

Phytotherapeutic potential of *Pterospermum acerifolium* L. in human cancer cell lines through mitochondrial mediated ROS generation

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Background- Natural products have provided a new direction for the treatment of many human cancer including lung and pancreatic cancer. Phytotherapeutics provide a better alternative over conventional chemotherapeutics as these have less side effects. *Pterospermum acerifolium* commonly called as ‘Kanak champa’ have a wide range of application in Indian traditional medicinal system. *P. acerifolium* has many medicinal properties; however, the anticancer potential of *P. acerifolium* is not well studied. In the present study, we have demonstrated the anticancer effect of *P. acerifolium* bark extract in lung and pancreatic cancer cells. **Objectives-** (1) To evaluate the *in vitro* cytotoxic potential of *Pterospermum acerifolium* bark extract in lung and pancreatic cancer cell lines. (2) To investigate the role of *Pterospermum acerifolium* in ROS generation and mitochondrial mediated apoptosis in lung and pancreatic cancer cells. **Methods-** The anticancer potential of *P. acerifolium* bark extract on A549 lung and PANC-1 pancreatic cancer cell lines were determined by MTT assay, flow cytometry, fluorescence staining, cell cycle analysis, clonogenic assay, scratch invasion assay and quantitative real time PCR study. **Results and Conclusion-** The cell viability by MTT assay showed that cytotoxicity was increased in a dose dependent manner in both lung and pancreatic cancer cell lines. Treatment with different concentrations of *P. acerifolium* bark extract showed clear visualization of change in cellular morphology, cell shrinkage, and cytoplasmic condensation in both A549 and PANC-1 cell lines. Scratch and Invasion assay showed that cell migration and invasion was significantly checked bark extract treated A549 and PANC-1 cells. Acridine orange/ethidium bromide and DAPI staining of *P. acerifolium* bark extract treated A549 and PANC-1 cells showed early and late apoptotic cells. Fluorescence and flow cytometry staining analysis showed that *P. acerifolium* bark extract reduces mitochondria membrane potential, induces ROS generation and increases the percentage of sub-G1 phase in both lung and pancreatic cancer cells confirming the apoptotic potential. In conclusion, we demonstrated that *P. acerifolium* bark extract showed cell cytotoxicity in lung and pancreatic cancer cells through modulating ROS generation, mitochondrial membrane potential and cell cycle checkpoints.

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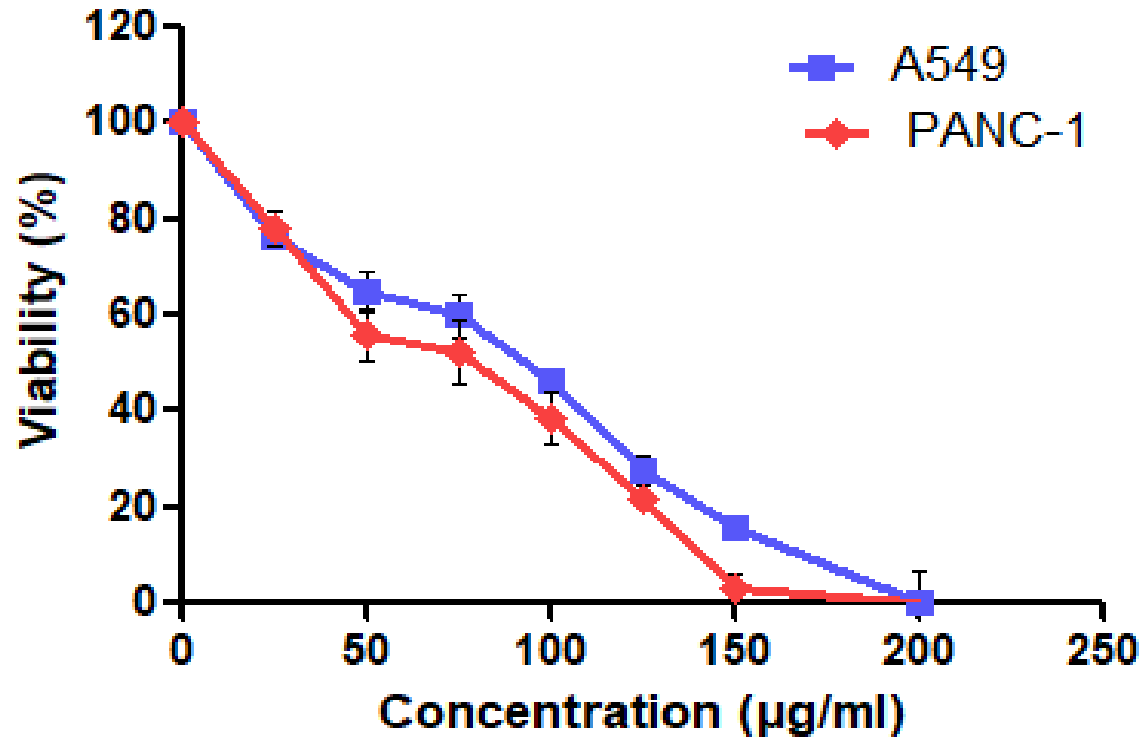
Introduction

- There are lots of research going on to find some new **natural compounds** to overcome chemotherapeutic side effects during cancer treatment.
- *Pterospermum acerifolium* is a shrub and ever green tree has very wide range of application in Indian traditional medicinal system.
- Studies suggested that bark extract of *P. acerifolium* has antimicrobial, antioxidant, anti-inflammatory, analgesic and anti-ulcer activity.
- However, the **anticancer effect of *P. acerifolium*** bark extracts is not well studied.
- We have demonstrated the anticancer potential of *P. acerifolium* bark extract in **lung and pancreatic cancer cell lines** by modulation ROS generation, mitochondrial deformation and cell cycle checkpoints.

