Deveci, Y, Gunal Sadik G, Akalin Urusak, E, Kuşoğlu Gültekin, S, Yanık, A, Atasever Arslan, B. International Journal of Science Letters (IJSL). 2021. 3(2): 121-128.
https://doi.org/10.38058/ijsl.983389

Research Article

Anti-diabetic effects of *Berberis cretica* extract in INS-1E cells

Yiğit Deveci[1], Gamze Gunal Sadik[2], Emine Akalin Urusak[3], Seda Kuşoğlu Gültekin[2], Ayşegül Yanık[2], Belkıs Atasever Arslan[2]*

[1]Department of Bioengineering, Faculty of Engineering and Natural Sciences, Üsküdar University, Istanbul/Turkey
[2]Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Üsküdar University, Istanbul/Turkey
[3]Department of Pharmaceutical Botany, Faculty of Pharmacy, İstanbul University, Beyazıt, Istanbul/Turkey

Abstract

Berberine has been used for the adjuvant treatment of type 2 *diabetes mellitus*, hyperlipidemia (high levels of fats), and hypertension (high blood pressure). Also, it has different effects on diarrhea, inflammation, and cancer. Berberine, is a profoundly common compound in Berberis species. Although *Berberis cretica* is one of the Berberis species, it is unknown whether it has anti-diabetic effects yet. Also, synergistic effects of various compounds together with berberin or similar chemical forms of berberine within Berberis species can lead to find new anti-diabetic agents. The aim of this study is to investigate possible drug potential of *Berberis cretica* extract containing berberine and, its potential signaling pathways on Rat Insulinoma (INS-1E) cells. According to our results, *Berberis cretica* extract has anti-apoptotic effects in INS-1E cells decreasing expression p53, p38 and Bax genes. Suppressive effects of *Berberis cretica* plant extracts on apoptotic signalling pathways in β cells show that the extract contents can have a drug potential for treatment of diabetes.

Article History

Received 02.08.2021
Accepted 23.08.2021

Keywords

Apoptosis,
Berberis cretica,
INS-1E,
Insulin

1. Introduction

Apoptosis is a form of programmed cell death regulated by genes. The Bcl-2 gene is an apoptosis inhibitor, and the Bax gene, an endogenous antagonist of Bcl-2, is an apoptosis

[2]Correspondence: belkis.arslan@uskudar.edu.tr

promoter. The ratio of Bax to Bcl-2 (Bax / Bcl-2) is accepted as an indicator of cell survival or death following an apoptotic stimulus (Oltvai et al., 1993).

Oxidative stress is the formation of cellular damage in the organism as a result of the deterioration of the balance between oxidant and antioxidants in favor of the oxidant system, lipid peroxidation and the release of free reactive oxygen products. When the antioxidant mechanisms of the organism against oxidative stress are insufficient, oxidative damage develops in the cells and functions are disrupted significantly. This mechanism is responsible for the aging process and progression of many diseases such as cardiovascular diseases, cancer, sepsis, degenerative neurological diseases, kidney failure, infertility, muscle and liver diseases (Tabakoğlu and Durgut, 2013).

Lipid metabolism, insulin secretion, inflammatory response, response to oxidative stress, and apoptosis are interrelated cellular processes. Fatty acid (FFA) oxidation is a major metabolic pathway in which fatty acids are catabolized by breaking down into 2-carbon units in the mitochondria, which is very important in meeting energy needs. Increased FFA oxidation has been shown to increase hyperglycemia and the dysfunction associated with diabetes in the sarcoplasmic reticulum. Fatty acids or toxic intermediates released during fatty acid metabolism; It leads to deterioration in mechanical functions, cellular damage, dysfunction of the sarcoplasmic reticulum Ca^{2+} pump, and a decrease in the activities of ATPase and myosin isoenzymes (Onay-Beşikçi and Güner, 2006). Na^{+}-K^{+} ATP'ase activity decreases in the cell and sodium accumulates in the cell. As a result, edema and dysfunction occur in the cell. These changes cause inflammation and necrosis in tissues and initiate apoptosis in cells (Duran-Salgado and Rubio-Guerra, 2014).

