APPA SHOWED SENOLYTIC CAPACITY IN SENESCENT CHONDROCYTES

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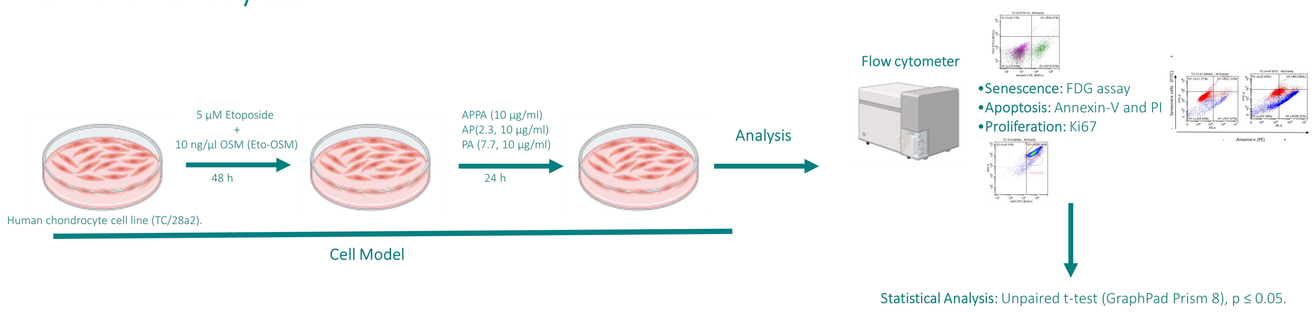
➢ *Purpose*.

Osteoarthritis (OA) is a joint disease where senescent cells contribute to disease progression. Targeting senescent cells with senolytics may provide therapeutic benefits. APPA, a combination of Apocynin (AP) and Paeonol (PA), has shown potential to reduce senescence and increase apoptosis in human chondrocytes.

➢ *Aim.*

This study investigates the potential of APPA, a combination of two bioactive compounds (apocynin (AP) and paeonol (PA)), in modulating senescence and apoptosis in human chondrocytes.

➢ *Method.*



➢ *Results.*

APPA, AP and PA did not affect overall cell viability. APPA (10 µg/ml) did not impact overall chondrocyte viability. Given that APPA is a combination of AP and PA in a 2:7 ratio, further testing of individual components revealed that neither AP (2.3 and 10 µg/ml) or PA (7.7 and 10 µg/ml) alone significantly affected cell viability (**Figure-1**).

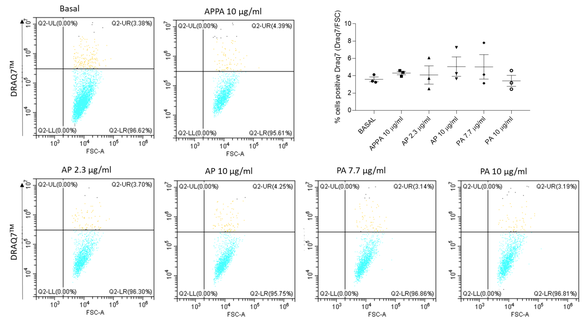


Figure-1. Drug effect on cell viability. Representative dot plots for cells treated with APPA, AP and PA for DRAQ7 viability assay. The quantification of percentage of cell positive to DRAQ7 was represented. Data are represented as the mean ± standard error of mean (SEM). All data were obtained from three independent experiments performed with two replicates.

APPA reduces the percentage of senescent cells and increases apoptosis . Senescence analysis demonstrated that APPA reduced senescent cells induced by Eto-OSM (**Figure 2-A**). Apoptosis analysis indicated that APPA, AP, and PA combining Eto-OSM with APPA significantly increased the cells in early apoptosis (**Figure 2-B**).

