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Effect Crud Alkaloids Extraction From Nicotina Tabacum In Same Cell Line

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Abstract. This study aimed to investigate some Chemical Detections of Some Compounds Nicotina tabacum in leaves of plant and the effects of the crude alkaloid extraction from Nicotina tabacum on the proliferation of MCF7 cancer cell line and MEF normal cell line. The Results the plant extraction showing alkaloid , Tannins and glycoside .the effect extract on growth of tumor cell lines MCF7 showed that crude extract revealed cytotoxicity on different cell line, and this effect depend on concentration and type of cells. The inhibition activity for tumor cell line increase with increase concentration of extract. The higher inhibition crud extraction rate in MCF7 cell line was % in concentration 15.1 µg\ml and in 500 µ g\ml was %, While in MEF cell line, the highest inhibition rate was % in 15.1 µ g\ml and % in concentration 500 µ g\ml. The study shows that crude alkaloids extract Nicotina tabacum have alkaloid and inhibition cell line and non inhibition normal cell.

Highlights:

1. Crude alkaloid extract from Nicotiana tabacum inhibited MCF-7 breast cancer cells in a dose-dependent manner.
2. MEF normal cells showed minimal sensitivity, indicating selective cytotoxicity.
3. Phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, and glycosides as active compounds.

Keywords: Nicotiana Tabacum, Alkaloids, MCF-7, Cytotoxicity, Selective Inhibition

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Introduction

Many perennial species in the genus Nicotiana are included, but the most famous is Nicotiana tabacum L. (Solanaceae), commonly referred to as tobacco. N. tabacum was found in the tropical Americas, Oceania and several South Pacific islands, but it later spread to Mexico and the West Indies [1]. The global spread of this valuable crop means it is frequently linked by experts to addictive substance use.Apart from being used in tobacco, all parts of N. tabacum have traditionally been employed in Chinese medicine as pesticides, tranquilizers, diaphoretics, anesthetics and anocathartic agents [2]. According to phytochemical studies, N. tabacum possesses a wide variety of parts, including terpenoids, alkaloids, lignans, flavonoids, phenylpropanoids, chromanones and coumarins [3]. Such compounds are not simple, but they do show possible usefulness for developing new pharmaceuticals as well as products for agriculture. Because natural products are key sources for drugs, examining unique chemicals from N. tabacum and thoroughly studying its uses has attracted considerable research interest [4].

Previous studies reveal that N. tabacum has a wide range of different chemical features. A wide range of studies have examined the Nicotiana genus' taxonomy, biology and ecology, but few reviews have extensively examined how its special phytochemical and pharmacological properties are used in medicines. Recent progress in the pharmaceutical industry has greatly encouraged natural product research, resulting in many new compounds with unique shapes and reliable bioactivity [7]. The specimen used in this study was Nicotiana tabacum, a member of the Solanaceae family commonly known as tobacco. The visual representation of the plant is provided in Figure 1, which shows the morphological features of the harvested specimen prior to alkaloid extraction.

Method

The plant used in these experiments Figure (1).

Figure 1. Nicotina tabacum plant

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A. Preparation of Crude Alkaloid Extract from Nicotiana tabacum Leaves

The alkaloids from Nicotiana tabacum (tobacco) leaves were extracted using the method mentioned in [8]. A solution was prepared with about 50 grams of powdered, air-dried tobacco leaves in 200 mL of distilled water in a flask. Magnetic stirring was done at 45°C for a duration of 24 hours without interruption. The filtrate from the solution was placed into pre-weighed glass Petri dishes. The foods went into an electric oven at 37°C and remained open for 2–3 days until they were completely dry. We calculated the extraction yield by comparing the amount extracted to the initial leaf mass.

B. Cancer Cell Lines

1. Michigan Cancer Foundation-7 (MCF-7)

A passage of 50 was given to the MCF-7 line which came from a biopsy of a 69-year-old female breast cancer patient (Soule, 1973). Cultures were carried out in RPMI-1640 medium containing 10% fetal calf serum (FCS). Trypsin/versene (EDTA) solution was used to subculture the cells after they had become confluent for further treatment.

2. Mouse Embryo Fibroblast (MEF) Cells

Mouse embryonic fibroblasts were included in this study as a standard example in stem cell and developmental biology at passage 15. The MEFs were kept in a standard culture and passed by trypsin/versene once they reached full growth. Cells in this passage usually do not change much in their physical and biological features, so they can be used well for experiments.

