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Original Article

Evaluation of Effectiveness of Ethanolic Extract of *Curcuma longa*, discretely and in Combination with Chloroquine against Chloroquine-Sensitive Strain of *Plasmodium berghei*

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Abstract

Background and Aim: Malaria is a parasitic disease and one of the most important public health problem. Chemical medications such as chloroquine, primaquine, pyrimethamine, mefloquine, artemisinin and fansidar are commonly used to treat malaria but most of the antimalarials have encountered with problem of drug resistance. Therefore, new therapeutic materials are promptly needed. During many years natural medicines, especially medicinal plants have been considered as the pivotal means for treatment of malaria in some endemic areas. In this study, we evaluated the effectiveness of ethanolic extract of *Curcuma longa L*, discretely and in combination with chloroquine against chloroquine-sensitive strain of *Plasmodium berghei*.

Materials and Methods: Fifty percent effective dose (ED_{50}s) of *C. longa* and chloroquine were determined according to peter' s method. Then based on the obtained ED50s combination of *Curcuma longa* and chloroquine with different ratios were examined against the strain.

Results: The results of ED50s for chloroquine and *C. longa* were 1.4mg/kg and 1250mg/kg, respectively. Combination of *C. longa* ethanolic extract with chloroquine in ratio of 80/20 showed the highest activity with 71.75% to inhibit the growth of *P. berghei* indicating synergistic interaction in the combination treatment.

Conclusion: It appears that turmeric (*C. longa*) extract is an effective anti-malarial substance especially in combination with chloroquine.

Keywords: Turmeric extract, *Plasmodium berghei*, Interaction, Chloroquine

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Introduction

Malaria is a parasitic disease and one of the most important public health problem, especially in tropical and subtropical countries around the world. The disease plays an important role in the history of medicine and considerably more than any other parasitic disease can cause injury to humans (1). According to World Health Organization suggestion that is necessary for malaria endemic countries to conduct some therapeutic efficacy studies of the herbal medicines as a probable replacement for old antimalarials to prevent the spread of drug resistance (2). Chemical medications such as chloroquine, primaquine, pyrimethamine, mefloquine, artemisinin and fansidar are commonly used to treat malaria in some endemic areas but drug resistance in some malaria parasites to the antimalarials is one of the major obstacles in the way of fighting against malaria. The ideal treatment for malaria seems to be free of toxicity, preventing relapses and removing gametocyte in humans and stops the cycle of transmission by *Anopheles* (3). During many years at the past natural medicines, especially medicinal plants were the only means of treatment in some infections. At present besides chemical substances some natural materials are used in the pharmaceutical industries as medicines due to their some medical and hygienic advantages (4). Since most chemical medicines utilizing against malaria parasites have many side effects and encounter with problem of drug resistance in the parasites, producing natural remedies including herbal materials is emphasized by malaria control policy makers such as WHO (5). Although many species of plants are used in traditional medicine worldwide, few of them have been registered in pharmaceutical lists (6). One of the plants is turmeric under the scientific name of *C. longa* (7). Extract of *C. longa* has been used as a flavor in food and also medicine for long time in Asian countries. It is reported that an alcoholic extract of turmeric has anti-bacterial property particularly against *Staphylococcus*, *Streptococcus*, *Clostridium* and *Corynebacterium* (8). The aim of this study was to evaluate the effectiveness of the alcoholic extract of *C. longa* in different concentrations and in combination with chloroquine against *P.berghei*.

Materials and Methods

Parasite

A chloroquine- sensitive NICD strain of *P. berghei* (from National Malaria Laboratory, School of Public Health, TUMS, Iran) was used in this study. Previous to main tests the parasite was rethawed from liquid nitrogen and injected intraperitoneally to reservoir Balb/c mice. Parasite – infected blood was prepared from reservoir mice and mixed with normal saline to make up a suspension with 10^6 parasites in 0.2 ml.

Herbal extracts and drugs

The rhizome of *C. longa* was prepared from trustable market and completely powdered, then 500 gram of the powder was soaked in 820 ml of ethanol 96°. After one hour stirring the produced crop was left at room temperature for overnight. The extraction process was repeated four times more and the result was purified with a vacuum distillation apparatus at 40°C. The purified extracts were placed at 37°C to evaporate the alcohol. Concentrations of 200, 400, 600, 800, 1000, 1250 mg /kg were made up with tween 20 and injected into mice for determination of antimalarial effect of *C. longa*. Also four concentrations of chloroquine as 1,3,10, 20 mg/kg were used for determination of ED50.

