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Evaluate the utility of Iraqi Ephedra Transitoria methyl extract on the proliferation of cervical cancer cell line

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Abstract

Objective: This study aimed to assess the inhibitory effect of a methanolic extract of Iraqi Ephedra transitoria on the proliferation of cervical cancer cells.
Materials and methods: To assess the anticancer properties of Ephedra transitoria, a HeLa cell line (derived from cervical cancer) was utilized. The concentration of the evaluated agent was varied between 0.1 and 1000 µg/ml, and the cells were incubated for 24 and 72 hours. The IC50 value was used to identify the potency of the examined substance.
Results: The study results demonstrated that the extraction ratio of E. transitoria was 8%. Additionally, a cytotoxicity study revealed that the plant extract can potentially inhibit the proliferation of human cervical cancer cells in a concentration- and time-dependent manner. The extract at a concentration of 1000 µg/ml inhibited growth by 88% after 72 hours.
The comparison between the cytotoxicity of a plant extract and traditional anticancer chemotherapy (cisplatin) revealed that the plant extract exhibited higher cytotoxicity than cisplatin in the HeLa cancer cell line after two incubation periods. Additionally, the IC50 value of the extract was lower than that of cisplatin.
Conclusion: Ephedra transitoria inhibits the proliferation of cervical cancer cells via cell cycle-specific and cell cycle-nonspecific mechanisms, thus demonstrating its anticancer properties. The study also revealed that, compared with cisplatin, the plant extract exhibited superior efficacy and potency in cancer treatment.

Keywords: *cervical cancer, cisplatin, Ephedra Transitoria, HeLa cell line*

Introduction

Throughout history, plants with medicinal properties have been utilized. Plants that can produce a wide range of chemical compounds are valuable for carrying out crucial biological processes. A minimum of 12,000 distinct compounds have been found and isolated. (1, 2)
Recent scientific studies on medicinal plants have made substantial advances in treating and controlling cancer progression. The main disadvantage of synthetic drugs is their concomitant side effects. Plant-based natural therapies have been shown to effectively eliminate cancer cells; investigations into anticancer compounds originating from plants commenced in the 1950s. (3) Further investigations have uncovered the capacity of vinca alkaloids (vinblastine and vincristine) and Prunus armeniaca kernels (apricot kernels) to display significant anticancer capabilities, as evidenced by numerous studies (4-9).
Following our research, several studies have been carried out. In 2017, a study evaluated the impact of an ethanolic extract obtained from the leaves and fruit juice of Ephedra foramina on colon cancer cells (HTC116) and breast cancer cells (MDA-MB-213). This study demonstrated that both the ethanolic extract of E. foramina and its fruit juice substantially lowered the viability of both cell lines. Additionally, this outcome was accomplished by triggering caspase-3-dependent apoptosis. (10). A separate study elucidated the anticancer efficacy of nanoparticles derived from the aqueous extract of E. sinica. The efficacy of the water extract was evaluated via a lecithin nanoencapsulation procedure to determine its ability to inhibit the formation of tumors generated in mice with sarcoma-180 cells. The nanoparticles significantly decreased the enlargement of internal organs, such as the spleen and liver, to 15~20%, resulting in a reduction in the solid tumor size to 20%. The anticancer efficacy of E. sinica can be augmented

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by the use of a nanoencapsulation technique involving lecithin, which facilitates improved penetration into cancerous cells (11).

A separate study revealed that the extract obtained from Ephedra Herb inhibited the movement of cancer cells driven by hepatocyte growth factor (HGF), presumably by obstructing the signaling pathway of HGF-c-Met. (9) Dysregulation of this pathway plays a significant role in the formation, expansion, progression, metastasis, and resistance to treatment of malignancies. (12, 13). Thus, the Ephedra Herb could be used as a unique c-Met inhibitor in cancer treatment. We recently discovered that herbacetin, a type of flavonoid found in Ephedra Herb, effectively blocked HGF/c-Met/Akt signaling and suppressed the HGF-induced movement of human MDA-MB-231 breast cancer cells. (14). Despite studies examining the anticancer qualities of many ephedra species, few studies have investigated the anticancer effects of Ephedra Transitoria spp. on cervical cancer. Our study evaluated the efficacy of methyl extraction from Iraqi Ephedra Transitoria in inhibiting the proliferation of human cervical cancer.