3. Cytotoxicity Assay of Crude Alkaloid Extract on Cancer Cell Lines

The ability of the alkaloid extract to kill cancer cells was assessed using crystal violet staining, as outlined in [9]. After being dissolved in DMSO, the dried alkaloid extract was diluted in SFM to create samples with concentrations of 15.1, 31.25, 62.5, 125, 250, 400 and 500 µg/mL. Both MCF-7 and MEF cells were added to 96-well microplates and grown for 24 hours at 37 degrees Celsius with 5% CO_2. Following incubation, the cell culture was treated with fresh SFM and specific levels of the extracts. They were kept under the same conditions for another 24 hours afterward. At the close of the exposure, the media were collected and the

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wells were treated with 100 µL/well of 0.5% crystal violet for 20 minutes at 37°C. Excess stain was removed from the wells by washing with PBS and the plates were left to dry in air for 15 minutes at room temperature. In the end, cell viability was measured by calculating the optical density (OD) at a wavelength of 492 nm using the ELISA plate reader.

Here's a corrected and professionally rewritten version of the Results and Discussion section, with improved structure, grammar, scientific tone, and clarity, along with a properly formatted table:

Results and Discussion

A. Phytochemical Screening of Nicotiana tabacum Leaf Extract

A total crude yield of 0.4 grams was obtained from 50 grams of dried Nicotiana tabacum leaf powder following the extraction process. Preliminary phytochemical analysis was performed to detect the presence of major bioactive constituents in the crude extract. The findings are summarized in Table 1.

Table 1. Phytochemical Composition of Nicotiana tabacum Leaf Extract

Chemical Compound	Detection Reagent	Result	Note
Alkaloids	Dragendorff's reagent	+	1
Flavonoids	1% Ferric chloride solution	+	2
Glycosides	Potassium hydroxide (KOH)	+	3
Tannins	1% Lead acetate solution	+	4

Figure 2. TLC Plate Showing Alkaloid Detection in Nicotiana tabacum Leaf Extract

The preliminary phytochemical screening revealed the presence of alkaloids, tannins, flavonoids, and glycosides in the crude extract. Detection of alkaloids was confirmed through thin-layer chromatography (TLC), as illustrated in Figure 2.

The presence of alkaloids, flavonoids, glycosides, and tannins in the extract

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indicates that N. tabacum leaves are rich in secondary metabolites, many of which are known to possess biological activity, including cytotoxic, antimicrobial, and antioxidant properties. The strong positive result for alkaloids is particularly notable, as these compounds are often implicated in anti-cancer mechanisms, including DNA intercalation and inhibition of cell division. These findings provide preliminary chemical evidence supporting the potential therapeutic application of the tobacco leaf extract in cancer research. the tropical regions of the Americas [10]. The aerial portions of this species are also used in traditional Chinese medicine as an insecticide, sedative, diaphoretic, anaesthetic, and emetic agent. This species is a valuable agricultural product that is traded all over the world. Over two thousand five hundred elements have been identified as a consequence of previous phytochemical research conducted on the Nicotiana genus [11]. These constituents include sesquiterpenoids, diterpenoids, alkaloids, and flavonoids. A previous study that was carried out by our group indicated that an ethanol extract that was obtained from the leaves of N. tabacum and contained 95% ethanol had significant antifungal activity against Valsa mali as well as eleven other phytopathogenic fungus [12].

B. Cytotoxic Effect of Crude Alkaloid Extract from Nicotiana tabacum Leaves on MCF-7 and MEF Cell Lines

To evaluate the cytotoxic potential of crude alkaloid extracts from Nicotiana tabacum, a cytotoxicity assay was conducted on the human breast cancer cell line (MCF-7) and the mouse embryonic fibroblast (MEF) cell line as a non-cancerous control. Cells were exposed to seven different concentrations of the extract (15.1, 31.2, 62.5, 125, 250, 400, and 500 µg/mL) for 24 hours. Cell viability was assessed using crystal violet staining, and growth inhibition was calculated as a percentage.

C. Effect on MCF-7 Cells

The crude alkaloid extract exhibited a dose-dependent inhibitory effect on MCF-7 cells. Significant growth inhibition was observed starting from the lowest tested concentration (15.1 µg/mL), with inhibition reaching 64.1% at 500 µg/mL. The results are presented in Table 2.

