Electronic Supplementary Material (ESI) for RSC Advances

Supporting information

Correlation of Biocapping Agents and Cytotoxic Effects of Silver Nanoparticles on Human Tumor Cells

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Cytovivo images of HeLa cells treated with Ag NPs

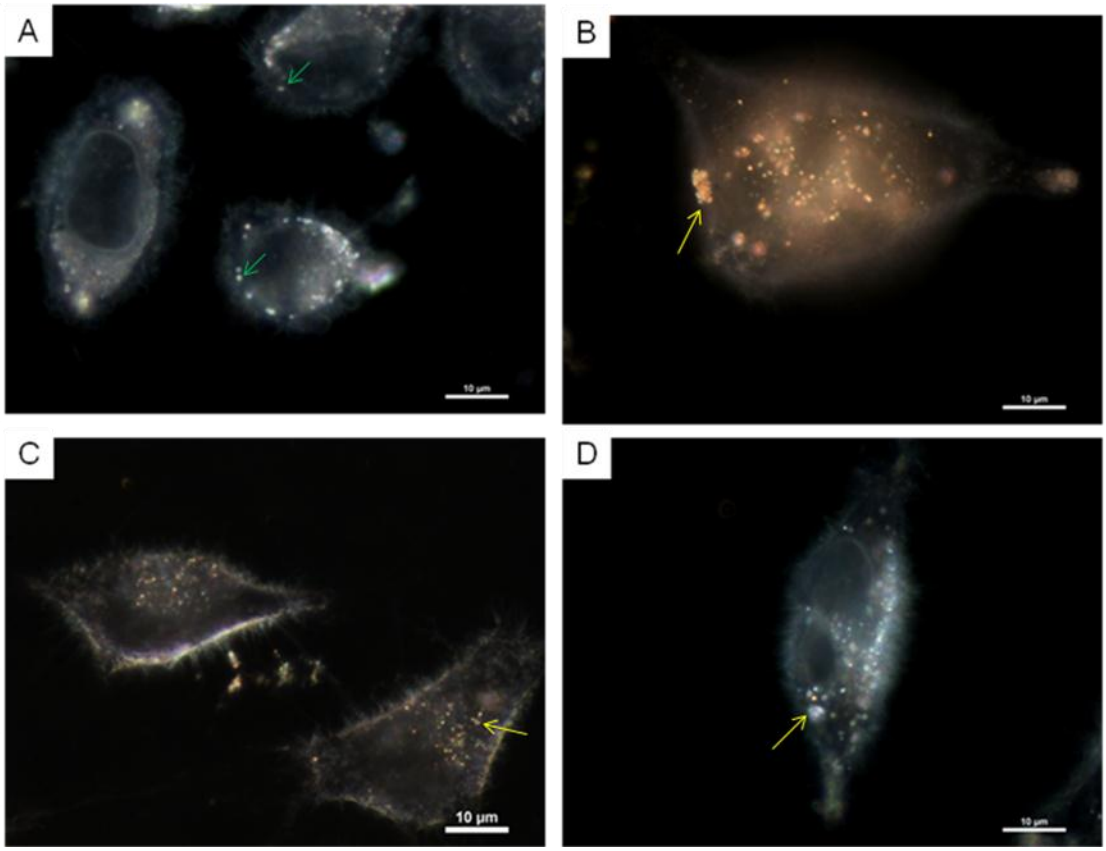

Figure S1: Optical images of HeLa cells untreated (A) and treated with Ag-mint (B), Ag-coffee (C) and Ag-ginger (D) NPs. Concentration of Ag NPs = 20 µg/mL. Green arrows point to cellular components such as endosomes and lysosomes, while yellow arrows point to big Ag NPs aggregates.

