Salvia officinalis-based green-mediated vanadium nanoparticles: Describing a modern chemotherapeutic drug for the treatment of colorectal carcinoma

Ruimin Xu[1]

[1]Hainan Medical University

November 16, 2022

Abstract

In this study, vanadium nanoparticles were green synthesized using the aqueous extract of Salvia officinalis. Different techniques such as FE-SEM, XRD, FT-IR, and EDS analysis were used to characterize VNPs@Salvia officinalis. A 25.6 nm was obtained for the crystal size of the vanadium nanoparticles using XRD analysis. The FE-SEM images show a spherical morphology for VNPs@Salvia officinalis with the range size of 11.28 to 40.74 for the synthetic nanoparticles. In the antioxidant test, the IC50 of VNPs@Salvia officinalis and BHT against DPPH free radicals were 300 and 265 μg/mL, respectively. For anticancer activity evaluation the treated cells with VNPs@Salvia officinalis were assessed by MTT assay for 48h about the cytotoxicity and anti-colorectal cancer properties on normal (HUVEC) and colorectal cancer cell lines i.e., Caco-2, COLO 320, DLD-1, HCT-15, HCT-116, and HT-29. The IC50 of VNPs@Salvia officinalis were 213, 210, 297, 204, 160, and 125 μg/mL against Caco-2, COLO 320, DLD-1, HCT-15, HCT-116, and HT-29 cell lines, respectively. The viability of malignant colorectal cell lines reduced dose-dependently in the presence of VNPs@Salvia officinalis. It appears that the anti-colorectal cancer effect of VNPs@Salvia officinalis is due to their antioxidant effects.

1. Introduction

Herbal medicines are more important in preventing all types of cancer due to the presence of fewer side effects than chemical medicines. For this purpose, extensive studies have been conducted on various plants and their cytotoxicity and anticancer effects have been investigated and evaluated [1-3]. In this regard, measuring cell survival and proliferation seems to be important in determining the effect of anticancer drugs on cells; In this regard, several methods have been standardized. *Salvia officinalis* is a flowering plant, sedum, dicotyledons with continuous petals, a shrub with woody and stable roots, 30 to 60 cm high, simple leaves [4-7]. This plant is native to the Middle East and Mediterranean regions, which has become common all over the world today. Due to its properties and unique taste, this plant is used in many foods and as an infusion [5,6]. In the traditional medicine of Asia and Latin America, *Salvia officinalis* is used to treat a variety of disorders such as seizures, wounds, inflammation and diarrhea, and in Europe to treat age-related cognitive disorders [7-9]. *Salvia officinalis* improves Alzheimer's and lowers blood sugar. In recent years, many studies have been conducted to find new biological effects for *Salvia officinalis* . These studies show a wide range of medicinal activities including antioxidant, anti-inflammatory, anti-cancer and analgesic effects [6-8]. The chemical components in the aqueous and hydroalcoholic extracts of this plant, such as cineole, penine, flavonoids, especially rosmarinic acid, saponins, vitamins E and C, etc., are responsible for these biological effects [3-6]. There is evidence that shows that rosmarinic acid, one of the compounds of*Salvia officinalis* , can suppress tumor development in several organs of the body, including colon, breast, liver, abdomen, as well as melanoma and leukemia cells. This substance can also increase the activity of catalase, superoxide dismutase and glutathione peroxidase enzymes [6-9].

Nanotechnology has grown rapidly in the manufacturing and production of nanoparticles with varied sizes, shapes and distribution [9,10]. Although physical and chemical methods may have known and successful pure production, they are generally hazardous to the environment, time-consuming, and expensive [10-12]. Therefore, considering the nanoparticle production environmental aspects, the use of plant biomass, plant extracts, plant oils and microorganisms can be a main alternative to the chemical and physical ways [10-13]. The biological production of nanoparticles greatly lowers the risk of danger to the environment and humans. The nanoparticle synthesis by biological materials has become the interest of researchers because of their new physical and chemical characteristics and their uses in several medical sciences, optics, electronics and mechanics [13-16]. Using physical ways needs high pressure and temperature as well as high cost. Also, in many chemical ways, chemicals are dangerous and toxic not only for the environment but also for biological systems [11-13]. The products of the chemical methods are so toxic. So, the need for a suitable way with low price, high efficiency, without environmental damage and toxic substances production is increasing [12-15]. Biological production is one of the ways of solving the above cases and attention to this way of producing nanoparticles is increasing. There is a big list of resources that are used in the metal nanoparticle biological production [13-16]. Things like microorganisms such as bacteria, actinomycetes, fungi and algae as well as plants and plant extracts are applied in the nanoparticle biological production [16-19]. The use of plants due to their compatibility with the environment and abundance are usually prioritized [17-21]. Also, due to their lack of need for special nutrients and conditions for growth, plants are considered the best option for the nanoparticle production by the biological method [19-21].

In the recent study, we investigated the vanadium nanoparticles green-synthesized by *Salvia officinalis* in the cytotoxicity studies against common human colorectal cancer cell lines i.e., Caco-2, COLO 320, DLD-1, HCT-15, HCT-116, and HT-29.

2. Methods and Materials

2.1. Preparation and extraction of aqueous extract

To prepare the aqueous extract of *Salvia officinalis* , 6 g of the dried part of the mill was poured into 150 ml of boiling water and boiled for ten minutes. Then the extract was allowed to cool down gradually. After complete cooling, the resulting extract was clarified by a high-speed centrifuge (20,000 rpm) for 15 minutes. The resulting sample was wrapped in aluminum foil and kept in the refrigerator.

2.2. Green synthesis and chemical characterization of VNPs@Salvia officinalis

VNPs@*Salvia officinalis* was green synthesized according to a reported procedure [21]. Briefly, a 50 mL of the plant extract (5 mg /10 mL of deionized water) was added to 50 mL of sodium metavenadatein at the concentration of 0.02 M. Then, the reaction mixture was stirred at room temperature 100 minutes. After the time, a yellow-orange precipitate was formed. Deionized water was used to wash the residue for three times and centrifuged interval at 10000 RPM for 15 minutes. The vanadium nanoparticles were put under a hood to dry.

