

***In vitro* effects and the related molecular mechanism of galangin and quercetin on human gastric cancer cell line (SGC-7901)**

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Abstract: Natural flavonoids are proven to be powerful against various cancers, but few studies have investigated the potential effects of two flavonoids galangin and quercetin on a human gastric cancer cell line (SGC-7901). *In vitro* growth inhibition and apoptosis of the two flavonoids on the SGC-7901 cells as well as potential mechanism about apoptosis induction are reported in the present study. The assaying results showed that the two flavonoids at 40-200 µmol/L for 24-72 hours conferred lower cell viability of 14.1-90.3% in dose- and time-dependent manner, and at 160 µmol/L for 24-48 hours enhanced the proportion of apoptotic cells into 13.3-27.4% and 40.6-65.6%, respectively. Galangin was more powerful than quercetin to inhibit cell growth, induce apoptosis and decrease mitochondrial membrane potential (MMP). Oligonucleotide micro array, real-time RT-PCR and Western-blot analyses revealed expression changes of the genes and proteins in the treated cells, clarifying a mechanism related to apoptosis induction. The two flavonoids activated caspase-8, which cleaved Bid into tBid; simultaneously, Bax transferred from cytosol into mitochondria to decrease MMP; consequentially, cytochrome c released from mitochondria activated caspase-9, and then caspase-9 activated caspase-3, which executed the apoptosis. That is, the apoptosis occurred via a mitochondrial pathway involving caspase-8/Bid/Bax activation.

Keywords: Galangin, quercetin, human gastric cancer cell line (SGC-7901), growth inhibition, apoptosis.

INTRODUCTION

Fruit and vegetable-rich diets are rich in phenol compounds, and have been found to be correlated with a lower incidence of some diseases (Riboli and Norat, 2003), because some studies have observed that phenol compounds and their extracts exert cytoprotection and anti-cancer property (Namvar *et al.*, 2012; Li *et al.*, 2013; Sakulnarmrat *et al.*, 2013). Flavonoids as one type of naturally phenol compounds are considered as disease-preventing dietary supplements (Moon *et al.*, 2006), among them are two flavones galangin and quercetin with structural similarity. Galangin (3,5,7-trihydroxyflavone) is rich in propolis and India root (*Alpinia officinarum*) (Heo *et al.*, 2001; Laskar *et al.*, 2010), while quercetin (3,3',4',5,7-pentahydroxyflavone) is widely found in many fruits, vegetables and herbs (Wach *et al.*, 2007). Both galangin and quercetin have *in vitro* cytotoxicity towards some cancer cells, including colon cancer (Psahoulia *et al.*, 2007), esophageal squamous cancer (Zhang *et al.*, 2008), leukemia (Niu *et al.*, 2011), breast cancer (Lee *et al.*, 2010), hepatoma (Granado-Serrano *et al.*, 2006) and lung cancer (Nguyen *et al.*, 2004) cells. Quercetin is also reported to increase sensitization of cancer cells to the drugs, for example, the lung cancer cells (Chen *et al.*, 2007).

Anti-cancer properties of galangin and quercetin include growth inhibition, differentiation and apoptosis induction

(Cao *et al.*, 1997). Related molecular mechanisms also have been revealed by the researchers. Galangin can induce apoptosis of human hepatoma HepG2 cells via mitochondrial pathway (Zhang *et al.*, 2010). Quercetin can induce apoptosis of human leukemia U937 cells by down-regulating Mcl-1 and activating Bax (Cheng *et al.*, 2010), or induce apoptosis of human hepatoma HepG2 cells by activating caspases, regulating Bcl-2, and inhibiting PI-3-Kinase/Akt and ERK pathways (Granado-Serrano *et al.*, 2006).

Gastric cancer is one of the common cancers found in the world (Alberts *et al.*, 2003). In 2008, the new cases and death of gastric cancer have been estimated about 989,600 and 738,000 (Jemal *et al.*, 2011). There is a great need to find out new ways especially dietary components for the prevention of gastric cancer (Bi *et al.*, 2010). Anti-cancer properties and possible mechanisms of dietary components should be well clarified. Two recent studies evidence that galangin and quercetin can induce apoptosis in gastric cancer SNU-484 (Kim *et al.*, 2012) and BGC-823 (Wang *et al.*, 2012) cells, respectively. However, apoptosis induction of galangin and quercetin on another gastric cancer (human gastric cancer line SGC-7901) is not studied yet.

In the present study, galangin and quercetin were investigated for their *in vitro* effects and molecular mechanism on the SGC-7901 cells. Growth inhibition and apoptosis induction of galangin and quercetin were evidenced by cell viability and flow cytometry analyses.

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Some genes and proteins of the cells related to the apoptosis were identified by oligonucleotide micro array, real-time RT-PCR and Western-blot analyses, to find out the possible mechanism. The aim of the present study was to clarify if galangin and quercetin are potential dietary components to prevent human gastric cancer and to show the related molecular mechanism.

MATERIALS AND METHODS

Materials

Galangin (>99% of purity) and quercetin (>98% of purity) were the products of Shanghai Yuanye Biotechnology Co. Ltd. and Shanghai Yousi Biotechnology Co. Ltd. (Shanghai, China), respectively. Fetal bovine serum (FBS) was obtained from Zhejiang Tianhang Biotechnology Co., Ltd. (Huzhou, Zhejiang, China). Annexin V-fluorescein isothiocyanate/propidium iodide (Annexin V-FITC/PI) detection kit was obtained from Beijing Biosea Biotechnology Co. Ltd (Beijing, China). Cytoplasmic and Mitochondrial Protein Extraction Kit Hoechst was obtained from Sangon Biotechnology Co. (Shanghai, China). Rhodamine 123 (Rh123), BCA Protein Assay Kit and antibodies (caspase-3, Bax) were obtained from Beyotime Institute of Biotechnology (Haimen, Zhejiang, China). Dimethyl sulfoxide (DMSO), 5-fluorouracil (5-FU), trypsin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), ethylenediamine tetraacetic acid (EDTA) and the materials for Western-blot analysis were obtained from Solarbio Science and Technology Co. Ltd. (Beijing, China). Other chemicals used were analytical agents.

