ORIGINAL ARTICLE

Anticancerous effects of Polygonum avicular exert through Fas ligand and Bcl2

Motahareh Mortazavi[1], Mehryar Habibi Roudkenar[2], Abbas Delazar[3]
Raheleh Halabian[2], Sara Ansari[1], Zohreh Alizadeh[1]
Amaneh Mohammadi Roushandeh[1,*]

ABSTRACT

Background: Herbal medicine is a new complementary strategy for treating diseases such as cancer. However, its mechanism of action is not clear yet. This study investigates antitumor efficacy of Polygonum avicular extract on MCF-7 cell line.

Methods: MCF-7 cells were treated with different concentrations of (50, 100, 150, 200, 250, 300,350 400 ng/µl) Polygonum avicular at different time intervals (6, 12, 24, and 48 hrs). In order to evaluate the expression level of FasL and Bcl2 genes, RT-PCR technique was employed.

Results: The obtained results demonstrate that expression of FasL gene was increased in Polygonum avicular treated cells in comparison to control group. Down-regulation of Bcl2 expression was observed during the treatment.

Conclusion: Polygonum aviculare herbal extract may induce apoptosis through up-regulation of FasL and down-regulation of Bcl2 gene, however, its complete mechanism of action must be elucidated.

Keywords: Breast cancer, Herbal medicine, Polygonum aviculare.

1. Anatomical Sciences Department, Medicine Faculty, Hamadan University of Medical Sciences, Hamadan, Iran.
2. Blood Transfusion Research Center, High Institute in Research Blood Transfusion Organization, Tehran, Iran.
3. Pharmacognosy Department, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.

***Corresponding Author:**
Amaneh Mohammadi Roushandeh
Email:
a.mohammadiroshandeh@umsha.ac.ir

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Introduction

Breast cancer originating from breast tissue, most commonly from the inner lining of milk ducts or the lobules that supply the ducts with milk is a disease of humans and other mammals; while the overwhelming majority of cases in humans are women, men can also develop breast cancer.[1, 2] The disease is now also the leading cause of cancer death among females in economically developing countries, a shift from the previous decade during which the most common cause of cancer death was cervical cancer. Further, the mortality burden for lung cancer among females in developing countries is as high as the burden for cervical cancer, with each accounting for 11% of the total female cancer deaths.[3]

Conventional breast cancer treatment may include surgery, drugs (hormonal therapy and chemotherapy), radiation and/or immunotherapy which have shown some effectiveness for reducing or eradicating malignancy; however, some of these therapies such as chemotherapy and radiation therapy can produce unpleasant side effects, e.g. nausea, vomiting, changes in bowel habits, fatigue and hair loss.[4]

Consumption of complementary and alternative medicines, herbal and multivitamin supplements, are common in the Asian countries such as Iran. In addition, the herbal medicinal interventions are widely used in all the developing world and rapidly growing in industrialized countries. These medicines are increasingly utilized as an adjunctive treatment option for cancer patients and a way of reducing or managing side effects of conventional cancer treatment.[5] Cui et al. study showed that patients who suffer from breast cancer commonly take herbal medicines for cancer treatment (81.5%), followed by immune system enhancement (12%), metastasis prevention or side effect management (7.9%), and the reduction of menopausal symptoms (4.7%).[6]

Apoptosis is the process of programmed cell death that may occur in multicellular organisms.[7] In mammals, activation of two signaling pathway which are called the extrinsic pathway and the intrinsic pathway, leads to apoptosis. Inhibition of apoptosis may contribute to the pathogenesis of a number of human disease including cancers, autoimmune diseases, inflammatory diseases, and viral infections. Mutations leading to loss of apoptosis can lead to tumorigenesis. Breast cancer, like other cancers, occurs because of an interaction between the environment and a defective gene. Cell cycle regulating genes (such as p53, ras or c-myc) are mutated or inactivated in diseased cells, and further genes (such as Bcl2) modify their expression in tumors.[8] Apoptosis plays a fundamental role in the regulation of the immune system, as well. Fas ligand (FasL or CD95L) binds with its receptor and induces extrinsic pathway of apoptosis. The ligand and Fas death receptor are type II transmembrane protein and belong to the tumor necrosis factor (TNF) family. Fas ligand/receptor interactions play an important role in the regulation of the immune system and the progression of cancer.[9]

