Original Article

Anti-*Toxoplasma* Activities of the Hydroalcoholic Extract of Some *Brassicaceae* Species

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Abstract

Background: *Toxoplasma gondii* (*T. gondii*) is a protozoan parasite that infects a wide range of warm-blooded animals and humans. The conventional anti-*Toxoplasma* treatments cause significant toxicity. *Brassicaceae* family contains several medicinal plants with anti-inflammatory, chemopreventive, insecticide, antibacterial, antiviral, and antiparasitic effects. In this study, the hydroalcoholic extract of some *Brassicaceae* species was investigated against *T. gondii in vitro*. **Materials and Methods:** Seeds of *Alyssum homolocarpum*, *Lepidium perfoliatum*, *Lepidium sativum,* and aerial parts of *Nasturtium officinale* and *Capsella bursa-pastoris* were extracted by maceration method using 80% ethanol. Vero cells were treated with different concentrations (5–600 µg/mL) of the extracts and pyrimethamine (as positive control), and the cellular viability was verified. Next, Vero cells were infected by *T. gondii* tachyzoites (RH strain), and the viability of the infected cells was measured by a colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. **Results:** The 50% inhibitory concentration values were 5.1, 14.67, 32.49, 37.31, 71.35, and 2.63 µg/mL, and the selectivity indices were 8.06, 2.59, 0.74, 0.78, 0.65 ($P < 0.05$ compared with positive control), and 3.03 for *L. sativum*, *L. perfoliatum*, *N. officinale*, *A. homolocarpum*, *C. bursa-pastoris,* and pyrimethamine, respectively. **Conclusion:** The results of this study demonstrated that the hydroalcoholic extracts of *L. sativum* and *L. perfoliatum* have the promising anti-*Toxoplasma* activity by growth inhibition of *T. gondii* tachyzoites in infected cells.

Keywords: *Brassicaceae, in vitro, Lepidium, Toxoplasma gondii*

Introduction

Toxoplasma gondii (*T. gondii*), an obligate intracellular protozoan, infects all mammals.[1] Human can be infected with *T. gondii* by ingestion of undercooked meat containing tissue cysts and also contaminated water or food with infected cat feces.[2,3] Toxoplasmosis is mostly asymptomatic, but some people may experience flu-like symptoms, swollen lymph glands, malaise, and pains which may last from a few days to several weeks. Toxoplasmosis can be life-threatening in immunocompromised patients.[4,5] In people with HIV infection, toxoplasmosis commonly infects the brain, causes encephalitis, and may also cause death.[6] During pregnancy, *T. gondii* can be transmitted through the placenta and may result in congenital toxoplasmosis and probably fetal death. Affected infants may suffer from mental retardation, neonatal growth retardation, ocular disorders, and blindness.[7] Combination of pyrimethamine and sulfadiazine is widely used to reduce the risk of clinical manifestation in patients; however, various side effects may occur such as allergic reactions, elevated serum creatinine and liver enzymes, and also bone marrow depression.[8-10]

In recent years, medicinal plants have been considered as effective and safe sources for drug discovery in the treatment of parasitic diseases.[11] Based on ethnobotanical surveys, it was reported that some *Brassicaceae* (*Cruciferae*) plants have been used traditionally for the treatment of parasitic diseases in Iran and other countries.[12-14] Moreover, the antiparasitic activities of *Brassicaceae* have been investigated in recent years. *Brassica*, *Lepidium*, *Raphanus,* and *Eruca* are the predominant plants in this family with anthelmintic, antitrypanosomal, and antileishmanial activities.[12,14-16]

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Glucosinolates (GSLs) and their hydrolysis products, isothiocyanates, are major groups of naturally occurring compounds found in the seeds, roots, stems, and leaves of *Brassicaceae* plants. Recently, there are several experimental evidence on the beneficial properties of GSLs against different types of parasites.[17-20]

According to ethnobotanical data and the reported antiparasitic effects from some *Brassicaceae* species, we aimed to evaluate *in vitro* anti-*Toxoplasma* activity of *Alyssum homolocarpum*, *Capsella bursa-pastoris*, *Lepidium perfoliatum*, *Lepidium sativum,* and *Nasturtium officinale* hydroalcoholic extracts.

