JOURNAL OF SURGICAL RESEARCH • JANUARY 2019 (233) 284–296

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

# Wound healing effect of kaempferol in diabetic and nondiabetic rats

Yusuf Özay, PhD,[a,*] Sevda Güzel, PhD,[b] Önder Yumrutaş, PhD,[a] Burçin Pehlivanoğlu, MD,[c] İbrahim Halil Erdoğdu, MD,[d] Zuhal Yildirim, PhD,[e] Bilge Aydın Türk, MD,[f] and Sinan Darcan, MSc[g]

[a] Department of Medical Biology, Faculty of Medicine, Adiyaman University, Adiyaman, Turkey
[b] Department of Pharmacognosy, Faculty of Pharmacy, Mersin University, Mersin, Turkey
[c] Department of Pathology, Adiyaman University Training and Research Hospital, Adiyaman, Turkey
[d] Department of Pathology, Adnan Menderes University, Aydin, Turkey
[e] Etimesgut Public Health Laboratory, Etimesgut, Ankara, Turkey
[f] Department of Pathology, Adiyaman University, Training and Research Hospital, Adiyaman, Turkey
[g] Gediz Voc.School of Health Services, Dumlupinar University, Kütahya, Turkey

ARTICLE INFO

*Article history:*
Received 14 September 2017
Received in revised form
6 June 2018
Accepted 2 August 2018
Available online xxx

*Keywords:*
Wound healing
Kaempferol
Diabetes
Surgical wound
Hydroxyproline

ABSTRACT

*Background:* Flavonoids have previously been suggested to play a role in wound healing. To date, however, limited information is available on the wound healing effect of kaempferol (KM), which belongs to the class of flavonoids. The objective of this study was to determine the wound healing effects of KM.

*Materials and methods:* The wound healing effects of KM with two different concentrations (0.5% and 1% [weight/weight, w/w]) were evaluated in incisional and excisional wound models on diabetic and nondiabetic rats by macroscopic, biomechanical, biochemical, and histopathological analyses. Diabetes was induced by streptozotocin. The KM ointments were prepared using a mixture of glycol stearate:propylene glycol:liquid paraffin (3:6:1); 0.5 g of the ointments were topically applied on the wounded areas once a day for 7 and 14 d. On days 0, 7, and 14, wounds were photographed, and macroscopic examination of the wounds was performed. After 7 and 14 d, hydroxyproline levels, biomechanical analysis, and histopathological parameters (reepithelialization, thickness of granulation tissue, angiogenesis, presence of inflammation, deposition of collagen, presence of fibrosis, degree of dermal inflammation, and number of mast cells) were assessed.

*Results:* The best wound healing effect was observed in the diabetic excisional and nondiabetic incisional wounds (92.12% and 94.17%, respectively) treated with 1% (w/w) KM ointment for 14 d according to macroscopic examination. The nondiabetic excisional (14th day) and incisional (7th day) wounds treated with 1% (w/w) KM ointment showed statistically higher levels of hydroxyproline than the control groups (2.84 and 2.07 μg/mg, respectively, $P < 0.01$). Reepithelialization scores of KM-treated diabetic and nondiabetic excisional wounds on both 7 and 14 d ($P < 0.05$ and $P < 0.01$) and incisional wounds on the day 14 ($P < 0.05$) were significantly higher than controls. The maximum tensile strength

* *Corresponding author*. Adiyaman University, Department of Medical Biology, Faculty of Medicine, Adiyaman 02040, Turkey. Tel.: +90 5366407893; fax: +90 416 223 3843.
E-mail address: yusufozay33@gmail.com (Y. Özay).
0022-4804/$ – see front matter 
https://doi.org/10.1016/j.jss.2018.08.009

was observed in nondiabetic and diabetic groups (0.92 and 0.82 g/s, respectively) treated with 0.5% (w/w) KM ointment on day 14.

*Conclusions:* Thus, KM appears to be an effective topical wound healing agent in the treatment of both nondiabetic and diabetic wounds.



