Article

Study Effects of Nano Chitosan-Paracetemol to Inhibition Spread of Colon Cancer

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Abstract

One of the gastrointestinal system's most frequent malignant maximums is colon cancer. Currently, surgical resection is the main course of treatment. Nevertheless, colon cancer is prone to metastasis and recurrence, making the development of an efficient anti-cancer medication imperative.

The present investigation employed FT-IR and ^{1}HNMR technique's to characterization of the synthesis and examination of nano Chitosan-Paracetemol composites. The cytotoxic potential of the new medicine (Nano Chitosan-Paracetemol composites) was evaluated, which had excellent results in our study, the number of infected cells decreased to (IC50=71.26 µg/ml). This work aimed to validate the cytotoxicity of nano Chitosan-Paracetemol on the colon cancer cell line HCT-29 as well as the efficacy of its reduction of metastasis of colon cancer.

Keyword: Colon cancer, Cytotoxicity, Nano Chitosan, Paracetemol, HCT-29,

1) Introduction

An important factor in millions of deaths worldwide each year is cancer[1]. Malignant tumors can be treated medically, however this is insufficient. Therefore, the main area of great concern is the discovery of new agents with appropriate treatments[2]. The optimization of antitumor medications with reduced toxicity necessitates targeting various aspects of anticancer treatments, such as tumor-specific cell signaling, drug resistance, energy metabolism, and gene expression. Chemical therapies are thought to primarily include heterocyclic chemicals. Once the desired

heterocyclic molecules have been synthesized, it is advised to use as few chemicals as feasible. For the synthesis reactions, it is preferable to use natural sources of biocatalysts[3, 4]. Given the advantages that using nanoparticulated drug delivery systems has over traditional drug administration, the use of nanomedicines for administration in cancer treatment has become increasingly important [5, 6]. Metallic nanoparticles, which are composed of nanoparticles, play roles in biology and medicine[7]. They include anticancer action, targeted gene delivery, and medication[8]. They offer numerous methods for regulating the encapsulated moieties' release profile. Numerous techniques, such as chemical reduction, laser ablation, microwave assistance, electro deposition, solvothermal, and green synthesis, can be used to synthesis NPs.

Chitosan is one of the natural polymers that have an intrinsic bioactivity advantage over its synthetic competitors. Because of cellular enzymatic action, they are able to have receptor binding ligands that bind to cells that are more susceptible to degradation[9, 10]. Chitosan is present in yeast, parasite cell walls, and the exoskeletons of arthropods like insects, crabs, and prawns. Chitosan has a large molecular weight, is pH-dependent, non-toxic, antibacterial, and readily bio absorbed. It is also biocompatible[11, 12].

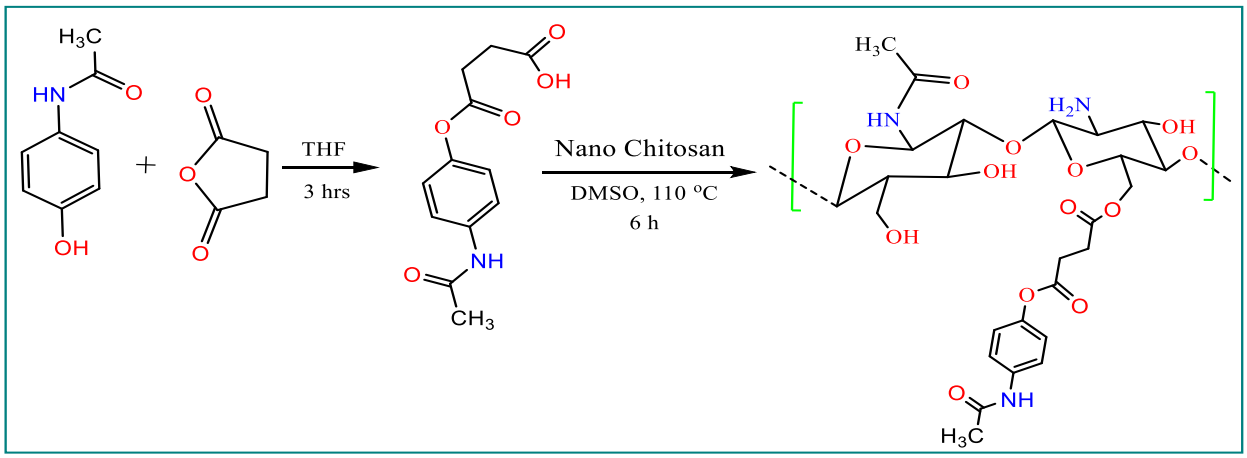
The remarkable quantity of research has been made possible by these polymers' biocompatibility and biodegradability, as well as their simplicity of chemical modification and blending[13]. When it came to delivering anticancer chemotherapy drugs to the intended tumor cells, chitosan demonstrated encouraging outcomes. Therapeutic-loaded nano-chitosan seems to have increased stability, permeability, and bioactivity[14]. Making natural nano Chitosan polymers using environmentally acceptable processes in order to use them as targeted anticancer medicines is the primary goal of this effort, where using neosynthesized nano Chitosan-Paracetemol to aid in this application is the main goal of this strategy.

