A comparative experiment on the anti-chronic myeloid leukemia capacities of $AgNO_3$, *Scrophularia striata* leaf aqueous extract, and silver nanoparticles containing natural compounds

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Abstract

Introduction: In the current study, silver nanoparticles were prepared and synthesized in aqueous medium using the *Scrophularia striata* leaf extract as stabilizing and reducing agents. Also, we investigated the anti-chronic myeloid leukemia potentials of silver nanoparticles against BV173 (chronic myeloid leukemia in blast crisis), CML-T1 (chronic myeloid leukemia in lymphoid blast crisis), EM-2 (chronic myeloid leukemia in blast crisis; relapse after bone marrow transplantation), and JOSK-M (chronic myeloid leukemia in myelomonocytic) cell lines.

Material and methods: Silver nanoparticles were characterized and analyzed using common nanotechnology techniques including UV-Vis and FT-IR Spectroscopy, Field Emission-Scanning Electron Microscopy (FE-SEM), Transmission Electron Microscopy (TEM), and Energy Dispersive X-ray Spectrometry (EDS). FT-IR analysis offered antioxidant compounds in the nanoparticles were the sources of reducing power, reducing silver ions to silver nanoparticles. FE-SEM and TEM images revealed a uniform spherical morphology in size of 19.72 nm for the green synthesized nanoparticles. DPPH test revealed similar antioxidant potentials for silver nanoparticles and butylated hydroxytoluene.

Results: Silver nanoparticles had very low cell viability and anti-chronic myeloid leukemia properties dose-dependently against JOSK-M, EM-2, CML-T1, and BV173 cell lines without any cytotoxicity on the HUVEC cell line. The best result of cytotoxicity properties of silver nanoparticles against the above cell lines was observed in the case of CML-T1 cell line.

Conclusions: After confirming in the *in vivo* and clinical trial studies, these nanoparticles can be administered to humans for the treatment of chronic myeloid leukemia.

Key words: silver nanoparticles, *Scrophularia striata* leaf extract, chronic myeloid leukemia.

Introduction

Leukemia or blood cancer is one of the most common types of cancer around the world [1]. Leukemia is divided into acute and chronic types depending on the range, severity, and progression rate of the disease. In leukemia, rapid growth is followed by the production of several immature white blood cells, and the interval between the incidence of disease and its progression is very short [2]. The symptoms of leukemia include anemia along with fatigue and paleness, cyanosis, swollen and bleeding gums, mild fever, swollen lymph nodes, bone pain, severe and consistent bleeding, and appearance of blood in the urine or feces [1, 2]. For the treatment of leukemia, chemotherapy, radiotherapy, bone marrow transplantation, and stem cells are used. The chemical drugs used for chemotherapy affect the cell division of cancer cells [1]. In recent years, researchers have always been looking for newer formulations of chemotherapy drugs of natural resources such as plants and plant nanoparticles to destroy more cancer cells in a shorter time. They have tried to combine nanotechnology with medicine for the synthesis of these drugs [3]. Metallic nanoparticles have gained significant attention in the area of biomedical technology [4, 5]. There are many methods for producing metallic nanoparticles including; a) physical method, b) chemical method, and c) biological method [6–8]. In biological nanoparticle synthesis, various microbes, enzymes, algae, and especially plants have been used and served as a suitable alternative method to physical and chemical procedures with high therapeutic potentials [9–12]. It has been revealed that metallic nanoparticles synthesized using plants have excellent non-cytotoxicity potential against human normal cells, antioxidant property against free radicals such as DPPH, antibacterial activities against Gram positive and negative bacteria and antifungal activities against *Candida* species [13]. Also, metallic nanoparticles were used for their anticancer properties against several cell lines such as MDAMB231 (human breast adenocarcinoma), human colorectal adenocarcinoma cells, MCF7 (human breast adenocarcinoma), HeLa (human cervical adenocarcinoma cells), HepG2 (human liver cancer cells), HCT-116 (colon cancer cells), SKBR3 (human breast adenocarcinoma cells), A549 (human lung carcinoma cells), and human chronic myelogenous cells [3]. In this regard, iron nanoparticles have a special role. Also, the study of Namvar *et al.* has indicated that iron nanoparticles synthesized using plant extracts have excellent cytotoxicity potentials against Jurkat cells (Human cell lines for leukemia), MCF-7 cells (breast cancer), HeLa cells (cervical cancer), and HepG2 cells (liver cancer) [14]. In a previous study, Klein *et al.* indicated that iron nanoparticles with range sizes of 9–20 nm could treat the breast and colon cancers. They investigated the Caco-2 and MCF-7 cell lines for analyzing the anti-breast and anti-colon cancer properties of iron nanoparticles, respectively [15]. In spite of the above studies, there are few reports about the therapeutic effects of iron nanoparticles on acute leukemia as a common leukemia in both developing and developed countries. However, the previous study indicated that ethno medicinal plants have a suitable ability in the treatment of acute leukemia. A list of medicinal plants that have been used for increasing the anti-acute leukemia activities includes *Maytenus boaria*, *Cephaelis acuminate*, *Barleria prionitis*, *Boswellia serrate*, *Lavandula officinalis*, *Cephalotaxus harringtonia* drupacea, *Tinospora cordifolia*, *Euphoria hirta*, *Lubinus perennis*, *Sophora subprostrata*, *Phyllanthus niruri*, and *Solanum seaforthianum* [16].

