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Karsinom Hücreleri Üzerinde Antiproliferatif Aktivite Sergileyen Biyouyumlu Hibrit Zencefil/Kitosan Karbon Nanodotunun Yeşil Sentezi

Hasan ILHAN*

Öne Çıkanlar:

- Ginger/chitosan@CD sentez ve karakterizasyonu
- Kanser hücre hatlarında ilk olarak denenmesi
- Zamana ve konsantrasyona bağlı olarak XTT test yapıldı

Anahtar Kelimeler:

- Karbon nanodot
- Zencefil
- Kitosan
- Kanser hücre hattı
- XTT

ÖZET:

Prostat kanseri hücre dizisi (PC-3), insan prostat adenokarsinomu hücre dizisi (LNCaP), meme kanseri hücre dizisi (MCF-7) üzerindeki çoğalmayı önleyici etkiyi değerlendirmek amacıyla karbon nanodotlar (CD'ler) ile modifiye edilmiş kitosan ve zencefil (ginger/chitosan@CD) bazlı biyouyumlu bir substrat oluşturulmuştur. CD'ler solvotermal sentez ile üretildi ve TEM, SERS ve UV-vis spektroskopisi ile karakterize edildi. Bu çalışmada, ilk kez sentezlenen zencefil/kitosan CD'sinin sitotoksik etkisi, kanser hücre hatlarında hem dozun (maksimum konsantrasyon 500 µg/mL) hem de zamanın (24 ve 48 saatte) bir fonksiyonu olarak XTT testi ile değerlendirilmiştir. 48 saat sonra, zencefil/kitosan@CD kombinasyonunun PC-3 hücre hattında 178,08 µg/mL, LNCaP prostat kanseri hücrelerinde 246.44 µg/mL ve MCF-7 kanser hücrelerinde 345.74 µg/mL'lik bir %50 inhibitör konsantrasyonuna (IC_{50}) sahip olduğu bulundu. Kanser hücresi proliferasyonu, zencefil/kitosan@CD'ler tarafından verimli bir şekilde bastırıldı.

Green Synthesis of Biocompatible Hybrid Ginger/Chitosan Carbon Nanodot Exhibiting Antiproliferative Activity on Carcinoma Cells

Highlights:

- Synthesis and characterization of ginger/chitosan@CD
- First trial in cancer cell lines
- XTT test was performed depending on time and concentration

Keywords:

- Carbon nanodot
- Ginger
- Chitosan
- Cancer cell lines
- XTT

ABSTRACT:

Carbon Nanodots (CDs)-modified chitosan and ginger (ginger/chitosan@CD) based biocompatible substrate was built with the purpose of assessing antiproliferation effect on prostate cancer cell line (PC-3), human prostate adenocarcinoma cell line (LNCaP), and breast cancer cell line (MCF-7). CDs were fabricated through a solvothermal synhesis process, and characterized with TEM, SERS, and UV-vis spectroscopy. In this study, the cytotoxic impact of ginger/chitosan CD, which was synthesized for the first time, was evaluated as a function of both dose (maximum concentration 500 µg/mL) and time (in 24 and 48 hours) in cancer cell lines by XTT assay. After 48 hours, the ginger/chitosan@CD combination was found to have a 50% inhibitory concentration (IC_{50}) of 178.08 µg/mL in the PC-3 cell line, 246.44 µg/mL in the LNCaP prostate cancer cells, and 345.74 µg/mL in the MCF-7. Cancer cell proliferation was efficiently suppressed by the ginger/chitosan@CDs.

