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Clinical assessment of an ointment obtained from *Alkanna orientalis* root extract in the management of burn wounds: a pilot cross-sectional clinical trial

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ABSTRACT:

Alkanna orientalis is known as a wound healing herb in Persian medicine. This study aimed to evaluate the effect of the ointment prepared from the extract of A. orientalis roots on the treatment of grade 1 and 2 burn injuries. Methods: This study was performed over one year in Sina hospital of Tabriz University of Medical Sciences. Patients, based on inclusion criteria, were divided into two categories of control and intervention groups. The control group received the current protocol of burn care of Sina hospital, while the case group received the routine treatment protocol alongside the herbal formulation for burn injury. The parameters indicating the efficacy of the herbal ointment were studied in both groups. Results: this study was performed on 60 patients (30 males and 30 females). The results showed that the patients who received herbal ointment formulation experienced a shorter duration of treatment and better therapeutic response. Also, the application of herbal formulation prevented the progression of wounds to higher grades. The duration of epithelial layer formation at the wound bed in control and intervention groups was 6.5±0.2 and 3.4±0.3 days (P-value<0.05), respectively. Successively, the rate of complete recovery of wound in control and intervention groups was 46.7% and 73.3% (P-value<0.05). The herbal ointment accelerated the recovery by 3.1±0.1 days (P-value<0.05). Conclusion: the results of this pilot study illustrated the efficacy of the prepared formulation on burn injuries which could be used alongside the conventional treatment protocol of burn care.

KEYWORDS: Alkanna orientalis; burn; wound healing; ointment; herbal extract .

1. INTRODUCTION

Burns are skin or other tissue injuries triggered by extreme heat, electricity, radiation, and chemical agents, etc. Consequently, the cells of the skin or other tissues are damaged. Burn injuries are considered a global problem since they give rise to 180000 mortalities annually. Clinics' high burn management cost imposes an economic burden on nations, especially low-income countries [1].

Burns fall into four categories based on the depth of injuries; superficial or first-degree burns, which involve the outer layer of skin or epidermis; second-degree burns involve outer and lower layers of skin, namely epidermis and dermis; and third and fourth-degree burns damage deep layers of skin and tissues [2]. Burn injuries and their care could be challenging and burdensome since the process of wound healing is generally low. Burn wound care is a multidisciplinary approach as reduces inflammation, infection prevention, and

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proliferation [3]. The ultimate goal of burn management and treatment is to repair and replace damaged tissue. The first stage of a treatment strategy for a burn injury is first aid; firstly, debridement is exercised, and the wound is cleansed by specific solutions to prevent infection. Secondly, antibiotic creams, such as silver sulfadiazine, mafenide acetate, and silver nitrate, are applied to wounds. Thirdly, the injury is dressed with gauze which is repeated daily until the healing phase [4].

Since mentioned topical creams cause several complications, several natural medicaments are widely evaluated to substitute creams mentioned above. Herbal products and medicaments are proper candidates owing to being cost-effective and less toxic. They could be extracted from traditional medicine manuscripts or herbs used by indigenous people for centuries.

Among suggested wound healing herbs, Alkanna species (Boraginaceae), known as "Shengar" in Persian, includes 50 species traditionally used to manage burn injuries. They wildly grow in the Mediterranean and Eastern Asia regions. This herb was applied to treat burn injuries from ancient times. It is traditionally professed to treat burn injuries, anal ulcers, hemorrhoids, infected and oozing wounds, crusts, bedsores, and dermatitis. Biologically active ingredients of plants belonging to this genus are responsible for wound healing effects. They include phenolic compounds such as tannins, flavonoids, and naphthoquinones. The mentioned ingredients have shown anti-inflammatory, anti-bacterial, anti-fungal, anti-oxidant, and wound healing activities [5]. Although these components act collaboratively to indicate an anti-ulcerogenic effect, naphthoquinones of Alkanna, alkannin and shikonin (hydroxyl naphthoquinones) are known as significant and potent phytoconstituents of the root of the plant [6].

