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Study of the Cytotoxic Effect of Nicotine Alkaloid Extracted from Nicotiana tabacum L. on Some Cancer Cell Lines

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Abstract

Cancer is a major health problem and is a cause of death in the world. There are many approaches to its treatment, but they have many side effects. Recently, the researchers turned to find new methods for treatment by using natural sources such as plants that contain compounds that have anticancer effects. The current study aimed to determine the presence of Nicotine alkaloids in the extract of Nicotiana tabacum L. and study its cytotoxic effect on MCF7 (Michigan Cancer Foundation-7), and Hela (Henrietta lacks) human cancer cell lines. Dragendroff's reagent and Gas Chromatography/ Mass Spectroscopy method is used to detect and measure the level of Nicotine. The cancer cell lines were treated with different concentrations of the extract. The results showed the effect of the extract on the growth of MCF7 that the lower rate of inhibition was 20.07% at (50 µg/ml), and the higher rate of inhibition was 96.67% at (800 µg/ml), but the inhibition rate of the extract on Hela was 34.87% at (50µg\ml), and the rate increased to 90.31% at (800 µg\ml).

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The study concluded that the Nicotine alkaloid extract of *Nicotiana tabacum L.* inhibits the growth of cancer cell lines making this plant a promising candidate for cancer treatment, but more research is needed to prove it.

Keyword: Gas Chromatography; Hela cancer cell line; MCF7 cancer cell line; *Nicotiana tabacum L.*; Nicotine alkaloid.

دراسة التأثير السمي للنيكوتين المستخلص من نبات التبغ في بعض خطوط الخلايا السرطانية

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الخلاصة

يعتبر السرطان مشكلة صحية كبيرة وأحد أسباب الوفاة في العالم. هناك العديد من الطرق لعلاجه، ولكن لها العديد من الآثار الجانبية. تحول الباحثون في الآونة الأخيرة إلى إيجاد طرق جديدة لعلاج السرطان باستخدام المصادر الطبيعية مثل النباتات حيث تحتوي على مركبات لها تأثيرات مضادة للسرطان. هدفت الدراسة الحالية إلى تحديد وجود قلويد النيكوتين في مستخلص نبات التبغ *Nicotiana tabacum L.* ودراسة تأثيرها السام للخلايا في خطوط الخلايا السرطانية البشرية وهي خط سرطان الثدي (MCF7)، وخط سرطان عنق الرحم (Hela).تم استخدام كاشف دراجيندروف وطريقة التحليل اللوني للغاز / التحليل الطيفي الكتلي لاكتشاف وقياس مستوى النيكوتين في المستخلص. تم علاج خطوط الخلايا السرطانية بتركيزات مختلفة من المستخلص. أظهرت النتائج تأثير المستخلص على نمو(MCF7) بأن أقل معدل تثبيط كان 20.07٪ عند (50 ميكروجرام / مل)، وأعلى معدل تثبيط كان 96.67٪ عند (800 ميكروجرام). / مل) لكن نسبة تثبيط المستخلص لنمو ((Hela كانت 34.87٪ عند (50 ميكروجرام / مل) وارتفع معدل التثبيط إلى 90.31٪ عند (800 ميكروجرام / مل). اوضحت الدراسة بأن مستخلص قلويد النيكوتين لنبات التبغ *Nicotiana tabacum L.* يثبط نمو خطوط الخلايا السرطانية، مما يجعل هذا النبات مرشحًا واعدًا لعلاج السرطان، ولكن هناك حاجة إلى مزيد من البحث لإثبات ذلك.

الكلمات المفتاحية: كروماتوغرافيا الغاز، الخط الخلوي Hela، الخط الخلوي MCF7، نبات التبغ، قلويد النيكوتين.

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Introduction

Cancer remains one of the major health problems and one of the leading causes of death in the world [1]. According to WHO the number of people living with cancer reached 21.2 million people in 2021[2]. There are many approaches to the treatment of cancer such as surgery, chemotherapy, gene therapy, and immunotherapy [3], but they didn't achieve the required results and they have many side effects [4]. In recent studies, the researchers turned to find new methods of treatment by using natural sources such as plants that contain many bioactive compounds [5] that have anti-inflammatory, antimicrobial, and anticancer effects [6]. More than 60% of drugs used in cancer treatment are taken from natural sources according to the Food and Drug Organization [7]. The therapeutic properties result from these plants, due to the presence of substances called secondary metabolites such as flavonoids, alkaloids, tannins, and steroids [8]. Recent studies have shown that the extract of some plants such as *Isatis tinctoria* and *Melia azedarach* contains alkaloids that have an inhibiting effect on the growth of some cancer cell lines [9][10].

