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Research Article

Evaluation of the wound healing potential of *Teucrioside*

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ABSTRACT

Herbal medicines are being used for primary health care due to their efficacy, safety and less side effects. *Teucrium* genus is a member of the Lamiaceae family, which is a medicinal plant have been used in traditional medicine, especially for wound healing and inflammatory conditions. *Teucroside*, 9′-decarboxyrosmarinic acid-4′-O-α-rhamnosyl-(1‴→6‴)-O-β-galactosyl-(1‴→4″)-O-α-rhamnoside is a natural phenolic compund which is isolated and identified from of *Teucrium genus*. In this study, because of bioactive properties of *Teucrioside*, it was decided to examine its potential wound healing effect. Wound healing process was investigated by *in vitro* scratch assay which was an easy, inexpensive and well developed method to measure cell migration. In this context, firstly, cell viability was determined by MTT assay and the results were evaluated to find effective concentration for wound healing. Then the cells were incubated for 48 h with extract with defined concentration. Finally, after 48 hours of incubation with teucroside, the wound healing was calculated as 47%. When the data were compared with untreated control (49%), it was concluded that *teucroside* had not wound healing potential.

Keywords: *Teucrioside*, *Lamiaceae*, wound healing, scratch assay.

Teukrosit'in yara iyileştirme potansiyelinin değerlendirilmesi

ÖZ

Bitkisel ilaçlar, etkinliği, güvenliği ve daha az yan etkileri nedeniyle primer sağlık hizmetinde kullanılmaktadır. *Teucrium* cinsi, özellikle yara iyileşmesi ve enflamatuar durumlar için geleneksel tıpta kullanılan bir tıbbi bitki olan Lamiaceae familyasının bir üyesidir. *Teukrosit,* 9′-dekarboksirosmarinik asit-4′-*O*-α-ramnozil- (1‴→6‴)- *O*-β-galaktozil- (1‴→4″)- *O*-α-ramnozid, *Teucrium* cinsinden izole edilen ve karakterize edilen doğal fenolik bir bileşiktir. Bu çalışmada, *Teukrosit*'in biyoaktif özelliklerden dolayı onun potansiyel yara iyileştirme etkilerini incelemeye karar verilmiştir. Yara iyileşme süreci; kolay, ucuz ve hücre migrasyonunu ölçmek için iyi geliştirilmiş bir metot olan in vitro çizik testi ile araştırılmıştır. Bu bağlamda ilk olarak, hücre canlılığı MTT analizi ile gerçekleştirildi ve sonuçlar, yara iyileşmesinde etkin dozu bulmak için değerlendirildi. Ardından, hücreler, belli konsantrasyona sahip özütleme ile 48 saatliğine inkübe edildi. Son olarak, *Teukrosit* ile 48 saatlik inkübasyon sonunda, yara iyileşmesi % 47 olarak hesaplanmıştır. Bu veriler, negatif kontrolle (%49) karşılaştırıldığında *teukrosit*'in, yara iyileşme potansiyeline sahip olmadığı sonucuna varıldı.

Anahtar Kelimeler: *Teukrosit*, *Lamiaceae*, yara iyileşme, çizik testi.

1. INTRODUCTION

The genus *Teucrium*, belonging to the *Lamiaceae* family is represented by about 300 species widespread all over the world, 27 of which are grown in Turkey.[1] Due to its pharmacological effects, various species of this genus are used widely in traditional medicine for their antioxidant, diuretic, antiulcer, antitumor, anti-inflammatory antispasmodic and antibacterial properties.[2] Therefore, the interest towards *Teucrium* species has increased recent years. One of the most common and highly investigated species in the genus is *Teucrium chamaedrys* (germander). Phytochemical constituents of this species comprise flavonoids, diterpenoids and glycosides.[1,3] Phenylethanoid glycosides are the main phenolic compounds in *Teucrium* species. Recently reports have shown the wide range of biological and pharmacological properties of these

components. *Teucrioside* (9′-decarboxyrosmarinic acid-4′-*O*-α-rhamnosyl-(1‴→6‴)-*O*-β-galactosyl-(1‴→4″)-*O*-α-rhamnoside) is L-lyxose containing phenylethanoid glycoside found in *Teucrium* genus. [4]

Wound healing is the general repair response of the body immediately after the disruption of skin integrity. Following injury, an inflammatory response occurs and the fibroblast cells in dermis produce collagen to regenerate connective tissue. Following this process, the epithelial cells of outer skin are repaired. Wound healing is a systematic and dynamic process which can be divided into four phases: hemostasis, inflammation, proliferation, and maturation. Although there has been an enormous development in pharmaceutical industry, the treatment of wounds is sometimes problematic by known wound healing drugs. Because they have low viability and various detrimental side effects.[5,6] Therefore, plant derived drugs is under great demand due to common belief that they are safe, reliable and effective. Agents with wound healing potential, which are obtained from natural and synthetic bioactive materials have the antioxidant, chelation and antimicrobial activities; and may act by one or more of these mechanisms. Because of these bioactive properties of *teucrioside*, it is decided to examine the potential wound healing effect of this phenolic compound.

