Clinical and Experimental Health Sciences

Assessment of the Effects of Quince Seed Mucilage and Wheat Germ Oil on Wound Healing in Rats

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ABSTRACT

Objective: People have used traditional herbal medicines for wound care since the dawn of time. This study aimed to assess the cutaneous wound healing effects of wheat germ oil (WGO) and quince seed mucilage (QSM) in rats.

Methods: Adult female Wistar albino rats were allocated to one of the three groups: rats treated with topical WGO (n=6); topical QSM (n=6); and topical saline (n=6) as the control group. Two circular, full-thickness wounds of 0.6 mm diameter were created on the dorsal thoracic region of each rat. Test and control solutions were applied twice daily for 14 days. Wound healing was assessed by measuring the wound contraction rate and the time needed for complete epithelialization.

Results: When compared with the control group, rats in the WGO group had reduced wound closure rates in the first four days, but considerably greater rates in the 8th, 10th, and 12th days, as well as a shorter duration of time needed to complete epithelialization (11 days vs. 13 days). The wound closure rates of the rats in the QSM group were not substantially different from the control rats and the duration of time needed for complete epithelialization was not significantly different from the control group.

Conclusion: WGO use has been shown to improve wound healing. It may be used as an alternative or complementary approach for wound treatment depending on the severity of the wounds. On the other hand, QSM was not found to improve wound healing.

Keywords: Wound healing, wheat germ oil, quince seed mucilage

1. INTRODUCTION

Wound healing is a complex body activity that helps to preserve the integrity of the skin after trauma (1). Wound healing has broad-reaching impacts that go far beyond medical, influencing many areas of personal and social life. Wound infections and delayed wound healing have become a major source of financial strain on healthcare systems around the world in the modern age (2). Wound healing consists of three sequential phases: hemostasis/inflammatory phase, proliferative phase, and remodeling phase. Aberration of wound healing disrupts normal physical activity (1).

Plants may help manage and heal wounds in a variety of different ways. In many cultures, tribal and traditional treatments use a wide range of herbs to heal wounds. Several plants have been utilized in research studies to treat skin problems and wound damage (3). New wound healing therapy research is a growing field in modern biomedical sciences. Various plant extracts were studied for significant wound healing activity using experimental models, and many of them have still to be investigated (4).

Traditional wound healing treatments are commonly applied to the wider population. Among the substances used by the public in wound healing are wheat germ oil (WGO) and quince seed mucilage (QSM). Linoleic acid (18:2 n6), an essential fatty acid, accounts for around 56% of WGO content. WGO has anticancer and antioxidant properties (5). Quince seeds have been shown to include phenolic compounds, organic acids, free amino acids, and pectin (6). QSM is known as cydonin, a condensed colloidal solution with quince seeds comprising 20%-22% of its weight (7).

There are few studies on the effect of QSM on wound healing. The present study aimed to assess how WGO and QSM influenced cutaneous wound healing in rats.

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2. METHODS

2.1. Animals

Adult female Wistar albino rats (n=18) weighing 200-300 g (Marmara University Experimental Research and Animal Laboratory) were used. Following the creation of the wound model, all animals were kept in separate sections under laboratory conditions with constant temperature and humidity. During the experiments, all animals were subjected to the same stress under the same conditions. The Marmara School of Medicine Animal Care and Use Ethics Committee authorized all methods for this study's experimental protocols (15.02.2013-99.2012.mar).

2.2. Experimental Groups

All rats were randomly allocated to one of the three groups: rats treated with topical wheat germ oil (WGO) (n=6); topical quince seed mucilage (QSM) (n=6); and topical saline [ie. sérum physiologique] (SP) (n=6) as the control group, and the study was conducted on these groups.

2.3. Materials

QSM: The seeds were extracted from the quince fruit and dried for one day at room temperature. 20 ml distilled water was added to 5 g of dried quince seeds. It was heated at 50–60 ◦C and mixed for 30 min as described by Hemmati et al. (7). The mucilage part of the mixture was used. QSM was prepared freshly every other day following the same procedures.

