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Brief reports

Ethanol Extracts of *Achillea millefolium* and *Hypericum perforatum* Low Anti-*Toxoplasma* Activity

Shagayegh Nozari[1], Abbas Azadmehr[2], Marjan Nassiri-Asl[3], Hasan Jahani-hashemi[4], Mohtaram Adine[1], Farzaneh Javadi[1], Mojtaba Shahnazi[1], Mehrzad Saraei[1,5]*

[1] Department of Parasitology and Mycology, Qazvin University of Medical Sciences, Qazvin, Iran
[2] Immunology Department, School of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran
[3] Department of Pharmacology, Qazvin University of Medical Sciences, Qazvin, Iran
[4] Children Growth and Development Center, Qazvin University of Medical Sciences, Qazvin, Iran
[5] Cellular and Molecular Research Center, Qazvin University of Medical Sciences, Qazvin, Iran

Key Words

Achillea millefolium, herbal medicine, *Hypericum perforatum*, *Toxoplasma gondii*

Abstract

Objectives: This study was performed to determine the lethal and the inhibitory effects of ethanol extracts of *Achillea millefolium* (*A. millefolium*) and *Hypericum perforatum* (*H. perforatum*) on *Toxoplasma gondii* (*T. gondii*) RH strain tachyzoites *in vitro*.

Methods: The tachyzoites were treated with concentrations of 10, 50, and 100 mg/mL of *A. millefolium* and *H. perforatum* extracts within 10, 30, and 45 minutes in the wells. The mortality rates of tachyzoites treated with extracts were determined by using alkaline methylene blue staining. Also, the tachyzoites in cell cultures were treated with concentrations of 50, 100, and 200 mg/mL of these extracts. The cell viability, inhibition concentration (IC_{50}), and selectivity were determined from MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assays.

Results: In the cell-free *in vitro* study, all of tachyzoites were killed at concentrations of 100 mg/mL of both extracts while at concentration 10 mg/mL, the mortality was 4.53% – 5.31%. In the cell culture study, the values of the effective concentration (EC_{50}) were 215 and 153 μg/mL and the selectivities were 0.73 and 0.69 for the *A. millefolium* and the *H. perforatum* extracts, respectively.

Conclusion: We conclude that neither extracts has any significant effect on the tachyzoites of *T. gondii* in cell cultures.

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1. Introduction

Toxoplasma gondii (*T. gondii*) is one of the most common parasitic zoonosis. It can be found worldwide [1]. Treatment of toxoplasmosis is commonly performed with the synergistic drugs pyrimethamine and sulfadiazine. Although, synthetic drugs have acceptable anti-*Toxoplasma* activities, their adverse effects are limiting factors, especially in pregnant women with acute toxoplasmosis. For example, pyrimethamine causes suppression of hematopoiesis in patients [2]. Therefore, production of an effective anti-*Toxoplasma* drug with low side effects is a priority in *Toxoplasma* research.

Based on a PubMed search, some *in vitro* studies have done on the effectiveness of herbal products in treating *T. gondii* [3-5]. However, only one report, a report from Iran, was found on the anti-*Toxoplasma* effect of herbal extracts; that research studied the effect of garlic extract on acute toxoplasmosis in mice [6]. This

*Corresponding Author
Mehrzad Saraei. Department of Parasitology and Mycology, Qazvin University of Medical Sciences, Qazvin-341197-5981, Iran.
Tel: +98-283-333-6001 Fax: +98-283-332-4971
E-mail: saraei56@yahoo.com

study was performed in order to determine the lethal and the inhibitory effects of ethanol extracts of *Achillea millefolium* (*A. millefolium*) and *Hypericum perforatum* (*H. perforatum*) on *T. gondii* RH strain tachyzoites *in vitro*.

2. Materials and Methods

A. millefolium and *H. perforatum* were obtained from herbal marketing Jahad Daneshgahi Karaj (Tehran, Iran) and were confirmed by a botanist. Voucher specimens of the herbal plants were saved at the Herbarium Center of Medicinal Plants of the Academic Center for Education, Culture and Research (ACECR), Karaj, Iran. Aerial parts of plants were dried at room temperature and were powdered. Fifty grams of each herb were extracted by using the percolation method with 80% ethanol at room temperature. The samples were stored at 4ºC until use (3 gr, 6% yield). The dried plant extracts were dissolved in 1% dimethyl sulfoxide (DMSO) and diluted with phosphate buffer solution (PBS) to different concentrations. Also, pyrimethamine (Sigma, USA) dissolved in methanol-acetone (50% v/v) and diluted with Roswell Park Memorial Institute (RPMI) 1640 medium was used as a positive control.

