

The Effects of the *Sideritis öztürkii* Extract on the Expression Levels of some Apoptotic Genes

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Abstract

Sideritis öztürkii (Lamiaceae) Aytac & Aksoy is an endemic species to Turkey and used as herbal tea and folk medicine in Central Anatolia. The purpose of this study is to reveal the apoptotic effects of different extracts derived from *S. öztürkii* on DLD-1 colorectal cancer cell line via Real Time PCR technique. By this aim, the expression levels of apoptotic genes for each extract were determined via Real Time PCR and then the expression levels were evaluated and compared respectively. Especially, leaf methanol extract was found to be more effective than leaf aqueous extract in terms of apoptosis induction. The results infer that the ironwort extracts have different effects on pro and anti-apoptotic gene expression levels. In conclusion, the leaf and flower extracts have apoptosis inducing activity in the different levels on DLD-1 cell line.

Keywords: DLD-1, endemic, Turkey

1. Introduction

Plants have been the source for medicinal treatments for thousands of years. Traditional medicine uses plants for both their curative and their preventive properties. When used for preventive purposes, i.e. for the maintenance of overall good health, medicinal plants can be classified as functional foods and/or nutraceuticals.

The genus *Sideritis*, a member of the Lamiaceae family, has more than 150 species which are distributed in temperate and tropical regions of the Northern Hemisphere (Tomas-Barberan et al 1988). 46 species and 53 taxa of the genus have existed and 40 of *Sideritis* taxa are endemic in Turkey (Davis 1982, Güner et al., 2012).

Sideritis taxa are traditionally used as teas, flavoring agents and for medicinal purposes in Turkey. The genus is commonly named as ironwort and contains antimicrobial and antioxidant polyphenolics such as flavonoids. In addition, different biological activities of *Sideritis* species have been reported before: anti-inflammatory, antiulcer, analgesic, antibacterial and antifungal (Barberan et al., 1987; Alcaraz et al., 1989; Zarzuelo et al., 1993; Ezer et al., 1994, 1995; Aboutabl et al., 2002; Hernández-Pérez et al., 2002; Basile et al., 2006; Küpeli et al., 2007; Charami et al., 2008). *Sideritis ozturkii* Aytac & Aksoy also locally known as “Dağ Çayı”, is endemic species and used as herbal tea and spice in Central Anatolia, Turkey. According to the literatures, there is no large-scale study of this species. As well as the phenolic contents and antioxidants with together antimicrobial activities, the anti-inflammatory and antinociceptive effects of *S. ozturkii* were searched by several researchers (Küpeli et al., 2007; Sagdic et al., 2008).

Previously, the cytotoxic effects of *S. öztürkii* extracts on colorectal cancer cells have been reported (Gelinci et al., 2017). Besides with, it is more important how the extract application promotes cell death. Apoptosis or necrosis? In the treatment of cancer, apoptotic cell death is more preferable than necrosis. Apoptosis is named as programmed cell death and it is highly desirable that the agent or extract applied might be induced apoptosis in cancer cells. The main objective of this study is to reveal the apoptotic effects of different extracts derived from *S. öztürkii* on DLD1 colorectal cancer cell line via Real Time PCR.

2. Material and Methods

Cell line: DLD1 a human colorectal cancer cell line was obtained from American Type Culture Collection via Gülhane Military Medical Academy. DLD1 cell line was routinely cultured in the media RPMI 1640 medium supplemented with 10% heat inactivated fetal bovine serum (FBS), 1% penicillin-streptomycin. Cells were grown under the conditions of 5% CO₂ at 37°C.

Preparation of plant extracts: The plant samples were dried without sun light and broken into small pieces under sterile conditions. Firstly, plant powders put on Pasteur oven at 110°C for 5h. Following, the prepared samples were extracted with methanol for 6-8h by using a Soxhlet apparatus. To prepare with aqueous leaf extract, leaf samples were poured into the bottle and distilled water was added. The mix was kept for 6 hours and after the extract was filtered using filter paper. The extracts were obtained and then the solvents were evaporated at 40°C by rotary evaporator. Finally, three extracts were obtained; *Sideritis ozturkii* leaf methanol extract (SLE), *S.ozturkii* flower methanol extract (SFE) and *S.ozturkii* leaf aqueous extract (SLA) respectively. The IC₅₀ doses of extracts were obtained from previous study (Gelinci et al., 2017).

