Dicle Üniversitesi Veteriner Fakültesi Dergisi
https://dergipark.org.tr/tr/pub/duvetfd
Araştırma Makalesi/Research Article
ISSN:1307-9972 Dicle Üniv Vet Fak Derg 2020;13(2):92-98 e-ISSN:1308-0679
DOI: 10.47027/duvetfd.735544

Investigation of the Effects of Topical Centaurium Erythraea on Full-Thickness Skin Wounds Healing in Diabetic Rabbits

Ünal YAVUZ[1,a,✉], Füsun TEMAMOĞULLARI[2,b], Akın YİĞİN[3,c], Nihat YUMUŞAK[4,d]

[1]Department of Surgery, Faculty of Veterinary Medicine, Harran University, Şanlıurfa, TURKEY
[2]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Harran University, Şanlıurfa, TURKEY
[3]Department of Genetic, Faculty of Veterinary Medicine, Harran University, Şanlıurfa, TURKEY
[4]Department of Pathology, Faculty of Veterinary Medicine, Harran University, Şanlıurfa, TURKEY

[a]ORCID: 0000-0002-4981-2355; [b]ORCID: 0000-0001-7738-1145; [c]ORCID: 0000-0001-9758-1697; [d]ORCID: 0000-0002-9299-2902

Geliş Tarihi/Received	Kabul Tarihi/Accepted	Yayın Tarihi/Published
11.05.2020	20.09.2020	31.12.2020

Abstract

Centaurium erythraea, a species of flowering plant in the Gentianaceae family, is a plant commonly used in the wound treatment. This study aims to investigate the effect of *Centaurium erythraea* for enhancing healing process in full-thickness skin wounds in diabetic rabbits. A total of 28 young female New Zealand rabbits were used and animals were divided into four equal groups randomly. Diabetes mellitus was created by injection of alloxan monohydrate. After 15 days following diabetes induction, two full-thickness wounds, a diameter of 2.6 cm, were created equidistantly from the dorsal median line under general anesthesia. For wounds, on a daily basis, oily homogenized form of *Centaurium erythraea* was administered in Group 1 (n=7), while pomade form of the titrated extract of *Centella asiatica* in Group 2 (n=7), pure olive oil in Group 3 (n=7) and normal saline was applied in Group 4 (n=7). Wound edges were measured on the 4th, 8th, 12th, 16th, 20th and 24th days, furthermore, histopathological and genetic examinations were performed on tissue samples taken on the same days. Examining the wound diameters, healing rates in the Group 1 and Group 2 were found statistically significant compared to Group 3 and Group 4 ($p<0,05$). It was noted that the increase of connective tissue, collagen proliferation and epitelogenesis were significant in Groups 1 and 2 compared to Groups 3 and 4 ($p<0.05$). IL-6, IL-8 and CXCR1 gene expressions were observed to be low in all diabetic groups. Although the expression values were low in groups 1 and 2, it was found that there was a significant difference in the expression amounts of the target genes on the 4th and 8th days compared to other groups ($p <0.05$). When the wound healing was examined in terms of wound diameters, histopathology and gene expression, it occurred in Group 2, Group 1, Group 3 and Group 4, respectively. In conclusion, oily homogenized form of *Centaurium erythraea* was found to have a positive effect on full-thickness wound healing of diabetic rabbits.

Key Words: *Centaurium erythraea*, *Centella asiatica*, gene expression, rabbit, wound healing

Diyabetik Tavşanların Tam Katlı Deri Yaralarının İyileşmesine Topikal *Centaurium Erythraea*'nın Etkisinin Araştırılması

Öz

Gentianaceae ailesine ait olan *Centaurium erythraea* yara tedavisinde halk arasında sık kullanılan bir bitkidir. Bu çalışmanın amacı *Centaurium erythraea*'nın diyabet oluşturulmuş tavşanların tam katlı deri yaralarında yara iyileşmesi üzerine etkisini araştırmaktır. Çalışmada 28 adet Yeni Zelanda tavşanı kullanıldı ve hayvanlar rastgele dört eşit gruba ayrıldı. Alloksan monohidrat enjeksiyonlarıyla diyabet oluşturuldu. Diyabet indüksiyonundan 15 gün sonra genel anestezi altında dorsal median hattan eşit uzaklıkta iki adet 2,6 cm çapında tam katlı deri yaraları oluşturuldu. Yaralara günlük olarak Grup 1 (n=7) *Centaurium erythraea*'nın yağlı homojenize formu, Grup 2 (n=7) *Centella asiatica*'nın titre edilmiş ekstraktının pomat formu, Grup 3 (n=7) saf zeytinyağı ve Grup 4 (n=7) serum fizyolojik uygulandı. Yara sınırları 4, 8, 12, 16, 20 ve 24. günlerde ölçülürken aynı günlerde alınan doku örneklerinde histopatolojik ve genetik incelemeler yapıldı. Çalışma sonunda yara çapları incelendiğinde Grup 1 ve Grup 2'de yara iyileşme hızı Grup 3 ve Grup 4'e oranla istatistiksel olarak önemli bulundu ($p<0,05$). Bağ doku artışı kollajen proliferasyonu ve epitelogenezisin Grup 1 ve 2'de Grup 3 ve 4'e oranla belirgin olduğu gözlendi ($p<0,05$). IL-6, IL-8 ve CXCR1 gen ekspresyonlarının tüm diabetli gruplarda düşük olduğu gözlendi. Grup 1 ve 2'de ekspresyon değerleri düşük olmasına rağmen 4 ve 8. günlerde diğer gruplara göre hedef genlerin ekspresyon miktarlarında anlamlı bir farklılık olduğu tespit edildi ($p<0.05$). Yara iyileşmesi yara çapı, histopatoloji ve gen ekspresyonu yönünden incelendiğinde Grup 2, Grup 1, Grup 3 ve Grup 4 sırasıyla gerçekleşti. Sonuç olarak Centaurium erythraea'nın yağlı homojenize formunun diyabetik tavşanların tam katlı deri yaralarında yara iyileşmesine olumlu etkisinin olduğu görüldü.

