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Research Article

Cytotoxicity of *Taxillus tomentosus* and *Spilanthes calva* in Different Cancer Cell Cultures *In-vitro*

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ABSTRACT

Cancer is one of the leading diseases to cause death globally. Most of the synthetic drugs used to treat cancer are having serious side effects. So, there is a need to develop the drugs which are used to treat cancer with little side effects. This can lead to investigating the plant products having fewer side effects, for their anti-cancer activity. The present investigation evaluated the in vitro toxicity of leaf extracts and fractions of *Spilanthes calva* and *Taxillus tomentosus* in 5 totally different neoplastic cell cultures, like HeLa (cervical cancer cells), HEK 293 (kidney cancer cells), MCF-7 (breast cancer cells), A549 (lung cancer cells) and Hep G2 (hepatic cancer cells) by 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and lactate dehydrogenase (LDH) release assays. Among all the test extracts and fractions, petroleum ether and n-hexane fractions of *S. calva*, and ethyl acetate and n-hexane fractions of *T. tomentosus* have been shown maximal cytotoxic activities against all tested cell cultures. The IC50 values of SCPEF, SCNHF, and TTEAF were found to be 41.79, 42.26 and 41.01 µg/mL on HEK 293 cells, which were very close to standard drug PCTXL has been found to be 35.22 µg/mL on HEK 293 kidney cancer cells. The TTNHF IC50 value was found to be 39.29 µg/mL which is comparable with the standard paclitaxel (PCTXL) 39.18 µg/mL on A549 lung cancer cells.

Introduction

Cancer is one of the most dangerous diseases found and is the second reason for death worldwide characterized by uncontrollable cellular growth.[1] Therapies for cancer malady embrace surgery, chemotherapy, radiation therapy, and hormonal therapy have shown restricted success and have some serious aspect effects. Thus, it's necessary to use different methodologies for the treatment of cancers.[2,3] The assumption that natural materials are safer than artificial medication has a basis for the extraordinary development in human exposure to natural medicines like plants, phytotherapeutic agents, and phytopharmaceutical products.[4] Research has developed into assessing the potential properties and uses of plant extracts for the treatment of cancers In parallel, there is huge evidence for the potential of plant-based products as inhibitors of various stages of cancers.[5,6]

To date, many plant-based compounds have been identified that have anti-cancer properties, such as, inhibition of cell proliferation and induction of apoptosis which finally reduce the cancer risk. Furthermore, many phytochemicals have been known that can inhibit tumor progression via various mechanisms.[7-10] Paclitaxel, Vincristine and vinblastine are well-known examples of derived from plants clinically useful anti-cancer drugs. Free radical scavenging properties of different plant extracts are also of great importance because herbal compounds with free radical scavenging activity can protect against different cancers.[5] Plant-derived natural drug merchandise have received important attention in recent years, thanks to their numerous pharmacologic assets as well as cytotoxic and cancer chemo-preventive effects. Therefore, knowing and assessing the potentials of plant-derived bioactive compounds is

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very important for additional drug development.[11,12] *Spilanthes calva* belongs to the family *Asteraceae*, is an herb having 60 cm in height. The plant is having high medicinal values and is used for the treatment of various diseases. This plant is having anti-ageing properties, antimicrobial and antifungal activities, alleviates toothache and dysentery, as well as enhances immunity. The plant leaves stimulate salivation, which is due to containing an active chemical spilanthol.[13,14] *T. tomentosus* is one of the plants that belong to the family *Loranthaceae*. Recent studies revealed that this plant have diverse pharmacological activities, such as antidiabetic, hepatoprotective, neuroprotective, cardioprotective, antistress, nootropic, and anti-urolithiasis activities.[15-17]

In this research, we tend to assess the *in vitro* toxicity of leaf extracts and fractions of *S. calva* and *T. tomentosus* using five different cancer cell cultures, such as HeLa (cervical cancer cells), (HEK 293) (Human embryonic kidney cancer cells), MCF-7 (breast cancer cells), A549 (lung cancer cells) and Hep G2 (hepatic cancer cells) by 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and lactate dehydrogenase (LDH) leakage assays.