Real Time PCR: In line with this objective, DLD1 colorectal cells were grown and prepared for RNA extraction. To determine the apoptotic effects of extracts, the IC₅₀ doses of *Sideritis* extracts were applied and RNA extractions were performed. After RNA extraction cDNA synthesis was performed. The expression levels of apoptotic gene regions were evaluated via Real Time PCR. PCR amplifications were performed using Biorad CFX Connect system. The data was analyzed by comparative CT method and the fold change was calculated by 2^{-ΔΔCT} method. Results obtained from three independent experiment and all the data are expressed as mean.

3. Results and Discussion

In this study, RT- PCR was used to analyze the mRNA expression levels of apoptotic gene regions in DLD1 cells exposed to *Sideritis* extracts at IC₅₀ dose for 24 h. The alterations of studied apoptotic gene regions were shown at Table 1. Firstly, APAF, apoptosis inducing factor, is very important gene region to promote apoptotic cell death. In our study, APAF gene expression was up regulated after the extract applications. The mRNA levels of APAF increasing from 8.6- 62 fold were measured for SLE, SFE and SLA, respectively. SLA extracts had been found more effective than the methanol extracts in terms of promote APAF expression (Fig 1A).

The expression levels of pro-apoptotic BAX gene compared with control cells had a significant increase at extract applied cells. BAX gene expression was raised importantly

around cells 7.5- 80 fold respectively (Fig 1B). SFE extract is more effective than the other extracts. Increased expression of BAX gene expression is a desirable condition for the apoptosis of cancer cells and causes increase the membrane permeability and it leads to release of cytochrome c. By this way, *Sideritis* extracts led DLD1 cells to apoptotic death. On the other hand, BCL2 gene expressions were down regulated with the extract applications except from SLA extract. BAX/BCL2 balance is the key factor for apoptotic cell death. When the equilibrium is deteriorated in the direction of BAX, cells become apoptotic. In case of it is deteriorated in the direction of BCL2, cells become immortal. The balance between BAX and BCL-2 expression levels help to determine the susceptibility of a cell to apoptotic stimuli (Oltvai et al., 1993; Eliopoulos et al., 1995; Chresta et al., 1996). The results indicated that BAX/BCL-2 ratio increased significantly in extract applied cells compared to control.

Table 1. The alterations of studied apoptotic gene regions (SLE: *Sideritis öztürkii* leaf extract, SFE: *S. öztürkii* flower extract, SLA: *S. öztürkii* leaf aqueous extract)

DLD-1 cell line	SLE	SFE	SLA
APAF	↑	↑	↑
BAX	↑	↑	↑
BCL2	↓	↓	↔
P53	↑	↑	↔
TNF	↑	↑	↑
HRK	↑	↑	↑
CASP3	↑	↑	↔

Activator of apoptosis, HRK regulates apoptosis through interaction with death-repressor proteins. In our study, the expression of HRK gene was up regulated with extract application 2.8-14 fold respectively (Fig 1C). The highest increase was observed in the treated group with SLE. The other gene TNF is the most potent inducer of apoptosis. The TNF expression was up regulated 7-28 fold with extract applications (Fig 1D). The highest increase was observed in the same group treated with SLE. p53, tumor suppressor gene is regulating apoptosis and is called “the guardian of genome”. p53 is important for cell cycle control and apoptosis. According to analyses extracts promote the expression levels of p53 gene except from SLA (Fig 1E). Lastly, caspase-3 is a frequently activated death protease, catalyzing the specific cleavage of many key cellular proteins. Activation of caspases located at the end of apoptosis is very important. The expression levels of caspase3 were up regulated by the SLE and SFE applications (Fig 1F) and this up regulation demonstrates that the extracts promote apoptosis. When we generally evaluate the Real Time PCR results it’s clear that the extracts show different but mostly positive effects on inducing apoptosis. Among the applied extracts, SLE is most effective one than the remaining in terms of inducing pro-apoptotic gene expressions such as BAX, HRK, TNF, p53 and casp3.

