RESEARCH ARTICLE

Inhibitory Effect of Ginseng on Breast Cancer Cell Line Growth Via Up-Regulation of Cyclin Dependent Kinase Inhibitor, p21 and p53

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Abstract

Objective: Breast cancer is global female health problem worldwide. Most of the currently used agents for breast cancer treatment have toxic side-effects. Ginseng root, an oriental medicine, has many health benefits and may exhibit direct anti-cancer properties. This study was performed to assess the effects of ginseng on breast cancer cell lines. **Materials and Methods:** Cytotoxicity of ginseng extract was measured by MTT assay after exposure of MDA-MB-231, MCF-10A and MCF-7 breast cancer cells to concentrations of 0.25, 0.5, 1, 1.5, 2 and 2.5 mg/well. Expression levels of p21WAF, p16INK4A, Bcl-2, Bax and P53 genes were analyzed by quantitative real time PCR. **Results:** The treatment resulted in inhibition of cell proliferation in a dose-and time-dependent manner. p53, p21WAF1and p16INK4A expression levels were up-regulated in ginseng treated MDA-MB-231 and MCF-7 cancer cells compared to untreated controls and in MCF-10A cells. The expression levels of Bcl2 in the MDA-MB-231 and MCF-7 cells were down-regulated. In contrast, that of Bax was significantly up-regulated. **Conclusion:** The results of this study revealed that ginseng may inhibit breast cancer cell growth by activation of the apoptotic pathway.

Keywords: Ginseng- MDA-231- MTT assay- gene expression- quantitative real time PCR

Asian Pac J Cancer Prev, **17 (11)**, 4965-4971

Introduction

Breast cancer is a worldwide health problem causing morbidity and mortality (Torre et al., 2015). Its incidence is raising all over the world as a result of changes in the dietary habits (Gonzalez and Riboli, 2010).Approximately 70% of breast cancers patients are estrogen receptor (ER) and/or progesterone (PR), human epidermal growth factor receptor-2 (HER2) positive and are typically responsive to hormonal therapies (Wu et al., 2015). However, about 15–20% of breast cancer cases, lack expression of these three molecules in their tumor cell and classified as triple negative breast cancers (TNBCs). TNBCs are often highly proliferative, poorly differentiated and aggressive subtypes of breast cancers (Brenton et al., 2005; Bauer et al., 2007). No effective therapies are for the TNBC treatment, and the systemic treatments are limited due to cytotoxic chemotherapy. This subtype of cancer cannot be treated with endocrine or anti-HER2 targeted therapies (Santana-Davila and Perez, 2010; Pal et al., 2011).

The natural products are sources for cancer treatment discoveries due to their great chemical diversity (Mann, 2002; Newman et al., 2002; Demain and Vaishnav, 2011). Analysis of some chemotherapeutic agents indicates that nearly 60% of approved drugs are derived from natural compounds. The importance of plant-derived drugs in cancer treatments was observed in different types of cancer (He et al., 2011).

Several species of genus Panax, such as Panax ginseng, are important for different medical conditions. Ginseng, Panax ginseng, has health benefits and may exhibit direct anti-cancer properties (Chang et al., 2003; King and Murphy, 2010; He et al., 2011). The major active components of ginseng are ginsenosides (such as Rg3, Rh2, Rg5, Rk1 and Rp1). Ginsenosides exhibit a broad range of biological activities, including anticancer, anti-proliferative and anti-angiogenic activities in vitro and in vivo (Kim et al., 2004; He et al., 2011). Some reports showed that Rg3, Rh2 and Rk1 can inhibit the NF-κB signaling pathway and cell proliferation, and to induce apoptosis in cancer cell lines (Kim et al., 2004; Wang et al., 2009; Zheng et al., 2013; Lee et al., 2014). However, ginsenosides mechanism of actions may be diverse and remain largely undefined.

Ginseng and ginsenoisdes exhibit multiple pharmacological activities via different mechanisms in vitro, in vivo, and clinical models (Ferguson et al., 2015). Therefore, scientists keep seeking for new naturally-product with no or the least toxicity to normal tissues or to reduce the chemotherapy dosage with better

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antitumor effects (Raina and Agarwal, 2007; Vibet et al., 2008).

