Cancer Nanotechnology

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Investigating the anticancer and anti-angiogenic effects of graphene oxide nanoparticles containing 6-gingerol modified with chitosan and folate

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Abstract

Objective: The use of nanocarriers to improve the targeting of treatment plays a key role in the treatment of many diseases, including cancer. This study was carried out to synthesize graphene oxide (GO) containing 6-gingerol (Ging) modified with chitosan (CS)-folic acid (FA) nanoparticles (Ging-GO-CS-FA) to improve the anti-cancer effects of Ging.

Methods: After the synthesis of nanoparticles, the average size, surface charge, and dispersion index (DPI) of nanoparticles were analyzed by the dynamic light scattering (DLS) method. Field emission scanning electron microscope (FESEM) and Fourier transform infrared spectroscopy (FTIR) were utilized to assess the morphology and functional groups of synthesized nanoparticles, respectively. The 2,5-diphenyl-2H-tetrazolium bromide (MTT) method was performed to assess the toxicity effect of nanoparticles on different types of cancer cells. The antioxidant power of nanoparticles was evaluated by ABTS and DPPH methods. In addition, the chorioallantoic membrane (CAM) test was conducted to investigate the anti-angiogenic effects of nanoparticles. Finally, the real-time quantitative PCR (qPCR) method was carried out to detect the changes in the expression of angiogenic and antioxidant genes in cancer cells.

Results: The nanoparticles have an average size of 73.21 nm, a DPI of 0.27, and a surface charge of 29.5. The encapsulation rate of Ging in nanoparticles was reported to be 81.7%. According to the MTT test, the most sensitive cell line to the Ging-GO-CS-FA nanoparticles was reported to be gastric cancer cells (IC_{50} ~ 27). The results of the antioxidant test showed the high antioxidant power of nanoparticles in the laboratory environment by inhibiting ABTS and DPPH free radicals. The pro-oxidant power of Ging-GO-CS-FA against cancer cells was confirmed by reducing the amount of the superoxide dismutase (SOD) gene in the treated cells. The decreasing effects of Ging-GO-CS-FA on angiogenesis were observed by reducing the average length, the number of blood vessels, average height, and weight of treated embryos. In addition, the decrease in the expression of VEGF and VEGF-R genes confirmed the anti-angiogenic of Ging-GO-CS-FA.

Conclusions: These results show the promising effect of Ging-GO-CS-FA on gastric cancer cells by inhibiting angiogenesis and increasing the level of oxidants.

Keywords: 6-Gingerol, Graphene oxide nanoparticles, Antioxidant, Anti-angiogenic, Anti-cancer

Introduction

The increase in the prevalence of various cancers due to the lack of effective treatments and the low efficacy of drugs has led to the development of clinical applications of Nanomedicine and drug delivery systems in the field of cancer therapy. Toward this end, various nanomaterials including polymeric nanoparticles, micelles, and liposomes have been synthesized and studied by researchers in order to deliver drugs to cancer cells (Farjadian et al. 2019; Zangabad et al. 2018). In addition, two-dimensional nanoparticles, including graphene, have received much attention from researchers due to their effective properties in the field of drug delivery.

Graphene and its derivatives have unique properties such as high biocompatibility, suitable physicochemical properties, high flexibility, and high thermal and electrical properties which lead to suitable carriers in drug delivery systems (Liu et al. 2008). However, oxidized derivatives of graphene such as graphene oxide (GO) have been developed by researchers to overcome the low dispersion of graphene in aqueous solutions (Campbell et al. 2019). A variety of nanocomposite materials can be developed using GO nanoparticles (Compton and Nguyen 2010). A variety of applications can be achieved using these materials to create nanofibers and nanotubes. The production of sensors and transistors is one of these activities (Gaur et al. 2021). A number of studies have been conducted on the potential benefits of GO nanoparticles in treating cancer. Carbon and oxygen atoms are arranged in a single layer in these particles, which can be programmed to target and destroy cancer cells (Feng et al. 2013). According to preclinical studies, GO nanoparticles are capable of selectively identifying and destroying tumor cells with minimal toxicity to healthy cells (Liu et al. 2013). Aside from inhibiting the growth of cancer cells, GO nanoparticles have also been shown to stimulate the growth of healthy cells. It is for this reason that they are considered to be potential candidates for cancer treatments in the future. The efficacy of GO nanoparticles in cancer treatment can also be enhanced by loading them with a variety of drugs and therapeutic molecules. Thus, GO nanoparticles offer a promising and innovative method of treating cancer (Itoo et al. 2022).

