# Effects exerted by Papaverine on proliferation, morphology, oxidative stress and cell cycle progression in cancer cell lines

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## INTRODUCTION

Cancer is a leading cause of mortality with 10 million deaths reported in 2020 with lung- and breast cancer the most common cancer diagnosed. Papaverine (PPV) is a natural occurring, non-narcotic alkaloid isolated from Papaver somniferum. Previous studies have indicated papaverine inhibits cell growth in tumorigenic cell lines; however, several questions remain regarding the influence of PPV in tumorigenic cells.

## MATERIALS and METHODS

**Cell lines:**

Triple negative breast tumorigenic cell line (MDA-MB-231)
Adenocarcinoma alveolar tumorigenic cell line (A549)
Prostate tumorigenic cell line (DU145)

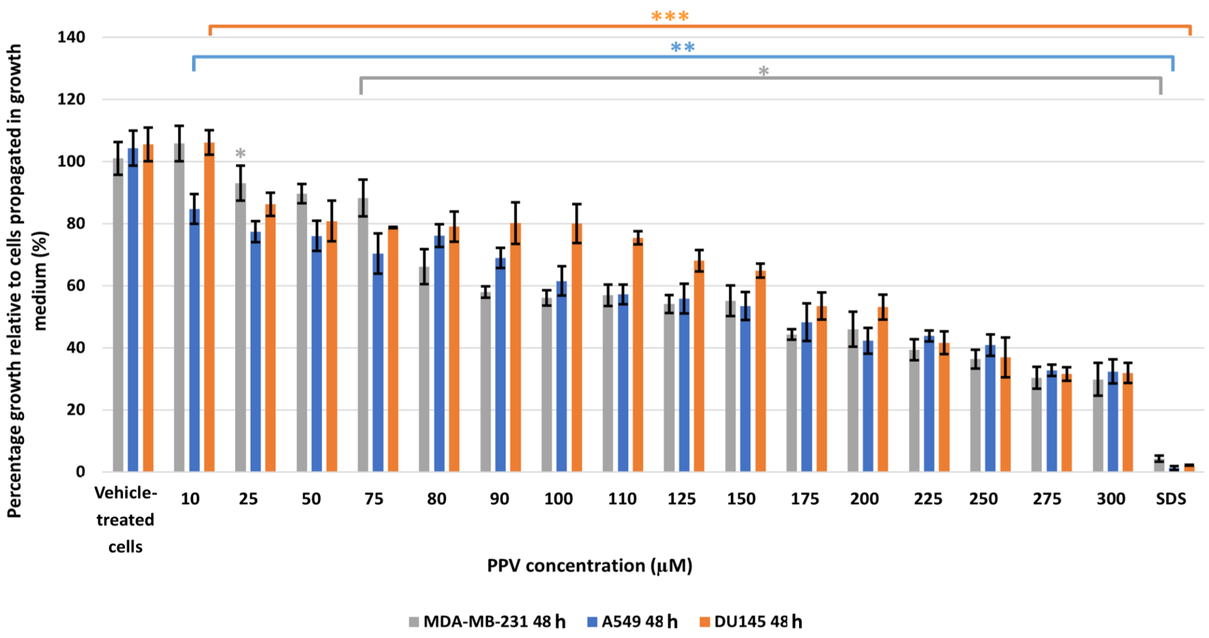
**Methods**

Cell proliferation: Spectrophotometry
Cell morphology: Light microscopy
Hydrogen peroxide generation: Fluorescent microscopy
Cell cycle progression: Flow cytometry

## RESULTS

### CELL PROLIFERATION

Crystal violet staining was used to investigate the effects of PPV on cell proliferation in MDA-MB-231-, A549- and DU145 cell lines. Data indicates that PPV induces antiproliferative activity that is specific to each cell line and is time-and doe-dependent.



