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Anti-Cancer of Leaves Extracts of Christ's thorn Jujube in Muthanna Province against Human Cervix Carcinoma Cells Line in Vitro

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Abstract: Rhamnaceae plants have been demonstrated to have anticancer properties. In this study, we investigated the cytotoxic potential of the leaves of the species from this family, Christ's thorn Jujube (C.T.J.), on cancer cell lines. The viability of HeLa cells (human cervix carcinoma cells) was significantly and concentration-dependently decreased by hexane and aqueous extracts of the leaves of Christ's thorn Jujube. The leaf extract from Christ's thorn Jujube was more effective than the other extracts in both cell lines. The results of this study suggest that Christ's thorn jujube is a promising candidate for further research into potential new cytotoxic agents.

Keywords: Cancer, Cervix Carcinoma, Tetrazolium Bromide Assay, Leaves of C.T.J.

1. INTRODUCTION

Squamous cell carcinoma is the most common type of cervical cancer; adenocarcinoma occurs less frequently. Infection with the human papillomavirus is the main cause of cervical cancer. The initial sign of cervical cancer is usually irregular, frequently postcoital vaginal bleeding; cervical neoplasia is frequently asymptomatic.
These reports confirmed the existence of significant oils such asmethyl octadecanoate, methyl hexadicanoate,peptide, granyl acetate and throat alkali such as spinanen , tannin, stimulants such as b-sitosterol, and flavonoids such as protein derivatives, kirositin, biological, and medical antibiotics.

[14, 16, 17] A few studies have suggested that C.T.J. raw extract is toxic to cancer cell lines' cells. [11] In this study, we used solvents with hexane and water to extract ingredients from Christ's thorn Jujube (C.T.J.) and determined how they affected the HeLa cancer cell line in terms of cytotoxicity.

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2. MATERIALS AND METHODS

Materials

Penicillin/streptomycin, sodium pyruvate, trypsin, 2,5-diphenyltetrazolium bromide (MTT), hexane, RPMI 1640, fetal calf serum (FCS), KCl, NaHCO3, Na2HPO4 (India), and sodium pyruvate (USA).

Plant material

The leaves of Christ's Thorn Jujube (C.T.J.) were obtained from Farms in Samawa City, Iraq's Muthanna Governorate.

Topotecan

A variety of human malignancies have been successfully treated with the anticancer medication topotecan. Recent research indicates that additional mechanisms involving the production of reactive free radicals and the induction of oxidative stress may also be involved in topotecan-dependent tumor cell death. Topoisomerase I interactions and DNA double-strand breaks are the primary mechanisms by which topotecan kills tumor cells. We have demonstrated that the one-electron oxidation of topotecan by a peroxidase-hydrogen peroxide system results in the production of the topotecan radical.
When this radical reacts with reduced glutathione and cysteine, the glutathiyl and cysteinyl radicals are produced. We have now investigated the impact of topotecan exposure for one hour and for twenty-four hours on overall gene expression patterns, including alterations in drug influx and efflux. high TS expression, enhanced deoxyuridinetriphosphatase activity, MLH1 gene methylation, and higher Bcl-2, Bcl-XL, and Mcl-1 expression. The proteins exhibited topotecan resistance, and several possible explanations were suggested.

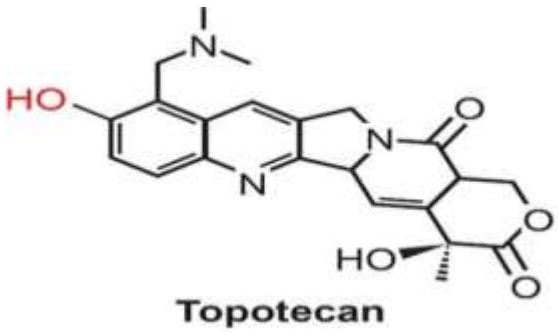

Figure 1: Topotecan's systemic design. Peroxidases oxidize the red-colored phenolic OH to start producing the corresponding phenoxy radical.

The Extractions

The maceration method was used to extract 200 grams of dried powder with 250 mL of hexane. [4]. The methanolic extract was then converted into butanolic and aqueous extracts. A rotary evaporator and freeze dryer concentrated the extracts. The extracts were vacuum-pumped dried and kept in an incubator at 40°C for six days. They were then kept at 4°C until they were used. 0.22-micron microbiological filters were used to filter 20 mg of the solid

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residues, which were then dissolved in 200ofDMSO, diluted to 4ml withPBS, and used as a sample. The final concentrations were 100, 500, 1000, 2000, and 4000 g/ml after further dilution.