Mono- and poly-clonal antibodies (caspase-9, Bid, cytochrome c and cleaved caspase-8) were obtained from Cell Signaling Technology Inc. (Beverly, MA, USA). β -Actin and horseradish peroxidase-conjugated goat anti-mouse and goat anti-rabbit antibodies were from Zhong Shan Gold Bridge Biotechnology Co. Ltd. (Beijing, China).

Cell line and culture conditions

A moderately differentiated human gastric adenocarcinoma cell line (SGC-7901) was obtained from Cell Bank of the Chinese Academy of Sciences (Shanghai, China), and used in the present study. Culture conditions of the cells were provided by the cell supplier. The cells were cultured in the RPMI-1640 medium (Gibco, Rockville, MD, USA) supplemented with heat-inactivated FBS of 10%. Both penicillin (100U/ml) and streptomycin (100 μ g/ml) were added into the culture. The cells were maintained at 37°C in the incubator of humidified air with 5% CO₂. Sub-confluent cells (about 80%) were passaged by a solution composing trypsin and EDTA about 0.25 and 0.02%, respectively.

Assaying of cell viability

Cell viability was measured by the classic MTT assay

(Mosmann, 1983), and used to reflect growth inhibition of the two flavonoids. SGC-7901 cells were plated in 96-well plates at 5×10^3 /well, and incubated for 24 hours before treatment with the two flavonoids. Each of the two flavonoids was dissolved in DMSO, and made up with the medium to ensure final concentration of the vehicle was lower than 0.1% DMSO. The cells were treated with galangin and quercetin at 10-200 μ mol/L for 24, 48 and 72 hours, respectively. The cells treated with 0.1% DMSO and 100 μ mol/L of 5-FU were served as negative and positive controls, respectively. MTT solution of 5mg/ml was prepared in phosphate-buffered saline (PBS) of 0.01 mol/L, added into each well, and incubated for 4 hours. After that, MTT solution was removed from the wells by aspiration, and 150 μ l of DMSO was added into each well. The plates were shaken for 10min. Absorbance of each well was detected by a microplate reader (Bio Rad Laboratories, Hercules, CA, USA) at 490nm. Growth inhibition of the cells was calculated and expressed as percent cell viability, while vehicle-treated cells were served as 100% viable.

Apoptosis analysis by flow cytometry

Proportion of the apoptotic cells was detected by flow cytometry with the Annexin V-FITC/PI double labeling method (Vermes *et al.*, 1995). Briefly, SGC-7901 cells were grown to 70-80% confluence in 6-well plates, and treated with galangin and quercetin at 160 μ mol/L for 24-48 hours, respectively. The cells treated with 0.1% DMSO were served as negative control. The cells were harvested, washed with cold PBS (0.01mol/L), and centrifuged at 110 $\times g$ for 5 min to discard the supernatant. The pellet was re-suspended gently in 200 μ l of Annexin V-FITC binding buffer, and incubated with 10 μ l of Annexin V-FITC for 15 min in the dark at room temperature. Binding buffer of 300 μ l and PI of 5 μ l were added into each well, and mixed gently. After filtration (400 apertures), the stained cells were analyzed with flow cytometry (FACS Calibur, Becton Dickson, San Jose, CA, USA), to record the percentage of intact, necrotic and apoptotic cells.

Determination of mitochondrial membrane potential

Rh123, which binds specifically to mitochondria, was used to estimate mitochondrial membrane potential (MMP) as per the method (Xiang *et al.*, 2006) with some modifications. Briefly, the cells in 6-well plates were grown to 70-80% confluence, and treated with galangin and quercetin at 160 μ mol/L for 24 and 48 hours, respectively. The cells were harvested, washed and incubated with Rh123 (5 μ g/ml) in PBS (0.01 mol/L) at 37°C for 60 min in the dark. Fluorescence was detected in a Hitachi F-4500 fluorescence spectrophotometer (Tokyo, Japan) using excitation and emission wavelengths of 507 and 529 nm, respectively. MMP of the treated cells was expressed as percent fluorescence value, while vehicle-treated cells were served as 100%.

Table 1: The primers used in real-time RT-PCR analysis

Genes	Primer sequence	PCR product (bp)
Bid	5'AACAACGGTTCCAGCCTCAG3'	153
	5'CTCATCGTAGCCCTCCCACT3'	
Caspase-6	5'AACTGGCTTGTTCAAAGGAGAC3'	107
	5'GCAGCGTGTAACGGAGG3'	
Caspase-9	5'TTGAGACCCTGGACGACA3'	88
	5'ACCGAAACAGCATTAGCG3'	
PAK1IP1	5'TCAGTGGAGCGGAAGATGGA3'	112
	5'AGATGGGTGAATAGAAAGGAAGGTC3'	
β-Actin	5'-AACACCCCAGCCATGTACG-3'	254
	5'-ATGTCACGCACGATTTCCTCC-3'	

Table 2: The alterations of gene expression in SGC-7901 cells treated with galangin

Genes	Description	Accession no.	Fold change
Bid	BH3-interacting domain death agonist	NM_197967	+2.47
Caspase-9	Caspase-9 precursor	NM_032996	+2.10
Caspase-6	Caspase-6 precursor	NM_005192	+1.58
PAK1IP1	P21-activated protein kinase-interacting protein 1	NM_017906	+1.96

Table 3: Effects of galangin and quercetin on gene expressions in SGC-7901 cells detected by real-time RT-PCR

Genes	Galangin	Quercetin
Bid	+4.16±0.08	+1.55±0.15
Caspase-6	+4.50±0.06	+1.08±0.25
Caspase-9	+2.28±0.07	+1.59±0.20
PAK1IP1	+2.49±0.84	+2.17±0.79

The cells treated with 0.1% DMSO for 24 hours are served as the control treatment. “+” denotes up-regulated expression.