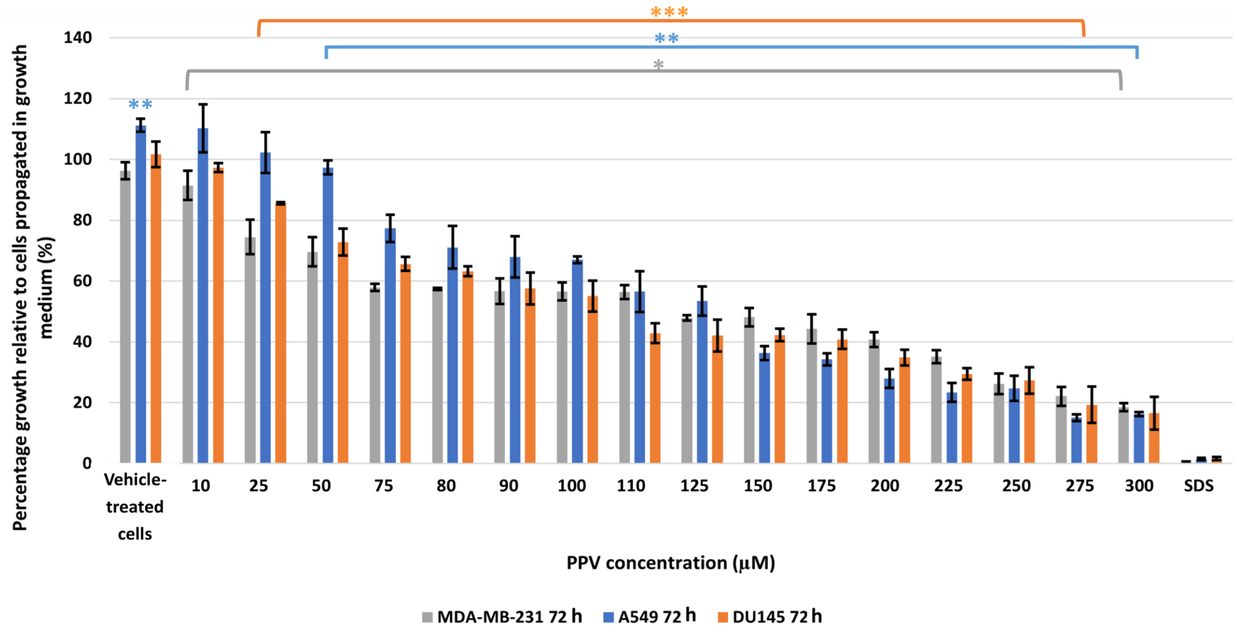




**Fig 1.** Spectrophotometry results of crystal violet staining demonstrating the effects of PPV (10-300 µM) on proliferation on MDA-MB-231 cells compared to A549- and DU145 cell lines at 48- and 72 h. The average of 3 independent experiments is represented by the graph with error bars indicating standard deviation. Statistical significance is represented by an * when using the student t-test with a P value of 0.05 compared to cells propagated in growth medium.

### CELL MORPHOLOGY

The effects of PPV on cell morphology was investigated using light microscopy on MDA-MB-231-, A549- and DU145 cell lines at 48 h.