2) Materials and methods

All the chemicals used were from analytical grade, Note that nano-chitosan is supplied by the company (Shaanxi Sangherb Bio-Techlnc) / China, the size of the particles was equal to ≤ 80 nm**, as in Appendix 1**

2.1) Preparation of Nano Chitosan-Paracetemol Composites [15]

Paracetemol (0.03 moles, 4.53 gm) was dissolved in (30 ml) THF with (1.0 mol.) of succinic anhydride, stirring and heating for 3 hours. Then the precipitate was filtered, and then the precipitate was dissolved in the solvent (DMSO) with the addition of drops of concentrated hydrochloric acid, and then it was added. To the nano-chitosan (0.000012 moles, 5.0 gm) and then we conducted the heating process at 110°C (with continuous stirring for 6.0 hours, and finally the precipitate was washed with a solution consisting of a ratio of (2:2) diethyl ether and (2.0 M) sodium hydroxide. Then leave it to dry for 16 hours, as in the following scheme:



Scheme (1) Synthesis of Nano Chitosan-Paracetemol Composite Drug

2.2) Biological activity [16]

Upkeep of cell culture: the HCT-29 cell line is maintained in RPMI-1640 supplemented with 10% bovine fetal, 100 units /mL penicillin, and 100 µg/mL strepto-mycin. Every single cell was passed through trypsin-EDTA, after which it was reseeded at 80% for duration of two weeks and kept at 37C° for incubation.

Cytotoxicity Assays: A 96-well plates were used to examine the MTT assay of the synthesized nanochitosan-paracetamol combination the HCT-29 cell lines was seeded in 1×10^4 cells/well for duration of 24 h. Following 72 h of medium removal and 28 µL of 2 mg / mL MTT solution addition, the cells were incubate for 2.5 hrs at 37C° to determine the viability of cells. To dissolve the remaining crystals on the wells, 130 µL of DMSO were added and incubation was left at 37°C for 15 min while shaking. A microplate reader operating at a constant 492 nm wavelength was used to measure the absorbance. Using equation, the inhibition for the growth rate was computed.

$$\text{Inhibition rate} = \frac{A-B}{A} \times 100$$

3) Result and discussion

3.1) Characterization of Nano Chitosan-Paracetemol Composite

Results of the FT-IR spectrum of Nano Chitosan-Paracetemol composite, shown in Figure (1), the presence of the hydroxyl group (OH) and the amine group (N-H) are joined to form an absorption peak at (3323.35 cm^{-1}), and the band (3030.17 cm^{-1}) represents the aromatic bond (C-H). In addition, the spectrum revealed a band Absorption at (2829.87 cm^{-1}), which represents the aliphatic (C-H) bond. In addition, the spectrum revealed an absorption band at (1714.72 cm^{-1}), which represents the ester group (O–C= O), and the absorption peak at (1676.14 cm^{-1}) represents the amide group (N–C=O), while the (C-O) peak appeared at (1271.09 cm^{-1}). On the other hand, the ^{1}H-NMR spectra, Figure (2) for the same displays a group of signals at (9.5 ppm), and we notice a signal representing the amide group in the nano chitosan regarding the two signals that appeared at (8.72 and 8.74 ppm), they represent the two amide groups present in the drug. As for the aromatic ring, a group of signals appeared (7.4-6.8 ppm) belonging to the hydrogen proton. Furthermore, a signal that indicated the hydroxyl group's existence in the chitosan nano polymer was detected at (4.45 ppm), the ^{1}H NMR spectrum also show a signal at (2.5 ppm), which indicates the presence of the solvent (DMSO-d^{6}), while the signal that appeared at (1.8 ppm) represents the C-H protons of the methylene group in the atoms. Finally, the ^{1}H NMR spectrum show a signal at (1.01 ppm) representing the methyl group in the chitosan nano polymer.