Materials and methods

Extraction of the plant:

a- Plant collection:

The leaves and branches of Iraqi Ephedra Transitoria were obtained from al-Samawa city. The plant material was dried, crushed, and pulverized using an electric blender. The dust was then stored in a dry, sealed container.

b- Extract preparation:

The preparation of the plant alcoholic extract was conducted as follows: A 100-gram sample of plant powder was immersed in 1000 cc of 70% ethanol. The extraction process was carried out using a Soxhlet device for 24 hours. A rotary evaporator was used to convert the extract into a dry powder. (15).

c- Chemotherapeutic agent:

A 1 mg/ml cisplatin vial (Mylan, USA) was used at different concentrations ranging from 0.1–1000 µg/ml after dilution with bovine serum-free medium.

Cell culture:

The HeLa cell line, derived from human cervical cancer, was obtained from the tissue culture unit at (ICCMGR) passage number (43). The cells were grown in tissue culture flasks with a surface area of 75 cm2 at 37°C in a moist environment with 5% carbon dioxide. The cells were cultured in RPMI-1640 media (Sigma Chemical, England) supplemented with 10% fetal bovine serum (FBS) and penicillin–streptomycin (100 U/mL penicillin and 100 µg/mL streptomycin).(16)

Cytotoxicity Assay

The cells were cultured in a microtiter plate with 96 wells. The plants were then exposed to a range of plant extracts and cisplatin. The concentration of cells increased during the logarithmic phase of growth. The cytotoxic effects of the study agents were assessed after 24 and 72 hrs. incubation periods. Each well contained 1X104 cells. Seeding was performed with calf serum medium at a concentration of 10%. The plates were incubated at 37°C for 24 hours to allow cell adhesion. A maintenance medium was subsequently used to prepare fourfold serial dilutions of the methanolic extract of Ephedra Transitoria and cisplatin at concentrations of 0.1, 1, 10, 100, and 1000 µg/ml.(16, 17)

After 24 hours of incubation, the cells were exposed. Each tested concentration was replicated six times, with 200 µl of maintenance medium delivered into each well in the control group. The exposure lasted for 24 and 72 hours. The plates were hermetically sealed with self-adhesive material and transported to the incubator. The cells were subsequently treated with MTT dye to induce staining.(16)

The optical density of each well was measured via a microtiter plate-ELISA reader at a transmitting wavelength of 550 nm, (18, 19). The growth inhibition rate was determined via the following mathematical equation. (19).

Growth inhibition % = ((optical density of control wells-optical density of treated wells)/(optical density of control wells))*100%

Research ethics:

This study did not involve any human participants.

Statistical analysis

Data from the MTT test, with six replicates, are presented as the mean ± standard error (SE). One-way ANOVA was used. The LSD test was used to compare groups. The investigation was conducted using (spss) statistical software version 20 and GraphPad Prism, with a level of significance of $p < 0.05$. (20).

Results

Plant extraction:

The product was dried via a rotary evaporator after hot methanolic extraction of Ephedra transitoria leaves and branches. The resulting material had a dark brown sticky appearance. The extraction ratio was approximately 8%.

Cytotoxic effects of the methanolic extracts of E. transitoria:

The cytotoxic effects of the methanolic extracts from E. transitoria on the HeLa cancer cell line revealed that higher extract concentrations resulted in more growth inhibition. Furthermore, longer incubation times led to greater growth inhibition. The results demonstrate a significant variation in the extent of growth inhibition during diverse incubation durations for all the tested doses, except for the lowest concentration. There was a significant difference among all the concentrations within each incubation period. (Table 1, Figure 1). The IC50 value was 876.47 µg/ml after 24 hours and decreased to 22.03 µg/ml after 72 hours. (Table 1).