Tumors may overexpress Fas ligand and induce the apoptosis of infiltrating lymphocytes, allowing the tumor to escape the effects of an immune response. The up-regulation of Fas ligand often occurs following chemotherapy, from which the tumor cells have attained apoptosis resistance. Defective Fas mediated apoptosis may lead to oncogenesis as well as drug resistance in existing tumors .[10]

Bcl2 is a cytoplasmic protein, which regulates the intrinsic pathway of apoptosis. Bcl2 has been shown to promote cell survival by inhibiting the process of apoptosis where this protein acts to inhibit apoptosis, Bax, another cytoplasmic protein, counteracts this protective effect. (Where this protein acts to inhibit pro-apoptotic protein, BAX. BAX is a cytoplasmic protein. Bcl2 and BAX proteins can bind to each other and inhibit each other's function.) Bcl2 is also thought to protect cells from apoptosis by dimerizing with BAX.[10]

It is shown that some natural plant extracts have chemopreventive activity. Polygonum or knotweed genus belongs to the Polygonaceae (Buckwheat) family. This genus contains 80 species such as knotweed, knotgrass, bistort, tear thumb, mile-a-minute, and several others. Modern herbalists use it to treat dysentery, excessive menstrual flow, lung disorders, bronchitis and jaundice, and gall and kidney stones. Scientific evidences do not support all of these uses. The plant is an astringent, coagulant, diuretic and expectorant herb.[11] Polygonum avicular extract has a high content of phenolics and flavonoid. In addition, it has DNA protective effect in hydroxyl radical-induced DNA strand scission assays. It is shown

that extracts of Polygonum have activity to phosphorylate Bcl2 and induce apoptosis in cancer cells. In addition, two dihydrobenzofuranones which promote Bcl2 phosphorylation in breast and prostate cancer cell lines have been isolated from the extract and their structures have been identified.[11]

Present study evaluates the anti-tumor and -apoptosis effect of Polygonum avicular extract on of breast cancer cell line, MCF-7.

Methods

Preparation of Polygonum avicular extract

Polygonum avicular plant was collected from Oscow Mountains in (Azarbaijan Province, Iran).The collection of voucher specimens (AD2009-1892) was deposited in the Herbarium of the Faculty of Pharmacy, Tabriz, Iran and the plant was recognized by Prof. A. Delazar. The extraction method was maceration. Aerial parts were dried at room temperature for 2 weeks. Herbal extraction was extracted from these dried parts with 70% methanol at room temperature followed by evaporation of combined hydromethanolic solutions through rotatory evaporation at 50° C under low pressure. This finally yielded the dried extract (The final dried yield was 11.23 g).

Cell culture

MCF-7 cells (the human breast cancer cell line) were obtained from the national cell bank of Iran ([NCBI], Pasteur Institute of Iran). The cells were cultured in RPMI-1640 (Sigma, USA) supplemented with 10% (v/v) FBS (Gibco) and 100 ug/ml penicillin and 100 µg/ml streptomycin. The cells were grown at 37°C in a humidified 5% (v/v) CO2 incubator for 2 weeks. After that, they were treated with herbal extract.

Assessment of cell viability

MCF-7 cells were cultured in RPMI-1640 and treated with different concentrations (50, 100, 150, 200, 250, 300,350 and 400 ng/µl) of Polygonum avicular for different length of time (6, 12, 24, and 48 hrs). Following incubation, number of viable cells was determined by trypan blue assay and counted using hemocytometer under a phase contrast microscope.

RT-PCR

MCF-7 cells were treated with Polygonum avicular herbal extract. Total RNAs were isolated using Tripure Isolation Reagent (Roche Applied Science, Germany) according to the manufacturer's recommendation. The extracted RNAs were suspended in 20 µl of RNase-free water and stored at -80˚ C for subsequent procedures. The quantity and quality of purified RNAs were verified by Nanodrop spectrophotometer (ND-1000, Wilmington, DE) and electrophoresis in 1% agarose gel (Cinnagene, Tehran, Iran) respectively. The total purified RNAs were used as templates for cDNA synthesis. Reverse transcription was carried out by SuperScript III reverse transcriptase (Invitrogen, Carlsbad, CA) followed by DNaseI (Invitrogen, Carlsbad, CA) treatment and heat inactivation, to eliminate any contamination with chromosomal DNA.