Iranian traditional medicine is one of the drug production sources in all of the world [17]. Every year, large numbers of pharmaceutical supplements and medications are produced from Iranian herbal medicine [18, 19]. One of these plants is *Scrophularia striata* from *Plantae* kingdom, *Tracheobionta* subkingdom, *Spermatophyta* superdivision, *Magnoliophyta* division, *Magnoliopsida* class, *Lamiales* order, *Scrophulariaceae* family, and *Scrophularia* genus. This species grows in the southwest of Asia especially in the west of Iran. In several countries, *S. striata* is known with other names such as Benjek mashineh, Benj ghan, and Teshneh dari. This species is rich in antioxidant compounds such as ethyl acetate, bis (2-ethylhexyl) phthalate, cinnamic acid, nepitrin, gallic acid, oleyl alcohol, isorhamnetin-3-O-rutinoside, acteoside, and quercetin. In traditional medicine, *S. striata* is used due to the antioxidant, antibacterial, antifungal, antiviral, anti-parasitic, antiproliferative, anti-inflammatory, neuroprotective, analgesic, anti-anxiety, preservative, and anticancer effects. In Iranian traditional medicine, people use *S. striata* for the prevention, control, and treatment of blood disorders such as iron efficiency, fauvism, hemolysis, hemolytic anemia, and thrombocytopenia [20–27].

Accordingly, the current study was conducted to evaluate the possible protective activity of synthesized silver nanoparticles using *S. striata* leaf aqueous extract against common cell lines of chronic myeloid leukemia including JOSK-M, EM-2, CML-T1, and BV173 in the *in vitro* condition.

Material and methods

Material

All materials were obtained from Sigma Aldrich chemicals.

Synthesis of AgNPs

Collected fresh fruits of *S. striata* were shade-dried at room temperature for 21 days. The dried fruits were then milled into fine powder using an electric mill. The powdered plant material was kept at room temperature away from direct sunlight in a dry airtight plastic container ready for extraction. For extraction, five hundred grams of the powdered *S. striata* fruit was soaked in 5 l of distilled water and swirled regularly for 24 h. The extract was decanted, filtered using muslin cloth into a different dry clean conical flask. The filtrate was concentrated under reduced pressure using a rotary evaporator at 40°C to obtain a semi-solid residue [17]. For synthesizing AgNPs, firstly, 2 g *S. striata* was dissolved in 20 ml de-ionized water. Then, 2.5 ml of the prepared solution was added to an aqueous $AgNO_3$ solution. In the subsequent stage, the obtained solution was heated up to 80°C in the oil bath under a certain stirring speed for 24 h. This led to the gradual formation of AgNPs. The similar reactions were also carried out using various concentrations of *S. striata* [18].

Characterization of AgNPs

In this study, to record the UV-Vis spectra, a Shimadzu UV spectrophotometer was used. JASCO (FT/IR-6200) spectrophotometer was utilized to record the FT-IR spectra. To evaluate the different morphological characteristics of nanoparticles such as size distribution, surface morphology and particle shape, MIRA3TESCAN-XMU FE-SEM was used to record Field Emission Scanning Electron Microscopy (FE-SEM) images. To investigate the size and morphology of AgNPs, Philips EM208S was employed to record transmission electron microscopy (TEM) images.

