BOLETIN LATINOAMERICANO Y DEL CARIBE DE PLANTAS MEDICINALES Y AROMÁTICAS
© / ISSN 0717 7917 / www.blacpma.ms-editions.cl

Articulo Original / Original Article

Anticancer activity of *Inula graveolens* by induction of ROS-independent apoptosis and suppression of IL6-IL8 in cervical cancer cells

[Actividad anticancerígena de *Inula graveolens* por inducción de apoptosis independiente de ROS y supresión de IL6-IL8 en células de cáncer de cuello uterino]

Onder Yumrutas & Ibrahim Bozgeyik

Department of Medical Biology, Faculty of Medicine, University of Adiyaman, Adıyaman-Turkey

Reviewed by:
José A. Salgado-Chavez
Universidad de La Guajira
Colombia

Ahmed Salah Naser
University of Mosul
Iraq

Correspondence:
Onder **YUMRUTAS**:
oyumrutas@adiyaman.edu.tr

Section Biological activity

Received: 26 February 2022
Accepted: 18 March 2022
Accepted corrected: 1 April 2022
Published: 30 May 2023

Citation:
Yumrutas O, Bozgeyik I
Anticancer activity of Inula graveolens by induction of ROS-independent apoptosis and suppression of IL6-IL8 in cervical cancer cells
Bol Latinoam Caribe Plant Med Aromat
22 (3): 314 - 325 (2023).
https://doi.org/10.37360/blacpma.23.22.3.23

Abstract: Cancer is an important disease that causing to deaths in the world. Cervical cancer is one of the most common among women and must be treated quickly to prevent cell proliferation. Natural products have been used for cancer treatment due to their antiproliferative and apoptosis induction properties. In the present study, we aimed to determine the antiproliferative and apoptosis induction activities of methanol extract of *Inula graveolens* (IGME) in the human cerival cancer cell line (HeLa). Antiproliferative activity of IGME was evaluated by using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) method. Apoptosis induction activity was determined by AnnexinV/propodium iodide staining with FACS. ROS induction was determined by DCFH-DA (2′,7′-Dichlorofluorescin diacetate) staining. Interleukin 6 and 8 levels (IL-6 and IL-8) were determined by the Elisa method. IGME exhibited antiproliferative effect and induced apoptosis in the HeLa cells. IL-6, IL-8 and intracellular ROS levels were decreased after treatment of IGME. Consequently, IGME was found to have antiproliferative and apoptosis induction activities as an indicator of the anticancer activity.

Keywords: *Inula graveolens*; Antiproliferation; Apoptosis; Intracellular reactive oxygene species; Phenolic

Resumen: El cáncer es una enfermedad importante que causa muertes en el mundo. El cáncer de cuello uterino es uno de los más comunes entre las mujeres y debe tratarse rápidamente para evitar la proliferación celular. Los productos naturales se han utilizado para el tratamiento del cáncer debido a sus propiedades anti proliferativas y de inducción de la apoptosis. En el presente estudio, nuestro objetivo fue determinar las actividades de inducción de apoptosis y antiproliferativas del extracto de metanol de *Inula graveolens* (IGME) en la línea celular de cáncer de cuello uterino humano (HeLa). La actividad antiproliferativa de IGME se evaluó utilizando el método MTT (bromuro de 3-[4,5-dimetiltiazol-2-il]-2,5-difenil-tetrazolio). La actividad de inducción de apoptosis se determinó mediante tinción con anexina V/yoduro de propodio con FACS. La inducción de ROS se determinó mediante tinción con DCFH-DA (diacetato de 2',7'-diclorofluoresceina). Los niveles de interleucina 6 y 8 (IL-6 e IL-8) se determinaron por el método Elisa. IGME exhibió un efecto antiproliferativo e indujo apoptosis en las células HeLa. Los niveles de IL-6, IL-8 y ROS intracelulares disminuyeron después del tratamiento con IGME. En consecuencia, se encontró que IGME tenía actividades antiproliferativas y de inducción de apoptosis como indicador de la actividad anticancerígena.

Palabras clave: *Inula graveolens*; Antiproliferación; Apoptosis; Especies reactivas de oxigeno intracelular; Fenólico

INTRODUCTION

Cervical cancer accounts for 6.6% of cancer cases in women and ranks fourth in cancer-related deaths (Bray *et al*., 2018). It is associated with infection with human papillomavirus. In 2018, 570,000 women were diagnosed with cervical cancer worldwide, and 311,000 of them died of this disease (WHO, 2022). Although immunotherapy, radiation therapy and chemotherapy are used in the treatment of cervical cancer, it is important to identify new complementary strategies such as natural compounds that will contribute to these treatments. As a source of natural compounds, plants play an important role in inhibiting and eliminating the proliferation of different tumor cells (Yumrutas *et al*., 2015; Iqbal *et al*., 2017; Yumrutas *et al*., 2018; Ege *et al*., 2020; Mohammed *et al*., 2021; Cocelli *et al*., 2021).

The *Inula* genus, which was represented by 27 species in Turkey, belongs to the Asteraceae and grows in many parts of the world, especially the Mediterranean. It has been determined in previous studies that Inula species have many important compounds (Silinsin & Bursal, 2018; El Omari *et al*., 2021). There are also many reports on biological activities of *Inula* species such as antiproliferative (Karan *et al*., 2018), antinociceptive (Zarei *et al*., 2018), analgesic, anti-inflammatory (Paliwal *et al*., 2017), antioxidant and antifungal (Mahmoudi *et al*., 2016). *Inula graveolens* (L.) Desf. is a fragrant plant belonging to this genus. In previous studies, it was mostly focused on the biological activities such as antioxidant and antimicrobial of essential oils of *I. graveolens*. (Topcu *et al*., 1993; Al-Fartosy, 2011). In addition, there are studies showing the cytotoxic and antiproliferative effects of extracts obtained from *I. graveolens*. Karan *et al*. (2018) reported that bornyl acetate and essential oil extracts of *I. graveolens* inhibit the proliferation of different cancer cells. However, studies showing the anticancer activity of *I. graveolens* in detail are very limited. Hence, in this study, the viability of HeLa cells, induction of apoptosis, the level of ROS, and levels of IL6 and IL8 thought to be involved in the pathogenesis of cervical cancer were evaluated to determine the possible anticancer activity of IGME (*I. graveolens* methanol extract). In addition, the antioxidant activity of IGME was also determined by cellular antioxidant activity (CAA) and DPPH scavenging activity tests.

