

Partial-thickness burn wounds healing by topical treatment

A randomized controlled comparison between silver sulfadiazine and centiderm

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Abstract

Background: Burns are common event and associated with a high incidence of death, disability, and high costs. *Centella asiatica* (L.) is a medicinal herb, commonly growing in humid areas in several tropical countries that improve wound healing. On the basis of previous studies, we compared the efficacy of Centiderm versus silver sulfadiazine (SSD) in partial thickness burning patients.

Methods: Study population comprised burn victims referred to Velayat Burning Hospital at Rasht, Iran. The intervention group received Centiderm and control group SSD cream. Burn wounds were treated once daily at home. All of the wounds were evaluated till complete healing occurred and at the admission, days 3, 7, 14 objective signs; visual acuity score (VAS) and subjective signs were recorded. Re-epithelialization time and complete healing days were recorded. We used random fixed block for randomization. The randomization sequence was created using the computer. Patients and burning specialist physician were blinded.

Results: Seventy-five patients randomized into 2 groups; (40 patients: Centiderm group; 35 patients: SSD group). The mean age of them was 30.67 ± 9.91 years and 19 of them were male (31.7%). Thirty patients in Centiderm and 30 patients in SSD group were analyzed. All of objective and subjective signs and mean of re-epithelialization and complete healing were significantly better in Centiderm group rather than SSD group ($P < 0.05$). There was no infection in Centiderm group.

Conclusions: We showed that use of Centiderm ointment not only improved the objective and subjective signs in less than 3 days, but also the re-epithelialization and complete healing rather than SSD without any infection in the subjects.

Abbreviations: %W/V = Percent Weight/Volume, $\mu\text{g/mL}$ = microgram per milliliter, eNOS = Endothelial nitric oxide synthase, iNOS = Inducible nitric oxide synthase, IRCT = Iranian Clinical Trial, MCP-1 = monocyte chemoattractant protein-1, mRNA = messenger ribonucleic acid, SEM = standard error of the mean, SSD = Silver Sulfadiazine, TBSA = total body surface area, TECA = titrated extract of *Centella asiatica*, TGF- β = Transforming growth factor beta, TIMP1 = tissue inhibitors of metalloproteinases-1, VAS = visual acuity score, VEGF = Vascular endothelial growth factor, VSS = Vancouver Scar Scale, WHO = World Health Organization.

Keywords: burning, *Centella asiatica*, partial thickness, silver sulfadiazine, wound

Editor: Thomas Biedermann.

Trial Registration: IRCT2013100614915N1

Funding/support: Funding for this study was provided by Guilan University of Medical Sciences.

The authors report no conflicts of interest.

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Medicine (2017) 96:9(e6168)

Received: 28 December 2015 / Received in final form: 24 January 2017 /

Accepted: 30 January 2017

http://dx.doi.org/10.1097/MD.00000000000006168

1. Introduction

Burn is still one of the emergency medicine affecting both genders and all age groups in both developed and developing countries leading to physical and psychological disabilities with an increasing trend in mortality and morbidity during pregnancy.^[1,2] Burns are a common event and are associated with a high incidence of death and disability, several surgery operations, prolonging of hospitalization, rehabilitation, and high health care costs.^[3] The second-degree burns are one of the most frequent burning in homes needed improvement to treatment.^[4] The incidence of burning in developing countries is more than developed countries. Burning is a chief cause of mortality worldwide.^[5] Healing is divided into 4 main parts: formation of granulation tissue, collagen deposition, re-epithelialization, and contraction.^[6,7] For many years, partial-thickness burns have treated by daily washing and cleaning of the wound, followed by a topical application of the antimicrobial cream. However, the pain suffered and damaged wound healing remain problems to be addressed. Superficial partial-thickness burns often heal in 3 weeks.^[8,9] In topical burn therapy, silver sulfadiazine was introduced as the gold standard having antibacterial properties.^[10] However, it has some disadvantages, including retardation of wound contracture, delayed and incomplete epithelialization, generation of black scars, limited penetration

the depth of the wound, hypersensitivity, neutropenia, silver toxicity, the inaccurate assessment of healing progress because of the presence of a layer of the slough, ineffectiveness against some microorganisms, and thrombocytopenia.^[11] Other topical antibiotics such as mafenide acetate and silver nitrate are also associated with side effects such as pain and hypersensitivity at the wound site, delayed wound healing, electrolytic imbalance, and hemoglobinemia.^[12,13] Currently, silver-foam dressings are being the gold standard used to manage the bio-burden of a wound, with or without a silicone skin interface such as Acticoat and Mepitel.^[14]

