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Wound Healing Potential of Crocin and Safranal, Main Saffron (*Crocus sativus* L.), the Active Constituents in Excision Wound Model in Rats

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Abstract

Background: Saffron is traditionally suggested for wound healing in Persian medicine. It is investigated for wound healing effect in multiple studies with promising results. It is not examined that which ingredient of saffron contributes more to this effect. This study was aimed to evaluate and compare the wound healing potential of saffron and its active constituents, crocin, and safranal, in rats. **Materials and Methods:** Forty female adult rats with induced excision wounds were randomly divided into four groups to receive topical formulation of saffron, crocin, safranal, and placebo for seven days. The Wound area and histopathologic stage of wound healing were evaluated as outcome measures. **Results:** The wound area was significantly lower in treatment groups (saffron, crocin, and safranal) compared to the control group on day 7 of the intervention. Compared to the control group, the wound in all treated groups showed a decreased inflammatory response and more progression to the proliferation phase. The saffron group showed more advanced healing phase with complete epithelialization of the wound on day 7 of study when partial and no epithelialization was observed in safranal and crocin groups. **Conclusion:** The study showed the wound healing properties of both safranal and crocin with the slight superiority of safranal. However, saffron seems to be more potent in wound healing effect compared to safranal and crocin as its main active constituents. **[GMJ.2021;10:e1900]**
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Keywords: Crocin; Safranal; Saffron; Wound Healing; Traditional Persian Medicine

Introduction

The wound is defined as a disruption of the normal skin structure and function, caused by various mechanisms, including but not limited to abrasion, crush, puncture, gunshot, burns, and surgery. There are four stages of the physiologic process of wound healing; hemostasis, inflammation, proliferation, and remodeling [1]. Many topical agents are avail-

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able to improve the wound healing environment. They are claimed to have different effects, such as preventing bacterial overgrowth, controlling exudate, enhancing granulation, providing proper fluid balance, and prevention of scar formation [2]. Many of these formulations are originated from traditional sources [3]. Among them, topical formulation of saffron is suggested in traditional Persian medicine by *Avicenna* [4]. Saffron is investigated for wound healing effect in multiple studies with promising results [5-8]. However, it is not examined that which ingredient(s) of saffron contributes to its wound healing effect. This study was aimed to evaluate the wound healing potential of saffron and its active constituents, crocin and safranal, in rats.

Materials and Methods

Ethics Considerations

The study protocols were reviewed and approved by the animal ethics committee of Shiraz University of Medical Sciences (approval code:95-01-01-13605). The standards for the protection of animals in scientific studies recommended by European Council were followed [9].

Animals

Forty healthy adult female Sprague-Dawley rats (*Rattusnorvegicus*) weighted 200-220 g, age 7–8 weeks, were colony-bred in the animal house of Shiraz University of Medical Sciences, Shiraz, Iran. They were kept in the animal room (five rats per cage) with a 12/12-hour light and dark cycle under constant temperature (22±2°C) and humidity (55±5%) during the study period. They were provided with free access to laboratory food and tap water.

Topical Formulations

Crocin and safranal were purchased from providers of Sigma-Aldrich (Germany). Saffron was purchased from Ghaenat Saffron co., which provides the saffron from Ghaenat-Khorasan, Northern Iran. Saffron, derived from the flower of *Crocus sativus*, was finely milled with size less than 75μm using sieve NO. 200. According to our previous topical formulation [10], the obtained fine powder was dispersed in distilled water and then geometric mixed with eucerin to prepare 5% w/w topical cream. Also, the 1% creams of safranal and crocin compounds responsible for the aroma and color of saffron, respectively, were prepared as previously. The hydrated eucerin using an equal volume of distilled water of saffron formulation was considered as placebo cream. The creams were applied once daily for a week to cover the whole area of the wound.