MATERIALS AND METHODS

Collection of *I. graveolens*

I. graveolens was collected in Adıyaman-Turkey in the flowering period.

Preparation of IGME

I. graveolens were air dried in a dark room and its aerial parts were pulverized. Then, 40 g of each plant powder was extracted by Soxhlet apparatus. For the preparation of methanol extract in the Soxhlet apparatus, *I. graveolens* was treated with methanol for 6 hours at 60°C. The methanol into the solution was removed by rotary evaporator at 50°C and remaining solid material was preserved at +4ºC until use.

Maintenance of cell culture

The HeLa cells were cultured in DMEM (Dulbecco's Modified Eagle Medium) with 10% of FBS (Fetal Bovine Serum) and 1% of penicillin/streptomycin and sustained at 37°C in a moistened atmosphere with consisting of 5% CO_2 and 95% air incubation.

Determination of IGME on HeLa Cell Viability by MTT (3- [4,5- dimethylthiazol- 2- yl]- 2,5- diphenyl- tetrazolium bromide) method

HeLa cells were cultured in 96-well plates and complete growth medium with FBS was replaced with serum free (SF) medium after cells reached 70-80% confluency and incubated for 24 hours. Subsequently, HeLa cells were incubated with different concentrations (25, 50, 100 and 200 µg/mL) of IGME for a period of 24 hours. As a positive control, cells were grown in the presence of 10% FCS were used. Cell viability was evaluated by using the MTT method. Culture medium was replaced with SF medium containing 1 mg/mL MTT (Sigma) and incubated at 37°C for 15 min. MTT solution was further removed and blue formazan crystals were dissolved by using DMSO (Sigma). The color change was read at 550 nm with a colorimetric reader (Biochrom, UK).

Determination of Apoptosis Induction

To determine the induction of apoptosis, HeLa cells were seeded with a $1x10^6$/mL density to 6-well plates and all doses of IGME were applied for a period of 24 hours. Annexin V/PI (Becton Dickinson, Pharmingen, UK) apoptosis detection kit was used to measure cellular apoptosis and the manufacturer' recommended protocols were followed. Results were measured in Becton-Dickinson FACSort flow cytometer.

Determination of IL6 and IL8 levels

HeLa cells were incubated for 24 hours after treatment of IGME. Then, supernatants were taken and interleukin 6 (IL6) and Interleukin 8 (IL8) levels were determined with the Elisa kits according to the manufacturer's protocol.

Determination of DPPH scavenging activity of IGME

DPPH free anion radical scavenging activities of IGME were determined according to the method of Saint-Cricq de Gaulejac *et al*. (1999). Briefly, 0.1 mL of different concentrations of IGME was taken and placed in a separate test tube and 2.9 mL of 6×10^{-5}. Then, these solutions were kept in the dark and at room temperature for 60 minutes and then measured at 517 nm in a spectrophotometer. Rosmarinic acid and quercetin, strong antioxidant compounds, were used as positive controls in the study.

Determination of CAA of IGME

Cellular antioxidant activity (CAA) of IGME on HeLa cells was assessed according to the method of Wolfe *et al*. (2008). Briefly, cells were dispersed to 96-well plates with a density of $6x10^4$ per milliliter and incubated for a period of 24 h. Further, the growth medium was removed, and cells were washed with PBS. Then wells were incubated with the 25 µM of 2′,7′-dichlorofluorescein diacetate (DCFH-DA) and 100 µL of SPE for 1 hour. Subsequently, wells were washed with 100 µL of PBS solution and 600 µM of 2,2′- azobis(2-ethylpropionamidine) dihydrochloride (ABAP) were added. Results were read at emission 538 nm and excitation 485 nm wavelengths every 5 minutes for 1 hour in florescence spectrophotometer (Termo, Germany).

Determination of Intracellular ROS Levels after Treatment of IGME

Determination of intracellular reactive oxygen species (ROS) production was achieved as described by Sreelatha *et al*. (2011). Shortly, cells were seeded with a 2×10^5 cells/well density and treated with the various concentrations of DCM and MeOH extracts for a period of 24 hours. Subsequently, cells were trypsinized and washed with HBSS and resuspended in 10µM DCFH-DA solution and incubated at 37°C for 30 minutes. Following incubation with DCFH-DA solution, 4mM H_2O_2 was added to each well and subjected to a second incubation at 37°C for 30 minutes. Lastly, measurements were performed using fluorescence spectrophotometer (Thermo, Germany).

Determination of phenolic compounds in IGME by LC-MS-MS

To determine phenolics of IGME were dissolved samples in methanol and then filtrated with 0.22 µM filter. The LC-MS-MS apparatus of Nexera UHPLC (Shimadzu) with LC-20AD two pumps, DGU-20A3R degasser, CTO-10ASVP column furnace and SIL-20AC autosampler was used for the study. C18 Intersil ODS-4 analytical colon (3.0 mm x 100 mm, 2 µm) was used. The injection volume was 2 µL and flow rate 0.3 mL/min. Mobile phase A (Water and 0.1% Formic acid) and mobile phase B (Methanol and 0.1% Formic acid) were used in a linear gradient flow and the column temperature was set at 40°C initially.

Statistical analysis

Statistical analyses and figures were performed and prepared using the Prism 7.01 program. The normal distributions of DPPH, MTT, and IL6 and IL8 were evaluated one-way analysis of variance (ANOVA). Dunnett test was used to compare the doses applied with controls. Tukey's pair-wise multiple comparison test was used to determine the differences between the groups of significant variables. The results were given as mean ± SD. The level of significance was accepted to be at least $p<0.05$.