Within the scope of this work, wound healing process was investigated by *in vitro* scratch assay which was an easy, inexpensive and well developed method for analysis of cell migration and proliferation in vitro. Two-dimensional in vitro cell migration assays are used to investigate the re-colonizing ability of cell populations.[7] During this experiment, cells are placed on a culture dish and growth cell monolayer. Then, artificial wound is created with a p200 pipette tip and the images of spreading of the resulting collective cell, driven by combined cell migration and proliferation, are captured over 24-48 h.[8]

2. MATERIALS AND METHODS

2.1. Materials

Commercial reagents were purchased from chemical suppliers. L929 cell lines were kindly provided by Ege University Animal Cell Culture and Tissue Engineering Laboratory. All cell culture reagents and products were obtained commercially.

2.2. Methods

2.2.1. Extraction, isolation and identification of Teucrioside

Teucrioside was extracted, isolated and identified with methods which were explained in previous study by Dr. Elmastaş and his team.[1]

2.2.2. Fibroblast cell culture

L929 mouse fibroblast cells were cultured with DMEM-high glucose supplemented with 10% FBS at 37°C in a humidified atmosphere of 95% air and 5% CO_2. Cultivating media was changed every 2 days.

2.2.3. MTT assay

In the present study, firstly MTT (3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide) assay was performed with L929 mouse fibroblast cells to determine effective concentration for *teucrioside*. The L929 cell suspension was prepared at a concentration of $4x10^4$ cell/ml and dispensed onto 96-well cell culture plates. The multiwell plates were incubated for 24 h. This test was performed at ten concentrations (from 200 µg ml^{-1} to 0.4 µg ml^{-1}) and cells left in contact with compound for 48 h. Stock solution (200 µg ml^{-1}) of the compund was prepared in DMSO (< 0.1% in culture medium) and filter sterilized prior to addition to the culture plate. After incubation, 100 µl of MTT solvent (5 mg ml^{-1}) was added into each well and then incubated at 37°C for 3 h. For the viability assay, MTT was removed and the formazan product was dissolved in 100 µl of DMSO and the absorbance was measured at 570 nm with a multimode microplate reader.

2.2.4. *In vitro* wound healing assay

In the present study, preliminary investigation was performed for wound healing effect of *teucrioside* by *in vitro* scratch assay. This assay is widely used when quantifying migration rate, as it provides a simple and economical set up in the hands of experienced users. The first step was creating an artificial wound in a cell monolayer. Then, capturing images at the beginning and at regular intervals during cell migration was performed. Finally the migration rate of the cells was quantified by comparing cell micrographs. [9-11]

For *in vitro* scratch assay, The L929 cell suspension was prepared at a concentration of $5x10^4$ cell/ml and dispensed onto 6-well cell culture plate. When the cells reached to 90% confluency at this plate, the cell monolayer was scraped with sterile p200 pipette tip in a straight line to create a scratch. The debris was removed and smoothed the edge of the scratch by washing the cells with culture medium.[11] Then cells were incubated with *teucrioside* containing media at 50 µg ml^{-1} concentration which was defined by MTT assay. After 24 and 48 h incubation, cell images were captured by phase contrast inverted microscope and analyzed quantitatively by image analysis software which was supplied by Olympus. Finally, percent wound healing was calculated and the results were plotted.

3. RESULTS AND DISCUSSION

To determine wound healing effect of *teucrioside in vitro*, L929 cells were incubated with this fenolic compound. Firstly, MTT assay was performed and cell viabilities were calculated to determine effective concentration for scratch assay. As a result, L929 cells which were incubated with *teucrioside* solution at 50 µg ml^{-1} concentration for 48 hours, demonstrated the highest cell viability against negative control (Figure 1).

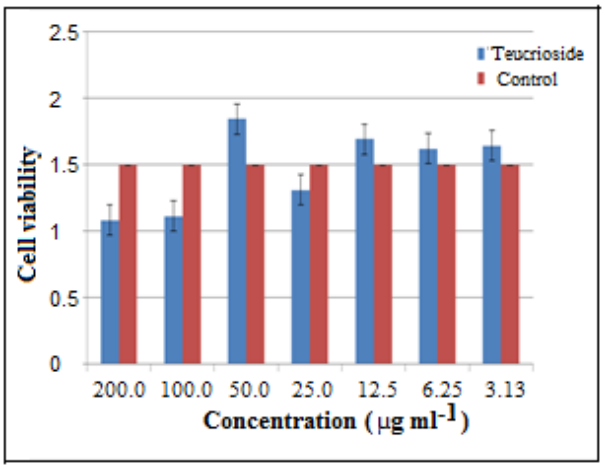

Figure 1. Effects of teucrioside on the proliferation of L929 cell lines.