Isolation of RNA and oligonucleotide microarray assay

Two independent cultured cells were treated with galangin (160 μmol/L) and DMSO (0.1%) for 24 hours, respectively, and then were used to isolate total RNA by the TRIzol reagent phenol chloroform procedure (Invitrogen). The prepared RNA samples were sent to CapitalBio Co. (Beijing, China) for human signal transduction oligonucleotide microarray assay, with the procedures reported on the website (<http://www.capitalbio.com>). The oligonucleotide chip (CapitalBio) used has 897 well-characterized Homo sapiens signal transduction related genes purchased from Operon Biotechnologies Inc. (Huntsville, AL, USA). Differently expressed genes, integrated ratios of replicate trials, were considered for >1.5-fold change in the ratios of expression levels between galangin treatment and DMSO control.

Real-time RT-PCR and Western-blot analyses

The cells grown to about 70% confluence in 6-well plates were treated with galangin and quercetin (160 μmol/L) for 24 hours, respectively. Two micrograms of total RNA from each sample were subjected to reverse transcription as per the procedure (Zhang *et al.*, 2008); however, 0.8 μl of each 10 μmol/L forward and reverse primers and 6.0 μl

of H₂O were used in analysis. The primers used are listed in table 1. The PCR program set for ABI PRISM 7500 Real-time PCR System (Applied Biosystems, Foster City, CA, US) was initiated by 10 seconds at 95°C before 40 thermal cycles, each of 5 seconds at 95°C and 34 seconds at 60°C. The data were analyzed based on the 2^{-ΔΔCt} method (Livak and Schmittgen, 2001), and normalized to β-actin expression. Melting curves for each PCR reaction were also generated to ensure purity of the amplification product.

The cells (70% confluent) were treated with galangin and quercetin (160 μmol/L) for 24 hours, respectively, harvested by centrifugation, treated with cold PBS (0.01mol/L) and the ice-cold lysis buffer (pH 7.4), and stored at -80°C as the previous study (Zhang *et al.*, 2008). Protein concentration was assayed by BCA Protein Assay Kit before Western-blot analysis. The proteins of 50μg from each sample was resolved over SDS-PAG of 12-15% and then transferred into a nitrocellulose membrane. Forthcoming treatments and densitometric measurements of the bands were the same to the previous study (Zhang *et al.*, 2008).

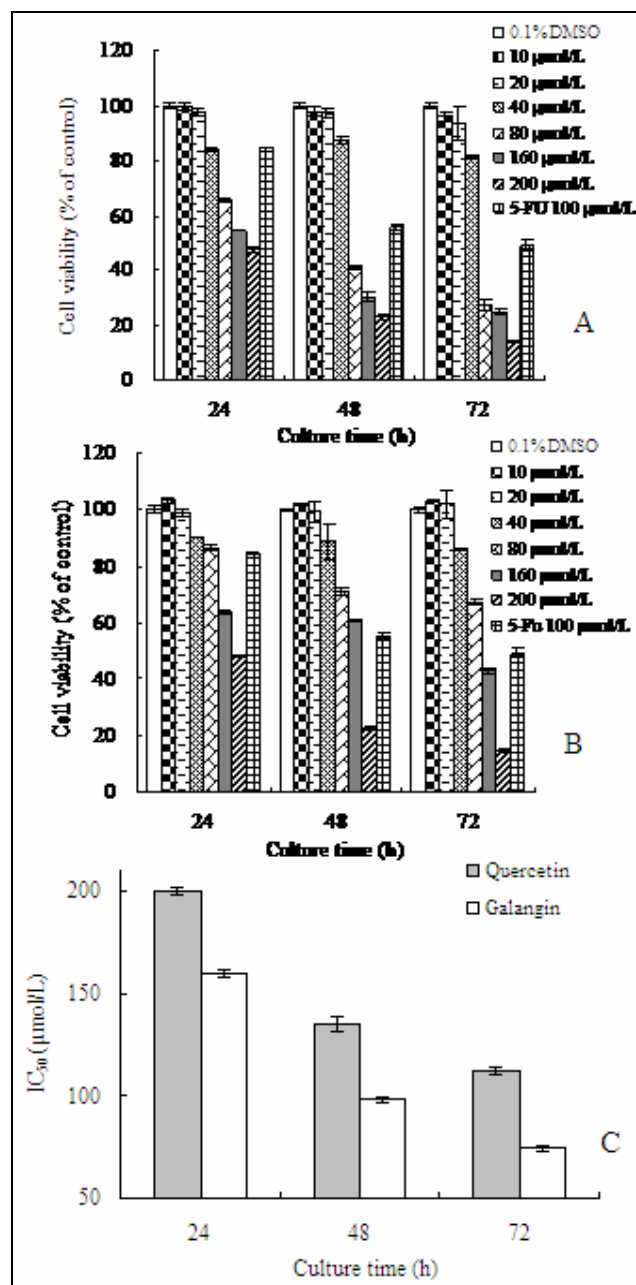


Fig. 1: Xu, Wang & Zhao

STATISTICAL ANALYSIS

All data were expressed as means $\pm$ standard derivations from at least three independent trials and measurements. The SPSS version 16.0 software (SPSS Inc., Chicago, IL, USA) was used in data analysis.