In addition, the use of compounds of natural origin as effective pharmaceutical agents or in combination with chemotherapy drugs has increased (Haque et al. 2016). In this regard, Ging (1-[4′-hydroxy-3′-methoxyphenyl]-5-hydroxy-3-decanone) derived from ginger was studied because of its antioxidant, anti-inflammatory, anti-angiogenic, and anti-tumor properties (Zhang et al. 2021). Various pieces of evidence have shown that Ging affects various cancer cells by regulating the cell cycle, inhibiting angiogenesis, and activating apoptosis (Wang et al. 2014).

The biopolymer chitosan (CS) has antimicrobial, antifungal, and antiviral properties and is derived from crustaceans' exoskeletons (Rabea et al. 2003). In addition to protecting nanoparticles from environmental stresses, such as temperature fluctuations and pH changes, it can also facilitate drug uptake and delivery within the body (Hamedinasab

et al. 2020). In addition, the CS coating prevents the body's immune system from attacking the particles (Jain and Jain 2020).

Overexpression of folic acid (FA) has been linked to cancer. Currently, FA is one of the most significant health concerns and one of the most active areas of research. Cancers such as colorectal, breast, and ovarian are associated with overexpression of FA (Tagde et al. 2020). The overexpression of FA may promote the growth of cancer cells by increasing the production of certain enzymes and proteins (Low et al. 2008).

Given these points, the present study was conducted in order to synthesize nanoparticles based on Ging-containing GO and modified with CS and FA which increases the efficiency of drug delivery to cancer cells. In addition, this study investigated the cytotoxic, oxidant, and anti-angiogenic influences on various cancer cells, including breast cancer, gastric cancer, pancreatic cancer, and liver cancer.

Materials and methods

Preparation of Ging-GO-CS-FA nanoparticles

To synthesize GO nanoparticles modified with 50 mg CS (high purity, MW 110,000-150,000, degree of deacetylation $\leq$ 40 mol%, Merck, Darmstadt, Germany) conjugated with 15 mg FA (kindly provided by Raha Pharmaceutical Company (Isfahan, Iran), a CS-FA combination was first prepared. Toward this end, a specific volume of FA dissolved in DMSO was combined with carbodiimide hydrochloride (EDC, Merck, Darmstadt, Germany) and N-hydroxysuccinimide (NHS, Merck, Darmstadt, Germany). Next, we added CS dissolved in 1% acetic acid dropwise to the prepared composition containing FA. After proper incubation, the pH of the resulting mixture was regulated to 8.5 and the formed precipitate was lyophilized after centrifugation. In the next step, GO dissolved in 1% acetic acid (pH 5.0) was added to EDC and NHS. The prepared CS-FA precipitate dissolved in 1% acetic acid was added to the solution containing GO. After incubation and centrifugation, the precipitate was dissolved in 20 ml of distilled water and added to the aqueous solution containing Ging (Golexir, Iran), and then centrifuged and lyophilized. At the time of use, the nanoparticles were dispersed in distilled water and sterilized using a 0.22 µm syringe filter.

Investigating the physicochemical properties of Ging-GO-CS-FA

To check the quality of synthesized nanoparticles, the average diameter and surface charge of nanoparticles were checked by the dynamic light scattering (DLS) (Malvern Instruments, Worcestershire UK) method and Zeta Sizer (Malvern Instruments Ltd, Worcestershire, UK), respectively (Mokhtareeizadeh et al. 2022). In addition, the Fourier Transform Infrared Spectroscopy (FTIR) technique was conducted to investigate the functional groups in the structure of prepared nanoparticles. In addition, morphological analysis of the samples was done with Field Emission Scanning Electron Microscope (FESEM) microscope (Mokhtareeizadeh et al. 2022).

Investigating the amount of encapsulation efficiency (EE) of Ging-GO-CS-FA

The absorbance of a specific amount of prepared nanoparticle was evaluated by UV spectrophotometer at 202 nm and the amount of EE (%) was calculated via the standard curve of Ging and the following formula:

$$EE\% = \text{amount of drug in NPs} / \text{total used drug} * 100$$

Investigating the antioxidant capacity of Ging-GO-CS-FA

Inhibition of ABTS and DPPH free radicals in the presence of several concentrations of 6 Ging-GO-CS-FA was investigated in order to investigate the antioxidant capacity of Ging-GO-CS-FA.

ABTS and DPPH methods

According to the previous protocols, we mixed 500 µL of ABTS solution prepared with potassium persulfate and deionized distilled water with 500 µL of different concentrations of Ging-GO-CS-FA and read their absorbance at 734 nm wavelength. Similarly, we added 500 µL of DPPH solution prepared with ethanol to 500 µL of different concentrations of Ging-GO-CS-FA and measured their absorbance at 517 nm. Finally, the amount of inhibition rate of free radicals was obtained through the following formula (Mokhtareeizadeh et al. 2022):

$$\text{Inhibition of free radical\%} = \left(A_{\text{Control}} - A_{\text{Sample}/A_{\text{Control}}}\right) \times 100$$

