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Anti-Cancerous Effect of Phytochemical Compound (Lectin) on A549 and NCI-H929 Cancer Cell Lines by Cytotoxicity Assays

Sayed Chandini*, Aneeta Blesna, and Shefali Raizada

Garden City University, Bangalore, Department of Molecular and Human Genetics.
*Corresponding author Email: Chandinisayed15@gmail.com

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ABSTRACT

Cancer is one of the leading reasons of death, worldwide. Cancer is a deadly disease, where the abnormal behaviour of a single cell type is challenging to treat via chemotherapy. It is essential in cancer therapy that the remedy targets only the affected cells, leaving the unaffected cells undisturbed, which is quite difficult, especially in chemotherapy. Anti-cancer drugs reachable in the modern-day market are not target-specific and elicit countless side-effects and issues encountered in the scientific management of a number of forms of cancer, which highlights the pressing need for novel effective and less-toxic therapeutic approaches. Recently, centre of attention has shifted from the usage of lectins to diagnose cancer to actually using lectins to combat cancer. Evidence is now emerging that lectins are dynamic contributors to tumour cell recognition (surface markers), cell adhesion and localization, signal transduction throughout membranes, mitogenic stimulation, augmentation of host immune defence, cytotoxicity, and apoptosis. In this study we have extracted lectin from plant Euphorbia Tithymaloide (ET). The predominant objective of this project is to check the anti-cancer property of lectins extracted from ET on A549 and NCI-H929 cell lines and a comparative analysis and confirmatory research of its cytotoxicity on these both cells lines.

Keywords: Euphorbia tithymaloide, lectin, anti-cancer, A549 cell line, NCI-H929 cell line, apoptosis, caspase3

INTRODUCTION

Cancer is one of the leading causes of death worldwide. Cancer is a deadly disease, where the abnormal behaviour of a single cell type is difficult to treat by chemotherapy. It is important in cancer therapy that the treatment targets only the affected cells, leaving the normal cells undisturbed, which is quite difficult, especially in chemotherapy. Anti-cancer drugs available in the current market are not target-specific and elicit several side-effects and complications encountered in the clinical management of various forms of cancer, which highlights the urgent need for novel effective and less-toxic

therapeutic approaches. Recently, focus has shifted from using lectins to detect cancer to actually using lectins to combat cancer.

Evidence is now emerging that lectins are dynamic contributors to tumor cell recognition (surface markers), cell adhesion and localization, signal transduction across membranes, mitogenic stimulation, augmentation of host immune defence, cytotoxicity, and apoptosis. A review by De Mejia and Prisecaru (2005) provides a comprehensive appraisal of the inhibitory effects of plant lectins on malignant cells in vitro and vivo. Lectins can be extracted from many sources such as soya bean, mushrooms, blue algae, leek tree, euphorbia tithymaloides etc, can be used as anti-proliferatory, tumor specific cytotoxicity, anti-viral therapies due to their therapeutic properties presence in them.

Anticancer functions of phytochemicals on cancer cells

Several plant species have been discovered to suppress the progression and development of tumors in cancer sufferers (Umadevi et al., 2013) and many phytochemicals have been identified as effective constituents in these plant species. Phytochemicals exert antitumor consequences via distinct mechanisms. They selectively kill rapidly dividing cells, target abnormally expressed molecular factors, remove oxidative stress, modulate cell growth factors, inhibit angiogenesis of cancerous tissue and result in apoptosis. For example some polyphenols (e.g., resveratrol, gallocatechins), flavonoids (e.g., methoxy licoflavanone, alpinumisoflavone), and brassino-steroids (e.g., homocatasterone and epibrassinolide) exert anticancer actions through apoptosis induction (Heo et al., 2014; Wen et al., 2014). Curcumin, thymol, rosmarinic acid, β-carotene, quercetin, rutin, allicin, gingerol, lectin, epigallocatechin gallate, and coumarin show anticancer functions via antioxidant mechanisms.

Anticancer drug development includes in vitro cytotoxicity on cancer cells, in vivo confirmation, and medical trial evaluation. Assessment of cytotoxicity toward most cancers cell lines is a trending strategy for the discovery of anticancer agents. Cell viability evaluation of cancer cells is a excessive throughput screening method via which numerous compounds can be screened in a quick period of time. Several such phytochemicals have been found from plants and dietary supplements. Crude phytochemical extracts additionally suppress the viability of cancer cells.

