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ORIGINAL ARTICLE

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# The potential effect of silver nanoparticles synthesized with *Coffea arabica* green seeds on *Leishmania major* proliferation, cytotoxicity activity, and cytokines expression level

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**Abstract** The goal of this study was to analyze the antileishmanial and antibacterial activity of *Coffea arabica* green seed biosynthesize silver nanoparticles (*C. arabica* AgNPs), as well as cytotoxicity and cytokine gene expression. UV–vis spectroscopy, FTIR, and FESEM methods used to examine the *C. arabica* AgNPs. MTT test was used to assess the antileishmanial and cytotoxicity effects. The gene expression level was assessed in NPs-treated J774 cells by qPCR. The synthesized *C. arabica* AgNPs were in the size range of 20–70 nm, through FESEM pictures. The $IC_{50}$ values of the NPs were 65. 4 and 47.70 µg/mL against promastigotes and amastigotes of *Leishmania major*, but these values were 580.1 and 171.1 µg/mL for Glucantime® as the control drug. *C. arabica* AgNPs represented a significant increase in IL-12P40, as a Th1 cytokine, in comparison to Glucantime® at high concentrations ($P < 0.01$), whilst IL-10 expression level showed a significant reduction between NPs-treated and Glucantime®-treated macrophages at 250–1000 µg/mL concentrations ($P < 0.001$). Moreover, the NPs were cytotoxic on cancer cell lines of Hek293, MCF7, and A172 with the $CC_{50}$ values of 437.2, 116.8, and 72.9 µg/mL, respectively. It showed a significant effect of these NPs against A172 ($P < 0.001$). Also, the lowest MIC values of the NPs were obtained for *Bacillus subtilis* and *Staphylococcus aureus* (204 µg/mL). According to the antileishmanial, anticancer, and antibacterial activity of these NPs, it can considered a bio-agent drug in the future in endemic countries.

**Keywords** *Coffea arabica* · Silver nanoparticles · Antileishmanial effects · Gene expression · Cytotoxicity activity

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## Introduction

Nanobiotechnology is a cross-disciplinary field that joins the mix of nanoparticles (NPs) and related activities. NPs have been used as unique administrators for remedial purposes. Nanomaterials are essentially particles, generally 1–100 nm in size, with different shapes and morphologies (Bawa 2019). Attractive nanoparticles range in size from a few nanometers to tens of nanometers, making them comparable to or smaller than a quality (2 nm wide and 10–100 nm long), a protein (5–50 nm), a cell (10–100 nm), and an infection (20–450 nm). Surely, they can be collaborated with or tied to an organic element by covering with natural particles, so given that controllable method for 'labeling' or tending to it (Pankhurst et al. 2003).

Due to the sensible convention, eco-friendly, quick, non-pathogenic, and unique advanced approach of biosynthetic processes, the use of plants as reducing agents in silver nanoparticle mixtures has recently received attention. The utilization of biosynthesized metal NPs, for example, selenium, zinc oxide, magnesium, titanium, gold, copper, silver, palladium, platinum, and magnetite NPs has just been assessed

against microorganisms as a proper alternative to conventional methods (Iravani et al. 2014). *Coffea arabica* green seeds silver nanoparticles (*C. arabica* AgNPs) are known to display mitigating, anticancer, antibacterial, antiviral, and against angiogenic movement (Jang 2015). The system of poisonousness of apoptosis incited by *C. arabica* AgNPs is all around affirmed in various cell lines (Yuan and Gurunathan 2017). The use of natural plant products to synthesize nanomaterials is becoming more popular due to its safety and pricing. Secondary plant metabolites such as flavonoids, ketones, aldehydes, terpenoids, amides, and carboxylic acid have been used as reducing agents in the bio-reduction reaction since its conception, due to the production of new metal nanoparticles (Sinha et al. 2018).

The caffeine-producing plant *Coffea arabica* (Rubiaceae) is a major cash crop in Cameroon, and a decoction of the leaves in water is used as an antimalarial treatment (Wahba et al. 2019). This is a big shrub with dark green oval leaves. It has four chromosomal arrays instead of two, which distinguishes it from other espresso species. The natural products are oval and grow in 7–9 months; they often contain two-level seeds (espresso beans), and when only one bean matures, it is referred to as a peaberry (Marcheafave et al. 2019; Silva et al. 2021). The availability and the administration of safe and effective drugs are critical in the treatment of infectious diseases. To increase the therapeutic index of infectious disease medicines and simplify their use, nanotechnology-based techniques have been the subject of extensive preclinical testing (Kirtane et al. 2021).

