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ORIGINAL PAPER

In Vitro and *In Vivo* Anti-parasitic Activity of *Sambucus ebulus* and *Feijoa sellowiana* Extracts Silver Nanoparticles on *Toxoplasma gondii* Tachyzoites

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Abstract

Background Current chemical treatments for toxoplasmosis have side effects, researchers are looking for herbal remedies with minimal side effects and the best effectiveness. This study aimed to evaluate the anti-toxoplasmic effects of silver nanoparticles based on *Sambucus ebulus* (Ag-NPs-*S. ebulus*) and *Feijoa sellowiana* (Ag-NPs-*F. sellowiana*) fruit extracts, *in vitro* and *in vivo.*

Methods Vero cells were treated with different concentrations (0.5, 1, 2, 5, 10, 20, 40 µg/mL) of extracts and pyrimethamine as a positive control. Vero cells were infected with *T. gondii* and treated with extracts. The infection index and intracellular proliferation of *T. gondii* were evaluated. The survival rate of infected mice with tachyzoites of *T. gondii* was examined after intraperitoneal injection of the extracts at a dose of 40 mg/kg/day for 5 days after infection.

Results The Ag-NPs-*S. ebulus* and Ag-NPs-*F. sellowiana*, almost similar to pyrimethamine, reduced proliferation index when compared to untreated group. Also, high toxoplasmicidal activity was observed with Ag-NPs-*S. ebulus* extract. Mice in the treatment groups of Ag-NPs-*S. ebulus* and pyrimethamine achieved better results in terms of survival than the others.

Conclusion The results indicated that Ag-NPs-*F. sellowiana* and *S. ebulus* have a significant growth effect on *T. gondii in vitro* and *in vivo*. Ag-NPs-*S. ebulus* extract has a more lethal effect on the parasite than Ag-NPs-*F. sellowiana*. It is suggested that in future investigate the induction of *Toxoplasma*-infected cell apoptosis using nanoparticles.

Keywords *Toxoplasma gondii* · Treatment · Silver nanoparticles · *Sambucus ebulus* · *Feijoa sellowiana* · Anti-parasitic activity

Introduction

Toxoplasmosis is a common disease in humans and animals. It is caused by an intracellular protozoan belonging to the Apicomplexa called *Toxoplasma gondii* (*T. gondii*) [1–3]. Most warm-blooded animals and humans are the intermediate hosts of this protozoan, but the main and definitive hosts are cats [4]. There are various ways for the infection transmission to humans, the most important of which is eating vegetables and water contaminated with oocysts excreted in the feces of cats, as well as eating uncooked meat containing tissue cysts [4]. There are other ways such as the insertion of a syringe infected with *T. gondii*, organ transplantation [5, 6]. *T. gondii* infection is generally asymptomatic in immunologically healthy adults. However, it can cause a variety of life-threatening clinical complications in immunocompromised patients, such as AIDS patients [7], organ transplant patients, and patients receiving

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chemotherapy as well as during pregnancy this parasite can cross the placental barrier to infect embryonic tissues [4]. The combination of pyrimethamine and sulfadiazine is used to treat or prevention of toxoplasmosis that had various side effects, such as teratogenic effects on the fetus, no effect on the cyst stage, hematological abnormalities, toxic hypersensitivity reactions, and mental complications in the patient [8–10].

There are also reports that people with AIDS have shown drug resistance to these drugs [11]. Therefore, the researchers are looking for natural plant products and more effective drugs as alternative materials which could be used to treat this disease. Medical plants and natural herb extracts are widely used as alternative treatment for various parasitic diseases and considered to be safe and to have low toxicity compared to synthetic drugs [12]. In recent years, silver nanoparticles and nanoparticles synthesized from plants have been considered by many researchers to treat various diseases, including parasitic diseases [13–15]. Nanoparticles are synthesized by physical, chemical, and biological methods. Researchers have always considered the synthesis of nanoparticles biologically using plant extracts. Plant extracts due to compounds such as alkaloids, terpenoids, and flavonoids can reduce silver ions to nanosilver metal and stabilize them. They also reduce the toxic effects of nanoparticles. Therefore, the biological method can be a better option compared to physical and chemical methods due to their ease of synthesis and environmental compatibility [16].

