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Original article

Evaluating the Healing Effects of *Ajuga chamaecistus* ssp. *tomentella*, on Second Grade Burn Wounds, a Double-Blind Randomized Controlled Clinical Trial

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Abstract

Background and objectives: Nowadays, the use of medicinal plants is on the rise mainly due to the fewer side effects and lower costs. Despite the traditional reports about the healing properties of *Ajuga* species, no clinical studies have been conducted. In this study, the effect of hydroalcoholic extract cream (3%) of *Ajuga chamaecistus* ssp. *tomentella*, one of the exclusive species of *Ajuga* in Iran, was compared with nitrofurazone cream (0.2%) on the second-degree burn wound healing. **Methods:** This clinical trial was performed at Motahhari Burns Hospital in Tehran. Fifty-two cases of second-degree burn patients were randomly assigned to two groups of 26 members, including nitrofurazone and *Ajuga*. We evaluated the wounds based on the onset of epithelialization, healing time, post-drug irritation, primary irritation, decreased irritation, post-drug pain, primary pain, decreased pain, allergy, infection parameters, and the Bates-Jensen Wound Assessment Tool. **Results:** *Ajuga* cream was more effective than nitrofurazone in terms of the mean day of epithelialization onset ($p = 0.007$), healing time ($p = 0.001$), post-drug irritation ($p = 0.007$), decreased irritation ($p < 0.001$), post-drug pain ($p = 0.018$) and decreased pain ($p = 0.001$). **Conclusion:** According to the results, the *Ajuga* cream (3%) can be a useful remedy for burn wounds due to the reduction in the onset of epithelialization, healing time, post-drug irritation, irritation reduction, post-drug pain, and pain reduction. However, further large clinical trials are needed to confirm these results.

Keywords: *Ajuga*; nitrofurazone; second degree burns; wound healing

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Introduction

The effects of skin wounds on patients' quality of life appear to be one of the most significant causes of physical disability [1]. These wounds can result from various factors such as burns, surgeries, trauma, and arterial disease, which can be acute or chronic [2]. Among the destructive injuries, burns and their complications are associated with the highest mortality rate. As a

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result, they are considered as public health problems, especially in developing countries [3].
Second-degree burns are the most common household incidents requiring care [4]. Most of the time there is no need for medical interventions or grafts [5] since it takes about two to three weeks for healing [5-7].
Burn lesions in the acute phase cause dehydration, hypothermia, and infection by creating an unsuitable environment and low immunity levels [8]. The speed of wound healing in skin burns is important regarding the cost of hospitalization, the healthcare system, and the individual cost. Any method that can reduce the recovery time will reduce the heavy burden of financial and psychological costs imposed on the patient and those around him. Daily dressings are applied with Vaseline or antibiotic creams such as Silver sulfadiazine cream [9]. Nitrofurazone is also used as a common treatment for second-degree burns, having side effects such as tenderness, itching, contact dermatitis, and reduce the healing process [10].
Research has shown that natural products and traditional medicine methods have spread worldwide for treating several diseases and wounds [11].
Ajuga species (Lamiaceae), commonly known as bugle, are found in China, Korea, and Japan [12].
Five species of *Ajuga* grow in Iran, *A. austro-iranica, A. chamaecistus, A. comate* (Syn.: *Ajuga chamaepitys* subsp. *chia*), *A. orientalis.* Also, some subspecies of *A. chamaecistus* including *Ajuga chamaecistuss* ssp. *tomentella* are exclusive to Iran [13].
Ajuga species have antibacterial [14], anti-tumor, anti-hyperglycemic [15-17], anti-inflammatory [18], anti-malarial [19], antioxidant [20], antimicrobial [21] and analgesic [22] effects. The species that grow in Asia and the Middle East are traditionally used to treat high blood pressure, rheumatism, joint pain, gouts, ascites, pain, jaundice, and wound healing [22-25].
According to a review article on the effective herbs in wound healing mentioned in traditional medicine sources, flowers and leaves of *Ajuga chamaepitys* (L) Schreb, were reported for wound healing properties [26]. *Ajuga* is mentioned in the references of Persian traditional medicine as "Kamafitos", "Khamanitos" and "Sanobar Al-Arz" which are referred to *Ajuga chamaepitys* (L) Schreb., and *Ajuga iva* L. [23,27].
In the references of Persian traditional medicine, "Kamafitos" or" Khamanitos" has a warm and dry temperament. The characteristics of this plant include anti-blockage feature, "Mofattih", as well as diuretic, "Modirr", cleansing "Monaqqi", and detergent, "Jali", properties according to Iranian traditional medicine concepts. It is used mainly to heal wounds [14,23,25].
Despite the traditional uses of *Ajuga* species for wound healing, no experimental studies have been performed to confirm the effects of *Ajuga* species on human skin burns; therefore, this study aimed to investigate the local effects of *Ajuga chamaecistus* ssp. *tomentella*, on second-degree wound healing burns of patients.

