Research in Pharmaceutical Sciences, October 2022; 17(5): 585-593
Received: 07-07-2021
Peer Reviewed: 23-08-2021
Revised: 13-03-2022
Accepted: 14-06-2022
Published: 08-09-2022

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Original Article

Ferula gummosa gum exerts cytotoxic effects against human malignant glioblastoma multiforme *in vitro*

Amir R. Afshari[1], Seyed Hadi Mousavi[2,3], Golrokh Mousavi[3], Sanam Daneshpour Moghadam[4], Abolfazl Maghrouni[5], Hossein Javid[6], Zahra Tayarani-Najaran[7], Bahram Bibak[1], Hamid Mollazadeh[1], and Azar Hosseini[3,*]

[1]Department of Physiology and Pharmacology, School of Medicine, North Khorasan University of Medical Sciences, Bojnurd, I.R. Iran.
[2]Medical Toxicology Research Center, Mashhad University of Medical Sciences, Mashhad, I.R. Iran.
[3]Pharmacological Research Center of Medicinal Plants, Mashhad University of Medical Sciences, Mashhad, I.R. Iran.
[4]Department of Biology, Tonekabon Branch, Islamic Azad University, Tonekabon, I.R. Iran.
[5]Department of Medical Genetics, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, I.R. Iran.
[6]Department of Medical Biochemistry, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, I.R. Iran.
[7]Department of Pharmacodynamics and Toxicology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, I.R. Iran.

Abstract

Background and purpose: *Ferula gummosa* (*F. gummosa*), a potent medicinal herb, has been shown to possess anticancer activities *in vitro*. The present examination evaluated the cytotoxic and apoptogenic impacts of *F. gummosa* gum on the U87 glioblastoma cells.
Experimental approach: MTT assay to determine the cell viability, flow cytometry by annexin V/FITC-PI to apoptosis evaluation, reactive oxygen species (ROS) assay, and quantitative RT-PCR were performed.
Findings / Results: The results revealed that *F. gummosa* inhibited the growth of U87 cells in a concentration- and time-dependent manner with IC_{50} values of 115, 82, and 52 µg/mL obtained for 24, 48, and 72 h post-treatment, respectively. It was also identified that ROS levels significantly decreased following 4, 12, and 24 h after treatment. The outcomes of flow cytometry analysis suggested that *F. gummosa* induced a sub-G1 peak which translated to apoptosis in a concentration-dependent manner. Further examination revealed that *F. gummosa* upregulated Bax/Bcl-2 ratio and p53 genes at mRNA levels.
Conclusion and implications: Collectively, these findings indicate that sub-G1 apoptosis and its related genes may participate in the cytotoxicity of *F. gummosa* gum in U87 cells.

Keywords: Apoptosis; Bax; Bcl-2; *Ferula gummosa*; Glioblastoma.

INTRODUCTION

Glioblastoma multiforme (GBM), the most common malignant tumor in the brain, has an abysmal prognosis with a 15-month median survival rate for patients despite developments in tumor mass resection, chemotherapy (alkylating agent temozolomide), and radiation therapy approaches (1,2). Recent therapeutic protocols for GBM suggest combination therapy to improve the patients' survival with lower adverse effects related to each modality (3). Therefore, finding new compounds which can potentially be combined with standard of care treatments such as chemotherapy with temozolomide and radiotherapy is indispensable.

Access this article online

Website: http://rps.mui.ac.ir

DOI: 10.4103/1735-5362.355215

*Corresponding author: A. Hosseini
Tel: +98-5138002283-, Fax: +98-5138828567
Email: HoseiniAZ@mums.ac.ir

Unfortunately, even when multidisciplinary approaches are used, most patients' GBM recur within one to two years which would be unresponsive to the standard therapies (3). As the present treatment modalities of GBM are far from perfect, it is urgently expected to look for novel methodologies for treating this malignant disease.

Traditionally, medicinal plants have been one of the most well-known sources of medication for different disorders for a long time. In folk medicine, many believe that drugs derived from plants are safer than chemically manufactured drugs due to their natural origin. Especially, natural medicinal plants' roles in cancer therapy have become more noticeable (4,5). Exhibiting antitumor activity, inhibiting the recurrence and metastasis of cancer, attenuating the adverse reaction of chemotherapy, and improving patients' quality of life are the advantages of herbal therapy (6,7). *Ferula gummosa* belongs to the Apiaceae plant family which is a medicinal plant that mostly grows in western Asia and Europe and is known for its antispasmodic, analgesic, antibacterial, antidiarrheal, antifungal, and anticough properties (8). Besides, the *Ferula* genus has been well-known for its antioxidant and apoptotic activities, and cytotoxic effects (9). Besides *F. gummosa* demonstrates cytotoxic and apoptotic activities on various cancer cells (9,10). The apoptotic and anti-proliferative effects of *F. gummosa* have been found in human gastric and leukemic cells (10-12). Phytochemicals such as coumarin derivatives, sesquiterpenes, and sulfur-containing compounds isolated from *F. gummosa* function as anti-inflammatory, cytotoxic, and antioxidant agents. Therefore, apoptosis induction and cell proliferation control in malignant cells are considered essential in applying medicinal plants for cancer treatment, particularly in GBM (13).