RESULTS

Determination of DPPH scavenging activity

DPPH scavenging activity of different doses of IGME was determined and given in the Figure No. 1. As can be seen the figure, DPPH scavenging activity of IGME increased in a dose-dependent manner. When compared with the control groups, it was determined that IGME extracts exhibited low antioxidant activity ($p>0.05$) and only moderate activity at 200 µg/mL ($p<0.01$).

Determination of CAA after treatment of IGME

In CAA test, the fluorescence level decreases due to the increase in the antioxidant activity of a substance. All doses of IGME were applied to HeLa cells for 60 min and the intercellular antioxidant level was determined. As can be seen in Figure No. 2, 25 and 50 µg/mL doses of IGME did not exhibit a CAA compared to control (DMSO). Moreover, 100 and 200 µg/mL doses strongly exhibited CAA. Especially, the 200 µg/mL dose of IGME was found to have higher CAA than that of other doses.

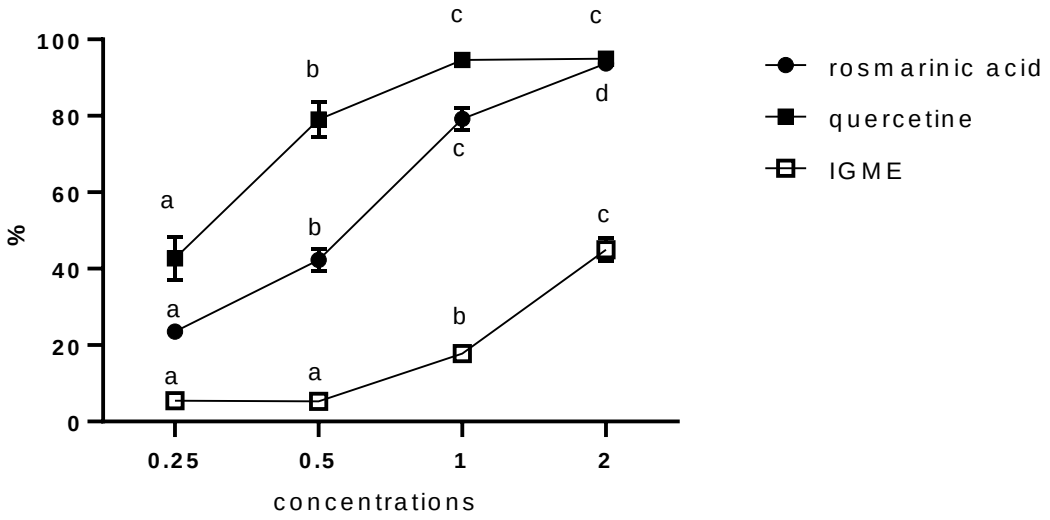

Figure No. 1
DPPH scavenging activity of IGME (mg/mL), rosmarinic acid and quercetin (µg/mL). The extract and positive controls were used as mg/mL. Tukey's pair-wise multiple comparison test was used to determine the differences between the doses of the samples themselves

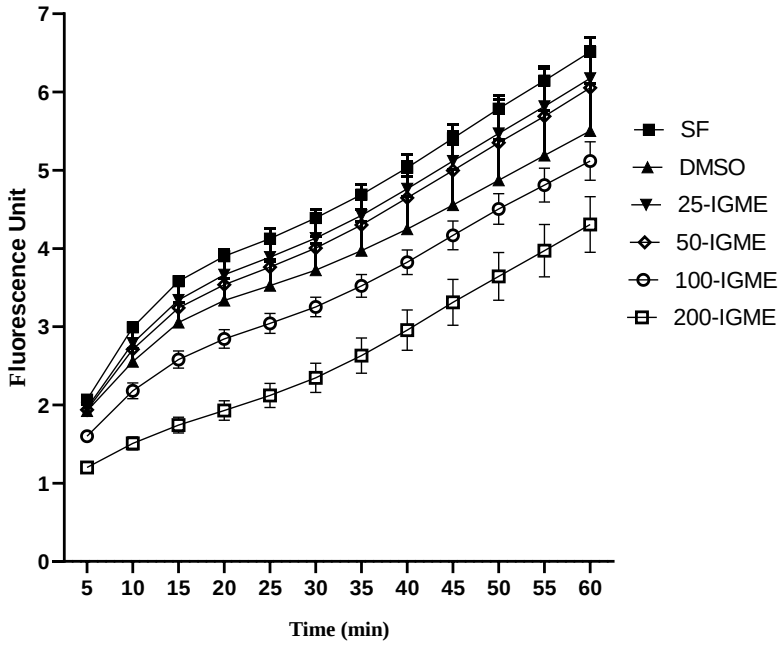

Figure No. 2
CAA of all the doses of IGME

Determination of intracellular ROS content in HeLa cells after treatment of IGME

Intracellular ROS levels were determined after treatment of all the doses of IGME for 1 h and the results were given in Figure No. 3. Similar to the CAA activity, it was observed that extracts at 25 and 50 µg/mL doses had no effect on ROS level. Furthermore, it was determined that the ROS level decreased slightly after the administration of 100 µg/mL dose of IGME, but significantly decreased in the application of 200 µg/mL dose