The leaf and fruit extracts of *Sambucus ebulus* (*S. ebulus*) contain compounds such as carbonyls, flavonoids, anthocyanins, and vitamin C, which have unique properties in the treatment of inflammation, gastric ulcer, cancer, etc. [17–19]. According to the literature review, this plant presented antiparasitic properties when tested against *Toxoplasma*, *Giardia* [20], hydatid cyst [21], and *Leishmania* [22].

Photochemical studies on *Feijoa sellowiana* (*F. sellowiana*) have shown that the leaves of this plant contain catechin derivatives such as epicatechin, gallocatechin, and epigallocatechin [23]. Numerous pharmacological studies of this plant have proven its antiparasitic, antimicrobial [24], and anticancer properties [25].

Due to the clinical importance of *T. gondii*, drug resistance and side effects of synthetic drugs, and that the anti-toxoplasmic effects of nanosilver of *S. ebulus* and *F. sellowiana* have not been reported so far; the purpose of this study was to investigate the anti-toxoplasmic effects of two nanosilver *in vitro* and *in vivo.*

Materials and Methods

Ethics Statement

The project was done according to the institutional animal ethics guidelines which were approved by the ethic committee of Mazandaran University of Medical Sciences (MUMSEC) (Ethics No. IR.MAZUMS.REC.1399.730).

Preparation of *F. sellowiana* Fruit Extract

F. sellowiana fruit was collected in early November 2020 from Ramsar city in Mazandaran province, northern Iran, and registered in the herbarium of Pharmaceutical Sciences Research Center, Mazandaran University of Medical Sciences, Sari, Iran.

To prepare the extract, the fruits were dried after cutting relatively thin in the oven on the first day at 45 °C and the second day at 40 °C. The efficiency of the extraction process was 26%.

Preparation of *S. ebulus* Fruit Extract

The fruit of *S. ebulus* was collected from Ghoroq village in near Sari city, and registered in the herbarium of Ghaemshahr Azad University. The fruits were then arranged on a drying rack to air dry over 3 weeks. The maceration process using methanol solvent extracted the powdered plant. Approximately 100 g of the fruit was mixed with about 250 mL of methanol, and the set was incubated for 24 h. The next day, methanol was discarded and new methanol was added. This process was repeated for 3 days. Solvents were evaporated *in vacuo* at 35 °C. Finally, the extract was freeze-dried (lyophilized) and the yield was 29 g.

Synthesis of Silver Nanoparticles

25 mg of *F. sellowiana* fruit extract was diluted in 25 mL of distilled deionized water (the pH of the solution was adjusted to 10). This solution was added to 25 of silver nitrate (4 mM) solution at 65 °C and under vigorous stirring for 30 min. The occurrence of dark brown color in the solution indicated the formation of Ag nanoparticles. Finally, the prepared nanoparticles were centrifuged at 14,000 rpm for 15 min and washed three times with methanol and distilled deionized water. Dried powder of silver nanoparticles was obtained by setting in an oven at 60 °C for 24 h for further characterization [16].

25 mg of *S. ebulus* fruit extract was diluted in 25 mL of distilled deionized water extract (the pH of the solution was adjusted to 10) and added to the aqueous solution of

$AgNO_3$ (25 mL; 20 mM) under vigorous stirring for 2 h. After 2 h, the light yellow colored mixture changed to dark brown, an evidence for the preparation of silver nanoparticles. The reduced mixture was centrifuged at 14,000 rpm for 15 min and washed three times with methanol and distilled deionized water. Dried powder of silver nanoparticles was obtained by setting in an oven at 60 °C for 24 h for further characterization [26].

Host Cells and Parasite Strain

Vero cells (ATCC No. CCL-81) were cultured in a RPMI-enriched cell culture medium with 10% FBS, 100 µg/mL penicillin, and streptomycin (Sigma, USA) in a sterile culture flask and finally preserved at 37 °C in 5% carbon dioxide for all *in vitro* assays.