Leishmaniasis is a vector-borne disease caused by 22 human parasite species that is found in 98 nations, with the tropics and sub-tropics having the highest prevalence (Choi et al. 2021). To date, there are neither available vaccines nor safe and effective drugs against all forms of visceral, cutaneous, and mucocutaneous leishmaniasis. Biological proceedings are not feasible due to the complexity of the life cycle and the existence of various vectors and reservoir species (Choi et al. 2021). Chemotherapy of pentavalent antimonial compounds such as meglumine antimoniate (Glucantime®, MA) is currently the only universal control measure against the disease, but it is limited due to a variety of side effects, poor treatment adherence, and the development of resistance against the causative agents (Ponte-Sucre et al. 2017). Maltreatment of anti-infection agents may cause the spread of safe microbial strains, which thusly prompts a mind-boggling and difficult issue in medication. Consequently, it is basic to investigate new antimicrobial substances and supplant them with basic anti-toxins. Nanoparticles have pulled in researchers' inclinations because of their solid antimicrobial properties (Tariq et al. 2020). To produce nanoparticles, researchers have looked at several plant resources so far. One of the most widely consumed plant items is *Coffea arabica* seed. The bean of this plant was previously found to have high quantities of phenolic chemicals (Geremu et al. 2016), which can be utilized as a bioreduction to make NPs. Silver nanoparticles produced from dried roasted coffee seed in the form of a hydroalcoholic extract were tested for antibacterial activity (Dhand et al. 2016). To the best of our knowledge, no investigation has been conducted on the cytotoxicity and antileishmanial efficacy of AgNPs produced from green *Coffea* seeds. Biogenic combination of *C. arabica* with AgNPs, as non-harmful synthetic mixtures, was carried out in this study to create a safe and natural agent in order to assess antileishmanial, antibacterial and cytotoxicity effects.

## Materials and methods

### Plant sampling, identification, and extraction

*Coffea arabica* was purchased from a market in Kerman province, southeast of Iran, in July 2019. A voucher specimen of the plant submitted to the Herbarium Center of Kerman University of Medical Sciences' Department of Pharmacognosy (KF2321). The green *Coffea* seed mashed, weighed (300 g), and extracted by the maceration method with 70% ethanol (40 °C). The maceration process conducted three times with periodic stirring. Stirring used to balance the concentration of the fluid outside the grain of the powder. The pulp and macerate separated by using a filter. The maceration procedure repeated and filtered again. The extractive solutions were concentrated under reduced pressure for ethanol elimination, and then lyophilized, yielding dry extracts from the green seed.

### Biosynthesis of silver nanoparticles

*C. arabica* AgNPs synthesized using $AgNO_3$ as a metal base. 300L of silver nitrate (0.1%) and 100 µg/mL of green *Coffea* seeds residual (10 mL) combined for this purpose. The mixture was oven dried at 40 °C and rinsed many times with deionized water before being dried at 50 °C. The shade change monitored by using UV–visible spectroscopy.

### Characterizations of silver nanoparticles

A UV–vis spectrophotometer (PerkinElmer, Germany) used to monitor the growth of silver nanoparticles at 300–600 nm. To determine the practical groups of the concentrate and characterize the unknown materials composition, the *C. arabica* AgNPs exposed to Fourier transform infrared spectroscopy (FTIR) analysis. Also, Field Emission-Scanning Electron Microscopy (FESEM) (Quanta 200, USA) in diameters of 500 and 10 nm was used to confirm the normal molecule size, shape, and appropriation of inserted silver nanoparticles.

### Cell cultures and parasite growth conditions

The cell lines of Hek293, MCF7 (Human breast cancer), A172 (Human glioblastoma), and murine macrophage (J774-A1 cells, ECACC 91051511) provided by the Pasteur Institute of Tehran, Iran. Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Invitrogen, U.S.A) used to culture cell lines, which contained 100 IU/ml penicillin, 100 µg/mL streptomycin (Gibco, U.S.A), 10% heat-inactivated FBS (Gibco, Invitrogen, Grand Island, NY, U.S.A.), and 1 mM sodium pyruvate at 37 °C in a $CO_2$ incubator (5%). In addition, leishmaniasis Research Center of Kerman University of Medical Sciences provided promastigotes of *Leishmania major* (MRHO/IR/75/ ER), then sub-cultured in RPMI-1640 medium completed with 10% FBS and incubated at 25 ± 1 °C.

### Cytotoxic assay

To determine the effect of *C. arabica* AgNPs on the viability of Hek293, MCF-7, A172, and J774-A1 cell lines, an MTT assay was used. On a 96-well plate, $10^4$ cells/well of each cell line plated and incubated for 24 h. The next day, the medium suctioned and 100 µl of various concentrations of the *C. arabica* AgNPs (1-500 µg/mL) poured into each well and brooded at 37 °C with 5% $CO_2$ for a normal period of 72 h. After incubation, 20 µL of MTT solution [3-(4, 5-dimethyl-2-thiazolyl)- 2, 5-diphenyl-2H-tetrazolium bromide] (5 µg/mL) added into each well. After 3 h, the absorbance was estimated at 490 nm by the ELISA reader (BioTek- Elx 800). Cell practicality communicated as 100% for control (untreated cells). All samples performed in triplicates and the survival rate (%) calculated as the following (Saduqi et al. 2019): Survival rate (%) = (OD in treatment group/OD in control group) × 100.

The inhibitory concentration required for half cytotoxicity ($CC_{50}$) value calculated utilizing the Probit test in the SPSS software (version 20).

