Effects of *Ardisia gigantifolia* Extract on Oxidative Stress and Apoptosis in AsPC-1 Pancreatic Cancer Cells

Jingyue ZHU[1△], Jing LI[1△], Yi OU[2△], Dabiao JING[3], Chen HUANG[3], Yijie JIANG[3], Zhiqiang HUANG[3], Yufei ZHANG[3*], Kaimei ZHU[3*], Yulin HE[3*]

1. The Second Affiliated Hospital of Guilin Medical University, Guilin 541199, China; 2. Guilin Sanjin Pharmaceutical Co., Ltd., Guilin 541199, China; 3. College of Pharmacy, Guilin Medical University, Guilin 54119, China

Abstract [**Objectives**] To investigate the effects of *Ardisia gigantifolia* extract on oxidative stress and apoptosis in pancreatic cancer AsPC-1 cells. [**Methods**] MTT assay was used to detect the inhibitory effects of different concentrations (0, 2, 4, 6, 8, 10 μg/mL) of *A. gigantifolia* extract on the proliferation of AsPC-1 cells after 48 h treatment, and the half maximal inhibitory concentration (IC_{50}) was calculated; Transwell assay was employed to evaluate the effects of the extract on cell migration and invasion; intracellular superoxide dismutase (SOD) activity and malondialdehyde (MDA) level were measured using commercial kits to reflect the oxidative stress status. [**Results**] *A. gigantifolia* extract significantly inhibited the proliferation of AsPC-1 cells (IC_{50} = 5.805 μg/mL), reduced their migration and invasion abilities, enhanced SOD activity, and decreased the oxidative stress level. [**Conclusions**] *A. gigantifolia* extract enhances SOD activity in pancreatic cancer AsPC-1 cells, reduces ROS levels, and induces apoptosis, thereby exerting an inhibitory effect on the viability of AsPC-1 cells.

Key words *Ardisia gigantifolia* extract, Pancreatic cancer, AsPC-1 cells, Oxidative stress, Cell apoptosis

1 Introduction

Pancreatic cancer is a digestive malignancy with high aggressiveness, subtle onset, rapid progression, and early metastatic potential. Because effective early detection methods are lacking, the majority of patients with locally advanced or metastatic disease at diagnosis, and the existing multimodal treatment approaches, including surgery, chemotherapy, radiotherapy, and targeted therapy, have shown limited overall effectiveness[1]. As the sixth leading cause of cancer-related mortality globally, pancreatic cancer has an overall 5-year survival rate of about 11.5%, a figure that declines sharply to below 5% in the metastatic setting. It has been predicted that by 2030, pancreatic ductal adenocarcinoma (PDAC) may become the second leading cause of cancer-related death, with an annual incidence increase of 2.4% in women and 1.2% in men[2]. Recent studies have shown that oxidative stress and the resulting disruption of intracellular redox homeostasis play a crucial role in the initiation and progression of pancreatic cancer[3]. Reactive oxygen species (ROS) function as important intracellular signaling molecules that regulate biological processes such as tumor cell proliferation, migration, invasion, and adaptive survival; however, when excessively accumulated, they can induce lipid peroxidation, organelle damage, and cell death. Pancreatic cancer cells often remodel their antioxidant defense systems to maintain ROS at a dynamic equilibrium that favors tumor survival[4-5].

Natural flavonoids are considered an important source for the development of novel anticancer drugs and have demonstrated great potential in this regard[6]. Flavonoids derived from flavonoid-rich herbs are a class of bioactive compounds that have been extensively studied and applied in traditional medicine[7].

Ardisia gigantifolia (also known as Matai, Shanzhuyao, and Dazijinniu in Chinese) is a perennial evergreen shrub belonging to the genus *Ardisia* of the family Myrsinaceae, and is a key protected wild plant species endemic to Guangxi[8]. Traditionally, it has been used to dispel wind and eliminate dampness, strengthen tendons and bones, and promote blood circulation to remove blood stasis. Modern studies have demonstrated that *A. gigantifolia* contains various chemical constituents including triterpenoids, flavonoids, coumarins, and quinones, and exhibits certain antitumor activities; recent research on this species has also suggested that its antitumor effects are characterized by multi-component and multi-target mechanisms[9]. However, systematic studies on the effects of *A. gigantifolia* extract on the biological behavior of pancreatic cancer cells and oxidative stress-related indicators are still lacking. In this study, we used pancreatic cancer AsPC-1 cells to investigate the effects of *A. gigantifolia* extract on cell proliferation, migration, invasion, superoxide dismutase (SOD) activity, and ROS levels, aiming to provide a theoretical basis for the experimental evaluation of its anti-pancreatic cancer effects and subsequent development and utilization.