We used *T. gondii* RH strain tachyzoites. HeLa cells were cultured and used to assay the anti-*Toxoplasma* effects of the herbal extracts. The assays were performed by treating a 50-μL tachyzoites suspensions containing nearly 5×10^5 tachyzoites with 50 μL of different concentrations of the extracts (10, 50, and 100 mg/mL) within 10, 30, and 45 minutes at room temperature. All treatments were assayed in triplicate.

The tachyzoites treated with both extracts that showed 100% mortality based on methylene-blue staining were bioassayed in mice. Fifty microliters of suspensions containing nearly 5×10^5 tachyzoites were inoculated intraperitoneally into 3 mice. All of the mice were monitored for up to one month after inoculation in terms of activity and mortality.

HeLa cell suspensions were cultured and within 24 hours after incubation, 100 μL of a suspension containing 3×10^5 fresh tachyzoites were added to each well. Six hours after the inoculation of the tachyzoites to wells, the cultures were washed twice with RPMI 1640 medium without fetal bovine serum (FBS) in order to remove non-adherent tachyzoites. Eighteen hours after incubation, the herbal extracts were individually added to the wells at a concentration of 50, 100, or 200 μg/mL. Twenty four hours after the addition of the herbal extracts, the anti-*Toxoplasma* activity and the cytotoxicity of those traditional medicines were examined using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay kits (Bio Idea Company, Tehran, Iran). Optical densities (ODs) were read using an enzyme-linked immunosorbent assay (ELISA) microplate reader (Epoch, USA) at a wavelength of 570 nm. All experiments were performed in triplicate. The results were expressed as percent cell viability, half maximal effective concentration (EC_{50}), and selectivity.

3. Results

Both herbal extracts showed toxoplasmacidal effects. The results for the mortality rates (%) of the tachyzoites are shown in Table 1. The mortality rate at a 100-mg/mL concentration of *A. millefolium* was significantly higher than that at a 50-mg/mL concentration ($P < 0.001$), but the difference in the mortality rates between of 10- and 50-mg/mL concentrations of the extract were not significant. The toxoplasmacidal effects of the *H. perforatum* extract were similar at concentrations of 50 and 100 mg/mL; however, compared to the concentration of 10 mg/mL, both the 50- and the 100-mg/mL concentrations of the extract showed significant increases in those effects. Also, a 100% mortality rate of the tachyzoites in mice treated with the extracts was confirmed by using bioassays, and all of the mice inoculated with the tachyzoites were alive and active one month after the inoculation. Both herbal extracts showed anti-*Toxoplasma* activity in the cell culture; however, the EC_{50} of *H. perforatum* extract (153 μg/mL) was lower than the EC_{50} of *A. millefolium* extract (215 μg/mL), (Table 2).

After *T. gondii*-infected HeLa cells had been incubated with different concentrations of the extracts, their viability decreased in a dose-dependent manner (Figs. 1, 2). The viability showed significant decreases, compared with the control, at all concentrations of the extracts ($P < 0.05$). On the other hand, the inhibitory effect of pyrimethamine on cell proliferation was significantly higher than the inhibitory effects of the two herbal extracts (Figs. 1, 2).

Table 1 Mortality (%) of *T. gondii* RH strain tachyzoites after treatment with ethanol extracts of *H. perforatum* and *A. millefolium*

Incubation time (minutes)	Herbal extract type	Concentration (mg/mL)		
		10	50	100
10	*H. perforatum*	5.01 ± 1.09	100	100
	A. millefolium	4.53 ± 0. 91	5.97 ± 2.43	100
30	*H. perforatum*	5.22 ± 0.77	100	100
	A. millefolium	4.63 ± 0.75	6.11 ± 2.17	100
45	*H. perforatum*	5.31 ± 0.89	100	100
	A. millefolium	5.11 ± 1.12	6.26 ± 2.70	100

T. gondii, *Toxoplasma gondii*; *A. millefolium*, *Achillea millefolium*; *H. perforatum*, *Hypericum perforatum*.

Table 2 *In vitro* anti-*Toxoplasma* activity and selectivity of *A. millefolium* and *H. perforatum* extracts and pyrimethamine

Herbal extract/ drug (μg/mL)	EC_{50}		Selectivity*
	HeLa	HeLa+ *T. gondii*	
A. millefolium	158	215	0.73
H. perforatum	105.9	153	0.69
Pyrimethamine	0.6	0.176	3.40

*Ratio of the EC_{50} value for HeLa cells to the EC_{50} value for *T. gondii* RH strain.
A. millefolium, *Achillea millefolium*; *H. perforatum*, *Hypericum perforatum*; EC_{50}, effective concentration; *T. gondii*, *Toxoplasma gondii*.

