Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Antitumor Effects and Mechanism of Protein from *Panax ginseng* C. A. Meyer on Human Breast Cancer Cell Line MCF-7

Xilin Wan[1,2], Xin Jin[3], Yuhe Ren[4], Yang Xiu[1], Yu Li[2], Changbao Chen[1], Shuying Liu[1]

[1]Key Laboratory of Ginseng Chemistry and Pharmacology of Jilin Province, Jilin Ginseng Academy, Changchun University of Traditional Chinese Medicine, [2]Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, [3]Key Laboratory of Molecular Epigenetics of the Ministry of Education (MOE), Northeast Normal University, [4]Institute of Special Animal and Plant Science, Chinese Academy of Agricultural Sciences, Changchun, Jilin, People's Republic of China

Submitted: 02-04-2019 Revised: 26-04-2019 Published: 19-09-2019

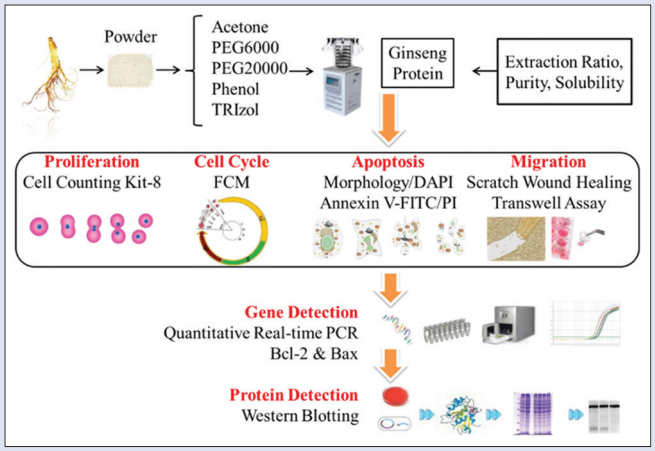
ABSTRACT

Context: *Panax ginseng* is well known for its various bioactivities, but specific knowledge on ginseng proteins (GPs) is limited. **Aims:** Protein components were extracted from ginseng and antitumor activity in human breast cancer cell line MCF-7 was investigated. **Settings and Design:** Five methods were applied to extract GP and the antitumor effects and mechanism of GP on MCF-7 were explored, including proliferation, cell cycle, apoptosis, and migration. **Subjects and Methods:** Five extraction methods were employed. MCF-7 cell proliferation was measured using a cell counting kit-8 with different GP concentrations (0, 0.25, 0.5, 1, 2, and 4 mg/mL) and half inhibitory concentration values were calculated. Cell cycle, morphology, and apoptosis were investigated using immunofluorescence staining and flow cytometry. Migration was probed by scratch wound healing and transwell assays. Quantitative-polymerase chain reaction and western blotting were performed to analyze apoptosis-associated gene/protein expression. **Statistical Analysis:** Experimental data were analyzed by Microsoft Excel and SPSS software. **Results:** Acetone extraction achieved the highest GP purity and yield. GP inhibited proliferation of MCF-7 in a time-dependent manner and induced cell cycle arrest at G1/S and apoptosis. Scratch wound healing and transwell assays showed that cell migration was also inhibited by GP and expression levels of Bcl-2 and Bax were affected. **Conclusion:** GP elicits antitumor activity by inhibiting cell proliferation and migration and inducing cell cycle arrest and apoptosis in MCF-7 cells and may act via the Bcl-2 and Bax apoptotic pathway.

Key words: Antitumor activity, bax, bcl-2, extraction, MCF-7, *Panax ginseng* protein

SUMMARY

- Acetone extraction was selected to prepare ginseng protein (GP) with high purity and activity
- GP affects the proliferation, cell cycle, apoptosis, and migration of MCF-7 breast cancer cells
- GP promotes apoptosis by regulating the expression of Bcl-2 and Bax in MCF-7 cells.



Abbreviations used: GP: Ginseng protein; CCK-8: Cell counting kit-8; FCM: Flow cytometry; q-PCR: Real-time fluorescence quantitative PCR.

Correspondence:

Prof. Shuying Liu,
Jilin Ginseng Academy, Changchun University of Chinese Medicine, Changchun 130117, Jilin, China.
E-mail: syliu@ciac.ac.cn
Prof. Changbao Chen,
Jilin Ginseng Academy, Changchun University of Chinese Medicine, Changchun 130117, Jilin, China.
E-mail: ccb2021@126.com

DOI: 10.4103/pm.pm_151_19

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

Breast cancer is a worldwide public health issue, currently the most common malignant tumor type and the leading cause of death in postmenopausal women, accounting for 23% of all cancer deaths.[1] Treatments for breast cancer treatment include chemotherapy, surgery, targeted, and radiotherapies. However, side effects are a problem, especially for chemotherapy. Therefore, finding natural bioactive components that can effectively prevent or diminish the occurrence and development of tumors and enhance the immune function of the body is a priority.

