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A novel protein fraction isolated from the leaves of *Abies webbiana* Lindl. Induces apoptosis in lung cancer cells via the intrinsic pathway

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Abstract
The genus *Pinaceae* is found to be rich in bioactive metabolites is used in Ayurveda for treating respiratory ailments. The aim of the study was to determine the effect of different protein fractions isolated from the leaves of the tree *Abies webbiana* Lindl, on adenocarcinomic human alveolar basal epithelial A549 cells. MTT assay was performed to check the ability of the fraction to induce cell death in cancerous A549 cells and normal NIH3T3 fibroblast cells. Acridine Orange/Ethidium Bromide staining, Hoechst staining and DNA fragmentation assays were performed. RT-PCR studies of pro-apoptotic BAX and anti-apoptotic Bcl-2 along with western blot of p-ATM, CHK-2, CDC25A, BAK and BAD were performed. Caspase-9 and 3 activities were determined using calorimetric assays. The fraction at lower concentrations was found to induce cell death in the lung cancer cells. Acridine Orange/Ethidium Bromide staining, Hoechst staining and DNA fragmentation assays were used to confirm the apoptosis inducing ability of the fraction on A549 cells. The transcriptional expression studies of the pro-apoptotic BAX and anti-apoptotic Bcl-2; and the western blot analysis of proteins that regulate apoptosis showed that the fraction upregulated the expression of pro-apoptotic factors and down regulated the expression of anti-apoptotic factors in the cancer cells. Caspase-9 and 3 activities were found to be upregulated in the calorimetric studies. With the above results, it could be concluded that the protein fraction (PF-2) induced apoptosis in the lung cancer cells via the intrinsic or the mitochondrial pathway.

Keywords: Protein fraction, apoptosis, mitochondrial pathway, cell cycle

1. Introduction
Nature has been the source of both nutrition and medicines for mankind from eternity. Phytochemicals have been used since time immemorial for the benefit of humans. *Pinaceae* family has been a rich source of bioactive compounds. *Abies* genus belongs to the family *Pinaceae* and is commonly referred to as Firs and are the coniferous trees found in high altitude areas. *Abies webbiana* Lindl. known as *Talispatra* in Ayurveda is used for treating ailments related to respiratory system as per Ayurvedic literature. Solvent extracts of the leaves of this plant has been reported to possess anti-oxidant, anti-bacterial, anti-fungal, anti-inflammatory and anti-tumor activities [1]. Cancer constitutes an enormous burden on society in more and less economically developed countries alike. Lung cancer is the most common cancer worldwide in incidence and mortality [2].
The objective of this study was to isolate protein fractions from the leaves of *Abies webbiana* Lindl. and to determine the effect of the fractions on the lung cancer cell line, A549 and normal NIH3T3 fibroblast cell line. The fraction showing highest activity was selected for further studies. The apoptosis inducing ability of the fraction was studied using Acridine Orange/Ethidium Bromide staining, Hoechst staining and DNA fragmentation assays. The study also tried to elucidate the mechanism by which the fraction induced apoptosis in the adenocarcinomic human alveolar basal epithelial A549 lung cancer cells by studying the expression of pro-and anti-apoptotic factors.

2. Materials and Methods
2.1 Preparation and collection of protein fractions
The leaves of *Abies webbiana* Lindl. were collected from the herbal garden of Arya Vaidya Pharmacy, Coimbatore and authenticated by Dr. Kunhikannan, Scientist E, Institute of Forest Genetics and Tree Breeding, Coimbatore, India (Voucher specimen no: IFGTB-25/03/14-006). The leaves were ground in phosphate buffered saline (PBS) in ice and the extract was filtered using cheese cloth and was further subjected to centrifugation at 2000 rpm for 10 minutes at 4 ºC. The clear supernatant was subjected to protein precipitation using ammonium sulphate

precipitation method and the three fractions (0–35% [PF-1], 35–70% [PF-2] and 70–100% [PF-3]) obtained were centrifuged, dialysed, passed through Sephadex G-25 column, lyophilized and used for the assays [3].

2.2 Cell culture

The Human adenocarcinomic alveolar basal epithelial A549 cells and normal (NIH3T3) cells were obtained from NCCS, Pune. The cells were maintained in DMEM supplemented with 10% FBS; and 100 mg/L streptomycin and 100 U/L penicillin. Cells were maintained at 37 ºC in a humidified atmosphere of 5% CO_2 and 95% air. All cell culture reagents were purchased from Hi-media (Mumbai, India).