### Anti-promastigote assessment

The effect of silver nanoparticles on *L. major* promastigotes assessed with a colorimetric test WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2*H*-5-tetrazolio]-1,3-benzene disulfonate) (Roche-Mannheim, Germany) as described elsewhere (Saduqi et al. 2019). In a 96-well small-scale titer plate, 100 µL of logarithmic stage promastigotes ($10^6$ cells/mL) inserted. Each well received 100 µl of various doses of *C. arabica* AgNPs as well as MA as a reference medication (1-500 µg/mL) and incubated at 25 °C for 72 h. Then, 10 µL of WST-1 added to each well and incubated at 25 °C for 4 h. Promastigotes with no *C. arabica* AgNPs defined as an untreated control. Finally, the absorbance measured at 490 nm using an ELISA reader (BioTek-ELX800, USA). The Probit test of the SPSS software used to calculate the 50% inhibitory concentration ($IC_{50}$ value).

### Anti-amastigote assessment

Drug susceptibility of the intra-macrophage amastigotes was determined (Chang 1980). 1$cm^2$ smear placed in the well of a 6-well slide (LabTek, NY, USA), then 200 µl J774A1 cells ($10^6$ cells/ml) added to the plate. After 2 h at 37 °C in 5% $CO_2$, fixed phase pre-flagella form added to the macrophages (ratio 10: 1) and incubated for 24 h. Free parasites eliminated by washing with RPMI-1640 medium and infected cells treated with 100 µl *C. arabica* AgNPs and Glucantime® (1–500 µg/mL) at 37 °C and 5% $CO_2$ for 72 h. Finally, the slides were dried, fixed with methanol, stained with Giemsa, and observed under a light microscope (Nikon, Japan). Furthermore, only macrophages and those containing amastigotes without any drugs used as negative and positive controls. The intracellular amastigotes of each concentration assessed by the mean number of amastigotes of 100 macrophages, which were implemented thrice (Sharifi et al. 2017, 2019). Finally, the $IC_{50}$ values calculated by the probit test of SPSS software.

### Evaluation of cytokines expression level

The expression levels of IL-12p40 (as the cytokine of the Th-1 pathway) and IL-10 (as the cytokine of the Th-2 pathway) evaluated in *C. arabica* AgNPs-treated and Glucantime®-treated macrophages by quantitative real-time PCR (qPCR) assays. The RNeasy Mini Kit (Cat. No. 74106, Qiagen, Germany) used to extract the total RNA from treated and untreated cells according to its protocol. For cDNA synthesize, RT reagent Kit (Takara, Japan) utilized by using 100 ng of extracted RNA in a thermal cycler. Finally, SYBR® Premix Ex TaqTM II (Takara, Japan) used for qPCR reaction in a total volume of 10 µl in the Rotor-Gene Q (Qiagen, Germany). Table 1 represented the target

**Table 1** The target primers and reference gene sequences

| Template | Forward and reverse sequences (5´-3´) | Product size (bp) |
|---|---|---|
| IL-12P40 | F-CTGGAGCACTCCCCATTCCTA | 160 |
| | R-GCAGACATTCCCGCCTTTG | |
| IL-10 | F-CTTACTGACTGGCATGAGGATCA | 101 |
| | R-GCAGCTCTAGGAGCATGTGC | |
| GAPDH | F-AGCTTCGGCACATATTTCATCTG | 89 |
| | R-CGTTCACTCCCATGACAAACA | |

primers and reference gene sequences. The experiments conducted in triplicate.

### Minimum inhibitory concentrations (MIC) determination

The antibacterial effect of *C. arabica* AgNPs determined through the broth microdilution method. One mL of *C. arabica* AgNPs suspension added to the test cylinder and two-fold serial dilutions were done for coming to the last concentrations between 12.4 mg/mL and 3.1 mg/mL. Four microorganism species including *Escherichia coli, Bacillus subtilis*, *Staphylococcus aureus,* and methicillin-resistant *S. aureus* (MRSA) utilized in this investigation. 10 µl of the bacterial suspension (concentration to 0.5 McFarland usual) was added to each test tube and incubated at 37 °C for 24 h. The lowest concentration of *C. arabica* AgNPs that caused the lack of growth in bacteria indicated as MIC of silver NPs. Besides, Vancomycin, Imipenem, Cefixime, and Ciprofloxacin used as the control standard antibiotics.

### Disk diffusion assay

All bacteria strains cultured on nutrient agar plates. Different concentrations of *C. arabica* AgNPs prepared in test tubes by diluting them with sterile water. Sterile blank antimicrobial disks immersed into prepared *C. arabica* AgNPs tubes and placed on the agar plates. In addition, the control standard antibiotic disks placed either. The plates incubated at 37 °C for one night and the inhibitory zone around the disks measured.

### Statistical analysis

The data presented as mean ± SD. The differences between the test and control groups analyzed by ANOVA. Also, a t-test was used to assess the difference between the $IC_{50}$ of the two groups. $P < 0.05$ as indicated significant. Prism 7.01 software (GraphPad Software, USA) was used to analysis data.