Materials and Methods

Chemicals

The fetal bovine serum, DMEM/RPMI 1640 culture media, L-glutamine, penicillin-streptomycin antibiotic solution, phosphate buffer saline (PBS), trypsin-EDTA, and ascorbic acid were purchased from Himedia, India. MTT was purchased from Sigma-Aldrich, Banglore, Karnataka, India. LDH assay kit was acquired from Cayman Chemicals, USA. All the neoplastic cell cultures were procured from National Centre for Cell Sciences (NCCS), Pune, Maharashtra, India.

Plant Collection, Extract Preparation and Fractionation

The recent healthy, sickness-free leaves of *S. calva* and *T. tomentosus* were collected from the hills of Tirumala, Tirupati, India, and were authenticated by Dr. M. Madhava Chetty, Department of Biological Sciences, Sri Venkateswara University, Andhra Pradesh, India. The leaves of each plant were shade dried for 2 weeks and ground into a rough powder employing a grinder. The 100 g of powder of all plant leaves were macerated for 24 hours with continuous stirred in 500 mL of ethyl alcohol employing a shaker at 28°C. Then, the supernatant was recovered by filtration through muslin cloth and Whatman paper.

Further, the filtrates were utterly dried by a rotary vacuum evaporator. The solvents were evaporated to dryness at room temperature to produce the ethanol extracts of both plants. The ethanol extracts of *S. calva* (SCEE) and *T. tomentosus* (TTEE) were subjected to fractionation by partitioning the aqueous suspension of the drug with petroleum ether, ethyl acetate, and n-hexane to get respective fractions such as petroleum ether fraction (SCPEF and TTPEF), ethyl acetate fraction (SCEAF and TTEAF), n-hexane (SCNHF and TTNHF) and aqueous fraction (SCAF and TTAF). Further, the extract and fractions were stored at -4°C until further use. Both plants' different concentrations of methanolic extract and their fractions (10, 20, 40, 80, and 160 µg/mL) were prepared in 1% DMSO for determining in vitro cytotoxicity.

Cell Culture and Treatment

HeLa, HEK 293, MCF-7, A549, and Hep G2 cells were obtained from NCCS, Pune. These cells were used between passages ten and fifteen. All these cells were fully grown in culture media (RPMI 1640/DMEM) along with 10% fetal bovine serum (FBS), 1% of L-glutamine, and 1% of the penicillin-streptomycin antibiotic solution. The neoplastic cells were planted at 2,50,000 cells/flask within the total volume of 10 mL. After sufficient growth, all the cells were trypsinized using trypsin-EDTA.

The cells were seeded in 96 well plates (Parsons, India) at the rate of 1.0 x 104 cells/100 µL. All the neoplastic cell lines were maintained in a 5% CO_2 + 95% O_2 incubator (WTC Binder, Germany) at a temperature of 37°C. The test extracts and their fractions were prepared in 1% DMSO. Various concentrations of test extracts, fractions, and standard drug, paclitaxel (PCTXL) in the corresponding culture medium were freshly ready and used for *in vitro* cytotoxic activity.

In-vitro Cytotoxicity by MTT Assay

The impact of *S. calva* and *T. tomentosus* test extracts and fractions on the cellular propagation and viability was decided by using the MTT assay technique. For performing this assay, the neoplastic cells were seeded in 96 well plates and each well solvent/extract/fraction solution (0.1 mL, in triplicate) in respected media was added.

This 96 well plate was subsequently incubated at 37 ± 2°C for 48 h in a 5% CO_2 incubator and the MTT (5 mg/mL) was added to all wells. Further, the 96 well plate was another time incubated for 2 hours, and DMSO (80 µL) was added to every well, the microtiter plate was enveloped with aluminum foil to avoid the oxidation of the MTT dye and the microtiter plate was kept on a rotary shaker (Remi equipment's, India) for 2 hours. The resulting absorbances were documented using the enzyme-linked immunoassay (ELISA) reader (Anthos, Germany) at 562 nm.[18,19] The absorbance produced by the test extracts and fractions compared with the solvent control to calculate the percent cytotoxicity.

