



The Roles of Apigenin Cream on Wound Healing in Rabbits Model

Ammar M. Rajab¹, Wael T. Al-Wattar² and Ghada A. Taqa³

¹Ministry of Health, Nineveh Health Directorate, Mosul, Iraq.

²Department of Oral and Maxillofacial Surgery, College of Dentistry, University of Mosul, Mosul, Iraq.

³Department of Dental Basic Science, College of Dentistry, University of Mosul, Mosul, Iraq.

*Corresponding Author: Ammar M. Rajab, E-Mail: ammar.dep4@student.uomosul.edu.iq

ABSTRACT

This study aims to evaluate the healing effect of topical Apigenin cream 2% in the skin of rabbits. Twenty Four healthy Newzealand adult male rabbits approximately at the same age were involved in this study. All animals were anesthetized using a mixture of 40 mg/kg ketamine injection and xylazine 4 mg/kg intramuscularly. The skin of the dorsum area of each rabbit was shaved; then, three incisions (1cm in length) were made. Wounds were left without suturing to heal by secondary intention healing, and each animal had three wounds (wounds divided into three groups according to the type of treatment): one treated with Apigenin cream twice a day, the other with cold cream twice a day, and the third wound was left untreated. The application of cream was repeated daily; the wound size and the ratio of wound contraction were assessed at the first, fourth, and seventh days of study. The results revealed that the group treated with apigenin showed significantly better wound healing capacity in the skin of rabbits; wound size and wound contraction ratio was superior in the Apigenin group compared to control positive and negative groups. It can be concluded that apigenin cream has beneficial value for improving the wound healing potential of skin in rabbits.

Keywords: Apigenin, Wound contraction ratio, Wound healing, Wound size.

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INTRODUCTION

A wound is a breakdown in the skin's protective function; the destruction of epithelium continuity, with or without destruction of underlying connective tissue (bone, muscle, and nerves), following damage to the skin or underlying tissues caused by surgery, a cut, heat/cold, chemicals, pressure, friction, or diseases, such as carcinomas or leg ulcers (Shankar *et al.*, 2014). Any damage or break in the skin initiates a series of events that culminate in the migration of specialized cells into the wound site to heal the wound (Khunger, 2017). Wound healing is a complicated process that requires various cells, growth factors, cytokines, and extracellular matrix (ECM) (Martin and Nunan, 2015).

Wound healing is a crucial physiological process for maintaining skin integrity. Normal wound healing consists of four sequential but overlapping phases: hemostasis, inflammatory phase, proliferative phase, and remodeling phase (Wang *et al.*, 2018). The fundamental aim of topical wound care is to restore structural and morphological features of the skin,

which is required to avoid external environment infections, keep wounds moist, and maintain internal environment homeostasis. This reduces edema and increases blood flow, saving time and money while improving quality of life. (Hunckler and De Mel, 2017; Khunger, 2017).

Apigenin has many biological properties of naturally occurring flavonoids, such as antithrombotic, hepatoprotective, antiviral, and anti-inflammatory activities. Many of them are thought to be related (at least in part) to their antioxidant and free radical scavenging properties (Rehecho *et al.*, 2011).

Apigenin, which is found in many plants, including chamomile, has been shown to function as a natural anti-inflammatory agent (Durate *et al.*, 2011). Its effectiveness in treating symptoms of gastritis, gastric ulcers, and other mucosal inflammatory diseases is attributed to the presence of apigenin glycosides in the plant. Recent research suggests that apigenin may be useful in treating skin inflammation caused by free radicals (Lopez Jornet *et al.*, 2014).

Consequently, this research aims to investigate the healing capacity of topical apigenin cream 2% on wound healing in rabbit skin.

MATERIALS AND METHODS

Animals:

The experiment randomly allocated 24 New Zealand adult male rabbits aged about 5-7 months, weighing 1.5 - 2 kg. The animals were kept in the animal's house of Collage of Dentistry, University of Mosul, Mosul, Iraq.

Preparation of Apigenin cream:

Apigenin powder has been bought from (Yanhuang Industrial Park, Guanxian, Liaocheng, Shandong, China). Apigenin cream is prepared by mixing pure apigenin powder with cold cream (beeswax, paraffin oil, borax and purified water) in measured doses of cream and apigenin powder, 2 gm. apigenin powder, and 98 gm. cold cream for obtaining 2% apigenin cream. The apigenin cream was stored in special containers and stored in a dry cooled environment (refrigerator at 4C^o) to be used later in this study. The same cold cream was used as control positive in the study.