2.3 Cell Viability Assay

The A549 and NIH3T3 cells were grown in 12-well culture plates. MTT assay was used to assess cell viability according to the manufacturer's instructions (Sigma-Aldrich, St. Louis, MO, USA). The assay was quantitated by measuring the absorbance at 570 nm [4]. NIH3T3 cells were used as control (noncancerous cells) to check the activity of the protein fractions on normal cells. After measuring the absorbance at 570 nm at the end of the MTT assay, the % growth inhibition was determined using the formula:

% growth Inhibition = 100 – (Abs (sample) / Abs (control) X 100)

2.4 Apoptosis studies

2.4.1 Acridine Orange/Ethidium Bromide Staining

The A549 cells, treated with the protein fraction (PF-2) for 24 h were washed with Phosphate buffered saline (PBS) and trypsinized. Twenty-five µL of cell suspension ($1x10^4$ cells/mL) was mixed with 1 µL of Acridine Orange/Ethidium Bromide solution (one part each of 100 µg/mL of Acridine Orange and 100 µg/ml of Ethidium Bromide in PBS) just prior to microscopy [5, 6]. The mixed suspension (10 µL) was placed on a microscope slide covered with a glass cover slip and examined under fluorescent microscope (Olympus 1X71) connected to a digital imaging system.

2.4.2 Hoechst Staining

A549 cells ($1 x 10^4$ cells/mL) were incubated with the protein fraction (PF-2) for 24 h. Apoptotic nuclear morphology was visualized using Hoechst staining [7, 8]. Cells were fixed with 4% p-formaldehyde and stained with Hoechst dye 33258 (working concentration of Hoechst was taken as 10 µg/mL, stock 1 mg/mL), incubated for 15 min and examined under fluorescent microscope (Olympus IX71) with excitation/emission at 300/380 nm, which was connected to a digital imaging system.

2.4.3 DNA Fragmentation Assay

A549 cells grown in 60 mm culture plates were treated with different concentrations (0, 5, and 10 µg/mL) of the protein fraction (PF-2) for 24 h [9]. Cells without treatment were taken as control. After 24 h, the cells were harvested and washed with PBS and pelleted by centrifugation at 300 rpm. To the cell pellet, 100 µL lysis buffer (50 mM Tris-HCl pH 8, 20 mM EDTA, 10 mM NaCl, 1% SDS) was added for 1 min and centrifuged at 2000 rpm for 5 min. The supernatant was collected and incubated with RNase A (5 µg/mL) for 1 h followed by digestion of proteins with proteinase K (100 µg/mL) for 5 h. Aliquots of lysate (20 µL) were loaded to 1.5% agarose prepared in TBE buffer (Tris-Borate 45 mM, 1 mM EDTA, pH 8) containing 3 mg/mL EtBr, and DNA was separated by electrophoresis. The separated DNA fragments in the gel were visualized using ChemiDoc XRS system (Bio-Rad Laboratories Inc., Hercules, CA).

2.5 Molecular studies

2.5.1 Reverse Transcriptase-PCR

The mRNA expression levels of B-cell lymphoma-2 (Bcl-2) and Bcl-2-associated X (BAX) were studied using reverse-transcriptase PCR [10, 11]. The A549 cells were grown in 60 mm culture plates. The first set of A549 cells was untreated. The second set of cells was treated with Phorbol 12-myristate 13-acetate (PMA) at a concentration of 50 ng/mL. The third set of cells was treated with the protein fraction (PF-2) alone at 10 µg/mL concentration. The last, fourth set of cells, was treated with both protein fraction PF-2 and PMA at 10 µg/mL and 50 ng/mL concentrations respectively. After incubation, total RNA was isolated from the treated and untreated cells using manufacturer's protocol (Chromous Biotech Ltd, Bangalore, India). cDNA was synthesized from 1 µg of total isolated RNA by incubation for 1 h at 37 ^{0}C with M-MLV reverse transcriptase (Promega, Madison, WI). After the 1 h incubation, the mixture was again incubated at 70 ^{0}C for 15 minutes to deactivate the reverse transcriptase. The cDNA synthesized (2 µL) was added with 5 µL reaction buffer (1X), 2 µL dNTPs (0.2 mM each), 1 µL each of Forward and Reverse primer (0.4 µM each), 3 µL $MgCl_2$, 0.2 µL Taq polymerase (Promega, Madison, WI, USA) and made up to 20 µL with nuclease free water for PCR reaction. Human specific primers were used for Bcl-2, BAX and beta-actin. The forward and reverse primers used for Bcl-2, BAX and beta-actin amplification are given in table. 1. The synthesized PCR products were separated on 1.5% agarose gels and analyzed using ChemiDoc XRS (Bio-Rad Laboratories Inc., Hercules, CA).