LDH Leakage Assay

Lactate dehydrogenase is a soluble enzyme that contains zinc and is situated in the cytosol of cells. This enzyme is released/leaked into the surrounding culture medium only upon cell death, may be used as a symbol of cell

wall integrity, and therefore a measurement of cell toxicity. For this assay, the cells were seeded in 96 well microtiter plates at a density of 1.0×10^4 cells/well in100 µL of corresponding culture medium. 100 µL of growing concentrations of test extracts/fractions/standard drug were added to each well in triplicate. Subsequently, the microtiter plate was incubated in a 5% CO_2 incubator at 37 ± 2°C for 48 hours.[20] The supernatants of microtiter plate continued shifted into new 96 well microtiter plates and assayed giving to the producer's protocol.

Statistical Analysis

The IC50 was calculated as soon as at least 2 viability values were beneath 50% of the control condition, utilizing the GraphPadPrism software. The statistical analysis remained performed employing one-way ANOVA subsequently Bonferroni posttests. The data were represented as mean ± standard deviation (S.D.) of 3 independent experiments. Test significance was designated by *p < 0.01 and ** p < 0.001 compared to control.

Results

MTT Assay

The *in vitro* toxicity of leaf extracts and fractions of *S. calva* and *T. tomentosus* using different cancer cell cultures by MTT assay were performed and calculated the IC50 values were shown in Table 1. The percent cytotoxicity induced by *Spilanthes calva* and *T. tomentosus* plant extracts and fractions were shown in Figs. 1 and 2. The percent cytotoxicity was increased in a dose-dependent manner related to all tested cancer cell cultures for both plant extracts and fractions tested.

LDH Leakage Assay

The cytotoxicity of *S. calva* and *T. tomentosus* using different cancer cell cultures by LDH leakage assay were performed and shown in Fig. 3 and 4, respectively. The leakage of LDH is increased in a dose-dependent manner.

Discussion

The plant-based most natural products efficiently induce cytotoxicity in neoplastic cells are promising to play a substantial role in managing and curing cancer. Various reports showed several extracts, fractions, and isolates derived from medicinal plants demonstrated their anti-cancer activities.[21] So, the present experiment investigated the potential in vitro cytotoxicity induced by the leaf extracts and fractions of *S. calva* and *T. tomentosus* using different cancer cell cultures, such as HeLa (cervical carcinoma cells), HEK 293 (Human embryonic kidney carcinomas cells), MCF-7 (breast carcinoma cells), A549 (lung carcinoma cells) and Hep G2 (hepatic carcinoma cells) by MTT and LDH leakage assays.

Our MTT assay study reveals that both plant extracts and fractions were shown increased cytotoxic activity in a dose-dependent manner. The SCPEF, SCNHF, TTEAF, and TTNHF fractions have been shown to promise cytotoxic activities in all the cell cultures tested. For *S. calva*, petroleum ether and n-hexane fractions showed the highest cytotoxic activities compared to other fractions and ethanolic extract. Also, both SCPEF and SCNHF have shown maximum cytotoxic activity towards HEK 293 cells so that these fractions have more cytotoxicity against kidney cancer cells. For *T. tomentosus*, fractions of n-hexane and ethyl acetate showed superior cytotoxic activities compared to other factions.