## Results

### Biosynthesis and characterization of silver nanoparticles

The synthesis of *C. arabica* AgNPs assessed by monitoring $AgNO_3$ color change from colorless to yellow–brown and its absorption spectrum appeared at 450 nm. The reduction of $Ag^+$ particles to silver particles upon contact with plant extract supernatants can lead to a color change from dull to bright yellow after reacting with $Ag^+$ particles. The UV–vis results showed that the assimilation peak (λmax) of silver observed at 440 nm (Fig. 1). The frequencies ranging from 400 to 4000 $cm^{-1}$ on the concentrate detected in Fig. 2. This shows the proximity of the IR homologs at 3356, 2923, 2852, 1654, 1516, 1383, 1126 (COC), 1072, 1050, and 834, 763, and 610 due to the $NH^2$ $cm^{-1}$ fluctuations. The absorption band at 3356 $cm^{-1}$ is because of extending of the N–H band of amino gatherings or is demonstrative of present O–H bunches because of the nearness of alcohols, phenols, sugars, and so forth. The presence of a top at 2923 and 2852 $cm^{-1}$ identified with that C–H, C=O, 1654 $cm^{-1}$ (carbonyl gatherings), 1516 $cm^{-1}$ (amide II), 1383 $cm^{-1}$ (C=O), 1072, 1050 $cm^{-1}$ (C–O–C) and 831, 763 and 610 $cm^{-1}$ because of C–Cl gathering of alkyl halides. The filtering electron microscopy (FESEM) picture indicated the sporadic molded *C. arabica* AgNPs of 20–70 nm in size (Fig. 3).

### Cytotoxic assay

The MTT results of different cell lines exposed to 1–500 µg/mL of *C. arabica* AgNPs represented the cytotoxic effect of these NPs on the cell lines of Hek293, MCF7, and A172 with the $CC_{50}$ values of 437.2, 116.8 and 72.9 µg/mL, respectively. These values indicated the potent cytotoxic activity of these NPs towards these studied human cell lines. The inhibition percentage of these NPs on these cell lines have shown in Fig. 4. But, *C. arabica* AgNPs showed less cytotoxic effect against the J774-A1 cells, as the macrophage model, with the $CC_{50}$ values of 742.4 µg/mL and Selectivity Index (SI) of 15.5 (Table 2).

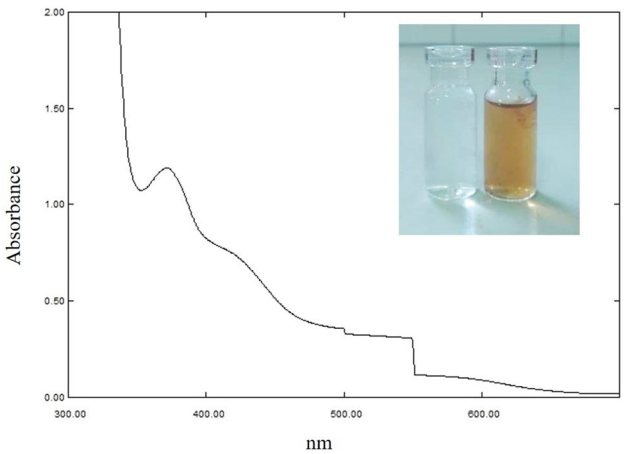


**Fig. 1** UV–vis spectra of the synthesized AgNPs (*C. arabica* silver nanoparticles)

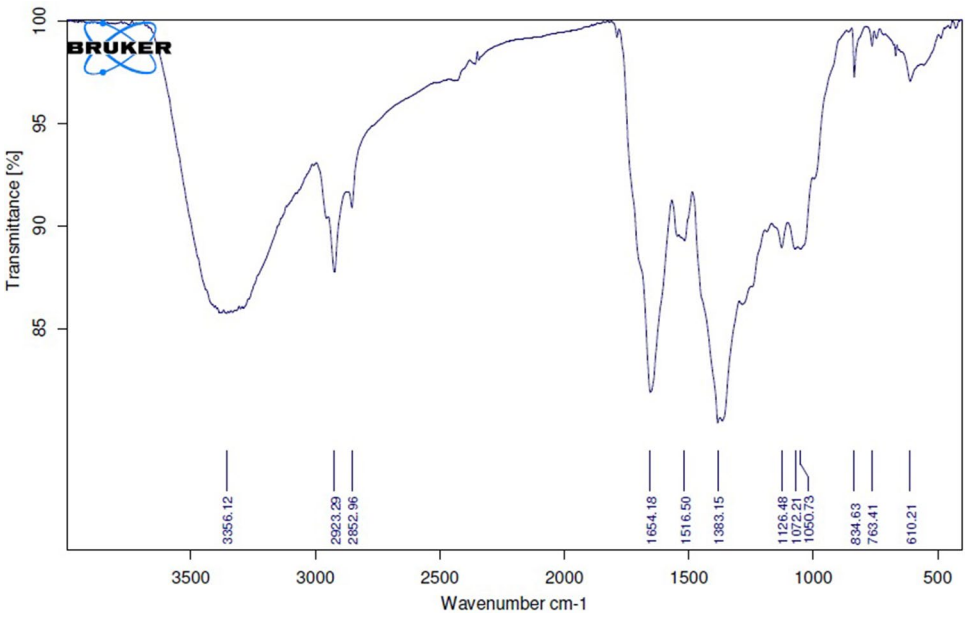


**Fig. 2** FTIR spectrum on the *C. arabica* AgNPs (*C. arabica* silver nanoparticles) in the range of 4000–400 cm.$^{-1}$

