Int J Cancer Manag. 2021 June; 14(6):e111319. doi: 10.5812/ijcm.111319.

Published online 2021 June 27. **Research Article**

Cytotoxic Effect of *Iris germanica* L. Rhizomes Extract on Human Melanoma Cell Line

Maryam Iranzadasl [1, 2], Parvin Pasalar [3], Mohammad Kamalinejad [4] and Mohammad Javad Mousavi [5, *]

[1] Department of Traditional Persian Medicine, School of Medicine, Shahed University, Tehran, Iran
[2] School of Persian Medicine, Iran University of Medical Sciences, Tehran, Iran
[3] Department of Clinical Biochemistry, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[4] Department of Pharmacology and Toxicology, School of Pharmacy, Shahid Beheshti University of Medical Sciences and Health Services, Tehran, Iran
[5] Department of Hematology, Faculty of Allied Medicine, Bushehr University of Medical Sciences, Bushehr, Iran

[*] *Corresponding author*: Faculty of Allied Medicine, Bushehr University of Medical Sciences, Rishehr St., Bushehr, Iran. P.O. Box: 7518759577, E-mail: M.j.mousavi@bpums.ac.ir

Received 2020 November 17; **Revised** 2021 February 16; **Accepted** 2021 March 07.

Abstract

Background: Melanoma is the leading cause of 80% of skin cancer worldwide due to its high proliferation rate, metastatic nature, and limited effective therapies. Given the rapid increase in its incidence compared to other skin cancers, new therapeutic agents are needed to control the disease. Scientists are interested in medicinal plants due to their anticancer properties. The rhizomes of the *Iris germanica* L., known as "Irsa", is one of the herbs used in traditional Persian medicine for the treatment of various skin cancers.
Objectives: This study aimed at investigating the cytotoxic effects of *Iris germanica* on A375 melanoma and AGO-1522 normal human fibroblast cell lines for the first time.
Methods: The ethanolic extract was prepared by the maceration method. Cell viability and cytotoxic activities were assessed through 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and flow cytometric assay, using annexin V/propidium iodide (PI) staining.
Results: IC_{50} values were estimated for the A375 melanoma and the AGO-1522 normal cell lines. We revealed that the IC_{50} for the A375 melanoma was 0.0438 mg/mL and for the AGO-1522 normal cell line was 0.8494 mg/mL after 48 hours of treatment. Furthermore, flow cytometry analysis illustrated that 0.125 mg/mL of the *Iris germanica* extract could lead to 55.24% apoptosis of the A375 melanoma cell line. The same concentration of the *Iris germanica* extracts only lead to 8.76% apoptosis in the AGO-1522 cell line.
Conclusions: *Iris germanica* extract has considerable cytotoxic effects on the human melanoma cell line. Further studies are required to demonstrate the therapeutic effects of *Iris germanica* on melanoma cancer.

Keywords: Melanoma, *Iris germanica*, A375 Melanoma Cell Line, Cytotoxicity

1. Background

Melanoma is a malignant tumor of the skin that is originated from melanocyte cells. Cells become proliferative, dysplastic, expand from epidermis to dermis, and can quickly metastasize to other organs (1). The prevalence of melanoma varies in different geographical regions and between races. The global incidence of melanoma has increased rapidly in recent decades and its prevalence is doubled every 10 to 20 years (2, 3).

Human skin melanoma is one of the most severe skin cancers and has a high prevalence and mortality rate. Currently, in the United States, melanoma is the 5th most common cancer in men and the 6th most common cancer in women (4), which is estimated to be even higher due to the lack of proper diagnostic criteria (5).

Melanoma has various risk factors including exposure to sunshine, genetic and epigenetic factors, immunosuppression, multiple and abnormal nevi, as well as occupational and nutritional factors (6-9). The most potent risk factor is exposure to type B ultraviolet radiation (UVB), while type A (UVA) such as tanning and psoralen plus ultraviolet A (PUVA) therapy can increase the chance of melanoma (10).

Many efforts have been made to achieve effective therapies for the disease. Common therapies are surgery, chemotherapy, hormone therapy, radiation therapy, gene therapy, and immunotherapy. Treatment for local lesions in the early stage is surgery, while in advanced cases, response to treatment is weak because they are metastatic and resistant to common medications such as single-drug chemotherapy regimens and radiotherapy, suggesting novel, effective, and low-risk therapies (11).

The use of medicinal plants for cancer treatment has always been an option for researchers (12, 13). Iranian scientists have provided different natural therapies for modulating cancers in traditional Persian medicine (14). *Iris germanica* L., known as "Irsa" in Persian medicine, is a herb and its rhizome has been used for the treatment of a skin cancer similar to melanoma (15-17).

The plant is a member of the Iridaceae family and has many varieties. This plant is popular in many parts of the world including Iran (18). The rhizome of *Iris germanica* is a rich source of flavonoids such as irigenin, nigricin, irilone, and irisolidone (19-21). Benzene derivatives have also been obtained from the methanolic extract of the Irsa rhizomes (22). The fresh rhizome of Irsa contains triterpenoids called "iridals", which are the precursor of iron. Irones are also extracted from the dried rhizome of Irsa and used for medical and perfumery purposes (23). In addition to these compounds, various types of sterols including sitosterol and stigmasterol have been extracted from the Irsa rhizomes (24). Furthermore, antioxidant, anti-inflammatory, anti-bacterial, and anti-ulcer properties of Irsa compounds have been investigated in several studies (18, 21, 25).