WGO: A commercial product (Tabia Pure Nature®) of WGO *(Triticum sativum)* that was obtained by supercritical carbon dioxide extraction technique was used.

Other materials used during the experiment: Physiological saline (Eczacibasi Pharmaceuticals), formaldehyde (Aksan), ether sulfuric (Pure Chemistry), distilled water, ketamine HCl (Ketalar®, Pfizer Pharmaceuticals), 0.2% chlorhexidine (Klorhex®, Drogsan Pharmaceuticals), chlorpromazine (Largactil ®, Eczacibasi Pharmaceuticals).

2.4. Wound Model and Treatment

Ketamine (100 mg/kg body weight, intraperitoneal [IP]) and chlorpromazine (5 mg/kg body weight, IP) were used to anesthetize the rats. The anesthetized rats' dorsal hair was then shaved using an electrical clipper, and the short hairs were removed with a depilatory cream. The wound site was cleaned with saline to remove any cream leftovers, then disinfected with a 0.2% percent chlorhexidine solution. Then, using a punch biopsy tool, two circular, full-thickness excisions of 0.6 mm in diameter were formed on the dorsal thoracic region of each rat. The wounding day was enumerated as day 0. Following wound excision, all groups received twice-daily applications of the test and control solutions (WGO, QSM, SP) for 14 days until the end of the study.

2.5. Wound Closure Measurements

The wound-healing rate was assessed by measurement of the wound contraction rate and the time needed for complete epithelialization by methods described by Apikoglu-Rabus et al. (8).

The wound area was measured daily planimetrically for each animal, resulting in a total of 12 wounds for each group per measurement day.

Wounds were first traced on transparent paper with a millimeter-scale to estimate the wound contraction rate. Then, traces on the transparent paper were transferred to photocopy papers of 80 g ⁄m2 weight. The wound traces were cut out and weighed by a precision balance to get more accurate measurements. The matching surface area values in mm2 were calculated using the weights. Wound closure was reported as percentage closure and calculated by the following formula: % closure = [(area on day 0 – open area on day n)/area on day 0] × 100; n represents the day of the measurement. The wound healing rate was calculated for days 2, 4, 6, 8, 10, 12, and 14.

Complete epithelialization time was the other wound healing parameter evaluated visually during the study. 'Complete epithelialization' was considered to be reached when the scar slid off the skin, leaving no raw wound behind. Whether or not complete epithelialization happened was evaluated on the 8th, 10th, and 12th days.

Signs of infection (redness, swelling, wound inflammation, the appearance of a red line around the wound), weight loss, and dehydration were visually assessed.

2.6. Histological Evaluation

At the end of the 14-day experiment, rats were sacrificed by cardiac puncture under ketamine anesthesia. Afterward, the wound tissues were excised and fixed in a 10% formaldehyde solution for light microscopic examination by immersion fixation. Tissues dehydrated by routine histological tissue follow-up by increasing alcohol series (70%, 90%, 96%, 100%) were later made transparent with toluene and blocked by embedding in paraffin. 5 µm sections were taken from the tissue blocks with a microtome (Leica RM2125RT). To evaluate the general structure of the histological sections, hematoxylin-eosin and Gomori's triple staining were applied to determine the changes in the connective tissue structure. All of the preparations were examined and photographed under a light microscope (Olympus BX-51 / Olympus DP72).

2.7. Statistical Analysis

Continuous variables were expressed as mean ± standard error (SE) of the mean, or median (min-max). The

difference between the two groups was analyzed using the nonparametric Mann–Whitney U test. Binomial data were expressed as n (%) and analyzed using Pearson's chi-square test. Various p values were used to detail the power of statistical significance ($p<0.001$, $p<0.01$, $p<0.05$). Statistical significance was set at $p<0.05$. The analyses were performed using the statistical software package SPSS (version 11.5; SPSS Inc., Chicago, IL, USA).

