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Original Research Article

ANTICANCER ACTIVITIES OF CHITOSAN AND CHITOSAN NANOPARTICLES AGAINST SOME CANCEROUS CELL LINES.

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ABSTRACT

Cancer is considering a major leading cause of mortality. Nanotechnology holds a gold promise to develop cancer drug delivery and treatment. The present study aimed to compare in-vitro anticancer activities of chitosan (CS) and chitosan nanoparticles (CSN) against MCF7, PC3, HCT116 and A549 cell lines. The nanoparticles physic-chemical properties were estimated by Transmission Electron Microscopy (TEM) and Fourier Transform Infrared analysis (FTIR) through size and zeta potential analysis. In the current study, the results revealed that, the average diameter and zeta potential of CSN were 116.5 nm and 6.43 mV, respectively. Subsequently, CSN showed the anticancer activity with the LC50 values of 101.28 µg/mL in MCF7, 367.65 µg/mL in PC3, 666.67 µg/mL in HCT116 and 681.82 µg/mL in A549, whereas CS gave the LC50 values of 102.67 µg/mL in MCF7, 694.44 µg/mL in PC3, 1470.59 µg/mL in HCT116 and 769.23 µg/mL in A549. This study concluded that CSN were better than those obtained from CS as anticancer agent.

Abbreviations: CS (Chitosan), CSN (Chitosan nanoparticles, Transmission Electron Microscopy (TEM) and Fourier Transform Infrared analysis (FTIR).

Running title: *Tahany et al*: In-vitro Estimation of chitosan and chitosan nanoparticles as anticancer**Key Words**: Cancer cell lines; Characterization; Chitosan; Chitosan Nanoparticles.

INTRODUCTION

Worldwide, Cancer was classified as a main reason of mortality. Nowadays, strong approaches have been made to investigating of cancer biology to explore advanced diagnostic tolls and better treatment methods. The combined surgical resection and radiotherapy with chemotherapy is the most common strategy for cancer treatment, the lack

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of use the selective targeted therapeutic agents which affects on healthy tissues and organs is consider big challenge [1]. Many disadvantages of chemotherapy such as its harmful on healthy tissues, many bad side effects and not full efficient as the chemotherapy cant distinguished between cancer cells and normal ones [2]. Nanotechnology and gene therapy approaches were applicable in different research area, hold a gold promise and develop drug delivery in cancer medicine and clinical applications [3]. Nanoparticles (NPs) molecules can be defined as the particles with typically dimensions less than hundreds nanometers and about 2-4 folds less magnitude than normal human cells. Due to these special chemical and physical properties, NPs are considered a promising target to interact on surface and inside the cancer cells. In addition, NPs have great applications for targeted delivery for diagnosis, treatment of cancer and minimized the unwanted side effects of traditional drugs [4, 5, and 6]

Chitosan (CS) is a cationic linear polysaccharide polymer that contains randomly β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine and also abundant in nature [7]. Due to amine and hydroxyl functional groups in CS make it ideal for chemical reactions and several special properties. Besides, CS is safe, and can be interacted with polyanions to form gels and complexes [8, 9]. Unsurprisingly, the application of natural compounds in chemopreventive fields has attracted a lot of interests in chemotherapy and cancer treatment. CS (a naturally occurring polymer) has many advantages and priorities to be used in pharmaceutical and biomedical applications such as biocompatibility, biodegradability and lower toxicity for mammalian cells [10], many biological activities like antimicrobial and antibacterial activities[11], antioxidant activity [12], and anti-cancer effects by suppressing the tumor cell growth and proliferation [13], which inducing cell apoptosis [14], also activating and modulating the immune functions [15]. This study was aimed to estimate the anticancer activities of CSN against MCF 7, HCT 116, A549 and PC3 cancerous cell lines compare with those activities of CS.

