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Antimalarial Activity of *Otostegia integrefolia* Leaf Extracts against Chloroquine Sensitive Strain of *Plasmodium berghei* in Mice

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Abstract

In the attempt to search for new plant based source of antimalarial drugs, the present study aimed at testing crude extracts from the leaves of *Otostegia integrefolia* against chloroquine (CQ) sensitive strain of *Plasmodium berghei In vivo* in Swiss albino mice. The standard 4-day suppressive test was employed to determine percent parasitaemia inhibition caused by extracts of the plant material. Aqueous, methanol and chloroform crude extracts were administered at doses of 200, 400 and 800 mg/kg. The extracts suppressed *Plasmodium berghei* parasitaemia significantly ($P<0.05$) with a maximum parasitaemia inhibition recorded in the methanol extract treated group (56.77%) followed by aqueous (44.45%) and chloroform (39.16%) at the highest tested dose of 800mg/kg. Toxicity study of the plant extracts on mice that received up to a single dose of 2000mg/kg did not show any sign of serious toxic symptoms during the follow up a week observation period. Thus, the result confirms that; the crude aqueous, methanol and chloroform extracts of the leaves of *Otostegia integrefolia* were nontoxic and possess potent antimalarial effects in mice. It is recommended that; further fractionation of the methanol and water extracts may lead to the isolation and identification of antimalarial active compounds.

KEY WORDS: ANTIMALARIAL ACTIVITY, *IN VIVO*, *OTOSTEGIA INTEGREFOLIA*, PARASITAEMIA, *PLASMODIUM BERGHEI*, TOXICITY

Introduction

Malaria remains one of the top most disastrous parasitic diseases in the tropical and subtropical regions of the world. It is the cause of death of more than 650,000 people every year with the majority affected groups being children under the age of five and pregnant women [8, 13]. According to World Malaria Report (WHO, 2010), malaria was prevalent in 106 countries of the tropical and semi tropical regions that are home to more than half of the world's people and it is a persistent problem in most of these areas [19]. The disease plays a tremendous burden on individual families and national health systems as well as an enormous global killer and places significant strains on economies around the world [4].

The spread of drug resistant malaria parasites are the major challenge in the control of the disease [17]. Drug resistant malaria parasites have been documented in all today's available antimalarial medicines [20] including the artemisinin derivatives [3, 10]. Therefore, there is an immediate need of developing new antimalarial drugs and one of the best sources is traditional medicinal plants [6].

Plants had been used for medicinal purposes long before documented histories [7]. Still the practices continue in many parts of the world and have made great contribution for the development of modern therapeutic agents through the application of modern technology in to the traditional knowledge [6, 16]. Many species of plants have played a critical role in the history of malaria with Peruvian bark (*Cinchona spp.*); being the first effective treatment for this impediment [21]. The long established use of quinine and the more recent introduction of artemisinin as highly effective antimalarials proved the importance of medicinal plants as sources for the discovery of new antimalarial drugs [2].

Otostegia integrefolia: "Tenjut" in the local language Amharic is an herbaceous plant belonging to the family Lamiaceae (Labiatae). It is commonly found in Eritrea, Ethiopia and Yemen. The plant grows in wild and it can be cultivated [1, 14]. It is a shrub with slender woody branches. Reports indicate that the leaves of the plant are used for the treatment of several complaints such as lung diseases, uvulitis, tonsillitis, stomach ache and mostly for malaria in the Ethiopian traditional medical system [5, 15, 25]. However, there is no scientific report on the biochemical activity of the plant in the literature. Thus, the aim of this study is to evaluate the antimalarial activity of the leaves of the plant *In vivo* against CQ-sensitive strain of *Plasmodium berghei* parasite in Swiss albino mice.

Materials and Methods

Plant material collection and authentication

Fresh leaves of *Otostegia integrefolia* were collected from "Dembecha woreda"; which is located in Amhara regional state and 350 km Northwest of Addis Ababa, Ethiopia during the months of September and October, 2011. The plant was identified and authenticated at the Ethiopian National Herbarium, Addis Ababa University, Addis Ababa, Ethiopia. And voucher specimen was deposited in collection number of (sy02/2011) for further references.

Crude extracts preparation of the plant material

The fresh leaves of the plant were cleaned, cut into pieces and air dried under shade in Biomedical Laboratory, College of Natural Sciences, Addis Ababa University. The dried leaves were ground in to coarse powder and crude extracts were prepared by cold maceration technique; soaking the plant powder in 1:8 w/v ratio with distilled water, methanol and chloroform in separate Erlenmeyer flasks.