Lectins' role in cancer

Plant lectins, a unique group of proteins and glycolproteins with robust organic activity, manifest in foods like wheat, corn, tomato, peanut, kidney bean, banana, pea, lentil, soybean, mushroom, rice, and potato. Thus, dietary intakes via humans can be significant. Many lectins withstand digestion, live on intestine passage, and bind to gastrointestinal cells and/or enter the circulation intact, preserving full biological activity. Several lectins have been observed to possess anticancer properties in vitro, in vivo, and in human case studies; they are used as therapeutic agents, preferentially binding to most cancers cell membranes or their receptors, inflicting cytotoxicity, apoptosis, and inhibition of tumor growth. These compounds can emerge as internalized into cells, inflicting most cancers cell agglutination and/or aggregation. Ingestion of lectins additionally sequesters the available body pool of polyamines, thereby thwarting most cancers cell growth. They additionally have an effect on the immune system via altering the production of a range of interleukins, or by activating certain protein kinases. Lectins can bind to ribosomes and inhibit protein synthesis. They also regulate the cell cycle with the aid of inducing non-apoptotic G1-phase accumulation mechanisms, G2/M phase cell cycle arrest and apoptosis, and can activate the caspase cascade. Lectins can additionally down regulate telomerase activity and inhibit angiogenesis. Although lectins appear to have remarkable potential as anticancer agents, similarly research is still needed and should consist of a genomic and proteomic approach. In this article we took lectin as our phytochemical compound to take a look at it's anti-cancer effect on human most cancers cells.

METHODOLOGY

Extraction of phytochemical compound

Lectin has been extracted from the leaves of *Euphorbia Tithymaloides* (ET), leaves has been dried for 7-8 days in 37 °C. Dried leaves were grinded using Pestle and Mortar with the help of liquid nitrogen (-196ºC). The finely grounded powder of 1g was transferred to the Conical flask and dissolved the entire contents in 50mL of 70% absolute ethanol and the entire contents thoroughly dissolved in the conical flask using magnetic stirrer for 48hours. Later, filtered the

dissolved extract using Fresh Filter paper with the help of funnel and transfer the filtered contents to sterile Petri plates. Kept the Petri plates in clean surface area to dry for 2-4 days at Room Temperature. Once the extract become powdery or pasty then scraped the extract and collected it into vials. Collected pasty or powder form is called as crude extract, yielding 24 milligram and stored at 4°C for further use.

CYTOTOXICITY TEST

The cytotoxicity effect of ET test compound on A549 and NCI-H929 cell lines by MTT assay.

MTT Assay

MTT assay is a colorimetric assay used for the determination of cell proliferation and cytotoxicity, based on reduction of the yellow-coloured water-soluble tetrazolium dye MTT to formazan crystals. Mitochondrial lactate dehydrogenase produced by live cells reduces MTT to insoluble formazan crystals, which upon dissolution into an appropriate solvent exhibits purple colour, the intensity of which is proportional to the number of viable cells and can be measured spectrophotometrically at 570nm. (Alley, M. C et al., 1986, Mosmann et al., 1983).

MTS Assay

MTS Cell Proliferation Assay is a colorimetric method for sensitive quantification of viable cells in proliferation and cytotoxicity assay. The method is based on the reduction of MTS tetrazolium compound by viable cells to generate a coloured formazan product that is soluble in cell culture media. This conversion is thought to be carried out by NAD(P)H-dependent dehydrogenase enzymes in metabolically active cells. The formazan dye produced by viable cells can be quantified by measuring the absorbance at 450nm. The assay can be used for the measurement of cell proliferation in response to growth factors, cytokines, mitogens, and nutrients, etc. It can also be used for the analysis of cytotoxic compounds like anticancer drugs and many other toxic agents and pharmaceutical compounds. MTS assay is performed by adding the reagent directly into the cell culture media without the intermittent steps, which are required in the routine MTT assay. In addition, this high-throughput assay requires no washing or solubilization step and can be performed in 96-well microtiter plate.

CELL LINES USED

- A549-Human alveolar lung adenocarcinoma cell line (From NCCS, Pune)
- NCI-H929-Human B-lymphocyte plasmacytoma cell line (From ATCC, USA)

MATERIALS REQUIRED

1. Cell culture medium:
- DMEM- High Glucose - (#AL111, Himedia)-For A549 cells
- RPMI-1640 media – (#AT150, Himedia)-For NCI-H929

2. Fetal Bovine Serum (#RM10432, Himedia)
3. MTT Reagent (5 mg/ml) (# 4060 Himedia)
4. MTS reagent (#CCK053, Himedia)
5. DMSO (#PHR1309, Sigma)
6. Cisplatin (#PHR1624, Sigma)
7. Doxorubicin (#D1515, Sigma)
8. D-PBS (#TL1006, Himedia)

ASSAY CONTROLS:

(i) Medium control (medium without cells)
(ii) Negative control (medium with cells but without the experimental drug/compound)
(iii) Positive control (medium with cells and 6uM of Cisplatin)-For A549 cells
(iv) Positive control (medium with cells and 4uM of Doxorubicin)-For NCI-H929 cells
Note: Extracellular reducing components such as ascorbic acid, cholesterol, alpha-tocopherol, dithiothreitol present in the culture media may reduce the MTT to formazan. To account for this reduction, it is important to use the same medium in control as well as test wells.

