Research Article

Wenli Gu#, Haining Li†, Xianyi Niu, and Jianyun Zhou*

Biological fabrication of zinc oxide nanoparticles from Nepeta cataria potentially produces apoptosis through inhibition of proliferative markers in ovarian cancer

https://doi.org/10.1515/gps-2022-0016
received October 10, 2021; accepted December 16, 2021

Abstract: This study evaluated the biological fabrication and characterization of zinc oxide nanoparticles (ZnONPs) using Nepeta cataria (NC) and their anticancer activity against ovarian cancer cells (SKOV3). This study synthesized ZnONPs using leaf extract of N. cataria through a biological method. The synthesized particles were characterized in several ways such as zeta potential, ultraviolet-visible (UV-Vis) spectrum, Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), and scanning electron microscopic (SEM) analysis. UV-Vis spectrum exhibited that maximum spectra were found to be 380 nm. The size of the material was shown to be 75.9 nm confirmed by dynamic light scattering measurement. Moreover, XRD, SEM, and transmission electron microscopic analysis were confirmed by the synthesized materials as crystal-based ZnONPs. FTIR studies represent that several biologically active functional groups existed in the synthesized nanoparticles. In addition, the anticancer ability and the inhibitory role of ZnONPs-NC against SKOV3 cells were investigated. We found that ZnONPs-NC causes efficient toxicity in SKOV3 cells by increasing cytotoxicity depending on reactive oxygen species production and nuclear fragmentation in SKOV3 cells. ZnONPs-NC activates Bax and Caspases while inhibiting Bcl-2 proteins in SKOV3 cells. Furthermore, we discovered that ZnONPs-NC inhibits the proliferative markers PCNA, cyclin-D1, matrix metalloproteinase (MMP)-2, and MMP-9 in SKOV3 cells. Thus, biologically synthesized ZnONPs-NC were found to be effectively inhibiting ovarian cancer cell growth.

Keywords: Nepeta cataria, ZnONPs, ovarian cancer, proliferation, apoptosis

1 Introduction

Epithelial ovarian cancer is considered one of the predominant kinds of deadliest cancer in women. It occurs in the epithelium, peritoneum, and fallopian tubes in the ovarian region [1]. Scientific data have shown that about 70% of ovarian cancer patients are diagnosed only during the advanced stage [2]. Ovarian cancer exhibited a peculiar property of invading quickly into the uterus and the pelvic cavity [3]. Chemotherapy and cytoreductive surgery combined with chemotherapy are the primary therapeutic procedures for ovarian cancer [4]. The improvement of the carboplatin-paclitaxel regimen as the first combined chemotherapeutic agent, administered by intraperitoneal cytostatic, resulted in significant advances in treating patients with advanced ovarian cancer [5]. Despite progress in the treatment protocol and massive increases in survival, 60–80% of cases had ineffective therapy and disease relapse within five years because of tumor resistance to cytostatic treatment [6]. Hence, identifying new therapeutic methods that are effective in treating ovarian cancer with clinical relevance is in progress. The nanotechnology-based cancer therapeutic approach is considered to develop a new drug delivery system [7]. Nanotechnology developments
with medicinal plant extracts show effective action mechanisms against the cancer cell lines [8].

Genus *Nepeta* belongs to Lamiaceae, comprising about 280 species widely distributed in Asia and Africa. *Nepeta cataria* is an edible plant that is also identified as catmints or catnips [9]. This plant has its place in folk medicine against various infections and diseases [10,11]. These plants are extensively used for antimicrobial activity against human pathogens [12]. The aromatic component present in these plants contains a wide range of secondary metabolites. The metabolite present in the plants is highly responsible for its biomedical properties [13]. *N. cataria* is exclusively studied for its secondary aromatic metabolite such as iridoids (terpenoids), neptelic acids, and nepetalactone, promising compounds that show biomedical activity against various illnesses. These metabolites have been extensively researched for their antiinflammatory, antibacterial, antifungal, antioxidant, anthelminthic, and anticancer properties, among other things [9,12].

Plant extract-mediated nanoparticle fabrication was shown to exhibit a variety of biological activities [14]. Zinc oxide nanoparticles (ZnONPs) belong to the metal particles and have enormous anticancer activity against numerous cancer [15,16]. There have been multiple advantages when using chemotherapy, such as a high degree of cancer cell selectivity at pathophysiological pH [17]. Also, ZnONPs have been believed to exhibit a wide range of biosafe, biocompatible, and noncytotoxic nanostructures [18]. ZnONPs are a crucial ingredient in several enzymes, ointments, and sunscreens with numerous biological and pharmacological properties [19]. Remarkably, Zn²⁺ triggers reactive oxygen species (ROS) production that induces cytotoxicity of cancer cells [20]. Moreover, ZnO protects macrophages from the cell toxic effects of chemotherapeutic drugs. Also, ZnONPs accumulated in several tissues when the administration of intravenous in the experimental model [21]. In this study, biological fabrication and characterization of ZnONPs using leaf extract of *N. cataria*. Furthermore, the synthesized ZnONPs from *N. cataria* inhibit the proliferation of SKOV3 ovarian cancer cells.

