Original Article

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Effect of Isolated Tricin from Arial Part of *Allium atrovoilaceum* Boiss. on Proliferation and Apoptosis in PC3 Cell Line

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ABSTRACT

BACKGROUND AND OBJECTIVE: and anti-proliferative effects in several cancer cell lines. Considering the importance of using natural anti-cancer drugs in treatment-resistant cancers such as prostate cancer, the aim of this study was to investigate the effect of tricin on cell growth and proliferation and induction of apoptosis in human PC3 prostate cancer cell line.

METHODS: In this experimental study, the PC3 cell line was prepared from the Pasteur Institute of Iran and cultivated. Extraction and purification of Tricin were performed using column chromatography and re-crystallization of *Alliuum atrovoilaceum* extract. The cytotoxic effect of Tricin in concentrations of 60,80,100,120, and 140 µM was evaluated by MTT method. Apoptotic effect was evaluated in the treated cell group with the IC_{50} concentration of Tricin and untreated cells (control group), using an Annexin-V kit and flow cytometry.

FINDINGS: The viability of cells at different tricin concentrations were 85.66±1.52, 76±3.60, 66.33±4.16, 44±3.60, and 36.66±3.21, respectively ($p<0.01$). The IC_{50} concentration of tricin was 117.5±4.4 µM for PC3 cell line. The apoptosis rate in PC3 cells after 48 hours of treatment with IC_{50} concentration of tricin was 24.3±0.58%, which was not significant in comparison with control cells (23.3±0.58%).

CONCLUSION: The results of this study showed that Tricin resulted in cell death in the PC3 cell line, but the cell death mechanism was not apoptosis.

KEY WORD: *Prostate Cancer, Tricin, Apoptosis.*

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Introduction

Prostate cancer is the second leading cause of death from cancer in men (1). Chemotherapy is one of the most commonly used treatments for prostate cancer. However, drug toxicity, adverse effects on healthy tissues and the relapse of disease caused by drug resistance are some of the disadvantages of chemotherapy (2, 3). Cancer research has indicated lower toxicity and fewer side effects caused by herbal medicinal compounds, and therefore, studying and recognizing herbal compounds effective in treating cancers is very important (4). Flavonoids of benzo[a]pyrene derivatives are composed of phenolic rings and pyran (5). Flavonoids have anti-oxidant properties, protect against heart disease and also have antiviral role. On the other hand, their antitumor effect does not have the harmful effects of chemotherapeutic drugs (6, 7). The anticancer mechanisms of flavonoids include induction of apoptosis, inactivation of carcinogens, inhibition of angiogenesis, cell growth inhibition and drug resistance (8). Tricin is a flavonoid from flavone category with 4′,5,7-Trihydroxy-3,6-dimethoxyflavone structure, and is found in many human food sources such as Palmaceae and Poaceae families and is considered a valuable compound due to its anti-oxidant effects and inhibition of cancer cell proliferation (9, 10). In this study, *Allium atrovoilaceum* with the Persian name, Purple Onion, and the local name, Sirdang, was used to extract the tricin (11). Due to the anti-cancer effects of tricin (12, 13), its effect on cell growth and proliferation and induction of apoptosis in PC3 was investigated for the first time.

Methods

Cell culture and isolation of tricin: In this experimental study with ethics code IR.SKUMS.REC.1394.135, the prostate cancer cell line PC3 was purchased from the Pasteur Institute of Iran. Cells were cultured in DMEM medium with 10% FBS and 1% penicillin / streptomycin (Gibco, USA) under the conditions of 37 ˚C, 5% CO2 and 90% humidity in the incubator. *Allium atrovoilaceum* was collected from the Rig Mountain in Lordegan city in Chaharmahal and Bakhtiari Province in spring 2014 and the species was confirmed by Dr. Keramatollah Saeidi in Shahrekord University. A voucher specimen (SKUMS-801) has been deposited in the Herbarium of Medical Plants Research Center, Shahrekord University of Medical Sciences. Extraction was done by percolation method using methanol solvent. After condensation by rotary, it was partitioned using liquid-liquid extraction method with ethyl acetate. Then, ethyl acetate fraction was Chromatography by silica gel (0.063 - 0.100 mesh), eluted with n-Hexane: ethyl acetate: methanol solvent system gradually increasing polarity. Similar fractions with TLC were detected by two chloroform solvent systems: ethyl acetate (60:40) and ethyl acetate: glacial acetic acid: formic acid: water (100: 11: 11: 27) and were mixed together. To observe the stains, ultraviolet light was used at 254 and 366 nm wavelengths and NP / PEG solution.