Based on previous findings, introducing *F. gummosa* with promising cytotoxic and apoptotic properties, this investigation aimed to evaluate the cytotoxic and apoptogenic impacts of *F. gummosa* gum on the GBM cells (U87) for the first time which paves the way for future studies towards characterizing active ingredients of this compound in the hope of administration in combination with standard chemotherapy agents to enhance their efficiency and minimize their adverse effects. This study revealed that *F. gummosa* induced cytotoxicity and U87 cell death probably through modulation of gene expression involved in GBM pathophysiology.

MATERIALS AND METHODS

Chemicals

The MTT powder (98%) was obtained from Thermo Fisher (Thermo Fisher Scientific Inc., MA, USA). Fetal bovine serum (FBS) and high glucose Dulbecco's modified eagle's medium (DMEM) were provided from Gibco (Grand Island, NY, USA). Sodium hydroxide, ethylenediaminetetraacetic acid (EDTA), triton X-100, and sodium bicarbonate were obtained from Merck (Darmstadt, Germany). Propidium iodide (PI), trypan blue, and penicillin/streptomycin were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Preparation of F. gummosa gum

F. gummosa was collected from Sabzevar, Khorasan Razavi, Iran. The gum separation was carried out by making three cuts in one-third of the roots. After a week, 100 g of *F. gummosa* gum was collected. A voucher specimen (Voucher No. 34577) was submitted to the herbarium of the Faculty of Pharmacy, Mashhad University of Medical Sciences (MUMS), Mashhad, Iran.

Cell culture and drug treatment

The U87 cell line, obtained from the National Cell Bank of Iran, NCBI; Tehran, Iran, was kept in a humidified atmosphere (95%) with 5% CO_2 at 37 °C by routine passage every two days. Fifty mg of *F. gummosa* gum was dissolved in 2 mL of dimethyl sulfoxide (DMSO). After the cells were grown as monolayers (confluency 80%), they were incubated with various concentrations of *F. gummosa* gum (0-600 µg/mL). All the treatments were carried out in triplicate.

Cell viability assay

About 8×10^3 U87 cells per well were seeded in a 96-well plate, incubated overnight,

and treated with *F. gummosa* (0-600 μg/mL) extract for 24, 48, and 72 h. Then, the impact of *F. gummosa* on cell viability was measured by MTT assay based on our previous studies (14,15). After 3 h, the formed formazan residues were dissolved in DMSO. Next, the absorbance was read at 570 and 620 nm (background) using a Stat FAX303 ELISA reader (USA).

Measurement of reactive oxygen species levels

According to the instructions, this method was performed and analyzed by the DCFDA/H2DCFDA cellular reactive oxygen species (ROS) detection assay kit purchased from Abcam (Cambridge, United Kingdom). The cells (20×10^3/well) were seeded overnight in a 96-well dark-sided culture plate. U87 cells were incubated with H2DCFDA (25 μM) solution for 30-45 min in the dark at 37 °C. The cells were then rewashed and treated with *F. gummosa* (50, 100, 200 μg/mL) or tert-butyl hydroperoxide (TBHP, as a positive control, 150 μM) for 4, 12, and 24 h. The fluorescence intensity was measured at an excitation of 485 nm and emission of 535 nm with the fluorescence-activated cell sorter (FACS, Becton Dickinson, San Jose, USA).

Cell cycle analysis

7×10^5 cells were incubated overnight, containing 100, 200, and 400 μg/mL of *F. gummosa* gum. Following 24 h, U87 cells were centrifuged at 2,000 rpm for 5 m at 4 °C. Next, the cells were fixed in 70% ethanol, and after that, the cells were washed and re-suspended in ice-cold phosphate-buffered saline. RNase A (100 μL) was added to the cells and incubated for 30 m at 37 °C. Next, the cells were re-suspended in 400 μL PI/triton-X100 solution. Then, cell cycle distribution was analyzed from 10^4 cells by a flow cytometer (Becton Dickinson, Mountain View, CA, USA). Finally, the cell cycle analysis of flow cytometry data was done using FlowJo® vX.0.7 software (Tree Star, Ashland, OR, USA).

Quantitative real-time polymerase chain reaction

Total RNA was extracted from the treated cells (7×10^5 cells/well) using the RNeasy® mini kit (Qiagen GmbH, Hilden, Germany). RNAse was reverse-transcribed (RT) using the Prime-Script™ RT reagent kit (TaKaRa Holdings, Inc., Kyoto, Japan). The quantitative real-time polymerase chain reaction (qRT-PCR) was conducted (*F. gummosa* concentrations of 60 and 120 μg/mL, after 24 h) utilizing specific primers for *p53*, *Bcl-2*, *Bax*, and *GAPDH* (Table 1), which were ordered from Macrogene (Macrogene Co., Seoul, Korea). The cDNA amplification was done by the Light Cycler 96 RT-PCR system (Roche Applied Science, Pleasanton, CA, USA). Gene expression data were normalized to the *GAPDH* gene. The $2^{-\Delta\Delta Ct}$ method was used to analyze the relative expression of target genes.