2. Objectives

Previous studies have demonstrated anti-mutagenic, anti-proliferative, and cytotoxic effects of the Irsa extract in different cancer cell lines (16, 25-27), while no studies have been performed so far on the impact of the whole extract of Irsa on A375 (human skin melanoma) cancer cell line. In this study with the inspiration of traditional Persian medicine, the effect of Irsa extract on proliferation and apoptosis of the A375 melanoma (cancer) cell line was investigated for the first time and compared with the AGO-1522 (normal human fibroblast) cell line.

3. Methods

3.1. Preparation of Plant Sample

The complete plant of *Iris germanica* with blue flowers and the Herbariums registration number of S-A1037-131 in the Plant Extract Bank was obtained from Karaj, Iran. The plant is also called "*Susan Assemanjooni*" according to the traditional and allopathic medicine references and used after confirmation by the Herbal Medicines Department of the Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran. The rhizome was separated from the rest of the plant, dried in shadow, chopped, and kept in a cold place until extraction.

3.2. Extraction and Condensation

Two hundred grams of the chopped rhizome was added into a mixture of water (480 mL) and (120 mL ethanol) for 72 hours. Extraction of the chopped rhizome by ethanol has the lowest damage to compounds such as alkaloids, saponins, carbohydrates, tannins, and flavonoids (28, 29). Next, the extract was filtered with a Whatman filter paper No. 1, incubated in a 70°C water bath for 12 hours, concentrated, and dried. The extract was stored in a refrigerator at 4°C for subsequent molecular experiments.

3.3. A375 and AGO-1522 Cell Lines

The A375 human melanoma cell line (C10141 IBRC, Iran) is originated from a 54-year-old woman with malignant melanoma in 1973.

The AGO-1522 cell line, which is a normal human fibroblast cell line, was used as a healthy control cell line. The cells were taken from the skin of a 3-day-old (male) newborn.

3.4. Cell Culture

A375 and AGO-1522 cell lines were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS).

Cells (10^4 cells) were cultured in each well of a 96-well plate with 100 μL of culture medium. In the next step, the plant extract was dissolved in dimethyl sulfoxide (DMSO) and cells were treated with different concentrations (0.031, 0.0625, 0.125, 0.25, 0.5, and 1 mg/mL). Then, cells were incubated in an incubator with 37°C, 5% CO_2, and humidified atmosphere for 48 hours.

In addition to the Irsa extract, the A375 and AGO-1522 cell lines were treated with 100, 250, 500, 750, and 1000 μM concentrations of dacarbazine (Sigma Chemical Co., St. Louis, MO, USA) as a chemotherapeutic agent and positive control. In previous studies, a range of 100 to 1000 μM was used for cytotoxicity (30, 31).

3.5. MTT Assay

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was prepared at a concentration of 5 mg in a serum-free RPMI medium. The supernatant of cell lines was removed and incubated with the different concentrations of the Iris extract in the 96-well plate. Then, the MTT solution was diluted with serum-free RPMI with a 1:1 ratio, and 100 μL of the obtained solution was added to each well of the plate and incubated at 37°C for 3 hours. Then, the solution was removed, and 100 μL of 0.04 HCl-containing acidic isopropanol was added. After the appearance of the violet color of formazan crystals, the

plates were read at 570 nm wavelength, using an enzyme-linked immunosorbent assay (ELISA) reader (BioTek ELISA reader, USA).

3.6. Flow Cytometry Analysis

Flow cytometry was performed to evaluate the viability of cells treated with 0.015, 0.0625, and 0.031 mg/mL concentrations of the Irsa extract. 1000 μM dacarbazine and 0.5% DMSO were used as positive and negative controls, respectively. Flow cytometry was carried out according to the BD kit instruction. Briefly, the supernatants of wells treated with the Irsa extract and dacarbazine were removed, and cells were isolated, using trypsin. Then, the cell suspension was divided into four 1.5 mL microtubes and centrifuged at 1500 rpm for 5 minutes. After removing the supernatant, 100 μL binding buffer was added to the cells. Five microliter Annexin V was added to 2 microtubes and incubated for 15 minutes; then, 5 μL propidium iodide (PI) was added to one of the two microtubes. Five microliter PI was added to one of the two primary microtubes that only had the binding buffer, and the other microtube remained intact. All microtubes were incubated for 2 minutes and, then, read by flow cytometry (BD FACSCalibur flow cytometer, USA). In addition to dacarbazine and Irsa extract, 0.5% DMSO was used as a negative control.

3.7. Statistical Analysis

Each MTT assay was performed with 8 repetitions. The growth curve was also obtained, using the equation of the concentration regression line. The cell growth rate was calculated, using the below formula:

$$Growth\,inhibition\ (\%) = \left[OD_{control} - \frac{OD_{sample}}{OD_{control}}\right] \times 100$$

Finally, IC_{50} (the drug concentration that destroys 50% of the cells) was obtained by using the plotted curve. Indeed, IC_{50} was used to quantify the measure that indicates how much of the Irsa extract is needed to apoptosis the cells by 50%. The effects of different levels of dacarbazine and Irsa extract on A375 and AGO-1522 cell lines were compared with one-way ANOVA, using SPSS release 25.0 (SPSS Inc., Chicago, IL, USA). P-values of < 0.05 were considered significant.

4. Results

4.1. MTT Assay on A375 Melanoma and AGO-1522 Control Cell Lines

According to the MTT assay, control and melanoma cancer cell lines had different sensitivities to the various concentrations of dacarbazine and Iris extract. The viability of the cells was evaluated, using MTT. The maximum inhibitory concentration (IC_{50}) of the Irsa extract for A375 melanoma cells was 0.0438 mg/mL after 48 hours of treatment. At the mentioned concentration, 50% of the cells in the plate lost their proliferation and viability. In addition, IC_{50} of the Irsa extract for the AGO-1522 control cell line was 0.8449 mg/mL and about 50% of the cultured cells lost their viability (Figure 1 and Table 1). Therefore, melanoma cancer cells were destroyed at 0.0438 mg/mL of Irsa extract, while the viability of control cells was not affected.

