

Evaluation of the Wound Healing Potency of *Myrtus communis* Aqueous Extract on Diabetic Rat

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ABSTRACT

Background: Diabetic patients have a main problem on treatment and management of diabetic wounds. Wound healing is accelerated by herbal material. Aim of the study was to investigate the wound healing potency of *Myrtus* extract in diabetic rat.

Methods and Materials: In this experimental study, 20 Wistar male rats were randomly divided into 2 groups as the control and the treatment by *M. communis* extract. Two days after streptozotocin injection all rats were tested for blood sugar and were kept for 4 weeks. A full-thickness circular excision was inflicted approximately 1 inch of diameter of the predetermined area on the dorsal side of each rat using a sterile surgical blade. Treatment group treated 3 times a day for 13 days with aqueous extract of *M. communis*. The control group was left untreated. The extract concentration was 200 ppm per litre and was made by a simple distillation system. The wound area was calculated on days 3,7,11,13. Finally, assessment of the cutaneous wound healing between the groups was carried out by histopathologic data and statistical tests.

Results: Fasting blood sugar in diabetic rats was 300 mg/dl \pm 30 after 48 hours and remained for four weeks. In control group, infection, secretion and inflammation were proved in wounds from 2nd day after inoculation, but without such evidences in test group. Histopathological evaluation indicated re-epithelialization, healing and reduced the inflammation on day 13 in comparison to control ($P < 0.001$). Area changes and wound healing in the test group treated with the extract was significantly better than control group ($P < 0.001$).

Conclusions: Our results proved that *Myrtus Communis* aqueous extract promoted wound healing and was controlled infection and proposes as a therapeutic material for wound treatment in clinical trial investigations.

Key words: *Myrtus communis* extract, Wound healing, Diabetic rat

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INTRODUCTION

Wound healing is an active process consists of a series of continuous related processes in involving cells and extracellular matrices [1].

This process is including coagulation, inflammation, proliferation and resuscitation skin lesions. Healing time is one of the most important aspects. The process of repairing and wound healing depends on factors such as production, storage, and how to connect collagen, connective tissues function, the amount of collagenase secretion, and other factors [2,3].

Diabetes, can delay the healing process of the wound because disturbances in local blood flow and oxygenation, along with age, obesity, malnutrition, infection, the use of certain drugs. Diabetic foot ulcer causes hospitalization of diabetic patients for several days per year. Diabetes also generally reduces immune defences. This causes widespread infection and delayed healing [3,4].

Medicinal plants are most important treatments for diabetic wounds because of their healing properties. *Myrtus* leaf has been used as an antiseptic and anti-inflammatory herbal medicine by reduction of hemorrhage, edema and also stimulates the formation of collagen and faster contraction of the wound, angiogenesis and vascular dilation [4,5].

The purpose of this study was to evaluate the effect of herbal extracts on the repair of infected wounds in diabetic rats.

MATERIALS AND METHODS

Preparation of herbal extract

Myrtus communis collected from the eastern province of Iran. The leaves were washed with tap water and parched in the traditional way in a dark place with ordinary room temperature. The leaves were grinded to powder. The extraction was accomplished by distillation of 20 g of obtained powder by 200 ml of Distilled water in distillation unit as reflux boiling equipped by 40 cm spiral condenser in 80°C for 120 minutes. After cooling the extract, it was filtrated by Whatman filter and was kept at 4°C.

Animal grouping

This research was conducted by permission from the Regional Medical Ethics Committee of Arak University of Medical Sciences. 14 Wistar male rats of both sex weighing 240 ± 20 g and 6-8 weeks old were divided into two groups. The first group was diabetic and infected with *Staphylococcus aureus* and treated with extract, the second was the same without any herbal treating. The rats were kept in 12 hours of darkness and 12 hours of brightness at the Arak Animal Hospital Maintenance Center. Food and water were freely available for two groups and were kept in separate cages. All experiments have been carried out based on protocols and procedures recommended by the National Institutes of Health (NIH) for the maintenance and use of laboratory animals and practical solutions.

Induction of diabetes

Single dose intraperitoneal injection of Streptozotocin (Sigma Alredrich) in 0.1 M citrate buffer was carried out at a dose of 55 mg/kg body weight and as solution in sodium citrate buffer with pH=4.5. The pH of the buffer solution was measured by pH meter before mixing buffer and streptozotocin. All injected rats were kept for 30 days to stabilize their diabetes. Blood glucose was measured to confirm the diabetic status by the glucometer and only animals with blood glucose greater than 500 mg/dl were included in the study [6].

