

Inhibitory Effect of Myricetin on Melanoma Cells (A375)

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Abstract

Background: Cancer, a major cause of mortality worldwide, is a group of diseases distinguished by uncontrolled growth and expansion of abnormal cells. According to American Cancer Society, melanoma, a kind of skin cancer, is one of the most prevalent cancers. The side effects of chemical treatment developed more demands on natural products. Flavonoids, polyphenol compounds, with anticancer and antioxidant activity attracted more attention to themselves.

Materials and Methods: Through this investigation the effect of myricetin on cell proliferation was determined by MTT (Methylthiazolyl diphenyl-tetrazolium bromide) assay. A375 cell lines were seeded in a 96 wells plate and were exposed to different concentrations of myricetin (10, 15, 20, 40, 60, 80, and 100 μ M). After considered times, the MTT solution was added, then the viability of cells was detected by measuring the absorbance on 570 and 630 nm.

Results: Our finding showed that low concentration of myricetin (up to 25 μ M) has no toxicity effect. Also the result confirmed the IC₅₀ of myricetin on melanoma cells for three ordered period (24, 48, 72 hours) as following: 50, 40, 35 μ M, respectively.

Conclusion: According to this research, myricetin has anti-proliferative effect on melanoma cells, which can be used as a therapeutic agent. We hope that this study could be used as a mile stone in future researches to acquire confirmative results.

Keywords: Melanoma, Flavonoids, Polyphenol, A375 cell line, Myricetin, Anti-proliferative

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Introduction

Cancer as a leading cause of death in most countries, especially in less developed countries is growing worldwide. The approval of mechanical lifestyle, poor diet, less activity, smoking and reproductive changes have increased the cancer burden globally¹. Among the list of common cancers reported by the American Cancer Society, melanoma, a kind of skin cancer, due to new lifestyles allocated high rate of mortality to itself².

The toxicity of current chemotherapeutics put all of efforts toward new anticancer drugs with greater efficacy and fewer side effects. Among natural compounds, low molecular weight product, flavonoids, rich in seeds, vegetables, and citrus fruits, attracted more attention according to their impressive pharmacological properties including antibacterial, antioxidant, antiangiogenic, anticancer and antiallergic activities³.

Chemically they are polyphenol with a phenyl benzopyrone structure (C₆-C₃-C₆), on the basis of

saturation level, C- ring substitution pattern and opening of the central pyron ring classified into different groups of flavonoids^{4,5} (Figure 1). There is a relationship between the activity of flavonoids and their structure, so myricetin based on the presence of an ortho-hydroxyl groups, a C₂-C₃ double bond in the C- ring is considered as one of the remarkable compounds among flavonoids (Figure 2)^{6,7}.

The role of myricetin to promote apoptosis, inhibition of signaling pathway, and cell cycle block is distinguished. Some studies showed that myricetin inhibited the proliferation of GC HGC-27 and SGC 7901 cells *in vivo* and *in vitro*⁸. However the function of myricetin in melanoma cells is not determined, in this study we determined the effect of myricetin on proliferation and viability of A375 cells.

As myricetin has presented the anti-inflammatory activity, a research exhibited that this flavonoid could suppress the activation of Akt, NF- κ B pathway that lead to the reduction of TNF- α -stimulated inflammatory mediator⁹. Another study showed that myricetin can be used as a potent inhibitor of purine synthesis pathway which is crucial for cancer development¹⁰. Beside these efficacies of myricetin another potency of myricetin is restoring iron homeostasis in patients with iron deficiency or anemia¹¹.

Methods

Myricetin, MTT (Methylthiazolyl diphenyl-tetrazolium bromide) powder was purchased from sigma Aldrich. A375 cell line was prepared from pasture institution. FBS (Fetal bovine serum), PBS (phosphate – buffered saline) tablet, penicillin-streptomycin, trypsin-EDTA were obtained from Invitrogen. Culture media (DMEM high glucose) was obtained from Biosera.

A375 cells were cultured in DMEM high glucose, containing 10% (v/v) FBS and 1% penicillin-streptomycin. The cells were incubated at 37°C in a humidified atmosphere of 5% CO₂. Confluent cultures of melanoma cells were rinsed in phosphate- buffered saline (PBS) and removed from the flask using 0.25% trypsin/EDTA, neutralized with FBS and the cell suspension were prepared.

