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Original Research Article

In vitro Anti-Gastric Tumor Activities and Possible Mechanisms of Action of Paederosidic Acid from *Paederia scandens* (Lour) Merrill

Li-lin Chen[1], Ru Zeng[1] and Yan-zhen Zhuang[2]*
[1]Department of Medical Oncology, [2]Department of Pathology, The First Affiliated Hospital of Xiamen University, Xiamen, 361001, China

For correspondence:* ***Email:* *yzhenzhuang@163.com*

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Abstract

Purpose: *To evaluate the anti-tumor activity and explore the possible mechanisms of action of paederosidic acid isolated from Paederia scandens (Lour.) Merrill.*
Methods: *Paederosidic acid (PA) was isolated from P. scandens and identified by spectroscopic methods. The cytotoxic effects of PA in gastric cancer cell lines (MGC-803, BGC-823, and SGC-7901 cells) were assayed using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT). Apoptosis of PA in SGC-7901 cells was evaluated by DAPI staining. To investigate the possible mechanisms of apoptosis, the effect of PA on C-caspase-3, C-caspase-9, Bcl-2 and Bax expressions in SGC-7901 cells were assayed by Western blot analysis.*
Results: *PA exerted significant inhibitory effects on MGC-803, BGC-823, and SGC-7901 cells with 50 % inhibitory concentration (IC50) values of 42.2, 43.7, and 30.5 μM, respectively, and in a concentration-dependent manner. Subsequently, SGC-7901 cells were selected for further studies. After treatment with PA, obvious condensation of the nucleus was observed in fluorescence photomicrographs, which is a characteristic of apoptosis. In addition, caspase-3, caspase-9 proteins and Bax were significantly up-regulated ($p < 0.05$), whereas Bcl-2 was significantly down-regulated ($p < 0.05$) by PA in a concentration-dependent manner.*
Conclusion: *PA has significant anti-tumor activity on SGC-7901 cells in vitro, and the possible mechanism of action may be related to PA-induced apoptosis via mitochondria-mediated apoptosis pathway.*

Keywords: *Paederosidic acid, Paederia scandens, Anti-tumor activity, Apoptosis, Gastric cancer*

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INTRODUCTION

Gastric cancer (GC) is a common cancer worldwide, and it is also the second leading cause of cancer mortality [1]. Chemotherapy is one of the primary treatments for patients with advanced GC [2,3], but for most patients, chemotherapy usually causes serious side effects and is ineffective against GC due to multi-drug resistance (MDR) [3-5]. Therefore, it is of great significance to find new approaches for treating GC.

For thousands of years, traditional Chinese Medicine (TCM) has been applied in many fields of human health. With its impressive achievements, TCM is being increasingly acknowledged all over the world [6,7].

Paederia scandens (Lour.) Merrill, known as "Ji Shi Teng" in Chinese, is a climbing plant belonging to the family Rubiaceae [8]. It is widely distributed in China, India, Vietnam, Japan, the Philippines, and the USA. In South-East Asia, the whole plant of *Paederia scandens* has been traditionally used as a folk medicine and food for thousands of years. Paederosidic Acid (PA) is an active component isolated from *P. scandens*, it has been reported to have some pharmacological activities, such as anti-convulsant effect, sedative effect [5], and anti-nociceptive effect [9], but there has been no report on its anti-tumor activity so far.

EXPERIMENTAL

Plant material

P. scandens was purchased from Hehuachi Market of Traditional Chinese Herbs (Chengdu, China), and Identified by an expert in the Traditional Chinese Medicine (TCM) Department of The First Affiliated Hospital of Xiamen University. A voucher specimen (no. S-20130802) was deposited in our laboratory herbarium.

Chemicals

The following reagents and drugs were used: Sephadex LH-20 [H&E Co, Ltd (Beijing, China)]; silica-gel (74-154 μm and 50-74 μm, Qingdao Haiyang Chemical Co Ltd. Qingdao, China); petroleum ether, analytical reagent grade (AR), ethyl alcohol (EtOH, AR), methyl alcohol (MeOH, AR), n-butanol (AR) and ethyl acetate (AR, Sinopharm Chemical Reagent Co, Ltd, Shanghai, China); 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), 4',6-Diamidino-2-phenylindole dihydrochloride(DAPI) and dimethyl sulfoxide reagent (Sigma Aldrich, St Louis, MO, USA); fetal bovine serum (FBS) and RPMI 1640 media (Invitrogen, Carlsbad, California, CA, USA); c-caspase-3, c-caspase-9, Bcl-2 and Bax monoclonal antibody (Beyotime, Jiangsu, China).

