Characterization and anti-acute myeloid leukemia and anti-acute T cell leukemia properties of zinc nanoparticles synthesized by a green approach for bioremediation applications

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Abstract

Introduction: Every year, many people die due to cancer in all of the world. So, the preparation and formulation of new chemotherapeutic supplements and drugs with remarkable effects to treat cancer are a priority of both developing and developed countries. Recently, zinc nanoparticles have been used as modern chemotherapeutic drugs to treat several cancers such as leukemia, lung cancer, breast cancer, prostate cancer, etc. In this study, zinc nanoparticles (ZnNPs) were synthesized in an aqueous medium using *Fumaria officinalis* leaf as stabilizing and reducing agents.

Material and methods: The green synthesized ZnNPs@*Fumaria officinalis* were characterized using different techniques including UV-visible and FT-IR spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), and energy dispersive X-ray spectrometry (EDS). The anticancer activity of ZnNPs@*Fumaria officinalis* was evaluated against acute myeloid leukemia and acute T cell leukemia**.** According to the XRD analysis, 20.44 nm was measured for the crystal size of ZnNPs@*Fumaria officinalis*.

Results: SEM images showed a uniform spherical morphology with an average size of 27.96 nm for ZnNPs@*Fumaria officinalis*. In the cellular and molecular part of the recent study, the cells treated with ZnNPs@*Fumaria officinalis* were assessed by MTT assay for 48 h regarding the cytotoxicity and anti-human acute leukemia properties on normal (HUVEC), acute myeloid leukemia (32D-FLT3-ITD and Human HL-60/vcr), and acute T cell leukemia (Jurkat, Clone E6-1 and J.RT3-T3.5) cell lines. In the antioxidant test, the IC_{50} values of ZnNPs@*Fumaria officinalis* and BHT against DPPH free radicals were 187 and 197 µg/ml, respectively. The IC_{50} values of ZnNPs@*Fumaria officinalis* were 227, 200, 250, and 336 µg/ml against 32D-FLT3-ITD, Human HL-60/vcr, Jurkat, Clone E6-1, and J.RT3-T3.5 cell lines, respectively. The viability of the malignant leukemia cell line decreased dose-dependently in the presence of ZnNPs@*Fumaria officinalis*.

Conclusions: It appears that the anti-human acute leukemia effect of ZnNPs@*Fumaria officinalis* is due to their antioxidant effects.

Key words: zinc nanoparticles, *Fumaria officinalis* leaf, green synthesis, chemical characterization, anti-acute myeloid leukemia, anti-acute T cell leukemia.

Introduction

Previous studies have indicated that when metallic nanoparticles are green-synthesized by ethnomedicinal plants rich in antioxidant molecules, their therapeutic properties such as anti-human cancer effects significantly increase. Many doctors use chemotherapy, immunotherapy, and radiation therapy to treat several types of cancers [1–3]. Chemotherapeutic drugs have a bad effect on the body, so today the formulation of an effective chemotherapy drug from metallic nanoparticles is important [2, 3].

Nanotechnology can yield modern systems, tools, and materials by taking control at the atomic and molecular levels using the features on those surfaces. Applications for nanotechnology in medical diagnostics, food, medicine, biotechnology, environment, energy, chemistry, physics, etc., introduce this technology in an interdisciplinary and cross-sectoral context. The interdisciplinary nature of nanoscience and nanotechnology that can yield modern systems, tools, and materials with precision atoms and molecules will sooner or later affect the health and medical sector [1–3]. Drug use is currently volumetric, so most cells in the body need medication. In the new method, the drug is directed directly to specific cells with new injection devices and delivered to the required location. By this mechanism, small and large diseases can be diagnosed and treated at the beginning of their development [1, 2]. The National Nanotechnology Project is being implemented in European countries, the United States, and Japan with high priority in various fields [3]. Emerging fields of nanotechnology and nanoscience can move materials very accurately, to understand and control unprecedented fundamental components of physical objects. It seems that these developments will change the way we design and build everything from vaccines to computers. The plan would increase investment in nanotechnology about twice as much each year as the previous year [2].

