Original Article *Iran J Ped Hematol Oncol. 2023, Vol 13, No 3, 166-171*

Examining the Effect of Extract of Oak Fruit Jaft on AGS Cell Lines

Ahmad Zare Bidaki PhD[1], Pouran Pourhakkak PhD[2], Fateme Montazeri PhD[3,4]*, Seyed Mehdi Kalantar PhD[3,4], Seyed Mehdi Hoseini PhD[3,4], Abolfazl Dehghanpour MSc[1], Majid Emtiazy PhD[1*]

1. Department of Persian Medicine, School of Persian Medicine, Shahid Sadoughi University of Medical Sciences, Ardakan, Yazd, Iran
2. Department of Chemistry, Payam Noor University, Tehran, Iran
3. Abortion Research Center, Yazd reproductive sciences institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
4. Research and Clinical Center for Infertility, Yazd reproductive sciences institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

*Corresponding authors: Dr Majid Emtiazy, Department of Persian Medicine, School of Persian Medicine, Shahid Sadoughi University of Medical Sciences, Ardakan, Yazd, Iran. Emails: dr.emtiazy@gmail.com. ORCID ID: 0000-0002-5984-3825
& Dr Fateme Montazeri, Abortion Research Center, Yazd reproductive sciences institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. Emails: f_montazeri@outlook.com. ORCID ID: 0000-0002-7739-5982

Received: 09 January 2023 **Accepted:** 06 June 2023

Abstract

Background: Gastrointestinal carcinoma comprises 5% of all pediatric cancer in children. Given that the possible and beneficial effect of the Jaft extract in the treatment of gastric cancer is not known and there is no comprehensive study in this regard, this study aimed to assess the effect of Jaft extract on gastric cancer cell lines.

Materials and Methods: In this case-control study, oak fruit was collected from the mountains of Lorestan province. A gastric cancer (AGS) cell line was obtained from the Institute Pasteur cell bank and was cultured. After the preparation of the ethanolic extract of Jaft, the cell viability of the gastric cancer cells treated with Jaft extract was investigated by MTT assay. Quantitative Reverse Transcription PCR was used for assessing the expression of *BAX*, and *BCL2* genes.

Results: The half maximal inhibitory concentration (IC50) value of Jaft extract was 162 µg/ml. The *BAX* gene expression was different between the case and control groups. In this regard, the expression of the *BAX* gene was increased in the concentration of 162 ($P<0.01$) and 250 µg/ml ($P<0.001$) of Jaft extract compared to the control group. The *BCL2* expression was different between the two groups ($P<0.05$). In this regard, the expression of the *BCL2* gene was decreased in the concentration of 162 and 250 µg/ml of Jaft extract compared to the control group.

Conclusion: It was found that Jaft extract increased the apoptosis of gastric cancer cells; therefore, it seems that the hydroalchoholic extract of Jaft is an appropriate anticancer medication.

Keywords: Apoptosis, Jaft, Gastric cancer.

Introduction

Gastrointestinal carcinoma comprises 5% of all pediatric cancer in children (1). It is the most frequent reason for cancer death and affects approximately 1 million individuals annually (2). The ratio of gastric cancer in men/women is about 2 to 1. The highest incidence of gastric cancer is seen in men in northeast Asia, including China, Korea, and Japan (2). Neoadjuvant and adjuvant treatments for gastric cancer are radiotherapy, chemoradiotherapy, chemotherapy, and targeted therapy.

Primary prevention strategies for decreasing gastric cancer involve avoidance of smoking, high use of fresh vegetables and fruits, improvement of sanitation, and safe food-preservation procedures (2). Recently, the role of herbal medicines in cancer treatment has been highlighted. On the other hand, one of the rich sources of natural anti-cancer components is herbal medicine. These components are used to treat gastric cancer (3, 4). The oak tree is considered as a

[Downloaded from ijpho.ssu.ac.ir on 2026-10-15]

[DOI: 10.18502/ijpho.v13i3.13128]

valuable traditional medicine. This plant is a large genus of the Fagaceae family with more than 450 species in Eurasia, northern Africa, and America (5).

Persian oak (Quercus castaneifolia C.A.Mey) belongs to the Fagaceae family (6) and is used in Iranian traditional medicine (6). The Quercus genus involves different components, including flavonoids, glycosides, phenolic acids (7), sterols, and tannins (7, 8). The Acorn fruit contains active biological components, including galic acid, tannins, and ellagic acid (9).