Materials and Methods

Plant material

The aerial parts of *N. officinale* and *C. bursa-pastoris* were collected from North of Iran, Mazandaran, and Golestan Provinces, respectively. *A. homolocarpum*, *L. perfoliatum,* and *L. sativum* seeds were purchased from a local market in Qaemshahr city, Mazandaran province.

The hydroalcoholic extract of the selected plants was obtained by mixing the powdered dry samples with 80% ethanol by maceration method. The resulting extracts were concentrated over a rotary evaporator at 35°C. The remaining semisolid materials were then freeze-dried at −50°C for 24 h and stored at −19°C for further use. For each sample, the stock solution was prepared by dissolving 0.08 g of the extract in 1 mL of dimethyl sulfoxide (DMSO) and diluted with Roswell Park Memorial Institute (RPMI) medium. Finally, it was filtered using a 0.22-micron filter. Different concentrations (5–600 µg/mL) of the extract were obtained by dilution of the stock solution.[21]

Toxoplasma gondii strain

Six-week-old female BALB/c mice, weighing 18–20 g, were used for this study. All mice were housed in cages under standard laboratory conditions including an average temperature of 20°C–25°C, given drinking water and regular diet according to the Ethics Committee of Mazandaran University of Medical Sciences.

The RH strain of *T. gondii* was provided by the Toxoplasmosis Research Center in Mazandaran University of Medical Sciences, Sari, Iran. *T. gondii* tachyzoites were harvested after 3–4 days intraperitoneal injection of 1×10^5 parasites to peritoneal cavity of mice. Tachyzoites were suspended in sterile phosphate-buffered saline (pH = 7.4) containing 100 U/mL penicillin and 100 µg/mL streptomycin and counted by hemocytometer under a light microscope (Olympus, Japan).[22]

Cytotoxicity tests

Vero cells from kidney fibroblast of African green monkey (ATCC No. CCL-81) were used for this assay. Vero cells were cultured in 96-well plates (2×10^4 cells/well/180 µL) for 24 h in RPMI 1640 medium with 10% inactivated fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL and incubated at 37°C and 5% CO_2. Then, the cells were exposed to the plant extracts at final concentrations of 5, 10, 25, 50, 100, 200, 400, and 600 µg/mL. Pyrimethamine (5–600 µg/mL) and RPMI 1640 were used as positive and negative controls, respectively. After 24 h, the cell viability was measured by adding MTT solution (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) to the cultures.[23] The absorbance of the supernatant was measured at 570 nm using an enzyme-linked immunosorbent assay microplate reader (Synergy H1/USA). Then the 50% cytotoxic concentrations (CC_{50}) were calculated using the GraphPad Prism 6.0 software (Graph Pad Software, Inc., San Diego, USA).

Effects of plant extracts on intracellular *Toxoplasma gondii*

For this purpose, Vero cells were cultured in 96-well plates (2×10^4 cells/well/180 µL) for 24 h in RPMI 1640 medium supplemented with 10% inactivated FBS at 37°C and 5% CO_2. Next, the cells were infected with *T. gondii* tachyzoites (parasite: cell ratio = 10:1). After 24 h, the medium was changed and the infected Vero cells were incubated with different concentrations (5–600 µg/mL) of the extracts. Pyrimethamine (5–600 µg/mL) and RPMI 1640 were also used as positive and negative controls, respectively. MTT solution (5 mg/mL) was added to the cultures and incubated for 4 h. Then, 200 µg/well of DMSO was added to all plates. After 15 min, the optical absorbance was measured at 570 nm wavelength. The growth inhibition concentration was calculated and the mean 50% inhibitory concentration (IC_{50}) was estimated from the dose-response curves of different concentrations of the extracts using the Graph Pad Prism 6.0 software. In addition, Selectivity Index (SI) of the samples was calculated using the IC_{50} and the host-cell cytotoxicity profiles ($SI = CC_{50}/IC_{50}$).

Determination of total glucosinolate content

The total GSL contents were evaluated as described by Jezek *et al*. and Makkar *et al*.[24,25] The method is based on spectrophotometric evaluation of GSLs after alkaline hydrolysis and reduction with potassium ferricyanide at 420 nm. Sinigrin was used for the preparation of standard curve.