Anahtar Kelimeler: *Centaurium erythraea*, *Centella asiatica*, gen ekspresyonu, tavşan, yara iyileşmesi

INTRODUCTION

Diabetes mellitus is a hyperglycemia-linked chronic metabolic disease originating from insufficient production or secretion of insulin. Today, it is estimated that more than 285 million people are affected by this disease and worldwide this number will double by 2030 (1-3). Delay in wound

healing is the most important complication of diabetic patients (4, 5). Wound healing is a dynamic, multicellular and normal biological process that takes place in four stages: hemostasis, inflammation, proliferation and remodeling. Harmful changes that affect one of these stages negatively affect wound healing. In wound healing studies where mice, rats and rabbits are used as experimental animals, it was determined that there were changes in RNA and protein amounts. Wound healing process, cell migration to the wound area (macrophages, neutrophils, monocyte and mast cells) and triggering of the acute inflammatory response, as well as cell division and differentiation, are associated with the growth factors of protein and enzyme production and secretion of inflammatory cytokines. (6, 7). Interleukin 6 (IL-6) gene is closely related to the differentiation and development of numerous dermal and epidermal cell types, and healing of skin wounds (8). Interleukin 8 (IL-8) gene is responsible for re-epithelization, tissue shaping, chemotaxis of neutrophil, proinflamation and the vessel development (9). The C-X-C Motif Chemokine Receptor 1 (CXCR1) gene is very important in the formation of angiogenesis with granulation tissue (6, 10, 11).

Although the mechanism of non-healing wounds cannot be fully made explicit, studies have shown that influencing leukocyte function, cytokines and disorders of growth factors, and neuropathy and vasculopathy have an important role (4, 12). Traditionally, many herbs with antihyperglycemic effects are used in diabetes and diabetes-related disorders (3, 13-15). *Centaurium*, a species of flowering plant in the *Gentianaceae* family, takes part in the pharmacopoeia of 23 different countries and it was named as the medicinal plant of the year 2004 (16, 19). *Centaurium erythraea* (CE), one of the most important pharmacological species of this family, has antidiabetic, antioxidant, anti-inflammatory, antipyretic, hepatoprotective and wound-healing properties (17, 19-22). CE grows in different regions around the world (17, 20, 23, 24) and is a traditional medicinal plant used in the treatment of various diseases (17, 20). As active compounds, *Centaurium erythraea* plant comprises the following substances: secoiridoids, secoiridoid alkaloids, xanthones, triterpenes, flavonoids, organic acids, phenolic acids and their derivatives (16, 20, 24-28).

The purpose of this study was to determinate the effect of *Centaurium erythraea* on wound healing in full-thickness skin wounds of diabetic rabbits.

MATERIALS AND METHODS

Plant Material and Preparation Processes

The buds of CE plant were gathered in May of 2016 in the countryside of Saimbeyli district of Adana province and were scientifically proven by Harran University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology.

The plant material prepared by the traditional method was gathered in bud in May. The buds of the plant were crushed into three parts. Raw, 10 portions of CE buds were placed in a glass jar, and 90 portions of fresh pure olive oil was added to the same jar, this mixture kept under sunlight for 40 days. During this period the direction of the glass jar was changed every 10 days. The oil obtained was made ready to use by filtering through the cloth.

Madecassol® (1% pomade, Bayer, İstanbul) preparation contains the titrated extract of 10 mg *Centella asiatica* (CA) in 1 g pomade. Madecassol® product was purchased from a local pharmacy.

Animals and Diabetes Induction

Ethics committee approval was obtained from the Dollvet A.Ş. Local Ethics Committee of Animal Experiments (Dollvet-Hadyek-2016/14) for the experimental animals used in the study. A total of 28 young (6 months and body weight of 2.5-3 kg) female New Zealand rabbits were used. Each of the rabbits was kept in standard cages and exposed to 12 hours of darkness and 12 hours of daytime. All rabbits were fed 160 grams of pellet rabbit feed (Nükleon Experimental Animal Feed, Ankara, Turkey) and ad libitum water daily.