TTEAF has been shown maximum cytotoxicity towards HEK 293 cells, whereas TTNHF has been shown maximum cytotoxic activity towards A549 cells. So that, TTEAF has more cytotoxic activity against kidney cancer cells, whereas TTNHF has more cytotoxicity against lung cancer cells. The IC50 values of SCPEF, SCNHF, and TTEAF were found to be 41.79, 42.26 and 41.01 µg/mL on HEK 293 cells, which were very close to standard drug PCTXL is 35.22 µg/mL on HEK 293 kidney cancer cells. The TTNHF IC50 value was 39.29 µg/mL, which is comparable with the

Table 1: IC50 values (µg/mL) of various extracts on different human cell cultures

	IC50 values in different cell lines (µg/mL)				
Plant extracts	*HeLa*	*HEK 293*	*A549*	*MCF 7*	*Hep G2*
SCEE	46.52	47.83	47.70	48.11	48.77
SCPEF	46.81	41.79	43.86	46.37	47.26
SCEAF	53.07	49.56	47.93	51.97	51.67
SCNHF	45.16	42.26	43.34	46.38	51.60
SCAF	50.18	52.27	56.26	48.85	48.52
TTEE	52.65	50.17	50.23	52.29	48.42
TTPEF	55.88	48.71	46.32	55.13	50.48
TTEAF	47.94	41.01	43.86	47.78	44.36
TTNHF	43.06	42.26	39.29	44.49	46.83
TTAF	54.56	54.30	59.73	51.56	49.66
PCTXL	40.84	35.22	39.18	41.04	39.94

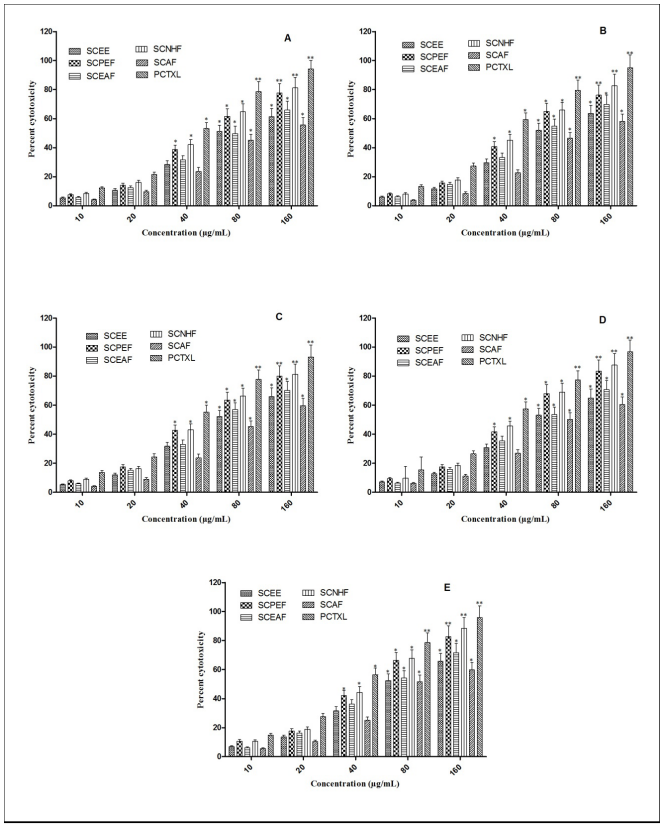

Fig. 1: Percent cytotoxicity measured by MTT assay on (A) HeLa, (B) HEK 293, (C) A549, (D) MCF-7, and (E) Hep G2 carcinoma cells, after treatment with different extracts of *Spilanthes calva* (10-160 µg/mL) for 48 h