Table (1): Impact of the plant extract on the growth of HeLa cancer cells at 24 and 72 hours

Concentration (µg/ml)	Inhibition of cellular viability (mean ± SE [a])		*P*- value
	24 hr.	72 hr.	
0.1	C 1.00 ± 0.577	C 3.00 ± 0.577	0.070

1	**C** 5.00 ± 1.732	**C** 13.00 ± 1.732	**0.031***
10	**B** 21.00 ± 0.577	**B** 33.00 ± 1.732	**0.003***
100	**B** 29.00 ± 2.309	**A** 70.00 ± 1.732	**0.0001***
1000	**A** 54.00 ± 2.309	**A** 88.00 ±2.309	**0.0001***
[b] LSD value	**10.68**	**10.8**	-
IC 50	**876.47 µg/ml**	**22.03 µg/ml**	

a: standard error, b: least significant difference, statistically significant differences are shown by variations in capital letters within the same column, *: significant at (P<0.05)

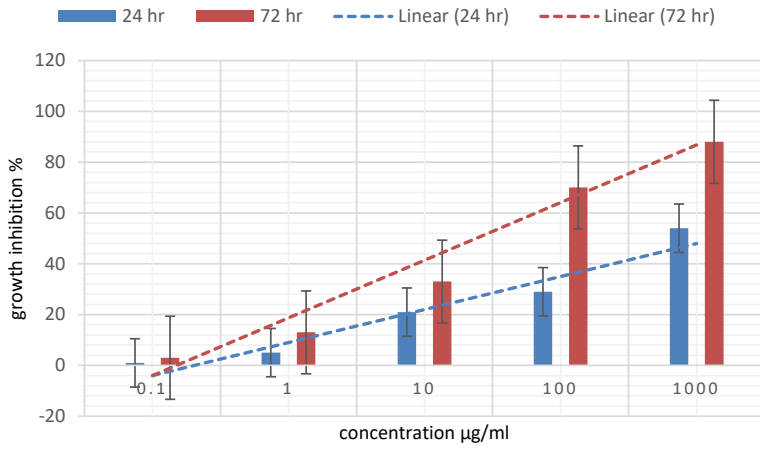

Figure (1): The cytotoxic impact of the plant extract on the HeLa cancer cell line at 24 and 72 hours.

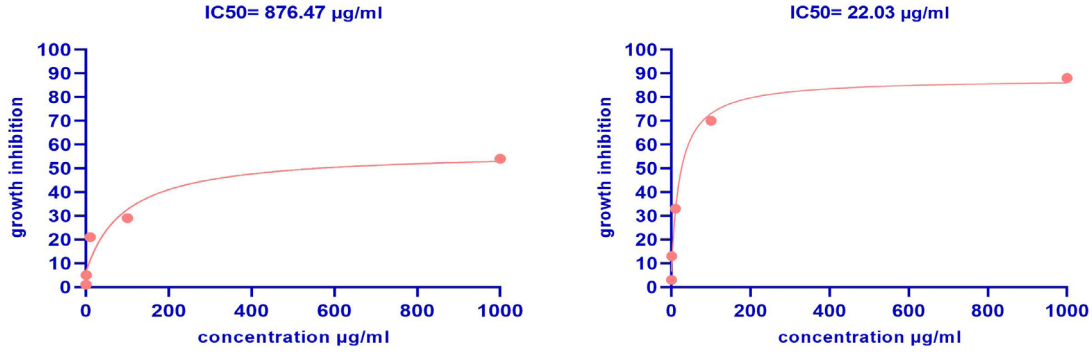

Figure (2): The IC50 values for the plant extracts are shown on the right at 24 hours and on the left at 72 hours.

