International Journal of Surgery 12 (2014) 843–847

Contents lists available at ScienceDirect

International Journal of Surgery

journal homepage: www.journal-surgery.net

Original research

Wound healing properties of quince seed mucilage: *In vivo* evaluation in rabbit full-thickness wound model

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HIGHLIGHTS

- Quince seed mucilage is a wound healing agent in Iranian traditional medicine.
- In the present study the wound healing properties of QSM were evaluated in details.
- QSM increased the wound fluid levels of growth factors involved in tissue repair.
- The results of this study support the traditional use of QSM in wound treatment.

ARTICLE INFO

Article history:
Received 26 February 2014
Received in revised form
11 June 2014
Accepted 23 June 2014
Available online 11 July 2014

Keywords:
Quince seed mucilage
Wound fluid
Growth factors
Rabbit
Wound healing

ABSTRACT

Objective: Quince seed mucilage (QSM) has been used in Iranian traditional medicine for the treatment of skin wounds and burns. Recent studies indicated that QSM accelerated wound healing. The present study was undertaken to investigate the healing efficiency of QSM formulated as 5%, 10%, and 20% creams in eucerin base with especial attention on growth factors involving in wound healing.
Methods: Full thickness wounds were created in Iranian male rabbits divided into five experimental groups ($n = 6$), as negative control, eucerin and treatments. Negative control group did not receive any treatment. Eucerin group received topical eucerin, twice a day. Treatment groups were treated topically by creams of QSM 5%, 10% and 20% (w/w) in eucerin base, twice daily. The efficacy of treatment was evaluated based on wound contraction, haydroxyproline content, tensile strength of wound tissue. The levels of epidermal growth factor (EGF), transforming growth factor-β1 (TGF-β1), vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF) were also determined in serum and wound fluid of tested animals.
Results: Results showed that there were statistically significant differences in wound contraction between QSM 10 and 20% creams treatments groups and control groups ($P < 0.05$) in most of the days. Rabbits treated with QSM 20% cream had the best results (completed healing in 13 days, higher hydroxyproline content, higher tissue resistance and higher wound fluid levels of evaluated growth factors).
Conclusion: We concluded tha QSM in 10–20% concentrations have a good potential for promote wound healing thus supports its traditional use.

1. Introduction

A wound is a physical injury that results in an opening or break of the skin [1]. Wound healing is a complex and orderly series of events involving cell migration, proliferation, differentiation and the formation of extracellular matrix [2]. Growth factors and the binding of these factors to specific transmembrane tyrosine kinase receptors control these events [3]. Such a controlled phenomenon can be disrupted in diseases like diabetes, immunocompromised persons, ischaemia, etc, thus leading to the development of a chronic wound. Chronic wounds are the result of an inadequate repair process that is unable to restore anatomic and functional

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http://dx.doi.org/10.1016/j.ijsu.2014.06.016
1743-9191/

integrity in an appropriate length of time [4]. Damaged skin can severely reduce quality of life and cause unwanted health problems when left untreated. In recent years natural products have been widely used as health promoting agents. Many medicinal plants and natural products have been reported to posses wound healing activity and found useful in the treatment of wounds. One of these natural products is quince (*Cydonia oblonga*) seed mucilage [5]. *Cydonia oblonga* Miller (Rosacea) is a small deciduous tree, also known as quince, is often related to apples and bears and has a pome fruit just like them. The fruit is green, with dense grey-white pubescence when immature and turns to bright golden yellow on maturity. The quince is native to Persia (Iran), but was also cultivated in Greece and Turkey. Today, the herb is cultivated throughout the world [6]. The plant has been used in Iranian folk medicine for a variety of diseases. The seed is a mild but reliable laxative, astringent and anti-inflammatory. When soaked in water, the seed swells up to form a mucilaginous mass. This has a soothing and demulcent action when taken internally [7] and is used in the treatment of respiratory diseases, especially in children [8]. This mucilage is also applied externally to minor burns [9]. Nowadays quince fruit is known as a good and low cost dietary source of health promoting compounds, due to it's antioxidant [10,11], antimicrobial [11,12] and anti ulcerative [13] properties.

The healing effects of quince seed mucilage on skin wounds in animal models and human who suffering from untreated wounds was evaluated in 2000 and 2006 respectively [5,14], and the results indicated that quince seed mucilage healed the wounds more rapidly compare to phenytoin cream (1%) as a standard agent. The results from another study showed that quince seed mucilage has more and better healing effects on dermal toxicity causing by T2-toxin comparing to control groups [15], but a detailed study dose not appear to have been undertaken. In the present study detailed evaluation of the wound healing activity of quince seed mucilage carried out using an *in vivo* model with more precise methods for evaluation.

