Experimental Parasitology 211 (2020) 107853

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

# Biogenic silver nanoparticles reduce adherence, infection, and proliferation of toxoplasma gondii RH strain in HeLa cells without inflammatory mediators induction

Laís Fernanda Machado[a], Raquel Arruda Sanfelice[a], Larissa Rodrigues Bosqui[a], João Paulo Assolini[a], Sara Scandorieiro[b], Italmar Teodorico Navarro[c], Allan Henrique Depieri Cataneo[d], Pryscilla Fanini Wowk[d], Gerson Nakazato[b], Juliano Bordignon[d], Wander Rogerio Pavanelli[a], Ivete Conchon-Costa[a], Idessania Nazareth Costa[a,*]

[a] Departamento de Patologia Experimental – Laboratório de Imunoparasitologia Das Doenças Negligenciadas e Câncer. Universidade Estadual de Londrina, 86057-970, Londrina, Paraná, Brazil
[b] Departamento de Microbiologia – Laboratório de Bacteriologia Básica e Aplicada. Universidade Estadual de Londrina, Paraná, Brazil
[c] Departamento de Medicina Veterinária Preventiva – Laboratório de Zoonoses e Saúde Pública. Universidade Estadual de Londrina, PR, Brazil
[d] Laboratório de Virologia Molecular. Instituto Carlos Chagas, ICC/ Fiocruz, Curitiba, Paraná, Brazil

ARTICLE INFO

*Keywords:*
Silver nanoparticles
Treatment
Toxoplasmosis
HeLa cells

ABSTRACT

The highlights of biogenic silver nanoparticles (AgNp-Bio) include low toxicity – depending on size and concentration – and efficient antiparasitic activity. Therefore, the objective of this study was to assess the action of the AgNp-Bio on HeLa cells in an infection with strain of RH *Toxoplasma gondii*. Firstly, we performed a cellular viability test and characterized the AgNp-Bio to proceed with the infection of HeLa cells with *T. gondii* to be treated using AgNp-Bio or conventional drugs. Subsequently, we determined the level of standard cytokines Th1/Th2 as well as the content of nitric oxide (NO) and reactive oxygen species (ROS). Results indicated a mean size of 69 nm in diameter for the AgNp-Bio and obtained a dose-dependent toxicity. In addition, the concentrations of 3 and 6 μM promoted a significant decrease in adherence, infection, and intracellular proliferation. We also found lower IL-8 and production of inflammatory mediators. Thus, the nanoparticles reduced the adherence, infection, and proliferation of ROS and NO, in addition to immunomodulating the IL-8. Therefore, our data proved relevant to introduce a promising therapeutic alternative to toxoplasmosis.

## 1. Introduction

Toxoplasmosis is an infection caused by the compulsory intracellular protozoan *Toxoplasma gondii* and represents a public health issue that affects around 30% to 50% of the world population (Montazeri et al., 2017). In 90% of the causes, the infection develops without symptoms or is benign (Robert-Gangneux and Dardé, 2012; Krueger et al., 2014). However, it is possible for immunocompromised individuals to develop a severe clinical condition (Sutterland et al., 2015; Atilla et al., 2015; Alday et al., 2017). The organism does not tolerate well the association of pyrimethamine and sulfadiazine used to treat symptomatic cases, which interact indistinctly with biochemical processes of both the parasite and the host (Sepúlveda-Arias et al., 2014) generating adverse effects, such as the suppression of bone marrow, which may lead to megaloblastic anemia, leukopenia, and granulocytopenia (Petersen, 2007).

In this context, investment has focused on the study of nanomaterial, whose actions involve carrying drugs, decreasing toxicity, modulating pharmacokinetics, and increasing bioavailability, in addition to releasing the drug directly into the specific target (Khalil et al., 2013; Torres-Sangiao et al., 2016). Silver nanoparticles (AgNp) are commonly used for a variety of medical applications, especially for their anti-inflammatory and antimicrobial activities (Pourali and Yahyaei, 2016) (Shrivastava et al., 2007; Adair et al., 2010; Scandorieiro et al., 2016) found in Gram-positive and Gram-negative bacteria (Shrivastava et al., 2007; Scandorieiro et al., 2016; Durán et al., 2016), filamentous fungi (Sanguiñedo et al., 2018), some types of viruses (Park et al., 2018; Sharma et al., 2019), and protozoa of the genus Leishmania

* Corresponding author. ,. Department of Pathological Sciences Center of Biological Sciences State University of Londrina, CEP, 86057-970, Londrina, PR, Brazil.
*E-mail address:* idessania@uel.br (I.N. Costa).

https://doi.org/10.1016/j.exppara.2020.107853
Received 24 October 2019; Received in revised form 17 January 2020; Accepted 8 February 2020
Available online 12 February 2020
0014-4894/ 

(Allahverdiyev et al., 2011; Fanti et al., 2018; Isaac-Marquez et al., 2018). Furthermore, this metallic nanoparticle has remarkable properties, such as accumulation in tissues – perhaps even reaching cysts of *T. gondii* (Adeyemi and Sulaiman, 2015) – and the production of reactive oxygen species (ROS), which are able to kill infectious agents (Butkus et al., 2004; Bhardwaj et al., 2012).

Metallic nanoparticles are known as promising anti-Toxoplasma agents (Gaafar et al., 2014; Assolini et al., 2017; Adeymi et al., 2017; Adeyemi et al., 2018), but studies on these protozoa and biogenically synthetized silver nanoparticles in human cells are yet to be developed. Therefore, silver nanoparticles have an attractive versatility as antiparasitic agents, particularly against *T. gondii*, which has encompassed a pioneer study on the use of biogenic silver nanoparticles as therapeutic alternative to toxoplasmosis. Thus, this paper assesses the antiproliferative and immunological actions of AgNp-Bio in HeLa cells in the context of an experimental infection with the *Toxoplasma gondii* RH strain.

## 2. Material and methods

### 2.1. HeLa cells culture

HeLa cells are a continuous cellular lineage that divides indefinitely – reason why they are called “immortal” – in addition to being the first human cells to have survived in vitro (Jones et al., 1971). Despite their cancerous nature, they share many basic characteristics with normal cells, such as the production of proteins, intracellular communication, and susceptibility to infection, which enables their use to study the basic functions of all human cells (Lucey et al., 2009).

We grown the cells in 75 $cm^2$ culture flasks (Ciencor Scientific, Brazil) in a Dulbecco Modified Eagle Medium (DMEM) (Gibco by life technologies) supplemented with 2% inactivated fetal bovine serum (FBS) (Sigma-Aldrich), 1% antibiotics (10,000U/mL penicillin and 10 mg/mL streptomycin solution) (Cultilab, Brazil), Lglutamine, sodium pyruvate, and 2-mercaptoethanol (complete medium for HeLa – MCH). Cell cultures were maintained in an incubator with 5% $CO_2$ at 37 °C. We used cells in the passage 21 for the experimental assays as well as to maintain the *T. gondii* strains.

### 2.2. Maintenance of T. gondii RH strain

Tachyzoites of the *T. gondii* RH strain were provided by Prof. Italmar Teodorico Navarro, State University of Londrina, and maintained in the peritoneal cavity of 60-day-old Swiss mice at intervals of 48–72 h. We collected the peritoneal exudates derived from mice in sterile PBS upon peritoneal massage and filtered to obtain the tachyzoite forms. This study was approved by the Ethics Committee for Animal Experimentation of the State University of Londrina 88/2017/CEUA.

### 2.3. Silver nanoparticles

AgNp-Bio were granted by Prof. Dr. Gerson Nakazato – Laboratory of Basic and Applied Bacteriology of the State University of Londrina (Paraná-Brazil) – and prepared according to a previously established method (Durán et al., 2005). Briefly, we obtained the AgNp-Bio after reducing the silver nitrate using F. oxysporum, strain 551, from the culture collection of the Molecular Genetics Laboratory of ESALQ-USP (Piracicaba, SãoPaulo, Brazil). We cultivated F. oxysporum in media containing 0.5% (w/v) yeast extract (Neogen), 2% (w/v) malt extract (Neogen), 2% (w/v) agar (Neogen), and distilled water at 28 °C for seven days. After the growth, the fungal biomass was added to distilled water at 0.1 g/mL and incubated at 28 °C for 72 h.

Afterwards, the solution components were separated by filtration. $AgNO_3$ (Nuclear) at 1 mM was added to fungal-free solution, and the system was incubated for several hours at 28 °C in the absence of light. Periodically, we removed aliquots of the solution system and measured the absorptions using an ultraviolet–visible spectrophotometry (VarianCary50Probe); the peak at 440 nm corresponded to the surface plasmon resonance of silver nanoparticles. Particle size distribution was measured through a nanoparticle tracking analysis (NTA) of 1 mM AgNp-Bio solution diluted in ultrapure water (1:600) using NanoSight LM10 system (Malvern Instruments Ltd, UK). We performed the analyses using default settings according to the manufacturer's protocol (Nanosight Software version 3.1).