Statistical analysis

The results are presented as the mean ± SD. The raw data were analyzed using GraphPad Prism® 7.01 (GraphPad Software, San Diego, CA, USA). The values were compared using a one-way analysis of variance (ANOVA) followed by the Dunnett test. *P*-values < 0.05 was considered statistically significant.

Table 1. The sequence of primers in the current study.

Gene Symbol	Gene name	Primer sequence (5'→ 3')	Accession number
TP53	Tumor protein P53	Forward: ACACGCTTCCCTGGATTGG Reverse: CTAGGATCTGACTGCGGCTC	NM_000546.6
Bax	Bcl-2-associated X protein	Forward: TGACGGCAACTTCAACTGGG Reverse: CTTCAGTGACTCGGCCAGGG	NM_001291428.2
Bcl2	B-cell lymphoma 2	Forward: GTCATGTGTGTGGAGAGCGTC Reverse: CCGTACAGTTCCACAAAGGCATC	NM_000633.3
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	Forward: TCAAGATCATCAGCAATGCCTCC Reverse: GCCATCACGCCACAGTTTC	NM_001357943.2

RESULTS

F. gummosa reduced the viability of U87 cells

U87 cells were influenced by different concentrations of *F. gummosa* (0-600 µg/mL) for 24, 48, and 72 h. As shown in Fig. 1, the inhibitory impacts of *F. gummosa* on the U87 cells increased with the time and concentration of treatment. The IC_{50} values, following *F. gummosa* treatment, were 115, 82, and 52 µg/mL for 24, 48, and 72 h, respectively (Fig. 1).

Impact of F. gummosa on ROS levels in U87 cells

ROS levels in cells treated with *F. gummosa* at 50, 100, and 200 µg/mL were measured using a fluorimeter (Epoch, BioTek® instruments, Inc, USA) after 4, 12, and 24 h of incubation. As presented in Fig. 2, the levels of ROS in the cells treated with *F. gummosa* showed a significant decrease in the early hours (4 and 12 h after treatment) at all concentrations, and a considerable decrease 24 h after treatment (only for 50 and 100 µg/mL) as compared to the control group, indicating that *F. gummosa* has antioxidant activity. Moreover, decrease in ROS level at 200 µg/mL was not significant.

F. gummosa induced sub-G1 peak apoptosis in U87 cells

The impact of *F. gummosa* on the cell cycle of U87 cells was examined by FACS analysis based on previous studies (15,16). The cells were incubated with 100, 200, and 400 µg/mL of *F. gummosa* for 24 h. As shown in Fig. 3, flow cytometry analysis of the cell cycle revealed that 24 h of treatment with *F. gummosa* remarkably elevated the percentage of cells (6.85 to 24.5 %) in the sub-G1 phase in a concentration-dependent manner compared to the control group.

Impact of F. gummosa on expression levels of Bax, Bcl-2, and p53 genes in U87 cells

The expression of genes involved in apoptosis in the cells treated with *F. gummosa* at 60 and 120 µg/mL was evaluated after 24 h using qRT-PCR. As shown in Fig. 4, we observed a considerable elevation in *Bax* and a reduction of *Bcl-2* expression. Also, the ratio of *Bax* to *Bcl-2* was significantly increased following treatment with *F. gummosa* compared to the control group in a concentration-dependent manner. Furthermore, the tumor suppressor p53 gene expression in the treated cells was significantly higher compared to the control group.