The *T. gondii* RH strain was supplied from the Toxoplasmosis Research Center (TRC) in Mazandaran University of Medical Sciences, Sari, Iran. Tachyzoites were maintained by intraperitoneal passages (IP) in female BALB/c mice (6–8 weeks, 25 g, female) in the Toxoplasmosis Research Center (TRC), Mazandaran University of Medical Science, Sari, Iran.

To this end, after 3–4 days, *T. gondii* tachyzoites were collected from the peritoneal cavities of the infected mice by the IP injection of 5 mL of phosphate-buffered saline (PBS), in addition to penicillin (100 U/mL) and streptomycin (100 µg/mL), for *in vitro* assays at a pH of 7.2.

Toxicity Survey of Different Concentrations of Silver Nanoparticles by MTT Assay

For toxicity survey, 180 µL of RPMI medium with 10% FBS containing 2×10^4 cells/well from Vero cell line was added to each 96-well plate and incubated at 37 °C and 5% CO_2 for 24 h. Subsequently, the cells were treated with *Sambucus ebulus* (Ag-NPs-*S. ebulus*) and *Feijoa sellowiana* (Ag-NPs-*F. sellowiana*) fruit extracts and pyrimethamine (Sigma-Aldrich, USA) incubated for 24 h at 37 °C at the concentrations of 0.5, 1, 2, 5, 10, 20, 40 µg/mL. The cells designated as controls were treated with RPMI 1640 medium that contained 10% FBS. Finally, tetrazolium salt colorimetric (MTT) was used to evaluate the viabilities of the Vero cells [25, 26]. For IC_{50}, Vero cells at concentrations of 2×10^4 Vero cells/mL, suspended in RPMI supplemented with 10% FBS, were seeded in 96-well plates. After 24 h of seeding, the cells were infected with the RH strain of *T. gondii* (2×10^5 tachyzoites/mL) and placed in a 37 °C incubator maintained at 5% CO_2 for 24 h. After that, the cells were exposed to Ag-NPs-*S. ebulus* and Ag-NPs-*F. sellowiana* and pyrimethamine for 24 h at the concentrations of 0.5–40 µg/mL; the culture medium was used as a negative control. The next day, the viabilities of the *T. gondii*-infected Vero cells were evaluated by using MTT assay. Then, the 50% inhibitory concentrations (IC_{50s}) were calculated using the Graph Pad Prism 6.0 software [27].

Investigation of the Effects of Silver Nanoparticles on Invasion and Replication of Parasites, Determination of Infection Index and Proliferation Index

Vero cells (2×10^4 cells/well/200 µL) were cultured on 13 mm round glass slides 24 cell culture plate. After 72 h of incubation in RPMI medium with 10% FBS at 37 °C and 5% CO_2, the cells were infected in a 10:1 ratio. Then, the culture medium was changed after 3 h to remove extracellular parasites. The cells were treated with the Ag-NPs-*F. sellowiana*, Ag-NPs-*S. ebulus* extract nanoparticles and pyrimethamine at concentrations of 20.93 µg/mL, 13.99 µg/mL and 2.93 µg/mL, respectively. In addition, the controls cells were subjected to infection without any subsequent treatment.

After 24 h, the slides containing the cells adhered to the wells were washed twice with cold PBS and fixed with 10% formalin buffer. In the next, the slides were stained with 1% toluidine blue for 10 s. Next, the coverslips were counted under a light microscope (Nikon, Japan) in order to determine the infection index (i.e., number of infected cells per 100 examined cells) and parasite intracellular proliferation (i.e., the total number of tachyzoites per 100 examined cells) [28].

In Vivo Evaluation of Silver Nanoparticles on *T. gondii* Infections

Six-week-old female Balb/c mice weighing 20–22 g were used for this experiment in the acute phase. All experimental animals were housed in cages under standard laboratory conditions with an average temperature of 20–25 °C, humidity ($60\pm10\%$), light (12 h per day), and were given drinking water and a regular mouse diet. Initially, for controlling the side effects of the drugs, a preliminary experiment was done on mice receiving the maximum dose at which no mortality or clinically significant toxicity was observed.