### 2.4. Compounds dilution

We performed the dilution of the biogenic silver nanoparticles (concentration of 10 mM) according to the following steps: 100 μL of the AgNp-Bio solution added with 900 μL of DMEM, resulting in the concentration of 1 mM; serial dilution to obtain the concentrations of 3 and 6 μM. The dilution to reach the concentrations of 50 and 25 μg/mL of sulfadiazine and pyrimethamine, respectively, was performed according to the descriptions of Sanfelice et al., (2017).

### 2.5. HeLa cell viability by MTT assay

We assessed the viability of HeLa cells after treatment with AgNp-Bio based on mitochondrial oxidation through a colorimetric MTT assay (3-[4,5-dimethylthiazol-2-yl] −2,5-diphenyltetrazolium bromide) (Sigma Chemical Co., Brazil) (Mosmann, 1983). HeLa cells were grown in 96-well plates ($3x10^4$ cells/well/200 μL) for 24 h in MCH at 37 °C and 5% $CO_2$. After this period, the cells received a 24-h treatment using the AgNp-Bio (3, 6, 9, and 12 μM); sulfadiazine, and pyrimethamine combination (50 and 25 μg/mL, respectively) used as positive control; cells that received only MCH were used as the negative control. After the treatment, we removed the medium and incubated the cells using a MTT solution (5 mg/mL) for 3 h under the same growth conditions.

The formazan crystals were solubilized in 10% sodium dodecyl sulfate (SDS) and 50% dimethyl formamide (DMF), and after a 30-min incubation, absorbance reading occurred at 570 nm using a plate reader (TP Reader, Thermo Plate). The results were expressed as percentage of MTT reduction relative to the control group, calculated with the following formula: viable cells (%) = (Abs of treated cells/Abs of untreated cells) X 100.

### 2.6. Experimental infection

We performed an experimental model of infection with pretreatment in order to assess the effect of the AgNp-Bio on adherence and an experimental model of invasion and proliferation of *T. gondii* in HeLa cells by applying a post-treatment experimental model.

We carried out the pretreatment model in 24-well plates containing culture of HeLa cells ($1x10^5$) adhered to 13 mm round coverslips (Ciencor Scientific, Brazil). These cells were pre-treated by associating sulfadiazine and pyrimethamine (50 and 25 μg/mL, respectively) (positive control) and AgNp-Bio at the concentrations of 3 and 6 μM for 24 h at 37 °C with 5% of $CO_2$. The cells treated only with DMEM were considered negative control.

After the treatment period, the cells were subjected to a 4%-paraformaldehyde fixation in phosphate buffered saline solution (PBS) for 30 min. Subsequently, the cells were washed to remove the excess of the fastener. Each pit was added with the *T. gondii* tachyzoites ($5x10^5$), which were subjected to a 4%-paraformaldehyde fixation after 3 h of infection for 30 min, and the glass slides were dyed with tolonium chloride for 5 s (Castanheira et al., 2015).

For the post-treatment model, the HeLa cells ($1x10^5$) maintained in 24-well plates containing 13 mm round coverslips (Ciencor Scientific, Brazil) were infected with $5x10^5$ tachyzoites of the *T. gondii* RH strain. After 3 h of infection, the cells were washed and treated with the association of sulfadiazine and pyrimethamine (50 and 25 μg/mL), respectively, and AgNp-Bio at the concentrations of 3 and 6 μM for 24 h.

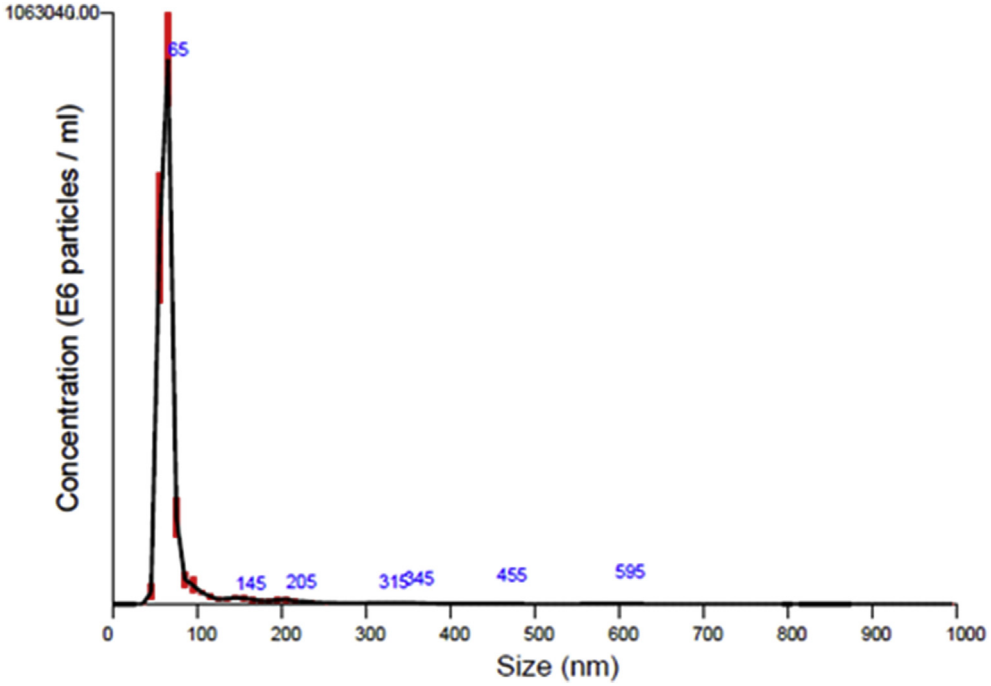


**Fig. 1.** AgNp-Bio size distribution characterization. Distribution of the size of the particles detected through the nanoparticles tracking analysis. Data represent the size distribution showing a mode of 65 nm of an independent analysis.

After the required time for both treatment models and upon coloring with tolonium chloride at 1% (Sigma Chemical Co.), we assembled the glass slides for light microscope analysis (e100, Nikon – led).

We analyzed the cells through light microscopy with immersion magnification to verify the parameters of adherence and number of tachyzoites adhered per cell, as well as the infection index (number of infected cells every 200 cells examined) and parasite intracellular proliferation (total number of parasites every 200 cells examined).

The percentages of inhibition of infection with *T. gondii* as well as inhibition of intracellular proliferation of *T. gondii* were calculated according to the following: mean index of infection or intracellular proliferation analyzed in non-treated cells, corresponding to 100% of the index of infection or intracellular proliferation. The inhibition percentages of these parameters undergoing treatments with AgNp-Bio were calculated by subtracting the percent values obtained for treated cells from those obtained for non-treated cells (Barbosa et al., 2012). The supernatant of the cells was collected and stored at −80 °C for further dosage of cytokines and nitric acid.

### 2.7. Determination of nitrite as estimative of NO levels

We applied the Griess method to determine nitric oxide (NO), according to the experiments previously conducted by our group (Tomiotto-Pellissier et al., 2018). Briefly, supernatant aliquots (60 μL) from the experimental infection supernatants were submitted to a 2-min centrifugation at 5000 rpm, and a volume of 50 μL of the supernatant was recovered and added with 50 μL of Griess reagent (1% sulfanilamide and 0,1% of naphthyl ethylenediamine dihydrochloride in orthophosphoric acid ($H_3PO_4$) 5%). After 10 min incubation at room temperature, the samples were placed in 96-well microplates. A calibration curve was generated using dilutions of $NaNO_2$, and the absorbance was determined at 550 nm on microplate reader (Multiskan GO, Thermo Scientific).

### 2.8. Estimative determination of the levels of reactive oxygen species (ROS)

We performed the test according to the experiments previously conducted by our group (Tomiotto-Pellissier et al., 2018). Therefore, the HeLa cells ($1x10^5$) were maintained in 24-well plates containing 13 mm round coverslips (Ciencor Scientific, Brazil) infected with $5x10^5$ tachyzoites of the *T. gondii* RH strain. Aiming at assessing ROS production, the HeLa cells ($1x10^5$) infected with $5x10^5$ tachyzoites of the *T. gondii* HR strain and treated for 24 h with AgNp-Bio at the concentrations of 3 and 6 μM were washed with PBS (pH 7.4) and charged with 10pM of a diacetate probe of 20, 70-dichlorofluorescein (H2DCFDA) (Sigma, St. Louis, MO, USA) diluted in DMSO and incubated in the dark (HeLa 30min, 37 °C, $CO_2$ at 5%).

We measured the reactive oxygen species (ROS) as the increase in fluorescence resulting from the conversion of the non-fluorescent dye into highly fluorescent 20,70-dichlorofluorescein with an excitation wavelength of 488 nm and an emission wavelength of 530 nm using a microplate fluorescence reader (Victor X3, PerkinElmer).