Diabetes mellitus (DM) is a chronic disorder depended on the absence or insensitivity of insulin secretion (Altinoz et al., 2015). Type 2 *diabetes mellitus* is a disorder characterized by progressive loss of pancreatic beta cell function and resistance to the effects of insulin in organs such as muscle, fat and liver (Ferranini, 1998). Chronic systemic inflammation is the main reason of vascular complications in DM (Salcini et al., 2016). Berberine has been used for the adjuvant treatment of type 2 *diabetes mellitus*, hyperlipidemia, and hypertension (Lan et al., 2015). Also, it has different effects on diarrhea, inflammation, and cancer (Abrams et al., 2019). It was demonstrated to repressed Nuclear Factor kappa B (NF-κB) and Signal Transducer and Activator of Transcription 3 (STAT3) pathways in cholangiocarcinoma

(Puthdee et al., 2017). On the other hand, it was shown that it can influence mitogen-activated protein kinase (MAPK) signaling pathways (Li, et al., 2016). These studies support to its anti-diabetic potential. Berberine, is a profoundly common compound in Berberis species. Although *Berberis cretic*a is one of the Berberis species, it is unknown whether it has anti-diabetic effects. Also, synergistic effects of various compounds together with berberin or similar chemical forms of berberine within Berberis species can lead to find new anti-diabetic agents. In view of the above lines of evidence, the aim of the study was to investigate effects of *Berberis cretica* extract on insulin secretion and apoptotic signaling pathways in INS-1E cells.

2. Materials and Methods

2.1. Preparation of B. cretica Extracts

Plants have been collected from the West Taurus Mountains at a height of 1500-1700 meters (Between Gevenalanı Plateau and Karumca Saddle) located on the north of Seydikemer district of Muğla Province, Turkey. Identification of species was done by Professor Emine Akalın Uruşak, from Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Botany. Roots of *B. cretica* were used for the extract preparation. Roots were air-dried at room temperature. Dried roots were extracted using methanol in a Soxhlet extractor.

2.2. Cell lines and Culture Conditions

INS-1E cells (a rat insulinoma cell line) were a kind gift from Professor Claes B. Wollheim from University of Geneva Medical Center, Switzerland. Cells were cultured in RPMI 1640 medium supplemented with 10 mM HEPES, $5x10^{-5}$ M ß-mercaptoethanol, 1 mM sodium pyruvate, 100 IU/mL penicillin and 100 µg/mL streptomycin. Cells were passed weekly by gentle trypsinization, 0.25% tripsin-EDTA. Experiments were carried out using cells between passages 2 and 9.

2.3. Real-time PCR

Cells were incubated with an extract concentration of 10 µg/mL for 24 hours and RNA was isolated subsequently. Controls were only cells without extract. After RNA isolation, cDNA synthesis was carried by using SensiFAST cDNA Synthesis Kit according to their manufacturer's instructions. Gene expression levels of p53, p38, Bax, and Bcl-2 were measured by using FastStart DNA Master SYBR Green I kit as stated in the manufacturer's instructions. The reverse primer 5' CCAGCCCATGATGGTTCTGAT 3' and the forward primer 5' CCCGAGAGGTCTTTTTCCGAG 3' were used to determine changes of Bax gene expression levels. For Bcl-2 gene, the reverse primer was 5' CGGTTCAGGTACTCAGTCATCC 3', and forward primer was 5'GGTGGGGTCATGTGTGTGG3'. For p38 gene, reverse primer was 5' CTGTAAGCTTCTGACATTTC 3', and forward primer was 5' GTGCCCGAGCGTTACCAGACC 3'. Also, the reverse primer 5' AGCTTCAAGAGCGACAAGTTTT 3', and forward primer 5' AACTGCGGGACGAGACAGA 3' were used to measure Bax gene expression levels (Kigili et al., 2019).

