ORIGINAL ARTICLE

The Effect of Saffron (*Crocus Sativus*) Extract for Healing of Second-degree Burn Wounds in Rats

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Abstract. The aim of this study was to evaluate the efficacy of pollen of saffron extract cream in the treatment of thermal induced burn wounds and to compare its results with silver sulfadiazine (SSD) in rats. Animals were divided into four groups and administrated a topical cream including control, base, saffron (20%) or SSD (1%) at 24 hour after a burn injury that was induced by hot water. In special days, according to a pre-planned schedule, animal's weight, wound size, as well as skin histo-pathology were determined in different groups under topical treatments. On day 25, average size of wound was 5.5, 4, 0.9 and 4.1 cm^2 in control, base, saffron and silver groups. The wound size of saffron group was significantly smaller than other groups. Histological comparison has shown that saffron significantly increased re-epithelialization in burn wounds, as compared to other cream-treated wounds. Although the exact mechanism of saffron is unclear, anti-inflammatory and antioxidant effects of saffron may have contributed to the wound healing. The results of this study raise the possibility of potential efficacy of saffron in accelerating wound healing in burn injuries. (Keio J Med 57 (4) : 190–195, December 2008)

Key words: burn, saffron, wound, silver sulfadiazine

Introduction

Thermal burn injury is a major cause of death and disability, with high cost in health care. Healing of burn wounds still remains a challenge to modern medicine, though many antiseptics have been discovered. Burn management entails significant duration of hospital stay, expensive medication, multiple operative procedures and prolonged period of rehabilitation. This makes burn care an expensive proposition and every effort should be made to provide a shorter in-patient care for the burn patients.[1] Recently we showed that a significant number of patients exposed to thermal and boiling burn injury in Northern Iran, in which they stayed for a long time in the hospital for the treatment. More than sixty percent (60%) stayed between 8 to 30 days.[2] Silver sulfadiazine (SSD) cream at 1% is the most widely used topical treatment for burn injury. The anti-microbial efficacy of SSD is probably the main reason why the use of this agent is so widespread.[3] Delayed wound healing following treatment is the most important clinical adverse effect of silver topical agent. The wound healing effects of silver sulfadiazine was compared with vaselinated tulle gras in burned patients. It was demonstrated that silver sulfadiazine delayed wound healing.[4] Several adverse reactions and side effects have been reported, such as resistance to silver sulfadiazine, renal toxicity, and leukopenia; therefore confirming that this topical cream should not be used for long periods on extensive wounds.[4] Sloughing of dead tissue in partial thickness burns is retarded be-

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cause silver sulfadiazine delays or prevents colonization by micro-organisms, and silver sulfadiazine might also slow down the healing of the wound.[5] Prolonged application of partial-thickness burn wounds results in high patient cares cost and complicates wound healing while a patient is being followed in the hospital. It is important to find more effective drugs with shorter time application.

Crocus Sativus (family: Iridaceae) is a herb with a long history of spicy food uses in many countries. The stigmas of this herb are used as a delicious additive on foods. This herb was assessed to have many pharmacological properties such as anti-inflammatory, antioxidant, anti-tumor and anti-depressant effects.[6-9]

The stigmas of *Crocus sativus* are expensive and exported mainly from Iran to other countries in the world. The pollen of stamens is an excessive part of Crocus sativus that is not used as a food additive and is very inexpensive. Fallah Hosseini *et al*, showed extract of pollen of this herb has a theraputic effect on wounds induced by mustard in animals. It was more effective than control group.[10] Realizing the potential use of pollen from this herb in wound healing, the present study was undertaken to study the effect of pollen extract of *Crocus Sativus*, in comparison with silver sulfadiazine on the rate of burn wound healing.

Materials and Methods

Preparation of Crocus Sativus extract

The pollen of *Crocus Sativus* was collected from Saraban in the Khorasan province of Eastern Iran. This province is the main producer of saffron in the world. The pollens of the saffron are dried at room temperature and powdered in a grinder. Aqueous ethanol (75%) was added to the powdered peels (500 g), and stirred for one hour. The mixture was kept at room temperature for 48 hours. Following filtration, ethanol was evaporated under reduced pressure at 40ºC. The remained water extract was dried at oven temperature of 50ºC. As a result, 150 grams of extract powder were obtained.