A branch of nanotechnology is the formulation of new drugs with metal nanoparticles [1–3]. Today, nanoparticles have become very popular due to their wide applications in biology and medicine. Structurally, their size is in the range of 100 nanometers. Several drugs such as small hydrophobic and hydrophilic drugs, molecules, and vaccines can be administered by these nanoparticles. They are widely used in improving the treatment and diagnosis of diseases. Nanoparticles in nanoliposomes, carbon nanotubes, nanofibers, and nanospheres have been widely used for drug carriers and in the manufacture of cell scaffolds [2]. Applications of nanoparticles in drug delivery include drug carriers in cancer, cardiovascular disease, and Alzheimer's. The use of these nanocarriers is very effective for neurological diseases such as Alzheimer's. Due to their size, these nanoparticles can cross the blood-brain barrier, which has always been a barrier to the passage of drugs to the affected area in this type of destructive brain disease. Due to their small size, nanoparticles can also be used in brain cancers [3]. The goal in making nanoparticles is to control the surface properties, particle size, and release of a specific and efficient drug in a specific place and time for the drug to be as effective as possible. Nanoparticles are widely used in tissue engineering scaffolds, targeted drug delivery, and disease diagnosis. At present, many drug delivery systems are made of nanoparticles and different materials have been used as drug stimulants or enhancers to ameliorate the effectiveness of treatment and the durability and stability as well as the safety of anticancer drugs [1, 2]. The substances used to release cancer drugs are divided into different polymers, magnetic, and biomolecules. These materials can also provide surface modifications such as binding to target antibodies and ligands to make the nanoparticles act purposefully to increase the effectiveness of the treatment [2].

Metallic nanoparticles containing medicinal plants have very significant anti-cancer effects. In recent years, these metal nanoparticles containing herbs have been used to treat various cancers of the ovaries, prostate, esophagus, stomach, lungs, and various leukemias [1–6]. Acute leukemia is one of the most common types of cancers in recent years, and is one of the most important cancers in the blood system. Radiation therapy, targeted therapy, immunotherapy, chemotherapy, and surgery are the therapeutic options of acute leukemia [4]. Another option to treat acute leukemia is using metallic nanoparticles green synthesized by medicinal plants. It is predicted that if metal nanoparticles are synthesized and formulated with these plants, their anti-cancer effects against acute leukemia cells will be much stronger [2–4].

Fumitory or *Fumaria officinalis* is an annual plant. The plant belongs to the family of Fumariaceae [7]. *Fumaria officinalis* comprises various secondary metabolites such as polyphenolic compounds (caffeic acid, rosmarinic acid), flavonoid compounds (isoquercitrin, quercetin, rutin, kaempferol), and alkaloid compounds (protopine, cryptopine, stylopine, N-methylsinactine, fumaritine, fumariline), saponins, carbohydrates, glycosides, and tannins [8–11]. The plant is known as a popular medicinal plant in different traditional medicine worldwide [7, 10]. *Fumaria officinalis* is used as an agent to cure hepatobiliary dysfunction, gastrointestinal diseases, diuretic problems, cancer, and skin disorders [12, 13]. Various organic compounds have a role in the plant responses

to the biological activity of *Fumaria officinalis* [14]. In the current research, the properties of zinc nanoparticles that were formed and conjugated to molecules from aqueous extract of *Fumaria officinalis* leaf against acute myeloid leukemia (32D-FLT3-ITD and Human HL-60/vcr) and acute T cell leukemia (Jurkat, Clone E6-1 and J.RT3-T3.5) were evaluated.

Material and methods

Preparation and extraction of aqueous extract

The aqueous extract of *Fumaria officinalis* was obtained by maceration of 120 g of the plant leaves in boiling water for 6 h. Then, a paper filter was used for separation of the plant part from the extract. Next, the diluted extract was evaporated. Finally, the produced crude extract was placed in a freeze dryer for 72 h.

Chemical characterization of ZnNPs@*Fumaria officinalis*

The green synthesized nanoparticles were characterized using different techniques such as UV-visible spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), and energy dispersive X-ray spectrometry (EDS).