Panax ginseng C. A. Meyer, a popular traditional Chinese herbal medicine, is used widely across Asia. It exerts cancer-preventive effects that are not specific to any organ.[2] Ginseng contains saponins, polysaccharides, ginseng proteins (GPs), volatile oils, and many other biologically active ingredients that exert memory enhancement,[3] antitumor,[4] immunity,[5] and cardiovascular system effects.[6] However, there exist few studies on GP components and most have focused

Cite this article as: Wan X, Jin X, Ren Y, Xiu Y, Li Y, Chen C, *et al*. Antitumor effects and mechanism of protein from *Panax ginseng* C. A. Meyer on human breast cancer cell line MCF-7. Phcog Mag 2019;15:715-21.

on extraction and purification, rather than biological activity. GP has neuroprotective effects,[7] but antitumor activity has not been widely explored, especially for breast cancer. In the present study, we investigated the antitumor activity of GP in MCF-7 breast cancer cells and explored the associated mechanisms.

SUBJECTS AND METHODS

Materials and reagents

Fresh ginseng was purchased in Fusong County, Jilin Province, China. Dulbecco's Modified Eagle Medium cell culture medium (Sigma-Aldrich, USA), Newborn Calf Serum (NBS) (Zhejiang Tianhang Biotechnology Co., Ltd.), a cell counting kit-8 (CCK-8) (MedChemExpress, China), an Annexin V-FITC/PI double-staining cell apoptosis analysis kit (KeyGENE BioTECH, China) and a cell cycle and apoptosis analysis kit (Beyotime, China) were employed. Doxorubicin (1 μmol/L; Sigma-Aldrich, USA) was used as a standard drug. All other chemicals and reagents were of analytical grade and supplied by Sigma-Aldrich.

Preparation of crude ginseng protein

Ginseng was dried, crushed into a coarse powder using a mill and 6.0 g was mixed with 60 mL of 10 mmol/L Tris-HCl buffer (pH 7.4) and stirred for 18 h. Samples were centrifuged at 4000 g for 30 min and another 60 mL of 10 mmol/L Tris-HCl (pH 7.4) was added and stirring (extraction) was continued for 18 h. After centrifuging at 4000 g for 30 min, the supernatant was divided equally into three portions for acetone, polyethylene glycol (PEG) 6000, and PEG 20,000 precipitation methods. Phenol extraction and TRIzol extraction methods were also employed. Details of different extraction methods are provided in Supplementary Data. GP powder was stored at −80°C refrigerator. The following formulae were applied:

Ratio of extraction (%) = (Total protein quantity/weight of ginseng crude powder) × 100%

Purity (%) = (Total protein quantity/weight of protein powder) × 100%

Cell culture

MCF-7 and MDA-MB-231 human breast cancer cells and MCF10A normal breast epithelial cells were obtained from Shanghai cell bank, Chinese Academy of Sciences. MCF-7 and MDA-MB-231 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) medium supplemented with 10% NBS 100 μg/mL streptomycin, and 100 units/mL penicillin G. MCF10A cells were cultured in DMEM/Ham's-F12 with 5% serum, 0.5 mg/mL hydrocortisone, 10 mg/mL insulin, and 20 ng/mL epidermal growth factor.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and western blot analysis

For sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), 15% separating and 5% stacking gels were prepared and 20 μL samples were separated. Coomassie Brilliant Blue R250 staining was performed for 30 min, followed by destaining for 8 h.

For western blotting analysis, cells were collected and lysed with radioimmunoprecipitation assay buffer. Protein samples (~45 μg) were separated by 12% SDS-PAGE, transferred to a polyvinylidene fluoride (PVDF) membrane for 3 h and blocked with 5% bovine serum albumin in Tris-Buffered Saline Tween20 (TBST) for 1 h. Primary antibodies, anti-β-actin, anti-Bax, and anti-Bcl-2 (1:1000), were then incubated for 2 h at the room temperature, membranes were washed three times, probed with secondary antibodies, and an Enhanced chemiluminescence (ECL) kit was used for visualization with a Tanon-5200 imaging system (Tanon, China).

Cell proliferation analysis

The proliferation capacity of cells was investigated with a CCK-8 kit to explore the influence on the logarithmic phase of MCF-7 cells. The absorbance value with and without GP treatment was measured and inhibition rate was calculated using the following formula:

Inhibition rate (%) = $([A_{\text{without drug}} - A_{\text{with drugs}}])/[A_{\text{without drugs}} - A_{\text{blank control}}]) \times 100\%$

Cell apoptosis analysis

MCF-7 cells (4×10^5/well) were cultured for 24 h, and the medium was replaced with medicated medium containing 0–4 mg/mL GP. Follow-on experiments were conducted in accordance with the instructions supplied with the Annexin V Fluoresceine Isothiocyanate/ Propidium Iodide (Annexin V-FITC and PI) cell Apoptosis Analysis Kits.

Cell cycle analysis

Cells were cultured for 48 h and follow-on experiments were conducted in accordance with the instructions supplied with the Cell Cycle and Apoptosis Analysis Kit.

4',6-diamidino-2-phenylindole staining

Cells were cultured for 48 h, washed, methanol and acetic acid (1:1) was added and incubated for 15 min and phosphate-buffered saline containing 0.5% Triton-100 was added and incubated for 15 min, and 4',6-diamidino-2-phenylindole (DAPI) staining was performed for 5 min. Samples were observed and imaged under a light microscope.

Cell migration assays

For scratch wound healing assays, cells were scraped with crosses in the center of wells. After culturing, healing was assessed at different time points using a light microscope. For transwell assays, cells were seeded in 24-well plates (1.2×10^5/well) and cultured for 24 h. The culture medium was then switched to medium containing GP and culturing was continued for a further 48 h. After staining with crystal violet dissolved in dimethylsulfoxide , the absorbance value was measured using a microplate reader.