To create diabetes, alloxane monohydrate (Sigma, A.B.D. CAS Number 2244-11-3) dissolved in normal saline was administered intravenously in different doses from the ear vein to all rabbits hungered overnight. After the application, blood glucose levels were measured (VivaChek, USA) and the dose of the alloxane monohydrate was determined as 160 mg/kg in the protocol to create diabetes. A preparation was made on ice of 160 mg/kg alloxane monohydrate in 30 ml saline and administered via an ear vein, using an IV catheter (26 G, Beybi Biomed), at a rate of 1.5 ml/minute. To prevent hypoglycemia after injection, water containing 1% dextrose was given to rabbits ad libitum for 48 hours orally. For blood glucose measurement, regular blood glucose level measurements were made with a glucometer device for 2 weeks. Rabbits with blood glucose above 250 mg/dL 7 days after alloxan administration were considered diabetic (29).

Surgical Procedure

Rabbits were premedicated with xylazine HCl (3 mg/kg, IM) (Alfazyne® %2, Egevet, Alfasan, Netherland), and anesthetized with ketamine HCl (30 mg/kg, IM) (Alfamyne® %10, Egevet, Alfasan, Netherland) 15 days after diabetes induction.

Each rabbit was placed in a sternal position and the dorsal area was shaved with an electric shaver. The skin surface was prepared aseptically with povidone iodine (Batticon®, Adeka, Istanbul, Turkey) and the area was limited to sterile patches. For each rabbit, two 2.6 cm wounds were created that were equidistant from the dorsal median line and behind scapula and cover full-thickness skin. Hemostasis was achieved by compression of sterile surgical tampons.

Treatment Protocol

Rabbits divided into four equal group randomly. For wounds, oily homogenized form of *CE* was administered in Group 1 (n=7), while pomade form of the titrated extract of *CA* (Madecassol®) (n=7) in Group 2, pure olive oil (n=7) in Group 3 and saline (n=7) was applied once a day in Group 4. After the applications, the wound areas were bandaged with sterile tampon and patch.

Measurement of Wound Size

Postoperatively, the edges of wound on the left side were measured on the 4th, 8th, 12th, 16th, 20th and 24th days using a transparent paper. The wound area was measured by transferring transparent paper onto millimeter paper (30).

Histopathology Examination

Histopathological samples taken clockwise from the wound on the right side on the 4th, 8th, 12th, 16th, 20th and 24th days were evaluated.

In histopathological evaluation, 10% buffered formaldehyde was detected in tissue samples. Samples taken for routine pathology tissue follow-up were blocked in paraffin. Serial sections of 5-micron thickness were taken from the prepared blocks with Leica RM 2125 RT microtome and passed through xylol and graded alcohol (50%, 75%, 96%, 100%) series for the purpose of dehydration, was stained with hematoxylin-eosin (H&E) and examined under a light microscope.

Molecular Methods

RNA isolation and cDNA

Tissue samples taken on the 4th, 8th, 12th, 16th, 20th and 24th days were taken into mRNA storage solution and stored at -80 ° C. For homogenization to tissue samples; they were weighted 15-20 mg and homogenized 350 µl High Pure RNA Tissue Kit Tissue Lysis/Binding Buffer Kit with a homogenizer. RNA isolation was performed in accordance with the protocol of the High Pure RNA Tissue (Roche 12033674001) commercial kit. RNA purity and integrity were checked with a Nano drop device. cDNA synthesis was performed in accordance with the Transcriptor First Strand cDNA Synthesis Kit (Roche 04897030001) protocol (31, 32).

Real-Time PCR

In Real-Time PCR study, for the IL6, IL8, CXCR1 target genes with the ACTB (Beta actin) reference gene, the following primary sequences were used (Table 1). LC DNA Master SYBR Green I (Roche 12239264001) kit used as master mix. RealTime PCR mixture protocol was performed as follows: ddH_2O 12,4 µL, Mg^{+2} (25Mm) 1,6 µL, LightCycler DNA Master SYBR Green I 10x conc 2.0 µL, Primer Forward (Target or ACTB) 1,0 µL, Primer Revers (Target or ACTB) 1,0 µL, total mixing volume 18 µL, 2,0 µL of each Target cDNA or ACTB was added to this mixture, final volume of concentration was 20 µL (7, 33).

Table 1. Primer sequences of genes with real-time PCR expressions

IL-6	5'- GTC AGC CTG ATG GAG AAC CT -3'	Pradhan et al (2011)
	5'- GGA TGA AGT GGA TCG TGG TC -3'	
IL-8	5'- CTC TGC TGG CTG CCC TAC -3'	
	5'- CTG ACA CGT CTC CTG GAT CA -3'	
CXCR1	5'- GGC GCT GTC TCT GAT TTT GT -3'	
	5'- GGC TGG AAT TGT TTG GAG AA -3'	
ACTB	5'-CTGGAACGGTGAAGGTGACA-3'	Seol et al (2011)
	5'-CGGCCACATTGCAGAACTTT- 3'	

For Realtime PCR protocol: The values were constantly noted for 1 cycle at 95 ^{0}C for 5 min, 45 cycles at 95 ^{0}C for 5 secs, at 59^{0}C for 10 secs, 72 ^{0}C for 5 secs and 1 cycle at 40 ^{0}C for 30 secs, at 95 ^{0}C for 5 secs, at 50 ^{0}C for 60 secs, and increased from 50 ^{0}C to 95 ^{0}C for 1 ^{0}C/secs for melting analysis and were analyzed on the Lightcycler Realtime PCR (Roche) instrument.