RESULTS

In vitro growth inhibition of galangin and quercetin on the cells

Growth inhibition of galangin and quercetin on SGC-7901 cells were first assayed in the present study. The results shown in fig. 1 indicate that if the cells were

treated with the two flavonoids at 10-20 $\mu\text{mol/L}$, the growth inhibition was not very clear as the measured values of cell viability were 93.4–99.7% and 98.9–103.3%, respectively. When the two flavonoids were used at higher levels (40–200 $\mu\text{mol/L}$), the growth inhibition became significantly as the measured values of cell viability were 14.1–84.1% and 14.4–90.3%, respectively. It is seen that *in vitro* growth inhibition of the two flavonoids on the cells behaved in a typical dose- and time-dependent manner (fig. 1A and 1B). Galangin exhibited a stronger inhibition than quercetin on the cells, as it had lower IC_{50} values than quercetin in the present assay (160 versus 200 $\mu\text{mol/L}$ at 24 hours, or 100 versus 135 $\mu\text{mol/L}$ at 48 hours, or 75 versus 112 $\mu\text{mol/L}$ at 72 hours) (fig. 1C); that is, galangin at lower level could decrease cell viability to 50%. Both galangin and quercetin at 160 $\mu\text{mol/L}$ were used in the later evaluation.

Inducing apoptosis of galangin and quercetin on the cells

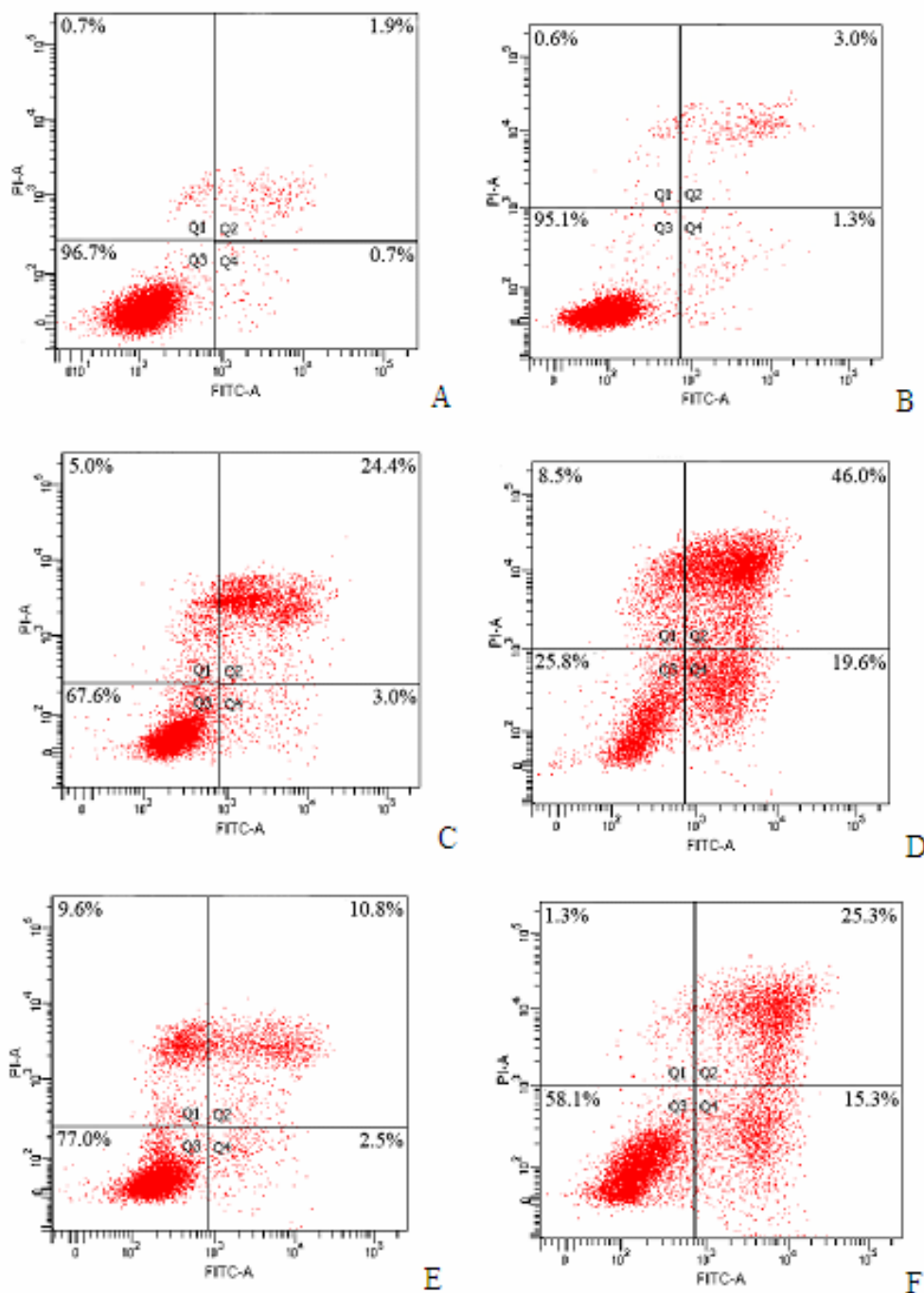
When the cells were treated with the two flavonoids of 160 $\mu\text{mol/L}$ for 24 and 48 hours, respectively, apoptosis induction was observed from the assaying results of flow cytometry (fig. 2). In the control group, only 0.1% DMSO was added into the culture, the proportions of apoptotic cells (Q2+Q4) were 2.6% (24 hours) and 4.3% (48 hours) (fig. 2A and 2B). When the cells were treated with galangin and quercetin for 24 hours, the proportions of apoptotic cells were increased to 27.4% and 13.3% (fig. 2C and 2E), respectively. If the cells were treated with galangin and quercetin for a longer time (48 hours), the proportions of apoptotic cells were increased to 65.6% and 40.6%, respectively (fig. 2D and 2F). Three results were obtained from the present evaluation: (1) the two flavonoids all could induce apoptosis of the cells; (2) galangin was more powerful than quercetin in apoptosis induction, as it was observed to bring about greater apoptosis in each case; and (3) longer treating time of the cells with the two flavonoids clearly led to greater apoptosis.