Quantitative-polymerase chain reaction analysis

Total RNA was extracted from each sample and used to prepare cDNA to measure gene expression by polymerase chain reaction (PCR) with primers as follows: Bcl-2, 5'-CGGTTCAGGTACTCAGTCATC-3' and 5'-CGGTGGGGTCATGTGTGTG-3'; Bax, 5'-CCTTT TCTACTTTGCCAGCAAAC-3' and 5'-GAGGCCGTCCCAACCAC-3'; β-actin, 5'-TGACGTGGACATCCGCAAAG-3' and 5'-CTGGAAGG TGGACAGCGAGG-3'.

Statistical analysis

All data are expressed as mean ± standard deviation, each experiment included at least three replicates, and all data were analyzed using Microsoft Excel (Microsoft Corporation, Redmond, Washington, dc, USA) and SPSS software (IBM Corp, Armonk, New York, USA). The significance of results was assessed using Student's *t*-tests and one-way analysis of variance (ANOVA). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the untreated group.

RESULTS

Efficiency and purity of ginseng protein extracted using different methods

To explore the purity of GP, five different extraction methods were employed: Acetone precipitation, PEG 6000 precipitation, PEG 20,000

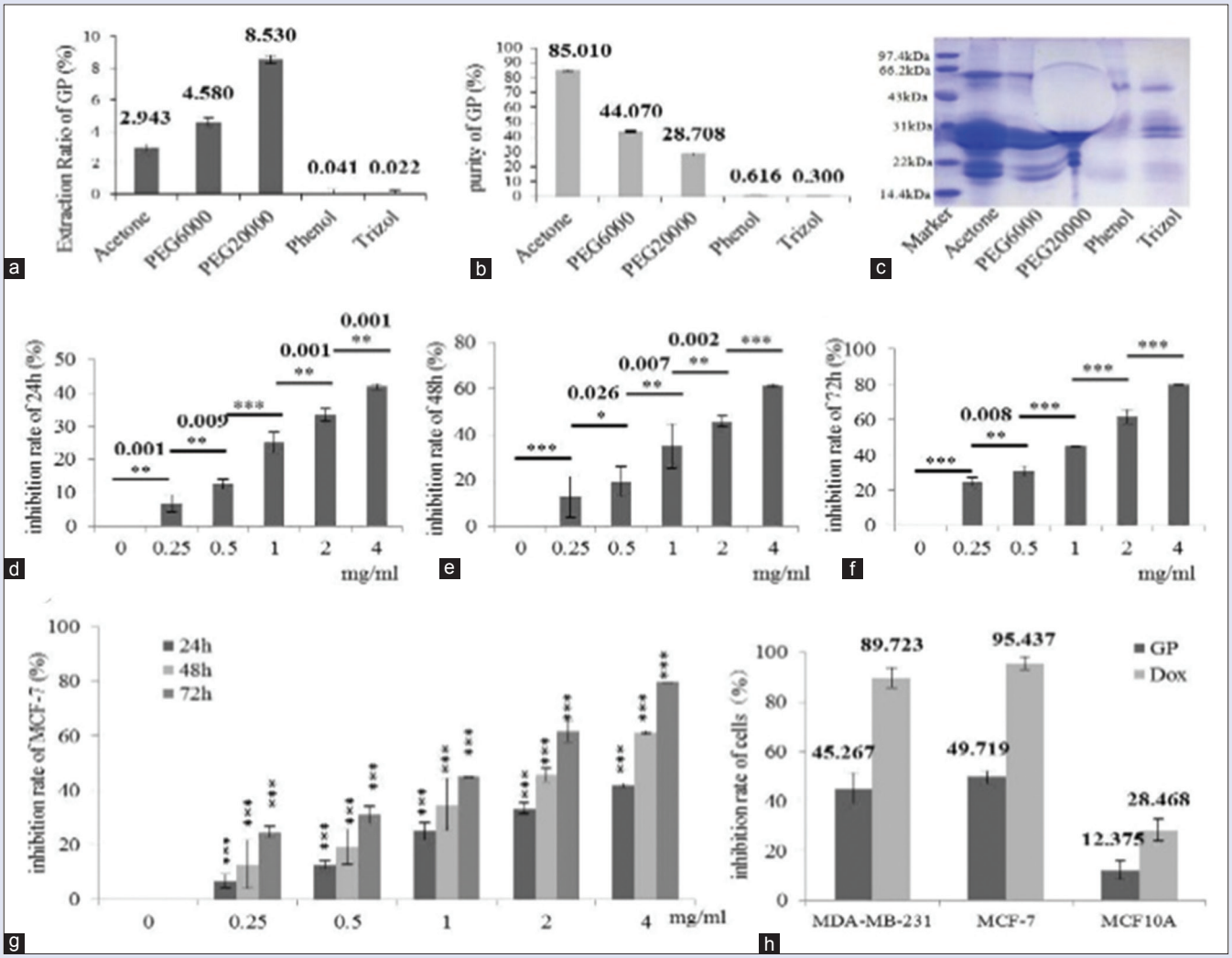


Figure 1: (a) Ginseng protein extraction ratio using five different extraction methods. (b) Ginseng protein extraction purity using five different extraction methods. (c) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of ginseng protein. (d-f) Inhibition rate in MCF-7 cells following ginseng protein treatment for 24–72 h. (g) Comparison of inhibition at different ginseng protein concentrations and different treatment durations. (h) Inhibition rate of ginseng protein on MDA-MB-231, MCF-7, and MCF10A cells after treatment with ginseng protein (half inhibitory concentration) and Dox (1 μmol/L) for 48 h. *T*-tests and one-way analysis of variance were used for statistical analysis. Data are expressed as means ± standard deviation of three independent experiments ($n = 5$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