3. RESULTS

3.1. Wound Healing in Rats Treated with WGO

In the first four days, rats treated with WGO exhibited poorer wound closure rates than the control group (significantly lower on the fourth day, $p=0.01$). However, it was found to be significantly higher than the control on the 8th, 10th, and 12th days ($p<0.05$, $p=0.01$, $p<0.05$, respectively). The median time of complete epithelialization was 11 days (ranging from 11 to 13) for the WGO-treated group and 13 days (ranging from 13 to 13) for the control group ($p<0.05$) (Table 1). WGO treatment improved wound healing by reducing the time required for full epithelialization compared to the control group.

3.2. Wound Healing in Rats Treated with QSM

There was no significant difference between SP and QSM groups in terms of complete epithelialization times ($p>0.05$). The QSM group had a median complete epithelialization time of 13 days (ranging from 11 to 13), which was not significantly different from the control group (Table 1).

Table 1. *Wound closure rates (wound closure rate percentage, median, (minimum-maximum))*

Group	2nd day	4th day	6th day	8th day	10th day	12th day	14th day
Control	48.0 (11.8-58.8)	53.4 (26.0-70.8)[a]	64.3 (31.2-83.9)	83.0 (70.5-93.7)[b]	91.8 (86.8-97.4)[a]	94.9 (92.4-98.5)[b]	100.0 (95.7-100.0)
WGO	31.0 (11.2-55.3)	27.0 (9.56-60.6)[a,b]	64.5 (24.1-100.0)	92.7 (82.9-100.0)[b,a]	97.7 (93.2-99.9)[a,b]	100.0 (92.8-100.0)[b]	100.0 (98.0-100.0)
QSM	28.5 (1.44-55.7)	46.7 (24.1-68.7)[b]	62.1 (37.9-78.1)	83.9 (69.5-91.4)[a]	94.4 (88.6-98.0)[b]	97.3 (92.0-100.0)	100.0 (91.6-100.0)

There is statistical significance between values marked with the same letter in each column. ap=0.01; bp<0.05; WGO: wheat germ oil; QSM: quince seed mucilage.

3.3. Histological Findings

After wound creation, it was observed that the general structure deteriorated only in the control group animals treated with SP. Papillae loss in the epidermis layer and thinning of the epithelium were detected. It was determined that the wavy distribution of collagen bundles accompanied the loss of connective tissue, which is observed intensely in the dermis layer. In addition, a decrease was observed in the cellular content of the tissue (Fig. 1-2).

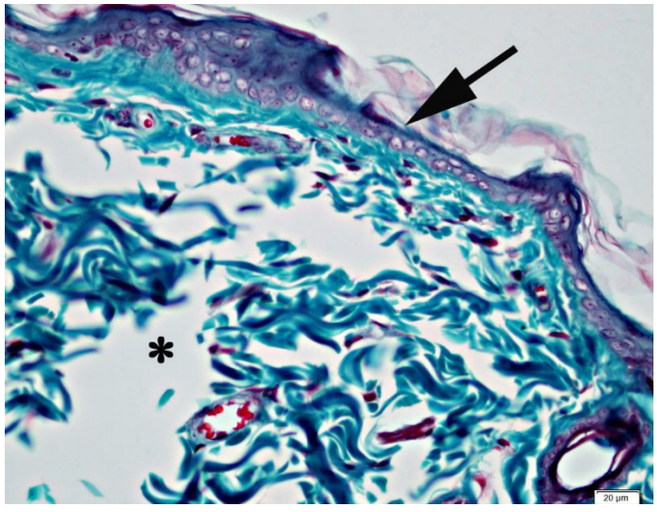

Figure 1. *Micrographs obtained from control group tissues are shown. Asterisk: scattered connective tissue loss in the dermis. Arrow: Distorted epidermis structure with irregular and thinned keratin layer. Stain: Gomori trichrome stain.*