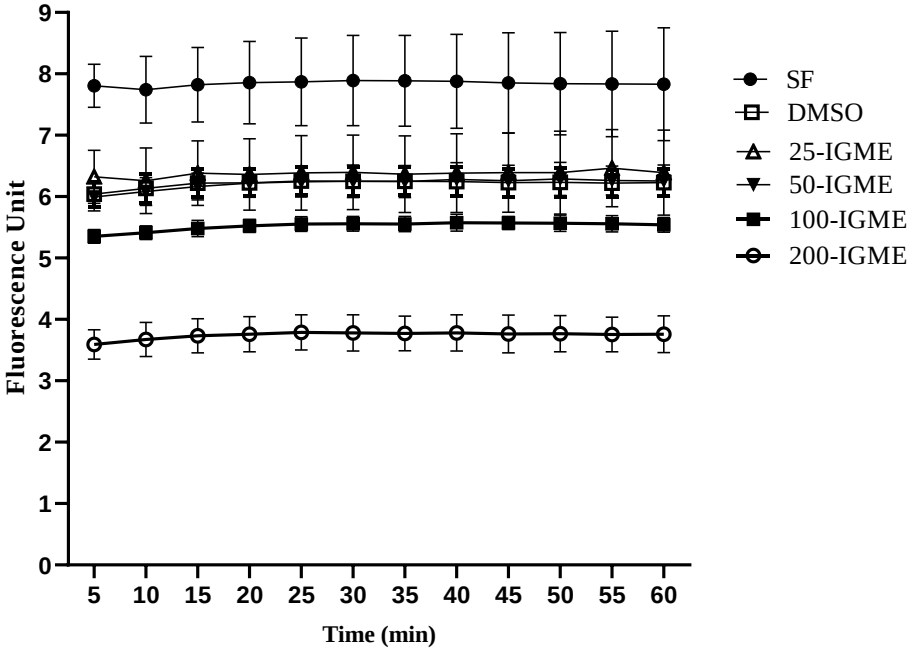

Figure No. 3
Intracellular ROS levels of cervical cancer cells after treatment of IGME

Determination of Antiproliferation activity of IGME
To determine the possible effects on the proliferation of HeLa cells, all the doses of IGME were exposed for 24 hours. Then, the viability of the cells was determined by MTT staining and the results were given in Figure No. 4. As can be seen in Figure 4, all the doses of IGME decreased the viability and proliferation of HeLa cells in a dose dependent manner (p<0.001). The highest antiproliferation activity was observed at a dose of 200 µg/mL (p<0.001).

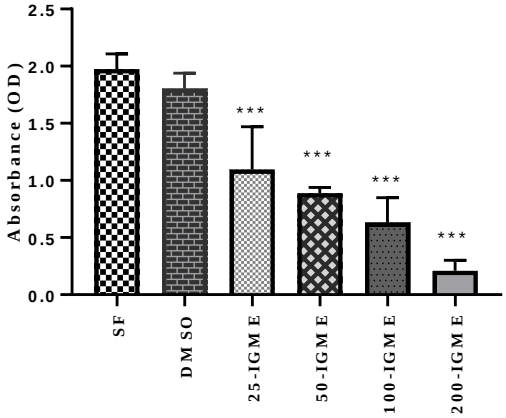

Figure No. 4
Viability of Hela cells after treatment of IGME.
Dunnett test was used to compare the doses applied with controls

Determination of Apoptosis in HeLa cells after IGME application

Only 25 and 200 mg /mL doses of IGME were used to determine apoptosis in HeLa cells. After 24 hours of IGME application, early and late apoptotic and necrotic cell percentages of HeLa cells were determined in a FACS. As can be seen Figure No. 5, no difference was observed in the percentages of early and necrotic cells, while the percentage of late apoptotic cells increased at both 25 (23.74%) and 200 µg/ml (24.25%) doses compared to the control.