Statistical Analysis

SPSS 24.0 (SPSS Inc., NY, USA) program was used for all statistical analyzes. The wound healing scores between the groups were evaluated by the Kruskal-Wallis test. The increase in connective tissue, collagen proliferation and epitelogenesis in the wound area were examined by using Mann-Whitney method. Data were presented as mean±standard deviation and $p<0.05$ and $p<0.01$ were considered statistically significant.

RESULTS

There was no statistically significant difference between Group 1 and Group 2 in terms of wound area on the 4th, 8th and 12th days ($p> 0.05$). Considering 16th, 20th and 24th day of measurements, all groups were found to be significantly different from each other ($p<0,05$). The difference between the groups in terms of wound diameters was found statistically significant ($p <0.05$). Accordingly, the fastest improvement in all measurement times occurred in Group 2, followed by Group 1. The statistical analysis of the wound size of all groups based on days is presented in Table 2 and the effect of topical application on wound healing is presented in Figure 1.

Table 2. Changes of wound surface areas in the Group 1 (Oily homogenized form of CE) (n = 7), Group 2 (Pomade form of the titrated extract of CA) (n = 7), Group 3 (Pure olive oil) (n = 7) and Group 4 (Saline) (n = 7) groups at measurement days (mean ± SD)

Days	Group 1 (CE)	Group 2 (CA)	Group 3 (POO)	Group 4 (Saline)
0	3,20±0	3,20±0	3,20±0	3,20±0
4	2,69±0,16^a	2,63±0,17^a	2,89±0,17^b	3,10±0,08^c
8	2,34±0,24^a	2,49±0,16^a	2,64±0,15^b	2,76±0,10^b
12	2,04±0,21^a	1,86±0,14^a	2,39±0,20^b	2,49±0,09^b
16	1,53±0,21^b	1,44±0,16^a	2,16±0,28^c	2,35±0,10^d
20	1,26±0,41^b	0,95±0,25^a	1,89±0,25^c	2,27±0,11^d
24	0,73±0,25^b	0,41±0,19^a	1,44±0,18^c	2,08±0,19^d

In histopathological examination, it was found that connective tissue increase, collagen proliferation and epitelogenesis in Groups 1 and 2 were significant compared to Groups 3 and 4 ($p <0.05$). In Group 3 and 4, it was observed that inflammatory cell infiltrations, hyperemia and necrosis were intense compared to Groups 1 and 2 ($p <0.05$). When the connective tissue increase, collagen proliferation and epitelogenesis changes in the wound area in Group 1 and 2 are examined statistically, a significant difference was found in days between the 4th and 20th day and ($p<0.05$) and between the 4th and 24th days ($p<0.001$). Histopathological evaluation is presented in Figure 2.

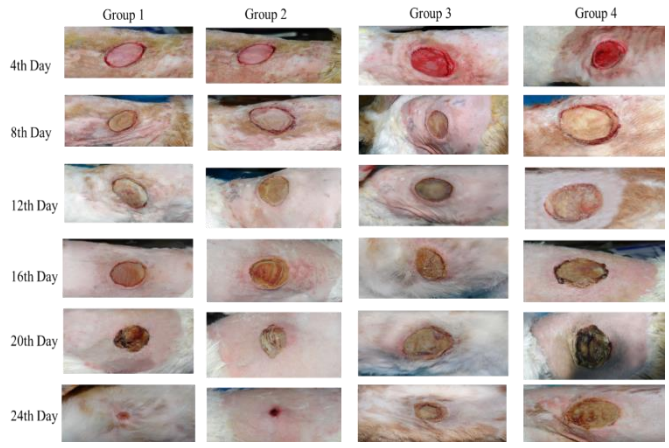

Figure 1. Effect of topical application of Group 1, Group 2, Group 3 and Group 4 on wound healing. Group 1: Oily homogenized form of *CE*, Group 2: Pomade form of the titrated extract of *CA*, Group 3: Pure olive oil, Group 4: Saline

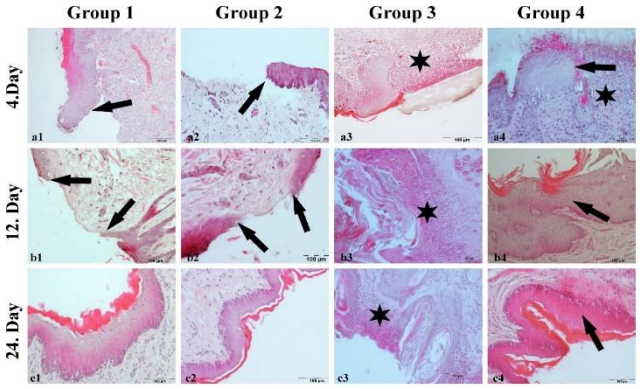

Figure 2. Histopathological findings of wound healing. Slight epithelialization (arrows) (a1, a2, a4) and severe inflammations (asterisks) (a3, a4) in the sections of the fourth day. Mild epithelialization in Group 1 (b1) and Group 2 (b2), severe inflammation (b3) (star) in Group 3, mild epithelialization (b4) (arrow) in Group 4 in sections on the twelfth day, Partial epithelialization and healing (c1, c2, c3, c4), mild inflammation (c3, c4) (star) and mild epithelization (arrow) in sections from the twenty-fourth day. Hematoxylin Eosin (HXE)