Table 1: Forward and reverse primers for the genes

Genes	Forward primer (5′ - 3′)	Reverse primer (5′ - 3′)
Bcl-2	ATGGACGGGTCCGGGGAG	TCAGCCCATCTTCTTCCA
BAX	CAGCTGCACCTGACG	ATGCACCTACCCAGC
β-actin	GTTTGAGACCTTCAACACCCC	GTGGCCATCTCCTGCTCGAAGTC

2.5.2 Western blot analysis

The protein expression levels of p-ATM (phosphorylated Ataxia telangiecectasia mutated [S1981]), CHK2 (Checkpoint kinase-2), CDC25A (Cell division cycle 25 homolog A), BAK (Bcl-2 homologous antagonist/killer) and BAD (Bcl-2-associated death promoter) were studied using western blotting assay. The A549 cells were grown in 60 mm culture plates. The first set of A549 cells was untreated. The second set of cells was treated with protein fraction PF-2 at 10 µg/mL. The last and third set of cells was treated with the fraction at 20 µg/mL. The protein was separated by SDS-PAGE on a 10% separating gel and transferred to nitrocellulose membrane [12-14]. Addition of primary mouse monoclonal anti-p-ATM, primary rabbit polyclonal anti-CHK2, primary rabbit polyclonal anti-CDC25A, primary rabbit monoclonal anti-BAK and rabbit monoclonal anti-BAD antibodies were done after the transfer of protein on to the membrane. The nonspecific protein binding sites on the

blotted nitrocellulose membrane were blocked with 5% non-fat dry milk in 0.1% Tween-20 in PBS (pH 7.4) at 4 °C overnight. The membrane was incubated with primary antibodies for p-ATM, CHK2, CDC25A, BAK, BAD and β-actin at the dilution of 1:1000 (in 3% BSA), 1:200 (in 3% BSA), 1:2000 (in 3% BSA), 1:1000 (in 3% BSA), 1:2000 (in 3% BSA) and 1:2000 (in 3% BSA) respectively at 4 ^{0}C overnight with gentle shaking. The membrane after incubation was washed thrice with PBS-Tween-20 (5 minutes each) and incubated with goat anti-mouse HRP antibody for p-ATM, goat anti-rabbit HRP antibody for CHK-2, mouse anti-rabbit HRP antibody for CDC25A, goat anti-rabbit HRP antibody for BAK and goat anti-rabbit HRP antibody for BAD (in 5% non-fat milk solution) (abcam, Cambridge, USA) at RT for 1 hour. After incubation, the membrane was washed twice with PBS-Tween-20 (5 minutes each) and for 10 minutes with the same. The protein bands were quantified by autoluminographs. The amount of protein in each well was confirmed by stripping the membrane with stripping buffer (at 70 °C for 1 hour) and reprobing with monoclonal antibody to β-actin by following the manufacturer's instruction (Santa Cruz Biotechnology, Inc., CA). Immunodetection of the protein expressions under study was performed using ECL prime western blotting detection reagent (Amersham Biosciences, Piscataway, NJ) and visualized in a ChemiDoc XRS system (Bio-Rad Laboratories Inc., Hercules, CA).

2.5.3 Caspase Activity Assay caspase-9 and 3 activities in the cell lysates were measured using the colorimetric assay with commercial kits according to the manufacturer's instructions (Sigma-Aldrich, St. Louis, MO). Cells were incubated with and without the protein fraction PF-2 for 24 h. After the incubation, the cells were collected by trypsinization and lysed using the lysis buffer (250 mM HEPES, pH 7.4 containing 25 mM CHAPS and 25 mM DTT). The assay was based on the hydrolysis of the peptide substrates Leu-Glu-His-Asp p-nitroaniline (LEHD-pNA) by caspase-9 and acetyl-Asp-Glu-Val-Asp p-nitroaniline (Ac-DEVD-pNA) by caspase-3 resulting in the release of p-nitroaniline (pNA) moiety. The cell lysates were mixed with the substrate and read at 405 nm in a microplate reader [15, 16]. The results were expressed as micromoles of pNA released per minute per mL.

2.6 Statistical Analysis

Results refer to mean ± standard deviation and are average of three values per experiment; each experiment was repeated at least three times. Statistical evaluations were assessed using the Student's *t* test, and $p < 0.05$ was considered significant.

3. Result and Discussion

3.1 Cell viability assay

Concentration-dependent study performed using MTT assay to check the effect of the protein fractions on the cell viability of A549 cancer cells showed that the fraction PF-2 exhibited maximum activity among the 3 fractions (Fig. 1). An increase in concentration of the PF-2 fraction (5, 10, 20 and 50 µg/mL) selectively reduced the cell viability significantly in the cancer cells than normal NIH3T3 cells (Fig. 2). The IC50 value of the protein fraction PF-2 was found to be 7.64 ± 0.83 µg/mL on the A549 cancer cells. Almost 100% growth inhibition was observed at a concentration of 50 µg/mL of the fraction on the A549 cancer cells whereas this concentration produced only 40% cell death in the normal NIH3T3 cells which showed that the fraction has reduced cytotoxic effect on non-cancerous normal cells.

