

Original Article

THE EFFECT OF TRAGACANTH MUCILAGE ON THE HEALING OF FULL-THICKNESS WOUND IN RABBIT

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Background: Wound repair is a natural reaction to injury, which results in restoration of tissue integrity. Wound healing occurs in 3 phases including inflammation, proliferation, and remodeling. There are common pathways in wound healing of human and certain animal species.

Objective: To examine the healing effect of tragacanth mucilage on excisional full-thickness wound in rabbit.

Methods: Mucilage was extracted from the dried tragacanth gum, using a mixture of water and glycerin (4:1) as the vehicle. Three percent, 6%, and 9% creams of tragacanth mucilage were prepared in the eucerin base. A full-thickness wound was made on the left flank of rabbits. Tragacanth creams were applied on the wound area twice daily. Control groups were treated with the drug-free cream base or commercial phenytoin cream 1%. Healing was determined quantitatively as the reduction in wound area. Histological study was performed using hematoxylin and eosin stain under light microscope.

Results: The wound healing profile of tragacanth-treated groups was significantly better than the nontreated groups. The best healing effect was observed with the 6% tragacanth cream, which exerted the lowest period for healing. This effect was significantly different from the control phenytoin or drug-free eucerin bases. The extent of tissue repairment was confirmed by histological examination.

Conclusion: Tragacanth mucilage exhibited a considerable potency for wound healing. This is probably due to an acceleration of collagenation and proliferation phases of the wound repair.

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Keywords: Mucilage • phenytoin • tragacanth • wound healing

Introduction

Wound healing is a complex process, which is commonly divided into three phases including the invasion of inflammatory cells, proliferation of tissue-repairing cells, and tissue remodeling.¹ The rate of wound healing depends upon many factors including the size of the wound, blood supply to the area, presence of foreign bodies, micro-organisms, age, health of the patient, nutritional status of the patient, use of drugs, and a variety of

systemic diseases.²

The effect of herbal remedies on wound healing has not still been completely investigated. Hemmati and Mohammadian³ studied the effect of quince seed mucilage on wound healing. In their study, a 10% quince seed mucilage caused complete healing of rabbit wound after 13 days of treatment. Such results encouraged us to examine the healing effect of tragacanth, which has been used in traditional medicine and also in modern therapeutic pharmaceuticals as a demulcent.⁴

Tragacanth is the dried gummy exudation flowing naturally or obtained by exudation from the trunk and branches of *Astragalus gummifer* and some other species of *Astragalus* (Leguminosae), found in western Asia. The powder forms a mucilaginous gel, with about ten times its weight of water.⁵ More than 500 species of *Astragalus*

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spread on different regions of Iran, e.g., Isfahan, Kurdistan, Kerman, Yazd, etc. Some of these species are the best types of tragacanth mostly exported to the United States of America, Europe, and other countries.⁶ The primary source of gum tragacanth, is the desert highlands of northern and western Iran, particularly the Zagros mountains region.⁷ In the Iranian traditional medicine, tragacanth is used as a demulcent for treating sore throat and hair loss due to seborrhea.⁶ It is widely used as a suspending agent in pharmaceuticals, cosmetics, and food products.⁴ In modern medicine, antiviral and antibacterial effects have been claimed for tragacanth.⁸ It contains 60% – 70% bassorin and 30% – 40% of a soluble gum, tragacanthin.

Tragacanthin yields glucuronic acid and arabinose, when hydrolyzed.⁹ So far, the effect of tragacanth on wound repair has not been studied extensively. This study was conducted to determine the efficacy of tragacanth topical formulations on wound healing.

Materials and Methods

Plant material condition and tests

Dried ribbon-like tragacanth, taken originally from Kerman, was purchased from the local market. It was stored in a dry, air-tight, and light-resistant container. A quality control test was performed on the tragacanth gum, based on USP 23.¹⁰

Preparation and formulation of the mucilage

Eighteen grams glycerin (Merck, Germany) was mixed with 75 mL deionized water and heated to boiling point. Six grams tragacanth and 0.2 g benzoic acid (BDH, England) were added to the mixture and macerated for 24 hr, while being stirred occasionally. Then, deionized water was added to make up a final weight of 100 g. The mucilage was stirred actively and strained forcibly through a muslin. Creams containing 3%, 6%, and 9% (w/w) of the mucilage in eucerin were prepared.

