

# Anti-Toxoplasma Activities of Zea Mays and Eryngium Caucasicum Extracts, In Vitro and In Vivo

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## Key Words

*Toxoplasma gondii*, *Zea mays*, *Eryngium caucasicum*, extracts, In vitro, In vivo

## Abstract

**Objectives:** Toxoplasmosis is a worldwide health problem that caused by intracellular apicomplexan parasite, *Toxoplasma gondii* (*T. gondii*). Considering that the available drugs for toxoplasmosis have serious host toxicity, the aim of the current study was to survey the in vitro and in vivo anti-Toxoplasma activity of *Zea mays* (*Z. mays*) and *Eryngium caucasicum* (*E. caucasicum*) extracts.

**Methods:** Four concentrations (5, 10, 25, and 50 mg mL<sup>-1</sup>) of *Z. mays* and *E. caucasicum* methanolic extracts for 30, 60, 120, and 180 min were incubated with infected macrophages and then the viability of RH strain of *T. gondii* tachyzoites was evaluated by trypan blue staining method. Also, we evaluated the survival rate of acutely infected mice with the extracts (100 and 200 mg kg<sup>-1</sup> day<sup>-1</sup>) intraperitoneally for 5 days after infection with 2 × 10<sup>4</sup>

tachyzoites of *T. gondii*.

**Results:** The anti-Toxoplasma effect of the methanolic extracts were extremely significant compared to the negative control group in all exposure times ( $P < 0.05$ ). The *Z. mays* (10, 25 and 50 mg mL<sup>-1</sup>) killed 100% of the parasites after 180 and 120 min exposure, respectively. Also, high toxoplasmacidal activity was observed with *E. caucasicum* extract. Furthermore, treatment of experimentally infected mice with the *Z. mays* (100, 200 mg kg<sup>-1</sup> day<sup>-1</sup>) and *E. caucasicum* (100 mg kg<sup>-1</sup> day<sup>-1</sup>) significantly increased their survival rate compared to untreated infected control ( $P < 0.05$ ).

**Conclusion:** These extracts are promising candidates for further medicine development on toxoplasmosis. However, further investigations are necessary to clarify effective fractions of the *Z. mays* and *E. caucasicum* extracts and the mechanisms of action.

## 1. Introduction

Toxoplasmosis is a worldwide health problem that caused by intracellular apicomplexan parasite, *Toxoplasma gondii* (*T. gondii*) [1]. It is estimated that more than 1 billion people are infected with *T. gondii* [2]. *T.*

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*T. gondii* prevalence in some countries is high (e.g. Brazil, 77.5%; Sao Tome and Principe, 75.2%; Iran, 63.9%; Colombia, 63.5%; and Cuba, 61.8%) [3]. Globally, the annual incidence of congenital toxoplasmosis is estimated to be 190,100 cases [4].

Although acute toxoplasmosis in healthy individuals is usually asymptomatic, it can lead to great mortality rates in immunocompromised hosts or congenitally infected infants [5, 6].

Currently, the first-line therapy for treatment or prophylaxis of toxoplasmosis is the combination of pyrimethamine and sulfadiazine in the clinic [7]. Unfortunately, these drugs have serious side effects. Pyrimethamine can lead to suppression of bone marrow and hematological toxicity [8]. In addition, azithromycin, clarithromycin, spiramycin, atovaquone, dapsone, and cotrimoxazole (trimethoprim-sulfamethoxazole), have been used for clinical toxoplasmosis. However, these drugs are poorly tolerated and have no effect on the bradyzoite form of the parasite [9-11].