Preparation and formulation of saffron cream

Two grams of liquid white paraffin, 7.5 grams of sterile alcohol, 3 grams of solid white paraffin, and propylene paraben (0.015 g) were mixed and heated to a boiling point as aqueous phase. Twenty grams of pollen saffron with 70 mL deionized water were added to the mixture of 7 grams of propylene glycol, 3 grams of sodium luryl sulfate and 0.025 grams methyl paraben. The mixture was heated as organic phase. Then, two separate phases were mixed continuously while being treated to a cooler temperature. Thus, the uniform cream was produced after cooling the cream filled in tube. Cream contained 20% of pollen. Our experimental research and formulations were carried out under sterile conditions. The final creams were tested for any probable contamination microbes.

Test animals

Male Wistar rats (n = 48) weighing 180 to 200 grams were used and housed under standard conditions at room temperature and given laboratory food and water, ad libitum throughout the study. The experimental protocol was approved by Research Committee of Mazandaran University of Medical Sciences prior to commencement of this study. The weights of the animals were measured. They were anesthetized intraperitoneally with thiopental (40 mg/kg body weight), while their backs were shaved. They were then placed in a supine position inside the bottom of hot water (90ºC for 6 seconds). This heat exposure caused a uniform second-degree burn on the back of the skin. In this experiment, approximately 10% total body surface (TBSA) was burned. The animals were resuscitated with an intraperitoneal injection of 5 ml of normal saline solution.

After 24 hours, the animals were assigned at random to four groups (n = 12/group). Group 1 was the control group and no topical agent was applied, group 2 was base cream, (treated group only with base cream without effective agent), group 3 was 1% SSD (silver sulfadiazine, Behvarzan Pharmaceutical Company, Rasht, Iran), group 4 was with a 20% saffron cream treated group. Treatment began 24 hours after the burn injury. Wounds were treated twice daily.

In order to quantify the rate of wound healing, the size of lesions was determined at 1, 3, 7, 10, 14, 20 and 25 days after burn injury be well apparent. At this time, each test animal was held in a good position and wound margin was traced on a transparent plastic sheet using a fine-tipped pen. Lesion body area was displayed as cm^2 on each experiment day. The area of the wounds on the first day was considered as 100% and wound areas on subsequent days were compared with the wound area on the initial days. The animals were weighed on the different days after the burn injury.

Histological study

Due to the wound healing of burn wounds, re-epithelialization was evaluated after 25 days and the last day of treatment period. For this purpose, skin tissue samples were taken for histological studies with a small excision containing part of the wound area. Tissues were fixed in 10% formalin. Paraffin-embedded sections (5-μm thick) were prepared and stained with hematoxylin and eosin. Light microscopy was used to evaluate the pathological

Table 1 Body weights of rats treated with base, saffron and silver sulfadiazine creams and control during the period of these experiments.

Groups	Days after burn injury						
	1	4	7	10	14	20	25
Control (g)	176±17	170±18	173±18	183±17	189±19	200±22	208±24
Base (g)	180±20	180±18	184±13	189±14	189±11	203±18	206±16
Saffron (g)	177±18	170±15	168±11	169±15	175±16	189±19	191±16
Silver sulfadiazine (g)	183±12	178±10	176±11	177±15	185±11	192±13	190±12

Table 2 Average size of wound in animals as control and treated with base, saffron and silver sulfadiazine creams.

