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# Morphological Change in Human Liver Cancer Cells HepG2 Induced by Ampicillin-Chitosan

Zahraa Ayad Ali[1*] , Zainab Nizar Jawad[2]

[1]Department of Biology, College of Education for Pure Sciences, University of Kerbala, Karbala, Iraq
[2]Department of Optical Techniques, Al-Zahrawi University College, Karbala, Iraq.

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*A B S T R A C T*

In this work, the effectiveness of the binding of ampicillin, one of the beta-lactam antibiotics, with nano-chitosan extracted from the skeletons of marine crustaceans was tested on human liver cancer cells Hep G2 ex vivo. The results were positive, as the binding was effective through the weakening of the vibration peaks of the active functional groups, according to the results of FTIR test, and the drug was toxic to the aforementioned cell lines and its effect increases with the dose, causing them to lose the integrity of their membranes, shrinkage of their cytoplasm, and fragmentation of their DNA, which indicates the activation of the programmed death pathway in them. This confirmed the synergistic role of chitosan in enhancing the therapeutic effectiveness of Ampicillin.

## 1. INTRODUCTION

Cancer remains an enormous challenge and one of the top causes of mortality in the entire world.It arises from a defect in natural biological processes that manage cell division, causing cells to grow up normally, which produces masses of tissue known as cancerous tumors [1,2] . Hepatocellular carcinoma (HCC) ranks third among cancer-related causes of mortality globally. The majority of cases involve people who already have liver disease, specifically fibrosis or a fatty liver, plus infection with hepatitis B and C virus [3]. Cancer has been cured by a variety of antibiotics, the phrase "antibiotics" is Known as bacterial-producing secondary metabolism products that have anticancer properties. They block the unchecked growth and destroy cancerous cells in all stages of the reproductive cycle including those in the (G0) phase by activating programmed death through apoptotic genes like TP53 gene [4]. As a beta-lactam antibiotic, ampicillin has effects on the cell wall by preventing the formation of peptidoglycan, which is crucial for cell wall synthesis. It also weakens the cell wall and causes cell lysis by preventing transpeptidase enzymes from cross-linking peptidoglycan chains, which causes lysis and cell death [5]. Antibiotic overuse increases bacteria resistance to many drugs, creating severe danger. As a result, it is essential to minimize antibiotic dosages while increasing ANTIBIOTIC bioavailability. It can be complexed with polymer by direct linkage or encapsulation to improve biocompatibility, stability, degradability, and other properties. Antimicrobial monomers like chitosan serve to enhance the effectiveness of ampicillin through covalent bonds [6]. Chitosan is a synthetic polymer that lies under the polysaccharides group. It is created via deacetylating chitin contained in the exoskeletons of various crustaceans. its exceptional characteristics include non-toxicity and biocompatibility. Chitosan-metal bio nanocomposites have been used in drug delivery applications [7]. One extremely interesting treatment option for fighting tumor cells is by restoring TP53 to its function, which can be activated in response to various stresses such as damage to DNA, oxidative overload, deficiency in nutrients, hypoxia, telomere attrition, or cellular dysfunction via transcription regulation. Additionally, p53 can either promote or block the expression of numerous particular genes that are responsible for halting the cell cycle, repairing DNA, apoptotic, and suicide, That is how the keeper of the genome determines cell fate [8].

*Corresponding Author Institutional Email:
Zahraa.ayad@uokerbala.edu.iq (Zahraa Ayad Ali)

**Objctive Of The Study**

The aim of the research is to load the antibiotic ampicillin onto nano-chitosan in order to improve its therapeutic properties and then target HepG2 liver cancer cells ex vivo to measure the toxicity of the drug and the extent of its effect on the HepG2 liver cell line.

## 2. MATERIALS AND METHODS

### 2.1. Preparation NanoChitosan

a specific amount of the powdery chitin (Shaanxi Sang herb Bio-Techlnc) disintegrated in fifteen milliliters of water solution including four percent of CH3CO2H at forty-five degrees Celsius.The chitosan then gradually disintegrated until the liquid turned clear by using high-frequency sound waves for six hours and then left at room heat all-nighter to create chitosan nanocrystals and dry out using filter paper [9].

### 2.2 Preparation of Nano Chitosan-Ampicillin

After dissolving (9.2 g, 0.03 moles) ampicillin medication obtained from (SDI Company/Samarra) into thirty milliliters of tetrahydrofuran (Thomas Baker) and a total of 3 drops hydrochloric acid, the medication is added to the nano chitosan (5.0 g, 0.0005 moles) and placed under reflex over one full day. Lastly, a mixture of diethyl ether and 2.0 M sodium hydroxide was used to wash the debris, and it was allowed to dry for sixteen hours [10].