Purification of the Tricin: Ethanol was used as a solvent for second crystallization fraction with number 81. After gradual cooling, the needle crystals were extracted and washed with cold absolute ethanol. The pure material was determined using (AVANCE-AV-30) ^{1}HNMR, ^{13}CNMR and MS techniques outside Iran. After extraction and purification, the tricin powder was dissolved in 80% ethanol solution and 20% DMSO and sterilized by 0.22 µ filter.

Cytotoxicity test using MTT assay: 5×10^3 cells were cultured in 96 – well plate and in five groups treated with Tricin at 60, 80, 100, 120 and 140 µM concentrations (12, 13) were incubated for 24 h.

The control group did not have tricin. The cell culture medium was then replaced with a new medium containing 20 µ tetrazolium bromide solution 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The cells were incubated in the dark and then the supernatant was removed and 150 ml DMSO was added. Optical absorption of 570 nm was assessed using ELISA reader (Stat Fax). Cell viability was calculated as follows:

100 × (Optical absorption of controls / Optical absorption of samples) = viability of cells (%)

Apoptosis: Apoptosis assay was performed using Annexin-V kit (BD, US) and based on flow cytometry between the two groups of cells treated with IC_{50} concentration of tricin for 48 h and control group (without treatment). The cells were fixed after treatment and analyzed according to the instruction of the kit using the above solution and Propidium Iodid staining solution and flow cytometry device (Partec).

Analysis of results: All experiments were performed three times. The results of cell growth and proliferation were analyzed with Prism V5 software using one-way ANOVA and, in the case of significance, they were analyzed using Tukey's post hoc test and apoptosis results were analyzed by t-test and Mann-Whitney test. $P<0.05$ was considered significant.

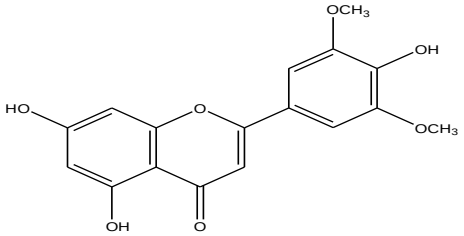
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Results

The results of the purification of the molecule with ZG-G code: The results of the spectroscopic studies were specified as follows. The material obtained from the interpretation of the spectra was identified as tricin (Fig. 1). ^{1}HNMR: (DMSO-d6،400MHz) δ7.32 (2H،s،H-2′،H-6′), 6.97 (1H،s،H-3), 6.57 (1H ،d،J= 2.0Hz،H-8), 6.21 (1H،d،J=2.0Hz،H-6), 3.89 (6H،s،OCH3×2)

^{13}CNMR: (DMSO-d6،300MHz)δ181.7(C،C-4)،164.2(C،C-2)،163.6(CH،C-7)،161.4(CH،C-9) ،157.3(C،C-5)،148.2(C×2،C-3′،C-5′)،139.8(C،C-4′)،120.3(C،C-1′)،104.3(CH×2،C-2′،C-6′) ،103.6(C،C-10) ،103.5(CH،C-3) ،98.8(CH،C-6) ،94.2 (CH،C-8)،56.3(-OCH3×2).

MS: m/z330 $[M]^{+}$ ،178 $[M-152]^{+}$ ،153 [M-152-26]



Tricin

Figure 1. Molecular structure of tricin

Cell growth and proliferation: By increasing the concentration of tricin, the death of PC3 cells increased in a dose-dependent manner (Fig 2). IC_{50} concentration of tricin in PC3 cells was found to be 117.5 ± 4.4 µM after 48 hours of cell treatment.

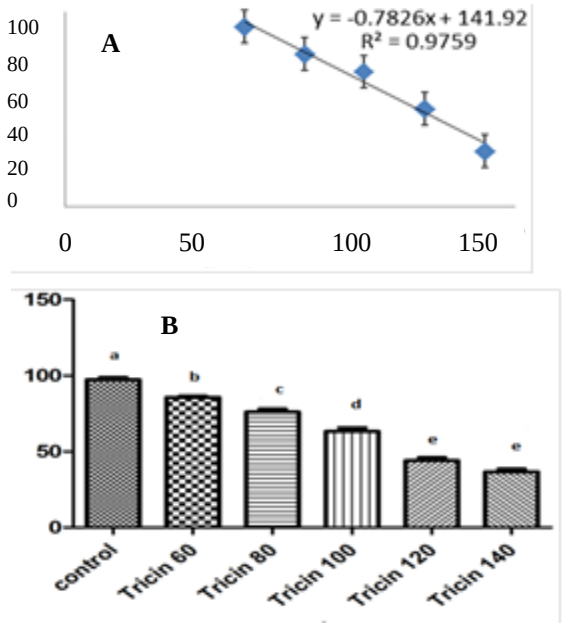


Figure 2. A: The apoptotic effect of tricin at different concentrations (IC_{50} tricin, 117.5 ± 4.4 µM). B: Dose-response of tricin on PC3 cell line. Non-similar letters are significant at the level of 0.05.

