into the infected mice. The ED_{50} concentrations of the *A. aucheri* and chloroquine against *P. berghei* were plotted on two ordinates and these values joined with a straight line. Interaction was measured according to the place of ratios points on, above or under the straight line indicating additive, synergism and antagonism respectively (11). The control sets, number of the mice in each group and calculating the percentage of parasitaemia in combination test were similar to the ED_{50} test.
The treated mice were followed up for 28 days after injection (on days 7, 14, 21 and 28), and survival time of the mice was recorded in each tests. For considering the toxicity of *A. aucheri*, 1000 mg/kg of the extract was daily administrated for two weeks and the mice were followed up for fifty days. Percentage inhibition and standard deviation (SD) were calculated for each test and all the results were analyzed using ANOVA test.

Results

The mean ED_{50} tests of *A. aucheri* extract and chloroquine resulted in 43.2% and 46.4% inhibition of chloroquine-sensitive *P. berghei* due to effectiveness of 1000 mg/kg and 1.6 mg/kg of the substances respectively. The results of interaction between *A. aucheri* extract and chloroquine against the *P. berghei* showed an antagonistic pattern particularly in ratio of 50% CQ + 50% Aa (Fig. 1). Survival time of the treated mice with 1000 mg/kg *A. aucheri* was longer than other concentrations. There were not any significant differences between combination ratios in survival time for the treated mice (Fig. 2-4). Toxicity assay of *A. aucheri* against the parasites did not revealed any clinical adverse manifestation until fifty days follow up.

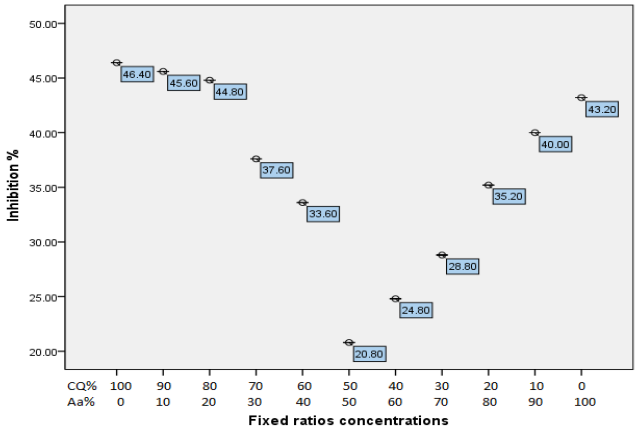

Fig.1: Interaction between *Artemisia aucheri* and chloroquine against chloroquine – sensitive strain of *Plasmodium berghei,* based on the fixed ratios method

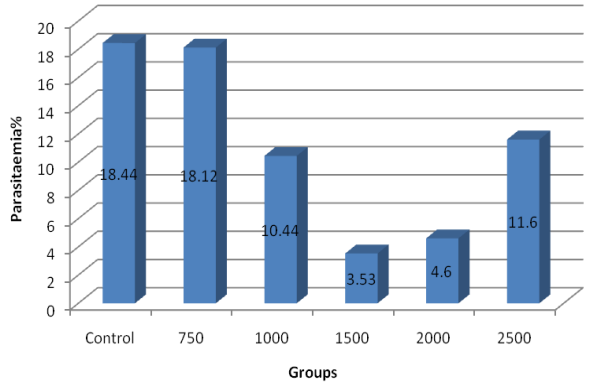

Fig. 2: Percentage of parasitaemia resulted from effectiveness of different concentrations of *Artemisia aucheri* against chloroquine – sensitive *Plasmodium berghei*

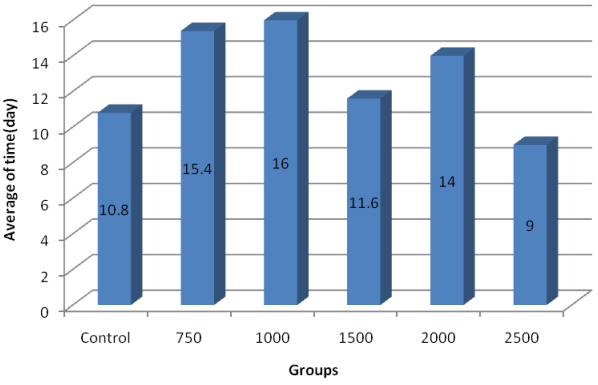

Fig. 3: Survival times of chloroquine – sensitive *P. berghei* infected mice after treating with different concentrations of *Artemisia aucheri*