Experimental animals and pathogen

Swiss albino mice of 6 to 8 weeks old weighing 23 to 32g were obtained from the Animal house of the College of Natural Sciences, Addis Ababa University. They were used in accordance with NIH Guide for the care and use of laboratory animals [11]. The antimalarial evaluation of the extracts were performed by using CQ-sensitive strain of the rodent malaria parasite; *Plasmodium berghei.*

In vivo acute toxicity tests

Acute toxicity test of the crude extracts were carried-out using twenty mice that were randomized into four groups of five mice per group and given 500, 1000 and 2000 mg/kg orally by dissolving each dose with 0.4ml of vehicles and the fourth group (control) was given 0.4ml of the respective vehicle for each mouse.

In vivo antimalarial suppressive tests

For the antimalarial suppressive tests of the plant extracts, a standard 4-day suppressive test was employed against CQ-sensitive strain of *P. berghei* infection in mice [12]. For each extract, twenty five (25) mice randomly in to five (5) groups were used. At day zero (D_0), each mouse in all groups were infected by *P. berghei* parasitaemia with standard inoculums ($1x10^6$ *P. berghei* infected RBCs) intraperitoneally [9]. Three hours after parasite inoculation, the three groups were administered with 200, 400 and 800 mg/kg of extract by dissolving the extract with 0.2 ml of the respective vehicle for each mouse orally by using gavage (an oral needle) for four consecutive days starting from D_0 in a 24hr schedule. The rest two groups were used as a positive and a negative control that were given with 25mg/kg body weight of CQ and 0.2 ml of the correct vehicle respectively.

Parasitological study

On the 5^{th}day (D_4) of the experiment, fresh blood samples were collected from the tail snip of each mouse and thin smear on to a microscope slide to make film [9]. Parasitaemia was examined under light microscopy [18]. Finally, percentage parasitaemia and suppression were calculated using the following formulas [24] respectively.

$$\%\text{Parasitaemia} = \frac{\text{Number of infected RBCs}}{\text{Total number of RBCs}} \times 100$$

$$\%\text{Suppression} = \frac{\text{Mean parasitaemia in study group}}{\text{Mean parasitaemia of negative control}} \times 100$$

Statistical analysis

Statistical analysis was undertaken by one way analysis of variance (ANOVA) to compare the level of parasitaemia of *P. berghei* infected mice between the controls and extract treated groups at a fixed time. All the results were presented as the Mean ± SEM (Standard Error of the Mean) and statistical significance was considered at a 95% confidence interval ($P<0.05$).

Results

Acute toxicity tests

Aqueous, methanol and chloroform crude extracts of the leaves of *O. integrefolia* showed no mortality of mice within 24 hours. But, gross physical and behavioral changes such as depression, decrease in feeding activities and hair erection were examined for the first 3 to 5 hours after being administered with 1000 and 2000 mg/kg of extracts; then, they returned to their normal conditions and have been physically active during the follow up a week observation period.

Antimalarial suppressive tests

Antimalarial suppressive test results of the three crude extracts against CQ-sensitive strain of *P. berghei* in Swiss albino mice caused varying degrees of suppressive effects in dose dependent manner even though they did not clear the parasite completely. Whereas, the positive control group (treated with 25 mg/kg of CQ) had no detectable parasitaemia on D_4 of post infection. In all extracts; the highest suppression of parasitaemia were recorded at the highest tested dose of 800 mg/kg.

The crude aqueous extract of the plant material resulted in significantly lower percent parasitaemia levels of 27.91±0.96, 27.17±2.30 and 25.36±1.28% at the oral tested doses of 200, 400 and 800 mg/kg extract respectively while the corresponding negative control (treated with dH_2O) parasitaemia level was 45.65±2.31%. Mice that received 200, 400 and 800mg/kg of the methanol extract showed parasitaemia suppression of 22.84, 41.35 and 56.77% respectively. On the other hand, all tested doses of chloroform extract administered mice had by far

lower parasitaemia level than the respective negative control. In these groups, percent parasitaemia were 21.09±0.87, 18.72±0.97 and 15.46±0.70% that resulted in percent suppression of 17.00, 26.33 and 39.16% at tested doses of 200, 400 and 800 mg/kg respectively while, the corresponding 20% DMSO treated group (negative control) parasitaemia level was 25.41±0.86%.

See Table 1.

See Fig.1

Discussion and Conclusion

The *In vivo* acute toxicity test of extracts in mice was conducted according to Center for Drug Evaluation and Research guideline for the testing of chemicals in rodents [23]. Evaluation of the safety of plant extracts is very important [16]. Absence of serious acute toxic symptoms such as mortality, impaired movement, listlessness and reduced motor activity within 24 hours and survival of mice for weeks indicated that; the estimated oral median lethal dose (LD_{50}=2000mg/kg) is not toxic. This indicates the extracts at 4000mg/kg is not lethal and suggesting that it is safe and this could be one ground for the use of the plant in traditional treatment of malaria in Ethiopian folk medicine [5].