Table 1. IC_{50} Values of *Iris germanica* L. on AGO-1522 (Normal) and A375 (Melanoma) Cell Lines [a]

Cell Line	Treatment	IC50 (mg/mL)
AGO-1522	*Iris* extract	0.8449
A375		0.0438

[a] MTT assay of 1000 μM of dacarbazine on the cell lines showed the highest toxicity. Therefore, this concentration was used as the positive control.

4.2. Flow Cytometry of Irsa Extract on A375 Melanoma and AGO-1522 Control Cell Lines

The viability of the AGO-1522 cells in 0.125 mg/mL of Irsa extract was similar to the negative control. Therefore, the 0.125 mg/mL concentration of Irsa extract was the most suitable recommended concentration with a low cytotoxic effect on the AGO-1522 cells (Figure 2). However, 0.25 mg/mL and 0.5 mg/mL concentrations of the extract led to 77.88% and 70.7% of apoptosis in the AGO-1522 cell line, respectively.

According to our results, 0.125 mg/mL of Irsa extract lead to 96.7% apoptosis in the A375 melanoma cell line, whereas the same concentration had the most viability and the least apoptosis rate (24.33%) in the AGO-1522 cell line (Figure 3 and Table 2).

Besides, the viability of the AGO-1522 control cells and the A375 melanoma cells treated with 1000 μM of dacarbazine were 18.4% and 8.53%, respectively. Moreover, the viability of the AGO-1522 control cell line and the A375 melanoma cell line treated with the 0.125 mg/mL of the Irsa extract was 72.1% and 2.87%, respectively (Figure 4).

4.3. Statistical Findings

Statistical analysis with ANOVA test showed a significant difference in growth inhibition of cancer cells treated with different levels of Iris extract ($P < 0.001$). In addition, there was a significant difference in growth inhibition of the AGO-1522 cell line treated with various levels of Irsa extract ($P < 0.001$).

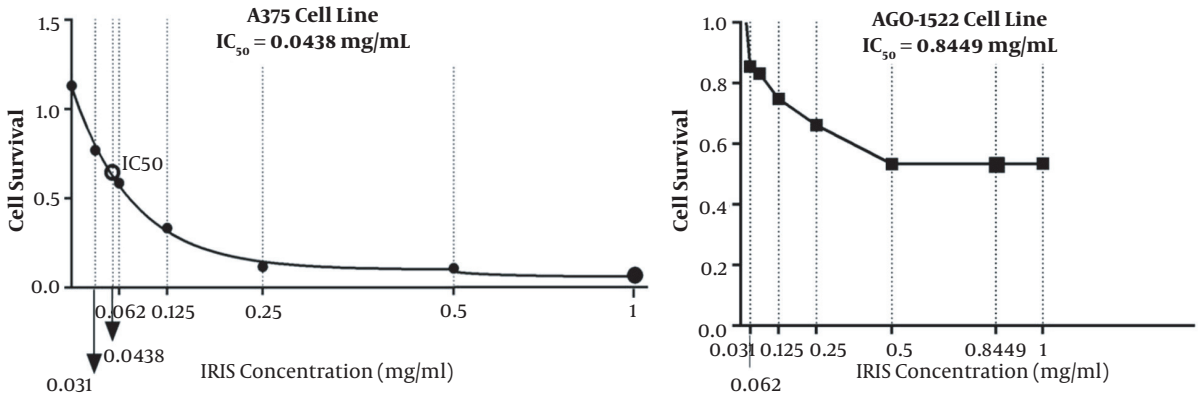

Figure 1. Measurement of IC_{50} values of Irsa extract on A375 melanoma cell line and AGO-1522 control cell line.