2 Materials and methods

2.1 Materials

2.1.1 Cell line. Human pancreatic cancer AsPC-1, purchased from the Shanghai Cell Bank, Chinese Academy of Sciences, catalog number TCHu 8.

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△These authors contributed equally to this work.
Jingyue ZHU, inspector; Jing LI, attending physician; Yi OU, engineer. *Corresponding author. Yulin HE, doctoral degree, master supervisor; Kaimei ZHU, master supervisor, professor; Yufei ZHANG, doctoral degree, master supervisor.

2.1.2 Reagents and drugs. Anhydrous ethanol (Tianjin Fuyu Fine Chemical Co., Ltd., batch No. 2022518); DMSO, PBS, MTT, and RIPA strong lysis buffer (Beijing Solarbio Science & Technology Co., Ltd.; batch No.: D8371, P1010, R0010, C8470, M8180, 11995); SOD and MDA assay kits (Shanghai Beyotime Biotechnology Co., Ltd., batch No.: S0086, S0131S). The medicinal material *Ardisia gigantifolia* was provided by the Guangxi Institute of Botany, Chinese Academy of Sciences, and identified as the rhizome of *Ardisia gigantifolia* (family Myrsinaceae) by researcher Tang Hui of the same institute.

2.1.3 Main instruments. 1900 Full-wavelength Epoch microplate reader (Shanghai Shanpu Biotechnology Co., Ltd.), 750A multi-function grinder (Yongkang Hongtaiyang Electromechanical Co., Ltd.), ME204E electronic balance (Shanghai Yaoxin Electronic Technology Co., Ltd.), Universal Hood II gel imaging luminescence system (Bio-Rad, USA), and 5810R refrigerated high-speed centrifuge (Eppendorf, Germany).

2.2 Methods

2.2.1 Extraction of active components from *A. gigantifolia* and drug preparation. Fresh *A. gigantifolia* was washed, dried, pulverized, and passed through a 40-mesh sieve for later use. A 100 g sample of the dried powder was mixed with 95% ethanol at a material-to-solvent ratio of 1∶10 (*w/v*) and subjected to reflux extraction in a 60 ℃ water bath for 2 h; the extraction was repeated three times. The extracts were filtered, combined, and concentrated under reduced pressure at 60 ℃ to obtain the active component extract of *A. gigantifolia*, which was stored at −20 ℃ for subsequent use. Before use, the extract was diluted with RPMI-1640 complete medium, and the concentrations corresponding to 1/2 IC_{50}, IC_{50}, and 2 IC_{50} were designated as the low-, medium-, and high-dose treatment groups, respectively.

2.2.2 Cell culture. Under aseptic conditions, pancreatic cancer AsPC-1 cells were seeded in RPMI-1640 complete medium and cultured in an incubator at 37 ℃ with 5% CO_2. Cell morphology and number were regularly observed, and AsPC-1 cells in good condition were selected for subsequent experiments.

2.2.3 MTT assay for cell proliferation. An appropriate number of AsPC-1 cells in good condition were adjusted to a density of 5×10^4 cells/mL, and seeded into a 96-well plate at approximately 5 000 cells per well. The cells were treated with the *A. gigantifolia* ethanol extract at concentrations of 0, 2, 4, 6, 8, and 10 μg/mL, with five replicate wells for each concentration. After 48 h of incubation, the culture medium was discarded, and 10 μL of 5 mg/mL MTT solution and 90 μL of RPMI-1640 medium were added to each well. The plate was incubated at 37 ℃ in a 5% CO_2 incubator for 4 h, after which the supernatant was removed, and 150 μL of DMSO was added to each well. Following dissolution by shaking, the absorbance (*OD* value) was measured at 490 nm.

2.2.4 Transwell migration and invasion assays for assessing cell migration and invasion abilities. Migration assay: According to the method described in reference[3], an appropriate number of AsPC-1 cells were taken and assigned to high-, medium-, and low-dose treatment groups and a control group. The cell density was adjusted to 5×10^5 cells/mL, and 200 μL of the cell suspension was seeded into the upper chamber of each Transwell insert, while 600 μL of complete medium was added to the lower chamber. After 48 h, the inserts were removed, and the cells were fixed, stained, washed, and photographed to calculate the cell migration rate. Invasion assay: According to the method described in reference[3], Matrigel was pre-coated onto the Transwell inserts, and the subsequent procedures were performed as in the migration assay. Finally, the cell invasion rate was calculated.