Animals

White male Balb/c mice (bred in School of Public Health, TUMS) with 20 ± 2 g weight were used in this study. The mice were kept in standard plastic cages at room temperature in normal light-dark cycle with access to standard food and tap water. This study acquired approval of Research Ethical committee of TUMS due to dealing with laboratory animals.

Antimalarial effects assay

To investigate the antimalarial effects of *C. longa* ethanolic extract, at the first step fifty percent of effective doses (ED50s) of the extract and chloroquine were calculated by using six groups of mice including five mice in each group for *C. longa* with concentrations of 200, 400, 600, 800, 1000 and 1250 mg /kg and four groups of mice as mentioned for chloroquine with concentrations of 1, 3, 10 and

20mg/kg. Moreover, two parasite – infected groups were adopted as control that one group received treatment and the rest was left without treatment. All groups received infected RBCs and were treated subcutaneously with the above mentioned agents two hours after receiving infected cells up to four days except one group. Parasitemia was determined under the light microscope via preparing thin blood smears from tail of the mice after staining the smears with Giemsa stain on days 4,7,21 and 28. Then growth inhibitory percentage was calculated to determine ED50 for each group via using a standard semi-log sheet.

Combination therapy assay

A fixed ratio method adopted by Nateghpour et al. for in vivo tests (9) was employed in this section of the study. Briefly, after preparing ED50s some ratios of *C.longa* and chloroquine ED50s were combined together contrarily and the mixed materials were injected into the relevant infected mice (Table 1). Results of parasite inhibition percent for each drug and ratio were plotted as a point ontwo ordinates. Points of ED50s were joined by a straight line and other points were scattered around the straight line. Effects of the two drugs on each other's activity were measured by the position of ratio points compared to the straight line. The points above, below and on the line indicated synergism (potentiation), antagonism and additive respectively.

Results and Discussion

ED50s for ethanolic extract of *C. longa* and chloroquine were obtained 1250 mg/kg and 1.4mg/kg respectively. The highest percentage of inhibition for *C.longa* was obtained in concentration of 400mg/kg with 77% inhibition. Different concentrations of ethanol extract of *C. longa* have been shown in Table 2. There is significant difference between *C. longa* effective doses and control group ($p<0.05$). Comparison among different doses of *C. longa* showed that there is significant difference in effectiveness between 400mg/kg concentration and others ($p=0.001$).

Interaction between *C. longa* and chloroquine against *P. berghei* in different ratios have been listed in Table 3. Combination of *C. longa* with chloroquine showed highest synergism in ratios of 80/20 (chloroquine/*C. longa*) and 40/60 (chloroquine/*C. longa*) with 68.34% and 67.62% inhibition of *P. berghei* respectively (Figure 2). Survival times of treated mice also are compatible with synergism ratios (Figure 3).

Since there are many side effects in chemical antimalarial drugs particularly for children and pregnant women, some medicinal herbs have been used in the treatment of malaria in recent years (10, 11). Indeed, one of the important strategies that has been recommended by WHO, is the use of herbs and natural productions for eliminating malaria parasites (12). Curcuminoids and curcumin agents have shown numerous biological efficacies such as anti-inflammatory, anticancer, antioxidant, wound healing, and antimicrobial effects (13). The results of this study indicated that alcoholic extract of *C. longa* in different concentrations could prevent growth of *P. berghei* about 55-78% especially at the concentration of 400 mg/kg. As mentioned already inhibitory activity of *C. longa* against *P. berghei* showed that concentration of 400 mg/kg was more active than other concentrations. Although the exact reason of this phenomenon is not clear, involving destructive activity of different fractions of the herb against each other in higher concentrations may induce such phenomenon. Indeed, fractional forms of the crude extract would be considered for more explanations. Synergism was observed in combination between *C. longa* and chloroquine in most ratios particularly between ratios of 40/60 and 80/20 (chloroquine/*C. longa*). It is important to be known that both the agents have very low side effects in the range of treating doses. Our study is the first study in the field of interaction between *C. longa* and chloroquine against *P. berghei*. So far several studies have been conducted in the field of antimalarial effects of *C. longa*. For the first time the effects of *C.longa* on *P. falciparum* showed moderate activity against *P.falciparum* (IC50: 3.5, 4.2 and 3.0 µg/m1) (14). In another study the effect of *C.longa* on *P. berghei* was evaluated and oral administration of 100 mg/kg *C.longa* led to 80 to 90 percent decrease in parasitaemia rate in infected mice (15). That is in agreement with results of our study. Also Nourizadeh *et al*. and some other authors evaluated the antibacterial effect of several plant compounds on *Helicobacter pylori* that among the