Objectives

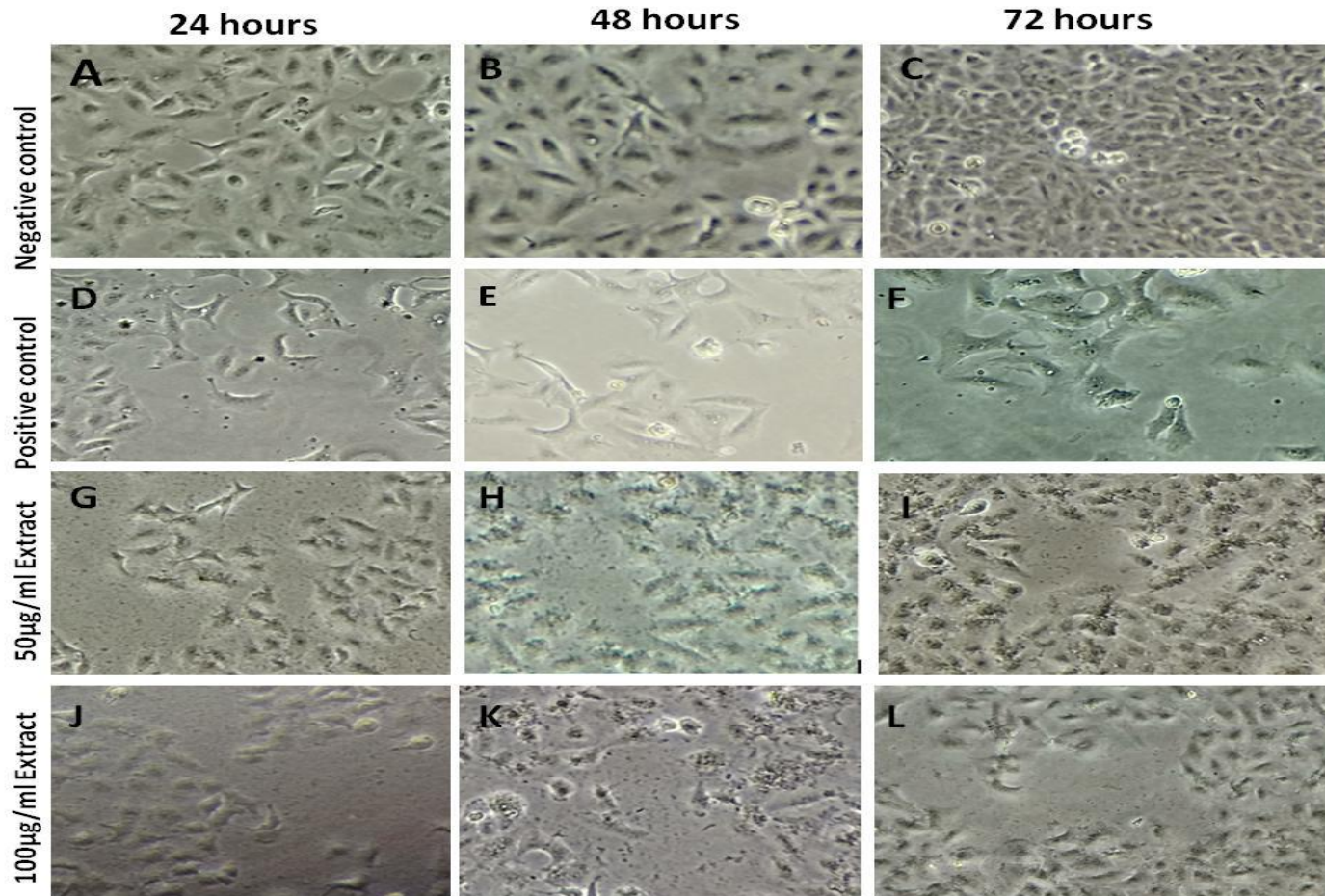
- 1. In vitro cytotoxic activity of *Pterospermum acerifolium* bark extract in lung and pancreatic cancer**
- 2. To investigate the effect of *Pterospermum acerifolium* bark extract in inducing ROS generation and mitochondrial mediated apoptosis in lung and pancreatic cancer**
- 3. To elucidate the mechanistic effect of *Pterospermum acerifolium* bark extract on ROS and mitochondrial mediated stress pathways**