Alkanna orientalis (L) Boiss. is a perennial herb endemic to Iran with the Persian name "Shengar sharghi". It was widely applied in Persian medicine to prepare medicaments for ulcer dressings. Although some research was conducted to reveal the pharmacological activities of A. orientalis [6, 7], to the best of our knowledge, there was no study to evaluate its effect on burns management. Thus, this study aimed to prepare an ointment from chloroform extract of roots of *A.orientalis* and evaluate its effects on burn injuries.

2. RESULTS

2.1. The results of evaluating the keratolytic activity of chloroform extract

The study of microscopic images indicated that immersion of feathers in 20% chloroform extract did not result in decomposition, while the feather soaked in the positive control (TCA) was completely decomposed.A clear zone was not observed around disks soaked in 20% chloroform extract and negative control in a culture medium containing keratin. In contrast, a clear zone could be observed around the disk of positive control (TGA).

2.2. The results of microbial tests

Max acceptable counts of TAMC and TYMC were 100 and 10 (CFU/ml), respectively, which were within an acceptable range according to USP guidelines.

2.3. Profile of patients in control and intervention groups

This study was carried out on 60 patients suffering from first and second-degree burns, belonging to both sexes (30 males and 30 females). In the control group, 43.3% of patients and in the intervention group, 56.7% of patients were female. As it is apparent in Table 1, there is no significant difference between patients of control and intervention groups in terms of gender, degree of burn injuries, percentage of burning area, age, weight, and underlying conditions.

Table 1. Profile of patients in study and intervention groups

Characteristics	Control	Treatment	P-value[1]
Gender			
Male	13	17	0.44
Female	17	13	
Average age (year)	39.7±2.5	43.7±2.7	0.29
Average weight (kg)	74.4±2.3	72.0±1.9	0.43
Wound degree			

First degree	3 (10%)	1 (3.3%)	0.61
Second degree	27 (90%)	29 (96.7%)	
Percentage of burning area (cm^{-2})	10.0±1.1	7.3±0.6	0.14
Underlying conditions			
None	18	13	
Hypertension	1	2	
Diabetes	2	2	
Anxiety	1	1	
PCO[2]	1	1	
Migraine	0	1	
Epilepsy	1	1	
Osteoarthritis	1	1	
Algeria	0	1	
Low blood pressure	0	1	
Mental retardation	0	1	
Fatty liver	1	1	
GERD[3]	0	1	
Paralysis	0	1	
Artificial heart valve	0	1	
Chronic heart disease	1	0	
Asthma and COPD[4]	2	0	
Drug addiction	1	0	
Endocrine disease	0	1	

[1] P-value <0.05 was considered statistically significant
[2] Poly Cystic Ovary
[3] Gastroesophageal reflux disease
[4] Chronic Obstructive Pulmonary Disease

2.4. Comparison of control and intervention groups

Table 2 indicates no statistically significant difference between two groups regarding topically-applied medicines. Table 3 compares two groups in terms of the parameters indicating the efficacy of herbal ointment. Figure 1 and 2 indicate the causes and the burn sites in two groups, respectively. As it is apparent from Figure 1, the main cause of burning in this study was boiling water and contact with a hot object. Figure 2 illustrates

Table 2. Comparison of two groups in terms of applying for topical medicines.

Drugs	Groups	Control	Intervention	P-value[1]
Silver Sulfadiazine		13	15	0.88
Mefenide		10	8	
Tetracycline		3	4	
Mupirocin		4	3	

[1]P-value<0.05 was considered statistically significant.

Table 3. Comparison of control and intervention groups in terms of the parameters indicating the efficacy of the herbal preparation.

Assessment of recovery	**Control**	**Intervention**	**P-value[1]**
Surface area (cm^{-1})	10.0±1.1	7.3±0.6	0.14
Complete recovery (%)	46.7	73.3	0.035
Mean hours of referral to hospital	35.1±14.4	104.1±33.0	0.001
Mean days of the formation of epithelium layer	6.5±0.2	3.4±0.3	0.000
Wound growth to higher stages (%)	20	0	0.024

[1]P-value<0.05 was considered statistically significant.