Nicotiana tabacum L. belongs to the family of Solanaceae and is commonly named tobacco [11]. It grows up to 2.5 m with large green leaves and terminal white-pinkish flowers and is cultivated worldwide [12]. It is rich in terpenoids, alkaloids, sterols, phenolic compounds [13], nicotine, nornicotine, anabasine, nitrate, and sorbitol [14]. It is widely used in the treatment of human infections such as cough, snake bite, ulcers, and respiratory tract infections [15]. The α-CBD isolated from tobacco can reduce the formation of cell clones and inhibits the proliferation of hepatocellular carcinoma cells in the S phase of mitotic division, change cell shape, and change the permeability of membranes [16]. The flavonoid isolated from tobacco leaves has cytotoxic activity on (MCF-7)cells and can be a potential agent for human breast cancer therapy [17].

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Materials and Methods

Extraction of Nicotine alkaloid

The extraction was done according to the method [18], by taking 10 grams of leaves of the plant and placing it in a thimble, is a cylindrical container, and then placing it in the designated place called Soxhlet, adding 500 ml of methanol 70% to it and the extraction was carried out for 5 hours at 50-60°C. Pour the extract into Petri dishes and concentrate for 24 hours at room temperature, and then filtrate with Whattman No. 1 filter paper. Then 100 ml of chloroform was mixed with 100 ml of the extract in the separating funnel, and then the pH was adjusted to [8] by adding a weak base and left for 24 hours. Two layers were obtained, the upper layer is the chloroform, which was disposed of, and the lower layer is the aqueous containing the alkaloid, which was mixed with petroleum ether in equal quantities and then filtered using a 0.22mm filter unit [19].

Detection of Nicotine alkaloid

The Nicotine alkaloids were detected by Dragendroff's reagent and gas chromatography/mass spectrometry (GC/MS) was used to identify the nicotine alkaloids. The Shimadzu apparatus (GCMS-QP2010Ultra) was used in the Ibn Al-Betar center, and it is obvious from its name that it is detrimental to two complementary processes. This method of analysis is used to check for the presence of alkaloids in plant extracts [20].

Cell Lines

In the current study, the breast cancer of human MCF7 cell line passage No. [50], and the human cervical cancer Hela cell line, which was utilized in passage No. [58].

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Effect of Nicotine alkaloid extract

1. Culturing and maintenance of cell lines

The cancer cell lines are grown according to the method [21]. The cells of each of the lines were placed in a culture plate with a diameter of 25 cm^2 containing RPMI-1640 culture medium and 10% Fetal Calf Serum (FCS). The plates containing the cell suspension and culture medium were incubated in a 5% CO_2 incubator at 37°C for 24 hours. After one day of incubation, the cells were examined by using an inverted microscope to ensure their viability, free from contaminants, and their growth. The cells were washed with PBS solution and then discarded, and the process was repeated twice. Then a trypsin-versin solution was added to the cells and incubated for 30-60 seconds at 37°C and then the enzyme was stopped by adding a new culture medium containing serum. The cells were collected in centrifugal tubes and placed in a centrifuge at 2000 rpm/min for 10 minutes at room temperature, to precipitate the cells. The filtrate was discarded and the cells were suspended in a fresh culture medium containing 10% serum. Then determine the number of cells by adding a specific volume of Trypan Blue stain with the same volume of the cell suspension and determine cells vitality by using a Hemacytometer slide.

2. Cytotoxic assay

The toxic effect of the extract of Nicotine alkaloid was tested in the cancer cell lines [Hela and MCF7) cell line by adding the solution of trypsin-versin to the plate of tissue culture (with a size of 25 cm^2). Then added (20 ml) of the RPMI-1640 to the Fetal Calf Serum (FCS) and mixed. Then (0.2 ml) of the mixture was added to every 96 wells in a plate of tissue culture by using a micro-pipette. The plates of tissue culture incubate for 24 hours at 37°C in an incubator until the cell adhesion to the well of the plate then removed from the medium from the wells, and added (0.2 ml) from each of (50, 100, 200, 400, 800) µg/ml concentrations of the extract

with three replicates for each one. The negative control was prepared by using three replicates of dimethyl sulfoxide (DMSO), while the positive control was prepared by adding three replicates of PBS supplemented with 0.2 ml of serum-free media and kept in the incubator for 24 hours at 37°C according to [21]. Then the plates were taken out of the incubator and disposed of the dead cells and old culture media from the holes, then added 100 µl of crystal-violet dye to each well, and then the plates were re-incubated for 20 minutes. Then the contents were removed and using PBS washed to remove the remnants of the dye, and then the cells were left to dry. A spectrophotometer was used to read the results at a wavelength of 492 nm. The rate of cell growth inhibition was determined by the following equation:

Percentage of inhibition rate (IR) % = (X - Y \ X) x 100

Since:

X = Reading (O.D) of control

Y = Reading (O.D) for each concentration treatment

Analytical statistics

The ANOVA test and the Graph Pad Prism Version 6 analytic tools were used to statistically examine the results. The Duncan Multiplex experiment was compared to the means, and significant differences were found at a probability level of $P \leq 0.05$ [22].

Results

Detection of Nicotine alkaloid

Using Dragendroff's reagent and the GC/MS technique to aid in chemical profiling and standardization of the extracts, we identified the chemicals responsible for the anticancer action

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of leaves of *N. tabacum* L. extracts in this work. The presence of alkaloids was detected by the emergence of a brown-orange blot after the extract was exposed to the reagent. The results of the GC/MS analysis revealed that the leaves contained a variety of bioactive secondary metabolites, including alkaloids, with the level of Nicotine alkaloid shown in peak 6 (Figure 1) accounting for 75% of the extract component and belonging to a compound with the fragmentation pattern shown in (Figure 2).

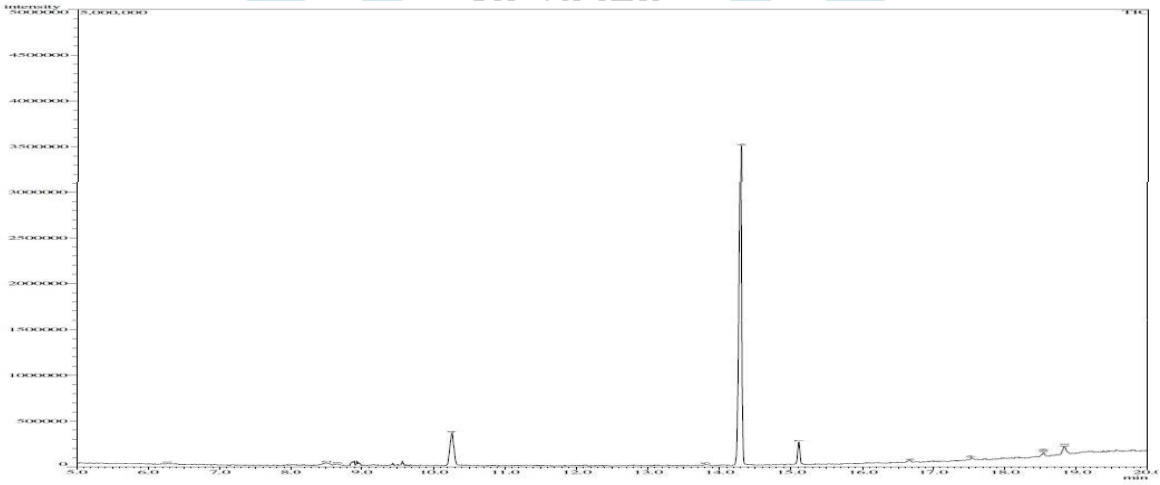

Figure 1: GC\MS analysis of the Nicotine alkaloid

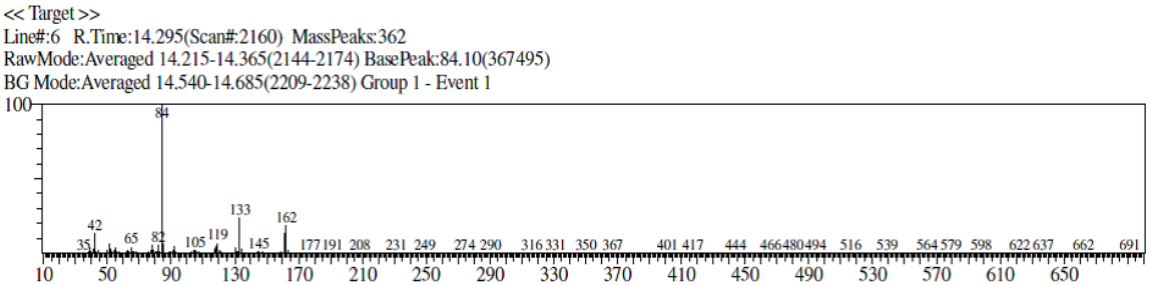

Figure 2: fragment of Nicotine

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Effect of Nicotine alkaloid extracted from N. tabacum L. on human cell lines

