**Houttuynia cordata** Thunberg exhibits anti-tumorigenic activity in human gastric cancer cells

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**ABSTRACT**

**Objectives** : Gastric cancer is a leading cause of cancer-related deaths, worldwide, *Houttuynia cordata* Thunberg (*H. cordata*) has been used as a medicinal plants and it has an anti-cancer activity in human colorectal cancer and leukemic cancer. However, the potential anti-cancer activity and mechanisms of *H. cordata* for human gastric cancer cells have not been tested so far. Thus, this study examined the biological effects of *H. cordata* on the human gastric cancer cell line SNU-1 and AGS.

**Methods** : Inhibition of cell proliferation and cell cycle by *H. cordata* was carried out by MTT assay and Muse cell cycle analysis and the expressions of protein associated with apoptosis and cell cycle regulation were investigated with Western blot analysis.

**Results** : In MTT assay, the proliferation of SNU-1 and AGS cells was significantly inhibited by *H. cordata* in a time and dose dependent manner, Inhibition of cell proliferation by *H. cordata* was in part associated with apoptotic cell death, as shown by changes in the expression ratio of Bax to Bcl–2 by *H. cordata*. Also, *H. cordata* regulated the expression of cell cycle regulatory proteins such as pRb, cyclin D1, cyclin E, CDK4, CDK2, p21 and p15.

**Conclusion** : The antiproliferative effect of *H. cordata* on SNU-1 and AGS gastric cancer cells revealed in this study suggests that *H. cordata* has intriguing potential as a chemopreventive or chemotherapeutic agent.

**Key words** : Anti-cancer activity; Apoptosis; Human gastric cancer; *Houttuynia cordata* Thunberg

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**Introduction**

Gastric cancer is one of the leading causes of cancer incidence and mortality around the world. Although surgery is the most effective therapy for gastric cancer, chemoprevention has been regarded as a promising strategy to reduce the incidence of gastric cancer. Thus, nowadays, many researchers have investigated the promising anti-cancer agents and found that natural compounds are important sources for cancer chemopreventive and chemotherapeutic agents.

*Houttuynia cordata* Thunberg (*H. cordata*) has been used as a vegetable and herbal plant and it is estimated that *H. cordata* has anti-inflammatory, anti-cestodal, anti-viral and anti-obesity effects, and induces apoptosis in human colorectal cancer cells and leukemic cancer cells. However, the potential anti-cancer activity and mechanisms of *H. cordata* for human gastric cancer cells have not been tested so far. Thus, in light of the therapeutic potential of *H. cordata* in human gastric cancer, this study was performed to elucidate the potential mechanism by which *H. cordata* induces the cell growth arrest and apoptosis in human gastric cancer cells. Here, for the first time, we report that *H. cordata* leads to cell growth arrest and apoptosis
which may be associated with the regulating cell cycle-regulatory proteins and Bax/Bcl−2 ratio in human gastric cancer cells.

Materials and Methods

1. Chemicals

Cell culture media, RPMI 1640 was purchased from Life Technologies (Grand Island, NY, USA). Antibodies against Bax, Bcl−2 and p21 were purchased from Santa Cruz Inc, (Santa Cruz, CA, USA) and other antibodies against cyclin D1, cyclin E, CDK2, CDK4, p15, phospho-Rb (ser780) and β-actin were purchased from Cell Signaling (Danvers, MA, USA). All chemicals were purchased from Fisher Scientific, unless otherwise specified.

2. Sample preparation

_H. cordata_ was kindly provided by Bonghwa Alpine Medicinal Plant Experiment Station, Korea. One kilogram of _H. cordata_ was extracted with 2L of 80% methanol with shaking for 24 hours. After 24 hours, the extract with 80% methanol was filtered, concentrated to approximately 400 ml volume using a vacuum evaporator, and subsequently fractioned with ethyl acetate in a separating funnel. The ethyl acetate fraction (42 g, yield rate: 4.2%) was separated from the mixture and evaporated by a vacuum evaporator. Ethyl acetate fraction was kept at −80 °C.

3. Cell culture and treatment

Human gastric cancer cell lines (SNU-1 and AGS) and human normal gastric epithelial cells were purchased from American Type Culture Collection (Manassas, VA, USA) and grown in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 µg/ml streptomycin. The cells were maintained at 37 °C under a humidified atmosphere of 5% CO2. Ethyl acetate fraction from _H. cordata_ was dissolved in dimethyl sulfoxide (DMSO) and treated to cells, DMSO was used as a vehicle and the final DMSO concentration was not exceeded 0.1% (v/v).

4. MTT assay

SNU-1, AGS and human normal gastric epithelial cells (1x10^5 cells) were plated onto 96–well plate and grown overnight. The cells were treated with 0, 5, 25 50 µg/ml of extracts in media for 24 and 48 hours at 37 °C under 5% CO2. Then, the cells were incubated with 50 µl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (1 mg/ml) for an additional 4 hours and then the formazan crystal produced was measured by an enzyme–linked immunosorbent assay reader at 570 nm. Cell growth inhibition was estimated as the reduction in values from a vehicle.

5. Cell cycle analysis

Cell cycle analysis was carried out using Muse™ Cell Cycle Kit (Millipore, Billerica, MA, USA) according to the manufacturer protocol. Briefly, the cells treated with ethyl acetate fraction from _H. cordata_ were harvested, centrifuged at 300 x g for 5 min, and then washed once with 1 x PBS. One milliliter of ice cold 70% ethanol was added to cells and then cells were incubated for 3 hours at − 20 °C. After 3 hours, 200 µl of fixed cells were transferred into new tubes, centrifuged at 300 x g for 5 min, and then washed once with 1 x PBS. After washing, cells were stained with 200 µl of Muse™ Cell Cycle reagent at the room temperature in the dark for 30 min, and subsequently cell cycle was analyzed using Muse™ Cell Analyzer.

6. SDS–PAGE and Western blot

The cells were washed with 1 x phosphated–buffered saline (PBS), and lysed in NP–40 lysis buffer supplemented with protease and phosphatase inhibitor cocktail (Sigma Aldrich, St.Louis, MO, USA), and centrifuged at 15,000 x g at 4 °C for 20 min. After protein concentration was determined by Bradford protein assay. Equal amounts of proteins were subjected to SDS–PAGE and then transferred onto PVDF membrane. The membranes were blocked for non-specific binding with 5% non-fat dry milk in tris-buffered saline containing 0.05% Tween 20 (TBS–T) for 1 hour at room temperature and then probed with the primary antibodies overnight at 4 °C, followed by incubation with horse radish peroxidase (HRP)–conjugated immunoglobulin G (IgG) for 1 hour at room temperature. Chemiluminescence was detected with ECL Western blotting substrate (GE Healthcare, Pittsburgh, PA, USA) and visualized in polaroid film.

7. Statistical analysis

Statistical analysis was performed with the Students unpaired t–test, with statistical significance set at *, P < 0.05.