Test animals

Male Iranian rabbits, weighing between 1.5 – 2.2 kg were used in this study. Before and during the experiments, the test animals were housed individually in aluminum cages (60 × 45 × 45 cm) and allowed to feed on a standard commercial pellet diet supplemented with fresh vegetable and

water *ad libitum*. The test animals were kept in a room illuminated with a mixture of fluorescent and day light from 7:00 AM to 8:00 PM at a temperature of $22 \pm 2^\circ\text{C}$ and a humidity of 50% to 55%.

Wound procedure

A full-thickness wound was made on the skin of the test animals, according to the model proposed by Cross et al,¹¹ and modified by Hemmati and Mohammadian.³ Hairs of the lower back and left flank of the test animals were fully shaved and sterilized with 70% ethanol. The desired area was locally anesthetized with a subcutaneous injection of 2% lidocaine. A metal template measuring $20 \times 20 \text{ mm}^2$ was placed on the stretched skin and an outline of the template was traced on the skin, using a fine-tipped pen. The wound was made by excising the skin, within the border of the template to the level of loose subcutaneous tissue (fascia), using a size 15 scalpel blade and forceps.

Animals were randomly divided into six groups ($n = 5 - 6/\text{group}$). The first group was left untreated. The other groups were treated with phenytoin, eucerin, or different concentrations of tragacanth mucilage. Animal wounds were treated topically with either eucerin cream containing 1% phenytoin (Darupakhsh Co., Iran) as a standard healing agent, or eucerin cream containing 0%, 3%, 6%, or 9% tragacanth mucilage. Wounds were treated twice daily. Based on the Ahwaz University Ethical Protocols on animal experiments, all the ethical issues were considered throughout the experiment. Experimental works and formulations were carried out under sterile conditions.

In order to quantify the rate of wound healing, every 24 hr each test animal was held in the standard crouching position and the wound margin was traced on a transparent plastic sheet using a fine-tipped pen. The area of the wounds on the first day was considered as 100% and the wound areas on subsequent days were compared with the wound area on the initial day.

Histological study

In order to observe the repairing process on the 7th day and the last day of the treatment period (the day in which the wound came to complete closure and evaluated as 100% healing, macroscopically), skin tissue samples were taken for histological studies. In order to prepare the tissue sample, a small excision containing part of the wound area

Table 1. The results of quality specifications of Iranian tragacanth gum.

No.	Test	Results (Mean \pm SEM)	Standard values
1	Arsenic	2.2 \pm 0.1 ppm	\leq 3 ppm
2	Heavy metals	0.003 \pm 0.0005%	\leq 0.004%
3	Lead	0.0008 \pm 0.0001%	\leq 0.001%
4	Karaya gum	Negative	Absent
5	<i>Salmonella</i> and <i>Escherichia coli</i>	Negative	Absent

and normal adjacent skin was made. Tissues were fixed in 10% formalin. Paraffin-embedded sections (5- μ m thick) were prepared and stained with hematoxylin and eosin. Light microscopy was used to evaluate the pathological changes, e.g., granulation tissue formation and reepithelization in wounds and their comparison with the normal tissue part.¹²

Statistical analysis

One-way analysis of variance (ANOVA) was used to compare group means. The results were expressed as mean \pm SEM. The differences were considered significant, when $P < 0.05$.

Results

The chemical specifications of tragacanth gum are shown in Table 1. Period of healing in non-treated and eucerin-treated groups were equal over 21 days. Healing in the group treated with eucerin containing 1% phenytoin was completed within 17 days (Figure 1). Healing in animals treated with eucerin containing 3%, 6%, and 9% tragacanth mucilage occurred after 17, 15, and 18 days,

respectively (Figures 2 – 4). The 6% tragacanth mucilage cream produced the best healing rate, which was better than phenytoin. A significant difference ($P < 0.05$) between the eucerin group and the 6% tragacanth mucilage cream was observed from the eighth treatment day onwards. Fifteen days after treatment with the 6% tragacanth mucilage cream, wound healing evaluated as 100%. The wound healing effect of the 6% tragacanth mucilage cream was more pronounced ($P < 0.05$) than those treated with phenytoin cream (Figures 5 and 6).

