Ethanolic extract of *Thuja occidentalis* blocks proliferation of A549 cells and induces apoptosis *in vitro*

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**OBJECTIVE:** To study the possible anticancer and antiproliferative activities of ethanolic leaf extract of *Thuja occidentalis* (TO) on A549 non-small lung carcinoma cells *in vitro*

**METHODS:** Cell viability was ascertained through 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay after deployment of TO in different doses. The half maximal inhibitory concentration (IC₅₀) dose (282 μg/mL) was determined, and two other doses for dose-dependence study, one below the IC₅₀ dose (IC₅₀ = 188 μg/mL) and one above the IC₅₀ dose (IC₅₀ = 376 μg/mL) were selected. Bromodeoxyuridine (BrdU) incorporation assay and migration studies were performed to elucidate antiproliferative activity of the drug, if any. Fluorescence-activated cell sorting analysis after annexin V-fluorescein isothiocyanate and propidium iodide (annexin V-FITC-Pi) dual staining method was done to ascertain early stage of apoptosis. If any, DNA fragmentation assay was done through Hoechst 33258 and acridine orange-ethidium bromide staining. DNA damage was quantified through comet assay. Bax-Bcl2 regulation and expression studies were performed through indirect enzyme-linked immunosorbent assay (ELISA). Caspase 3 activity was measured at gene level through reverse transcription-polymerase chain reaction (RT-PCR) analysis. Its activation at protein level was analyzed through indirect ELISA and Western blot analysis.

**RESULTS:** TO demonstrated a dose-dependent decrease in viability of A549 cells after 24 h of exposure. Cell proliferation was reduced in a time-dependent manner of drug exposure as revealed from BrdU incorporation and migration studies. Annexin-V-FITC positivity of cells up to 11.72% as compared to the untreated control revealed early state of TO-induced apoptosis. Occurrence of comet tail and increased fluorescence of Hoechst after 24 h of drug exposure revealed significant

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DNA nick generation and chromatin condensation. Bax up-regulation and Bcl-2 down-regulation suitably altered ratio of Bax/Bcl-2 in favor of apoptosis. From RT-PCR, indirect ELISA and Western blot studies, caspase 3 activity was also found to be significantly increased along with cleaved poly ADP-ribose polymerase expression.

**CONCLUSION**: Ethanolic leaf extract of TO demonstrated apoptotic and antiproliferative potentials against A549 cell line.

**KEYWORDS**: *Thuja*, plant extracts, carcinoma, non-small-cell lung, cell proliferation, apoptosis, antineoplastic agents, phytoxic, in vitro

Lung cancer is one of the major threats among all types of cancer, aggregating to almost 1.4 million cases per year\(^1\). Lung cancer is mainly differentiated into two categories, namely, small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC).

Although several chemotherapeutic approaches can be attempted against SCLC, therapeutic scope for NSCLC is limited as it has a very narrow range of chemotherapeutic sensitivity. The drugs which are found to be relatively effective are generally cytotoxic against normal cells, precluding their therapeutic use. So new drugs which are relatively less cytotoxic to normal cells but have anticancer and antiproliferative effects on NSCLC are highly sought after.

*Thuja occidentalis* (TO) is one of the major herbs largely produced in part of Europe for its medicinal value. In homoeopathy, ethanolic leaf extract of this plant is used as a mother tincture for treating several diseases including cancer. Pharmacological studies revealed that *Thuja* extract is rich source of terpenoids\(^2\), flavonoids, and tannin-like components\(^3\). Many of these components have been reported to have anticancer property individually. TO has been reported earlier to have anticancer property against skin melanoma cell line\(^4\). It has also been reported as an antitumorigenic agent\(^5\). But activity of TO against NSCLC had not been reported so far.

Apoptosis is a common phenomenon through which a cell undergoes programmed death of itself. Extrinsic and intrinsic pathways either alone or in combination, regulate this apoptotic signalling pathway\(^6\). However, the basic target of any anticancer drug is to induce these apoptotic pathways in a cancer cell. They serve such a function by targeting apoptotic signalling genes and the respective proteins\(^7\).

Thus, the hypotheses to be tested in the present study are: if TO has demonstrable antiproliferative and anticancer potentials *in vitro* against an NSCLC cell line A549; if it had, whether it was cytotoxic to the cancer cells only, but not so against normal human embryonic lung cells (L-132 cell line); whether it is possible to ascertain the signalling pathway by which it accomplished its functions. Incidentally, A549 cells, derived from NSCLC, have several mutations in the genes like KRAS, CDKN2A, etc. that make them more resistant to any anticancer chemotherapeutic drugs, thereby denying practically all chemotherapeutic approaches made so far through the orthodox drug regimen\(^8\).