Survival Study

In this study, female BALB/c mice were categorized into six groups ($n=6$). To develop the acute experimental toxoplasmosis model, mice were IP with 1×10^3 tachyzoites of *T. gondii* (the RH strain) into six main groups, including non-infected mice (group I), PBS (group II), Ag-NPs-*F. sellowiana* extract (group III), Ag-NPs-*S. ebulus* extract (group IV), Ag-NPs-*S. ebulus* + pyrimethamine (group V), pyrimethamine (group VI). These agents were injected 6 h after challenge at regular 24-h intervals for 4 days as follows:

non-infected mice (no injected material), PBS (0.5 mL), Ag-NPs-*F. sellowiana* extract (40 mg/kg/day), Ag-NPs-*S. ebulus* extract (40 mg/kg/day), Ag-NPs-*S. ebulus* + pyrimethamine (40 mg/kg/day of each material), pyrimethamine (40 mg/kg/day). The survival periods of all mice were monitored daily.

Statistical Analysis

The data were analyzed in Graph Pad Prism software, version 6.0 (GraphPad Software, Inc., San Diego, CA, USA). Differences between the test and control groups (positive and negative) were analyzed using repeated measures ANOVA test. Also, the Kaplan–Meier method was used to compare the survival time between the test and control groups. Kaplan–Meier survival curves were investigated with the log-rank tests. *P*-values of less than 0.05 were considered statistically significant.

Results

HPLC Analysis

The phenolic compounds present in the extracts were analyzed by the HPLC. *F. sellowiana* extract contained five phenolic compounds including catechin, gallic acid, caffeic acid, rutin, and *p*-coumaric acid where catechin was the main component (188.5 mg/g of extract) [16]. *S. ebulus* extract contained 6 phenolic compounds including caffeic acid, catechin, rutin, *p*-coumaric acid, ferulic acid and gallic acid where rutin was the main component (13.6 mg/g of extract). HPLC profiles and precise contents of extracts have been reported elsewhere [26]. These phenolic compounds are the most important components in the plants for formation of nanoparticles. It has been suggested that the mechanism of NPs synthesis in presence of a flavonoid is related to the ortho-hydroxyl groups. The suggested mechanism for the formation of nanoparticles in the presence of different phenolic acids: gallic acid [29], rutin [26, 30], chlorogenic acid [15] and catechin [31] have been reported recently.

Characterization of Silver Nanoparticles

The size and morphology of the green synthesized silver nanoparticles using *S. ebulus* fruit extract were analyzed by field emission scanning electron microscopy (FE-SEM). Transmission electron microscopy (TEM) analysis was used to determine particle size distributions and morphological characteristics of AgNPs. The TEM image showed that the morphology of the synthesized AgNPs was spherical and oval with particle size about 35–50 nm [26]. SEM showed well-defined spherical AgNPs for green synthesized silver nanoparticles using *F. sellowiana* fruit extract. TEM image shows that AgNPs were in the size range of 15–30 nm and crystallized in a spherical shape [16].

Cell Viability Assays *In Vitro*

In order to evaluate the possible cytotoxicity of Ag-NPs-*S. ebulus* and Ag-NPs-*F. sellowiana*, pyrimethamine in Vero cells, the MTT assay was performed. Ag-NPs-*S. ebulus,* Ag-NPs-*F. sellowiana* did not change the cellular viability in Vero Cells when compared to untreated cells ($P < 0.05$; Fig. 1). Vero cell treatment with pyrimethamine showed a significant reduction in the cell viability at all concentrations of 0.5–40 µg/mL, when compared to untreated cells ($P < 0.05$; Fig. 1).

Figure 1 presents cellular viability in Vero cells treated with Ag-NPs-*S. ebulus* and Ag-NPs-*F. sellowiana*, pyrimethamine. According to the results presented in Fig. 1, Ag-NPs-*S. ebulus* and Ag-NPs-*F. sellowiana* were less cytotoxic than pyrimethamine. In addition, based on the amount of IC_{50} obtained for, the concentrations of 13.99 µg/mL, 20.93 µg/mL and 2.93 µg/mL were chosen for Ag-NPs-*S. ebulus* and Ag-NPs-*F. sellowiana*, pyrimethamine, respectively, to continue the further studies.

