



P2.100: Evaluation of Anticancer Activity of Beta-Escin Against A549 and LLC1 Cell Lines of Lung Cancer



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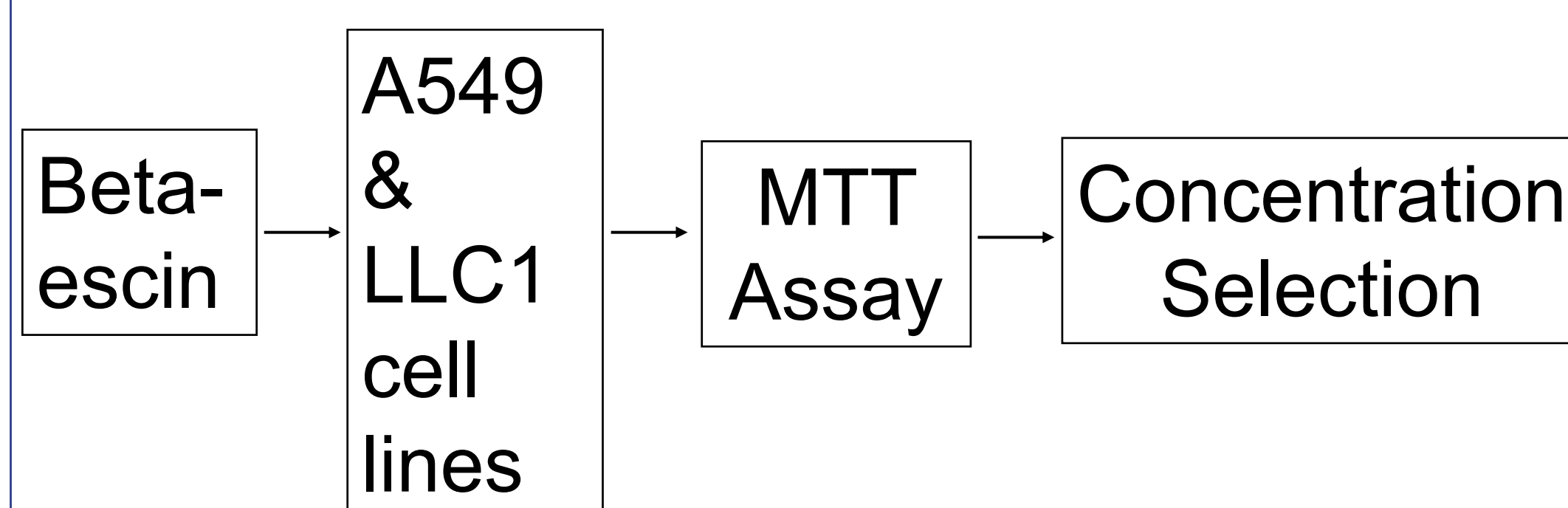
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INTRODUCTION

Lung cancer is a leading cause of cancer-related mortality globally¹. Despite the introduction of newer modalities, survival of lung cancer has only reached to approximately 20%². Beta-escin, a natural saponin derived from seeds of Aesculum Hippocastanum (horse chestnut), has shown anticancer activity against breast³, colon⁴, and ovarian cancer⁵. In this *in vitro* study, beta – escin was being tested for anticancer activity against human lung adenocarcinoma (A549) and mouse originated Lewis Lung carcinoma (LLC1) cell lines.

METHODS

Design: *In vitro* Preliminary Study



Apoptosis Confirmatory and Mechanistic Assays

(at selected concentrations)

- Annexin V/ Propidium iodide Assay
- Cell cycle analysis
- Nitric Oxide release
- Hoechst staining
- Ultrastructural change determination

DISCUSSION AND CONCLUSION

Beta – escin showed concentration – dependent anticancer activity against cell lines from two species (human and mouse) and is a promising agent for drug repurposing.

Further studies will be required to confirm the anticancer activity of beta-escin in mouse model of lung cancer

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RESULTS

A549 cell line

- IC₅₀ values were 24.8 μ M, 21.5 μ M and 21.8 μ M at 24 h, 48 h and 72 h, respectively.
- Beta-escin increased concentration-dependent dead cells (p<0.0001) and arrested cells in G0-G1 phase (p<0.05).
- Beta-escin increased integrated density of Hoechst stain and the nuclear damage was observed through TEM.
- Also, the release of nitric oxide was reduced.