Cell culture

Three tumor cell lines, MGC-803, BGC-823 and SGC-7901 were purchased from American Type Culture Collection (Manassas, Virginia, VA, USA). All cell lines were cultured in RPMI-1640 medium with 10 % fetal bovine serum, 100 U/mL penicillin and 100 mg/mL streptomycin. All these cells were incubated in the presence of 5 % CO_2 at 37 ^{o}C.

Isolation and purification of paederosidic acid (PA) from *P. scandens*

The whole plant of *P. scandens* (45 kg) was dried by airing at room temperature and powdered, extracted with 60 % EtOH by reflux. The EtOH extract was partitioned with n-butanol, ethyl acetate, and petroleum ether, respectively. A residue of the n-butanol fraction was obtained by reduced pressure drying (RE52CS-1 rotary evaporator, YaRong biochemical instrument, Shanghai).

The n-butanol fraction was eluted through silica-gel (100–200 mesh) with $CHCl_3$:MeOH (15:1, 10:1, 8:1, 5:1, 2:1, 1:1) and a series of sub fractions (I-VI) were obtained. Columns of silica gel (200–300 mesh), Sephadex LH-20 (GE) and a reverse phase preparative-HPLC [solvent = MeOH:H_2O (60:40)] with flow rate 1.0 mL/min were used to purify PA . Finally, the purified product of 3.56 g was obtained from fraction II.

Identification of the compound isolated from *P. scandens*

The identification of PA (Fig 1) was accomplished by spectroscopic methods (MS, ^{1}H NMR and ^{13}C NMR) and the spectral data of PA are corresponding to literature values [8,10]. PA was purified with purity not less than 98 % (HPLC assay).

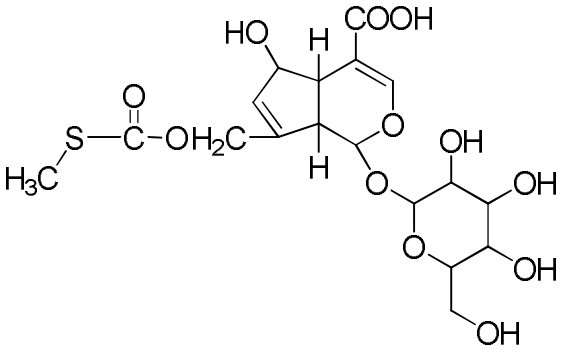

Fig. 1: Structure of the Paederosidic acid (PA)

MTT reduction assay

Cells (1 × 10^{4}/0.2 mL) were seeded into 96-well plates and indicated concentrations of PA for 48 h. The standard protocol was used to carry out MTT assay, 20 μL of 5 mg/mL MTT solution was added to each well and the plates were incubated for 4 h. Subsequently, the supernatant was aspirated and 150 μL DMSO was added to each well. A 96-well plate reader (Bio-rad Microplate Reader Model 550) was used to read the absorbance at 570 nm. The viability of the cells was measured by the level of activity

because reduction of MTT only occurs in metabolically active cells. Cell inhibition (C) was computed as in Eq 1.

C (%) = {(Ac – At)/Ac}100 …… (1)

where Ac and At are the absorbance of control and treated cells, respectively.

Apoptosis assay

The cells (1 × 10^4/0.2 mL) were seeded in 96-well plates and treated with PA at concentrations of 10, 20, 40 μM for 48 h. Then cells were stained by DAPI, observed and photographed by using a fluorescence microscope (×200, BX41-32PO2-FLB3, Olympus, Tokyo, Japan).

Western blot analysis

Total proteins were extracted from cells or tumor tissues, and then separated by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE) and electro transferred onto polyvinylidene difluoride (PVDF). Proteins were probed with corresponding primary monoclonal antibody respectively and with goat anti-rabbit/HRP subsequently, and then detected by using the chemiluminescence peroxidase reagents. Antibodies directed against β-actin were used to measure protein loading.

Statistical analysis

Data are shown as mean ± SEM (n=3). The scientific statistic software (SPSS13.0 software, SPSS, USA) was used to evaluate the significance of differences between groups. Results were analyzed by one-way analysis of variance (ANOVA) and $p < 0.05$ was considered significant.

RESULTS

Cytotoxic effect of paederosidic acid (PA) on MGC-803, BGC-823, and SGC-7901 cells

As shown in Fig 2, PA exerted significant inhibitory effects on MGC-803, BGC-823, and SGC-7901 cells with IC_{50} values of 42.2, 43.7, and 30.5 μM, respectively, in a concentration-dependent manner. Then, the SGC-7901 cell line was selected from three cell lines for further research due to PA possess the best anti-proliferative effect on SGC-7901 among the three tested cell lines.

Effect of paederosidic acid (PA) on apoptosis in SGC-7901 cells

To explore the induction of apoptosis by PA in SGC-7901 cells, fluorescence photomicrographs were recorded after staining with DAPI. After treatment with PA, significant condensation of the nucleus was induced as shown in Figure 3, which meets the characteristics of apoptosis.