This is one of the reasons why we became interested to test the efficacy of TO leaf extract on NSCLC cell line A549.

## 1 Materials and methods

### 1.1 Chemicals and reagents

Ethanolic extract of TO, which is also available in the market as a homoeopathic mother tincture, was procured from Boiron Laboratory, France. For culture of A549 and L132 cells, Dulbecco’s modified Eagle’s medium (DMEM) with fetal bovine serum (FBS), penicillin-streptomycin-neomycin (PSN) antibiotic, trypsin and ethylene diamine tetraacetic acid (EDTA) were purchased from Gibco BRL (Grand Island, USA).
NY, USA). Tissue culture plastic wares were obtained from TARSONS. All the antibodies used were obtained from Santa Cruz Biotechnology (USA). All organic solvents used were of high-performance liquid chromatography grade. Hoechst 33258, acridine orange (AO), 3-(4, 5-dimethyl-thiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), ethidium bromide (EB) and all other chemicals used were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Polyvinylidene fluoride (PVDF) membrane for Western blot was procured from Bio-Rad.

1.2 Cell lines Human NSCLC cell line A549 and human normal embryonic cell line L-132 were collected from National Centre for Cell Science, Pune, India. Cells were cultured in DMEM with 10% heat-inactivated FBS and 1% PSN and maintained at 37°C with 5% CO₂ in a humidified incubator. Cells were harvested with 0.025% trypsin and 0.52 mmol/L EDTA in phosphate buffer saline (PBS), plated at required cell numbers and allowed to adhere for 24 h before treatment.

1.3 Cell viability assay A549 cells were dispensed in 96-well flat bottom microtiter plates at a density of 1×10⁴ cells per well for 24 h. They were then treated with various concentrations of TO for 24 h to determine the optimum concentration of TO at which the percentage of cell death was nearly 50% (the IC₅₀ value of the drug). Based on IC₅₀ dose, two doses, one below and one above the IC₅₀ dose, were selected for further study.

The control received no drug. After the incubation 10 μL of MTT solution (1 mg/mL) was added to each well. The intracellular formazan crystals formed were solubilised with acidic isopropanol and the absorbance of the solution was measured at 595 nm using an enzyme-linked immunosorbent assay (ELISA) reader (Multiscan EX, Thermo Electron Corporation, USA). The relative percentage of viability was calculated.

Furthermore, L-132 cells were treated in the same manner and cell viability was analyzed after MTT assay to ascertain whether the drug has any cytotoxicity on the normal cells or not.

1.4 Bromodeoxyuridine incorporation assay A549 cells were dispensed in 96-well flat bottom microtiter plates at a density of 1×10⁴ cells per well. After 24 h of incubation, they were treated with bromodeoxyuridine (BrdU) solution along with three different drug concentrations for 6, 12 and 24 h of exposure, respectively.

After their respective hour of exposure, cells were washed with PBS and fixed with 1% paraformaldehyde solution for 1 h; after washing it again with PBS it was incubated with primary anti-BrdU antibody (1:500 dilution) for 4 h. After that it was incubated with anti-mouse monoclonal secondary antibody (1:500 dilution) for 2 to 3 h. Thereafter, para-nitrophenylphosphate (pNPP) was used as a colour developer and colour intensity was measured at 405 nm with respect to blank.

1.5 Wound-healing assay Cell migration was examined by using wound-healing assay. Cells were cultured in a 6-well plates to almost 100% confluence. At the center of the well a wound was generated by scratching it with a plastic pipette tip. Cell debris was removed by washing it with PBS. After the drug treatment with less than IC₅₀ dose (188 μg/mL) for 6, 12 and 24 h, the plate was placed under a phase-contrast microscope. Reference point was matched, the photographed regions of the first image were aligned and then the second image was obtained.

1.6 Observation of morphological changes A549 cells (1×10⁴ per well) were plated in 6-well culture plates in DMEM supplemented with 10% FBS. Cells were treated with or without TO at a specified concentration. After 24 h, the cells were observed under inverted phase-contrast microscope (Axioscope plus 2, Zeiss, Germany) and the photographs were taken.

