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Original Article

Effect of aqueous extract of fig fruit (*Ficus Carica*) on wound healing in albino rabbits

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Abstract

Background and aims: Skin ulcers are caused by various reasons such as physical, chemical, and biological damages. Wound healing has long been considered one of the most important issues in surgery. Extensive research has been employed in this field and various chemical, herbal, homeopathic, and physical methods such as laser therapy have been used for treatment. Nowadays, wound healing is one of the most important aims of medical science. Furthermore, people prefer to use natural drugs because of fewer side effects. Therefore, the present study was designed considering these issues.

Methods: For this study, 6 albino rabbits with an average weight of 3 kg were purchased. After preparing the rabbits, two pairs of full-thickness wounds were created in a completely round shape with a diameter of almost 6.30 mm on both sides of the spine in two anterior and posterior parts at a distance of 3 cm from the midline of the body. The animals were divided into 2 groups of 3 rabbits, including 1 experimental group and 1 control group. The wounds of the experimental group were treated with fig fruit extract while the wounds of the control group received no treatment. The wounds were macroscopically examined at 4, 7, 12, 16, and 20 days after surgery.

Results: The results of *t*-test showed a significant difference ($P<0.05$) among the groups on all days. Meanwhile, the average wound area significantly decreased in the group which was treated with the extract of fig fruit.

Conclusion: This study showed that fig fruit extract contains phenolic compounds with antioxidant, antimicrobial, and anti-inflammatory properties which can accelerate the wound healing process.

Keywords: Fig fruit extract, Skin wound, Albino rabbit, Surgery

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Introduction

A wound is a ruptured tissue that has lost its connectivity due to various factors. Several reasons can cause skin ulcers, including physical, chemical, and biological damages (1). Wound healing is an essential process to repair the structure and function of tissues that have been injured by physical, chemical, bacterial, or viral damage. It is also a complex process that causes restoration of structural and functional integrity after injury (2). Wound healing involves coordinated repair responses of blood cells, extracellular matrix, and parenchymal cells that occur after surgery or traumatic injury to the body (3). Wound healing has long been considered one of the most important issues in surgery. Extensive research has been employed in this field and various chemical, herbal, homeopathic, and physical methods such as laser therapy have been used for treatment. The goal of these methods is fast treatment, low complication rate as well as low cost (4).

Today, standard topical antibiotics such as silver nitrate, mafenide acetate, and silver sulfadiazine are commonly used for treating the wounds. The use of mafenide acetate can soothe pain or burning at the site of medication while frequent consumption of silver nitrate due to the presence of silver leads to permanent skin discoloration. Currently, the use of sulfadiazine cream is one of the most common methods of healing burn wounds; however, it has several side effects, including non-penetration into the wound, resistance to Gram-negative microorganisms, and delay in the healing of small burn wounds. Reducing regimentation and epithelialization is one of the goals of medical wound healing in a shorter time, as well as reducing side effects (5).

Studies have shown that fig fruit as a laxative is useful in the treatment of various diseases such as hemorrhoids, gout, and epilepsy (6). Figs are also a good source of potassium and thus are effective in lowering blood pressure. Other benefits of figs include weight loss, prevention of breast cancer, improvement in bone density, prevention of muscle weakness, and improvement in nervous system function (7). Although figs are known as edible fruits, their leaves, roots, and sap are used as medicine, the medicinal value of which has been proven through

scientific research (8). The biological activities of fig juice are very diverse. In traditional medicine, fig juice is used to treat stomach ulcers and skin infections such as viral warts. Additionally, bioactive substances called 6-1-acetyl-beta-glucose-betacitosterol and lupeol acetate have been isolated from fig juice, which inhibit the proliferation of cancer cells (9). Fig tree sap, which is a type of secretion from the leaves and fruits of *Ficus carica*, has therapeutic effects due to its antioxidant, antihyperlipidemic (10), anticancer (11), and antimicrobial activities (12). It has also been investigated by researchers due to the presence of proteolytic enzymes, amino acids, minerals, sugars, terpenes, and organic acids. Figs are free of fat, sodium, and cholesterol. Among fruits, it has the highest amount of minerals and especially contains a lot of calcium and fiber (7). Figs can increase the metabolic activity of the kidneys, which leads to an increase in the excretion of harmful substances in the urine in patients who consume figs. Uric acid combines with malic acid in figs to increase bile secretion, which is effective in the treatment of liver disease (13). Nowadays, wound healing is one of the most important aims of medical science. Furthermore, people prefer to use natural drugs because of fewer side effects. Therefore, the present study was designed considering these issues.