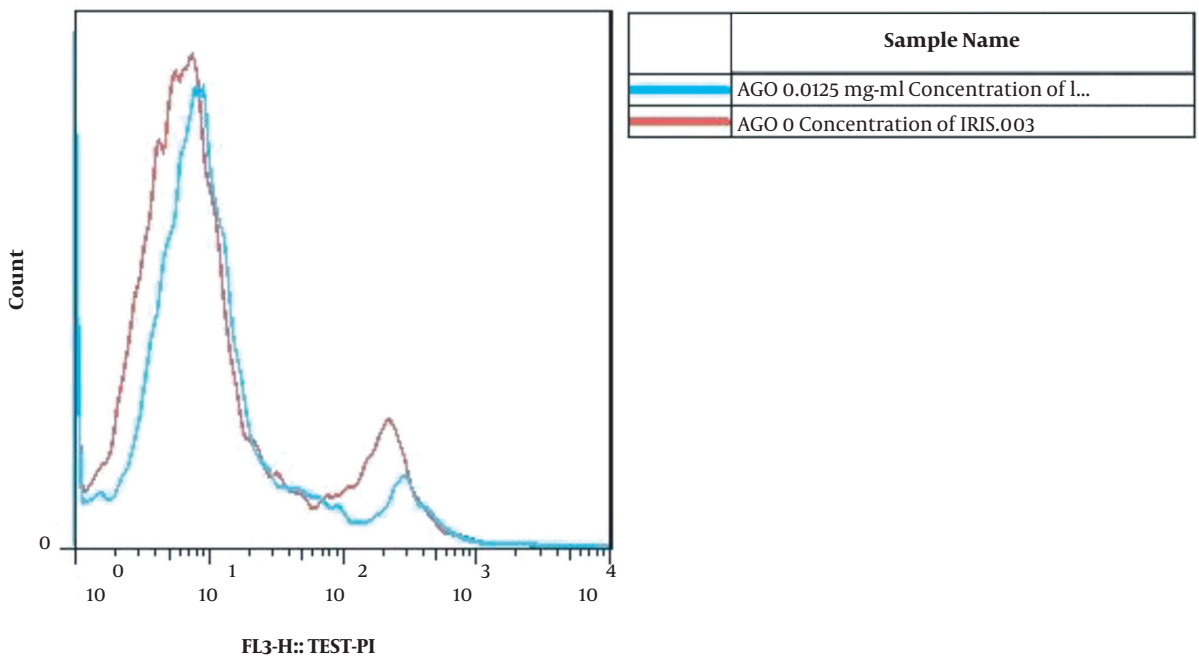

Figure 2. Flow cytometric analysis and comparison between the 0.125 mg/mL with zero concentration (negative control) of the Irsa extract in AGO-1522 cells. At the 0.125 mg/mL of Irsa extract, the viability of the cells was similar to the negative control. Therefore, the 0.125 mg/mL of Irsa extract is the most suitable recommended concentration that has a lower cytotoxic effect on AGO-1522 cells.

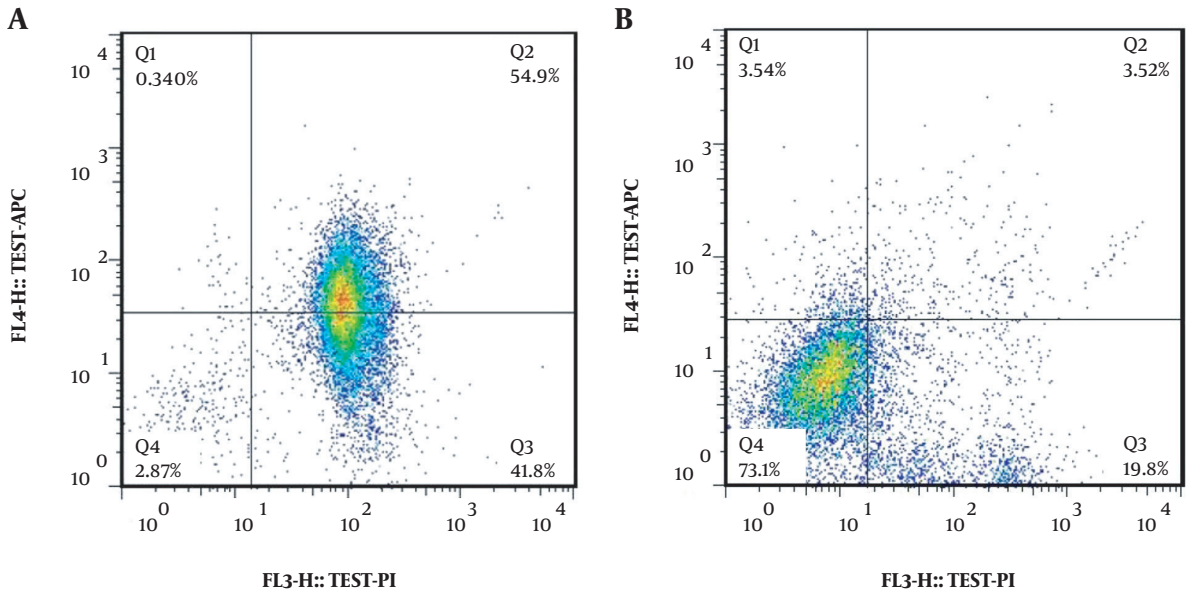

Figure 3. A. In the 0.125 mg/mL of Irsa extract on the A375 melanoma cell line, 96.7% of the cells were apoptotic. B. However, this rate was 24.33% for the AGO-1522 cell line.

Table 2. Evaluation of the Different Concentrations of the Irsa Extract on Apoptosis Induction in A375 Melanoma Cells and AGO-1522 Healthy Cells

Category	Treated Cells						Non-treated (DMSO) (Control -)		Dacarbazine (Control+)	
	0.031 mg/mL	0.0625 mg/mL	0.125 mg/mL	0.125 mg/mL	0.25 mg/mL	0.5 mg/mL				
Cell Type	A375	A375	A375	AGO-1522	AGO-1522	AGO-1522	A375	AGO-1522	A375	AGO-1522
Apoptotic Cells	5.51	48.45	55.24	8.76	11.06	48.9	4.26	8.72	91.2	77.9
Live Cells	83.9	3.11	2.87	72.1	19.9	18	84.8	71.9	8.53	18.4
Necrosis	10.6	48.4	41.8	19.2	69	33.1	10.9	18.7	0.230	3.75

Moreover, a significant difference was observed between the inhibitory effects of Irsa extract on induction of apoptosis in the A375 cell line compared to the AGO-1522 cell line ($P < 0.001$).

5. Discussion

Melanoma is one of the most common and malignant forms of skin cancer that originates from melanocyte cells. Melanocytes are pigment-including skin cells that produce pigment in response to irritations such as UV radiation. These cells are responsible for the color of skin, eyes, and hair. Environmental and genetic factors are involved in their development (32).

According to the World Health Organization (WHO), the incidence of melanoma is rapidly increasing in comparison to other skin cancers (33). The conventional treatments for melanoma including immunotherapy, chemotherapy, radiotherapy, and surgery did not show promising results and had their limitations (34). Recently, few effective treatments have been developed including immunotherapy with interferon (IFN) alfa-2b, interleukin (IL)-2, anti-CTLA-4, ipilimumab, and BRAF and MEK inhibitors. These treatments had many side effects, long-term responses, and were cost-effective (35-37). Due to these problems, alternative therapies are highly welcomed in this field (38). In the present study, *Iris germanica* L., known as Irsa, was selected to achieve an effective and less toxic treatment for melanoma.