### 2.9. Determination of cytokine levels

We analyzed the levels of the cytokines IL-1β, TNF-α, IL-12p70, IL-8, IL-6, IL-10 present in the supernatant of the infected cells through a Cytometric Bead Array (CBA) according to the instructions in the kit (BD CBA Human Th1/Th2) using the equipment FACS Canto II by Becton Dickinson. The analysis of the cytometry data was performed on the software FCAP Array, v1.0.1, by Soft Flow Hungary Ltd. and generated the concentrations of the cytokines in pg/mL.

### 2.10. Statistical analysis

All data represent the mean and standard deviation of three independent experiments performed in triplicate. Differences between treatments and controls were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test, using GraphPad Prism software 5.0 (GraphPad Software, Inc., San Diego, CA. USA). Statistical significance was considered when $P \leq 0.05$.

## 3. Results

### 3.1. Characterization of biogenic silver nanoparticles

A recent analysis on the production of biogenic silver nanoparticles (AgNp-Bio) by Fusarium oxysporum revealed that the silver nanoparticles coexist with chloride nanoparticles (AgCl) – named Ag@AgCl (Durán et al., 2016; Picoli et al., 2016). However, our study used the term AgNp-Bio to which our nanoparticle tracking analysis (NTA) revealed a mean diameter of 69.0 nm (Fig. 1).

### 3.2. Treatment using biogenic silver nanoparticles (AgNp-Bio) has dose-dependent toxicity in HeLa cells

The treatment of the HeLa cells at the concentrations of 9 and 12 μM reduced cellular viability in 17% and 31%, respectively, in relation to the negative control. However, the concentrations of 3 and 6 μM for 24 h maintained the cells viable, thus justified as the concentrations selected for the subsequent experiments. Additionally, the positive control (association of sulfadiazine and pyrimethamine at the concentrations of 50 and 25 μg/mL, respectively) was not able to decrease cellular viability for 24 h (Fig. 2).

### 3.3. Biogenic silver nanoparticles reduce adherence, infection, and proliferation of tachyzoites in HeLa cells infected with T. gondii RH strain

The efficiency of the treatment using AgNp-Bio at the concentrations of 3 and 6 μM was evaluated according to the processes of adherence through pretreatment test and indices of intracellular infection and proliferation of the tachyzoites of *T. gondii* at the post-treatment stage.

We found in the pretreatment experiments a decrease of 51% and 66% in the number of cells with tachyzoites adhered upon treatment with AgNp-Bio at the concentrations of 3 and 6 μM, respectively (Fig. 3A) ($p < 0.0001$) and 26% in the treatment using conventional drugs ($p < 0.05$) in relation to the negative control. Regarding the amount of tachyzoites adhered per cell, we found a reduction of 67% and 76% in the treatment using AgNp-Bio (3 and 6 μM respectively) in relation to both the negative and positive controls ($p < 0.0001$) as well as a decrease of 42% in the treatment using conventional drugs in relation to the negative control ($p < 0.0001$) (Fig. 3B).

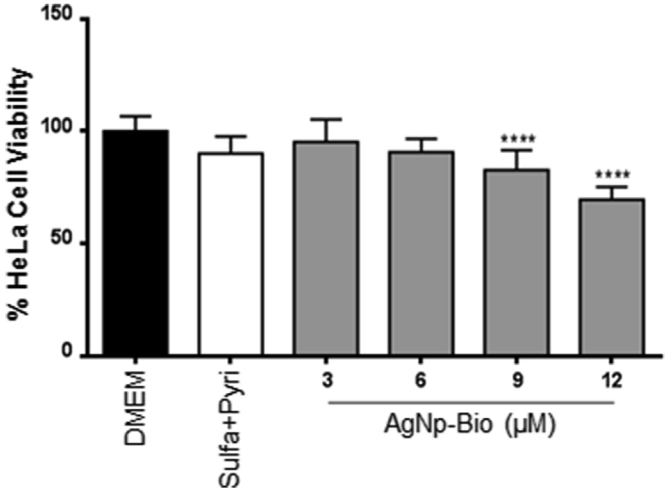


**Fig. 2.** The viability of HeLa cells after treatment with AgNp-Bio at the concentrations of 3 and 6 μM and the association of sulfadiazine and pyrimethamine (50 and 25 μg/mL respectively) for 24 h. Cells treated with DMEM were applied as negative control. ****Significantly different from the negative control ($p < 0.0001$). Three independent experiments were performed in triplicate (One – way ANOVA followed by Tukey test).

For the post-treatment experiments, the infection indices showed a decrease of 78% and 90% in the cells infected regarding the concentrations of 3 μM and 6 μM, respectively, in relation to the negative control ($p < 0.0001$). In turn, the treatment using the association of sulfadiazine and pyrimethamine was responsible for a reduction of 56% in relation to the control ($p < 0.0001$) (Fig. 3C).

Regarding the proliferation of tachyzoites in the HeLa cells, we detected a decrease of 82% upon treatment with AgNp-Bio (3 μM) and 94% at the concentration of 6 μM in relation to the negative control ($p < 0.0001$). We found a 86% lower proliferation of infected cells for the treatment using the association of sulfadiazine and pyrimethamine (50 μg/mL and 25 μg/mL) ($p < 0.0001$) in relation to the negative control (Fig. 3D).

### 3.4. Reduced replication of T. gondii is independent of NO and ROS

We assessed the treatment capacity to modulate the production of NO and ROS and found that the treatment using AgNp-Bio reduced the production of NO at the concentration of 6 μM ($p < 0.005$), while the positive control also revealed a reduction in the levels of NO in relation to the negative control ($p < 0.05$) (Fig. 4A). In addition, we carried out a ROS test using a fluorescent probe H2DCFDA in HeLa cells infected with *T. gondii* and treated at different concentrations of AgNp-Bio. The results indicated that the concentrations tested were not able to induce ROS production (Fig. 4B).

### 3.5. Cytokines

The cytokines IL-1β, IL-6, IL-10, IL-12p70 and TNF-α did not present statistical differences in relation to the infected control or the positive control, as demonstrated in Fig. 5.

However, the production of cytokine IL-8 presented statistically reduced indices having reached values of 50% and 89% at the concentrations of 3 and 6 μM, respectively, in relation to the infected control. In turn, the treatment associated with sulfadiazine (50 μg/mL) and pyrimethamine (25 μg/mL) in relation to the infected control, with a reduction of 92% ($p < 0.0001$) (Fig. 5C).

### 3.6. Photomicroscopy

Fig. 6 presents illustrative images of light microscopy resulting from the dyeing of the infected HeLa cells treated with DMEM (negative control), conventional drugs (positive control) or AgNp-Bio and dyed with tolonium chloride. Image 6A – infected HeLa cells without treatment – shows many infected cells with parasitic vacuoles containing a large amount of intracellular tachyzoites. Image 6B – infected HeLa cells treated with the association of sulfadiazine and pyrimethamine at the concentrations of 25 and 50 μg/mL, respectively, – reveals fewer vacuoles and intracellular parasites in relation to 6A. Imagens 6C and 6D – infected HeLa cells treated with AgNp-Bio at the concentrations of 3 and 6 μM, respectively, – presents fewer infected cells in relation to both the negative and positive controls.

## 4. Discussion

The AgNp-Bio have a large surface area, single chemical properties, and small size, which provides them with the ability to surpass many biological barriers to transport therapeutic agents into the target tissue (Riehemann et al., 2009). Thus, they contribute significantly to an interdisciplinary field of sciences by offering a greater specificity as well as reduced collateral effects in relation to other conventional treatments existing in nanomedicine (Khanna et al., 2015).