There are many reports that have confirmed the use of medicinal plants for dressing wounds described by traditional medicine.^[15] *Centella asiatica* (L.) is a Stolonerous constant herb, commonly growing in humid areas in several tropical countries. In Asia, it has long been used in traditional medicine in the treatment of different diseases. This plant is commonly found in many parts of the world,^[16–18] including Asia and the Middle East.^[19] In Iran, it just found in a local wetland at Bandar-Anzali and it is growing and blossoms in April, May, and June.^[20] Extracts and active constituents are found in many herbal drugs and cosmetic preparations worldwide, especially for venous circulation and skin care.^[21,22] The main active principles of *C. asiatica* are triterpene glycosides and their respective aglycones.^[23] *C. asiatica* contains triterpene glycosides such as centellasaponin, alkaloids, volatile and flavonoids compounds, steroids, asiaticoside, scelefoleoside, phenolic acid, and madecassic acid.^[24,25] Asiaticoside is the most major triterpene glycoside in the water extract and it is transformed in vivo by hydrolysis into asiatic acid, which was shown to be effective in induction of apoptosis in different types of cancer.^[26] The therapeutic substances in *C. asiatica* are saponin-containing triterpene acids and their sugar esters, of which asiatic acid, madecassic acid, and asiaticosides are considered to be the most important.^[27] It has been reported that 1% *C. asiatica* extract cream improves wound healing of chronic ulcer in width, length, and depth after 7, 14, and 21 days of use of the product.^[28] Phytoconstituents present in *C. asiatica* have been found to be responsible for these wound healing properties. Asiaticoside isolated from *C. asiatica* increased hydroxyproline content, tensile strength, collagen content, and epithelialization in a punch wound model.^[29] Further, triterpenes from *C. asiatica* were shown to increase remodeling of the collagen matrix and stimulate glycosaminoglycan synthesis in a rat wound chamber model.^[30] Oral administration of madecassoside isolated from *C. asiatica* herbs promoted burn wound healing in mice through its antioxidative effect, collagen synthesis, and angiogenesis.^[31] All extracts of *C. asiatica* promote the wound healing in both incisional and burn wounds. Asiatic acid in the ethyl acetate extract is the most active ingredient for healing the wound. The extracts of this plant have antibacterial effects, too.^[32–34] The final purpose in burn management is promotion of wound healing and epithelialization as soon as possible to avoid infection and lesser side effects. Given the adverse effects of currently used topical drugs, innovation of novel topical agents that could effectively and safely promote wound healing and relieve the associated pain is desirable.^[35]

On the basis of the previously studies that showed promoting healing in animal studies for burn and surgical wounds and antibacterial effects of *C. asiatica*, in this study, we compared the efficacy of Centiderm versus SSD in partial thickness burning patients referred to Velayat Hospital _referral hospital of Guilan province in Northern_ Rasht, Iran.

2. Methods

2.1. Participants

Our study population comprised burn victims who were treated in Velayat Burning Hospital at Rasht, Iran. Period of study was from October 2014 to February 2015.

2.2. Eligibility criteria

The inclusion criteria were partial thickness, burning wound less than 10% of total body surface area (TBSA) and in the limbs, burning event was fewer than 48 hours, no other concurrent injury except burning, general physical and mental health, between 14 and 60 years old.

Exclusion criteria were the existence of any cerebrovascular disease, cardiovascular disease, concurrent endocrine, hepatic or renal disease, pregnancy, history of alcohol or drug abuse, concurrent use of antibiotic.

A total of 134 patients with second-degree burn wounds on their limbs were referred to Velayat Hospital emergency ward. Seventy-five patients had the eligibility and entered the study, 35 of them in SSD group and 40 of them in Centiderm group. Fifteen patients have excluded the study because of lost follow-up and incomplete data. Thirty patients in both groups were evaluated. One burning specialist physician evaluated the patients at admission and chose and classified them on the basis of inclusion criteria and ruling out the exclusion criteria based on scientific definitions of partial thickness burning wounds.^[36] Bedside clinical evaluation was used to assess burn wound depth based on the subjective assessment of visual and tactile characteristics of a burn wound, namely wound appearance, capillary blanching and refill, capillary staining, and burn wound sensibility to light touch and pinprick.^[37] Lund and Browder's chart were used for evaluating TBSA.^[38] If the wounds were mixed superficial, deep and full thickness, they were excluded from the study because of not confounding the results.