In addition, the fruit of the Quercus species is a rich source of energy, including protein, fat, and carbohydrate. In the western area of Iran, the extract of the various parts of the oak tree, including fruit of the oak tree, and the extract of the inner layer of oak fruit (Jaft in Persian) (10) was used to treat microbial infections from the past.

After exposure to light, the color of Jaft converts from yellow to brown due to oxidation (10, 11). Based on Iranian indigenous information, this plant is applied for diseases, including acute diarrhea inflammation, gastropathy, burns, cuts, and cancers (10). In addition, Jaft or Jaft-E-Baloot has biological characteristic (12), including tissue protective effects, wound healing, anti-microbial, anti-tuberculosis, albumin sedimentation, and anti-proliferative effects (13).

Due to the high incidence of gastric cancer in our country (14), the possible and beneficial effect of Jaft extract on the treatment of cancer, and no comprehensive study regarding the effect of Jaft on gastric cancer cell lines, this study aimed to assess the effect of Jaft extract on gastric cancer cell lines.

Materials and Methods

Sample selection and cell culture preparation

This study was a case-control study. Oak fruit was collected from the mountains of Lorestan province. A gastric cancer (AGS) cell line was obtained from the Institute Pasteur cell bank and was cultured in RPMI 1640 medium, containing 10% Fetal Bovine Serum (FBS) and 1% penicillin/streptomycin antibiotics.

Preparation of ethanolic extraction

The process of extraction, filtration and desolvation to prepare the alcoholic extract is carried out. For the preparation of alcoholic extract, 200 gr of ground Jaft was mixed with 400 ml ethanol. It was placed in the darkroom for 72 hours. During this period, the mixture was well stirred 3 times a day. After 72 hours, the mixture was filtered and finally, extraction was done by Rotatory Evaporator.

MTT assay

A gastric cancer cell line (AGS) was obtained from the Institute Pasteur cell bank and was cultured in RPMI 1640 medium, containing 10% FBS and 1% penicillin/streptomycin antibiotics. Cells were seeded in 96-well tissue culture plates (10, 000 cells/well) in the presence of 50 µl RPMI 1640 medium. Then plates were incubated with ethanolic extracts of Jaft (75,150, 250, 500, 750, and 1000 µg/ml) for 48-72 hours, twice as triplicates. The control plates received DMSO.

In the next step, a 3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) solution was added to each well. Then the cell culture plate was placed in the incubator for 3 to 4 hours (at 37°C and 5% CO2). In the next step, the liquid on the cells was removed and 80 µL DMSO was added to each well. The optical absorbance of the samples was read at 570 nm using a spectrophotometer (EPOCH). The percentage of viability of cells was calculated by the following formula.

Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

Subsequently determining the appropriate concentration of Jaft, 10000 cells were

[Downloaded from ijpho.ssu.ac.ir on 2026-10-15]

[DOI: 10.18502/ijpho.v13i3.13128]

cultured in each plate as triplicate in two rounds with 162 and 250 µg/ml of Jaft extract. In this regard, after 48 hours of treatment, the total RNA was extracted. This process was performed using the RNeasy Mini Kit (Qiagen, Germany) based on the protocol of the manufacturer. RNA concentration and purification was determined using a Biophotometer (Eppendorf). Total RNA was reverse-transcribed to cDNA based on the protocol using the RevertAid™ First Strand cDNA Synthesis Kit (Fermentas).

The real-time procedure was done using the Add SYBR Master high Rox (addbio). Thermal cycling conditions were done for initial denaturation, denaturation, annealing, and extension at 95°C for 10 minutes, 95˚C for 15 seconds, 60˚c for 20 seconds, and 72˚c for 20 seconds, respectively (40 cycles).

Analysis of data was performed by the comparative Ct ($2^{-\Delta ct}$) method. These experiments were carried out in duplicate and *GAPDH* gene was used as a reference gene for internal control. The primer sequence of *BAX* and *BCL2* genes is indicated in Table I.

Ethical Consideration

The Ethics Committee of Shahid Sadoughi University approved this study (number: IR.SSU.REC.1399.215).

Statistical analysis

Student-t- test was used for the expression level of genes between groups. In tables with a smaller number of data frequencies; Fisher exact test with less power was used. P-value ≤0.05 (*), P-value ≤0.01 (**), and P-value ≤0.001 (***) were considered as statistical significance. The diagrams were plotted using GraphPad Prism 7.3 software.

Results

The cell viability of the gastric cancer cells treated with oak (Jaft) extract was investigated by MTT assay as explained in the method part. As shown in Figure 1, the half maximal inhibitory concentration (IC50) value of the Jaft extact was 162µg/ml with R^2=0.9886. Figure 1 shows the comparison of the case and the control groups regarding the relative expression of the *BAX* gene.