2.4. Real-time PCR

Insulin ELISA kit (ThermoFisher Scientific, Cat. No: ERINS) was used to analyze secretion of insulin from INS-1E cells incubated with *B. cretica* root extracts according to the manufacturer's instructions.

3. Results and Discussion

It is known that the aqueous extract of the roots of Berberis species is used as an anti-diabetic, and the cure obtained from the roots is used as a wound healer among the people (Durmuş et al., 2016). To further investigate the effects of *B. cretica* root extracts on insulin secretion of INS-1E cells, insulin protein levels were measured. A significant difference in insulin secretion was not observed after the extract treatment on INS-1E cells (Figure 1).

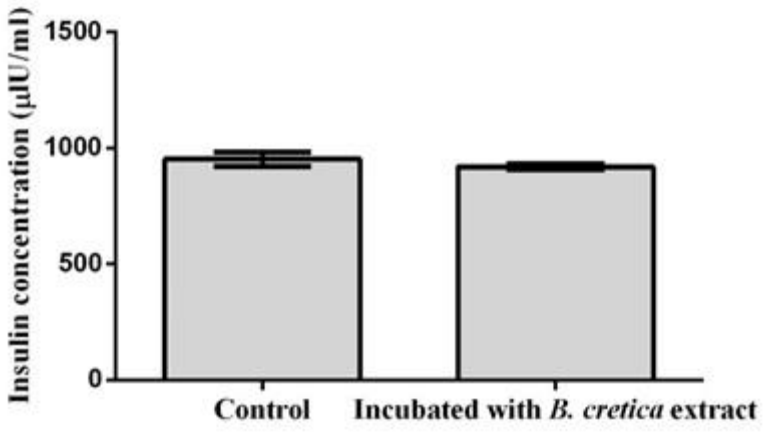

Figure 1. Insulin concentrations of control and *B. cretica* treated INS1-E cells are shown as bar graph.

In vivo and *in vitro* studies with *B. cretica*, which has been used in traditional medicine for many years, have shown that the root of *B. cretica* contains high levels of berberine and has anti-bacterial and anti-tumor activities (Alemardan et al., 2013). In a study with MCF-7 breast cancer and M4A4 metastatic breast cancer cell lines, berberine-chloride was reported to have an anti-cancer effect. In the same study, anti-bacterial properties of berberine were also demonstrated against various bacterial strains (*Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Enterococcus faecalis*) that are the source of common bacterial infections (Altundağ et al., 2020). Chu et al. (2016) showed that berberine administration in the presence of a caspase-1 inhibitor can reduce the viability and invasiveness of cancer cells by inducing apoptosis in liver cancer cells.

In addition to its anti-cancer properties, berberine is known to have high antioxidant properties (Kukula-Koch et al., 2013). Berberine has been shown to alleviate oxidative stress by increasing enzymatic and non-enzymatic anti-oxidant levels (İleritürk et al., 2021).

To understand anti-apoptotic effects of the extract, gene expression levels of common apoptotic and anti-apoptotic proteins for type 1 and type 2 diabetes after *B. cretica* root extract treatment were analyzed. According to the results, while *B. cretica* root extract significantly decreased expression levels of p53, Bax, and p38 genes, it did not change anti-apoptotic Bcl-2 gene expression (Figure 2). Following *B. cretica* incubation, p53 (4-fold), Bax (2-fold), and p38 (2-fold) gene expressions were reduced in INS-1E cells. This result implies that *B. cretica* root extracts can have diminishing effects on p53-dependent apoptotic

activity. Fatty acid (FFA) oxidation activates p53/Bax-mediated mitochondrial apoptosis (Li et al., 2015) Therefore, it's the 4-fold decreasing effect on p53 and Bax genes expression can suppress FFA-dependent p53/Bax-mediated mitochondrial apoptosis in INS-1E cells.

