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Original Research Article

In vivo Antimalarial Activity of Methanol and Water Extracts of *Eryngium thorifolium* Boiss (Apiaceae Family) against *P. berghei* in Infected Mice

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Abstract

Purpose: *To investigate the in vivo antimalarial effect of Eryngium thorifolium, an endemic plant in Turkey.*
Methods: *The methanol and water extracts were prepared and phytochemical analysis conducted on the extracts. Twenty four healthy Balb/c male mice, divided into 4 groups (n = 6), were infected intravenously with Plasmodium berghei and 100 - 250 mg/kg plant extracts administered orally in a single dose per day for 5 days. The untreated group of mice received normal saline solution and chloroquine (standard drug) served as reference drug.*
Results: *The water extract group (250 mg/kg) prolonged the survival of the mice by 6 days compared with the untreated mice while the mice that received choloroquine treatment remained alive at the end of the study of the mice. In the untreated control group, maximum parasitaemia was observed on the 10th day of infection whereas The water extract exhibited some degree of antiplasmodial activity compared to untreated control group. The mice of chloroquine treated group remained alive at the end of the study with 100 % chemosuppression ($p < 0.05$). In the untreated control group, maximum parasitaemia was observed on the 10th day of infection whereas in the water extract group maximum parasitaemia was attained on the 16th day of infection. The water extract of the plant showed 45.85 % chemosuppression. Phytochemical screening of the water and methanol extracts revealed the presence of flavonoids, terpenoids and tannins. Anthraquinones were positive for water extract.*
Conclusions: *The possible active compounds responsible for the observed chemosupression may be flavonoids, terpeneoids and anthraquinones which are present in the extract. This is the first report on the in vivo antimalarial activity of E. thorifolium.*

Keywords: *Antimalarial, Eryngium thorifolium, Plasmodium berghei, flavonoids, terpeneoids, anthraquinones*

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INTRODUCTION

Malaria is one of the most infectious diseases caused by protozoa species of *Plasmodium* genus. Approximately 219 million malaria cases and 660 thousand deaths with the majority being children and pregnant women were recorded in 2010, according to the recent report of World Health Organisation [1]. Malaria is widespread in Sub-Saharan Africa, Asia and America and it is

estimated that 40 % of the world population live in these areas [2].

Plants represent valuable sources to search for new active principles for the treatment of several diseases. As a result of the recognition of the global importance of fighting against malaria, efforts to discover and develop new antimalarials from plant sources has increased [3].

Eryngium genus from Apiaceae family represented by 317 taxa is widely distributed in the world. In Turkey ten of 23 species of this genus were reported to be endemic [4-5]. The phytochemical searches on *Eryngium* species revealed that this genus contained phenolic compounds, monoterpenes, sesquiterpenes, triterpenoids as well as saponins, flavonoids, coumarin, steroids and acetylenes. Cytotoxic, antimutagenic, anti-inflammatory, antioxidant, antihyperglycemic, anti-snake and scorpion venoms, antibacterial and antifungal activities were previously reported for this genus [6]. In a review of the plants of the American continent with antimalarial activity, *E. foetidum* water extract, *E. nudicaule* ethanol extract and *E. yuccaefolium* water extract were reported to possess antiplasmodial activities. The aqueous extract of *Eryngium foetidum* was reported to be active on *Plasmodium gallinaceum* which infects chickens [7]. However, no work has been reported on the *in vivo* antimalarial activity of *Eryngium* species from Turkey. Hence this study was designed to evaluate the *in vivo* antimalarial potential of this plant. Preliminary phytochemical analysis and acute toxicity studies of the water and methanol extracts prepared from *Eryngium thorifolium* were also conducted.

EXPERIMENTAL

Plant material

The aerial parts of *Eryngium thorifolium* were collected at Kandil Mountain, Köyceğiz, Muğla in June 2012. The plant material was identified and authenticated by Dr. Cenk Durmuşkahya, Celal Bayar University, Faculty of Education, Demirci, Manisa. A voucher specimen of the plant material (no. 1446) was deposited at the Herbarium of Ege University, Faculty of Pharmacy, Department of Pharmacognosy, Izmir, Turkey.

Extraction of plant material

The aerial parts of the plant material were air dried at room temperature and ground into fine powder using a ball mill (Retsch). The powdered material was macerated with methanol (1:20) for 24 h with constant shaking. The water extract was prepared by 2 % infusion of plant material. The extracts were filtered through Whatman no. 1 filter paper and the extraction solvent evaporated *in vacuo* to yield methanol and water extracts.