Assessment of apoptotic/antiapoptotic genes Expression

Based on published sequences in the GenBank database (http://www.ncbi.nlm.nih.gov/) with an alignment algorithm (BLAST) specific primers were designed by primer3 software (Input 0.4.0), (http://frodo.wi.mit.edu/). Semiquantitative PCR of Fas ligand and Bcl2 genes was performed using recombinant Taq DNA polymerase (Cinnagene, Tehran, Iran). In order to normalize RT-PCR β-actin gene sequence was amplified. Subsequent to initial denaturation at 94º C for 5 minutes, cDNA was subjected to 33 cycles of PCR consisting of denaturation at 94° C for 30 seconds, annealing at 59° C for 30 seconds, and extension at 72° C for 59 seconds followed by a 5 minutes terminal extension cycle at 72° C. PCR products were electrophoreses on a 2 % agarose gel (Cinnagene, Tehran, Iran) to determine successful amplification.

Statistical analysis

Results were presented as mean ± SD in triplicate experiment. Differences were determined using ANOVA with the Tukey–Kramer multiple comparisons test.

Results

Effects of Polygonum avicular on cell viability and prolifration

Cytotoxicity analysis indicated that the extract at the

concentrations of 300 and 350 ng/µl after 24 hrs had highest effects on vitality in treated groups compared to the control. %99 of cell death occurred at the concentration of 400 ng/µl after 24 hrs.

Induction of apoptosis after treatment with Polygonum avicular

RT-PCR results revealed that 100 ng/µl of Polygonum avicular extract induced an increase in expression level of Fas L mRNA of whereas the expression level of the Bcl2 antiapoptotic gene was reduced (**Fig 1**).

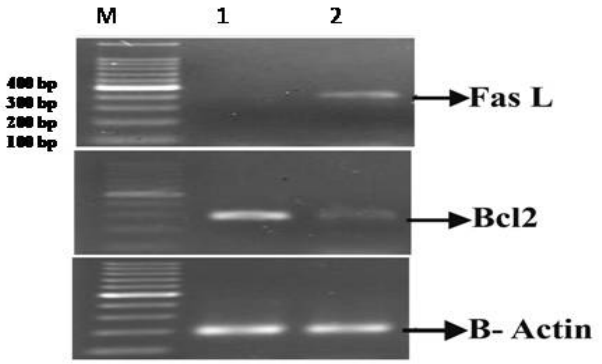

Fig 1: Expression of Fas L and Bcl-2 in MCF-7 cell line in experimental and control groups. Expression of Fas L and Bcl-2 was determined by semiquantitative RT-PCR. Fas L Expression was Up- regulated in Polygonum group compared with the control. Bcl-2 expression was down-regulated in Polygonum group compared with the control. Poly (Polygonum avicular), Cont (Control).

Discussion

The herbal medicinal interventions are widely used in all the developing world and rapidly growing in industrialized countries. These medicines are increasingly utilized as an adjunctive treatment option for cancer patients and a way of reducing or managing side effects of conventional cancer treatment.[5]

Present study evaluates the anti-tumor and -apoptosis effect of Polygonum avicular extract on of breast cancer cell line, MCF-7. Our results showed that Polygonum aviculare extract induced Fas L expression as an apoptotic gene progression and inhibited Bcl2 as an anti-apoptotic gene in MCF-7 cells.