About 500 mg of the powdered seeds was added to 7.5 mL solution of near-boiling acetate buffer (pH 4.2, 0.2 M). The mixture was kept in a boiling water bath (15 min). After cooling, the extracts were mixed with 1.5 mL of barium and lead acetate solution (0.5 M). Then, 0.4 g of polyvinylpolypyrrolidone was added, and the mixture was stirred for 15 min. Finally, 1.5 mL of sodium sulfate solution (2 M) was added, and the mixture was centrifuged (10,000 rpm, 5 min).

Alkaline treatment and reaction with ferricyanide

The clear supernatant was mixed with an equal volume of 2 M sodium hydroxide solution. Concentrated hydrochloric acid (HCL) (37%) was added to neutralize the solution and incubated for 30 min in room temperature. The final mixture was centrifuged (10,000 rpm, 3 min), and the supernatant was mixed with an equal volume of 2 mM ferricyanide prepared in phosphate buffer (pH 7, 0.2 M). The absorbance of the solution was measured quickly against the blank sample.

Preparation of calibration curve

2 M sodium hydroxide solution was added to 500 µL of the sinigrin stock solution (5 mg sinigrin in 1 mL distilled water) and incubated for 30 min at room temperature. Concentrated HCl (37%) was added to neutralize the solution. From this, 0–500 µL was taken and phosphate buffer was added to reach the final volume of 500 µL. After that, 500 µL of potassium ferricyanide solution was added in each tube and mixed thoroughly then centrifuged at 10,000 rpm for 3 min. The supernatant absorbance was measured against the phosphate buffer.

Statistical analysis

Statistical analysis was performed on all data using GraphPad Prism 6.0 software. Differences between the test and control groups were analyzed by analysis of variance and the Newman–Keuls multiple comparison test. P <0.05 was considered statistically significant.

Results

Cellular viability

For evaluating the toxicity of different concentration of the extracts and pyrimethamine on Vero cells *in vitro*, CC_{50} were calculated. The results showed diverse degrees of toxicity on Vero cells. Pyrimethamine was the most toxic treatment for the host cells. The extracts showed less toxicity in comparison with pyrimethamine [Table 1].

The effects of plant extracts on intracellular *Toxoplasma gondii*

IC_{50} and SI of each extract and pyrimethamine were calculated. Two *Lepidum* species (*L. sativum* and *L. perfoliatum*) showed the best anti-*Toxoplasma* activity with the IC_{50} of 5.1 and 14.67 µg/mL and SIs of 8.06 and 2.59, respectively. The IC_{50} and SI of pyrimethamine were determined to be 2.63 µg/mL and 3.03, respectively. The data are shown in Table 1.

Total glucosinolate content

GSLs are one of the main compounds in the seeds of *Brassicaceae* family. In our study, the seed extracts of *L. sativum* and *L. perfoliatum* showed the best anti-*Toxoplasma* activity. The total GSL content in the seeds of these plants was determined from the sinigrin standard curve and expressed as mmol of GSLs/kg dry weight of seeds [Table 2].

Discussion

Typically, pyrimethamine alone or combined with sulfadiazine or atovaquone is used to treat *T. gondii* infections. Treatment with the mentioned drugs, especially pyrimethamine, is associated with toxic side effects such as suppression of the bone marrow, cutaneous rash, leukopenia, and thrombocytopenia.[26] Therefore, research to find effective compounds with less toxicity in the treatment of toxoplasmosis is needed. The antiparasitic activities of some *Brassicaceae* species have been reported in recent years.[15,16] It is noteworthy that some of the certain species are used commonly in Iran as vegetables or for medicinal purposes.[27] In the present study, the anti-*Toxoplasma* activity of *A. homolocarpum*, *C. bursa-pastoris*, *L. perfoliatum*, *L. sativum*, and *N. officinale* has been investigated for the first time.

According to the results, the SIs of the different extracts were obtained in the following order: *L. sativum* > *L. perfoliatum* > *N. officinale* > A. *homolocarpum* > *C. bursa-pastoris*. Thus, the hydroalcoholic extracts of *L. sativum* and *L. perfoliatum* were the most effective ones against growth of *T. gondii*-infected cells ($P < 0.05$).