1.7 Determination of early apoptosis after annexin V-fluorescein isothiocyanate and propidium iodide dual staining fluorescence-activated cell sorting analysis A549 cells (1×10⁴ per well) were taken into small centrifuge tubes after drug treatment. Cells were spun and pellets were washed with 500 μL binding buffer (10 mmol/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.4; 150 mmol/L sodium chloride; 5 mmol/L potassium chloride; 1 mmol/L magnesium chloride and 1.8 mmol/L calcium chloride). Then cells were harvested and cell pellet was resuspended in 100 μL binding buffer. Annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI)-labelling solutions were added and the cell suspension was incubated for 15 min at room temperature in the dark using Calibur flow cytometer (BD FACS ARIA III) and analyzed by flow cytometric cyclophycyanine software.

1.8 Determination of nuclear fragmentation Cells of 1×10⁴ were treated and then were stained separately with Hoechst 33258 (10 μg/mL) and AO-EB. Then the cells were analyzed under a fluorescence microscope (Axioscope plus 2, Zeiss, Germany) and representative photographs were taken for further qualitative analysis.

1.9 Comet assay After treatment and incubation, the cells were trypsinized and washed in ice-cold PBS. The cell suspension was mixed with an equal amount of 0.75% low-melting agarose and kept at 37°C. The suspension (100 μL) was pipetted on to agarose-precated microscope slide, then covered with cover slip and placed on a glass tray on ice. Thereafter, the slides were immersed in cold lysis solution (2.5 mol/L sodium chloride, 100 mmol/L EDTA, 10 mmol/L Tris, pH 10, with freshly added 1% Triton X-100, 2% dimethyl sulfoxide (DMSO)) followed by incubation at 4°C for 1 h. The electrophoresis in weak alkali (0.3 mol/L sodium hydroxide, 1 mmol/L EDTA, pH 12) at 1 V/cm and 30 mA for 15 min was preceded by a
20 min immersion of the slides in electrophoresis buffer for chromatin unwinding. After electrophoresis the slides were neutralized in 0.05 mol/L Tris buffer. Then it was stained with ethidium bromide (50 μg/mL) for 10 min, washed in distilled water and examined under a fluorescence microscope (Lycal, USA)\(^{11}\).

1.10 Reverse transcription-polymerase chain reaction

Reverse transcription-polymerase chain reaction (RT-PCR) method was performed to evaluate mRNA expression level of caspase 3 taking glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as a housekeeping gene control. Total RNA was extracted according to manufacturer’s instructions using TRIzol reagent. RNA concentration was determined by Orcinol method. One microgram of total RNA was subjected to a RT reaction. Preparation of cDNA and amplification with primer sequences (caspase 3, forward: 5'-AGGGGTCAATTATGCGACA-3', reverse: 5'-TACGGGAACTCTGTTCTTTG-3'; GAPDH, forward: 5'-CAATGATCTCCATGCGTCTAG-3', reverse: 5'-GCGATGAGGGGATGATG-3') were done according to the method of Chakraborty et al\(^{13}\). Fluorescence intensity of band on the agarose gel was measured by using the Image J software.

1.11 Indirect ELISA

Cells of 1×10⁵ were seeded in 60 mm petri dish and allowed to adhere for 24 h. Then TO was treated for 18 h in case of Bax/Bcl2 protein expression and 24 h in case of caspase 3 activity assessment. The assay was done according to the manufacturer’s protocol (Santa Cruz Biotechnology Inc, USA). Protein activity level of Bcl-2, Bax, and caspase 3 was measured by using an ELISA reader. GAPDH served as the housekeeping gene. pNPP was used as a colour developer and colour intensity was measured at 405 nm with respect to blank.

1.12 Western blot analysis

Cells of 1×10⁵ were seeded and allowed to adhere for 48 h. Then the cells were treated with TO for 24 h and homogenates were used for Western blot analysis. Equal amounts (70 μg) of protein were run on sodium dodecyl sulfate polyacrylamide gel electrophoresis and electrophoretically transferred to PVDF membrane. Bound antibodies of caspase 3 (1:1 000) and poly ADP-ribose polymerase (PARP) (1:1 000) were developed by 5-bromo-4-chloro-3-indolyl-phosphate-nitro blue tetrazolium and densitometric quantification of proteins was done by using image J software. GAPDH (1:1 000) was used as housekeeping gene\(^{14}\).

1.13 Statistical analysis

The data were presented as mean±standard deviation. Statistical analysis was performed by one-way analysis of variance using SPSS software. \(P<0.05\) was considered significant.