2.3. DPPH Assay Protocol

In the recent experiment, 1ml of DPPH methanol solution was added to 1ml of concentrate to 3 ml of nanoparticles and the resulting mixture was stirred vigorously. The test tubes were placed in a dark place for 30 minutes. After this period, the absorbance at the wavelength of 517 nm was read. It should be noted that in the control sample, the nanoparticles was replaced with 3 ml of methanol. Finally, the DPPH radical's inhibition percentage was calculated with this formula [22,23]:

$$\text{Inhibition } (\%) = \frac{\text{Sample A.}}{\text{Control A.}} x100$$

2.4. MTT test protocol

The Caco-2, COLO 320, DLD-1, HCT-15, HCT-116, and HT-29 colorectal cancer cells in DMEM culture medium (Gibco, USA) with 10% FBS (Gibco, USA) and penicillin/streptomycin (100 µL/100 µg/ml) in an incubator containing 5 % Carbon dioxide with 90% humidity was stored at 37 °C.

Cancer cell lines were placed in 1640-RPMI medium from GiBco manufacturer and were cultured after adding 10% bovine serum, 1% streptomycin and penicillin antibiotics and 2% glutamine. At this stage, the cell culture flasks were kept in an incubator with 5% CO_2 and 95% humidity at a temperature of 37°C, and the culture medium was replaced every three days. In this step, flasks with 80% cell density were used (flasks filled with cells up to 80% of the bottom). First, the culture medium was removed from the surface of the cells and by adding 1 ml of trypsin for 3 minutes and then adding the same volume of medium to neutralize the effect of trypsin, all the cells were separated from the flask bottom. This cell suspension was centrifuged at 1200 rpm for 4 minutes. The liquid above the sediment was discarded and 1 ml of culture medium was added to the sediment. By taking 10 µl of the cell suspension and adding the same amount of trypan blue on the surface of the neobar slide, the number of living cells was counted. The number of 10,000 cells from this cell suspension was added to each well of 96-well plates and 180 µl of culture medium was added to it. In the next step, 20 µl different concentrations of nanoparticles were added to the wells. In this research, based on the conventional concentrations of nanoparticles at the 0-1000 µg/ml, they were added to cancer cells. Another group of cells were tested as a control, without adding nanoparticles and only by adding water instead of nanoparticles, and each experiment was done in four replicates. After 24, 48 and 72 hours, the medium on the cells was replaced with a new medium. Then 20 µl MTT solution was added to each well and placed in a greenhouse for 4 hours in the dark in a CO2 incubator. During this time, the mitochondrial succinate dehydrogenase enzyme of living cells changes the yellow MTT solution into purple formazan crystals, which are insoluble in water. In the next step, 200 µl of DMSO (Dimethylsulfoxide) was added to the empty medium and shaken for 20 minutes to dissolve the light-producing crystals. In the last step, the absorbance was read with a wavelength of 492 and then 630 nm in an ELISA reader. Finally, the percentage of cell viability was calculated after dividing the optical absorbance (OD) of treated cells compared to control cells and multiplying by 100 (22,23).

The collected data were analyzed using SPSS ver22 statistical software and a one-way ANNOVA test. P <0.01 was considered remarkable.

3. Results and Discussion

XRD analysis:

Figure 1 displays the XRD diffractogram of VNPs@*Salvia officinalis* . The pattern confirms the well crystallizing of the synthesized NPs. The data are matched to the standard database of JCPDS no: 85–2422 for vanadium oxide. The signals at 2ϑ values of 25.18, 27.79, 44.99, 49.43, 66.67, and 70.43, are indexed as (202), (103), (401), (205), (406), and (125) planes. A similar data has been reported for V_2O_5 previously [24]. The Scherer's equation was applied to calculate the crystal size of nanoparticles, that revealed 25.60 nm for the crystal size of VNPs@*Salvia officinalis* .

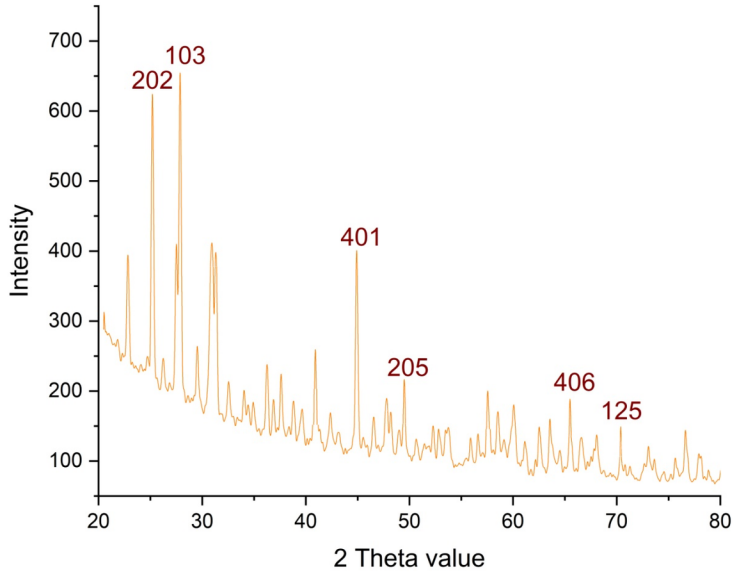

Fig. 1. XRD Pattern of VNPs@Salvia officinalis

SEM analysis:

SEM analysis is a member of the scanning electron microscope family and is used to examine the surface characteristics and morphology of different samples. In this method, electron beams with specific energy and wavelength sweep the sample surface [25-27]. By the detector data that have collected the return sample surface electrons, benefits data is obtained from the sample surface. It should be noted that image quality and high resolution in the images have a direct relationship with the structure of the sample and the quality of synthesis and the absence of contamination and unwanted particles, and samples with a specific structure provide acceptable images [28-32].

Figure 2 presents the FE-SEM images of images VNPs@*Salvia officinalis* . According to the results, a spherical shape is observed for the morphology of the synthetic NPs with the range size of 11.28 to 40.74 nm. The vanadium nanoparticles exhibit a propensity to aggregate that has been reported as a property for green synthetic metallic NPs including vanadium and others such as titanium, silver, and nickel [24-27]. The reported size for the green synthesized vanadium is in a range of 10 to 100 nm in the literature [28-32].