Expression of IL-6, IL-8 and CXCR1 as target gene and ACTB expression as reference gene were examined in diabetic rabbits. Since wound healing was significantly slower in diabetic rabbits, target gene expression was significantly low in all groups from the first days of the wound (days 4 and 8) to day 24. Since all research groups were diabetic, the differences of target genes with each other were examined. It was observed that there were very low differences between the groups in the expression of IL-6, IL-8 and CXCR1 genes, which have an important role in wound healing. In the last days of wound healing, it was found that expression values decreased for all genes compared to the first days. In the first days of wound healing (days 4 and 8), Group 1 and Group 2 were found to be statistically higher in terms of CXCR1 gene expression compared to other groups ($p < 0.05$) (Figure 3). Although the expression of IL-8 increased in Group 1 and Group 2 compared to Group 4, no statistical difference was observed ($p > 0.05$) (Figure 4).

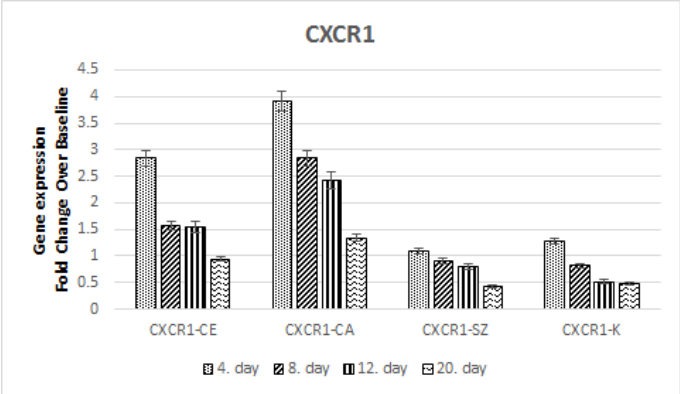

Figure 3. C-X-C Motif Chemokine Receptor 1 (CXCR1) gene expression values by days (CE: Centaurium erythraea, CA: Centella asiatica, SZ: Pure olive oil, K: Saline)

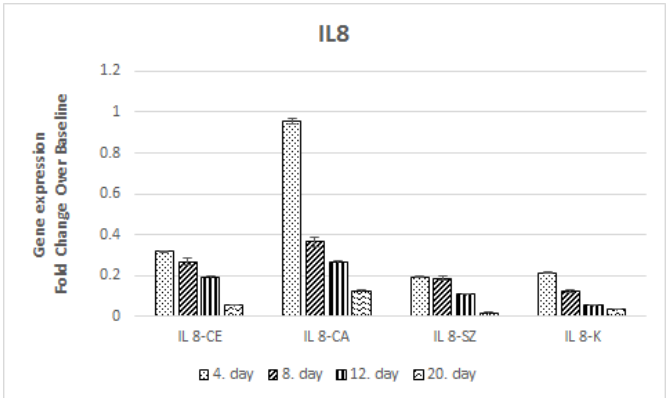

Figure 4. IL-8 gene expression values by days (CE: Centaurium erythraea, CA: Centella asiatica, SZ: Pure olive oil, K: Saline)

DISCUSSION AND CONCLUSION

In the current study, in which CE extract was examined in the treatment of full-thickness skin wounds created in diabetic rabbits, differences were determined between the groups in wound healing. Hamza et al. (1), it was found that CE extract administered orally against harmful effects caused by Type 2 diabetes decreases insulin resistance and reduces hyperglycemia. Hamza et al. (34), in another study investigating the therapeutic effectiveness of CE in Type 2 diabetes, it was stated that the hydroalcoholic extract of CE has antihyperglycemic, antihypercholesterolemic and antihypertriglyceridemic effect. In our study, it is thought that the acceleration of wound healing in diabetic rabbits with CE extract may be due to the antihyperglycemic effect of the plant.

It was shown that topical applications of compounds with free radical scavenging feature significantly increase wound healing in patients and protect tissues from oxidative damage (35, 36). Tuluce et al. (20) reported that CE has antioxidant effect. In our study, it is thought that the faster healing of the CE extracted wounds may have resulted from the

antioxidant properties of the plant. In a study conducted by Shukla et al. (36), it was found that aciaticosidine isolated from CA had a positive effect on wound healing by increasing tissue antioxidant capacity. It has been shown by Maquart et al (37) that CA has fibroblast enhancing properties. In a study conducted by Valentao et al (16), it was determined that CE showed antioxidant properties by noncompetitive inhibition of the superoxide radical scavenging capacity and xanthine oxidase. In the current study, it is thought that CA pomade has fibroblast enhancer and antioxidant properties, accelerates wound healing.

In recent years, gene and protein expressions related to healing mechanisms of diabetic wounds have been studied frequently. In studies conducted in diabetic rabbits, gene expression of pro-inflammatory cytokines and expression of neuropeptides and cytokines in diabetic neuroischemic wound healing were investigated (7, 38). The findings obtained in both studies are consistent with our study findings. Although there were changes in the expression of IL6, IL8 and CXCR1 target genes in the wound healing process, there was no difference in protein expressions.