In this study we have tried to check the growth inhibitory activity of protein fractions isolated from the leaves of *Abies webbiana* Lindl. and its potential to induce apoptosis in A549 cells. Further, we elucidated the mechanism by which the protein fraction PF-2, which was found to have higher growth inhibitory activity compared to the other fractions, induces apoptosis in the A549 cells. Our studies revealed that the PF-2 fraction triggered factors that regulate cell cycle and also induced the intrinsic or the Mitochondria-mediated pathway of apoptosis. The growth inhibitory activity studies using MTT assay showed that, among the three protein fractions isolated, the second fraction PF-2 showed maximum growth inhibitory activity in A549 cells with an IC50 value of 7.64 ± 0.83 µg/mL. MTT assay is generally used as a preliminary assay to measure the growth inhibitory activity of a chemical on cells. The result showed that the protein fraction PF-2 almost completely inhibited the growth of A549 cells at 50 µg /mL. Further, the fraction was found to induce lower growth inhibition in normal NIH3T3 cells. At 50 µg /mL, the fraction was found to induce only 40% growth inhibition in the NIH3T3 cells.

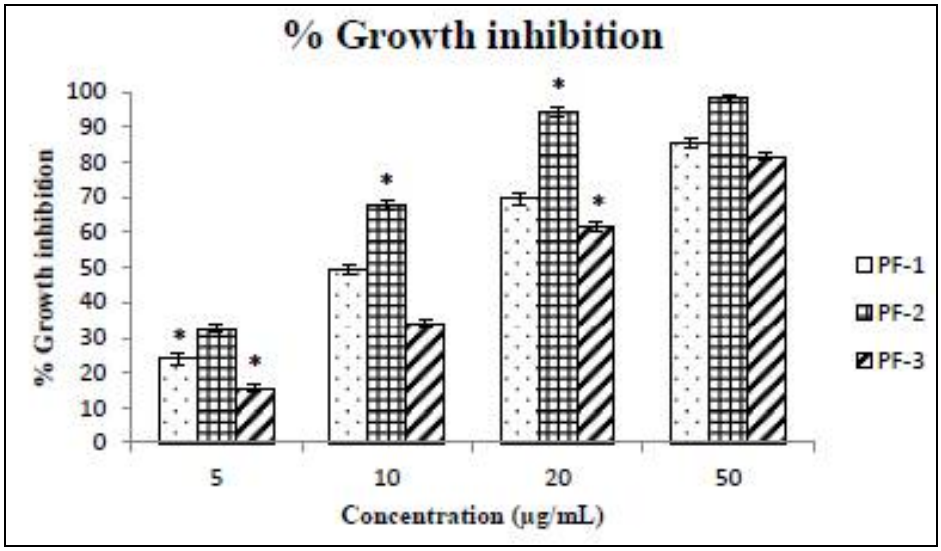


Fig 1: Dose dependent response of Protein fractions on the cell viability of A549 and normal NIH3T3 cells

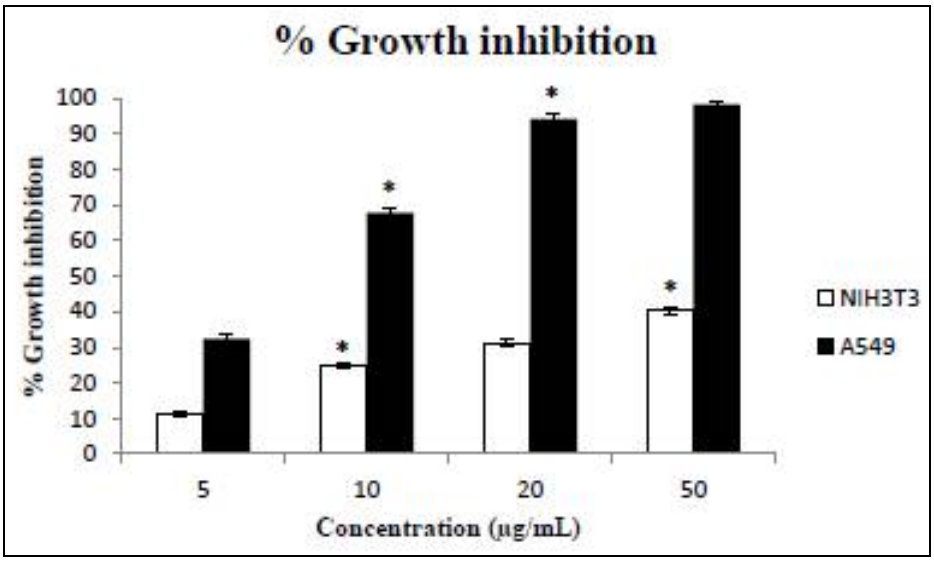


Fig 2: Dose dependent response of Protein fraction PF-2 on the cell viability of A549 and normal NIH3T3 cells