Induced apoptosis of paederosidic acid in SGC-7901 cells

In the present study, SGC-7901 Cells were treated with PA. As a result, the caspase-3 and caspase-9 proteins were significantly up-regulated (at the concentrations of 10, 20, 40 μM) by treatment with PA, in a concentration-dependent manner (Fig 4).

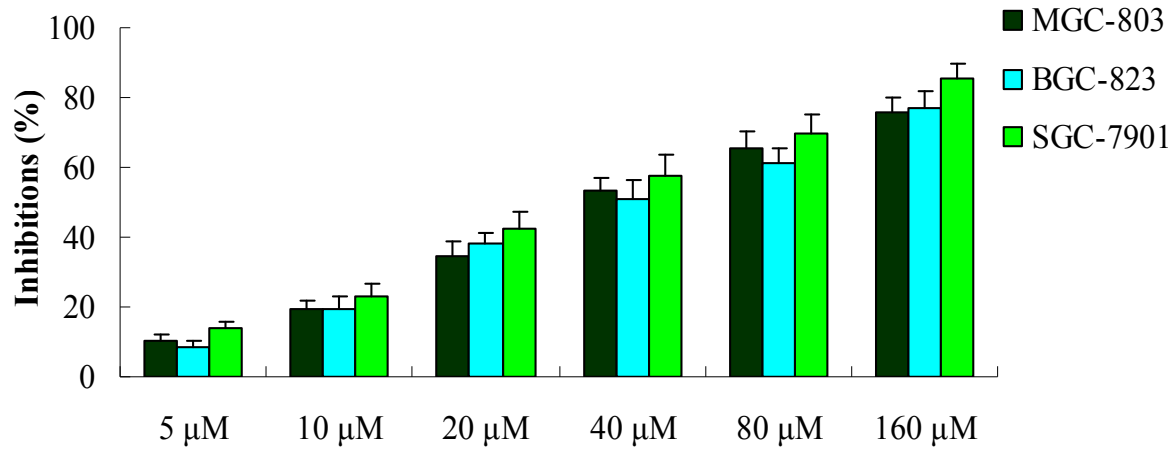

Fig 2: Cytotoxic effects of PA on gastric carcinoma cells (n=4). Cells were treated with PA at the concentrations range from 5-160 μM for 48 hours, the cells viabilities were determined by the MTT assay. IC_{50} values of PA on MGC-803, BGC-823, and SGC-7901 cells were 42.2, 43.7, and 30.5 μM, respectively

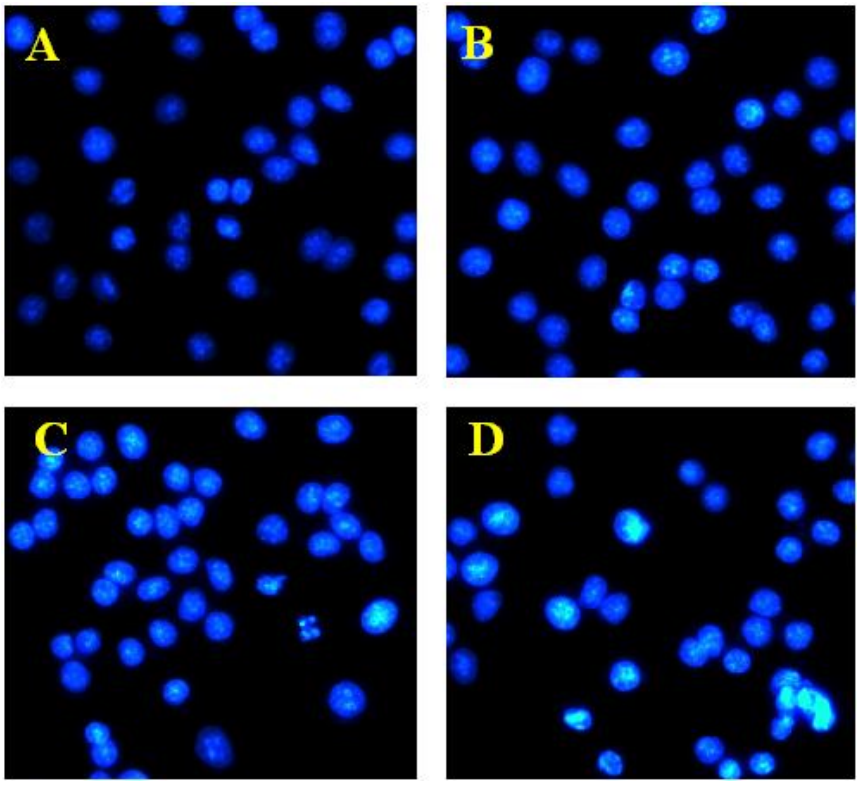

Fig 3: Determination of apoptosis in SGC-7901 cells by DAPI staining. Cells were treated with PA at concentrations of 10, 20, and 40 μM for 48 hours, then cells were stained by DAPI and observed by fluorescence photomicrography (× 200). A–D represented the control, 10 μM, 20 μM, and 40 μM, respectively

