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# Comparison of antimalarial activity of *Artemisia turanica* extract with current drugs *in vivo*

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## ABSTRACT

*Background & objectives:* The purpose of this study was to compare antimalarial activity of *Artemisia turanica* Krasch as Iranian flora with current antimalarial drugs against *Plasmodium berghei in vivo* in mice.

*Methods:* Air-dried aerial parts of Iranian flora *A. turanica* were collected from Khorasan, northeastern Iran, extracted with $Et_2O$/MeOH/Petrol and defatted. Toxicity of herbal extracts was assessed on male NMRI mice, and their antimalarial efficacy was compared with antimalarial drugs [artemether, chloroquine and sulfadoxine-pyrimethamine (Fansidar)] on infected *P. berghei* animals. All the groups were investigated for parasitaemia, body weight, hepatomegaly, splenomegaly and anemia. The significance of differences was determined by Analysis of Variances (ANOVA) and Student's *t*-test using Graph Pad Prism software.

*Results:* The inhibitory effects of *A. turanica* extract on early decline of *P. berghei* parasitaemia highlights its antimalarial activity, however, this effect no longer can be observed in the late infection. This may be due to the metabolic process of *A. turanica* crude extract by mice and reduction of its concentration in the body. Crude extract of *A. turanica* represented its antisymptomatic effects by stabilization of body, liver and spleen weights.

*Conclusion:* This study confirmed antimalarial effects of *A. turanica* extracts against murine malaria *in vivo* during early infection, however, there are more benefits on pathophysiological symptoms by this medication.

**Key words** Artemether; *Artemisia turanica*; chloroquine; Fansidar; *in vivo*; parasitaemia; *Plasmodium berghei*

## INTRODUCTION

Malaria is one of the most serious and widespread health problem in many parts of the world, particularly in Africa and Latin America with a high mortality rate. The situation is further complicated by the spread of drug-resistant parasites in many parts of the world where *Plasmodium falciparum* is endemic. The multi-drug resistance in *P. falciparum* is a major problem in many countries and the number of drugs available, effective and affordable is very limited[1]. The malaria situation is aggravated by the appearance of strains of *P. falciparum* resistant to antimalarial drugs as well as by the resistance of vector *Anopheles* mosquitoes to DDT and other insecticides[2].

As malaria vaccines remain problematic, chemotherapy is the most important weapon in the fight against the disease[3, 4]. The antimalarial drugs including chloroquine, quinine, mefloquine and artemisinin are currently used to prevent and treat human malaria. Part of the reason for the failure to control malaria, is the spread of resistance to the first line antimalarial drugs, cross-resistance between the limited number of drug families available, and some multidrug resistance[5]. Resistance has emerged to all the classes of antimalarial drugs except artemisinin, an endoperoxide antimalarial drug derived as the active component of *Artemisia annua*, a herbal remedy used in Chinese folk medicine for 2000 years "qinghaosu"[6–9]. Artemisinin is a powerful antimalarial drug with significant activities, against *Plasmodium* strains which are resistant to chloroquine. It is a natural product, which has the characteristics of high potency against the parasite whilst possessing low toxicity during treatment of malaria infections[10–12].

The genus *Artemisia* (Asteraceae tribe Anthemideae) belongs to the important family of medicinal and aromatic plants which comprises about 1000 genera and over 20,000 species[13, 14]. Within this family, *Artemisia* is included into the trible Anthemideae and comprises itself over 500 species. The 500 species of *Artemisia* are mainly

found in Asia, Europe and North America. In all, 35 species of this genus are found in Iran, among those two are dominating: *A. melanolepis* Boiss and *A. kermanensis* Podl[15, 16].

This genus including some Iranian species has been studied chemically and present of monoterpenes[16], sesquiterpenes[17, 18], especially sesquiterpene lactones[19, 20] and essential oils[21–25] were reported.