Materials and Methods

Preparation of fig fruit

Fresh fig fruit was collected from the gardens of Mehriz city. It was identified and approved by the relevant experts in the Medicinal Plants Research Centre.

Preparation of fig fruit extract

First, fig fruit was crushed with a mixer. Then, 100 g of the crushed fruit was added to water (1 L) and kept in a dark place for 72 hours. The resulting liquid was distilled in a rotary evaporator by double vacuum distillation. Afterwards, it was incubated at 38°C until finally a viscous aqueous extract (10%) was obtained.

Animal preparation

In this experiment, 6 Albino rabbits with an average weight of 3 kg were used. The animals were obtained from the Animal Care Centre of Medical School and kept in propylene cages. Animals were kept separately in special cages under the same condition in terms of temperature (20 ± 2°C) and light-dark cycle (12 hours). They were fed with lettuce and carrots, and the drinking water was provided ad libitum.

Making a wound

For surgery, the animals were anesthetized with xylazine at a dose of 5 mg/kg and ketamine at a dose of 35 mg/kg by intramuscular injection. After induction of anesthesia, the animals were put in the prone position and the hairs on the back on both sides of the thoracic and lumbar spines were completely shaved. A skin punch was used to create wounds of equal sizes (Figure 1 and Figure 2).

In the present study, two pairs of full-thickness wounds were created on the skin on both sides of the spine. Wounds on both sides (left and right) were created at a distance of 3 cm from the midline of the body and the distance between the wounds on both sides was 4 cm.

After scarring, the lesion areas were covered with sterile dressings to prevent infection, and the rabbits were examined until the end of full recovery. Then, each of them was kept and treated in a separate cage.

Animal grouping

Rabbits were divided into two groups of three animals. In the experimental group, fig ointment was applied to the wounds while the control group received no treatment. A

Figure 1. Pre-operative preparation.

Figure 2. Sampling and wounding steps.

sufficient amount of fig ointment (1 mm thickness) was applied daily to the wound.

Treatment duration

The wounds were treated for 21 days. The investigation was initiated on day 0. Tissue sampling was performed on histopathological sections on days 4, 9, and 21.

Macroscopic examination

Left anterior and posterior wounds were used for macroscopic examination. Graphs were analyzed at days 0, 4, 8, 12,16, and 20 by a digital camera with a calibrated ruler next to the wound. The images were then transferred to a computer, and the area of the wounds was calculated using TCapture software (Figure 3 and Figure 7).

Microscopic examination

Right anterior and posterior wounds were used for microscopic evaluation of wounds. On days 4, 9, and, 21, a part of the skin tissue with completely thick edges of the wound, including healthy and damaged skin, was removed by scalpel and after washing with physiological serum, it was fixed in a container containing 10% formalin buffer.

Then, 5-µm sections of these samples were prepared by microtome and stained with Fiber hematoxylin and eosin. In the prepared slides, the histopathological evaluation of the wound healing process was done based on evidence such as inflammation, granulation tissue formation, fibroblast cell maturation, collagen fiber arrangement, vascularization, epithelial tissue rearrangement, and wound healing rate.

Statistical analysis

In order to analyze the data obtained from macroscopic evaluation, the average wound area in the treatment and control groups was evaluated using student's *t* test in SPSS version 25.0. The significance level was considered to be $P < 0.05$.

Results

On day 4, the damaged epidermal tissue (ep) was observed in the control group. Furthermore, a large distance was observed between the epidermis and the dermis. No trace of granulation tissue was observed. Tissue hyperemia was evident. Collagen fibers were disordered and the inflammatory process was observed in this tissue. A slow healing process was observed. Granulation tissue was obvious. Inflammation was observed in the tissue. On day 4, the repair of the epidermis (ep) was not complete in the treatment group. The fibroblasts matured. The number of blood vessels (v) was small. Hair follicle formation was observed. On day 4, granulation tissue was observed. Inflammation was also observed in the tissue (Figure 3 and Figure 4).