The chorioallantoic membrane (CAM) assay

CAM assay was performed to assess the effect of Ging-GO-CS-FA on angiogenesis. In the present study, a certain number of ROSS eggs were prepared and located in the incubator. After 48 h, the eggs were removed from the incubator and a window was created on the surface of the eggs and they were blocked with paraffin and glue. After 6 days of incubation, the gelatin sponge prepared from egg white and agar was placed on the formed chorioallantoic membrane, and then 10 µL of the solution containing Ging-GO-CS-FA was loaded on the membrane. Next, the windows were blocked again with paraffin and glue and transferred to the incubator. On the 12th day of incubation, the area of the sponge and its surrounding areas were photographed with a stereomicroscope and analyzed with Image J software. In addition, changes in the length and weight of the fetuses were measured with calipers and digital scales (Mokhtareeizadeh et al. 2022).

Cytotoxic effect of Ging-GO-CS-FA

To investigate the cytotoxicity effects of Ging-GO-CS-FA on cancer cells, the MTT test was performed on MCF-7 (breast cancer), AGS (gastric cancer), PANC (pancreatic cancer), and HepG2 (liver cancer) cell lines. It is noteworthy that HFF cell lines were used as normal cells. After exposing the cells to different concentrations of Ging-GO-CS-FA (7.5, 15.6, 31.2, 62.5, 125, 250, 500 µg/mL) for 24 h, the prepared MTT solution was added to the wells. After 1.5 h of incubation at 37 °C, the medium of the wells was removed and 100 µL of DMSO was added to reveal the purple color resulting from the formation of formazan crystals (Zarei et al. 2022). Finally, the absorbance of the sample was measured at 570 nm using an ELISA reader.

RNA extraction and real-time qPCR

The real-time qPCR method was performed to evaluate the effect of Ging-GO-CS-FA on the expression of angiogenesis-associated genes such as VEGF-R and VEGF and antioxidant enzymes including superoxide dismutase (SOD) and glutathione peroxidase (GPx). The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was also considered a housekeeping gene (Table 1). After 24 h of cultivated cells in the flask, the cells were treated with Ging-GO-CS-FA (26 and 36 µg/mL). After 48 h of treatment, the total RNA was obtained with the help of a commercial RNA extraction kit (BIOFACT, South Korea). The concentration of obtained RNA was evaluated by a nanodrop. The obtained RNA was reversed cDNA using the cDNA Synthesis Kit (Pars Touse, Iran), according to its guideline. Finally, the cycle threshold (CT) of the samples was evaluated by a real-time thermal cycler (BIRAD, CFX96, USA) by SYBR Green real-time qPCR Master Mix (No ROX) (Ampliqon, Denmark). The data analysis was performed by the $2^{-\Delta\Delta Ct}$ formula (Table 1).

Statistical analysis of data

SPSS statistical software was utilized to analyze the obtained data. Toward this end, we used one-way ANOVA and LSD tests. $P < 0.05$ was considered a meaningful criterion.

Results

Physicochemical properties of Ging-GO-CS-FA

The results of examining the physicochemical properties of the synthesized Ging-GO-CS-FA indicated an average size of 73.21 nm, a hydrodynamic diameter of 207.45 nm, and a polydispersion index (PDI) of 0.27, which was in line with the results of FESEM. Besides, the data of Zeta Sizer implied that the surface charge of the nanoparticle is about + 30 mV. The FTIR results implied NHCO stretching vibrations at 3394 and 2921 cm^{-1} associated with the GO-CS spectrum. According to the figure, the absence of the peak at 1731 cm^{-1} which is associated with the carboxylic acid group of graphene oxide indicates the reaction of carboxylic groups with NH2 groups in CS to form amide bonds (Fig. 1) (Dimiev and Tour 2014).

Entrapment efficiency of Ging-GO-CS-FA

As mentioned previously, we obtained the amount of Ging loading in the synthesized nanoparticle by indirect method and standard graph. According to the figure, the amount of encapsulated drug was calculated as 86.2% (Fig. 2).

Table 1 The list of primers used for real-time qPCR.

Gene	Forward	Reverse	Amplicon length (bp)	Genebank No.
GAPDH	TGCTGGTGCTGAGTATGTCG	GCATGTCAGATCCACAACGG	131	NM_002046
SOD	AGCATGGGTTCCACGTCCA	CACATTGGCCACACCGTCCT	129	NM_000454.5
GPx	GTGCTCGGCTTCCCGTGCAAC	CTCGAAGAGCATGAAGTTGGGC	142	NM_001329503.2
VEGF	GACCTGTAAATGTTCCTGCAA	AGAAATCAGGCTCCAGAAACA	148	NM_205042.3
VEGF-R	GCACAAGAATGAGAGCACCA	ACCATTTTGCCTCTGGAGAA	141	NM_001004368.2