Somewhat the mechanisms associated with apoptosis have been explained and still need more studies especially in cancer cells. P53, p21WAF1 , Bcl-2 and Bax are some genes that are involved in apoptosis (Chiarugi et al., 1994). P53 is a tumor suppressor gene that regulates the cell cycle by making cell cycle arrest to permit DNA repair or apoptosis (Polyak et al., 1997). p21WAF1 is activated by the p53 protein, and the increased level of p21WAF1is associated with decreasing cyclin-dependent activity in damaged cells (el-Deiry et al., 1994). Bcl-2 is a suppressor of apoptosis that is triggered by a variety of signals. Bax expression is speed up apoptosis (Gai et al., 2015; Naseri et al., 2015). In an attempt to find compounds that could have anti-cancer effect with no toxicity, the current study was designed to study the effect of ginseng on breast cancer cell line.

Materials and Methods

Ginseng and Trypan blue solution were purchased from Sigma Aldrich (Sigma Aldrich, USA). SYBR® Green PCR Master Mix kit was purchased from Applied Biosystems (Life Technologies, Grand Island, NY, USA). The primers were designed using Primer Express 3.0 software (Applied Biosystem, Life Technologies, Grand Island, NY, USA) and were synthesized by Metabion Company (Metabion international AG, Germany).

Cell culture

MDA-MB-231, MCF-7 (human breast adenocarcinoma cell line) and MCF-10A non-tumorigenic epithelial cell line were a generous gift from college of science, King Saud University and were cultured in DMEM/F12 medium (Gibco, Life Technology, USA) supplemented with 10% heat-inactivate fetal bovine serum (Gibco, Life Technology, USA), penicillin/streptomycin (10,000 U/mL penicillin and 10,000 µg/mL streptomycin; Invitrogen). All cultures were maintained at 37oC in a humidified 5% CO2 atmosphere. The cells were seeded at a density of 104 cell/well in a ninety-six well culture dish. After 24 h, the cells were treated with 0, 0.25 mg/well, 0.5 mg/ well, 1 mg/well, 1.5 mg/well, 2 mg/well and 2.5 mg/well of ginseng. After incubation periods of 24-h, 48-h and 72-h the cells were harvested and were treated with diluted trypsin-EDTA (Gibco Life Technology, USA) to obtain the cells for molecular studies.

Cytotoxicity assay

Trypan Blue solution: Trypan Blue is an essential dye, use in estimating number of viable cells present in a population (Kumar et al., 2015). In brief, cell suspension in a fixed volume of cells was made (e.g. 1ml). Cell suspension of 50ul were taken and were mixed with an equal volume of trypan blue (final concentration 0.4%). The solution was mixed well then was transferred to a hemocytometer for counting (in<5 minutes). Calculate the number of cells per ml, and the total number of cells, using the following formula: % viability = (live cell count/ total cell count) *100

MTT assay: The cytotoxicity was determined by quantifying the relative cell number using MTT assay as described previously (Wang et al., 2006). Briefly, 100 µl aliquots of the cell suspension were transferred to 96-well micro-plates (1x 105cells/well) and incubated for 24 hours at 37ºC in a humidified 5% CO2 atmosphere. Ginseng extracts were dissolved in phosphate-buffered saline, and the final concentrations were adjusted to 0.25, 0.5, 1, 1.5, 2 and 2.5 mg/well. Cells were incubated with the extracts for 24 hours at 37ºC. At the end of the incubation, MTT (5 mg/mL in phosphate-buffered saline) was added, and the plate was incubated for an additional 4 hours. The supernatant was then aspirated, then to dissolve the colored formazan crystals produced from the reaction of cells with MTT, 200 µL of dimethyl sulfoxide (DMSO) was added to each well. Optical density values were then measured using a microplate reader at 570 nm. All conditions were performed in triplicate and the concentration of ginseng that is lethal to 50% of cells (LC50) was calculated.