2.3. Interventions

The intervention group subjected to use topical Centiderm ointment. The control group used silver sulfadiazine 1% cream (routine treatment) on the area of burning wound. Burn wounds were treated once daily in the home after getting information about how to use the drugs. Equal shape and boxes of ointment were used in both groups. Patient compliance was checked at the first visit after inclusion on the basis of jobs and ability to referring to the hospital for follow-up. After enrolling patients in the study, they were given a remembering checklist for using the drugs at home. They brought the remember checklist in follow-up sessions. Also, for checking true use, we called them during the period they used the ointment, and in every visit, they showed the drug boxes to make confidence true using.

The herbal product was Centiderm ointment that was a derivative of *C. asiatica*. This product is cheaper than SSD. The formulation of ointment was made by pharmacognosist of the pharmacy faculty in Guilan University of Medical Sciences and confirmed by the University and registered and licensed as a drug patent in Iranian Intellectual Property Office (Patents) with the registration number of 86035 and international classification patent of A61K36/00; A61J3/04.^[39]

The fresh leaves of *C. asiatica* were collected in May 2014 from a local wetland at Bandar-Anzali, Iran. The accuracy of plant selection was proved and authenticated by Mr. Asaadi, a senior

botanist of the Department of Botany, University of Guilan, with adopting the scientific herbarium information. Fresh leaves of the plant were air-dried at 40°C and ground to powder by mixer. Then, they were subjected to exhaustive extraction using ethanol (96%) and distilled water with the relation of 60:40. The dark green liquid extract was concentrated under rotary evaporator and vacuum. The resulting dried extract was partitioned between butanol and petroleum ether. It made 2 separate portions in which Butanol fraction was concentrated under rotary evaporator and vacuum. Dried butanol fraction was mixed with Vaseline and Glycerin to make the emulsified final based contained about 3% of the final extract which named Centiderm ointment. All chemical ingredients were purchased from Merck Co., Germany. The last formulation was tested physically and chemically to select the most stable and desirable product thermodynamically or physiologically. Samples were sterilized by gamma radiation.

2.4. Pharmacokinetic and safety

C. asiatica is in World Health Organization (WHO) medicinal plants monographs and its safety and pharmacokinetic is validated there.^[46] It contains several active constituents, of which the most important are the triterpenoid saponins, including asiaticoside, centelloside, madecassoside, and asiatic acid. In addition, it contains other components, including volatile oils, flavonoids, tannins, phytosterols, amino acids, and sugars.^[46] A pharmacokinetic study suggests that the active ingredients in total triterpenoid fraction of *C. asiatica* are well absorbed in human volunteers. After single oral administration of 30 and 60 mg of the extract, maximum plasma levels of asiatic acid were reached at 4.5 and 4.2 hours, respectively. Plasma half-lives were 2.2 hours in the 30-mg dose and 3.4 hours in the 60-mg dose, with no detectable levels of the saponin present 24 hours after single dosing. Seven-day treatment with the herb at the same dosing schedule resulted in higher peak plasma concentrations, longer half-lives, and greater area-under-the curve values,^[47] which ensured its external usage safety. Its external use is validated by European Committee on Herbal Medicinal Products. In this committee, reports about *C. asiatica* mentioned that the cream 1% for cutaneous use is authorized in Belgium since 1969 for the treatment of moderate or benign problems in wound formation such as atonic wounds, hypertrophic scars, keloids in active phase, in France, since 1975 to aid in the local treatment for cutaneous ulcerations and since 2000 to aid in the local treatment for granulation phase of wounds, cutaneous ulcers, and cutaneous gangrene. The ointment 1% is used in Greece as a potent wound healing agent (induce collagen biosynthesis).^[48]

C. asiatica is an important medicinal herb that is widely used in traditional medicine. The safety of this plant extracts mentioned in many medical references and clinical trials. Therefore, using this product in our research based on such references is safe.^[40–44] We carried out the determination of total triterpenoid content for the product (Centiderm) during the research and findings showed that the total triterpenoid content of this product is constant during the trial. The content of triterpenoids obtained by the aforementioned method was determined according to the study by Lu et al,^[45] with a slight modification and then expressed as milligrams ursolic acid equivalent/gram dry weight. Briefly, after a 200- μ L sample solution in a 10-mL volumetric flask was heated to evaporation in a water-bath, 1 mL new mixed 5% (W/V) vanillin-acetic solution and 1.8 mL sulfuric acid was added, mixed, and incubated at 70°C for 30 minutes. Then, the mixed solution was cooled and diluted to 10 mL with acetic acid. The

absorbance was measured at 573 nm against blank using a spectrophotometer. The blank consisted of all reagents and solvents without sample solution. The content was determined using the standard ursolic acid calibration curve. The calibration equation for ursolic acid was $Y=0.0725X - 0.0207$ ($R^2=0.9991$), in which Y was the absorbance value; X was the concentration of ursolic acid (μ g/mL). The linear range of ursolic acid was 1 to 10 μ g/mL.