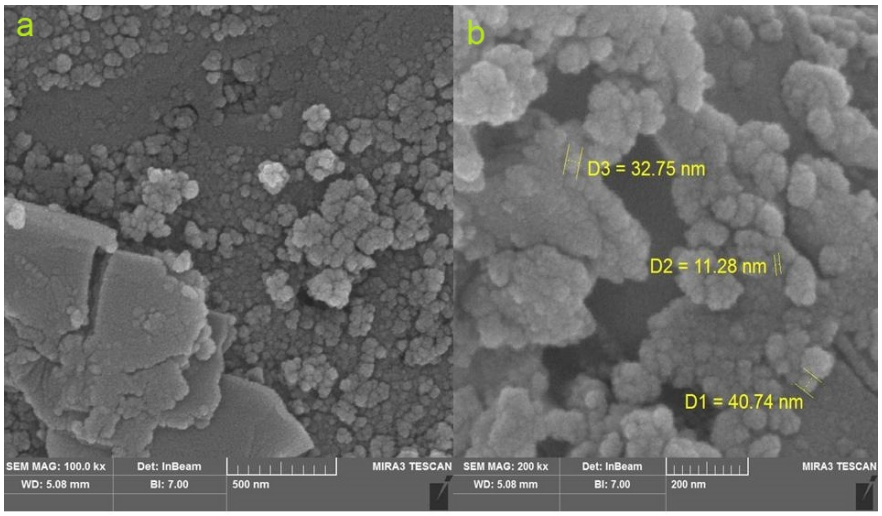

Fig. 2. FE-SEM images of VNPs@Salvia officinalis

FT-IR analysis:

Spectrophotometers are devices that determine the absorption or passage of special radiant energy wavelengths (light) from an analyte in a solution. Due to the difference in the number and arrangement of groups, the double bonds of carbon atoms in each molecule absorb light at specific wavelengths with a specific spectrum pattern. According to the Beer-Lambert law, the light absorbed amount at this specific wavelength is directly proportional to the chemical sample concentration [32].

In laboratories, a large part of the measurements is based on absorbance reactions. The activity of most cholesterol, triglycerides, enzymes, lipoproteins, creatinine, urea, sugar and a wide range of analytes with research and clinical applications, drugs and metabolites can be measured by spectrophotometry. Investigating the molecular structure, identifying compounds, comparing structures and finding the maximum absorption wavelength are other spectrophotometry applications in research problems [32].

Figure 3 shows the FT-IR spectrum of VNPs@*Salvia officinalis* . In the FT-IR results of metallic nanoparticles the peaks in 400 to 700 cm^{-1} usually attributes to metal-oxygen bonds. Accordingly, for VNPs@*Salvia officinalis* , the peaks at 462, 538, and 725 cm^{-1} can be assigned for V-O bond. Similar peaks with a little difference in wavenumbers have been reported for green synthetic vanadium nanoparticles [32]. There are many points of similarity between vanadium nanoparticles spectrum and that of plant extract. The peaks at 3205 m^{-1} (of O-H), 1424 to 1665 cm^{-1} (C=C and C=O s), and 1120 cm^{-1}(for the stretching vibration of C-O), which belong to various functional groups, reveal the linkage of different organic compounds to the surface of VNPs@*Salvia officinalis* . These compounds have been reported as *Salvia officinalis* secondary metabolites previously [7-9].

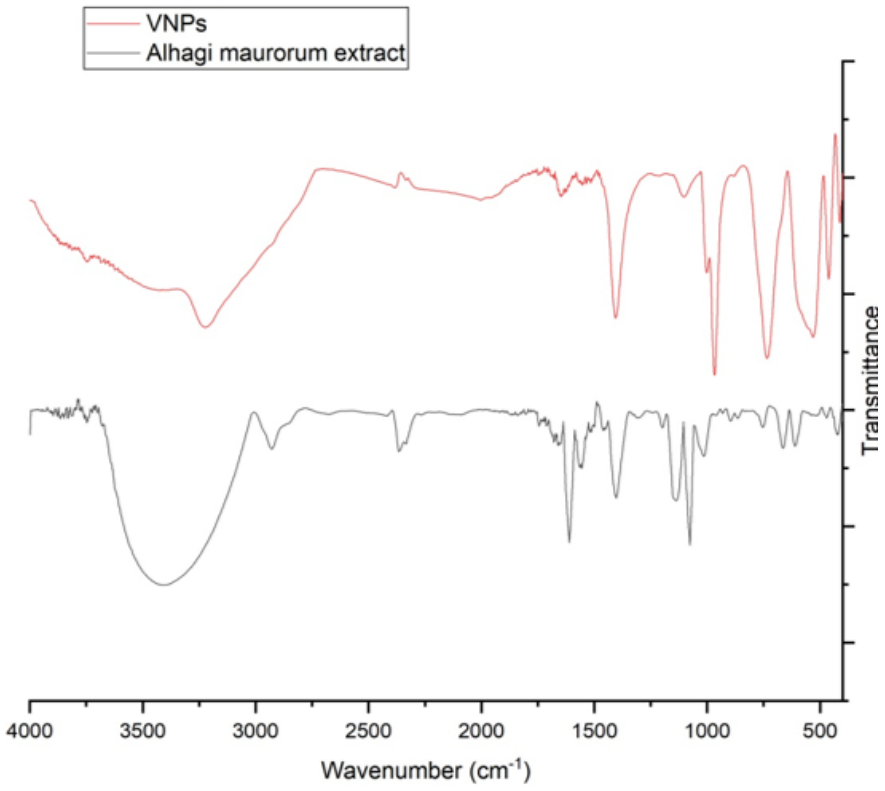

Fig. 3. FT-IR Spectrum of VNPs@*Salvia officinalis* .

EDS analysis :

Figure 4 exhibits the EDS diagram for VNPs@*Salvia officinalis* as a qualitative elemental analysis. The signals at 0.52 Kev (VLα), 4.95 (VKα), and 5.43 (VKβ) approve the presences of vanadium in VNPs@*Salvia officinalis* . The signals for vanadium nanoparticles have been recorded previously [30].