Materials and Methods

Ethical considerations

The current study was a double-blind, randomized controlled clinical trial. The study was approved by the ethics committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1397.483) (2018/09/04) and then it was registered at the clinical trial center (IRCT) (IRCT20180908040966N1). The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. All patient information in this study remained confidential. Written informed consent was obtained from the patient due to the intervention of the study (with a full explanation of all study conditions). In receiving the consent, behaviors such as threats, temptations or coercion were not imposed on the patient and did not endanger the patient in any way. The participants could leave the study at any time and were informed and supported about the risks and potential harms of early withdrawal. Participants were explained that they might enter into one of the case and control groups during the study following a random division. After obtaining the patient's consent to participate in the relevant project the research was conducted at Motahhari Trauma and Burn Hospital of Tehran, Iran, from February to April 2019.
According to the traditional medical sources [23] and a recent study [20] use of *Ajuga* had no serious side effects.

Plant material

The aerial parts of *Ajuga chamaecistus* ssp. *tomentella* (Boiss.) Rech.f.,were collected in June 2018 from Sorkheh Hessar around Tehran. The plant sample was verified by Prof. GH. Amin and a sample was kept at the Herbarium of School of

Pharmacy, Tehran University of Medical sciences, Tehran, Iran with the voucher no. 6697-THE.
The aerial parts were dried in shade at room temperature. Two kg of the dry plant was ground and extracted by maceration method with 70% ethanol solvent (1:6; plant: solvent ratio), three times, with an interval of at least three days. The resulting extract was concentrated with a rotary evaporator apparatus (temperature 40 °C) and completely dried in vacuum oven.

Preparation of the cream
The concentrated hydroalcoholic extract was mixed with cold cream (ORAND®) for 1 hour using a mixer till it became completely homogeneous. The cream was filled in 30 g tubes. The *Ajuga* cream contains 3% (w/w) hydro alcoholic extract of the plant. According to previous study, it is estimated that hydroalcoholic extract of this plant contains 2.58% of 20-hydroxyecdysone [28].

Microbial control of the product
Microbiological quality control of the cream was carried out at the Food and Drug Deputy Laboratory of Tehran Medical University according to WHO protocols [29].

Study design
Patients were randomly assigned to two groups receiving the cream, *Ajuga*, and nitrofurazone. If there were second-degree burn wounds in two similar organs or two different places in one limb, each limb or each part of the limb was accidentally treated with either nitrofurazone or *Ajuga*. Two experienced specialists confirmed the degree of burns. The patients could be included in the study in case they met the criteria for entering the project.
In this study, the patients and two experienced specialists responsible for visiting and treating burned patients were kept uninformed regarding the type of medications used for treating the wounds.

Participants
The target population included Iranian patients aged 18-60 years with second-degree burns having a burn rate of less than 3% that referred to Motahhari Hospital in Tehran with no underlying disease and having the inclusion criteria.

Inclusion criteria
In the current study, the burning incident had happened in the last 24 hours, and the burn wound was not stained with contaminants. The patients also had not used topical immunosuppressants for four weeks. The lack of pregnancy and lactation, obsession and over-washing with water and detergents, and the desire to participate in the research and answer the questions were among the conditions for entering the study. Also, burns on the face, hands, and genital area were not included in the study due to the sensitivity of these areas and people in need of skin grafts.