3.2 Apoptosis studies

Typical morphological changes were observed after AO-EtBr staining in A549 cells after treatment with protein fraction PF-2. The A549 cells stained with acridine orange/Ethidium bromide showed that the live cells took up the acridine orange stain and fluoresced in bright green color (Fig. 3). As the concentration of the protein fraction increased, cellular shrinkage and membrane blebbing was observable and the degree of membrane damage also increased and the apoptotic cells stained as orange/red as a result of co-staining with ethidium bromide. Hoechst staining for evaluating changes to nuclear morphology after treatment with different concentrations of the protein fraction showed that bright blue dots appeared in the cells which represent chromatin condensation in the treated A549 cells. Nuclear condensation was observable at lower concentration of the protein fraction PF-2 and significantly increased at higher concentrations (Fig. 4). The results clearly indicated significant changes to the nuclear morphology associated with apoptosis in the A549 cells after treatment with different concentrations of the protein fraction. Typical DNA ladder formation was visible in the protein fraction treated cells which is the result of the degradation of DNA into fragments of 200 bps or its multiples. DNA fragmentation was observed to increase in the A549 cells when treated with increasing concentrations of the protein fraction in a dose dependent manner, confirming induction of apoptosis in the cancer cells (Fig. 5).

The Acridine orange-Ethidium bromide staining showed that the protein fraction PF-2 altered the membrane stability of the fraction treated cells, whereas the membranes of untreated cells were unaffected. The untreated cells fluoresced green due to the binding of Acridine orange to the intact cell membrane. But as the concentration of the fraction increased from 10 µg/mL to 50 µg/mL, the cells were found to take up more Ethidium bromide and fluoresce red due to the disturbance in the membrane integrity caused by the action of the protein fraction. Apoptosis induction studies using Hoechst staining assay, revealed that the nuclear condensation, which is a feature of apoptosis, was visible prominently and increased in a dose dependent manner in the PF-2 fraction treated A549 cells [17]. The results showed that the nuclei of untreated control cells emitted lower fluorescence intensity compared to the fraction treated cells which showed typical features of apoptosis like chromatin condensation which leads to the increase in emission of intense fluorescence from the nucleus. Finally when the DNA fragmentation assay was performed, apoptosis induction in protein fraction PF-2 treated cells was confirmed when the DNA isolated from the treated cells formed a ladder like pattern in the gel. The DNA which gets cut into approximately 180-200 base pairs or its multiples forms a ladder like pattern when electrophoresed. The DNA gets cut at the internucleosomal linker sites by the action of Caspase activated DNase (CAD) and forms the ladder pattern which is a classic feature of apoptosis [18, 19].