Cytotoxic potential of *P. acerifolium* in lung and pancreatic cancer



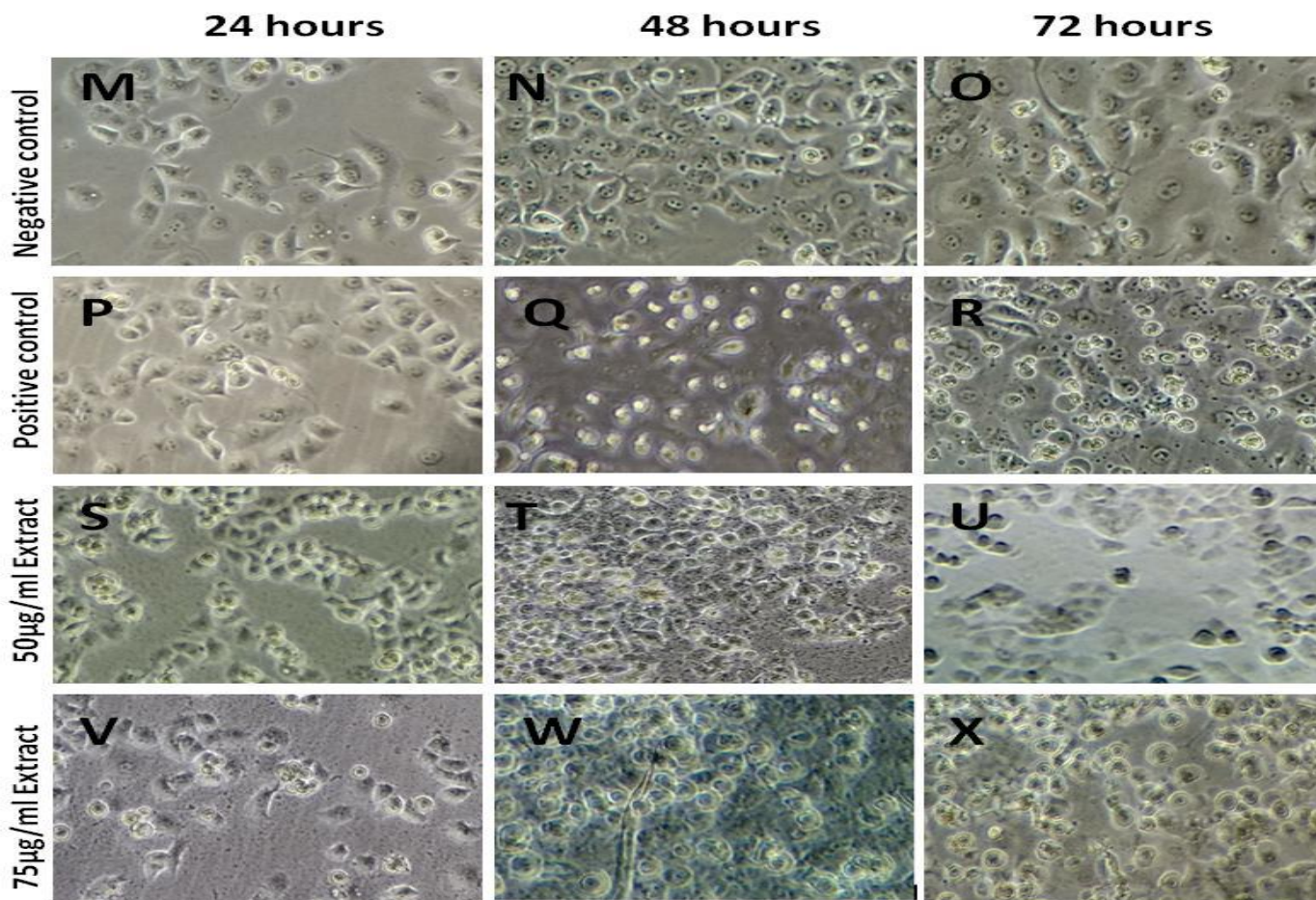
Cell viability assay of PaEBE in lung (A549) and pancreatic (PANC-1) cancer cells. A549 and PANC-1 cells were treated with different concentrations of PaEBE for 24 h and cell viability was done by MTT assay.

Alteration in cellular morphology by *P. acerifolium* treatment in lung cancer



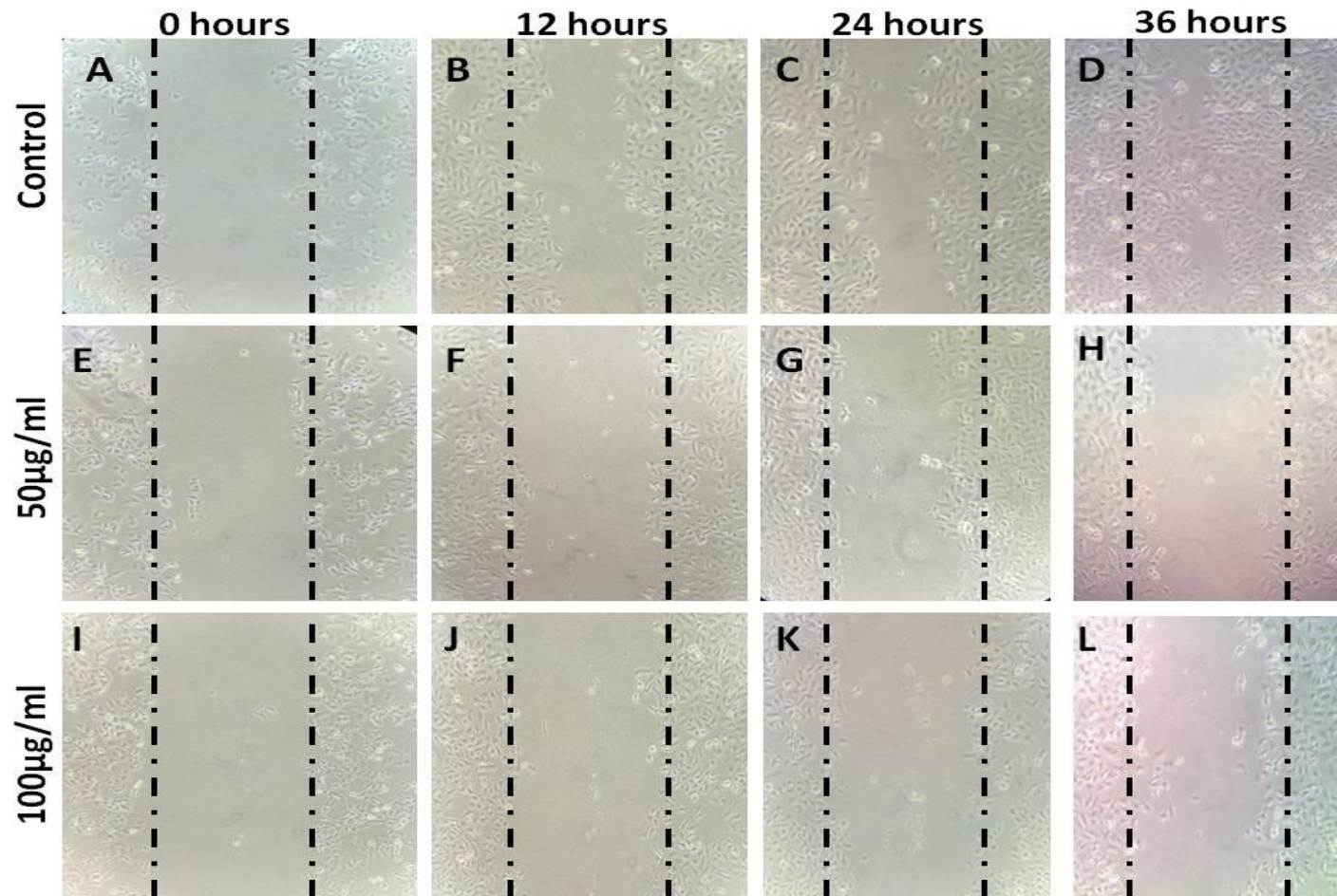
The morphological changes of A549 cells treated with PaEBE at 50 and 100 µg/ml concentration. Erlotinib (20 µM) and ethanol (20 µl) were used as positive and negative control respectively.