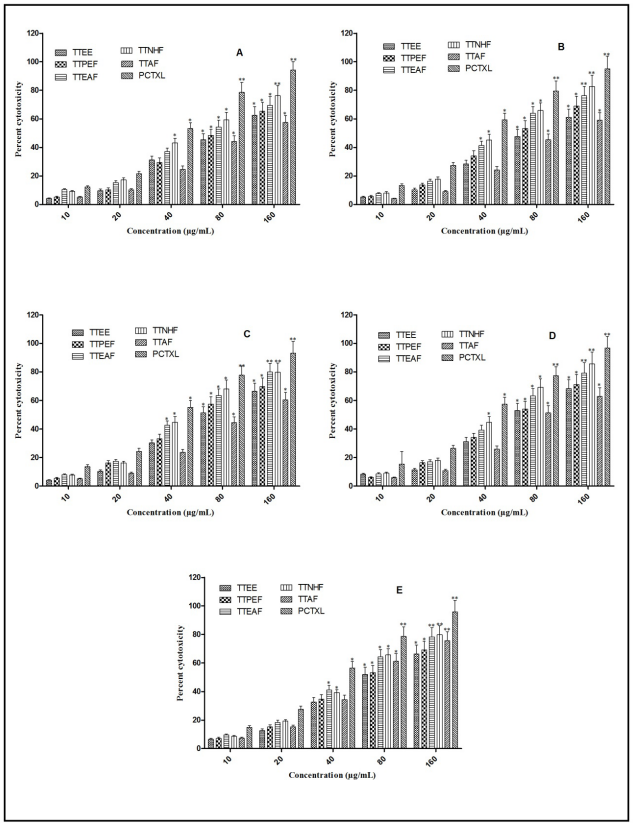

Fig. 2: Percent cytotoxicity measured by MTT assay on (A) HeLa, (B) HEK 293, (C) A549, (D) MCF-7, and (E) Hep G2 carcinoma cells, after treatment with different extracts of *Taxillus tomentosus* (10-160 µg/mL) for 48 h.

standard PCTXL 39.18 µg/mL on A549 lung cancer cells.

Effect of anti-cancer activity raised considered by using MTT assay on HT- 29 cell lines of ethanolic extract of *Reissantia indica*. Different quantities of plant extracts and standard remained taken, and existing into cancer cells remained recorded at 24 hours respectively. It visibly showed U's the dose needed for the inhibition of cells. The utmost potent anti-cancer activity is shown colon cancer cell line at the concentration of 1000 µg/mL of *R. indica* extract on HT-29.[5] Dikamaliartane, a plant product assessed for the cytotoxic activity by MTT assay on HeLa and MCF 7 cell lines, and the study revealed the potential for its cytotoxic activity against tested cancer cell lines. These studies support our cytotoxic activity results using MTT assay with various cell cultures.

S. calva and *T. tomentosus* extracts and fractions upon treatment with different cancer cell cultures revealed the dose-dependent leakage of LDH from the cells leading to cytotoxicity. As LDH is an enzyme present in the cytoplasm in cells, can release into extracellular sites only after cell membrane damage leading to cell mortality, an indication of cytotoxicity.[18] Similar to cytotoxicity induced through MTT assay, SCPEF, SCNHF, TTEAF, and TTNHF fractions have been shown increased leakage of LDH in all the cell cultures tested. For *S. calva*, petroleum ether and n-hexane fractions showed the highest LDH leakage, whereas, for *T. tomentosus*, ethyl acetate and n-hexane fractions showed superior LDH leakage, which was comparable to the standard drug paclitaxel.

One of the studies investigated the in vitro cytotoxic activity of methanol extracts of *Morus alba*, *Eucalyptus camaldulensis*, *Zataria multiflora* and *Cichorium intybus* against P19 embryonal cancer cells using various cytotoxic assays like MTT, LDH leakage, etc. In this investigation, *Morus alba* showed the highest cytotoxicity, whereas *C. intybus* showed the least cytotoxicity.[2] Other studies also revealed that the plant extracts and their fractions have shown the in vitro toxicity against different types of carcinoma cell lines using MTT and LDH leakage assays[22,23] supports our results. Upon treatment with five different carcinoma cells, *S. calva* and *T. tomentosus* extracts and fractions showed the potent in vitro cytotoxic activities in a concentration-dependent manner. Among all the test extracts and fractions, SCPEF, SCNHF, TTEAF, and TTNHF fractions have been shown maximal cytotoxic activities against tested cell cultures by using both MTT and LDH assays. These fractions upon further research may be useful for future drug candidates to treat cancer.