MATERIALS AND METHODS

Chemicals and reagents

CS was obtained from Acros Organic Co. (B-2440 Geel, Belgium) with 170 kDa molecular weight, the degree of deacetylation = 82 ± 2%, water content = 6 % and ash contents = 0.8 %. Sodium hydroxide and glacial acetic acid were obtained from El-Nasr for Chemicals Co. The other reagents, buffers and chemicals were obtained from Sigma chemical Co. (London, Lab. Poole), England company (Cairo branch).

Chemical Preparation of CSN

Chitosan, CS powder was dissolved in glacial acetic acid at 4.6-4.8pH. To prepare chitosan nanoparticles, CNS, an aqueous triphosphate solution was added to CS solution with stirring. CNS was purified by centrifugation, rinsed with distilled water and freezing dry [16].

Physical properties of CSN

Photon correlation spectroscopy and laser Doppler anemometry, respectively, were used to determine CNS particle size and zeta potential (Central lab, Faculty of Pharmacy, Ain-shams University). Transmission electron microscopy (TEM, model JEM-2100, JEOL) at the central lab of National Research Center (NRC), Dokki, Giza, Egypt was used to examine the morphological properties of CS and CSN.

Fourier Transform Infrared

An anhydrous potassium bromide (KBr) was used to prepare CS and CSN ultra-fine powers. The Fourier-transform infrared (FTIR) spectroscopy analyzer (Model JASCO FTIR-6100) was used to detect the infrared spectra.

MTT assay to detect the cytotoxic effects of CN and CSN on human cancerous cell lines

Cells viability (MCF7, HCT116, A549 and PC3) were evaluated by MTT assay to purple formazan [17].

Cell culture

Cells were suspended in RPMI 1640 medium for MCF7 and HCT116 and DMEM for A549 and PC3 with supplemented antibiotics and fetal bovine serum and kept at 37 ºC under 5% CO2. (Sheldon, TC2323, Cornelius, OR, USA).

Cell Cytotoxicity Assay

Cell cytotoxicity assay was determined by MTT method according to protocol guidelines [17].

Data Analyses and statistics

Data were analyzed according to Snedecor and Cochran [18] and LSD (Least squared difference) test [19]. Values were expressed as Mean ± SE and $P<0.05$ considered significant.

Note: More details in materials and methods were mentioned in supplementary data file.

RESULTS

Characterization of CSN

The mean size and size distribution of CSN suspension was analyzed using the Zetasizer analysis as shown in Figure (1).

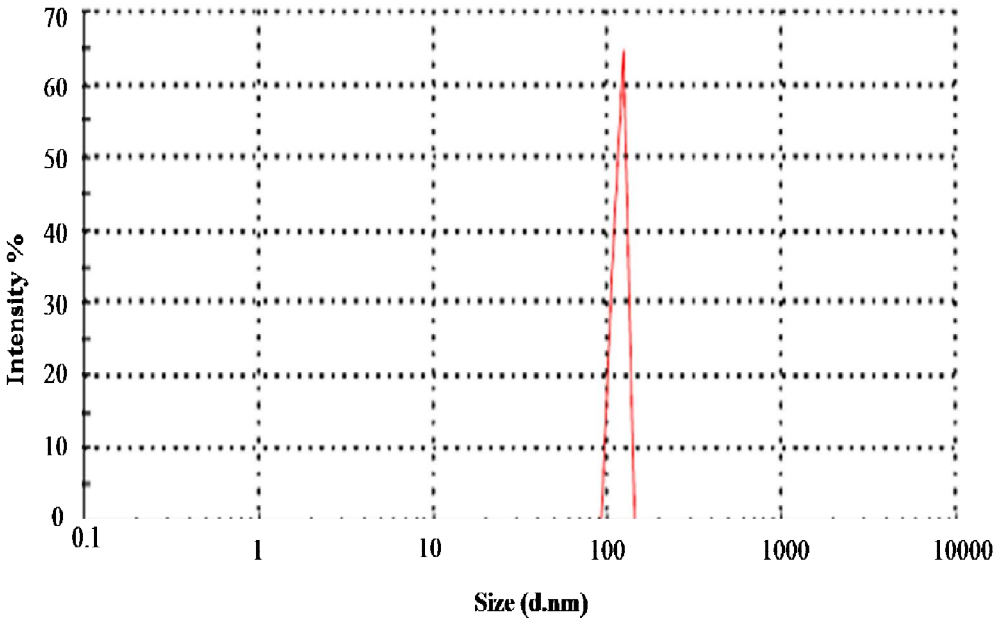

Figure 1. The size distribution by intensity of CSN.