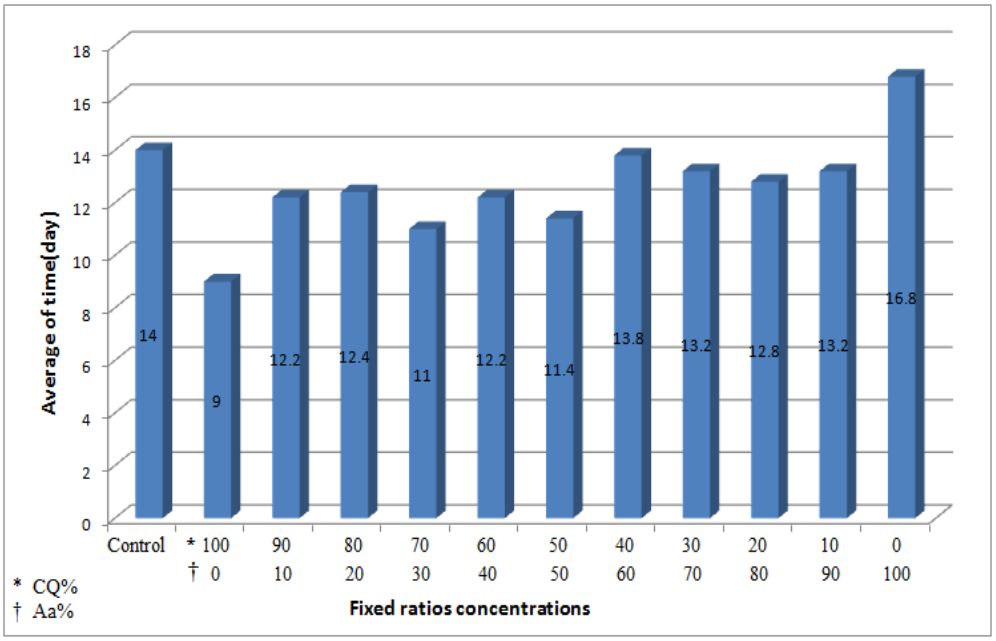

Fig. 4: Survival times of chloroquine – sensitive *P. berghei* infected mice after treating with combination of *Artemisia aucheri* and chloroquine

Discussion

Prevalence of antimalarial drug resistance in *P. falciparum* in most of the malarious countries and in *P. vivax* in some areas (12-16) makes serious problem to combat malaria infection. On the other hand, production of an effective vaccine against malaria disease encounters some difficulties. Therefore, discovering the new, efficient and cost benefit compounds is a crucial effort for control of malaria parasites.

Investigation on the effectiveness of herbal medicines alone or in combination with current antimalarial drugs opens a promising window for combating malaria infection. Although many herbal extracts were proved against malaria parasites, particularly *P. berghei,* genus *Artemisia* was found to be more effective against the parasites. According to the ancient texts employing traditional herbal extracts on curative remedies in Iran has a historical life, but based on our knowledge the scientific studies about effectiveness of the plants on malaria parasites have commenced from two decades ago. Antimalarial activity of *A. khorassanica* and *A. sieberi* against *P. berghei* was proved by Nahrevanian and colleagues (17, 18). Motevalli – Haghi and colleagues examined the effectiveness of ethanolic extract of *Peganum harmala* on *P. berghei.* Extract of the plant showed a considerable effect on the parasite in 100 mg/kg dosage (19). Although employment of some herbal medicines in combination with antimalarial drugs could not prevent the establishment of drug resistance in malaria parasites, it could delays the phenomenon of drug resistance in the parasites and also enhances effectiveness of antimalarial drugs on the parasites.

Using *Otostegia persica* (OP) in combination with chloroquine (CQ) against chloroquine – sensitive and chloroquine – resistant strains of *P. berghei* revealed potentiation against the chloroquine – sensitive parasites in ratios of 70% CQ +30% OP, 50% CQ + 50% OP and 30% CQ + 70% OP , but additive effect on chloroquine – resistant strain in the all ratios. Such results can be explained based on the similar mechanisms of action for both chloroquine and *O. persica* against *P. berghei* (11). Chawira and colleagues previously found marked synergism between artemisinin as a herbal remedy and mefloquine, tetracycline or spiramycin

against normally susceptible strain of *P. berghei* (20). In a randomized controlled trial conducted by Sutherland and colleagues combination of chloroquine with artesunate could not sufficiently reduce the Parasitaemia of *Plasmodium falciparum* among the Gambian malaria-infected children those who received the combination medicine (21). In our study interaction between *A. aucheri* and chloroquine resulted in antagonism effect against chloroquine – sensitive *P. berghei*. This event may be happened due to adverse mode of action of *A. aucheri* and chloroquine on the parasites.

Conclusion

Although, using combination of *A.aucheri* and chloroquine, in this study revealed an antagonistic effect on chloroquine – sensitive *P. berghei* more studies are needed to prove other species of *Artemisia* in combination with chloroquine against both chloroquine – sensitive and chloroquine – resistant strains of *P. berghei*.

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.

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