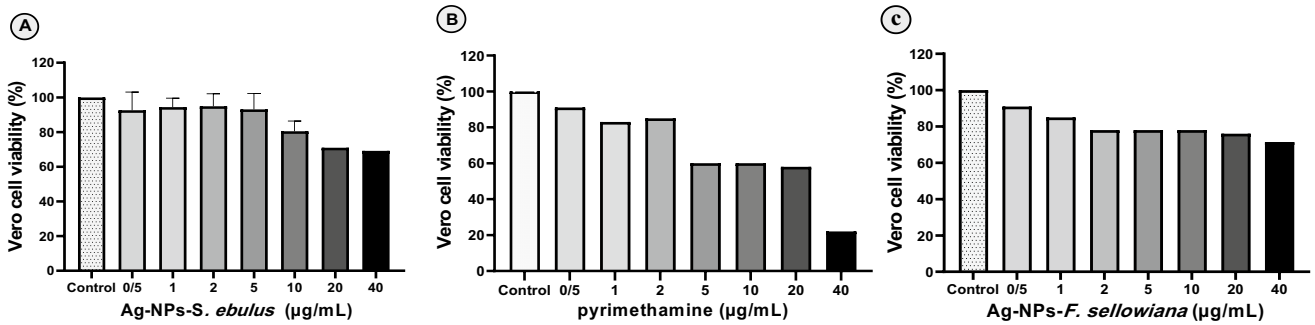

Fig. 1 Cellular viability determined by MTT assay in Vero cells treated with Ag-NPs-*F. sellowiana, Ag*-NPs-*S. ebulus* and pyrimethamine in several concentrations ranging from 0.5 to 40 µg/mL. Significant differences in relation to untreated cells (control) (*$P < 0.05$)

Invasion and Replication Assays *In Vitro*

The results of the effects of silver nanoparticles of Ag-NPs-*F. sellowiana* and Ag-NPs-*S. ebulus* extracts on parasite invasion show that Ag-NPs-*F. sellowiana* and Ag-NPs-*S. ebulus* had a similar effect to pyrimethamine (positive control) and significantly reduced infection index (67.15 ± 3.03, 65.65 ± 2.33, and 64.3 ± 1.15, respectively) when compared to the untreated cells (76.5 ± 1.5) ($P < 0.05$).

Also, the results of the effects of Ag-NPs-*F. sellowiana* and Ag-NPs-*S. ebulus* extracts on parasite proliferation showed that Ag-NPs-*F. sellowiana* and Ag-NPs-*S. ebulus* acted almost similar to pyrimethamine and significantly reduced parasite proliferation ($191 \pm 9 \pm 5.29$, $156 \pm 4 \pm 12.02$, and $135.5 \pm 5.5 \pm 5$, respectively) when compared to the untreated cells (360 ± 10) ($P < 0.05$), but in comparison between Ag-NPs-*S. ebulus* and Ag-NPs-*F. sellowiana*, Ag-NPs-*S. ebulus* inhibited proliferation of the parasite was more effective (Fig. 2).

Survival Rates *In Vivo*

Non-infected mice (normal control) were alive until the last day of the experiment. The survival rate in PBS (negative control) group started a declining trend from the sixth day post-infection and all mice died until the eighth day post-infection. Mice of other treatment groups started to die on the seventh day and all of them died until on the eleventh day (Fig. 3). Mice in the treatment groups of Ag-NPs-*S. ebulus* + Pyrimethamine and Pyrimethamine (positive control) showed a statistically higher survival rate compared to untreated infected control ($P < 0.0001$).