Detection of apoptosis

Nucleosomal DNA fragmentation analysis

Apoptotic DNA fragments were isolated according to the previously described method (Herrmann et al., 1994). Briefly, cells were plated at 106 in 6 wells plates and were treated with 0.25, 0.5, 1, 1.5, 2 and 2.5 mg/well ginseng. After 24 hours, attached and floating cells were harvested, washed with PBS and pelleted by centrifugation. The cell pellets were then treated for 10 seconds with lysis buffer (1% NP-40 in 20 mM EDTA, 50 mM Tris-HCl, and pH 7.5). After centrifugation for 5 minutes at 1,600 g, the supernatant was collected and the extraction was repeated with the same amount of lysis buffer. The supernatants were brought to 1% SDS and treated for 2 hours with RNaseA (final concentration 5 mg/ml) at 56°C followed by digestion with proteinase K (final concentration 2.5 mg/ml) for 2 hours at 37°C. After addition of 1/2 vol. 10 M ammonium acetate, the DNA was precipitated with 2.5 vol. ethanol, after centrifugation the DNA was dissolved in TE buffer (10mMTris-HCl and 1mM EDTA), and separated by electrophoresis in 2% agarose gels.

Total RNA isolation and quantitative real-time PCR

To determine the ginseng effect on genes expression levels the quantitative real-time polymerase chain reaction (qRT-PCR) assay was conducted: MDA-MB-231, MCF-7 and MCF-10A cell lines were treated with ginseng extract (1.5 mg- 2.5 mg) for 48 hours as these doses and time were chosen depending on the cytotoxicity results. The total RNA was obtained from cells using TRIzol reagent according to the standard protocol as previously described (Chomczynski, 1993). The extracted RNA has 260/280 ratio of 1.9-2.1. First-strand cDNA was synthesized from 1µg total RNA by reverse transcription with a SuperScript™ first-strand synthesis system kit (Invitrogen, CA, USA), using oligo (dt) according to the manufacturer's instructions, the quantity was characterized using a UV spectrophotometer (NanoDrop 8000, Thermo Scientific, USA).

Real time quantitative PCR was performed to detect the gene expression levels of p21WAF1, p16INK4A,Bcl-2,

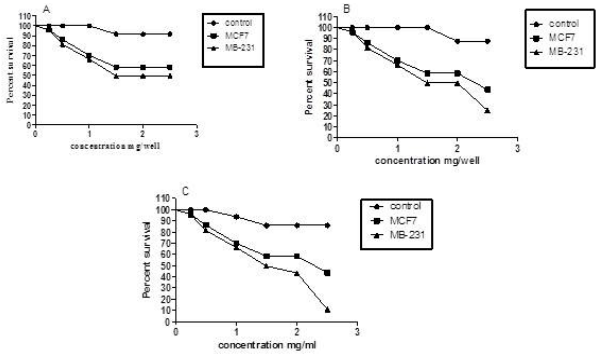

Figure 1. The Cytotoxic Effects of Ginseng Extract on Different Breast Cancer Cells. Exponentially growing cells were cultured in 96-well plates and were treated with ginseng extract for 24 h (A), 48 h (B) and 72 h (C). Cell viability was determined by MTT assay. %cell survival= {(Absorbance value of treated cells- Absorbance value of blank)/ (Absorbance value of untreated cells - Absorbance value of blank)} x100.

Bax and P53 by using SYBR Green master mix (Applied Biosystems, CA, USA) and the reaction was performed on ABI PRISM 7500 Detection System (Applied Biosystems, USA). The program was set to run for one cycle at 95°C for 2 min, followed by 40 cycles at 95°C for 15s and at 60°C for 1min. The specificity of PCR amplification was confirmed by agarose gel electrophoresis and melting curve analysis. glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as internal control for qRT-PCR. The primers sequences were listed in table 1 (Phalke et al., 2012). Expressions were normalized to GAPDH. Each sample was analyzed in triplicate, and representative data sets are shown. Results of gene expression were analyzed using 2−ΔΔCT method. Data were expressed as the mean fold changes ± SEM for three independent amplifications.

Statistical Analyses

Data were expressed as mean ± standard error (SEM) of the mean of n observations. All experiments were repeated at least three times, and all data were compiled from a minimum of triplicate experiments. Significant differences between treated and untreated cells lines and between each other were analyzed by two ways ANOVA. A P value<0.05 was accepted as a significant difference between each pair of compared groups."

