

## In vivo evaluation of *Berberis vulgaris* extract on acute toxoplasmosis in mice

Hossein Mahmoudvand, Amir Tavakoli Kareshk, Amir Keyhani, Naser Zia-Ali, Mohammad Reza Aflatoonian

### ABSTRACT

Since last centuries, herbal medicines have been considered as the main source for prevention and treatment a broad spectrum of illnesses as well as infectious diseases. Here we evaluated the *in vivo* activity of *Berberis vulgaris* methanolic root extract against acute toxoplasmosis induced by *Toxoplasma gondii*, RH strain. Male NMRI mice orally treated with *B. vulgaris* methanolic extract at the dose of 1 and 2 g/kg once a day for 14 days after 24 h mice were infected intraperitoneally with  $10^4$  tachyzoites of *T. gondii*, RH strain. The mortality rate in all infected mice and also parasite load were determined to assess the efficacy *B. vulgaris* methanolic extract against acute toxoplasmosis in mice model. The obtained results demonstrated that the mortality rate of

infected mice was 100%, in 9<sup>th</sup> day after orally administration of *B. vulgaris* at the concentrations of 1 and 2 g/kg; while all mice in the control group were death in 5th day. Moreover obtained findings revealed that the mean number of tachyzoites was  $131 \times 10^4$  and  $79 \times 10^4$  for infected mice treated with *B. vulgaris* at the concentrations of 1 and 2 g/kg, respectively, whereas in control group the mean number of tachyzoites was  $288 \times 10^4$  parasite. The obtained findings demonstrated the potential of *B. vulgaris* as a natural source for the production of new prophylactic agent for use in toxoplasmosis.

**Keywords:** *Toxoplasma gondii*; *Berberis vulgaris*; Prophylactic; *In vivo*

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### Introduction

*Toxoplasma gondii* is a universal prevalent protozoan parasite which infected all the warm-blooded animals such as humans (1). Human, usually, can be infected via three chief ways of transmission: consumption of tissue cysts in infected meat, consumption of food or water contaminated with oocysts and congenitally (2, 3). Toxoplasmosis in immunocompetent persons is seldom symptomatic. However, the disease happening in the fetus or immunocompromised individuals (transplant recipients and patients with acquired immunodeficiency syndrome) might cause serious symptoms or even passing away (4, 5). Today, the preferred treatment for toxoplasmosis is combination of pyrimethamine and sulfadiazine. Nevertheless, the use of these drugs is associated with some serious complications including osteoporosis, sepsis and also teratogenic effects (6, 7). Since last centuries, herbal drugs have been considered as the main source for prevention and treatment a broad spectrum of illnesses as well as infectious ones (7).

*Berberis vulgaris* L. called in Persian as “Zereshk” (Berberidaceae family) broadly growth in most countries in the world such as Iran (8). In traditional medicine, a variety of portions of *B. vulgaris* such as root, leaf, bark and fruit have been extensively applied for the healing a number of diseases such as skin, respiratory, cardiovascular, digestive, and infectious ones (9). Nowadays, reviews have reported a range of pharmacological possessions of *B. vulgaris* including antinociceptive, antioxidant, anti-inflammatory and antimicrobial effects (10). To the best of our knowledge and according to the literature review, there is no study on the effects of *B. vulgaris* against toxoplasmosis. Therefore, the current investigation aims to evaluate the efficacy of *B. vulgaris* root methanolic extract on infected mice with acute toxoplasmosis.

## Materials and methods

### Plant Collection

The *B. vulgaris* roots were obtained from the farms in the Baft district of Kerman province, Iran, in May 2016. The identities were confirmed by the botanist at the Botany Department of Shahid Bahonar University, Kerman, Iran. Voucher specimen (kf 585) of the plant materials was deposited at the Herbarium of Department of Pharmacognosy of the School of Pharmacy, Kerman University of Medical Science, Kerman, Iran.

### Preparing of methanolic extract

Extraction of the dried roots (200 g) of *B. vulgaris* was performed percolation technique with methanol for 72 h in room temperature. The extract was moved among filter paper (Whatman No.3, Sigma, Germany) to take out plant remains. The extract was lastly concentrated in vacuum at 50°C using a rotary evaporator (Heidolph, Germany) and kept at -20°C, until testing (11).