Assessment of the antioxidant property of AgNPs by DPPH

The modified Brand-Williams method was followed in order to study antioxidant activity using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay [17].

Generally, the DPPH exhibits purple color with a highly stable free radical. The donation of electron to DPPH free radical by antioxidant results in variation in the absorbance which can be analyzed spectrophotometrically. The stoichiometric amount of DPPH (39.4 mg) was well dissolved in 100 ml of methanol resulting in 0.14 mM solution. The methanol was diluted 1 : 1 with distilled water to get 50% methanol. The different concentrations of $AgNO_3$, *S. striata*, and AgNPs, i.e. 0–1000 µg/ml were considered. The 140 µl of 1 mM DPPH was thoroughly mixed with above resultant solutions and incubated at 37°C for 30 min. The absorbance data were recorded at 517 nm against 50% methanol blank, a control reaction was performed without any test sample. In this experiment, butylated hydroxytoluene (BHT) was used as a positive control. The percentage radical scavenging activity was calculated according to the following formula [17]: DPPH free radical scavenging (%) = (Control – Test/Control) × 100.

The actual absorbance was considered as the absorbance difference of the control, the test sample and IC_{50} value was determined.

Measurement of cell toxicity of AgNPs

In this experiment, the following cell lines have been used for investing the cytotoxicity effects of the $AgNO_3$, *S. striata*, and AgNPs using an MTT assay:

1) Normal cell line: HUVEC;
2) Chronic myeloid leukemia cell lines:
   a) JOSK-M (chronic myeloid leukemia in myelomonocytic),
   b) EM-2 (chronic myeloid leukemia in blast crisis; relapse after bone marrow transplantation),
   c) CML-T1 (chronic myeloid leukemia in lymphoid blast crisis),
   d) BV173 (chronic myeloid leukemia in blast crisis).

These cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% (w/v) FBS, 100 U/ml penicillin, and 100 µg/ml streptomycin. Then, cells were distributed at 10,000 cells/well in 96-well plates. The cells were grown under a humidified incubator with 5% CO_2 at 37°C until reaching confluency (typically after 24 h). The cells were treated with $AgNO_3$, *S. striata*, and AgNPs at concentrations of 0, 1, 2, 3, 7, 15, 31, 62, 125, 250, 500, and 1000 µg/ml and subsequently incubated for 2 and 24 h. $AgNO_3$, *S. striata*, and AgNPs were sterilized using UV radiation for 1 h. Finally, the MTT solution (5 mg/ml in PBS) was added to each well and incubated for 4 h at 37°C. The medium with MTT was removed and the formazan crystals formed in the living cells were dissolved in 100 µl DMSO per well. All tests were run in the triplicates. The relative viability (%) was calculated based on the absorbance at λ = 570 nm determined using a microplate reader [17]: Percentage of cell viability (%) = (Sample absorbance/Control absorbance) × 100.

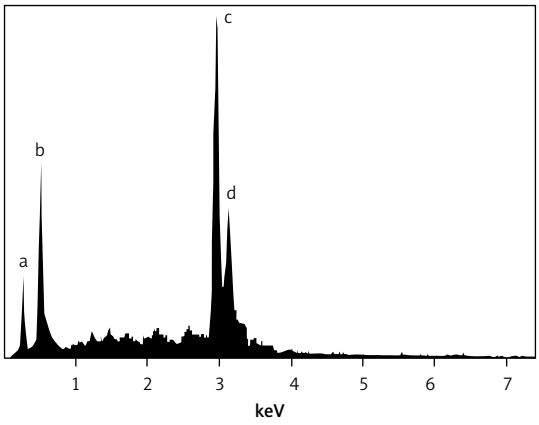
The percentage of cell viability was then plotted against various concentrations and the IC_{50} (half maximal inhibitory concentration) was determined graphically.

This research was approved by Heji Hospital Affiliated to Changzhi Medical College animal ethical committee, Approval No. CZMC-202008.