Exclusion criteria
To assess the condition of the wound, both groups were evaluated for the signs of infection during the study and at each day of dressing change. In the presence of any of the following symptoms, the wound was considered infectious, the patient was removed from the study, and the treatment changed according to the physician. In cases of swelling in the burned area during the study and the inflammation of the wound edges, discoloration of the burnt wound to dark red, brown, purple, or black, presence of purulent, foul-smelling, green and abundant discharge and fever during the treatment period, the treatment would be stopped. During the treatment period, if any of the patients from both groups stopped participating in the study for any reason, they were removed and replaced with another patient.

Intervention
Following initial treatment for burn injuries, including washing with normal saline and drying with sterile gauze, the wound was randomly bandaged with *Ajuga* cream or nitrofurazone.
Patients referred to the hospital every day to change their dressings and receive recovery period examinations to assess possible complications and continue treatment. They were followed up daily by the researcher (physician), and they were dressed with the treatment. Healing was considered when the apparent wound was pink and clear, having no discharge and a covering texture. Patients were evaluated in six stages by two experienced specialists, one session before starting the treatment, other session were in the third and seventh day, at the day of epithelialization onset, at the day of wound healing, and at the fourteenth day after

starting the treatment. The wound condition was also assessed for granulation and epithelialization tissue each time the dressing was changed. In addition, wounds were photographed at each meeting. The tools used in this study included the demographic profile form, and the Bates-Jensen Wound Assessment Tool. The Bates-Jensen questionnaire has 15 phrases, two of which are localized and the shape of the wound is not categorized, but the other 13 phrases are based on a 5-part Liker scale.

The Bates-Jensen Wound Assessment Tool parameters regarding burn wounds include: wound width, wound degree, wound depth, wound edge, subcutaneous tissue destruction, necrotic tissue type, exudate type, exudate value, skin color around the wound and peripheral tissue. During the study, environmental tissue of edema, granular texture, and epithelialization were examined. According to the obtained scores, the wound healing process was evaluated. A higher score on this questionnaire indicated further wound destruction. However, a lower score indicated suitable wound healing. Digital photographs of patients' wounds were taken during the treatment, and they were examined for healing. At the earliest opportunity after the observation, the observer studied the observed material to avoid inconsistency and ambiguity due to the time interval between observation and study. To measure the size of the wound, a plastic tape measure was used, having an accuracy of about 1.1000. At the beginning of the patient's visit to the clinic and during the treatment period the extent of the wound was measured by having contact with a tape measure.

Outcome measurement

Primary outcomes included post-drug irritation, decreased irritation, post-drug pain, and decreased pain. Secondary outcomes could be observed at the epithelialization onset and healing time.

Due to the high prevalence of burns and their complications, such as infection or complications of existing treatments, it is better to take a drug which is more effective in a shorter period to improve the condition; this had to be more effective in terms of epithelialization and showing fewer side effects. This study aimed to reduce the days required for epithelialization and healing.

Sample calculation method

The samples size required for this study was 52 based on the sample size formula in intervention studies and comparing the mean of the two communities.

$$n = \frac{\left(z1 - \frac{a}{2} + z1 - B\right)^2 * (\sigma 1^2 + \sigma 2^2)}{d^2}$$

Blinding method

The wound were dressed with *Ajuga* or nitrofurazone cream by the researcher (physician) onto the patients' wounds who were unaware of the treatment. The two experienced specialists (those who evaluated wound appearance and the onset of epithelialization and wound healing) were blind to the type of drug and knew the treatments as A and B.

Randomization method

The simple randomization method was performed by lottery. To use the drug for a patient with one burn wound site, the lottery was held between drug A (nitrofurazone) and drug B (*Ajuga*). In patients with two burn wounds, if both were in the same limb or the upper and lower extremities, the drug was used for the upper wound lottery, and another drug was used for the lower wound. If the two wounds were on the same limb or surface, the lottery was drawn for the right wound and another drug was used for the left wound. People with more than one wound were included in the study having wounds in two different organs, or if they were on one organ, they were separated. This method was used to minimize the synergistic effect of drugs.