2 Materials and methods

Dulbecco’s modified eagle’s medium (DMEM), 2,7-dichlorofluorescin diacetate (DCFH-DA), streptomycin, Hoechst, fetal bovine serum, penicillin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), and zinc nitrate were purchased from Sigma Merck, St. Louis, MO, USA). China’s cell signaling company distributes primary antibodies such as Bax (Catalog No. #2774), cyclin-D1 (Catalog No. #2922), Bcl-2 (Catalog No. #15071), proliferating cell nuclear antigen (PCNA) (Catalog No. #2866), matrix metallo proteinase-2 (MMP-2) (Catalog No. #4022), MMP-9 (Catalog No. #2270), and β-actin (Catalog No. #6967).

2.1 Plant sample collection and ZnO NP preparation

*N. cataria*, also commonly called catnip, was collected from the local market of Ningxia, China. The 5 g quantity of fresh leaves of *N. cataria* was finely chopped and rinsed in sterilized molecular grade water. The leaves were drained and boiled using sterilized molecular grade water (Merck, USA, Catalog No. 693520) for 15 min at 70°C. The boiled extract sample was filtered through Whatman No. 1 filter paper. Then, 10 mL of collected extract was added in 90 mL of 1 mM zinc nitrate solution, and this mixture was allowed to stand overnight. The confirmation of ZnONPs has been observed by the mixture conversion from colorless to pale yellow.

2.2 Characterization studies to confirm the ZnONPs-NC

The synthesized ZnONPs-NC have been evaluated for several characterization studies to ensure ZnONPs. UV-vis absorption spectrum was taken in ranges between 200 and 700 nm by UV-Vis spectrophotometer (Hitachi, Japan). The size of the ZnONPs and their zeta potential value were studied by the dynamic light scattering (DLS) technique. The DLS results were interpreted based on the size, core, structure, and type of ion present in the sample. Fourier transform infrared (FTIR) spectroscopy evaluated the biologically active functional groups in the ZnONPs-NC. This spectrum was recorded from 500 to 4,000 cm⁻¹ (FTIR-ALPHA interferometer, Bruker, Germany). X-ray diffraction (XRD) analysis extensively studied the crystalline nature of the ZnONPs. The diffraction pattern of the samples was obtained by subjecting them to Copper K-alpha (CuKα) radiation with 40 kV to 40 mA of voltage, respectively. The diffraction pattern was recorded at 2θ angle from 20° to 80° (XRD-6000-Shimadzu). The morphological features of ZnONPs-NC were studied by scanning electron microscope and transmission electron microscope.

2.3 Cell culture

The American Type Culture Collection has been distributed to human ovarian cancer cell lines (SKOV3) for
ZnONP-mediated anticancer studies. This cell line was grown in DMEM nutrient with 2 mM L^{-1} glutamine, 10% FBS, and 1% penicillin or streptomycin in a 5% CO₂ atmosphere at 37°C.

### 2.4 Cytotoxicity testing

The cytotoxicity ability of ZnONPs-NC against SKOV3 cells was determined by MTT colorimetric assay [22]. The cells were mixed with medium and equally distributed in 96 well plates, incubated for 24 h at 37°C in 5% CO₂. Next, cells were treated with appropriate concentrations of ZnONPs-NC and incubated for 24 and 48 h, respectively. After the treatment, cells were rinsed with PBS buffer, added the 200 μL of MTT yellow color reagent, and incubated for 4 h in a dark environment. In addition, 200 μL of DMSO was evenly distributed to all wells to solubilize the formazan crystals. Finally, the absorbance was measured at 570 nm by a microplate reader.

### 2.5 DCFH-DA staining

The role of ZnONPs-NC on ROS production in SKOV3 cells was determined using DCFH-DA stained spectrofluorimetric method [23]. Briefly, 1 × 10⁶ cells were cultured in 6 well plates. Then cells were exposed with 25 μg mL⁻¹ of ZnONPs-NC and incubated for 24 and 48 h, respectively. After the treatment, cells were rinsed with buffer, added 5 μg mL⁻¹ of DCFH-DA fluorescent probe, and incubated for 1 h in a dark environment. Then the intensity of the fluorescence probe was measured by spectrofluorimetry in the excitation, and emission levels were 490/540 nm.