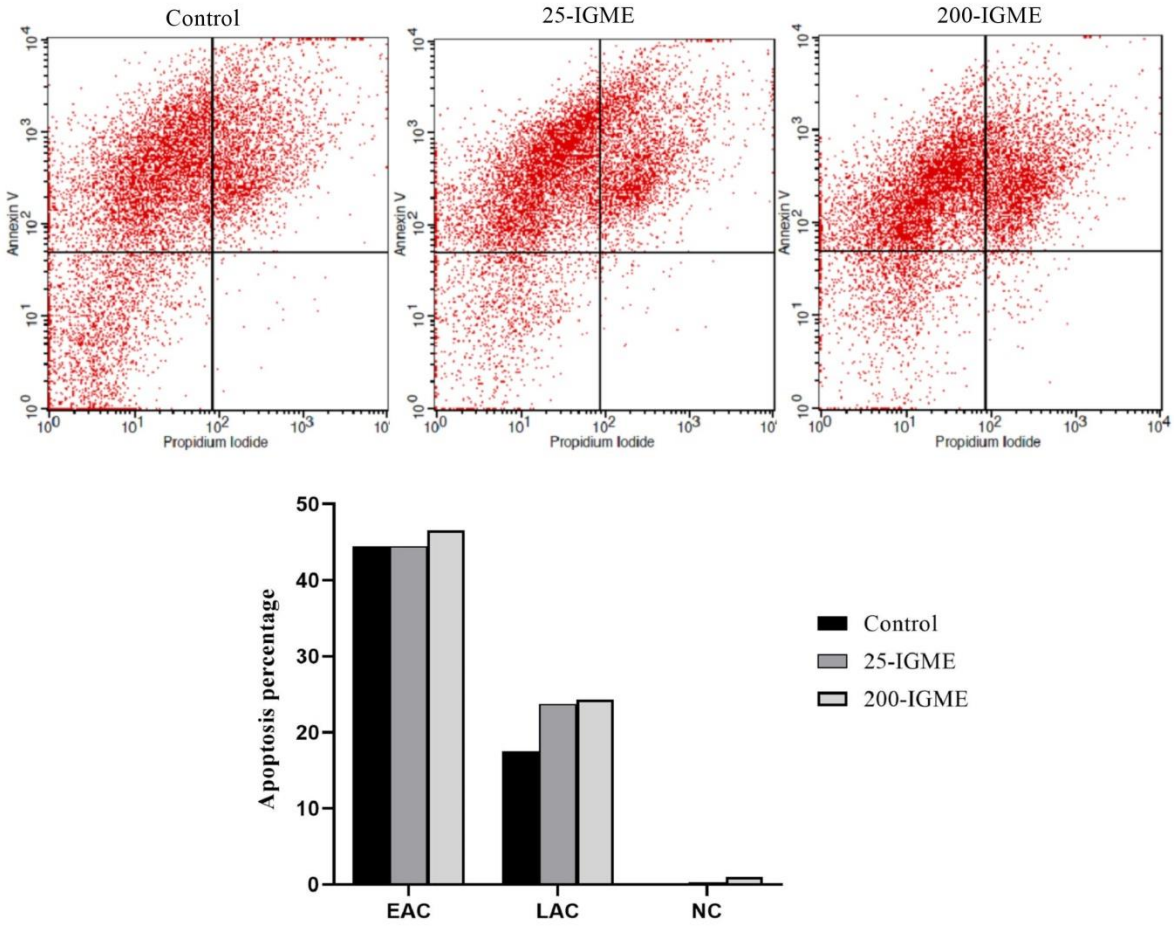

Figure No. 5
Apoptosis inducing in HeLa cells after treatment IGME

Determination of IL 6 and IL 8 levels after treatment of IGME

After applying all the doses of IGME to HeLa cells, supernatants were taken and IL6 and IL8 levels were determined. As can be seen in Figure 6, IL6 and IL8 levels decreased in a dose-dependent manner compared to control (DMSO), excepting 100 µg/mL dose in IL6 treatment group.

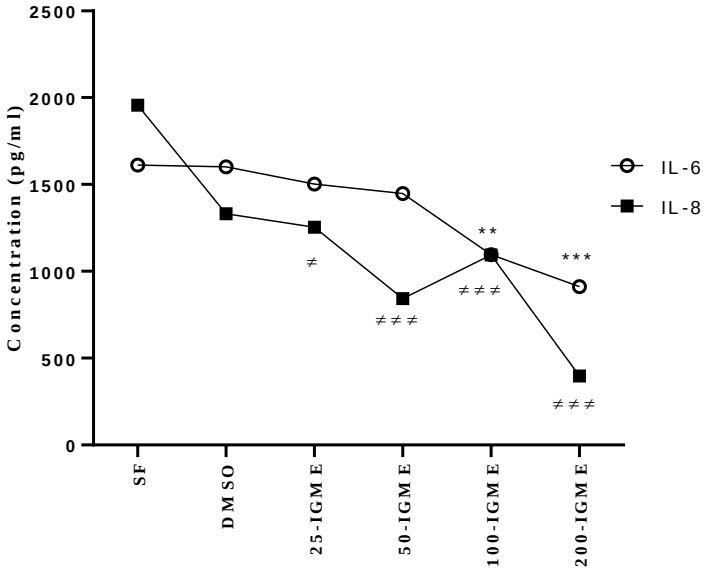

Figure No. 6
IL6 and IL8 levels in HeLa cells after administering of IGME. Tukey's pair-wise multiple comparison test was used to determine the differences between the doses of the samples

Ç

Determination of phenolic compounds of IGME by LC-MS

In this study, phenolic components of IGME were screened by LC-MS-MS. As a result of the screening, a total of 20 compounds were determined and these compounds and their amounts were given in Table No. 1. As a result of the screening, it was determined that amount of quercetin, which is a flavonoid, was highest (44.34 mg/L) in the methanol extract. Myricetin, kaempferol, oleuropein, naringenin, butein, luteolin, catechin hydrate, thymoquinone and phloridzin are also among the other determined flavonoids. In addition, fumaric, syringic, caffeic, gallic, acetohydroxamic, protocatechuic, salicylic, ellagic and hydroxybenzoic acids were determined.

Table N. 1
Phenolic compounds of IGME determined by LC-MS

NO	Compounds	Amount (mg/L)
1	Acetohydroxamic acid	0.454
2	Catechin hydrate	<0.01
3	Syringic acid	<0.01
4	Resveratrol	<0.01
5	Fumaric acid	4.483
6	Gallic acid	0.051
7	Caffeic acid	1.165
8	Phloridzin	0.57
9	Oleuropein	0.178
10	Protocatechuic acid	<0.01
11	Salicylic acid	0.352
12	Ellagic acid	0.120
13	Myricetin	2.858
14	Hydroxy benzoic acid	0.449
15	Quercetin	44.347
16	Naringenin	0.105
17	Butein	<0.01
18	Luteolin	0.120
19	Kaempferol	0.523
20	Thymoquinone	<0.01

DISCUSSION

There are some studies in the literature reporting that Inula species have activities such as antioxidant, antibacterial, analgesic, anti-inflammatory (Paliwal *et al*., 2017). In addition to these activities, Inula species have also been found to have important anticancer activities. In previous studies, anticancer activities of *I. viscosa* (Brahmi-Chendouh *et al*., 2019; Bar-Shalom *et al*., 2019), *I. japonica* (Wu *et al*., 2016), *I. helenium* (Zhang *et al*., 2018; Lee *et al*., 2019), *I. lineariifolia* (Qin *et al*., 2013) and *I. bratinnica* (Fischedick *et al*., 2013) was reported. Considering the Inula species in the literature, it is thought that more information about their anticancer activities is required. In this context, our study focused on the anticancer activity of *I. graveolens*. In our study, after treatment of the different doses of IGME*s* on cervical cancer cells HeLa, the proliferation of the cells decreased in a dose-dependent manner. The vitality of majority of cells decreased especially at the dose of 200 µg/mL. In a previous study, Karan *et al*. (2018), reported that bornyl acetate extract and essential oil of *I. graveolens* on different cancer cells reduced the proliferation and viability of HeLa cells in a dose-dependent manner. Consistent with the literature, it has been proven that *I. graveolens* reduces the viability of HeLa cells *in vitro*.

Apoptosis has been defined as controlled cell death characterized by chromatin condensation, DNA fragmentation, and cell morphological changes. It is controlled through two main pathways controlled by many proteins playing a role in the extrinsic-death receptor pathway and the intrinsic-mitochondrial pathway (Elmore, 2007). Apoptosis is a process that works under normal conditions of physiological development, aging and disease. In addition, when mutated/damaged cells cannot be repaired by repair mechanisms, these cells are destroyed by apoptosis. Apoptosis mechanisms in cells, however, are suppressed during the process of cancer. Therefore, inducing apoptosis of a compound or extract tested in anticancer studies is one of the main aims. According to the results, it was determined that the percentage of apoptosis increased in cervical cancer cells to which IGME was applied. Therefore, IGME was thought to have apoptosis inducing activity. Similar to the

results of our study, it was reported in previous studies that different species of *Inula* induced the apoptosis in cancer cells. Talib *et al*. (2012), reported that flavanoids obtained from *I. viscosa* induce apoptosis in MCF7 breast cancer cells. In another study, it was reported that *I. viscosa* inhibits colorectal cancer cells by inducing apoptosis (Bar-Shalom *et al*., 2019). Pal *et al*. (2010), also determined that the apoptosis was induced in leukemia HL-60 cells after the application of *I. racemosa*.

