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Original Article

Inhibition of aqueous extracts of *Solanum nigrum* (AESN) on oral cancer through regulation of mitochondrial fission

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ABSTRACT

The present study is designed to investigate the anti-oral cancer properties of *Solanum nigrum* on oral squamous cell carcinoma. *S. nigrum* is a Chinese herb used for suppression of various cancers. However, the inhibition of *S. nigrum* on oral cancer is unclear. Therefore, human oral squamous cancer cells (SCC)-4 were used to evaluate the effect of aqueous extracts of *S. nigrum* (AESN) on cancer cell proliferation, cell cycle, mitochondrial function and apoptosis. The SCC-4 cells were treated by AESN to evaluate the inhibition of cell proliferation and mitochondrial function *in vitro*. Our results suggested that AESN markedly increased reactive oxygen species production. AESN also promoted caspase-9 and caspase-3 activation and subsequent triggering of the mitochondrial apoptotic pathway. The inhibition of glucose uptake was alleviated mediated by a dose-dependent manner in SCC-4 cells with AESN treatment for 24 h, resulting in mitochondrial fission. These results suggested that AESN has potential to be used as a functional food in adjuvant chemotherapy for treating human oral cancer by suppression of mitochondrial function.

1. Introduction

Oral cancer is the fifth most common neoplasm worldwide, accounting for more than 500,000 cases annually.[1] In Taiwan it has the fastest-rising incidence and mortality rate of any cancer and is the sixth most common cause of cancer death, being more prevalent in males than in females. Tobacco and alcohol consumption have been reported to be the major factors in the development of oral cancer.[2] Diets low in carotenoids and vitamin A, poor oral hygiene and indoor air pollution are also recognized as factors in oral cancer.[3,4] However, betel quid chewing is one of the most important causes of oral cancer in Taiwan, with high mortality and poor prognosis. Therefore, in an effort to improve patient survival and quality of life, new therapeutic approaches focusing on the molecular target and mechanism that mediate tumor cell growth or cell death has gained much attention.

Oxidative damage to cellular macromolecules can arise through the overproduction of reactive oxygen species (ROS) and faulty antioxidant and/or DNA repair mechanisms that result in cancer.[5] Chronic inflammation can lead to the production of chemical intermediates such as nitrogen oxide, which in turn can mediate DNA damage and block the DNA repair system.[6–8] Chemoprevention has evolved as a novel approach to control the incidence of oral cancer. Therefore, it is important to establish chemoprevention in an experimental animal tumor model that mimics specific characteristics of human oral squamous cell carcinoma. Chemoprevention by

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dietary agents has evolved as an effective strategy to control the incidence of oral cancer. The present study was designed to evaluate the inhibitory effects of the water extract from *Solanum nigrum* (AESN) on squamous cancer cells-4 (SCC-4).

2. Materials and methods

2.1. Sample isolation

The *S. nigrum* was collected in Tainan market (Taiwan) on January. The leaf of *S. nigrum* (1 kg) was extracted with water (10 L) three times at room temperature. After evaporating the solvents under vacuum at 40 °C and frozen-dried, a residue powder was obtained (179.4 g).

2.2. Cell culture

Human SCC-4 cell lines were obtained from the Bioresource Collection and Research Center (Hsinchu, Taiwan). SCC-4 cells were maintained in DMEM/Ham's F-12 (1:1 v/v) medium supplemented with 100 mL/L FBS, 1.5 g/L sodium bicarbonate, 400 ng/mL hydrocortisone and 10 mL/L antibiotic solution. Cells were incubated in 5% CO_2 and 95% humidified atmosphere at 37 °C.