On day of 9, the collagen fibers moved towards parallelism and the maturation of fibroblasts was observed in the control group. The repair of epidermal epithelial tissue was still not complete. On day 9, increased blood vessel formation, granulation tissue formation, and maturation of fibroblasts were detected in the treatment group (B). Collagen fibers had a more regular arrangement. Angiogenesis (V) and rearrangement of epithelial tissue were observed (Figure 5).

On day 21, granulation tissue was rarely seen in the control group (A). The collagen fibers were more regular and parallel. Limited evidence of angiogenesis (V) and rearrangement of the epithelial tissue could be observed. On day 21, angiogenesis was not observed in the treatment group (B). The epithelial tissue was completely repaired and limited traces of granulation tissue were observed (gt) (Figure 6).

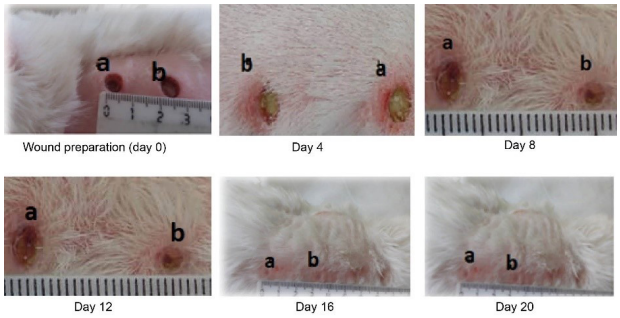

Figure 3. Results of Macroscopic Examination of the Control (a) and Treatment Groups (b).

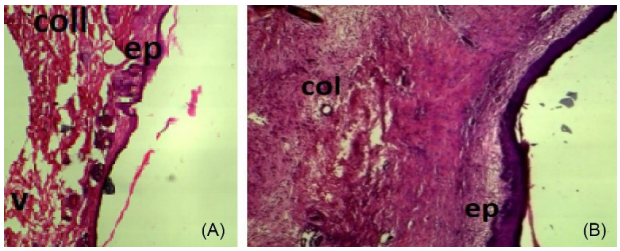

Figure 4. Results of microscopic examination of the control (A) and treatment (B) groups (H&E, × 50) on day 4.

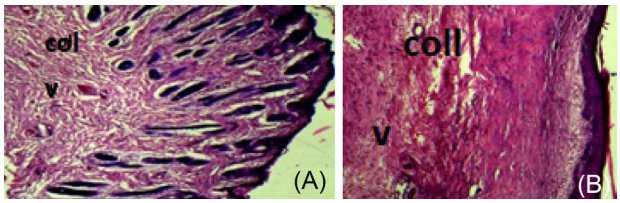

Figure 5. Results of Microscopic Examination of the Control (A) and Treatment (B) Group (H&E, × 50) on Day 9.

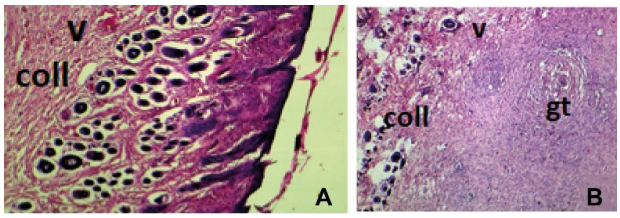

Figure 6. Results of microscopic examination of control (A) and treatment (B) group (H&E, × 50) on day 21.