Results of the histological studies indicated an improvement in wound healing by the passage of time. This improvement was quicker with the tragacanth mucilage. On the 7th day of treatment with tragacanth mucilage, in most samples reconstruction of the epidermis was initiated (Figure 7), whereas in the nontreated or eucerin groups, foci of necrosis were still observable (Figure 8). However, in most groups, the presence of inflammatory cells in the dermis were evident. On the day when the wound closure was completed macroscopically, the histological studies of wound section confirmed the formation of epidermis, and

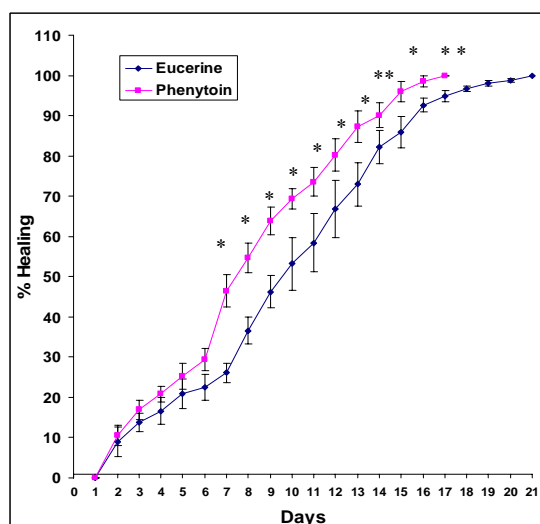


Figure 1. Comparison of wound healing in phenytoin- and eucerin-treated groups. Values significantly different from eucerin treatment are indicated as * ($P < 0.05$) or ** ($P < 0.01$).

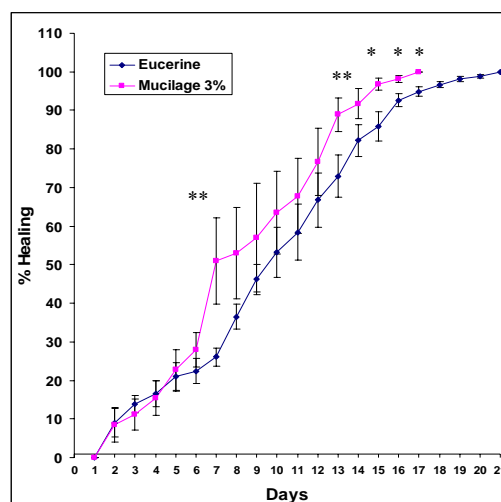


Figure 2. Comparison of wound healing in tragacanth mucilage (3%)- and eucerin-treated groups. Values significantly different from eucerin treatment are indicated as * ($P < 0.05$) or ** ($P < 0.01$).

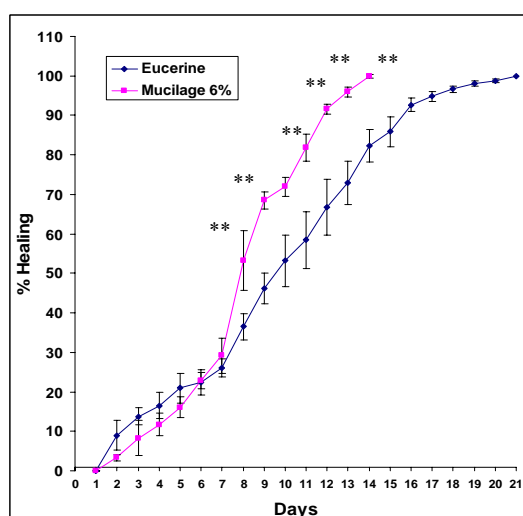


Figure 3. Comparison of wound healing in tragacanth mucilage (6%)- and eucerin-treated groups. Values significantly different from eucerin treatment are indicated as $^*(P < 0.05)$ or $^{**}(P < 0.01)$.

less inflammatory cells infiltrated the dermis. With the 6% mucilage the epidermis was close to, a normal structure (Figure 9).