Green synthesis and chemical characterization of ZnNPs@*Fumaria officinalis*

The green synthesis of ZnNPs@*Fumaria officinalis* was carried out according to a previous study with some modification [15]. First, 0.4 g of the dried plant extract was dissolved in 50 ml of deionized water and added to 15 ml of $Zn(NO_3)_2 \times 6\ H_2O$ (0.04 M). The pH was adjusted to 8. Then, the container was placed in an ultrasonic bath for 1 h at 60°C. During the reaction time, the nanoparticles were formed. The precipitate was washed with ethanol : water (1 : 1) 3 times, centrifuged at 12000 rps for 15 min, and subsequently dried in an oven at 50°C The obtained powder was kept in a vial for the chemical characterization and evaluation of its biological activity.

Anti-human acute leukemia effects of ZnNPs@*Fumaria officinalis*

MTT is a colorimetric technique. Based on the fact that living cells can carry out oxidative metabolism, as a result, oxidation breaks down the MTT dye and produces a dye ranging from purplish to blue. This test determines the number of living cells [16].

In this research, we used the following cell lines to evaluate anti-human acute leukemia and cytotoxicity effects of $Zn(NO_3)_2$, *Fumaria officinalis* leaf aqueous extract, and ZnNPs@*Fumaria officinalis* using an MTT method.

a) Normal cell line:
   – HUVEC;
b) Human acute leukemia cell lines:
   – Acute myeloid leukemia (32D-FLT3-ITD and Human HL-60/vcr),
   – Acute T cell leukemia (Jurkat, Clone E6-1 and J.RT3-T3.5).

These cell lines were cultured in appropriate numbers (10,000 cells/well) in a 96-well microplate. They were then incubated at 37°C and 5% CO_2 for 24 h to form a cell monolayer. Then, out of the greenhouse and under the hood, different concentrations of $Zn(NO_3)_2$, *Fumaria officinalis* leaf aqueous extract, and ZnNPs@*Fumaria officinalis* were added to the normal and cancerous cells. Control wells also included a cell control containing complete cell and culture medium and a blank control containing no cells and complete culture medium, and the microplates were again placed in the oven at 37°C and 5% CO_2 until the required time (24–48–72 h). After the test times, the cells were washed with PBS saline phosphate, MTT dye was added to the wells and the microplates were incubated for 3 h [16].

Finally, the optical absorption of cells at 570 nm was read by the ELISA reader (model: Tek Bio Elx800). The cell viability percentage was calculated by the following formula [16]: Cell viability (%) = (Sample A/Control A) × 100.

Antioxidant properties of ZnNPs@*Fumaria officinalis*

The free radical scavenging test was first performed by Blois in 1958, and after some modification by numerous studies in its current form. The DPPH method is one of the most widely used methods for estimating antioxidant content. DPPH is a stable radical that reacts with hydrogen atom compounds. This test is based on the inhibition of DPPH, which causes the decolorization of DPPH solution by adding radical species or antioxidants. DPPH changes color from purple to yellow by taking an electron from the antioxidant compound. The free radicals in DPPH are adsorbed at 517 nm, which follows Beer Lambert's law, and decreased absorption is linearly related to the amount of antioxidants; the higher the amount of antioxidants, the more DPPH is consumed and the more purple turns yellow [6]. In a recent study, the degree of inhibition of DPPH radicals was evaluated by Shaneza *et al.* [6]. For this purpose, solutions with different samples of the $Zn(NO_3)_2$, *Fumaria officinalis* leaf aqueous extract, and ZnNPs@*Fumaria officinalis* of variable concentrations (0–1000 µg/ml) as well as synthetic antioxidant BHT in methanol solvent were prepared. The test method was that 1 ml

of DPPH methanolic solution (at a concentration of 1 mM) was added to 4 ml of the extract and the resulting mixture was stirred vigorously [6].

The test tubes were placed in a dark place for 60 minutes. After this period, the absorbance was read at 517 nm. Finally, the DPPH radicals' inhibition percentage of the $Zn(NO_3)_2$, *Fumaria officinalis* leaf aqueous extract, and ZnNPs@*Fumaria officinalis* was calculated by the formula below [6]: Inhibition (%) = (Sample A/Control A) × 100.

IC_{50} was used to evaluate better the antioxidant activity, which indicates the concentration of $Zn(NO_3)_2$, *Fumaria officinalis* leaf aqueous extract, and ZnNPs@*Fumaria officinalis* that can reduce the concentration of free radical DPPH. The initial is 50% of the initial value, and the lower the amount, the greater the antioxidant activity [6].