Statistical analysis

After collecting the statistical data, the results were recorded and analyzed as a questionnaire in SPSS, version 20, (SPSS, Inc., USA) software. This study used descriptive statistics and Kolmogorov-Smirnov (data normality test) test, T-test, and Mann-Whitney in normal and non-normal data to compare the two groups, respectively. Paired t-test was also used to compare the results before and after using the treatments, and a chi-square test was used to compare the qualitative data in two types of intervention. A p-value less than 0.05 was considered significant.

Results and Discussion

Results of the microbiological quality control of the cream were in agreement with WHO protocols [29].

In this study, 72 samples having second-degree burn wounds were included in the study. Fourteen samples were removed after starting the treatment due to traveling, lack of cooperation, dressing in another center due to the distance of Motahhari Hospital, and a positive pregnancy test result (Figure 1).

According to the findings, both groups were homogeneous in terms of age (p-value=0.375) (Table 1) and gender (p-value=0.622). There were 19 women and seven men in each group (Table 2). There was a statistically significant difference between the two types of drugs, according to the results of the onset of epithelialization, healing time, post-drug irritation, decreased irritation, post-drug pain, and pain reduction (Table 3).

By comparing the mean values of wound size in six measurements, Ajuga's average recovery time was expected to be two days less than nitrofurazone.

According to the Kolmogorov-Smirnov test, the onset of epithelialization and healing time was abnormal in both groups. Both groups were regular for post-drug irritation, primary irritation, reduced irritation, post-drug pain, primary pain, decreased pain.

Mann-Whitney test and T-test were used to compare the abnormal data, and normal data, respectively. Paired t-test was used to compare the groups before and after variables of post-drug burning, primary burning, post-drug pain, and primary pain. In each test, if the p-value was less than 0.05, it would be considered as significant.