The patients were requested to inform investigators about any adverse events or complaint during the trial. If there were any symptoms, they were checked and recorded at the beginning and at each baseline visit. Also, possible side effects were checked and recorded via telephone call every day and the physician was responsible for continuing or discontinuing the drugs. The adverse effects check list was completed by independent raters.

2.5. Objectives & outcomes

We evaluated the burn wounds based on objective and subjective criteria in both groups and our hypothesis was that burning wound in Centiderm group cannot heal them better and faster than the SSD group. All the wounds were evaluated at the admission to the emergency ward of the hospital by objective signs, including burning wound VSS score (Pliability, Pigmentation, Height, Vascularity of the wound), VAS, Subjective criteria (Itching, Dryness, and Irritation) and re-epithelialization, complete healing, and existence of infection^[49] by 1 burning specialist physician. Re-epithelialization was defined as noting the number of days required for the Escher to fall off from the burn wound surface without leaving a raw wound behind in examination.^[50,51] The patients followed up till complete healing daily with a burning specialist physician, and on days 3, 7, 14, the subjective and objective variables and VAS were recorded. Also, the pathological changes, for example, granulation tissue formation and re-epithelialization in wounds and their comparison with the normal tissue part were inspected medically by him. Patients were visited daily for compliance of drug consumption and evaluation of re-epithelialization and probable wound infection till complete healing of wound reached and the day of finishing re-epithelialization and complete wound healing were recorded for each patient. We evaluated patients in all of the days (even weekends) during the week on the basis of remember chart.

2.6. Sample size

For the purpose of sample size calculation, the difference in duration of treatment for complete wound healing was considered as the primary outcome measure. It was calculated that 30 subjects would be required per group in order to detect a difference of 5 days in this parameter with 80% power and 5% probability of type I error. This calculation assumed a standard deviation of 1.5 days for the complete wound healing feature.

2.7. Randomization

The study was designed as a prospective, parallel group, randomized controlled trial. We used the foursome random fixed block for randomization. After allocation of patients into 2 different groups, SSD and Centiderm ointment were administered topically once a day. Seventy-five subjects were randomized of whom data were analyzed for 30 patients treated with 1% SSD cream and 30 patients treated with Centiderm. The sequence was concealed until interventions were assigned. The randomization