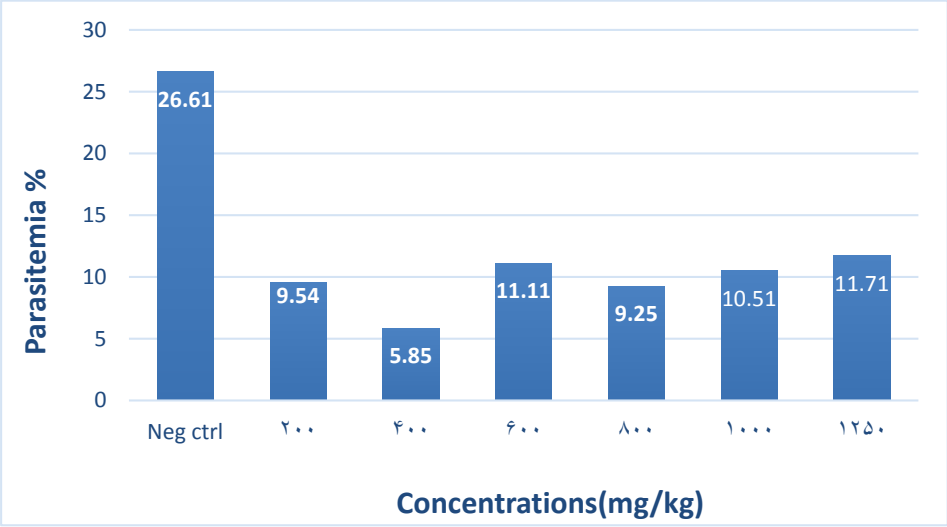

Figure 1. Trend of parasitemia with different concentrations of *C.longa* in treated mice on day 7.

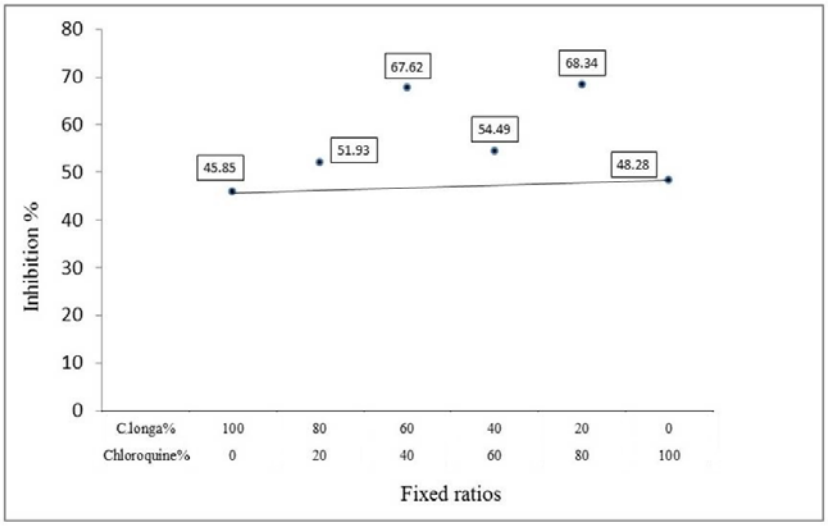

Figure 2. Interaction between chloroquine and *C. longa* on the chloroquine–sensitive strain of *P. berghei* in different ratios.

studied plants, extract of *C. longa* showed a significant effect on the bacteria and has been introduced as an appropriate option for treatment of *Helicobacter pylori* (7, 16, 17). In 2006, Nanda Kumar *et al*. examined the combination of *C. longa* and β -arteether *on P. berghei* –infected mice with 3 oral doses of curcumin which was followed with a single injection of β-arteether. The treatment resulted in 100% survival time of infected mice (18). Studies of Wongsansee *et al*. showed that *C. longa* and *Andrographis paniculata* could not suppress growth and multiplication of *Plasmodium yoelii* in infected mice (19). A similar result to our result was obtained by Martinelli *et al*. that combination of artemisinin and *C. longa* against artemisinin- resistant *Plasmodium chabaudi* resulted in a synergism interaction (20). Differentiation between our results and those results obtained by Wogsansee et al. may be due to different species of murine Plasmodia. As we see combination between *C. longa* and *A. paniculata* against *P. berghei* in their study resulted in 40% decline in parasitemia rate (21). Nevertheless Kettawan et al based on their studies believe that antioxidant and antimalarial efficacy of *C. longa* can repress the growth of *P. yoelii* (22). Our findings are, more or less, similar to those results that have been obtained by Rasoanaivo *et al*.