LLC1 cell line

- IC₅₀ values were 25.5 μ M, 22.9 μ M and 20.1 μ M, at 24 h, 48 h and 72 h, respectively.
- The percentage of cells in G0-G1 phase reduced (p<0.0001).
- Also, the release of nitric oxide was reduced.

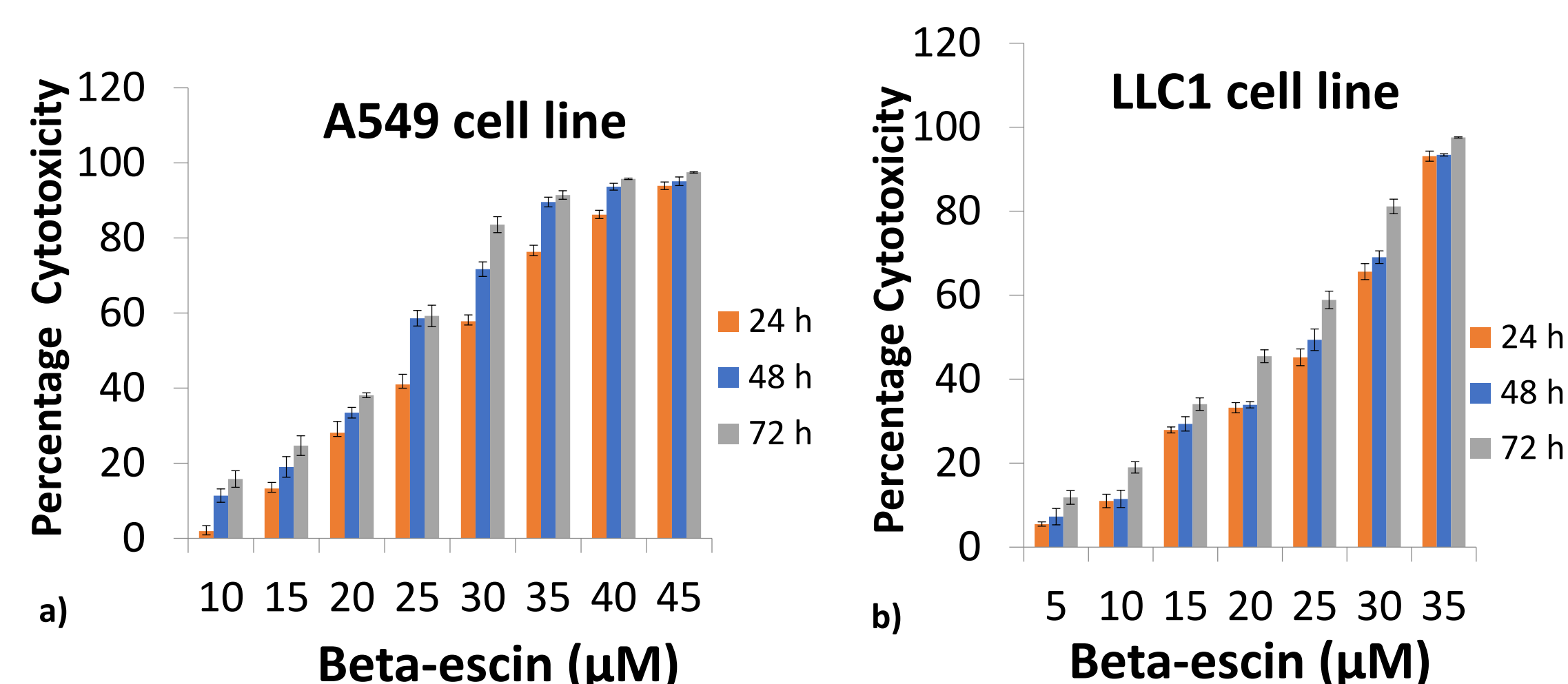


Figure 1: MTT assay of Beta-escin in a) A549 and; b) LLC1 cell lines