### 2.6 Hoechst staining

The role of ZnONPs-NC on nuclear fragmentation mediated apoptosis in SKOV3 cells was studied by Hoechst staining. Briefly, 1 × 10⁶ cells were cultured in 6 well plates. Then cells were exposed with 25 μg mL⁻¹ of ZnONPs-NC and incubated for 24 and 48 h, respectively. After the treatment, cells were rinsed with buffer, added to the 5 μg mL⁻¹ Hoechst fluorescent probe, and incubated for 1 h in a dark environment. Then the treated cells were examined under a fluorescent microscope using a blue filter (Leica-microscope, Germany).

### 2.7 Western blot

The role of ZnONPs-NC on protein expression of proliferation and apoptosis markers was studied by western blotting. After treatment with ZnONPs-NC, SKOV3 cells were collected and digested using radio-immunoprecipitation assay buffer. The digested samples were considered as protein sample, and it has been used for western blot analysis. The protein samples were dispersed in sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the gel was reallocated to the membrane (PVDF). The transferred membrane was blocked and exposed with appropriate primary and secondary antibodies. The brief methodology was followed by Balupillai et al. [24].

### 2.8 Statistical analysis

All experiments were done in six independent repetitive experiments. The value of the results provided as mean ± standard deviation (SD) using one-way analysis of Duncan’s multiple range test (DMRT). P < 0.05 standards were indicative of significant alterations and modifications.

### 3 Results

#### 3.1 UV-vis spectral, DLS, and zeta potential analysis of ZnONPs

The synthesized ZnONPs-NC were studied for their spectral properties using UV-Vis spectroscopy. Figure 1a shows the presence of a peak at 300–400 nm confirming the presence of ZnO nanoparticles. In the study, the peak at 380 nm indicates the presence of ZnONPs. The synthesized ZnONP size was studied using a DLS particle size analyzer and Zeta potential using standard analysis time. The average size of the synthesized nanoparticles was found to vary between 20 and 130 nm (Figure 1b). The zeta potential for the ZnONPs was −2.5 mV, representing that the synthesized nanomaterials are partially stable, and susceptible to agglomeration, which may be due to the presence of capping molecules present over the ZnONPs (Figure 1c). These results could moderate the stability of the ZnONPs.

#### 3.2 XRD and FTIR analysis of ZnONPs-NC

XRD analysis was performed to study the nature of ZnONPs-NC. The crystalline nature of the nanoparticles
was studied using the dried powdered nanoparticles. Figure 2a shows that the diffraction pattern of the samples was obtained by subjecting them to CUKα radiation with 40 kV to 40 mA of voltage and current, respectively. The diffraction pattern was observed that 100, 102, 103, 107, 110, and 112, respectively. Moreover, the ZnONPs synthesized using *N. cataria* leaf extract were subjected to FTIR to determine the spectral properties and functional groups present in the green synthesized nanoparticles (Figure 2b). The FTIR results showed peaks at 824, 1,080, 1,383, 1,633, 1,762, 2,732, 2,926, and 3,392 cm$^{-1}$. Medium stretch at 824 cm$^{-1}$ indicated the presence of alkyl halides (C−Cl). The peak at 1,080 and 1,633 cm$^{-1}$ indicates the presence of aliphatic amines (C−N) and primary amines (N−H). The small sharp peak at 1,762, 2,732, and 2,926 cm$^{-1}$ indicated the presence of carbonyl group (C═O), aldehydes (N−C═O; C−H), and alkanes (C−H), respectively. A broad peak at 3,392 cm$^{-1}$ indicated the presence of alcohols and phenolic compounds (O−H).

3.3 Scanning electron microscopic (SEM) and transmission electron microscopic (TEM) analysis of ZnONPs-NC

SEM analysis was used to determine the structure and morphology of the synthesized nanoparticles (Figure 3a). The particles exhibited agglomeration due to aromatic and other secondary metabolites present in the leaf extract of *N. cataria*. The SEM image revealed that the particles were
spherical in structure. The image also represented spongy-shaped and oval-shaped particles. The lower particle size was exhibited by the ZnONPs is 200 nm on agglomeration. Homogenous distribution of the nanoparticles could result in broad knowledge about the structural and morphological arrangement of the particles. Furthermore, TEM analysis was carried out to determine the size and clear morphology of the synthesized ZnONPs-NC (Figure 3b). TEM micrography of the present study exhibited the average size of ZnONPs-NC which was found to be 20–50 nm. The shape of the nanoparticles was found to be spherical and hexagonal on higher magnification. The selected area electron diffraction pattern of the synthesized nanoparticles showed the crystalline nature of the particles.

3.4 ZnONPs-NC induces significant cell toxicity and ROS production in SKOV3 cells

The ZnONPs-NC-mediated cell death was evaluated by MTT assay. As Figure 4a shows, the administration of ZnONPs-NC has significantly enhanced the SKOV3 cell proliferation in dose-associated way. The IC50 concentration of ZnONPs-NC was provided as 25 µg/mL for 24 h incubation time. Based on the results, ZnONPs-NC (25 µg/mL) for two different incubation times such as 24 and 48 h used for further studies. ZnONPs-NC treatment-associated ROS production in SKOV3 cells were studied by DCFH-DA staining. As Figure 4b shows, ZnONPs-NC (25 µg/mL)
induced ROS production in SKOV3 in 24 and 48 h incubation, respectively. The positive control paclitaxel (PTX) 500 ng/mL enhanced ROS production in SKOV3 cells.