Qualitative measurement

After collecting data, Minitab statistical software was used for statistical analysis. For evaluation of antioxidant results in a completely randomized design and comparison of means Duncan's post-hoc test with a maximum error of 5% was used. To measure the percentage of cell survival in factorial experiments with the original design of completely randomized blocks and compare the means, a Duncan's post-hoc test with a maximum error of 5% was used. The 50% cytotoxicity (IC_{50}) and 50% free radical scavenging (IC_{50})) were estimated with ED50 plus software (INER, V: 1.0). Measurements were reported as mean ± standard deviation.

Results and Discussion

Chemical characterization of ZnNPs@*Fumaria officinalis*

XRD analysis

The crystallinity of ZnNPs@*Fumaria officinalis* was studied using the XRD diagram that is shown in Figure 1. The formation of ZnNPs@*Fumaria officinalis* (is approved to this result). The obtained data were compared with the standard database ICDD PDF card no. 01-080-3002. According to the results, the peaks at 31.96, 33.01, 36.44, 47.51, and 56.35 correspond to diffraction planes of (100), (002), (101), (102), and (110) respectively. The peaks at different degrees for green synthesized zinc nanoparticles have been reported previously [17]. The diffractogram shows that despite the small size of zinc nanoparticles, they are well crystallized. The average size of the crystal was calculated with a value of 20.44 nm, which was obtained using Scherrer's formula.

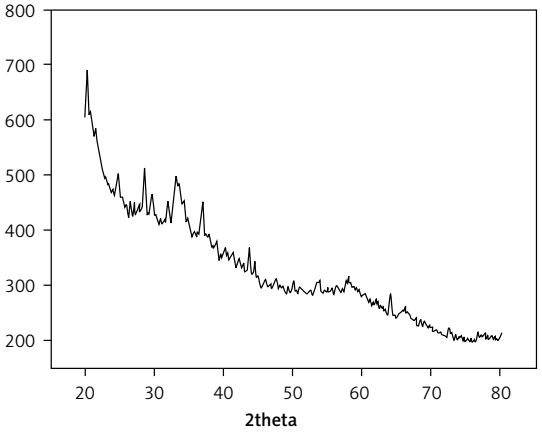

Figure 1. XRD Pattern of ZnNPs@*Fumaria officinalis*

SEM analysis

In this study, we used FE-SEM imaging to evaluate the morphology and shape of the synthesized zinc nanoparticles. According to the FE-SEM images of ZnNPs@*Fumaria officinalis* (Figures 2 A, B), the nanoparticles are synthesized in spherical morphology with good dispersion. Additionally, the NPs tend to aggregate, which is a common property for green synthesized metallic nanoparticles [18, 19]. As shown in Figure 2 and according to the findings, 27.96 nm was the average diameter of ZnNPs@*F. officinalis*. *C. officinalis* has not been defined.

EDS analysis

To evaluate the elemental composition of ZnNPs@*Fumaria officinalis*, we used the EDS technique, which is known as a sufficient quality method for this proposal. Figure 3 presents the EDS analysis ZnNPs@*Fumaria officinalis*. The presence of zinc in the synthesized ZnNPs@*Fumaria officinalis* is indicated by the presence of signals at 1 keV for ZnLα, below 9 keV for ZnKα; and below 10 keV for ZnKβ; the other signals below 0.5 keV are related to oxygen and carbon of organic compounds that are linked to the surface of ZnNPs@*Fumaria officinalis*. These signals have been reported for the green synthesized ZnONPs previously [20].

UV-visible analysis

The UV-Vis. spectrum of the green-synthesized ZnNPs using the aqueous extract of *Fumaria of-*