According to the results obtained in this study by using the MTT test, the biogenic silver nanoparticles (AgNp-Bio) presented dose-dependent toxicity in relation to the HeLa cells. Accordingly, a study by Mnkandhala et al., (2018) using *Plasmodium falciparum* – parasite of the

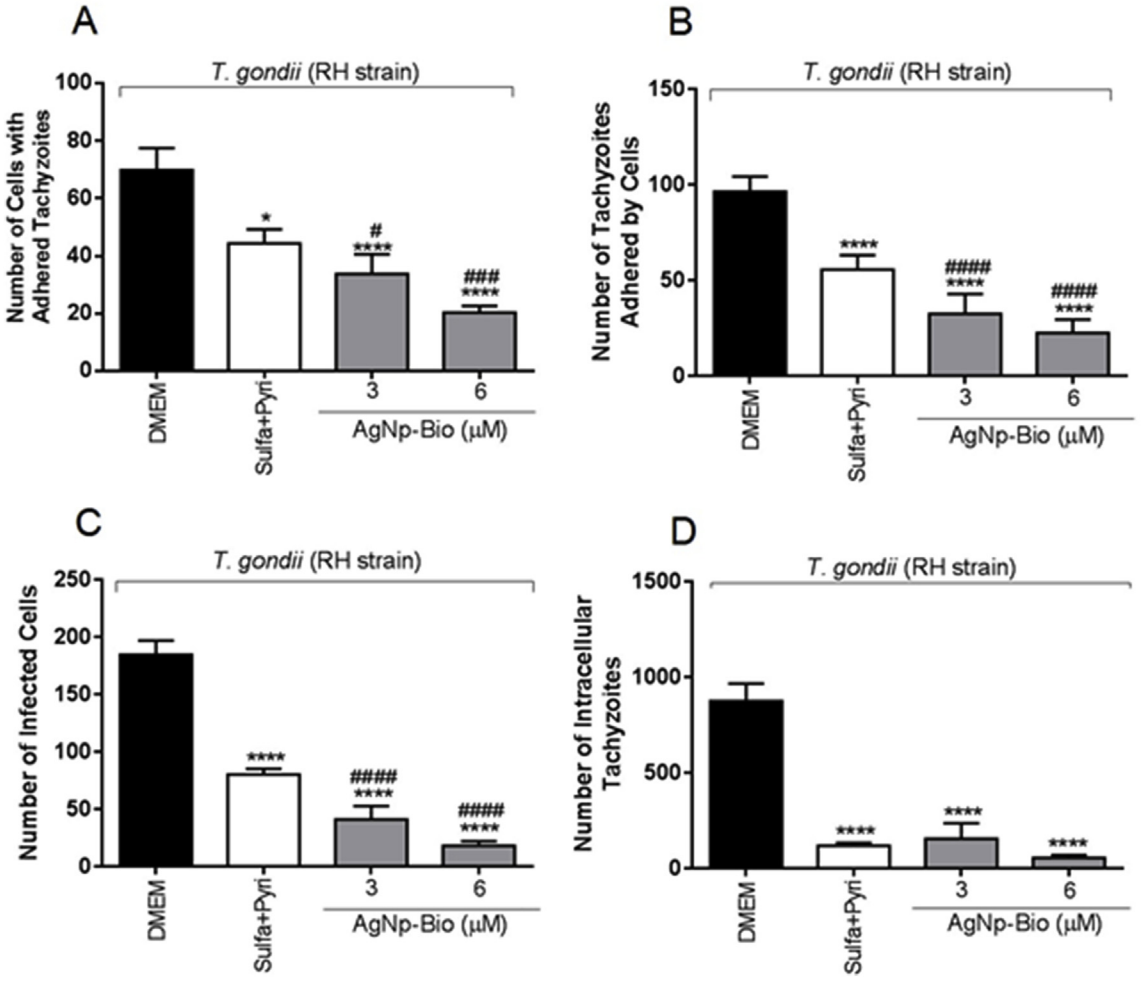


**Fig. 3.** Treatment with AgNp-Bio reduce the index of adhesion, infection and proliferation. Infection test in HeLa cells with tachyzoites of *T. gondii* in vitro in the models of pre- and post-treatment with biogenic silver nanoparticles. HeLa cells infected with tachyzoites of *T. gondii* were treated with AgNp-Bio for 24 h (3 and 6 μM). The following parameters were assessed adherence (A and B), number of infected cells (C), and intracellular proliferation of *T. gondii* (D). Tachyzoites treated with DMEM were considered as negative control and the treatment using the association of sulfadiazine and pyrimethamine (50 and 25 μg/mL respectively) was considered as positive control. Data represent the mean ± SEM of the three independent experiments performed in triplicate. *Significantly different from the negative control ($p < 0.05$). ****Significantly different from both the negative and positive controls ($p < 0.0001$) #Different from both the positive and negative controls ($p < 0.05$); ### ($p < 0.0005$); #### ($p < 0.0001$) (One-Way ANOVA followed by Tukey test).

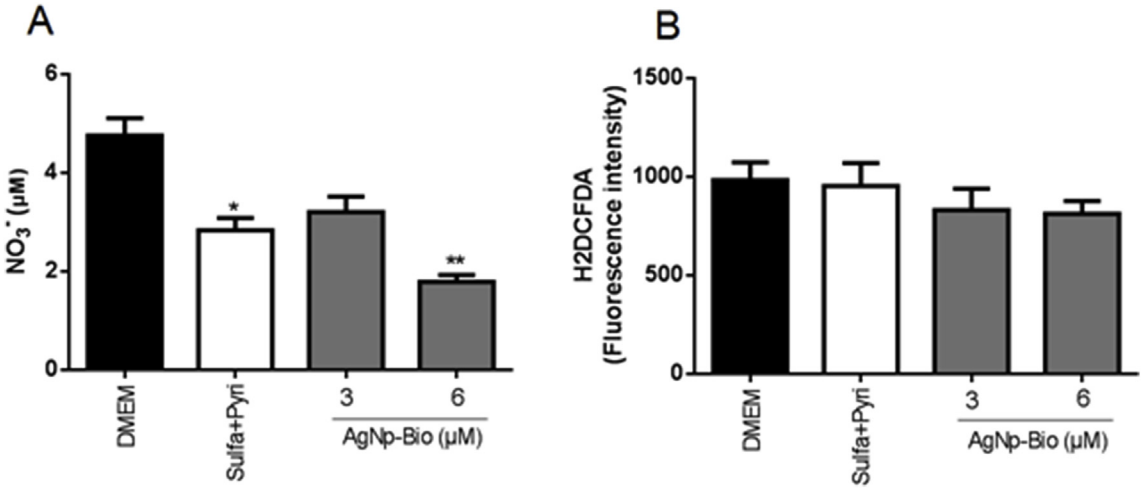


**Fig. 4.** Death of *T. gondii* is dependent on NO and independent of ROS. The following methods were applied to assess the HeLa cells infected with *T. gondii* and subjected to the treatment using AgNp-Bio: (A) Griess method for nitrite levels; (B) fluorescent probe H2DCFDA for reactive oxygen species mediation. Data represent the mean ± SEM of the three independent experiments performed in triplicate. *Significant difference in relation to the negative control ($p < 0.05$), ** ($p < 0.005$).