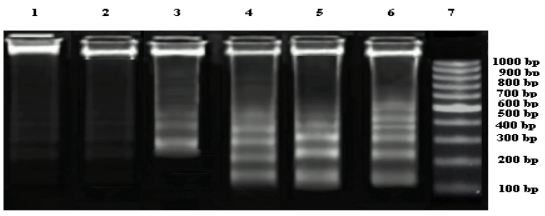

Figure 2. DNA Fragmentation Induced by Ginseng Extract in Breast Cancer Cell Lines. The extracted DNA was run on 2% agarose gel and the image was documented using Syngene bio imaging system. Lane 1: un-treated cells, lane 2 treated MCF-10A, lane3&4 MCF-7 treated with ginseng extract at 2&2.5 mg/well respectively and lane 5&6 MDA-MB-231 treated with ginseng extract at 2 and 2.5 mg/well, respectively and lane 7: 1 kb DNA ladder

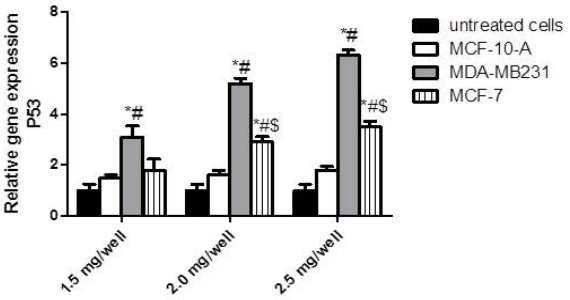

Figure 3. The Effect of Ginseng Extract on Centration at 48 Hours on the P53 Gene Expression in MCF-10A, MDA-MB-231 and MCF7 Cell Line. Data were presented as mean ± SEM. *, # and $ indicate a significant change from untreated cells, MCF-10A and MDA-MB-231, respectively, at $P < 0.05$ using two-way ANOVA test

Results

To find the scientific basis for using ginseng extract as therapeutic agent against breast cancer, this study investigated the inhibitory mechanism for ginseng on breast cancer cell lines.

Cytotoxic activities of ginseng

To test the effect of ginseng extract on the apoptotic pathway in different breast cancer cell lines, first the anti-proliferative effects of ginseng extract were assessed by MTT on breast cancer cell lines cultured with increasing concentrations of ginseng extract for periods of 24, 48, or 72 hours. Breast cancer cells were seeded in triplicates into 96 well plates and treated with different concentrations of ginseng extract (0.5, 1, 1.5, 2 and 2.5 mg/well) for 24 h ,48h and 72 h and viable cells were measured by MTT assay. Ginseng extract caused a time-dependent and dose-dependent reduction in cell viability in MDA-MB-231

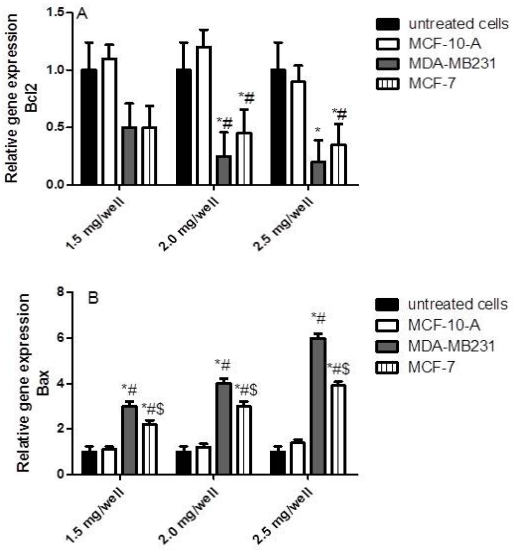

Figure 4. The Effect of Different Concentration of Ginseng Extract at 48 hours on the BCl2 (A) and Bax (B) Genes Expression in MCF-10A, MDA-MB-231 and MCF7 Cell Line. Data were presented as mean ± SEM. *, # and $ indicate a significant change from untreated, MCF-10A and MDA-MB-231, respectively, at $P < 0.05$ using two-way ANOVA test

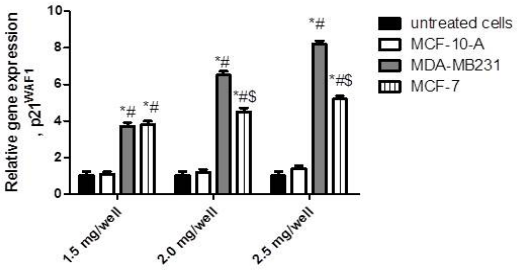

Figure 5. The Effect of Different Concentration of Ginseng Extract at 48 Hours on the P21WAF1 Genes Expression in MCF-10A, MDA-MB-231 and MCF7 Cell Line. Data were presented as mean ± SEM. *,#and $ indicate a significant change from untreated, MCF-10A and MDA-MB-231, respectively, at P < 0.05 using two way ANOVA test