In 2016, scientists at the Applied Medical Center of Korea showed that the ethanolic extract of *Gardenia jasminoides Ellis* roasted fruit inhibited migration of metastatic melanoma pulmonary tumor cells and reduced angiogenesis (39). In another study, herbal medicine from the ginseng family (Ginsenoside Rg3) was introduced to the scientific community as a unique product for the treatment of melanoma. This herbal medicine was able to inhibit mediators involved in tumor growth such as fucosyltransferase IV (FUT4) and induced apoptosis in a melanoma cell

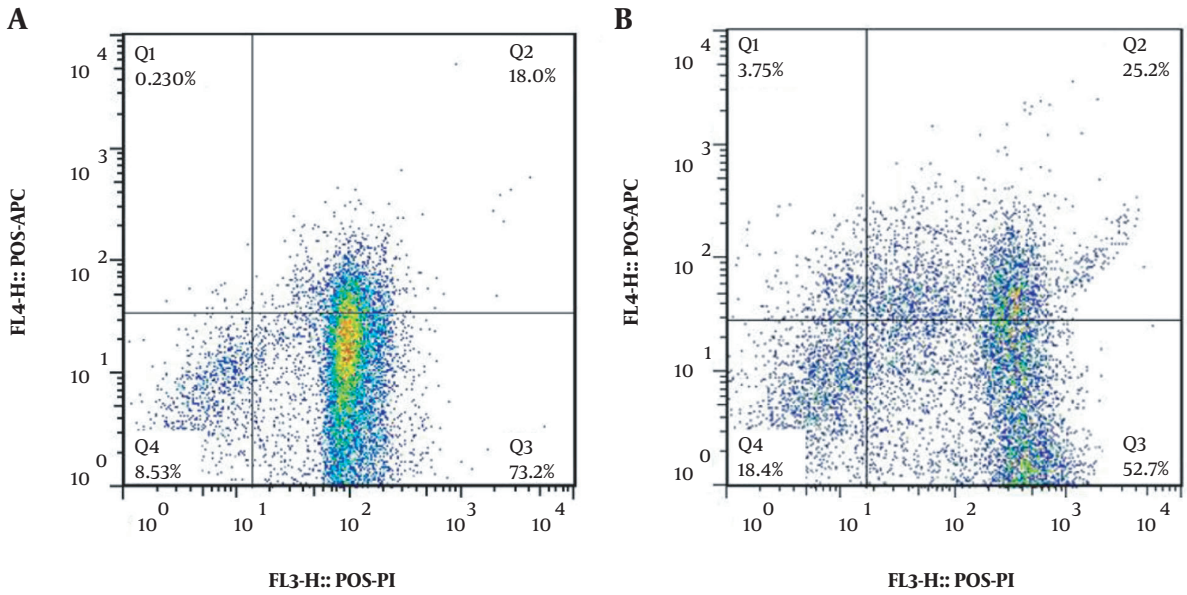

Figure 4. The effect of 1000 μM treatment with dacarbazine on the induction of apoptosis in A375 melanoma (A.) and AGO-1522 control (B.) cell lines. The rate of apoptotic cells to living cells was 91.2% to 8.53% and 77.9% to 14.4%, respectively.

line (40). Furthermore, Chinese scientists identified the molecular mechanisms implicated in cell cytotoxicity of the Physalin A on the A375-S2 human melanoma cell line. This compound is an active withanolide that is derived from the *physalis alkekengi* L. and the Makino shrub called Jin Deng Long in Chinese traditional herbal medicine. The cytotoxic effect of the Jin Deng Long on the melanoma cell line has been investigated in previous studies (41). In recent years, Chinese researchers have also investigated the cytotoxic effect and anti-tumor mechanism of Chinese traditional herbs and their derivatives on melanoma and pulmonary metastatic melanoma (42-45).

None of the studies have investigated the cytotoxicity effect of the herbs extract on the A375 cell line; therefore, no comparison can be performed with the current study. However, the cytotoxic effects of certain compounds of the Irsa extract on different cancer cell lines other than melanoma have been investigated in previous studies.

The protective effects of isoflavonoids of the Irsa rhizome extract on the inflammatory condition of the Hepa-1c1c7 cell line were evaluated. It was revealed that the compounds present in the Irsa extract could inhibit the activity of cytochrome P450 1A. In addition, isoflavone 2, 3, and 5 can induce NAD(P)H quinone reductase activity. The effects of Irsa on cancer cells are exerted through the activation of anti-inflammatory mechanisms (46). The anticancer effects of isoflavonoids of the Irsa extract on the EAC cancer cell line have also been studied, and the results showed that this product could inhibit the proliferation of the cancer cells (25).

In another study, the therapeutic effects of lipid compounds of the Irsa extract on the Hela cancer cell line were investigated. The study showed that the Irsa extract could inhibit migration of the cells and maintain cells in the G1 phase of the cell cycle. The Irsa extract prevents the migration of cancer cells by increasing adhesion molecules and producing more actin fibers (47).

The superiority of triterpenoids of the Irsa extract to doxorubicin was demonstrated in the A2780 and K562 cancer cell lines. Although these cell lines are resistant to a large number of chemotherapy drugs, they respond properly and showed cytotoxicity against the Irsa extract (48).