Chemical compounds analyses
A colorimetric test was used to determine whether the leaves of Christ's thorn jujube contained lipids, alkaloids, flavonoids, tannins, and saponins. [20, 21]

Cell line
Hela cancer cell line (human cervix carcinoma) were purchased from the Iraqi Center for Cancer and Medical Genetic research.

Prepare of cell line
The cells were increased in RPMI 1640, which was supplemented with 10% FCS, 50 IU/ml and 500 g/ml of penicillin and streptomycin, sodium pyruvate, 1 mM of NaHCO3, and 2 mM of L-glutamine, respectively. Before use, finished media was stored at 4 °C after sterilization with 0.22 m microbiological filters. For up to 15 subcultures, cells were expanded and kept alive in RPMI 1640. The sample of each cell line was frozen and kept in liquid nitrogen for some further explanation.

Cytotoxicity test
Using an MTT-based fast colorimetric test, the cytotoxic efficacy of the extracts with different polarity against HeLa human carcinoma cell lines was evaluated. [22] Viable tumor cells' mitochondrial enzyme activity converts soluble MTT in this experiment into an insoluble, colored formazan product. The formazan was then dissolved in DMSO and spectrophotometrically quantified at 540 nm. A 96-well microplate was seeded with a 200 ul cell suspension and incubated for 24 h at 37 °C, 5% CO2, and humidified air. Then, 20 ul of each concentration of the various extracts were added, and microplates containing cells and extracts were incubated for a further 48 h under the same circumstances. The drug topotecan served as a positive control(Hycamtin).
In the negative control wells, just the media-bound cells were present; neither extracts nor topotecan were. Each well was treated with 30 of the MTT solution (10 mg/ml in PBS) before being incubated for 3 hours to determine cell survival. Then, any formazan crystals were gently dispersed by pipetting 150 ofDMSO into the old medium containingMTT, replacing anyMTT that had crystallized. A reader for ELISA plates was used to measure the absorbance at 540 nm (enzyme-linked immunosorbent assay). Three measurements of each extract concentration in 4 wells were taken. For a cervical carcinoma cell line, standard curves (absorbance versus number of cells) were plotted. It was anticipated that some cells would survive.

Statistical Analysis
The results of study data are shown as means and standard errors of the means. Data comparison between treatment groups was done using a one-way analysis of variance. At P

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0.05, the data were deemed statistically significant. The analysis was conducted using the GraphPadPrism 6program (GraphPadSoftware, Inc., San DiegoCalifornia).

3. RESULTS

Phyto-chemical studies

Table 1 displays the phytochemical analysis of leaf extracts from Christ's Thorn Jujube. These results showed the presence of lipids, alkaloids, flavonoids, and saponin tannins.

NO.	Chemical compounds	Colorimetric test	Results (+ / -)
1-	Tannin	aquamarine	Positive +
2-	lipids	Thick red sediment color	Positive +
3-	Alkaloids	Wight residuum	Positive +
4-	Flavonoids	Yellow	Positive +
5-	Saponins	Bubble reddish	Positive +

Table (1): Colorimetric test for identification of chemical compounds of leaf plant extract

Cytotoxic effect of Christ's thorn Jujubeextracts

The absorbance and cell count had a good linear relationship, as indicated by the standard curves for cervix carcinoma cell lines (r2 = 0.992 and 0.980, respectively) [Figures 2]. As a positive control, the well-known cytotoxic antibiotic topotecan (Hycamtin) (20 g/ml) significantly reduced the growth of the Hela cell lines to less than 25%. When cell viability dropped to less than 50%, extracts were deemed to be cytotoxic.

The MTT-based cytotoxicity method was used to assess the cytotoxic effects of various Christ's thorn Jujube extracts on HeLa cells. Hela's viability was significantly and concentration-dependently decreased by hexane and aqueous extracts of Christ's thorn jujube (P 0.05, Figure 3). Christ's Thorn Jujube extracts were more effective in these cell lines at these concentrations