3.5 ZnONPs-NC inhibits cell proliferation and invasive markers in SKOV3 cells

ZnONPs-NC treatment-mediated cell proliferation marker (cyclin-D1 and PCNA) and invasion marker (MMP-2 and MMP-9) were analyzed by western blotting. As Figure 5 shows, ZnONPs-NC magnificently inhibited the over-expression of cyclin-D1, PCNA, MMP-2, and MMP-9 in SKOV3 cells in a time-dependent way. ZnONPs-NC (25 µg/mL) for 48 h incubation showed better activity and is relatively similar to PTX-treated SKOV3 cells.

3.6 ZnONPs-NC induces nuclear fragmentation mediated apoptosis in SKOV3 cells

ZnONPs treatment associated nuclear condensation was studied by SKOV3 cells stained with Hoechst dye. Figure 6a shows that the microscopic images of ZnONPs-NC treatment increase the nuclear condensation in SKOV3 cells. ZnONPs-NC (25 µg/mL) for 48 h incubation showed highly condensed nuclei, and it was relatively similar to PTX-treated SKOV3 cells. Moreover, ZnONPs-NC treatment-associated proapoptotic gene expression was studied by western blotting. As shown in Figure 6b and c, ZnONPs-NC magnificently enhanced the protein expression of Bax and decreased the expression of Bcl-2 in SKOV3 cells in time on the dependent way. ZnONPs-NC (25 µg/mL) for 48 h incubation showed better activity and was relatively similar to PTX-treated SKOV3 cells.

Figure 3: SEM and TEM analyses of ZnONPs-NC: (a) SEM microscopic image shows the structure and morphology of the ZnONPs-NC; (b) TEM microscopic image shows the size and clear morphology of the synthesized ZnONPs-NC.

Figure 4: ZnONPs-NC induce significant cell toxicity and ROS production in SKOV3 cells. (a) The ZnONPs-NC-mediated cell death was evaluated by MTT assay. (b) ZnONPs-NC treatment-associated ROS production in SKOV3 cells was studied by DCFH-DA staining. Values from the plot are expressed as mean ± SD calculated by DMRT.
4 Discussion

In this study, biological fabrication and characterization of ZnONPs from N. cataria leaf extract (ZnONPs-NC) and their inhibitory role of SKOV3 cell proliferation were investigated. Synthesis of nanoparticles using plant extract has more advantages over other means of nanoparticle synthesis. Plant sources are more effective since they contain numerous natural bioactive components such as flavonoids and other phenolic components, which reduce metallic zinc into zinc ions [25]. In this study, the leaf extract is considered as one of the essential sources for the synthesis of nanoparticles. The conversion of metallic to ionic form was carried out using several leaf extracts as a reducing agent [26]. N. cataria, also known as catnip, has a wide range of active phytochemicals such as polyphenols, flavonoids, and terpenoids [27]. These plant extracts have numerous pharmacological activities, such as antidiabetic, antioxidants, anticancer, and antimmune deficiency [28]. In this study, synthesized ZnONPs-NC were characterized by several UV-Vis, FTIR, XRD, DLS, SEM, and TEM to confirm the ZnONPs.

The synthesized ZnONPs were studied for their spectral properties using UV-Vis spectroscopy. The presence of a peak at 300–400 nm confirms the presence of ZnONPs. In this study, the peak at 302 nm shows the presence of ZnONPs. UV-Vis spectroscopic results of the study reveal zinc nanoparticles when excited at 355 nm [29]. The average size of the synthesized nanoparticles was found to vary between 20 and 130 nm, and DLS confirmed it. The zeta potential for the ZnONPs was −2.5 mV, representing that the synthesized nanomaterials are partially stable and are also susceptible to agglomeration, which may be due to the presence of capping molecules present over the ZnONPs. This also results in the moderate stability of the ZnONPs.

Similarly, the comparative study between chemically synthesized ZnONPs and green synthesized nanoparticles shows a broad range of differences in the zeta potential values where chemical-mediated synthesis showed greater stability between −32.06 and −17.89 mV, whereas biosynthesized nanoparticles exhibited lesser values which are due to the capping molecules present on the surface of the nanoparticles during green synthesis [30]. This study synthesized ZnONPs-NC was characterized by FTIR studies to confirm the biologically active molecules that exist in the nanoparticles. FTIR results confirm the presence of alkyl halides, aliphatic amines, a primary amines carbonyl group, aldehydes, alkanes, alcohols, and phenolic compounds. Previously, FTIR studies revealed that methanolic extract of N. cataria contains numerous organic compounds such as citral, α-humulene, geraniol, rosmaric acid, β-elemene, myrcene, caryophyllene oxide, and thymol.