In a study to clarify the role of the interleukin IL-6 gene in wound healing, wild-type (WT) and BALB/c [knockout (KO)] mice with IL - 6 deficiency were used (39). At the end of the study, they emphasized that IL-6 had important roles in wound healing, possibly by regulating leukocyte infiltration, angiogenesis and collagen deposition. McCarthy et al. (6) showed that IL-6 gene was effective in anti-inflammation, IL8 gene in granulation tissue formation and angiogenesis in wound healing study. Although the expression values were low in Groups 1 and 2 in the current study, it was observed that there was a significant difference in IL-6, IL-8 and CXCR1 gene expression levels on the 4th and 8th days compared to other groups. In the first days of wound healing (days 4 and 8), CXCR1 gene expression value was found to be statistically higher in Group 1 and 2 than Group 4 ($p < 0.05$). In a study, it was emphasized that IL-8 production and neutrophil infiltration in diabetic wounds increased in an environment with high glucose level, thus the disruption of wound healing process in diabetic tissue accelerates. (40). In the current study, although IL-8 expression increased in Groups 1 and 2 compared to Group 4, there was no statistically significant difference ($p > 0.05$). As noted in the above studies (6, 39, 40), since inflammation was significantly high in non-diabetic wounds, target gene expressions were found to be quite low in all groups from the first days of the wound (days 4 and 8) to the last day (day 24).

Polyphenols obtained from plants (especially flavonoids and phenolic acids) are the most commonly reported compounds as antioxidants. They can remove free radicals by giving them a hydrogen atom or electron to the free radical molecule, so they can inhibit oxidative chain reaction mechanisms (41). In the study conducted by Sefi et al. (42) in streptozosin-induced diabetic rats, CE extract was found to decrease blood glucose level, increase serum insulin level and have antioxidant potential. The same study has shown that CE extract protects pancreatic β cells from free radical-mediated oxidative stress, thereby stimulating the synthesis of residual pancreatic β cells and strengthening the state of the antioxidant system, providing more insulin secretion. It has been shown by Stefkov G et al (27) that CE has antihyperglycemic and antilipidemic effects in diabetes induced by streptozosine induction in rats. In the current study, the antioxidant and antihyperglycemic effects of CE extract are thought to accelerate wound healing.

In a study by Shetty et al (43) with *O. sanctum* extracts, flavanoids with free radical scavenger activity in the structure of the plant were reported to provide better collagenization in the wound. In the same study, *O. sanctum* plant, which has antioxidant properties, has been found to accelerate wound healing. In his *in vitro* study investigating the relationship between flavonoids, xanthine oxidase and superoxide radical scavenger as a structure-activity relationship, Cos et al (44) determined the important structure-activity relationship between flavonoids and antioxidant activity and emphasized that CE demonstrates antioxidant properties by noncompetitive inhibition of xanthine oxidase with the ability to remove superoxide radicals. Tuluce et al (20) created gastric mucosal damage in rats using acetyl salicylic acid. They showed that the harmful effects of acetyl salicylic acid disappeared by oral supplementation of CE extraction and that the gastro-protective effect of CE is associated with antioxidant properties, suppression of lipid peroxidation level, the effect of scavenging free radicals and its reducing effect on ulcer index. As stated by the authors (20, 43, 44) in our study, it is thought to accelerate wound healing thanks to the flavonoids contained in the CE extract and its antioxidant content.

At the end of study, it was noted that the wound healing rate increased between the wound diameters among the oily homogenized form of CE (Group 1) and the pomade form of the CA titrated extract (Group 2) compared to other groups. Wound healing rate was slower in pure olive oil (Group 3) and normal saline (Group 4) group.

In conclusion, oily homogenized form of *Centaurium erythraea* was found to have a positive effect on full-thickness wound healing in diabetic rabbits.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest with respect to the publication of this manuscript.

REFERENCES

1. Hamza N, Berke B, Cheze C, et al. (2010). Prevention of Type 2 Diabetes Induced by High Fat Diet in the C57BL/6J Mouse by Two Medicinal Plants Used in Traditional Treatment of Diabetes in the East of Algeria. J Ethnopharmacol. 128(2): 513-518.
2. Perez-Nieves M, Ivanova JI, Hadjiyianni I, et al. (2017). Basal Insulin Initiation Use and Experience Among People with Type 2 Diabetes Mellitus with Different Patterns of Persistence: Results from a Multinational Survey. Curr Med Res Opin. 33(10): 1833-1842.
3. Yadav M, Sharma N, Garg A, et al. (2017). Herbal Drugs and Phytoconstituents Useful for the Management of Diabetes. Int J Green Pharm. 11(1): 21-29.