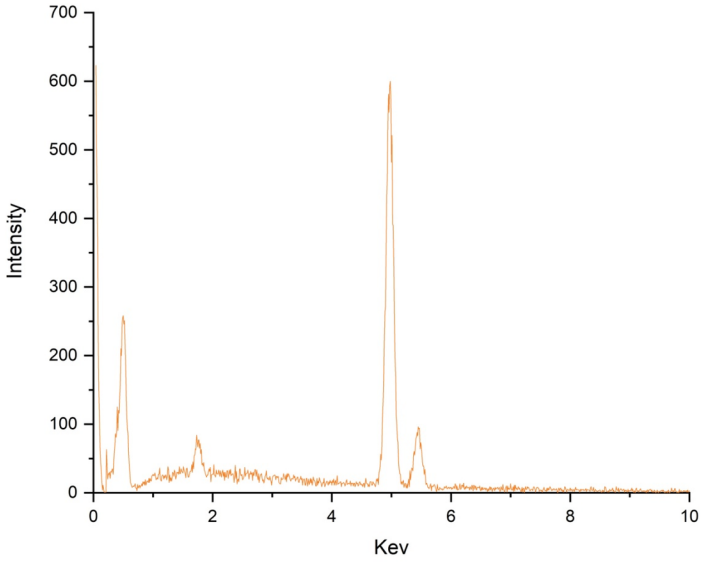

Figure 4. EDS analysis of VNPs@*Salvia officinalis*

In this study, the scavenging capacity of VNPs@*Salvia officinalis*and BHT at different concentrations expressed as percentage inhibition has been indicated in Figure 5.

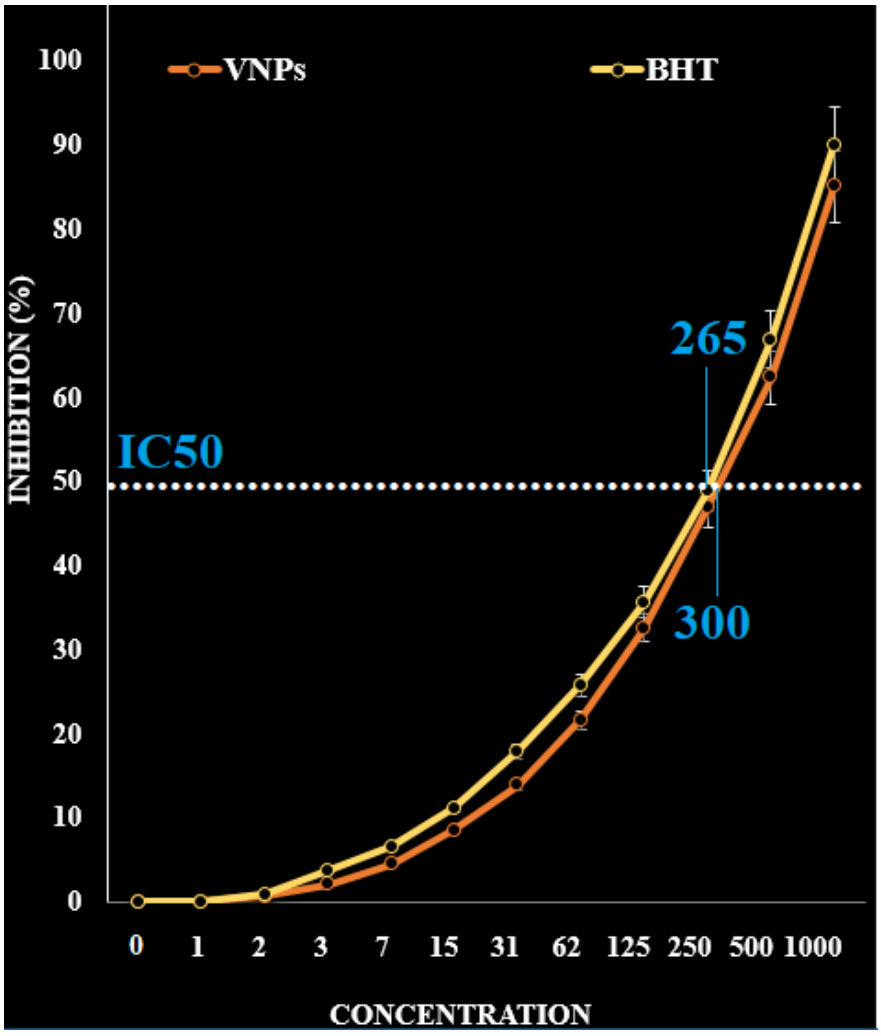

Fig. 5. The antioxidant properties of VNPs@*Salvia officinalis* and BHT against DPPH.

In the process of apoptosis, dangerous, damaged, and unwanted cells are removed without damaging the surrounding tissues or cells. Cancer cells escape from programmed death, one of the reasons for which is the convert in the gene expression that is involved in the process regulation. Most anticancer agents exert their remedial activities by inducing apoptosis [27-31]. Programmed death induction is a main important method to kill tumor cells without complications. In recent research, by the MTT colorimetric method, it was indicated that nanoparticles have a concentration-dependent lethal activity on tumor cells. In addition, it was found that nanoparticles increased apoptosis in cancer cells. Today, the focus of cancer studies is the search for anti-cancer agents with a higher safety factor and greater acceptability for patients. In addition, nanoparticles can act synergistically or redundantly in combination with radiation therapy or chemotherapy for anticancer activity [31-33]. With the advent of nanotechnology, the paradigm of drug delivery systems has been developed, which can carry many drugs with chemical properties and actively target specific cells. Also, it can pass through biological barriers and transfer its cargo to the target location without pharmaceutical interventions. Today, the development of nanoparticles as effective drug carriers is at the center of attention. In these effective carriers, the drug is at the center of attention [33-35]. In this study, nanoparticles were used for cytotoxic purposes by the MTT method. One of the reasons that nanoparticles had a significant effect on cancer cells is that this phenomenon is due to their direct effect on the cell's respiratory system