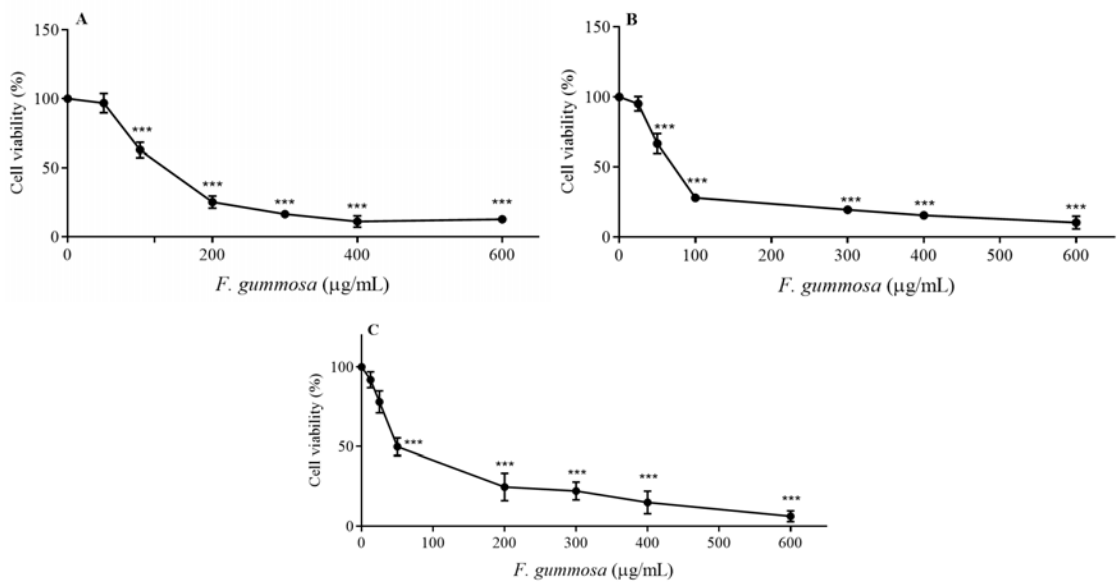

Fig. 1. Cytotoxic impacts of *Ferula gummosa* (*F. gummosa*) on U87 cells after (A) 24, (B) 48, and (C) 72 h of treatment. The data representing mean ± SD, n = 3. ***$P < 0.001$ Indicates significant differences compared to the control group.

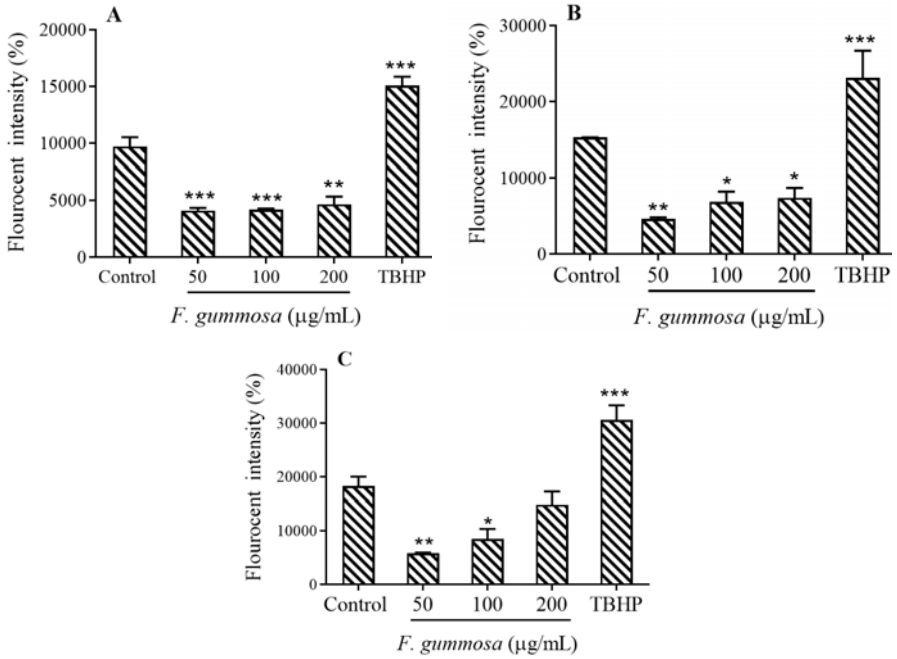

Fig. 2. The effects of *Ferula gummosa* (*F. gummosa*) on ROS levels in the U87 cell line. The cells were treated for (A) 4, (B) 12, and (C) 24 h with *F. gummosa* at 50, 100, and 200 µg/mL, and the ROS was measured by a fluorimeter. TBHP (150 µM) was used as the positive control. The data represent mean ± SD, n = 4. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 indicate significant differences compared to the control group. TBHP, Tert-butyl hydroperoxide; ROS, reactive oxygen species.

