Turkish Journal of Agriculture - Food Science and Technology, 9(4): 803-806, 2021
DOI: https://doi.org/10.24925/turjaf.v9i4.803-806.4238

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Licorice Root Ethanol Extract Induces Cell Proliferation in Human Osteoblast Cells

Sema Misir[1,a,*]

[1]*Department of Biochemistry, Faculty of Pharmacy, Sivas Cumhuriyet University, 58140 Sivas, Turkiye*
[*]*Corresponding author*

ARTICLE INFO

Research Article

Received : 01/02/2021
Accepted : 10/02/2021

Keywords:
Licorice
Proliferation
Osteoblast
MTT assay
Osteoporosis

ABSTRACT

Licorice, also known as the root of *Glycyrrhiza glabra,* has been used for many years in traditional medicine to treat various diseases. Licorice root has remarkable pharmacological properties and these biological effects are predominantly attributed to its content of polyphenols and flavonoids. The aim of this study was to determine the proliferative effect of licorice root extract on human osteoblast cells. The study groups were exposed to various concentrations of licorice root extract on 31.25, 62.5, 250, 500, 1000 µg/mL for 24, and 48 h. The proliferative effect of the extract on human osteoblast cells was assessed using the MTT assay. After 24 and 48 h, cell proliferation of groups treated were increased statistically significant compared to the control cells, and also all concentrations showing no cytotoxic effects on osteoblast cells. Phytomedical applications of licorice root may represent a promising approach in the treatment of periodontal regeneration and osteoporosis.

[a] *semay-61@hotmail.com* *https://orcid.org/0000-0002-5919-3295*

Introduction

Licorice, also known as the root of *Glycyrrhiza glabra*, has been used in herbal medicine in Asia and Europe (Choi et al., 2011). Licorice species have been used against different human diseases, such as cancer, atherosclerosis, gastric ulcers, hepatitis, bacterial infections, and immunodeficiency (Messier et al., 2012; Isbrucker et al., 2006; Shen et al., 2007; Nassiri et al., 2008). Licorice has many secondary metabolites such as saponins, alkaloids, polysaccharides, polyamines, flavonoids. These bioactive components have remarkable pharmacological properties. Among them, Glycyrrhizin, is a triterpenoid saponin, glabridin, which is an active isoflavone, Liquitigenin, licochalcone A, and licorisoflavan A are main active components (Asl et al., 2008; Hosseinzadeh et al., 2015; Malvania et al., 2019). Bioactive compounds have antioxidant, proliferative, anti-inflammatory, antibacterial, antiviral, anti-cancer and anti-ulcer properties (Choi et al., 2011; Hosseinzadeh et al., 2015; Wang et al., 2013). In literature, many studies indicated that licorice has anti-adherence (Messier et al., 2012), anti-microbial (Fatima et al., 2009; So derling et al., 2006), anti-inflammatory (Garlet et al., 2010; Sasaki et al., 2010), anti-caries (Hu et al., 2011), anti-bacterial (He et al., 2006) properties of the compounds in oral diseases.

Especially in recent years, many studies have focused on periodontal regeneration and osteoporosis (Oringer et al., 2002; Kızıldağ et al., 2020). Osteoblast cells have been shown to play a vital role in bone metabolism and periodontal regeneration. Increasing osteoblast proliferation is very important for osteoporosis and the regeneration of destroyed tissues as a result of periodontal disease (Huttner et al., 2009, Cekici et al., 2000). Licorice can increase the proliferation rate of osteoblast cells due to its proliferative and antioxidant properties (Wang et al., 2013). Thus, while the periodontal tissue regeneration can be increased and osteoporosis can be prevented. Although its widespread use in the medical field, few articles have been published on the use of therapeutic benefits of licorice in dentistry. Therefore, the aim of this study is to investigate the proliferative effect of licorice ethanolic extract on osteoblast cells.