Trial on animals:

Twenty-four rabbits participated in this study. Each rabbit received a 40 mg/kg ketamine injection intramuscularly in the thigh muscle, combined with 4 mg/kg of xylazine of rabbit weight (Ahirwar *et al.*, 2021). The rabbit's weight was recorded using electronic digital scales, and the rabbit's reflexes were checked after 5-10 minutes to confirm that anesthesia was properly administered.

Animals in all groups were prepared aseptically. A full-thickness longitudinal skin incision was made, and the defect was 1cm in length. All the Defective wounds left without suturing to heal by secondary intentional healing.

Three stab incisions were made at the dorsum area through the skin of each rabbit. The first incision treated with apigenin cream 2%, the second incision treated with cold cream alone, and the last incision left without treatment. Wounds were divided randomly into three experimental groups; each group consisted of twenty-four wounds according to the treatment material used.

G1: (Apigenin group): 24 wounds treated with topical apigenin 2% cream twice daily.

G2: (Control positive group): 24 wounds treated with topical cold cream alone two times daily.

G3: (Control negative group): 24 wounds did not receive any treatment.

The application of apigenin and cold cream was repeated twice daily. The wounds were totally covered with cream for 7 days. The wound

measurement was carried out on the 1st, 4th, and 7th Days of the experiment.

Vernia device was used to calculate the changes in wound size in mm² by multiplying the longest and widest measurement in the wound bed in millimeters. Percentage of wound Contraction ratio (WCR) was calculated taking the initial size of the wound as 100% using the following formula: (Taqa *et al.*, 2014; Nagar *et al.*, 2016)

$$WCR = \frac{\text{Initial wound area} - \text{Specific day wound area}}{\text{Initial wound area}} \times 100$$

Statistical analysis:

The data were collected to find the means and standard deviation of each group. These data were used to analyze the difference between groups by using one-way analysis of variance (ANOVA) with Duncan Multiple Analysis Rang Test, $p \leq 0.05$ has relied as significance value.

RESULTS

In the present research, one day postoperatively, the value of the wound size mean was 26.66 mm² and the wound contraction ratio was 11.11 % in the positive control group. In contrast, the control negative wound size mean was 27 mm² and the wound contraction ratio was 10 % which was the lowest value on this day of study; the wound size mean of apigenin 2% group was 24 mm² with wound contraction ratio equals 20% which was the best healing score between all groups. But there was no significant difference between all groups at p -value ≤ 0.05 (Table 1,2 and Figs. 1,2).

Table 1: Wound size findings of skin comparing between control positive, control negative and apigenin groups:

Duration	Group	Wound size (Mean + SD)	P-value
1 st Day	Control positive	26.666 ± 4.618	0.002
	Control negative	27.000 ± 0.00	
	Apigenin 2%	24.000 ± 0.00	
4 th day	Control positive	24.3333 ± 3.511	0.001
	Control negative	26.6667 ± 4.618	
	Apigenin 2%	12.6667 ± 4.041	
7 th day	Control positive	14.3333 ± 4.041	0.004
	Control negative	17.0000 ± 4.582	
	Apigenin 2%	7.3333 ± 1.154	

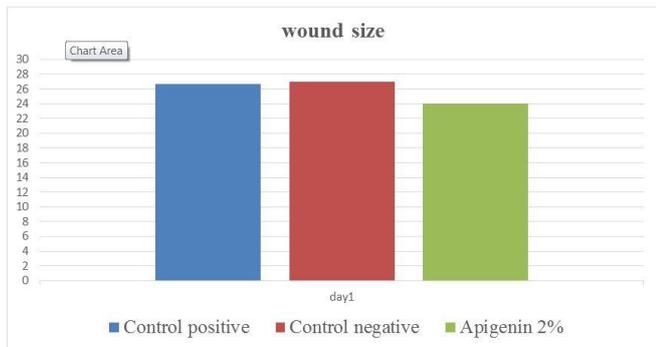


Fig. 1: Mean rank difference of skin wound size between groups treated topically on the first day.

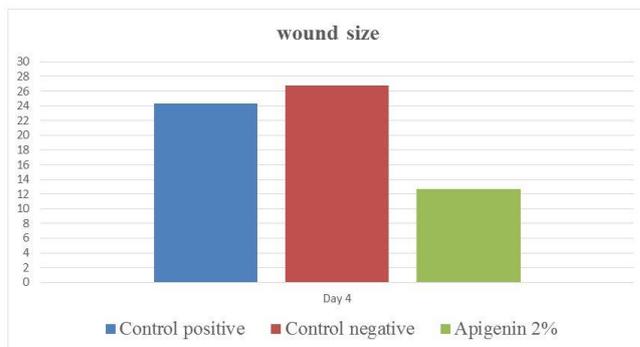


Fig. 3: Mean rank difference of skin wound size between groups treated topically on the fourth day.