The extract of the aerial parts of *A. diffusa* Krasch ex P. Poljakov collected in the Province of Khorassan (Iran) afforded, in addition to several eudesmanolides, a new type of sesquiterpene lactone (Tehranolide)[20], with anendoperoxide group that probably has the same effect as the antimalarial agent artemisinin. The antimalarial properties of the extract and the fraction which contains sesquiterpene lactones including Tehranolide of the same species (*Artemisia diffusa*)[26]. The results specifically indicated the inhibitory effects of the *A. diffusa* crude extracts and the fraction which contains sesquiterpene lactones including Tehranolide, on the developmental stages of *P. berghei* by decreasing parasitaemia[27].

The aerial parts of Iranian flora *A. khorasanica* were collected at flowering stage from Khorassan Province, northeastern Iran in 2008. Toxicity of herbal extracts was assessed on naïve NMRI mice, and its antimalarial efficacy was investigated on infected *P. berghei* animals. This is the first application on *A. khorassanica* extract for treatment of murine malaria. The herbal extract was successfully tested *in vivo* for its antiplasmodial activity through artemisinin composition, which is widely used as a standard malaria treatment[28].

The extract of the aerial parts of *A. turanica* Krasch was collected from Esfarayen in the province of Khorasan, northeastern Iran. The study especially examined the inhibitory effects of the extracts on the developmental stages *in vivo* of *P. berghei* in mice. In the preliminary experiments, the toxicity of the *A. turanica* extract was tested and from the high doses that were tolerated without significant overt mortality or signs of toxicity, it was estimated that the extract of plant is of relating low toxicity.

The pharmacological and biochemical evaluation on antimalarial effects of *A. turanica* and *A. oliveriana* on *P. berghei in vivo*, showed that two extracts were effective against malarial agent[29]. Last study especially examined the inhibitory effects of the extracts on the developmental stages *in vivo* of *P. berghei* in mice but in this work, the extract of *A. turanica* was successfully tested *in vivo* for its antimalarial activity and compared with other current antimalarial drugs (artemether, chloroquine and fansidar) which are widely used as a standard malaria therapy.

## MATERIAL & METHODS

### *Plant material*

The aerial parts of *A. turanica*, were collected from Baam village, after Gahreman abad in Esfarayen, Province of Khorasan, northeastern Iran, in 2011. Voucher specimens have been deposited at the Herbarium of the Research Institute of Forests and Rangelands (TARI), Tehran, Iran.

### *Extraction*

The aerial parts of *A. turanica* were air-dried at room temperature and were then powdered. The herbs powder (150 g) was extracted with $Et_2O$/MeOH/Petrol (1:1:1) (2 × 250 ml) at room temperature for 48 h. Evaporation at reduced pressure furnished crude extract (7 g), which was suspended in EtOH (120 ml), diluted with water (100 ml) and extracted successively with hexane (2 × 100 ml) and chloroform (2 × 100 ml). Evaporation of the $CHCl_3$ extract at reduced pressure furnished 4 g of residue.

### *Animals*

Male outbred NMRI mice, supplied by the Karaj Laboratory Animal Unit, Pasteur Institute of Iran were used in this study. The mice were housed at room temperature (20–23°C) on a 12 h light and 12 h dark cycle, with unlimited access to food and tap water. Experiments with animals were done according to the ethical standards formulated in the Declaration of Helsinki, and adequate measures were taken to protect animals from pain or discomfort. The study has been approved by the Institutional Ethical Review Board (Ethical Committee of the Pasteur Institute of Iran).

### *Malaria parasites*

*Plasmodium berghei* NY strain was kindly donated by Dr M.J. Dascombe from the School of Life Sciences, University of Manchester, UK. Malaria parasite was maintained by blood passage in NMRI mice when active parasites were required; otherwise it was stored at –70°C in Alserver's solution (2.33% glucose, 0.525% NaCI and 1% sodium citrate in deionised water) and glycerol (9:1 parts by volume).

### *Inoculation of malaria parasites*

Mice were inoculated (0.2 ml, i.v.) into a tail vein with blood from a donor mouse (41% parasitaemia *P. berghei*) diluted with 0.85% saline to contain $2\times10^7$ parasitised red blood cells (PRBCs).