Discussion

Tragacanth gum has an ancient history and is widely used in Chinese and Iranian folk medicine.⁵ - 7 There are a number of published medicinal

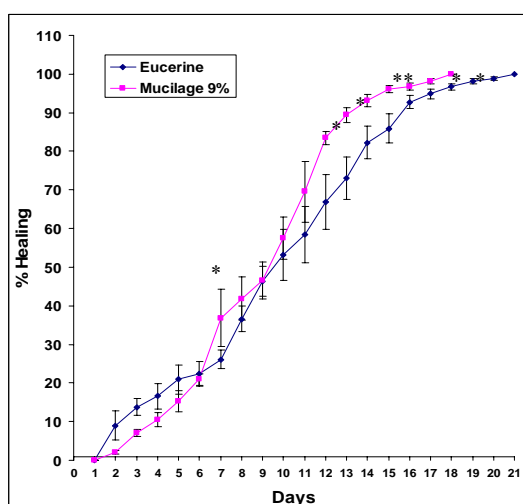


Figure 4. Comparison of wound healing in tragacanth mucilage (9%)- and eucerin-treated groups. Values significantly different from eucerin treatment are indicated as $^*(P < 0.05)$ or $^{**}(P < 0.01)$.

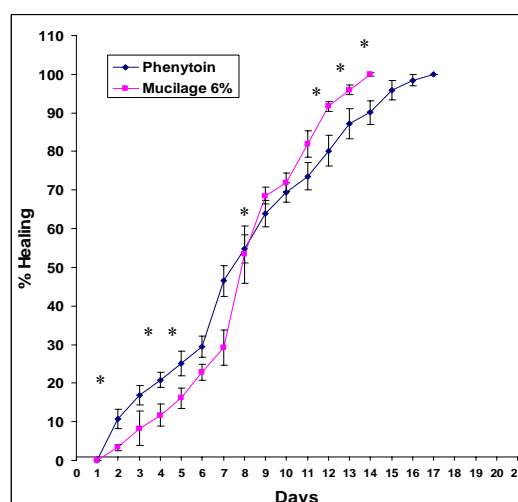


Figure 5. Comparison of wound healing in tragacanth mucilage (6%) and phenytoin-treated groups. Values significantly different from phenytoin treatment are indicated as $^*(P < 0.05)$ or $^{**}(P < 0.01)$.

applications for tragacanth, either alone or in combination with other herbs for the treatment of diabetes mellitus, cancers, and constipation.^{13 - 14} Complex glucoarabinan polysaccharides isolated from a related Asian species (*A. mongholicus*) have been shown to stimulate the production of T-cells and antibody-producing plasma cells.^{15 - 16} Although pharmacological effects of tragacanth have been investigated in different diseases, its effect on wound healing has not been studied adequately.

In the present study, excisional wound model for contraction and epithelization was employed. Our results indicated that tragacanth used in this

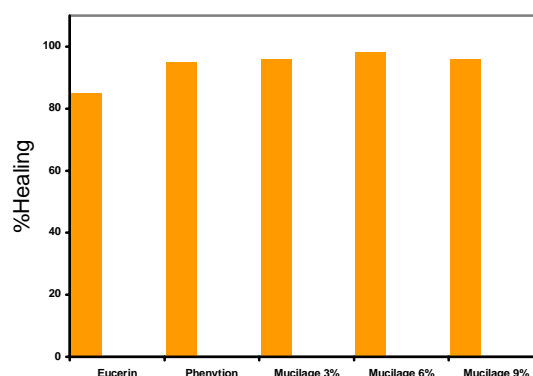


Figure 6. Comparison of wound healing in phenytoin- and eucerin-treated groups. Values significantly different from eucerin treatment are indicated as $^*(P < 0.05)$ or $^{**}(P < 0.01)$.