Apoptosis: Apoptosis in PC3 cells treated for 48h with IC_{50} concentration of tricin was 24.3 ± 0.58% and apoptosis in the control group (cells not treated with tricin) was 23.3±0.58%. There was no significant difference between the two groups (Fig 3).

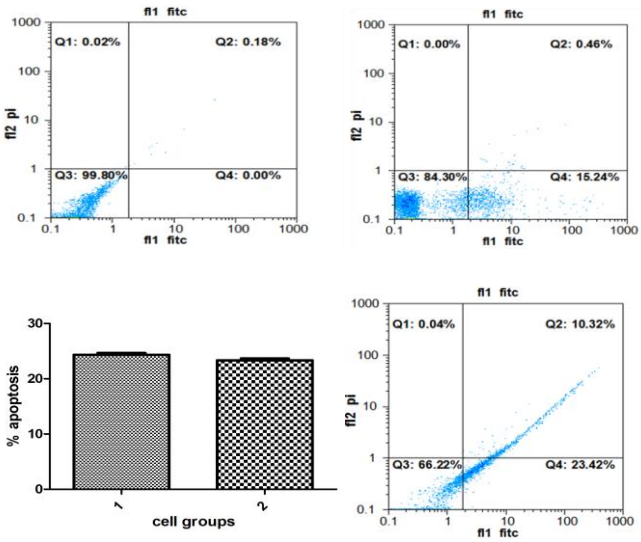


Figure 3. Cell apoptosis at 117.5 ± 5 µM (IC50) concentration of tricin. The results showed that the mechanism of death of PC3 cells treated with IC_{50} concentration of tricin after 48 h was not due to apoptosis and cell death probably occurred through another mechanism (P = 0.157).

Discussion

The present study showed that tricin extracted from *Allium atrovoilaceum* has inhibitory effect on dose-dependent proliferation in the PC3 cell line. This result is consistent with previous studies in this regard. Cia et al. showed that tricin has an inhibitory effect on proliferation and cell cycle in MDA – MB – 468 breast cancer (12). The findings of Oyama et al. indicated the effect of tricin on inhibition of the inflammation and carcinogenesis of the colon, as well as a significant reduction in the growth and proliferation of adenocarcinoma cells in male CD-1 rats (13).

However, the rate of apoptosis induced in PC3 cells treated with IC_{50} concentration of tricin (117.5 µM) in 48h was not significantly different from that of control cells ($p > 0.05$). This finding is consistent with the findings of Cia et al. who showed that tricin (≥ 5 µM) led to the discontinuity of MDA – MB – 468 breast cancer cells in the G2 / M phase without inducing apoptosis (12). According to previous studies, the structure of flavonoids has an effect on their activity, and flavonoids, depending on the molecular structure, can act as kinase inhibitors in signal transmission. Studies have shown that the position and number of

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hydroxyl groups of 2-phenyl ring (ring B) have a powerful effect on inhibitory effect of these molecules (14, 15). The total number of hydroxyl groups not only affects the antioxidant activity but also affects the pro-oxidant activity of some flavonoids. Flavonoids with more than two hydroxyl groups, especially on the ring B, lead to a significant increase in the production of hydroxyl radicals in the Fenton system, which is responsible for the cytotoxic and pro-apoptotic effects of flavonoids extracted from many medicinal plants. Methylation of hydroxyl groups may reduce the pro-oxidant behavior of flavonoids (5). Wang et al. reported that the chance of activation of Caspase-3 and the induction of apoptosis by flavonoids depends on the number of hydroxyl groups in the 2-phenyl ring and the absence of 3-hydroxyl group, and the presence of the 3-hydroxyl group inhibits the induction of cell apoptosis. On the other hand, the greater the number of hydroxyl groups in the 2-phenyl ring, the greater the power to regain the ability to induce cell death inhibited by the 3-hydroxyl group (15).

Therefore, it seems that in the present study, the lack of induction of apoptosis is due to the presence of 3′ and 5′ methoxy groups in tricin molecule that prevent the necessary hydrogen bonding, and the presence of a small number of hydroxyl groups on ring B of tricin can also play a role in this process. Overall, the results of this study showed that, despite the significant effect of tricin on reducing the proliferation of PC3 cells, but cell death mechanism induced by tricin was not apoptosis. In this concentration and this period, tricin did not result in significant apoptosis compared to the control group. Since molecular structure plays a role in the function of flavonoids, more studies are needed in this regard.

Suggestions: Since the PC3 cell line is a metastatic category, it may be possible to help treat or control the progression of prostate cancer by conducting studies on complementary therapies with flavonoids, such as tricin. It is suggested that interventional studies with larger sample size be conducted on the effect of different concentrations of tricin in other cancer cell lines, animal models, and human.

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