*Study on toxicity of herbal extracts on naive mice*

Three different concentrations of herbal extracts (50, 5 and 0.05 mg/ml) were tested in addition to a control (vehicle). All the animals were injected s.c. every day for 8 days with 100 µl solutions. Four groups of mice (n = 10) were investigated for assessment of pathology including body weight, physiological activities, hepatomegaly and splenomegaly to assess the toxicity of the extracts.

*Antimalarial effects of herbal extract on Plasmodium berghei infected mice*

Animals were divided into five groups (n=10 mice/group), namely a control (Drug vehicle), *A. turanica* extract, artemether, chloroquine and fansidar. All the groups were infected with murine malaria parasite, *P. berghei*. *Artemisia turanica* extract in 50 mg/ml concentration and control (100 µl) were injected s.c. into control and test groups respectively every day with 100 µl of solutions for the period of 12 days after infection in malaria mice. Artemether in 4 mg/kg concentration and chloroquine in 5 mg/kg concentration (100 µl) were injected i.m. every day for 12 days after infection in malaria mice. Fansidar in 25 mg/kg concentration (100 µl) were fed by gavage every day for 12 days after infection in malaria mice. After the injections period, five groups of mice (n=10 mice/group), were investigated for antimalarial efficacy, degree of parasitaemia, assessment of pathology including body weight, physiological activities, hepatomegaly, splenomegaly and anemia.

Parasitaemia was measured every other day by counting Giemsa-stained blood smears from end tail cutting. Diagnosis of anemia by measuring Hematocrit (Hct), hepatomegaly, splenomegaly was measured last day. The body weight was measured on first and last day.

Following toxicity assay, the highest dose of herbal extract (50 mg/ml concentration) was selected to apply for its antimalarial activity on male NMRI mice infected with *P. berghei*.

**Assessment of pathology**

*Parasitaemia*

The clinical diagnosis was confirmed by laboratory demonstration of the malaria parasite in the stained smears. In all animals, parasitaemia was determined on different days after infection using blood smears stained with Giemsa stain (Sigma Chemical Co., USA). Parasitized red blood cells (PRBC) were counted in five different fields, each of approximately 200 cells. Results expressed as the mean percentage of erythrocytes containing Giemsa positive bodies. Experiments were licensed under the Animals (Scientific Procedures) Act 1986. In compliance with the conditions of this license, infected animals were humanely killed at the onset of the terminal phase of malaria (*P. berghei* NY) infection.

*Assessment of degree of hepato/splenomegaly*

Entire livers and spleens were removed during *post-mortem* at the end of the experimental period from mice after induction of terminal general anaesthesia by inhalation of diethyl ether (Sigma Co., Germany). Organ wet weights were measured and compared with controls as indices for degree of hepatomegaly and splenomegaly.

*Diagnosis of anemia by measuring hematocrit (Hct)*

Hematocrit is a screening test to determine anemia. The hematocrit measured manually by centrifugation. A thin capillary tube called a microhematocrit tube is filled with blood and sealed at the bottom. The tube is centrifuged at 10,000 RPM (revolutions per minute) for five min. The RBCs have the greatest weight and are forced to the bottom of the tube. The WBCs and platelets form a thin layer, called the buffy coat, between the RBCs and the plasma, and the liquid plasma rises to the top. The height of the red cell column is measured as a percent of the total blood column. The higher the column of red cells, the higher the hematocrit.

$$\% \text{ Hct} = \frac{\text{RBC}_{\text{volume}}}{\text{RBC}_{\text{volume}} + \text{Plasma}_{\text{volume}}} \times 100$$

*Body weight*

Body weight was measured initially and at different times of experiment using a top pan balance (OHAUS Scale Corp., USA).

*Statistical analysis*

Values are presented as the mean ± SEM for groups of samples.

The significance of differences was determined by analysis of variances (ANOVA) and Student's *t*-test using Graph Pad Prism Software (GraphPad, San Diego, California, USA) (*$p$ <0.05, **$p$ <0.01, $p$ <0.001).