Groups*	Days after burn injury				
	7	10	14	20	25
Control (cm^2)	28.9±2.9	26.7±5.6	21±8	7.5±4	5.5±3
Base (cm^2)	28.4±8.3	25.3±6	12.3±5.6	6.8±2.2	4±2.3
Saffron (cm^2)	30.1±6.5	24.3±7	16±7	4±1.7	0.9±1.9**
Silver sulfadiazine (cm^2)	34±4.4	28±6	18.6±8.5	6.6±3.7	4.1±3.6

* Values are means ± SD for each group of ten mice.
** $P<0.05$ comparison of saffron extract cream compare to other groups.

changes, e.g., granulation tissue formation and re-epithelialization in wounds and their comparison with the normal tissue part. The severity of inflammation in healed areas was evaluated by counting the mean number of inflammatory cells infiltration per high field (PHF) (×400 magnification) of three samples of each groups.

Statistical analysis

One-way analysis of variance (ANOVA) and tukey post test were used to compare group means. The differences were considered significant, when $p<0.05$.

Results

Average weight of animals in four groups of control, base, saffron and silver sulfadiazine before beginning of this study was 176, 180, 177 and 183 grams, and after 4 days of burning was decreased to 170, 180, 170 and 178 grams respectively. There were no significant differences between groups in the reduction of weight. After the treatment period (25 days), average weight of animal increased (Table 1).

Wound condition on the 7^{th} day after the burn, was assessed macroscopically in all groups for facilitation of exact determination of wounds size. Skin lesions were measured on 7, 14, 20 and 25 days after burn injury and its ratio to first day of burn was evaluated. Average size of wound after 25 days was 5.5, 4, 0.9 and 4.1 cm^2 in control, base, saffron and silver groups (Table 2). There were significant differences between saffron group and the other three groups in this aspect ($p<0.05$). There was no observed statistical difference among control, base and silver sulfadiazine in wound size on the 25^{th} day of treatment. Trends of wound size ratios were showed in the Figure 1 in control and treatment groups. The photograph of burned mice on the 4^{th} and 10^{th} of treated with saffron cream is demonstrated in Figure 2.

Results of the histological studies indicated an improvement in wound healing on the 25^{th} day after treatment. The slides from saffron treated wounds were showed significant wound healing with completed re-epithelialization of epidermis, fibrosis of dermis and mild inflammatory cell infiltration. (Fig 3). The severity of inflammation in healed areas was approximately 12, 20 and 28 inflammatory cell infiltration for saffron, silver and base cream treated groups respectively. The slides of wounds treated with SSD showed lesser degree of wound healing compared to saffron and basic creams treated groups. Bacteria were found in the wound treated with basic creams. Histological samples of non-treated control group showed negligible wound healing and many bacteria.

Discussion

Saffron has a food and medicinal history usage in the world and is widely used as a food additive. Saffron is the most precious and expensive spice in the world. The saffron filaments, or threads, are actually the dried stig-

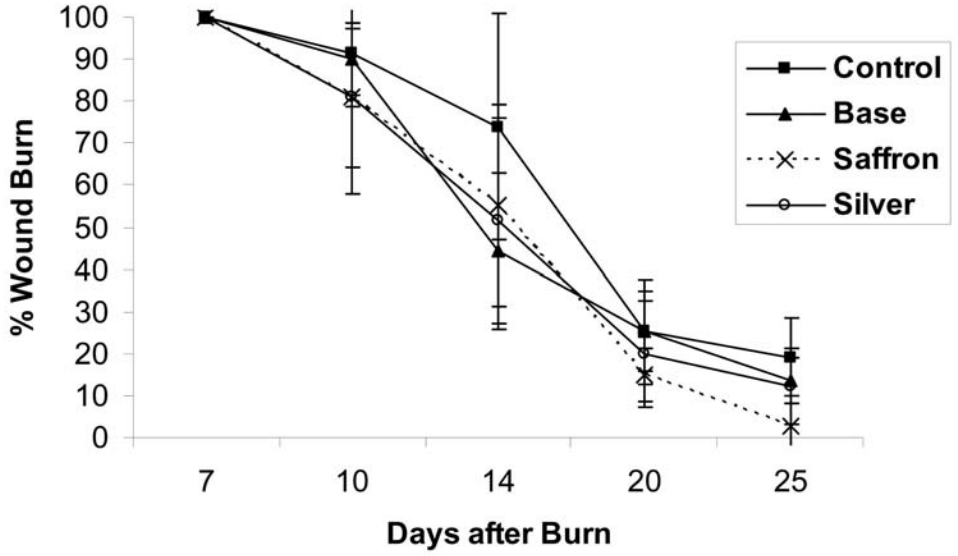

Fig. 1 Comparison of wound burn size in control, base, saffron and silver sulfadiazine. The wound size was stated as 100% on the 7th day and wound size on other days calculated compare to the 7th day.