As shown in Figure 2, the expression of the *BAX* gene was increased in the intervention groups with a concentration of 162 (p<0.01) and 250 µg/ml (P<0.001) compared to the control group. Figure 3 shows the comparison of the case and control groups in terms of *BCL2* gene expression.

As shown in Figure 3, *BCL2* gene expression was different between the case and control groups (P<0.05).

In this regard, the expression of the *BCL2* gene was decreased in the intervention groups with the concentration of 162 and 250 µg/ml compared to the control group (p<0.05).

Table I: The primer sequences of BAX, and BCL2 and reference gene GAPDH

Primer	Primer sequences (5' to 3')	product length
BAX	F: AGATCATGAAGACAGGGGCC R: AGACACTCGCTCAGCTTCTT	136 bp
BCL2	F: GCCCTGTGGATGACTGAGTA R: GAAATCAAACAGAGGCCGCA	117 bp
GAPDH	F:TGGTATCGTGGAAGGACTCA R:CCAGTAGAGGCAGGGATGAT	101 bp

[Downloaded from ijpho.ssu.ac.ir on 2026-10-15]

[DOI: 10.18502/ijpho.v13i3.13128]

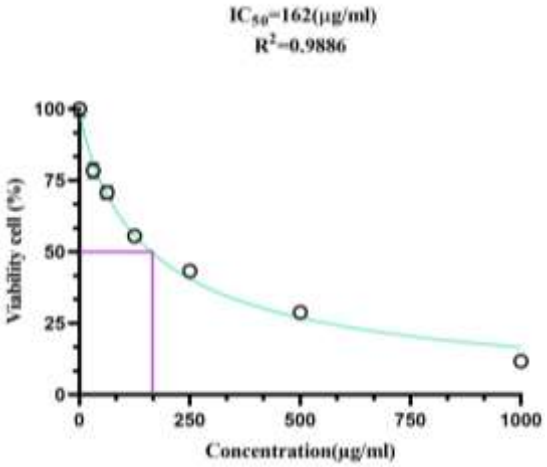

Figure 1: The effect of oak (Jaft) extract on cell viability

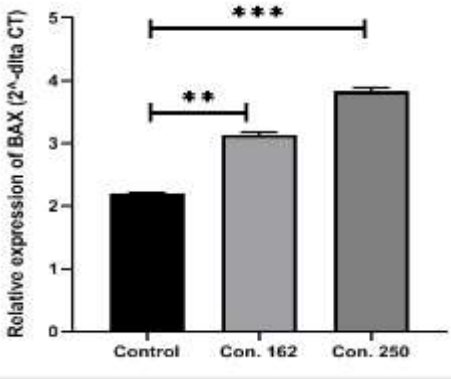

Figure 2: The comparison of *BAX* gene expression between the control group and AGS cell line under treatment with 162 and 250 µ/ml of Jaft extract (*p<0.01; *** p<0.001).

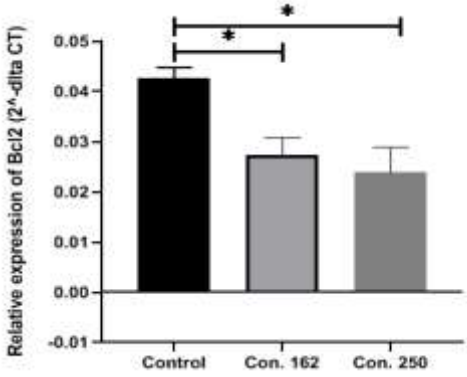

Figure 3: The comparison of *BCL2* gene expression between the control group and AGS cell line under treatment with 162 and 250 µ/ml of Jaft extract (*p<0.05).

Discussion

The IC50 value of Jaft extraction was 162 µg/ml. Moreover, the findings showed that the *BCL2* gene expression was decreased in the intervention groups (at concentrations 162 and 250 µg/ml) compared to the control group. In addition, the expression of the *BAX* gene was increased in the concentration of 162 and 250 µg/ml of Jaft extraction compared to the control group, indicating the increase of apoptosis of gastric cancer cells in the presence of Jaft extract.

No comprehensive study has been conducted regarding the extract of the Jaft on the cancer cell line, especially gastric cancer cells. Heidary et al., assessed the cytotoxic activity of the Jaft extract containing AgNPs on the MCF-7 cell line and investigated that the IC50 was 0.04 µg/mL at 24 h incubation, and revealed that the Jaft extract containing AgNPs may be a potential alternative agent for the treatment of patients with breast cancer (15). The findings of this study indicated that the AgNPs with Jaft extract (IC_{50}: 0.04 µg/ml) exert a stronger cytotoxic effect on cells than the Jaft extract of our study (162µ/ml). Some studies have also shown the anticancer effects of different Quercus species which are also known as oak (16-18).