## RESULTS

The results of toxicity assay in naïve mice indicated no significant pathophysiological changes in hepatomegaly and splenomegaly in test groups as compared with those in control after injection of low, average and high doses of *A. turanica* crude extracts. There was a little increase ($p$ <0.05) in body weight of test groups injected

with high dose of herbal crude, emphasising the anti-symptomatic effects of *A. turanica*.

As shown, no toxicity was observed *in vivo* even with high dose of *A. turanica* total extract. Pathophysiological signs including body weight, hepatomegaly, and splenomegaly represented no side effects of total extract (Fig. 1).

The clinical diagnosis of *P. berghei* infection was confirmed by laboratory demonstration of the malaria parasite in the stained smears. Parasitaemia was determined using blood smears stained with Giemsa stain from mice (Fig. 2). The observations specifically indicated the

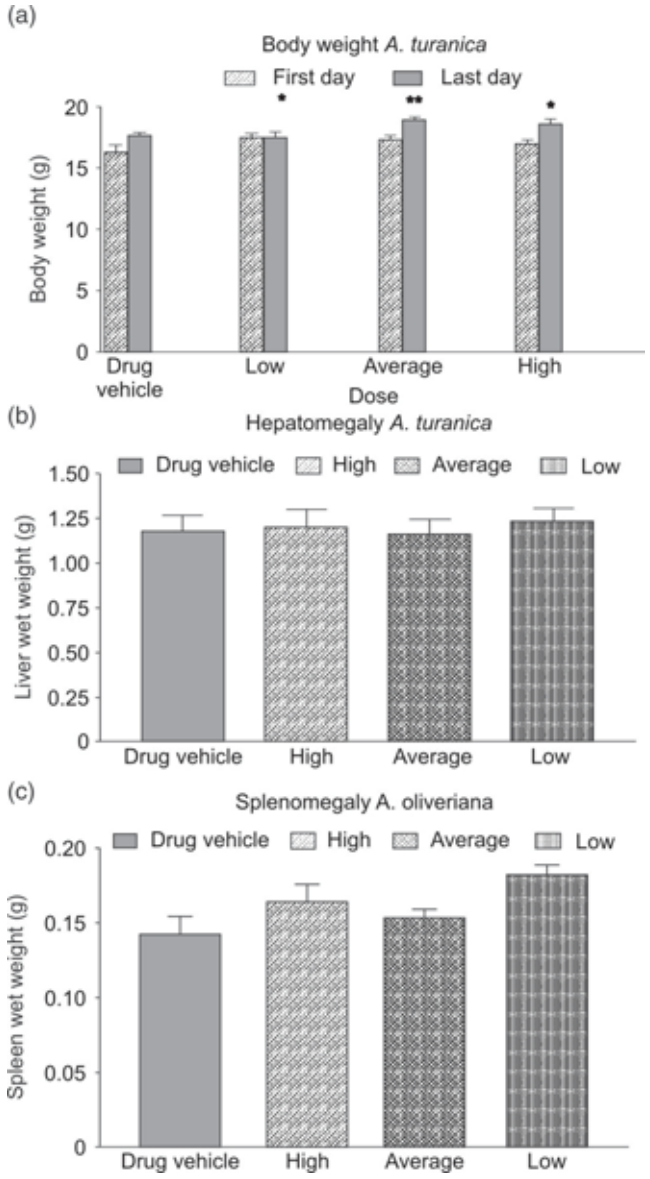


*Fig. 1:* Toxicity assay and pathophysiological changes induced by *A. turanica* crude extract in naive animals. Pathophysiological changes including body weight (a); hepatomegaly (b); and splenomegaly (c); were evaluated in control and test groups as toxicity assay induced by injection of low, average and high doses of *A. turanica* crude extract (n=10, *$p$ <0.05, ANOVA).