Moreover, N. cataria exhibits various types of secondary metabolites and primarily essential oils. The diverse vibrational stretching values have confirmed these reports attained for extracts of N. cataria through FTIR analysis [31]. Similarly, ZnONPs synthesized using Calotropis gigantea show major peaks at 4,307, 3,390, 2,825, 871, 439, and

Figure 5: ZnONPs-NC inhibit cell proliferation and invasive markers in SKOV3 cells. (a) Western blot studies of ZnONPs-NC treatment-mediated cell proliferation marker (cyclin-D1 and PCNA) and invasion marker (MMP-2 and MMP-9) in SKOV3 cells; (b) quantification of protein was evaluated by image-J software, and β-actin has served as positive control; it can normalize the interest of protein. Values from the plot are expressed as mean ± SD calculated by DMRT.
420 cm\(^{-1}\), representing \(-\)OH stretch and \(-\)CH stretch. The band at 871 cm\(^{-1}\) is due to the asymmetrical and symmetrical stretch of zinc. The spectrum also shows the presence of proteins in the leaf extract [32].

XRD analysis was performed to study the nature of the crystalline integrity of the ZnONPs. This result confirmed the crystalline structure in the synthesized nanoparticles from *N. cataria*. SEM and TEM analyses were used to determine the structure and morphology of the synthesized nanoparticles. The particles exhibited agglomeration which is due to the presence of aromatic and other secondary metabolites present in the leaf extract of *N. cataria*. The SEM image reveals that the particles were spherical in structure. TEM micrography of this study exhibits the shape of the nanoparticles was found to be spherical and hexagonal on higher magnification. The aggregations in the particles were due to the synthesis of nanoparticles in the aqueous medium [33]. Similar results were reported in green synthesized ZnONPs using the leaf extract of *Atalantia monophylla* exhibited 30 nm as average particle size, and the particles were found to be crystalline [34].

After synthesized ZnONPs-NC were intended to evaluate the biomedical application on ovarian cancer cell proliferation inhibition in SKOV3 cells. Ovarian cancer is a lethal disease in gynecologic-related malignancies. The main treatment strategies are chemotherapy; if it fails, affected ovarian tissue would be removed by surgical operation [35]. ZnONPs are reported that biologically safer, and they produce toxicity in several cancer models [36,15]. In our study, first, we observed that ZnONPs-NC produce cytotoxicity in a concentration-associated way in SKOV3 cells. The induction of elevated free radical production, which seems to be the crucial process for inducing apoptosis in tumor cells, can extend the proposed mechanism of nanoparticle-induced cell death in cancerous cells [37]. In the present investigation, the
administration of ZnONPs-NC resulted in increased ROS production in SKOV3 cells. Green synthesis of ZnONPs from several plant sources has been documented to produce ROS-mediated cytotoxicity in numerous cancer models [38,39]. Several factors influence biological metal nanoparticles such as size distribution, shape, and surface chemistry that lead to producing cytotoxic potential of nanoparticles in cancer cell lines [40]. Generally, in a biosynthetic approach, the biomolecules are accountable for the bioreduction of metal cations to their nano-forms. This could be conjugated to the surface of biosynthesized nanoparticles that act as stabilizers to prevent the aggregation of metal nanoparticles. [41]. Moreover, biomolecules that could be attached to the surface of the metal nanoparticles might change the surface chemistry of metal nanoparticles and interfere with behavior in response to their biological activities [40–43].

Ovarian cancer cell proliferation and metastasis have been directly associated with the over-expression proliferative marker expressions such as cyclin-D1, PCNA, and MMPs [44,45]. MMPs belong to the family of proteolytic enzymes or zinc-rich endopeptidases that accelerate the damage of extracellular cell matrix (ECM) resulting in cell migration [46]. Various cancer models have observed that overexpression of MMP-2 and MMP-9 resulted in invasion, metastasis, and migration [47,48]. In this study, ZnONPs-NC magnificently inhibited the overexpression of cyclin-D1, PCNA, MMP-2, and MMP-9 in SKOV3 cells in a time-dependent way. Apoptosis is the most crucial pathway for eliminating cancer cells through drug treatment [49]. Proapoptotic mediators such as Bax and antiapoptotic mediators in Bcl-2 have stimulated the apoptotic machinery. A high pattern of Bax expression and a common way of Bcl-2 expression can induce apoptosis in cancer cells [50]. The current study found that ZnONPs-NC treatment increased nuclear condensation in SKOV3 cells. Furthermore, ZnONPs-NC significantly increase Bax protein expression while decreasing Bcl-2 expression in SKOV3 cells over time. This report correlated with the ZnONPs-NC-mediated inhibition of proliferative marker expressions of cyclin-D1, PCNA, MMP-2, and MMP-9 in SKOV3. Previously, ZnONPs were synthesized and characterized using root extract of Euphorbia fischeriana, which induces apoptosis in lung cancer cells by modulating Bax and Bcl-2 expression [51].