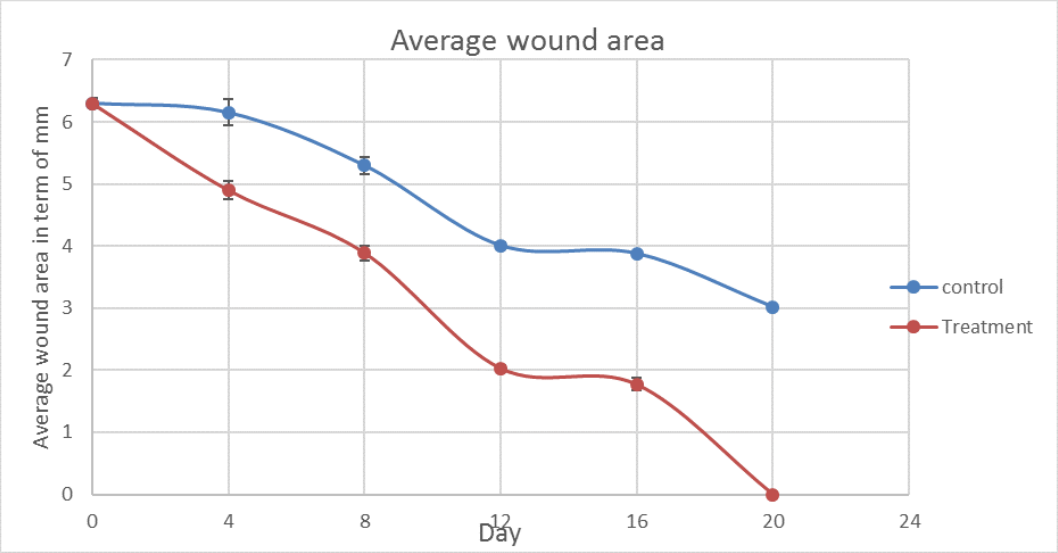

Figure 7. Comparison of the average wound area between the control and treatment groups during different days.

Statistical analysis

In the diagram, the average wound area was plotted with standard error in terms of days in the control and experimental groups. Then, the area under the curve was calculated.

Table 1 shows the statistical characteristics of the level variable below the wound area chart in terms of days at the experimental and control groups. In this table, the average level below the chart is 48.355 for the control group and 40.605 for the target group. It can be said that the wound area in terms of day in the target group is lower compared to the control group. To determine the significance and non-significance of the difference, *t* test with two independent samples was used (Figure 7 and Table 1).

In this diagram, the average wound area was plotted with standard error in terms of days in the control and experimental groups. Then, the area under the curve was calculated.

To determine the significance and non-significance of the difference between the average surface variable below the wound area diagram in comparison to day, *t* test with two independent samples was used.

According to the *t* test, it was observed that at the error level of less than 5%, the significance level of the Loon test for the variable of the wound area was less than 5%. ($P=0.023$). Therefore, the condition of inequality of variance was accepted. Due to the significant level of t-test for this variable at an error level of less than 5%, the mean wound area per day in the experiment and control groups were significantly different from each other, which was equal to 7.750. As a result, the mean difference between the two groups was significant, so it could be said that the wound area in the study period in the target group compared to the control group had a significant decrease (Table 1 and Figure 7).

Table 1. The Results of t-test

	Group	Number	Mean	SD
Wound Area Based on Day	Treatment	6	40.605	1.288
	Control	6	48.355	1.188

Discussion

In this study, the effect of aqueous extract of fig fruit on the skin wound healing process in albino rabbits was investigated. The results of macroscopic examinations (measurement of wound area) at days 0 (day of wound formation), 4, 8, 12, 16, and 20 in the control group and the group treated with fig fruit showed that the wound area in the whole period of time had a significant decrease in the experiment group compared to the control (less than 5%) and therefore this treatment was successful in this group. Additionally, in the microscopic examination performed in this study, the wound healing process during the 4, 9, and 21 days was evaluated histologically and it was shown that the speed and process of wound healing in the group treated with fig fruit extract was higher than in the control group. Wound healing is one of the most fundamental issues that human beings have faced since the beginning of creation. Numerous drugs and ointments are used to repair open wounds, each of which has several drawbacks, limitations, and side effects (3-5). Today, the importance of healing is due to the high prevalence of wounds in human and animal communities. Major common wounds include traumatic, burn, chemical, physical, and biological wounds. Research in the field of wound healing is very important in terms of accelerating the healing process of the wounds that heal slowly or are under pressure and movement. The use of plant extracts in the healing process of skin wounds has been common from ancient times in traditional medicine. The herbal medicine selected for wound healing based on its physical and chemical properties should be able to ideally improve one or more healing phases without causing side effects. Numerous studies have shown positive effects of herbal medicines on wound healing, some of which are mentioned. The microscopic results of the present study