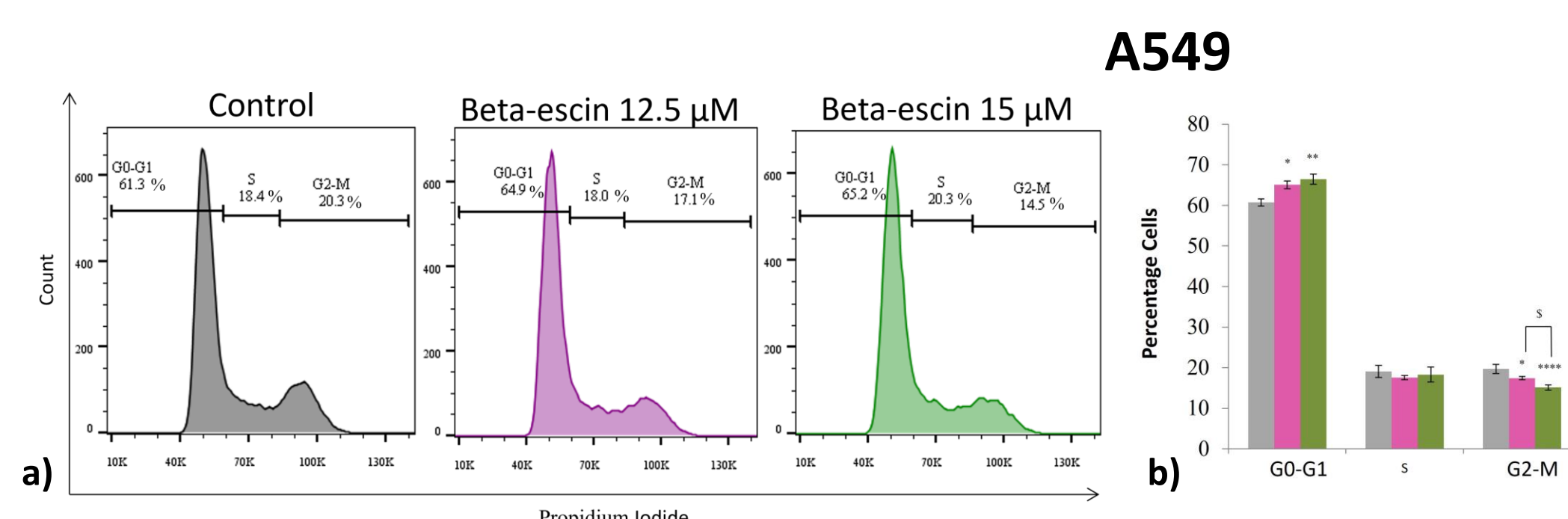


Figure 3a): Cell cycle analysis of A549 cells treated with different concentrations of beta-escin; b) quantitative comparison of percentage cells of beta-escin treated groups in different stages of cell cycle with control group