Ismaeil et al., also assessed the anticancer effect of Quercus infectoria and observed that Quercus infectoria is a candidate for the prevention and therapy of cervical cancer (16). Abdalan et al., evaluated the effect of anticancer effect of hydroalcoholic and aqueous extract of Quercusus infectoria Leaf (a species of oak) in the HT29 cell line and demonstrated its potential role in cancer HT29 cell, regarding apoptosis (17). Moradi et al., evaluated the antiproliferative activity of crude ethyle alcohol extract of Quercus brantii *L*. acorn and revealed that it suppresses the proliferation of cancer cells using induction of apoptosis (18). Abdollah et al., examined the effect of *Q.*

[Downloaded from ijpho.ssu.ac.ir on 2026-10-15]

[DOI: 10.18502/ijpho.v13i3.13128]

infectoria aqueous on HeLa cervical cancer cells and observed that both aqueous and supercritical extraction inhibited the growth of HeLa cells (19). According to studies, the hydroalchoholic extract of Quercus persica L. can be a promising and appropriate herbal medicine due to the high efficacy and low toxicity of Quercus persica L (20).

Other characteristics of extracts of Jaft (Quercus Persica) have been reported in some studies. The antibacterial effect of the hydroalcoholic extract of Jaft has been seen in some studies (21, 22). Another study has demonstrated the anti-inflammatory effect of extract of galls of Quercus infectoria (23). The extract of this component inhibits the release of mediators of inflammation, such as O_2^- PGE_2, NO, and lytic enzymes. Moreover, gall extract inhibited formyl-Met-Leu-Phe (fMLP) stimulated degranulation in neutrophils. Therefore, the potency of gall extract may be relevant to cure various inflammatory pathologies (23).

Additionally, the antimicrobial effect of Oak Jaft (Quercus Persica) (10), wound healing and collagen synthesis of the internal layer of Iranian oak (24) have also been reported in various studies. The potent antioxidant activity of aqueous and methanolic extracts of Jaft is also revealed (25). Mirzaei et al., evaluated the hepaprotective effect of Jaft fruit and grape seed extracts and reported that the hepatoprotective effect of the mixture of Jaft and grape seed extract is due to antioxidant components (26).

Mirzaei et al., assessed the toxicity of Iranian Jaft of oak component. In this regard, animals were treated by Jaft extract (250, 500 and 1000 mg/kg for 28 days) and the findings showed that all hematological parameters were not changed between the two groups, except white blood cell. Additionally, no significant difference was seen in triglyceride, urea, cholesterol, alkalin phosphatase, and oxaloacetate transaminase between the two groups. According to these findings, it seems that Jaft extract can be applied safely in individuals, especially by the oral route (12).

Conclusion

It was found that Jaft extract increased the apoptosis of gastric cancer cells; therefore, it seems that the hydroalchoholic extract of Jaft is a promising anticancer medication.

Conflict of interest

The authors declare no conflict of interest.

References

1.Vivek Subbiah, Gauri Varadhachary,Gastric Adenocarcinoma in Children and Adolescents. Pediatr Blood Cance 2011; 57(3): 524–527.

2. HHartgrink H, Edwin PMJansen E, CTvan N. Gasteric Cancer. The Lancet 2009; 374; 9688: 8–14 477-490.

3. Lee Y , Bae K, Yoo H. Benefit of adjuvant traditional herbal medicine with chemotherapy for resettable gastric cancer. Integr Cancer Ther 2018; 17(3): 619–627.

4. Ghazanfari T. Cytotoxic effect of four herbal medicines on gastric cancer (AGS) cell line. Food Agric Immunol 2011; 1-7.

5. Ding W. Quercus acutissima Carruth. root extract triggers apoptosis, autophagy and inhibits cell viability in breast cancer cells. Journal of Ethnopharmacology 2022; 289(10): 115039-11542.

6. Malayeri A. Evaluating the effictiveness of aquous extract of Persian Oak (quecus castaneifolia C.A.Mey) fruit hull on dermal wound healing in rat model. Jundishapur J Nat Pharm Prod 2022; 1-9.

7. Taib M. Green Medicinal Uses, Phytochemistry, and Pharmacological Activities of *Quercus* Species. Evid Based Complement Alternat Med 2020; 2020: 1920683.

