**Materials and methods**

**Reagents**

The *C. adamanteus* LAAO and catalase were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Cell culture reagents were obtained from Life Technologies (Carlsbad, CA, USA). Real time fluorescence quantitative PCR reagents were purchased from Vazyme Biotech Co., Ltd (Nanjing, China). Western blotting-related chemicals were bought from Dingguo Changsheng Biotech Co. Ltd (Beijing, China).

**Cell culture**

Human ovarian cancer cell line CAOV3 was purchased from Xiangf Biotech (Shanghai, China) and OVCAR3 was from Dingguo Changsheng Biotech Co.Ltd (Beijing, China). CAOV3 and OVCAR3 were cultured in RPMI-1640 medium containing 10% fetal bovine serum and 100 unit/mL penicillin/streptomycin at 37°C in a humidified incubator with 5% CO2.

**Cell viability, morphological alteration and apoptosis**

Cell viability is a simple yet important parameter when working with cell-based research such as toxicology, immuno-oncology, bioprocessing and so on [1]. In this part, we performed three experiments: observation of morphological changes under a light microscope, Cell Counting Kit-8 (CCK-8) assay for cytotoxicity and acridine orange (AO)/propidium iodide (PI) double fluorescence staining experiment. CCK-8 serves as a tool for studying induction and inhibition of cell proliferation or cytotoxicity in any in vitro model. AO/PI fluorescence detection has been used for direct cell counting and viability measurement [2].

**CCK-8 assays for cytotoxicity**

CCK-8 was exploited to evaluate the cytotoxic effect of svLAAO on CAOV3 and OVCAR3 cells. Before the CCK-8 experiments, we treated the CAOV3 and OVCAR3 cells with different concentrations of svLAAO (1, 3, 5 and 7 μg/mL), and then observed at different time points (6, 12, 24 and 36 hr) to preliminarily select the experimental time point and concentration according to the changes of cell morphology. And then, the two ovarian cancer cells were seeded with a volume of 100 μL cell suspension (8000 cells/well) into 96-well plates, and incubated the plate for 12 hr in a humidified incubator at 37 °C. After the adherent cell growth, the medium was changed and cultured for 12 hr in new culture medium. Next, the 1, 3, 5 and 7 μg/mL svLAAO were added into the tested plate and incubated the plate for 24 hr in the humidified incubator. Finally, 10 μL of CCK-8 solution was added into each well of the plate and incubated for 2.5 hr in the incubator. The cytotoxicity parameters were determined through the absorbance of the solution measured at 450 nm by a microplate reader (Bio-Rad, USA). And then the inhibitory concentration 50% (IC50) of svLAAO in CAOV3 and OVCAR3 cell apoptosis was determined by CCK-8.

**AO/PI double fluorescence-staining tests**

The IC50 was calculated by the results of CCK-8 cytotoxicity test, which was 6.725 μg/mL in CAOV3 cells and 6.364 μg/ mL in OVCAR3 cells. In this work, the combination of AO and PI (AO/PI double-staining reagents, BestBio, China) was used to assess the integrity of morphological features of cells exposed to 5 μg/mL svLAAO and different concentrations of catalase. In order to comply with the subsequent molecular experiments, cell samples were collected and controlled the number of cells within 5.0×105. Firstly, 5.0×105 cells were seeded in 6-well microplates and cultured for 24hr in RPMI 1640 medium with 10% FBS. Secondly, we changed the medium containing different concentrations of catalase enzyme (0 mg/mL, 0.025 mg/mL, 0.05 mg/mL, 0.1 mg/mL, 0.15 mg/mL catalase) and pre-incubated for 1 hr. Thirdly, 5 μg/mL svLAAO was added into the above wells and continued to incubate for 24hr. Fourthly, the cells were digested with trypsin and washed twice by PBS, then resuspended with 500 μL staining buffer. Next, we added 5 μL AO and 10 μL PI staining solution into the cell suspension, mixed gently and incubated for 15 minutes at 4°C in the dark environment. Finally, the cells were washed with PBS again and verified by fluorescence microscope. The cell survival status was evaluated by analyzing the results of cell staining and morphological changes under fluorescence microscope.

**Statistical analysis**

Statistical analyses were performed using PASW Statistics 18.0. The average and standard deviation were calculated and expressed as Mean ± SD. The comparisons of experimental data between the groups and the significance of variance were analyzed by a one-way ANOVA test. The p＜0.05 was considered to indicate statistical significance.

**References**

1. Chan LL, Zhong X, Qiu J, Li PY, Lin B. Cellometer Vision as an alternative to flow cytometry for cell cycle analysis, mitochondrial potential, and immunophenotyping. Cytometry Part A the Journal of the International Society for Analytical Cytology. 2011;79A:507-17.

2. Chan LY, Kuksin D, Laverty DJ, Saldi S, Qiu J. Morphological observation and analysis using automated image cytometry for the comparison of trypan blue and fluorescence-based viability detection method. Cytotechnology. 2015;67:461-73.