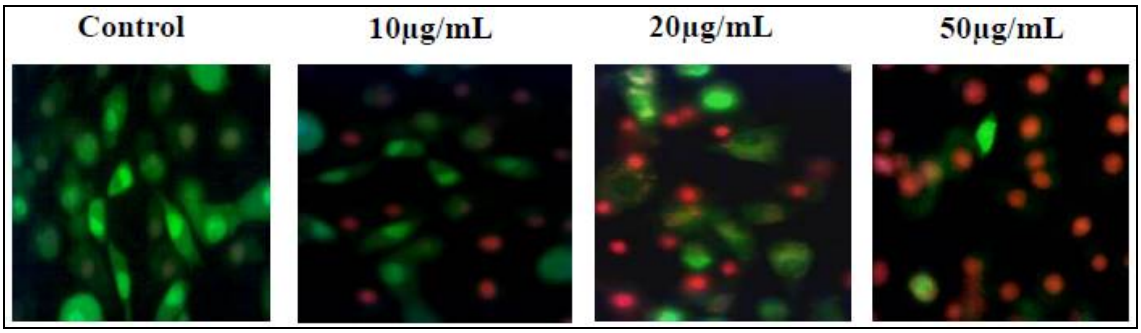


Fig 3: AO-EtBr stained A549 cells after treatment with fraction PF-2

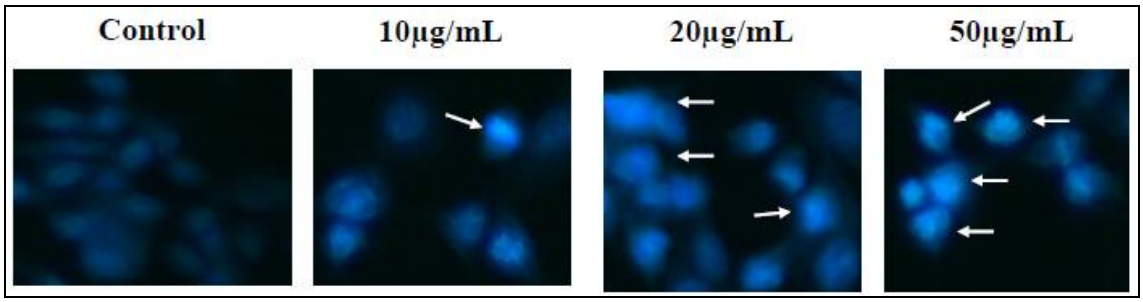
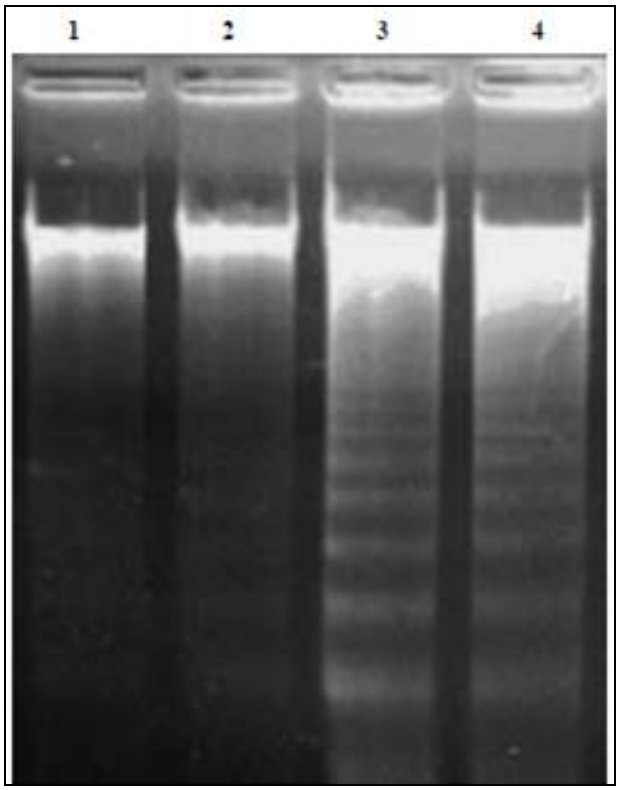


Fig 4: Hoechst dye stained A549 cells after treatment with fraction PF-2



Lane 1: Control, Lane 2: 10 µg/mL, Lane 3: 20 µg/mL, Lane 4: 50 µg/Ml

Fig 5: DNA fragmentation assay gel after treatment with fraction PF-2 on A549 cells.

3.3 Molecular studies

The mRNA expression level of pro-apoptotic BAX in the protein fraction treated A549 was found to increase whereas the expression of anti-apoptotic Bcl-2 mRNA was found to decrease (Fig. 6). It was evident that as the concentration of the protein fraction increased, the expression of pro-apoptotic BAX increased and the expression of anti-apoptotic Bcl-2 decreased, resulting in increased BAX/Bcl-2 ration which causes mitochondrial membrane disruption leading to the release of apoptosis inducing factors which triggers the caspase cascade.

Molecular studies of pro-apoptotic BAX and anti-apoptotic BCL-2 mRNA showed that the protein fraction PF-2 regulated their expressions. The expression of BAX was found to be upregulated in the fraction treated cells whereas the expression of BCL-2 was found to be down-regulated. Mitochondrial membrane permeability changes when the ratio of BCL-2 family members changes [20, 21]. When the pro-apoptotic members are up-regulated and anti-apoptotic members are down-regulated, this leads to the release of cytochrome-c from mitochondria, which activates the caspase cascade [22]. Here, the protein fraction was found to decrease the expression of anti-apoptotic BCL-2 mRNA and also increase the expression of pro-apoptotic BAX mRNA thus shifting the ratio towards the pro-apoptotic events which induces apoptosis in the A549 cells.

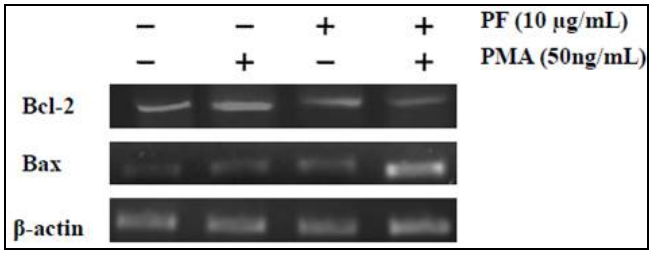


Fig 6: RT-PCR gel showing mRNA expression of anti-apoptotic Bcl-2 and pro-apoptotic BAX after treatment with fraction PF-2

The protein expression studies of p-ATM (S1981), CHK2, CDC25A, BAK and BAD showed that the protein fraction PF-2 induced increased expression of all the pro-apoptotic factors and decreased expression of anti-apoptotic factors in a dose dependent manner (Fig. 7); which can be compared to the expression of house-keeping beta-actin protein providing a relative expression pattern (Fig. 8). All the selected pro-apoptotic and anti-apoptotic factors are reported to play a very important role in cell cycle arrest leading to disruption of mitochondrial membrane integrity and thereby causing induction of intrinsic pathway of apoptosis through caspase cascade.