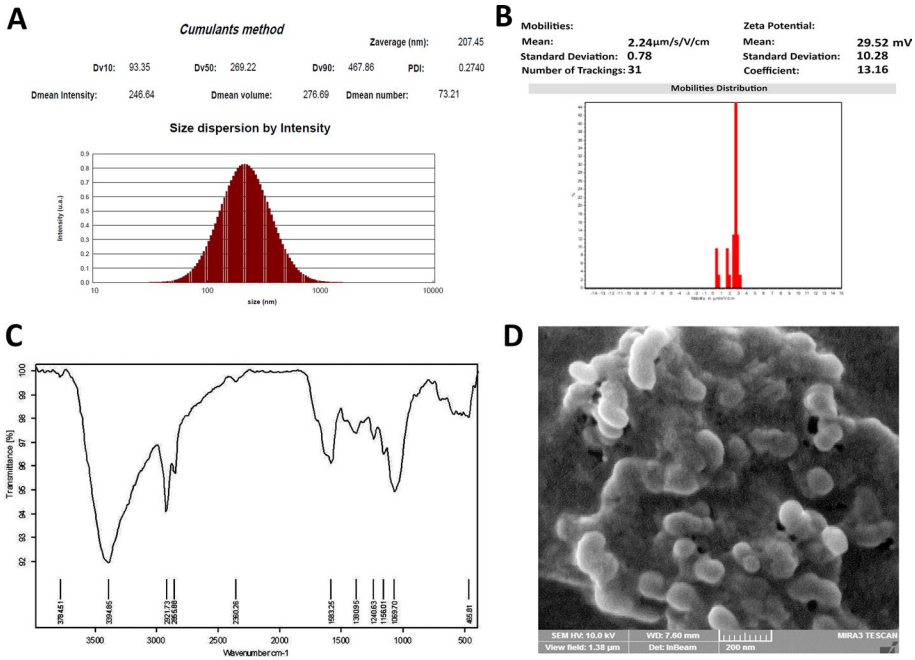

Fig. 1 Physicochemical characteristics of Ging-GO-CS-FA. **A** Average size and PDI. **B** Zeta potential of Ging-GO-CS-FA. **C** FTIR spectra of Ging-GO-CS-FA. **D** FESEM figure of Ging-GO-CS-FA

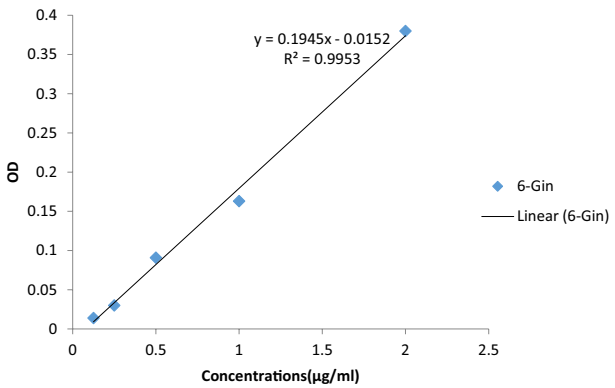

Fig. 2 The percentage of encapsulation efficiency (EE%) of Ging-GO-CS-FA

Antioxidant capacity of Ging-GO-CS-FA

The results of DPPH and ABTS scavenging free radicals by Ging-GO-CS-FA showed that Ging-GO-CS-FA have antioxidant ability by inhibiting free radicals. As shown in Fig. 3, the IC_{50} of nanoparticles in scavenging ABTS and DPPH free radicals is 68 µg/mL and 723 µg/mL, respectively. According to the data, it shows more inhibitory effects of Ging-GO-CS-FA on ABTS free radicals compared to DPPH.

Effect of Ging-GO-CS-FA toxicity on cell viability

Examining the toxicity effects of Ging-GO-CS-FA against breast (MCF-7) and gastric cancer cells (AGS) showed that Ging-GO-CS-FA decrease the survival of cancer cells in

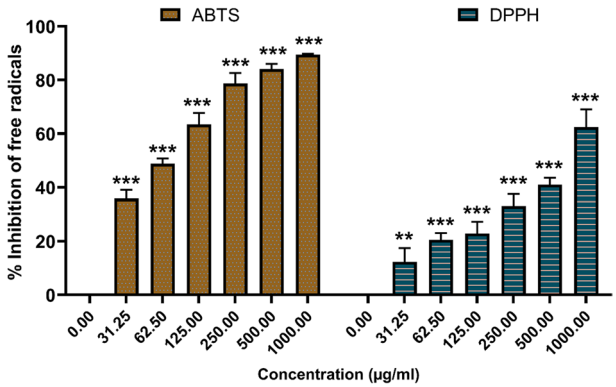

Fig. 3 Inhibition effect of Ging-GO-CS-FA by scavenging of ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) and DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radicals. (** $p < 0.01$ and *** $p < 0.001$). The data are presented as mean ± SD. The test was performed in triplicate