Results and Discussion

Chemical characterization of AgNPs

The result of the EDS (Figure 1) demonstrates the clear elemental composition profile of the bio-



a = CKα, b = OHα, c = AgLα, d = AgLβ

Figure 1. EDS pattern of the AgNPs

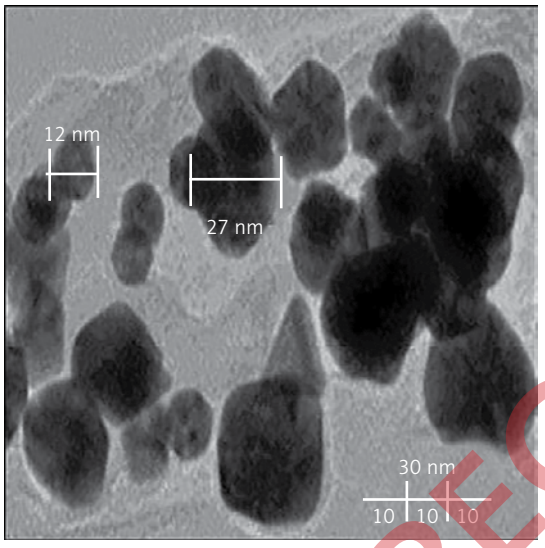


Figure 3. TEM image of AgNPs

synthesized silver nanoparticles. The presences of silver in synthesized nanoparticles was approved by the observed signals including AgLα and AgLβ around 3 keV. These signals are as well as match to a previous study on synthesized AgNPs [17]. The other signals including OKα and CKα belong to the organic molecules present in *S. striata* aqueous extract which are linked to AgNPs.

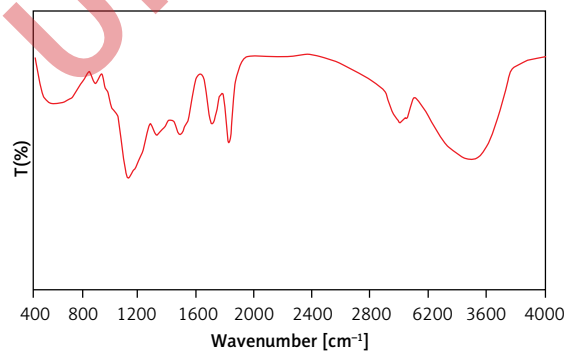


Figure 4. FT-IR pattern of AgNPs

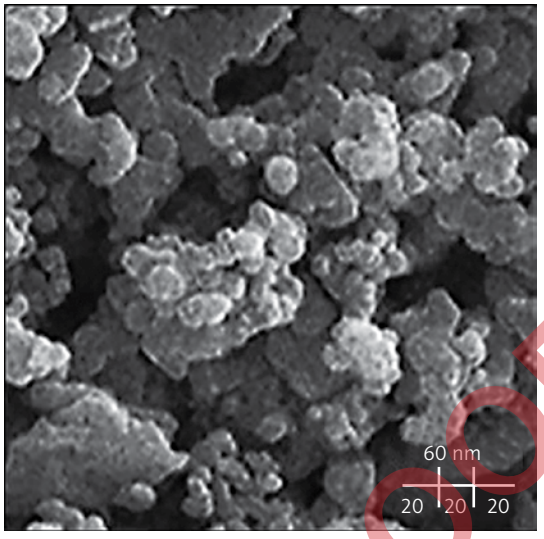


Figure 2. FE-SEM image of AgNPs

The morphology of the synthesized silver nanostructures was studied by recording the FE-SEM image of synthesized AgNPs, which enumerated the formation of homogeneous and relatively spherical silver nanoparticles by stabilizing and capping agents (Figure 2). The biomolecule layer of the silver nanoparticles was observed in the FE-SEM images. This layer confirms the plant extract metabolites' role in the synthesis and stabilizing of the silver nanoparticles. Moreover, the average size of some selected silver nanoparticles was found to be 19.72 nm.

In the TEM image, the particles formed were spherical (Figure 3). The nanospherical particles formed were shown to have a high surface area. Formed nanoparticles were in the average range of 19.72 nm in size. The particles were monodispersing with thin layers of the extract on their surface.

In our review of the literature, a range size of 10–50 nm has been reported for the silver nanoparticles biosynthesized using plant extracts [18].

To identify the biomolecules responsible for the stabilizing of AgNPs, FT-IR was performed on biosynthesized AgNPs (Figure 4). The 556 cm^{-1} absorption band is relevant to the Ag-O functional group resonance. This confirms that nano-sized silver particles are present in the nanocomposite.

Sp^2-Carbon groups are generally the reason for the band around 1081 cm^{-1}, while carbonyl functional groups include the 1831 cm^{-1} band. An intense and thick band emerged in the 3000–3800 cm^{-1} region, matching the hydroxyl functional groups stretching mode.