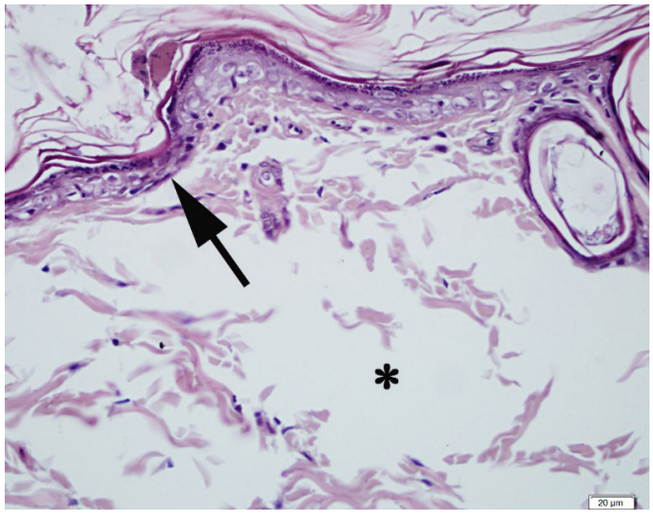

Figure 2. *Micrographs of the tissues taken from the control group are shown. Asterisk: Collagen bundles with irregular organization and intense loss of connective tissue in the dermis. Arrow: The flattened and quite thinned epidermis layer. Stain: Hematoxylin-Eosin stain.*

When tissues from the group that received WGO for wound treatment were analyzed, it was discovered that the overall structure was more regular than in the control group. In terms of histological structure, however, there was no significant difference between the groups in which QSM was administered. In addition to epidermis layer loss and flattening, an epithelial structure with degraded cells was identified. The epithelial layer has been found to produce papillae in the dermis on occasion. The collagen fiber bundles in the dermis layer, on the other hand, were found to be unevenly dispersed (Fig. 3-4)

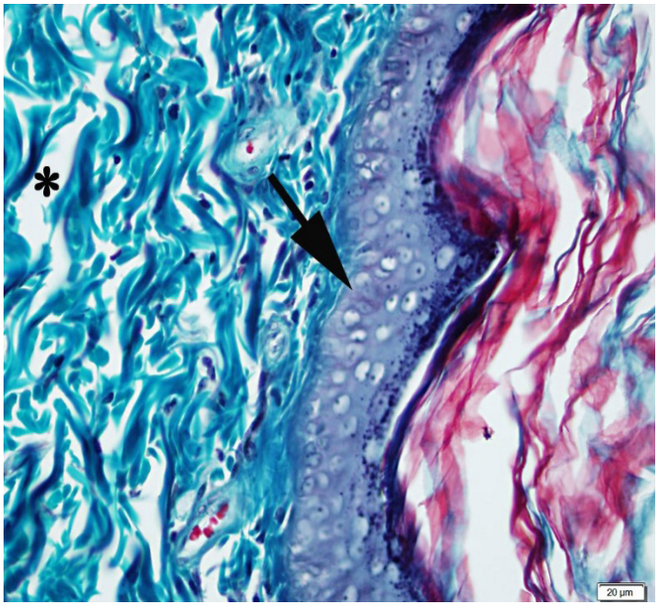

Figure 3. *Micrographs of tissues taken from the wheat germ oil group are shown. Arrow: Flattened endothelial layer. Asterisk: The dermis layer with wavy collagen bundles and localized loss of connective tissue. Stain: Gomori trichrome stain.*