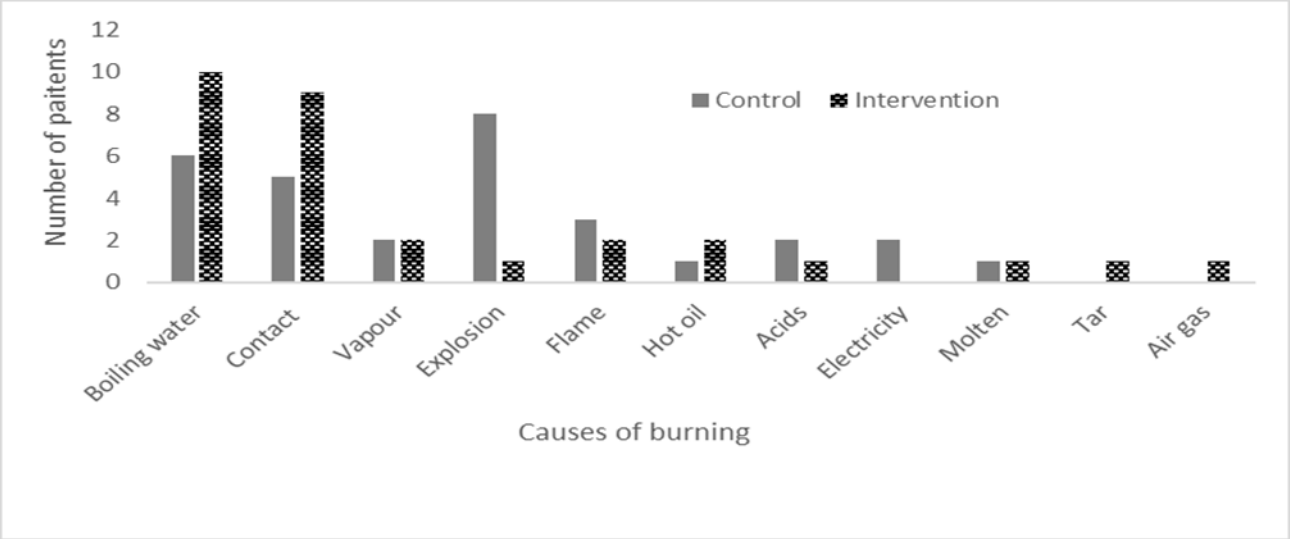

Figure 1. Comparison of causes of burning in two groups.