2 Results

2.1 Effects of TO extract on viability of A549

The effect of TO on cell viability of A549 cells was measured by MTT assay. The results showed viability reduction in response to increasing drug concentrations; the IC\(_{50}\) value was determined to be 282 μg/mL after 24 h of exposure (Figure 1A). Ethanol was used as a drug vehicle control and no significant viability reduction was observed on exposure of it in A549 cells. Meanwhile, exposure of TO on L-132 cells showed cytotoxicity at a negligible level (Figure 1B). For further study on dose-dependence, 188 μg/mL (IC\(_{50}\) designated as D1), 282 μg/mL (IC\(_{50}\) designated as D2) and 376 μg/mL (IC\(_{50}\) designated as D3) of drug doses were taken.

![Figure 1](image-url) Effects of *Thuja occidentalis* ethanolic extract on viability of A549 cell line (A) and L-132 cell line (B)

Cells were exposed to *Thuja occidentalis* ethanolic extract for 24 h at different concentrations and the cell viability was determined by MTT assay. Results are expressed as percentage of viability and data are expressed as mean±standard deviation, \(n=6\); *\(P<0.05\), vs control group. MTT: 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide.
2.2 Effects of TO on proliferating activity of A549 cells. Proliferation activity was blocked after drug induction as revealed from BrdU incorporation assay in a time-dependent manner, rather than in a dose-dependent manner. While there was a palpable amount of inhibitory effect of TO on proliferating activity of A549 cells at 6, 12 and 24 h of exposure, apparently increasing in magnitude with the time of exposure, there was a concomitant destruction of BrdU activity (Figure 2) at a significant level ($P<0.05$). From wound-healing assay it was further evident that TO administration blocked migration of A549 cells successfully and in a time-dependent manner (Figure 3), thus proving evidence of antiproliferative activity of TO extract.

![Figure 2](image)

**Figure 2** Effects of Thuja occidentalis ethanolic extract on cellular proliferation tested by BrdU incorporation assay.

Cells were exposed to three different concentration of *Thuja occidentalis* ethanolic extract for 6, 12 and 24 h along with incorporation of BrdU. Incorporated BrdU was labelled with anti-BrdU antibody and intensity was measured at 405 nm using PNPP as a colour developer. Data are expressed as mean ± standard deviation, $n=6$, *P* < 0.05, vs normal control group. D1: 188 µg/mL; D2: 282 µg/mL; D3: 376 µg/mL; BrdU: bromodeoxyuridine; PNPP: paranitrophenylphosphate.

2.3 Effects of TO on cellular morphology of A549 cells. The morphological changes of A549 cells treated with TO were observed with respect to untreated ones. Cell shrinkage and blebbing, which are the hallmark features of apoptotic cell death, were observed after drug induction (Figure 4).

2.4 TO-induced apoptosis shown by annexin-V positivity. The early state of apoptosis was evaluated by flow cytometry after annexin-V-FITC assay. To confirm the preceding observations, we found an increase of annexin-V-positive cells in TO-treated A549 cells as compared to the untreated control. This increase is dose-dependent in nature as the highest drug dose shows higher level of annexin-V positivity than the others (Figure 5).

![Figure 4](image)

**Figure 4** Analysis of cellular morphology by a phase-contrast microscope (Light microscopy, ×20).
D1: 188 µg/mL; D2: 282 µg/mL; D3: 376 µg/mL.

![Figure 5](image)

**Figure 5** FACS analysis after annexin V-fluorescein isothiocyanate and propidium iodide dual staining assay
D1: 188 µg/mL; D2: 282 µg/mL; D3: 376 µg/mL.
2.5 Effects of TO on nick generation and fragmentation of DNA. Hoechst 33258 staining, which is sensitive to DNA, was used to quantify DNA nick generation, if any, after drug administration. An increase in fluorescence of Hoechst 33258 staining was observed after exposure of A549 cells to different drug doses for 24 h (Figure 6A).

Comet assay revealed an increased length of comet tail (Figure 6B). AO-EB staining produced changes in fluorescence pattern from green (normal cellular DNA) to orange (nicked cellular DNA) along with increased fluorescence intensity of EB (Figure 6C). Collectively, these results indicate that on administration of TO, cellular DNA gets fragmented and nick generation occurs in a dose-dependent manner.

2.6 Effects of TO administration on Bax-Bcl2 protein expression. ELISA assay showed significant \( (P < 0.05) \) up-regulation of Bax and down-regulation of Bcl2 (Figure 7) after drug induction for 18 h; this kind of change in Bax-Bcl2 ratio leads the cells into apoptosis\(^{12}\).

