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Effects of *Aloe vera* and *Eucalyptus* methanolic extracts on experimental toxoplasmosis *in vitro* and *in vivo*

Bahareh Mirzaalizadeh[a,b], Mehdi Sharif[a,c], Ahmad Daryani[a,d], Mohammad Ali Ebrahimzadeh[e], Mehryar Zargari[f], Shahabeddin Sarvi[a,d], Saeed Mehrzadi[g], Mohammad Taghi Rahimi[h], Zahra Mirabediny[a,b], Monireh Golpour[b,i], Mahbobeh Montazeri[a,b,d,*]

[a] Toxoplasmosis Research Center, Mazandaran University of Medical Sciences, Sari, Iran
[b] Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran
[c] Department of Parasitology, School of Medicine, Sari Branch, Islamic AZAD University, Sari, Iran
[d] Department of Parasitology and Mycology, Sari Medical School, Mazandaran University of Medical Sciences, Sari, Iran
[e] Pharmaceutical Sciences Research Center, School of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran
[f] Department of Biochemistry, Biophysics and Genetic, Cellular and Molecular Research Center, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
[g] Razi Drug Research Center, Iran University of Medical Sciences, Tehran, Iran
[h] School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran
[i] Molecular and Cell Biology Research Center, Mazandaran University of Medical Sciences, Sari, Iran

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ABSTRACT

Toxoplasmosis is a worldwide disease caused by the protozoan parasite *Toxoplasma gondii* (*T. gondii*), which is most commonly treated by pyrimethamine and sulfadiazine. However, this treatment presents several adverse side effects; Thus, new drugs with lower toxicities are urgently needed. In this study the anti-*T. gondii* activity of *A. vera* and *Eucalyptus* extracts were evaluated *in vitro* using a MTT (3-(4, 5-dimethylthiazol-2-yl) 2, 5-diphenyltetrazolium bromide) assay and *in vivo* by measuring the survival rates of mice infected with 2×10^3 tachyzoites of RH strain of *T. gondii* and then injected intraperitoneally by different concentrations of extracts for 4 days. Biochemical parameters such as Ferric Reducing Antioxidant Potential (FRAP) and malondialdehyde (MDA) assay were also evaluated. As results, in the *in vitro* assay, the IC_{50} values were 13.2, 24.7, 2.63 μg/ml, and the selectivity indexes were 3.3, 2.4, 3.03 for the *A. vera, Eucalyptus* and pyrimethamine, respectively. The mice treated with *Eucalyptus* showed a better survival rate than others ($P < 0.05$). The increased weight of liver and spleen, due to infection, was reduced by treatments. In FRAP assay *Eucalyptus* showed a better antioxidant activity than the other extracts. MDA levels in both liver and spleen were reduced by treatment. The results show that *A. Vera* and *Eucalyptus* possess anti-*T. gondii* activities *in vitro* and *in vivo*, in addition, *Eucalyptus* shows antioxidant activity with a higher survival rate. Therefore, *Eucalyptus* may be a useful candidate for treating *Toxoplasma* infection. Moreover, further studies are required to investigate the fractionations of this plant against *T. gondii*.

1. Introduction

Toxoplasma gondii (*T. gondii*) is an obligate intracellular protozoan parasite of the Apicomplexa phylum that infects a wide range of hosts, including humans and other warm blooded animals (Faucher et al., 2012). There are three infective stages of *T. gondii*: a rapidly dividing invasive tachyzoite, a slowly dividing bradyzoite in tissue cysts, and an environmental stage, in which the sporozoites is protected inside an oocyst (Robert-Gangneux and Dardé, 2012). People typically become infected by three principal routes of transmission: food borne, animal to human (zoonotic) and mother-to-child (congenital), and rarely as post-solid organ transplant or blood transfusion (Scallan et al., 2011).