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INTRODUCTION

Carbon nanodots (CDs) are the most recent addition to the family of carbon nanomaterials. CDs were found by accident in 2004 during the process of purifying single-walled carbon nanotubes and separating them (X. Xu et al., 2004). Since their discovery, CDs have attracted significant interest due to their electronic, biochemical, and optical properties (Baker & Baker, 2010) such as high solubility (H. Liu, Ye, & Mao, 2007; Zhai et al., 2012), green synthetic roots (H. Liu et al., 2007; Sahu, Behera, Maiti, & Mohapatra, 2012; J. Wang, Wang, & Chen, 2012; Zhai et al., 2012; Zhao et al., 2008), stable fluorescence, simple functionalization, low toxicity (Zhao et al., 2008), high electrochemical response (Sarkar, Bohidar, & Solanki, 2018), and high biocompatibility (J. Wang et al., 2012). CDs have considerable promise for use in bioimaging (Cao et al., 2007), sensing (S. Liu et al., 2012), drug delivery (Brindhadevi, Garalleh, et al., 2023), photoelectrochemistry (Huang et al., 2017), electro-chemiluminescence (J. Xu et al., 2018), and optoelectronics (Stepanidenko, Ushakova, Fedorov, & Rogach, 2021) throughout the years. Laser ablation (Sun et al., 2006), discharge technique (X. Xu et al., 2004), combustion/thermal supported root (Dong et al., 2010), electrochemical oxidation root (M. Liu, Xu, et al., 2016), and microwave pyrolysis method (Zhai et al., 2012) are all methods for synthesizing CDs. The microwave pyrolysis approach is superior to the others in terms of suitability, speed, cost-effectiveness, absence of harsh chemicals, and mass yield [3]. Microwave-assisted pyrolysis was used to produce CDs from citric acid (CA) and ethylenediamine (EDA). These CDs had an abundance of primary amine groups (single bond NH2) and carboxyl groups (single bond COOH) on their surfaces, rendering them extremely water-soluble and ideal for biological applications and other analyses (Zhang, Pu, et al., 2002). The CDs are extremely soluble in water and are provided with a very low yield, making it difficult to obtain a thin film/electrode for applications such as electrochemical biosensing. There is a chance that incorporating these CDs into an appropriate matrix would preserve their electrochemical capabilities.

Due to its antioxidative, anti-inflammatory, and anticarcinogenic characteristics, ginger is not only one of the spices that is consumed in the greatest quantities all over the globe, but it is also a traditional medicinal herb in eastern such as China. (Shukla & Singh, 2007). Due to their cheap cost, nontoxicity, biodegradability, and nonimmunogenic qualities, chitosan, lignin, and starch are all examples of naturally occurring polymers that have found several applications in the medicinal and biotechnology fields. (Bhattarai, Gunn, & Zhang, 2010; Builders & Arhewoh, 2016; Ma, Dai, et al., 2017). Many studies have looked at the feasibility of using pH-responsive Ch based compound nanocarriers for the sustained release of genes, proteins, and medicines (C.-K. Chen et al., 2014; R. Chen et al., 2013; Shao et al., 2013; H. Wang et al., 2015). Quantum dots composed of carbon or graphene oxide have been mixed into a variety of nanomaterials and polymer matrices to create high-performance membranes, catalysts, drug carriers, MRI contrast agents, and nanodevices (Ge et al., 2015; Jung et al., 2013; X. Li, Rui, et al., 2015; Lim, Shen, & Gao, 2015; H. Wang, Revia, et al., 2017). In this particular investigation, we synthesized carbon nanodots from ginger and chitosan. We demonstrated for the first time that the as-prepared hybrid ginger/chitosan carbon nanodots (without any modification of anchoring medicines) limit the development of human cancer cells PC-3, LNCaP, and MCF-7.

MATERIALS AND METHODS

Chemicals

All the chemicals and solvents were used without sürter purification. Chitosan and citric acid (CA) were obtained from Sigma-Aldrich and were in reagent grade. Ginger (*Zingiber officinale*) was obtained from a local supplier in Turkiye. Dialysis tube was obtained from Spectra/Por with 2 kDa of MW. The other chemicals used in the cell culture and cytotoxicity experiments were mentioned in relevant sections. In all experiments, deionized water was used at 18.2 MOhm.