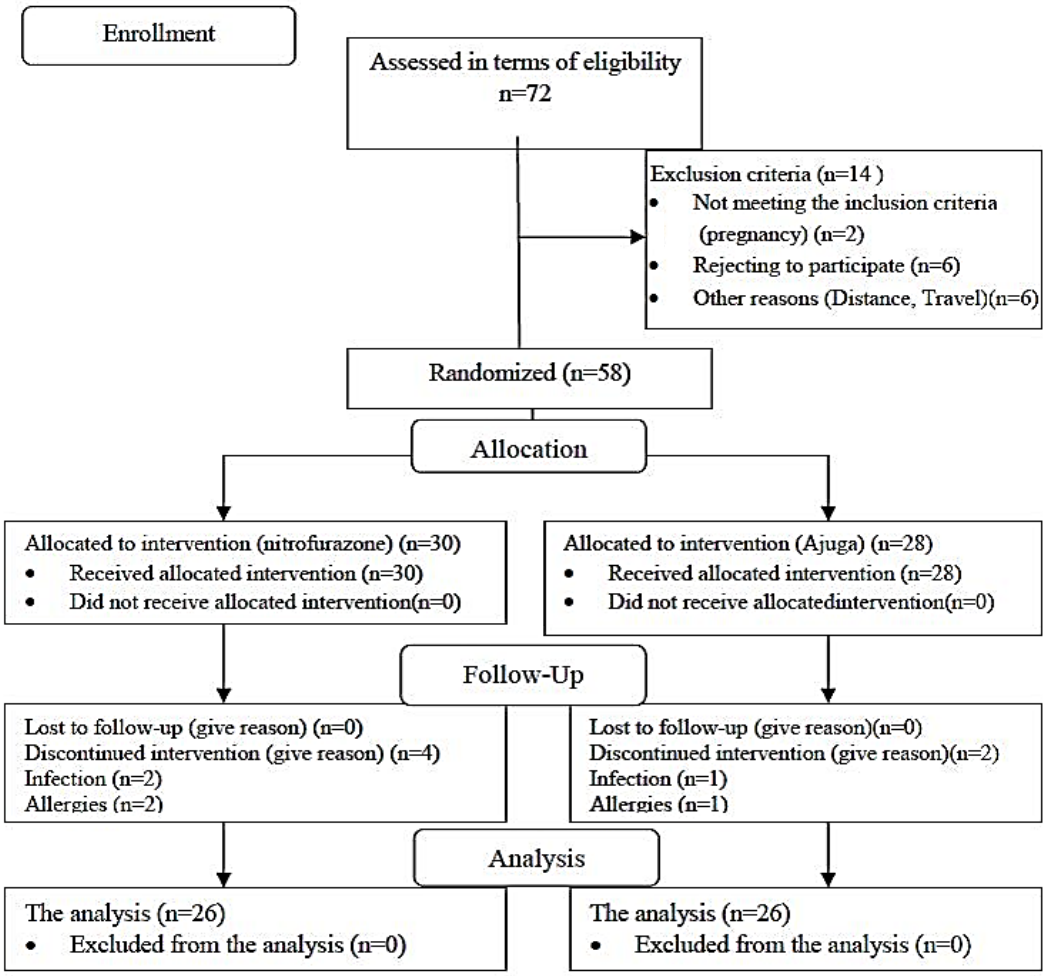

Figure 1. CONSORT flow diagram of the study

Table1. Comparison of age (year) in nitrofurazone group and *Ajuga* group

	Group statistics					
	Group	**N**	**Minimum**	**Maximum**	**Average ± SD**	**P-value**
Age	Nitrofurazone cream	26	19	59	37.76±2.41	0.375
	Ajuga cream	26	19	59	41.07±2.31	

Table 2. Comparison of gender of nitrofurazone group and *Ajuga* group

			Group		Total	P-value
			Nitrofurazone	***Ajuga***		
Gender	Woman	Count	19	19	38	0.622
		% Within Group	73.1%	73.1%	73.1%	
	Man	Count	7	7	14	
		% Within Group	26.9%	26.9%	26.9%	
Total		Count	26	26	52	

Table 3. Comparison of the epithelialization onset, healing Time, primary & post-drug irritation and pain of groups

	Group	**Average ± SD**	**P-value**
Onset of Epithelialization (Day)	A	7.31±0.10	0.007*
	B	6.92±0.09	
Healing time (Day)	A	10.19±0.13	0.001*
	B	9.54±0.13	
Post-drug irritation	A	2.85±0.26	0.007*
	B	2.00±0.20	
Primary irritation	A	5.50±0.21	0.325
	B	5.77±0.21	
Decreased irritation	A	2.69±0.22	<0.001*
	B	3.77±0.19	
Post-drug pain	A	3.92±0.27	0.018*
	B	2.96±0.18	
Primary pain	A	6.77±0.22	0.498
	B	6.92±0.17	
Decreased pain	A	2.85±0.22	0.001*
	B	3.96±0.22	

*: Significant difference ($p<0.05$); A: nitrofurazone cream; B: *Ajuga* cream; n=26 in each group; total: 52