The cytotoxic effect of cisplatin:
The cytotoxic impact of cisplatin on the HeLa cancer cell line demonstrated an increase in growth inhibition with increasing concentrations of the drug and increased incubation durations. The results show a notable variance in growth inhibition between each incubation period for all concentrations

except the smaller one. Additionally, there was a significant difference in growth inhibition among the concentrations within each incubation period. (Table 2, Figure 3). The IC50 values were 1275.5 µg/ml and 53.39 µg/ml after 24 and 72 hours of incubation, respectively. Table (2).

Table (2): The impact of cisplatin on the growth of HeLa cancer cells at 24 and 72 hours

Concentration (µg/ml)	Inhibition of cellular viability (mean ± SE [a])		*P*- value
	24 hr.	72 hr.	
0.1	**B** 1.00 ± 0.577	**D** 2.00 ± 0.577	**0.288**
1	**B** 1.00 ± 0.577	**D** 5.00 ± 1.155	**0.036***
10	**B** 4.00 ± 1.155	**C** 16.00 ± 1.732	**0.004***
100	**A** 28.00 ± 2.887	**B** 50.00 ± 2.887	**0.006***
1000	**A** 39.00 ± 2.309	**A** 73.00 ± 1.732	**0.0001***
[b] **LSD value**	**11.16**	**11.28**	-
IC 50	**1275.5 µg/ml**	**53.39 µg/ml**	-

a: standard error, b: least significant difference, statistically significant differences are shown by variations in capital letters within the same column, *: significant at (P<0.05)

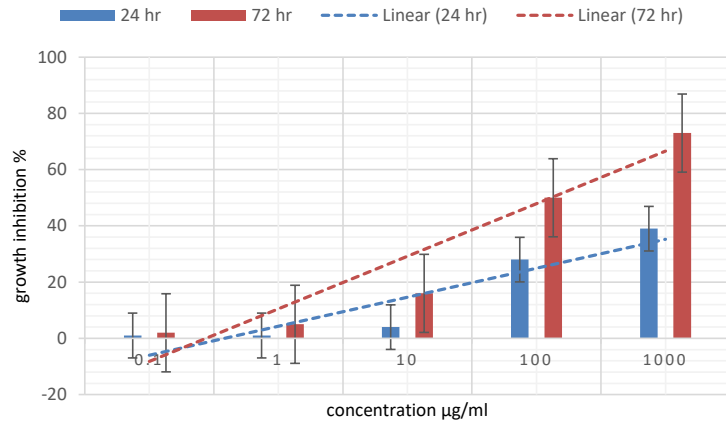

Figure (3): The impact of cisplatin on the growth of HeLa cancer cells at 24 and 72 hours

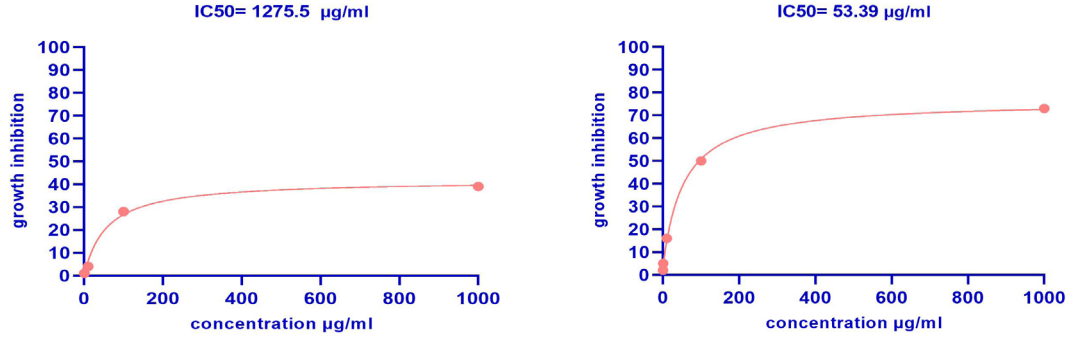

Figure (4): The IC50 values for cisplatin are shown on the right at 24 hours and on the left at 72 hours.

