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Research Article

The Effect of Fisetin on KATO-III cell proliferation and invasion

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ABSTRACT:
Fisetin is a natural chemical that widely found in different plants. The aim of this study is to investigate the role of fisetin on proliferation and invasion on KATO-III human gastric carcinoma cell. KATO-III cells were seeded into the plates and 24h after dose-dependent effects of fisetin (50 and 100µM) were tested on cultured KATO-III cells. Real-time cell proliferation impedance analysis was performed up to 72h and the Transwell insert assay was performed. Relative mRNA expressions of MMP2 and MMP9, which are markers of invasion, were measured. Data are presented as fold-change in expression of any group compared to that of control group, using the 2-ΔΔCt method. Statistical comparisons were made using one-way ANOVA followed by Tukey's test. Fisetin exert anti-proliferative and anti-invasion effect on KATO-III cells. According to the cell proliferation impedance results, it was shown that proliferations of all fisetin treated groups were suppressed in dose dependent manner. According to transwell invasion results, 100µM fisetin group showed important inhibitory effects on invasion of KATO-III cells compared to the Control group. Regarding to the mRNA expression results of MMP2 and MMP9, it was shown that 50 and 100µM fisetin treatments significantly decreased these expressions compared to Control group. We conclude that fisetin negatively regulates the invasion and proliferation potentials of KATO-III cells via the MMP9 and MMP2 suppression. Thus, our findings implicate fisetin a potential therapeutic target in KATO-III cells.

Keywords: Fisetin, invasion, KATO-III, proliferation

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1. INTRODUCTION

Cancer is a disease characterized by uncontrolled proliferation of cells and spread to other parts of the body [1]. Cancer cells surround healthy cells and blood vessels to form a new microenvironment. This microenvironment also leads to the formation of new blood vessels that provide oxygen and nutrients necessary for tumor growth, namely angiogenesis [2]. Such uncontrolled division and growth of cancer cells complicates the treatment of the disease.

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Around 14 million people worldwide are affected by cancer in a year, and about 9 million of them die [3] The most common types of cancer are breast cancer in women and prostate cancer in men. The highest death rates are seen in lung cancer patients [4]. This situation in Turkey is similar to the world in general. In our country, approximately 175 thousand people are diagnosed with cancer in a year and breast and lung cancer take the first place [5].

Fisetin (3,7,3′,4′ -tetrahydroxy flavone) is a polyphenolic flavonoid, commonly found in fruits and vegetables such as onions, cucumbers, apples, grapes, persimmon, nuts and strawberries. Fisetin is known to have broad pharmacological effects such as anti-tumor [6], neurotrophic [7], anti-inflammatory [8], antioxidant [9] and antiangiogenic [10].

The aim of this study is to investigate the role of fisetin on proliferation and invasion on KATO-III human gastric carcinoma cell.

2. MATERIAL AND METHOD

Cells were seeded into the plates and 24 h after Dose-dependent effects of fisetin (50 and 100 μM) were tested on cultured KATO-III cells. Relative mRNA expressions of MMP9, which are markers of invasion, were measured. Statistical comparisons were made using one-way ANOVA followed by Tukey's test.

KATO-III cell lines will be cultured in suitable culture media containing 10% serum and 1% penicillin-streptomycin (Life Technologies, USA). Cell lines will be incubated in 370C, 90% humidity and 5% CO2 medium.

Experimental Groups;

1. Control (Cancer Cell lines)
2. Fisetin (at doses of 50, 100 μM)

2.1. Invasion Test

Invasion levels will be performed using Transwell Chambers wells and 24-well cell culture dishes. 750 μl serum medium will be placed in the lower wells and 200 μl (250.000 cells/ml) serum-free medium cells will be planted on the upper side. Appropriate doses of drug applications will be made. Incubate at 37^{o}C for 12 hours. Cell numbers migrating to the membrane will be taken using a Leica invert light microscope.

2.2. Real Time PCR Analysis

RNA Isolation: Tissue samples Total RNA isolation steps in Qiaqube (Qiagen RNA isolation device) using RNeasy Mini Kit (Qiagen) will be performed as recommended by the manufacturer.

Reverse Transcriptase Reaction and cDNA Synthesis: High Capacity cDNA Reverse Transcription Kit will be used to synthesize cDNA from total RNA. Each reaction is carried out with 10 ll RNA and cDNA synthesis will be performed with Veriti 96 Well Thermal Cycler (Applied Biosystem) according to the following temperature values. The amount of cDNA will be determined by nano drop spectrophotometer (EPOCH Take3 Plate, Biotek) and stored at -20˚C.