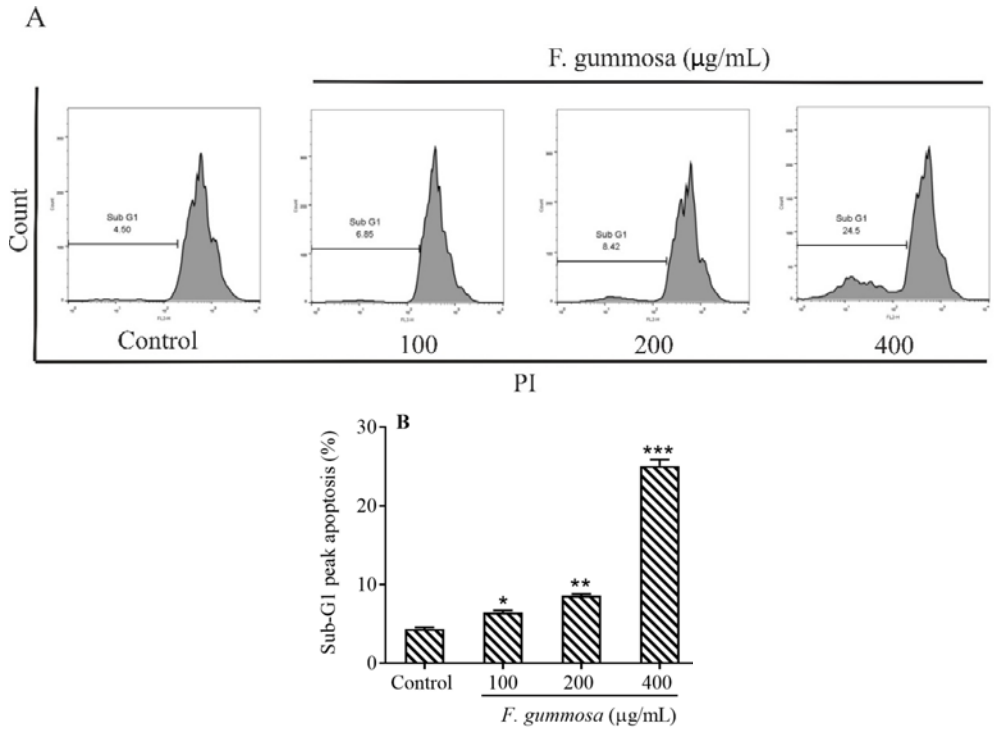

Fig. 3. Flow cytometry analysis of sub-G1 peak apoptosis in U87 cells. (A) Histograms of cell cycle distribution are depicting apoptosis in U87 cells treated for 24 h with *Ferula gummosa* (*F. gummosa)* at 100, 200, and 400 µg/mL. (B) Quantification of sub-G1 phase and cell population in each phase were analyzed by FlowJo software 24 h treatment with *F. gummosa*. Data are expressed as mean ± SD. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 indicate significant differences compared to the control group.

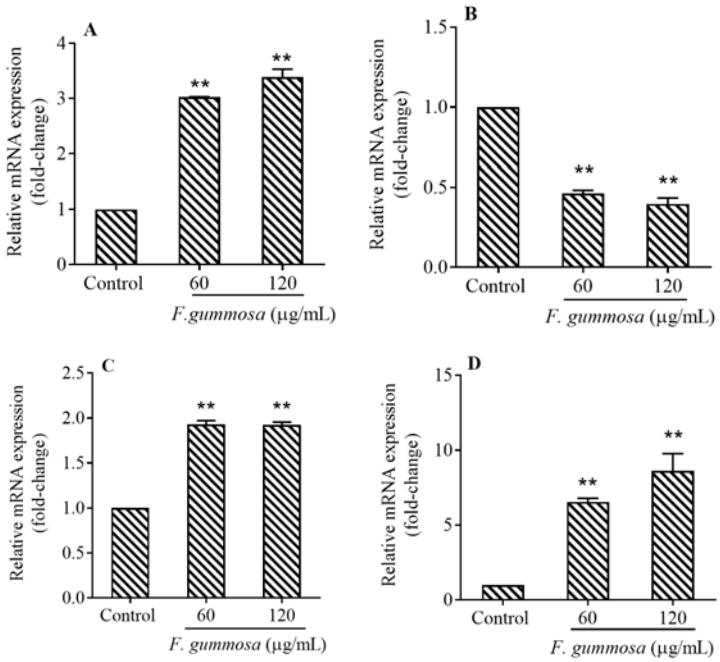

Fig. 4. The relative genes expression levels of (A) *Bax*, (B) *Bcl-2*, (C) *p53*, and (D) *Bax/Bcl-2* ratio in U87 GBM cells treated with *Ferula gummosa* (*F. gummosa*) at 60 and 120 µg/mL for 24 h were evaluated using the quantitative real-time polymerase chain reaction. Results were normalized against GAPDH in the samples. The data represent mean ± SD. $^{**}P < 0.01$ Indicates significant differences compared to the control group.

DISCUSSION

To our knowledge, the current study, for the first time, showed that *F. gummosa* might be a beneficial medicinal herb against GBM by triggering apoptosis *via* downregulation of involved genes and inducing cytotoxicity in U87 cells (Fig. 5).

Despite developments in neurosurgery, radiation therapy, and chemotherapy, GBM remains one of the most lethal astrocytic tumors (17). Due to several adverse effects of conventional treatments, the present research focused on harnessing the potential of natural products to develop novel anti-GBM medicines (18). Many studies have shown the growth inhibitory effects of biological agents against GBM (19). Thus, the current study demonstrated the cytotoxic and apoptogenic effects of *F. gummosa* on GBM cancer cells' growth for the first time.

It is accepted that a significant number of particular natural products have the potential to act as antitumor agents in humans. Natural phytochemicals are used as promising assets for chemotherapeutic drugs to raise their efficacy and reduce their adverse effects and toxicity. In this regard, *F. gummosa* is a well-known medicinal herb, which represents various pharmacological effects, including antinociceptive, expectorant, antihemolytic, antioxidant, antispasmodic, anti-epilepsy, antibacterial, wound healing, and anticancer (20).

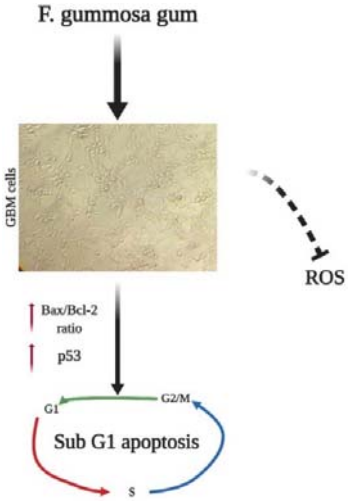

Fig. 5. The proposed mechanisms of *Ferula gummosa* on U87 cells.