4. Chouhan D, Janani G, Chakraborty B, Nandi SK, Mandal BB. (2018). Functionalized PVA–Silk Blended Nanofibrous Mats Promote Diabetic Wound Healing via Regulation of Extracellular Matrix and Tissue Remodelling. J Tissue Eng Regen Med. 12(3): 1559-1570.
5. Yadollah-Damavandi S, Chavoshi-Nejad M, Jangholi E, et al. (2015). Topical Hypericum perforatum Improves Tissue Regeneration in Full-Thickness Excisional Wounds in Diabetic Rat Model. Evid Based Complement Altern Med. Article ID 245328: 1-4.
6. McCarthy ME, Brown TA, Bukowska J, et al. (2018). Therapeutic Applications for Adipose-Derived Stem Cells in Wound Healing and Tissue Engineering. Curr Stem Cell Rep. 4(2): 127-137.
7. Pradhan L, Cai X, Wu S, et al. (2011). Gene Expression of Pro-Inflammatory Cytokines and Neuropeptides in Diabetic Wound Healing. J Surg Res. 167(2): 336-342.
8. Luckett-Chastain LR, Gipson JR, Gillaspy AF, Gallucci RM. (2018). Transcriptional Profiling of Irritant Contact Dermatitis (ICD) in a Mouse Model Identifies Specific Patterns of Gene Expression and İmmune-Regulation. Toxicology. 410: 1-9.
9. Law JX, Chowdhury SR, Aminuddin BS, Ruszymah BHI. (2017). Role of Plasma-Derived Fibrin on Keratinocyte and Fibroblast Wound Healing. Cell Tissue Bank. 18(4): 585-595.
10. Shi HX, Lin C, Lin BB, et al. (2013). The Anti-Scar Effects of Basic Fibroblast Growth Factor on The Wound Repair in Vitro and in Vivo. PloS one, 8(4): e59966.
11. Xu C, Rosler E, Jiang J, et al. (2005). Basic Fibroblast Growth Factor Supports Undifferentiated Human Embryonic Stem Cell Growth Without Conditioned Medium. Stem cells, 23(3): 315-323.
12. Vatankhah N, Jahangiri Y, Landry GJ, Moneta GL, Azarbal AF. (2017). Effect of Systemic Insulin Treatment on Diabetic Wound Healing. Wound Repair Regen, 25(2): 288-291.
13. Arulselvana P, Abdul Ghofara HA, Karthivashana G, et al. (2014). Antidiabetic Therapeutics from Natural Source: A Systematic Review. Biomed Prev Nutr. (4): 607–617.
14. Arumugam G, Manjula P, Paari N. (2013). A Review: Anti Diabetic Medicinal Plants Used for Diabetes Mellitus. J Acute Dis. 2(3): 196-200.
15. Grover JK, Yadav S, Vats V. (2002). Medicinal Plants of India with Anti-Diabetic Potential, J Ethnopharmacol. 81 (1): 81-100.
16. Valentao P, Fernandes E, Carvalho F, Andrade PB, Seabra RM, Bastos ML. (2001). Antioxidant Activity of Centaurium erythraea Infusion Evidenced by Its Superoxide Radical Scavenging and Xanthine Oxidase Inhibitory Activity. J Agric Food Chem. 49: 3476−3479.
17. Tahraoui A, Israili ZH, Lyoussi B. (2010). Acute and Sub-Chronic Toxicity of a Lyophilised Aqueous Extract of Centaurium erythraea in Rodents. J Ethnopharmacol. 132(1):48-55.
18. Springefeld K. Centaurium erythraea-The medicinal plant of the year 2004. Pharm Ztg. 149: 30.
19. Šiler B, Mišić D. (2016). Biologically Active Compounds from the Genus Centaurium sl (Gentianaceae): Current Knowledge and Future Prospects in Medicine. In: Studies in Natural Products Chemistry. Vol. 49, pp. 363-397. Elsevier, USA.
20. Tuluce Y, Ozkol H, Koyuncu I, Ine H. (2011). Gastroprotective Effect of Small Centaury (Centaurium Erythraea L) on Aspirin-Induced Gastric Damage in Rats. Toxıcol Ind Health. 27(8): 760-768.
21. Berkan T, Üstünes L, Lermioglu F, Özer A. (1991). Antiinflammatory, Analgesic, and Antipyretic Effects of an Aqueous Extract of Erythraea centaurium. Planta Med. 57: 34-37.
22. Moore N, Hamza N, Berke B, Umar A. (2017). News from Tartary: an Ethnopharmacological Approach to Drug and Therapeutic Discovery. Br J Clin Pharmacol. 83(1): 33-37.
23. Schouppe D, Brys R, Vallejo-Marin M, Jacquemyn H. (2017). Geographic Variation in Floral Traits and the Capacity of Autonomous Selfing Across Allopatric and Sympatric Populations of Two Closely Related Centaurium Species. Sci Rep. 7: 46410.
24. Kumarasamy Y, Nahar L, Cox PJ, Jaspars M, Sarker SD. (2003). Bioactivity of Secoiridoid Glycosides from Centaurium erythraea. Phytomedicine. 10(4): 344-347.
25. Valentão P, Fernandes E, Carvalho F, Andrade PB, Seabra RM, Bastos ML. (2003). Hydroxyl Radical and Hypochlorous Acid Scavenging Activity of Small Centaury (Centaurium erythraea) Infusion. A Comparative Study with Green Tea (Camellia sinensis). Phytomedicine. 10: 517–522.
26. Kumarasamy Y, Nahar L, Sarker SD. (2003). Bioactivity of Gentiopicroside from the Aerial Parts of Centaurium erythraea. Fitoterapia. 74: 151–154.
27. Stefkov G, Miova B, Dinevska-Kjovkarovska S, et al. (2014). Chemical Characterization of Centaurium Erythrea L. and its Effects on Carbohydrate and Lipid Metabolism in Experimental Diabetes. J Ethnopharmacol. 152: 71-77.
28. Banjanac T, Dragićević M, Šiler B, et al. (2017). Chemodiversity of Two Closely Related Tetraploid Centaurium Species and Their Hexaploid Hybrid: Metabolomic Search for High-Resolution Taxonomic Classifiers. Phytochemistry. 140: 27-44.
29. Breen A, Mc Redmond G, Dockery P, O'Brien T, Pandit A. (2008). Assessment of Wound Healing in the Alloxan-Induced Diabetic Rabbit Ear Model. J Invest Surg 21(5): 261-9.
30. Han MC, Durmuş AS, Sağlıyan A, et al. (2017). Effects of Nigella sativa and Hypericum perforatum on Wound Healing. Türk J Vet Anım Sci. 41: 99-105.
31. Hassani FV, Naseri V, Razavi BM, Mehri S, Abnous K, Hosseinzadeh H. (2014). Antidepressant Effects of Crocin and its Effects on Transcript and Protein Levels of CREB, BDNF, and VGF in Rat Hippocampus. DARU. 22(1): 16.
32. Karaca ZM, Ozen H, Akgoz M. Cigremis Y. (2018). Effect of Caffeic Acid Phenethyl ester (CAPE) on Vascular Endothelial Growth Factor a (VEGF-A) Gene Expression in Gentamicin-Induced Acute Renal Nephrotoxicity. Medicine. 7(4): 805-9.
33. Słotwiński R, Sarnecka A, Dąbrowska A, et al. (2015). Innate Immunity Gene Expression Changes in Critically Ill Patients with Sepsis and Disease-Related Malnutrition. Cent Eur J Immunol. 40(3): 311.
34. Hamza N, Berke B, Cheze C, et al. (2011). Treatment of High Fat Diet Induced Type 2 Diabetes in C57BL/6J Mice by Two Medicinal Plants Used in Traditional Treatment of Diabetes in The East of Algeria. J Ethnopharmacol. 133(2): 931-933.
35. Gopinath D, Ahmeda MR, Gomathia K, Chitraa K, Sehgalb PK, Jayakumara R. (2004). Dermal Wound Healing Processes with Curcumin Incorporated Collagen Films. Biomaterials. 25: 1911–1917.
36. Shukla A, Rasik AM, Dhawan BN. (1999). Asiaticoside-Induced Elevation of Antioxidant Levels in Healing Wounds. Phytother Res. 13: 50–54.
37. Maquart FX, Bellon G, Wegrowski Y, Barel JP. (1990). Stimulation of Collagen Synthesis in Fibroblast Culture by a Triterpene Extracted from *Centella asiatica*. Conn. Tissue Res. 24: 107-120.