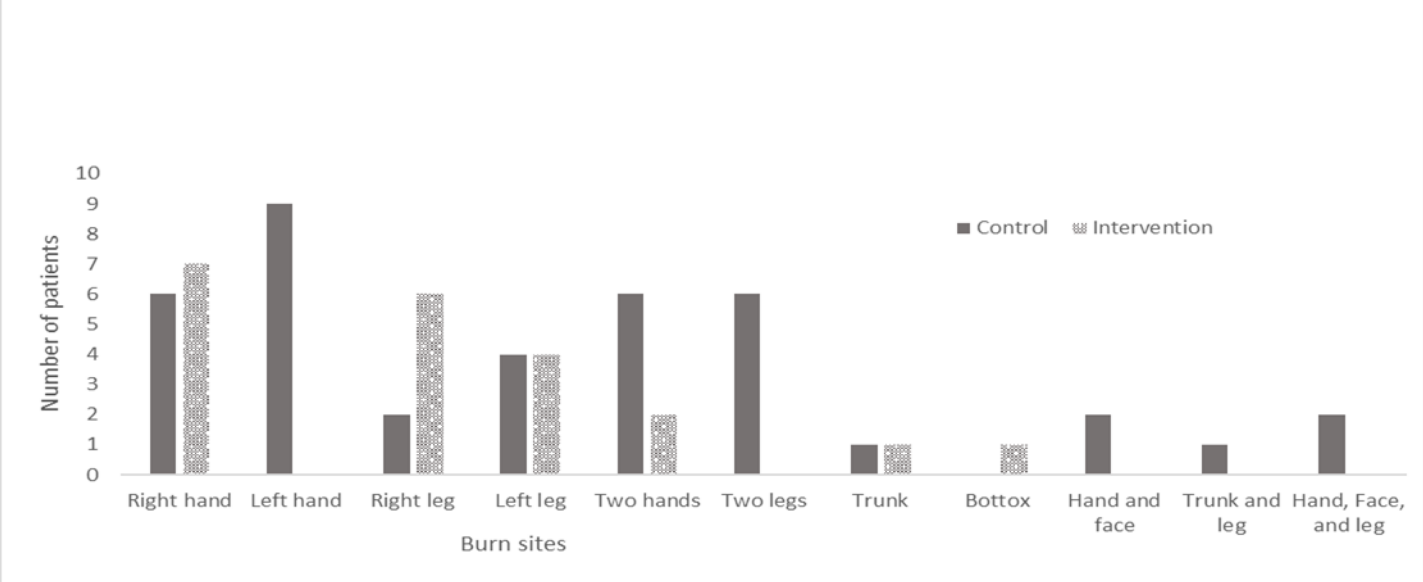

Figure 2. Comparison of burn sites in two groups.

3. DISCUSSION

In ethnomedicine, several herbal preparations are applied to cure ulcers. Therefore, it is important to ascertain their efficacy and validity through well-conducted experimental models. The wound healing activity of the different *Alkanna* species was validated in modern medicine. However, this is the first clinical trial to validate the effect of herbal ointment prepared from the root extract of this herb on burn injuries. Previous studies have indicated the bacteriostatic and/or bactericidal effects of *Alkanna orientalis* root extract against pathogenic bacteria [12]. In prior studies, the wound healing effect of extracts obtained from different *Alkanna* species was confirmed by various experimental models. Hatipoglu et al. conducted research applying 16% extract of *A. tinctoria* solved in olive oil on burn wounds of rabbits. The results depicted acceptable dermal and epidermal junctions; however, severe burns did not respond to the mentioned medicament [13]. As it seemed, the formulations containing less than 16% Alkanna extract did not show adequate efficacy. Another experiment was planned to assess the healing effect of dressing containing *A. tinctoria* extract, olive oil, and beeswax on second-degree burn wounds. The results of this study indicated that the mentioned dressing could significantly accelerate epithelization, decrease the pain while changing the dressing, and lower the duration of hospitalization compared to the control group who received the routine dressing at the hospital [14]. Also,

research was conducted to compare the effect of the ointment prepared from the root of A.tinctoria and vaseline on cutaneous wounds of rats. The results revealed the significant anti-inflammatory effects of the herbal ointment, which reduced the intensity and severity of inflammation in wounds and assisted in wound healing [15].

In this study, the patients in the control group received the conventional treatment protocol of Sina hospital, including washing the burn injury with normal saline and applying the topical medicines of mupirocin, tetracycline, mafenide, and silver sulfadiazine. In the intervention group, herbal ointment was included in the current treatment protocol. According to the results of this study, the patients had no significant difference in terms of gender, age, weight, and stage of burn wounds. However, at the trial's termination, the duration of formation of epithelial layer was significantly shorter in the intervention group. The required days for epithelium formation were 6.5±0.2 and 3.4±0.3 (P-value<0.05) in the control and intervention groups, respectively. Furthermore, the complete recovery rate was 46.7% and 73.3% (P-value<0.05) in the control and intervention groups, successively. Thus, applying herbal ointment of A.orientalis accelerated the complete recovery by 3.1±0.1days and improved treatment process by 26.6% (P-value<0.05). Moreover, in contrast, in the control group, the burn wounds did not develop to upper degrees in intervention group.

The effects mentioned above of A.orientalis root extract could be attributed to the natural enantiomeric naphthoquinones, alkannin, and shikonin. The pharmacological effects of these pigments could be ascribed to the prevention of bacterial topoisomerase and DNA gyrase, which leads to anti-bacterial activity [16]. Hence, they could inhibit the synthesis of prostaglandins bringing about the analgesic effects [17]. Therefore, this herb's anti-bacterial and anti-inflammatory effects could justify this plant's wound-healing activity.