Effects of quercetin and galangin on MMP

Mitochondrial function and integrity are dependent on maintenance of the MPP. As the results shown in fig. 3, the cells treated with galangin and quercetin at 40-200 $\mu\text{mol/L}$ for 24-48 hours showed decreased values of MMP, which also was behaved in a dose- and time-dependent. For example, if the cells were treated with galangin and quercetin at 80 $\mu\text{mol/L}$ for 24-48 hours, the MMP were 63.2-45.9% and 82.8-56.4%, respectively; when the cells were treated with the two flavonoids at 160 $\mu\text{mol/L}$ for 24-48 hours, the measured MMP values were 55.5-38.2% and 77.6-45.3%, respectively (fig. 3). Galangin was observed more effective than quercetin to decrease MMP, as it resulted in the cells with much lower MMP values. It is thus demonstrated that the two flavonoids might induce apoptosis of the cells via the

Treating times 24 hours

48 hours

**Fig. 2:** Xu, Wang & Zhao

mitochondrial pathway.

Effects of quercetin and galangin on the four genes related to apoptosis

Galangin was chosen as a representative to assess its effect on the alterations of gene expression in the cells, aiming to screen those genes related to apoptosis via microarray assay. Detailed information about expression

changes of genes in the cells is not reported here. It was found from the assay results that four genes (Bid, caspase-9, caspase-6, and PAK1IP1) were up-regulated by +2.47, +1.58, +2.10 and +1.96 folds, respectively (table 2). As those described by GenBank, functions of these genes are related to cell apoptosis (<http://www.ncbi.nlm.nih.gov/genbank>).

Real-time RT-PCR analysis was applied to confirm the results from microarray assay. The obtained results indicated that Bid, caspase-9, caspase-6, and PAK1IP1 were up-regulated by +4.16, +2.28, +4.50 and +2.49 folds (galangin treatment), and by +1.55, +1.59, +1.08 and +2.17 (quercetin treatment), respectively (table 3). The RT-PCR results were consistent with the microarray assay results, although the measured alteration folds of gene expression were not exactly same. RT-PCR analysis results therefore point out that apoptosis induction of the two flavonoids on the cells depended on the expression levels of the four genes.

Suggested molecular mechanism related to the apoptosis of the cells

Based on the up-regulation at mRNA levels and suggested functions of Bid and caspase-9, a mechanism related to the apoptosis induction of galangin and quercetin on the cells was speculated. That is, Bid integrated intrinsic and extrinsic signaling pathways.

A series of Western-blot analysis were thereof carried out for caspase-8, Bid, Bax, caspase-9, caspase-3 proteins, and cytochrome c. Caspase-8 protein level was also detected to see if the two flavonoids induced apoptosis via triggering extrinsic pathway in the cells. The obtained results are shown in fig. 4, and indicate that: (1) the two flavonoids induced the cleavage of procaspase-8 into active caspase-8, and then enhanced the cleavage of caspase-8, as protein expression of caspase-8 had nearly an one-fold increase (fig. 4A), which suggests an extrinsic pathway; (2) the two flavonoids induced cleavage of procaspase-9 and procaspase-3 (two mitochondria-derived caspases) to form activated caspase-9 and caspase-3 (fig. 4B and 4D), respectively, suggesting mitochondria-dependent apoptosis; (3) Bid protein (22 kDa) was cleaved to form tBid (15 kDa) (*i.e.* activation of Bid) (fig. 4C), resulting in increased translocation of Bax from cytosol to mitochondria (*i.e.* Bax decreased in cytosol but increased in mitochondria) (fig. 4G and 4H) and increased translocation of cytochrome c from mitochondria to cytosol (*i.e.* cytochrome c decreased in mitochondria but increased in cytosol) (fig. 4E and 4F). The two flavonoids thus induced mitochondria-dependent apoptosis via activation of caspase-8/Bid/ Bax in the cells. It was Bid that integrated the intrinsic and extrinsic pathways.

DISCUSSION

As naturally flavonoids, both galangin and quercetin are focused on their potential bioactivities including growth inhibition and/or apoptosis induction (Zhang *et al.*, 2008; Lee *et al.*, 2010). Sharing similarity to the past studies, the present study also found that the two flavonoids had *in vitro* cytotoxicity towards the SGC-7901 cells, and galangin had greater cytotoxicity than quercetin (fig. 1). Cytotoxicity of flavonoids has been postulated to be compound-type-specific (Kuntz *et al.*, 1999). Two

previous results have indicated that the investigated flavonoids exhibited different *in vitro* growth inhibition on esophageal squamous OE33 cells (Zhang *et al.*, 2008), and galangin was more powerful than other three flavonoids (Wang *et al.*, 2011). These results pointed out a fact that growth inhibition of flavonoids on cancer cells is dependent on both chemical nature of the flavonoids and the cell line investigated.