Reactive oxygen species (ROS) may activate Jnk/P38 in β-cells increasing insulin receptor substrate 2 (IRS-2) serine/threonine phosphorylation, and its degradation. Suppression of IRS-2 signaling, can cause β- cell apoptosis as well as insulin resistance (Rhodes, 2005). In our study, *B. cretica* root extract is found to reduce p38 gene activity by 2-fold in INS-1E cells (Figure 2). Therefore, contents of the extract have a potential for preventing p38 mediated IRS2 degradation.

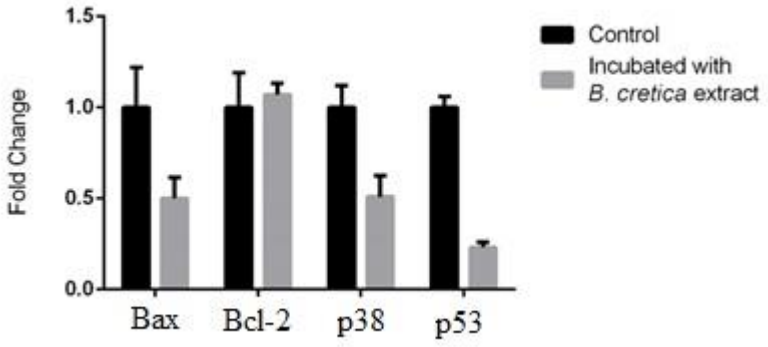

Figure 2. p38, Bax, p53, and Bcl-2 gene expression levels in control and *B. cretica* treated INS1-E cells.

Although the extract did not change Bcl-2 gene expression, its suppressive effects on expressions of apoptotic genes can lead to indirectly Bcl-2 activation on INS-1E cells. The apoptotic gene expressions results indicate that the extract can contain the molecules interacting with these pathways. It is known that berberine is a major compound of the extract. However, the extract also may have some new agents except from berberine and synergistic and antagonistic effects of their combinations can induce the differences in the apoptotic gene expressions.

4. Conclusion

Suppressive effects of *B. cretica* plant extracts on apoptotic signaling pathways in β cells show that the extract contents can have a drug potential for treatment of diabetes. Further

isolation of the molecules from the extract and investigation of their specific activity can give rise to identification of new drug molecules towards treatment of this disease.

Acknowledgements

This study was supported by the Scientific and Technological Research Council of Turkey (TUBITAK) 2209-A (Project No: 1919B011603443).

References

Abrams, S. L., Follo, M. Y., Steelman, L. S., Lertpiriyapong, K., Cocco, L. et al. 2019. Abilities of berberine and chemically modified berberines to inhibit proliferation of pancreatic cancer cells, Advances in Biological Regulation, 71: 172-182.

Alemardan, A., Asadi, W., Rezaei, M., Tabrizi, L., Mohammadi, S. 2013. Cultivation of Iranian seedless barberry (Berberis integerrima 'Bidaneh'): A medicinal shrub, Industrial Crops and Products, 50: 276-287.

Altinoz, E., Taskin, E., Oner, Z., Elbe, H., Atasever Arslan, B. 2015. The effect of saffron (Its active constituent, crocin) on The cardiovascular complication and dyslipidemia in streptozotocin induced diabetic rats, African Journal of Traditional, Complementary and Alternative Medicines, 12(5): 1-7.

Altundağ, E. M., Becer, E., Şanlıtürk, G., Güran, M., Vatansever, H. S. 2020. Berberin-Klorürün meme kanseri hücreleri üzerindeki sitotoksik etkilerinin değerlendirilmesi ve antimikrobiyal aktivite analizi, Turkiye Klinikleri Journal of Medical Sciences, 40(3): 342-348.