### Ethical statements

This survey was done in line with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Bam University of Medical Science, Iran (No.218/2017)

## Establishment of acute toxoplasmosis

Forty male NMRI mice (6–8 weeks old) weighing from 20 to 25 g for establishing animal model of *T. gondii* were obtained from the Animal House of Kerman University of Medical sciences, Iran. Animals were housed in a colony room with a 12:12 h light/ dark cycle at  $21 \pm 2^\circ\text{C}$  and were handled according to standard protocols for the use of laboratory animals. They were divided into four groups; every group contained ten mice. Animal model of acute toxoplasmosis was established according to the method described by Asgari et al (2013) using intraperitoneally injection  $10^4$  tachyzoite of *T. gondii*, RH strain (12).

## Efficacy of *B. vulgaris* on acute toxoplasmosis

Mice were divided into four main groups (10 mice per each group) including: (1) non-treated control, (2) mice treated with normal saline for 14 days, (3) orally treated with *B. vulgaris* methanolic extract at the dose of 1 g/kg once a day for 14 days, (4) orally treated with *B. vulgaris* methanolic extract at the dose of 2 g/kg once a day for 14 days. In 15<sup>th</sup> day mice in all tested groups were infected *T. gondii*, RH strain as mentioned above. The selections of extract doses was based on primary experiments that showed *B. vulgaris* had no toxicity in mentioned doses. The subsequent factors were performed to determine the efficacy *B. vulgaris* methanolic extract against acute toxoplasmosis in mice model.

### Mortality rate

The mortality rate was determined according to the following formula:

$$\text{MR} = \frac{\text{Number of dead mice}}{\text{Number of mice at the beginning of the experiment}} \times 100$$

### Parasite load

Counting the mean number of *T. gondii* tachyzoites in the peritoneal fluid of mice. The extracellular tachyzoites in each mouse were counted in 10 high power field (HPF) and then the mean number of tachyzoites 10/HPF was estimated. Then the mean number of tachyzoites in each group of infected mice was calculated (13).

### Statistical analysis

Data analysis was carried out using SPSS statistical package version 17.0 (SPSS Inc., Chicago, IL, USA). Differences

between test and control groups were analyzed by *t*-test. In addition,  $p < 0.05$  was considered statistically significant.

## Results

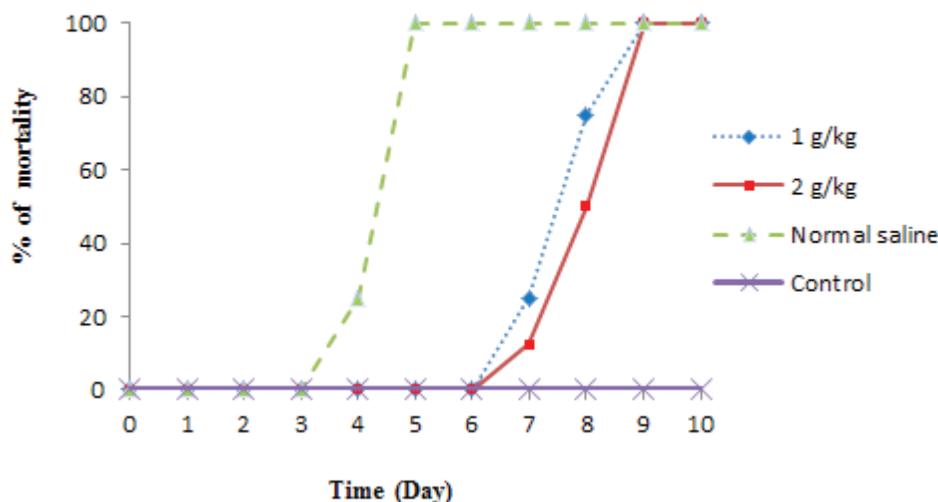
In the present study we evaluated efficacy of *B. vulgaris* root methanolic extract against acute toxoplasmosis in mice model. As shown in Figure 1, the mortality rate of infected mice in the control group was 100% after 5<sup>th</sup> after establishment. The mortality rate of infected mice in group 3 was 100%, in 9<sup>th</sup> day after orally administration of *B. vulgaris* at the concentration of 1 g/kg; indicating that *B. vulgaris* significantly ( $p < 0.05$ ) increased the time of death in the infected mice. Similarly, the mortality rate of infected mice in group 4 was 100% in 9<sup>th</sup> day after orally administration of *B. vulgaris* at the concentration of 2 g/kg which demonstrated that *B. vulgaris* significantly ( $p < 0.05$ ) increased the time of death in the infected mice.