So far, no studies have been carried out to evaluate the Irsa extract therapeutic effects on the A375 melanoma cell line. Our study was performed for the first time to study the effects of the total Irsa extract on proliferation and apoptosis in the A375 melanoma cell line compared these effects with the AGO-1522 as a control cell line.

According to the present study, 0.0438 mg/mL of the Irsa extract destroys 50% of the A375 melanoma cell line, while no significant destructive effect on proliferation and viability of the AGO-1522 cell line was not observed.

Based on the flow cytometry results, the Irsa extract, similar to dacarbazine, lead to apoptosis in the melanoma cancer cells. Nonetheless, the Irsa extract, unlike dacarbazine, had no cytotoxic effect on normal cells. In addition,

flow cytometry results showed that 0.125 mg/mL of Irsa extract had the most cytotoxic effect on the A375 cell line and the highest rate of apoptotic and necrotized cells were observed. According to the results, the best-recommended concentration for A375 melanoma and AGO-1522 control cell lines is 0.125 mg/mL of the Irsa extract. This concentration not only had the highest cytotoxic effects on the A375 melanoma cell line but also had the lowest cytotoxic effects on the AGO-1522 control cell line.

5.1. Conclusions

We evaluated cytotoxic effects and apoptosis induction of the *Iris germanica* L. total extract on the human skin melanoma cell line (A375) for the first time. Our results showed that 0.125 mg/mL of the Irsa extract significantly increase apoptosis in melanoma cancer cells, while it was entirely safe for the normal cell lines. Therefore, this compound can be considered a novel, side-effect-free treatment for human skin melanoma.

Footnotes

Authors' Contribution: Study concept and design: P. P. and M. K.; analysis, interpretation of data, and drafting of the manuscript: M. I.; critical revision of the manuscript for important intellectual content: M. J. M. All authors read and approved the final manuscript.

Conflict of Interests: The authors declared no conflict of interest.

Funding/Support: This study was partially supported by the Tehran University of Medical Sciences (TUMS) and performed as a part of a Ph.D. dissertation.

References

1. Habif TP. *Clinical Dermatology E-Book*. Elsevier Health Sciences; 2015.
2. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*. 2010;**127**(12):2893–917. doi: 10.1002/ijc.25516. [PubMed: 21351269].
3. Garbe C, Leiter U. Melanoma epidemiology and trends. *Clin Dermatol*. 2009;**27**(1):3–9. doi: 10.1016/j.clindermatol.2008.09.001. [PubMed: 19095149].
4. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin*. 2018;**68**(1):7–30. doi: 10.3322/caac.21442. [PubMed: 29313949].
5. Harris RB, Koch SM, Newton C, Silvis NG, Curiel-Lewandroski C, Giancola J, et al. Underreporting of Melanoma in Arizona and Strategies for Increasing Reporting: A Public Health Partnership Approach. *Public Health Rep*. 2015;**130**(6):737–44. doi: 10.1177/003335491513000624. [PubMed: 26556947]. [PubMed Central: PMC4612184].
6. Caini S, Masala G, Saieva C, Kvaskoff M, Savoye I, Sacerdote C, et al. Coffee, tea and melanoma risk: findings from the European Prospective Investigation into Cancer and Nutrition. *Int J Cancer*. 2017;**140**(10):2246–55. doi: 10.1002/ijc.30659. [PubMed: 28218395]. [PubMed Central: PMC6198927].
7. Chen T, Fallah M, Forsti A, Kharazmi E, Sundquist K, Hemminki K. Risk of Next Melanoma in Patients With Familial and Sporadic Melanoma by Number of Previous Melanomas. *JAMA Dermatol*. 2015;**151**(6):607–15. doi: 10.1001/jamadermatol.2014.4777. [PubMed: 25671687].