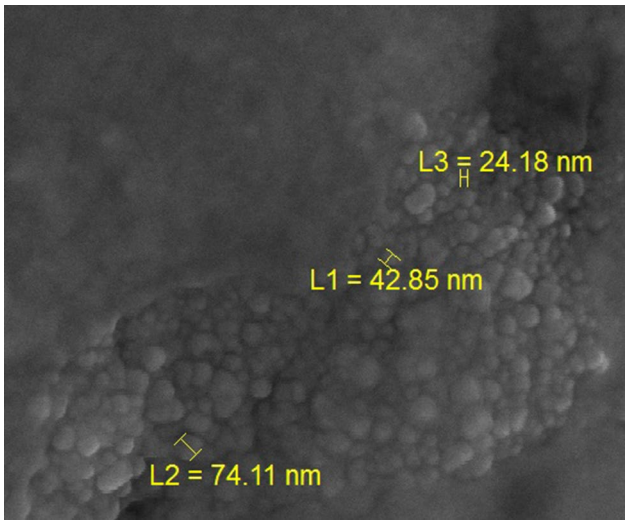


**Fig. 3** Field Emission-Scanning Electron Microscopy (FESEM) image and size distribution of *C. arabica* AgNPs (*C. arabica* silver nanoparticles)

### Anti-promastigote assay

The findings showed the critical antileishmanial action of these NPs against the promastigote phase of *L. major* in a dose-dependent manner ($P < 0.05$). Figure 5 represented a significant decrease in promastigote viability compared to Glucantime®, as the reference drug, especially at two concentrations of 250 and 500 µg/mL. Also, the $IC_{50}$ values for *C. arabica* AgNPs and Glucantime® against promastigotes were $65.39 \pm 0.05$ and $580.1 \pm 0.16$ µg/mL, respectively; which showed lower $IC_{50}$ compared to Glucantime® ($P < 0.001$) (Table 2).

### Anti-amastigote assay

The results showed that *C. arabica* AgNPs significantly ($P < 0.05$) decreased the mean number of *L. major* amastigotes in J774-A1 cells in comparison to Glucantime® (Table 3). Moreover, these NPs indicated a 3.5-fold reduction in the $IC_{50}$ value compared to the reference drug ($47.7 \pm 0.03$ vs. $171.01 \pm 0.01$ µg/mL) (Table 2).

### Gene expression

The expression of cytokines assessed in the *C. arabica* AgNPs and Glucantime® groups. IL12P40 gene expression increased in NP-treated macrophages, especially at the last three concentrations; while the expression level of IL-10 represented a significant decrease with increasing drug concentration, especially at concentrations of 250, 500 and 1000 µg/mL ($P < 0.001$) compared with Glucantime® (Fig. 6).

### Antibacterial activity

The prepared *C. arabica* AgNPs represented a significant antibacterial effect against all four bacteria species by elevating concentration, especially at 750 and 1000 µg/mL, as *S. aureus* showed the highest inhibition zone (100 mm) (Fig. 7A). NPs at 1000 µg/mL caused the same inhibitory effect as Cefixime and a higher effect than Vancomycine against *S. aureus*. Also, at this concentration of NPs, more inhibition zone was identified against *B. subtilis* compared to all antibiotics. A more inhibitory effect observed against MRSA at 1000 µg/mL of NPs in comparison to two antibiotics of Ciprofloxacin and Cefixime ($P < 0.01$) (Fig. 7B).