Materials and Methods

Chemicals and Reagents

Ethanol, phosphate buffer saline, trypan blue solution, dimethyl sulfoxide, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) were purchased from Sigma (St. Louis, MO). All chemicals used in cell culture studies were supplied from Lonza (Verviers, Belgium) and Biological Industries (Kibbutz Beit Haemek, Israel).

Preparation of Licorice Extracts

Roots of licorice were purchased from the Arifoglu trading company. Briefly, samples were grinded, 100 g licorice powder was extracted with 1000 mL pure ethanol in a mechanical shaker (150 rpm) at 45°C for 72h. Then, samples were filtered by a filter of 0.2 mm. The stored extract was stored at -20°C until used for experiments (Turan et al., 2015).

Cell Culture

Osteoblast cell was supplied by the Atlas biotechnology. Osteoblast was cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum and 1% antibiotic solution with a 5% CO_2 supply at 37°C.

Cell Proliferation

The cell proliferation effects of licorice root extracts on osteoblast cells was analyzed using MTT method with 24 and 48 h treatment (Mosmann, 1983). Briefly, all cells were cultured into plates with a density of $5x10^3$ cells each well. All the cells were treated with different concentrations of licorice extracts on osteoblast, and incubated for 24, 48 h. Then, MTT solution was added to each well for 2 h incubation. After incubation, DMSO was added to dissolve composed crystals. Finally, the optical density values were measured by a microplate reader (Versamax, MolecularDevices, Sunnyvale, CA, USA) at 570 nm. Optical densities (ODs) were used to detect % cell viabilities (Frion-Herrera et al., 2013) Cell viability (%) was calculated used with the following formula. (Shanmugapriya et al., 2019). Cell viability (%) = (OD treatment group / OD control group) × 100.

Statistical Analysis

Dates were expressed as arithmetic mean and standard deviation (x ± SD). Kolmogorov-Smirnov test was used to evaluate the compatibility of the variables with the normal distribution. One-way analysis of variance (ANOVA) was performed by SPSS 22.0. Intergroup comparisons were followed by the post-hoc Tukey's test. Differences were considered significant for $P<0.05$. At least three independent data were obtained in the experiment.

Results

We determined that the proliferative effects of the ethanolic licorice root extracts on osteoblast cells. The results of the proliferation analysis of osteoblast cells are shown in Figure 1, 2. Ethanolic licorice extracts increased the cell viability in a dose-dependent manner for 24 and 48 h. Moreover, it has also been shown that not all concentrations have any cytotoxic effect on osteoblast cells.

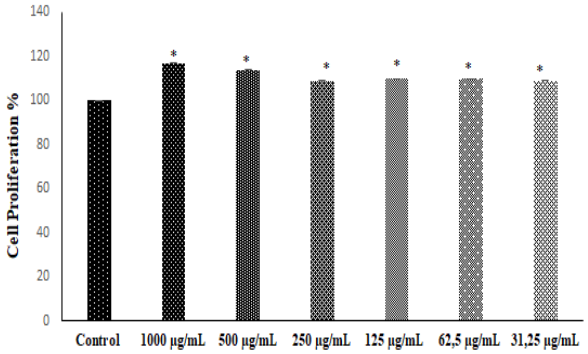

Figure1. Proliferation analysis of human osteoblast cells treated for 24h with licorice root extracts at different concentrations. Represents significant results ($P<0.05$) compared with untreated cells.

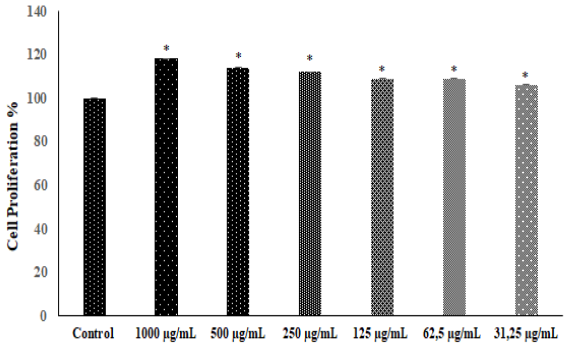

Figure 2. Proliferation analysis of human osteoblast cells treated for 48 h with licorice root extracts at different concentrations. Represents significant results ($P<0.05$) compared with untreated cells.