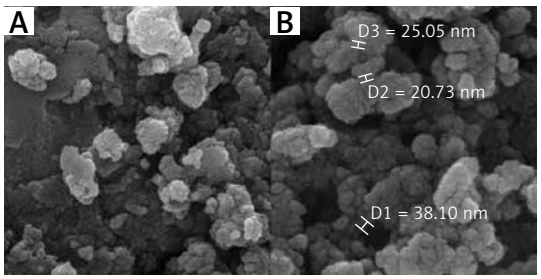

Figure 2. Surface morphology of ZnNPs@*C. officinalis*

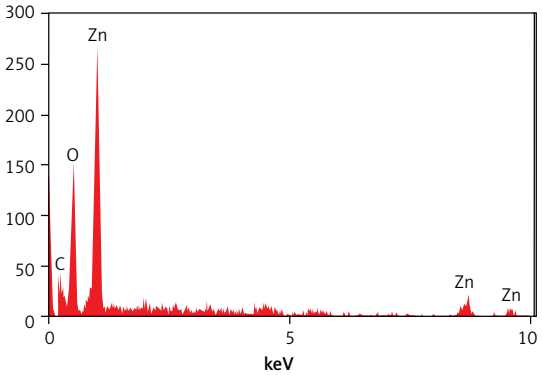

Figure 3. EDS analysis of ZnNPs@*Fumaria officinalis*

ficinalis is shown in Figure 4. The surface plasmon resonance of ZnNPs@*Fumaria officinalis* was confirmed by UV-Vis. analysis. The bands at the wavelengths of 225, 274, and 387 nm indicate the formation of zinc nanoparticles. Similar bands for the green synthetic zinc nanoparticles have been reported previously [21].

FT-IR analysis

Figure 5 presents the FT-IR spectrum of ZnNPs@*Fumaria officinalis*. According to the results, the peaks at 505 and 609 cm^{-1} are related to the vibration of the Zn-O band. Peaks with similar wavenumber have been reported for the green synthesized zinc nanoparticles [22]. The other peaks can be considered for various functional groups of organic compounds linked to the surface of ZnNPs@*Fumaria officinalis*. The organic compounds in the extract of *C. officinalis* are known as the main capping and reducing agents for the green synthesis of ZnNPs@*Fumaria officinalis*. The bands at 3404 and 2937 cm^{-1} related to O-H and aliphatic C-H stretching; the peaks at the range of 1434 to 1668 cm^{-1} correspond to C=C and C=O stretching, and peaks at 1253 and 1088cm^{-1} could be ascribed to -C-O and -C-O-C stretching. The presence of these peaks can be considered to represent secondary metabolites such as phenolic, flavonoids, saponins, carbohydrates, and tannins in the plant extract that have been reported previously [8–11].

Antioxidant properties of ZnNPs@*Fumaria officinalis*

In this study, we assessed the antioxidant properties of *Fumaria officinalis* leaf aqueous extract green-synthesized ZnNPs@*Fumaria officinalis* by using the DPPH test as a common free radical. Free radicals are atoms, molecules, or ions with unpaired electrons and are therefore very active, unstable, and highly reactive. Free radicals are formed by breaking a bond of a stable molecule. Free radicals collide with other molecules to achieve stability and can separate electrons from