Table 2: Apigenin cream effect on Wound contraction ratio in comparison to control groups.

group	Wound contraction ratio		
	Day 1	Day3	Day7
Control positive	11.11 %	18.9 %	52.23 %
Control negative	10 %	11.13 %	43.33 %
Apigenin 2%	20 %	57.8 %	75.56 %

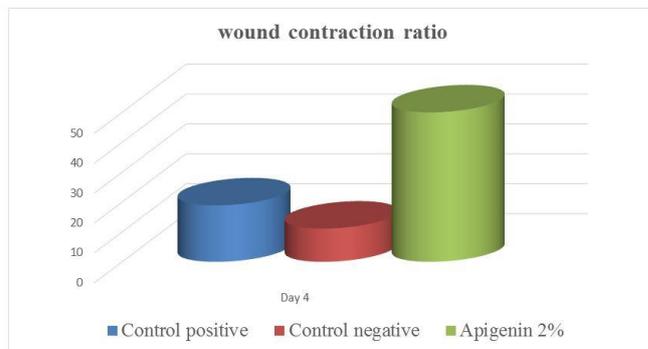


Fig. 4: Effect of apigenin 2% cream on wound contraction ratio in rabbits after four days.

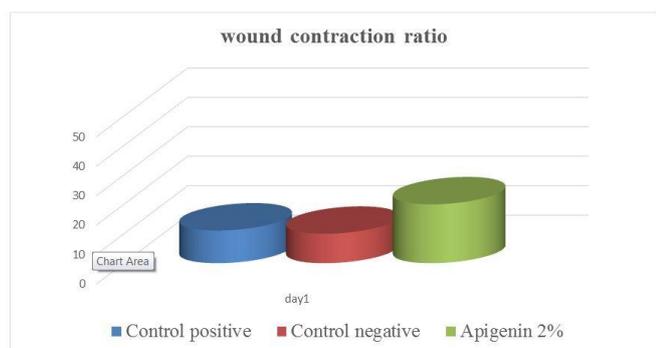


Fig. 2: Effect of apigenin 2% cream on wound contraction ratio in rabbits after the first day.

On Day Four of this study, the value of the wound size mean was 24.33 mm² and the wound contraction ratio was 18.9 % in the positive control group, while in the negative control group; the value of wound size mean 26.66 mm² with wound contraction ratio 11.13%, apigenin group also maintained the best result between groups, with contraction ratio 57.8 % and mean value of wound size 12.66 mm².

Apigenin group showed a significant difference in comparison to control groups at a p-value ≤ of 0.05. It was found that the control positive and control negative groups had similar values. (Table 1,2 and Figs. 3,4).

On Day seven of the study, the wound size mean values were measured and the wound size for the positive control group was 14.33 mm². The wound contraction ratio was 52.23%. The control positive wound size was 17 mm² with a wound contraction ratio of 43.33%. In the apigenin group, the healing score was also the best among all groups. The wound size mean was 7.33 mm² and the wound contraction ratio 75.56 %.

There was a significant difference between the groups treated with apigenin and control groups, whereas there was no significant difference between control positive and control negative groups (Table 1,2 and Figs.5,6).

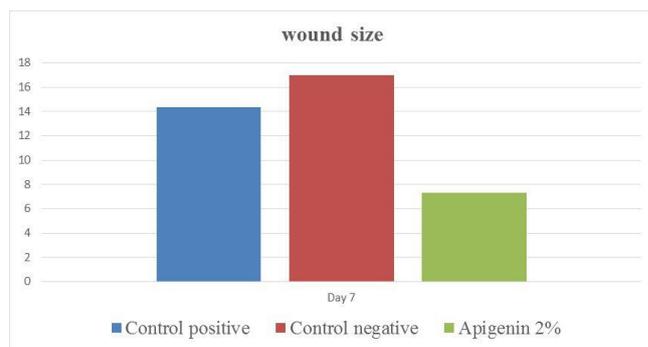


Fig. 5: Mean rank difference of skin wound size between groups treated topically on the seventh day.