precipitation, phenol extraction, and TRIzol extraction. The extraction ratio and purity of the different methods are presented in Figure 1a and b and the results of SDS-PAGE and Coomassie Brilliant Blue R250 staining are shown in Figure 1c. Extracted GPs mainly ranged between 14.4 and 66.2 kDa (64, 29, 27, 21, 18, and 15 kDa) and the most abundant proteins had molecular weights of 27–29 kDa. The PEG 20,000 method achieved the highest extraction ratio (8.530%), but we repeated SDS-PAGE at least five times and bands were deformed. Therefore, we concluded that the PEG 20,000 method may suffer from contamination and low purity. Thus, we selected the acetone method that achieved the highest purity (up to 85.010% purity) for extraction of GP in large quantities.

Inhibitory effect of ginseng protein on proliferation of MCF-7 cells

Many existing natural medicines inhibit the proliferation and migration of MCF-7 breast cancer cells, including *Larrea nitida* extracts,[8] berberine, and evodiamine.[9] To analyze the biological role of GP, we performed a series of activity tests using breast cancer cells. The inhibitory effect of GP on MCF-7 proliferation was measured by CCK-8

Table 1: Data of *n* inhibition ratio on proliferation on MCF-7 after treated by ginseng protein

Concentration (mg/ml)	Inhibition ratio (%)		
	24 h	48 h	72 h
Control	0.00	0.00	0.00
0.25	6.92±2.50**	12.88±8.90***	24.84±2.17***
0.5	12.71±1.55***	19.52±6.47***	31.01±3.07***
1	25.12±3.20***	34.81±9.66***	44.92±0.10***
2	33.50±1.98***	45.64±2.37***	61.75±4.01***
4	41.89±0.78***	61.29±0.50***	79.77±0.10***

Only 0.25 mg/ml treated at 24 h was $^{**}P<0.01$ consisted with controls other groups are $^{***}P<0.001$. *T*-tests and one-way ANOVA were used for statistical analysis. Data are expressed as means±SD of three independent experiments ($n=5$). SD: Standard deviation; ANOVA: Analysis of variance; MCF-7

assays [Figure 1d-g and Table 1]. The inhibition rate was significant for GP concentrations of 0.25, 0.5, 1, 2, and 4 mg/mL ($P < 0.05$). The inhibition rate gradually increased with increasing duration from 24, 48, and 72 h ($P < 0.01$ and $P < 0.001$) and was also dependent on concentration. The highest inhibition rate was 79.77% ±0.10% and half inhibitory concentration (IC_{50}) values at 24, 48, and 72 h were 5.207, 2.594, and 1.109 mg/mL, respectively.

Hence, we used the IC_{50} concentration (2.594 mg/mL) for follow-up experiments and an incubation time of 48 h. MCF-7 is an estrogen receptor (ER)-positive cell line and we also selected MDA-MB-231 (ER-negative) and MCF10A (normal) breast cell lines as controls. The results [Figure 1h] show that GP inhibited both MCF-7 and MDA-MB-231 breast cancer cell lines but had little inhibitory effect on the proliferation of MCF10A normal breast cells. Doxorubicin (1 μmol/L) was used as a positive control drug.

Effect of ginseng protein on morphological changes in MCF-7 cells

Next, we observed the cell status after treatment with different GP concentrations (0–4 mg/mL) for 48 h, and the results are shown in Figure 2a. Compared with the control group, the number of cells decreased significantly and the number of suspended cells increased gradually with increasing GP concentration. Changes in cell morphology and structure were apparent, with cell shrinkage, changes in direction, and occasional vacuolar cytoplasm. Nuclear morphological changes observed by DAPI staining are shown in Figure 2b. Nuclear chromatin was unevenly distributed, nuclei gathered toward the center, blue fluorescence was enhanced, and apoptotic bodies appeared.

Effects of ginseng protein on cell cycle distribution in MCF-7 cells

To investigate the factors affecting cell proliferation, cell cycle changes were analyzed by flow cytometry, and the results are shown in Figure 2c. At a GP concentration of 1–4 mg/mL, cells accumulated in the G0/G1 phase compared with the untreated group, from 38.37% to 41.64% initially and G0/G1 phase then decreased with a concomitant increase in S phase cells from 55.67% to 68.24% and G2 phase cells disappeared. These results suggested that GP mainly arrested MCF-7 cells at the G0/G1 phase.