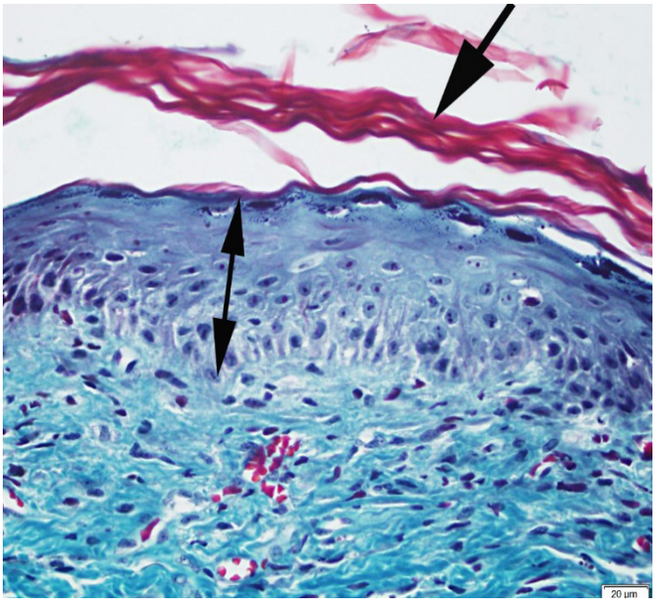

Figure 5. *Micrographs of the sections obtained from the quince seed mucilage group are shown. Arrow: A more uniform keratin layer compared to the control group. Double-headed arrow: Epithelial formation similar to normal structure. Stain: Gomori trichrome stain.*

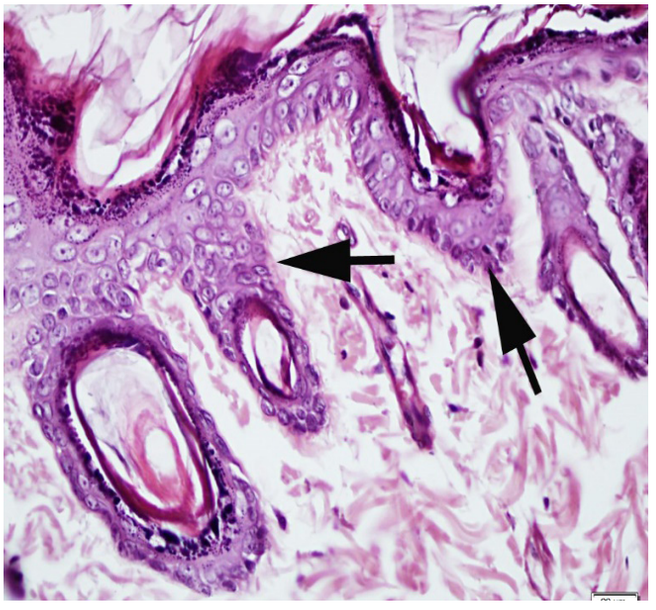

Figure 4. *Micrographs obtained from the wheat germ oil group are shown. Arrow: Papillae formation in the epidermis layer. Stain: Hematoxylin-Eosin stain.*

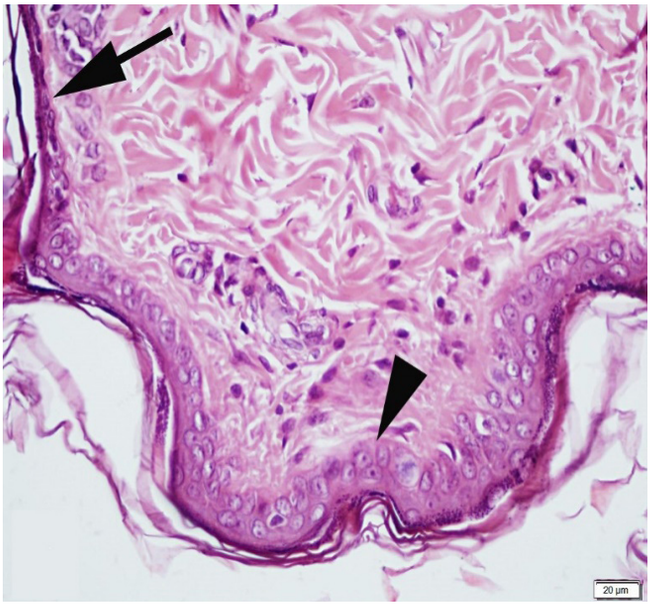

Figure 6. *Micrographs of the tissues obtained from the quince seed mucilage group are shown. Arrow: Thinning and degeneration of the epidermis. Arrowhead: Epithelial structure consisting of several cell layers observed in some parts of the epidermis. Stain: Hematoxylin-Eosin stain.*

It was observed that the samples taken from the QSM group for wound treatment had a more uniform structure than the control group. It was observed that the keratin layer was more regular in the epidermis layer and the epithelium contained more cell lines. It was observed that papillae structures began to form into connective tissue in the epithelial layer. While less connective tissue loss was in the dermis layer compared to the control group, an irregularly wavy sequence was observed in the collagen fiber bundles (Fig. 5-6).