Several studies have been conducted on parasitic activity of *Lepidium* species. In a recent ethnoveterinary study about the treatment of different ailments in dairy animals in Pakistan, some *Brassicaceae* plants, especially *L. sativum,* were effective for ectoparasites and endoparasites infections, mastitis, diarrhea, bloating, fever, and anorexia in bovine and bubaline.[14] There is experimental evidence

Table 1: In vitro anti-Toxoplasma activity of the extracts of selected Brassicaceae species and pyrimethamine

Extract/drug	CC_{50} (µg/mL)	IC_{50} (µg/mL)	SI[a,*]
Lepidium sativum	41.11	5.1	8.06
Lepidium perfoliatum	37.99	14.67	2.59
Nasturtium officinale	24	32.49	0.74
Alyssum homolocarpum	29.1	37.31	0.78
Capsella bursa-pastoris	46.38	71.35	0.65
Pyrimethamine	7.97	2.63	3.03

Results were shown as the mean IC_{50} and CC_{50} values obtained from three independent experiments. [a]SI=CC_{50}/IC_{50}, *P<0.05 compared with positive control. SI: Selectivity index, CC_{50}: 50% cytotoxic concentration, IC_{50}: 50% inhibitory concentration

Table 2: Total glucosinolates in the hydroalcoholic extracts of *Lepidium sativum* and *Lepidium perfoliatum* seeds

Samples	Total glucosinolates (mmol/kg dry weight)
Lepidium sativum	27.4±0.735*
Lepidium perfoliatum	20.33±0.928

*The values are mean±SD of three replicates. SD: Standard deviation

on the beneficial properties of GSLs against different types of parasites. Based on Calzada *et al*., the GSL isolated from *Lepidium virginicum* showed a significant antiamebic activity. The methanol extract of the roots and isolated Benzyl GSL exhibited *in vitro* antiprotozoal activity against *Entamoeba histolytica* trophozoites (IC_{50} of 100.1 µg/mL).[28]

Recently, a series of novel thiohydantoins (1-benzyl-3-aryl-2-thiohydantoin derivatives) originated from GSLs was isolated from *Lepidium meyenii*. These compounds showed a variety of activities such as anti-*Trypanosoma brucei* properties.[29,30] By the comparison of IC_{50} and SIs values of the extracts in this study with the results of the previous studies on other plant extracts (*Allium paradoxum*, *Aloe vera*, *Eucalyptus*, *Feijoa sellowiana*, *Quercus castaneifolia,* and *Sambucus nigra*), the seed extract of *L. sativum* was the most effective (SI: 8.06, $IC_{50:}$ 5.1 µg/mL, $CC_{50:}$ 41.11 µg/mL) sample against the growth of *Toxoplasma*-infected cells with the same methods.[21,31,32]

Lepidium species are rich in aromatic GSLs which are converted to isothiocyanates such as benzyl isothiocyanates (BITCs) by hydrolysis.[33] Previous studies indicated that BITCs are the main bioactive compounds responsible for the observed anthelmintic and antinematode activities.[34,35]

Steverding *et al*. reported that dietary isothiocyanates (BITC, phenylethyl isothiocyanate, sulforaphane, erucin, and iberin) have significant *in vitro* trypanocidal activities against *T. brucei*. All isothiocyanates showed a dose-dependent effect on the growth of trypanosomes.[15] In our study, the considerable anti-*Toxoplasma* activity of *L. sativum* and *L. perfoliatum* may be related to the presence of GSLs such as BITCs in their seeds.

Conclusion

This is the first report on anti-*Toxoplasma* activity of some species of the *Brassicaceae* family. Based on our findings, the hydroalcoholic extracts of *L. sativum* and *L. perfoliatum* seeds have the promising anti-*Toxoplasma* activity by direct growth inhibition of mice cells infected with *T. gondii* tachyzoites. It seems that *Lepidium* species can be considered as anti-*Toxoplasma* agents in future researches.

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Conflicts of interest

There are no conflicts of interest.

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