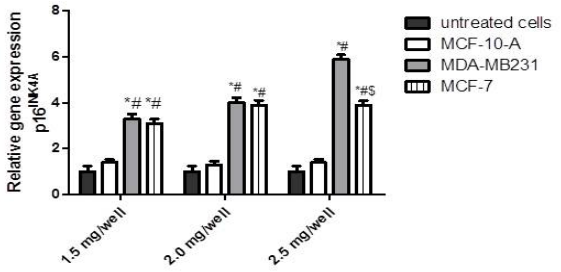

Figure 6. The Effect of Different Concentration of Ginseng Extract at 48 Hours on the P16 Genes Expression in MCF-10A, MDA-MB-231 and MCF7 Cell Line. Data were presented as mean ± SEM. *, # and $ indicate a significant change from untreated, MCF-10A and MDA-MB-231, respectively, at P < 0.05 using two way ANOVA test.

and MCF-7 cells (Figure 1). An evident inhibition in cell viability was observed as early as 24 hours in both cell lines, and LC50 was achieved after 48 hours of incubation. The effective concentrations were between 1.5-2.5 mg/well and time was at 48h and these were selected for all the further molecular studies.

DNA fragmentation

DNA fragmentation is a key feature of programmed cell death, toxicity and certain stages of necrosis. The percentage of DNA fragmentation was more pronounced at high concentration than in low concentration of ginseng extract (Figure 2). The presence of DNA cleavage bands in ginseng treated cells indicated the cytotoxic effects of ginseng on MDA-MB-231 and MCF-7 cells. Moreover, MDA-MB-231 cells were more sensitive to damage than MCF-7.

The effect of ginseng on apoptotic pathway

The inhibition of cell proliferation could be the result of the induction of apoptosis. Therefore, the current study investigated the alterations in genes expressions involved in the apoptotic pathways in respond ginseng extract treatment.

The expression of p53: The effect of ginseng extract on P53 expression levels at 48 hours was showed in figure 3. In MCF-10-A cells, there was no significant difference observed in P53 expression levels in correlation to different concentrations (P>0.05). In treated MDA-MB-231 cells a significant different was observed at concentrations 1.5, 2 and 2.5 mg/well compared to MCF-10-A and untreated cells (P<0.05). MCF-7 cells showed a significant difference at 2.0 and 2.5 mg/well compared to untreated cells and treated MCF10-A (P<0.05). At high concentrations 2.0 and 2.5 mg/well a highly significant difference was observed in P53 expression levels in MDA-MB-231 compared to MCF-7 cell lines (P<0.001).

The Bcl2 and Bax expression

The effect of ginseng extract concentrations on Bcl2 expression levels at 48 hours was showed in figure 4A. There was no significant difference observed in Bcl2 expression levels in treated and untreated MCF-10A with different concentration of ginseng extract (P>0.05). The expression levels of Bcl2 in MDA-MB-231 and MCF-7 cells were significantly suppressed by 0.25, 0.2 folds respectively in treated cell with 2 mg/well and by 0.45 and 0.35 fold respectively in treated cells with 2.5 mg/well compared to untreated cells. Also significant down-regulation in Bcl2 expression level was observed in MDA-MB-231 and MCF-7 at 2.0 and 2.5 mg/well concentrations of ginseng extract compared to MCF-10A cell line. Figure 4B showed the effect of different concentration of ginseng on the expression level of Bax at 48 hours. The expression levels of Bax were significantly increased by 2.1, 3, 4 and 6 folds compared to untreated cells (P<0.001). The expression levels of Bax in MDA-MB-231 and MCF-7 was significant increase by dose of 1.5, 2.0 and 2.5 mg compared to MCF-10 A treated cells, whereas, in MDA-MB-231 Bax was significantly over-expression compared to MCF-7 cell lines.