2.2.5 SOD level measurement. An appropriate number of AsPC-1 cells in good condition were seeded into a 6-well plate and assigned to high-, medium-, and low-dose treatment groups as well as a control group. After 48 h of culture, the cells were collected, and SOD levels were determined using the SOD assay kit according to the manufacturer's instructions. The SOD level of each group was then calculated.

2.2.6 MDA level measurement. An appropriate number of AsPC-1 cells in good condition were seeded into a 6-well plate and assigned to high-, medium-, and low-dose treatment groups as well as a control group. After 48 h of culture, the cells were collected, and MDA levels were determined using the MDA assay kit according to the manufacturer's instructions. The MDA level of each group was then calculated.

2.2.7 Data processing. Statistical analysis was performed using GraphPad Prism 11.0.0 and other statistical software. Comparisons of means among multiple groups were conducted using one-way analysis of variance (one-way ANOVA). Statistical significance was denoted as $P<0.05$, $^{*}P<0.01$, $^{**}P<0.001$.

3 Results and analysis

3.1 Inhibition of AsPC-1 cell proliferation The extract was diluted to a concentration gradient of 0, 2, 4, 6, 8, and 10 μg/mL and applied to the designated treatment groups. After 48 h of treatment, the *OD* values of each concentration group were measured, and the survival rate of AsPC-1 cells was calculated. As shown in Fig. 1, the IC_{50} of the extract against AsPC-1 cells at 48 h was calculated to be 5.805 μg/mL. These results indicate that the active components of *A. gigantifolia* inhibit the proliferation of pancreatic cancer AsPC-1 cells in a dose-dependent manner.

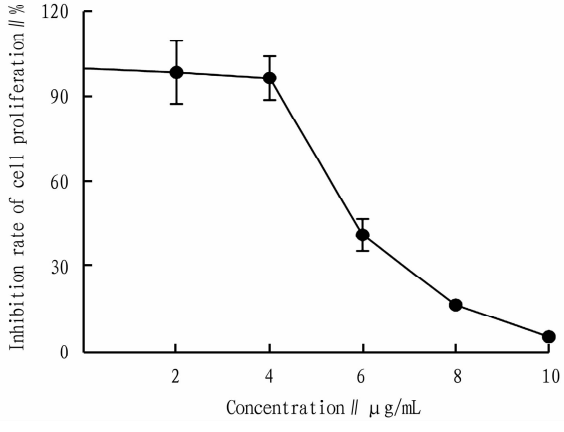
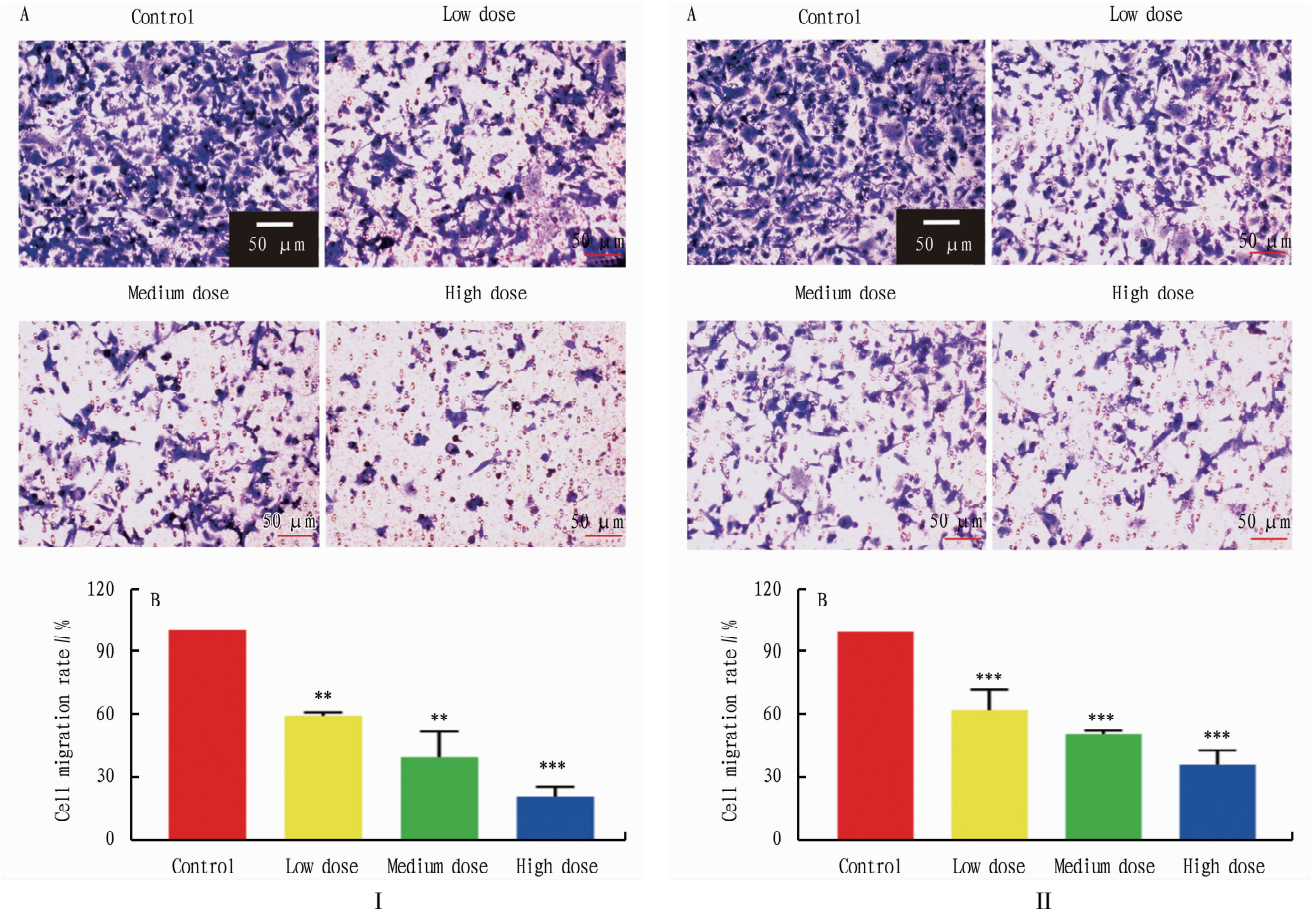