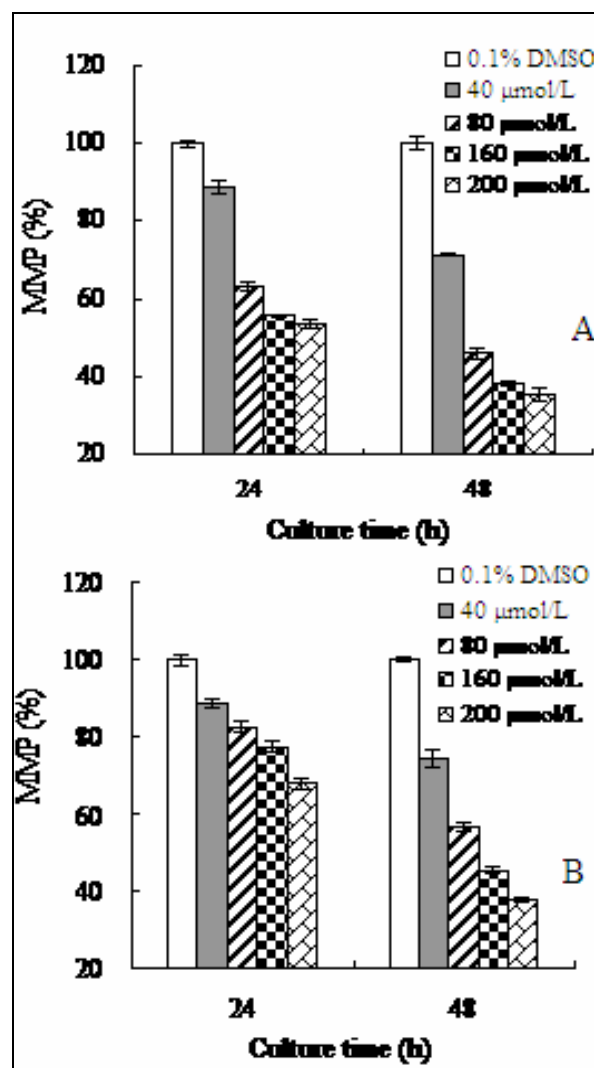


Fig. 3: Xu, Wang & Zhao

Growth inhibition of flavonoids on mammalian cells might be mediated via apoptosis induction. Some studies have reported apoptosis induction of galangin and quercetin on several cancer cells. If human gastric cancer BGC-823 cells were treated with quercetin at 120μmol/L for 48 hours, the ratio of apoptotic cells was enhanced to 35% (Wang *et al.* 2012). When human leukemia HL-60 cells were treated with quercetin at 100μmol/L for 48 hours, the proportion of apoptosis cells was about 59.97% (Niu *et al.*, 2011). Galangin was capable of inducing apoptosis in human hepatocellular carcinoma cells (Zhang *et al.* 2010). Treating of human gastric cancer SNU-484

cells with galangin at 100 μ mol/L, typical apoptosis was detected (Kim *et al.* 2012). In the present study, treatment

and Bad), resulting in mitochondria-mediated apoptosis in human glioblastoma GBM 8401 and U87 cells (Tsai *et al.*,

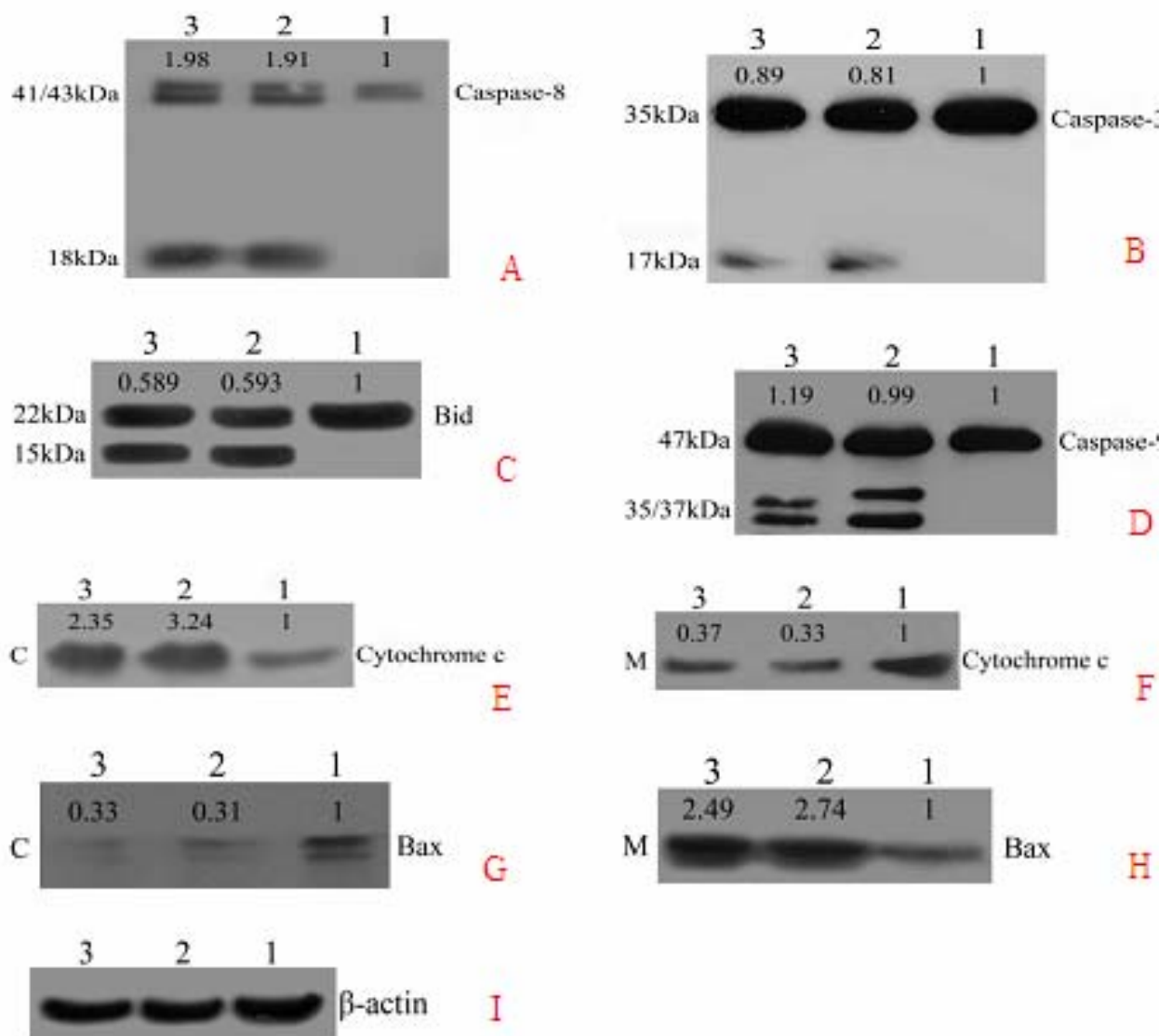


Fig. 4: Xu, Wang & Zhao

of the SGC-7901 cells with galangin and quercetin significantly increased the proportion of apoptotic cells (fig. 2), sharing a consistence to these reported studies.