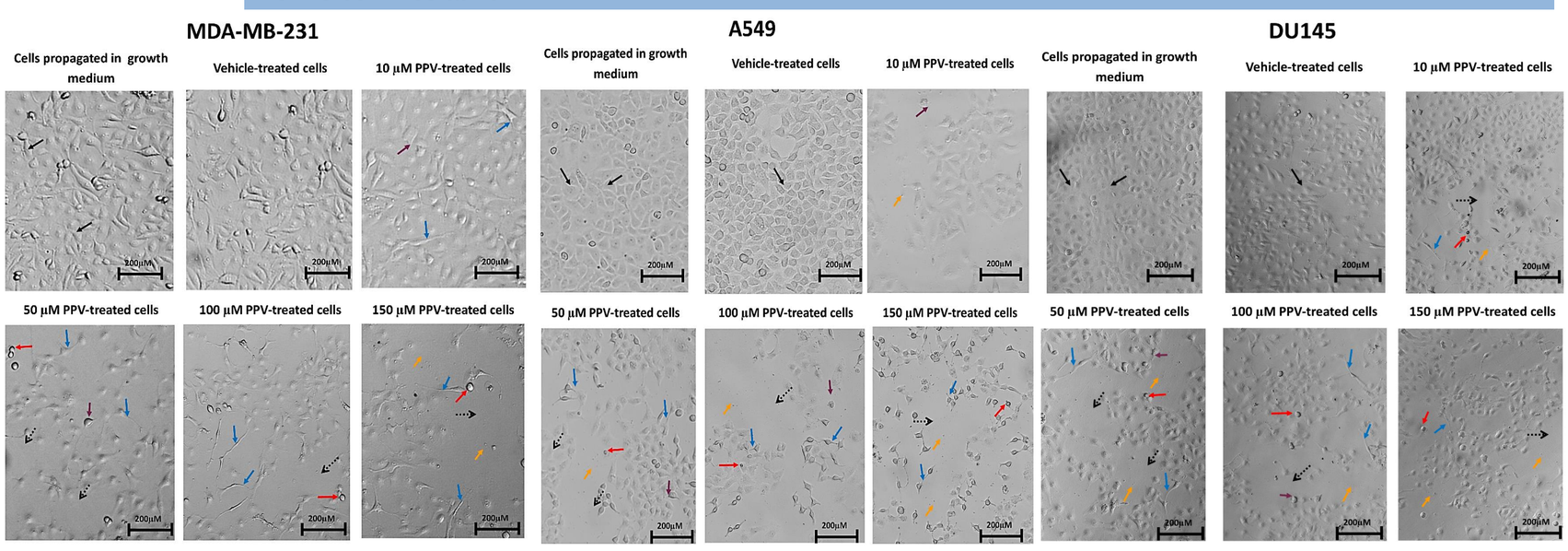



**Fig 2.** Light microscopy images of cell morphology demonstrating the effects of PPV ((10-150 µM) on cell morphology on MDA-MB-231-, A549- and DU145 cell at 48 h at a magnification of x10. Blue arrows: lamellipodia-like protrusions, green arrows: rounded cells, red arrows: shrunken rounded cells, purple arrows: cells exhibiting membrane blebbing, black solid arrows: cells with no abnormal morphology, yellow arrows: cell debris and black dashed arrows: areas exhibiting reduced cell density.

### HYDROGEN PEROXID GENERATION

PPV induces oxidative stress as indicated by aberrant quantities of hydrogen peroxide ($H_2O_2$).

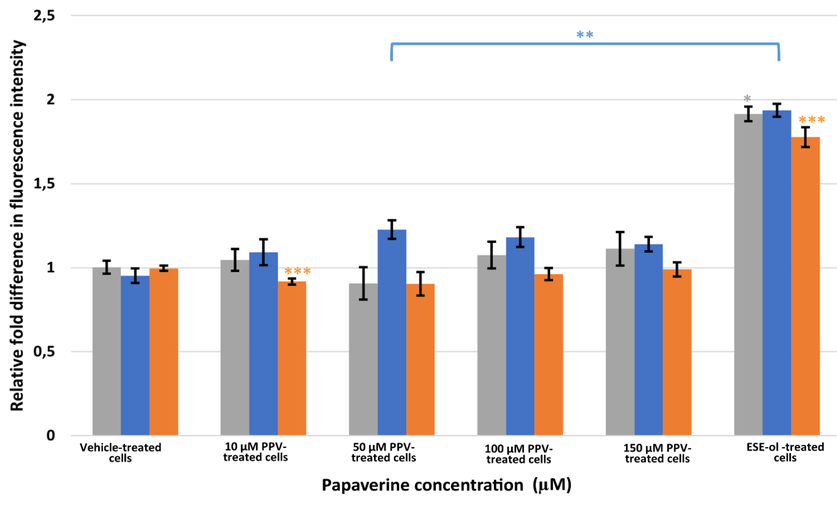



**Fig 3.** Fluorescence microscopy results of DCFDA staining demonstrating the effects of PPV (10-150 µM) on $H_2O_2$ production on MDA-MB-231 cells compared to A549- and DU145 cell lines at 48 h. The average of 3 independent experiments is represented by the graph with error bars indicating standard deviation. Statistical significance is represented by an * when using the student t-test with a P value of 0.05 compared to cells propagated in growth medium

### CELL CYCLE PROGRESSION

PPV induces abnormal cell cycle changes and endoreduplication.