Table 2: Data of ginseng protein effects on different phases of the MCF-7 cell cycle at 48 h

Concentration (mg/ml)	G0/G1 phase (%)	S phases (%)	G2 phases (%)
Control	44.04±4.56	44.04±4.76	8.58±0.97
2.594	55.92±4.80	43.10±5.28	0.98±0.78
P	0.040*	0.829	0.000***

T-tests and one-way ANOVA were used for statistical analysis. Data are expressed as means±SD of three independent experiments (n=3). *P<0.05; ***P<0.001. SD: Standard deviation; ANOVA: Analysis of variance; MCF-7

Table 3: Data of ginseng protein effects on different type of apoptosis cells after treated by ginseng protein

Concentration (mg/ml)	Q2-2 (%)	Q4-2 (%)	Q2-2 + Q4-2 (%)
Control	1.60±0.26	2.53±1.59	4.13±1.60
2.594	0.8±0.26	71.73±9.29	72.53±9.06
P	0.021*	0.000***	0.000***

T-tests and one-way ANOVA were used for statistical analysis. Data are expressed as means±SD of three independent experiments (n=3). *P<0.05; ***P<0.001. ANOVA: Analysis of variance; SD: Standard deviation

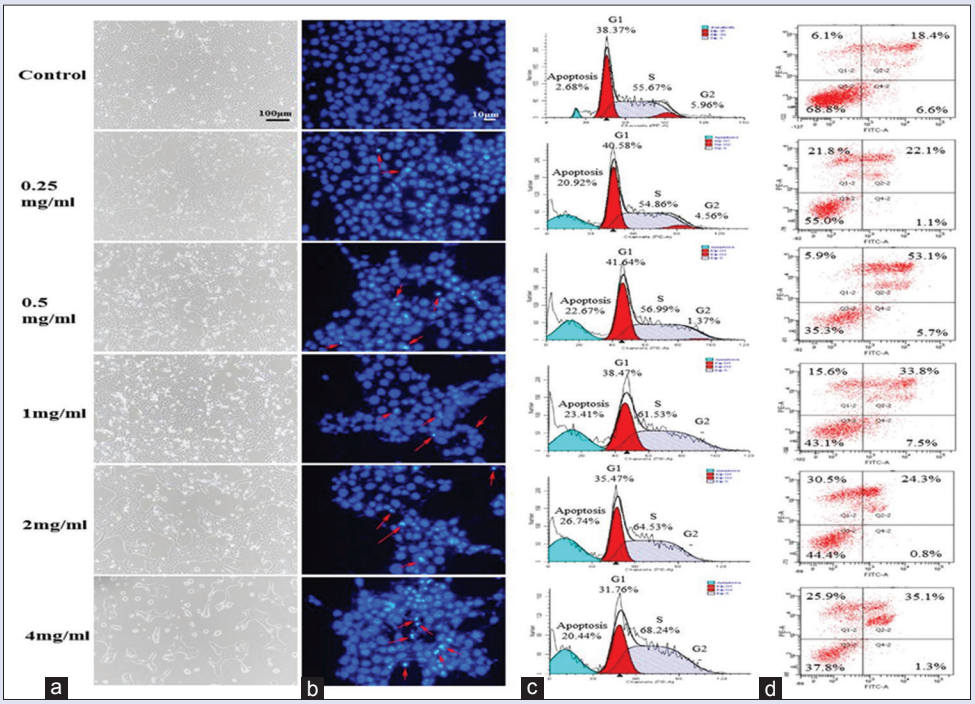


Figure 2: (a and b) Morphological changes (×200) and 4′,6-diamidino-2-phenylindole staining (×400) of MCF-7 cells treated with 0–4 mg/mL ginseng protein for 48 h. Cell observation and image capture were performed using a Nikon Ri2 inverted fluorescence microscope. (c) Cell cycle analysis using a cell cycle and apoptosis analysis kit and flow cytometry. (d) Apoptosis analysis using an Annexin V-FITC/PI Apoptosis Analysis Kit and flow cytometry after ginseng protein treatment for 48 h (n = 3)

In addition, the proportion of apoptotic cells increased from 2.68% to 26.74%, indicating that GP promotes the apoptosis of MCF-7 cells. We therefore set the GP concentration at 2.594 mg/mL (the IC_{50} value for 48 h). After 48 h of GP treatment, the results [Table 2] indicated that GP mediated G0/G1 phase cell cycle arrest in MCF-7 cells ($P < 0.05$ and $P < 0.001$).

Effects of ginseng protein on apoptotic induction of MCF-7 cells

In addition to cell cycle changes, apoptosis is another factor affecting cell proliferation. In our study, the results of apoptotic analysis are shown in Figure 2d and Table 3. Cells were mostly alive in the control group with very few dead cells (6.1%). After treatment with GP, the apoptosis rate was significantly higher ($P < 0.05$ and $P < 0.001$) and the proportion of living cells decreased from 68.8% to 37.8%. These results suggested obvious proapoptotic effects of GP in MCF-7 cells. At a GP concentration of 2.594 mg/mL and treatment for 48 h (in triplicate), the percentage of Q2–2, Q4–2 and their sum increased from 4.13 ± 1.60 to 72.53 ± 9.06 [$P < 0.001$; Figure S1a]. This suggested that GP mediated the apoptosis of MCF-7 after 48 h of treatment.

Effect of ginseng protein on the expression of apoptosis-related proteins

To explore the potential mechanism of GP-induced apoptosis in MCF-7 cells, we examined the expression of several apoptosis-related genes and proteins. We selected apoptosis-related genes for quantitative-PCR (Q-PCR) analysis. As shown in Figure 3a, expression of bax increased, while bcl-2 expression decreased following GP treatment ($P < 0.01$). The results of protein expression were consistent with gene expression [$P < 0.05$; Figures 3b and S1b and Table 4], further indicating that GP induced apoptosis of MCF-7 cells, probably via the bcl-2/bax pathway.