Discussion

In recent years, natural products and their bioactive components are becoming famous as complementary and alternative medicines worldwide (Wang et al., 2013). It is believed that bioactive compounds in natural products can prevent different diseases and show potential clinical benefits (Kaur et al., 2001). Therefore, natural products are regarded as exciting raw materials for new drug discovery (Azizsoltani et al., 2018) One of the most widely used natural products in traditional medicine is licorice (Wang et al., 2013). Licorice and its bioactive ingredients have biological activities such as detoxification, anti-inflammatory, antiviral, antiatherogenic, anticarcinogenic, and antioxidant properties (Park et al., 2010; Seon et al., 2012; Vaya et al., 1997; Wang and Nixon, 2001). It has been used to treat metabolic syndrome, chronic liver diseases, asthma, coughing, lung diseases, dyspnea, spasms, and for relieving drug toxicity. Moreover, it is useful to relieve for mouth ulcerations, kidney stones, neuralgia, skin, and eye diseases (Fiore et al., 2005; Wang et al., 2013; Nazari et al., 2017).

Osteoblast cells are very important cells for bone formation and periodontal tissue (Kim et al., 2019). It is vital to promote osteoblast activity, therefore, it is necessary to identify natural products that trigger osteoblast activity. Recently, with the advent of global interest in alternative medicinal foods, researchers' interest

in natural products has increased (Cho et al., 2010). There are many studies, both *in vitro* and *in vivo*, showing that natural products and their compounds may have useful effects by protecting and promoting bone health (Che et al., 2016). Many studies indicated that licorice root extracts showed a protective effect on osteoporosis. Choi et al. showed that glabridin increases osteocalcin secretion and has an effect on bone metabolism by enhancing the proliferation of MC3T3-E1 cells (Choi, 2005). Another study demonstrated that ethyl acetate extract of licorice root (10-50 µg/mL) increased proliferation and osteogenic differentiation on human bone marrow mesenchymal stem cells (Azizsoltani et al., 2018). Cho et al. reported that *Glycyrrhiza uralensis* extracts significantly increased osteoblast proliferation in osteoblastic MC3T3-E1 cells (Cho et al., 2018; La et al., 2011).

In this study, the effects of ethanolic licorice root extract, which has antioxidant properties, on osteoblast cells at different concentrations were evaluated. We found that ethanolic licorice root extract markedly increased osteoblast proliferation in concentration 31.25-1000 µg/mL. According to the results, ethanolic licorice extract did not exhibit dose-dependent cytotoxicity in the osteoblast cells studied. The results from the present study were generally similar to those of other studies in the literature. Small differences may arise due to the type of extraction methods and solvents. Further studies are needed to determine the phytochemical and pharmacological effects of licorice root based on its therapeutic potentials.

Conclusion

According to the results of this study, ethanolic licorice root extract showed proliferative effects on osteoblast cells. Licorice root ethanol extracts can be a good candidate for protecting against

periodontal diseases and osteoporosis. Further studies are needed to reveal the effect of licorice root ethanol extracts on signaling pathways in bone formation.

Funding

The authors received no funds for this paper.

Conflict of interest

The author declared no conflict of interest.

Ethical approval

Not applicable

References

Asl MN, Hosseinzadeh H. 2008. Review of pharmacological effects of Glycyrrhiza sp. and its bioactive compounds. Phytother Res, 22:709e24.

Azizsoltani A, Piri K, Behzad S, Soleimani M, Nekouei M, Mahmoudi Z, Kazemi A. 2018. Ethyl Acetate Extract of Licorice Root *(Glycyrrhiza glabra)* Enhances Proliferation and Osteogenic Differentiation of Human Bone Marrow Mesenchymal Stem Cells. Iran J Pharm Res, 17(3):1057-1067.