2. Materials and methods

2.1. Plant materials

Quince fruits (*Cydonia oblonga* Miller) were collected during late summer from a garden in the city of Isfahan, Iran. The plant was taxonomically identified at the Botany Department of Shahid Chamran University, Ahvaz, Iran.

2.2. Preparation of quince seed mucilage

Seeds were separated from fresh pulps. The prepared seeds were dried in shed at temperature of 25–30 °C. 200 g of quince seeds were added in 2500 ml of distilled water then heated at 50–60 °C and mixed for 30 min. After 30 min the beaker containing quince seed mucilage was left to reach near 40 °C. Afterwards it was filtered through a clean cotton cloth and mucilage was separated. The mucilage was heated in an oven at 40 °C. 40 g of dried powder was obtained from the 200 g mucilage. For use in experiment, the dried powder of mucilage was mixed with eucerin for preparing the 5%, 10% and 20% creams.

2.3. Animals

Healthy Iranian male rabbits(*Oryctolagus cuniculus*) weighting 1800–2200 g were used for the study. They were individually housed in stainless steel cages at a 12-h cycle of light and dark, room temperature was kept at 24 ± 2 °C and humidity maintained at 50%. Standard food, vegetables and water were provided *ad libitum*. Animal procedures were in accordance to the guidelines for animal care prepared by the Animal Ethical Committee of Ahvaz Jondishapur University of Medical Sciences (approval code no. 05/2013/CPCSEA/AJUMS).

2.4. Wound procedure and experimental design

The dorsal hair of the rabbits were shaved and sterilized with 70% ethanol, and then marked area was locally anaesthetized using lidocaine 2%. Full thickness excision wound of 20 × 20 mm was created along the markings using scalp blade and forceps [16].

Animals were randomly divided into five groups ($n = 6$).

Group 1 served as negative control and did not receive any treatment.

Group 2 served as vehicle control group and treated with eucerin base.

Group 3 served as test group and treated with cream containing QSM 5%.

Group 4 served as test group and treated with cream containing QSM 10%.

Group 5 served as test group and treated with cream containing QSM 20%.

QSM, quince seed mucilage.

2.5. Wound contraction

The surface area of healing wound was measured by tracing the boundary of still open wound on transparent paper and calculation of area was done by using a scale graph paper. The percent wound contraction was calculated using the following formula:

$$\frac{\text{Initial wound size} - \text{specific day wound size}}{\text{Initial wound size}} \times 100$$

2.6. Hydroxyproline assay

Wound tissues were analysed for hydroxyproline content, which is a basic constituent of collagen. After complete healing, tissue samples taken from animals were dried in a hot air oven at 60–70 °C to constant weight and were hydrolysed in 6 N HCl at 130 °C for 4 h in sealed tubes. The hydrolysate was neutralized to pH7.0 and was subjected to chloramine-T oxidation for 20 min. The reaction was terminated by addition of 0.4 M perchloric acid and colour was developed with the help of Ehrlich reagent at 60 °C [17] and measured at 557 nm using UV/Vis spectrophotometer. Hydroxyproline concentrations of the unknown samples were calculated from a linear standard curve and presented as μg/g dry tissue weight.

2.7. Tensile strength

The tensile strength of a wound represents the degree of tissue integrity. The tensile strength increment indicates better wound healing stimulation by the applied drug. At the end of healing period, a strip of repaired tissue measuring 20 × 5 mm was isolated and the tensile strength was measured with tensiometer. Tensile strength was calculated using the following formula [18]:

$$\text{Tensile strength} = \frac{\text{Breaking strength(g)}}{\text{Cross-sectional area of skin } (\text{mm}^2)}$$

2.8. Blood sampling

Blood samples were collected using rabbit marginal vein method. The dorsal surface of the ear was shaved, and then blood was taken from the tip of the ear, away from the base of the ear using a 20-gauge needle and 1 ml syringe. Serial blood samples were taken by moving towards the base of the ear on the same vein and by alternating ears. The technique was carried out aseptically. After centrifugation for 15 min at 1000 g, serum samples were removed, aliquoted and finally stored in −80 °C [19]. The blood samples were collected on day 2, 7 and 14 after wounding.