in the mitochondria. Therefore, due to the high level of mitochondrial activity in the respiration process of cancer cells compared to normal cells, a suitable substrate is provided for nanoparticles to destroy cancer cells. Another reason is the morphological differences between the membranes of cancer cells in terms of the size of their pores [35-40]. Various researches have been conducted to investigate the cytotoxic activities of nanoparticles on cancer cell lines [38-40]. The results of this study showed that metal nanoparticles in low concentrations do not have significant cytotoxic effects, but in higher concentrations, they have significant cytotoxic effects. The results of this research showed that the cytotoxicity of nanoparticles is dependent on dose and time and suggested that this nanoparticle can have biomedical applications. In general, the results of our study are consistent with other studies in the field of cytotoxicity of nanoparticles. Another mechanism of cytotoxicity of nanoparticles is the toxic oxygen radical's generation [38-41]. Another mechanism of nanoparticles cytotoxicity is the toxic oxygen radicals (ROS) generation that disturb the cellular redox balance, which is called oxidative stress. Oxidative stress destroys cellular antioxidant enzymes, destruction of cellular DNA structure, oxidation of cellular proteins and membrane lipids, and finally cell death. One of the important issues in the field of using metal nanoparticles in cancer treatment is the non-toxicity of nanoparticles on normal cells [29-34]. Studies show that nanoparticles have a greater effect on cancer cell lines, which is due to their direct effect on the cell's respiratory system in the mitochondria. Therefore, due to the high level of mitochondrial activity in the respiration process of cancer cells compared to normal cells, a suitable platform is provided for nanoparticles to destroy cancer cells [24-28]. Another reason is the morphological differences between the membrane of normal and cancer cells in terms of the difference in the size of their pores. Also, the difference in the shape, size and surface charge of nanoparticles is another factor in the difference in toxicity of nanoparticles between cancer and normal cells [28-31]. It is important to identify the importance of the apoptotic genes expression pattern in response to the anticancer drugs metastasis activity. Therefore, more investigations are necessary to prove that the mRNA expression profile of this gene can be in response to treatment [27-31]. The results obtained from this study showed the therapeutic use of nanoparticles in cancer cells. According to these reviews, clinical studies on human and animal models are necessary to prove the effect of nanoparticles and also the effect of these nanoparticles on healthy and normal cell lines. So, the nanoparticle's use can be efficient in enhancing the expression of some proliferative and apoptotic genes [32-36]. According to this study and previous research, it can be concluded that nanoparticles have powerful anticancer effects on cancer cells and derivatives of this compound can be used in the treatment of cancer. Therefore, if the clinical process of this nanoparticle is confirmed, these nanoparticles can be used in clinical cases for cancer patients in the future.

In this study, the treated cells with different concentrations of the present VNPs@*Salvia officinalis* were assessed by MTT assay for 48h about the cytotoxicity properties on normal (HUVEC) and colorectal malignancy cell lines i.e. Caco-2, COLO 320, DLD-1, HCT-15, HCT-116, and HT-29. The absorbance rate was evaluated at 570 nm, which represented viability on normal cell line (HUVEC) even up to 1000µg/mL for VNPs@*Salvia officinalis* (Table 1 and Figures 6-9).

The viability of malignant colorectal cell lines reduced dose-dependently in the presence of VNPs@*Salvia officinalis* . The IC50 of VNPs@*Salvia officinalis* were 213, 210, 297, 204, 160, and 125 µg/mL against Caco-2, COLO 320, DLD-1, HCT-15, HCT-116, and HT-29 cell lines, respectively (Table 1 and Figures 6-9).

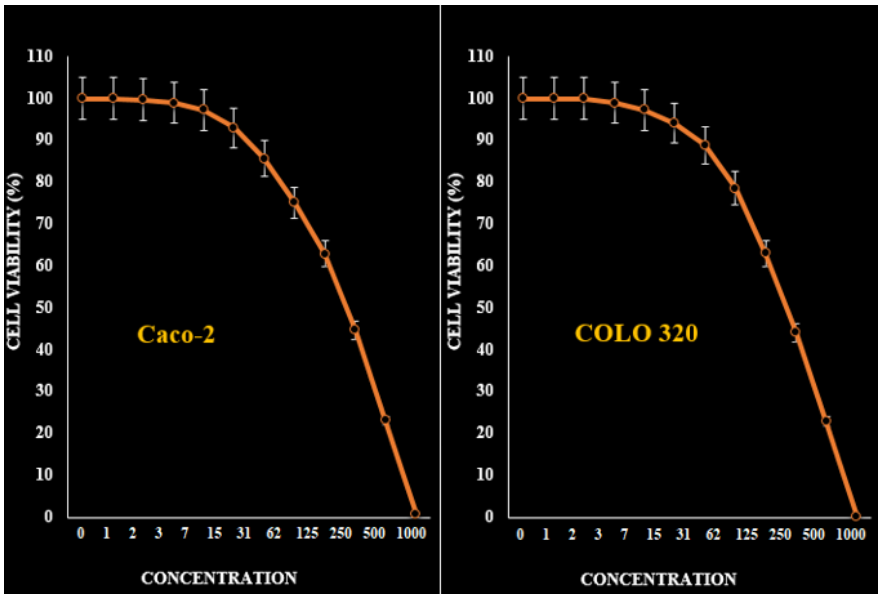

Fig. 6. The anti-colorectal cancer properties of VNPs@*Salvia officinalis* against Caco-2 and COLO 320.

