Licorice Root Ethanol Extract Induces Cell Proliferation in Human Osteoblast Cells

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ABSTRACT

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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.

Keywords:
Licorice
Proliferation
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MTT assay
Osteoporosis

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, polyanines, flavonoids. These bioactive components have remarkable pharmacological properties. Among them, Glycyrrhizin, is a triterpenoid saponin, glabridin, which is an active isoflavone, Liquiritigenin, 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% CO2 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. (Shannonupriya et al., 2019). Cell viability (%) = (OD treatment group / OD control group) x 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. It has also been shown that not all concentrations have any cytotoxic effect on osteoblast cells.
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