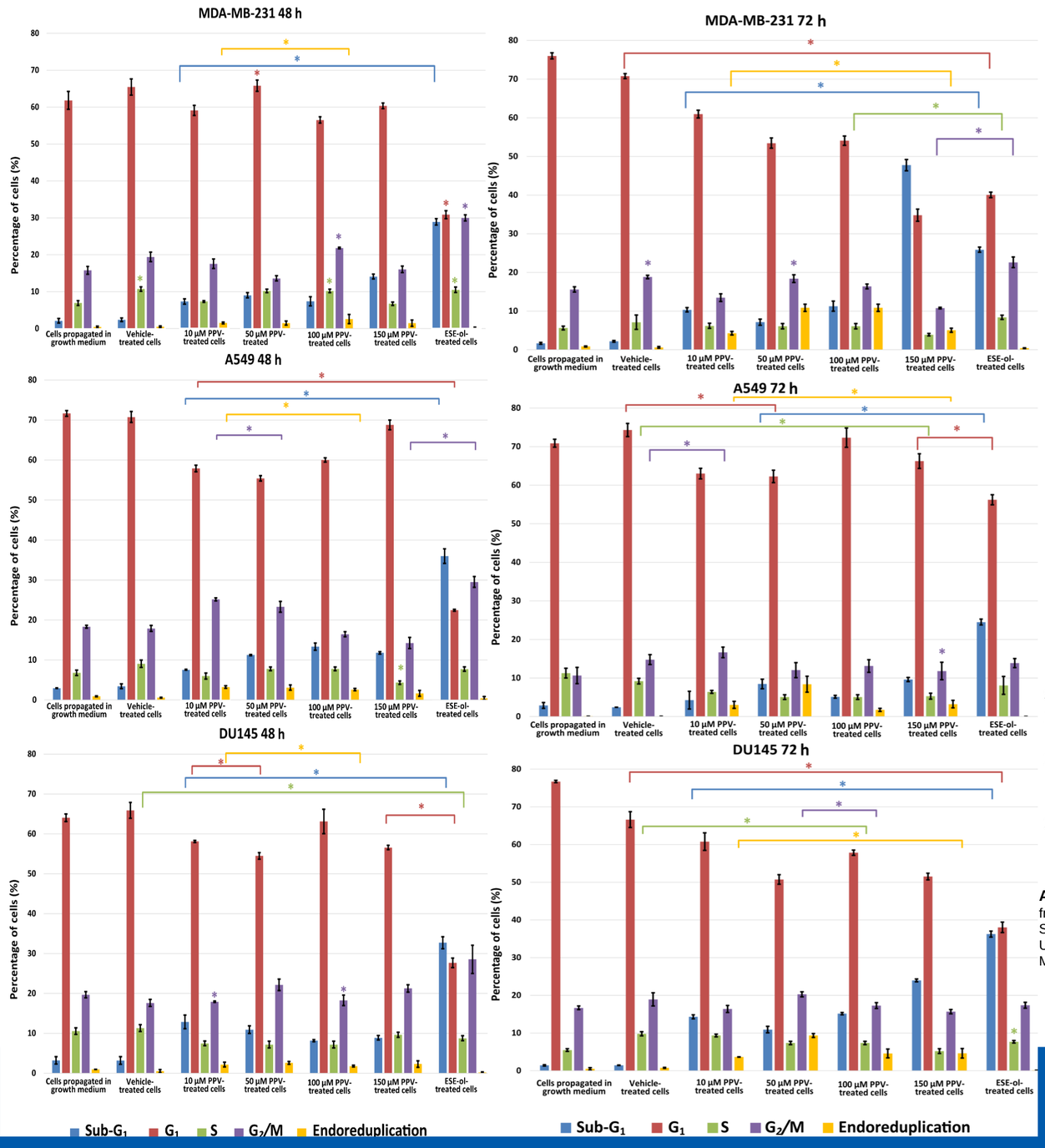



**Fig 4.** Fluorescence microscopy results of DCFDA staining demonstrating the effects of PPV (10-150 µM) on $H_2O_2$ production on MDA-MB-231 cells compared to A549- and DU145 cell lines at 72 h. The average of 3 independent experiments is represented by the graph with error bars indicating standard deviation. Statistical significance is represented by an * when using the student t-test with a P value of 0.05 compared to cells propagated in growth medium

**Fig 5.** Flow cytometry results demonstrating the effects of PPV (10-150 µM) on the cell cycle on MDA-MB-231-, A549- and DU145 cells at 48- and 72 h. Blue bar: cells in Sub-G1 phase, red bar: G1 phase, the green bar: S phase, the purple bar: cells in G2/M phase and the yellow bar: cells undergoing endoreduplication. Statistical significance is represented by an * when using the student t-test with a P value of 0.05 compared to cells propagated in growth medium.

## DISCUSSION and CONCLUSION

This study Indicates that PPV induces time and dose dependent activity in MDA-MB-231-,A549-, and DU145 cells. Data obtained in indicates that PPV exerts time-and dose-dependent antiproliferative activity in all three cell lines accompanied by lamellipodia-like protrusions, oxidative stress and endoreduplication and sub-G1 phase increases. This study contributes to the understanding regarding the influence of phytomedicinal alkaloid compounds in cancer cell lines.

**Selected References:**

1. Sajadian S, Vatankhah M, Majdzadeh M, Kouhsari SM, Ghahremani MH. Cell cycle arrest and apoptogenic properties of opium alkaloids noscapine and papaverine on breast cancer stem cells. Toxicology Mechanisms Methods. 2015; 25(5):388-395.
2. Goto T, Matsushima H, Kasuya Y, Hosaka Y, Kitamura T, Kawabe K, Hida A, Ohta Y, Simizu T, Takeda K. The effect of papaverine on morphologic differentiation, proliferation and invasive potential of human prostatic cancer Incap cells. International Journal of Urology. 1999; 6(6):314-319.
3. Huang H, Li L-J, Zhang H-B, Wei A-Y. Papaverine selectively inhibits human prostate cancer cell (pc-3) growth by inducing mitochondrial mediated apoptosis, cell cycle arrest and downregulation of nf-κb/pi3k/akt signalling pathway. Journal of BU ON.: official journal of the Balkan Union of Oncology. 2017; 22(1):112-118.

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