Like other apicomplexa such as *Plasmodium* spp. resistance to anti-Toxoplasma drugs has also been reported in *T. gondii* [12]. Despite great progress in pharmacological and immunological researches, there is no available drug for treatment of chronic toxoplasmosis. In addition, there is no effective vaccine for prevention of infection in human [8, 13, 14]. Accordingly, new drugs with lack of toxicity and teratogenicity, effective penetration in the placenta, and parasiticidal effect against the different stage of *Toxoplasma* particularly cystic form are critically needed [15]. There are increasing studies of therapeutic potential of natural or herbal products and medicinal plants are considered to be generally safe and have low toxicity compared to synthetic drugs [16]. Natural products are the source of most drugs in clinical use. Plants have been used as a base or precursors to the development of new synthetic or semi-synthetic drugs with anti-infectious activity, such as antiprotozoal and antibacterial [16, 17], or immunomodulatory activity [18]. Based on studies, approximately 70% of new anti-infective drugs are of natural origin, including 14 approved antiparasitics [19]. Natural products provide a unique structural variety, and a valuable opportunity for the discovery of new active compounds of low molecular weight [20].

Considering the aforementioned side effects of drugs against toxoplasmosis, currently multitude efforts are concentrated on the use of plant extracts to improve the therapies and many researchers have focused on therapeutic potential of natural products against *T. gondii* [21]. According to our previous study, the extracts of *Feijoa sellowiana* (leaves and fruits), *Quercus castaneifolia* (fruits), and *Allium paradoxum* (leaves) were evaluated against *T. gondii* [22]. Currently no natural products exist that have been patented for use in the treatment of toxoplasmosis [20].

*Zea mays* (*Z. mays*), a traditional medicine, was used for the treatment of interstitial cystitis, diuretic, edema, kidney stones, prostate disorder, and urinary infections in many parts of the world [23]. Also, *Eryngium caucasicum* (*E. caucasicum*) is a new cultivated vegetable plant in northern Iran and the antioxidant activity of leaves and inflorescence has recently been shown [24, 25]. No data are available on the effects of these valuable herbs on *T. gondii* and other parasitic infections. Therefore, the current study

was performed to evaluate the in vitro and in vivo effects of methanolic extract from *Z. mays* and *E. caucasicum* against RH strain of *T. gondii*.

## 2. Material and Methods

### 2.1. Plant material

Dried cut stigmata of *Z. mays* L, Poaceae flowers, used for this investigation. Plant specimen was collected from Mazandaran province, Iran, in January 2015 and authenticated by Dr. Bahman Eslami (Department of Biology, Islamic Azad University of Qhaemshahr, Iran) and the voucher specimen was deposited in the Sari School of Pharmacy herbarium (No. HS280). *E. caucasicum* leaf was collected from khazar abad area, Mazandaran province, Iran, and identified by Dr. Bahman Eslami. A voucher (No. 987) has been deposited in the Sari School of Pharmacy herbarium. The plants dried under shade, and powdered mechanically using a commercial electrical blender in the Sari School of Pharmacy.

### 2.2. Extracts preparation

To obtain the *Z. mays* and *E. caucasicum* methanolic extracts, 150 g of dry powder was added to 350 mL of pure methanol and mixed gradually for 1 hour using a magnetic stirrer. The solution was left at room temperature overnight and filtered through Whatman No. 1 filter paper after sterilization. Then the solvent was removed at 40 °C with a rotary evaporator. The remaining semi solid material was freeze-dried at -50 °C for 24 h. The final crude extracts (14.5 g) was placed into a sterile glass container and kept at 4 °C for further use [26].

### 2.3. *T. gondii* strain

Tachyzoites of *T. gondii* virulent RH strain was maintained in Swiss Webster mice. Parasites were propagated intraperitoneal (i.p.) every 3-4 days. The tachyzoites were purified in sterile Phosphate-Buffered Saline (PBS) containing penicillin and streptomycin, 100 IU mL<sup>-1</sup> and 100 mg mL<sup>-1</sup>, respectively [22]. Number of tachyzoites was determined by counting in a hemocytometer under light microscopy.

### 2.4. Mice

Female inbred Balb/c mice weighing 18-20 g (six-week old) were used for the present study. This research underwent ethical review and was approved by the Research Ethics Committee of Mazandaran University of Medical Sciences. Care and use of laboratory animals complied with local animal welfare laws, policies, and guidelines. All experimental mice were housed in cages (n=5) under standard laboratory conditions (with an average temperature 20-25 °C, given drinking water and regular mouse diet) [27].