2.9. Wound fluid sample collection and preparation

Wound fluid collection was performed using a standard protocol, as described previously [20]. In brief, the wound area was covered by tegaderm, a clear adhesive dressing (3 M, St.paul, Minn), any fluid accumulated under the dressing after 2 h was aspirated using a hypodermic needle and syringe. The samples were centrifuged within 30 min at 13,000 g for 10 min to separated particular matter, then aliquoted and finally stored at −80 °C until use. The wound fluid samples were collected on day 2 and 7 after wounding as representative days for inflammatory and proliferation phases, respectively. On day 7 after wounding an incision was made by a single cut with scissors, then the wound area was covered by tegaderm and the wound fluid was collected.

2.10. Growth factors assay

The serum and wound fluid levels of EGF, TGF-β_1, VEGF and PDGF were determined by enzyme-linked immunosorbent assay (ELISA) kits (Bioassay technology laboratory, China) in accordance to the manufacturer's instructions. According to the manufacturer's descriptions the sensitivity of ELISA kits is 5.12 ng/L, 2.56 pg/ml, 5.21 ng/L and 0.25 ng/ml for EGF, TGF-β_1, VEGF and PDGF respectively. The assay was carried out in three independent experiments.

2.11. Statistical analysis

Data were expressed as mean ± SE and statistical significance between experimental and control values were analysed by one-way analysis of variance (ANOVA) and followed by Dunnett's test to identify differences between groups. Values of $P < 0.05$ were considered as statistically significant.

3. Results

3.1. Wound contraction

Skin of different groups were inspected on daily basis and the percentage of wound contraction was recorded. Period of healing in no treatment and eucerin treatment groups was equal to 20 days. Trend of healing in these groups was very similar without any significant differences. Wound closure in animals treated with 5, 10 and 20% w/w creams of QSM was completed within 17, 15 and 13 days respectively. 20% w/w cream of QSM produced the best healing rate. In most days of treatment there were significant differences ($P < 0.05$) between QSM (10 and 20%) and no treatment or eucerin group. QSM 20% cream healed the wounds in a shorter period (13days) as compared to the QSM 10% cream (15days), but the percentage of wound contraction in these two groups was not significantly different (Fig. 1).

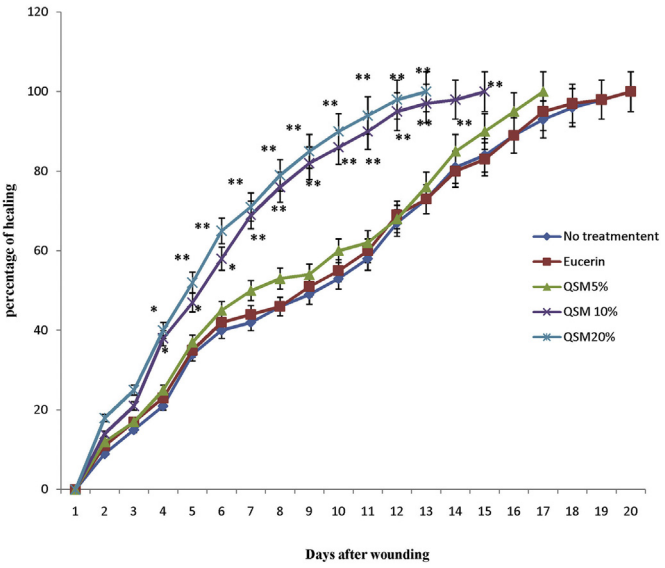

Fig. 1. Comparison of the wound healing in experimental groups. Data are expressed as mean ± SE. Values significantly different from eucerin-treated or no-treatment are indicated as $^{*}P < 0.05$, $^{**}P < 0.01$.

3.2. Tensile strength

The wounds treated with QSM 10% and 20% creams showed significant increase in tensile strength ($P < 0.05$) as compare to other groups (Table 1).

3.3. Hydroxyproline content

The hydroxylproline content were significantly increased in QSM (10 and 20%) treated groups as compared to no treatment or eucerin treated groups (Table 2).

3.4. Changes in the serum levels of growth factors

No significant difference was found among serum levels of growth factors (EGF, TGF-β_1, VEGF and PDGF) in experimental groups in different days of the interval.

3.5. Changes in the wound fluid levels of growth factors

In QSM (10 and 20%) treated groups the wound fluid levels of EGF, TGFβ_1, VEGF and PDGF were significantly higher compared to those in no treatment or eucerin group at 2nd and 7th day after wounding (Fig. 2A–D).

Table 1
Comparison of tensile strength of wound tissue samples after treatment period.