Figure 2 indicates the parasite load (mean number of tachyzoites) in tested groups. The mean number of tachyzoites was  $131 \times 10^4$  and  $79 \times 10^4$  for infected mice treated with 1 and 2 g/kg, respectively, whereas in control group the mean number of tachyzoites was  $288 \times 10^4$  parasite. The obtained results showed that the difference in mortality rate between of *B. vulgaris* at the concentrations of 1 and 2 g/kg in comparison to the control group was statistically significant ( $p < 0.05$ ).

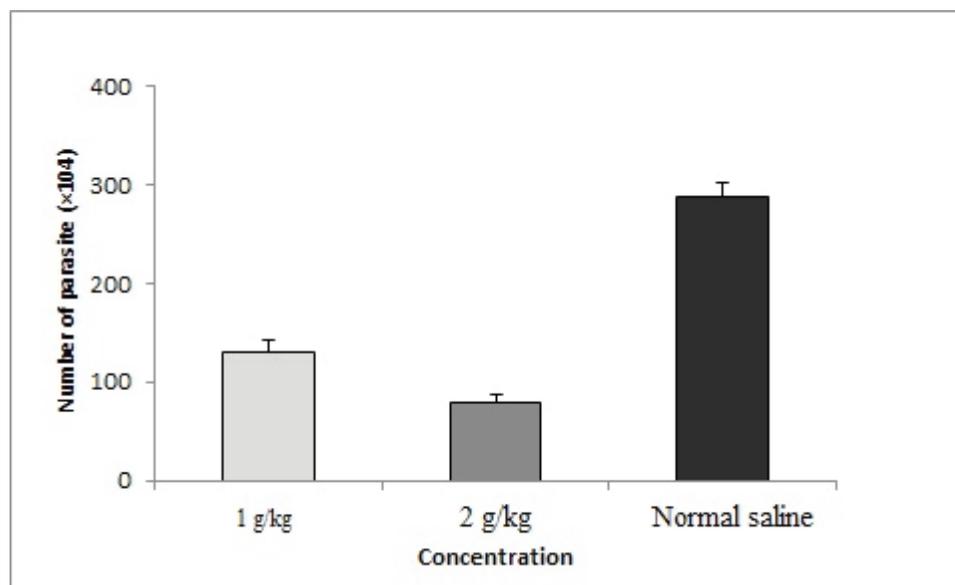
## Discussion

At present, there are a restricted number of agents for the treatment of toxoplasmosis and the combination of pyrimethamine and sulfadiazine is the most common (3). However, these drugs may results in some adverse effects, including osteoporosis, sepsis and also teratogenic effects particularly in persons with immunocompromised patients (2, 3). So far, efforts to decrease the toxicity of these compounds have been failed, that reinforces the requirement for novel anti-*Toxoplasma* drugs. Thus, studies that could offer a substitute therapy, condense dosages, treatment time and side effects for toxoplasmosis, would be greeting. Here we evaluated efficacy of *B. vulgaris* root methanolic extract against acute toxoplasmosis induced by RH strain *T. gondii* in mice model.

The obtained results demonstrated that the mortality rate of infected mice was 100%, in 9<sup>th</sup> day after orally administration of *B. vulgaris* at the concentrations of 1 and 2 g/kg; indicating that oral administration of *B. vulgaris* for 14 days especially at the aforementioned concentrations had potent prophylactic effects against acute toxoplasmosis in mice and their survival was prolonged to 9 days, while all mice in the control group were death in 5<sup>th</sup> day. Moreover obtained findings revealed that the mean number of tachyzoites was  $131 \times 10^4$  and  $79 \times 10^4$  for infected mice treated with *B. vulgaris* at the concentrations of, respectively, whereas in control group the mean number of tachyzoites was  $288 \times 10^4$  parasite; which indicated that *B.*



**Figure 1.** Effects of orally administration of *Berberis vulgaris* methanolic root extract on mortality rate of infected mice with acute toxoplasmosis.



**Figure 2.** The mean number of *T. gondii* tachyzoites in the peritoneal fluid of infected mice following orally administration of *Berberis vulgaris* methanolic root extract

*vulgaris* significantly reduce parasite load in infected mice with acute toxoplasmosis.