4. CONCLUSION

This herbal formulation accelerated the formation of epithelia, reduced the duration of complete recovery, and improved the process of recovery by preventing the progression of burn injuries to higher degrees. Including this formulation could assist in bettering the treatment of burn injuries.

5. MATERIALS AND METHODS

5.1. Collection of herb, extraction, and quantitative determination of the extract

The roots of *Alkana orientalis* (L.) Tausch (Boraginaceae) were gathered from Khodaafarin region, East Azarbaijan, Iran, early in the spring. The voucher specimen (TbzMed-FPh 4003) was deposited in the herbarium of the faculty of pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran. The dried, cleaned, and powdered roots were extracted by soxhlet apparatus using chloroform as a solvent within 14 hours. In the next step, prepared extract was filtered, and a rotary evaporator was employed for the evaporation of the solvent at 45°C (Figure 3). Quantitative determination of alkannin/shikonin derivatives was performed using a UV–Visible spectrophotometer (Shimadzu UV-1650 PC) at 620 nm. Shikonin was used as a standard solution.

Figure 3. The chloroform extract of *Alkanna orientalis* roots.

5.2. Extraction of keratin from chicken feather

For the extraction of keratin from chicken feather Wawrzkiewicz et al. method was employed. Firstly, 1 g of the chicken feather was suspended in DMSO. Afterward, the mixture was heated at a temperature of 100°C for 1 hour. Cold acetone (-20°C) was gradually added to the mixture to precipitate keratin in DMSO. Next, the solution was filtered with filter paper to separate the feathers. The keratin gathered on filter paper was washed with phosphate buffer and collected. The solution was added to 10% TCA (Tri Chloric Acetic acid) and incubated at four °C for 24h to separate keratin from acetone. The remaining keratin in microtubes was centrifuged for 10min at 8000 rpm and at a temperature of 4°C for 20 min. The residue was twice washed with distilled water, suspended in phosphate buffer (0.1M, pH=7), and lyophilized [8,9].

5.3. Assessment of extract effect on feather

This experiment was designed to evaluate the microscopic effects of the extract on skin keratin. A feather was immersed in a solution comprising 10cc extract, 1cc DMSO, and 99 cc phosphate buffer for 24 hours. The positive control solution was five cc TGA solved in a 45 cc phosphate buffer. The blank container was comprised of feather immersed in distilled water.

5.4. Study of extract effect on culture medium containing keratin

Initially, 0.25 g peptone, 0.15 g yeast extract, and 0.6 g agar were mixed and solved in 45 cc phosphate buffer (0.1M), and heated up to boiling point. Before completely cooling the solution, 600 mg lyophilized keratin was solved in a five cc phosphate buffer, and the solution was homogenously added to the plates. Afterward, 20mg of extract was solved in 20cc acetone (1mg/ml). Disks were categorized into three groups; disks immersed in extract solution, soaked in phosphate buffer 0.1M (negative control), and disks immersed in TGA solution (positive control). Plate's surface was divided into three regions to place disks. Finally, the plates were incubated at 37°C for 24h. After 24h, clearing zones were measured and compared with zones of positive and negative control [8,9].

5.5. Fabrication of the ointment

For the preparation of 20% ointment, 6 g of CERAO was solved in 3 g of transcutol. Afterward, the mixture was heated to 30°C till a transparent solution was prepared. Next, 18 g of white petroleum and 3 g (10%) of stearyl alcohol were added to the solvent, mixed, and heated up to 80°C till the homogenous ointment was obtained.