Groups	Tensile strength (g/cm$^{2)}$
No-treatment	985 ± 33
Eucerin	988 ± 32
QSM5%	1070 ± 36
QSM10%	1211 ± 45*
QSM20%	1223 ± 41*

QSM, quince seed mucilage.
*$P < 0.001$ (significant differences from no-treatment or eucerin treated groups).

Table 2
Comparison of hydroxyproline content of wound tissue samples after the treatment period.

Groups	Hydroxyproline content (μg/g tissue)
Non-treatment	765 ± 21
Eucerin	786.8 ± 32
QSM5%	790 ± 26
QSM10%	980.6 ± 44*
QSM20%	1087 ± 31*

QSM, Quince seed mucilage.
*$P < 0.001$ (significant differences from no-treatment or eucerin treated groups).

4. Discussion

Wounds and particularly chronic wounds are the major concerns for the patient and clinician alike. Research on wound healing agents is one of the developing areas in modern biomedical sciences. Plant-based medicines enjoy a respectable position today, especially in the developing countries, where modern health services are limited. QSM has been used as a traditional medicine for burns and skin wounds. Previous studies have shown that this natural product has a strong potential for treatment of skin wounds [5,14], but the mechanism by which this agent affects cells involved in wound healing process is unknown. In the present study topical application of QSM in normal animals enhanced the rate of wound healing as assessed by increase in collagen synthesis and tensile strength of the wound tissues. QSM also increased the wound fluid levels of four growth factors (EGF, TGF-β_1, VEGF and PDGF) that play a crucial role in the wound healing process. Growth factors are agents that promote cell proliferation and induce the migration of cells [21]. As wound healing is a well orchestrated and complex series of events involving cell–cell and cell–matrix interactions, it has become clear that growth factors serve as messengers that regulate the various process involved [22]. Because of important role of these growth factors in all phases of wound healing, QSM may be effective in all phases of healing process by increasing the concentrations of some growth factors at the wound site. Quince seed contains phenolic compounds (including: Caffeoylquinic, 4-O-caffeoylquinic, 5-O-caffeoylquinic, 3,5-dicaffeoylquinic acids, lucenin-2, vicenin-2. …), organic acids (including: Citric, Ascorbic, Malc, Quinic, Shikimic and Fumaric acids) and amino acids (including: Glutamic and Aspartic acids and Asparagine.) [23]. This phytochemical profile of quince seed may explain the wound healing activity of this product. Ascorbic acid and 5-O-caffeoylquinic acid are potent antioxidants. The antioxidant activity of quince seed has been investigated and reported by Magalhaes et al.