Table 2. Cytotoxic Effect of Crude Alkaloids on MCF-7 Cell Line After 24 Hours

Concentration (µg/mL)	Inhibition (%) ± SD	Statistical Significance
15.1	20.07 ± 3.6	c
31.2	26.45 ± 4.1	c
62.5	29.41 ± 5.8	c
125	33.32 ± 6.9	b
250	36.67 ± 7.2	b

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400	56.83 ± 8.1	a
500	64.10 ± 9.8	a

Different letters indicate statistically significant differences at P ≤ 0.05.

D. Effect on MEF Cells

In contrast, the MEF normal cell line showed minimal sensitivity to the crude alkaloid extract. Growth inhibition remained statistically insignificant at lower concentrations and peaked at only 11.3% at the highest concentration (500 µg/mL), indicating selective toxicity towards cancer cells. The results are shown in Table 3.

Table 3. Cytotoxic Effect of Crude Alkaloids on MEF Cell Line After 24 Hours

Concentration (µg/mL)	Inhibition (%) ± SD	Statistical Significance
15.1	0.00 ± 0.0	c
31.2	0.00 ± 0.0	c
62.5	0.00 ± 0.0	c
125	0.00 ± 0.0	c
250	10.20 ± 1.5	c
400	10.20 ± 1.5	b
500	11.30 ± 1.6	a

Different letters indicate statistically significant differences at P ≤ 0.05.

Upon exposure to 500 µg/mL of crude alkaloid extract, MCF-7 breast cancer cells displayed notable cytotoxic effects including cell rounding, detachment, and evidence of apoptosis. These morphological changes are shown in Figure 3.

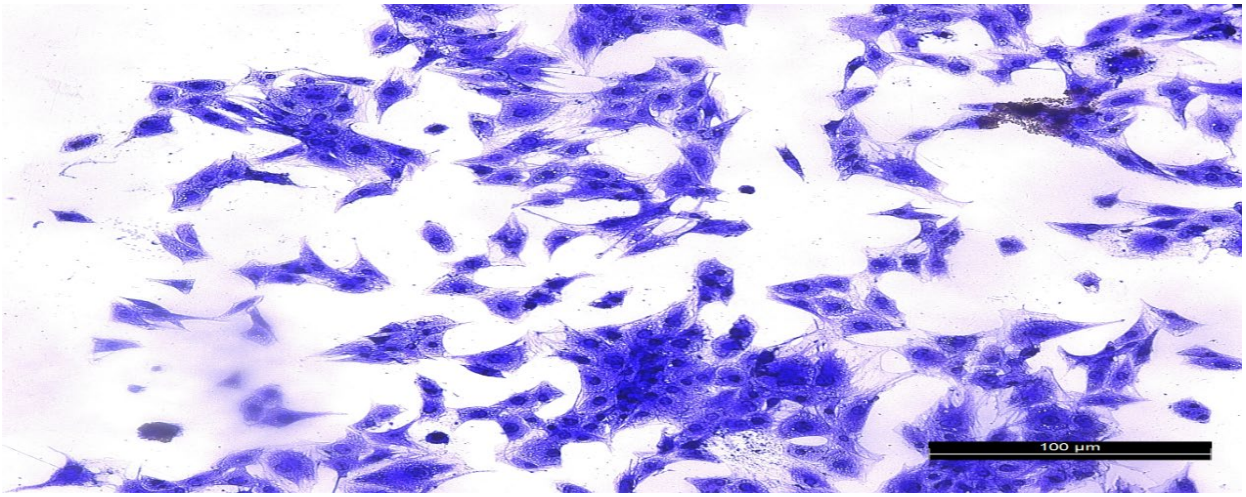

Figure 3. MCF7 Cells Treated with crude alkaloids at a Concentration of 500 µg/ml for 24 hrs (100X) showing dead cells and intercellular spaces.

In contrast, MEF cells showed relatively preserved morphology under identical treatment conditions, with only minor changes in cell density and intercellular spacing. This is evident in Figure 4.