Alteration in cellular morphology by *P. acerifolium* treatment in pancreatic cancer



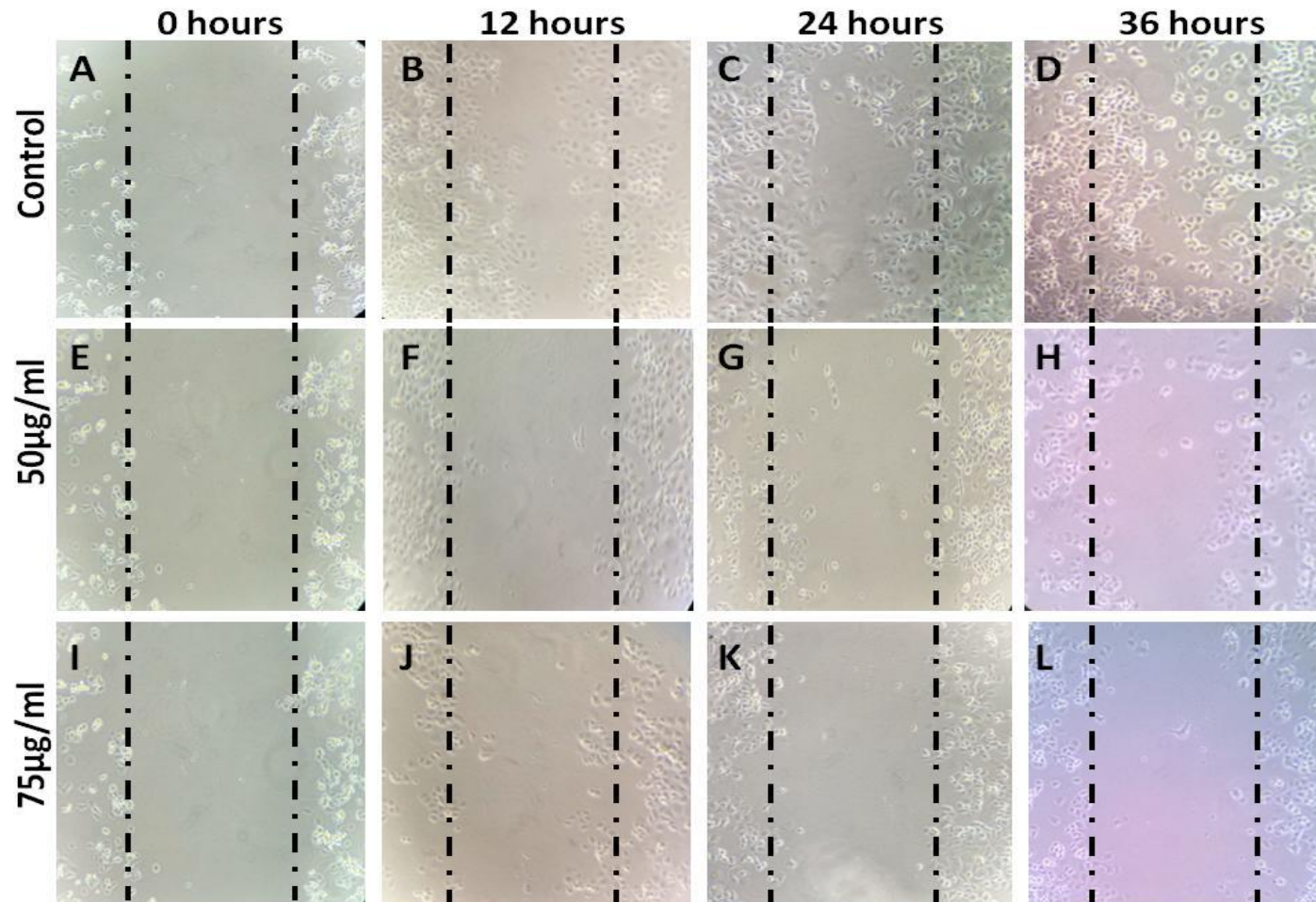
The morphological changes of PANC-1 cell line treated with PaEBE at 50 and 75 µg/ml concentration. Gemcitabine (20 µM) and ethanol (15 µl) were used as positive and negative control respectively.