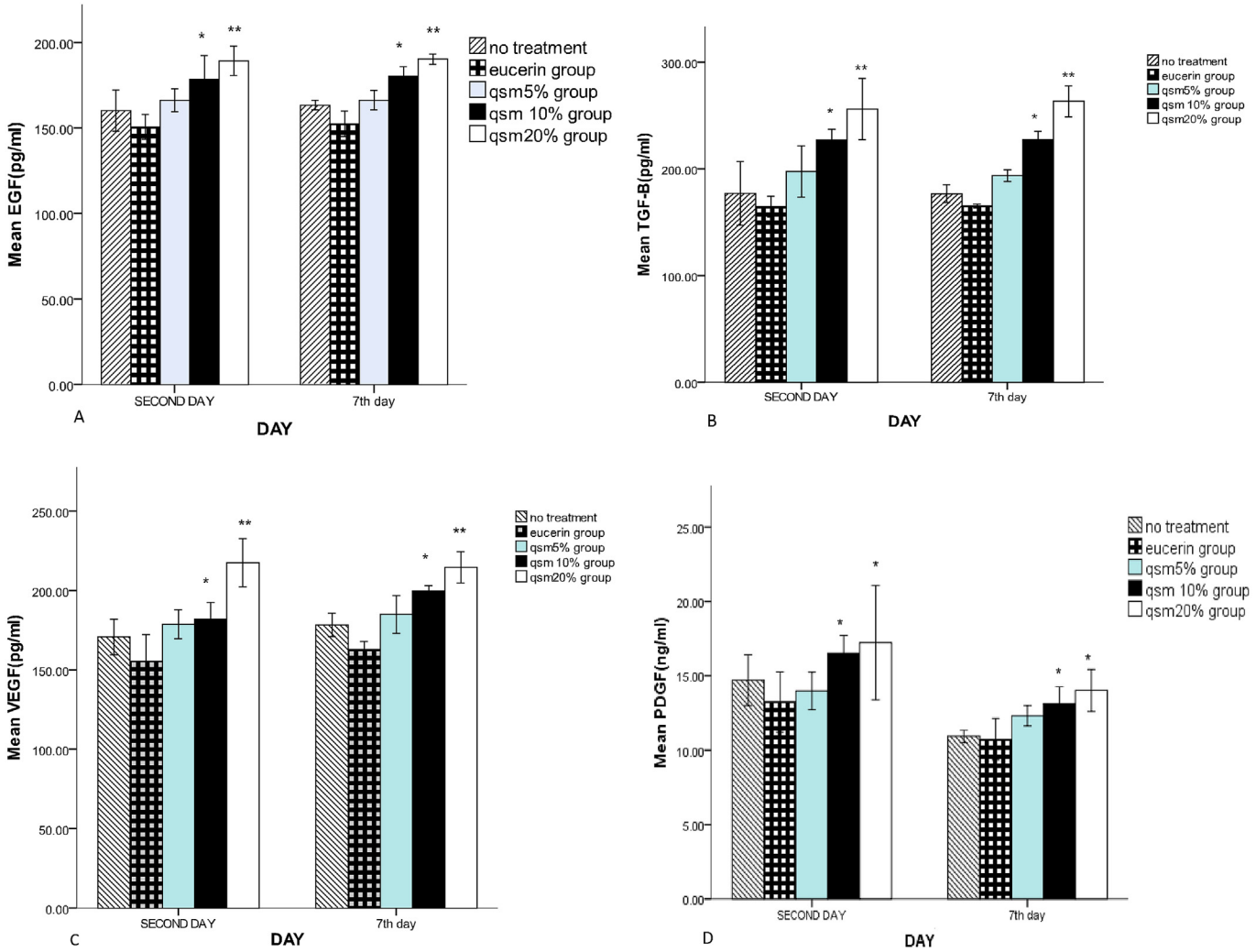

Fig. 2. Comparison of wound fluid levels of EGF (A), TGF-β_1 (B), VEGF(C) and PDGF (D) in experimental groups. Data are expressed as mean ± SE. Values significantly different from eucerin-treated or no-treatment are indicated as *$P < 0.05$, **$P < 0.01$.

and Joucki et al. [24,25]. Molecular oxygen plays a central role in the pathogenesis and therapy of chronic wounds. Wound sites are rich in free radicals [26]. The presence of these radicals will result in oxidative stress leading to cytotoxicity and delayed wound healing. Therefore, elimination of reactive oxygen species (ROS) could be an important strategy in healing of chronic wounds [27]. Lipid peroxidation is considered responsible for the impairment of endothelial cells, keratinocyte capillary permeability, fibroblast and collagen metabolism. Therefore, inhibition of lipid peroxidation by QSM may result in enhancing the synthesis of collagen and fibroblasts. Fibroblasts promote neo-angiogenesis, secrete all the components of the extra cellular matrix (glycosaminoglycan, proteoglycans, glycoproteins and collagen) and produce several cytokines and growth factors [28].

Mucilage is a long chain mucopolysccharide and can work as a reservoir because of high ability in water absorption, and when it is applied to a wound, the wound remains moist.

As we know in a moist environment, exudate provides the cells involved in wound repair with nutrients, controls infections, and provides the best environment for healing [29].

Finally QSM potentially altered the amount of growth factors involved in wound healing process positively. Such interesting finding could help us to consider QSM as an ideal wound promoter agent.

5. Conclusion

In conclusion, it can be interpreted that topical application of QSM exhibited significant wound healing activity in full thickness wound model.

Ethical approval

The experimental procedure has been approved by the Animal Ethical Committee of Ahvaz Jundishapur University of Medical Sciences (approval code no. 05/2013/CPCSEA/AJUMS).

Funding

This research has been financially supported by Physiology Research Center, Ahvaz Jundishpur University of Medical Sciences, (Grant No: PRC123), Ahvaz, Iran.

Author contribution

Aliasghar Hemmati: Study design and supervision of research.

Mehri Ghafourian Boroujerdnia: Manuscript editing and proofreading.

Pari Tamri: Study design, data collection, data analysis, writing of manuscript.

Conflicts of interest

Authors declare that there is no conflict of interest.

Acknowledgement

We would like to express our special thanks to the Physiology Research Center of Ahvaz jundishapur University of Medical Sciences for financial support. This study is from PhD Thesis of Dr Pari Tamri (Grant no. PRC 123).

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