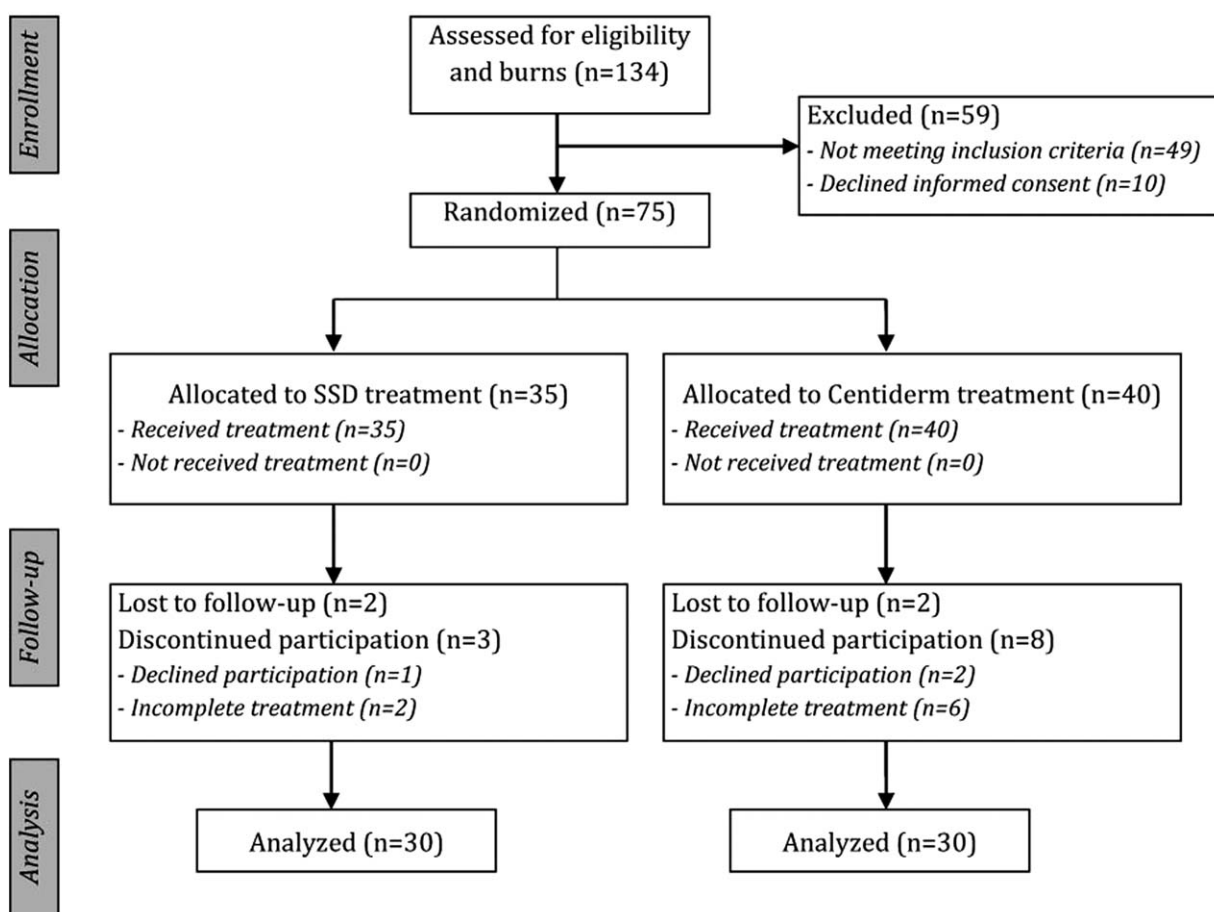


Figure 1. Study flow chart.

sequence was created using Excel 2010 (Microsoft, Redmond, WA) with a 1:1 allocation using random block sizes of 2 and 4 by an independent doctor. Patients and burning specialist, and physician were blinded.

2.8. Statistical analysis

Analysis was performed per protocol. The data statistically were analyzed using SPSS 18.0 for Windows (SPSS Inc., Chicago, IL). The means of the experimental groups were compared with the control group using the repeated-measure General Linear Model test and Student *t* and independent *t* tests. Data were expressed as mean \pm SEM and $P < 0.05$ was considered as a significant difference.

2.9. Ethics statement

The study design protocol and ethical issues were reviewed and approved by Guilan University of Medical Sciences (2013.12.7) with ethical code of 1920341317. Ethical issues were considered throughout the experiment. The patient followed the approval of the ethics committee of the Guilan University of Medical Sciences and Velayat Hospital. We described the risks and benefits of the treatment. Then, written informed consent was obtained from each participant. The IRCT (Iranian Clinical Trial) code was registered at 2013.10.06, which is IRCT2013100614915N1.

3. Results

3.1. Demographic data

A total of 134 participants were recruited, and 75 patients met the inclusion criteria. Of these, 40 patients were placed in the Centiderm group and 35 patients placed in the SSD group. During the study, 10 participants in the Centiderm group and 5 participants in the control group dropped out (Fig. 1) due to incomplete participation and declining participation during the clinical trial. The mean age of the participants was 30.67 ± 9.91 years and 19 of them were male (31.7%) and 41 of them were female (68.3%). Other demographic data are summarized in Table 1.

3.2. Objective indexes

All of the objective indexes, that is, pliability, vascularity, pigmentation, height, and VAS, significantly led to better healing of the burning wound in Centiderm group rather than SSD group ($P < 0.05$). Although the mean of pigmentation score was better in Centiderm group rather than the SSD group, it was not significant on day 7 ($P = 0.52$). One of the characteristics of Centiderm was this pigmentation on day 7 (Table 2). The mean of VSS score in Centiderm group in days 0, 3, 7, 14 was 7.2 ± 1.9 , 2.5 ± 1.6 , 1.4 ± 1.2 , and 0.23 ± 0.43 , respectively. The mean of VSS score in SSD group in days 0, 3, 7, 14 was 7.1 ± 1.3 , 5.8 ± 1.7 , 4.9 ± 1.3 , and 2.86 ± 1.19 , respectively. There was a significant

Table 1
Demographic data in the Centiderm and Silver sulfadiazine groups.

| Characteristics | Centiderm | SSD* | P | |
|-----------------------|------------------|------------|------------|-------|
| Age, y | 29.5±9.7 | 31.8±10 | 0.36 | |
| Sex MF | 9/21 | 10/20 | 0.78 | |
| Cause of burning | Hot water | 8 (26.7%) | 16 (53.3%) | 0.001 |
| | Heater | 4 (13.3%) | 7 (23.3%) | |
| | Hot oil | 3 (10%) | 6 (20%) | |
| | Fire | 2 (6.7%) | 1 (3.3%) | |
| | Water steam | 13 (43.3%) | 0 | |
| Site of burning wound | Right upper limb | 18 (60%) | 13 (43.3%) | 0.46 |
| | Left upper limb | 12 (40%) | 10 (33.3%) | |
| | Left lower limb | 0 | 6 (20%) | |
| | Right lower limb | 0 | 1 (3.3%) | |
| | | | | |

* Silver sulfadiazine.

difference between VSS score in all days between 2 groups ($P=0.0001$).