Excision Wound Model

Rats were anesthetized with ketamine (30 mg/kg, intraperitoneal). An area of 1.75 cm^2 on the back of the rat was marked, and then a full-thickness skin excision was made carefully. The wound diameters were measured on the 1st, 4th, and 7th day of wounding and the wound area was calculated by ImageJ software (version 1.52p released June 2019, NIH). The rats were then randomly divided into four groups to receive topical formulation of saffron, crocin, safranal, and placebo for seven days. They were kept in the animal room with standard conditions as mentioned above during the treatment (five rats of the same group per cage).

Histopathology

At the end of the study (7th day), the animals were sacrificed under general anesthesia with diethyl ether (Merck, German) and the complete wound area was excised as full-thickness skin specimens for the histopathological evaluation. Samples were fixed in 10% buffered formalin and after processing and blocking with paraffin, sectioned into 5-μm sections. Hematoxylin and eosin staining method [11] was applied on all sections.

Statistical Analysis

The descriptive quantitative results were presented as means± standard error of the mean (SEM). The analysis of results was made by SPSS software (Version 16; Chicago, IL, USA). One-way analysis of variance (ANOVA) followed by post hoc multiple comparisons test with Bonferroni correction was applied to the comparison between different treatment groups, considering $P<0.05$ as statistically significant.

Results

There was a significant decrease in wound area of all groups in 4th and 7th days after the start of treatment. The wound area was significantly lower in treatment groups (saffron, crocin, and safranal) compared to the control group on the 7th day of intervention (Figure-1). There was no significant difference between treatment groups in the term of wound area in 4th and 7th days of study (Table-1). In the control group, basal and spinosum layer were formed in wound area with 4-5 cell layers. The granulose, lucidom, and keratinized layers were not formed. An inflammatory response involving the accumulation of neutrophils and red blood cells can be observed in dermis layer. The wound was in the inflam-

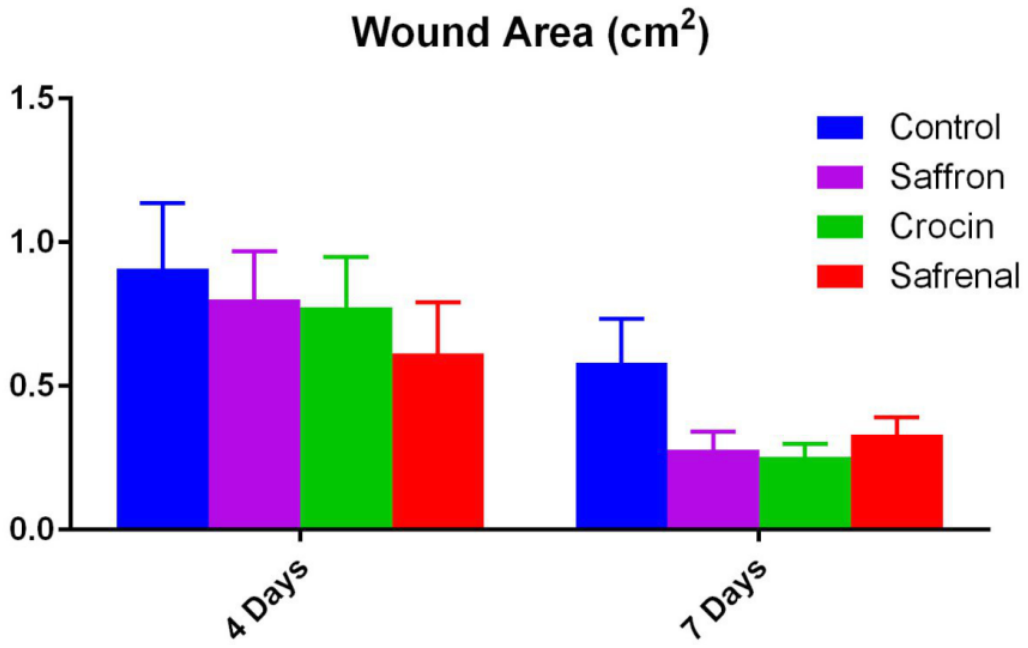

Figure 1. The comparison of wound area between study groups after 4 and 7 days of treatment

Table 1. Pairwise Multiple Comparison of Wound Area between Study Groups with Bonferroni Correction.