8. Xu J, Cao J, Yue J, Zhang X, Zhao Y. New triterpenoids from acorns of Quercus liaotungensis and their inhibitory activity against α -glucosidase, α -amylase and

[Downloaded from ijpho.ssu.ac.ir on 2026-10-15]

[DOI: 10.18502/ijpho.v13i3.13128]

protein-tyrosine phosphatase 1B. J Functional Foods 2018; 41:232–239.
9. Bahmani M. Oak (quercus brant): An over-review. J Chemical Pharmaceutical Res 2015, 7(1):634-639.
10. Cheraghi M. Antibacterial Effects of Oak Fruit, Jaft, and Jaftex Herbal Mouthwash: A Review. Research Square 2019; 1-9.
11. Khosravi A.D, Behzad A. Evaluation of the antibacterial activity of the seed hull of Quercus brantii onsome gram-negative bacteria. Pak. J. Med. Sci 2006; 22: 429-432.
12. Mirzaei A, Mirzaei N. Acute and Chronic of Iranian Jaft on Oak Fruit Component. Res. J. Biol. Sci 2012; 7 (9-12): 350-354.
13. Mahbobi M. Quercus infectoria fruit hulls and galls and female genital disorders. Clin. Phytoscience 2020; 6: 44; 1-9.
14. Nourozinia F, Rasmi Y, Otarod M, Golizadeh M, Khadem-Ansari M. Epidemiology and Histopathology of gastric cancer in Urmia. Studies in Medical Sciences 2013; 24 (3): 170-175.
15. Heidary R. Green Synthesis of Silver Nanoparticles Using Extract of Oak Fruit Hull (Jaft): Synthesis and In Vitro Cytotoxic Effect on MCF-7 Cells. International J Breast Canc 2015; 1-9.
16. Ismaeil I. A review of anticancer potential of quercus infection and its bioactive compounds. BIOMEDICINE 2021; 41(4):1-9.
17. Abdalan S, Baghbani-Arani F, Sadat Shandiz S A. Evaluation of Anticancer Effect of Aqueous and Hydroalcoholic Extracts of Quercusin Fectoria Leaf against Colon Cancer HT29 Cell Line. J Arak Uni Med Sci 2018; 21 (4): 48-57.
18. Moradi M. In vitro antiproliferative and apoptosis-inducing activities of crude ethyle alcohole extract of Quercus brantii L. acorn and subsequent fractions. Chinese J Natural Med 2016; 14 (3): 196-202.
19. Abdullah H, Ismail I, Suppian R. Induction of Apoptosis in HeLa Cervical Cancer Cells Treated with Aqueous and Supercritical Fluid Extracts of Quercus infectoria. RJP 2021; 8(4): 63-77.
20. Karimi A. Antiviral activity of Quercus persica L.: High efficacy and low toxicity. Adv. Biomed. Res 2013; 2(1):36-40.
21. Anaam M. Antimicrobial Effect of Hydroalcoholic Extracts of Oak Jaft (Quercus Persica) and Echium Amoenum on Shigella Flexneri. Qom J Univ Med Sci 2021; 15(5): 352-357.
22. Karimi Poor fard M, Mirzaei A, Kargar M, Khosravani SAM, Mohamadi R. Antibacterial Activities of Thymus Denaensis, Jaft and Hydro-Alcoholic Extract of Green hull Pistacia Atlantica on Listeria Monocytogenes. Armaghane-danesh, YUMS 2011; 17(1): 68-77.
23. Kaur G, Hamid H, Ali A, Alam M. S., Athar M. Antiinflammatory evaluation of alcoholic extract of galls of Quercus infectoria. J. Ethnopharmacol 2004; 90(2-3): 285–292.
24. Kakoie A , karimi A, Toghiani Sh. The Effect of Hydroalcoholic Extract Internal Layer of Iranian Oak (Quercus persica) on the Process of Wound Healing and Collagen Synthesis in Wistar Rats. Anim. Biol. J 2019; 1-9.
25. Toori M. Mirzaee M. Antioxidant and hepatoprotective effects of the internal layer of oak fruit (Jaft). J. Med. Plant Res 2013; 7(1): 24-28.
26. Mirzaei A. Hepatoprotectiv effect of Iranian grape seed and Jaft (a part of oak fruit) extracts against CCL4 induced liver toxicity in rats. Iran South Med J 2011; 14 (4): 230-239.

[Downloaded from ijpho.ssu.ac.ir on 2026-10-15]

[DOI: 10.18502/ijpho.v13i3.13128]