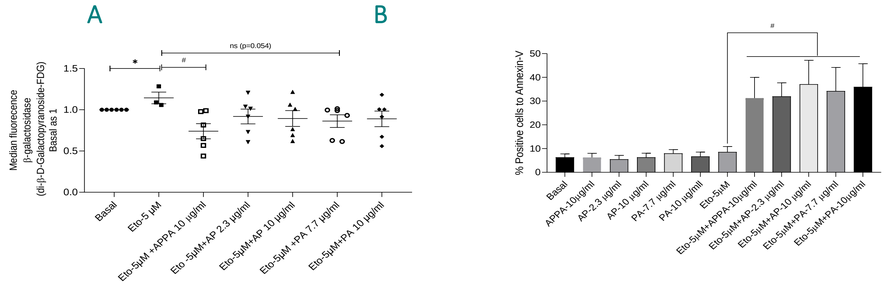


Figure-2. T/C-28a2 human chondrocytes treated with 5µM Eto + 10 ng/µl OSM during 48 h and then APPA 10 µg/ml, AP 2.3 and 10 µg/ml or PA 7.7 and 10 µg/ml for 24 h was added and senescent cells was evaluated by FDG (A) and apoptotic cells were analyzed (B).

Senolytic Effect: APPA, AP and PA elevated senescent cell apoptosis Double-marker analysis (FDG and Annexin-V) showed that APPA, AP and PA elevated the proportion of senescent cells undergoing apoptosis (**Figure-3**). These findings establish the senolytic potential of APPA.

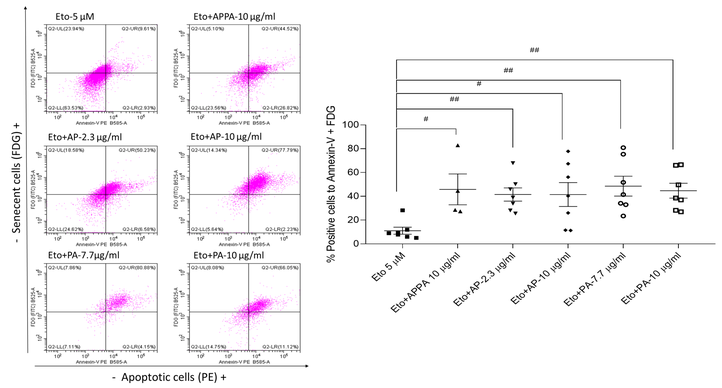


Figure-3. Drugs increased apoptosis in senescent chondrocytes. Representative dot plot of the percentages of FDG/Annexin-V double-positive (upper-right quadrant_UR_). FDG (FITC)/Annexin V (PE) double-staining flow cytometry analysis of senescent and apoptotic cells. UL: Senescent cells; UR: Senescent and apoptotic cells; LL: Normal cells; LR: Apoptotic cells. Data are represented as mean ± SEM and analyzed by unpaired Mann Whitney test (*, # p<0.05, ##p<0.01). * relative to basal # relative to 5 µM Etoposide + OSM.

APPA reduced Ki67+ cells in senescent conditions but increased them in non-senescent cells. The percentage of Ki67+ cells in senescent conditions showed that APPA reduced this proliferative marker in senescent cells. Conversely, APPA, AP and PA increased percentage of cells positive for Ki67 in non-senescent populations (**Figure-4**).

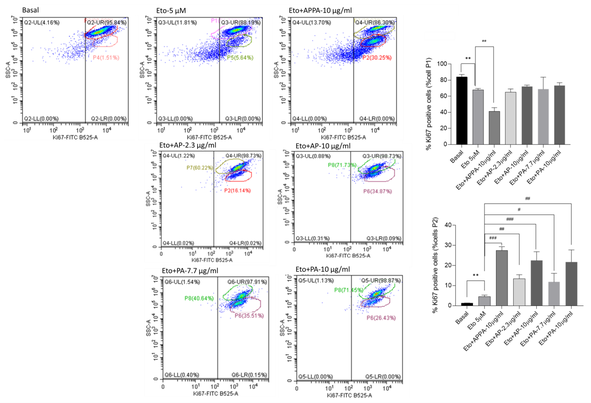


Figure-4. APPA effect on cell proliferation. Flow cytometry plots of Ki67 expression. The percentage of Ki67+ cells were measured and analyzed in each population (P1 and P2). All data were obtained from six independent experiments performed with two replicates. Values are presented as mean ± SEM and analyzed by Mann Whitney test (* # p≤0.05; **, ## p≤0.01; ***, ### p≤0.0005). * relative to basal # relative to Eto-OSM.

➢ *CONCLUSIONS.*

- ✓ APPA reduces senescent cells and promotes apoptosis of senescent chondrocytes
- ✓ APPA increases proliferation markers in non-senescent cells
- ✓ APPA has senotherapeutic activity against senescent human chondrocytes suggestive of both senomorphic and senolytic effects