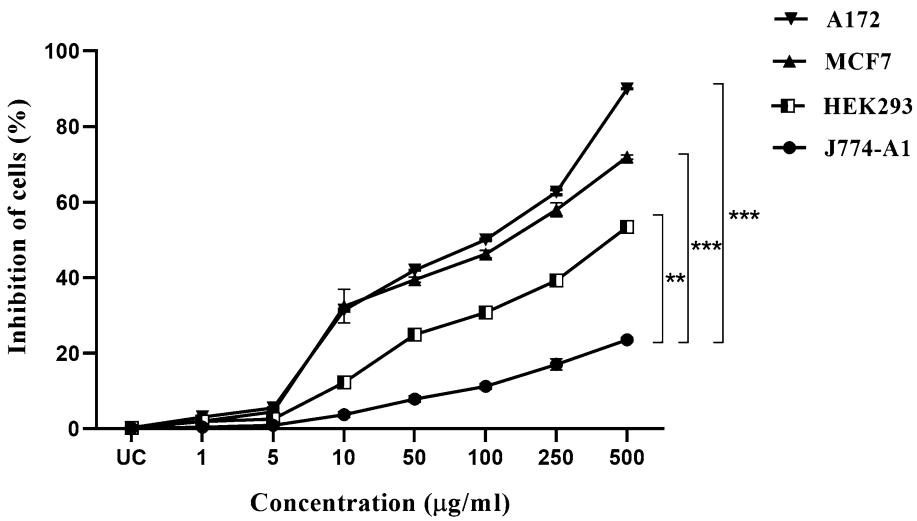


**Fig. 4** Inhibition percentage of different concentrations of *C. arabica* AgNPs (*C. arabica* silver nanoparticles) in Hek293, MCF7, and A172 cell lines compared to J774-A1 cells (** $P<0.01$ and *** $P<0.001$)

**Table 2** Evaluating the $IC_{50}$ values of *C. arabica* AgNPs (*Coffea arabica* silver nanoparticles) in both forms of *L. major* compared to the positive control of Glucantime® as well as the $CC_{50}$ value of macrophage

| Drugs | Amastigote | | Promastigote | | Macrophage | [c]SI |
|---|---|---|---|---|---|---|
| | [a]$IC_{50}$ ± SD (µg/mL) | *P* -value | [a]$IC_{50}$ ± SD (µg/mL) | *P* -value | [b]$CC_{50}$ (µg/mL) | (Selectivity Index) |
| Glucantime® | 171.1 ± 0.01 | NR | 580 ± 0.02 | NR | 1095 ± 0.69 | 6.40 |
| *C. arabica* AgNPs | 47.7 ± 0.03 | $P<0.001$ | 65.4 ± 0.01 | $P<0.001$ | 742.4 ± 0.71 | 15.5 |

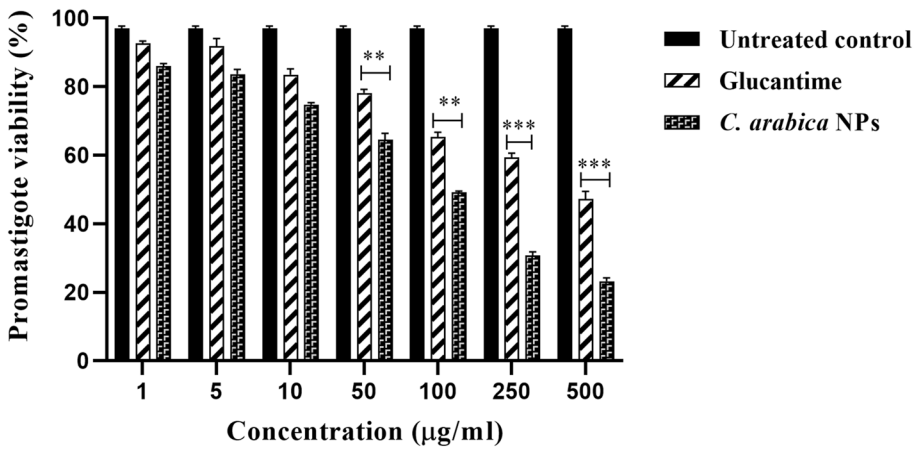


**Fig. 5** Effect of *C. arabica* AgNPs (*C. arabica* silver nanoparticles) concentrations on the viability of *L. major* promastigotes in comparison with Glucantime® and untreated control. Error bars are SD (** $P<0.01$ and *** $P<0.001$). Each test conducted in triplicate

The *C. arabica* AgNPs showed the lowest MIC against *B. Subtilis* and *S. aureus* (204 µg/mL). These NPs indicated decreased MIC levels against *B. Subtilis* and *S. aureus* compared to the other antibiotics (Table 4).

## Discussion

The proximity of a large variety of natural synthetic compounds such as starch, fat, proteins, catalysts and coenzymes, phenols flavonoids, terpenoids, alkaloids, gum, and others capable of delivering electrons for the reduction of $Ag^+$ particles to $Ag^0$ explains why *C. arabica* AgNPs are combined with natural components (Allahverdiyev et al. 2011). The dynamic fixing is liable for the decrease of $Ag^+$ particles changes relying on living being/remove utilized. For nano-change of *C. arabica* AgNPs, electrons should be gotten from dehydrogenation of acids (ascorbic corrosive) and alcohols (catechol) in hydrophytes, keto to enol transformations (cyperaquinone,