Viability of HDF cells treated with Ag NPs

The toxicity of Ag NPs capping with coffee, ginger and mint extracts was also explored on human normal cells: human dermal fibroblast (HDF, Gibco, Life technologies) cell line. Cells were cultured in minimum essential medium (MEM, Gibco, Life technologies) supplemented with 10% fetal bovine serum (FBS, Hyclone), 1% penicillin streptomycin (PAN Biotech GmbH), 1% MEM non-essential amino acids (100X, Gibco, Life technologies) and 1% MEM vitamin solution (100X, Gibco, Life technologies) and maintained in an incubator containing 5% CO_2 at 37 °C. Cells were sub-cultured at 80-90% confluent using trypsin-EDTA (10X, PAA) and seeded in 96 well plate at a density of 5000 cells per well together with 100 µL medium. The next day, the spent medium were removed and replaced with fresh medium with Ag-coffee, Ag-ginger and Ag-mint NPs at different concentrations (0, 10, 25, 50, 75 and 100 µg/mL). The cells were treated with Ag NPs for 24 h and the viability of cells was detected by CellTiter-Glo viability assay following manufacturer's instructions. Experiments were performed in triplicates and summary data were shown below.

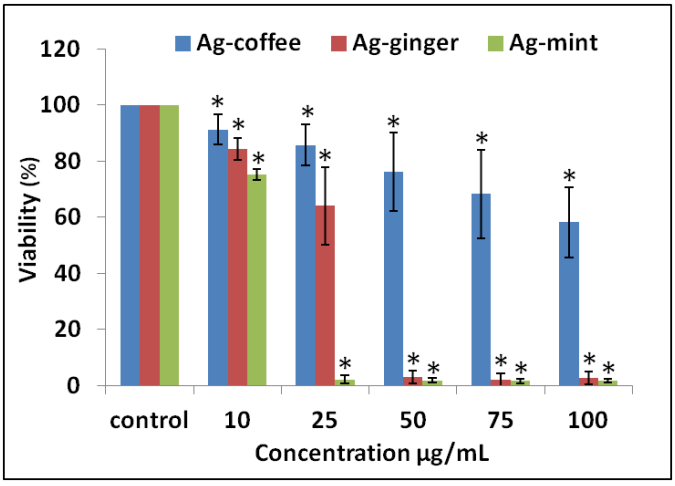

Figure S2: Viability of HDF cells treated with Ag-coffee, Ag-ginger and Ag-mint NPs at different concentrations for 24h, * represents $P < 0.05$.