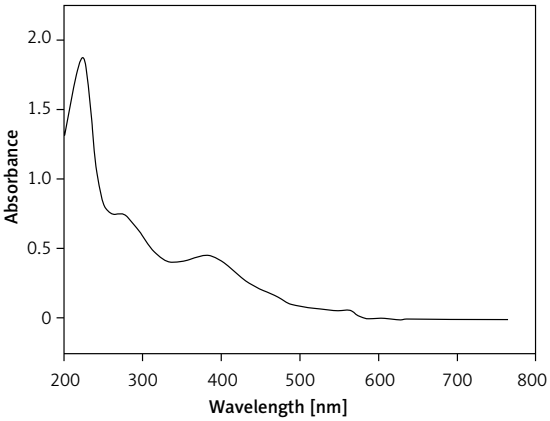

Figure 4. UV-Vis. spectrum of ZnNPs@*Fumaria officinalis*

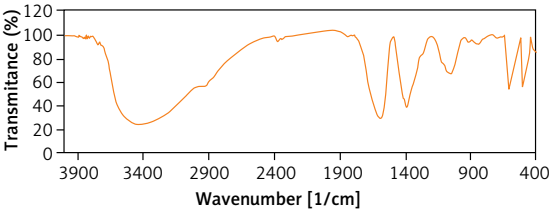

Figure 5. FT-IR Spectrum of ZnNPs@*Fumaria officinalis*

them; as a result, they form a chain of more unstable molecules. A free radical can have a positive, negative, or neutral charge [6]. During the body's natural metabolism or under conditions such as smoking, pollution, the entry of unnecessary chemicals into the body in any way, radiation, and stress, free radicals are produced in the body. The most important free radical in the human body is oxygen, damaging DNA and other molecules. Oxidative stress is the victory of free radicals over the body's antioxidant defense and is a biological attack on the body [2, 3]. Antioxidants are molecules that can donate an electron to a free radical without destabilizing themselves. This stabilizes the free radical and makes it less reactive. The result of oxidative stress in the body is various degeneration, eye damage, premature aging, muscle problems, brain damage, heart failure, diabetes, cancer, and overall weakness of the immune system [1]. Oxygen radicals are continuously produced in all living organisms and with destructive effects, leading to cell damage and death. The production of oxidant species under physiological conditions has a controlled rate, but this production increases under oxidative conditions [6]. Various studies have shown that antioxidant compounds have very significant anti-cancer effects through elimination of free radicals. Herbs are rich in antioxidant compounds and reduce the risk of some chronic diseases such as cataracts, rheumatoid arthritis, memory loss, stroke, heart disease, and cancer by protecting cells and increasing the pow-

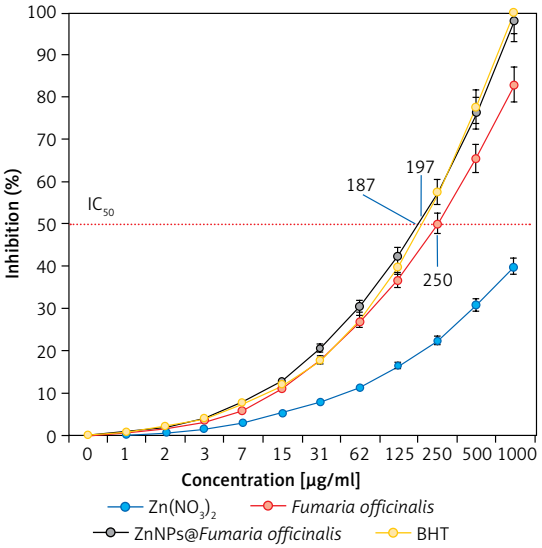

Figure 6. Antioxidant properties of $Zn(NO_3)_2$, *Fumaria officinalis* leaf aqueous extract, ZnNPs@*Fumaria officinalis*, and BHT against DPPH

er of plasma antioxidants. Flavonoids and alkaloids commonly found in medicinal plants have high antioxidant activity [2, 3].

The scavenging capacity of *Fumaria officinalis* leaf aqueous extract green-synthesized ZnNPs@*Fumaria officinalis* and BHT at different concentrations expressed as percentage inhibition is indicated in Figure 6. In the antioxidant test, the IC_{50} values of ZnNPs@*Fumaria officinalis* and BHT against DPPH free radicals were 187 and 197 µg/ml, respectively (Figure 6).

Cytotoxicity and anti-human acute leukemia activities of ZnNPs@*Fumaria officinalis*

One of the cytotoxicity test methods to measure the rate of cell death is the MTT method,

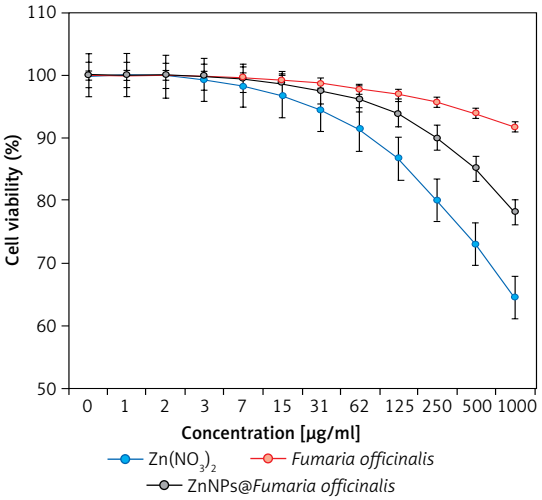

Figure 7. Cytotoxic properties (cell viability (%)) of $Zn(NO_3)_2$, *Fumaria officinalis* leaf aqueous extract, and ZnNPs@*Fumaria officinalis* (concentrations of 0–1000 µg/ml) against human normal (HUVEC) cell line