Two cancer cell lines were used to assess the effect of the extract of Nicotine alkaloid on the capacity of cells of cancer to proliferate. Cancer cell lines were treated with five concentrations and three replicates for each concentration of the alkaloid extract for 24 hours at 37°C by using different concentrations that ranged between 50, 100, 200, 400, and 800 µg/ml, and the cytotoxicity test was done to determine each concentration of the extract effect on the cancer cells growth, in a term of the percentage of the rate of inhibition of growth. The extract of Nicotine alkaloid had an inhibitory effect on the growth of cancer cells of the MCF7 line, starting with a concentration of 50µg/ml, the inhibition rate was 20.07%, and this rate is increased for the concentrations 100, 200, 400, and 800 µg/ml. A significant difference was observed between concentrations as shown in (Figures 3, and 4).

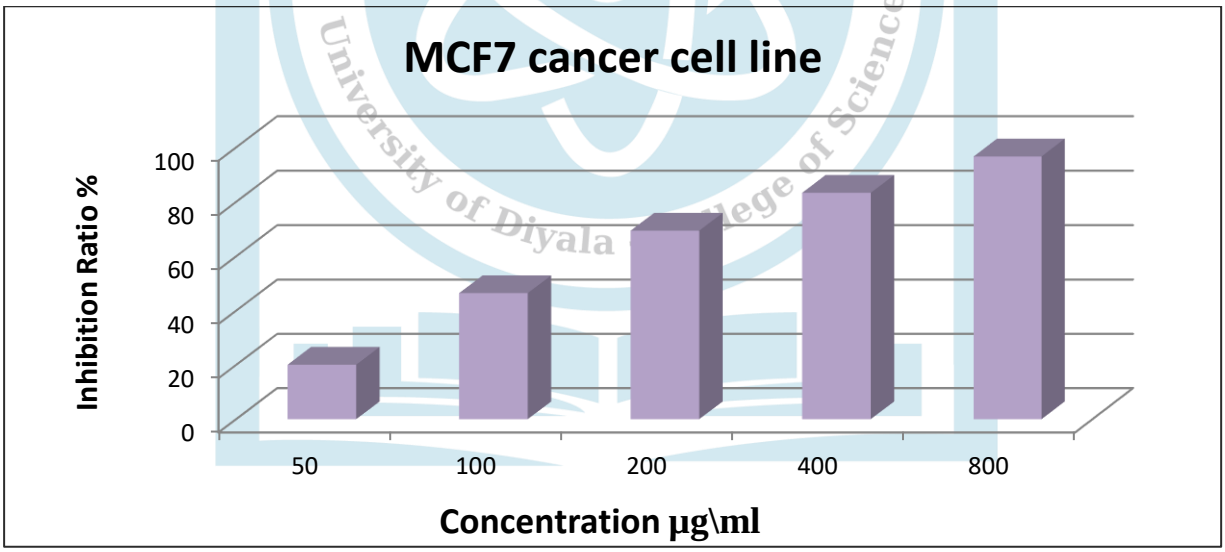

Figure 3: Different Concentrations of Nicotine Alkaloid Extract Affect the Rate of Inhibition in MCF7 Cancer cell line at 37°C after 24 Hours of Exposure.

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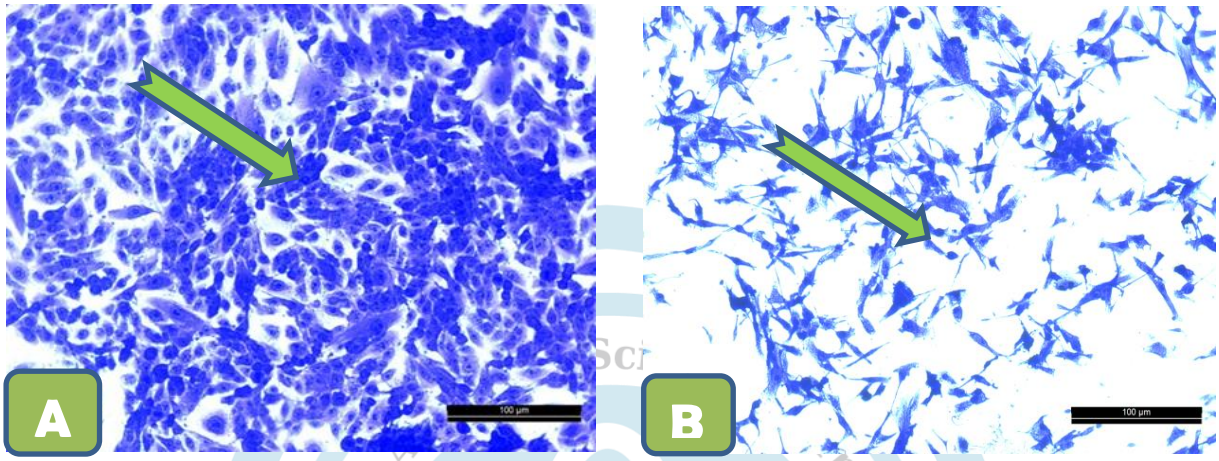