Annexin-V propidium iodide staining of Ag NPs treated cancer cells

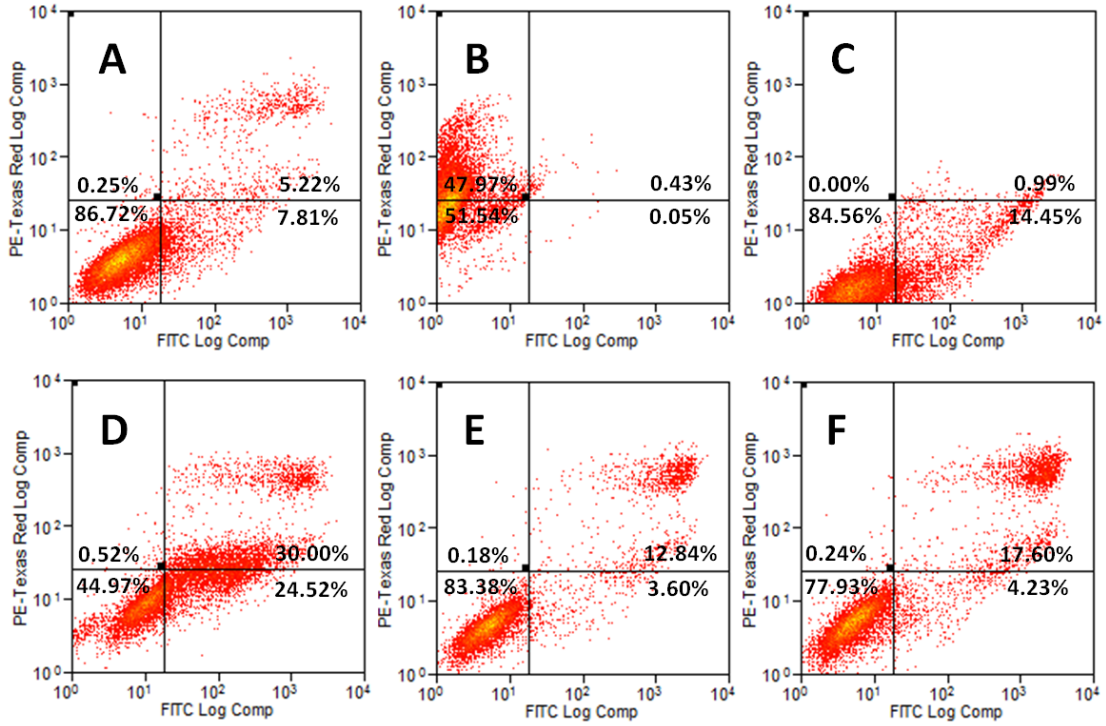

Figure S3: Dot plots from Annexin- FITC staining of HepG2 cells. Untreated cells (A) are used to calculate % of change in population. Cells accumulating at lower left window represent live cells. (B) shows necrosis positive controls (H_2O_2 treated) which accumulate on upper left window for red fluorescent cells.(C) shows apoptosis positive controls (Staurosporine treated) accumulating on lower right window. Late apoptotic cells will accumulate in upper right window. (D) shows cell population for cells treated with Ag-mint (E) represents cells treated with Ag-ginger and (F) represents cells treated with Ag-coffee NPs (20 µg/mL). The % of cells under each category (live, early and late apoptosis, necrosis) is represented in respective windows.