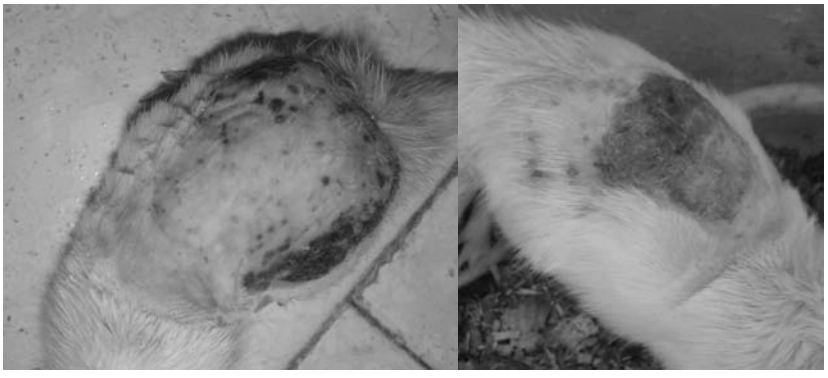

Fig. 2 Photograph of burned mice on the 4th (left) and 10th (right) of treated with saffron cream.

mas of the saffron flower and have red colors. These components are often dried and used in cooking as a seasoning and coloring agent and it is most expensive. There are several reports showing that saffron has pharmacological effects including anti tumor, antidepressant, neuroprotective and antioxidative effects.[6,7,9,11,12] The stamens parts of the flower do not have any taste and are less expensive and yellow in color. Stamens of saffron flower were used in this study. Assessment of wound size and microscopical examination were employed for evaluating efficacy of pollen saffron in the treatment of burn injuries. Our results indicated that saffron cream is able to accelerate the rate of wound healing and shortens the time of healing, compared with those treated with silver sulfadiazine in burned rats. The extent of healing with saffron was significantly higher than silver sulfadiazine and base creams. In our study, microscopic examination of the histo-pathological slides, prepared after the completion of treatment, revealed that the wounds treated with saffron cream contained fewer inflammatory cells than other groups. Wound closure was complete macroscopically, and the histological studies of wound section confirmed the formation of epiderm and less inflammatory cells infiltrated the dermis. After treatment with 20% saffron cream, the epidermis was close to normal structure.

Chemical constituents of saffron include crocins, crocetin, picrocrocin, β-carotene and safranal.[9,12] These