Cekici A, Kantarci A, Hasturk H, Dyke TEV. 2000. Inflammatory and immune pathways in the pathogenesis of periodontal disease. Periodontol, 64(1): 57-80.

Che CT, Wong MS, Lam CW. 2016. Natural products from Chinese medicines with potential benefits to bone health. Molecules, 21(3): 239.

Cho YE, Alcantara E, Kumaran S, Son KH, Sohn HY, Lee JH, Choi CS, Ha TY, Kwun IS. 2010. Red yeast rice stimulates osteoblast proliferation and increases alkaline phosphatase activity in MC3T3-E1 cells. Nutr Res, 30(7): 501-510.

Cho YE, Kwun IS. 2018.Glycyrrhiza uralensis (licorice) extracts increase cell proliferation and bone markerenzyme alkaline phosphatase activity in osteoblastic MC3T3-E1 cells. Journal of Nutrition and Health, 51(3): 316.

Choi EM. 2005. The licorice root derived isoflavan glabridin increases the function of osteoblastic MC3T3-E1 cells. *Biochem. Pharmacol*, 70: 363-368.

Choi EM. 2011. Glabridin protects osteoblastic MC3T3-E1 cells against antimycin A induced cytotoxicity. Chem Biol Interact,193 (1): 71-78.

Fatima A, Gupta VK, Luqman S, Arvind SN, Kumar JK, Shanker K, Saikia D, Srivastava S, Darokar MP, Khanuja SPS. 2009. Antifungal activity of Glycyrrhizia glabra extracts and its active constituent glabridin. Phytother Res, 23: 1190-1193.

Fiore C, Eisenhut M, Ragazzi E, et al. 2005. A history of the therapeutic use of liquorice in Europe. J Ethnopharmacol, 99: 317-324.

Frion-Herrera Y, Diaz-Garcia A, Rodriguez-Sanchez H, Ruiz-Fuentes J, Monzote-Fidalgo L, et al. 2013. Cytotoxic effect of Cuban propolis extracts against tumor cells lines. Am JEssen Oils Nat Prod, (1): 112-117,

Garlet GP. 2010. Destructive and protective roles of cytokines in periodontitis: a re-appraisal from host defense and tissue destruction viewpoints. J Dent Res, 89: 1349-1363.

He J, Chen L, Heber D, Shi W, Lu QY. 2006. Antibacterial compounds from Glycyrrhiza uralensis. J Nat Prod, 69: 121-124.

Hosseinzadeh H, Nassiri-Asl M. 2015. Pharmacological effects of Glycyrrhiza spp. and its bioactive constituents: update and review. Phytother Res, 29(12):1868-1886.

Hu CH, He J, Eckert R. et al. 2011. Development and evaluation of a safe and effective sugar-free herbal lollipop that kills cavity-causing bacteria. Int J Oral Sci, 3: 13-20.

Huttner EA, Machado DC, Oliveira RB de, Antunes AGF, Hebling E. 2009. Effects of human aging on periodontal tissues. Spec Care Dentist, 29(4):149-55.

Isbrucker RA, Burdock GA. 2006. Risk and safety assess-ment on the consumption of licorice root (Glycyrrhiza sp.), its extract and powder as a food ingredient, with emphasison the pharmacology and toxicology of glycyrrhizin. RegulToxicol Pharmacol, 26:167-192.

Kaur P, Kaur S, Kumar N, Singh B, Kumar S. 2009. Evaluation of antigenotoxic activity of isoliquiritin apioside from Glycyrrhiza glabra L. Toxicol. In vitro: Int. J. Publ. Assoc. BIBRA 23: 680-686.

Kim JH, Kim M, Jung HS, Sohn Y. 2019. Leonurus sibiricus L. ethanol extract promotes osteoblast differentiation and inhibits osteoclast formation. Int J Mol Med, 44(3):913-926.