To find the doubling time of melanoma cells, the 8000 cells were placed on each well in a 96 wells plate. During one week in different periods of time (24, 32, 48, 56, 72, 76, 80, 96, 100, and 122h) the cells were counted and the

data were recorded. The experiments were done quadruplicate. Three 96 well plate for three period of time (24, 48, 72 hours) were chosen. The cells were placed at a density of 8000 cell/well with culture media for an overnight to attach the plate.

Cell viability was detected by use of micro culture tetrazolium technique (MTT). This quantitative measure provides the number of viable cells by determining the amount of formazan crystals produced by metabolic activity in treated versus control cells. After 24 h, the media was replaced with different concentrations of myricetin as following: negative control, 0.1% ethanol control, 10 μ M, 15 μ M, 20 μ M, 40 μ M, 60 μ M, 80 μ M, 100 μ M, and DMSO 30% as positive control. After incubation in determined time (24, 48, 72 hours) the media was removed, new media along with MTT reagent in PBS (5 mg/ml) was added to each well. The plates were incubated for 4 hours in a humidified atmosphere of 5% of CO₂ at 37°C. After removing the media, DMSO was added and the absorbance was measured at 570 nm relative to 630 nm. All experiments were done 5 times.

Statistical analysis was performed using PRISM software. Differences between the control and treated group were analyzed by mean \pm SD using one-way ANOVA test with considering significant at p<0.05 and p<0.0001. This study is investigated according to the Helsinki declaration approved by our regional institution number: IR.QUMS.REC.1394.155 Qazvin University of medical science.

Results

After recording the data in Excel software, the result showed that the doubling time of melanoma (A375) cells was about 17 hour (Figure 3). To ascertain the inhibitory effect of myricetin on melanoma cells, A375 cells were treated with different concentrations in three period of time (24, 48, and 72 Hours). As shown in Fig4, the more concentration of myricetin, the more mortality of cells was seen, so that in high concentration like 100 μ M, almost all cells were inhibited. Also Fig5 verified that low concentration (up to 25 μ M) has no significantly cytotoxic effect and the IC₅₀ (50% inhibitory concentration) for 24 h, 48 h, and 72h was 50 μ M, 40 μ M and 35 μ M respectively.

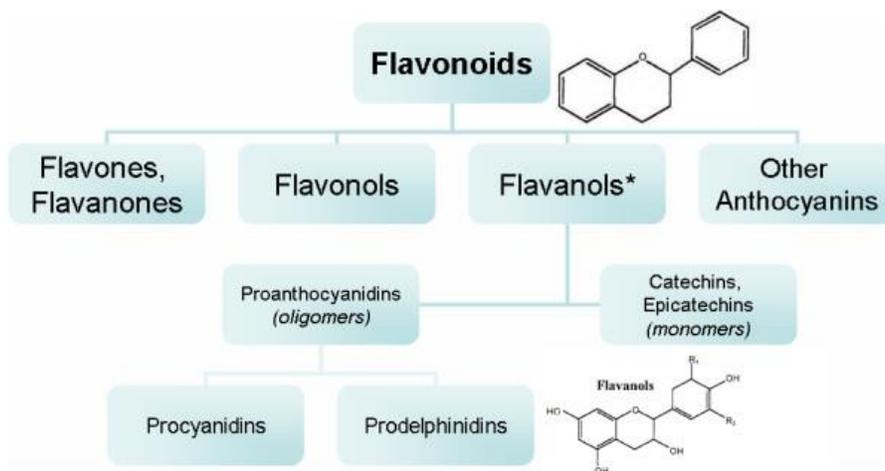


Figure 1. The backbone structure of flavonoid and its subclasses categorized according to their hydroxyl groups (4).

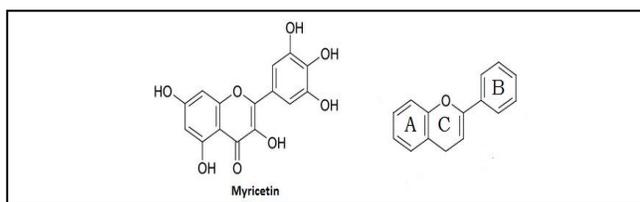


Figure 2. The structure of Myricetin (7).