Smolarz et al reported that methanol juice of Polygonum amphibium L. was able to induce apoptosis in the tested human leukaemic cells. These compounds penetrate through cytoplasm to the cellular nucleus of the cultured cells and they give intensive apoptotic responses in the stimulated leukaemic cells.[13]

Breast cancer, like other cancers that occurs because of the apoptosis process in the organ's cells is inhibited. Cells become cancerous when mutations destroy their ability to stop dividing, to attach to other cells and to stay where they belong. The malignant cells experience an abnormal response to apoptosis induction; therefore, apoptotic induction for the treatment of cancers is necessary.[8] Anticancer drugs eliminate tumor cells by inducing their apoptosis, as well. The results reported in our previous study revealed that induction of apoptosis by different concentrations of Polygonum avicular extract has occurred in MCF-7 cells.[14] In the present study expression level of Fas L as an apoptotic gene has been investigated to confirm our previous results about apoptosis induction.

It is shown that Bcl2 promotes cell survival by inhibiting the process of apoptosis.[10] Therefore, in this study, our attempts focused on the up-regulation of Fas-ligand and down-regulation of Bcl2 by Polygonum avicular juice that could act as an apoptotic inducer agent. The results of the cell viability test confirmed that the cytotoxicity property of Polygonum avicular juice. Furthermore, RT-PCR results showed that Polygonum avicular juice induces up-regulation of Fas-ligand and down-regulation of Bcl2.

In conclusion, up-regulation of Fas-ligand as an apoptotic gene accompanied with down-regulation of Bcl2 as an anti-apoptotic gene suggests that Polygonum avicular juice leads to apoptosis induction in MCF-7 cells. Therefore, it is suggested that Polygonum avicular is a cancer chemopreventive agent and can apply for breast cancer or other cancers treatment. Although, in vitro investigation showed antitumoral activity of Polygonum avicular but, it is not sufficient and in vivo studies are necessary in future.

Conclusion

Polygonum aviculare herbal extract may induce apoptosis through up-regulation of FasL and down-regulation of Bcl2 gene, however, its complete mechanism of action

must be elucidated.

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References

1. Sariego J. Breast cancer in the young patient. The American surg. 2010; 76 (12): 1397–1401.

2. Giordano SH, Cohen DS, Buzdar AU, Perkins G, Hortobagyi GN. Breast carcinoma in men: a population-based study. Cancer. 2004;101(1): 51–7.

3. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011; 61(2): 69-90.

4. Florescu A, Amir E, Bouganim N, Clemons M. Immune therapy for breast cancer in 2010—hype or hope?. Cur Oncol. 2011; 18 (1): e9–e18.

5. Carmady B, Smith C. Use of Chinese medicine by cancer patients: a review of surveys. BioMed central. 2011; 6(22): 1-8.

6. Cui Y, Shu XO, Gao Y, Wen W, Ruan ZX, Jin F, Zheng W. Use of complementary and alternative medicine by Chinese women with breast cancer. Breast Cancer Res Treat. 2004; 85: 263-270.

7. Lacroix MA. Concise History of Breast Cancer. Nova Science Publishers: USA; 2011; 59–68.

8. Wajant H. The Fas signaling pathway: more than a paradigm. Science. 2002; 296 (5573): 1635–6.

9. Igney FH, Krammer PH. Tumor counterattack: fact or fiction?. Cancer Immunol Immunother. 2005; 54 (11): 1127–1136.

10. Elmore S. Apoptosis: A Review of Programmed Cell Death. Toxicol Pathol. 2007; 35:495–516.

11. Chin-Yuan H, Yu-Pei C, Jeli C. Antioxidant activity of extract from Polygonum aviculare L. Biol Res. 2007; 40: 13-21.

12. Labi V, Erlacher M, Krumschnabel G, Manzl C, Tzankov A, Pinon J, et al. Apoptosis of leukocytes triggered by acute DNA damage promotes lymphoma formation. Genes Dev. 2010; 24: 1602-1607.

13. Smolarz HD, Budzianowski J, Bogucka-Kocka A, Kocki J, Mendyk E. Flavonoid glucuronides with anti-leukaemic activity from Polygonum amphibium L. Phytochem Anal. 2008; 19(6):506-13.

14. Habibi Roudkenar M, Mohammadi Roushandeh A, Delazar A, Halabian R, Soleimani Rad J, Mehdipour A, Bagheri M, et al. Effects of polygonum aviculare herbal extract on proliferation and apoptotic gene expression of MCF-7. DARU. 2011; 19(5): 326-331.