3.3. Subjective indexes

All of the subjective indexes, that is, dryness, itching, and irritation, significantly led to better healing of the burning wound both in time and efficacy in Centiderm group rather than SSD group ($P < 0.05$) (Table 2).

3.4. Re-epithelialization

Mean of re-epithelialization in burning wounds was 13.7 ± 1.48 days in Centiderm versus 20.67 ± 2.02 days in SSD group. Starting time of re-epithelialization in Centiderm group patients was on the 10th day, which was the 16th day in SSD group. It means that the first patient in Centiderm group reached faster to re-epithelialization than the SSD group. Finishing time of re-epithelialization was

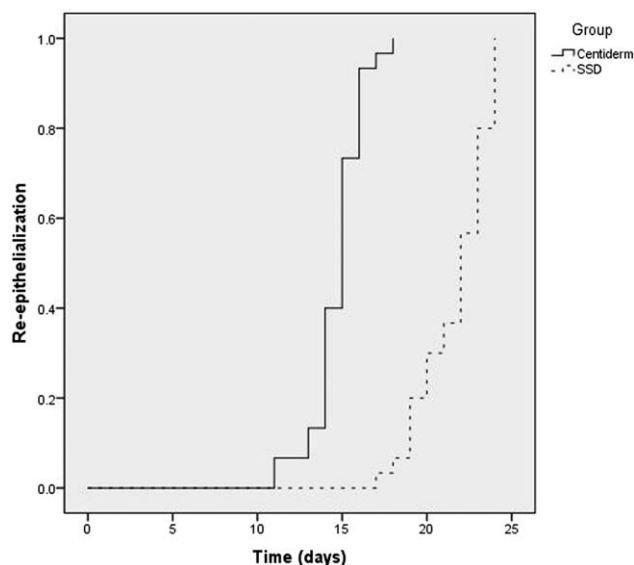


Figure 2. Re-epithelialization in Centiderm versus SSD groups.

the 17th day, whereas it was finished on 23rd day in SSD group patients. It means that the last patient in Centiderm group reached faster to re-epithelialization than the SSD group. The average time of re-epithelialization was 6.9 days sooner in Centiderm group versus SSD group patients. Multivariate analysis showed a significant difference in re-epithelialization between Centiderm and SSD groups ($P=0.001$) (Fig. 2).

3.5. Complete healing

The mean of complete healing in burning wounds was 14.67 ± 1.78 days in Centiderm versus 21.53 ± 1.65 days in SSD group.

Table 2
Objective and subjective information in patients with partial thickness burning wound.

| Day characteristics | Groups | 0 | 3 | 7 | 14 |
|---------------------|-----------|-----------|-----------|-----------|-----------|
| Pliability | Centiderm | 2.37±0.85 | 1.10±0.60 | 0.53±0.57 | 0.23±0.43 |
| | SSD | 2.30±0.53 | 1.90±0.48 | 1.63±0.55 | 1.00±0.37 |
| | P | 0.718 | 0.001 | 0.001 | 0.001 |
| Height | Centiderm | 1.63±0.49 | 0.27±0.45 | 0.07±0.25 | 0.00±0.00 |
| | SSD | 1.77±0.43 | 1.63±0.49 | 1.47±0.50 | 0.70±0.46 |
| | P | 0.267 | 0.001 | 0.001 | 0.001 |
| Vascularity | Centiderm | 1.77±0.50 | 0.73±0.52 | 0.23±0.43 | 0.00±0.00 |
| | SSD | 1.63±0.49 | 1.47±0.50 | 1.07±0.25 | 0.70±0.53 |
| | P | 0.303 | 0.001 | 0.002 | 0.001 |
| Pigmentation | Centiderm | 1.47±0.68 | 0.47±0.57 | 0.63±0.80 | 0.00±0.00 |
| | SSD | 1.40±0.49 | 0.87±0.62 | 0.77±0.43 | 0.47±0.50 |
| | P | 0.667 | 0.012 | 0.52 | 0.001 |
| VAS | Centiderm | 8.10±1.51 | 1.70±2.46 | 0.17±0.64 | 0.07±0.35 |
| | SSD | 8.43±1.04 | 5.60±1.63 | 3.27±1.70 | 1.17±0.95 |
| | P | 0.335 | 0.001 | 0.001 | 0.001 |
| Dryness | Centiderm | 0.80±0.40 | 0.17±0.37 | 0.27±0.45 | 0.07±0.25 |
| | SSD | 0.83±0.37 | 0.70±0.46 | 0.60±0.49 | 0.40±0.49 |
| | P | 0.744 | 0.001 | 0.009 | 0.002 |
| Itching | Centiderm | 0.93±0.25 | 0.27±0.45 | 0.37±0.49 | 0.20±0.40 |
| | SSD | 0.97±0.18 | 0.97±0.18 | 0.73±0.45 | 0.70±0.46 |
| | P | 0.561 | 0.001 | 0.004 | 0.001 |
| Irritation | Centiderm | 0.93±0.25 | 0.30±0.46 | 0.20±0.40 | 0.03±0.18 |
| | SSD | 0.97±0.18 | 1.00±0.00 | 0.77±0.43 | 0.43±0.50 |
| | P | 0.561 | 0.001 | 0.001 | 0.001 |