The size distribution profile was shown in Figure (2), the mean diameter is 116.5 nm with polydispersity index <1 and indicated that CSN surfaces have a positive charge about 6.43

mV.

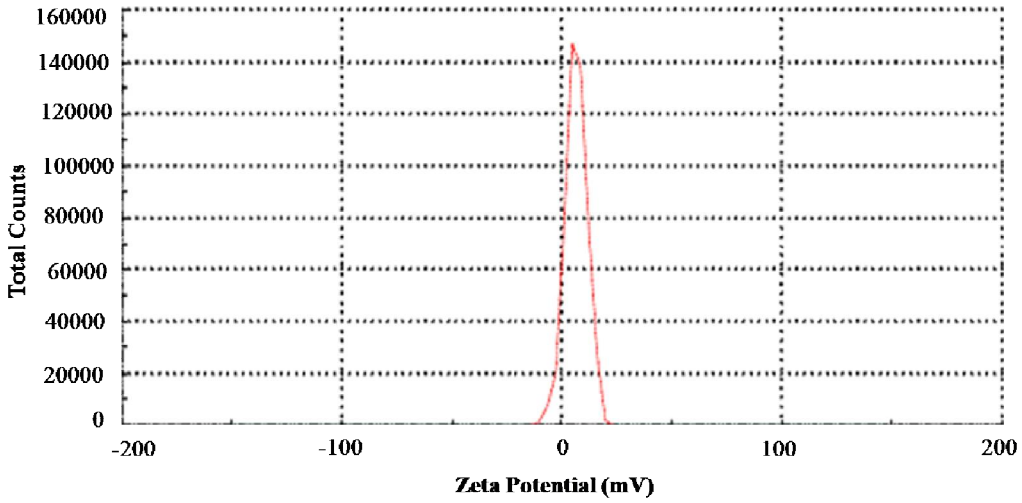

Figure 2. Zeta potential distribution of CSN

Transmission Electron Microscopy (TEM) of CSN

As shown in Figure (4),

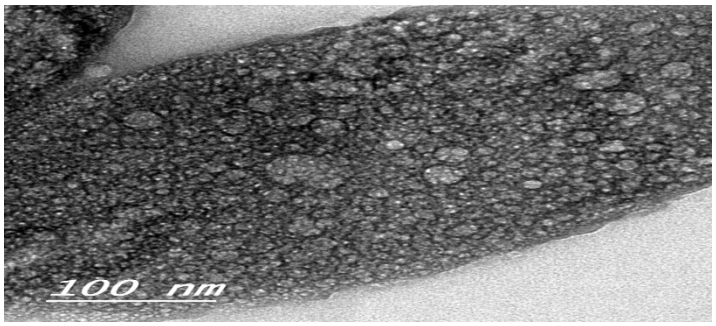

Figure 4. The transmission electron microscope micrographs of CS.

CS has a granular shape but CSN has a spherical shape with size ranged from 25-60 nm as shown in Figure (3).

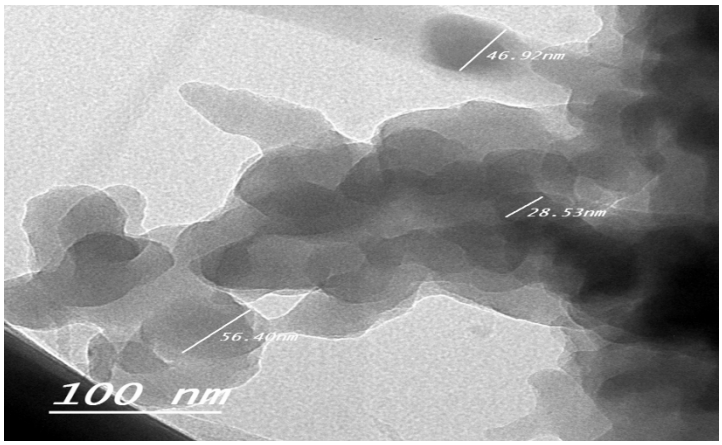

Figure 3. The transmission electron microscope micrographs of CSN.