Figure 4: Crystal Violate stain comparison between MCF7 cells treated and untreated with Extract of Nicotine alkaloid extract at a concentration of 800 g/ml for 24 hours of exposure at 37°C (x100).
(A) MCF7 cancer cell line, which serves as a control and displays dense cells.
(B) MCF7 cell line with dead cells and voids between cells after treatment with alkaloid extract at a dose of 800 g/ml

In the Hela cell line, the Nicotine alkaloid extract inhibited their growth that started with a concentration of 50µg/ml, and the rate of inhibition was 34.87% and increased for the concentrations of 100, 200, 400, and 800 µg/ml. There is no significant difference was observed between 400 and 800µg/ml as in (Figures 5, and 6).

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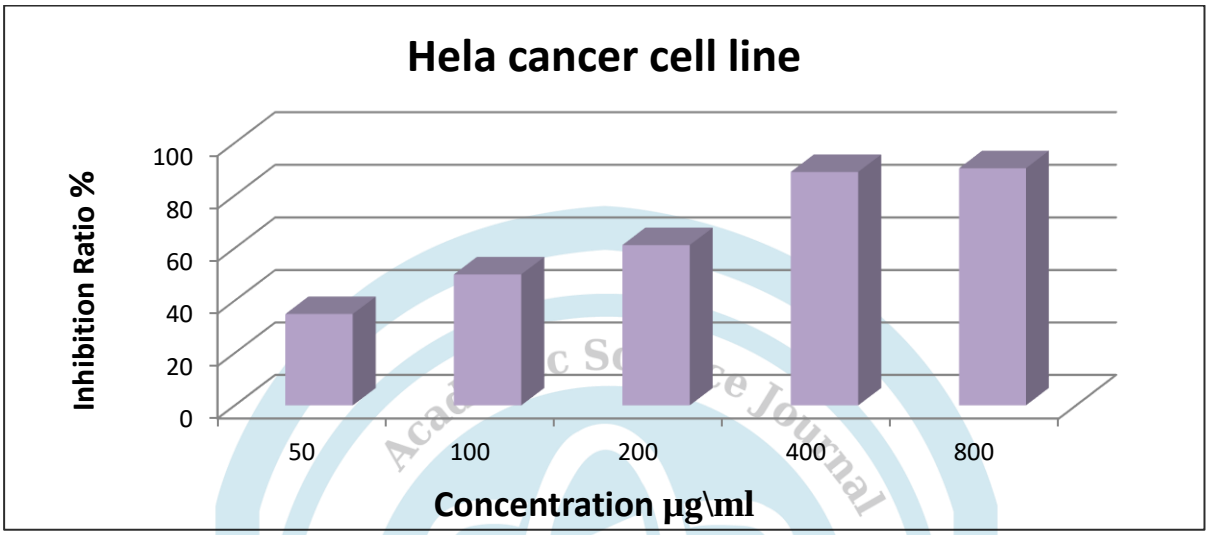

Figure 5: Different Concentrations of Nicotine Alkaloid Extract Affect the Rate of Inhibition in Hela Cancer Cell Line at 37°C after 24 Hours of Exposure.