5 Conclusion

This study concluded that synthesized ZnONPs-NC have been characterized by different techniques that conform to the particles. UV-Vis absorption spectra confirm ZnONPs by observed the maximum peak was 380 nm. The size of the material was shown to be 75.9 nm which was confirmed by DLS measurement. Moreover, the synthesized materials confirmed SEM and TEM microscopical analyses as crystal-based polygonal and spherical structured ZnONPs. FTIR studies represent that several biologically active functional groups existed in the synthesized nanoparticles. XRD intensity has confirmed that the particles are crystalline-based ZnONPs. In addition, ZnONPs-NC induces cytotoxicity-associated ROS production, nuclear fragmentation in SKOV3 cells. Moreover, ZnONPs-NC inhibit proliferative markers such as cyclin-D1, PCNA, MMP-2, and MMP-9 in SKOV3 cells. Also, ZnONPs-NC induce proapoptotic factors in SKOV3 cells.

Acknowledgment: Authors acknowledge that Obstetrics and Gynecology, Affiliated Haian Hospital of Nantong University, Nantong, Jiangsu, 226600, China for providing lab facilities to carry out this work.

Funding information: Authors state no funding involved.

Author contributions: Wenli Gu and Haining Li: experiments, analysis; Xianyi Niu: methodology, formal analysis; Jianyun Zhou: writing – original draft, project administration, resources.

Conflict of interest: Authors state no conflict of interest.

Data availability statement: All the data are available in the corresponding author. It will be provide upon the reasonable request.

References

[1] Lisio MA, Fu L, Goyeneche A, Gao ZH, Telleria C. High-grade serous ovarian cancer: basic sciences, clinical and therapeutic standpoints. Int J Mol Sci. 2019 Feb 22;20(4):952.
[2] Torre LA, Trabert B, DeSantis CE, Miller KD, Samimi G, Runowicz CD, et al. Ovarian cancer statistics, 2018. CA A Cancer J Clinicians. 2018;68(4):284–96.
[3] Lengyel E. Ovarian cancer development and metastasis. Am J Pathol. 2010;177(3):1053–64.
[4] Li X, Du X. Neoadjuvant chemotherapy combined with interval cytoreductive surgery in ovarian cancer. J Buon. 2019;24(5):2035–40.
[5] Kunit KC, Fleming GF, Lengyel E. Updates and new options in advanced epithelial ovarian cancer treatment. Obstet Gynecol. 2021;137(1):108–21.
[6] Hanif F, Muzaffar K, Perveen K, Malhi SM, Simjee ShU. Glioblastoma multiforme: a review of its epidemiology and
pathogenesis through clinical presentation and treatment. Asian Pac J Cancer Prev. 2017;18(1):3–9.

[7] Pantshwa JM, Kondiah PPD, Choona YE, Marimuthu T, Pillay V. Nanodrug delivery systems for the treatment of ovarian cancer. Cancers (Basel). 2020;12(1):213.

[8] Rao PV, Nallappan D, Madhavi K, Rahman S, Jun Wei L, Gan SH. Phytochemicals and biogenic metallic nanoparticles as anticancer agents. Oxid Med Cell Longev. 2016;2016:3685671.

[9] Birkett MA, Hassanali A, Hoglund S, Pettersson J, Pickett JA. Repellent activity of catmint, Nepeta cataria, and iridoid nepetalactone isomers against Afro-tropical mosquitoes, ixodid ticks and red poultry mites. Phytochemistry. 2011 Jan;72(1):109–14.

[10] Zomorodian K, Saharkhzij MJ, Shariatifard S, Pakshir K, Rahimi MJ, Khashei R. Chemical composition and antimicrobial activities of essential oils from nepeta cataria L. against common causes of food-borne infections. ISRN Pharm. 2012;2012:591953.

[11] Sharma A, Nayik GA, Canoo DS. Pharmacology and toxicology of Nepeta cataria (Catmint) species of genus Nepeta: A review. Plant Hum Health. 2019;3:285–99.

[12] Zomorodian K, Saharkhzij MJ, Rahimi MJ, Shariatifard S, Pakshir K, Khashei R. Chemical composition and antimicrobial activities of essential oil of nepeta cataria L. against common causes of oral infections. J Dent (Tehran). 2013;10(4):329–37.

[13] Zhu J, Berkebile DR, Dunlap CA, Zhang A, Boxler D, Tangtrakulwanich K, et al. Nepetacetolones from essential oil of Nepeta cataria represent a stable fly feeding and oviposition repellent. Med Vet Entomol. 2012;26(2):131–8.