PROCEDURE

1. Seeded 200µl (for NCI-H929), 100µl (for A549) cell suspension in a 96-well plate at required cell density (20,000 cells per well), without the test agent. Allow the cells to grow for about 24 hours.
2. Added appropriate concentrations 12.5, 25, 50, 100, 200µg/ml of the test compound (ET) in different wells respectively and standard drug compounds as control.
3. Incubated the plate for 24hrs at 37°C in a 5% CO2 atmosphere.
4. After the incubation period, takeout the plates from incubator, and remove spent media and add MTT reagent to a final concentration of 0.5mg/mL of total volume.

5. Wrapped the plate with aluminium foil to avoid exposure to light.
6. Returned the plates to the incubator and incubate for 3 hours. (Note: Incubation time varies for different cell lines. Within one experiment, incubation time should be kept constant while making comparisons.)
7. Removed the MTT reagent and then add 100µl of solubilisation solution (DMSO).
8. Gently stirred the plate in a gyratory shaker will enhance dissolution. Occasionally, pipetting up and down may be required to completely dissolve the MTT formazan crystals especially in dense cultures.
9. Read the absorbance on a spectrophotometer or an ELISA reader at 570nm wavelength.
10. After the readings have been taken the p value has been analysed for the 3 assays of MTT assays for both cell lines.

CONFIRMATION STUDIES

1. APOPTOSIS-NECROSIS STUDY

FITC Annexin V is used to quantitatively determine the percentage of cells within a population that are actively undergoing apoptosis. It relies on the property of cells to lose membrane asymmetry in the early phases of apoptosis. In apoptotic cells, the membrane phospholipid phosphatidylserine (PS) is translocated from the inner leaflet of the plasma membrane to the outer leaflet, thereby exposing PS to the external environment. Annexin V is a calcium-dependent phospholipid-binding protein that has a high affinity for PS, and is useful for identifying apoptotic cells with exposed PS. Propidium Iodide (PI) is a standard flow cytometric viability probe and is used to distinguish viable from nonviable cells. Viable cells with intact membranes exclude PI, whereas the membranes of dead and damaged cells are permeable to PI. Cells that stain positive for FITC Annexin V and negative for PI are undergoing apoptosis. Cells that stain positive for both FITC Annexin V and PI are either in the end stage of apoptosis, are undergoing necrosis, or are already dead. Cells that stain negative for both FITC Annexin V and PI are alive and not undergoing measurable apoptosis.

MATERIALS:

- **Cell lines:**

a. **A549–Human alveolar lung adenocarcinoma cell line (NCCS, Pune)**

- Cell culture medium:

a) DMEM-high glucose- (#AL-111, Himedia)

- Adjustable multichannel pipettes and a pipettor (Benchtop, USA)
- Fetal Bovine Serum (#RM10432, Himedia)
- D-PBS (#TL1006, Himedia)
- FITC Annenxin V (Cat No: 51-65874X, BD Biosciences)
- Propidium Iodide (PI) (Cat No. 51-66211E, BD Biosciences)
- Test compounds: **1 Samples with IC_{50} concentration**
- Cisplatin (Cat No: PHR 1624, Sigma)
- 6 well cell culture plate (Biolite - Thermo)
- 50 ml centrifuge tubes (# 546043 TORSON)
- 1.5 ml centrifuge tubes (TORSON)
- 10 ml serological pipettes (TORSON)
- 10 to 1000 ul tips (TORSON)

PROCEDURE

1. Culture cells in a 6-well plate at a density of 0.5 x 10^6 cells/2 ml and incubate in a CO_2 incubator overnight at 37°C for 24 hours.
2. Aspirate the spent medium and treat the cells with ET extract 113.46ug/ml and standard control (cisplatin-6uM/ml), in 2 ml of culture medium and incubate the cells for 24hours.
3. At the end of the treatment, remove the medium from all the wells and give a PBS wash. Remove the PBS and add 200µl of trypsin-EDTA solution and incubate at 37°C for 3-4 minutes. Add 2 ml culture medium and harvest the cells directly into 12 x 75 mm polystyrene tubes.
4. Centrifuge the tubes for five minutes at 300 x g at 25°C. Carefully decant the supernatant.
5. Wash the cells twice with PBS. Decant the PBS completely.
6. Add 5µl of FITC Annexin V.
7. Gently vortex the cells and incubate for 15 min at RT (25°C) in the dark.
8. Add 5µl of PI and 400 µl of 1X Binding Buffer to each tube and vortex gently. Analyze by flow cytometry immediately after addition of PI.