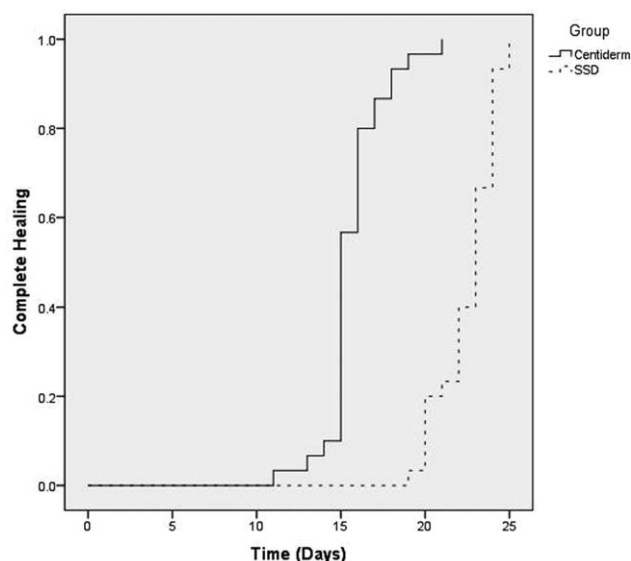


Figure 3. Complete healing in Centiderm versus SSD groups.

Starting time of complete healing in Centiderm group patients was on the 10th day, which was the 18th day in SSD group. It means that the first patient in Centiderm group reached faster to complete healing than the SSD group. Finishing time of complete healing was 20th day, whereas it was finished on 27rd day in SSD group patients. It means that the last patient in Centiderm group reached faster to complete healing than the SSD group. The average time of re-epithelialization was 6.8 days sooner in Centiderm group versus SSD group patients. Multivariate analysis showed a significant difference in complete healing between Centiderm and SSD groups ($P=0.001$) (Fig. 3). Figure 4 shows the pre and post treatment with Centiderm in 1 of 4 patients.

3.6. Adverse events

There was no adverse reaction such as severe itching, hypersensitivity, systemic symptoms in the Centiderm group during the trial, while in the SSD group, 4 patients were infected and received antibiotic therapy and conservative treatment.

4. Discussion

C. asiatica has a powerful history in traditional medicine in many diseases. Its effects on healing surgical and burning wound have

mentioned and evaluated in many documents.^[23,29–32] Antioxidant^[34] and antibacterial^[35] effects of this medicinal plant had proved previously. These properties are based on this plant constitutes as seen in previous studies.^[22–25] Burns are the most common forms of trauma. They are physical and chemical phenomena and cause many morbidities and mortalities in the world. The best goal of all the current burn treatments is to speed up skin healing and prevent wound infection.^[15] One standard antimicrobial topical ointment is silver sulfadiazine with advantages such as easy and convenient use. It is not causing pain during administration, yielding low toxicity and sensitivity, and having antibacterial effects. These properties have made it known as the gold standard among antimicrobial topical drugs for the patients with burns and turned it to the main consumed drug in treating burn wounds around the world.^[9]