**Table 3** Comparing the effect of various concentrations of *C. arabica* AgNPs (*Coffea arabica* silver nanoparticles) and Glucantime® as the positive control on the mean number of *L. major* amastigotes in each macrophage

| Concentration (µg/mL) | *C. arabica* AgNPs | | Glucantime® | |
|---|---|---|---|---|
| | Mean ± SD | *P* value | Mean ± SD | *P* value |
| 0.0 (Control) | 96.9 ± 1.2 | NR | 96.9 ± 1.2 | NR |
| 1 | 83.1 ± 1.1 | $P > 0.05$ | 94.9 ± 1.05 | $P > 0.05$ |
| 5 | 80.2 ± 1.3 | $P < 0.05$ | 90.6 ± 1.2 | $P > 0.05$ |
| 10 | 75.1 ± 1.2 | $P < 0.05$ | 85.9 ± 1.1 | $P > 0.05$ |
| 50 | 67.6 ± 1.3 | $P < 0.01$ | 73.2 ± 0.9 | $P < 0.05$ |
| 100 | 32.1 ± 1.5 | $P < 0.001$ | 62.8 ± 1.5 | $P < 0.05$ |
| 250 | 25.2 ± 0.9 | $P < 0.001$ | 44 ± 1.2 | $P < 0.001$ |
| 500 | 15.1 ± 1.2 | $P < 0.001$ | 27.9 ± 1.2 | $P < 0.001$ |

dietchequinone, remirin) in mesophytes or the two systems in xerophytes plants.

The decrease of $Ag^+$ to $Ag^0$ affirmed by the shading change of the response blend from colorless to brownish yellow. The absorption spectra of spherical *C. arabica* AgNPs have a maximum between 420 and 450 nm, according to the literature (Abdi et al. 2019). FTIR study demonstrates that likely the C–H, carboxyl (–C=O), hydroxyl (–OH), and (C–O–C) bunches in seed exudates are fundamentally associated with the decrease of $Ag^+$ particles to $Ag^0$ nanoparticles (Javed et al. 2020).

NPs such as *C. arabica* AgNPs contain special physicochemical properties such as size and shape (1–100 nm in diameter) and excellent surface-to-volume ratio resulting in higher chemical reactivity (Flores-López et al. 2019). In this study, the FESEM image indicated the sporadic mold of *C. arabica* AgNPs of 20–70 nm in size.

For decades, leishmaniasis overlooked, leading to serious health problems such as death, scarring, stigma, and

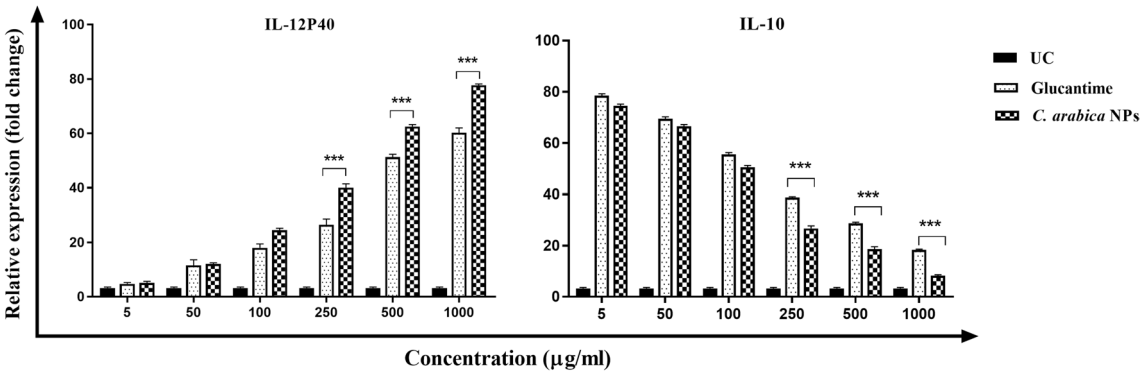


**Fig. 6** Gene expression profiles of IL-12P40 and IL-10 in macrophages treated with different concentrations (5-1000 µg/mL) of *C. arabica* AgNPs (*C. arabica* silver nanoparticles) in comparison to the untreated group (UC) and Glucantime®. Error bars are SD ***$p < 0.001$). Each test conducted in triplicate