4. DISCUSSION

Wounds are undesirable life events that might occur as a result of harm, but healing is a survival strategy that reflects an attempt to repair the tissue's natural anatomical structure and function. Various plant extracts were studied for significant wound healing activity using experimental

models, and many of them have still to be investigated. Hence, wound-healing medications that can improve the healing process are still limited (4).

This study evaluated the possible wound-healing effects of WGO and QSM rats using planimetric measurements and histological analysis in a full-thickness wound model on Wistar albino rats. When compared with the control group, although WGO-treated rats exhibited lower wound closure rates in the first four days, the rates were significantly higher after the 8th day. It was also observed that WGO treatment significantly reduced the time required for complete epithelialization when compared with the control group.

In a 2020 research by Sui et al., the macroscopic view of wound healing on days 0, 4, 7, and 10 administered by a wheat germ-derived peptide (YDW) and epidermal growth factor (EGF) showed dramatically increased wound closure compared to the control group. On the 10th day after the injury, the wound areas of the EGF-treated animals were entirely healed, the wound areas of the YDW-treated animals were nearly completely closed, and the wound areas of the control animals were still 19.3% ± 2.4% ($p<0.01$) of the original wound area (9).

Early wound cellular processes trigger acute inflammatory responses. However, chronic activation of the pro-inflammatory factors is probable to arise in persistent tissue damage, wound repair inhibition, and pathological or excessive wound healing (1). Fatty acids may regulate the healing process, which includes sequential stages (inflammation, new tissue production, and tissue remodeling) (10).

When compared to the control group, WGO prolonged the inflammatory phase, which is the initial step of wound healing. WGO's anti-inflammatory activities may reduce inflammation, which is a critical stage in wound healing, causing the process to be extended. After the extended anti-inflammatory phase, epithelialization and granulation tissue development begin with the start of the proliferative phase. WGO may have improved healing by acting at this stage, which is sensitive to oxidative damage and boosting epithelialization.

Antioxidants are utilized in wound healing to neutralize the damaging effects of free oxygen radicals. Antioxidants make the ultimate sacrifice by cleaning up free radicals and becoming less reactive and hence less damaging than the radicals themselves. As a result, antioxidants enhance wound healing (11). The antioxidant properties of cereal products often are associated with the phenolic components that comprise the cereals. In their study, Zou et al. observed that the phenolic components in WGO exhibit antioxidant effects (12).

Wheat bran (WB) and wheat germ (WG) contain 3%–4% and 7%–9% oil, respectively. An oil high in unsaturated fatty acids and a high concentration of minor components is considered to provide health benefits (13). WGO has diverse biological activities, including anticancer and antioxidant capabilities (5).

Unsaturated fatty acids are precursors of arachidonic acid. In the absence of essential fatty acids, cutaneous wound healing is hampered in mice, rats, and infants (14) but linoleic acid and other essential fatty acids can help prevent and treat pressure sores (14,15). With its chemotactic and neutrophil stimulating effects, linoleic acid, which has been linked to cell proliferation and inflammatory processes, operates as a modulator of leukocyte activities (16). Because of their impacts on pro-inflammatory cytokines, linoleic and oleic acids have been shown to speed up wound healing (14). The active component linoleic acid methyl ester extracted from Chinese and German calendula extracts has an enhancing effect on wound healing and epithelialization, according to Zimmer et al. (17).

Fatty acids can affect inflammation, lymphocyte function, and cell-mediated immunity (18). On the other hand, unsaturated fatty acids, such as omega-3, can influence the gene production of pro-inflammatory cytokines by affecting cellular membrane fluidity, cell-to-cell signaling, cell mobility, receptor interaction with their agonist, membrane function, and secondary signal generation (19).