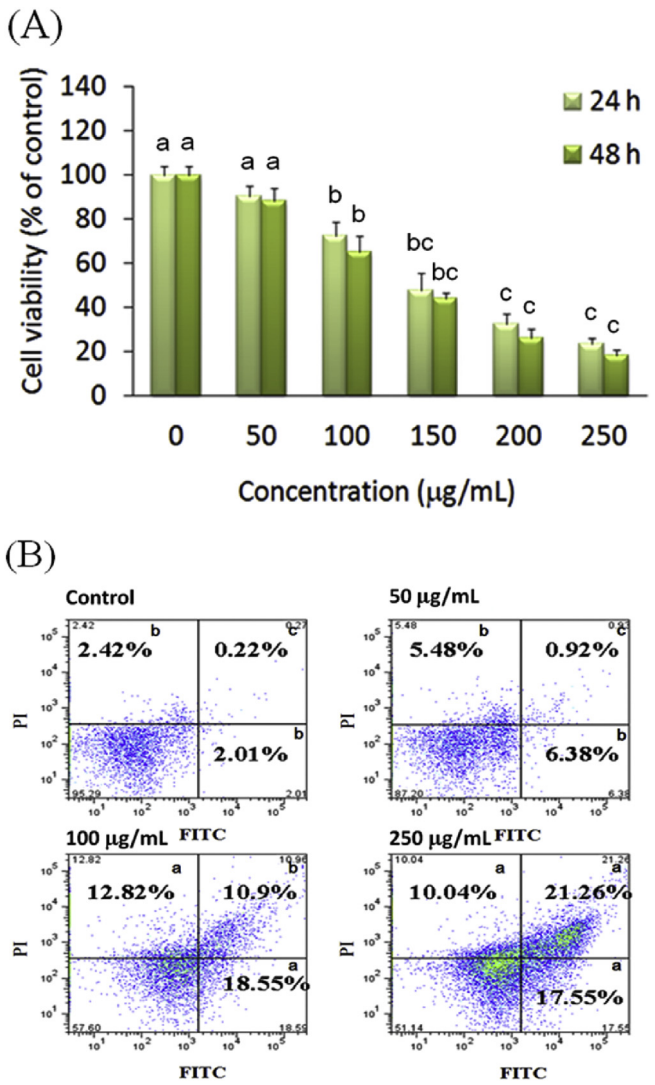

Fig. 1. (A) Inhibitory effect of AESN on cell viability of SCC-4 after 24 h and 48 h treatment. Data were shown as mean ± SD (n = 3). Significantly difference was shown as various letters (between a, b, bc, c in 24 h or 48 h) ($P < 0.05$). (B) Induction of cell apoptosis and necrosis by AESN in SCC-4 cells. After 24 h treatment of AESN, the apoptotic event was detected by co-staining with Annexin V and PI using flow cytometry. Untreated cells were used as the control for double staining. [a,b,c]Values with one different letter superscript are significantly different from each other ($P < 0.05$).

2.3. Cell viability

The cell-killing effect of AESN against oral cancer cells was measured using the crystal violet staining assay. Cells were seed on 24-well plates (3×10^4 cells per well) and treated with various concentrations of AESN for 24 and 48 h, respectively. The medium was then removed, washed with phosphate buffered saline (PBS) and stained with 2 g/L crystal violet in 100 mL/L phosphate-buffered formaldehyde for 20 min before being washed with water. The crystal violet bound to the cells was dissolved in 20 g/L SDS solution and its absorbance at 600 nm was measured.

2.4. Apoptosis analysis

For apoptosis detection, floating cells in the medium and adherent cells were collected after 24 h of AESN treatment. Cells were harvested, washed in ice-cold PBS and resuspended in 200 μL of binding buffer before being incubated in 5 μL of Annexin V-fluorescein isothiocyanate (FITC) (BD Biosciences) solution and 5 μL of propidium iodide (PI) at room temperature for 15 min in the dark. Then 300 μL of binding buffer was added. Cells were analyzed by flow cytometry. Untreated cells were used as the control for double staining.

2.5. Assay for caspase-3

After treatment of AESNfor 12 h, SCC-4 cells were treated with anti-caspase-3 and caspase-9 antibody with fluorescent dyes for 30 min. After PBS wish, cells were analyzed by flow cytometry. Untreated cells were used as the control for double staining.

2.6. Assay for oxidative stress

The level of oxidative stress was monitored by the measurement of ROS. Collected cells were suspended in 500 μL of PBS and mixed with 10 μM (final concentration) of dichloro-dihydro-fluorescein diacetate (DCFH-DA) to incubate for 20 min at 37 °C. The cells were washed thrice with phosphate-buffered saline (PBS) to remove redundant DCFH-DA. The cell pellet was mixed with 500 μL

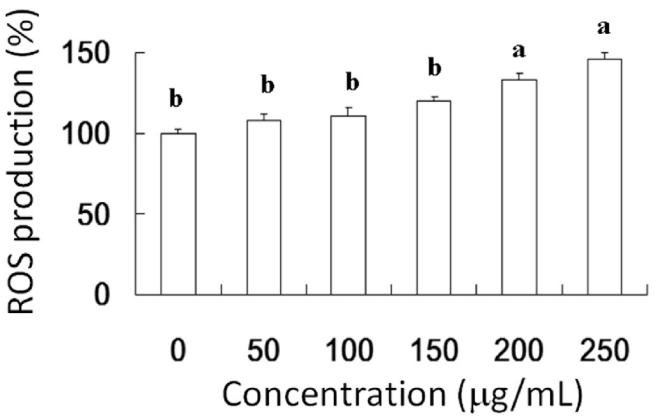

Fig. 2. Induction of oxidative stress by AESN in SCC-4 cells. ROS level of SCC-4 cells treated with AESN for 24 h was measured by flow cytometry. Data were shown as mean ± SD (n = 3). [a,b]Values with one different letter superscript are significantly different from each other ($P < 0.05$).

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