38. Nabzdyk LP, Kuchibhotla S, Guthrie P, et al. (2013). Expression of Neuropeptides and Cytokines in a Rabbit Model of Diabetic Neuroischemic Wound Healing. J Vasc Surg. 58(3): 766-775.
39. Lin ZQ, Kondo T, Ishida Y, Takayasu T, Mukaida N. (2003). Essential Involvement of IL-6 in the Skin Wound-Healing Process as Evidenced by Delayed Wound Healing in IL-6-Deficient Mice. J Leukoc Biol. 73(6): 713-721.
40. Lan CCE, Wu, CS, Huang SM, Wu, IH, Chen GS. (2013). High-Glucose Environment Enhanced Oxidative Stress and Increased Interleukin-8 Secretion from Keratinocytes: New Insights into Impaired Diabetic Wound Healing. Diabetes. 62(7): 2530-2538.
41. El Euch SK, Cieśla Ł, Bouzouita N. (2014). Free Radical Scavenging Fingerprints of Selected Aromatic and Medicinal Tunisian Plants Assessed by Means of TLC-DPPH• Test and Image Processing. J AOAC Int. 97(5): 1291-1298.
42. Sefi M, Fetoui H, Lachkar N, et al. (2011). Centaurium erythrea (Gentianaceae) Leaf Extract Alleviates Streptozotocin-Induced Oxidative Stress and B-Cell Damage in Rat Pancreas. J Ethnopharmacol. 135(2): 243-250.
43. Shetty S, Udupa S, Udupa L. (2008). Evaluation of Antioxidant and Wound Healing Effects of Alcoholic and Aqueous Extract of Ocimum Sanctum Linn in Rats. Evid Based Complement Altern Med. 5(1): 95-101.
44. Cos P, Ying L, Calomme M, et al. (1998). Structure−Activity Relationship and Classification of Flavonoids as Inhibitors of Xanthine Oxidase and Superoxide Scavengers. J Nat Prod. 61(1): 71-76.

✉ **Corresponding Author:**
Ünal YAVUZ
Department of Surgery, Faculty of Veterinary Medicine, Harran University, Şanlıurfa, TURKEY
Email: unalyavuz@harran.edu.tr