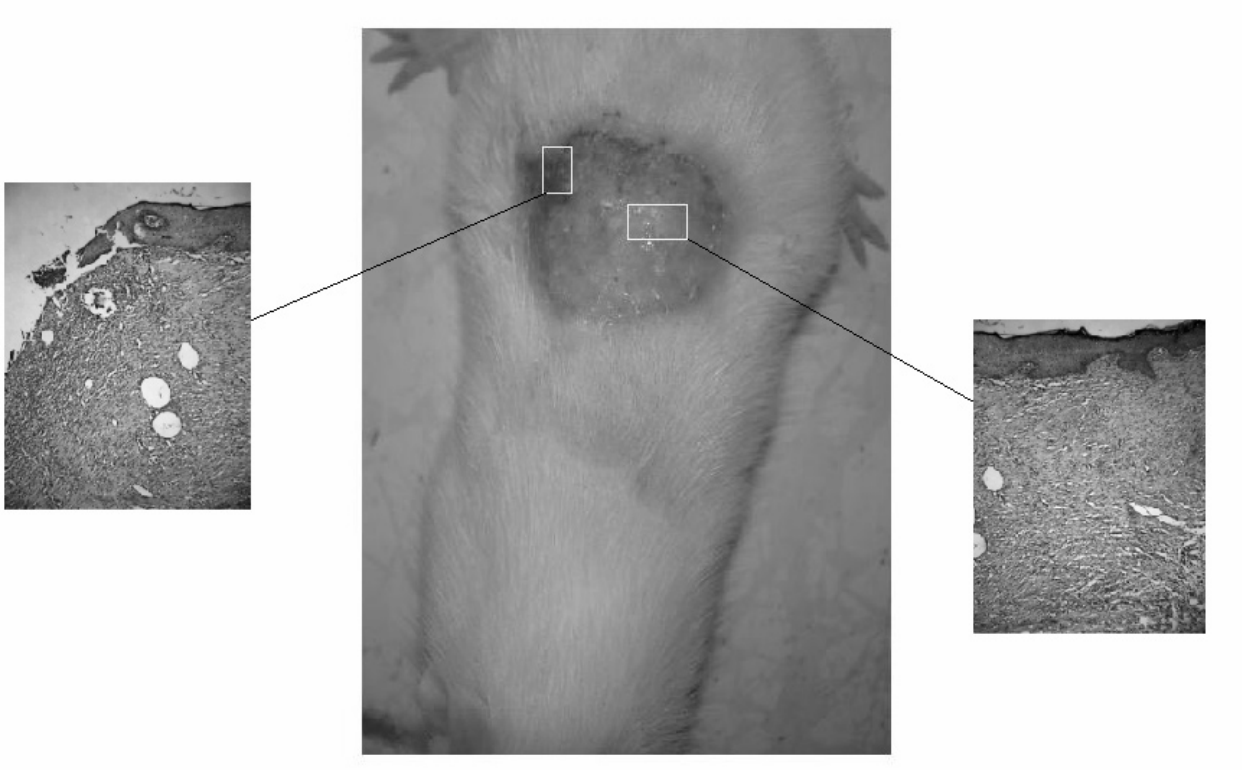

Fig. 3 Macroscopic view and photomicrograph of skin wound section of rat on the 25th day of treated with saffron. Left pathologic slide is ulcer and healing junction, right pathologic slide is healing skin. Most part of skin was healing with growing hair on skin. The specimens stained with Hematoxylin and Eosin.

compounds have antioxidant activity.[6,9,12] Saffron inhibited the growth of tumors in brain of mice.[13] Ethanolic extracts of Crocus sativus showed anti-inflammatory effects in animal model,[8] Crocetin modified the inflammatory markers such as tumor necrosis factor (TNF) and interlukin (IL).[14] The release of pro-inflammatory mediators such as IL and TNF and reactive oxygen species are increased in patients with burn injuries.[15] Thus, saffron may affect the healing of burns with its antioxidant and anti-inflammatory effects.

Silver sulfadiazine cream is the most widely used topical treatment for burn injury and the antimicrobial efficacy of SSD is probably the main reason for the usage of this agent.[7] Although SSD is considered a gold standard for treatment of burn injuries, prolonged application of this agent may result in a longer hospitalization.

We found that the severity of inflammation in SSD group was more than that in saffron and also base cream. The inflammatory effect of SSD may lead to a delay in re-epitalization of epidermis and dermal fibrosis. Both fibroblasts and keratinocytes are susceptible to lethal damage when exposed to concentration of silver which are lethal for bacteria. In other words, silver-based products can not discriminate healthy cells involved in wound healing and bacteria.[16]

As in our experimental study, delayed wound healing is often observed clinically following the use of silver-containing topical antimicrobial agents.[17] Based on the result of this study, it is probable that saffron cream could lead to a shorter hospitalization of burned patients compared with SSD.

We showed that saffron has better wound healing effects in burn animals compared to SSD. For instance, at the 25th day of our study, the wound size in saffron group was 0.9 cm^2 compared to 4.5 cm^2 in SSD group. In fact, the re-epithelialization process in animals treated with saffron was better than those that received SSD. Although the antioxidant and anti-inflammatory effects of saffron were suggested, further studies are needed to elucidate the exact mechanism of saffron in wound healing.

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