a dose-dependent manner. In this regard, the IC_{50} value for MCF-7 and AGS cell lines was calculated as 56.7 µg/mL and 27 µg/mL, respectively. However, the results of investigating the toxicity of Ging-GO-CS-FA against pancreatic cancer cells (PANC) indicated that the inhibitory effect against these cells was not observed until the concentration of 62.5 µg/mL and the IC_{50} was about 253 µg/mL. Similarly, high concentrations of Ging-GO-CS-FA showed a significant inhibitory effect on pancreatic cancer cells. However, by comparing the toxicity effect of Ging-GO-CS-FA against cancer and normal cells (HFF), we found that Ging-GO-CS-FA have a higher inhibitory effect against the mentioned cancer cells compared to normal cells, which shows the safety of Ging-GO-CS-FA for clinical applications. Due to the higher effect of Ging-GO-CS-FA against gastric cancer cells, this cell line (AGS) was selected for further experiments (Fig. 4).

The results of gene expression analysis

In order to investigate the antioxidant property and anti-angiogenic effects of Ging-GO-CS-FA on the AGS cell line, the level of gene expression of GPx, SOD, VEGF, and VEGF-R was investigated. According to the figure, the treatment of the AGS cell line by Ging-GO-CS-FA leads to a decrease in the expression of GPx, SOD, VEGF, and VEGF-R genes. These results implied the pro-oxidant and anti-angiogenic properties of Ging-GO-CS-FA in gastric cancer cells (Fig. 5).

CAM test results

The examination of the blood vessel morphology resulting from CAM showed a considerable reduction in the density and number of vessels in the treated groups with several concentrations of Ging-GO-CS-FA compared to the untreated group. In addition, the average length of blood vessels in the treated groups was significantly reduced compared to the untreated sample. Besides, by examining the weight and height of the fetuses, we found that Ging-GO-CS-FA have significant inhibitory effects on the height and weight of the fetuses compared to the untreated group (Fig. 6).

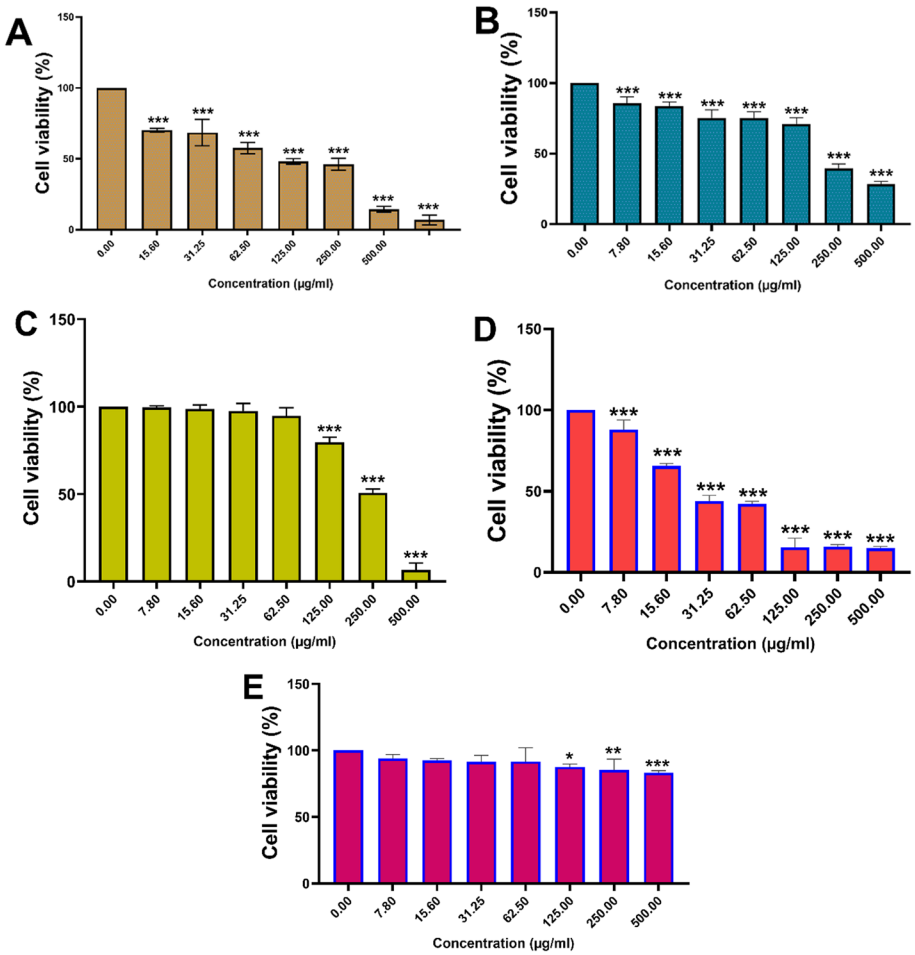

Fig. 4 Investigating the cytotoxic effect of Ging-GO-CS-FA on MCF-7 (**A**), HepG2 (**B**), PANC (**C**), AGS (**D**), and HFF (**E**) cell lines. (*$P < 0.05$ vs. control; **$P < 0.01$ vs. control; ***$P < 0.001$ vs. control)