Ginger extraction and preparation

A trip to the neighborhood market resulted in the acquisition of ginger. Following the removal of the ginger's skin, it was subsequently hacked up and crushed with a porcelain pestle and mortar. Around fifty grams of curcumin was given a thorough washing under running water, meticulously chopped into small pieces, and then mechanically crushed to a fine powder. In a typical hydrothermal synthesis, the powder of ginger was added to a solution containing 100 mL of water. It was followed by stirred vigorously at 80 °C for 2 hours. In order to remove any solid residues, the ginger juice that had been extracted (20 milliliters) was attained an RCF of 12.000 g in a centrifuge for ten minutes. After that, the pH of the extract (the supernatant) was brought down to 7.4 with NaOH. In the final step, the supernatant was passed through a membrane with a pore size of 0.22 millimeters to eliminate any remaining trace solid residues.

Chitosan solution preparation

In order to obtain the chitosan solution with a concentration of 0.2%, 0.2 grams of chitosan in the ginger solution (sln) with a degree of deacetylation of 90% were diluted in 100 milliliters of acetic acid with a concentration of 1%, and the mixture was stirred with a magnetic stirrer for a period of two hours.

Synthesis of ginger/chitosan@carbon dot

The mixing of extracted ginger solution (25 mL) containing the chitosan solution (0.2%) and citric acid as a carbon source were treated hydrothermally at a temperature of 200 °C for two hours in a drying oven. The resulting carbonized sln became dark yellow, and it was cooled to 25 °C. In order to remove the big particles, the solution that had been reacted was centrifuged with RCF at a speed of 12.000 g for ten minutes. In order to remove any remaining big particles, the C-dot-containing supernatant was passed through a membrane with a pore size of 0.22 millimeters. After that, the supernatant, which was brownish yellow in color and contained 18 mL, was dialyzed for two hours in ultrapure water using a dialysis membrane. Every half an hour, ultrapure water would replace the existing water in the tank. The as-prepared solution that contained purified CDs were divided into aliquots of 18 milliliters each and freeze-dried in a lyophilizer for twenty-four hours. After the sln was purified, the CD content was found to be 12.1 mg/mL.

Cell culture

In this study, prostate cancer cells (PC-3 and LNCaP) were grown in RPMI 1640 medium, and breast cancer cells (MCF-7) were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 20 μg/mL streptomycin and 20 units/ml penicillin, 1 mM sodium pyruvate, and 0.01 mM amino acid solution.

The prostate and breast cancer cells were treated with our novel compound ginger/chitosan@CD at a variety of concentrations, including 100-500 μg/mL, in order to evaluate the anti-proliferative activity at 24 and 48 hours according to a time and dosage based approach.

XTT- cell proliferation assay

Our novel compound, ginger/chitosan@CD, was tested for its anti-proliferative effects on PC-3, LNCaP prostate cancer, and MCF-7 breast cancer cells at a concentration of 1104 cells per well in 96-well plates using the XTT (2,3-bis(2-methoxy-4 nitro-5- sulfophenyl)-2H-tetrazolium-5-carboxanilide) assay. After the appropriate dosing intervals had passed, the XTT combination was given in accordance with the guidelines provided by the manufacturer. An automated microplate reader and a spectrophotometer set to 450 nm (reference 630 nm) were used to analyze the color and intensity of farmazon produced in the lab (Multiskan GO microplate spectrophotometer, Termo). As stated by Alur and groups (Alur et al., 2016), absorbance measurements were utilized to determine cell viability (%) using the specified method.

Viability (%) = Absorbance of experiment well / Absorbance of control well × 100

The AAT bioquest online tool was used to assess the IC_{50} dosages of ginger/chitosan@CD on cancer cells (https://www.aatbio.com/tools/ic50-calculator).

Characterization:

For this study, we used an Agilent/Cary 60 spectrophotometer to collect UV-vis absorption spectra. Surface Enhancement Raman Spectroscopy (SERS) was used to gather spectra from at least ten distinct locations throughout the whole dried region after the SERS spectra were obtained (Jasco NRS-4500). The Raman spectra were collected using an objective lens of numerical aperture 20, a laser spot diameter of 3 micrometers, and a laser power of 30 milliwatts.Transmission electron micrographs (TEM) were obtained from FEI TALOS F200S TEM electron microscope using an acceleration voltage of 2.