2.7 Effects of TO on expression level of caspase 3 and PARP activity. Results of RT-PCR analysis revealed that caspase 3 gene activity was up-regulated after drug induction (Figures 8A and 8B). This was further confirmed by results of the ELISA assay (Figure 8C) and Western blot analysis (Figure 9).

Subsequently, increased expression of cleaved PARP was also observed in the drug-treated cells as compared to the untreated ones (Figure 9). The expression of cleaved PARP was generally found to be increased in the drug-treated lots when compared with the untreated ones, but when they were further critically analyzed, the degree of increase was not found to be strictly dose-dependent, as the higher doses did not produce proportionately greater effect when compared with the lower doses. This would possibly imply that the initiation of DNA fragmentation could be triggered at all the concentrations of the drugs, including the one at the lowest dose.

3 Discussion

Complementary and alternative medicines are now considered to play an emerging role as cancer chemopreventive therapy\(^{13}\). Since cancer cells are known to attain immortalization, induction of apoptosis in them by a drug is considered to have a great anticancer impact, and a drug showing specific killing effect on the cancer cells, but sparing the normal ones, is the one which is highly solicited in anticancer drug formulation. In the present study, the administration of TO appeared to increase the incidence of apoptosis in A549 cells, but it was not significantly cytotoxic to the normal human embryonic lung cell line L-132. Therefore, we were tempted to study the exact signalling

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**Figure 6** Assessment of DNA damage by Hoechst staining, comet assay, and acridine orange-ethidium bromide staining assay (Fluorescence microscopy, \( \times 40 \))

DNA nick generation was indicated by Hoechst 33258 (A), increasing comet tail length (B), and acridine orange-ethidium bromide fluorescence (C). Changing colour pattern of acridine orange from green (control) to slightly orange (drug treated) indicates significant DNA fragmentation in a dose-dependent manner. White arrows indicate fragmented nuclei of the apoptotic cells. D1: 183 \( \mu \text{g/mL} \); D2: 283 \( \mu \text{g/mL} \); D3: 373 \( \mu \text{g/mL} \).
Figure 7  Effects of TO on Bax/Bcl2 expression at protein levels

Significant Bax up-regulation and Bcl2 down-regulation were observed by enzyme-linked immunosorbent assay after drug treatment for 18 h in A549 cells. Results are expressed as percentage of control and data are expressed as mean±standard deviation. n=6; * P<0.05, vs control group. D1: 188 µg/mL; D2: 282 µg/mL; D3: 378 µg/mL.

Figure 8  Caspase 3 expression analysis after drug treatment at different doses

Caspase 3 gene expression was elevated after drug treatment as revealed from RT-PCR band (A) and their relative intensities (B). Caspase 3 expression at protein level was also increased as revealed from indirect ELISA analysis (C). Band intensities of RT-PCR and protein expression by ELISA assay were expressed as percentage of control and data are expressed as mean±standard deviation, n=6; * P<0.05, vs normal control group. GAPDH was used as a housekeeping gene control. D1: 188 µg/mL; D2: 282 µg/mL; D3: 378 µg/mL; RT-PCR: reverse transcription-polymerase chain reaction; ELISA: enzyme-linked immunosorbent assay; GAPDH: glyceraldehyde 3-phosphate dehydrogenase.

Figure 9  Immunoblot analysis of caspase 3 and PARP

Expression of caspase 3 and PARP was increased as revealed from Western blot band (A) and their relative intensities (B). Band intensities of Western blot analysis were expressed as percentage of control and data are expressed as mean±standard deviation, n=6; * P<0.05, vs normal control group. GAPDH was used as a housekeeping gene control. D1: 188 µg/mL; D2: 282 µg/mL; D3: 378 µg/mL; PARP: poly ADP-ribose polymerase; GAPDH: glyceraldehyde 3-phosphate dehydrogenase.
mechanism by which TO accomplishes this task. TO administration showed cytotoxicity against A549 cells in vitro in a dose-dependent manner. It showed membrane blebbing and distinct morphological changes as observed from cell morphological analysis, suggesting thereby that the cytotoxicity originated possibly more due to apoptosis than due to necrosis. This contention could be verified by the data of FACS analysis after annexin-V-FITC-PI staining which confirms early stage of apoptosis and furthermore, DNA fragmentation, as suggested in earlier studies\(^\text{[17]}\). Successful DNA nick generation after drug exposure was revealed from increased fluorescence of Hoechst and AO-EB staining against untreated control. Additionally, comet tail formation showed significant amount of DNA fragmentation as well.