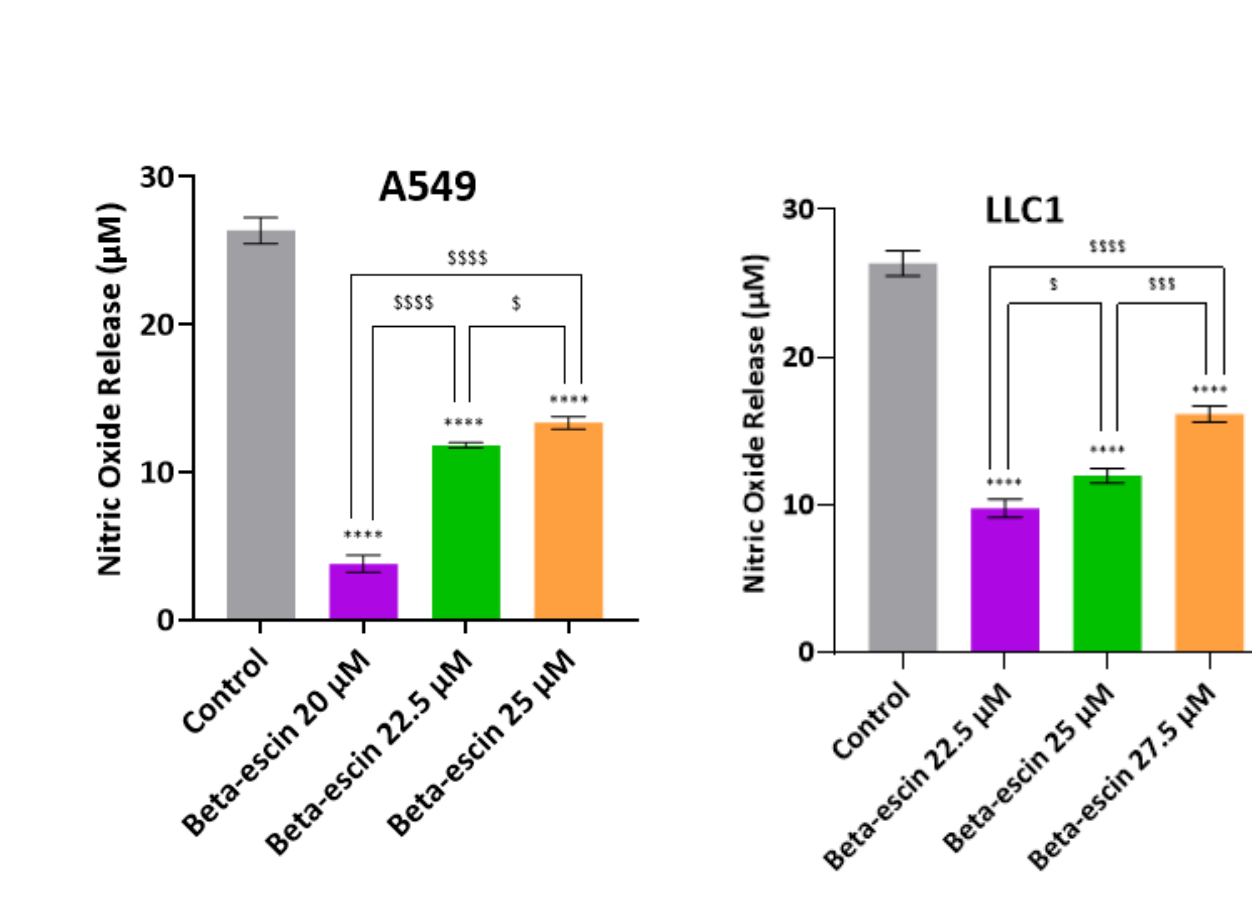


Figure 5: Quantitative comparison of nitric oxide release in A549 and LLC1 cells treated with different concentrations of beta-escin with control group