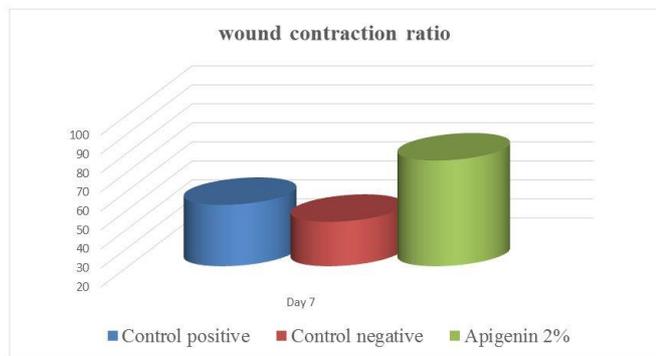


Fig. 6: Effect of apigenin 2% cream on wound contraction ratio in rabbits after seven days.

DISCUSSION

Wound healing is a complicated and dynamic process involving replacing devitalized and lost cellular components and tissue layers. There are four overlapping phases of wound healing: hemostasis, inflammatory phase, proliferative phase, and remodeling phase. These stages occur sequentially as a result of the integration of dynamic processes, including cellular mediators, blood cells, and parenchymal cells (Strodtbeck, 2001; Gonzalez *et al.*, 2016; Sorg *et al.*, 2017).

As a primary indicator of healing, wound size assessment may provide an effective treatment protocol, provide an objective form of analysis, anticipate healing, improve quality of care, contribute to more accurate professional communication, and improve wound management (Humbert *et al.*, 2004; Williams *et al.*, 2017).

In our study, apigenin significantly improved wound healing after the fourth and seventh days in comparison to other groups in this study, this effect of apigenin on wound healing may return to many pharmacological effects of apigenin; apigenin may enhance the re-epithelialization process and collagen fiber deposition in the dermis, as well as the efficiency of granulation tissue formation (Motealleh *et al.*, 2014).

Another apigenin function is that it has an anti-inflammatory and chemo-preventive impact; apigenin directly reduced (Src) activity. Src (a non-receptor tyrosine kinase) is an oncogenic kinase whose activity is connected to inflammatory reactions, apigenin directly bind to src leading to Src inhibition and decreased inflammatory changes; Src inhibition is thought to be an important factor for avoiding inflammation and cancer because it suppresses downstream signaling pathways to decrease COX-2 expression which is implicated in skin inflammation and carcinogenesis (Byun *et al.*, 2013).

Apigenin inhibits acute inflammation by improving epidermal permeability barrier function, and topical apigenin partly inhibits changes in stratum corneum hydration and skin surface pH by lowering transepidermal water loss, which could be attributed to apigenin's anti-inflammatory and antioxidant properties (Man *et al.*, 2012).

Apigenin also suppresses the production of inflammation-related molecules produced by TNF-alpha and IL-1alpha, such as intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin (Lim and Kim., 2007).

Some researchers believe that apigenin inhibits inflammation by decreasing matrix metalloproteinase-1 expression and inhibiting the proinflammatory cytokines TNF- alpha and IL-6 at very low micromole levels via inhibiting NF-κB activation and phosphorylation of p38 and JNK in macrophages antioxidants (Man *et al.*, 2012; Xie *et al.*, 2012), As well as its positive role in activating the factor Nuclear Factor Erythroid 2-Related (NrF-2) that regulates the process of cloning the genes responsible for the manufacture of antioxidant enzymes like glutathione (Ibrahim *et al.*, 2020).

Trauma illnesses have oxidative stress as an important part of their pathophysiology. After tissue damage, reactive oxygen species (ROS) production frequently increases in the wounded cells. Antioxidant biomaterials could effectively prevent ROS damage to cells so that they promote wound healing (Liu *et al.*, 2018; Ibrahim *et al.*, 2020). Many previous studies established the antioxidant effects of apigenin so that we could attribute the enhanced wound healing in the apigenin group to their antioxidant properties (Huang *et al.*, 2019; Xu *et al.*, 2020).

Apigenin has a well known antioxidant effect. According to several studies, antioxidants suppress the production of inflammatory mediators such as leukotrienes, histamine, IL-8, IL-6, and TNF from mast cells. As a result, It is possible that the antioxidant properties of apigenin are responsible for its anti-inflammatory effects. (Weng *et al.*, 2012).

Apigenin's lipid peroxidation inhibition effect is thought to improve collagen fibril viability by stimulating DNA synthesis and minimizing cell damage resulting in enhanced wound healing in the proliferation phase, with increased blood vessels, collagen fibers, and fibroblast cell growth in wounds treated with apigenin (Lopez Jornet *et al.*, 2014; Shukla *et al.*, 2016).

CONCLUSION

The data analysis of this study revealed a positive effect of apigenin in the acceleration of wound healing in rabbits.

Declaration of Conflicting Interests

The authors revealed that there is no potential conflicts of interest.

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