Comparison of the cytotoxic effects of the methanolic extracts of E. transitoria and cisplatin:

At 24 hours of incubation, the results of the comparison revealed a significant difference in the cytotoxicity of the plant extract compared with that of cisplatin at concentrations of 10 and 1000 µg/ml (Table 3) (Figure 5).

The results of the comparison revealed a significant difference in the cytotoxicity of the plant extract compared with that of cisplatin at all concentrations except for the lowest concentration (Table 4) (Figure 6) (Figure 7).

Table (3): cisplatin vs. plant extract for cervical cancer growth suppression at 24 h

Concentration (µg/ml)	Inhibition of cellular viability (mean ± SE [a])		*P*- value
	Plant extract	cisplatin	
0.1	**C** 1.00 ± 0.577	**B** 1.00 ± 0.577	**1.000**
1	**C** 5.00 ± 1.732	**B** 1.00 ± 0.577	**0.094**
10	**B** 21.00 ± 0.577	**B** 4.00 ± 1.155	**0.0001***
100	**B** 29.00 ± 2.309	**A** 28.00 ± 2.887	**0.800**
1000	**A** 54.00 ± 2.309	**A** 39.00 ± 2.309	**0.010***
[b] LSD value	**10.68**	**11.16**	-
IC 50	**876.47 µg/ml**	**1275.5 µg/ml**	-

a: standard error, b: least significant difference, statistically significant differences are shown by variations in capital letters within the same column, *: significant at (P<0.05)

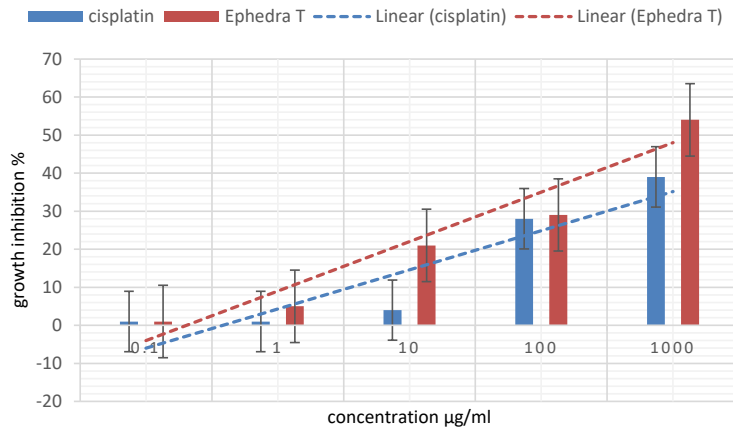

Figure (5): Cervical cancer growth suppression by cisplatin vs. plant extracts at 24 h

Table (4): Cisplatin vs. plant extracts for cervical cancer growth suppression at 72 h

Concentration (µg/ml)	Inhibition of cellular viability (mean ± SE [a])		*P*- value
	Plant extract	cisplatin	
0.1	**C** 3.00 ± 0.577	**D** 2.00 ± 0.577	**0.288**
1	**C** 13.00 ± 1.732	**D** 5.00 ± 1.155	**0.018***
10	**B** 33.00 ± 1.732	**C** 16.00 ± 1.732	**0.002***
100	**A** 70.00 ± 1.732	**B** 50.00 ± 2.887	**0.004***
1000	**An** 88.00 ±2.309	**A** 73.00 ± 1.732	**0.007***
[b] LSD value	**10.8**	**11.28**	-
IC 50	**22.03 µg/ml**	**53.39 µg/ml**	-

a: standard error, b: least significant difference, statistically significant differences are shown by variations in capital letters within the same column, *: significant at (P<0.05)