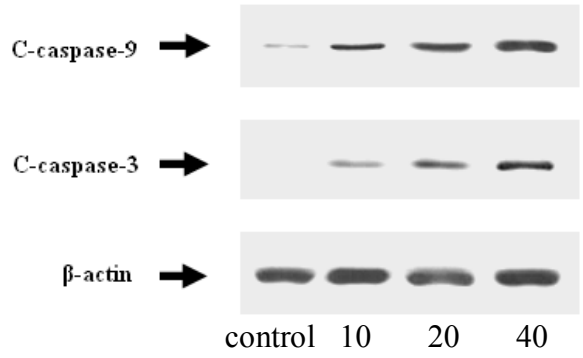

Fig 4: Effect of PA on C-caspase-3 and C-caspase-9 expressions in SGC-7901 cells. Cells were treated with PA at the concentrations of 10, 20, and 40 μM for 48 h, and the whole cell lysates were determined by western blot analysis using antibodies for C-caspase-3 and C-caspase-9 as indicated

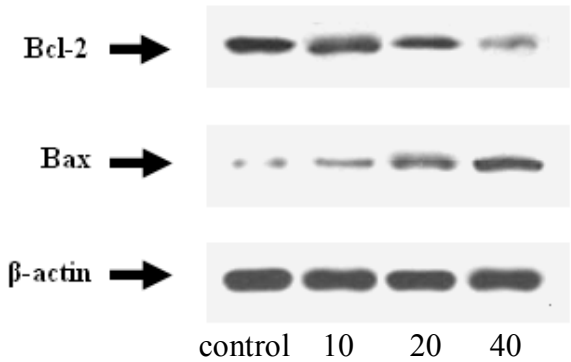

Fig 5: Effect of PA on Bcl-2 and Bax expressions in SGC-7901 cells. Cells were treated with PA at the concentrations of 10, 20, and 40 μM for 48 h, and the whole cell lysates were determined by western blot analysis using antibodies for Bcl-2 and Bax as indicated

The results indicate that PA can significantly down-regulate the expressions of Bcl-2 (at the concentrations of 10, 20, 40 μM, $p < 0.05$), and significantly up-regulate the expression of Bax(at the concentrations of 10, 20, 40 μM, $p < 0.05$), in a concentration-dependent manner (Fig 5). In addition, the ratio of Bax/Bcl-2 also increased after treatment with PA.

DISCUSSION

In this paper, we investigated the *in vitro* anti-tumor activity of PA in SGC-7901 cells, and this is the first report of the related research to our knowledge. In addition, we made an attempt to support mitochondria-mediated intrinsic apoptosis as one of the major apoptotic pathways in SGC-7901 cells.

Apoptosis is a cell-intrinsic programmed suicide mechanism for damaged cells and mitochondria-mediated intrinsic apoptosis is one of the major apoptotic pathways of apoptosis [8,11]. Caspase proteins play pivotal roles in initiation and execution of apoptosis. Caspase-3 is one of the effectors in initiation of apoptosis and caspase-3 can be commonly activated by caspase-9 [12,13]. The B-cell lymphoma/leukemia-2(Bcl-2) family can significantly regulate apoptosis as an activator or as an inhibitor. Bax is a well-known pro-apoptotic protein and Bcl-2 is an anti-apoptotic protein of Bcl-2 family, so the Bax/Bcl-2 ratio is a key factor in apoptotic regulation process. The mitochondria-mediated apoptotic cell death pathway can be activated when the ratio of Bax/Bcl-2 increase [14-16].

In our present study, the caspase-3, caspase-9 proteins and Bax were significantly up-regulated ($p < 0.05$), Bcl-2 was significantly down-regulated ($p < 0.05$) by treatment with PA, in a concentration-dependent manner. Therefore, this indicates that the possible mechanism of anti-tumor activity may be related to apoptosis induced by PA via a mitochondria-mediated apoptosis pathway. More investigations are necessary to elucidate the complete mechanism of apoptosis induced by PA.

CONCLUSION

PA has significant antitumor activity on gastric cancer cell lines, and the possible mechanism of action may be related to apoptosis induced by PA via mitochondria-mediated apoptosis pathway. PA is an interesting apoptosis inducer with a potential to be developed into a therapeutic agent for gastric cancer. However, more investigations are necessary to elucidate its complete mechanism(s) of action.

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