### 2.5. Anti-Toxoplasma activity of the extracts in vitro

Four concentrations (5, 10, 25, and 50 mg / mL<sup>-1</sup>) of the *Z. mays* and *E. caucasicum* extracts were accessed for 30, 60, 120 and 180 min. Two mL of each concentration and 4×10<sup>6</sup> fresh *T. gondii* tachyzoites were put into a test tube. The contents of the tubes were mixed gently and incubated at 37 °C for 30, 60, 120 and 180 min. After the end of each incubation time, two mL of 0.5% (w/v) trypan blue dye was added to the settled tachyzoites. The remained pellet of tachyzoites was smeared on a glass slide. The percentage of dead tachyzoites was determined by counting 200 tachyzoites under a light microscope. Tubes containing pyrimethamine in concentration of 100 mg mL<sup>-1</sup> and PBS were considered as positive and negative control groups, respectively. All experiments were performed in triplicate in this study.

## 2.6. Anti-Toxoplasma activity of the extracts in vivo

For assaying of anti-Toxoplasma activity in vivo, 30 female Balb/c mice were infected i.p. with 2×10<sup>4</sup> tachyzoites of *T. gondii* RH strain and distributed into six groups, each with 5 mice, that were treated on the same day of inoculum, during 5 days i.p. at regular 24-h intervals as follows: *Z. mays*, *E. caucasicum* (100 and 200 mg / kg<sup>-1</sup> / day<sup>-1</sup>), pyrimethamine (50 mg / kg<sup>-1</sup> / day<sup>-1</sup>) (positive control) and PBS (negative control). The mice were monitored daily for mortality and the morbidity. The survival periods were recorded daily until all mice died. Initially, for controlling of drug side effects, a preliminary experiment was done on Balb/c mice receiving the same dose of drugs and no mortality or clinically significant toxicity was observed.

## 2.7. Statistical analysis

Statistical analysis was performed with SPSS-14. Differences between the test and control groups were analyzed using repeated measures ANOVA test. Also, the Kaplan-Meier curve was used to show the survival time and by using log-rank test, the survival rates among different groups were compared. Differences were considered statistically significant when P<0.05.

## 3. Results

### 3.1. Effects of the extracts on *T. gondii* in vitro

Based on in vitro results, the extract of *Z. mays* at the concentration of 5 mg mL<sup>-1</sup> killed 97.08% of tachyzoites after 180 min. Also, *Z. mays* (10, 25 and 50 mg / mL<sup>-1</sup>) after 120 and 180 min killed 100% of the parasites. It is notable that extract of *E. caucasicum* (5 and 25 mg / mL<sup>-1</sup>) destroyed 91.58% and 96.29% of the parasites after 180 min, respectively. The anti-Toxoplasma effect of the methanolic extracts were statistically significant compared to the negative control group in all exposure times (P < 0.05). Furthermore, pyrimethamine (50 mg / mL<sup>-1</sup>) after 180 min destroyed 98.64 % of the tachyzoites. The anti-Toxoplasma effects of the *Z. mays* and *E. caucasicum* extracts are summarized in Table 1, 2.

### 3.2. Effects of the extracts on *T. gondii* in vivo

Clinically, the numbers of mice in untreated infected groups (negative control) started to reduce on the seventh day of study and all mice died before the eighth day. Mice

**Table 1** Anti- Toxoplasma activity of *Zea mays* extract in vitro.

Groups	Concentration (mg/ mL)	Time				P-value
		30 min	60 min	120 min	180 min	
Case	5	56.73±5.26	70.40±8.59	96.46±1.39	97.08±2.19	0.003*
	10	82.02±0.21	85.56±2.74	97.61±1.21	100±0.00	<0.01*
	25	86.78±2.48	90.26±3.16	98.28±1.33	100±0.00	<0.01*
	50	90.54±5.14	95.17±1.97	100±0.00	100±0.00	>0.05
Pos control	100	15.59±1.96	69.37±8.36	95.46±0.48	98.64±1.92	0.001*
Neg control	-	3.5±0.14	3.6±0.07	4±0.21	4.8±0.21	>0.05

Pos control: Positive control group receiving 100 mg mL<sup>-1</sup> pyrimethamine, Neg control:

Negative control group receiving PBS, \* Statistically significant compared to control group.