## Introduction

Chronic wounds cause severe health problems; worldwide, chronic wounds are typically related to various diseases such as *diabetes mellitus* (DM).[1,2] Patients with DM develop serious complications such as chronic ulcers,[2,3] which are defined as impaired, persistent, and nonhealing wounds.[3,4] Skin wounds of diabetic patients require a longer treatment time than those of nondiabetic patients. The longer treatment duration in patients with DM is because of the impaired inflammatory response and the defects in collagen deposition and differentiation of the extracellular matrix.[5] In addition, oxidative stress plays a role in the delayed recovery of wounds in diabetic patients; phenolic compounds decrease the level of oxidation in diabetic rats and accelerate wound healing.[6]

Ideal wound healing is defined as the successful closure of a wound in a very short time without any side effects.[7] Wound healing is considered to be mostly related to the antioxidant activity of the therapeutic agent used; antioxidants significantly accelerate wound healing by removing the free oxygen radicals and by increasing colloid synthesis.[8] Several studies have investigated the therapeutic effects of medicinal plants and/or plant-derived substances such as phenolic compounds on wound healing.[9,10] Flavonoids are phenolic compounds that are naturally synthesized by plants,[11] and these compounds have many biological activities,[12] including neuroprotective, cardioprotective,[13] antioxidant, antiinflammatory,[13,14] antimicrobial, and antidiabetic activities.[15] Kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one) (KM), which has anticancer,[11,16] antiproliferative,[17] antiinflammatory,[18] antibacterial,[19] and antioxidant[20] activities, is a flavonol that belongs to the class of flavonoids. Several studies on wound healing effects of medicinal plants containing KM have been reported.[21] To date, however, limited information is available on the wound healing effect of KM. Therefore, in this study, we aimed to examine the *in vivo* wound healing potential of KM by using macroscopic, biomechanical, biochemical, and histopathological methods on excisional and incisional dermal wounds in nondiabetic and diabetic rats.

## Materials and methods

### *Preparation of the KM ointment*

Glycol stearate, 1,2 propylene glycol, and liquid paraffin were mixed in a 3:6:1 ratio and KM (Sigma-Aldrich, St. Louis, MO) was added at a concentration of 0.5% and 1% (w/w) to prepare the ointments. The wounds of the rats were treated with 0.5 g of the ointment daily during the course of the experiment.

### *Animals and experimental protocol*

Three- to four-month-old male Wistar rats weighing 180-240 g were used for the experiments; the rats were fed *ad libitum* with standard industrial pellet feed and tap water under standard conditions (in well-ventilated rooms with normal night and day rotation) at the Biology Department, Faculty of Arts and Sciences, Dumlupinar University (Kutahya, Turkey). The study was approved by the Animal Ethics Committee of Dumlupinar University.

The rats were randomly divided into two main study groups (diabetic and nondiabetic, $n = 112$) and placed in individual cages. Subsequently, each main group was randomly divided into four groups with 14 animals (seven animals each for 7- and 14-d applications) in each group; the experimental design of the study is shown in Table 1.

### *Induction of DM*

DM was induced by a single intraperitoneal injection of streptozotocin dissolved in saline (45 mg/kg).[22] After 3 d of administration of streptozotocin, blood was taken from the tail vein of the rat and fasting blood glucose levels were

**Table 1 – Experimental design of animal groups ($n = 112$).**

| | |
|---|---|
| Nondiabetic groups ($n = 56$) | |
| Group 1 | |
| C | Control ($n = 14$)* (nondiabetic group without any intervention/non-DM control) |
| V | Vehicle ($n = 14$)* (nondiabetic group treated with a mixture of glycol stearate:propylene glycol:liquid paraffin (3:6:1)/non-DM vehicle) |
| KM-05 | Nondiabetic group treated with 0.5% (w/w) kaempferol ointment-0.5% KM ($n = 14$)* |
| KM-1 | Nondiabetic group treated with 1% (w/w) kaempferol ointment-1% KM ($n = 14$)* |
| Diabetic groups (n = 56) | |
| Group 2 | |
| DM/C | Diabetic control ($n = 14$)* (diabetic group without any intervention/DM-control) |
| DM/V | Diabetic vehicle ($n = 14$)* (diabetic group treated with a mixture of glycol stearate:propylene glycol:liquid paraffin (3:6:1)/DM-vehicle) |
| DM/KM-05 | Diabetic group treated with 0.5% (w/w) kaempferol ointment-DM-0.5% KM ($n = 14$)* |
| DM/KM-1 | Diabetic group treated with 1% (w/w) kaempferol ointment-DM-1% KM ($n = 14$)* |

* $n = 7$ for 7-day treatment and $n = 7$ for 14-d treatment.