2. CASPASE 3 EXPRESSION STUDY

The caspase family of cysteine proteases plays a key role in apoptosis and inflammation. Caspase-3 is a key protease that is activated during the early stages of apoptosis and, like other members of the caspase family, is synthesized as an inactive pro-enzyme that is processed in cells undergoing apoptosis by self-proteolysis and/or cleavage by another protease. The processed forms of caspases consist of large (17-22

kDa) and small (10-12 kDa) subunits which associate to form an active enzyme. Active caspase-3, a marker for cells undergoing apoptosis, consists of a heterodimer of 17 and 12 kDa subunits which is derived from the 32 kDa pro-enzyme. Active caspase-3 proteolytically cleaves and activates other caspases, as well as relevant targets in the cytoplasm, e.g., D4-GDI and Bcl-2, and in the nucleus (e.g. PARP). This antibody has been reported to specifically recognize the active form of caspase-3 in human and mouse cells. It has not been reported to recognize the pro-enzyme form of caspase-3.

MATERIALS:

- Cell lines: **A549-Human alveolar lung cancer cell line (NCCS, Pune)**
- DMEM-High Glucose Medium (Cat No: AL111, Himedia)
- FBS (Cat No:RM10432, Himedia)
- 1X Dulbecco's Phosphate-buffered saline (Cat No: TL1006, Himedia)
- 70% pre-chilled absolute ethanol (China grade)
- Dimethyl Sulfoxide (Sigma-Aldrich, catalog number: D4540)
- Cisplatin (#PHR1624, Sigma)
- Caspase-3 FITC antibody (Cat No:560901, BD Biosciences, USA)

PROCEDURE:

1. Culture cells in a 6-well plate at a density of 0.5x 10^6 cells/ ml and incubate in a CO_2 incubator overnight at 37°C for 24 hours.
2. Aspirate the spent medium and wash with 1ml 1X PBS.
3. Treat the cells with ET extract 113.46uG/ml and incubate the cells for 24 hours.
4. At the end of the treatment, collect the cells by trypsinization and harvest the cells directly into 5ml storage vials.
5. Centrifuge at 1800rpm for 5min at RT.
6. Wash the cells with 1x PBS and centrifuge again at 1800rpm for 5min at RT.
7. Fix the cells with 1m of pre-chilled absolute ethanol for 1hour in -20°C deep Freezer to fix and permeabilize the cells.
8. Centrifuge the cells again at 1800rpm for 5min and remove ethanol.
9. To remove excess of ethanol, wash the cells with 1ml of 1x PBS and centrifuge at 1800rpm for 5mins at RT.
10. Remove PBS completely and stain the pelleted cells with 20ul of Caspase-3 antibody and incubate for 30mins at RT in the absence of light.
11. Analyse the CASPASE-3 expression in cells using the 488 nm laser for excitation and detection at 535nm (FL1) by flow cytometry and data analysis was done by using BD Cell Quest Pro software. (ver.6.0)

RESULTS AND DISCUSSIONS

Cytotoxicity Assays

In this study, 1 test compound is evaluated to analyse the cytotoxicity effect on A549 and NCI-H929 cells. The concentrations of the test compound used to treat the cells are as follows [table 1].

The results of cytotoxicity study performed by MTT assay [Fig2; Fig3] suggests that the given test compound, ET extract is significantly cytotoxic in nature against A549 cells than NCI-H929 cells. We can consider ET ethanolic extract as anticancer in nature on A549 cells due to its lower IC_{50} values. On the other hand, Same extract shows moderate cytotoxicity on NCI-H929 cells respectively

Table 1: Details of drug treatment to respective cell lines used for the study

Sl.No	Test Compound	Cell Lines	Concentration treated to cells
1	Untreated	A549 & NCI-H929	No treatment
2	Cisplatin	A549	6uM
3	Doxorubicin	NCI-H929	4uM
4	Blank	-	Only Media without cells
5	Test compound	A549 & NCI-H929	12.5, 25, 50, 100, 200µg/ml