Reduction of silver ions was seen in the reaction solution by measuring the UV absorption range of the solution using an optical spectrometer (X-ma 2000, UV-vis, Humancorp) at the wavelength of 350–650 nm. The absorption of the reaction solu-

tion was observed at a pH range of 5–10 and volumetric ratios of 0.05–0.6 at several periods for the formation of silver nanoparticles.

Figure 5 shows an absorption band at 414 nm that is related to the surface plasmon resonance of AgNPs. Also, we could find that the intensity of the surface plasmon resonance band raises by raising the amount of *S. striata* extract solution. It reveals that using a higher concentration of *S. striata* extract, the average size of AgNPs reduces and AgNPs concentration raises.

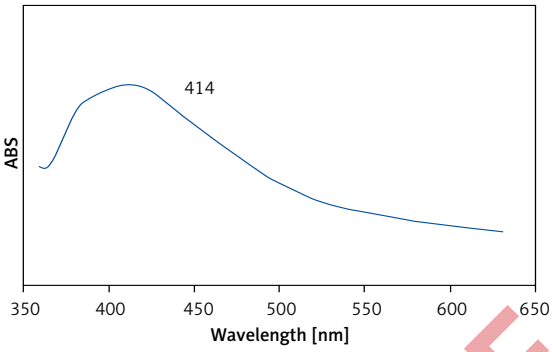


Figure 5. UV-Vis pattern of AgNPs

Antioxidant potential of AgNPs

Medicinal plants have important antioxidant components with an eminent property of improving oxidative stress-related degenerative ailments with minimal cytotoxicity [28]. Their relative potency is largely proportional to their interactions and synergistic effects with endogenous antioxidants in the eradication of free radicals [29, 30]. The aqueous extract of *Allium saralicum* [31], *Falcaria vulgaris* [32], and *Thymus kotschyanus* [33] were found to exhibit antioxidant effects through the degradation of free radicals. Besides, *A. saralicum, F. vulgaris*, and *T. kotschyanus* contain phytocompounds such as alkaloid, anthraquinone, flavonoid, phenolic, saponin, steroids, and tannin which have been revealed to confer antioxidant effects in cellular systems [31–33]. One option for raising the antioxidant activity of plants is combining them with metallic salts that are called herbal nanoparticles. The previous studies have reported that when plants are combined with gold, titanium, copper, iron, silver, and zinc salts, their antioxidant potentials significantly raise [17].

In the current experiment, the DPPH free radical scavenging potential of *S. striata* and *AgNPs* in many concentrations (0, 1, 3, 7, 15, 31, 62, 125, 250, 500, and 1000 µg/ml) revealed impressive prevention similar to BHT (Figure 6).

Metallic nanoparticles such as silver nanoparticles have excellent potential in inhibiting free radicals such as DPPH. In agreement with our contents, Hemmati *et al*. have followed the synergistic effect between *Thymus vulgaris* and metals for raising the antioxidant capacities [19].

The antioxidant effect shown by *S. striata* can be attributed to the presence of various phytochemicals that are thought to function interactively and synergistically to neutralize free radicals [34]. These bioactive compounds have been revealed to maintain the redox homeostasis through multiple-step processes of antioxidant reactions, which involve initiation, propagation, branching, and termination of free radicals [34].

Cytotoxicity potential of AgNPs

In our study, the treated cells with several concentrations of the $AgNO_3$, *S. striata*, and AgNPs present were examined by MTT test for 48 h regarding the cytotoxicity property on normal (HUVEC) and chronic myeloid leukemia (JOSK-M, EM-2, CML-T1, and BV173) cell lines (Figures 7–11). The absorbance rate was determined at 570 nm, which indicated extraordinary viability on the normal cell line (HUVEC) even up to 1000 µg/ml for $AgNO_3$, *S. striata*, and AgNPs. In the case of

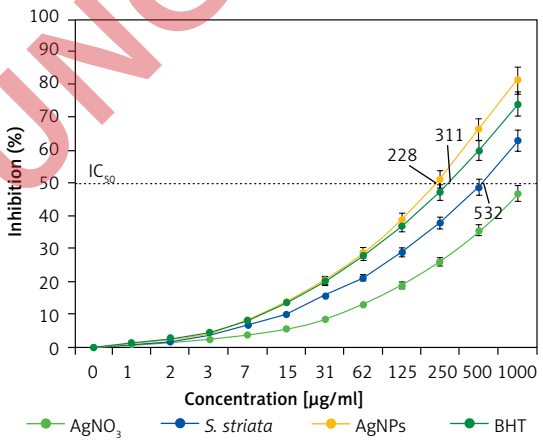


Figure 6. The antioxidant effects of $AgNO_3$, *S. striata*, AgNPs, and BHT against DPPH