Approximately one third of humans have been infected with *T. gondii* (Demar et al., 2012). In immune competent hosts the acute infection is mild, self-limited and persisting in a latent form as tissue cysts (Mozzatto and Procianoy, 2003). However, the infection in immune compromised patients, represents serious problems for general health and it may cause retinochoroiditis and fatal encephalitis if not treated (Kim and Weiss, 2004). Additionally, *T. gondii* infection during pregnancy may cross the placental barrier, causing neurological damage to

* Corresponding author. Department of Parasitology and Mycology, Sari Medical School, Mazandaran University of Medical Sciences, PC 48168-95475, Sari, Iran.
E-mail address: m.montazeri2012@yahoo.com (M. Montazeri).

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the fetus and miscarriage (Mozzatto and Procianoy, 2003). There is no vaccine available to prevent human infection with this pathogen (Ahmadpour et al., 2017a, 2017b).

Currently, toxoplasmosis is being treated with a combination of sulfadiazine and pyrimethamine (PRY) (Wei et al., 2015). Unfortunately, the available treatments have significant toxicity, including suppression of the bone marrow and teratogenic effects in the first trimester of pregnancy (Değerli et al., 2003; Schmidt et al., 2006). Moreover, these therapies are effective against tachyzoites in the acute stage of the disease and have no effect over the bradyzoite form (Montazeri et al., 2017). Thus, development of new treatment options for toxoplasmosis with less toxic effects are extremely important (Montazeri et al., 2016). Natural herb extracts and medical plants are widely used as alternative treatment for various parasitic diseases and considered to be safe and to have low toxicity compared to synthetic drugs (Ebrahimzadeh et al., 2017).

Aloe vera (A. vera) is one of the Liliaceae family of which there are about more than 400 species and it grows wild in tropical climates around the world and it is also cultivated for agricultural and medical uses (Newall et al., 1996). This plant is one of the oldest medical herb that has been used to treat wounds and reduce fever (Ahlawat and Khatkar, 2011). Pharmacologically the properties of *A. vera* plants have been shown to be anti-bacterial, anti-inflammatory, anti-arthritic, anti-diabetic, anti-fungal, anti-cancer, wound healing and gastro protective (Jani et al., 2007; Newall et al., 1996; Yadav et al., 2015).

Eucalyptus, a large genus of Myrtaceae family, represented by 900 species and subspecies, is native to Australia and can be found all around the world. Native Australians used *Eucalyptus* leaves for wound healing and treating fungal infections (Gilles et al., 2010). According to previous studies, *Eucalyptus* has beneficial biological effects such as, anti-microbial, anti-hyperglycemic, anti-oxidant activities and anti-trichomonas activity (Mahdi et al., 2006; Takahashi et al., 2004; Youse et al., 2012). Thus, we were interested in exploring whether *Eucalyptus* and *A. vera* plants possess anti-*T. gondii* activity. The aim of this study was to evaluate the effects of *Eucalyptus* and *A. vera* methanol extracts on *T. gondii* infections *in vitro* and *in vivo*.

2. Materials and methods

2.1. Plant material

The fresh leaves of *A. vera* and *Eucalyptus* were purchased from herbal drug stores in Mazandaran province and were confirmed by Dr. B. Eslami, Assistant Professor of Plant Systematic and Ecology (Department of Biology, Islamic Azad University of Qhaemshahr, Iran). The plant serial numbers for *A. vera* are as follows: 34–93 and for *Eucalyptus*: 18–93. Fresh leaves were cleaned with water and only sound leaves were dried at room temperature and then powdered. Each herb was extracted by the percolation method, with methanol at room temperature. The air-dried powdered leaves were extracted with 97% methanol for 3 days at 50 °C temperature and filtrated with sterile cotton gauze. The filtrate was evaporated to dryness under reduced pressure with rotary evaporator (Heidolph-4000, made in Germany), and then lyophilized using freeze Dry Vacuum System. The yields of the extracts based on their dry weights (100 g) were 17.1% for *Eucalyptus* and 10% for *A. vera*.