5.6. Stability, microbial limit test, and packaging

Initially, the product was prepared to perform a microbial limit test in which 1g of ointment was mixed with 9cc of 80% polysorbate and heated up to 40°C until a homogenous emulsion was obtained. First, the ointment was solved in polysorbate 80 to obtain a well-dispersed emulsion. Then, one cc of the prepared sample was mixed with soybean casein digest agar on a plate. Next, the prepared product was assessed to monitor pathogenic species of Pseudomonas aeruginosa and Staphylococcus aureus. For monitoring S. aeruginosa, 10cc of the product was added to the culture medium of Soybean Casein Digest Agar (SCDA) and incubated at 35°C for 24h. After that incubation period, the bacterial colonies were added to the selective medium of Cetrimide Agar and incubated at 35°C for 24h. For monitoring S. aureus, 10cc of the product was added to the culture medium of SCDA and incubated at 35°C for 24h. Afterward, the bacterial colonies were added to the selective medium of Mannitol Salt Agar and incubated at 35°C for 24h.

Physical stability testing was performed to assess the effect of environmental factors on the quality of the product. The ointments were retained at three varied temperatures of 25°C (room temperature), 4°C (refrigerator temperature), and 40°C (oven temperature) for one month. The optimum physical stability was observed below 25°C. Ultimately, the ointments were packaged in 30g aluminum tubes [10].

5.7. Study setting and population

This clinical trial was a pilot single-center study that enrolled 60 patients diagnosed with first or second-degree burn injuries. The patients were randomly assigned to intervention or control groups (30 patients in each group) in Sina hospital, Tabriz, Iran, during Aug 2017 to Aug 2018. Initially, the patients' history was recorded, and their general health was evaluated. Then, the degree of the burn was determined according to the scoring system indicated in Table 4[11]. The control group received the routine burn treatment protocol of Sina hospital, including applying topical mupirocin, tetracycline, mafenide, and silver sulfadiazine. In the intervention group, the patients received the herbal ointment plus the routine treatment, daily.

The study duration was six months, and the efficacy of the herbal ointment was evaluated weekly for two months, every two weeks for four months, and monthly for six months. The ethics Committee of Tabriz University of Medical Sciences approved the protocol of this study (IR.TBZMED.REC.1395.1054). The work described has been performed under the Code of Ethics of the World Medical Association (Declaration of Helsinki). The study protocol was declared to the study population, and the volunteers obtained a written consent form. Subjects included in this study were males and females, 18 years of age and older, presenting burning wounds of first and second-degree. The exclusion criteria were pregnancy and lactation, organ failure, corticosteroid therapy, weakened or suppressed immune system, and those aged under 18.

Table 4. Classification of burn wounds according to the depth of injury.

Burning degree	Depth of injury	Appearance of burn site	Sensation	Duration of healing
First-degree	Epidermis	Redness, dryness, blanch with pressure	Painful	3-6 days
Second-degree	Superficial and deep partial thickness	Blisters, moist, red, weeping, blanches with pressure,	Painful to temperature and air and touch	7-21 days usually requires surgical treatment
Third-degree	Full-thickness	No blanching with pressure, Waxy white to leathery gray to charred and black, Dry and inelastic	Deep pressure only	Rare, unless surgically treated
Fourth-degree	Deeper injury	Extends into fascia and/or muscle	Deep pressure only	Rare, unless surgically treated

5.8. Efficacy assessment

Since wound healing process is dynamic and might be switched to deeper worse stages, efficacy assessment was thoroughly performed by considering the complete recovery and closure of the wound area (area of ulcer surface), the mean hours passed before hospital admission, the mean number of days spent for the generation

of new epithelial tissue, and the percentage of wound progression to higher (worse) stages. Also swab test results was also included in our evaluation.

5.9. Statistical analysis

Values of this study were expressed by descriptive statuses; Mean±SD, frequency, and percentage. Fisher's exact and chi-square tests were used to compare qualitative values. In addition, the Kolmogorov-Smirnov test was applied to evaluate whether data comes from a normal distribution. For the comparison of quantitative values with normal distribution, a parametric test (t-test) was used, and for data with not-normal distribution, non-parametric test (U-Mann Whitney) was applied. P-values less than 0.05 were considered statistically significant. SPSS version 20 software was used for statistical analysis.

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