The expression of p21WAF1

The effect of ginseng extract concentrations on the p21WAF1 expression levels at 48 hours was showed in Figure 5. There was no significant difference observed in p21WAF1expression levels in treated and untreated MCF-10A with different concentration of ginseng

Table 1. The Primers Sequences

Gene name	Forward	Reverse
p21WAF1	5'-GGC CTG GAC TGT TTT CTC TCG-3'	5'-GAG AAA CGG GAA CCA GGA CAC-3'
p16INK4A	5'-GAG CAG CAT GGA GCC TTC-3'	5'-CCT CCG ACC GTA ACT ATT CG-3'
p53	5'-GCT GCT CAG ATA GCG ATG GTC T-3'	5'-CAT CCA AAT ACT CCA CAC GCAA-3'
Bcl2	5'-GAAGGTTTCCTCGTCCCTGG-3'	5'-CTGTGTTGAAACAGGCCACG-3'
Bax	5'-CCCCGATTCATCTACCCTGC-3'	5'-GAGCTAGGGTCAGAGGGTCA-3'
GAPDH	5'-GAA GGT GAA GGT CGG AGT C-3'	5'-GAA GAT GGT GAT GGG ATT TC-3'

extract (P>0.05). The expression levels of p21WAF1 in MDA-MB-231 and MCF-7 cells were significantly increased by3.7, 3.8 fold respectively in treated cell with 1.5 mg/well and by 6.5 and 4.5 fold respectively in treated cell with 2.0mg/well compared to untreated cells. The expression levels of p21WAF1 in MDA-MB-231 and MCF-7 cells were significantly increased by 8.2, 5.2 fold respectively in treated cell with 2.5 mg/well compared to both the untreated cells and MCF-10A cells (P<0.001.

The expression of p16INK4A

The expression levels of p16INK4A in MDA-MB-231, MCF-7 and MCF-10A cells were investigated in ginseng-treated and -untreated cells (Figure 6). There was a significant over-expression of p16INK4A in treated cells with 1.5, 2.0 and 2.5 mg ginseng for 48 h compared to untreated cells. The expression levels of p16INK4A was over-expressed by 4 and 5.9 folds in MDA-MB-231 and by 3.5 and 3.9 folds in MCF-7 cells compared to 1.3 and 1.4 folds in MCF-10A cells treated with ginseng at concentration 2.0 and 2.5 mg/well, respectively. The expression levels of p16INK4A in MDA-MB231 cells with 2.5 mg ginseng was significant increased compared to MCF-7.

Discussion

Breast cancer is the most common leading cause of death among women (Azevedo et al., 2015) due to aggressiveness and distant metastases. Breast cancer is a global health problem (Torre et al., 2015).Triple negative breast cancers (TNBCs) are an aggressive subtypes of breast cancers (Brenton et al., 2005; Bauer et al., 2007) with no effective targeted therapies and cannot be treated with endocrine or anti-HER2 targeted therapies (Santana-Davila and Perez, 2010; Pal et al., 2011). At the present time more attentions have been directed to natural products in cancer treatment. Natural products showed several anticancer effects (Mann, 2002; Newman et al., 2002; Demain and Vaishnav, 2011). Several studies have shown the anticancer effect of ginseng against several type of cancers (Nishino et al., 2001; 2007; Lee et al., 2009; Saw et al., 2010; Jang et al., 2014; Kim and Kim, 2015; Sharma and Goyal, 2015; Wong et al., 2015). The present study investigates the effect of ginseng extract on breast cancer cell line.

The current study revealed that ginseng inhibits breast cancer cells growth in a dose-dependent manner. The decreased cell numbers in the treated groups may be due to apoptosis and/or growth inhibition.

The main target of anti-cancer agents is their ability to trigger cancer apoptosis. Several mechanisms associated with apoptosis have been explained but more studies especially in cancer cells still needed. P53, p21WAF1, Bcl-2 and Bax are involved in cell apoptosis (Chiarugi et al., 1994). P53, tumor suppressor gene, regulates the cell cycle via making cell cycle arrest to permit DNA repair or apoptosis (Polyak et al., 1997).In ginseng treated MDA-MB-231 and MCF7 cells, p53 expression levels was up-regulated compared to the untreated groups in dose- and time-dependent manner. Similar studies showed the apoptotic effect of some natural products via p53 expression levels regulation (Prasad et al., 1997; Liu and Zhang, 1998; Kralj et al., 2003; Kurata et al., 2008; Munagala et al., 2011; Al Dhaheri et al., 2013; Hassan et al., 2014). The effect of ginseng on cell growth inhibition and induction of apoptosis may be depend on the p53 status. The p53 up-regulation in treated group may be due to the possible anti-tumorigenic effect of ginseng. p53 can down-regulate Bcl-2 which protects cells from apoptosis (Sano et al., 1995; Kane et al., 1994).