Cell migration inhibitory potential of *P. acerifolium* in lung cancer



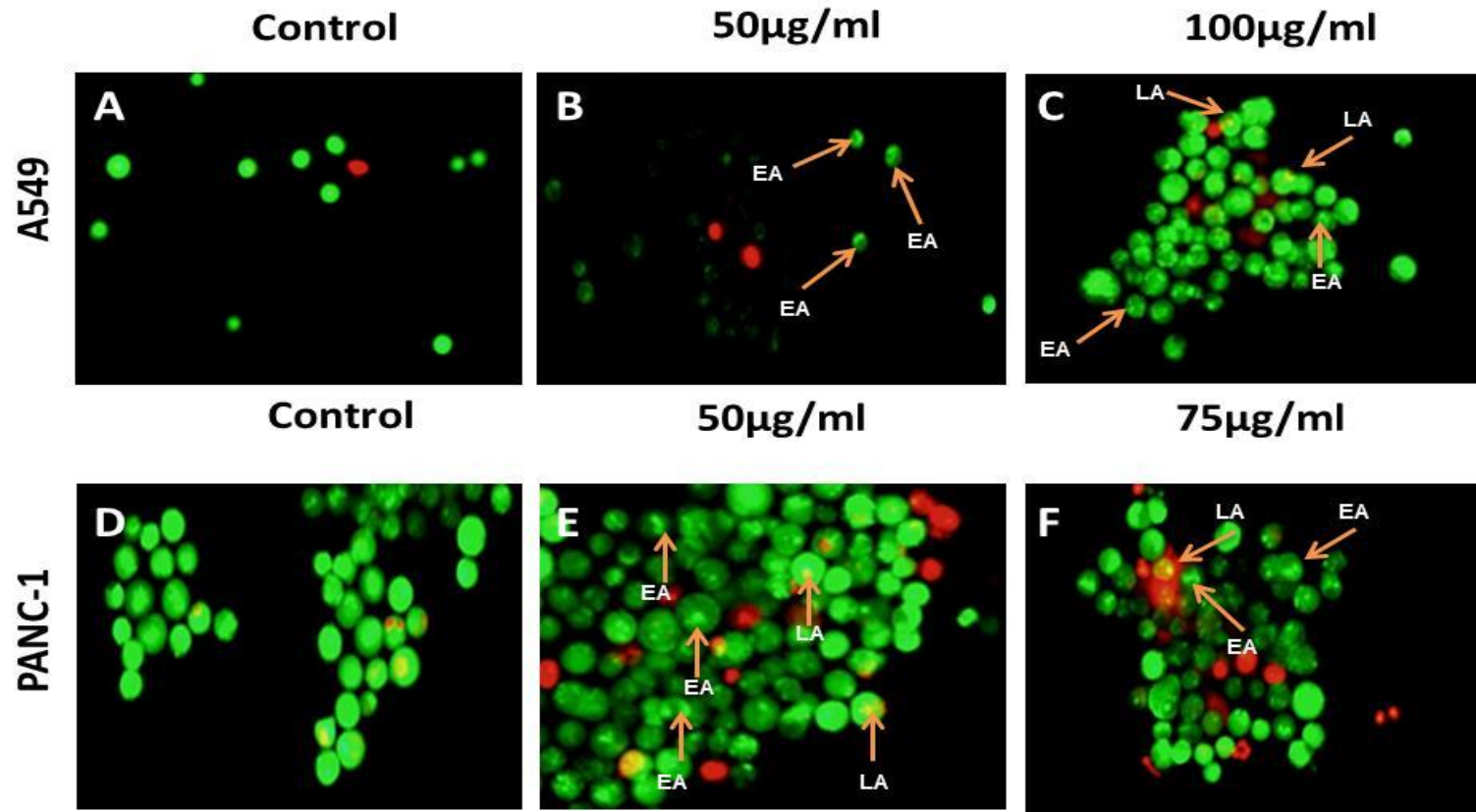
In-vitro scratch motility assay of PaEBE on A549 cells. Wound closure ability of treated lung cancer cell line after creation of scratch wound in control and treated wells.