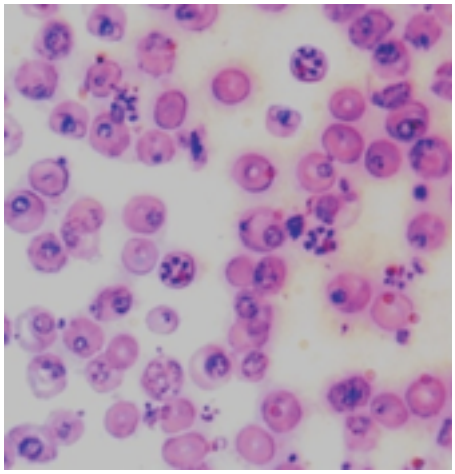

*Fig. 2: Plasmodium berghei* blood-stage forms in Giemsa stained smears from mice.

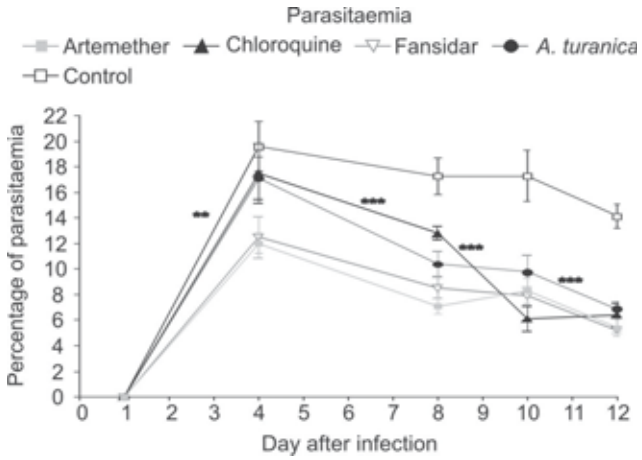


*Fig. 3:* Percentage of parasitaemia in smears from blood of malarial mice test, *A. turanica* crude extract; control and antimalarial drugs (n=10 mice/day/group, Student's *t*-test, **$p$ <0.01).

inhibitory effects of *A. turanica* extract on the early developmental stages of *P. berghei* by decreasing parasitaemia ($p$ <0.01). This may suggest that the active constituents in the herbal extract may be toxic for *P. berghei*, thereby inhibiting their development to the erythrocytic stages (Fig. 3).

No pathophysiological changes including body weight, hepatomegaly and splenomegaly were detected in control and malarial groups as induction markers for toxicity after injection of crude extract of *A. turanica* in malarial infected animals (Fig. 4).

## DISCUSSION

Although, malaria is still one of the most common infectious diseases and an enormous public health problem and as malaria vaccines remain problematic, chemotherapy is the most important weapon against the disease.