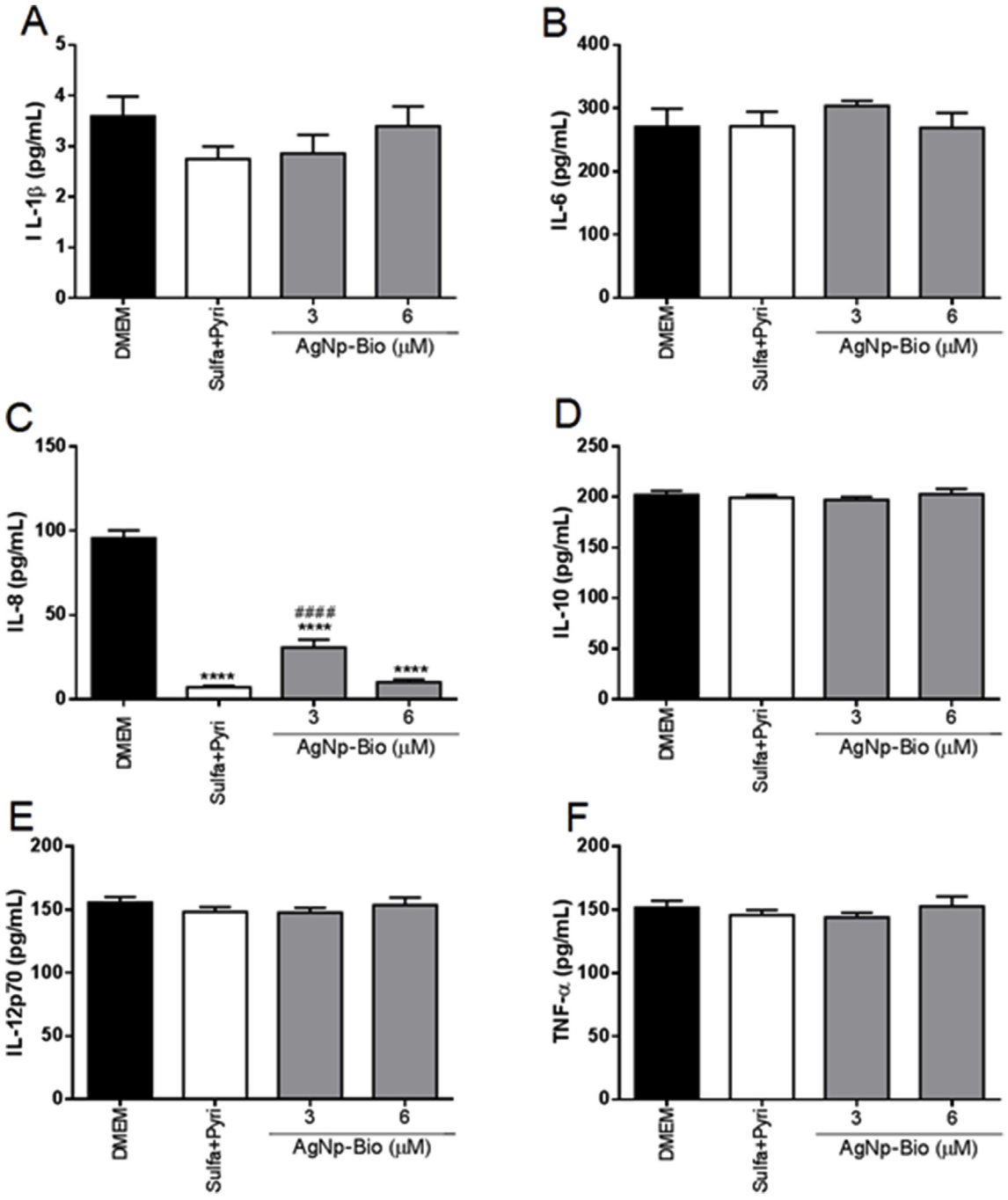


**Fig. 5.** Levels of cytokines dosed from the supernatant of the infected HeLa cells cultures treated with AgNp-Bio and positive control (sulfadiazine and pyrimethamine) according to the instructions in the kit (BD CBA). Control was considered: HeLa cells infected with tachyzoites of *T. gondii* (infected control) and HeLa cells infected with *T. gondii* treated with sulfadiazine and pyrimethamine (50 and 25 μg/mL) (positive control) **** Different from the infected control ($p < 0.0001$); #### Different from the positive control ($p < 0.0001$) (One-Way ANOVA followed by Tukey test).

phylum Apicomplexa as well as Toxoplasma – demonstrated that the treatment with AgNp has a more pronounced toxic effect on the parasite than the HeLa cells. Rahul et al., (2015), in turn, found that microbial pigments associated with AgNp did not present significant toxicity in breast cancer cells (MCF7) as well as in HeLa cells.

Furthermore, a study by Kim et al, (2012) revealed that over a period of 24 h, the cytotoxic effect in HeLa cells proved dose-dependent and size-dependent. Thus, the size of nanoparticles is regarded as a significant contributing factor to toxicity since smaller nanoparticles have more pronounced toxic effects due to a larger surface area (Oberdörster et al., 2005). In addition, many studies did not find toxic effects in different cultures of human cells with the incubation of silver nanoparticles of diameters from 6 to 80 nm (Hussain et al., 2005; Asharani et al., 2008; Singh et al., 2010; Lu et al., 2010; Foldbjerg et al., 2011). As our study found a mean size of 69.0 nm for the silver nanoparticles, we believe it to be a factor to justify the low toxicity of this nanomaterial in HeLa cells.

Our study revealed that the AgNp-Bio promoted a significant decrease in the number of tachyzoites adhered to the HeLa cells as well as the number of infected cells in addition to having fostered an inhibition up to 94% in the parasite intracellular proliferation. The images in Fig. 6 corroborate these data through light microscopy with reduced

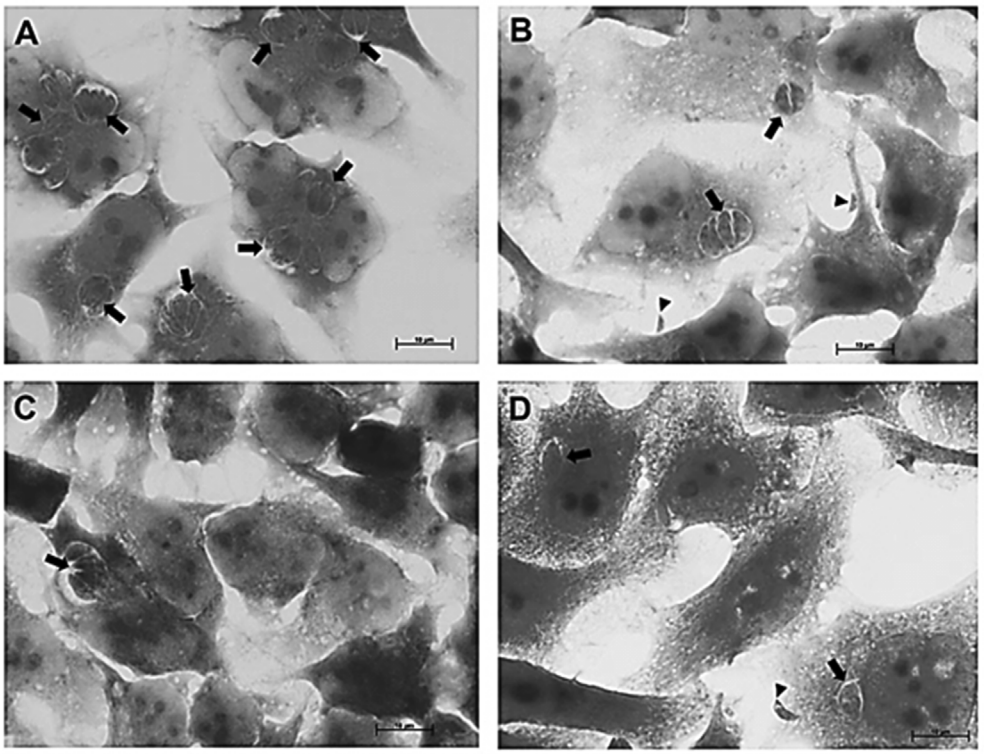


**Fig. 6.** Photomicroscopy of experimental infection in HeLa cells treated for 24 h and dyed with tolonium chloride. HeLa cells infected with the *T. gondii* RH strain treated with DMEM medium (negative control) (A). HeLa cells infected and treated with the association of pyrimethamine and sulfadiazine (50 and 25 μg/mL respectively) (positive control) (B). HeLa cells infected with *T. gondii* and treated with AgNp-Bio at the concentrations of 3 μM (C) and 6 μM (D). Arrows indicate tachyzoites of *T. gondii* inside the parasitic vacuoles; edges of arrows point to tachyzoites adhered to the cells. Magnification of 1000x.

parasitic vacuoles and tachyzoites in the cells treated with AgNp-Bio.

In this context, the in vitro study by Rahul et al (2015) also corroborates our data by demonstrating that prodigiosine – a bioactive microbial pigment – associated with phytosynthetic AgNp inhibit the growth of *P. falciparum* and *Trypanosoma brucei gambiense* in vitro. The authors also found a significant reduction in the values of IC50 for both parasites (2.7–3.6 times) without an increase in cytotoxicity in the cells.

Specifically regarding the protozoan *T. gondii*, a study carried out by Gaafar et al, (2014) demonstrated that the AgNp had an anti-*Toxoplasma in vivo* effect since all animals that received this compound had a significant reduction in parasitic load in the liver and spleen in relation to the control group – infected animals and without treatment. Furthermore, this treatment caused the paralysis of the parasite and deformations in the tachyzoites with the formation of multiple irregular grooves and disorder of the conoid observed through scanning electron microscopy.

Additionally, s study by Adeyemi and collaborators (2017) assessed the anti-*T.gondii* action of gold, silver and platinum nanoparticles and detected a decrease in the parasite invasion and replication, which suggests that the treatment interrupts the replication of *T. gondii* through unknown mechanisms. Jointly, the antiparasitic actions promoted by the AgNp are consistent with the study by Yah and Simate (2015) on the antimicrobial actions of these nanoparticles and stimulate the investigation of AgNp as a promising anti-Toxoplasma alternative.

Although the parasitic invasion processes prevent in this study are yet to be revealed, it is known that the AgNp can act by causing deformations and disorder in the plasmatic membrane as well as damages in the ultrastructures, which interferes in the infectivity potential of parasites (Gaafar et al., 2014; Fanti et al., 2018). Additionally, according to the literature, it is known that the AgNp can be stored inside mitochondria, thus hampering the synthesis of adenosine triphosphate (ATP) (Zheng et al., 2008) as well as causing damages to the mitochondrial membrane (Adeyemi et al., 2017). It was also demonstrated that the *T. gondii* has the leucine aminopeptidase (LAP) enzyme (Jia et al., 2010), which has fundamental roles on physiological processes in the parasite, such as catabolism of proteins and modulation of genic expression (Matsui et al., 2006).

Zheng et al., (2015) demonstrated its importance by studying knockout parasites of this enzyme and finding a reduction in intracellular invasion, infection, and proliferation. It is known that the AgNp are able to interact with LAP fragmenting its protein structure and leading to a loss of function (Mnkdhala et al., 2018) and consequently able to impair the parasitic activity of *T. gondii*. We believe these to be the main reasons pointed out in our data regarding both the adherence and the indices of parasite intracellular infection and proliferation.