RESULTS AND DISCUSSION

Characterization and Properties of Ginger/Chitosan@CD

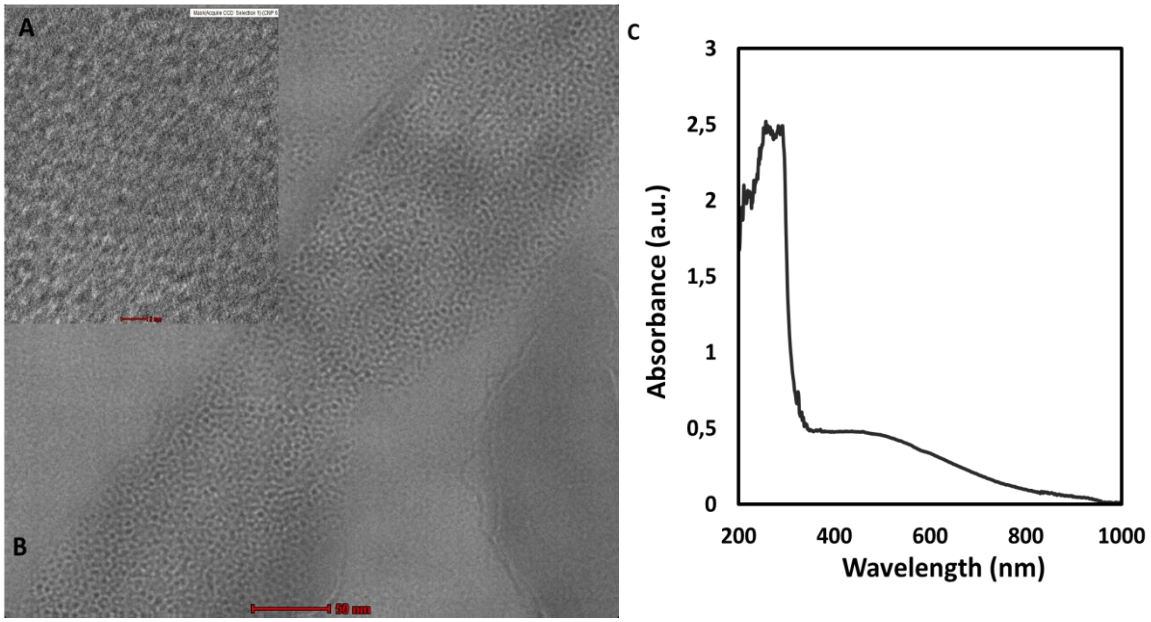

Figure 1. A) and B) TEM İmages of Ginger/Chitosan@Carbon Nanodot, C) UV-Vis Spectrum of Ginger/Chitosan@CD

The ginger/chitosan@CD that were synthesized from chitosan and ginger were evaluated using a transmission electron microscope (TEM), a Surface Enhancement Raman Spectroscopy (SERS), and a UV-vis Spectrscopy. Nanoscale size and shape of the new ginger/chitosan@CD were characterized using TEM. The ginger/chitosan@CD are spherical, monodisperse particles, as shown in Fig. 1A and B. These nanoparticles were found to have a limited diameter range of 1.5–4.3 nm, with an average particle diameter of 3 nm, according to the findings of a statistical analysis of particle dots. UV-vis spectroscopy was performed to study the structural characterization of ginger/chitosan@CD. UV-vis absorption spectrum of ginger/chitosan@CD is shown in Fig. 1C. Carbon based nanoparticles solution showed broad absorption band around wavelength 488 nm and low band spectra 325 nm.