The cytotoxic effects of *F. gummosa* have been shown in some studies, as well (9). It was demonstrated by Eslami *et al.* that *F. gummosa* has cytotoxic impacts on breast and oral cancer cells (21). The cytotoxicity of the hydroalcoholic extract of *F. gummosa* root was evaluated on Hela cells (22). However, the cytotoxic effects of *F. gummosa* on GBM remain unknown. In our study, the cytotoxic effect of *F. gummosa* gum was assessed on U87 cells. It was found that the viability of U87 cells was influenced by different concentrations of *F. gummosa* (0-600 µg/mL), time- and concentration-dependently. Also, the IC_{50} (50% inhibition of cell proliferation) values of 115, 82, and 52 µg/mL, following 24-, 48-, and 72-h treatment, were observed, respectively, in a time-dependent manner. These results indicated the higher cytotoxicity of *F. gummosa* in the last time point. A recent chemical analysis of *Ferula* species reported the presence of various phytochemicals, such as sulfur-containing compounds, sesquiterpenes, coumarin derivatives, and galbanic acid, inducing anticancer effects. Hence, the anticancer impact of *F. gummosa* gum might be through its active ingredients, including coumarins and galbanic acid.

Oxidative stress, mitochondrial dysfunction, and oncogenic stimulation cause ROS overproduction, promoting cellular damage and death. In fact, a high amount of intracellular ROS is one of the internal stimuli that can activate the intrinsic apoptotic pathway rendering it a compelling target of conventional chemotherapeutic agents (23). Hence, altering the intracellular ROS concentration and thus cellular redox homeostasis plays a crucial role in cancer cell annihilation (24). In the present study, ROS levels decreased after 4-, 12-, and 24-h treatment with *F. gummosa*, which could suggest that the cytotoxic effects of this plant are not through ROS-mediated pathways. An alternative explanation for this observation is that as most cancer cells are dependent on increased levels of ROS, F. *gummosa* could modulate this effect and therefore reduces cancer cells' survival or at least their aggressiveness through unknown mechanisms (25).

The cell cycle is one of the most disrupted cellular mechanisms in cancer cells, particularly in brain tumors (26,27). Thus, modulation of cell cycle mechanisms in cancer cells might be a beneficial strategy for controlling tumor proliferation and cell growth (28). The anticancer potential of *Ferula* genus in some cancer cell lines has been studied (29). Gharaei *et al.* exhibited that *F. gummosa* suppressed gastric cancer cells' proliferation through apoptosis induction (10). The cytotoxic impact of *F. gummosa* ethanolic extract on the cell cycle of BHY (a human oral squamous cell carcinoma) cells was through cell cycle arrest (9). The present study revealed *F. gummosa* (100, 200, and 400 µg/mL) significantly elevated the sub-G1 peak after 24-h treatment concentration-dependently, indicating it blocks cell cycle progression and consequently induces apoptosis.

Deregulation of the Bax to Bcl-2 proteins ratio is presented as a major player in apoptosis regulation (30). *F. gummosa*, in the present study, triggered apoptosis by increasing Bax/Bcl-2 expression ratio in the U87 GBM cell line, in line with the cell cycle arrest. Furthermore, p53, as a tumor suppressor, is the typically mutated pathway in numerous cancers (31). It regulates tumor growth, cell development, and cell cycle progression. The elevation in p53 gene expression could help to control cancer by apoptosis induction (*i.e.* through Bax/Bcl-2 upregulation) (32). Studies have shown that the activation of the p53 pathway is related to the development of GBM (33). The current research exhibited *F. gummosa* elevated the gene expression of p53 at the transcriptional level in GBM cells, which might consequently promote apoptosis and cell cycle disruption.

CONCLUSION

We confirmed that the results of this study provide the first report that *F. gummosa* gum diminished ROS levels and induced cytotoxicity, and also blocked the cell cycle progression in U87 cells. This apoptotic response might be related to the modulation of the expression of the Bcl-2 gene family. In summary, our study's outcomes call for further

investigation on *F. gummosa* and its main ingredients, such as coumarins and galbanic acid, as sources of natural pharmacological products. After further mechanistic studies, we revealed that *F. gummosa* could be a promising medicinal plant for treating GBM. Further studies on *F. gummosa* affect the internal or external pathways of apoptosis, as well as its effect on the cell cycle, are highly recommended.

Acknowledgments

This work was a part of a *Pharm. D* thesis which was submitted by A.R. Afshari and financially supported by the Vice-Chancellor of Research, Mashhad University of Medical Sciences, Mashhad, Khorasan Razavi, Iran through Grant No. 951404. We appreciate all members of the Research Laboratory of Medical Biochemistry, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran., for their support in this study.

Conflict of interest statement

All authors declared no conflict of interest in this study.