Time Intervals	(I) Group	(J) Group	Mean Difference (I-J)	P-value	95% Confidence Interval	
					Lower Bound	Upper Bound
4th Day	Control	Safranal	0.29± 0.11	0.1	-.03	0.62
		Crocin	0.13± 0.11	1	-0.19	0.46
		Saffron	0.10± 0.11	1	-0.22	0.43
	Safranal	Crocin	-0.16± 0.11	1	-0.48	0.16
		Saffron	-0.18± 0.11	0.7	-0.51	0.14
	Crocin	Saffron	-0.02± 0.11	1	-0.35	0.30
7th Days	Control	Safranal	0.25± 0.05	0.00	0.09	0.40
		Crocin	0.32± 0.05	0.00	0.17	0.48
		Saffron	0.30± 0.05	0.00	0.14	0.46
	Safranal	Crocin	0.07± 0.05	1	-0.07	0.23
		Saffron	0.05± 0.05	1	-0.10	0.21
	Crocin	Saffron	-0.02± 0.05	1	-0.18	0.13

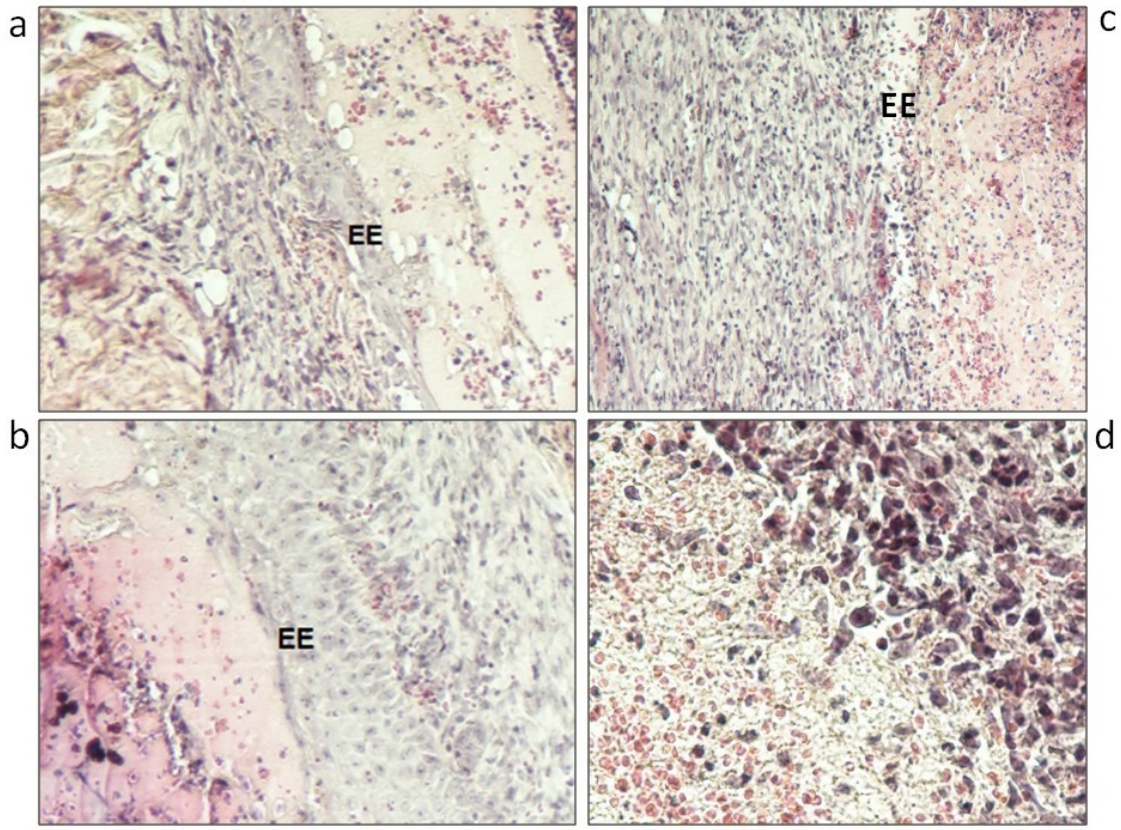

Figure 2. Histopathology of wound healing in rats treated with saffron **(a)**, safranal **(b)**, crocin **(c)**, and placebo **(d)**. All treated groups were progressed to the proliferation phase when the control group was in the inflammation phase. The saffron group **(a)** was in more advanced healing phase showing complete epithelialization of the wound (EE) when partial and no epithelialization was observed in safranal **(b)** and crocin **(c)** groups, respectively.

mation phase (2nd stage of healing, Figure-2). Compared to control group, the wound in all treated groups (saffron, safranal, and crocin) showed a decrease in inflammatory response and progress to proliferation phase indicated by accumulation of fibroblasts, collagen fibers, extracellular matrix, and granulation tissue. The saffron group was in a more advanced healing phase showing complete epithelialization of the wound when partial and no epithelialization was observed in safranal and crocin groups, respectively (Figure-2).

Discussion

In this study, we explored the effect of a topical formulation of safranal and crocin, the main active ingredients of saffron, on the excision wound healing model in rats. Compared with the control group, treatment groups showed significantly lower wound area after seven days of treatment. Histopathology studies revealed the best response in saffron and safranal, respectively. Saffron is a spice that originated from the flower of *C. sativus*L., besides being used as a spice, it is known for different medicinal applications throughout history. In traditional Persian medicine, it was recommended for wound (*Ghorhe*) healing by Rhazes (865–925 AD) in *Al-Havi*, Avicenna (980-1037 AD) in *al-Qanoon* and