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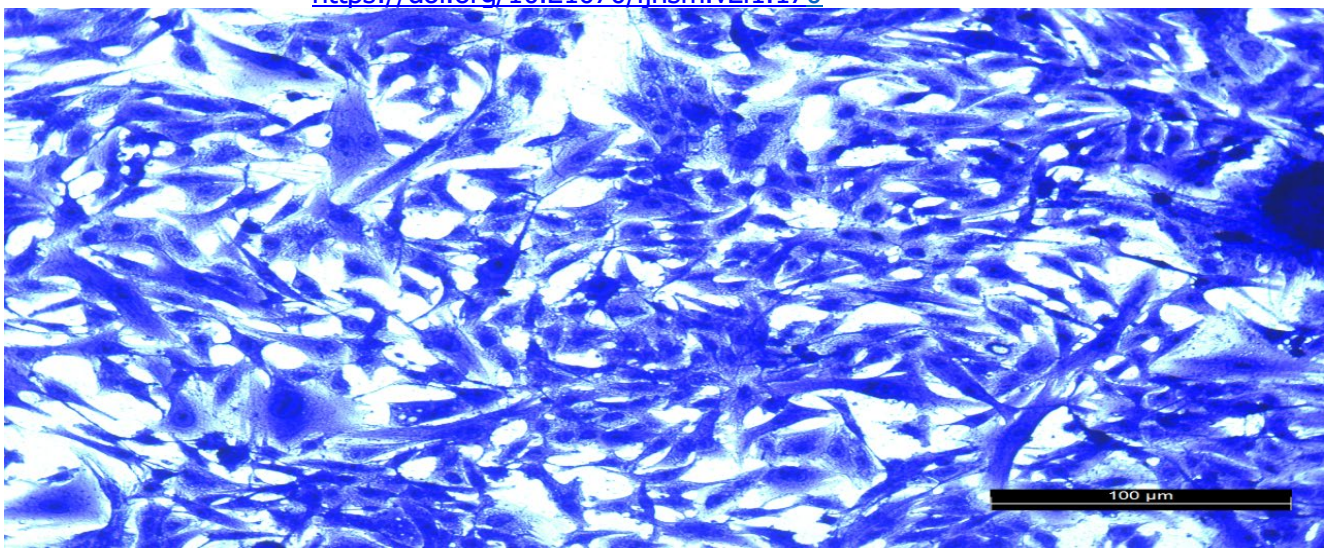

Figure 4. MEF Cells Treated with crude alkaloids at a Concentration of 500 µg/ml for 24 hrs (100X) showing cells and intercellular spaces.

In recent decades, medicinal plants have been extensively investigated for their antitumor pharmacological activity [13]. The use of medicinal plants and natural products in cytotoxicity studies against tumor cell lines is predicated on their potential as sources of anticancer agents with reduced side effects compared to conventional chemotherapeutic drugs. *Nicotiana tabacum*, the most prominent species of the tobacco plant genus *Nicotiana* (Solanaceae), which comprises over sixty species [14], presents a complex duality. While tobacco smoke is associated with numerous detrimental health effects, research on *N. tabacum* leaf extracts has revealed potential medicinal applications, including antibacterial, anti-inflammatory, cardiovascular, and anticancer properties [15]. These leaves contain a variety of organic alkaloid compounds present throughout the plant [16].

Studies have demonstrated varying levels of antioxidant activity in tobacco products, ranging from moderate to high, with a significant positive correlation observed between overall antioxidant capacity and total phenolic content. [17].This high antioxidant activity has been proposed as a potential explanation for the lower cancer risk associated with long-term use of certain tobacco products. [18] reported the presence of phenolics and flavonoids, contributing to antioxidant activity, in methanol and aqueous extracts of *N. tabacum* leaves. [19] identified chlorogenic acid in tobacco leaves as a compound capable of inducing cytotoxic effects against cancer formation. [20] showed that in vitro treatment of A549 lung epithelial cells with nicotine resulted in a significant increase in apoptosis. Furthermore, combined treatment with taxol and nicotine synergistically enhanced apoptosis, particularly in

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detached cells, and led to a reduction in mitochondrial cytochrome C coupled with an increase in cytosolic cytochrome C after 4 hours of incubation, indicative of increased tumor cell death. [21] investigated the cytotoxic activity of tobacco extracts against human oral squamous cell carcinoma cell lines, demonstrating that tobacco contains compounds capable of inducing apoptosis in cancer cells. However, no studies were found investigating the effects of aqueous and methanol crude extracts from locally sourced Iraqi *N. tabacum* leaves on the proliferation of tumor cell lines. Therefore, this study was designed to evaluate the cytotoxic effects of crude extracts of *N. tabacum* on MCF7 and MEF cell lines, as well as the cytogenetic effects of the methanol crude extract on the mitotic index (MI) of these cell lines.

Conclusions

Extracting only alkaloids from Nicotiana tabacum leaves made them highly toxic to MCF-7 breast cancer cases, yet harmless to MEF normal cells, proving they can be promising for treating cancer. Because the plant contains both alkaloids and flavonoids, it is considered a useful herb for therapy. These reports form the base for further work targeting specific chemicals and understanding their roles to create new cancer treatments.

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