Two major signaling pathways involve in caspase-mediated apoptosis, the death receptor (extrinsic) and the mitochondrial (intrinsic) pathways, mediated distinctively by cleaving (*i.e.* activating) caspase-8 and caspase-9, respectively (Fan *et al.*, 2005; Gupta, 2003). Based on the present results (figs. 3 and 4), both galangin and quercetin were evidenced to mediate both extrinsic and intrinsic pathways of the apoptosis of the SGC-7901 cells, and Bid integrated the two pathways. Similar results have been found by other researchers. Some examples are listed here to give supports to the present results. Luteolin increases the expression of pro-apoptotic proteins (Bid, Bak, Bax

2013). The flavonoids isolated from stem bark of *Erythrina suberosa* induce apoptosis of human leukemia HL-60 cells by Bax-mediated mitochondrial pathway (Kumar *et al.*, 2013). Caspase-8 cleavage (aspase-8 activation) activates Bid (Li *et al.*, 1998). The active form of Bid (tBid) thus translocates into mitochondria, and then triggers oligomerization of Bax, which translocates from cytosol into mitochondria (Roucou *et al.*, 2002). tBid induces mitochondrial membrane permeabilization (*i.e.* decreased MMP) and release of apoptotic molecules (*e.g.* cytochrome c) from mitochondria into cytosol via Bax channels (formed by the interaction between tBid and Bax) (Donovan and Cotter, 2004). Once cytochrome c is released, it associates with Apaf-1 to promote the activation of caspase-9, which thereof activates caspase-3 to execute the apoptosis of the cells (Donovan *et al.*,

2004). One important fact pointed out here is that Bid serves as a “bridge” between the two-apoptotic pathways. Both galangin and quercetin belong to flavones, and have the same chemical nature but different contents of hydroxyl groups. The present study evidenced that both galangin and quercetin had proliferative inhibition, and also were capable of inducing caspase-dependent apoptosis in human gastric cancer SGC-7901 cells. The apoptosis induction is suggested via mitochondrial pathway involving caspase-8/Bid/Bax activation. Other potential pathways remain unclear in the present time, and will be identified in future study.

CONCLUSION

Both galangin and quercetin exerted clear *in vitro* growth inhibition and apoptosis induction on the SGC-7901 cells, and galangin was more powerful than quercetin. By assaying the expression changes of the related genes and proteins in the treated cells, a molecular mechanism related to apoptosis induction of the two flavonoids was clarified, and was evidenced involving in activation of upstream caspase-8, cleavage of Bid, translocation of Bax, release of cytochrome c and activation of caspase-9 and caspase-3. That is, the two flavonoids induced apoptosis in the cells via a mitochondrial pathway by caspase-8/Bid/Bax activation.

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REFERENCES

- Alberts SR, Cervantes A and Van de Velde CJH (2003). Gastric cancer: epidemiology, pathology and treatment. *Ann. Oncol.*, **14**(Supplement 2): 31-36.
- Bi S, Liu JR, Li Y, Wang Q, Liu HK, Yan YG, Chen BQ and Sun WG (2010). γ -Tocotrienol modulates the paracrine secretion of VEGF induced by cobalt (II) chloride via ERK signaling pathway in gastric adenocarcinoma SGC-7901 cell line. *Toxicol.*, **274**: 27-33.
- Cao G, Sofic E and Prior RL (1997). Antioxidant and prooxidant behavior of flavonoids: Structure-activity relationships. *Free Radical Bio. Med.*, **22**: 749-760.
- Chen WS, Wang X, Zhuang JG, Zhang L and Lin Y (2007). Induction of death receptor 5 and suppression of survivin contribute to sensitization of TRAIL-induced cytotoxicity by quercetin in non-small cell lung cancer cells. *Carcinogenesis*, **28**: 2114-2121.
- Cheng S, Gao N, Zhang Z, Chen G, Budhraj A and Ke Z (2010). Quercetin induces tumor-selective apoptosis through down regulation of Mcl-1 and activation of Bax. *Clin. Cancer Res.*, **16**: 5679-5691.
- Donovan M and Cotter TG (2004). Control of mitochondrial integrity by Bcl-2 family members and caspase-independent cell death. *Biochim. Biophys. Acta.*, **1644**: 133-147.
- Fan TJ, Han LH, Cong RS and Liang J (2005). Caspase family proteases and apoptosis. *Acta. Bioch. Biophys. Sinica.*, **37**: 719-727.
- Granado-Serrano AB, Martín MA, Bravo L, Goya L and Ramos S (2006). Quercetin induces apoptosis via caspase activation, regulation of Bcl-2 and inhibition of PI-3-kinase/Akt and ERK pathways in a human hepatoma cell line (HepG2). *J. Nutr.*, **136**: 2715-2721.
- Gupta S (2003). Molecular signaling in death receptor and mitochondrial pathways of apoptosis (Review). *Int. J. Oncol.*, **22**: 15-20.
- Heo MY, Sohn SJ and Au WW (2001). Anti-genotoxicity of galangin as a cancer chemo preventive agent candidate. *Mutat. Res. Rev. Mutat. Res.*, **488**: 135-150.
- Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D (2011). Global cancer statistics. *CA: Cancer J. Clin.*, **61**: 69-90.
- Kim DA, Jeon YK and Nam MJ (2012). Galangin induces apoptosis in gastric cancer cells via regulation of ubiquitin carboxy-terminal hydrolase isozyme L1 and glutathione S-transferase P. *Food Chem. Toxicol.*, **50**: 684-688.
- Kumar S, Pathania AS, Saxena AK, Vishwakarma RA, Ali A and Bhushan SS (2013). The anticancer potential of flavonoids isolated from the stem bark of *Erythrina suberosa* through induction of apoptosis and inhibition of STAT signaling pathway in human leukemia HL-60 cells. *Chem. Biol. Interact.*, **205**: 128-137.
- Kuntz S, Wenzel U and Daniel H (1999). Comparative analysis of the effects of flavonoids on proliferation, cytotoxicity and apoptosis in human colon cancer cell lines. *Eur. J. Nutr.*, **38**: 133-142.
- Laskar RA, Sk I, Roy N and Begum NA (2010). Antioxidant activity of Indian propolis and its chemical constituents. *Food Chem.*, **122**: 233-237.
- Lee YK, Hwang JT, Kwon DY, Surh YJ and Park OJ (2010). Induction of apoptosis by quercetin is mediated through AMPK α 1/ASK1/p38 pathway. *Cancer Lett.*, **292**: 228-236.
- Li H, Zhu H, Xu CJ and Yuan J (1998). Cleavage of BID by caspase-8 mediates the mitochondrial damage in the Fas pathway of apoptosis. *Cell*, **94**: 491-501.
- Li T, Zhu J, Guo L, Shi XL, Liu YF and Yang XB (2013). Differential effects of polyphenols-enriched extracts from hawthorn fruit peels and flesh on cell cycle and apoptosis in human MCF-7 breast carcinoma cells. *Food Chem.*, **141**: 1008-1018.
- Livak KJ and Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. *Methods*, **25**: 402-408.