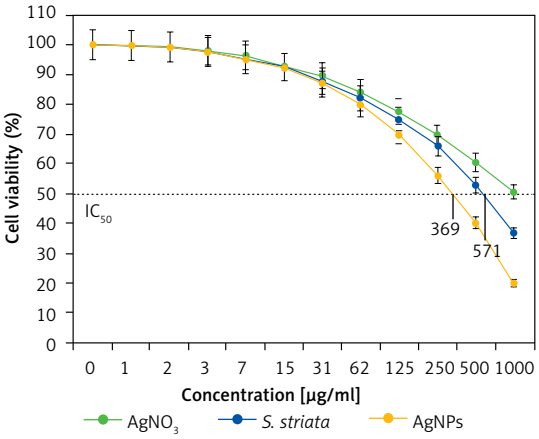


Figure 7. The anti-chronic myeloid leukemia effects of $AgNO_3$, *S. striata*, and AgNPs against JOSK-M

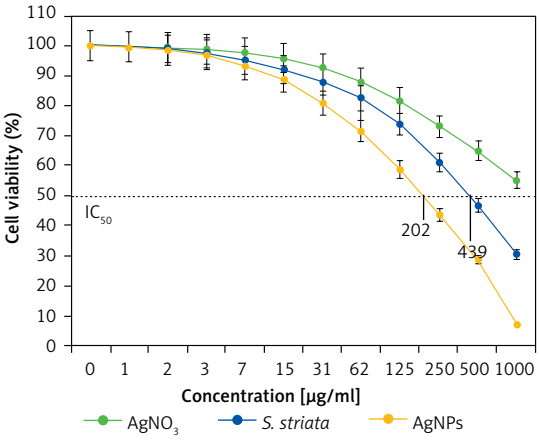


Figure 8. The anti-chronic myeloid leukemia effects of $AgNO_3$, *S. striata*, and AgNPs against EM-2

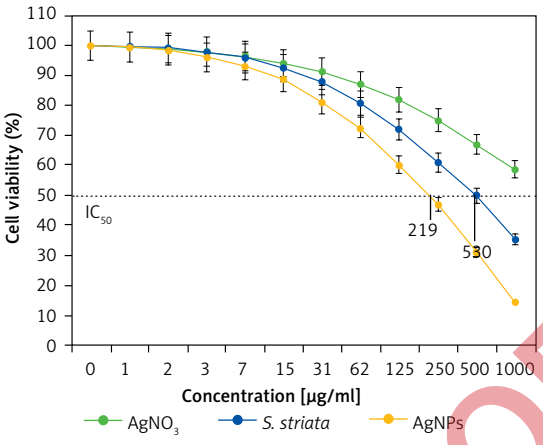


Figure 9. The anti-chronic myeloid leukemia effects of $AgNO_3$, *S. striata*, and AgNPs against CML-T1

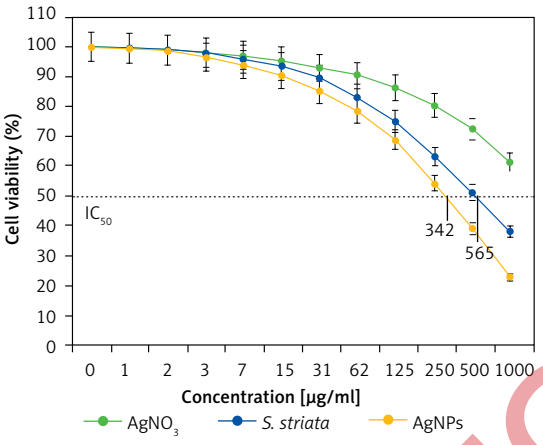


Figure 10. The anti-chronic myeloid leukemia effects of $AgNO_3$, *S. striata*, and AgNPs against BV173