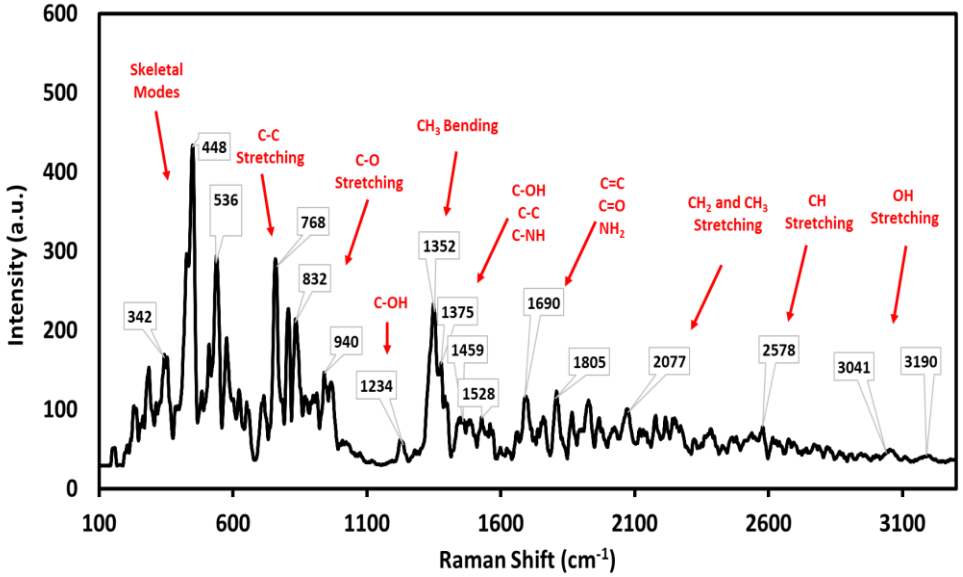

Figure 2. SERS Spectrum of Ginger/Chitosan@CD

SERS is a promising method for quick and sensitive chemical and biological analysis. Fig. 2 shows ginger/chitosan@CD SERS spectra. Raman spectroscopy is particularly useful for recognizing groups of rather big moleculesThe new band at 1690 1/cm shows that carbonyl groups react to generate imino groups in modified chitosan and ginger, which practically eliminates this band. Thus, the modified ginger/chitosan@CD SERS spectrum is dominated by chitosan and ginger SERS bands, with few bands (1234, 1352, 1528 1/cm) that easily correspond to free base bands. The main SERS peaks include the C-C stretching area (800-1200 cm^{-1}), the C-NH bending region (1234-1375 1/cm). Besides the CO stretching vibration (832 and 940 1/cm) and the CH wagging vibration (1375 1/cm), the other SERS bands in the spectrum are likely attributable to the CH in-plane ring deformation vibration (536 1/cm), the aliphatic chain C-C vibration (768 and 832 1/cm), the CH through vibrations (767 and 802 1/cm).

XTT assay

Under in vitro parameters, the vitality of human prostate cancer (PC-3 and LNCaP) and breast cancer (MCF-7) cells was evaluated following treatment with ginger/chitosan@CD. A time- and dose-dependent decline in the viability of cancer cells was found.

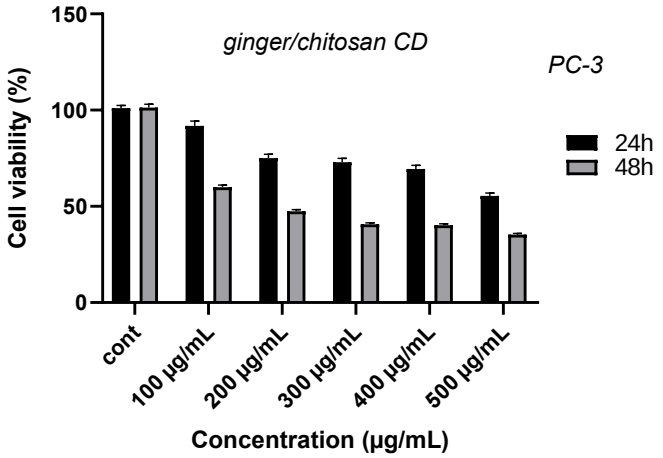

Figure 3. The Effects of Ginger/Chitosan@CD Treatment on PC-3 Prostate Cancer Cell Viability Were Determined Using the XTT Test at A Range of Doses and Time Points. The Data Represents the Average Findings of Three individual Studies. For PC3 Prostate Cancer Cells, the IC_{50} Dosage of Ginger/Chitosan CD Was Determined to Be 178.08 µg/mL After 48 Hours of Treatment