2.2. T. gondii strain

The RH strain of *T. gondii* was provided by the Toxoplasmosis Research Center (TRC) in Mazandaran University of Medical Sciences, Sari, Iran. Tachyzoites were maintained by intraperitoneal (IP) passages in female Balb/c mice (8-10 week-old, 20–25 g weight), 3–4 day after IP injection with 1×10^5 of the parasite. All mice were housed in cages under standard laboratory conditions including an average temperature (20–25 °C), humidity (60 ± 10%), light (12 h per day), given drinking water, and regular diet in the animal center of Mazandaran University of Medical Sciences, Sari, Iran.

The tachyzoites were collected from the peritoneal exudates of infected mice, washed three times, and then got diluted with phosphate -buffered saline (PBS) PH: 7.4, containing 100 IU/ml of penicillin and 100 µg/mL of streptomycin (Montazeri et al., 2015). The animal protocols used in this research were approved by the Mazandaran University of Medical Sciences Ethics Committee (MUMSEC) (Permit number 492).

2.3. Cytotoxicity tests

Vero cells, kidney fibroblast from African green monkey (ATCC No. CCL-81), were used for *in vitro* assays; cultured in RPMI-1640, supplemented with 10% inactivated fetal bovine serum (FBS), 100 µg/ml penicillin and streptomycin and maintained at 37 °C in 5% CO2. All agents were dissolved in complete culture medium RPMI 1640 with less than 1% dimethyl sulfoxide (DMSO). Vero cells were plated in 96-well plates (cell suspensions 2×10^4 cell/ml in complete culture medium RPMI 1640) and incubated at 37 °C in 5% CO_2 for 24 h. Next the cells were exposed to the *A. vera*, *Eucalyptus*, and PRY at final concentrations of (5- 10- 25- 50- 100- 200- 400–600 µg/ml) and RPMI 1640 was used as a control. 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 (Choi et al., 2013). The absorbance of the supernatant was measured at 570 nm using an enzyme-linked immunosorbent assay (ELISA) microplate reader (Synergy H1/USA). Then the 50% cytotoxic concentrations (CC_{50s}) were calculated using the Graph Pad Prism 6.0 software (Graph Pad Software, Inc., San Diego, USA).

2.4. Effects of A. vera and Eucalyptus extracts on intracellular T. gondii in vitro

Vero cells were cultured in 96-well plates (2×10^4 cell/well) in complete culture medium RPMI 1640 for 24 h at 37 °C and 5% CO2. Next, the cells were infected with the RH strain of *T. gondii* tachyzoites (parasite: cell ratio = 10:1). After 24 h the medium was changed in order to remove extracellular parasites and then incubated with different concentrations (5–600 µg/ml) of *A. vera* and *Eucalyptus* extracts. PRY and RPMI 1640 culture medium were used as positive and negative control, respectively. After 24 h, MTT solution (5 mg/ml) was added to the culture wells and then incubated for 4 h at 37 °C in 5% CO_2 atmosphere. Finally, 200 µg/well DMSO were added to all plates. After 15 min, the optical absorbance was measured at the 570 nm wavelength. The growth inhibition concentration was calculated and the mean 50% growth inhibition concentration (IC_{50}) was estimated from the dose-response curves of *A. vera* and *Eucalyptus's* different concentrations by using the Graph Pad Prism 6.0 software. In addition, selectivity index (SIs) of the drugs were calculated using the IC_{50} and the host-cell cytotoxicity profiles ($SI = CC_{50}/IC_{50}$).

2.5. Effects of A. vera and Eucalyptus extracts on T. gondii infections in vivo

Female Balb/c mice, were divided into 7 groups (n = 6). One group did not get inoculated with any tachyzoites (uninfected control). Six groups were IP inoculated with *T. gondii* (RH strain, 2×10^3 tachyzoites per mouse); after 4 h post-infection, mice got injected with different