Fig. 1 Effects of *Ardisia gigantifolia* extract on the proliferation of AsPC-1 cells

3.2 Inhibition of AsPC-1 cell migration and invasion Cells were divided into high-, medium-, and low-dose treatment groups and a control group. After treatment with the corresponding concentrations of the extract for 48 h, the number of cells that passed through the Transwell inserts was determined by 0.01% crystal violet staining. As shown in Fig. 2, compared with the control group, the migration and invasion abilities of AsPC-1 cells were inhibited in the low-, medium-, and high-dose groups, and the inhibitory effect became more pronounced as the drug concentration increased.



NOTE $P<0.05$, $^{*}P<0.01$, $^{**}P<0.001$ compared with the control group. The same below.

Fig. 2 Effects of *Ardisia gigantifolia* extract on the migration (I) and invasion (II) abilities of AsPC-1 cells

3.3 Effects on SOD levels in AsPC-1 cells After 48 h of treatment, the SOD levels in the high-, medium-, and low-dose groups and the control group were measured using an SOD assay kit. As shown in Fig. 3, compared with the control group, different concentrations of *A. gigantifolia* extract significantly increased the SOD levels in AsPC-1 cells, and the effect was more pronounced at higher concentrations.

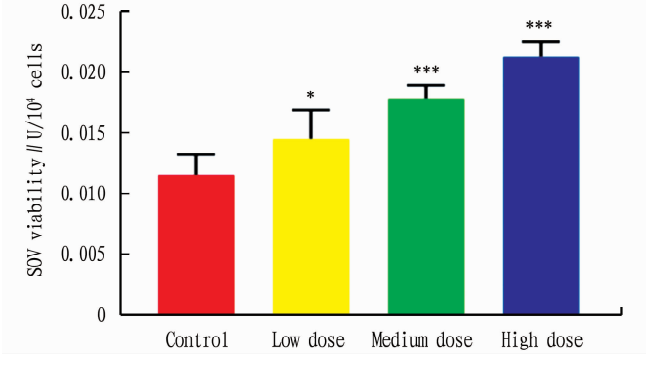


Fig. 3 Effects of *Ardisia gigantifolia* extract on SOD levels in AsPC-1 cells

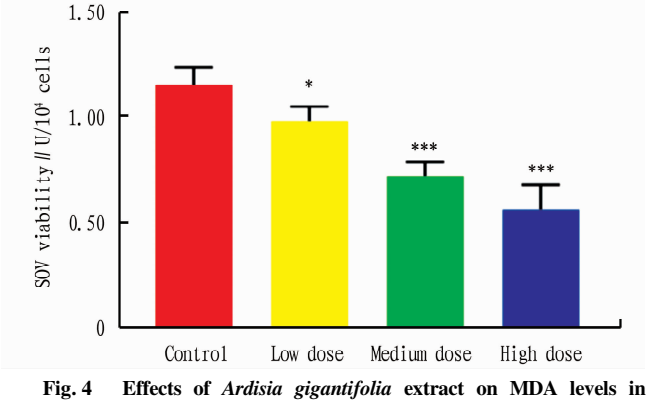


Fig. 4 Effects of *Ardisia gigantifolia* extract on MDA levels in AsPC-1 cells

3.4 Effects on MDA levels in AsPC-1 cells MDA levels in the high-, medium-, and low-dose groups and the control group were measured using an MDA assay kit after 48 h of treatment. As shown in Fig. 4, compared with the control group, different concentrations of *A. gigantifolia* extract significantly decreased the MDA levels in AsPC-1 cells, and the effect was more pronounced at higher concentrations.

4 Discussion

A. gigantifolia is an edible medicinal plant used by ethnic minorities, and its traditional effects mainly include dispelling wind and eliminating dampness, strengthening tendons and bones, and promoting blood circulation to remove blood stasis. Related studies have shown that *A. gigantifolia* contains various chemical constituents such as triterpenoids, flavonoids, coumarins, and quinones, and possesses extensive pharmacological activities. As a traditional Chinese medicine, *A. gigantifolia* exerts anticancer effects by regulating autophagy, apoptosis, cell growth, and oxidative stress[10]. The results of this study demonstrated that *A. gigantifolia* extract could inhibit the proliferation, migration, and invasion of AsPC-1 cells. In this study, the MTT assay determined that the IC_{50} of the extract at 48 h was 5.805 μg/mL, exhibiting a dose-dependent effect; the Transwell assay showed that the extract significantly inhibited the migration and invasion abilities of AsPC-1 cells, and the inhibitory effect became stronger with increasing drug concentration. These findings indicate that *A. gigantifolia* extract not only suppresses the growth of pancreatic cancer cells but also attenuates their invasion and metastasis-related capabilities to a certain extent.