Phytochemical screening

The extracts were investigated for phytochemical constituents using standard procedures [8].

Acute toxicity study

In order to determine LD_{50}, the acute oral toxicities of water and methanol extracts of *E. thorifolium* were evaluated according to OECD Guideline no. 423 [9].

Experimental animals

Male Balb/c mice (20 – 25 g) were used for the study. They were fed on standard diet and water *ad libitum* according to NIH Guide for care and use of laboratory animals [10]. The approval for the present work was obtained from Ethics Committee of the Faculty of Medicine, Celal Bayar University, Manisa (CBUTF – HADYEK no. 75, dated on 18/09/2013).

Evaluation of *in vivo* antimalarial activity

Parasite inoculation

Rodent malaria agent *P. berghei* (MRA - 311) strain was obtained from American Type Culture Collection (ATCC), Malaria Research and Reference Reagent Resource Center (MR4). The blood solution was thawed rapidly at 37 ºC and the existence of *P. berghei* schizogonic phases (young and mature trophozoite and schizont stages) in erythrocytes were confirmed by microscopic examination of thin blood smears. This solution was injected intraperitoneally to mice to perform donor mice. After injection, blood smear preparations were made by cutting the tail tip of each infected mouse every other day and infection percentages were determined and parasitemia was viewed. The preferred ratio of 20 % parasitemia was reached on 7th day and blood was collected from mice by cutting axillary venous in a sterile cabinet on this day. This blood was diluted with sterile saline in 107 infected erythrocytes/ml as infection ratio. Then, the diluted blood was injected to each mouse in 0.25 ml (2.5×10^7 *P. berghei*) by intravenous tail vein. Mouse groups were regulated as; *E. thorifolium* water extract, *E. thorifolium* methanol extract,

Chloroquine reference drug control and untreated control group with sterile saline. 4 groups of 6 mice in each were placed in separate cages and tagged.

Drug administration

After the fourth day of experimental mouse infection, water and methanol extracts (at 100 and 250 mg/kg dilutions prepared with sterile saline) was administered by intragastric gavages to each mouse of treatment groups and untreated control group received equivalent volume of saline solution, for five consecutive days. Chloroquine tablets were crushed and diluted with sterile distilled water and administered by intragastric gavages to each mouse (50 mg/kg) to perform reference drug control group. On the fifth day of treatment, thin blood smears were prepared from tail blood, stained with Giemsa and the percentage of parasitaemia was determined by calculating the infected erythrocyte percentages. This process was repeated every other day for 20 days.

Statistical analysis

Data obtained from the studies were analyzed statistically by one-way variance analysis with comparison tests (Tukey's test) using Windows SPSS v15.0. Differences at $p < 0.05$ were considered significant.

RESULTS

Phytochemical profile

The extraction yield was 6 and 12.92 % for methanol and water extracts, respectively.

The phytochemical screening of the water and methanol extract revealed the presence of flavonoids, terpenoids and tannins. Anthraquinones were positive for water extract but negative for the methanol extract. Alkaloids were absent in both extracts.

Acute toxicity

The acute toxicity results for the extracts indicate that none of the extracts caused mortality even up to 5000 mg/kg. The results support the safe use of *Eryngium thorifolium* in traditional medicine.

Antimalarial activity

Methanol extract (100 and 250 mg/kg) and water extract administered at 100 mg/kg showed similar results with the infected but untreated control group (sterile saline group) and was not active on *Plasmodium berghei* infected mice. Maximum parasitaemia was reached on the 10th day of observation for untreated control group (Figure 1).

Methanol extract at 100 and 250 mg/kg and water extract at 100 mg/kg showed similar results with the untreated control group ($p > 0.05$). However, maximum parasitaemia (14.90 %) was reached on the 16th day for *E. thorifolium* water extract at 250 mg/kg while on the 10th day, it was 14.62 % for the untreated control group, 14.63 % for *E. thorifolium* methanol extract. Mean parasitaemia data for chloroquine, untreated as well as for the water and methanol extract (250 mg/kg) groups are shown in Table 1.