Discussion

Recently, the use of nanomaterials science, including polymer nanoparticles, micelles, liposomes, and inorganic nanoparticles, in the field of medicine and treatment of many diseases, including various cancers, has attracted the attention of many researchers. In addition, due to the properties of some two-dimensional nanomaterials, such as a suitable surface area, biocompatibility, and easy surface modification have attracted attention in the field of nano-biotechnology (Liu et al. 2013). Graphene and its derivatives are one of the two-dimensional materials that have been selected as a potential candidate for use in the field of drug delivery systems due to their high biocompatibility, optical properties, and suitable biological performance (Liu et al. 2008; , Farjadian et al. 2020; –Kovalchuk et al. 2020). However, the lack of solubility of graphene in an aqueous environment is one of the most serious limitations of its use in the field of drug delivery. That is why graphene derivatives such as GO and reduced GO (rGO) have been proposed as functional graphene derivatives to compensate for the limitations of using graphene in drug/gene delivery (Campbell et al. 2019). Given these points, we synthesized GO nanoparticles

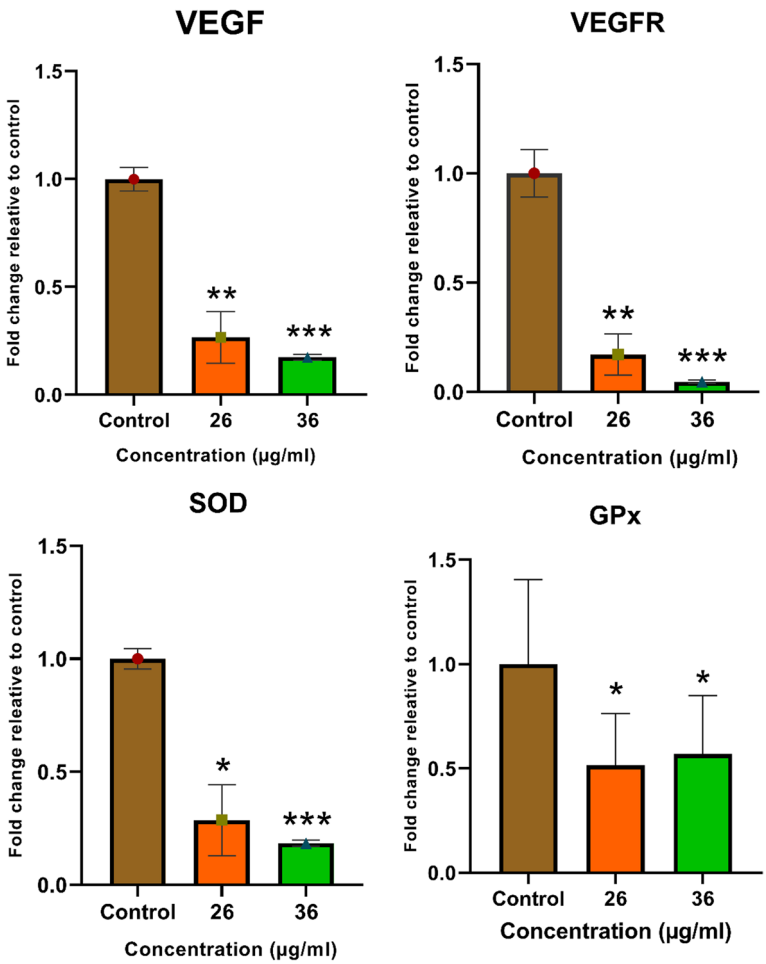

Fig. 5 Investigating the anti-angiogenic and pro-oxidant effect of Ging-GO-CS-FA by detecting the expression of related genes such as VEGF, VEGF-R, SOD, and GPx. (* $p < 0.05$ vs. control; ** $p < 0.01$ vs. control; *** $p < 0.001$ vs. control)

modified with CS-FA and used them to deliver Ging to cancer cells. CS was used to functionalize GO nanoparticle surfaces in this study. Combining CS with GO nanoparticles increases its strength and stability by providing mucoadhesive properties, biocompatibility, and biodegradability (Mura et al. 2022). The results of examining the properties of the synthesized nanoparticles showed that the nanoparticles have an average size of 73.21 nm, a surface charge of + 29.5 mV, and a PDI of 0.27 (Fig.1). Evidence reported that the positive charge of CS on the surface of nanoparticles causes more nanoparticles to be absorbed by cancer cells (Chen et al. 2016; –Lu et al. 2019). In addition, due to the increased level of the FA receptor on the surface of tumor cells, the use of FA on the surface of nanoparticles leads to improvements in the efficiency of the drug delivery system for tumor cell therapy (Unger et al. 2012). Similarly, several studies have confirmed the increase in efficiency of nanoparticles modified with CS and FA in the drug delivery system (Keklikcioglu Cakmak and Eroglu 2023; –de Sousa et al. 2018). For instance, Cakmak et al. demonstrated that the use of graphene oxide-based nanoparticles modified CS-FA containing