These results confirmed the data of Zetasizer assay about the nanoscale of CSN.

Fourier Transform Infrared (FTIR) of CS and CSN

As shown in Figures (5 and 6).

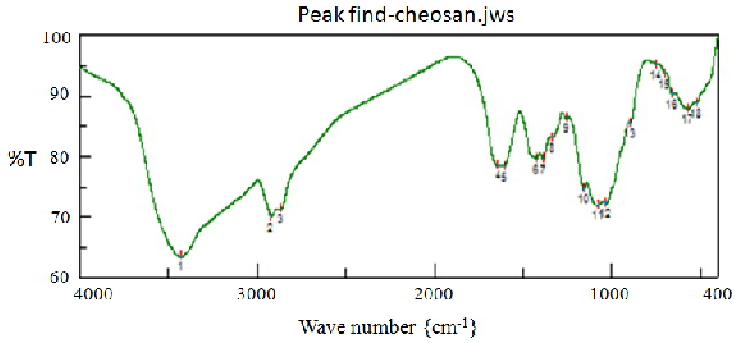

Figure 5. FTIR spectra of CS.

CS was appeared as one band at 3432 cm-1 which revealed the combined peaks of amino and hydroxyl group and another band at 1641 cm-1 was matched with CONH2 group. Otherwise, sharper CSN band at 3432 cm-1 which indicate enhanced hydrogen bonding.

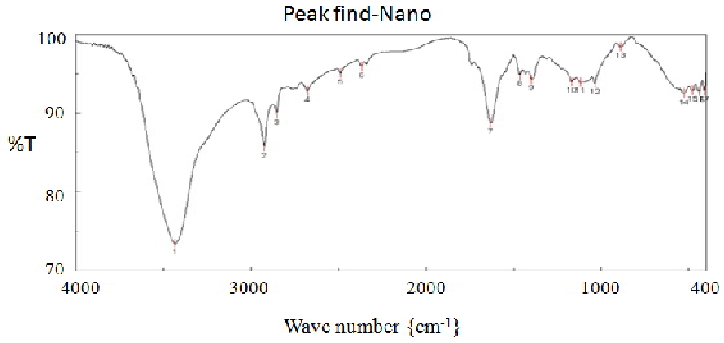

Figure 6. FTIR spectra of CSN

Cytotoxic effects of CN and CSN on human cancerous cell lines

The anticancer effect of CS and CSN was conducted in cultured MCF 7, HCT116, A549 and PC3 cell lines. LC50 values were evaluated by MTT assay and the results were shown that CS and CSN suppressed the cell growth and viability at (50 – 2000 µg/ml) for 48 hours. As shown in Table (1), CS and CSN increased the cell death. CS showed a potent cytotoxic effect with LC50 values (102.67 µg/ml, 694.44 µg/ml, 1470.59 µg/ml and769.23 µg/ml) in MCF7, PC3,HCT116 and A549 cell lines, respectively. On the other hand, CSN showed LC50 values (101.28 µg/ml, 367.65 µg/ml, 666.67 µg/ml and 681.82 µg/ml) in MCF7, PC3, HCT116 and A549 cell lines, respectively. LC50 values showed that CSN has a significantly higher anticancer activity more than CS against studied cell lines. In addition, Doxorubicin antiproliferative activity was found more effective against MCF7 (LC50 = 26.1 µg/ml), PC3 (LC50 = 23.8 µg/ml), HCT116 (LC50 = 37.6 µg/ml) and A549 (LC50 = 28.3 µg/ml) as shown in (Table 1).