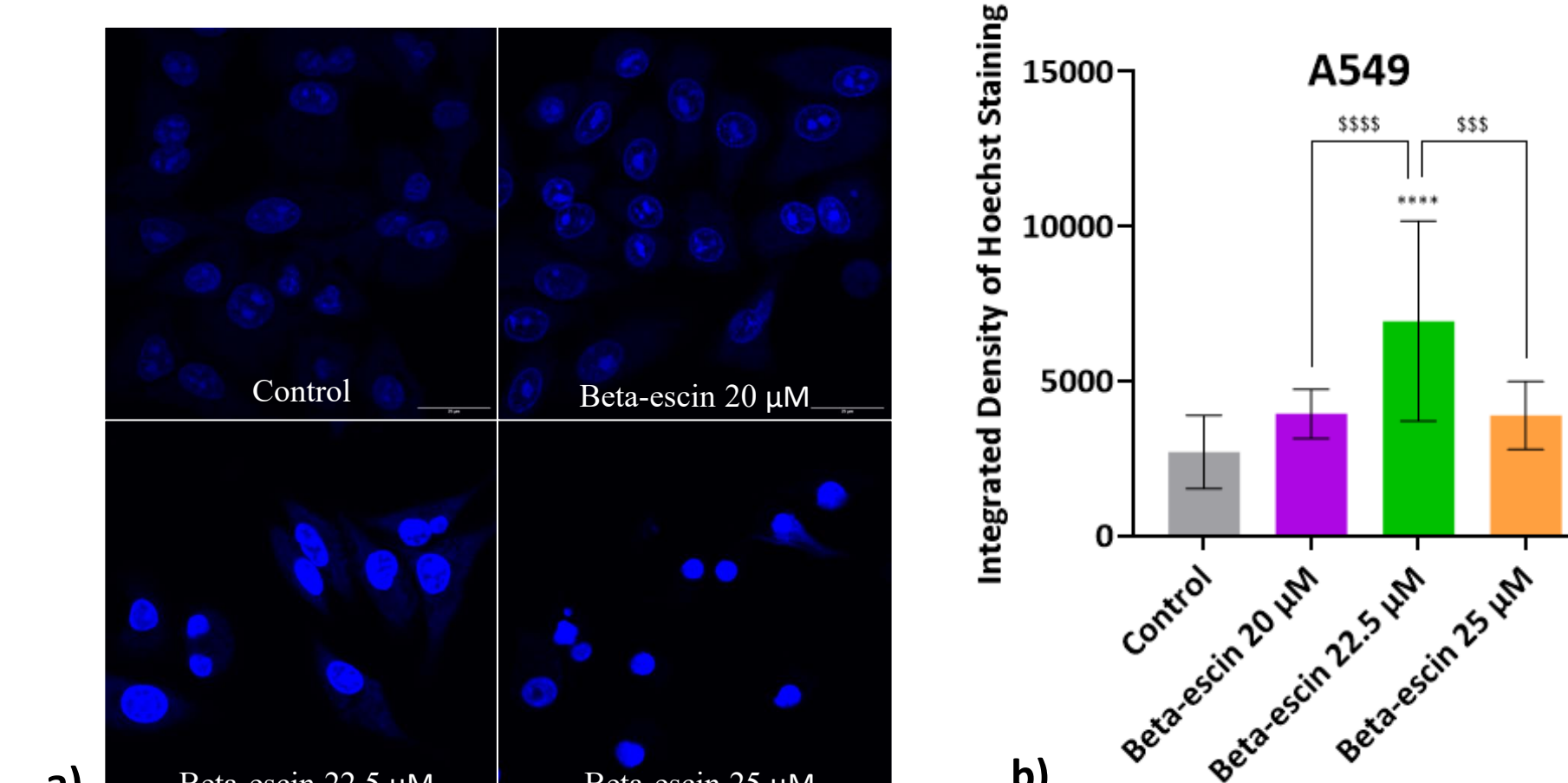


Figure 6a): Hoechst staining of A549 cells treated with different concentrations of beta – escin; b) quantitative comparison of integrated density of beta-escin treated groups with control group