In previous studies it was reported that an increase in IL6 and IL8 levels plays a role in the pathogenesis of cervical cancer (Wei *et al*., 2001a; Wei *et al*., 2001b; Palena *et al*., 2012; Abdelwahab *et al*., 2012; Jia *et al*., 2018). The increase in level of IL6 suppressed the apoptosis in cervical cancer cells and the apoptosis was induced due to the decrease in level of IL-6 after the administration of an anticancer agent. Jia *et al*. (2018), reported that IL8 increases the proliferation and migration of cervical cancer cells. In our study it was determined that the levels of both IL6 and IL8 decreased when IGME was applied to cervical cancer cells. In addition, Cocelli *et al*. (2021), found that apoptosis in HeLa cells was induced due to the decrease in level of IL6 and IL8 after treatment of medical plant extract. In the light of these findings, it can be said that the increase in percentage of apoptosis in HeLa cells by IGME may be associated with the decrease in level of IL6 and IL8.

It is known to have the degenerative effects of Reactive Oxygen Species (ROS) on carbohydrates, lipids, proteins and nucleic acids in cells. Moreover, ROS, which are created naturally in oxygen metabolism, are also used as signal molecules in physiological events such as proliferation and differentiation of cells. It has been stated that apoptosis is induced related to the induction of ROS (Simon *et al*., 2000). It has also been reported that ROS acts as a signal in the induction of apoptosis and it also plays a role in activating the autophagy pathway with inhibition of MTORC1 (Xu *et al*., 2017). However, in contrast to these studies, it was determined in some studies that apoptosis was induced in cancer cells despite suppression of ROS. 5,7-dihydroxy-4-methyl-6-(3-methylbutanoyl)-coumarin, a coumarin derivative, was showed to play a proapoptotic role in colon cancer without the induction of ROS (Lin *et al*., 2014). In another study, it was reported that diphenyleneiodonium, used as a flavoenzyme inhibitor, induced apoptosis in RPE cells despite suppression of ROS by NAC, which is an antioxidant compound (Park *et al*., 2007). Moreover, it was reported that the antioxidants such as NAC and DPI did not prevent caspase-3 activity and apoptotic body formation (Lin *et al*., 2014). In our study, it was observed that the intracellular ROS level in Hela cells decreased related to the application of IGME. ROS level was significantly suppressed especially in the administration of 200 mg/mL dose. In line with the results obtained, it was thought that IGME shows anticancer activity by inducing ROS-independent apoptosis due to the induction of apoptosis despite the decrease in ROS level.

In this study, the antioxidant activity of IGME was determined by DPPH scavenging and CAA test systems. Both DPPH scavenging activity and CAA increased after treatment of IGME in a dose-dependent manner. It has been determined that IGME has a moderate DPPH scavenging and a strong CAA. When the increase in CAA activity and decrease in ROS level is evaluated, the results were found to be consistent. Kaplan *et al*. (2019), determined to have a significant DPPH scavenging activity of *I. graveolens* methanol and aqueous extracts. In another study it was reported the DPPH scavenging activity of water and ethanol extracts of *I. graveolens* due to its rich phenolic compounds (Silinsin & Bursal, 2018). Although the in vitro antioxidant activities of *I. graveolen*s were reported in previous studies, no information about its CAA activity could be determined. Therefore, IGME is thought to be the first findings of the CAA results.

In scanning of LC-MS, twenty of phenolic compounds were detected. Of these, quercetin was the most abundant compound. Quercetin is a flavonoid found in many plants and exhibits powerful antioxidant and anticancer activity (Baghel *et al*., 2012). It has been reported in previous studies that quercetin inhibits the proliferation of HeLa cells and induces apoptosis. (Wang *et al*., 2016). Therefore, it is thought that quercetin has a role in the antioxidant and anticancer activity exhibited by IGME. The fact that phenolics determined by LC-MS were not evaluated individually in the tests performed is a limitation of our study. As these compounds may have individual roles, it is also thought that a more precise evaluation will be made by determining the synergistic or antagonistic effects. Unlike our results, in a previous study, Silinsin & Bursal, (2018), also determined a 27 of phenolic compounds by UHPLC-ESI-MS-MS and found the chlorogenic and quinic acids as major phenolic compounds. The different

content of the same plant may be due to the harvesting time, region and extraction methods.

In the light of all the information discussed above, it is considered to have the anticancer and antioxidant activities of *I. graveolens* due to its chemical content. Due to the dose-dependent decrease in IL6 and IL8 levels playing a role in the pathogenesis of cervical cancer, it is recommended to investigate the effects of *I. graveolens* on cell signaling pathways in which these cytokines take part.

REFERENCES

Abdelwahab SI, Abdul AB, Zain ZNM, Hadi AHA. 2012. Zerumbone inhibits interleukin-6 and induces apoptosis and cell cycle arrest in ovarian and cervical cancer cells. **Int immunopharmacol** 12: 594 - 602. **https://doi.org/10.1016/j.intimp.2012.01.014**

Al-Fartosy AJ. 2011. Antioxidant properties of methanolic extract from *Inula graveolens* L. **Turk J Agric For** 35: 591 - 596. **https://doi.org/10.3906/tar-1010-1268**

Baghel SS, Shrivastava N, Baghel RS, Agrawal P, Rajput S. 2012. A review of quercetin: antioxidant and anticancer properties. **World J Pharm Pharmaceut Sci** 1: 146 - 160.