4. Discussion

In the present *in vitro* study, ethanol extracts of *A. millefolium* and *H. perforatum* showed toxoplasmacidal activities in RH strain tachyzoites exposed to those extracts and had inhibitory effects on the parasite in cell cultures. However, the anti-*Toxoplasma* activity of the *H. perforatum* extract was stronger than that of the *A. millefolium* extract, but their selective toxicities were low. Other studies on herbal extracts have also shown that *Glycyrrhiza glabra* L., *Acorus gramineus Soland* and *Dryopteris crassirhizoma* have anti-*Toxoplasma* activities [7, 8].

Previous studies have been shown that some extracts/ fractions of medicinal plants have remarkable anti-*Toxoplasma* activities. The extracts of *Sophora flavescens Aiton* and *Zingiber officinal*e have high anti-*Toxoplasma* activities (EC_{50} = 0.20 and 0.18) with high selectivities (selectivity = 4.6% and 10.1%, respectively) [8]. Furthermore, in cell cultures, the inhibitory effects of *Torilis japonica* and *Sophora flavescens* on *T. gondii* and *Neospora caninum tachyzoites* have been shown to be higher than those of *Pulsatilla koreana*, *Ulmus macrocarpa*, and *Sinomenium acutum* [3]. Furthermore, the study of Youn *et al* [4] also showed that some high-performance liquid chromatography (HPLC) fractions of those plants have more efficient on tachyzoites in cell cultures [4].

5. Conclusion

Our study showed that the anti-*Toxoplasma* activities of *A. millefolium* and *H. perforatum* extracts were significantly lower than that of pyrimethamine which is a common synthetic drug for the treatment of toxoplasmosis. Therefore, these plants do not seem to be good candidates for continuance of anti-*Toxoplasma* studies. However, further studies to clarify the anti-*Toxoplasma* activities of other herbs are recommended.

Conflict of interest

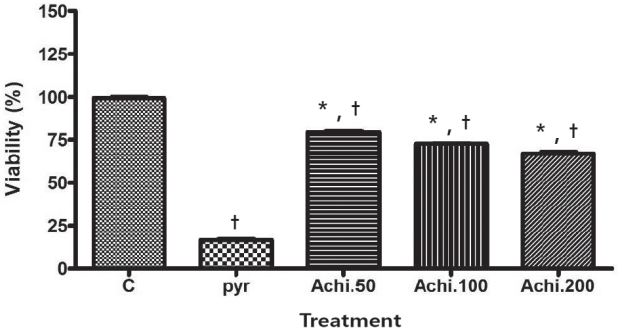

Figure 1 Inhibitory effect of *A. millefolium* extract on *T. gondii* RH strain tachyzoites in cell cultures.

*P < 0.01, compared to pyrimethamine and †P < 0.001 compared to control (Tukey Kramer test).
A. millefolium, *Achillea millefolium*; *T. gondii*, *Toxoplasma gondii*; C, control; Pyr, pyrimethamine; Achi, *Achillea millefolium*.

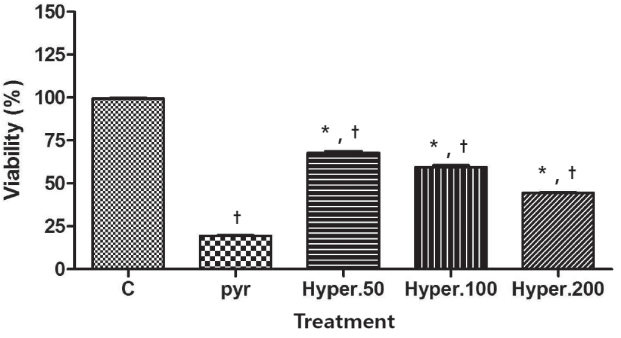

Figure 2 Inhibitory effect of *H. perforatum* extract on *T. gondii* RH strain tachyzoites in cell cultures.

*P < 0.01 compared to pyrimethamine and †P < 0.001 compared to control (Tukey Kramer test).
H. perforatum, Hypericum perforatum; *T. gondii*, *Toxoplasma gondii*; C, control; Pyr, pyrimethamine; Hyper, Hypericum perforatum.

The authors declare that there are no conflict of interest.

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