8. Gandini S, Sera F, Cattaruzza MS, Pasquini P, Abeni D, Boyle P, et al. Meta-analysis of risk factors for cutaneous melanoma: I. Common and atypical naevi. *Eur J Cancer*. 2005;**41**(1):28–44. doi: 10.1016/j.ejca.2004.10.015. [PubMed: 15617989].
9. Jiang AJ, Rambhatla PV, Eide MJ. Socioeconomic and lifestyle factors and melanoma: a systematic review. *Br J Dermatol*. 2015;**172**(4):885–915. doi: 10.1111/bjd.13500. [PubMed: 25354495].
10. Noonan FP, Zaidi MR, Wolnicka-Glubisz A, Anver MR, Bahn J, Wielgus A, et al. Melanoma induction by ultraviolet A but not ultraviolet B radiation requires melanin pigment. *Nat Commun*. 2012;**3**:884. doi: 10.1038/ncomms1893. [PubMed: 22673911]. [PubMed Central: PMC3621412].
11. Guy GJ, Thomas CC, Thompson T, Watson M, Massetti GM, Richardson LC, et al. Vital signs: melanoma incidence and mortality trends and projections - United States, 1982-2030. *MMWR Morb Mortal Wkly Rep*. 2015;**64**(21):591–6. [PubMed: 26042651]. [PubMed Central: PMC4584771].
12. Curiel-Lewandrowski C, Chen SC, Swetter SM, Melanoma Prevention Working Group-Pigmented Skin Lesion S. Screening and prevention measures for melanoma: is there a survival advantage? *Curr Oncol Rep*. 2012;**14**(5):458–67. doi: 10.1007/s11912-012-0256-6. [PubMed: 22907282]. [PubMed Central: PMC3517928].
13. Gladfelter P, Darwish NHE, Mousa SA. Current status and future direction in the management of malignant melanoma. *Melanoma Res*. 2017;**27**(5):403–10. doi: 10.1097/CMR.0000000000000379. [PubMed: 28800028].
14. Cragg GM, Newman DJ. Plants as a source of anti-cancer agents. *J Ethnopharmacol*. 2005;**100**(1-2):72–9. doi: 10.1016/j.jep.2005.05.011. [PubMed: 16009521].
15. Emami SA, Sahebkar A, Tayarani-Najaran N, Tayarani-Najaran Z. Cancer and its Treatment in Main Ancient Books of Islamic Iranian Traditional Medicine (7th to 14th Century AD). *Iran Red Crescent Med J*. 2012;**14**(12):747–57. doi: 10.5812/ircmj.4954. [PubMed: 23482830]. [PubMed Central: PMC3587862].
16. Nobili S, Lippi D, Witort E, Donnini M, Bausi L, Mini E, et al. Natural compounds for cancer treatment and prevention. *Pharmacol Res*. 2009;**59**(6):365–78. doi: 10.1016/j.phrs.2009.01.017. [PubMed: 19429468].
17. Iranzadasl M, Mansouri P, Kamalinejad M, Gorji N. Review of skin cancers terminology, etiology and treatment from ancient Persian medicine view point. *J Med Plants*. 2020;**1**(73):27–36. doi: 10.29252/jmp.1.73.27.
18. Naghibi F, Khalaj A, Mosaddegh M, Malekmohamadi M, Hamzeloo-Moghadam M. Cytotoxic activity evaluation of some medicinal plants, selected from Iranian traditional medicine Pharmacopoeia to treat cancer and related disorders. *J Ethnopharmacol*. 2014;**155**(1):230–9. doi: 10.1016/j.jep.2014.05.025. [PubMed: 24911336].
19. Rabizadeh F, Okhovat AR. [Improving certainty in employment of medicinal plants of traditional medicine by determination of their scientific names]. *J Islamic Iran Trad Med*. 2010;**1**(3 (3)). Persian.
20. Ibrahim SR, Mohamed GA, Zayed MF, Ross SA. 8-Hydroxyirilone 5-methyl ether and 8-hydroxyirilone, new antioxidant and alpha-amylase inhibitors isoflavonoids from Iris germanica rhizomes. *Bioorg Chem*. 2017;**70**:192–8. doi: 10.1016/j.bioorg.2016.12.010. [PubMed: 28069265].
21. Rahman AU, Nasim S, Baig I, Jalil S, Orhan I, Sener B, et al. Anti-inflammatory isoflavonoids from the rhizomes of Iris germanica. *J Ethnopharmacol*. 2003;**86**(2-3):177–80. doi: 10.1016/s0378-8741(03)00055-2. [PubMed: 12738083].
22. Roger B, Jeannot V, Fernandez X, Cerantola S, Chahboun J. Characterisation and quantification of flavonoids in Iris germanica L. and Iris pallida Lam. resinoids from Morocco. *Phytochem Anal*. 2012;**23**(5):450–5. doi: 10.1002/pca.1379. [PubMed: 22213588].