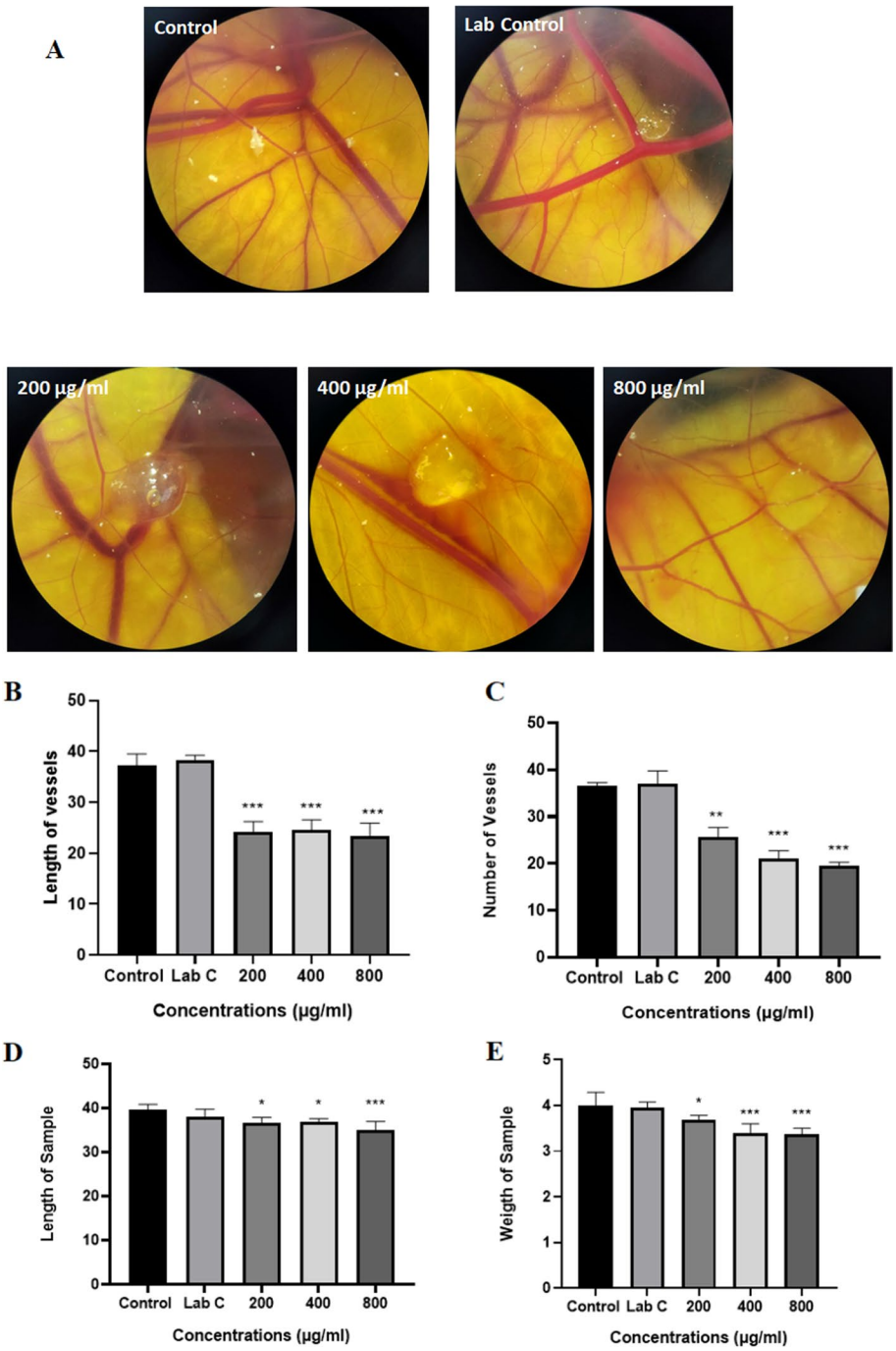

Fig. 6 Investigating the anti-angiogenic property of Ging-GO-CS-FA by chorioallantoic membrane (CAM) test. Comparison of stereomicroscopic images between treated and control groups (**A**). Investigation of blood vessel length (**B**), number of blood vessels (**C**), fetal length (**D**), and fetal weight (**E**) in different doses of Ging-GO-CS-FA in comparison with the control group. (* $P < 0.05$ vs. control; ** $P < 0.01$ vs. control; *** $P < 0.001$ vs. control). Lab C: Laboratory control

the chemotherapeutic drugs doxorubicin (DOX) and tamoxifen (TAM) resulted in increased drug release at acidic pH of cancer cells and increases the effectiveness of drugs (Keklikcioglu Cakmak and Eroglu 2023).