Discussion

Toxoplasmosis is a common infection in humans and animals and had clinical importance, especially in immunocompromised individuals and pregnant women. Pyrimethamine, sulfonamides, spiramycin, clindamycin, and tetracycline

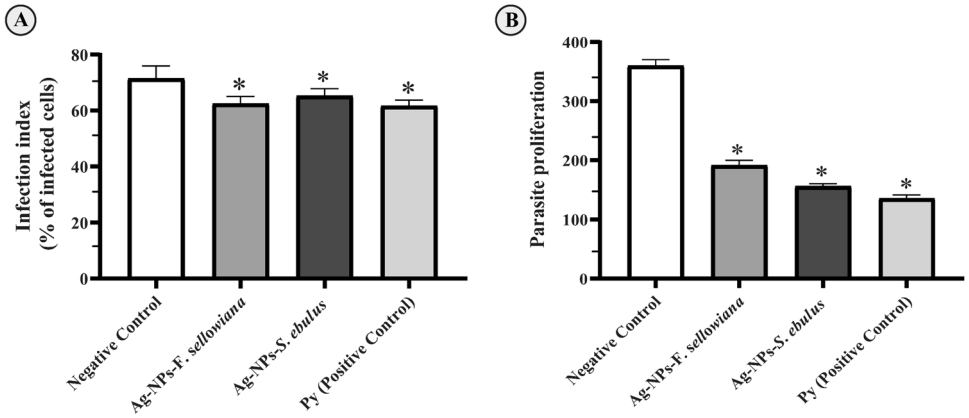

Fig. 2 **A** Infection index (% of infected cells) and; **B** parasite proliferation (total number of tachyzoites per 100 cells) in the Vero cells treated with Ag-NPs-*F. sellowiana* and Ag-NPs-*S. ebulus* as well as pyrimethamine at the concentration of 20.93 µg/mL, 13.99 µg/mL and 2.93 µg/mL, respectively, using blue toluidine staining. The control cells were infected, but not treated. Data of the two independents experiments performed in triplicates are presented as (mean ± SEM). *$P < 0.05$ compared with control

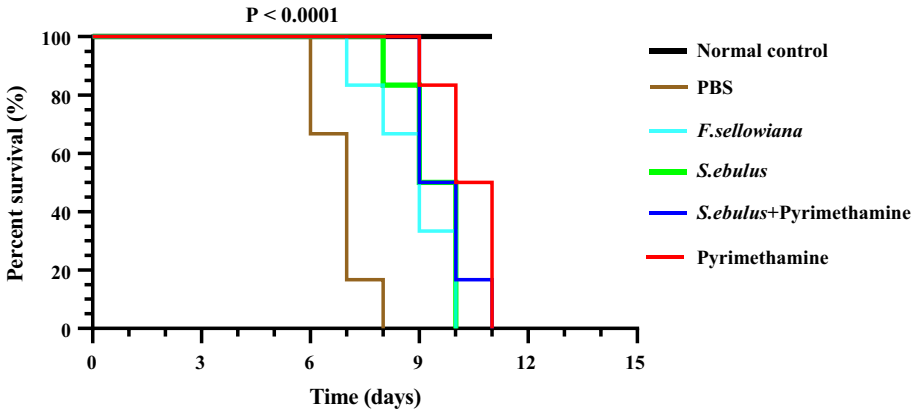

Fig. 3 Survival rate in the study groups including normal control (non-infected mice), PBS (negative control), silver nanoparticles using extract of *F. sellowiana*, silver nanoparticles using extract of *S. ebulus*, silver nanoparticles using extract of *S. ebulus* + pyrimethamine, pyrimethamine (positive control)

are drugs currently used to treat [32]. Due to the low effect of these drugs in eliminating the parasite and having side effects, especially in people with immune deficiency diseases like AIDS and patients on immune-suppressing drugs [33], producing appropriate drugs with minimal side effects is one of the priorities in replacing previous drugs by researchers. In recent years, nanoparticles and nanoparticles synthesized from plants have played an important role in the diagnosis and treatment of diseases. Several studies have shown that *F. sellowiana* and *S. ebulus* have inhibitory effects on the growth of parasites, viruses, fungi, and bacteria. However, no research has been done on the anti-parasitic effect of Ag-NP based on *F. sellowiana* and *S. ebulus* on *T. gondii*. Therefore, the aim of this study was to evaluate the anti-parasitic effects of Ag-NPs-*S. ebulus* and Ag-NPs-*F. sellowiana* plants on the growth of *T. gondii* tachyzoites *in vitro.*

In the current investigation, the results of viability assessment by MTT assay showed that treatment with Ag-NPs-*S. ebulus*, Ag-NPs-*F. sellowiana*, and pyrimethamine resulted in the reduction in cellular viability in a dose-dependent manner. According to the *in vitro* results, Ag-NPs-*S. ebulus* and Ag-NPs-*F. sellowiana* showed higher CC_{50} in Vero cells than pyrimethamine.