Table 4: Data of the expressions of apoptosis-related genes and proteins after treated by ginseng protein

Gene and protein	Control	2.594 mg/ml	*P*
Bax gene	1	1.19±0.06	0.006**
Bcl-2 gene	1	0.36±0.12	0.001**
Bax protein	177.78±62.22	65.75±1.02	0.021*
Bcl-2 protein	87.91±1.49	155.30±31.72	0.036*
Bax/Bcl-2	0.55±0.23	2.36±0.45	0.003**

t-tests and one-way ANOVA were used for statistical analysis. Data are expressed as means±SD of three independent experiments (n=3). *P<0.05; **P<0.01; ***P<0.001. ANOVA: Analysis of variance; SD: Standard deviation

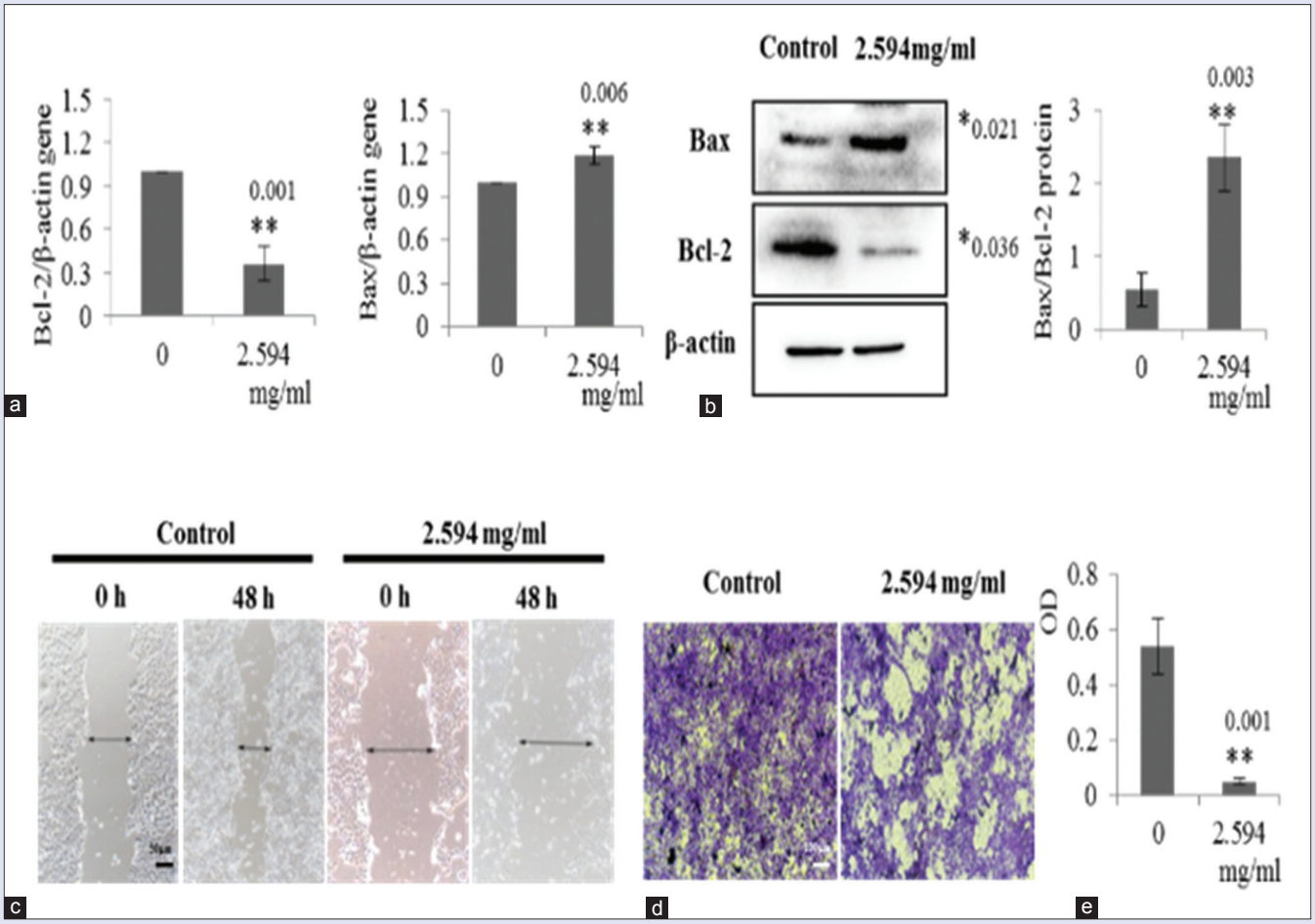


Figure 3: (a) Quantitative analysis of apoptosis-related genes bcl-2 and bax in MCF-7 cells normalized against β-actin. (b) Western blot analysis of bax and bcl-2 expression in MCF-7 cells normalized against β-actin as an internal control. (c and d) Migration analysis using scratch wound healing and transwell assays. (e) Statistical analysis of cell migration by *t*-tests and one-way analysis of variance. Data are expressed as means ± standard deviation of three independent experiments (n = 3). *P < 0.05 and **P < 0.01 compared with the untreated group

Ginseng protein inhibits the migration of MCF-7 cells

Malignant metastasis of tumor cells is a typical feature of tumors, hence we further tested the antitumor cell migration effect of GP. Migration was probed by scratch wound healing and transwell assays. Compared with the untreated group, the scratch distance did not decrease and the proportion of dead cells increased [Figure 3c]. This proved that GP had a significant effect on cell migration and invasion. As shown in Figure 3d and e, transwell assay results were consistent with those of scratch wound healing assays ($P = 0.001$), revealing a significant antitumor function. The inhibitory effect of GP on cell migration needs further study in future work.