Recently, evidence has shown that the compounds of phytochemicals, including Ging derived from ginger due to its antioxidant, anti-inflammatory, antiplatelet, antimicrobial, and anticancer agents are used in the treatment of various diseases (Zhang et al. 2021). Anti-angiogenic effect of Ging is one of the important hypotheses of the effect of this compound in the prevention of carcinogenesis (Wang et al. 2014). A recent study confirmed the anti-angiogenic influence of Ging in in vitro and in vivo by inhibiting the expression of VEGF in endothelial cells and the development of vessels in the rat aorta (Kim et al. 2005). Vascular endothelial cells express the VEGF receptor, which is primarily responsible for angiogenesis. VEGF plays an important role in promoting angiogenesis in healthy humans, both during embryonic development and wound healing. Angiogenesis is initiated by the expression of oncogenes, a variety of growth factors, as well as hypoxia in cancer cells (Carmeliet 2005; , Mashreghi et al. 2018). In addition to their anti-angiogenic properties, GO nanoplatforms have also been studied for their angiogenic properties. It is during angiogenesis that the primary vessels are formed. The primary vessels are responsible for feeding and oxygenating proliferating cells as well as removing waste products (Xiong et al. 2014; , Khurana et al. 2005). There has been considerable evidence that angiogenesis plays an important role in wound healing, the treatment of cardiovascular disease, and the growth of tumors. Angiogenesis is induced by GO by phosphorylating Akt and regulating nitric oxide synthase (NOS) to increase the production of intracellular nitric oxide (NO). This angiogenic process can be reversed by reversing the concentration of GO and the amount of ROS in the cells (Mukherjee et al. 2015). Similarly, we found that the synthesized nanoparticles containing Ging have antioxidant and anti-angiogenic effects by inhibiting free radicals and anti-angiogenic power by the CAM method (Fig.6) and inhibiting the expression of VEGF and VEGF-R (Fig. 5a, b). However, we observed that Ging-GO-CS-FA contain pro-oxidant power in cancer cells, which can activate apoptotic pathways (Fig. 5c). In addition, it was found that the cytotoxic effect of Ging-GO-CS-FA on gastric cancer cells was higher than on other studied cancer cells (Fig. 4). In this regard, it has been found that Ging can control cancer cell proliferation by inhibiting the expression of cyclin-dependent protein kinases in the cell cycle (Wang et al. 2014). In addition, the apoptotic and pre-apoptotic effect of Ging has been observed on various cancer cells, including ovarian and skin cancer (Pashaei-Asl et al. 2017). Moreover, it has been reported that Ging is able to suppress the cell cycle, increase ROS production and programmed cell death in breast cancer cells (Sp et al. 2021). Furthermore, the composition of GO also has anti-angiogenic properties, which may be effective in increasing the anti-angiogenic properties of Ging in Ging-GO-CS-FA. It is well known that graphene oxide-based nanoparticles are able to increase the production of ROS and reactive nitrogen species (RNS), which varies according to the cell type and the concentration of graphene oxide (Mukherjee et al. 2020).

Conclusion

In this study, synthesized graphene oxide nanoparticles containing Ging modified with CS-FA have an average size of 73.21 nm, PDI of 0.27, and positive surface charge. In addition, the percentage of drug encapsulation was reported about 81.7%. The high antioxidant power of nanoparticles in the laboratory environment and their pro-oxidant power in cancer cells showed the dual role of nanoparticles in the external and internal

environment to remove free radicals and induce apoptosis and eliminate cancer cells. It also confirmed the effect of the high toxicity of nanoparticles on cancer cells compared to normal cells and their immunity for clinical applications. Besides, the effects of Ging-GO-CS-FA in inhibiting angiogenesis in the CAM model and molecular analysis suggest the possibility of using Ging-GO-CS-FA for preclinical and clinical studies, although further basic and preclinical studies are required to clarify the molecular mechanism of Ging-GO-CS-FA in inducing apoptosis and inhibiting the proliferation of tumor cells.

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Author contributions
AHAAl-J: Investigation, Methodology, Investigation and Writing-Original draft. NHR and MHT: Supervision, Data curation, Conceptualization Software, Validation and Writing- Reviewing.

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This reserch was performed at personal expense in the laboratory of Islamic Azad University of Tehran.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
All institutional and national guidelines for the care and use of laboratory animals were followed.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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