Wound excision

All rats were anaesthetized with intraperitoneally injection of ketamine and xylazine (Sigma Aldrich) combination as 100 mg/kg and 15 mg/kg respectively [7]. Back side of each rat was shaved and was outlined for area of the wound with a marker. Excision was created as 1 Inch² of full thickness included all skin layers using a sterile surgical scissor and considered as the day zero. Wound area was calculated by Sigma Scan Pro ver. 5.0.

Wound infection

Drug resistant *Staphylococcus aureus* (MRSA) strain was obtained from the microbial bank of the Infectious Disease Research Center of Arak University of Medical Sciences for induction of infection in the wounds. Whole surface of wound was inoculated by a concentration of 1.5×10^8 cfu/ml of fresh cells with a sterile cotton swab. Infection and inflammation induction were proved by microscopic examination of wound surface and microbiological culture after 24 h of contamination.

Herbal treatment

Myrtus communis aqueous extract was poured on the wounds of test group 3 times daily by one investigator. The control group did not receive any treatment. On days 3,7,11 and 14 post-surgery the wounds areas were assessed for all rats.

Statistical analysis

All analyses were performed by Sigma Scan Pro ver. 5.0. Analysis of variance was accomplished to compare two groups. $P < 0.05$ was considered as significant.

RESULTS

Diabetes confirmation

After 48 h fasting blood sugar in all rats reached to 300 ± 30 mg/dl and remained constant to start of experiments in 4th week.

Wound area evaluation

A statistically significant difference was observed between the two groups in open wound area from 2nd days to end of experiments (Table 1).

Table 1: Effect of aqueous extract of *Myrtus communis* on infected diabetic wounds

Treatment	Parameter				Period of complete healing (days)
	Wound area (cm ²) on day				
	3	7	11	13	
<i>M. communis</i> aqueous extract	5.62 ± 0.11	2.03 ± 0.06**	0.23 ± 0.01***	0.00 ± 0.00***	13.00 ± 0.00***
none	5.95 ± 0.08	4.01 ± 0.12	2.71 ± 0.08	1.89 ± 0.06	24.50 ± 0.5

Values represent the mean ± S.E.M. Statistical differences were determined by One-way ANOVA, Turkey test. *** $P < 0.001$, ** $P < 0.05$ as compared to respective non- treated group.

The percentage of wound healing in the third day was 4.8% in the control group and 9.968% in the herbal treatment test group, which showed a significant difference ($P < 0.001$).

The percentage of wound healing on day 14 in the diabetic control group have a significant difference as 69.76% against test group by 100% ($P < 0.001$).

The area of the first day's wound was 6.25 cm². The area of the wound in the test group was reduced in the following days and the healing was showed more rapid than the control group. As a very small scar (0.23 cm²) was calculated at eleventh day that disappeared completely on the 14th day.

The results showed that the wound healing process was very slow in the control group so that on the eleventh day the wound was not completely treated (Table 1, Figure 1).

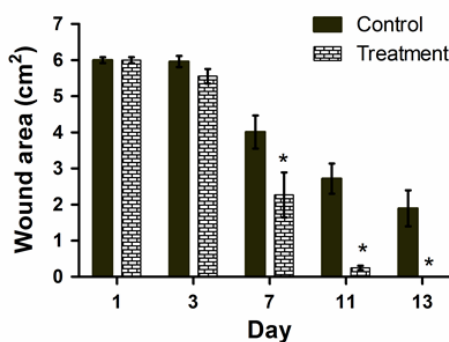


Figure 1: Wound areas of herbal treatment test and control groups (cm²)

The area of the wound in the test group was significantly different from that of the control group ($P < 0.001$) (Tables 2 and 3, Figure 2).

Table 2: Effect of aqueous extract of *Myrtus communis* on infected diabetic wounds

Treatment	Parameter			
	Wound closure (%) compared to day 0			
	3	7	11	13
<i>M. communis</i> aqueous extract	9.9 ± 0.06%	67.4 ± 0.04%**	96.3 ± 0.02%***	100.0 ± 0.00%***
none	4.8 ± 0.01%	35.8 ± 0.02%	56.6 ± 0.04%	69.7 ± 0.06%

Values represent the mean ± S.E.M. Statistical differences were determined by One-way ANOVA, Turkey test. *** $P < 0.001$, ** $P < 0.05$ as compared to respective non-treated group.