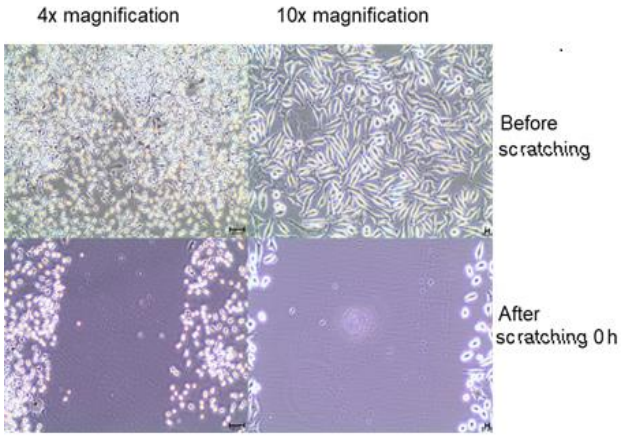

Figure 2. Inverted light microscope images of L929 cell lines before scratching and after scratching (0h). The scale bars indicated 10 µm.

According to MTT assay results, *in vitro* scratch assay was carried out with L929 cells and *teucrioside* solution at 50 µg ml^{-1} concentration. It was clearly observed that following 48 hours of incubation of the *teucrioside* with L929 cells, the percent wound healing was calculated as 47% (Figure 2 and 3).

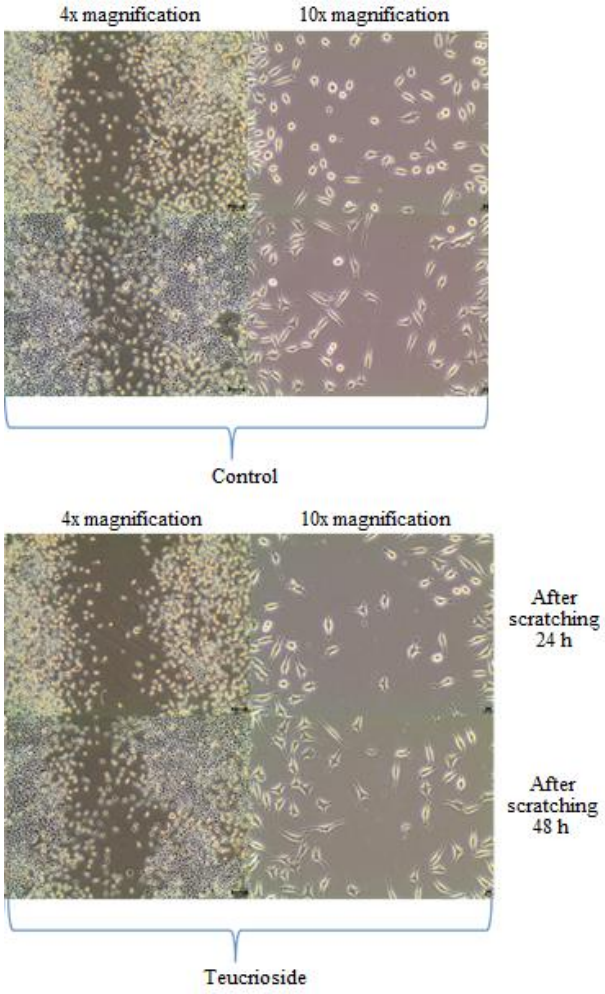

Figure 3. Inverted light microscope images of L929 cell lines after scratching (24 and 48 hours). The scale bars indicated 10 µm.

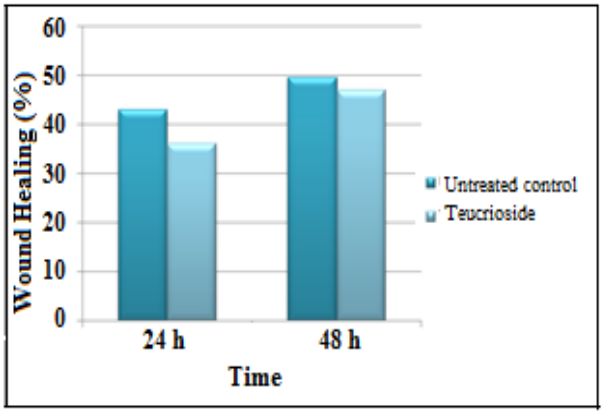

Figure 4. Wound healing effects of teucrioside against untreated control.

When this data was compared with untreated control (49%), it was concluded that *teucroside* had not wound healing potential due to fibroblast stimulation (Figure 4).

4. CONCLUSIONS

Wounds can be defined as the disruption of tissue integrity because of several intrinsic and extrinsic factors. Complex reconstruction of the wound tissue requires cell activation to arrange proper transportation of nutrients and oxygen. In the current study, *teucrioside* which was isolated from *Teucrium chamaedrys* was shown had not wound healing potential due to fibroblast stimulation, considered as important factor in dermis regeneration.

Conflict of Interest

Authors declare that there is no a conflict of interest with any person, institute, company, etc.

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