DISCUSSION

Natural products have been used for the treatment of many bodily ailments for centuries. However, the associated mechanisms remain poorly understood. With significant advances in research, we are now able to investigate the molecular mechanisms underlying the effects of compounds present in herbs, shrubs, roots, leaves, flowers, and other natural products.[10] Ginseng extract has been investigated for many biological activities, including antiviral, anti-inflammation, cell apoptosis, and cell cycle arrest. Plant proteins have specific regulatory effects on the human body,[11-13] and GP can ameliorate cognitive impairment in Alzheimer's disease rats.[14]

In the current study, we chose the acetone extraction method for obtaining GP and tested it against breast cancer (MCF-7 and MDA-MB-231) and non-breast cancer (MCF10A) cell lines to assess anticancer activity. Our results showed that GP inhibited proliferation, led to cell cycle arrest, promoted apoptosis, and inhibited the migration of MCF-7 cells. Normal breast cells and some breast cancer cells contain receptors that attach to estrogen and progesterone receptor (PR). These are called ER-positive and PR-positive cells.[15] MCF-7 cells are ER^+ and PR^+, while MDA-MB-231 are ER^- and PR^-, hence we choose these two cell lines to investigate inhibition of proliferation by GP, along with MCF10A cells as normal controls. GP might contain certain ingredients that act as antitumor agents, possibly by binding to ER and exerting antagonistic effects that inhibit the growth of tumor cells.

A more detailed approach is needed to determine how GP regulates cancer cell growth. This depends on the effects of drugs on the cell cycle and apoptosis, as well as the distribution of related proteins such as cdk4, cdk6, cyclin B, cyclin E, bax, bcl-2, p53, p21, and Poly ADP-Ribose Polymerase (PARP). The G0/G1 phase is the first phase of the cell cycle, during which cells synthesize mRNA and proteins to prepare for subsequent steps leading to mitosis. The S phase is when DNA replication occurs and the G2/M phase is when cells divide into two daughter cells.[16] In the present study, GP induced cell cycle arrest in MCF-7 cells. Future studies should focus on the molecular mechanisms by which GP regulates the cell cycle in MCF-7 cells.

Apoptosis is characterized by a series of biochemical and morphological changes. It has been reported that some cells in which p53 is activated undergo apoptosis. Bax, an apoptosis-inducing member of the bcl-2 protein family, is a potential mediator of p53-induced apoptosis and is directly activated by p53-binding sites in the regulatory region of the gene.[17] Our results indicated that GP induced apoptosis in MCF-7 cells and Q-PCR experiments revealed significant changes in the expression of bcl-2 and bax. Western blot analysis indicated that GP induced cytostatic effects and promoted cellular apoptosis, as evidenced by the expression of bax and bcl-2.

Antitumor activity has been reported for ginseng sapogenins,[18] ginsenosides,[19] and polysaccharides.[20] PI3K/AKT/mTOR pathway-mediated apoptosis may be one of the potential mechanisms of 20(S)-protopanaxadiol treatment.[21] GP exerts anticancer effects in MCF-7 breast cancer cells, but the underlying mechanisms remain unclear. More detailed information on the genomic and proteomic responses underlying the GP-induced anticancer effects remains to be elucidated.

CONCLUSION

P. ginseng is one of the most popular herbs originating from Eastern countries.[22] The present study demonstrated that GP had antitumor effects on MCF-7 cells. It disrupted the proliferation, cell cycle, apoptosis, and migration of MCF-7 breast cancer cells and promoted apoptosis by interfering with the expression of bcl-2 and bax in tumor cells. Our studies provide evidence that supports the use of GP as an antitumor adjuvant in breast cancer patients.

Acknowledgements

We would like to thank the research group of Prof. Min Wei and Prof. Xiaojuan Zhu from the School of Life Sciences, Northeast Normal University, for providing laboratory space and equipment. We also thank the Jilin Ginseng Academy of Changchun University of Chinese Medicine for building the cytology experiment platform and providing equipment and space for follow-up experiments.

Finanacial support and sponsorship

The study was financially supported by the National Natural Science Foundation of China (grant numbers 21475012); Jilin Administration of Traditional Chinese Medicine, TCM Science and Technology Project (grant numbers 2017015, 2018026), Natural Science Foundation of Jilin province (20190201297JC).

Conflicts of Interest

The authors declare no conflict of interest.