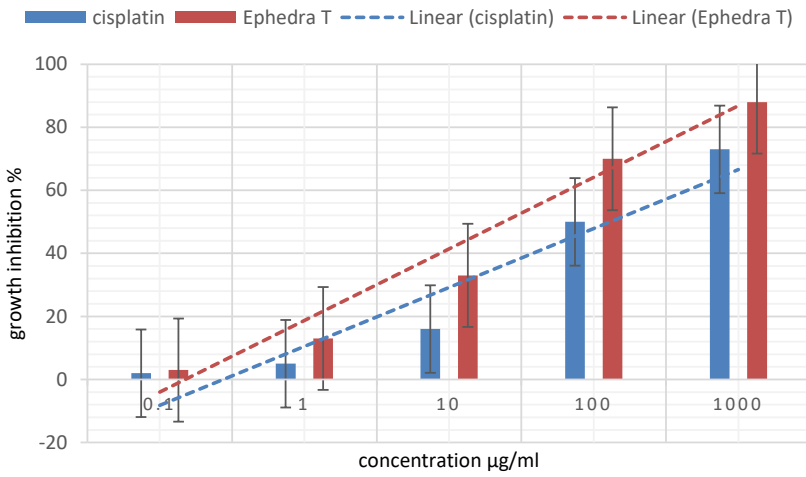

Figure (6): Cisplatin vs. plant extracts for cervical cancer growth suppression at 72 h

Discussion

The extraction technique employed 70% methanolic solution to enhance the retrieval of chemical constituents from Ephedra transitoria by isolating the lipophilic and hydrophilic chemical components of the plant.(21)

The cytotoxicity of the methanolic extract of Ephedra transitoria on cervical cancer cells was directly correlated with both the concentration and period of incubation. These findings suggest that the ability of the plant extract to kill cells is influenced by the amount of extract and the duration of exposure rather than being unique to a particular phase of the cell cycle.

Multiple investigations have been conducted on identical research subjects. A study on Jordanian plants revealed that Ephedra alata (70% ethanol maceration extract) had a notable cytotoxic effect on PC3 (prostate cancer cell line) and MCF-7 (human breast cancer cell line) (22).

Several investigations have clarified the mechanism by which plants have an anticancer influence. These investigations revealed that a flavonoid aglycone found in the herbaceous 7-O-hesperidin in Ephedra inhibits the proliferation of cancer cells and possesses analgesic qualities. (23-25). Additionally, it can potentially hinder the excessive growth of cancer cells caused by hepatocyte growth factor (HGF) by suppressing the phosphorylation of C-Met and its tyrosine kinase activity through the PI3K/Akt pathway. (25, 26). Furthermore, the activity of tyrosinase genes in B16F10 melanoma cells was considerably and dose-dependently suppressed by ephedrine A and B (27).

The plant extract is rich in long-chain fatty acids and contains high proportions of ephedrine. Every chemical has a cytotoxic effect. (28, 29). Long-chain fatty acids, such as n-tetradecanoic acid, n-dodecanoic acid, and n-octadecanoic acid, exhibit differentiation, cytotoxic, and apoptotic effects on breast cancer cells. (30, 31). N-octadecanoic acid exhibits cytotoxic properties against prostate cancer. (32, 33). N-hexadecanoic acid impacts the proliferation of colon cancer cells, inhibits DNA topoisomerase I, and induces death in leukemia and neuroblastoma cells. (34, 35). Phytol extracts exhibit cytotoxic effects on a broad spectrum of cancer cell types. (36, 37). In addition, phenolic substances such as isovanillin and (E)-coniferyl alcohol were extracted. These compounds share a similar core (benzene-3,4-OR), which is a fragment found in various molecules with antiproliferative properties, such as lignins and benzaldehydes. (38, 39). In contrast, long-chain alkanes, including n-heptadecane, n-triacontane, and n-hexatriacontane, exhibit cytotoxic effects on several cancer cell lines, particularly breast cancer cells. (40, 41).