Bar-Shalom R, Bergman M, Grossman S, Azzam N, Sharvit L, Fares F. 2019. *Inula viscosa* extract inhibits growth of colorectal cancer cells *in vitro* and *in vivo* through induction of apoptosis. **Front Oncol** 9: 227. **https://doi.org/10.3389/fonc.2019.00227**

Brahmi-Chendouh N, Piccolella S, Crescente G, Pacifico F, Boulekbache L, Hamri-Zeghichi S, Akkal S, Madani K, Pacifico S. 2019. A nutraceutical extract from *Inula viscosa* leaves: UHPLC-HR-MS/MS based polyphenol profile, and antioxidant and cytotoxic activities. **J Food Drug Anal** 27: 692 - 702. **https://doi.org/10.1016/j.jfda.2018.11.006**

Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. 2018. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. **CA Cancer J Clin** 68: 394 - 424. **https://doi.org/10.3322/caac.21492**

Cocelli G, Pehlivan M, Yumrutas O. 2021. *Sideritis perfoliata* inhibits cell proliferation, induces apoptosis and exhibits cellular antioxidant activity in cervical cancer cells. **Bol Latinoam Caribe Plant Med Aromat** 20: 394 - 405. **https://doi.org/10.37360/blacpma.21.20.4.29**

Ege B, Yumrutas O, Ege M, Pehlivan M, Bozgeyik I. 2020. Pharmacological properties and therapeutic potential of saffron (*Crocus sativus* L.) in osteosarcoma. **J Pharm Pharmacol** 72: 56 - 67. **https://doi.org/10.1111/jphp.13179**

Elmore S. 2007. Apoptosis: a review of programmed cell death. **Toxicol Pathol** 35: 495 - 516. **https://doi.org/10.1080/01926230701320337**

El Omari N, El Menyiy N, Zengin G, Goh BH, Gallo M, Montesano D, Naviglio D, Bouyahya A. 2021. Anticancer and anti-inflammatory effects of tomentosin: cellular and molecular mechanisms. **Separations** 8: 207. **https://doi.org/10.3390/separations8110207**

Fischedick JT, Pesic M, Podolski-Renic A, Bankovic J, de Vos RC, Perić M, Todorovic S, Tanic N. 2013. Cytotoxic activity of sesquiterpene lactones from *Inula britannica* on human cancer cell lines. **Phytochem Lett** 6: 246 - 252. **https://doi.org/10.1016/j.phytol.2013.02.006**

Iqbal J, Abbasi BA, Mahmood T, Kanwal S, Ali B, Shah SA, Khalil AT. 2017. Plant-derived anticancer agents: A green anticancer approach. **Asian Pac J Trop Biomed** 7: 1129 - 1150. **https://doi.org/10.1016/j.apjtb.2017.10.016**

Jia L, Li F, Shao M, Zhang W, Zhang C, Zhao X, Luan H, Qi Y, Zhang P, Liang L, Jia X, Zhang K, Lu Y, Yang Z, Zhu X, Zhang Q, Du J, Wang W. 2018. IL-8 is upregulated in cervical cancer tissues and is associated with the proliferation and migration of HeLa cervical cancer cells. **Oncol Lett** 15: 1350 - 1356.

Kaplan AU, Duysak L, Gulaboğlu M, Dogru U, Çetın M. 2019. **Determination of antioxidant capacity of methanol and aqueous extracts of *Inula graveolens*.** In 4th Internatıonal Conference on advances in natural & applıed scıences, Agry, Turkey.

Karan T, Yildiz I, Aydin A, Erenler R. 2018. Inhibition of various cancer cells proliferation of bornyl acetate and essential oil from *Inula graveolens* (Linnaeus) Desf. **Rec Nat Prod** 12: 273 - 283.

Lee KM, Shin JM, Chun J, Song K, Nho CW, Kim YS. 2019. Igalan induces detoxifying enzymes mediated by the Nrf2 pathway in HepG2 cells. **J Biochem Mol Toxicol** 33: e22297. **https://doi.org/10.1002/jbt.22297**

Lin MH, Cheng CH, Chen KC, Lee WT, Wang YF, Xiao CQ, Lin CW. 2014. Induction of ROS-independent JNK-activation-mediated apoptosis by a novel coumarin-derivative, DMAC, in human colon cancer cells. **Chem Biol Interact** 218: 42 - 49.

Mahmoudi H, Hosni K, Zaouali W, Amri I, Zargouni H, Hamida NB, Kaddour R, Hamrouni L, Nasri MB, Ouerghi Z. 2016. Comprehensive phytochemical analysis, antioxidant and antifungal activities of *Inula viscosa* aiton leaves. **J Food Saf** 36: 77 - 88.

Mohammed FS, Pehlivan M, Sevindik E, Akgul H, Sevindik M, Bozgeyik I, Yumrutas O. 2021. Pharmacological properties of edible *Asparagus acutifolius* and *Asparagus officinalis* collected from North Iraq and Turkey (Hatay). **Acta Alimentaria** 50: 136 - 143. **https://doi.org/10.1556/066.2020.00204**

Pal HC, Sehar I, Bhushan S, Gupta BD, Saxena AK. 2010. Activation of caspases and poly (ADP-ribose) polymerase cleavage to induce apoptosis in leukemia HL-60 cells by Inula racemosa. **Toxicol in vitro** 24: 1599 - 1609. **https://doi.org/10.1016/j.tiv.2010.06.007**

Palena C, Hamilton DH, Fernando RI. 2012. Influence of IL-8 on the epithelial–mesenchymal transition and the tumor microenvironment. **Future Oncol** 8: 713 - 722. **https://doi.org/10.2217/fon.12.59**

Paliwal SK, Sati B, Faujdar S, Sharma S. 2017. Antioxidant and antibacterial activities of various extracts of Inula cuspidata CB Clarke stem. **Beni-Suef Univ J Basic Appl Sci** 6: 97 - 105. **https://doi.org/10.1016/j.bjbas.2016.10.003**