**Table 2** Table 2 Anti- Toxoplasma activity of *Eryngium caucasicum* extract in vitro.

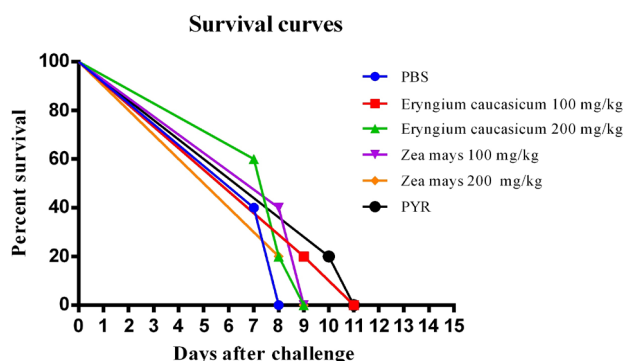
Groups	Concentration (mg/ mL)	Time				P-value
		30 min	60 min	120 min	180 min	
Case	5	57.51±8.88	73.37±0.12	85.36±1.57	91.58±1.75	<0.05*
	10	66.88±11.23	80.87±1.81	87.79±2.69	94.16±0.33	<0.05*
	25	67.15±3.71	87.45±0.91	90.12±1.01	96.29±1.50	0.007*
	50	80.42±10.10	94.44±3.93	94.20±0.16	94.91±2.96	>0.05
Pos control	100	15.59±1.96	69.37±8.36	95.46±0.48	98.64±1.92	0.001*
Neg control	-	3.5±0.14	3.6±0.07	4±0.21	4.8±0.21	>0.05

Pos control: Positive control group receiving 100 mg mL<sup>-1</sup> pyrimethamine, Neg control:

Negative control group receiving PBS, \* Statistically significant compared to control group.

of *Z. mays*, *E. caucasicum* and pyrimethamine groups started to die on the seventh day until the eleventh day. The treatment with *Z. mays* and *E. caucasicum* (100 mg / kg<sup>-1</sup> / day<sup>-1</sup>) lead to better results in mice survival than treatment with *Z. mays* and *E. caucasicum* (200 mg / kg<sup>-1</sup> / day<sup>-1</sup>) (Fig. 1).

Mice in the treatment groups of *Z. mays* (100 and 200 mg kg<sup>-1</sup> day<sup>-1</sup>) and *E. caucasicum* (100 mg / kg<sup>-1</sup> / day<sup>-1</sup>) showed statistically higher survival rate compared to untreated infected control (P<0.05). There was significant difference between *Z. mays* (100 and 200 mg / kg<sup>-1</sup> / day<sup>-1</sup>) and *E. caucasicum* (200 mg / kg<sup>-1</sup> / day<sup>-1</sup>) groups with the positive control (P < 0.05).



**Figure 1** The survival curves of mice following acute toxoplasmosis. BALB/c mice (n=5) infected with 2×10<sup>4</sup> tachyzoites of the *T. gondii* RH strain were treated with the *Z. mays* and *E. caucasicum* extract 100, 200 mg / kg<sup>-1</sup> / day<sup>-1</sup>, pyrimethamine (50 mg / kg<sup>-1</sup> / day<sup>-1</sup>) (as the positive control) and PBS (as the negative control) for 5 days via the intraperitoneal route.

#### 4. Discussion

In the present study, we evaluated the efficacies of *Z. mays* and *E. caucasicum* on *T. gondii* infection in vitro and in vivo for the first time. In the in vitro tests, the anti-Toxoplasma effect of the methanolic extracts were statistically significant compared to the negative control group in all exposure times (P < 0.05), and they did not show any significant difference compared to pyrimethamine (positive control). Interestingly, the *Z. mays* (10, 25 and 50 mg / mL<sup>-1</sup>) after 180 and 120 min killed 100% of the tachyzoites, respectively. Also, the *E. caucasicum* (25 mg / mL<sup>-1</sup>) after 180 min killed 96.29% of the tachyzoites indicating that both *Z. mays* and *E. caucasicum* had been shown high toxoplasma-cidal activity.