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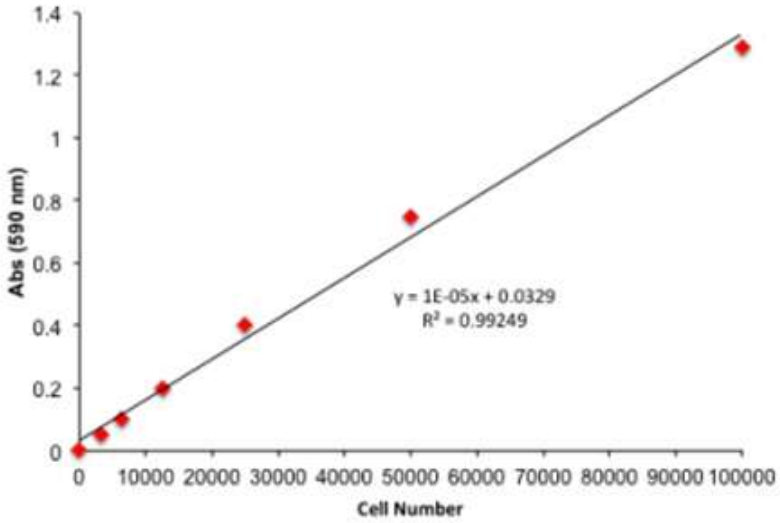

Figure 2: MTT cell replication assay HeLa cervix carcinoma cell line after incubation,cells were treated with MTT stop solution ,as described in the protocol.

4. DISCUSSION

Most disease causes death in world; particularly in many developed nations, is cancer. Thorne Jojobi has been found to have anti-cancer properties in some varieties. [2] In any case, scientists have been very interested in the announcement of new anti-cancer medications over the past few years. There are several types of cancer treatments and various anti-cancer drug categories. One of these drug classes is cytotoxic medication, which is crucial due to the presence of various and highly complex chemicals found in plants, such as alkalis, flavonoids, fat, saponins, and tannins, which are significant sources of cytotoxic agents. Anti-cancer compounds have been found in more than 3,000 different plant species. [7]

In this investigation, we used the MTT test to examine the cytotoxic effects of various Christ's thorn jujube extracts on Hela. Hela and other well-known tumor lines are frequently used to assess cellular toxicity. [22 25] HeLa cell standard curves were created, and they demonstrated a strong correlation between cell number and absorption. The efficacy of the approach was demonstrated by the fact that the well-known cytotoxic medication topotecan severely reduced the vitality of both lines. Hexane and water were used for extraction to break up the various polarizations of the compounds in this plant. The HeLa cell line used to model human cervical cancer was highly toxic to all of the extracts tested.
According to some earlier studies, plant extracts may be able to cause Bax-mediated programmed cell death in addition to caspase-mediated mitochondrial death (Independent BAX) (caspases 3 and 9 were stimulated) as the primary pathway for cell death (18). Similar

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outcomes were seen when quercetin was administered for 24-72 hours to the human HT-29 and COLO-201 colorectal cancer cell lines. Total reactive oxygen species (ROS) were measured in cells using the cellular flow measurement techniques detailed in Annex V, and caspase 3/8 and cleft PARP were measured in cells using western blotting. The information showed that total reactive oxygen species and the programmed death marker were linearly correlated over time (31). The importance of this process at clinically relevant low concentrations has not yet been fully evaluated. In general, relatively high concentrations of alkaloids and other substances appear to be required to induce cultured cell death.

However, numerous studies have demonstrated that various tumor cell lines respond differently to cytotoxic agents. [22,26] The following extracts were most effective against HeLa cells in the following order: HCH and liquid Christ's spinal grape extracts in chloroform and methanol may have cytotoxic effects due to the presence of triterpenoidsaponin, phytonutrients like rutin and quercetin, cyclopeptide alkaloids like spinanine A, and triterpenoidsaponin. [18,27] Better chloroform and methanol chloroform extraction of alkaloids like jubanin A, amphibians E, and spinanin A may account for the extracts' greater effects on the two cell lines under study. ([28]) Numerous cell lines have demonstrated the potent cytotoxic action of certain alkaloids. [29.30] The saponins, including betulic acid, are responsible for this butanolic extract's poisonous effects. [31]

Christ's Thorn Jujube is a viable option for further study to develop new cytotoxic medicines since, according to the study's findings, it includes chemicals with cytotoxic activity.

5. CONCLUSIONS

We concluded by reporting that Iraqi Christ's Thorn Jujube L. extract is a very promising candidate for cancer therapy for clinical application because it is a selective broad-spectrum anti-cervix carcinoma agent and has no toxic effect on normal cells.

Ethical Clearance

The Ministry of Environment and Health, as well as the Ministries of Higher Education and Scientific Research in Iraq, have ethically approved the research conducted there.

Conflict of Interest: The authors affirm that they do not have any competing interests.
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