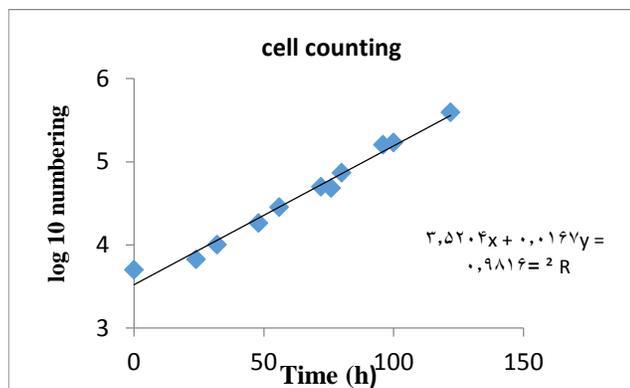


Figure 3. Cell counting curve.

Discussion

Myricetin as a kind of flavonoid, widely spread in vegetables and fruits, has multiple biological effects. In the present study our primary result indicated that myricetin could inhibit cell proliferation. We determined that the inhibitory effect (IC50) of Myricetin on melanoma cells (A375) for 24, 48, and 72 h was 50, 40, and 35µM, respectively.

Although melanoma displays less than 10% of skin cancer, it is accountable for more than 75% of skin

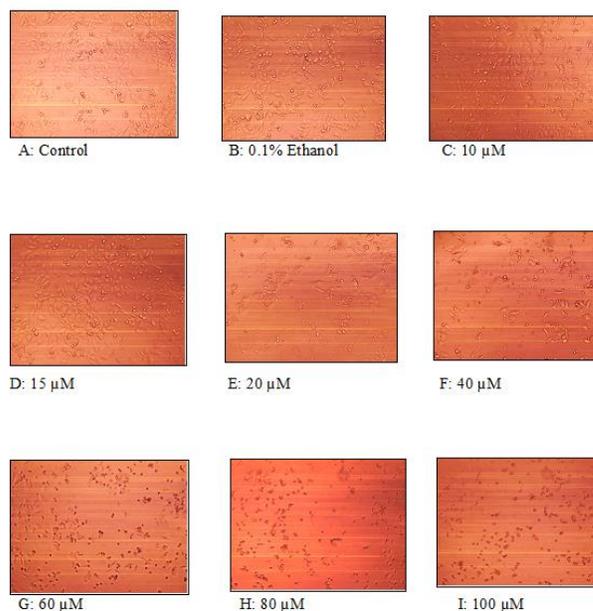


Figure 4. After treatment with different concentrations of myricetin. The groups were A, B, C, D, E, F, G, H, and I, with different concentrations as: control, 0.1% ethanol, 10, 15, 20, 40, 60, 80, and 100µM myricetin respectively.

cancer- related death¹². Over the past decade, according to the significant acceleration of cancer, substantial compounds include flavonoids of human diet raised more demand¹³. Studies have suggested that myricetin could inhibit tumor growth¹⁴, and block signal transduction¹⁵. Several studies investigated that flavonoids could be inhibitors of kinases involving protein kinase C¹⁶ and tyrosine kinase¹⁷. A research demonstrated that structurally related flavonoids can activate apoptosis in HL-60 cells¹⁸.