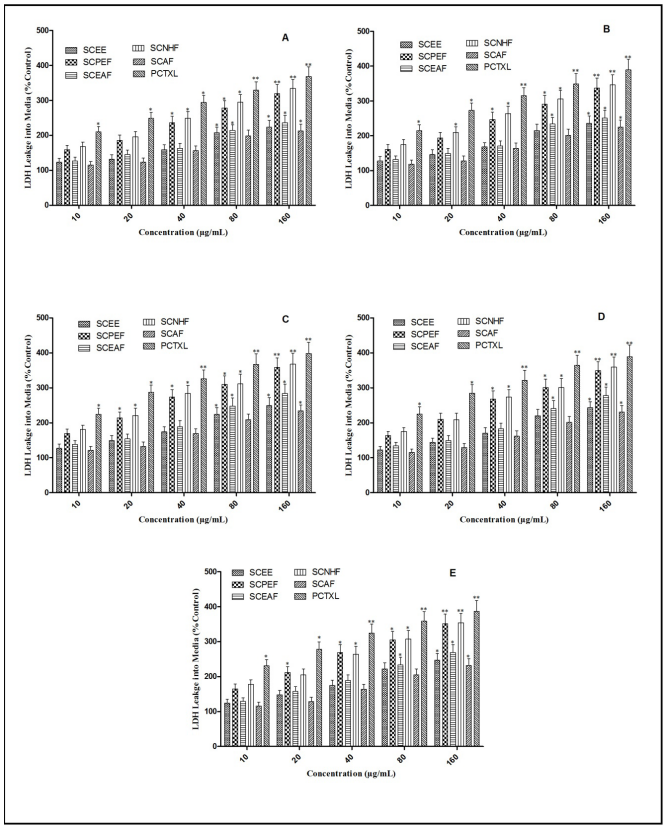

Fig. 3: LDH leakage from (A) HeLa, (B) HEK 293, (C) A549, (D) MCF-7 and (E) Hep G2 carcinoma cells, after treatment with different extracts of Spilanthes calva (10-160 µg/mL) for 48 hours.

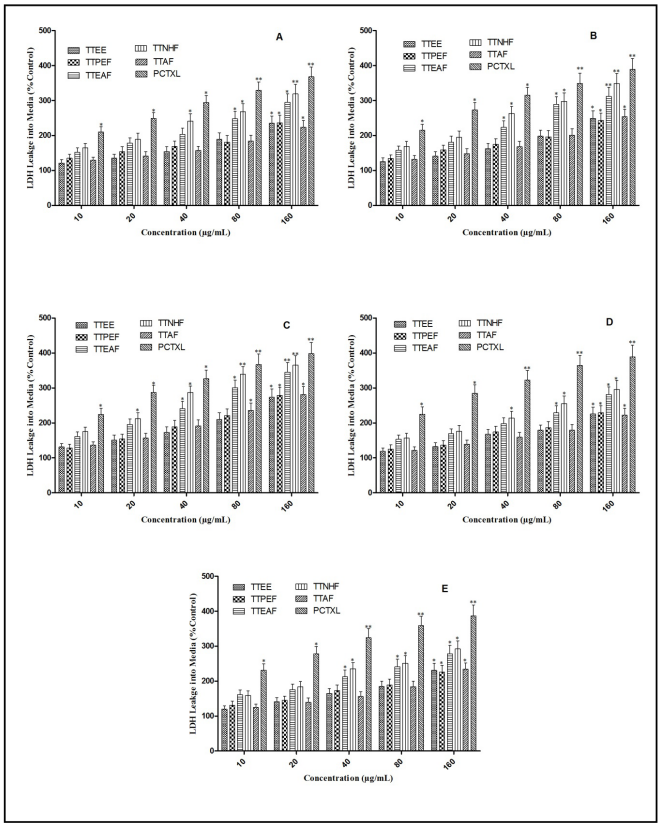

Fig. 4: LDH leakage from (A) HeLa, (B) HEK 293, (C) A549, (D) MCF-7 and (E) Hep G2 carcinoma cells, after treatment with different extracts of Taxillus tomentosus (10-160 µg/mL) for 48 hours.

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