Table 3: Wound healing parameters in two groups

Treatment	Parameters				
	Number of Macrophage (cell/HPF)	Neovascularization	Number of fibroblast	Collagen synthesis	Edema
<i>M. communis</i> aqueous extract	10.50 ± 0.92***	81.00 ± 0.81***	111.66 ± 0.71***	+++	-/+
none	50.2 ± 0.87	42.66 ± 0.84	49.00 ± 0.96	+	+

Values represent the mean ± S.E.M. Statistical differences were determined by One-way ANOVA, Turkey test. *** $P < 0.001$, ** $P < 0.05$ as compared to respective non-treated group. - low, +/- low to mild, +/- very mild, + mild, ++ mild to moderate, +++ moderate, ++++ severe

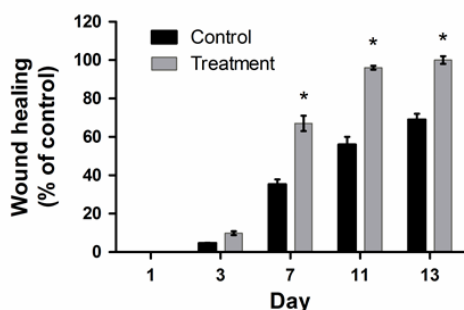


Figure 2: Wound healing percent in 13 days in test and control groups (%)

DISCUSSION

In this study, significant effect of *Myrtus* extract on treatment of diabetic foot ulcer was proved.

There was a significant difference in the percentage of wound healing and its area reduction in the experimental group and the control group in 4th, 7th and 11th. However, the completion of the healing process in the test group was 11th, while in the control group this time was 16th. These findings have shown positive effects of the herbal material on the repair of deep skin lesions.

This plant has a variety of effects due to the variety of its well-known and unknown compounds.

Rezaie et al. investigated the effects of plant extract treatment on wound healing in Wistar rats [8]. Their results showed that complete recovery of 22 mm wounds in rats by daily admission of 5% leaves extract was

achieved in 28 days. While in our research, this time was much less and around 11 days in the group of rats receiving daily extract of the plant. However, the size of the wound in the present study was 25 mm.

Our findings were based on the antimicrobial properties of the herbal, which have been investigated in numerous studies [9-11].

Rossi et al. examined the anti-inflammatory effects of a plant [12]. They reported that the compounds present in the plant leaf could inhibit lipoxygenase and cyclooxygenase, and inhibit the formation of free oxygen in the leukocytes. Also, plant compounds can prevent edema and inflammation in mice. In our experiments, it was also observed that the plant extract inhibits inflammation and prevents further tissue damage and other damage to the tissue.

Kumar et al. showed that flavonoids and tripepenoids increase wound contraction and epithelization [13]. Azaizeh et al. also showed in 2002 that quercetin (flavonoid) has an anti-inflammatory activity, which inhibits the production and secretion of histamine and other inflammatory and allergic mediators [14].

Dweck et al. showed that plants with anti-inflammatory properties have a high level of flavonoids [15].

Considering the fact that the plant used in the present study also has the mentioned compounds, based on the results of the present study about wound healing and skin recovery, it may be caused by flavonoids or tritrophenoids.

Choudhary in 2008 proved that healing of the wound was influenced by the ethanolic extract of the *Terminalia bellirica* fruit and showed that tannins are one of the most important compounds of this plant [16]. It was mainly responsible for wound contraction, increased epithelization, formation of capillary ducts and increased fibroblasts.

Due to the fact that the plant contains tannin, this compound is probably responsible for wound healing due to its contractile and antimicrobial properties. Although in a whole extract of a plant, the cumulative effects of different compounds may have different results in comparison with the individual effects of each compound; therefore, the findings of this study seem to be consistent with the mechanisms of tannins, flavonoids and Tripepenoids in other studies.

Latiff et al. showed that the *Momordica charantia* extract of the bitter mildew caused an increase in the rate of wound healing in diabetic patients, probably due to photochemicals such as polyphenols, flavonoids and tertphenoids [17].

Ruiz et al. showed that quercetin, a flavonoid type, inhibits the expression of inflammatory cytokines, and, as a result, suppresses inflammation [18].

It seems that in the present study, due to the presence of flavonoids in the plant, in accordance with the research of these scientists, probably with the anti-inflammatory

effects that through the control of relevant mediators, by reducing the duration of inflammation, accelerating the healing of diabetic wounds in the group Treatment has been observed.

CONCLUSION

It was proved that the *Myrtus comminis* could significantly decrease the duration of wound healing in diabetic rats. It was probably due to Anti-inflammatory and cellular proliferation effects that have been accelerated in several repair processes. Clinical trial experiments on patients with diabetic foot ulcer by this extract are recommended.

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CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this paper.

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