The results of the infection index showed that Ag-NPs-*F. sellowiana* and Ag-NPs-*S. ebulus* acted almost similarly to pyrimethamine. Although the result of Ag-NPs-*S. ebulus* was better than Ag-NPs-*F. sellowiana* in this test. The results of the amplification index showed that Ag-NPs-*F. sellowiana* and Ag-NPs-*S. ebulus* acted as the positive control, and Ag-NPs-*S. ebulus* had the greatest effect in preventing the parasite from reproducing. In general, the results showed that Ag-NPs-*S. ebulus* had the greatest effect compared Ag-NPs-*F. sellowiana*. Daryani *et al*. evaluated the inhibitory effect of methanolic extract of *S. nigra* fruit and leaves on *T. gondii* tachyzoites; the results of this study showed that the extract of *S. nigra* fruit has a better lethality than the leaves of this extract. This study is consistent with the MTT results of our study in terms of the anti-parasitic effect [34]. The results of Ebrahimzadeh *et al*., showed that *F. sellowiana* leaf extract had the highest anti-toxoplasmic effect compared to the fruit extract of this plant and pyrimethamine. In *in vivo* investigation, mice treated with *F. sellowiana* extract had the longest survival time compared to other extracts [27]. Diego Vergara-Duque *et al*. investigated the effects of nanosilver on the morphology of *T. gondii* by fluorescence microscopy and scanning microscopy. The results showed changes in the structure of *T. gondii* oocysts and reduced half-life of this parasite [35].

Sambucus spp. was primarily used as an antiviral agent for colds, flu [36], and herpes virus [37] infections. Many researchers have also shown that *Sambucus* spp. has an immune system modification, antioxidants, and in traditional medicine, traditionally recommended as a remedy for diabetes [38, 39]. Among the chemicals, quercetin, anthocyanin, cyanidin-3-samobiozide, hemagglutinin protein agglutinin III (SNA-III), cyanogenic glycosides including sambunigrin, vibronic acid, vitamins A and C have been reported [38]. Numerous studies have shown that *F. sellowiana* has various biological properties such as antibacterial, antifungal [40] antioxidant [41, 42], nephroprotective [43], hepatoprotective [44], antidepressant [45], protective effect against testicular toxicity [46], anti-inflammatory and anti-nociceptive [47], It is rich in vitamin C, polyphenols, terpenes, tannins, flavonoids and steroidal saponins [40–42]. Polyphenol compounds, such as flavonoid, proanthocyanidin and ellagitannin in leaves and fruits, have been shown to prevent blood clotting and regulate blood pressure [48]. Zhang *et al*. showed that the activity and selectivity index of oxymatrine and matrine, the major alkaloids of legume sophora, were higher than spiramycin against the RH strain of HeLa cells infected with *T. gondii* [49].

Karimipour *et al*. conducted a study on the anti-toxoplasmic effects of silver nanoparticles synthesized with ginger extract *in vitro*. They studied the toxic effects of this extract on macrophage cells by MTT and evaluated the apoptotic potential of *T. gondii* by silver nanoparticles by flow cytometry technique. The results showed that silver nanoparticles synthesized with ginger led to apoptosis in about 55.22% of *T. gondii* tachyzoites. The results also showed that this extract has a lethal effect on *T. gondii* and induces apoptosis in this parasite [1].

Algmi *et al*. carried out a study entitled anti-toxoplasmic activity of silver nanoparticles synthesized with *Phoenix dactylifera*, and *Ziziphus spina-christi*. The results showed that silver nanoparticles synthesized by inhibiting enzymatic activity reduced liver damage caused by toxoplasmosis. Histopathological tests also showed significant changes in infected mice compared to the control group in the pretreatment test of silver nanoparticles and plant extracts. These changes included reduction in hepatocyte polymorphism and reduction in degeneration as well as the reduction in TGFB and N-FKB activities. The researchers concluded that silver nanoparticles synthesized with extracts of these plants could be an alternative and useful treatment option for *Toxoplasma* infection [50].