DISCUSSION

As in our results, CSN was prepared by gelatin interaction between CS with positive charges

and tripolyphosphate with negative charges at RT [20], Wen et al. [21] reported that the size of CSN ranged from 63.16 to 101.70 nm and confirmed by another study [22] that reported the cross-linked CS with sodium tripolyphosphate (TPP) with (100–200 nm hydrodynamic diameters) for delivery of riboflavin. Qi et al. [23] prepared CSN with a mean diameter of 40 nm, but these results not matched with Zhang et al. [24] and Zhang et al. [25] who prepared CSN with different sizes ranged from 400–700 nm and 600–1000 nm in size, respectively. NPs with cationic coronas may have an easier time getting into cells [26]. Qi et al. [23] and Wen et al. [21] has reported that CSN surfaces are charged positively with intensities of 1641cm-1 and 1606 cm-1 for CONH2 and NH2 band, respectively, these can be shown obviously in pure CS, decrease dramatically, and two new sorption bands at 1631 and 1464 cm-1 added, which increased the NH2 groups cross-linking with tripolyphosphate molecules [20]. Otherwise, the enhancement of inter and intra molecular interaction in CSN was due to binding between the polyphosphoric groups of sodium polyphosphate and NH2 groups of CS [27]. CS showed a moderate anti-proliferative, cytotoxic and anti-tumor activity on cancerous cell lines [28, 29 and 30]. El-Awady et al [31] reported that doxorubicin has anti-proliferative effect on different cancer cell lines by cytotoxicity assay but, doxorubicin may be toxic for cardiac and myelosuppression which decreased the therapeutic effect [32].

Different mechanism of CS as anti-tumor through interfering with cell metabolism, suppress cellular growth and, or enhancing the cell death , activating the body's immune response and neutralizing effect due to selective targeted adsorption of positively charged CS [33, 34]. Moreover, CS has the ability to remove cancer cells by apoptosis effect and that explained the membrane change of phosphatidylserine during apoptosis [26] and enhancing apoptotic changes in a human bladder tumor cell line [35]. It was postulated that, CS has a cell killing functions as CS-treated cancer cells revealed DNA fragmentation and chromatin condensation, which are considered a diagnostic markers for apoptotic cells that confirmed by inter-nucleosomal DNA cleavage on agarose gel electrophoresis

In addition, CSN has smart properties to bind with higher affinity to negative charge functions at the biological membranes that leads to site-specific targeting in-vivo. Moreover, CSN also has dose-dependent suppressing effects on the growth of various cancer cell lines and low toxic against normal hepatocytes [36]. Also, the unique features of CSN exhibit more superior activities than CS (Table 1). Qi and Xu reported that CSN has antitumor efficacy against Sarcoma-180 subcutaneous tumor in ICR mice [37].

A hot spot could explained the high anticancer efficacy of CSN such as enhancing cell death and inhibiting the growth, that has been understood that CSN works with different levels to induce MCF 7, HCT116, A549 and PC3 cell death, involving destruction the cell membrane, decreasing the charge negativity and mitochondrial membrane potency, including oxidative stress and peroxides production from cellular lipids membranes and DNA fragmentation [38]. All these concepts could be introducing CSN as a novel drug for cancer therapy [39]. Other in-vitro studies have reported that, CSN hasn't killing effect on normal healthy cells, it clearly inhibit cell viability and enhance DNA fragmentation in-vitro, that's mean it has a potent standard and targeted cytotoxic effects on cancer cells, activating the immune functions and antimicrobial activity of CS [23, 40].

In Conclusion, the present study showed that CS and CSN may be potentially used as anticancer against MCF 7, HCT116, A549 and PC3 cell lines. The overall results obtained from CSN were better than those obtained from CS. In addition, CS and CSN are more effective on MCF 7 cell line than the other cell lines. The reported anticancer activities of CS and CSN could be having a powerful approach for next studies to investigate their natural

antimicrobial and antiviral activities. However, more studies in-vivo should be carried out to evaluate and underatsnd the mechanism of anticancer functions of CS and CSN and to determine the detailed metabolic pathways involved in their degradation.

Authors' contributions

The authors have equally contribution in this study

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