The fact that the AgNp-Bio interfere in the mitochondrial membrane can contribute to the increase in oxidative stress promoting enhanced ROS and free radicals, such as NO (Lodge et al., 2006; Misawa and Takahashi, 2011; Manke et al., 2013). However, our study found that the treatment with AgNp-Bio decreased NO production and did not alter ROS production. Thus, by knowing that the AgNp is able to diffuse in cellular membranes (Busch et al., 2011) and associating it with the results of low production of the molecules necessary to the death of intracellular pathogens, we can infer that the action of the silver nanoparticles is more direct in the parasite. A similar study conducted by our own group found a reduced proliferation of amastigotes of *Leishmania amazonensis*, but without the induction of inflammatory mediator synthesis (Fanti et al., 2018). Nonetheless, the heterogeneity of the in vitro cellular models generates a great diversity of results in the literature regarding the effects of the AgNp for the production of ROS and NO.

Regarding the cytokines, Stępkowski et al. (2014) demonstrated that the treatment using AgNp generated an inducibility of the NF-kB signaling in lung cancer cells (A549) approximately five times lower than in liver cancer cells (HepG2), which proves that cellular type influences the cellular response mediated by the NF-kB. This is clear in the case of *T. gondii* in the studies by Butcher et al (2001) demonstrating that the *T. gondii* tachyzoites obstructs the NF-kB activation route preventing its translocation to the core and allowing the parasite to invade the macrophages without triggering the induction of pro-inflammatory

cytokines, such as IL-12 and TNF-α.

Such interference caused by the parasite was also found by Shapira et al (2002) in fibroblasts, which proves that the *T. gondii* is able to interfere in the NF-KB route escaping the immune system and fostering its survival inside the host cell. In contrast, Kim et al, (2001) concluded that HeLa cells infected with *T. gondii* produce a higher amount of IL-8 (chemokine that help the migration of leukocytes, especially neutrophils) due to the NF-kB activation, which is in accordance with our results. In addition, some authors demonstrated the regulating role of NO regarding the IL-8 by stimulating its production, while the NO inhibition decreases its production (Villarete and Remick, 1995; Andrew et al., 1995), which was proven in our results that show reduced NO in the treatment using AgNp-Bio, thus a reduction in IL-8 is expected.

Furthermore, aiming at overcoming the anti-inflammatory action triggered by the parasite, the AgNp can have an important role in the anti-inflammatory field (Zhang et al., 2016). In this context, Wong et al (2009) use *in vivo* and in vitro models to obtain evidence on the anti-inflammatory properties of AgNPs and found that these nanoparticles are able to reduce the quantity of inflammatory markers, which was also corroborated by David et al (2014), who reported an ability of AgNp-Bio to inhibit the production of pro-inflammatory cytokines in an immortalized lineage of keratinocytes (HaCaT) as well as decrease the levels of edema in in vivo experiments.

It is known that Toxoplasma is a highly successful parasite that establishes a chronic infection along its life by carefully regulating the immune activation and effector mechanisms of the host cell. Consequently, *T. gondii* has the ability to soften or inciting the immune response of the host through effector proteins (Melo et al., 2011). Thus, it is possible to infer that the combinations of these effectors present in certain *T. gondii* strain work to maintain an optimum parasitic load in different hosts to guarantee the parasite transmission. However, even though an important advance has been reached over the last decade regarding the understanding on the action of *T. gondii* to modulate immune responses, the exact mechanisms mediated by the cytokines as an initial response to inflammation upon infection with *T. gondii* are yet to be further explored.

## 5. Conclusion

Considering all the above-mentioned remarks, our study was pioneer at highlighting the anti-*T. gondii* action of biogenically synthetized. Our results indicate that these nanoparticles proved able to reduce the parasite proliferation without producing ROS or NO. In addition, we found that the AgNp-Bio had the ability to immunomodulate the IL-8. Therefore, the data found in this study have proved relevant for presenting promising results as a therapeutic alternative for toxoplasmosis.

## CRediT authorship contribution statement

**Laís Fernanda Machado:** Conceptualization, Methodology, Software. **Raquel Arruda Sanfelice:** Conceptualization, Methodology, Software. **Larissa Rodrigues Bosqui:** Data curation, Writing - review & editing, Writing - original draft. **João Paulo Assolini:** Conceptualization, Methodology, Software. **Sara Scandorieiro:** Conceptualization, Methodology, Software. **Italmar Teodorico Navarro:** Conceptualization, Supervision, Methodology, Software. **Allan Henrique Depieri Cataneo:** Conceptualization, Methodology, Software. **Pryscilla Fanini Wowk:** Data curation, Writing - original draft. **Gerson Nakazato:** Conceptualization, Supervision, Methodology, Software. **Juliano Bordignon:** Data curation, Writing - original draft. **Wander Rogerio Pavanelli:** Visualization, Investigation. **Ivete Conchon-Costa:** Visualization, Investigation. **Idessania Nazareth Costa:** Data curation, Visualization, Investigation, Writing - original draft, Writing - review & editing.

## Acknowledgments

The authors acknowledge the funding agencies Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). The authors thank the Program for Technological Development in Tools for Health, PDTIS-FIOCRUZ, for use of its facilities, microscopy and flow cytometry (PDTIS/Instituto Carlos Chagas- Fiocruz-PR).

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.exppara.2020.107853.

## References

Adair, J.H., Parette, M.P., Altınoglu, E.I., Kester, M., 2010. Nanoparticulate alternatives for drug delivery. ACS nan 4, 4967–4970. https://doi.org/10.1021/nn102324e.

Adeyemi, O.S., Sulaiman, F.A., 2015. Evaluation of metal nanoparticles for drug delivery systems. J. Biomed Res. 29, 145. https://doi.org/10.7555/JBR.28.20130096.

Adeyemi, O.S., Murata, Y., Sugi, T., Kato, K., 2017. Inorganic nanoparticles kill *Toxoplasma gondii* via changes in redox status and mitochondrial membrane potential. Int. J. Nanomed. 12, 1647. https://doi.org/10.2147/IJN.S122178.

Adeyemi, O.S., Molefe, N.I., Awakan, O.J., Nwonuma, C.O., Alejolowo, O.O., Olaolu, T., Maimako, R.F., Suganuma, K., han, Y., Kato, K., 2018. Metal nanoparticles restrict the growth of protozoan parasites. Artificial cells, nanomedicine, and biotechnology 14, 1–9. https://doi.org/10.1080/21691401.2018.1489267.

Alday, P.H., Bruzual, I., Nilsen, A., Pou, S., Winter, R., Mamoun, C.B., Riscoe, M.K., Doggett, J.S., 2017. Genetic evidence for cytochrome b qi site inhibition by 4 (1H)-quinolone-3-diarylethers and antimycin in *Toxoplasma gondii*. Antimicrob. Agents Chemother. 61https://doi.org/10.1128/AAC.01866-16. e01866-16.

Allahverdiyev, A.M., Abamor, E.S., Bagirova, M., Ustundag, C.B., Kaya, C., Kaya, F., Rafailovich, M., 2011. Antileishmanial effect of silver nanoparticles and their enhanced antiparasitic activity under ultraviolet light. Int. J. Nanomed. 6, 2705. https://doi.org/10.2147/IJN.S23883.

Andrew, P.J., Harant, H., Lindley, I.J.D., 1995. Nitric oxide regulates IL-8 expression in melanoma cells at the transcriptional level. Biochem. Biophys. Res. Commun. 214, 949–956. https://doi.org/10.1006/bbrc.1995.2378.

Asharani, P.V., Wu, Y.L., Gong, Z., Valiyaveettil, S., 2008. Toxicity of silver nanoparticles in zebrafish models. Nanotechnology 19, 255102 10.1088/0957-7 4484/19/25/255102.

Assolini, J.P., Concato, V.M., Gonçalves, M.D., Carloto, A.C.M., Conchon-Costa, I., Pavanelli, W.R., Melanda, F.N., Costa, I.N., 2017. Nanomedicine advances in toxoplasmosis: diagnostic, treatment, and vaccine applications. Parasitol. Res. 116, 1603–1615. https://doi.org/10.1007/s00436-017-5458-2.

Atilla, A., Aydin, S., Demirdöven, A.N., Kiliç, S.S., 2015. Severe toxoplasmic hepatitis in an immunocompetent patient. Jpn. Infect. Dis. 68, 407–409. https://doi.org/10.7883/yoken.JJID.2014.422.

Barbosa, B.F., Gomes, A.O., Ferro, E.A.V., Napolitano, D.R., Mineo, J.R., Silva, N.M., 2012. Enrofloxacin is able to control *Toxoplasma gondii* infection in both in vitro and in vivo experimental models. Vet. Parasitol. 187, 44–52. https://doi.org/10.1016/j.vetpar.2011.12.039.

Bhardwaj, R., Saudagar, P., Dubey, V.K., 2012. Nanobiosciences: a contemporary approach in antiparasitic drugs. Mol. Cell. Pharmacol. 4, 97–103.