Cell migration inhibitory potential of *P. acerifolium* in pancreatic cancer



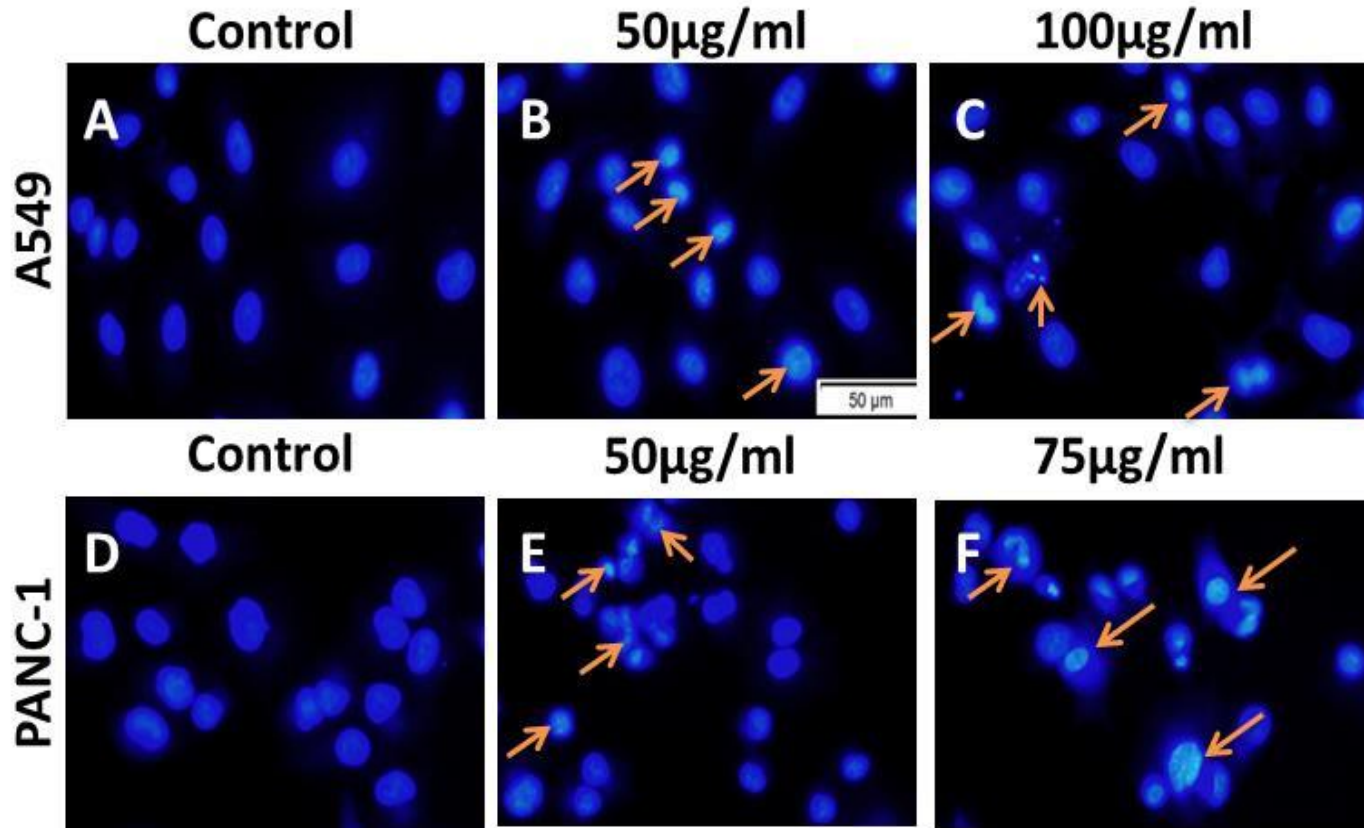
In-vitro scratch motility assay of PaEBE on PANC-1 cancer cells. Wound closure ability of treated PANC-1 cell line after creation of scratch wound in control and treated wells.

Acridine orange(AO)/ Ethidium bromide(EtBr) staining for apoptosis detection



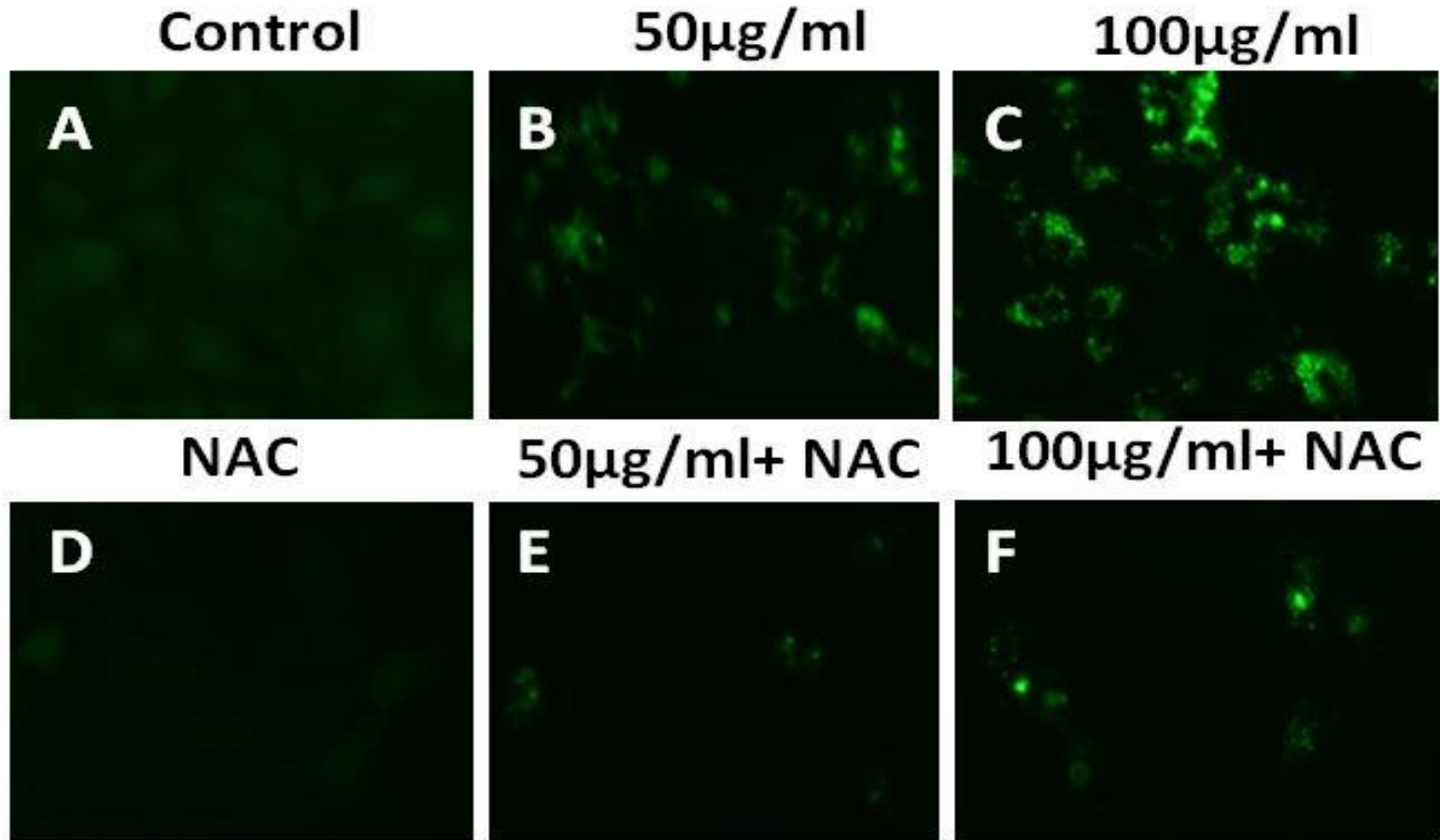
Morphological observation of AO/EtBr stained A549 and PANC-1 cell lines treated with PaEBE for 24 h. Early apoptotic and late apoptotic cells were visualized under a fluorescence microscope.