Quantitative Determination of mRNA Expression by Real Time PCR MMP9 mRNA expression will be quantified using the TaqMan Gene Expression Master Mix kit. Amplification and quantification will be performed on the StepOne Plus Real Time PCR System (Applied Biosystems). B-actin as housekeeping gene will be pipetted as follows and carried out with 40 cycles. Ct values will be taken from the instrument and converted to delta Ct by formula and the findings will be evaluated statistically in IBM SPSS 20.0 package program.

3. RESULTS

Fisetin exert anti-proliferative and anti-invasion effect on KATO-III cells. According to the cell proliferation impedance results, it was shown that proliferations of all fisetin treated groups were suppressed in dose dependent manner. According to transwell invasion results, 100μM fisetin group showed important inhibitory effects on invasion of KATO-III cells compared to the Control group. Regarding to the mRNA expression results of MMP2 and MMP9, it was shown that 50 and 100μM fisetin treatments significantly decreased these expressions compared to Control group. MM9 mRNA expression results are shown figure 1. Real-time cell proliferation impedance analysis results are shown figure 2.

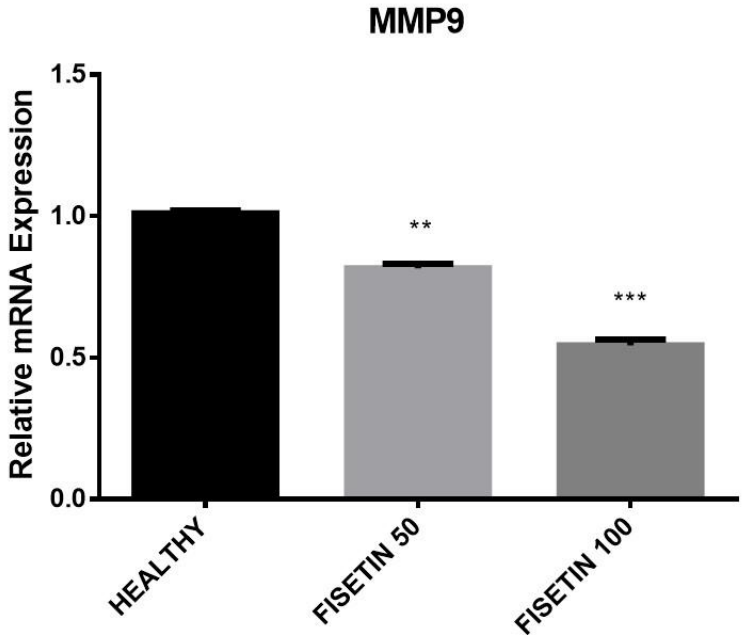

Figure 1. MM9 mRNA Expression Results

Results present as mean±S.D. Data are presented as fold-change in expression of any group compared to that of control group, using the 2-ΔΔCt method. ($p<0,001$).

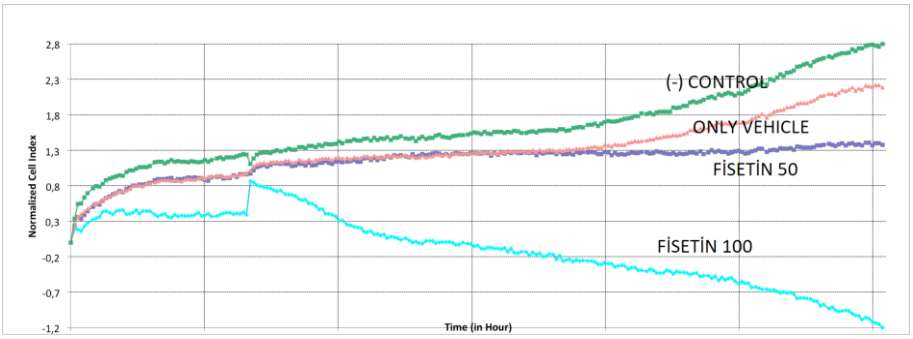

Figure 2. Real-time cell proliferation impedance analysis results

4. CONCLUSION

It was found that fisetin reduces the level of inflammatory cytokines such as TNFα, IL6 and IL-1β [11]. Fisetin inhibited NO, PGE2, IL-1β, and IL-6 production, iNOS and COX-2 expression, NF-κB activation [12]. On the other hand, fisetin has been found to reduce angiogenesis by reducing eNOS, VEGF, EGFR, COX-2 expressions [13].