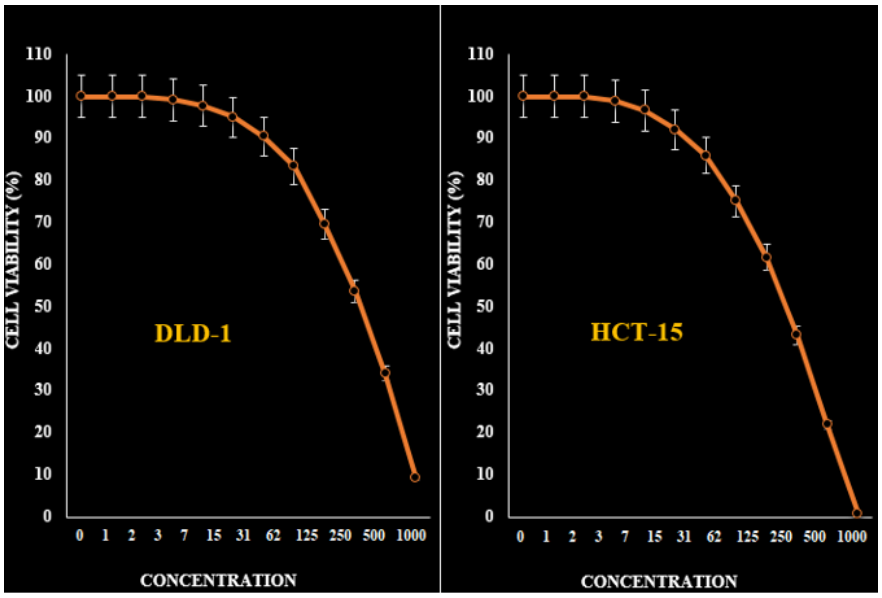

Fig. 7. The anti-colorectal cancer properties of VNPs@*Salvia officinalis* against DLD-1 and HCT-15.

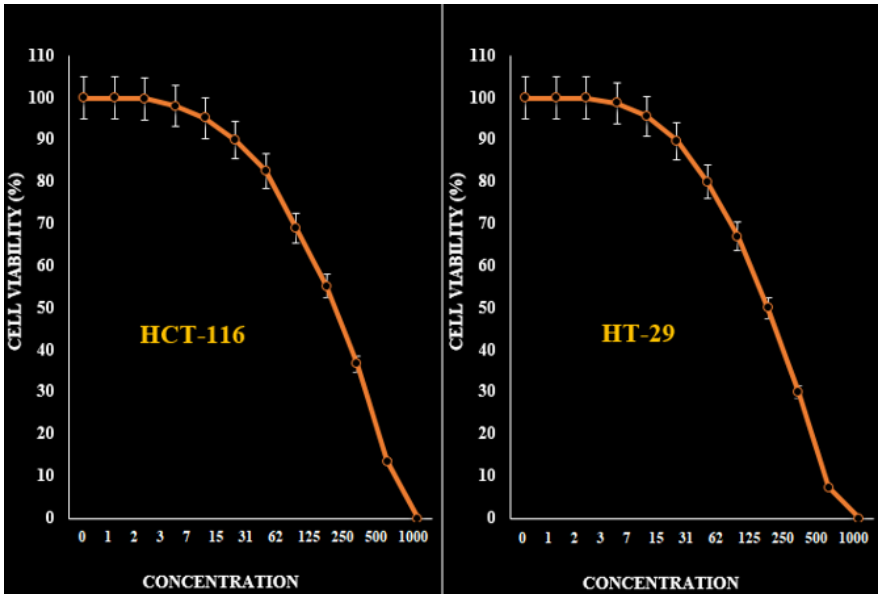

Fig. 8. The anti-colorectal cancer properties of VNPs@*Salvia officinalis* against HCT-116 and HT-29.

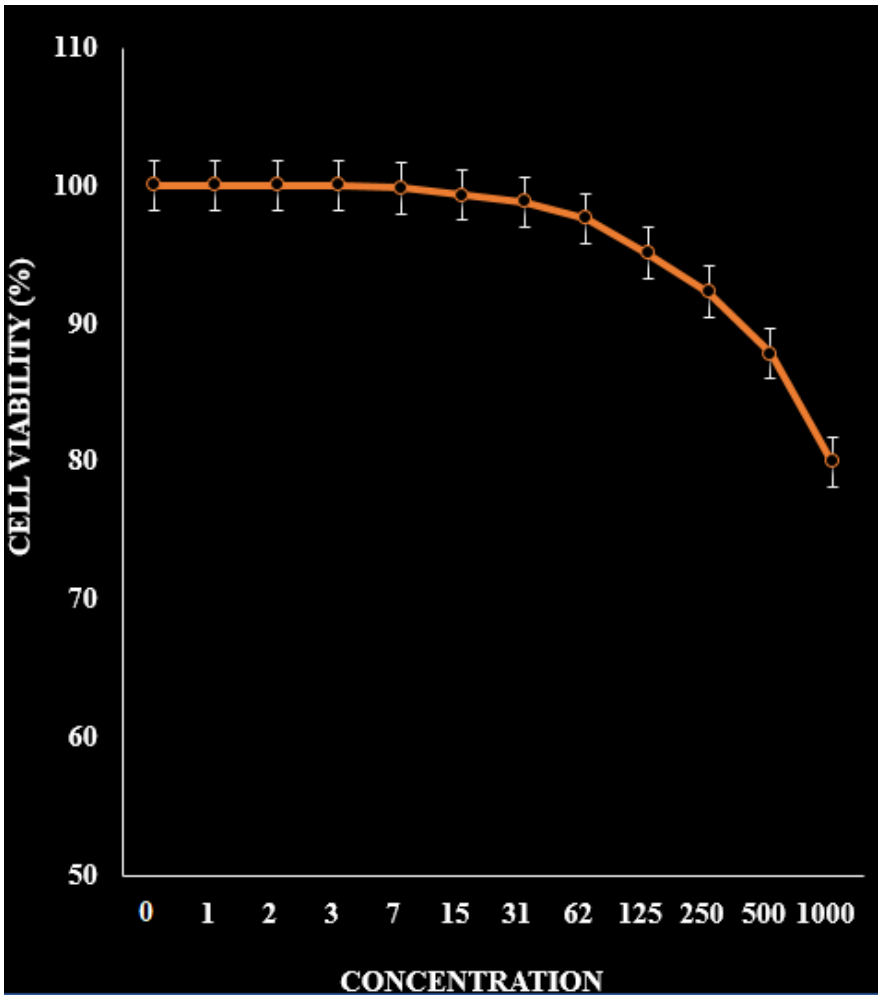

Fig. 9. The cytotoxicity effects of VNPs@*Salvia officinalis* against normal (HUVEC) cell line.

Table 1. The IC50 of VNPs@*Salvia officinalis* in the anti-colorectal cancer test.