### 2. 3. Maintenance cell culture

The HepG2 cells of liver tumors was cultured in cell culture plates obtained from Santa Cruz Biotechnology in the USA. The cells were kept in 10 milliliters of Roswell Park Memorial Institute -1640 supplemented with 10% Fetal bovine serum, 100 units/mL penicillin, and 100 µg/mL streptomycin ( Capricorn / Germany ) The cells were cultured in a CO2 incubator (Cypress Diagnostics/Belgium) at 37 °C with 5% CO2 for 24 hours after being passaged using Trypsin-EDTA prepared from (Capricorn / Germany). The cells were then checked under a microscope to confirm their viability. Subsequently, albumin serum was added to halt the trypsin's action. After that, cancer cells (HepG2) were split using a centrifugal technique (200 revolutions per minute) and albumin serum was added after removing the filtrate,[11].

### 2. 4. Cytotoxicity Test

The purpose of the test was to gauge the malignancy cells' survival. To preserve the tetrazolium dye MTT ( Invitrogen / USA ) , until usage, 50 mg of its powder had to be dissolved in a hundred ml of phosphate buffer solution. HepG2 cells were grown in a plate with 96 wells and incubated to obtain a single layer of cells. The aforementioned cells were then treated with ampicillin, an antibiotic, loaded on nano chitosan at the following concentrations: ( 12.5 , 25 , 50 , 100 , 200 , 400 g/ml ) . after 48 hours passed, the cells were washed and treated with MTT dye. After using dimethyl sulfoxide to remove the crystals, it was briefly incubated before being checked using a microplate reader, [12]. The tumor cell inhibition rate was calculated according to the equation below(1):

Percentage of inhibition = (optical density of the control sample -optical density of the studied sample )/(optical density of the control sample) X 100

### 2.5. Detection of Morphological Change

To examine the morphological change with an inverted microscope ( Olympus / Japan ), human liver cancer ( HepG2 ) cells were placed into 24-well micro-titration plates and kept for 24 hours at 37 °C. Next, for a whole day, cells were exposed to nano chitosan-ampicillin. Following the treatment period, the plates were incubated for between ten and fifteen minutes at thirty-seven degrees Celsius after being colored with a violet crystal dye (Switzerland / Fluka), [13]. The stain was carefully cleaned using ordinary water till all traces of the pigment were gone. At a zoom level of 100×, HepG2 cells were studied with an inverted microscope, and by a digital camera, images were taken. Every analysis is done in triplicate.

## 3. RESULTS AND DISCUSSION

### 3.1 FTIR analysis

The FTIR technique had been used to determine the bonding of antibiotic ampicillin with polysaccharide. Functional groups including the secondary amine (N-H) (3135.78 cm-1), alcoholic (O-H) (3399.65cm-1), aromatic (3080.32cm-1), and aliphatic (C-H) (2976.16cm-1), ester group (1726.29cm-1), amide group (1681.93cm-1), and (C-O) band (1263.37cm-1), are responsible for the vibrations seen in figure (1). This indicated the efficiency of cross-linked bonds, Among the considerations that contributed to the selection of chitosan for loading with drugs were the existence of a functional amino group, a large surface area linked to the c=c group, and hydroxyl and carboxyl groups at the surface or border of the chitosan. These properties offer opportunities for medicine loading via hydrogen-bonded and electric interactions,[14].

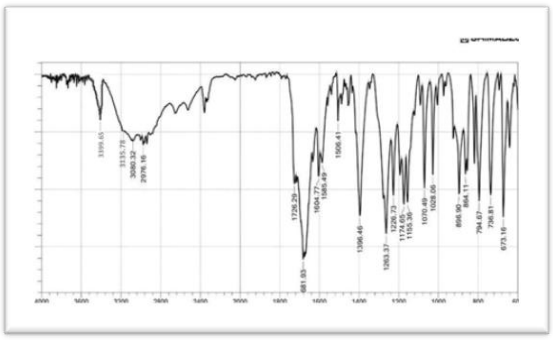

**Figure 1.** FTIR for Chitosan- ampicillin

### 3.2. Cytotoxicity Test

The results of the test are shown in (Figure2) , where a decrease in the number of cells can be observed coinciding with an increase in the dose and based on the basic principle of the work of the MTT dye.The mitochondria of healthy cells have the ability to convert this yellow-colored dye into purple, water-insoluble crystals, the percentage of which is determined. Color intensity of healthy cells is measured and this is what cells, that have lost the integrity of their membranes, cannot do.This confirms the damage to the membranes of cancerous liver cells which is due to the effect of the ampicillin-chitosan drug [15,16] .