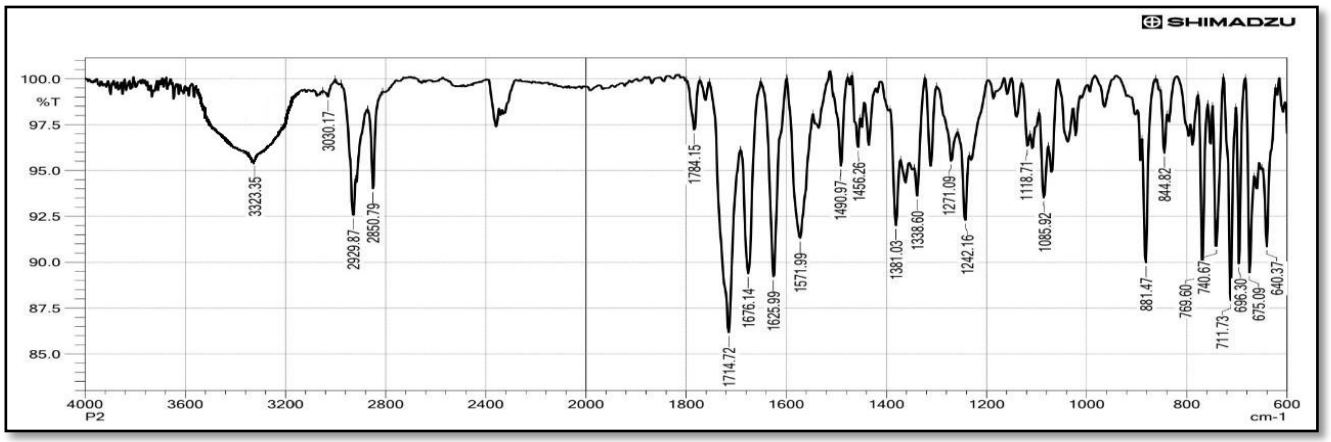


Figure (1) FT-IR spectra of nano Chitosan-Paracetemol composite

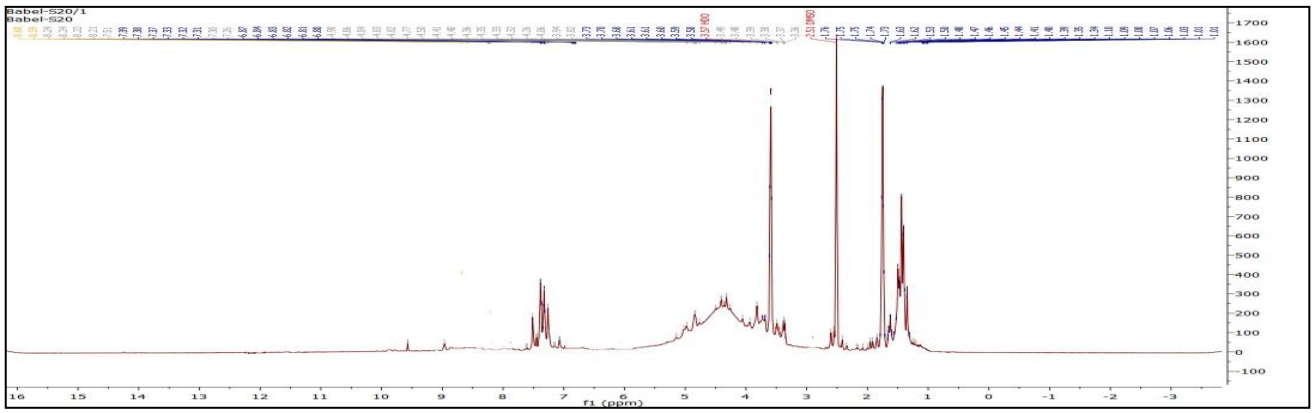


Figure (2) ^{1}HNMR spectra of nano Chitosan-Paracetemol composite

2.3) Biological activity

Study examined the cytotoxicity of the paracetamol-bound nano-chitosan medication on the colon cancer cell line HCT-29, both prior to and following exposure. The activities are expressed as the median growth inhibitory concentration (IC50), where the X axis represent the drugs concentrations and the Y axis represent the toxicity cells of cancer. According to the study, Paracetamol-Nano Chitosan possesses potent biological action that stops colon cancer cells from proliferating. The way that Nano Chitosan - Paracetamol interacts with colon cancer cells depends on concentration. Figure (3) presented the study's findings.