Kızıldağ A, Dalyanoğlu Mm, Yazkan B, Kara R. 2020. Effects of Different Concentrations of Crithmum Maritimum L. on Periodontal Ligament Fibroblast Cells. Turkiye Klinikleri J Dental Sci, 26(1): 31-35

La VD, Tanabe S, Bergeron C, Gafner S, Grenier D (2011). Modulation of matrix metalloproteinase and cytokine production by licorice isolates licoricidin and licorisoflavan A: potential therapeutic approach for periodontitis. J Periodontol 82: 122–128.

Malvania EA, Sharma AS, Sheth SA, Rathod S, Chovatia NR, Kachwala MS. 2019. In Vitro Analysis of Licorice (Glycyrrhiza glabra) Root Extract Activity on Streptococcus mutans in Comparison to Chlorhexidine and Fluoride Mouthwash. J Contemp Dent Pract, 20(12):1389-1394.

Messier C, F Epifano, S Genovese, D Grenier. 2012. Licorice and its potential beneficial effects in common oro-dental diseases. Oral Dis, 18(1):32-9.

Mosmann T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods, 65: 55-63.

Nassiri Asl M, Hosseinzadeh H. 2008. Review of pharmacological effects of Glycyrrhiza sp. and its bioactive compounds. Phytother Res, 22: 709-724.

Nazari S, Rameshrad M, Hosseinzadeh H. 2017.Toxicological Effects of Glycyrrhiza glabra (Licorice): A Review. Phytother Res, 31(11):1635-1650.

Oringer RJ. 2002. Biological mediators for periodontal and bone regeneration. Compendium, 23: 501-503.

Park SY, Lim SS, Kim JK, Kang IJ, Kim JS, Lee C, Kim J, Park JH. 2010. Hexane-ethanol extract of *Glycyrrhiza uralensis* containing licoricidin inhibits the metastatic capacity of DU145 human prostate cancer cells. Br J Nutr, 104(9): 1272-1282.

Sasaki H, Suzuki N, AlShwaimi E. et al. 2010. 18b-Glycyrrhetinic acid inhibits periodontitis via glucocorticoid-independent nuclear factor-jB inactivation in interleukin-10-deficient mice. J Periodontal Res, 45: 757-763.

Seon MR, Park SY, Kwon SJ, Lim SS, Choi HJ, Park H, Lim DY, Kim JS, Lee CH, Kim J, Park JH. 2012. Hexane/ethanol extract of *Glycyrrhiza uralensis* and its active compound isoangustone A induce G1 cycle arrest in DU145 human prostate and 4T1 murine mammary cancer cells. J Nutr Biochem, 23(1): 85-92.

Shanmugapriya K, Kim H, Kang HW. 2019. In vitro antitumor potential of astaxanthin nanoemulsion against cancer cells via mitochondrial mediated apoptosis. Int J Pharm, 560: 334-346.

Shen XP, Xiao PG, Liu CX. 2007. Research and applicationof Radix Glycyrrhizae. Asian J Drug Metab Pharmacokinet, 7: 181-200.

So derling E, Karjalainen S, Lille M, Maukonen J, Saarela M, Autio K. 2006. The effect of liquorice extract-containing starch gel on the amount and microbial composition of plaque. Clin Oral Invest, 10: 108-113.

Vaya J, Belinky PA, Aviram M. 1997Antioxidant constituents from licorice roots: isolation, structure elucidation and antioxidativecapacity toward LDL oxidation. Free Radic Biol Med, 23(2): 302-313.

Wang ZY, Nixon DW. 2001. Licorice and cancer. Nutr Cancer, 39(1): 1-11.

Wang X, Zhang, Chen L, Shan L, Fan G, Gao X. 2013. Liquorice, a unique "guide drug" of traditional Chinese medicine: a review of its role in drug interactions. J Ethnopharmacol, 150(3):781-90.