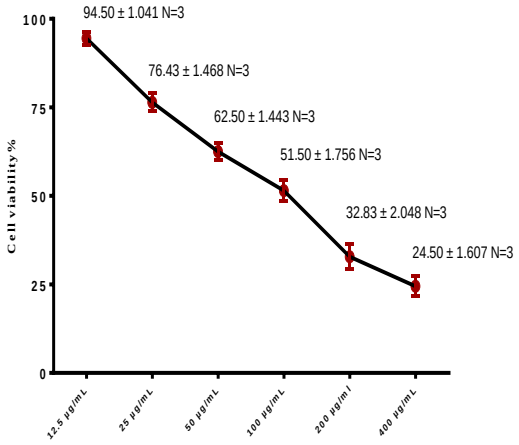


**Figure 2.** Cytotoxicity of Chitosan-Ampicillin in HepG2 cells. IC50=69.09 µg/ml

### 3.3. Morphological Change in HepG2 cells

The HepG2 cells were subjected to the half-lethal dose for over a day before being painted with crystal violet dye and examined with a 100 x magnification microscope. Morphological variations in the liver malignant cells treated with ampicillin are loaded on chitosan as Figure (4) shows.This is in contrast to the changes seen in Figure (3) of non-drug exposed cancer cells.

Regarding the untreated cells' membrane integrity, there is a noticeable difference. The cell's cytoplasm has been stained violet in color and its nucleus looks tiny and transparent. They resemble small cell aggregates with dull-colored cytoplasm, as opposed to the cancer cells that were treated with the medication. Furthermore, there seems to be a lack of unique borders on the outermost layer of the cell's membrane , Which shows the effect of nano drug on them. The principles behind the usage of antibiotic as antitumor therapy are based on their capacity to suppress cell division in addition to its pro-apoptotic and anti-epithelial-to-mesenchymal transition actions. A variety of antibiotics have found application in the treatment of cancer. Research studies have validated our findings, showing that ampicillin inhibits the growth of blood vessels in cancer cells, hence having an anti-cancer impact [17].

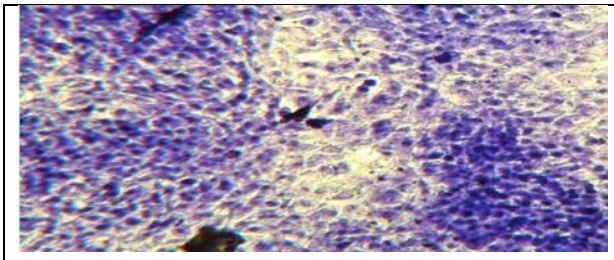

**Figure 3 .** Non-drug exposed cancer cells HepG2 cells

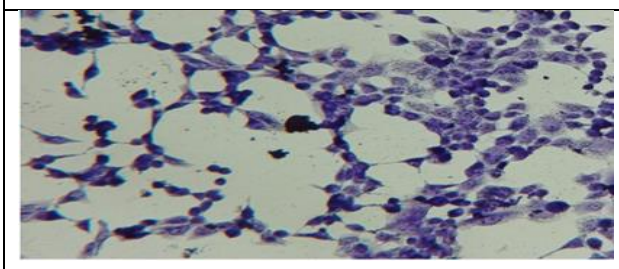

**Figure 4.** HepG2 cells that exposed to Chitosan-Ampicillin

### 4. CONCLUSION

After treating HepG2 liver cancer cells with the antibiotic ampicillin loaded on nanochitosan and measuring its toxic effects and the changes it caused on the aforementioned cell line, we concluded that the drug led to the killing of cancer cells and caused the activation of p53, which stimulated the programmed death pathway in them.This in turn opens promising doors for the treatment of liver cancer.

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## Arabic Abstract


في هذا العمل تم اختبار فعالية ارتباط الامبسلين احد مضادات بيتا لاكتام الحيويه مع الكيتوسان النانوي المستخلص من هياكل القشريات البحريه على خلايا سرطان الكبد البشري خارج الجسم الحي اما النتائج كانت ايجابيه حيث كان الربط فعال من خلال ضهور قمم اهتزاز المجاميع الوظيفية الفعاله حسب نتائج اختبار *FTIR*وكان العقار ذو سميه على خط الخلايا المذكورة وبتأثير متزايد مع الجرعه فتسبب في فقدانها سلامة اغشيتها وانكماش السيتوبلازم وتجزء حمضها النووي مما يشير إلى تفعيل مسار الموت المبرمج فيها. مما أكد الدور الفعال للكيتوسان في تعزيز فعالية الامبسلين العلاجية.