REFERENCES

1. Akram M, Iqbal M, Daniyal M, Khan AU. Awareness and current knowledge of breast cancer. Biol Res 2017;50:33.
2. Yun TK. *Panax ginseng* – A non-organ-specific cancer preventive? Lancet Oncol 2001;2:49-55.
3. Lin T, Liu Y, Shi M, Liu X, Li L, Liu Y, *et al*. Promotive effect of ginsenoside Rd on proliferation of neural stem cells *in vivo* and *in vitro*. J Ethnopharmacol 2012;142:754-61.
4. Hwang JW, Oh JH, Yoo HS, Lee YW, Cho CK, Kwon KR, *et al*. Mountain ginseng extract exhibits anti-lung cancer activity by inhibiting the nuclear translocation of NF-κ B. Am J Chin Med 2012;40:187-202.
5. Huang CW, Lee TT, Shih YC, Yu B. Effects of dietary supplementation of Chinese medicinal herbs on polymorphonuclear neutrophil immune activity and small intestinal morphology in weanling pigs. J Anim Physiol Anim Nutr (Berl) 2012;96:285-94.
6. Cheung LW, Leung KW, Wong CK, Wong RN, Wong AS. Ginsenoside-Rg1 induces angiogenesis via non-genomic crosstalk of glucocorticoid receptor and fibroblast growth factor receptor-1. Cardiovasc Res 2011;89:419-25.
7. Li H, Kang T, Qi B, Kong L, Jiao Y, Cao Y, *et al*. Neuroprotective effects of ginseng protein on PI3K/Akt signaling pathway in the hippocampus of D-galactose/$AlCl_3$ inducing rats model of Alzheimer's disease. J Ethnopharmacol 2016;179:162-9.
8. Ahn HN, Jeong SY, Bae GU, Chang M, Zhang D, Liu X, *et al*. Selective Estrogen Receptor Modulation by *Larrea nitida* on MCF-7 Cell Proliferation and Immature Rat Uterus. Biomol Ther (Seoul) 2014;22:347-54.
9. Du J, Sun Y, Lu YY, Lau E, Zhao M, Zhou QM, *et al*. Berberine and evodiamine act synergistically against human breast cancer MCF-7 cells by inducing cell cycle Arrest and apoptosis. Anticancer Res 2017;37:6141-51.
10. Saba E, Jeong D, Irfan M, Lee YY, Park SJ, Park CK, *et al*. Anti-inflammatory activity of Rg3-enriched Korean red ginseng extract in murine model of sepsis. Evid Based Complement Alternat Med 2018:6874692.

11. Chalvon-Demersay T, Azzout-Marniche D, Arfsten J, Egli L, Gaudichon C, Karagounis LG, *et al.* A systematic review of the effects of plant compared with animal protein sources on features of metabolic syndrome. J Nutr 2017;147:281-92.
12. Paula PC, Oliveira JT, Sousa DO, Alves BG, Carvalho AF, Franco OL, *et al.* Insulin-like plant proteins as potential innovative drugs to treat diabetes-the *Moringa oleifera* case study. N Biotechnol 2017;39(Pt A):99-109.
13. Malekzad H, Mirshekari H, Sahandi ZP, Moosavi BS, Baniasadi F, Sharifi AM, *et al.* Plant protein-based hydrophobic fine and ultrafine carrier particles in drug delivery systems. Crit Rev Biotechnol 2018;38:47-67.
14. Zhao C, Su G, Wang X, Zhang X, Guo S, Zhao Y. Antitumor activity of ginseng sapogenins, 25-OH-PPD and 25-OCH_3-PPD, on gastric cancer cells. Biotechnol Lett 2016;38:43-50.
15. Li H, Song J, Zhang J, Wang T, Yan Y, Tao Z, *et al.* Ginseng protein reverses amyloid beta peptide and H_2O_2 cytotoxicity in neurons and ameliorates cognitive Impairment in AD Rats Induced by a combination of D-galactose and $AlCl_3$. Phytother Res 2017;31:284-95.
16. Chou SM, Lai WJ, Hong TW, Lai JY, Tsai SH, Chen YH, *et al.* Synergistic property of cordycepin in cultivated *Cordyceps militaris*-mediated apoptosis in human leukemia cells. Phytomedicine 2014;21:1516-24.
17. Yuan B, Ma N, Zhao L, Zhao E, Gao Z, Wang W, *et al. In vitro* and *in vivo* inhibitory effects of a *Pleurotus eryngii* protein on colon cancer cells. Food Funct 2017;8:3553-62.
18. Xu H, Zou S, Xu X, Zhang L. Anti-tumor effect of β-glucan from *Lentinus edodes* and the underlying mechanism. Sci Rep 2016;6:28802.
19. Wu W, Zhou Q, Zhao W, Gong Y, Su A, Liu F, *et al.* Ginsenoside Rg3 inhibition of thyroid cancer metastasis is associated with alternation of actin skeleton. J Med Food 2018;21:849-57.
20. Zhou X, Shi H, Jiang G, Zhou Y, Xu J. Antitumor activities of ginseng polysaccharide in C57BL/6 mice with Lewis lung carcinoma. Tumour Biol 2014;35:12561-6.
21. Zhang H, Xu HL, Wang YC, Lu ZY, Yu XF, Sui DY. 20(S)-Protopanaxadiol-induced apoptosis in MCF-7 breast cancer cell line through the inhibition of PI3K/AKT/mTOR signaling pathway. Int J Mol Sci 2018;19. pii: E1053.
22. Sabouri RS, Sahebkar A, Tayarani NZ. Ginseng in dermatology: A review. Curr Pharm Des 2017;23:1649-66.