Table 2: IC_{50} values of the test compound, ET extract against A549 and NCI-H929 cell lines. [fig 2; fig3]

Cell line	IC50 (ug/ml)
A549	113.46
NCI-H929	197.45

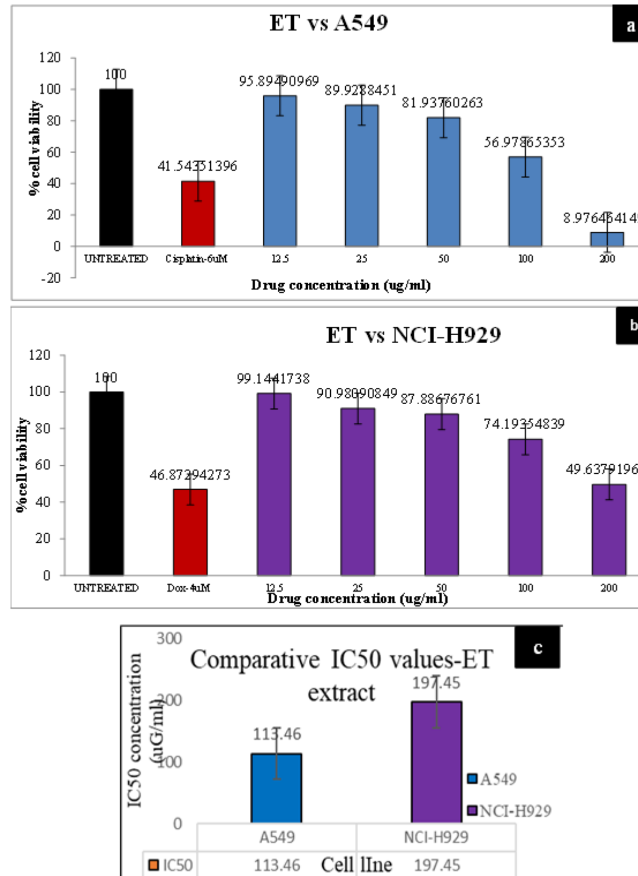
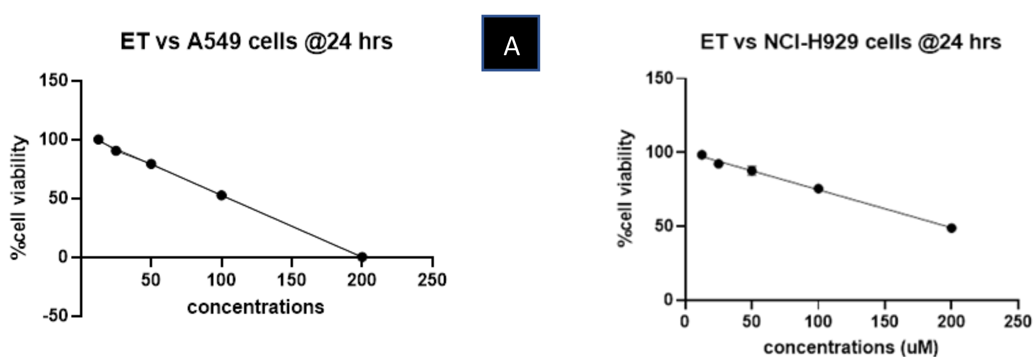


Figure 1: a- Comparative % cell viability of ET extract treated A549 cells; b- Comparative % cell viability of ET extract treated NCI-H929 cells; c- Comparative IC50 values of ET extract treated A549 and NCI-H929 cells



Graph 1 A: Cell viability of A549 cells after 24 hours of incubation of test drug ET (n=3) (p=<0.0001); B: cell viability of NCI-H929 cells after 24 hours of incubation of test drug ET (n=3) (p=<0.0001).