Nuclear morphology study by DAPI staining



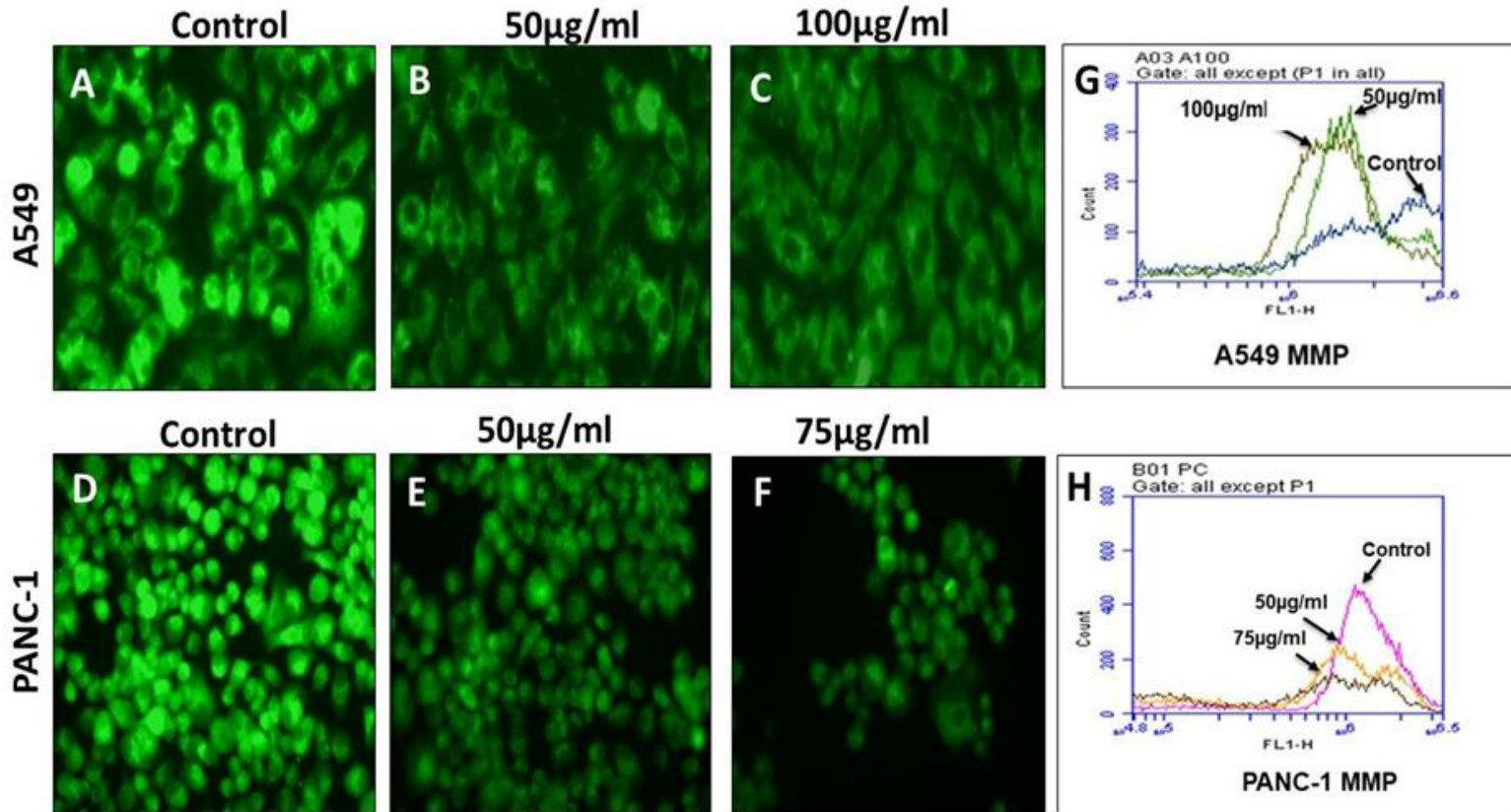
Morphological observation of DAPI-stained A549 and PANC-1 cell lines treated with PaEBE for 24 h. The morphological changes in the cells were visualized under a fluorescence microscope.