Reviews have reported that the main components of *B. vulgaris* are isoquinoline alkaloids such as berbamine, palmatine, and particularly berberine (10, 14). Previous investigations have shown antibacterial, anti candidal, and anti-dermatophyte activity of this plant due to having high amount of isoquinoline alkaloids (15-17). Furthermore, in several study anti-parasitic effects of *B. vulgaris* root extract have been demonstrated; for example in the study conducted by Mahmoudvand *et al*, it has been proven that *B. vulgaris* methanolic extract and berberine alone have high protoscolicidal effects against protoscoleces of *Echinococcus granulosus* in low concentration and short exposure time on *in vitro* model (18). Fata *et al* (19) have demonstrated that ethanolic extract of *B. vulgaris* considerably reduced ulcer size of cutaneous leishmaniasis of BALB/c mice after 14 days.

Kaneda *et al* (20) have demonstrated that berberine notably condensed growth of *Entamoeba histolytica*, *Giardia lamblia*, and *Trichomonas vaginalis* on an *in vitro* model and result in morphological changes in their structure. Sheng *et al* (21) showed that, in chloroquine resistant malaria, the combination of berberine and pyrimethamine have a synergistic effect in the removal of parasites and it was further efficient than other drugs including tetracycline or cotrimoxazole. Moreover, Vennerstrom *et al* (22) have reported that berberine derivatives considerably concealed

the parasite load in liver or ulcer size in golden hamsters infected with *Leishmania donovani* and *Leishmania braziliensis* when compared with meglumine antimoniate.

Regarding antimicrobial mechanisms of berberine, reviews have demonstrated that antifungal activity of berberine on *Candida* growth was most likely because of hang-up of both sterol and cell wall biosyntheses in this yeast (8). Moreover, Peng *et al* (23) reported that berberine can break the structure of bacterial cell wall and restrain synthesis of protein and DNA. With respect to toxicity effects of *B. vulgaris* and berberine reviews have shown that they are not considered toxic at the doses used in clinical studies, nor have they been revealed to be cytotoxic or mutagenic, whereas, their side effects can be increased to dose development (5, 24, 25).

## Conclusion

The obtained results delivered the scientific suggestion that natural plants might be used in traditional medicine for the prevention and treatment of parasitic infections such as acute toxoplasmosis. However, additional investigations are needed to assess exact effect of *B. vulgaris* principally its immunomodulatory on acute toxoplasmosis.

## Declaration of Interest

The authors declare that there is no conflict of interest in this study.

***Berberis vulgaris* ekstraktının farelerde oluşturulan akut toksoplazmozis üzerine etkileri****ÖZ**

Son birkaç yüzyıldır bitkisel kaynaklardan elde edilen ilaçlar çeşitli hastalıklarla birlikte enfeksiyon hastalıklarının da tedavisinde ve profilaksisinde kullanılmaktadır. Bu çalışmada; *Berberis vulgaris*'in köklerinden hazırlanan matanollü ekstrenin, *Toxoplasma gondii* RH suşuyla enfekte edilerek akut toksoplazmozis oluşturulmuş farelerdeki etkisi araştırıldı. Erkek NMRI fareleri intraperitoneal yoldan *Toxoplasma gondii* RH suşuna ait  $10^4$  taşızoitle enfekte edildikten 24 saat sonra bu farelere oral yoldan *Berberis vulgaris*'in ekstresi 1 ve 2 g/kg dozlarında, günde 1 kez, 14 gün boyunca uygulandı. Tüm farelerde

ölüm oranı ve parazit yükü tespit edilerek akut toksoplazmoziste *B. vulgaris*'in metanollü ekstresinin etkisi belirlendi. *Berberis vulgaris*'in 1 ve 2 g/kg dozlarında oral yoldan uygulanmasının takip eden 9. günde farelerde ölüm oranı %100 olarak belirlendi, kontrol grubundaki bütün farelerin 5.günün sonunda öldüğü gözlemlendi. *Berberis vulgaris*'in 1 ve 2 g/kg dozlarında uygulandığı gruplarda ortalama taşızoit değeri sırasıyla  $131 \times 10^4$  ve  $79 \times 10^4$  olarak saptanırken kontrol grubunda taşızoit sayısı ortalama  $288 \times 10^4$  parazit olarak belirlendi. Elde edilen sonuçlar dikkate alınarak *B. vulgaris*'in toksoplazmozis profilaksisinde kullanılabilir bir ilacın üretiminde doğal kaynaklı bir bileşen olarak kullanılabilirliği gösterilmiştir.

**Anahtar kelimeler:** *Toxoplasma gondii*; *Berberis vulgaris*; Profilaksi; *In vivo*

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