SOD is an important class of antioxidant enzymes that scavenge harmful intracellular free radicals and mitigate oxidative stress-induced cellular damage, thereby exerting cytoprotective effects. The SOD family mainly includes the copper/zinc-containing SOD1 and SOD3, as well as the manganese-containing SOD2. Their primary mechanism of action involves catalyzing the conversion of superoxide anion radicals into relatively stable molecular oxygen and hydrogen peroxide, thus reducing free radical-mediated oxidative damage[11]. The SOD assay results demonstrated that as the concentration of *A. gigantifolia* extract increased, the SOD levels in AsPC-1 cells gradually rose, with a more pronounced effect observed at higher concentrations.

Oxidative stress is a pathological state resulting from the excessive accumulation of intracellular ROS, leading to an imbalance between the oxidative and antioxidant systems. Previous studies have shown that ROS not only participate in the regulation of processes such as apoptosis, autophagy, and necrosis, but also serve as important second messengers mediating multiple signal transduction pathways, thereby affecting the expression of related genes and cell fate[12]. MDA is isan end product of lipid peroxidation formed when ROS attack unsaturated fatty acids in lipids, and its level can reflect, to a certain extent, the degree of cellular lipid peroxidation damage. The MDA assay results showed that after treatment with *A. gigantifolia* extract, the intracellular MDA levels in AsPC-1 cells decreased. Considered together with the SOD assay results, this indicates that *A. gigantifolia* extract intervention induces alterations in the redox status of AsPC-1 cells; collectively, the SOD and MDA findings demonstrate that *A. gigantifolia* extract increases SOD activity in AsPC-1 cells, scavenges excessive intracellular ROS, and reduces the level of oxidative stress, with the effect becoming more pronounced as the drug concentration increases.

In summary, *A. gigantifolia* extract exerts anti-pancreatic cancer effects by inhibiting the proliferation, migration, and invasion of AsPC-1 cells. Further investigation into the mechanism underlying the effects of *A. gigantifolia* extract on AsPC-1 cell viability revealed that the extract enhances SOD activity in cancer cells, thereby improving their ROS-scavenging capacity. These results provide a theoretical basis for the further development and utilization of *A. gigantifolia* and for research on pancreatic cancer treatment.

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