Busch, W., Bastian, S., Trahorsch, U., Iwe, M., Kühnel, D., Meibner, T., Springer, A., Gelinsky, M., Richter, V., Ikonomidou, C., Potthoff, A., Lehmann, I., Schirmer, K., 2011. Internalisation of engineered nanoparticles into mammalian cells in vitro: influence of cell type and particle properties. J. Nano Res. 13, 293–310. https://doi.org/10.1007/s11051-010-0030-3.

Butcher, B.A., Kim, L., Johnson, P.F., Denkers, E.Y., 2001. *Toxoplasma gondii* tachyzoites inhibit proinflammatory cytokine induction in infected macrophages by preventing nuclear translocation of the transcription factor NF-κB. J. Immunol. 167, 2193–2201. https://doi.org/10.4049/jimmunol.167.4.2193.

Butkus, M.A., Labare, M.P., Starke, J.A., Moon, K., Talbot, M., 2004. Use of aqueous silver to enhance inactivation of coliphage MS-2 by UV disinfection. Appl. Environ. Microbiol. 70, 2848–2853. https://doi.org/10.1128/AEM.70.5.2848-2853.2004.

Castanheira, L., De Souza, D.L.N., Silva, R.J., Barbosa, B., Mineo, J.R., Tudini, K.A., Rodrigues, R., Ferro, E.V., De Melo Rodrigues, V., 2015. Insights into anti-parasitism induced by a C-type lectin from Bothrops pauloensis venom on *Toxoplasma gondii*. Int. J. Biol. Macromol. 74, 568–574. https://doi.org/10.1016/j.ijbiomac.2014.11.035.

David, L., Moldovan, B., Vulcu, A., Olenic, L., Perde-Schrepler, M., Fischer-Fodor, E., Florea, A., Crisan, M., Chiorean, I., Clichici, S., Filip, G.A., 2014. Green synthesis, characterization and anti-inflammatory activity of silver nanoparticles using European black elderberry fruits extract. Colloids Surf. B Biointerfaces 122, 767–777. https://doi.org/10.1016/j.colsurfb.2014.08.018.

Durán, N., Durán, M., De Jesus, M.B., Seabra, A.B., Fávaro, W.J., Nakazato, G., 2016. Silver nanoparticles: a new view on mechanistic aspects on antimicrobial activity. Nanomed. Nanotechnol. Biol. Med. 12, 789–799. https://doi.org/10.1016/j.nano.2015.11.016.

Durán, N., Marcato, P.D., Alves, O.L., De Souza, G.I., Esposito, E., 2005. Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains. J. Nanobiotechnol. 3, 8. https://doi.org/10.1186/1477-3155-3-8.

Fanti, J.R., Tomiotto-Pellissier, F., Miranda-Sapla, M.M., Cataneo, A.H.D., De Jesus Andrade, C.G.T., Panis, C., Rodrigues, J.H.S., Wowk, P.F., Kuczera, D., Costa, I.N., Nakamura, C.V., Nakazato, G., Duran, N., Pavanelli, W.R., Conchon-Costa, I., 2018. Biogenic silver nanoparticles inducing *Leishmania amazonensis* promastigote and amastigote death in vitro. Acta Trop. 178, 46–54. https://doi.org/10.1016/j.actatropica.2017.10.027.

Foldbjerg, R., Dang, D.A., Autrup, H., 2011. Cytotoxicity and genotoxicity of silver nanoparticles in the human lung cancer cell line, A549. Arch. Toxicol. 85, 743–750. https://doi.org/10.1007/s00204-010-0545-5.

Gaafar, M.R., Mady, R.F., Diab, R.G., Shalaby, T.I., 2014. Chitosan and silver nanoparticles: promising anti-*Toxoplasma* agents. Exp. Parasitol. 143, 30–38. https://doi.org/10.1016/j.exppara.2014.05.005.

Hussain, S.M., Hess, K.L., Gearhart, J.M., Geiss, K.T., Schlager, J.J., 2005. In vitro toxicity of nanoparticles in BRL 3A rat liver cells. Toxicol. Vitro 19, 975–983. https://doi.org/10.1016/j.tiv.2005.06.034.

Isaac-Márquez, A.P., Talamás-Rohana, P., Galindo-Sevilla, N., Gaitan-Puch, S.E., Díaz-Díaz, N.A., Hernández-Ballina, G.A., Lezama-Dávila, C.M., 2018. Decanethiol functionalized silver nanoparticles are new powerful leishmanicidals in vitro. World J. Microbiol. Biotechnol. 34, 38. https://doi.org/10.1007/s11274-018-2420-0.

Jia, H., Nishikawa, Y., Luo, Y., Yamagishi, J., Sugimoto, C., Xuan, X., 2010. Characterization of a leucine aminopeptidase from *Toxoplasma gondii*. Mol. Biochem. Parasitol. 170, 1–6. https://doi.org/10.1016/j.molbiopara.2009.11.005.

Jones, H.W., Mc Kusick, V.A., Harper, P.S., Wuu, K.D., 1971. George Otto Gey (1899–1970): the HeLa cell and a reappraisal of its origin. Ob/Gyn. 38, 945–949.

Khalil, N.M., Do Nascimento, T.C.F., Casa, D.M., Dalmolin, L.F., De Mattos, A.C., Hoss, I., Romano, M.A., Mainardes, R.M., 2013. Pharmacokinetics of curcumin-loaded PLGA and PLGA–PEG blend nanoparticles after oral administration in rats. Colloids Surf. B Biointerfaces 101, 353–360. https://doi.org/10.1016/j.colsurfb.2012.06.024.

Khanna, P., Ong, C., Bay, B., Baeg, G., 2015. Nanotoxicity: an interplay of oxidative stress, inflammation and cell death. Nanomaterials 5, 1163–1180. https://doi.org/10.3390/nano5031163.

Kim, J., Oh, Y.K., Kim, Y.J., Cho, S., Ahn, M.H., Cho, Y.J., 2001. Nuclear factor-kappa B plays a major role in the regulation of chemokine expression of HeLa cells in response to *Toxoplasma gondii* infection. Parasitol. Res. 87, 758–763. https://doi.org/10.1007/s004360100447.

Kim, T.H., Kim, M., Park, H.S., Shin, U.S., Gong, M.S., Kim, H.W., 2012. Size dependent cellular toxicity of silver nanoparticles. J. Biomed. Mater. Res. 100, 1033–1043. https://doi.org/10.1002/jbm.a.34053.

Krueger, W.S., Hilborn, E.D., Converse, R.R., Wade, T.J., 2014. Drinking water source and human *Toxoplasma gondii* infection in the United States: a cross-sectional analysis of NHANES data. BMC Publ. Health 14, 711. https://doi.org/10.1186/1471-2458-14-711.

Lodge, R., Diallo, T.O., Descoteaux, A., 2006. *Leishmania donovani* lipophosphoglycan blocks NADPH oxidase assembly at the phagosome membrane. Cell Microbiol. 8, 1922–1931. https://doi.org/10.1111/j.1462-5822.2006.00758.x.

Lu, W., Senapati, D., Wang, S., Tovmachenko, O., Singh, A.K., Yu, H., Ray, P.C., 2010. Effect of surface coating on the toxicity of silver nanomaterials on human skin keratinocytes. Chem. Phys. Lett. 487, 92–96. https://doi.org/10.1016/j.cplett.2010.01.027.

Lucey, B.P., Nelson-Rees, W.A., Hutchins, G.M., 2009. Henrietta Lacks, HeLa cells, and cell culture contamination. Arch. Pathol. Lab Med. 133, 1463–1467. https://doi.org/10.1043/1543-2165-133.9.1463.

Manke, A., Wang, L., Rojanasakul, Y., 2013. Mechanisms of nanoparticle-induced oxidative stress and toxicity. BioMed Res. Int. 2013, 1–15. https://doi.org/10.1155/2013/942916.

Matsui, M., Fowler, J.H., Walling, L.L., 2006. Leucine aminopeptidases: diversity in structure and function. JBC 12, 1535–1544 14 10.1515/BC.2006.191.

Melo, M.B., Jensen, K.D., Saeij, J.P., 2011. *Toxoplasma gondii* effectors are master regulators of the inflammatory response. Trends Parasitol. 27, 487–495. https://doi.org/10.1016/j.pt.2011.08.001.

Misawa, M., Takahashi, J., 2011. Generation of reactive oxygen species induced by gold nanoparticles under x-ray and UV Irradiations. Nanomedicine: NBM (NMR Biomed.) 7, 604–614. https://doi.org/10.1016/j.nano.2011.01.014.

Mnkandhla, D., Marwijk, J.V., Hoppe, H., Wilhelmi, B.S., Whiteley, C.G., 2018. In vivo; in vitro interaction of silver nanoparticles with leucine aminopeptidase from human and *Plasmodium falciparum*. J. Nanosci. Nanotechnol. 18, 865–871. https://doi.org/10.1166/jnn.2018.13966.