[14] Ahmed S, Ahmad M, Swami BL, Ikram S. A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise. J Adv Res. 2016;7(1):17–28.

[15] Anjum S, Hashim M, Malik SA, Khan M, Lorenzo JM, Abbasi BH, et al. Recent advances in zinc oxide nanoparticles (ZnO NPs) for cancer diagnosis, target drug delivery, and treatment. Cancers (Basel). 2021;13(18):4570.

[16] Rasmussen JW, Martínez E, Louka P, Wingett DG. Zinc oxide nanoparticles for selective destruction of tumor cells and potential for drug delivery applications. Expert Opin Drug Deliv. 2010;7(9):1063–77.

[17] Kundu M, Sadhukhan P, Ghosh N, Chatterjee S, Manna P, Das J, et al. pH-responsive and targeted delivery of curcumin via phenylboronic acid-functionalized ZnO nanoparticles for breast cancer therapy. J Adv Res. 2019;18:161–72.

[18] Jiang J, Pi J, Cai J. The advancing of zinc oxide nanoparticles for biomedical applications. Bioinorg Chem Appl. 2018;2018:1062562.

[19] Siddiqui KS, Ur Rahman A, Tajuddin, Husen A. Properties of zinc oxide nanoparticles and their activity against microbes. Nanoscale Res Lett. 2018;13(1):141.

[20] De Berardinis B, Civitelli G, Condello M, Lista P, Pozzi R, Arancia G, et al. Exposure to ZnO nanoparticles induces oxidative stress and cytotoxicity in human colon carcinoma cells. Toxicol Appl Pharmacol. 2010;246(3):116–27.

[21] Fujihara J, Tongu M, Hashimoto H, Yamada T, Kimura-Kataoka K, Yasuda T, et al. Distribution and toxicity evaluation of ZnO dispersion nanoparticles in single intravenously exposed mice. J Med Investigation. 2015;62(1.2):45–50.

[22] Sabitha R, Nishi K, Gunasekaran VP, Agilan B, David E, Annamalai G, et al. p-Coumaric acid attenuates alcohol exposed hepatic injury through MAPKs, apoptosis and Nrf2 signaling in experimental models. Chem-Biol Interact. 2020;321:109044.

[23] Balupillai A, Nagarajan RP, Ramasamy K, Govindasamy K, Muthusamy G. Caffeic acid prevents UVB radiation induced photocarcinogenesis through regulation of PTEN signaling in human dermal fibroblasts and mouse skin. Toxicol Appl Pharmacol. 2018;352:87–96.

[24] Balupillai A, Kaninimogi H, Khan HA, Alhumida AS, Prasad NR. Opuntiin prevents phlogistoaging of mouse skin via blocking inflammatory responses and collagen degradation. Oxid Med Cell Longev. 2020;2020:5275178.

[25] Makarov VV, Love AJ, Sinitsyna OV, Makarova SS, Yaminsky IV, Taliysky ME, et al. “Green” nanotechnologies: synthesis of metal nanoparticles using plants. Acta Naturae. 2014;6(1):20.

[26] Jain S, Mehta MS. Medicinal plant leaf extract and pure flavonoid mediated green synthesis of silver nanoparticles and their enhanced antibacterial property. Sci Rep. 2017;7(1):15867.

[27] Reichert W, Villani T, Pan MH, Ho CT, Simon JE, Wu Q. Phytochemical analysis and anti-inflammatory activity of Nepeta cataria accessions. J Med Active Plants. 2018;7(1):19–27.

[28] Adiguzel A, Ozher H, Sokmen M, Gulluce M, Sokmen A, Kilic H, et al. Antimicrobial and antioxidant activity of the essential oil and methanol extract of Nepeta cataria. Pol J Microbiol. 2009;58(1):69–76.

[29] Talam S, Karumuri SR, Gunnam N. Synthesis, characterization, and spectroscopic properties of ZnO nanoparticles. Int Sch Res Not. 2012;2012:372505.

[30] Singh A, Gautam PK, Verma A, Singh V, Shivapriya PM, Shivalkar S, et al. Green synthesis of metallic nanoparticles as effective alternatives to treat antibiotics resistant bacterial infections: A review. Biotechnol Rep (Amst). 2020;25:e00427.

[31] Petenatti ME, Gette MD, Camí GE, Popovich MC, Marchevsky EJ, del Vitto LA, et al. Quantitative micrograph, HPLC and FTIR profiles of Melissa officinalis and Nepeta cataria (Lamiaceae) from Argentina. Rev de la Facul de Cienc Agrarias. 2014;46(2):15–27.