Authors' contribution

A.R. Afshari and S.H. Mousavi designed and supervised the project; G. Mousavi, S. Daneshpour Moghadam, A. Maghrouni, and H. Javid performed all the experiments; Z. Tayarani-Najaran provided the *Ferula gummosa* for cellular treatment; B. Bibak and H. Mollazadeh co-wrote the paper; A. Hosseini worked out almost all of the statistical analyses and funded the project. The last version of the article was approved by all the authors.

REFERENCES

1. Hou LC, Veeravagu A, Hsu AR, Tse VCK. Recurrent glioblastoma multiforme: a review of natural history and management options. Neurosurg Focus. 2006;20(4):E3,1-9. DOI: 10.3171/foc.2006.20.4.2.
2. Jalili-Nik M, Sabri H, Zamiri E, Soukhtanloo M, Roshan MK, Hosseini A, *et al.* Cytotoxic effects of *Ferula Latisecta* on human glioma U87 cells. Drug Res (Stuttg). 2019;69(12):665-670. DOI: 10.1055/a-0986-6543.
3. Zhao M, Straten DV, Broekman MLD, Preat V, Schiffelers RM. Nanocarrier-based drug combination therapy for glioblastoma. Theranostics. 2020;10(3):1355-1372. DOI: 10.7150/thno.38147.
4. Khajeh E, Rasmi Y, Kheradmand F, Malekinejad H, Aramwit P, Saboory E, *et al*. Crocetin suppresses the growth and migration in HCT-116 human colorectal cancer cells by activating the p-38 MAPK signaling pathway. Res Pharm Sci. 2020;15(6):592-601. DOI: 10.4103/1735-5362.301344.
5. Iranmanesh M, Mohebbati R, Forouzanfar F, Karimi Roshan M, Ghorbani A, Jalili Nik M, *et al. In vivo* and *in vitro* effects of ethanolic extract of *Trigonella foenum-graecum* L. seeds on proliferation, angiogenesis and tube formation of endothelial cells. Res Pharm Sci. 2018;13(4):343-352. DOI: 10.4103/1735-5362.235161.
6. Zhou GS, Song LJ, Yang B. Isoliquiritigenin inhibits proliferation and induces apoptosis of U87 human glioma cells *in vitro*. Mol Med Rep. 2013;7(2):531-536. DOI: 10.3892/mmr.2012.1218.
7. Afshari AR, Jalili-Nik M, Soukhtanloo M, Ghorbani A, Sadeghnia HR, Mollazadeh H, *et al*. Auraptene-induced cytotoxicity mechanisms in human malignant glioblastoma (U87) cells: role of reactive oxygen species (ROS). EXCLI J. 2019;18:576-590. DOI: 10.17179/excli2019-1136.
8. Sahebkar A, Iranshahi M. Biological activities of essential oils from the genus *Ferula* (*Apiaceae*). Asian Biomed. 2010;4(6):835-847. DOI: 10.2478/abm-2010-0110.
9. Gudarzi H, Salimi M, Irian S, Amanzadeh A, Mostafapour Kandelous H, Azadmanesh K, *et al*. Ethanolic extract of *Ferula gummosa* is cytotoxic against cancer cells by inducing apoptosis and cell cycle arrest. Nat Prod res. 2015;29(6):546-550. DOI: 10.1080/14786419.2014.951854.
10. Gharaei R, Akrami H, Heidari S, Asadi MH, Jalili A. The suppression effect of *Ferula gummosa* Boiss. extracts on cell proliferation through apoptosis induction in gastric cancer cell line Eur J Integr Med. 2013;5(3):241-247. DOI: 10.1016/j.eujim.2013.01.002.
11. Moradzadeh M, Sadeghnia HR, Mousavi SH, Mahmoodi M, Hosseini A. *Ferula gummosa* gum induces apoptosis *via* ROS mechanism in human leukemic cells. Cell Mol Biol (Noisy-le-grand). 2017;63(11):17-22. DOI: 10.14715/cmb/2017.63.11.4.
12. Gharaei R, Ghobadi S, Akrami H. Study of apoptosis inducing effects of ethanol extract of Ferula gummosa leaf. Clin Biochem. 2011;44(13):S332. DOI: 10.1016/j.clinbiochem.2011.08.819.
13. Valdes-Rives SA, Casique-Aguirre D, German-Castelan L, Velasco-Velazquez MA, Gonzalez-Arenas A. apoptotic signaling pathways in glioblastoma and therapeutic implications. Biomed Res Int. 2017;2017:7403747,1-12. DOI: 10.1155/2017/7403747.
14. Sadeghnia HR, Jamshidi R, Afshari AR, Mollazadeh H, Forouzanfar F, Rakhshandeh H. *Terminalia chebula* attenuates quinolinate-induced oxidative

PC12 and OLN-93 cell death. Mult Scler Relat Disord. 2017;14:60-67.
DOI: 10.1016/j.msard.2017.03.012.