showed that the number of blood vessels and granular tissue of the group treated with fig fruit extract was significantly different from the control, which is consistent with a study by Yaghmaei et al. Yaghmaei et al showed that the number of blood vessels in the group treated with green tea extract was significantly different from control group because green tea extract accelerated the healing process, which is consistent with the present findings (14). Additionally, the study by Rezvanipour et al on the effect of mummy on some indicators in wound healing of mice showed that the number of blood vessels in the treated group was significantly different from the control group and mummy could accelerate the healing process, which is consistent with the present study (15). A study by Azhdari-Zarmehri et al on the effect of hydroalcoholic extract of *Scrophularia striata* on burn wounds in rats showed that the granulation tissue and blood vessels of the treated group were significantly different from the control group and hydroalcoholic extract of this plant accelerated the healing process; their results are consistent with the present study (16). The obtained results of this study showed the quality and speed of wound healing and the amount of shrinkage of wound edges in the treatment group were higher compared to the control group. Avijgan et al investigated the effect of aloe vera gel on chronic ulcers and showed that aloe vera gel was much more effective and less expensive than conventional treatments, which is similar to the present study (17). Tramer et al in 2012, by studying medicinal plants, stated that figs are plants whose different parts are medically important and have been used extensively in traditional Iranian medicine. They indicated that fig ointment has an anti-inflammatory effect on tumors and can be used to prevent cancer due to its antibacterial and antifungal properties. Figs contain antioxidants that prevent damage to cells and tissues. All antioxidant properties along with antimicrobial properties are found in figs (18). A study conducted by Hashemi and Abediankenari on mice confirmed the anti-inflammatory effects of lavender. They reported that the use of lavender was effective in healing burns (19). Another study found that *Ficus carica* stem extract contained large amounts of polyphenols and carotenoids. On the other hand, the antioxidant activity of aqueous extract and polysaccharide extracted from figs was studied and researchers found that both aqueous extract and polysaccharide obtained from figs had high amounts of phenolic compounds and flavonoids with remarkable inhibitory properties (2, 2 diphenyl-1-picryl hydroxyl), which can be beneficial for human health (20). In another study, the levels of phenolic compounds including gallic acid, chlorogenic acid, epicatechin, and catechins as well as the total antioxidant capacity of fig juice were investigated, and it was found that fig juice can be useful in preventing damage induced by free radicals (21). Raj and Joseph stated that figs contain antioxidant compounds including flavonoids and polyphenols and some biologically active compounds such as arabinose, β-amyrin, β-carotene, glycosides, etc (22). The results of the phytochemical analysis of *Ferula assa-foetida* resin show that this plant contains compounds such as ascorbic acid, phenolic acid, oxalic acid, uric acid, maleic acid, coumarins, alanine, tannins, alpha linoleic acid, and glycoside. All these compounds have anti-inflammatory effects and can accelerate the wound healing process, which is consistent with the present study (23). Moreover, Laitiff et al in 2010 showed that bitter gourd increases the wound healing rate in diabetics due to the presence of photochemical substances such as polyphenols, flavonoids, and triterpenoids (24).

Conclusion

Wound healing is one of the most fundamental issues that mankind has faced since the beginning of creation. Numerous medications and ointments are used to heal open wounds, each of which has several drawbacks, limitations, and side effects. The use of herbal extracts in the healing process of skin wounds has been common in traditional medicine since ancient times. The herbal medicine selected for wound healing based on its physical and chemical properties should be able to ideally improve one or more healing phases without causing side effects. According to the present study, the aqueous extract of fig fruit can improve the wound healing process.

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Authors' Contribution

MM, ES, and ZMZ contributed to the conception and design of experimental work. APA contributed to surgical operations. All authors read and approved the final manuscript.

Conflict of Interests

No conflict of interest exists.

Ethical Approval

This study was approved by the Ethics Committee of Ardakan University (IR.YAZD.REC.1400.005).

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