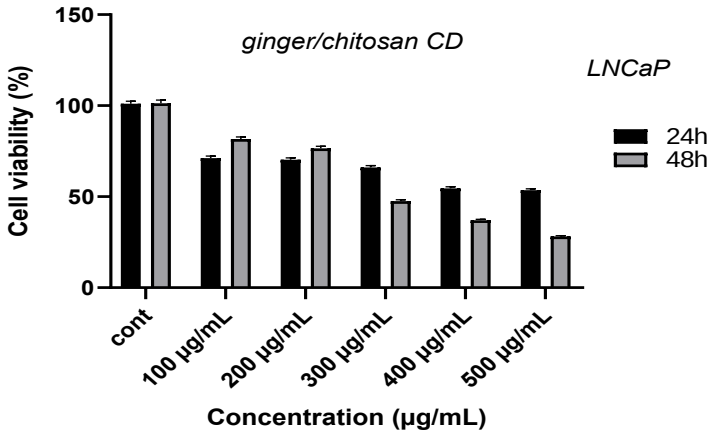

Figure 4. The XTT Test Was Used to Determine Whether or not LNCaP Prostate Cancer Cells Were Still Alive After Being Treated with Ginger/Chitosan@CD at Several Doses and Times. The Data Depicts the Average Values of Three İndividual Studies. LNCaP Prostate Cancer Cells Had an IC_{50} of 246.44 µg/mL for Ginger/Chitosan CD at 48 Hours

At 24 hours, the increasing dose of ginger/chitosan@CD reduced the cell viability of ginger/chitosan@CD-treated cells, while at 48 hours, the treatment had a greater impact. The 50% inhibitory concentration (IC50) of ginger/chitosan@CD was determined to be 178.08 µg/mL in the PC-3 cell line after 48 hours (Figure 3), 246.44 µg/mL in the LNCaP prostate cancer cells after 48 hours (Figure 4), and 345.74 µg/mL in the MCF-7 breast cancer cells after 48 hours (Figure 5).

All of these XTT data demonstrate to us that ginger/chitosan@CD reveals antiproliferative action in cancer cells depending on the dose and the amount of time that it is exposed to them. Even at the highest dose concentration (500 µg/mL), none of the three distinct cell lines had a reduction in cell proliferation of less than fifty percent within the first twenty-four hours. On the other hand, it was discovered that cell death occurred due to increasing dose concentrations dependent on the time in the 48-hour treatments. In breast cancer cell lines, higher concentrations of the compound were required to achieve the same level of antiproliferative activity that was observed in prostate cancer cell lines. It indicates that it performs different effects on cells that are derived from various sources.

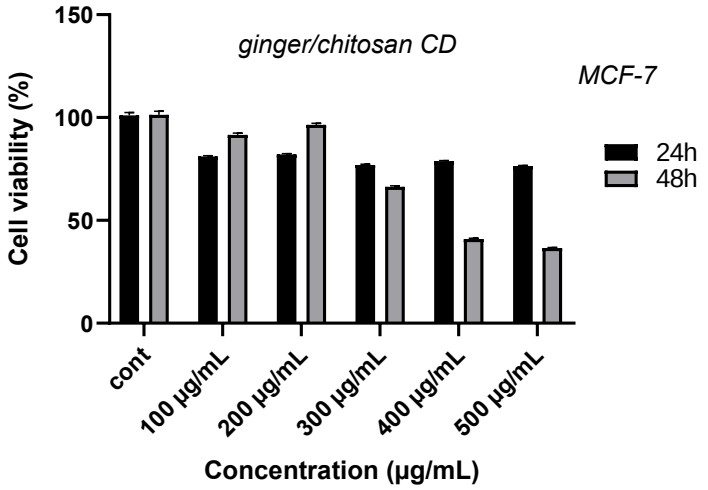

Figure 5. The XTT Test Was Utilized to Assess the Viability of MCF-7 Breast Cancer Cells After Treatment with Ginger/Chitosan@CD at Several Doses and Times. The Data Represent the Average Findings of Three İndividual Studies. At 48 Hours, the IC_{50} for Ginger/Chitosan@CD in MCF-7 Breast Cancer Cells was Determined to be 345.74 µg/mL

In our study, it has been shown that the ginger/chitosan@CD employed in our research inhibits cell growth in PC-3, LNCaP, and MCF-7 cells in vitro. Although cell proliferation declined, particularly in the first 24 hours, it did not fall below 50%; nevertheless, at the end of the 48th hour, cell proliferation had also reduced owing to the rising dosage. These findings show that our nanoparticle, which we designed specially, has antiproliferative effect in cancer cell lines based on dosage and duration.