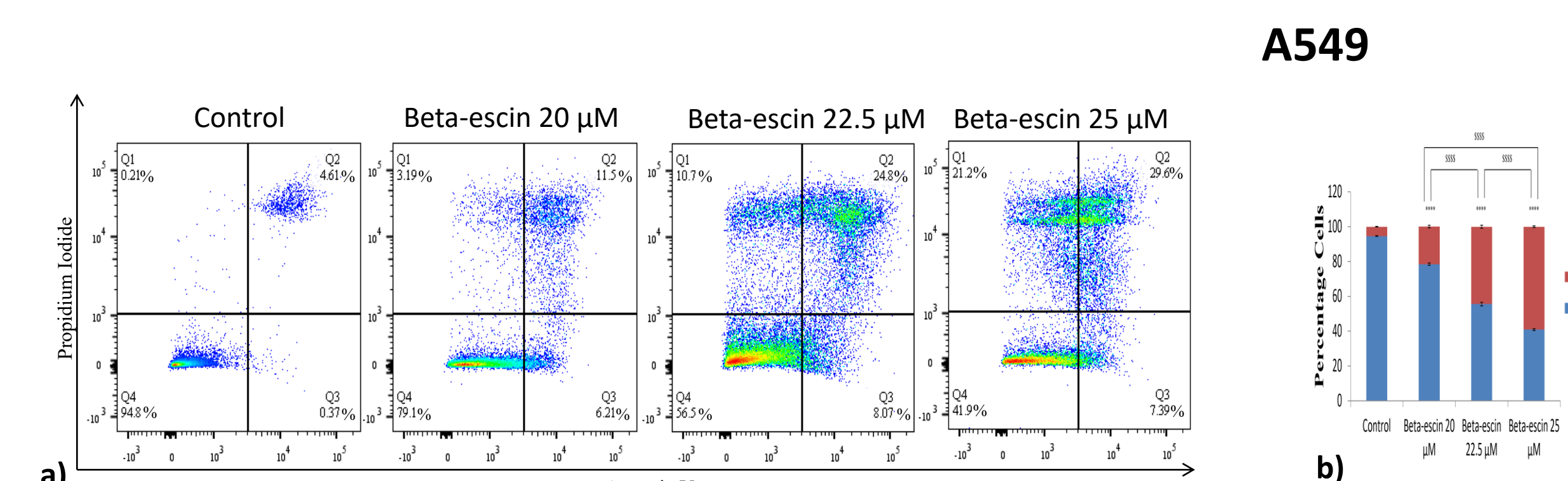


Figure 2a): Annexin V/ propidium iodide staining of A549 cells treated with different concentrations of beta-escin; b) quantitative comparison of percentage cells of beta-escin treated groups in different stages of viability (live v/s dead) with control group

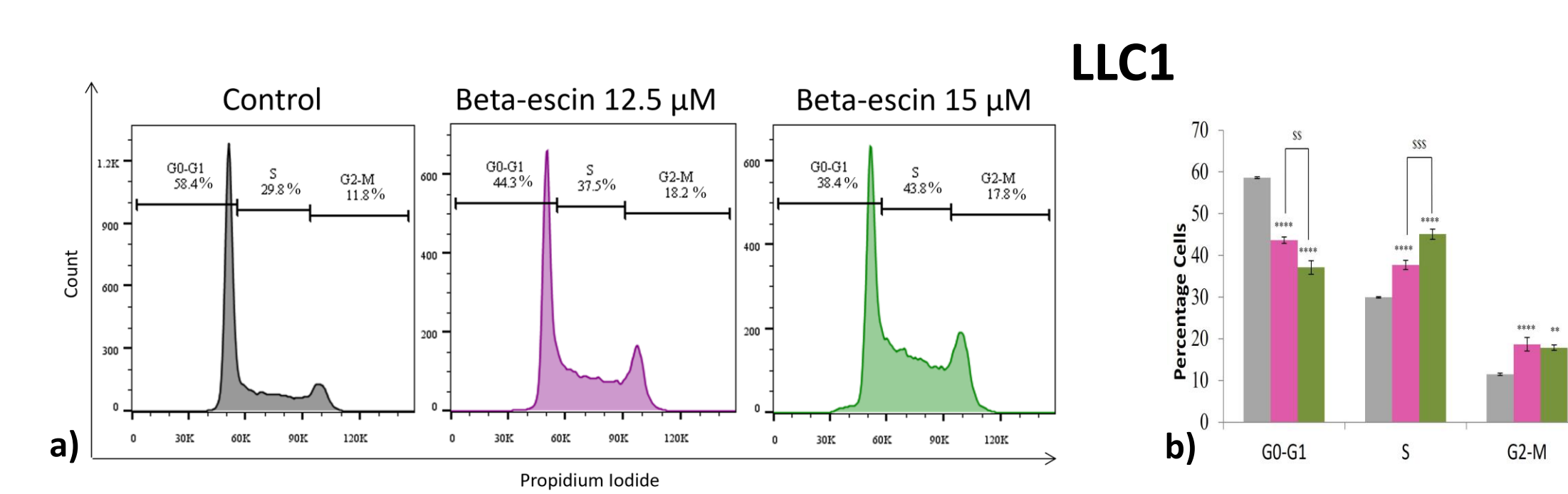


Figure 4a): Cell cycle analysis of LLC1 cells treated with different concentrations of beta-escin; b) quantitative comparison of percentage cells of beta-escin treated groups in different stages of cell cycle with control group