which is based on the formation of formazan dye by reducing the substance MTT (dimethyl thiazole 2 and 5 diphenyltetrazolium bromide) or other tetrazolium salts [23–25]. By breaking the MTT tetrazolium ring by mitochondrial enzymes in living cells, insoluble purple formazan crystals are formed. The formation of these crystals indicates the activity of respiratory chain enzymes and is a measure of cell viability. By measuring the absorption by spectrophotometry at specific wavelengths, the number of living cells can be determined. This test is performed according to ISO 10993-5 and its purpose is in vitro evaluation of cytotoxicity. The cytotoxicity test is performed according to the ISO10993-5 standard and in four ways: the NRU test, the CFU test, the MTT test and the XTT test. The most common method for assessing cytotoxicity is to measure cell survival by MTT [24–26]. The MTT method is based on the intensity of dye produced by the mitochondrial activity of cells, measured at a wavelength of 540 to 630 nm and directly proportional to the number of living cells; the increase or decrease in the number of living cells is linearly related to the activity of cell mitochondria. MTT tetrazolium dye is revived in active (metabolically) cells. Mitochondrial dehydrogenases in living cells produce NADH and NADPH, leading to an insoluble purple precipitate called formazan. This precipitate can be dissolved by isopropanol or dimethyl sulfoxide [24–27]. Dead cells, on the other hand, are unable to perform this conversion due to the inactivity of their mitochondria and therefore do not show a signal. In this method, dye formation is used as a marker for living cells [27, 28]. In recent years, MTT testing has been the most important measurement method to evaluate the toxicity and anti-cancer effects of metal nanoparticles [16, 27, 28].

In this investigation, the cells treated with different concentrations of the present $Zn(NO_3)_2$, *Fumaria officinalis* leaf aqueous extract, and ZnNPs@*Fumaria officinalis* were assessed by MTT assay for 48 h regarding the cytotoxici properties towards normal (HUVEC) and leukemia malignancy cell lines, i.e. acute myeloid leukemia (32D-FLT3-ITD and Human HL-60/vcr) and acute T cell leukemia (Jurkat, Clone E6-1 and J.RT3-T3.5).

The absorbance rate was evaluated at 570 nm, which represented viability on a normal cell line (HUVEC) even up to 1000 µg/ml for $Zn(NO_3)_2$, *Fumaria officinalis* leaf aqueous extract, and ZnNPs@*Fumaria officinalis* (Figure 7).

The viability of malignant leukemia cell line decreased dose-dependently in the presence of $Zn(NO_3)_2$, *Fumaria officinalis* leaf aqueous extract, and ZnNPs@*Fumaria officinalis*. The IC_{50} values of ZnNPs@*Fumaria officinalis* were 227, 200, 250, and 336 µg/ml against 32D-FLT3-ITD, Human HL-

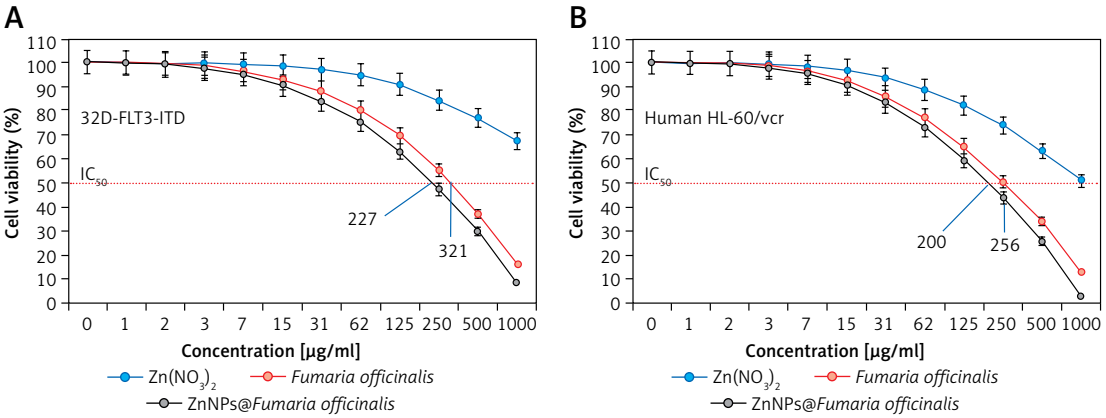

Figure 8. Anti-acute myeloid leukemia properties (cell viability (%)) of $Zn(NO_3)_2$, *Fumaria officinalis* leaf aqueous extract, and ZnNPs@*Fumaria officinalis* (concentrations of 0–1000 µg/ml) against acute myeloid leukemia (32D-FLT3-ITD and Human HL-60/vcr) cell lines

60/vcr, Jurkat, Clone E6-1, and J.RT3-T3.5 cell lines, respectively (Figures 8, 9).