The impact of cisplatin on cervical cancer cell viability is attributed to various mechanisms. These include the capacity of cisplatin to form covalent bonds with nucleophilic sites on DNA and its ability to react with electron-rich regions, such as sulfhydryls, and different sites on DNA. This results in the formation of intrastrand and interstrand cross-links. The DNA–platinum adducts reduce replication and transcription, causing single- and double-strand breaks and errors in coding. If p53 and other checkpoint proteins are detected, this leads to the initiation of apoptosis. (42, 43).

The results of our investigation revealed that the efficacy of the plant extract was greater than that of traditional chemical agents. Specifically, when the growth inhibition of the plant extract was compared with that of cisplatin, the plant extract demonstrated superior efficacy, particularly after 24 hours. Alternatively, the plant extract had greater potency than did cisplatin, as evidenced by its lower IC50 value of 22.03 µg/ml (Figure 2), whereas the IC50 value of cisplatin was 53.39 µg/ml (Figure 4).

The study limitations did not rely on a narrow range of concentrations. Instead, owing to the absence of specific and reliable concentration data, we relied on a diverse array of concentrations.

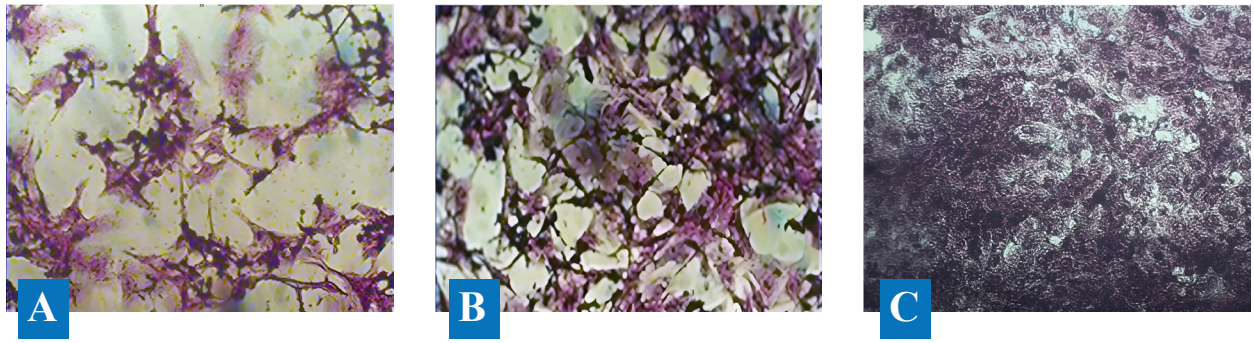

Figure (7): Morphology of the HeLa cells. (B) HeLa cells treated with 1000 µg/ml cisplatin for 72 hr. (A) HeLa cells treated with 1000 µg/ml plant extract for 72 hr. (C) HeLa cells in the control groups without any treatment.

Conclusion:

The study revealed that the methanolic extract of Iraqi Ephedra transitoria demonstrated increased cytotoxicity against human cervical cancer cells (a HeLa cancer cell line) as the concentration and incubation period increased. The potency of the plant extract was greater than that of cisplatin, as indicated by the IC50 values.

Author Contributions:

Design and development: Azal Hamoody.
Gathering and organizing data: Azal Hamoody, Montadher Ali, Yasser Jassim.
Data analysis/interpretation: Azal Hamoody, Montadher Ali, Yasser Jassim.
Article composition: Azal Hamoody, Montadher Ali.
Critique the essay for significant ideas: Azal Hamoody, Kawakeb NA.
Statistical analysis expertise: Azal Hamoody, Montadher Ali.
Ultimate article endorsement and guarantee: Azal Hamoody, Montadher Ali, Kawakeb NA.

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Conflicts of interest:

The authors declare no conflict of interest.

Declaration of Generative AI and AI-assisted technologies in the writing process:

The authors affirm that this work does not require the use of generative AI or AI-assisted technologies.

Abbreviations:

(ICCMGR): The Iraqi Centre for Cancer and Medical Genetics Research.
MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide stain
RPMI: Roswell Park Memorial Institute medium
SAS: Statistical analysis system
LSD: Least significant difference

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