Chu, Q., Jiang, Y., Zhang, W., Xu, Du, W. et al., 2016. Pyroptosis is involved in the pathogenesis of human hepatocellular carcinoma, Oncotarget, 7: 84658-665.

Duran-Salgado, M. B., Rubio-Guerra, A. F. 2014. Diabetic nephropathy and inflammation, World J Diabetes, 5(3): 393-398.

Durmuş, R., Şahin, E., Bireller, S. 2016. Gestasyonel diyabette hipoglisemik etkili bitkilerin kullanımı, Deneysel Tıp Araştırma Enstitüsü Dergisi, 6(11): 3-16.

Ferranini, E. 1998. Insulin resistance versus insulin deficiency in noninsulin dependent *diabetes mellitus*: Problems and prospects, Endocrine Reviews, 19: 447-458.

İleritürk, M., Doğan, T., Kandemir, O. 2021. Investigation of the effect of berberine with arginase activity and oxidantantioxidant parameters on bortezomib-induced spleen injury in rats, Kocatepe Veterinary Journal, 14(1): 6-15.

Kigili, F., Ozen, F., Catal, T., Atasever Arslan, B. 2019. Androgen receptor (NR3C4) regulator potential of *Ceratonia siliqua* extract and its signaling pathways, Pharmacognosy Magazine, 15(62): 1-4

Kukula-Kocha, W., Aligiannis, N., Halabalaki, M., Skaltsounis, A. L., Glowniak, K., Kalpoutzakis, E. 2013. Influence of extraction procedures on phenolic content and antioxidant activity of cretan baberry herb, Food Chemistry, 138: 406-413.

Lan, J., Zhao, Y., Dong, F., Yan, Z., Zheng, W. et al. 2015. Meta-analysis of the effect and safety of berberine in the treatment of type 2 D*iabetes mellitus*, hyperlipemia and hypertension, Journal of Ethnopharmacol, 161: 69-81.

Li, H. L., Wu, H., Zhang, B. B., Shi, H.L., Wu, X.J. 2016. MAPK pathways are involved in the inhibitory effect of berberine hydrochloride on gastric cancer MGC 803 cell proliferation and IL-8 secretion in vitro and in vivo, Molecular Medicine Reports, 14(2): 1430-1438.

Li, J., He, W., Liao, B., Yang, J. 2015. FFA-ROS-P53-mediated mitochondrial apoptosis contributes to reduction of osteoblastogenesis and bone mass in type 2 *diabetes mellitus*, Scientific Reports, 31(5): 12724.

Oltvai, Z. N., Milliman, C. L., Korsmeyer, S. J. 1993. Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programed cell death, Cell, 74: 609-619.

Onay-Beşikci, A., Güner, Ş. 2006. Diyabetik kalpte görülen mekanik ve metabolik değişimler ve bunlarin tedavisinde metabolik yaklaşım, Ankara Eczacılık Fakültesi Dergisi, 35(4): 297-317.

Puthdee, N., Seubwai, W., Vaeteewoottacharn, K., Boonmars, T., Cha'on, U. et al. 2017. Berberine induces cell cycle arrest in cholangiocarcinoma cell lines via inhibition of NF-kappaB and STAT3 pathways, Biological & Pharmaceutical Bulletin, 40(6): 751-757.

Rhodes, C. J. 2005. Type 2 diabetes-a matter of beta-cell life and death? Science, 307(5708): 380-384.

Salcini, C., Atasever-Arslan, B., Sunter, G., Gur H., Isik, F. B. et al. 2016. High plasma pentraxin 3 levels in diabetic polyneuropathy patients with nociceptive pain, The Tohoku Journal of Experimental Medicine, 239(1): 73-79.

Tabakoğlu, E., Durgut, R. 2013. Veteriner hekimlikte oksidatif stres ve bazı önemli hastalıklarda oksidatif stresin etkileri, Adana Veteriner Kontrol ve Araştırma Enstitüsü Dergisi, 3(1), 69-75.