Park SE, Song JD, Kim KM, Park YM, Kim ND, Yoo YH, Park YC. 2007. Diphenyleneiodonium induces ROS-independent p53 expression and apoptosis in human RPE cells. **FEBS Lett** 581: 180 - 186. **https://doi.org/10.1016/j.febslet.2006.12.006**

Qin JJ, Jin HZ, Huang Y, Zhang SD, Shan L, Voruganti S, Nag S, Wang W, Zang WD, Zhang R. 2013. Selective cytotoxicity, inhibition of cell cycle progression, and induction of apoptosis in human breast cancer cells by sesquiterpenoids from *Inula linearifolia* Turcz. **Eur J Med Chem** 68: 473 - 481. **https://doi.org/10.1016/j.ejmech.2013.07.018**

Saint-Cricq de Gaulejac N, Provost C, Vivas N. 1999. Comparative study of polyphenol scavenging activities assessed by different methods. **J Agric Food Chem** 47: 425 - 431. **https://doi.org/10.1021/jf980700b**

Silinsin M, Bursal E. 2018. UHPLC-MS/MS phenolic profiling and in vitro antioxidant activities of *Inula graveolens* (L.) Desf. **Nat Prod Res** 32: 1467 - 1471. **https://doi.org/10.1080/14786419.2017.1350673**

Simon HU, Haj-Yehia A, Levi-Schaffer F. 2000. Role of reactive oxygen species (ROS) in apoptosis induction. **Apoptosis** 5: 415 - 418. **https://doi.org/10.1023/a:1009616228304**

Sreelatha S, Jeyachitra A, Padma PR. 2011. Antiproliferation and induction of apoptosis by *Moringa oleifera* leaf extract on human cancer cells. **Food Chem Toxicol** 49: 1270 - 1275. **https://doi.org/10.1016/j.fct.2011.03.006**

Talib WH, Zarga MHA, Mahasneh AM. 2012. Antiproliferative, antimicrobial and apoptosis inducing effects of compounds isolated from *Inula viscosa*. **Molecules** 17: 3291 - 3303. **https://doi.org/10.3390/molecules17033291**

Topçu G, Öksüz S, Shieh HL, Cordell GA, Pezzuto JM, Bozok-Johansson C. 1993. Cytotoxic and antibacterial sesquiterpenes from *Inula graveolens*. **Phytochemistry** 33: 407 - 410.

Wang Y, Zhang W, Lv Q, Zhang J, Zhu D. 2016. The critical role of quercetin in autophagy and apoptosis in HeLa cells. **Tumor Biol** 37: 925 - 929. **https://doi.org/10.1007/s13277-015-3890-4**

Wei LH, Kuo ML, Chen CA, Chou CH, Cheng WF, Chang MC, Su JL, Hsieh CY. 2001a. The anti-apoptotic role of interleukin-6 in human cervical cancer is mediated by up-regulation of Mcl-1 through a PI 3-K/Akt pathway. **Oncogene** 20: 5799 - 5809. **https://doi.org/10.1038/sj.onc.1204733**

Wei LH. Kuo ML, Chen CA, Cheng WF, Cheng SP, Hsieh FJ, Hsieh CY. 2001b. Interleukin-6 in cervical cancer: the relationship with vascular endothelial growth factor. **Gynecol Oncol** 82: 49 - 56. **https://doi.org/10.1006/gyno.2001.6235**

Wolfe KL, Kang X, He X, Dong M, Zhang Q, Liu RH. 2008. Cellular antioxidant activity of common fruits. **J Agric Food Chem** 56: 8418 - 8426. **https://doi.org/10.1021/jf801381y**

WHO. 2022. (World Health Organisation). **Cervical cancer**. **https://www.who.int/health-topics/cervical-cancer#tab=tab_1**

Wu M, Li T, Chen L, Peng S, Liao W, Bai R, Zhao X, Yang H, Wu C, Zeng H, Liu Y. 2016. Essential oils from *Inula japonica* and *Angelicae dahuricae* enhance sensitivity of MCF-7/ADR breast cancer cells to

doxorubicin via multiple mechanisms. **J Ethnopharmacol** 180: 18 - 27. **https://doi.org/10.1016/j.jep.2016.01.015**

Xu J, Wu Y, Lu G, Xie S, Ma Z, Chen Z, Shen HM, Xia D. 2017. Importance of ROS-mediated autophagy in determining apoptotic cell death induced by physapubescin B. **Redox Biol** 12: 198 - 207. **https://doi.org/10.1016/j.redox.2017.02.017**

Yumrutas O, Oztuzcu S, Pehlivan M, Ozturk N, Eroz Poyraz I, Igci YZ, Cevik MO, Bozgeyik I, Aksoy AF, Bagis H. 2015. Cell viability, anti-proliferation and antioxidant activities of *Sideritis syriaca*, *Tanacetum argenteum* sub sp. *argenteum* and *Achillea aleppica* subsp. *zederbaueri* on human breast cancer cell line (MCF-7). **J Appl Pharm Sci** 5: 1 - 5. **https://doi.org/10.7324/JAPS.2015.50301**

Yumrutaş Ö, Pehlivan M, Güven C, Bozgeyik I, Bozgeyik E, Yumrutas P, Temiz E, Üçkardeş F. 2018. Investigation of cytotoxic effect of *Salvia pilifera* extracts and synthetic chlorogenic and caffeic acids on DU145 prostate cancer cells line.**KSU J Agric Nat** 21: 141 - 147. **https://doi.org/10.18016/ksudobil.302249**

Zarei M, Mohammadi S, Komaki A. 2018. Antinociceptive activity of *Inula britannica* L. and patuletin: *In vivo* and possible mechanisms studies. **J Ethnopharmacol** 219: 351 - 358. **https://doi,org/10.1016/j.jep.2018.03.021**

Zhang B, Zeng J, Yan Y, Yang B, Huang M, Wang L, Zhang Q, Lin N. 2018. Ethyl acetate extract from *Inula helenium* L. inhibits the proliferation of pancreatic cancer cells by regulating the STAT3/AKT pathway. **Mol Med Rep** 17: 5440 - 5448. **https://doi.org/10.3892/mmr.2018.8534**