[32] Chaudhuri SK, Malodia L. Biosynthesis of zinc oxide nanoparticles using leaf extract of Calotropis gigantea: characterization and its evaluation on tree seedling growth in nursery stage. Appl Nanosci. 2017;7(8):501–12.

[33] Iravani S, Korbekandi H, Mirmohammadi SV, Zolfaghari B. Synthesis of silver nanoparticles: chemical, physical and biological methods. Res Pharm Sci. 2014;9(6):385–406.

[34] Vijayakumar S, Mahadevan S, Arulmozhi P, Sriman S, Praseetha PK. Green synthesis of zinc oxide nanoparticles using Atalanta monophylla leaf extracts: Characterization and antimicrobial analysis. Mater Sci Semiconductor Process. 2018;82:39–45.

[35] Matulonis UA, Sood AK, Fallowfield L, Howitt BE, Sehoul J, Karlan BY. Ovarian cancer. Nat Rev Dis Primers. 2016;2:16061.

[36] Bisht G, Rayamajhi S. ZnO nanoparticles: a promising anticancer agent. Nanobiomed (Rij). 2016;3:9.

[37] Mohammadinejad R, Moosavi MA, Tavakol S, Vardar DÖ, Hosseini A, Rahmati M, et al. Necrotic, apoptotic and autophagic cell fates triggered by nanoparticles. Autophagy. 2019;15(1):4–33.
Hameed S, Iqbal J, Ali M, Khalil AT, Abbasi BA, Numan M, et al. Green synthesis of zinc nanoparticles through plant extracts: establishing a novel era in cancer theranostics. Mater Res Exp. 2019;6(10):102005.

Abbasi BA, Iqbal J, Ahmad R, Zia L, Kanwal S, Mahmood T, et al. Bioactivities of Geranium wallichianum leaf extracts conjugated with zinc oxide nanoparticles. Biomolecules. 2020;10(1):38.

Barabadi H, Vahidi H, Kamali KD, Rashedi M, Saravanan M. Antineoplastic biogenic silver nanomaterials to combat cervical cancer: a novel approach in cancer therapeutics. J Clust Sci. 2020 Jul;31(4):659–72.

Barabadi H, Vahidi H, Kamali KD, Hosseini O, Mahjoub MA, Rashedi M, et al. Emerging theranostic gold nanomaterials to combat lung cancer: a systematic review. J Clust Sci. 2020;31(2):323–30.

Barabadi H, Vahidi H, Mahjoub MA, Kosar Z, Damavandi Kamali K, Ponnurugan K, et al. Emerging antineoplastic gold nanomaterials for cervical cancer therapeutics: a systematic review. J Clust Sci. 2020 Nov;31(6):1173–84.

Khatua A, Prasad A, Priyadarshini E, Patel AK, Naik A, Saravanan M, et al. Emerging antineoplastic plant-based gold nanoparticle synthesis: a mechanistic exploration of their anticancer activity toward cervical cancer cells. J Clust Sci. 2020;31(6):1329–40.

Hu X, Li D, Zhang W, Zhou J, Tang B, Li L. Matrix metalloproteinase-9 expression correlates with prognosis and involved in ovarian cancer cell invasion. Arch Gynecol Obstet. 2012;286(6):3537–43.

Quintero-Fabían S, Arreola R, Becerril-Villanueva E, Torres-Romero JC, Arana-Argáez V, Lara-Riegos J, et al. Role of matrix metalloproteinases in angiogenesis and cancer. Front Oncol. 2019;9:1370.

Jablonska-Trypuć A, Matejczyk M, Rosochacki S. Matrix metalloproteinases (MMPs), the main extracellular matrix (ECM) enzymes in collagen degradation, as a target for anticancer drugs. J Enzyme Inhibition Med Chem. 2016;31:177–83.

Webb AH, Gao BT, Goldsmith ZK, Irvine AS, Saleh N, Lee RP, et al. Inhibition of MMP-2 and MMP-9 decreases cellular migration, and angiogenesis in in vitro models of retinoblastoma. BMC Cancer. 2017;17(1):434.

Li H, Qiu Z, Li F, Wang C. The relationship between MMP-2 and MMP-9 expression levels with breast cancer incidence and prognosis. Oncol Lett. 2017;14(5):5865–70.

Pfeffer CM, Singh ATK. Apoptosis: a target for anticancer therapy. Int J Mol Sci. 2018;19(2):448.

Redza-Dutordoir M, Averill-Bates DA. Activation of apoptosis signalling pathways by reactive oxygen species. Biochim et Biophys Acta (BBA)-Molecular Cell Res. 2016;1863(12):2977–92.

Zhang H, Liang Z, Zhang J, Wang WP, Zhang H, Lu Q. Zinc oxide nanoparticle synthesized from Euphorbia fischeriana root inhibits the cancer cell growth through modulation of apoptotic signaling pathways in lung cancer cells. Arab J Chem. 2020;13(7):6174–83.