23. Schutz C, Quitschau M, Hamburger M, Potterat O. Profiling of isoflavonoids in Iris germanica rhizome extracts by microprobe NMR and HPLC-PDA-MS analysis. *Fitoterapia*. 2011;**82**(7):1021–6. doi: 10.1016/j.fitote.2011.06.005. [PubMed: 21708227].
24. Asghar SF, Habib ur R, Atta ur R, Choudhary MI. Phytochemical investigations on Iris germanica. *Nat Prod Res*. 2010;**24**(2):131–9. doi: 10.1080/14786410802435950. [PubMed: 20077306].
25. Basgedik B, Ugur A, Sarac N. Antimicrobial, antioxidant, antimutagenic activities, and phenolic compounds of Iris germanica. *Ind Crops Prod*. 2014;**61**:526–30. doi: 10.1016/j.indcrop.2014.07.022.
26. Borhani M, Sharifzadeh M, Farzaei MH, Narimani Z, Sabbaghziarani F, Gholami M, et al. Protective effect of Iris germanica L. in β-amyloid-induced animal model of alzheimer's disease. *Afr J Tradit Complement Altern Med*. 2017;**14**(4):140–8. doi: 10.21010/ajtcam.v14i4.17. [PubMed: 28638877]. [PubMed Central: PMC5471461].
27. Ibrahim SR, Mohamed GA, Al-Musayeib NM. New constituents from the rhizomes of Egyptian Iris germanica L. *Molecules*. 2012;**17**(3):2587–98. doi: 10.3390/molecules17032587. [PubMed: 22388969]. [PubMed Central: PMC6268570].
28. He H, Feng YS, Zang LH, Liu WW, Ding LQ, Chen LX, et al. Nitric oxide induces apoptosis and autophagy; autophagy down-regulates NO synthesis in physalin A-treated A375-S2 human melanoma cells. *Food Chem Toxicol*. 2014;**71**:128–35. doi: 10.1016/j.fct.2014.06.007. [PubMed: 24952311].
29. Xie GY, Qin XY, Liu R, Wang Q, Lin BB, Wang GK, et al. New isoflavones with cytotoxic activity from the rhizomes of Iris germanica L. *Nat Prod Res*. 2013;**27**(23):2173–7. doi: 10.1080/14786419.2013.796468. [PubMed: 23662687].
30. De Monte C, Carradori S, Granese A, Di Pierro GB, Leonardo C, De Nunzio C. Modern extraction techniques and their impact on the pharmacological profile of Serenoa repens extracts for the treatment of lower urinary tract symptoms. *BMC Urol*. 2014;**14**:63. doi: 10.1186/1471-2490-14-63. [PubMed: 25112532]. [PubMed Central: PMC4136420].
31. Dhanani T, Shah S, Gajbhiye NA, Kumar S. Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of Withania somnifera. *Arab J Chem*. 2017;**10**:S1193–9. doi: 10.1016/j.arabjc.2013.02.015.
32. Samulitis BK, Dorr RT, Chow HH. Interaction of dacarbazine and imexon, in vitro and in vivo, in human A375 melanoma cells. *Anticancer Res*. 2011;**31**(9):2781–5. [PubMed: 21868520].
33. Lin JY, Fisher DE. Melanocyte biology and skin pigmentation. *Nature*. 2007;**445**(7130):843–50. doi: 10.1038/nature05660. [PubMed: 17314970].
34. Burke KE, Wei H. Synergistic damage by UVA radiation and pollutants. *Toxicol Ind Health*. 2009;**25**(4-5):219–24. doi: 10.1177/0748233709106067. [PubMed: 19651790].
35. Jones RR. Ozone depletion and its effects on human populations. *Br J Dermatol*. 1992;**127 Suppl 41**:2–6. doi: 10.1111/j.1365-2133.1992.tb16980.x. [PubMed: 1390182].
36. Maverakis E, Cornelius LA, Bowen GM, Phan T, Patel FB, Fitzmaurice S, et al. Metastatic melanoma - a review of current and future treatment options. *Acta Derm Venereol*. 2015;**95**(5):516–24. doi: 10.2340/00015555-2035. [PubMed: 25520039].
37. Ma C, Armstrong AW. Severe adverse events from the treatment of advanced melanoma: a systematic review of severe side effects associated with ipilimumab, vemurafenib, interferon alfa-2b, dacarbazine and interleukin-2. *J Dermatolog Treat*. 2014;**25**(5):401–8. doi: 10.3109/09546634.2013.813897. [PubMed: 23763243].
38. Menaa F. Latest approved therapies for metastatic melanoma: what comes next? *J Skin Cancer*. 2013;**2013**:735282. doi: 10.1155/2013/735282. [PubMed: 23533766]. [PubMed Central: PMC3595667].
39. American Cancer Society. *Complementary and alternative methods and cancer*. 2011. Available from: https://www.cancer.org/content/dam/CRC/PDF/Public/8077.00.pdf.
40. Colleen Lee CD, Zia F, Olaku O, Michie J, White JD. Survey of complementary and alternative medicine practitioners regarding cancer management and research. *J Soc Integr Oncol*. 2009;**7**(1):26–34. doi: 10.2310/7200.2009.0003. [PubMed: 19476732]. [PubMed Central: PMC2719964].
41. Bae K, Park JH, Kim J, Cho CK, Oh B, Costa D, et al. Traditional Oriental Herbal Medicine and Natural Killer Cells for Cancer Patients: A Systematic Review and Meta-analysis. *Phytother Res*. 2017;**31**(4):519–32. doi: 10.1002/ptr.5781. [PubMed: 28198055].
42. Chinembiri TN, du Plessis LH, Gerber M, Hamman JH, du Plessis J. Review of natural compounds for potential skin cancer treatment. *Molecules*. 2014;**19**(8):11679–721. doi: 10.3390/molecules190811679. [PubMed: 25102117]. [PubMed Central: PMC6271439].
43. He H, Zang LH, Feng YS, Chen LX, Kang N, Tashiro S, et al. Physalin A induces apoptosis via p53-Noxa-mediated ROS generation, and autophagy plays a protective role against apoptosis through p38-NF-kappaB survival pathway in A375-S2 cells. *J Ethnopharmacol*. 2013;**148**(2):544–55. doi: 10.1016/j.jep.2013.04.051. [PubMed: 23684722].
44. Im M, Kim A, Ma JY. Ethanol extract of baked Gardeniae Fructus exhibits in vitro and in vivo anti-metastatic and anti-angiogenic activities in malignant cancer cells: Role of suppression of the NF-kappaB and HIF-1alpha pathways. *Int J Oncol*. 2016;**49**(6):2377–86. doi: 10.3892/ijo.2016.3742. [PubMed: 27779658].
45. Shan X, Aziz F, Tian LL, Wang XQ, Yan Q, Liu JW. Ginsenoside Rg3-induced EGFR/MAPK pathway deactivation inhibits melanoma cell proliferation by decreasing FUT4/LeY expression. *Int J Oncol*. 2015;**46**(4):1667–76. doi: 10.3892/ijo.2015.2886. [PubMed: 25672851]. [PubMed Central: PMC6903901].
46. Chen L, Zheng SZ, Sun ZG, Wang AY, Huang CH, Punchard NA, et al. Cryptotanshinone has diverse effects on cell cycle events in melanoma cell lines with different metastatic capacity. *Cancer Chemother Pharmacol*. 2011;**68**(1):17–27. doi: 10.1007/s00280-010-1440-8. [PubMed: 20820782]. [PubMed Central: PMC3032829].
47. Lee KJ, Kim JY, Choi JH, Kim HG, Chung YC, Roh SH, et al. Inhibition of tumor invasion and metastasis by aqueous extract of the radix of Platycodon grandiflorum. *Food Chem Toxicol*. 2006;**44**(11):1890–6. doi: 10.1016/j.fct.2006.06.009. [PubMed: 16890340].
48. Matsuda T, Maekawa K, Asano K, Hisamitsu T. Suppressive effect of juzen-taiho-to on lung metastasis of b16 melanoma cells in vivo. *Evid Based Complement Alternat Med*. 2011;**2011**:743153. doi: 10.1093/ecam/nen081. [PubMed: 19131394]. [PubMed Central: PMC3138676].