Actually, the current anti- *T. gondii* chemotherapy is deficient [8]. Natural compounds and traditional herbal medicine may be developed as a source of valuable pharmacologically active agents that improve the treatment of toxoplasmosis. These novel therapeutic agents have high availability and lower side effects, compared with the current chemical drugs [22]. There are many herbal compounds against fungi, protozoa and helminthes, and some have anti- *T. gondii* properties such as *Curcuma longa* [28], *Eurycoma longifolia* Jack [29, 30], and *Myristica*

*fragrans* Houtt [31], etc. However, *Z. mays* and *E. caucasicum* extracts have not been examined for their potential anti- *Toxoplasma* properties. Herbal extract of *Z. mays*, as traditional medicine, was used for the treatment of cystitis, kidney stones, edema, diuretic, and urinary infections in many parts of the world [23]. Also, *E. caucasicum* is a new cultivated vegetable plant in northern Iran and the antioxidant activity of leaves and inflorescence has recently been shown [24, 25].

Previously we have shown that the fruits and leaves of *Sambucus nigra* at the concentrations of 5, 10 and 25 mg / mL after 180 min, and concentration of 50 mg / mL after 60 min, resulted with the highest efficacy [26]. Also, Leesombun et al. reported that 25 µg / mL<sup>-1</sup> of the Piper beetle extract eradicated *T. gondii* in vitro [32]. Similarly, our data indicated that extract of *Z. mays* and *E. caucasicum* had high toxoplasma-cidal activity in vitro.

Considering the in vitro anti-*T. gondii* activity of *Z. mays* and *E. caucasicum* extracts, we evaluated the effects of these extracts in acute *T. gondii* infection model with virulent RH strain in Balb/c mice. Treatment of experimental mice with the *Z. mays* (100, 200 mg kg<sup>-1</sup> day<sup>-1</sup>) and *E. caucasicum* (100 mg / kg<sup>-1</sup> / day<sup>-1</sup>) for 5 days after infection with 2×10<sup>4</sup> tachyzoites of the *T. gondii* RH strain increased statistically their survival rate than untreated infected control statistically (P < 0.05). Moreover, the mice treated with *E. caucasicum* (100 mg / kg<sup>-1</sup> / day<sup>-1</sup>) also achieved better effect in survival compared with other groups. Similarly, Zhang et al. have shown that oxymatrine and matrine, two Sophora alkaloids, have unique properties against *T. gondii* tachyzoites in vitro and in vivo [33].

In our study, *Z. mays* and *E. caucasicum* were more effective at doses (100 mg kg<sup>-1</sup> day<sup>-1</sup>) in the acute phase of infection. However, the anti-toxoplasma-cidal mechanism of the extracts is not known. Similar effects were reported for endochin-like quinolone: ELQ-271 and ELQ-316 at low doses were highly active against *T. gondii* in mice [34]. Leesombun et al. performed a mouse survival study and reported that Piper beetle extract was highly effective against *T. gondii* in vivo [32]. Accordingly, *E. caucasicum* extract (100 mg / kg<sup>-1</sup> / day<sup>-1</sup>) was effective as pyrimethamine for control of infection. However, there is no difference between *E. caucasicum* and pyrimethamine.

Previously we have shown that the propranolol and ketotifen combined with pyrimethamine was more effective in inhibiting growth of tachyzoites when compared with propranolol and pyrimethamine alone on murine toxoplasmosis [35, 36, 37]. Therefore, further studies should be performed to compare the efficacy of *Z. mays* and *E. caucasicum* combination in inhibiting growth of *T. gondii*.

#### 5. Conclusion

The present results clearly indicated that the methanolic extracts have promising efficacies on tachyzoites of *T. gondii* in vitro. Also these extracts were effective for acute toxoplasmosis of RH strain of *T. gondii* in vivo more investigations are required to determine active compounds of *Z. mays* and *E. caucasicum* in which act as anti-Toxoplasma agents. However, further study should be conducted to investigate potential bioactives of these extracts through bioactivity guided fractionation.

## Conflict of interest

There is no conflict of interests.

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