AghiliShirazi (1670-1747 AD) in *Makhzan-al-Advieh* [12, 13]. Previous studies have suggested different mechanisms for the wound healing effect of saffron. The enhancement of fibroblasts proliferation and migration is reported to be made by the increase in TGF-β1 level in wound bed of saffron treated rats [8]. Enhanced epitheliogenesis and wound closure is also suggested to be due to enhanced hydroxyproline, and dry matter content in the saffron treated wounds. Saffron and its ingredients also showed anti-inflammatory and antioxidant activity, which improves the wound

healing process [8]. Most medicinal effects of saffron are evaluated in its powder or water extract form. A small proportion of studies investigated the medicinal benefits of crocin and safranal as the main active ingredients of saffron. For example, antidepressant and aphrodisiac properties of saffron is considered to be more secondary to the crocin content [14, 15], while anti-asthmatic and anti-cancer effects are reported to be more contributed to safranal [16, 17]. Our study showed that despite the observed improvement in all treated groups' wound healing process compared to control, there was a marked superiority in the histopathologic stage of wound healing in saffron treated rats compared to safranal and crocin treated ones. Safranal demonstrated slightly better results compared to crocin. The main limitation of our study was the lack of dose-response assessment. The dose was selected based on the suggested dose in previous studies [8]. The next limitation was that the histopathologic evaluation of the wound was done only in only one time period (7th day of wounding). This protocol was selected to decrease the number of studied animals. The 7th day of wounding was selected for histopathologic evaluation of the wound based on previous studies that showed the maximum difference between study groups in this time interval.

Conclusion

The present study showed the wound healing properties of both safranal and crocin with the slight superiority of safranal. However, saffron seems to be more potent in wound healing effect compared to safranal and crocin as its main active constituents.

Acknowledgment

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Conflict of Interest

Nothing to declare.

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