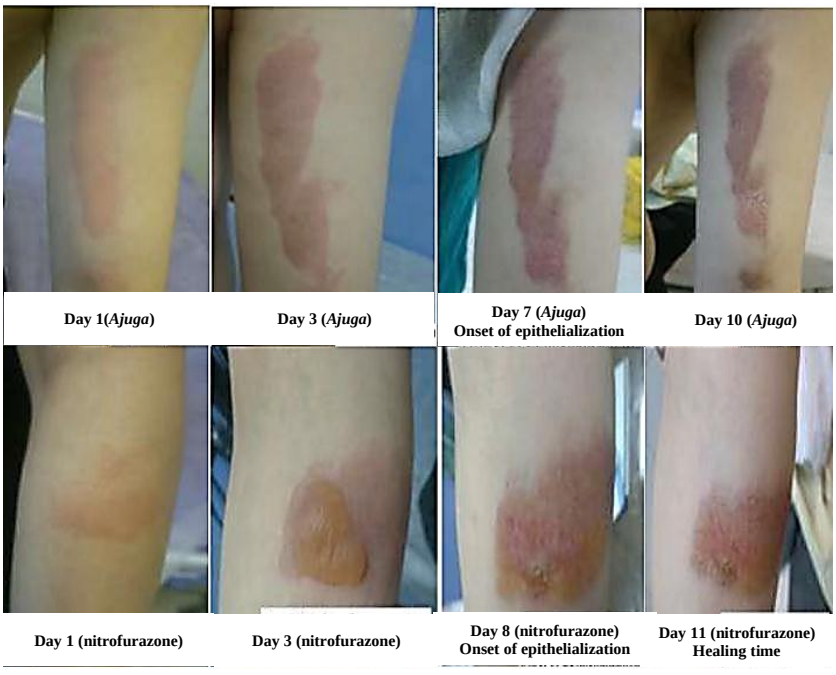

Figure 2. General appearance of burn wounds in *Ajuga* and nitrofurazone groups; a 30-years-old female with mixed (first and second) degree burn in right and left upper limbs

According to the results, decreased pain (p-value<0.001) and decreased irritation (p-value<0.001) were observed in patients after using drugs, but the effect of drug B was significantly more suitable compared to treatment A (Table 3).

Two cases from the control group and one case from *Ajuga* were excluded due to drug allergy. Two cases from the control group (7.6%) and one case from the *Ajuga* group (3.6%) were excluded due to wound infection and required other treatments; nineteen cases were women (73%) in both groups, and 7 were men (27%), (Figure 1).

Regarding the Bates-Jensen Wound Assessment Tool, the mean values of burn wound widths during the first, third, seventh, fourteenth, and start-up days of epithelialization, and the day of wound healing were compared; the wound size decreased over time and became significant. However, there were no significant differences between the two groups. Changes in the effect of treatment groups on the extent of burns over time were not significant.

Figure 2 shows two wounds in a 30-year-old woman that were dressed with *Ajuga* cream and nitrofurazone, respectively.

Adverse events: Two cases were removed from the nitrofurazone group, and one from *Ajuga* due to drug sensitivity. *Ajuga*'s sensitivity included itching, erythema, and redness around the wound, which appeared on the eighth day of dressing. Two cases from the nitrofurazone group and one from the *Ajuga* group were excluded from the study due to the infection of the wound site and the need to use other treatments.

The study ended with 52 cases, having 26 samples in each group (Figure 1). Eight patients had one wound and 22 patients had more than one wound. *Ajugachamaecistus* ssp. *tomentella* contains chemical compounds with different structures, including phytoecdysteroids and phenylpropane glycosides [30,31]. Due to its antioxidant [20,21,33], anti-microbial [14,21,33], and anti-inflammatory [18,22] effects, it can be a suitable option to accelerate wound healing.

Phytoecdysteroids have shown wound healing properties by stimulating the differentiation of Keratinocytes. The use of these compounds in treating superficial wounds and burns and psoriasis has also been suggested [34].

This study was performed cross-sectionally to consider the drug's therapeutic effect on epithelialization and healing time. Accordingly, it was difficult to conclude the ultimate effect of the drug on wound scarring. Another limitation was that the results of the present study were related to patients with second-degree burns. The use of this drug in patients with higher degrees of burns should be done with caution and sufficient awareness.

Conclusion

According to the results, the *Ajuga* cream (3%) can be a useful remedy for burn wounds due to the reduction in the onset of epithelialization, healing time, post-drug irritation, and pain. However, further large clinical trials are needed to confirm these results.

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Author contributions

Aysan Rahiminiya performed the wound healing study, data analysis and drafting of the manuscript; Mohammad Hossein Ayati and Leila Shirbeigi were involved in conception and design of the study; Seyed Hamid Salehi designed the study and evaluated the second grade burn wounds, onset of epithelialization , and wound healing; Seyyed Mohammad Bagher Fazljou reviewed the manuscript; Seyede Nargess Sadati Lamardi prepared herbal drug and was involved in critical revision; Hamideh Herizchi Ghadim was involved in designing the study, the acquisition and analysis of data, and critical revision.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

ssp: sub species; Syn: synonym; WHO: World Health Organization