It has been proven in some previous scientific studies that fisetine decreases the amount of increased MDA and that it decreases the levels of decreased SOD, CAT, GSH and GST to a level close to normal values [14, 15]. Recent findings indicate that administration of fisetin significantly increases antioxidant response element activity and nuclear translocation of Nrf2 [16]. In addition, Fisetin has been shown to inhibit NADH oxidation and ATPase activity [9].

We conclude that fisetin negatively regulates the invasion and proliferation potentials of KATO-III cells via the MMP9 and MMP2 suppression. Thus, our findings implicate fisetin a potential therapeutic target in KATO-III cells.

Acknowledgment

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Conflict of Interest

Author has no personal financial or non-financial interests.

REFERENCES

1. Burtis CA, Bruns DE, Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics. 7. baskı. 2015, St. Louis Missouri: Elsevier Saunders.

2. Cai C, Chen Y, Zhong S, Ji B, Wang J, Bai X, et al, Anti-inflammatory activity of N-butanol extract from Ipomoea stolonifera in vivo and in vitro. PLoS One. 2014; 9; e95931.

3. World Healty Organisation. Cancer. 2017 [21.10.2017]; Available from: http://www.who.int/mediacentre/factsheets/fs297/en/.

4. Bignold L, Principles of Tumors. 1. baskı. 2015, New York: Academic Press.
5. T.C.Sağlık Bakanlığı. Türkiye Kanser İstatistikleri. 2016 [21.10.2017]; Available from: http://kanser.gov.tr/daire-faaliyetleri/kanser-istatistikleri.
6. Suh Y, Afaq F, Khan N, Johnson JJ, Khusro FH, Mukhtar H, Fisetin induces autophagic cell death through suppression of mTOR signaling pathway in prostate cancer cells. Carcinogenesis. 2010; 31; 1424-33.
7. Maher P, A comparison of the neurotrophic activities of the flavonoid fisetin and some of its derivatives. Free Radic Res. 2006; 40; 1105-11.
8. Higa S, Hirano T, Kotani M, Matsumoto M, Fujita A, Suemura M, et al, Fisetin, a flavonol, inhibits TH2-type cytokine production by activated human basophils. J Allergy Clin Immunol. 2003; 111; 1299-306.
9. Kashyap D, Sharma A, Sak K, Tuli HS, Buttar HS, Bishayee A, Fisetin: A bioactive phytochemical with potential for cancer prevention and pharmacotherapy. Life Sci. 2018; 194; 75-87.
10. Prasath GS, Subramanian SP, Fisetin, a tetra hydroxy flavone recuperates antioxidant status and protects hepatocellular ultrastructure from hyperglycemia mediated oxidative stress in streptozotocin induced experimental diabetes in rats. Food Chem Toxicol. 2013; 59; 249-55.
11. Pal HC, Athar M, Elmets CA, Afaq F, Fisetin inhibits UVB-induced cutaneous inflammation and activation of PI3K/AKT/NFkappaB signaling pathways in SKH-1 hairless mice. Photochem Photobiol. 2015; 91; 225-34.
12. Sakai E, Shimada-Sugawara M, Yamaguchi Y, Sakamoto H, Fumimoto R, Fukuma Y, et al, Fisetin inhibits osteoclastogenesis through prevention of RANKL-induced ROS production by Nrf2-mediated up-regulation of phase II antioxidant enzymes. J Pharmacol Sci. 2013; 121; 288-98.
13. Suh Y, Afaq F, Johnson JJ, Mukhtar H, A plant flavonoid fisetin induces apoptosis in colon cancer cells by inhibition of COX2 and Wnt/EGFR/NF-kappaB-signaling pathways. Carcinogenesis. 2009; 30; 300-7.
14. Prakash D, Gopinath K, Sudhandiran G, Fisetin enhances behavioral performances and attenuates reactive gliosis and inflammation during aluminum chloride-induced neurotoxicity. Neuromolecular Med. 2013; 15; 192-208.
15. Hemanth Kumar B, Arun Reddy R, Mahesh Kumar J, Dinesh Kumar B, Diwan PV, Effects of fisetin on hyperhomocysteinemia-induced experimental endothelial dysfunction and vascular dementia. Can J Physiol Pharmacol. 2017; 95; 32-42.
16. Lee SE, Jeong SI, Yang H, Park CS, Jin YH, Park YS, Park. Fisetin induces Nrf2-mediated HO-1 expression through PKC-delta and p38 in human umbilical vein endothelial cells. J Cell Biochem. 2011; 112; 2352-60.