Montazeri, M., Sharif, M., Sarvi, S., Mehrzadi, S., Ahmadpour, E., Daryani, A., 2017. A systematic review of in vitro and in vivo activities of anti-*Toxoplasma* drugs and compounds (2006–2016). Front. Microbiol. 8, 25. https://doi.org/10.3389/fmicb.2017.00025.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65, 55–63. https://doi.org/10.1016/0022-1759(83)90303-4.

Oberdörster, G., Oberdörster, E., Oberdörster, J., 2005. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ. Health Perspect. 113, 823–839. https://doi.org/10.3390/nano5031163.

Park, S., Ko, Y.S., Lee, S.J., Lee, C., Woo, K., Ko, G., 2018. Inactivation of influenza A virus via exposure to silver nanoparticle-decorated silica hybrid composites. Environ. Sci. Pollut. Res. 25, 27021–27030.

Petersen, E., 2007. Toxoplasmosis. formerly.Semin. Neonatol. 12, 214–223. https://doi.org/10.1016/j.siny.2007.01.011.

Picoli, S.U., Durán, M., Andrade, P.F., Duran, N., 2016. Silver nanoparticles/silver chloride (Ag/AgCl) synthesized from *Fusarium oxysporum* acting against Klebsiella pneumouniae carbapenemase (KPC) and extended spectrum beta-lactamase (ESBL). Front. Nanosci. Nanotech. 2, 107–110. https://doi.org/10.15761/FNN.1000117.

Pourali, P., Yahyaei, B., 2016. Biological production of silver nanoparticles by soil isolated bacteria and preliminary study of their cytotoxicity and cutaneous wound healing efficiency in rat. J. Trace Elem. Med. Biol. 34, 22–31. https://doi.org/10.1016/j.jtemb.2015.11.004.

Rahul, S., Chandrashekhar, P., Hemant, B., Bipinchandra, S., Mouray, E., Grellier, P., Satish, P., 2015. In vitro antiparasitic activity of microbial pigments and their combination with phytosynthesized metal nanoparticles. Parasitol. Int. 64, 353–356. https://doi.org/10.1016/j.parint.2015.05.004.

Riehemann, K., Schneider, S.W., Luger, T.A., Godin, B., Ferrari, M., Fuchs, H., 2009. Nanomedicine: challenge and perspectives. Angew. Chem. Int. Ed. 48, 872–897. https://doi.org/10.1002/anie.200802585.

Robert-Gangneux, F., Dardé, M.L., 2012. Epidemiology of and diagnostic strategies for toxoplasmosis. Clin. Microbiol. Ver. 25, 264–296. https://doi.org/10.1128/CMR.05013-11.

Sanfelice, R.A., Machado, L.F., Bosqui, L.R., Miranda-Sapla, M.M., Tomiotto-Pellissier, F., De Alcântara Dalevedo, G., Ioris, D., Reis, G.F., Panagio, L.A., Navarro, I.T., Bordignon, J., Conchon-Costa, I., Pavanelli, W.R., Almeida, R.S., Costa, I.N., 2017. Activity of rosuvastatin in tachyzoites of *Toxoplasma gondii* (RH strain) in HeLa cells. Exp. Parasitol. 181, 75–81. https://doi.org/10.1016/j.exppara.2017.07.009.

Sanguiñedo, P., Fratila, R.M., Estevez, M.B., De La Fuente, J.M., Grazú, V., Alborés, S., 2018. Extracellular biosynthesis of silver nanoparticles using fungi and their antibacterial activity. Nano Biomed. Eng. 10, 165–173.

Scandorieiro, S., De Camargo, L.C., Lancheros, C.A., Yamada-Ogatta, S.F., Nakamura, C.V., De Oliveira, A.G., Andrade, C.G.T.J., Duran, N., Nakazato, G., Kobayashi, R.K., 2016. Synergistic and additive effect of oregano essential oil and biological silver nanoparticles against multidrug-resistant bacterial strains. Front. Microbiol. 7, 760. https://doi.org/10.3389/fmicb.2016.00760.

Sepulveda-Arias, J.C., Veloza, L.A., Mantilla-Muriel, L.E., 2014. Anti-*Toxoplasma* activity of natural products: a review. Recent Pat. Anti-Infect. 9, 186–194.

Shapira, S., Speirs, K., Gerstein, A., Caamano, J., Hunter, C.A., 2002. Suppression of NF-κB activation by infection with *Toxoplasma gondii*. J. Infect. Dis. 185, S66–S72. https://doi.org/10.1086/338000.

Sharma, V., Kaushik, S., Pandit, P., Dhull, D., Yadav, J.P., Kaushik, S., 2019. Green synthesis of silver nanoparticles from medicinal plants and evaluation of their antiviral potential against chikungunya virus. Appl. Microbiol. Biotechnol. 103, 881–891.

Shrivastava, S., Bera, T., Roy, A., Singh, G., Ramachandrarao, P., Dash, D., 2007. Characterization of enhanced antibacterial effects of novel silver nanoparticles. Nanotechnology 18, 225103. https://doi.org/10.1088/0957-4484/18/22/225103.

Singh, S., D'Britto, V., Prabhune, A.A., Ramana, C.V., Dhawan, A., Prasad, B.L.V., 2010. Cytotoxic and genotoxic assessment of glycolipid-reduced and-capped gold and silver nanoparticles. New J. Chem. 34, 294–301. https://doi.org/10.1039/B9NJ00277D.

Stępkowski, T.M., Brzóska, K., Kruszewski, M., 2014. Silver nanoparticles induced changes in the expression of NF-κB related genes are cell type specific and related to the basal activity of NF-κB. Toxicol. Vitro 28, 473–478. https://doi.org/10.1016/j.tiv.2014.01.008.

Sutterland, A.L., Fond, G., Kuin, A., Koeter, M.W.J., Lutter, R., Van Gool, T., Yolken, R., Szoke, A., Leboyer, M., De Haan, L., 2015. Beyond the association *Toxoplasma gondii* in schizophrenia, bipolar disorder, and addiction: systematic review and meta analysis. Acta Psychiatr. Scand. 132, 161–179. https://doi.org/10.1111/acps.12423.

Tomiotto-Pellissier, F., Alves, D.R., Miranda-Sapla, M.M., de Morais, S.M., Assolini, J.P., da Silva Bortoleti, B.T., Gonçalves, M.D., Cataneo, A.H.D., Kian, D., Madeira, T.B., Yamauchi, L.M., Nixdorf, S.L., Costa, I.N., Conchon-Costa, I., Pavanelli, W.R., 2018. Caryocar coriaceum extracts exert leishmanicidal effect acting in promastigote forms by apoptosis-like mechanism and intracellular amastigotes by Nrf2/HO-1/ferritin dependent response and iron depletion: leishmanicidal effect of *Caryocar coriaceum* leaf exracts. Biomed. Pharmacother. 98, 662–672 9 10.1016/j.biopha.2017.12.083.

Torres-Sangiao, E., Holban, A., Gestal, M., 2016. Advanced nanobiomaterials: vaccines, diagnosis and treatment of infectious diseases. Molecules 21, 867. https://doi.org/10.3390/molecules21070867.

Villarete, L.H., Remick, D.G., 1995. Nitric oxide regulation of IL-8 expression in human endothelial cells. Biochem. Biophys. Res. Commun. 211, 671–676. https://doi.org/10.1006/bbrc.1995.1864.

Wong, K.K., Cheung, S.O., Huang, L., Niu, J., Tao, C., Ho, C.M., Che, C.M., Tam, P.K.H., 2009. Further evidence of the anti inflammatory effects of silver nanoparticles. ChemMedChem 4, 1129–1135 22 10.1002/cmdc.200900049.

Yah, C.S., Simate, G.S., 2015. Nanoparticles as potential new generation broad spectrum antimicrobial agents. Daru 23, 43. https://doi.org/10.1186/s40199-015-0125-6.

Zhang, X.F., Liu, Z.G., Shen, W., Gurunathan, S., 2016. Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches. Int. J. Mol. Sci. 17, 1534. https://doi.org/10.3390/ijms17091534.

Zheng, J., Wu, X., Wang, M., Ran, D., Xu, W., Yang, J., 2008. Study on the interaction between silver nanoparticles and nucleic acids in the presence of cetyltrimethylammonium bromide and its analytical application. Talanta 74, 526–532. https://doi.org/10.1016/j.talanta.2007.06.014.

Zheng, J., Jia, H., Zheng, Y., 2015. Knockout of leucine aminopeptidase in *Toxoplasma gondii* using CRISPR/Cas9. Int. J. Parasitol. 45, 141–148. https://doi.org/10.1016/j.ijpara.2014.09.003.