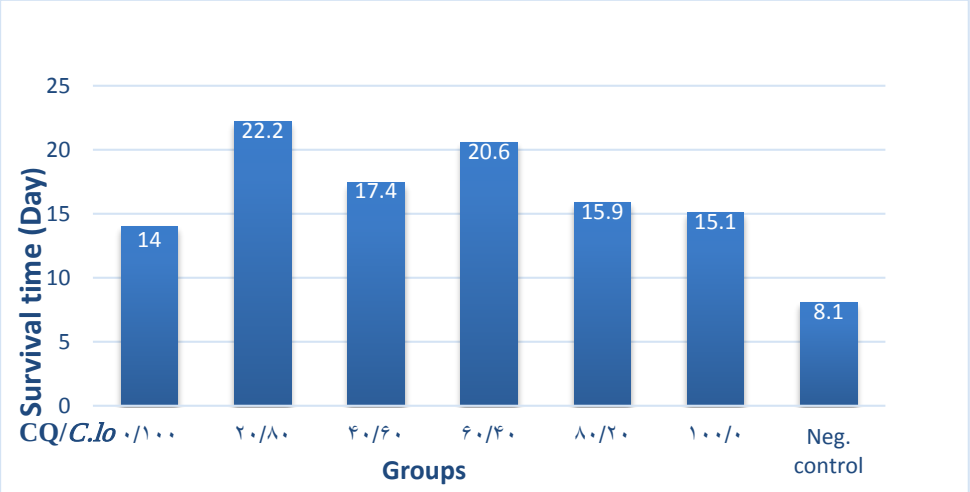

Figure 3. Mean survival time of treated mice with different ratios of *C.longa* and chloroquine.

Table 1: Combination ratios of chloroquine and *C. longa* based on their ED50s.

Drugs	Groups and percent of ratios					
	1	2	3	4	5	6
Chloroquine	0	20	40	60	80	100
C. longa	100	80	60	40	20	0

Table 2: Inhibitory activity of *C. longa* against *P. berghei* in different concentrations on 7th day.

Groups	Concentrations	Inhibition % (Mean ± SD)
1	200 mg/ kg	64.14 ± 7.1
2	400 mg/ kg	78.01 ± 2.6
3	600 mg/ kg	58.24 ± 6.3
4	800 mg/ kg	65.23 ± 4.8
5	1000 mg/ kg	60.50 ± 2.9
6	1250 mg/kg	55.99 ± 3.7

between *C. longa* and artemisinin using in vivo and in vitro tests (23).

In current study mean survival time in infected mice that received combination of *C. longa* with chloroquine was higher than control group without any treatment.

Resistance in human and rodent plasmodia against the numerous existing antimalarials particularly chloroquine and its derivatives encourage malaria policy makers to search novel antimalarials especially in combination forms.

Conclusion

It appears that *C. longa* extract is an effective anti-malarial substance especially in combination with antimalarial drugs, such as chloroquine and artemisinin. Because of the abundance of this plant in nature, non-toxicity and the therapeutic effects against some kinds of microorganisms, this can be effective in solving problems such as the high cost, relapse and drug resistance. Alcoholic extract of *C. longa* can be used as a low-cost anti-malarial of the drug substance in the treatment of this disease in the pharmaceutical industry and combinations including extract of *C. longa* can be great candidate in order to organizing the clinical trials.

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Table 3: Fixed ratios of chloroquine and *C. longa* in combination form and percentage of their inhibition activities against *P. berghei*.

Groups	Fixed ratios	Inhibition % (Mean ± SD)
1	100% *C. longa*	45.85 ± 3.3
2	20% CQ+ 80% *C. longa*	51.93 ± 5.2
3	40% CQ+ 60% *C. longa*	67.62 ± 2.4
4	60% CQ+ 40% *C. longa*	54.49 ± 3.7
5	80% CQ+ 20% *C. longa*	68.34 ± 4.1
6	100% CQ	48.28 ± 3.6

Conflict of Interest

The authors declare that they have no conflict of interest.

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