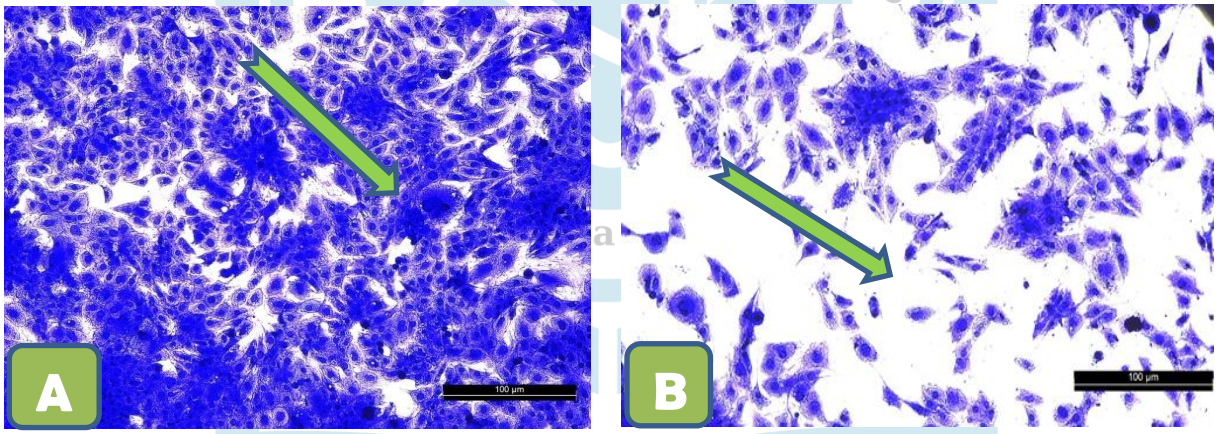

Figure 6: Crystal Violate stain comparison between Hela cells treated and untreated with Extract of Nicotine alkaloid at a concentration of 800 g/ml for 24 hours of exposure at 37°C (x100).
(A) Hela cancer cell line, which serves as a control and displays dense cells.
(B) Hela cell line with dead cells and voids between cells after treatment with alkaloid extract at a dose of 800 g/ml

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Discussion

A brown-orange color that appeared after exposing the extract to Dragendroff's reagent means to refer to the presence of Nicotine alkaloid in the extract [23]. The GC\MS results showed the presence of Nicotine alkaloids [24]. The study results reinforced the findings of many researchers about plant extracts having anti-cancer activity. This effectiveness depends on the type and concentration of extract, in addition to the sensitivity of cancer cells [25]. The results showed that the Nicotine alkaloid extract of *N. tabacum L.* has a toxic effect on the cancer cell lines (MCF7 and Hela) growth for all the concentrations that were used as shown in tables [1] and [2]. The inhibitory effect of the extract on cancer cells is due to its chemical content, especially nicotine alkaloid, which is effective in inhibiting or stopping the growth of cancer cells [26]. These alkaloids have an important effect by preventing the polymerization of proteins of microtubules that form the mitotic spindle and this leads to impedes the division and differentiation of cancer cells [27]. The study of Al-Lahham [12] showed that the aqueous and methanol extracts of *N. tabacum* L. roots have an anti-proliferative effect on HeLa cervical adenocarcinoma. Also, the flavonoid extract of *N. tabacum* L. leaves has a potent cytotoxic effect on MCF7 human breast cancer cells [17]. The study conducted by Obare [28] showed that the aqueous acetone extract of *N. tabacum* L. stems has a toxic effect on some human cancer cell lines such as human alveolar basal epithelial cells adenocarcinoma (A549), prostate cancer cell line (PC3), and MCF7. Also [29] showed that the alkaloid extract of *Coptis Chinensis Franch* has an inhibitory effect on the proliferation of breast and liver cancer cells by stopping the cell cycle and causing stress in the endoplasmic reticulum and leading to inhibition of cancer cell metastasis.

Another study used ether, chloroform, and aqueous extracts of *Cynanchum acutum* L. seeds to evaluate the effects of alkaloid extract on the growth of lung cancer (A549), breast cancer

(MCF-7), hepatocellular carcinoma (HepG-2), and normal human fetal lung fibroblast (WI-38) cell lines found that they had an inhibitory effect on their growth. [30]. The cytotoxic effects of the alkaloid extract of *Stachys pilifera* were evaluated against human colon adenocarcinoma (HT-29) by inducing programmed cell death which is characterized by morphological changes such as cell shrinkage, apoptotic bodies formation, and cell debris [31].

Also, the methanolic extract of *Phyllodium elegans* shows morphological changes in the brain (U251-MG), melanoma (A375), and colorectal (HCT116) cancer cell lines when treated with it, these changes include shrinkage of the cells, damaged and condensed nuclei, and increased in the number of dead cells [32].

The study concluded that at high concentrations, the Nicotine alkaloid extract of *Nicotiana tabacum* L. inhibits the growth of MCF7 and Hela cancer cell lines making this plant a promising candidate for cancer treatment, but more research is needed to prove it.

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