It seems that the anti-human acute leukemia effect of recent nanoparticles is due to their antioxidant effects. Because tumor progression is so closely linked to inflammation and oxidative stress, a compound with anti-inflammatory or antioxidant properties can be an anticarcinogenic agent [29–31].

Many nanoparticles have pharmacological and biochemical properties, including antioxidant and anti-inflammatory properties, which appear to be involved in anticarcinogenic and antimutagenic activities [6, 16]. Today, nanoparticles synthesized by biological methods play a vital role in treating many diseases, including cancer [30, 31].

Nanoparticles synthesized by biological methods are no longer the only ones in traditional medicine; in addition, they have been able to adopt an industrial line of natural products for treating various cancers. Various cell lines from cancers of the prostate, ovary, lung, liver, and pancreas have been treated with synthesized herbal nanoparticles [29–31].

In conclusion, in this research, the zinc nanoparticles were green-synthesized using an aqueous medium and *Fumaria officinalis* leaf extract. A few techniques such as UV-Vis. and FT-IR spectroscopy, FE-SEM, XRD, and EDS analysis were used to characterize ZnNPs@*Fumaria officinalis*. The obtained results revealed that ZnNPs@*Fumaria officinalis* had been successfully synthesized with an average size of 27.96 nm. The viability of the malignant leukemia cell line decreased dose-dependently in the presence of ZnNPs@*Fumaria officinalis*. The IC_{50} values of ZnNPs@*c* were 227, 200, 250, and 336 µg/ml against 32D-FLT3-ITD, Human HL-60/vcr, Jurkat, Clone E6-1, and J.RT3-T3.5 cell lines, respectively. The ZnNPs@*Fumaria officinalis* showed the best antioxidant activities against DPPH. The IC_{50} values of ZnNPs@*Fumaria officinalis* and BHT against DPPH free radicals were 187 and 197 µg/ml, respectively. After the clinical study, ZnNPs@*Fumaria officinalis* containing *Fumaria officinalis* leaf aqueous extract can be used as an efficient drug/supplement to treat acute leukemia in humans.

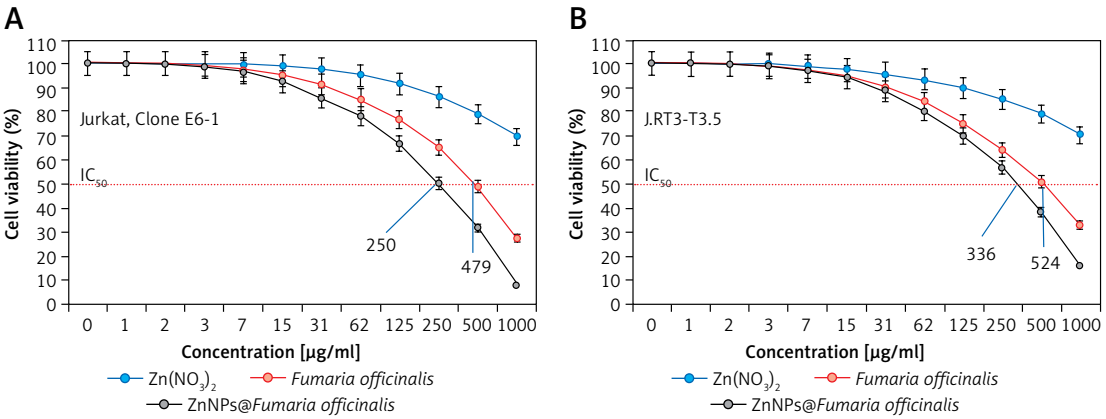

Figure 9. Anti-acute T cell leukemia properties (cell viability (%)) of $Zn(NO_3)_2$, *Fumaria officinalis* leaf aqueous extract and ZnNPs@*Fumaria officinalis* (concentrations of 0–1000 µg/ml) against acute T cell leukemia (Jurkat, Clone E6-1 and J.RT3-T3.5) cell lines

Conflict of interest

The authors declare no conflict of interest.

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