There is no research regarding the pharmacological activity of this nano-sized substance that has been published in the scientific literature since it is a novel compound that has never been synthesized yet. On the other hand, research conducted on ginger and chitosan, both of which are components that are used in the production of the molecule, resulted in the discovery of the following results.

In literature, Carbon nanodots (CDs) are a relatively new kind of carbon nanomaterial that have inspired a significant amount of attention as a result of their many desirable characteristics, including as chemical stability, high water solubility, tuneable fluorescence qualities, cheap cost, low toxicity, strong biocompatibility, and environmental friendliness. In the study of Mohammadi et al. (Mohammadi, Mohammadi, & Salimi, 2021) reported to detect microRNA-21 in MCF-7 cancer cells, carbon dots synthesized from various aldehyde precursors were reacted with chitosan to create CDs chitosan nanocomposite hydrogels. The schiff base reaction, which included the formation of imine bonds between the amine in chitosan and the aldehyde groups on the surface of the CDs, was used to produce three luminous hydrogels. This is the most significant finding. The authors (H. Wang, Mukherjee, et al., 2017) of this study describe to achieve simultaneous near-infrared (NIR) imaging and NIR/pH dual-responsive drug release, chitosan CDs hybrid nanogels were developed by combining pH-sensitive chitosan with fluorescent CDs in a single nanostructure. These CCHNs were manufactured by forming chitosan-CD complexes into colloidal nanoparticles in the presence of EDTA compounds in an aqueous environment without the use of any solvents. Nano scaled CDs materials in the Ch networks may be immobilized by the selective cross-linking of chitosan chains in the nanoparticles. Colloidal stability, loading capacity for doxorubicin (DOX), brilliance and stability of fluorescence from the UV to NIR range, NIR photothermal conversion efficiency, and intelligent drug release in response to NIR light and change in pH are just a few of the many impressive features of these CCHNs. Human safety of CCHNs has been shown in both cell culture tests and examinations

of tissues from animal models. Doxorubicin (DOX)-loaded CCHNs may migrate to and distribute throughout tumors injected in animals, where they can block tumor growth by releasing the medication. Due to the effectiveness of NIR photothermal conversion of CCHNs, further photothermal therapies with NIR irradiation may further restrict tumor growth. The shown CCHNs show promising results toward a multifunctional intelligent nanoplatform for highly effective imaging-guided cancer treatment with little adverse effects. In the study by Li et al., (C.-L. Li et al., 2014), CDs (fluorescent carbon nanoparticles; 4.3 nm) isolated from tender ginger significantly inhibit the proliferation of human hepatocellular carcinoma (HepG2) while having little effects on MCF-10 and FL83B. HepG2 is the only kind of cancer cell for which this inhibition is specific; it does not affect the human lung cancer cell line (A549) or the human breast cancer cell line (MDA-MB-231) or the human cervical cancer cell line (HeLa) (HeLa). The expression of p53 protein is increased by the C-dots, but only in the HepG2 cell line, as shown by Western blotting. C-dots have an IC50 of 0.35 mg/mL when used to treat HepG2 cells.

CONCLUSION

In this pioneering work, the cytotoxic effect of ginger/chitosan CD, which was synthesized for the first time, was examined in cancer cell lines as a function of both dosage and time. The identification of novel pharmacological agents in cancer treatment processes and the development of successful treatments will be enhanced by research into the anticancer effects of natural substances acquired from species like plants and fungi with new formulations and by the clarification of their molecular effect mechanisms. The antiproliferative activity of our unique compound synthesized in this research was tested for the first-time in vitro cell culture conditions and cancer cell lines, and the first data were presented for furthermore comprehensive molecular biological studies. Experiments should be performed both in vitro and in vivo to get a comprehensive understanding of the pharmacotherapeutic potential of the compound that we have produced.

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