- Moon YJ, Wang X and Morris ME (2006). Dietary flavonoids: Effects on xenobiotic and carcinogen metabolism. *Toxicol. in Vitro.*, **20**: 187-210.
- Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods*, **65**: 55-63.
- Namvar F, Mohamed S, Fard SG, Behravan J, Mustapha NM, Alitheen NBM and Othman F (2012). Polyphenol-rich seaweed (*Eucheuma cottonii*) extract suppresses breast tumour via hormone modulation and apoptosis induction. *Food Chem.*, **130**: 376-382.
- Nguyen TTT, Tran E, Nguyen TH, Do PT, Huynh TH and Huynh H (2004). The role of activated MEK-ERK pathway in quercetin-induced growth inhibition and apoptosis in A549 lung cancer cells. *Carcinogenesis*, **25**: 647-659.
- Niu GM, Yin SM, Xie SF, Li YQ, Nie D, Ma LP, Wang X and Wu Y (2011). Quercetin induces apoptosis by activating caspase-3 and regulating Bcl-2 and cyclooxygenase-2 pathways in human HL-60 cells. *Acta. Biochim. Biophys. Sinica.*, **43**: 30-37.
- Psahoulia FH, Drosopoulos KG, Doubravska L, Andera L and Pintzas A (2007). Quercetin enhances TRAIL-mediated apoptosis in colon cancer cells by inducing the accumulation of death receptors in lipid rafts. *Mol. Cancer Ther.*, **6**: 2591-2599.
- Riboli E and Norat T (2003). Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk 1-4. *Am. J. Clin. Nutr.*, **78**: 559S-569S.
- Roucou X, Montessuit S, Antonsson B and Martinou JC (2002). Bax oligomerization in mitochondrial membranes requires tBid (caspase-8-cleaved Bid) and a mitochondrial protein. *Biochem. J.*, **368**: 915-921.
- Sakulnarmrat K, Fenech M, Thomas P and Konczak I (2013). Cytoprotective and pro-apoptotic activities of native Australian herbs polyphenolic-rich extracts. *Food Chem.*, **136**: 9-17.
- Tsai YD, Chen HJ, Hsu HF, Lu K, Liang CL, Liliang PC, Wang KW, Wang, HK, Wang CP and Hwang JY (2013). Luteolin inhibits proliferation of human glioblastoma cells via induction of cell cycle arrest and apoptosis. *J. Taiwan Ins. Chem. Eng.*, **44**: 837-845.
- Vermes I, Haanen C, Steffens-Nakken H and Reutellingsperger C (1995). A novel assay for apoptosis Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. *J. Immunol. Methods*, **184**: 39-51.
- Wach A, Pyrzyńska K and Biesaga M (2007). Quercetin content in some food and herbal samples. *Food Chem.*, **100**: 699-704.
- Wang P, Zhang K, Zhang Q, Mei J, Chen CJ, Feng ZZ and Yu DH (2012). Effects of quercetin on the apoptosis of the human gastric carcinoma cells. *Toxicol. in Vitro.*, **26**: 221-228.
- Wang, ZJ, Zhang Q, Li TJ and Zhao XH (2011). A possible molecular mechanism of two flavones and two flavonols on the induction of differentiation in a human oesophageal adenocarcinoma cell line (OE33). *J. Med. Plants Res.*, **5**: 2652-2664.
- Xiang M, Qian ZY, Zhou CH, Liu J and Li WN (2006). Crocetin inhibits leukocyte adherence to vascular endothelial cells induced by AGEs. *J. Ethnopharmacol.*, **107**: 25-31.
- Zhang HT, Luo H, Wu J, Lan LB, Fan DH, Zhu KD, Chen ZY, Wen M and Liu HM (2010). Galangin induces apoptosis of hepatocellular carcinoma cells via the mitochondrial pathway. *World J. Gastroenterol.*, **16**: 3377-3384.
- Zhang Q, Zhao XH and Wang ZJ (2008). Flavones and flavonols exert cytotoxic effects on a human oesophageal adenocarcinoma cell line (OE33) by causing G2/M arrest and inducing apoptosis. *Food Chem. Toxicol.*, **46**: 2042-2053.