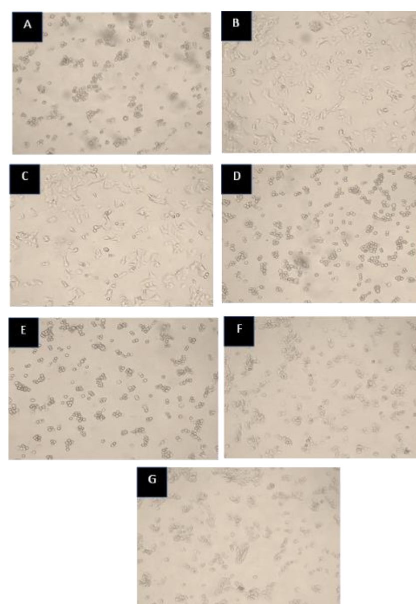
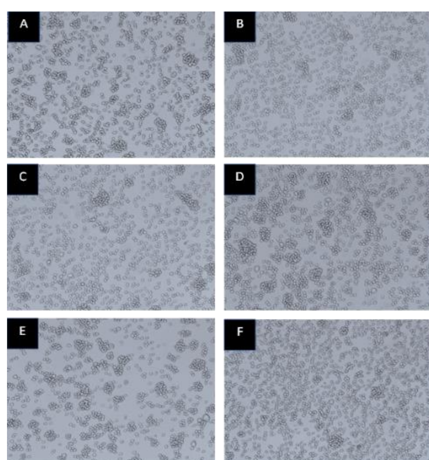


Figure 2: A596 cells in MTT assay after 24hours incubation of treatment of test drug ET. **A:** cells treated with Standard 6uM cisplatin; **B:** Cells without any drug only medium; **C:** 12.5 ug of ET on cells; **D:** 25ug of ET; **E:** 50ug of ET on cells; **F:** 100ug of ET on cells; **G:** 200ug of ET on cells.

Apoptosis and Necrosis Study

Apoptosis is a normal physiologic process which occurs during embryonic development as well as in maintenance of tissue homeostasis. The apoptotic program is characterized by certain morphologic features, including loss of plasma membrane asymmetry and attachment, condensation of the cytoplasm and nucleus, and inter nucleosomal cleavage of DNA. Loss of plasma membrane is one of the earliest features.

In apoptotic cells, the membrane phospholipid phosphatidylserine (PS) is translocated from the inner to the outer leaflet of the plasma membrane, thereby exposing PS to the external cellular environment. Annexin V is a 35-36 kDa Ca2+ dependent phospholipid-binding protein that has a high affinity for PS, and binds to cells with exposed PS. Annexin V may be conjugated to fluorochromes including FITC. Since externalization of PS occurs in the earlier stages of apoptosis, FITC Annexin V staining can identify apoptosis at an earlier stage than assays based on nuclear changes such as DNA fragmentation.



FITC Annexin V staining precedes the loss of membrane integrity which accompanies the latest stages of cell death resulting from either apoptotic or necrotic processes. Therefore, staining with FITC Annexin V is typically used in conjunction with a vital dye such as propidium iodide (PI) or 7-Amino-Actinomycin (7-AAD) to allow the investigator to identify early apoptotic cells (PI negative, FITC Annexin V positive). Viable cells with intact membranes exclude PI, whereas the membranes of dead and damaged cells are permeable to PI. For example, cells that are considered viable are FITC Annexin V and PI negative. Cells that are in early apoptosis are FITC Annexin V positive and PI negative; and cells that are in late apoptosis or already dead are both FITC Annexin V and PI positive. This assay does not distinguish between cells that have undergone apoptotic death versus those that have died as a result of a necrotic pathway because in either case, the dead cells will stain with both FITC Annexin V and PI. However, when apoptosis is measured over time, cells can be often tracked from FITC Annexin V and PI negative (viable, or no measurable apoptosis), to FITC Annexin V positive and PI negative (early apoptosis, membrane integrity is present) and finally to FITC Annexin V and PI positive (end stage apoptosis and death). The movement of cells through these three stages suggests apoptosis. In contrast, a single observation indicating that cells are both FITC Annexin V and PI positive, in of itself, reveals less information

Fig 3: NCI-H919 cells in MTT assay after 24hours incubation of treatment of test drug ET. A: cells treated with Standard 4uM of Doxorubicin; B: Cells without any drug only medium; C: 12.5 ug of ET on cells; D: 25ug of ET; E: 50ug of ET on cells; F: 100ug of ET on cells; G: 200ug of

about the process by which the cells underwent their demise.

In this study, 1 Compound with IC_{50} concentration with 2 controls (untreated and std control) is used to study the Annexin V/PI expression Study on the 1 cell line namely, A549. The used concentrations of the compound to treat the cells as follows [table 3]

Sl. No	Test Compounds	Cell lines	Concentration treated to cells
1	Untreated	A549	No treatment
2	Std control	A549	6uM/ml
3	ET	A549	113.46ug/ml

Table 3: Details of the experimental conditions used for the study

Annexin V/PI expression Study of the ET extract against the A549Cell line:

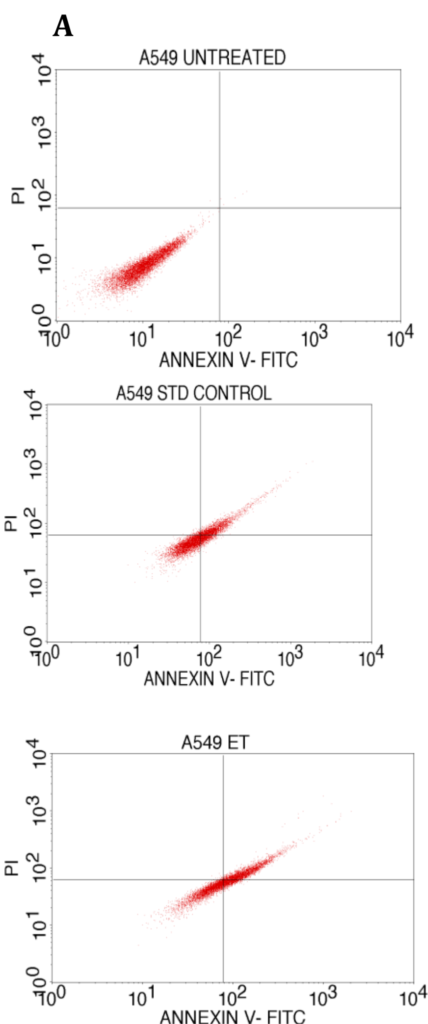


Fig 4. Quadrangular plots representing the Annexin V/PI expression in A549 cells upon culturing in the presence and absence of test compound ET. Analysis was done by using BD FACS Calibur, Cell Quest Pro Software (Version: 6.0)*. Here, Annexin V-FITC - Primary Marker, PI- Propidium Iodide (Secondary fluorescence Marker). Cisplatin is used as a std control. Ref-table-4.* ***A- untreated Cell lines depict viable cells; B- treated cells with standard drug. Depict early and late apoptosis; C- treated cells with ET depicts early and late apoptosis.***

UL - Upper left: % of Necrotic Cells	UR - Upper right: % l
LL- Lower left: % Viable Cells	LR- Lower right: % cells

Table 4: Meaning of the quadrant layout (FIG - 4) in Apoptosis/necrosis study.

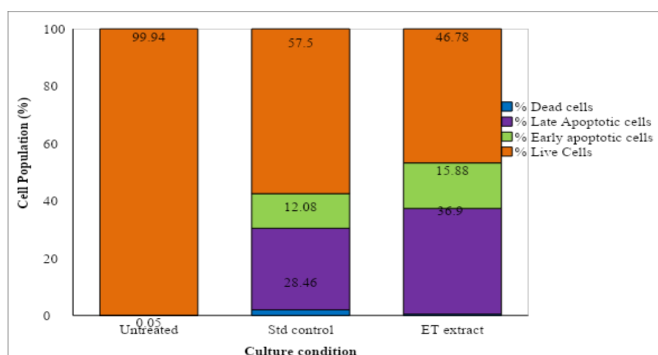
Observations & Graphs

Annexin V/PI expression study in A549cell line:

Quadrant	%Necrotic cells	%Late apoptotic cells	%Viab
Label	**UL**	**UR**	**LL**
Untreated	0.01	0.05	99.94
Std control	1.96	28.46	57.5
ET extract	0.44	36.9	46.78

Table 5: Table showing the % of cells of undergone Apoptosis in untreated, std control and ET extract treated A549 cells [fig 4].

As the result shows the A549 cells on treating with 113.46ug/ml of ET extract shows 0.44% of necrotic cells, 36.9% of late apoptotic cells, 15.88% early apoptotic cells and 46.78% viable cells [Fig 4; table 4], whereas standard control (cisplatin) percentages are 1.96% necrotic cells, 28.46% late apoptotic cells, 12.08% early apoptotic cells, 57.5% viable cells.



Graph-2: *Bar graph showing the % of live, apoptotic and necrotic cells*

The result & observations [fig4; table4; Graph2] depicted that the test compound, ET extract induces the significant apoptosis in human lung cancer (A549) cells. The compound, ET may have therapeutic potential against human lung cancer cells and further preclinical studies have to be done to confirm the mechanism of action on Human lung cancer cells.

Caspase 3 expression study

Active caspase-3, a marker for cells undergoing apoptosis, consists of a heterodimer of 17 and 12 kDa subunits which is derived from the 32 kDa pro-enzyme. Active caspase-3 proteolytically cleaves and activates other caspases, as well as relevant targets in the cytoplasm, e.g., D4-GDI and Bcl-2, and in the nucleus (e.g. PARP). This antibody has been reported to specifically recognize the active form of caspase-3 in human and mouse cells. It has not been reported to recognize the pro-enzyme form of caspase-3. Caspase-3 is a key protease that is activated during the early stages of apoptosis

In this study cisplatin 6uM/ml is taken as standard and ET extract 113.46uG/ml is taken as test compound on A549 cell lines. [table 6].