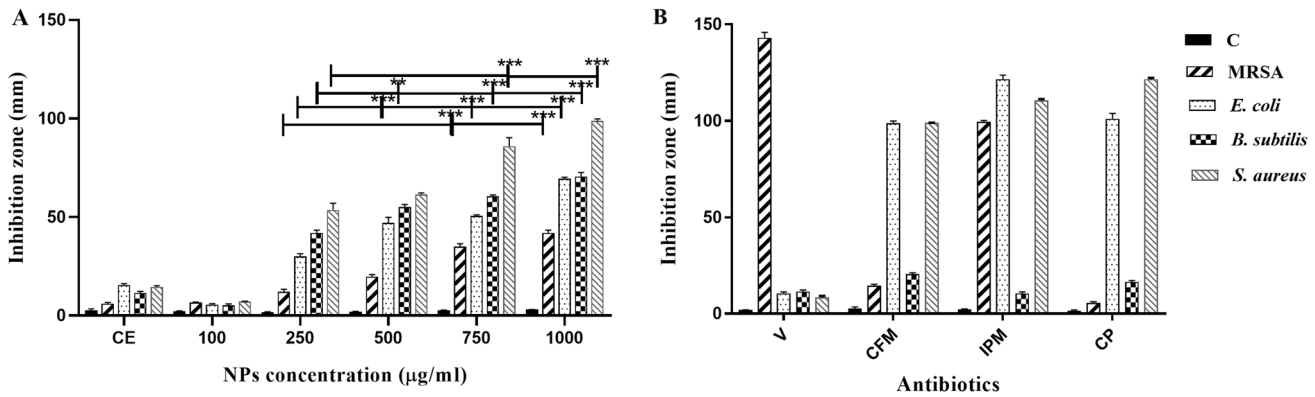


**Fig. 7** Inhibition zone of different concentrations of *C. arabica* AgNPs (*C. arabica* silver nanoparticles) and CE (crude extract) (**A**), as well as IMP (Imipenem), CP (Ciprofloxacin), CFM (Cefixime) and V (Vancomycin) antibiotics (**B**) against *E. coli*, *S. aureus, B. subtilis,* and MRSA compared to the control. Error bars are SD (** $P < 0.01$ and *** $P < 0.001$)

**Table 4** MIC level of *C. arabica* AgNPs and four antibiotics against *E. coli, B. Subtilis*, *S. aureus* and MRSA

| Isolate | MIC (µg/mL) | | | | |
|---|---|---|---|---|---|
| | *C. arabica* AgNPs | Imipenem | Vancomycin | Ciprofloxacin | Cefixime |
| *E. coli* | 275 | 212 | 404 | 210 | 201 |
| *B. subtilis* | 204 | 456 | 412 | 322 | 321 |
| *S. aureus* | 204 | 212 | 430 | 271 | 203 |
| MRSA | 402 | 216 | 110 | 412 | 356 |

severe depression (Bailey et al. 2019). As a result, creative ways for developing more effective and less harmful nanomedicine to treat and manage *Leishmania* parasites are high on the priority list for research (Ismail et al. 2019). One of the effective ways is to combine nanometals with bioactive chemicals to create a dynamic anti-leishmaniasis agent with good biocompatibility (Akbari et al. 2017). According to the findings, the $IC_{50}$ values of *C. arabica* AgNPs was 65.4 and 47.7 µg/mL against promastigote and amastigote stages of *L. major*, whereas these values were 580.1 and 171.1 µg/mL for Glucantime® as control drug. Also, these NPs induced a significant reduction in the mean number of amastigotes in J774-A1 cells in comparison to Glucantime®, especially at the last two concentrations. Besides, IL-12P40 and IL-10 genes expression showed increased and decreased levels, respectively, in *C. arabica* AgNPs-treated macrophages compared to Glucantime®-treated cells, especially at 250, 500, and 1000 µg/mL concentrations.

The synergetic action of both silver nanoparticles and bioactive phytochemicals produced from *C. arabica* green seed residues linked to the surface of *C. arabica* AgNPs could explain the high anticancer activity of these biogenic NPs (Farah et al. 2016). In addition, nanoformulation of organic chemicals can cause higher stability and cell penetration, leading to reduced stress, greater cytotoxicity, and cell death (Kajani et al. 2016). Various studies have established the cytotoxic effects of compounds biosynthesized using different plant extracts in contradiction to different cancer cell lines (Ogur 2014; Romeilah 2016). In our results, these NPs had the cytotoxic activity on all three cancer cell lines, with the best inhibitory effect on A172 cells (92%).

Silver nanoparticles are widely employed in medicine, food storage, textile coatings, public health, and environmental applications due to their antibacterial qualities (Gao et al. 2015). Some mechanisms for *C. arabica* AgNPs' antibacterial action have been proposed. Silver ions produced from these NPs thought to increase their bactericidal efficacy by inhibiting DNA replication, bacteria growth, respiration, and ATP synthesis, ultimately leading to cell death (Feng et al. 2000; Sondi and Salopek-Sondi 2004; Morones et al. 2005). *C. arabica* AgNPs are able to penetrate the bacterial cell wall and get into the cytoplasm (Alsammarraie et al. 2018). According to the obtained results, it seems that *C. arabica* AgNPs immediately crossed *S. aureus*, *B. subtilis,* and *E. coli* cell walls, but the MRSA cell wall showed resistance to low concentrations of the NPs. Also, more inhibition zone was identified against *B. subtilis* in NPs-treated compared to the antibiotic-treated bacteria. Moreover, *C. arabica* AgNPs showed the lowest MIC against *B. Subtilis* and *S. aureus* (204 µg/mL) compared to the other antibiotics.

## Conclusions

Visual confirmation of Ag-NPs synthesis obtained by watching the color change in the solution. To our knowledge, this is the first manuscript that demonstrated notable antileishmanial activity of synthesized *C. arabica* AgNPs against the promastigotes and amastigotes of *L. major* in a dose-dependent manner as well as the significant cytotoxic effect on studied cell lines. These prepared NPs displayed a remarkable bactericidal activity; so, it can considered as a bio-agent drug in the future. Because clinical evidence is currently limited, more research is need.

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**Data availability** All data used to support the findings of this study are included in the article.

**Declarations**

**Conflict of interest** The authors declare that they have no conflicts of interest.

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