Cell growth and cell division are known to be controlled by several genetically defined cell cycle checkpoints to ensure the correct progression of cell cycle through different stages [23]. Cell cycle checkpoints would also induce cell cycle arrest or activate DNA repair response when cells are simulated by varieties of stimuli [24]. Ataxia telangiecectasia mutated (ATM) is a nuclear kinase which gets phosphorylated and activated in response to DNA damage in eukaryotic cells. The phosphorylated ATM further phosphorylates and activates Checkpoint kinase-2 (CHK2) which phosphorylates Cell division cycle 25 homolog A (CDC25A) marking it for ubiquitination and degradation. This blocks the progression for cells from G1 to S phase. The CHK2 also phosphorylates p53 and stabilizes it which induces upregulation of pro-apoptotic factors like BAX, BAD and BAK; and also down regulates the expression of anti-apoptotic factors like Bcl-2 through activation of p53 upregulated modulator of apoptosis (PUMA) and Bcl-2-like protein 11 (BIM) [25-29]. The mitochondria-anchored BAK and BAD proteins are found to be pro-apoptotic in nature and induce apoptosis in the cancer cells [30, 31]. The BAK and BAD proteins induce apoptosis by destabilizing the mitochondrial membrane and creating pores in the mitochondrial outer membrane thereby initiating

release of cytochrome-c into the cytosol which triggers caspase cascade [32, 33]. The BAK and BAD protein expression was found to increase in protein fraction treated A549 cells. This increase in expression in BAK and BAD proteins initiates apoptosis by forming pores in the mitochondrial outer membrane. This release of mitochondrial contents further activates caspase-9 and caspase-3 bringing about apoptosis.

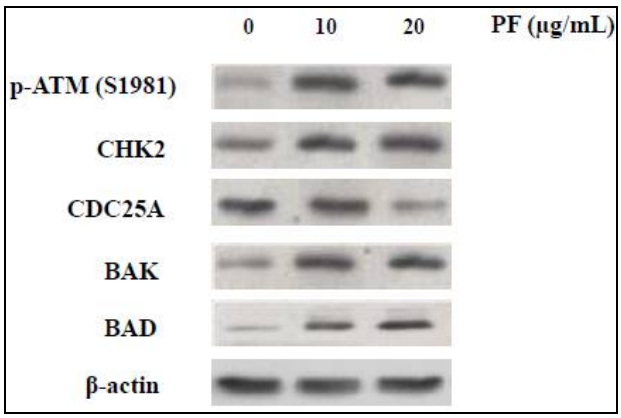


Fig 7: Western blot of p-ATM (S1981), CHK2, CDC25A, BAK and BAD proteins after treatment with fraction PF-2

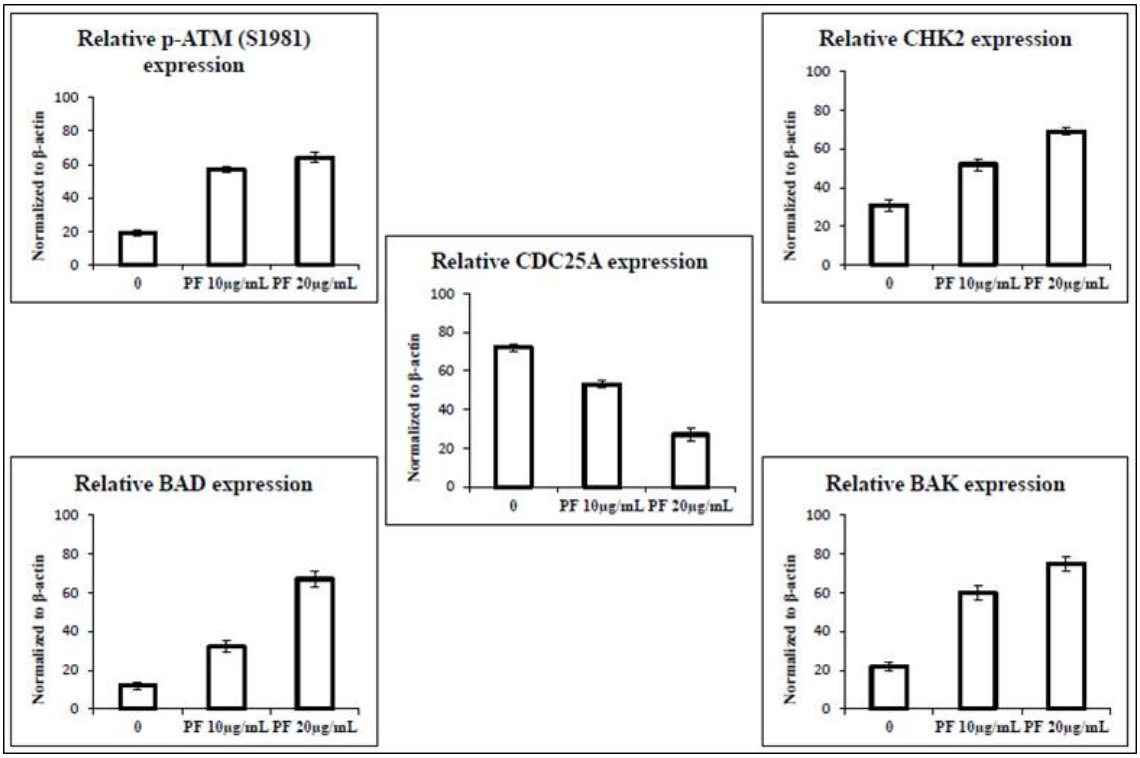


Fig 8: Relative protein expression of p-ATM (S1981), CHK2, CDC25A, BAK and BAD compared to β-actin