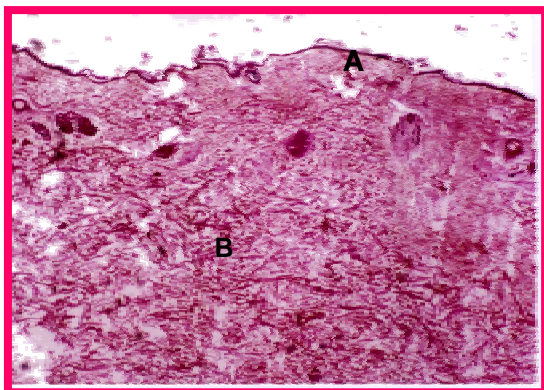


Figure 7. Photomicrograph of skin wound section of rabbit on the 7th day of the treatment with tragacanth mucilage (6%). A) Recently-formed epidermis. B) Dermis with slight inflammation (H & E x100).

study met the requirements of USP 23 regulations for quality control (Table 1).¹⁰ This preparation is able to accelerate the rate of wound healing, presumably due to a faster contraction of wounds as compared with the control or untreated wounds. Tragacanth mucilage was found to be more potent than phenytoin, which is commercially available for clinical uses.¹⁷ The course of healing with the 6% tragacanth mucilage was 15 days, which is two days shorter than those treated with phenytoin. This is considered as a great advantage for this mucilage. The extent of healing with tragacanth mucilage was significantly higher than eucerin from the eighth treatment day onwards.

With respect to the wound healing mechanism

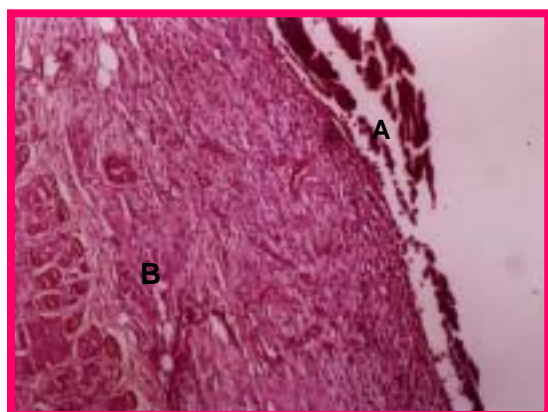


Figure 8. Photomicrograph of skin wound section of rabbit on the 7th day of treatment with eucerin. A) Necrosis. B) Dermis infiltrated with inflammatory cells (H & E x100).

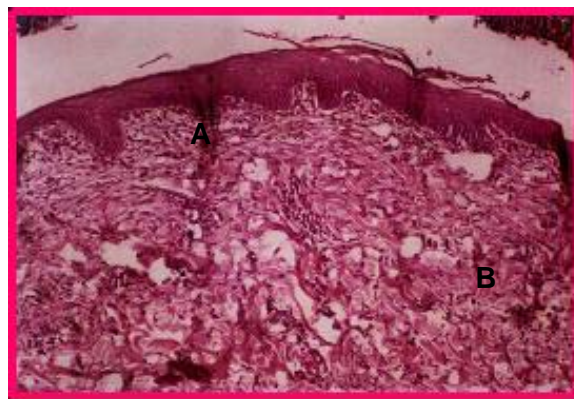


Figure 9. Photomicrograph of skin wound section of rabbit on the 15th day of the treatment with tragacanth mucilage (6%). A) Epidermis. B) Dermis with normal structure (H & E x100).

of tragacanth mucilage, the exact mechanism could be proposed. However, the significant differences observed with the tragacanth mucilage-treated groups suggest that it could be effective on the proliferation and remodeling phases of wound healing. It is probably capable of stimulating the myofibroblasts contraction, for a faster closure of the wound.³ Active ingredients of tragacanth mucilage, such as bassorin and tragacanthin,⁴ may contribute to the healing effects of tragacanth mucilage. Hydrolysis of tragacanthin into arabinose and glucuronic acid⁹ may cause coagulation of surface proteins, and prevent wound infection that causes a faster wound healing.¹

However, further studies are needed to elucidate the exact mechanism of tragacanth mucilage in wound healing.

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