	˝NПσ˜Σαλια οφφιςιναλις (μγ/μΛ)
IC50 against HUVEC	**-**
IC50 against Caco-2	213 ± 0^{c}
IC50 against COLO 320	210 ± 0^{c}
IC50 against DLD-1	297 ± 0^{d}
IC50 against HCT-15	204 ± 0^{c}
IC50 against HCT-116	160 ± 0^{b}
IC50 against HT-29	125 ± 0^{a}

4. Conclusion

In Conclusion, the present study reports the green synthesis of vanadium nanoparticles using the aqueous extract of *Salvia officinalis* as the reducing agent. The NPs has been characterized using FT-IR, XRD, FE-SEM, and EDS techniques. The obtained results demonstrate a spherical morphology for the vanadium

nanoparticles in a range size of 11.28 to 40.74 nm.

The viability of malignant cancer cell lines reduced dose-dependently in the presence of VNPs@*Salvia officinalis* . The VNPs@*Salvia officinalis* showed the best antioxidant activities against DPPH. So, the findings of the recent research show that biologically synthesized VNPs@*Salvia officinalis* might be used to cure colorectal cancer. In addition, the current study offer that VNPs@*Salvia officinalis*could be a new potential adjuvant chemopreventive and chemotherapeutic agent against cytotoxic cells.

Conflict of Interest statement

There is no conflict of interest

References

1. Garcia CS, Menti C, Lambert AP, Barcellos T, et al. Pharmacological perspectives from Brazilian Salvia officinalis (Lamiaceae): antioxidant, and antitumor in mammalian cells. An Acad Bras Cienc. 2016; 88(1): 281-92.

2. Kozics K, Klusova V, Srancikova A, Mucaji P, et al. Effects of Salvia officinalis and Thymus vulgaris on oxidant-induced DNA damage and antioxidant status in HepG2 cells. Food Chem. 2013; 141(3): 2198-206.

3. Zare Shahneh F, Valiyari S, Baradaran B, Abdolalizadeh J, et al. Inhibitory and cytotoxic activities of salvia officinalis L. Extract on human lymphoma and leukemia cells by induction of apoptosis. Adv Pharm Bull. 2013; 3(1): 51-5.

4. Zhang Y, Smuts JP, Dodbiba E, Rangarajan R, et al. Degradation study of carnosic acid, carnosol, rosmarinic acid, and rosemary extract (Rosmarinus officinalis L.) assessed using HPLC. J Agric Food Chem. 2012; 60(36): 9305-14.

5. Zhou R, Long H, Zhang B, Lao Z, et al. Salvianolic acid B, an antioxidant derived from Salvia militarize, protects mice against gammaradiationinduced damage through Nrf2/Bach1. Mol Med Rep. 2019; 19(2): 1309-17.

6. Jiang Y, Zhang L, Rupasinghe HP. Antiproliferative effects of extracts from Salvia officinalis L. and Saliva miltiorrhiza Bunge on hepatocellular carcinoma cells. Biomed Pharmacother. 2017; 85: 57-67.

7. Ghorbani A, Esmaeilizadeh M. Pharmacological properties of Salvia officinalis and its components. J Tradit Complement Med. 2017; 7(4): 433-40.

8. Poulios E, Giaginis C,Vasios GK. Current Advances on the Extraction and Identification of Bioactive Components of Sage (Salvia spp.). Curr Pharm Biotechnol. 2019; 20(10): 845-57.

9. Naimi M, Vlavcheski F, Shamshoum H, Tsiani E. Rosemary Extract as a Potential Anti-Hyperglycemic Agent: Current Evidence and Future Perspectives. Nutrients. 2017; 9(9): 968.

10. Thevenot P., Cho J., Wavhal D., Timmons R.B., Tang L. Surface chemistry influences cancer killing effect of TiO_2nanoparticles. Nanomedicine. 2008;4:226–236.

11. Colon J., Hsieh N., Ferguson A., Kupelian P., Seal S., Jenkins D.W., Baker C.H. Cerium oxide nanoparticles protect gastrointestinal epithelium from radiation-induced damage by reduction of reactive oxygen species and upregulation of superoxide dismutase 2. Nanomedicine. 2010;6:698–705.

12. Wason M.S., Colon J., Das S., Seal S., Turkson J., Zhao J., Baker C.H. Sensitization of pancreatic cancer cells to radiation by cerium oxide nanoparticle-induced ROS production. Nanomedicine. 2013;9:558–569.

13. Tarnuzzer R.W., Colon J., Patil S., Seal S. Vacancy engineered ceria nanostructures for protection from radiation-induced cellular damage. Nano Lett. 2005;5:2573–2577.

14. Ali D., Alarifi S., Alkahtani S., AlKahtane A.A., Almalik A. Cerium oxide nanoparticles induce oxidative stress and genotoxicity in human skin melanoma cells. Cell Biochem. Biophys. 2014;71:1643–1651.

15. Neri D., Supuran C.T. Interfering with pH regulation in tumors as a therapeutic strategy. Nat. Rev. Drug Discov. 2011;10:767–777.

16. Seo J.W., Chung H., Kim M.Y., Lee J., Choi I.H., Cheon J. Development of watersoluble single-crystalline TiO_2nanoparticles for photocatalytic cancer-cell treatment. Small. 2007;3:850–853.

17. Hou Z., Zhang Y., Deng K., Chen Y., Li X., Deng X., Cheng Z., Lian H., Li C., Lin J. UV-emitting upconversion-based TiO_2photosensitizing nanoplatform: Near-infrared light mediated *in vivo* photodynamic therapy via mitochondria-involved apoptosis pathway. ACS Nano. 2015;9:2584–2599.

18. Cui S., Yin D., Chen Y., Di Y., Chen H., Ma Y., Achilefu S., Gu Y.*In vivo* targeted deep-tissue photodynamic therapy based on near-infrared light triggered upconversion nanoconstruct. ACS Nano. 2013;7:676–688.

19. Lucky S.S., Idris N.M., Li Z., Huang K., Soo K.C., Zhang Y. Titania coated upconversion nanoparticles for near-infrared light triggered photodynamic therapy. ACS Nano. 2015;9:191–205.

20. Idris N.M., Lucky S.S., Li Z., Huang K., Zhang Y. Photoactivation of core-shell titania coated upconversion nanoparticles and their effect on cell death. J. Mater. Chem. B. 2014;2:7017–7026.