Finally, a significant increase in the caspase-9 and 3 activities were observed in protein fraction PF-2 treated cells compared to the untreated control, which indicated the activation of caspase-9 and 3 in fraction treated cells thereby inducing apoptosis in the A549 cells. The activity of the protein fraction at 10 µg/mL was 114.74 ± 3.82 µmols of pNA released/min/mL in caspase-9 assay and 125.51 ± 4.73 µmols of pNA released/min/mL in caspase-3 assay which decreased when the concentration of the protein fraction was reduced (Fig. 9).

The activities of both caspase-9 and 3 were found to increase in the A549 cells when treated with the protein fraction PF-2. Caspase-9 and 3 are the major enzymes involved in the mitochondria-mediated or intrinsic pathway of apoptosis [22, 34]. The activity of both these enzymes which was studied calorimetrically proved categorically that the protein fraction PF- 2 has the ability to activate these enzymes and thus induce apoptosis in the A549 cancer cells. The mitochondria-mediated or the intrinsic apoptotic pathway is often activated by various stimuli such as chemotherapeutic agents and radiation, which leads to altered ratio of Bcl-2 family members, promoting the release of cytochrome c as well as apoptotic protease activating factor-1 (Apaf-1) from mitochondria, and subsequently activating caspase-9 [35, 36]. Activated caspase-9 sets off further activation of downstream effector caspase-3, resulting in apoptotic cell death as elucidated in our study.

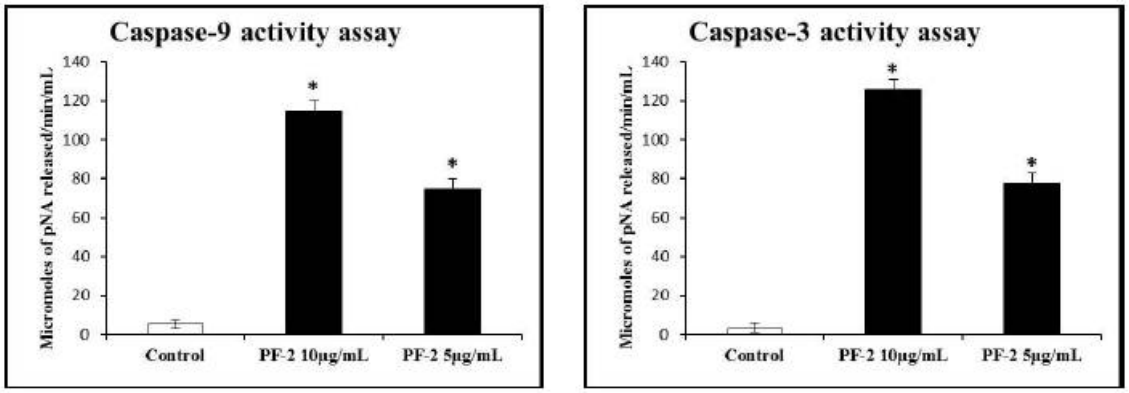


Fig 9: Effect of fraction PF-2 on the Caspase-9 and 3 release in A549 cells when treated with different concentrations

Members of *Pinaceaee* family and Genus Abies are reported to possess anti-inflammatory and anti-proliferative activity but no study has been conducted till date on the proteins isolated from the leaves of *Abies webbiana* Lindl. The protein fraction PF-2 induced considerable lower percentage of cell death in normal NIH3T3 cells compared to the cancerous A549 cells. Our study shows that the protein fraction induces apoptosis in the lung cancer cells via the intrinsic or the mitochondria mediated pathway by down regulating the anti-apoptotic factors and up regulating the pro-apoptotic factors.

4. Conclusion

In this study, the protein fraction PF-2, isolated from the leaves of *Abies webbiana* Lindl. was found to induce apoptosis in the A549 cancer cells via the mitochondria-dependent pathway. However, the fraction induced lower growth inhibitory activity on the normal NIH3T3 cells. This is the first report on the mechanism of apoptosis induction by the protein fraction isolated from the leaves of *Abies webbiana* Lindl. and these findings would provide yet another method of targeting lung cancer cells and further studies are underway to isolate and characterize specific protein molecules and to explore their apoptosis inducing ability in cancer cells.

5. Acknowledgement

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6. References

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