Table 6: Details of Samples received

Sl. No.	Sample Name/Code	Concentrations	Cell line
1	ET extract	113.46uG/ml	A549
2	Cisplatin	6uM/ml	A549

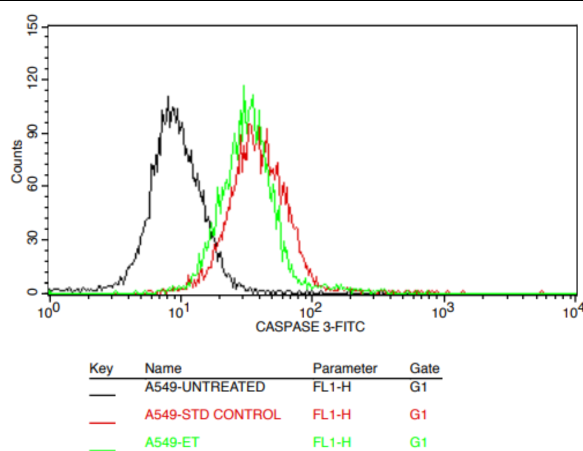


Figure 3: Results depicts the expression and regulation of Caspase 3 on A549 cells on untreated, standard (cisplatin) and treated (ET) cells by using Flow cytometry (BD FACS Callibur) , Software: BD Cell Quest Pro (ver.6.0).

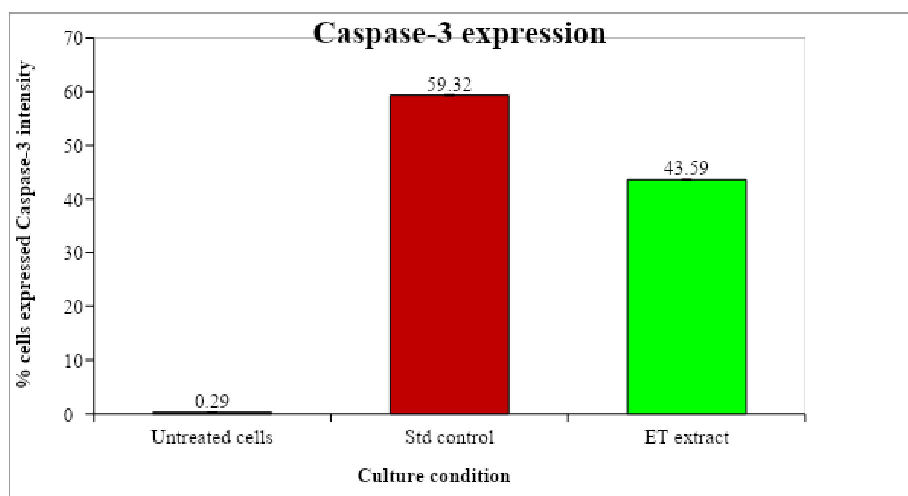


Figure 4 : % cells expressed caspase 3 intensity.

Table 7: % cells expressed Caspase-3 intensity in treated and untreated cells.

Culture conditions	% Cells expressed Caspase 3 intensity
Untreated cells	0.29
Std control	59.32
ET extract-113.46ug/ml	43.59

SUMMARY & CONCLUSION

In this study, A549 and NCI-H929 cell lines are treated with a phytochemical compound lectin extracted from plant Euphorbia tithymaloide. The lectin in the extract by performing MTT assay found to have anticancer action on cells lines we have selected. The effectiveness of the extract was more on the A549 cell lines than compared to NCI-H929 cell line. For further confirmatory studies we have taken A549 cells, to study which kind of cell death has been occurred due to the extract used. The confirmatory studies were apoptotic death or necrotic death confirmation by using FITC ANNEXIN V staining and analysed by FACS calibur and Caspase-3 regulation and expression was confirmed by BD FACS Calibur, these both studies had confirmed that cells had undergone programmed cell death that is Apoptotic cell death via Caspase 3 mediated pathway. This proves that the lectin extracted from the plant Euphorbia Tithymaloides has anti-cancer property and it is more affective on lung cancer cells (A549), it works on Myeloma cell line (NCI-H929) but not that effective in the potencies that we have selected, further research can be done on these cell lines using higher potencies.

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