Proliferation is the major property of cancer cells that pushes them to migrate and invade other tissue. Therefore, the therapy can be effective if this proliferation of cells can be blocked, and apoptosis can be induced in them as a result of drug treatment\(^\text{[18]}\). In this investigation, TO was also found to block proliferation significantly in a time-dependent manner, as revealed from BrdU incorporation study. TO appeared to block proliferation at 6, 12 and 24 h time points of drug exposure. This modulation is very much time-dependent in action, rather than dose-dependent. Migration assay helps us also to conclude that at different time points of fixation, the drug blocks A549 cell proliferation and migration.

Pro- and anti-apoptotic proteins regulate cell survivability by maintaining its proper balance in a regulated manner. Therefore creating alteration in major pro- and anti-apoptotic proteins is the major target area of any anticancer drug\(^\text{[19]}\). Consistent with this, from indirect ELISA assay, it was revealed that up-regulation of Bax and down-regulation of Bcl-2 occurred after drug exposure in A549 cells.

Caspace activation is also a marked phenomenon of apoptotic cell death\(^\text{[20]}\). From RT-PCR, indirect ELISA assay and Western blot analysis it was found that expression of caspase 3 gene and its protein product in A549 cells increased significantly after drug treatment. Subsequently cleaved PARP, which is the downstream effectors of caspase 3 was also found to be increased in its expression after drug induction. Similarly, results of comet assay and Hoechst fluorescence study also revealed that DNA fragmentation was possibly enhanced due to caspase 3 and cleaved PARP expression after administration of TO.

In summary, our study shows that TO extract induces apoptotic cell death by altering the balance between Bax/Bcl2 expression and activation of caspase 3. TO also blocks cell proliferation in a time-dependent manner. Through blocking proliferation and activating caspase 3 it induced significant DNA fragmentation that could lead to A549 cell death. Therefore, it could be concluded that TO extract has antiproliferative and anticancer activities against A549 cells having several somatic mutations which raise their immortality and resistance against several drugs. Thus, the results of this study would validate its use as a supportive medicine against NSCLC. However, further indepth studies are needed to ascertain if any particular isolated compound from this plant extract could have more specific anticancer property or not.

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5 Competing interests

The authors declare that they have no competing interests.

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北美香柏叶的乙醇提取物阻断 A549 细胞增殖
并引起细胞凋亡的体外研究

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目的: 研究北美香柏叶的乙醇提取物对非小细胞肺癌 A549 细胞的抗肿瘤及抗增殖作用。

方法: 喷雾蒸馏法提取不同溶剂北美香柏叶的乙醇提取物对细胞活性的影响。确定半数最大抑制浓度为 282 μg/mL，另外选择两个浓度 188 μg/mL 和 376 μg/mL 进行剂量依赖性检测。进行脱氧尿苷结合实验和细胞迁移实验检测细胞的抗肿瘤细胞增殖活性。膜联蛋白 V-异硫氰酸荧光素-碘化丙啶双染色后采用荧光激活细胞分类检测仪对细胞早期凋亡进行检测。Hoechst 33258 及叮啶橙-溴化乙啶荧光染色法进行 DNA 片段分析。间接流式细胞仪分析法对 Bax-Bcl2 的调节和表达情况。逆转录聚合酶链反应检测 caspase 3 基因表达情况，其活性水平与表达水平的变化则使用间接流式细胞仪分析法和蛋白印迹法进行检测。

结果: A549 的细胞活性在暴露于北美香柏叶的乙醇提取物 24 h 后呈剂量依赖性下降。脱氧尿苷结合实验和细胞迁移实验表明细胞增殖活性与暴露于药物的时间有时间依赖性关系。11.72% 的细胞在单染色后呈阳性反应，说明药物引起了细胞的早期凋亡。药物作用 24 h 后 DNA 片段呈星状的出现以及 Hoechst 33258 荧光染色的增加提示显著的 DNA 缺口出现以及染色质凝聚。Bax 的上调及 Bcl2 的下调表明了细胞凋亡的出现。逆转录聚合酶链反应、间接流式细胞仪分析法和蛋白印迹法的检测结果表明 caspase 3 的活性随着抗 (A) Bap-2-磷酸-核糖) 聚合酶的表达的增加而增加。

结论: 北美香柏叶的乙醇提取物能够促进 A549 细胞凋亡并抑制其增殖活性。

关键词: 香柏属植物；提取物；癌，非小细胞肺；细胞增殖；细胞凋亡；抗肿瘤药；植物；体外研究