15. Afshari AR, Karimi Roshan M, Soukhtanloo M, Ghorbani A, Rahmani F, Jalili-Nik M, *et al*. Cytotoxic effects of auraptene against a human malignant glioblastoma cell line. Avicenna J Phytomed. 2019;9(4):334-346.
PMID: 31309072.
16. Poonaki E, Ariakia F, Jalili-Nik M, Shafiee Ardestani M, Tondro G, Samini F*, et al*. Targeting BMI-1 with PLGA-PEG nanoparticle-containing PTC209 modulates the behavior of human glioblastoma stem cells and cancer cells. Cancer Nanotechnol. 2021;12:5,1-20.
DOI: 10.1186/s12645-021-00078-8.
17. Boussiotis VA, Charest A. Immunotherapies for malignant glioma. Oncogene. 2018;37(9):1121-1141.
DOI: 10.1038/s41388-017-0024-z.
18. Sathornsumetee S, Reardon DA, Desjardins A, Quinn JA, Vredenburgh JJ, Rich JN. Molecularly targeted therapy for malignant glioma. Cancer. 2007;110(1):13-24.
DOI: 10.1002/cncr.22741.
19. Hajimehdipoor H, Esmaeili S, Ramezani R, Jafari Anaraki M, Mosaddegh M. The cytotoxic effects of *Ferula persica* var. *persica* and *Ferula hezarlalehzarica* against HepG2, A549, HT29, MCF7 and MDBK cell lines. Iran J Pharm Sci. 2012;8(2):115-119.
20. Sadraei H, Asghari GR, Hajhashemi V, Kolagar A, Ebrahimi M. Spasmolytic activity of essential oil and various extracts *of Ferula gummosa* Boiss. on ileum contractions. Phytomedicine. 2001;8(5):370-376.
DOI: 10.1078/0944-7113-00052.
21. Eslami Jadidi B, Dehpouri A, Nemati F, Rezaei B. Cytotoxicity effects of the *Ferula gummosa* extract on the cancer cell line Mcf7. J Anim Biol. 2013;5: 4,1-7.
22. Forouzmand SH, Mousavi SH, Vazifedan V, Nourbakhsh M, Chamani J, Hoseini A, *et al.* Synergistic effects of *Ferula gummosa* and radiotherapy on induction of cytotoxicity in HeLa cell line. Avicenna J Phytomed. 2018;8(5):439-477.
PMID: 30345231.
23. Ivanova D, Zhelev Z, Aoki I, Bakalova R, Higashi T. Overproduction of reactive oxygen species - obligatory or not for induction of apoptosis by anticancer drugs. Chin J Cancer Res. 2016;28(4):383-396.
DOI: 10.21147/j.issn.1000-9604.2016.04.01.
24. Trachootham D, Alexandre J, Huang P. Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? Nat Rev Drug Discov. 2009;8(7):579-591.
DOI: 10.1038/nrd2803.
25. Perillo B, Di Donato M, Pezone A, Di Zazzo E, Giovannelli P, Galasso G, *et al.* ROS in cancer therapy: the bright side of the moon. Exp Mol Med. 2020;52(2):192-203.
DOI: 10.1038/s12276-020-0384-2.
26. Sharma A, Kaur M, Katnoria JK, Nagpal AK. Polyphenols in food: cancer prevention and apoptosis induction. Curr Med Chem. 2018;25(36):4740-4757.
DOI: 10.2174/0929867324666171006144208.
27. Piperigkou Z, Manou D, Karamanou K, Theocharis AD. Strategies to target matrix metalloproteinases as therapeutic approach in cancer. Methods Mol Biol; 2018;1731:325-348.
DOI: 10.1007/978-1-4939-7595-2_27.
28. Massague J. G1 cell-cycle control and cancer. Nature. 2004;432(7015):298-306.
DOI: 10.1038/nature03094.
29. Asemani Y, Azadmehr A, Hajiaghaee R, Amirghofran Z. Anticancer potential of *Ferula hezarlalehzarica* Y. Ajani fraction in Raji lymphoma cell line: induction of apoptosis, cell cycle arrest, and changes in mitochondrial membrane potential. Daru. 2018;26(2):143-154.
DOI: 10.1007/s40199-018-0219-z.
30. Kaufmann SH, Earnshaw WC. Induction of apoptosis by cancer chemotherapy. Exp Cell Res. 2000;256(1):42-49.
DOI: 10.1006/excr.2000.4838.
31. Harris BRE, Wang D, Zhang Y, Ferrari M, Okon A, Cleary MP*, et al.* Induction of the p53 tumor tuppressor in cancer cells through inhibition of cap-dependent translation. Mol Cell Biol. 2018;38(10):e00367-17,1-39.
DOI: 10.1128/MCB.00367-17.
32. Da Costa DCF, Fialho E, Silva JL. Cancer chemoprevention by resveratrol: the p53 tumor suppressor protein as a promising molecular target. Molecules. 2017;22(6):1014,1-24.
DOI: 10.3390/molecules22061014.
33. Naumann U, Wolburg SKH, Wick W, Rascher G, Schulz JB, *et al*. Chimeric tumor suppressor 1, a p53-derived chimeric tumor suppressor gene, kills p53 mutant and p53 wild-type glioma cells in synergy with irradiation and CD95 ligand. Cancer Res. 2001;61(15):5833-5842.
PMID: 11479223.