As far as known, this is the first study revealing apoptotic effects of *S. öztürkii* extracts. Although there have been previous studies on the the plant content and cytotoxicity, there is no any study carried out at the molecular level about this topic. On the other hand, there are studies about different *Sideritis* extracts applications on cancer cells, as literature. The anti-proliferative properties of *S. libanotica* were reported on Vero, C6 and HeLa cell lines by Demirtaş et al., (2009) and it is noted that the extracts have

statistically significant antiproliferative activities. The cytotoxic effects of *S. scardica* extracts were reported against B16 mouse melanoma and human leukemia HL-60 cell lines (Tadic et al., 2012).

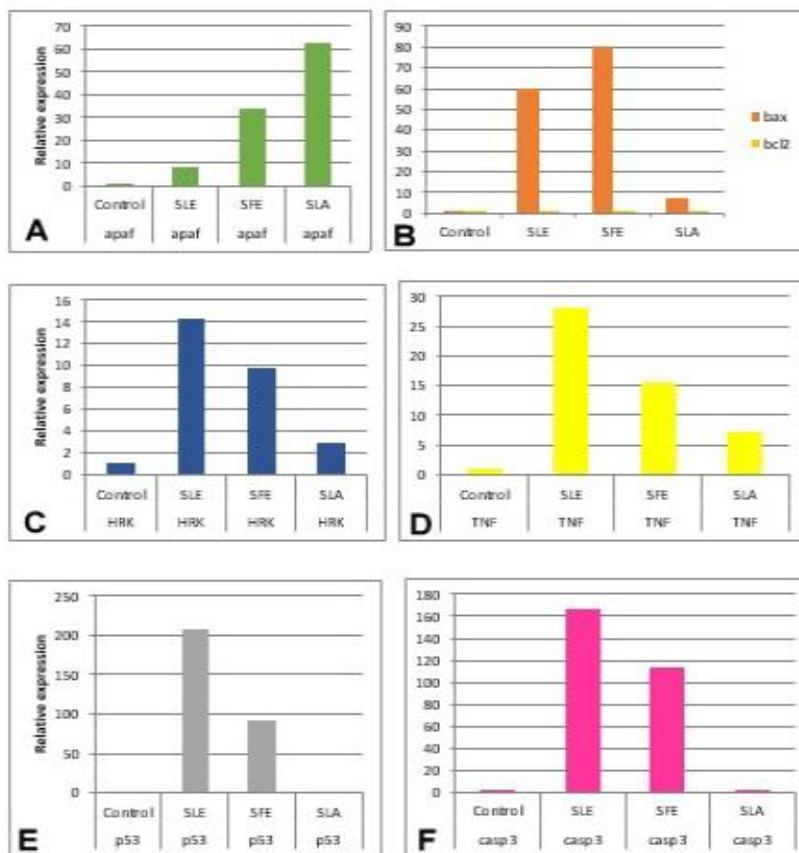


Figure 1. Relative expressions of studied apoptotic gene regions (SLE: *Sideritis öztürkii* leaf extract, SFE: *S. öztürkii* flower extract, SLA: *S. öztürkii* leaf aqueous extract)

The cytotoxic activity of different *S. scardica* extracts were studied against the rat glioma C6 line and rat astrocytes in primary culture by Jeremic et al., (2013) and it is reported that the extracts decreased viability of rat C6 glioma cells with cell cycle arrest and finally that induces apoptosis and autophagy. Additionally, it is reported that the methanol extracts of *S. syriaca* affected significantly the proliferation and cell viability of MCF7 cells in a dose dependent manner (Yumrutaş et al., 2015). As a contribution to all these, it is determined that *S. öztürkii* extracts induces similarly apoptosis on DLD1 cells. Even though the cell lines and plant species used are different, all refer to a consensus that *Sideritis* species have anti-cancer potential.

4. Conclusion

As a general remark, the genus *Sideritis* provides a wide range of research possibilities. We suggest carrying out pharmacological studies over untouched species belonging to this genus. The findings point out that these extracts would be having a potential in cancer therapy for future studies. This study is pioneer study for future targets and our studies will continue for determine the active phytochemical in the extracts content and to find phytoterapeutic agents.

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