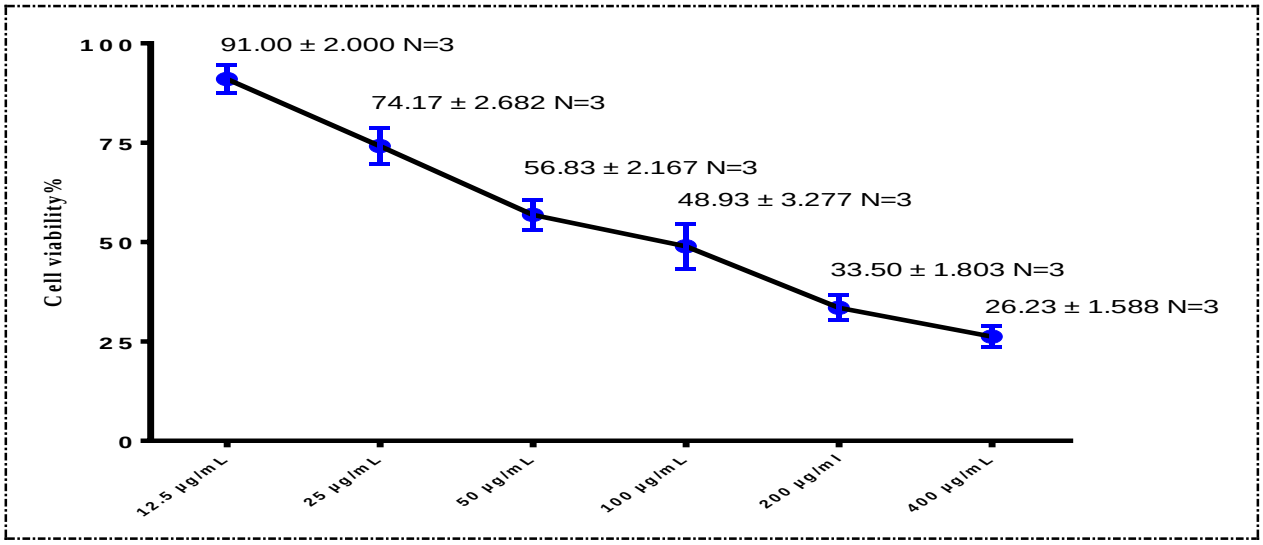


Figure (3) Cytotoxicity of Nanochitosan-Paracetamol in HCT-29 cells. IC50=71.26 µg/ml

4) Conclusion

Many techniques were used to study the generated nanoparticles drug composite, and were how well Paracetemol drug composite worked on cancer cell lines. Their concentration-dependence was shown by the evaluation of their cytotoxicity using in vitro anti-proliferative activity in this cancer cell line. The results demonstrated the strong biological activity of the Paracetemol linked with nano Chitosan composite, which stops colon cancer cells from growing. This study has brought attention to the necessity of using suitable selection of green organic chemicals and synthesis methodologies for the creation of nanoparticles for various applications.

References

[1] H. Sung et al., "Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries," CA: a cancer journal for clinicians, vol. 71, no. 3, pp. 209-249, 2021.

[2] M. R. Al-Zubaidi, H. T. Thwiny, and M. N. Al-Biati, "Modulation of chitosan nanoparticles properties for sheep pox mucosal vaccine delivery with cytotoxicity and release Studies-in vitro," Iraqi Journal of Veterinary Sciences, vol. 37, no. Supplement I-IV, pp. 111-119, 2023.