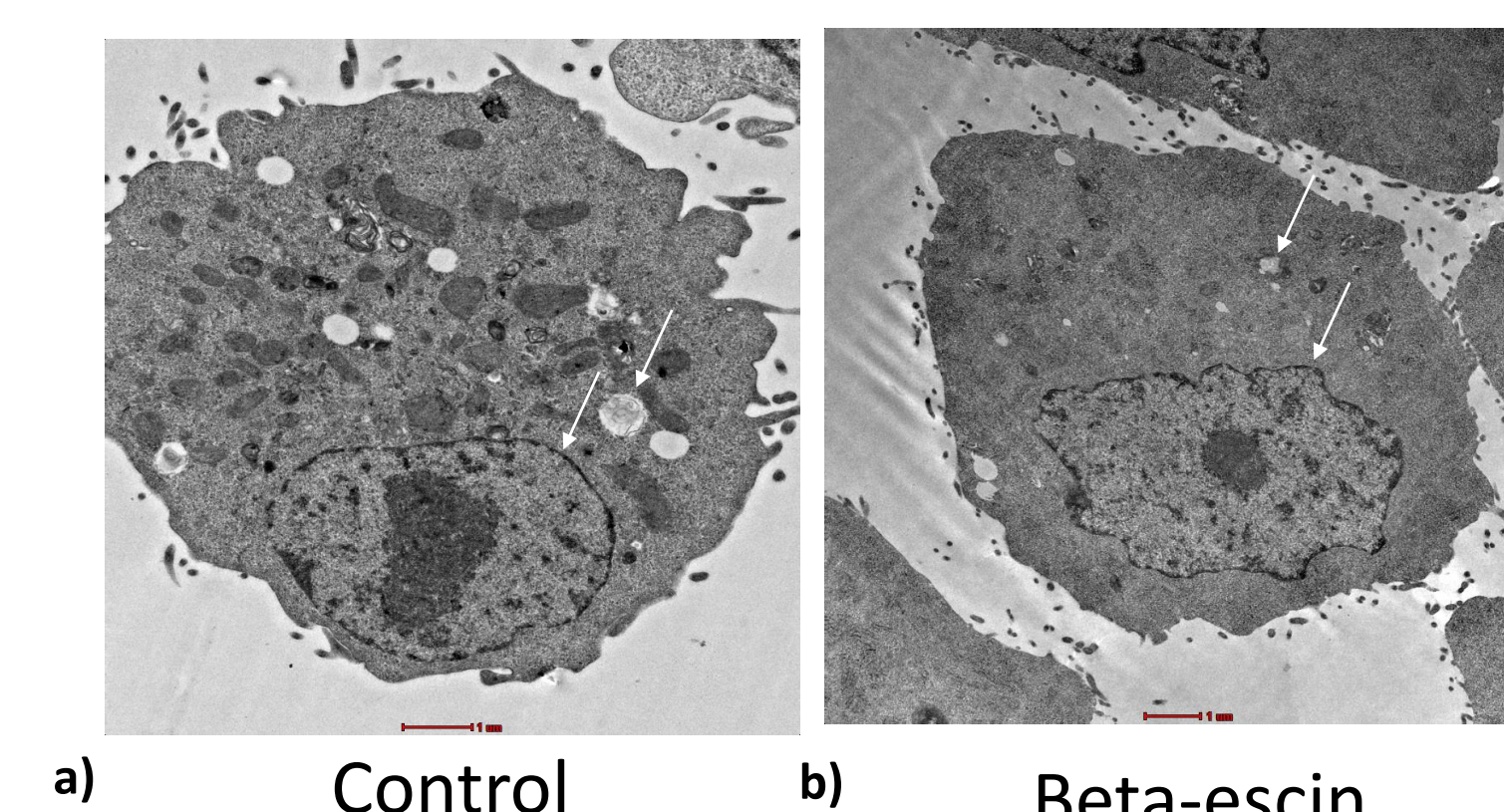


Figure 7: Transmission electron microscopy image of A549 cells showing ultrastructural changes in a) control and; b) beta-escin treated group

Note: (*p<0.05; **p<0.01, ***p<0.001, ****p<0.0001) along with comparison of percentage cells of different groups of beta-escin (\$p<0.05, \$\$p<0.01, \$\$\$p<0.001, \$\$\$\$p<0.0001).