Table 1: Parasitaemia data for treated mice

Day	Mean parasitaemia (%)			
	EW	**EM**	**C**	**U**
2	1.07	1.18	1.08	1.15
4	2.28	2.07	2.18	2.13
6	3.70	4.9	1.13	4.90
8	6.30	10.29	0.15	10.28
10	7.92	14.63	0.00	14.62
12	10.73	d	0.00	d
14	13.20	d	0.00	d
16	14.90	d	0.00	d
18	d	d	0.00	d

Treatment groups: *EW = water extract (250 mg/kg); EM = methanol extract (250 mg/kg); C = standard reference drug (chloroquine); U = untreated (sterile saline solution).* ***Note:*** *d = death of mice*

The chemosupression percentages were determined for the water extract which had longer survival time than the methanol extract. Mean chemosuppression (A) was calculated as in Eq 1.

$$A = (A - B/A)100 \quad \text{..... (1)}$$

where A is the mean parasitaemia of untreated control group and B is the mean parasitaemia of water extract, chloroquine or untreated control group.

Chemosuppression data for water extract (250 mg/kg dose) are given in Tables 2. The range of p data for the treatment groups was 0.975 - < 0.001.

Mice treated with 250 mg/kg water extract remained alive for 16 days while untreated group of mice (control) given normal saline solution stayed alive for only 10 days. The mice in the chloroquine-treated group remained alive at the end of the study and demonstrated 100 % chemosuppression.

Table 2: Chemosuppression and survival time of treated mice

Extract/drug	Chemosuppression (%)					Survival (days)
	Day 2	Day 4	Day 6	Day 8	Day 10	
Water extract (250mg/kg)	8.00±0.08	12.5±0.15	24.49±0.26	38.73±0.25	45.85±0.07	**16**
Chloroquine (50mg/kg)	5.82±0.00	9.16± 0.03	76.93±0.32	98.57±0.25	100.0±0.1	**20**
Normal saline	**0**	**0**	**0**	**0**	**0**	**10**

**Data are expressed as mean ±SD (n = 6); p < 0.05 when compared with Chloroquine*

DISCUSSION

P. berghei, a chloroquine sensitive parasite, was employed in this study. The Rane test which relies on the ability of standard inoculation of *P. berghei* to kill mouse, is a standard test used for antimalarial screening. The survival time longer than 12 days by the test compound is regarded as active [11]. In this preliminary phytochemical screening and antimalarial activity investigation, the water extract of *Eryngium thorifolium* was found to possess moderate antimalarial effect on mice infected with *Plasmodium berghei*. The treatment group had longer survival time than the untreated control group.

The extraction yield of plant material depends on various conditions such as solvent pH values, temperature and type of matrix. The higher extraction yield of the water extract may be attributed to temperature as it was prepared as 2 % infusion with boiling water. The solubility of a substance in a liquid is determined by intermolecular interactions (ionic forces, dipole-dipole interactions etc.) The non polar compounds with weak intermolecular bonding tend to be soluble in solvents with high polarity. In addition, ionic compounds are generally most soluble in polar solvents. In the present study water was chosen as an extraction solvent as the plant was used traditionally in infusion forms Methanol is used for both compounds with polar and non polar compounds. The compounds classified as tannins, terpenoids, and antraquinones may possess both polar and non polar characteristics. In addition, the identified class of metabolites can also be in glycosidic forms which can readily dissolve in polar solvents.

The possible active compounds responsible for the observed chemosupression may be flavonoids or terpeneoids. Flavonoids comprising a large group of polyphenolic secondary metabolites possess antimalarial activities. Triterpenoids including saponins have been found to be active on *P. falciparum* [3]. The observed antimalarial activity of water extract might also be attributed to the presence of anthraquinones which were absent in the methanol extract. The *in vitro* antiplasmodial activity of *Cassia nigricans* was reported due to presence of anthraquinones in this plant. Anthraquinones, emodin, citreoresin and emodic acid were also isolated as insecticidal principles from this plant [12].

The aerial parts of *E. campestre*, *E. creticum*, *E. maritimum* and *E. ternatum* were investigated and no significant *in vitro* antiplasmodial or cytotoxic activity was recorded for the mentioned species [13]. To the best of our knowledge, there is only one scientific report on biological activity of *E. thorifolium*. Essential oil of this plant was reported to have significant antibacterial activity on methycillin-resistant *Staphyloccoccus aureus* strains [14].

CONCLUSION

This is the first report on the in vivo antimalarial activity of Turkish endemic plant *Eryngium thorifolium*. The results of this work confirm the use of plants from *Eryngium* genus for searching new sources of plant derived antimalarials. Further studies are, however, needed to identify the active metabolites and elucidate its mechanism of action.

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