Quan *et al*. in a study showed that silver nanoparticles induce mitochondrial apoptosis in human cells through dysregulation and autophagy. Numerous studies showed that silver nanoparticles induce cytotoxic responses, including increased production of toxic oxygen radicals (ROS) and apoptosis, DNA damage, and pre-inflammation at the cellular and molecular levels. Due to the cytotoxicity of silver nanoparticles, this particle induces size-dependent cytotoxicity and reduces cell viability in a concentration-dependent manner. The strong oxidative activity of nanoparticles

causes the release of silver ions which induces cytotoxicity, gene toxicity, immunological response, and even cell death [51]. Cerutti *et al*. evaluated the effect of nanoparticles on bradyzoites of *T. gondii*. The results showed that nanoparticles eliminate bradyzoites and reduce the load of infection. These findings confirm the anti-parasitic potential of nanoparticles [52].

Said *et al*. measured the anti-parasitic effect of silver nanoparticles, chitosan, and curcumin as anti-Giardiasis. The results of these researches showed that silver nanoparticles have a very good performance and efficiency for the treatment of giardiasis *in vitro* [53].

Allahverdiyev *et al*. also evaluated the anti-chemical effect of silver nanoparticles on *Leishmania tropica*. The results of these researchers showed that silver nanoparticles have an inhibitory effect on the growth of promastigotes and *leishmania* amastigotes, and on the other hand, the effects of silver nanoparticles on parasite growth increased by UV radiation [54].

Also, treatment of experimental mice with Ag-NPs-*S. ebulus* + pyrimethamine for 4 days after infection with 1×10^3 tachyzoites of the *T. gondii* RH strain statistically increased their survival rates when compared to pyrimethamine were not statistically significant, indicating that the activity of Ag-NPs-*S. ebulus* + pyrimethamine cannot increase survival time compared to pyrimethamine alone. Moreover, Ag-NPs-*S. ebulus* increased the survival rate of the mice compared to that of the mice in the PBS group interestingly. Rahimi-Esboei showed that *S. ebulus* extract at the concentration of 100 mg/mL for 60 min had the most anti-*Giardia* activity compared to lower concentrations [20].

Gholami *et al*. demonstrated that *S. ebulus* extract displayed a high scolicidal activity *in vitro* ($P < 0.0001$) [21]. As mentioned above, silver nanoparticles synthesized with *F. sellowiana* and *S. ebulus* have anti-parasitic effects separately. On the other hand, the extracts of *F. sellowiana* and *S. ebulus* plants due to the presence of compounds such as flavonoids and phenol through the hydroxyl and carboxyl groups in phenolic compounds, are attached to metals and therefore reduce the toxicity of silver nanoparticles and make it a useful and effective compound. In this study, by adding and synthesizing silver nanoparticles with *F. sellowiana* and *S. ebulus*, we sought the synergistic and anti-parasitic effect of this compound while reducing the toxicity of nanoparticles.

Conclusion

Based on the results obtained in the current study, it was found that Ag-NPs-*S. ebulus* and Ag-NPs-*F. sellowiana* have a significant growth effect on *T. gondii in vitro* and *in vivo*. Also, in comparison between these two nanoparticles, the results showed that the nanoparticles of *S. ebulus* fruit extract have a more lethal effect on the parasite than silver nanoparticles of *F. sellowiana*. It is suggested that researchers in future studies investigate the induction of *toxoplasma*-infected cell apoptosis using nanoparticles.

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Author Contributions SAH, MAE, AD and MS were involved in designing the research. AH, MS carried out the experiments. MS, SAH and TN drafted the article. SAH and AD critically revised the manuscript. All authors read and approved the final manuscript.

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Declarations

Conflict of Interest The authors declare no conflict of interest.

Ethical Approval The project was done according to the institutional animal ethics guidelines which were approved by the ethic 301 committee of Mazandaran University of Medical Sciences (MUMSEC) (Ethics No. 302 IR.MAZUMS.REC.1399.730).

Consent of Publication Not applicable.

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