[3] A. H Abbas and M. N. Al-Baiati; 2023; Using of Nano-poly Chitosan Cephalexin Drug to Inhibit Spread Cancer Cells; International J.of Drug Delivery Technology; vol. 13(1), 29-32

[4] Zainab N. Jawad, K. I. Abd, B. K. AL-Ghanimi, Mohammad N Al-Baiati; 2023; Using A Novel Nano Chitosan-Ampicillin Drug to Study the Effective Range of Drug Level Outsidethe Affected Cells; HIV Nursing; vol.23(2); 953-956

[5] Khawla I. Abd, Aliaa H. Abbas, Ahmed S. Abed , Mohammad N. Al-Baiati and Emad S. Abood; 2022; Nano-Poly Chitosan-Ampicillin Drug: Synthesis, Characterization and Cytotoxicity, Egypt J. Chem. Vol. 65, No. SI:13, pp. 1313 - 1318

[6] C. Pacheco, F. Sousa, and B. Sarmento, "Chitosan-based nanomedicine for brain delivery: Where are we heading?," Reactive and Functional Polymers, vol. 146, p. 104430, 2020.

[7] F. Khademi, R.-A. Taheri, A. Y. Avarvand, H. Vaez, A. A. Momtazi-Borojeni, and S. Soleimanpour, "Are chitosan natural polymers suitable as adjuvant/delivery

system for anti-tuberculosis vaccines?," Microbial pathogenesis, vol. 121, pp. 218-223, 2018.

[8] N. D. Al-Jbour, M. D. Beg, J. Gimbun, and A. M. Alam, "An overview of chitosan nanofibers and their applications in the drug delivery process," Current drug delivery, vol. 16, no. 4, pp. 272-294, 2019.

[9] J. Sharifi-Rad et al., "Chitosan nanoparticles as a promising tool in nanomedicine with particular emphasis on oncological treatment," Cancer Cell International, vol. 21, no. 1, pp. 1-21, 2021.

[10] M. Fathi, S. Majidi, P. S. Zangabad, J. Barar, H. Erfan□Niya, and Y. Omidi, "Chitosan□based multifunctional nanomedicines and theranostics for targeted therapy of cancer," Medicinal research reviews, vol. 38, no. 6, pp. 2110-2136, 2018.

[11] A. W. Pan, B. B. Wu, and J. M. Wu, "Chitosan nanoparticles crosslinked by glycidoxypropyltrimethoxysilane for pH triggered release of protein," Chinese Chemical Letters, vol. 20, no. 1, pp. 79-83, 2009.

[12] K. Divya and M. Jisha, "Chitosan nanoparticles preparation and applications," Environmental chemistry letters, vol. 16, pp. 101-112, 2018.

[13] G. A. Martău, M. Mihai, and D. C. Vodnar, "The use of chitosan, alginate, and pectin in the biomedical and food sector—biocompatibility, bioadhesiveness, and biodegradability," Polymers, vol. 11, no. 11, p. 1837, 2019.

[14] N. Gull et al., "Designing of biocompatible and biodegradable chitosan based crosslinked hydrogel for in vitro release of encapsulated povidone-iodine: A clinical translation," International Journal of Biological Macromolecules, vol. 164, pp. 4370-4380, 2020.

[15] Aliaa H. Abbas, Mohammad N Al-Baiati; (2022); MSc. Thesis; Using Chitosan Nano Polymer Grafted with Some Drugs in Inhibiting Breast Cancer Type AMJ13; University of Kerbala, College of Education for Pure Sciences; pp. 54

[16] Z. M. A. Al-Aama, H. Q. AL-Masoudi, A. A. Abdulridha, K. I. Abd Nusaif, and M. N. AL-Baiati, "Synthesis of a smart nano graft co-polymer as a drug delivery," in AIP Conference Proceedings, 2023, vol. 2414, no. 1: AIP Publishing.

Appendix (1), Specifications of the nano Chitosan used

HAANXI SANGHERB BIO-TECHLNC

CERTIFICATE OF ANALYSIS

Product: Nano Chitosan **Batch No.: SH180314N**
Manufacturing Date: 20220314 **Quantitles: 200**

(Items)	(Specifications)	(Results)
(D.A.C) %	**≥ 95**	**95.7**
Viscosity (Cps)	**≤ 100**	**25**
Insoluble (%)	**≤ 1**	**0.10**
(Ash) %	**≤ 1**	**0.73**
Moisture (%)	**≤ 10**	**9.45**
Fineness (nm.)	**≤ 80 nm**	**(Pass)**
Heavy metals (ppm)	**≤ 10**	**< 1.0**
Arsenic (ppm)	**≤ 0.5**	**0.02**
Density (g/ml)	**0.28**	**0.32**
Appearance	**White Powder**	**(Pass)**

Analyst: WangZunhua **Verification: ZhangXuelin**