21. Prasad, K.S., Shivamallu, C., Shruthi, G., Prasad, M., 2018. A Novel and One-pot Green Synthesis of Vanadium Oxide Nanorods Using a Phytomolecule Isolated from Phyllanthus amarus. ChemistrySelect 3, 3860-3865.

22. Zangeneh, M. M., Zangeneh, A., Pirabbasi, E., Moradi, R., Almasi. M. (2019). *Appl. Organometal. Chem.33* , e5246

23. Jalalvand, A. R., Zhaleh, M., Goorani, S., Zangeneh, M. M., Seydi, N., Zangeneh, A., Moradi, R. (2019). J. Photochem. Photobiol. B.: Biol.*192* , 103–112.

24. Karthik, K., Nikolova, M. P., Phuruangrat, A., Pushpa, S., Revathi, V., & Subbulakshmi, M. (2020). Ultrasound-assisted synthesis of V2O5 nanoparticles for photocatalytic and antibacterial studies. *Materials Research Innovations* , *24* (4), 229-234.

25. Seydi, N., Mahdavi, B., Paydarfard, S., Zangeneh, A., Zangeneh, M.M., Najafi, F., Jalalvand, A.R., Pirabbasi, E., 2019. Preparation, characterization, and assessment of cytotoxicity, antioxidant, antibacterial, antifungal, and cutaneous wound healing properties of titanium nanoparticles using aqueous extract of Ziziphora clinopodioides Lam leaves. Applied Organometallic Chemistry 33, e5009.

26. Baghayeri, M., Mahdavi, B., Hosseinpor-Mohsen Abadi, Z., Farhadi, S., 2018. Green synthesis of silver nanoparticles using water extract of Salvia leriifolia: Antibacterial studies and applications as catalysts in the electrochemical detection of nitrite. Applied Organometallic Chemistry 32, e4057.

27. Mahdavi, B., Paydarfard, S., Rezaei-Seresht, E., Baghayeri, M., & Nodehi, M. (2021). Green synthesis of NiONPs using Trigonella subenervis extract and its applications as a highly efficient electrochemical sensor, catalyst, and antibacterial agent. *Applied Organometallic Chemistry* , *35* (8), e6264.

28. Aliyu, A., Garba, S., Bognet, O., 2017. Green synthesis, characterization and antimicrobial activity of vanadium nanoparticles using leaf extract of Moringa Oleifera. International Journal of Chemical Sciences 16, 231.

29. de Oliveira Carvalho, H., Goes, L.D.M., Cunha, N.M.B., Ferreira, A.M., Fernandes, C.P., Favacho, H.A.S., Junior, J.O.C.S., Ortiz, B.L.S., Navarrete, A., Carvalho, J.C.T., 2018. Development and standardization of capsules and tablets containing Calendula officinalis L. hydroethanolic extract. Revista Latinoamericana de Quimica 46, 16-27. 30. Deepika, P., Vinusha, H., Muneera, B., Rekha, N., Prasad, K.S., 2020. Vanadium oxide nanorods as DNA cleaving and anti-angiogenic agent: Novel green synthetic approach using leaf extract of Tinospora cordifolia. Current Research in Green and Sustainable Chemistry.

31. Talavera, N., Navarro, M., Sifontes, A., Diaz, Y., Villalobos, H., Nino-Vega, G., Boada-Sucre, A., Gonzalez, I., 2013. Green synthesis of nanosized vanadium pentoxide using Saccharomyces cerevisiae as biotemplate. Recent Research Developments in Materials Science 10, 89.

32. Zhang, Y., Zhang, X., Zhang, L., Alarfaj, A. A., Hirad, A. H., & Alsabri, A. E. (2021). Green formulation, chemical characterization, and antioxidant, cytotoxicity, and anti-human cervical cancer effects of vanadium nanoparticles: A pre-clinical study. *Arabian Journal of Chemistry* , *14* (6), 103147.

33. Hilger I., Kaiser W.A. Iron oxide-based nanostructures for MRI and magnetic hyperthermia. Nanomedicine. 2012;7:1443–1459.

34. Orel V., Shevchenko A., Romanov A., Tselepi M., Mitrelias T., Barnes C.H., Burlaka A., Lukin S., Shchepotin I. Magnetic properties and antitumor effect of anocomplexes of iron oxide and doxorubicin. Nanomedicine. 2015;11:47–55.

35. Van Landeghem F.K., Maier-Hauff K., Jordan A., Hoffmann K.-T., Gneveckow U., Scholz R., Thiesen B., Bruck W., von Deimling A. Post-mortem studies in glioblastoma patients treated with thermotherapy using magnetic nanoparticles. Biomaterials. 2009;30:52–57.

36. Silva A.C., Oliveira T.R., Mamani J.B., Malheiros S.M., Malavolta L., Pavon L.F., Sibov T.T., Amaro E., Jr., Tann_us A., Vidoto E.L. Application of hyperthermia induced by superparamagnetic iron oxide nanoparticles in glioma treatment. Int. J. Nanomed. 2011;6:591–603.

37. Johannsen M., Thiesen B., Wust P., Jordan A. Magnetic nanoparticle hyperthermia for prostate cancer. Int. J. Hyperthermia. 2010;26:790–795.

38. Banobre-Lopez M., Teijeiro A., Rivas J. Magnetic nanoparticle-based hyper-thermia for cancer treatment. Rep. Pract. Oncol. Radiother. 2013;18:397–400.

39. Klein S., Sommer A., Distel L.V., Hazemann J.L., Kroner W., Neuhuber W., Muller P., Proux O., Kryschi C. Superparamagnetic iron oxide nanoparticles as novel X-ray enhancer for low-dose radiation therapy. J. Phys. Chem. B. 2014;118:6159–6166.

40. Chatterjee D.K., Fong L.S., Zhang Y. Nanoparticles in photodynamic therapy: An emerging paradigm. Adv. Drug Deliv. Rev. 2008;60:1627–1637.

41. Zhang A.P., Sun Y.P. Photocatalytic killing effect of TiO_2 nanoparticles on Ls- 174-t human colon carcinoma cells. World J. Gastroenterol. 2004;10:3191–3193.