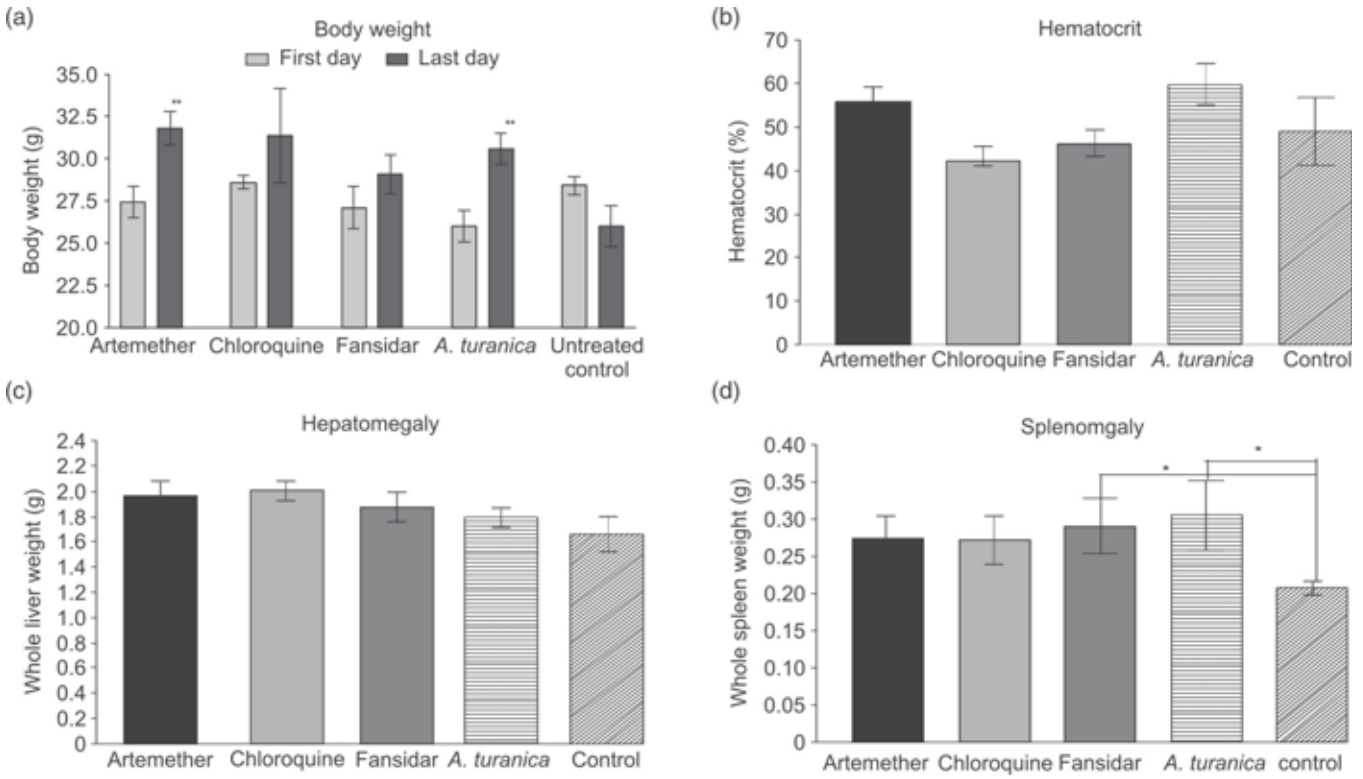


*Fig. 4:* Toxicity assay and pathophysiological changes induced by *A. turanica* crude extract in malarial animals. Pathophysiological changes including: (a) body weight; (b) hematocrit; (c) hepatomegaly; and (d) splenomegaly were evaluated as indices of toxicity by crude extract of *A. turanica* in control and malarial groups (n=10 mice/day/group, Student's *t*-test).

The current antimalarial drugs have their own problems to prevent and treat human malaria.

The results of this study indicated no toxicity in naïve mice with even high dose of *A. turanica* crude extracts, which confirm its lowest effects. Although, the inhibitory effects of the *A. turanica* extract on the early decline of *P. berghei* parasitaemia highlight its antimalarial activity, but effect no longer can be observed in the late infection. This may be due to the metabolic process of *A. turanica* crude extract by mice and reduction of its concentration in body. Malaria parasite actually decreases body weight and increases hepatomegaly and splenomegaly. Crude extract of *A. turanica* represented its antisymptomatic effects by stabilization of body, liver and spleen weights.

This is the first report on comparison of *in vivo* antimalarial activity of Iranian flora *A. turanica* crude extract and other antimalarial drugs (artemether, chloroquine and fansidar) against *P. berghei* in mice model.

The herbal extract was successfully tested *in vivo* for its antimalarial activity and compared with other current antimalarial drugs (artemether, chloroquine and fansidar) which is widely used as a standard malaria therapy. Although, this study confirmed antimalarial effects of *A. turanica* extracts against murine malaria *in vivo* during early infection, however, there are more efficacies on pathophysiological symptoms by this medication. As it shown in hepatomegaly diagram, *A. turanica* extract decreases the anemia better than others. These observations provide the basis for the traditional use of this herb in treatment of malaria disease. The route of inoculation is an important factor to determine herbal efficacy. More investigations on different *Plasmodia* and animal hosts are needed to clarify antimalarial activity of Iranian flora *A. turanica* and analysis of its natural components more effectively.

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