AMELIORATION OF NECROSIS FORMATION IN GINGIVA BY LICORICE ROOT EXTRACT

SHIVANNI SS, VISHNU PRIYA V*, GAYATHRI R

1Department of Biochemistry, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India, 2Department of Biochemistry Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Tamil Nadu, India. Email: drvishnupriyav@gmail.com

INTRODUCTION

Plants serve as potential storage of various secondary metabolites (phytochemicals). These phytochemicals cover diverse and important biochemical components that serve as a raw material for the development of pharmacologically active natural products. Plants still serve as the base for the existence and development of traditional medicine system for 1000 of years in India [1]. Gingivitis is a reversible condition associated with a bacterial plaque that resolves in about 1 week after the reinstitution of oral hygiene procedures [2,3]. The genus name Glycyrrhiza comes from the ancient Greek word for “sweet root” that was later called as licorice [4]. In Chinese traditional medicine, licorice remains one of the oldest and most commonly prescribed herbs and has been used in the treatment of numerous ailments ranging from tuberculosis to peptic ulcers [5]. Licorice has been found to be effective against fevers, liver ailments, dyspepsia, gastric ulcers, sore throats, asthma, and bronchitis [6]. Matricaria chamomilla (chamomile), Eclipta alba (false daisy), and Glycyrrhiza glabra (licorice) are the well-known traditional medicinal plants used in the treatment of various oral infections. The flowers of chamomile, leaves of false daisy, and roots of licorice were selected for our study due to its various medicinal properties and based on its use in ancient traditional medicines [7,8].

The pharmaceutical importance of licorice lies in their capacity to produce a great variety of secondary metabolites. Depending on the modern studies, the most important bioactive compounds in licorice are triterpenes, flavonoids, and polysaccharides [9]. Inflammation responses with Celsius’ four cardinal signs, namely calor, dolor, rubor, and tumor have attracted increasing attention [10]. Inflammation responses play an important role in multiple diseases with a high prevalence among the population, such as hepatitis, lung disease, and Alzheimer’s disease. Moreover, they are also centrally related to the pathogenesis of a large number of acute and chronic diseases, such as rheumatoid arthritis, colonic inflammatory response, and periodontitis [11-16]. The conventional therapies for inflammation, including steroids and nonsteroidal anti-inflammatory drugs have shown many side effects and deficiencies [17].

Licorice is an excellent alternative choice, due to the fact that it causes minimal disorders in the physiological functions of the organism, has a non-specific action and exerts a therapeutic action regardless of the direction of the pathological state. Furthermore, it is especially suitable for children, since glycyrrhizin (GC), a compound isolated from licorice, is 50 times sweeter than sugar that makes it much easier for children to accept [18]. The three original plants of licorice are G. uralensis, G. inflata, and G. glabra. They contain many natural active compounds, including more than 20 triterpenes and 300 flavonoids. 73 bioactive compounds and 91 potential targets are identified for this medicinal herb [19]. The large number of metabolites indicated that licorice was an ideal option for obtaining anti-inflammatory compounds [3]. Licorice is a perennial herb which possesses sweet taste [20]. Liquorice is the name applied to the extract from roots and shoots of Glycyrrhiza species. It is an herb native to the Mediterranean and certain areas of Asia having varied application in chronic hepatitis, peptic ulcer, and lichen planus. Its usage dates back centuries [21,22].

METHODS

Preparation of licorice root ethanolic extract

The collected plant materials were shade dried and ground to a coarse powder. The 20 g of powdered material was weighed and extracted with methanol in a Soxhlet apparatus [8].

Human gingival fibroblasts

Cells were obtained at passage one and propagated in T-75 flasks. Cells were trypsinized and repeated as needed for experiments. The cells
were treated with licorice root extract (100 µg/ml) and incubated for 48 h.

**Assay of tumor necrosis factor-alpha (TNF-α)**

The wells of microtiter plates were coated with 100 µl of cell lines appropriately diluted in carbonate buffer. The plates were kept at 4°C overnight and then washed thrice with phosphate buffered saline (pH 7.4) containing 0.05% Tween 20 and blocked with 3% BSA in phosphate buffered saline (100 µl/well), kept at 37°C for 1 hr. After washing 100 µl of serially diluted antiserum or purified TNF-alpha antibody or the preimmune serum was added and incubated at 37°C for 1 hr. It was then washed with PBS and Tween 20. The wells were charged with 100 µl of anti-rabbit IgG, conjugated with horseradish peroxidase. The plates were incubated at 37°C for 1 hr and washed. The activity of bound horseradish peroxidase was monitored by addition of 50 µl of substrate (1 µl of hydrogen peroxide, 0.5 mg OPD in 1ml of citrate phosphate buffer, pH 5.0). After 20 min in the dark at room temperature, the reaction was arrested by the addition of 50 µl of 2N sulfuric acid. The intensity of the color was recorded on ELISA reader at 490 nm. The TNF-α concentrations were expressed as picogram/ml.

**C-reactive protein test by agglutination method**

C-reactive protein (CRP) is made by the liver and released into the blood within a few hours after onset of gingivitis. 1 drop of positive control and negative control was placed on separate reaction circle on a glass slide. 1 drop of CRP latex reagent was added to each of the circles. Mixed with separate mixing sticks and the fluid was spread over the entire area of the cell. Visible agglutination was observed.

**Statistical analysis**

Results were expressed as mean±standard deviation. The statistical analysis was done using one-way ANOVA by SPSS software, and post hoc test was conducted using Dunnett’s t3 test.

**RESULTS AND DISCUSSION**

The results of this study show the anti-inflammatory activity of licorice root extract and its role in the amelioration of necrosis formation in human gingival tissues. Fig. 1 show the reduction in the concentration of TNF-alpha, an inflammatory mediator when the tissue is treated with Licorice root extract. Fig. 2 shows the presence of agglutination in C reactive protein test in non-treated gingivitis tissue and absence of agglutination when the tissue is treated with Licorice root extract. A study by Rui Yang et al. showed that three triterpenes and 13 flavonoids were mainly responsible for the anti-inflammatory activity of licorice through a variety of mechanisms, especially downregulation of mediators, such as TNF-alpha, MMPs, PGE2, and oxidative stress on the progression of inflammation-related diseases [20]. A study by Fu et al. found that the anti-inflammatory effects of GC may be attributable to its ability to activate ATP-binding cassette transporter A1. GC might be a useful therapeutic reagent for the treatment of mastitis and other inflammatory diseases [23]. In a study done by Asn et al., the cell toxicity of licorice root extract on normal human gingival fibroblast cells was tested using a methyl thiazolyl tetrazolium assay. These results suggest that DG-LRE can be used in developing oral hygiene products, such as dentifrice to prevent human dental caries [24]. The results of Cho et al. indicate that HEGU, which contains licorizin, is a potent anti-metastatic agent, which can markedly inhibit the metastatic and invasive capacity of malignant prostate cancer cells [25,26] which support the protection against HGF by licorice root extract treatment.

**CONCLUSION**

TNF-alpha and CRP levels are raised during necrosis formation and these levels were decreased when treated with licorice root extract treatment. Thus, licorice root extract causes amelioration of necrosis formation in gingiva.