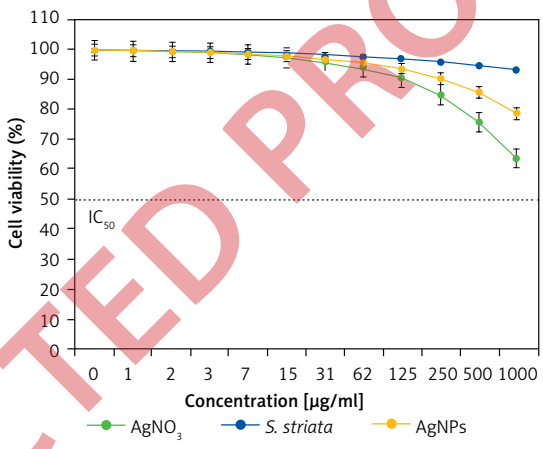


Figure 11. The cytotoxicity effects of $AgNO_3$, *S. striata*, and AgNPs against HUVEC

chronic myeloid leukemia cell lines, their viability reduced dose-dependently in the presence of $AgNO_3$, *S. striata*, and AgNPs. The best result of the cytotoxicity property of AgNPs against above cell lines was seen in the case of EM-2 cell line.

The cytotoxicity and anti-proliferative activities of *S. striata* are indicated in several cancers including liver, colon, and breast cancers but its molecular mechanisms of action have not been yet established [35–37]. Also, other studies found that *S. striata* has an oxidizing effect (production of reactive oxygen-derived ROS) against tumor cells such as acute leukemia cells [38–40]. As to the anticancer properties of silver nanoparticles, they have been used for the treatment of several cancers including human lung cancer, mammary carcinoma, uterus cancer, lung epithelial cancer, Lewis lung carcinoma, colon cancer, and human glioma [5]. The anticancer properties of silver nanoparticles were found to be highly dependent on a range of factors related to their physical characteristics, such as surface coating, shape, and size. As to the size, it has been reported that silver nanoparticles with a small size can transfer of cell membrane of tumor cells and remove them. In the larger size, the above ability significantly is confined [41]. As can be observed in Figures 2 and 3 of our study, silver nanoparticles had uniform spherical morphology in a size of 19.72 nm. The size of silver nanoparticles lower than 50 nm is very suitable for the killing of tumor cell lines *in vivo* and *in vitro* [41].

Likely the significant anti-leukemia potentials of silver nanoparticles synthesized by *S. striata* aqueous extract are linked to their antioxidant activities. The similar research studies have revealed that the antioxidant materials such as metallic nanoparticles especially silver nanoparticles and ethno medicinal plants reduce the volume of tumors by removing free radicals [42, 43]. In detail, the high presence of free radicals in the normal cells make many mutations in their DNA and RNA, destroy their gene expression and then accelerate the proliferation and growth of abnormal cells or cancerous cells [42–44]. The free radicals high presences in all cancers such as breast, gallbladder, stomach, rectal, liver, gastrointestinal stromal, esophageal, bile duct, small intestine, pancreatic,

colon, parathyroid, thyroid, bladder, prostate, testicular, fallopian tube, vaginal, ovarian, hypopharyngeal, throat, lung, and skin cancers indicate a significant role of these molecules in angiogenesis and tumorigenesis [43, 44]. Many researchers reported that silver nanoparticles synthesized by ethno medicinal plants have a remarkable role in the removing of free radicals and growth inhibition of all cancerous cells [45, 46].

In conclusion, the *Scrophularia striata* leaf harvested from China was used for synthesizing of silver nanoparticles as a suitable and safe material. After silver nanoparticle synthesizing, they were characterized and analyzed by UV-Vis and FT-IR Spectroscopy, Field Emission-Scanning Electron Microscopy (FE-SEM), and Transmission Electron Microscopy (TEM), and Energy Dispersive X-ray Spectrometry (EDS). The above tests indicated that these nanoparticles were synthesized as the best possible form.

In the biological experiments, silver nanoparticles revealed excellent antioxidant and cytotoxicity activities against chronic myeloid leukemia cell lines including BV173 (chronic myeloid leukemia in blast crisis), CML-T1 (chronic myeloid leukemia in lymphoid blast crisis), EM-2 (chronic myeloid leukemia in blast crisis; relapse after bone marrow transplantation), and JOSK-M (chronic myeloid leukemia in myelomonocytic cells). It looks that silver nanoparticles may be administered as chemotherapeutic supplements or drugs.

Conflict of interest

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