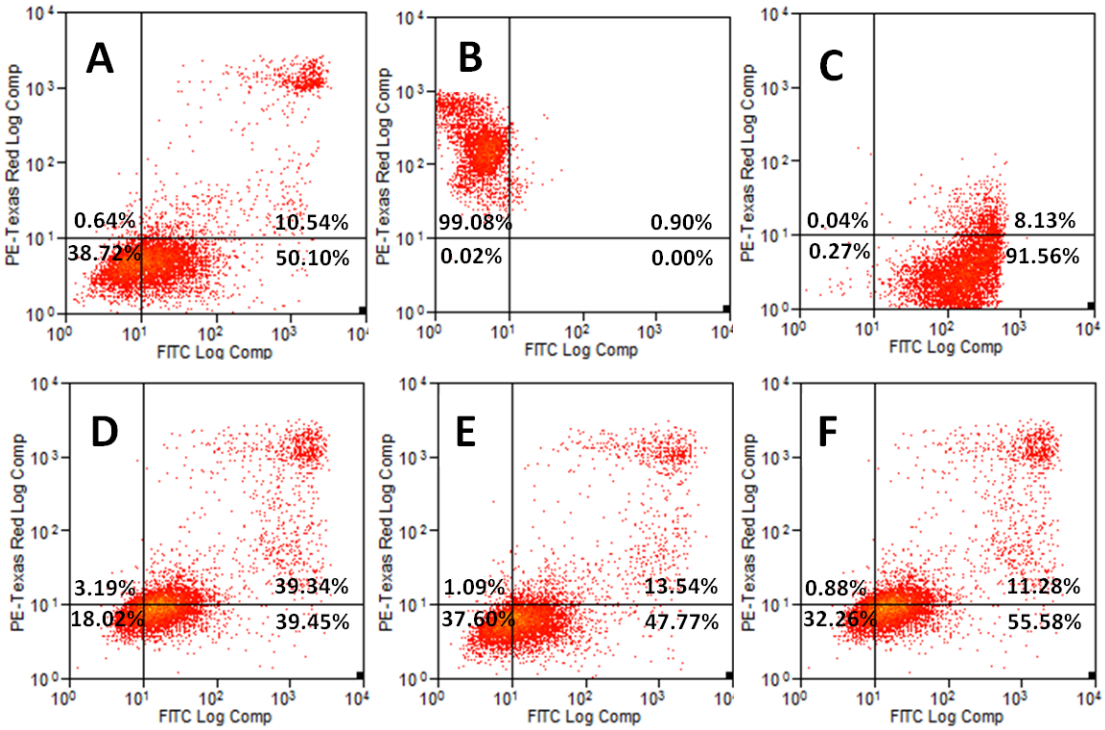

Figure S4: Dot plots from Annexin- FITC staining of HeLa cells. Untreated cells (A) are used to calculate % of change in population. Cells accumulating at lower left window represent live cells. (B) shows necrosis positive controls (H_2O_2 treated) which accumulate on upper left window for red fluorescent cells.(C) shows apoptosis positive controls (Staurosporine treated) accumulating on lower right window. Late apoptotic cells will accumulate in upper right window. (D) shows cell population for cells treated with Ag-mint (E) represents cells treated with Ag-ginger and (F) represents cells treated with Ag-coffee NPs (20 µg/mL). The % of cells under each category (live, early and late apoptosis, necrosis) is represented in respective windows.

Cell cycle analysis of Ag NPs treated cancer cells

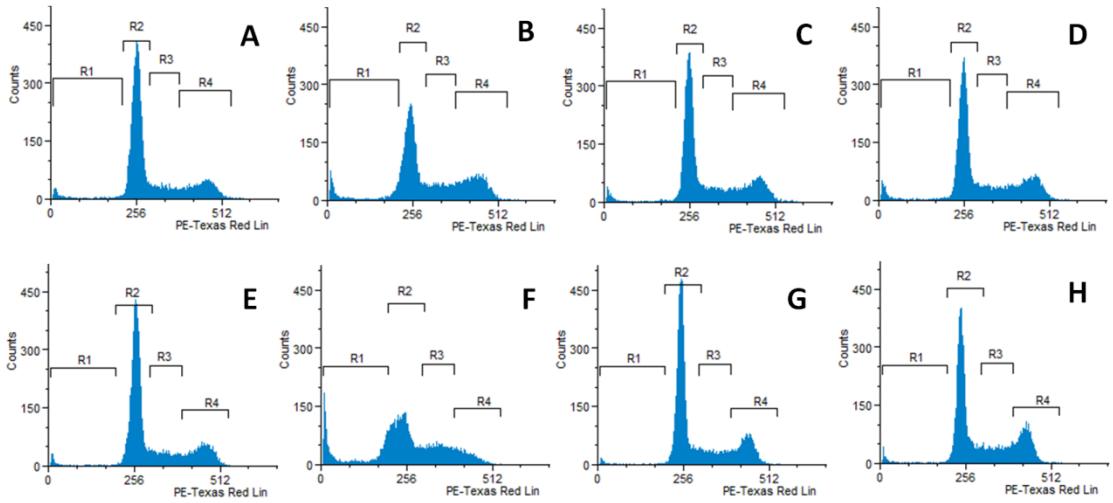

Figure S5: Histograms representing cell cycle analysis of HepG2 (A-D) and HeLa (E-H) cells. The control showed normal distribution of sub G1, G1, S and G2/M (A and E). The cells treated with 20 µg/mL Ag mint (B and F), Ag-ginger (C and G) and Ag-coffee (D and H) showed increases in S/G2

population indicating S/G2 arrest while the presence subG1 population of cells treated Ag mint (B and F) indicates cell death through apoptosis. Markers were drawn on regions of interest (subG1, G1, S and G2/M) to generate statistics of cells under each region. Corresponding statistics were generated using Summit V4.3.02 software.