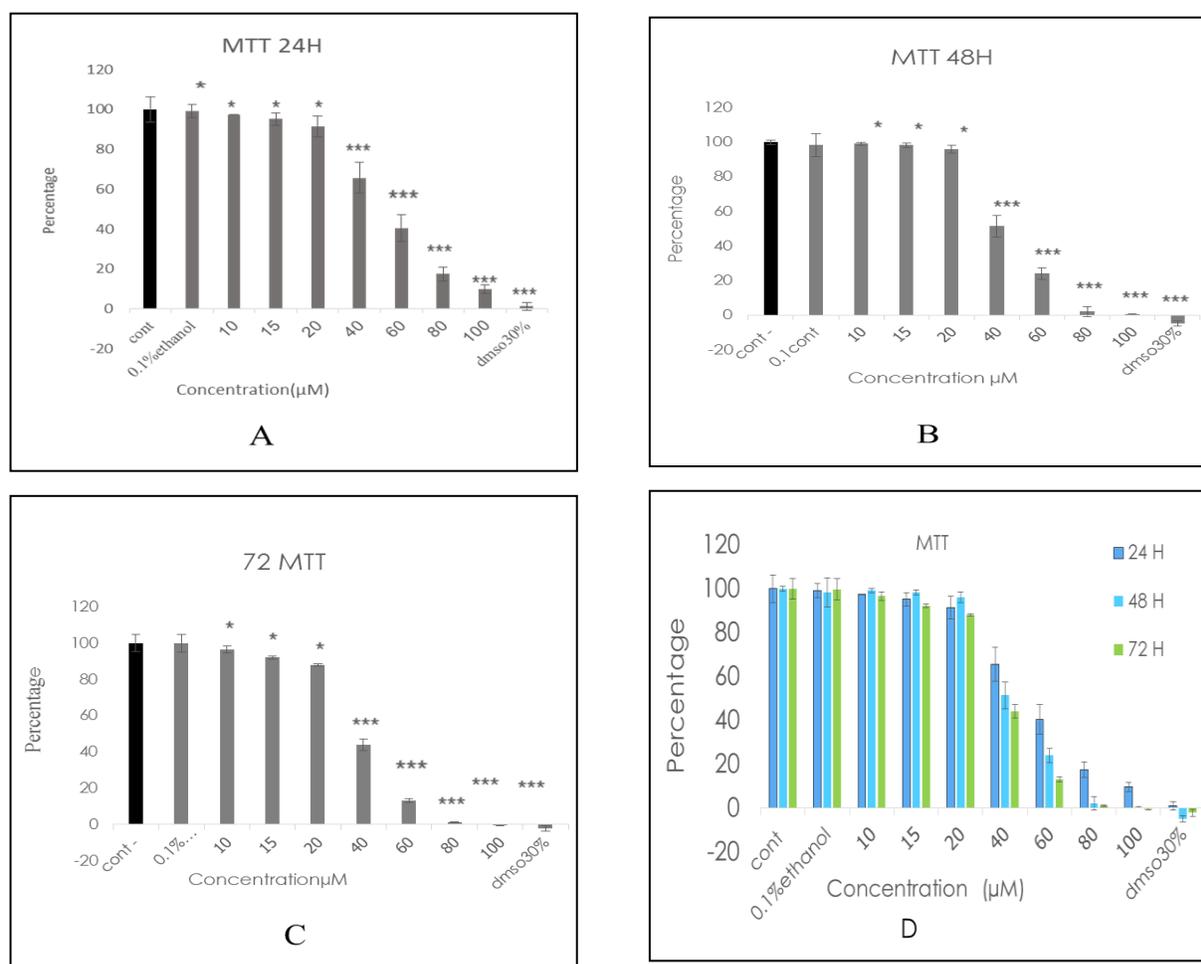


Figure 5. Myricetin inhibits proliferation of melanoma cells. A, B, and C were respectively 24, 48, and 72 hour post treatment with myricetin. Data were presented as the mean \pm SD of 5 times experiments. (* $P < 0.05$, *** $P < 0.0001$) versus control group.

Another study showed that Quercetin, a kind of flavonoid, can motivate apoptosis in tumor cells containing K 562, Molt-4, gastric and lung cells¹⁹.

Since there is a relationship between the structure and function of flavonoids, three hydroxyl group in B-ring of myricetin, can modulate its inhibitory effect and exhibit brilliant anticancer, antioxidant, anti-inflammatory properties²⁰ (Figure 2). Myricetin has been investigated to obtain cytotoxic effects on several cancer cells such as colon²¹, breast²². Another study determined that the concentration of myricetin up to 25 μM has no significantly effect on HaCaT cell viability⁹.

Our finding in this manuscript showed that myricetin greatly decreased melanoma cell viability (Figure 5). Our result verified that low concentration of myricetin had no cytotoxicity effect on cells, but high dosage of myricetin can cause cell mortality (Figure 4). We also detected that myricetin IC_{50} was 50 μM , 40 μM , and 35 μM in order

for 24, 48, and 72h (Figure 5).

Another research has demonstrated that myricetin can inhibit different signaling pathway such as PI3 kinase/ Akt5²³. Some studies have shown that myricetin can inhibit proliferation of cells (GC HGC-27 and SGC7901) *invitro* and *invivo*⁸.

This work once again demonstrated the importance of these natural products as the potential chemopreventive agents.

Conclusion

This work in approval of other researches acknowledged the inhibitory effect of myricetin and showed that myricetin in low concentration (up to 25 μM) had no toxicity effect; also the inhibitory effect (IC_{50}) of myricetin for 24, 48, and 72h was respectively 50, 40, and 35 μM . These preliminary results showed that MTT

assay could be used as a primary technique to determine the cytotoxicity effect of drugs and to evaluate the resistance of cells to different agents.

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