The water, methanol and chloroform crude extracts of the plant material showed statistically significant ($P<0.05$) parasitaemia suppression in dose dependent manner. Extracts of the plant part did not totally clear the parasitaemia up to the highest tested doses. It could be possible that, complete clearance of the parasites may be obtained within the higher range of the extracts.

The antimalarial action of the plant material might be implicated with the presence of antimalarial phytochemical constituents such as terpinoids; which are identified as the major phytochemical constituent of the plant [15]. In view of the fact that; terpinoids are attributed as having antiplasmodial activities of many other plants including the potent source of artimesinin, *Artemisia annua* (wormwood) [15, 22]. In addition, the presence of other metabolites such as flavonoids and steroids that are identified as major phytochemical constituents in the genus *Otostegia* might also be involved in the suppression of the parasite [1].

According to the review by Krettli et al., (2009), an antimalarial compound is considered as an active compound when it suppress percent parasitaemia by ≥ 30%; that supports the result of the present study. Methanol and water extracts showed the highest antimalarial effects, justifying the traditional usage of this plant as malaria remedy and using of water and ethanol (local alcohols) as common solvents. As a recommendation; further phytochemical fractionation of the water and the methanol extracts of the plant material could permit the isolation and identification of active antimalarial compounds and other secondary metabolites.

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Treatment	Dose (mg/kg)	%Parasitaemia	%Suppression
a) dH_2O extract	dH_2O (NC)	45.65±2.31	0.00
	200	27.91±0.96	38.86
	400	27.17±2.30	40.48
	800	25.36±1.28	44.45
b) MeOH extract	20%DMSO (NC)	31.39±1.94	0.00
	200	24.22±1.08	22.84
	400	18.41±1.01	41.35
	800	13.57±0.70	56.77
c) $CHCL_3$ extract	20%DMSO (NC)	25.41±0.86	0.00
	200	21.09±0.87	17.00
	400	18.72±0.97	26.33
	800	15.46±0.70	39.16
CQ	25 (PC)	0.00±0.00	100.00

Table 1: In vivo antimalarial suppressive test of crude aqueous, methanol and chloroform extracts of the leaves of *O. integrefolia*
Values are expressed as Mean ± SEM, SEM = Standard error of mean

Legend:
DMSO: dimethylsulfoxide
dH2O: distilled water
CHCL3: chloroform
MeOH: methanol
CQ: chloroquine
NC: negative control
PC: positive control

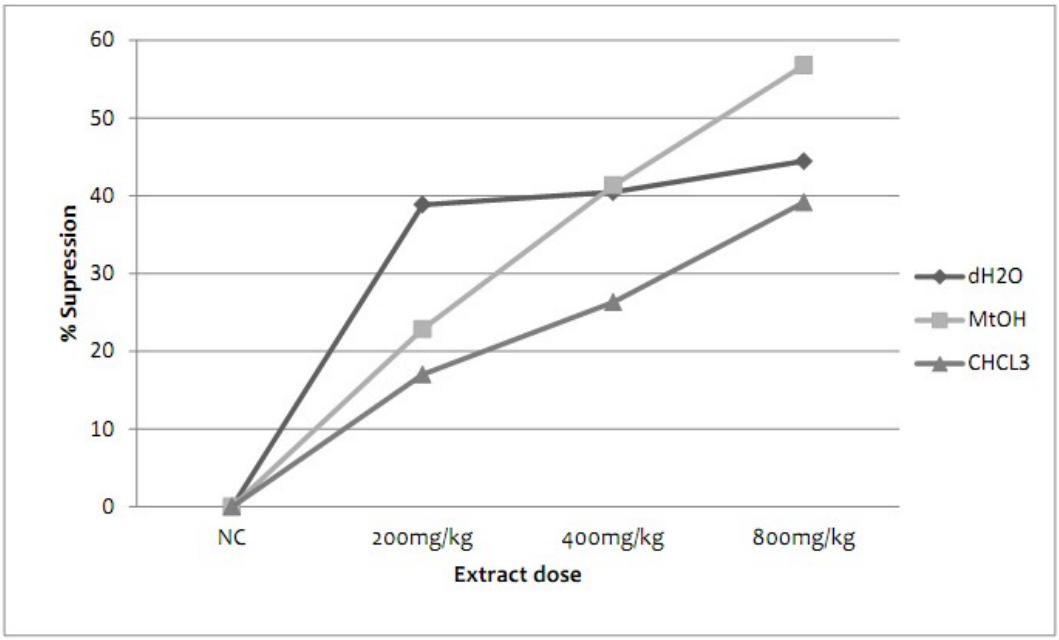

Fig.1. Comparison of antimalarial suppressive effect of aqueous, methanol and chloroform crude extracts at all tested doses