Determination of intracellular ROS generation



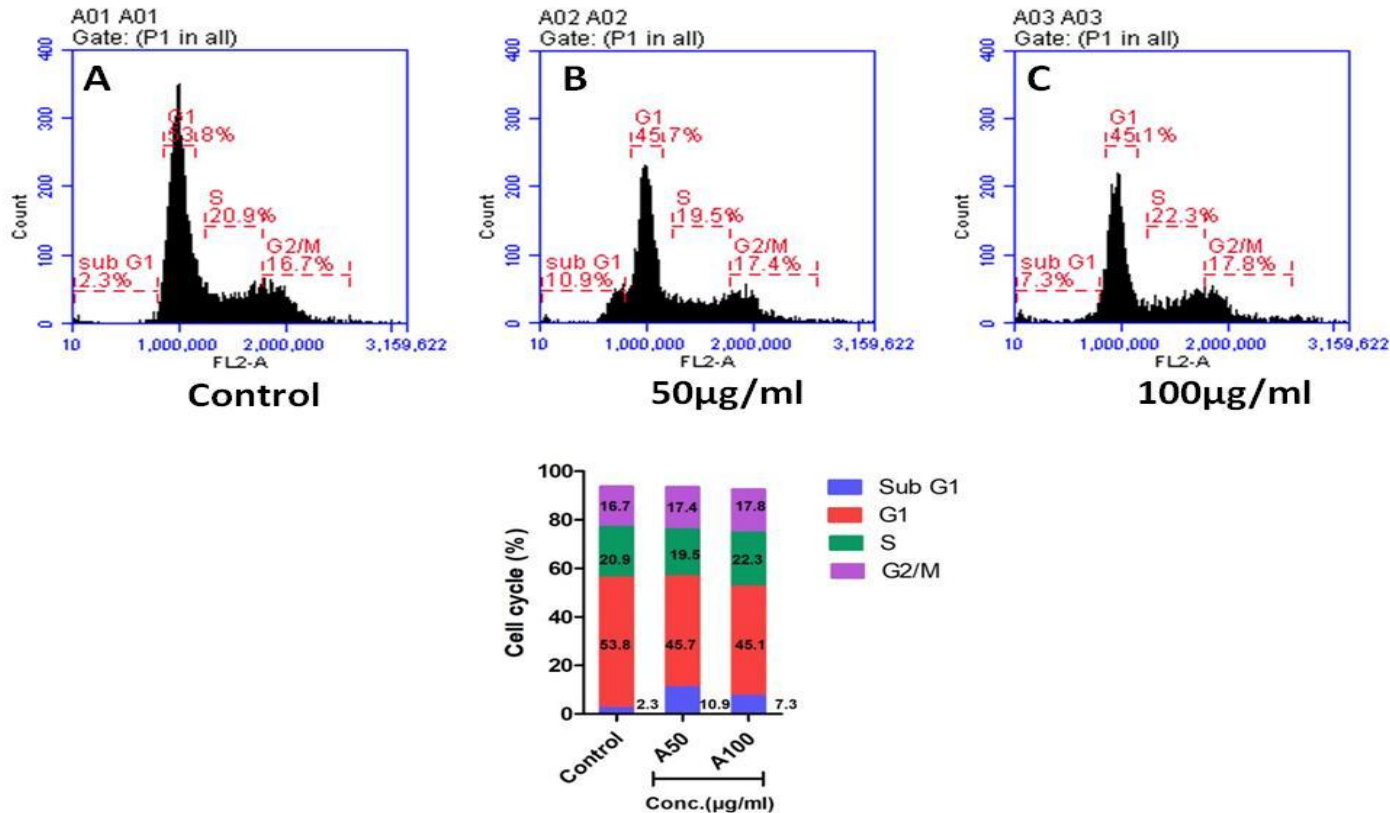
Qualitative measurement of intracellular ROS levels using the fluorescent probe DCF-DA in A549 cells after treatment with PaEBE for 24 h.

Measurement of mitochondrial membrane potential (MMP)



Effect of *P. acerifolium* bark extract on MMP in A549 and PANC-1 cells using Rho-123. Effect on MMP intensity analysis by flow cytometry in A549 and PANC-1 cell lines.

Cell cycle analysis of lung cancer (A549) cell line



Effect of *P. acerifolium* bark extract on cell cycle of lung cancer cell line. Graphical representation of changes in different cell cycle stages after drug treatment with respect to control.

Conclusion

- *P. acerifolium* bark extract shows cytotoxic activity in lung and pancreatic cancer cell lines. However, PANC-1 cells are more sensitive than A549 cells.
- *P. acerifolium* bark extract shows anti-proliferative and anti-metastatic property.
- Our results showed the apoptotic inducing property of *P. acerifolium* bark extract through mitochondrial mediated ROS generation.

Acknowledgement

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Thank You