The skin absorbs Vitamin E acetate quickly and transforms it into vitamin E (tocopherol) with the enzymes in its structure. Topical formulations of vitamin E are thought to accelerate wound healing due to their ability to suppress collagen formation and reduce fibroblast proliferation and inflammation (20). A study in diabetic rats found that tocopherol treatment significantly increased wound closure rate and total protein amount, positively affecting the wound healing process, and epithelial and collagen fibers were better organized with this therapy (21).

According to the studies, the level of vitamin E in different tissues increased immediately, and there were changes in lipid peroxidation in rats fed WGO (22). One of the components of WGO, beta-sitosterol, has been shown to have antioxidant (23,24) and anti-inflammatory (25,26) properties. Furthermore, beta-sitosterol promotes epithelialization (27). Several studies have shown that antioxidant compounds are beneficial to wound healing. In accordance with these findings, the components of WGO have antioxidant activity and are thought to have a favorable effect on wound healing in the current study as well (5,13,28).

According to a study on WG by Brandolini and Hidalgo, the pharmaceutical importance of WGO would increase considerably and gain value (28). More research is needed to produce higher-quality WGO, such as the use of additional creative and optimal ways to increase its utilization after more health benefits have been explored.

QSM was another component whose impact was investigated in the current research. Quince seed has been popularly used in some communities to heal skin wounds and reduce pain and inflammation (29).

The mucilage and gum produced from quince seeds are used to treat wounds. It's worth mentioning that extracting gums or isolating secondary plant components requires higher ethanol levels of 70%-90%, whereas mucilage is formed when the seeds are immersed in water, and a large amount of clear gelatinous substance is immediately released (30). According to Tamri et al., mucilage is a long-chain mucopolysaccharide that can act as a reservoir due to its high water absorption capacity, and when applied to a wound, it keeps the wound moist (31). Hydration improves wound healing by providing a wet wound surface on which epithelial cells may move more easily than a dry scab, protecting protease and growth factors in the exudate fluid, and increasing oxygen partial pressure (32).

Although studies have shown that mucilage can aid in healing skin wounds (closed wounds) (7,29,30), it cannot be used on exudative wounds due to its impermeable character and the risk of bacteria growth and infection spread. Mucilage reduces cell-to-cell interactions and inhibits wound healing. An impermeable structure is formed as the saturation of the gel with water affects the pores in the quince seed gel matrix. It is worth mentioning that long-term storage of QSM is inconvenient due to mold growth in the gel (30).

Quince seeds have been shown to include phenolic compounds, organic acids, free amino acids, and pectin (6). The increased proliferation of fibroblasts indicates that QSM may include growth factor(s). According to Ghafourian et al., these components are potential candidates for the effects of QSM on fibroblast growth (29). Quince seed contains phenolic compounds. One of these components, caffeoylquinic acid, is a potent antioxidant. The acceleratory impact of QSM on fibroblast proliferation might be explained by the radical scavenging and antioxidant activities of quince seed.

The wound closure rate in rats administered QSM was similar to those of control rats in our study. While the components in it are expected to improve wound healing, the finding that it has similar effects to physiological saline may be due to the insufficiency of the applied concentration.

According to a study using the microculture tetrazolium test, QSM increased the proliferation of human skin fibroblasts after 48 hours, even at low concentrations (50 g/mL) (29). Additionally, Tamri et al., studied 5%, 10%, and 20% Quince seeds mucilage cream (QSMC) with eucerin on white Iranian rabbits and reported that QSMC (20%) cream completely healed skin wounds after a 13-day therapy (31).

5. CONCLUSION

The current study revealed that WGO improved wound healing. It may be used as an alternative or complementary approach for wound treatment depending on the severity of the wounds. On the other hand, QSM was not found to improve wound healing.

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Author Contributions:
Research idea: CÇ, SA
Design of the study: CÇ, SA
Acquisition of data for the study: CÇ
Analysis of data for the study: CÇ, SA
Interpretation of data for the study: CÇ, ZÜG, AS
Drafting the manuscript: CÇ, ZÜG, AS, SA
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