Bax and Bcl-2 play major role in apoptosis under experimental conditions that promote cell death (Li et al., 1999).Bcl-2 is apoptosis suppressor but Bax expression is speed up apoptosis (Gai et al., 2015; Naseri et al., 2015). In the current study, Bcl2 expression levels were down-regulated in treated cancer cell lines in dose- and time-dependent manner. Conversely, Bax expression was significantly up-regulated with dose and time-dependent manner. These data suggest that the stimulation of Bax is fast, and the effect is reduced at 72-h cells via apoptosis. Similar studies showed that increased expression of Bax can induce apoptosis, and others showed that Bcl-2 defends cells from apoptosis (Brambilla et al., 1996; Farid et al., 2001; Thees et al., 2005; Teijido and Dejean, 2010; Naseri et al., 2015).

The ratio of Bax:Bcl-2 is essential in apoptosis (Thees et al., 2005; Naseri et al., 2015).This study showed that the ratios of Bax:Bcl-2 expression were overexpressed then gradually decreased in time-dependent in treated cells with 2 and 2.5 mg/well. Bax forms heterodimers with Bcl-2 (Li et al., 1999) and antagonizes the anti-apoptotic function of Bcl-2. The over-expression of Bax and suppression of Bcl-2 may be one mechanism through which ginseng induces apoptosis.

An important stage in cancer is the movement of cells from G0 to enter cell cycle (Nurse et al., 1998; Szallasi and Liang, 1998). p21WAF1, tumor suppressor gene, has apoptotic, growth inhibition and effect the cell-cycle checkpoint (Grana and Reddy, 1995). Induction of p21 expression has been linked to growth inhibition by p53 (el-Deiry et al., 1993). p21WAF1 is activated by the p53 protein, and an increased level of p21WAF1 is associated in cyclin-containing complexes with decreasing cyclin-dependent activity in damaged cells destined to apoptosis (el-Deiry et al., 1994). In the current study there was over-expression of p21WAF1 in treated MDA-MB-231 and MCF-7 cells with ginseng for 48 h. A non-significant increase in the expression was observed in MDA-MB-231 and MCF-7 cells compared to MCF-10A. The p21WAF1up-regulation was directly correlated with the inhibition of cell growth. Similar study found that p21WAF1 up-regulation may be one mechanism through which ginseng inhibits cancer cell growth and induces apoptosis (Duda et al., 2001).

p16INK4A is independent cyclin-dependent kinase inhibitor encoded by the CDKN2A gene and is involved in anti-cancer processes (Al-Khalaf and Aboussekhra, 2013). p16INK4A plays important roles in tumor suppression (Al-Mohanna et al., 2007; Li et al., 2011) and belongs to pRB cancer-related pathway. p16 is involved in cell cycle control and interact with CDK4

(Coleman et al., 1997). In the current study p16INK4A was overexpressed in ginseng treated MDA-MB-231 and MCF-7 cells in dose- and time-dependent manner. p16INK4A up-regulation expression was directly correlated with cell growth inhibition. Similar studied reported that p21 and p16 were down-regulated in various breast cancer-associated fibroblasts as compared to their adjacent counterparts (Al-Ansari et al., 2012). Similarly, the correlation between p16 and p21 expression was reported with cancer (Al-Khalaf and Aboussekhra, 2013).

In conclusion, this data demonstrated that ginseng has inhibitory effect on breast cancer cell growth via transcriptional up-regulation of cyclin dependent kinase inhibitor, p21 and p53.

Conflict of Interests

The authors declare that they have no conflict of interests.

Authors' Contribution

All authors have the same contribution. All authors read and approved the final manuscript.

Acknowledgments

Authors thank the Deanship of Scientific Research at KSU for funding this work through the research group project no. RGP-142.

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