Investigation of our results showed that indexes of the burning wound healing with Centiderm after 3 days of treatment healed better than routine treatment of 1% silver sulfadiazine ($P < 0.001$) that was surprising in the few first days of the burn and its maximum effects seen in the first 3 days. The range of re-epithelialization was between 10 and 16 days after administrating the Centiderm ointment that was significantly better than the SSD ($P < 0.001$). Also, because of good antioxidant and antibacterial activity of Centiderm, patients showed no adverse reaction and infection in comparison with SSD. Some clinical studies about external use of *C. asiatica* will be mentioned. Basset^[52] assessed the effects of impregnated dressing of Madecassol derivate from *C. asiatica* on ulcer cicatrization and burns recovery were investigated and Madecassol individual gauze (10 x 10 cm) impregnated with a mixture of titrated extract of *C. asiatica* (TECA) was used on 76 patients who had experienced previous allergic dermatitis or were affected by dermatosis of different kinds. The results were favorable after a treatment period of 8 to 10 days.^[52] Haftek et al^[53] performed a randomized, double-blind study on photo-aged skin of 20 female volunteers with actinically aged facial, neck, and forearm skin to investigate the effects of topically applied 5% vitamin C and 0.1% madecassoside on the clinical, biophysical, and structural skin properties, and their results indicated a functional and structural remodeling of chronically sun-damaged skin. Ryu et al^[54] performed a study to evaluate the efficacy of wrinkle improving lipstick containing asiaticoside (0.2% concentration) and showed that by using the lipstick containing asiaticoside for 8 weeks, the change of visual grading scores and replica analysis indicated the wrinkle-improving effect. The results of our study were better than other natural products used for burning wound. Panahi^[33] evaluated the herbal cream consisting of Aloe vera, Lavandula stoechas, and Pelargonium roseum as an alternative for silver sulfadiazine in burn management. They showed that



Figure 4. Patient pictures before and after treatment with Centiderm. (A) Patient with partial thickness burning wound; (B) 3 days after treatment with Centiderm; (C) 7 days after treatment with Centiderm; (D) 10 days after treatment with Centiderm showed complete healing.

both herbal and SSD groups experienced a significant reduction in the pain severity at day 14 compared with baseline ($P < 0.001$) and there was a significantly greater reduction from baseline to the 7 ($P = 0.014$) and 14 ($P = 0.05$) day in the herbal cream compared with control group. However, the frequency of skin dryness was not significantly different between the groups at any of the assessed time points ($P > 0.05$) and there was a single case of infection in the herbal cream group.^[133] Akbari et al^[151] showed that use of nettle extract treatment led to shorter wound healing time, fewer dressing changes, and shorter hospital stay, than silver sulfadiazine treatment. But no difference in the incidence of wound infection or grafting was found.^[151] Malik et al^[551] compared honey with SSD in the treatment of superficial partial-thickness burns. They showed that the rate of re-epithelialization and healing of superficial and partial-thickness burns was significantly faster in the sites treated with honey than in the sites treated with SSD (13.47 vs 15.62 days, respectively; $P < 0.0001$) and the site treated with honey healed completely in less than 21 versus 24 days for the site treated with SSD. Six patients had positive culture for *Pseudomonas aeruginosa* in honey-treated site.^[551] Khorasani et al^[561] evaluated Aloe versus SSD creams for second-degree burns on 30 patients with similar types of second-degree burns at 2 sites on different parts of the body. They showed that the rate of re-epithelialization and healing of the partial thickness burns was significantly faster in the site treated with aloe than SSD (15.9 vs 18.73 days, respectively; $P < 0.0001$).^[561] There was no clinical trial similar to our work. However, animal and in vitro studies were performed on this subject that are in line with our results. Somboonwong et al^[521] investigated wound healing activities of different extracts of *C. asiatica* in incision and burn wound models. They showed that similar to our study, the degrees of healing in the burn wound with the 4 extracts were significantly higher than that of the control on Days 3, 10, and 14. Also, histopathological findings on Day 14 after burn injury revealed prominent fibrinoid necrosis and incomplete epithelialization in the control and untreated groups, whereas fully developed epithelialization and keratinization were observed. They referred these effects to the phytoconstituents β -sitosterol, asiatic acid, and asiaticoside and madecassoside.^[521] Shukla et al^[291] showed that asiaticoside in streptozotocin diabetic rats, where healing is delayed, improved by topical application of 0.4% solution of asiaticoside. They indicated that asiaticoside exhibits significant wound healing activity in normal as well as delayed healing models and is the main active constituent of *C. asiatica*. Lu et al^[571] explained the mechanism of asiaticoside by using cDNA microarray technology and showed that alternation of genes expression profiles was determined in a human dermal fibroblast in vitro in the presence of 30 $\mu\text{g}/\text{mL}$ asiaticoside, and 54 genes, with known functions for cell proliferation, cell-cycle progression, and synthesis of the extracellular matrix, were significantly upregulated. They showed that there is a close correlation among the gene profile, mRNA, and protein production in the cells response to asiaticoside stimulation.^[571] Ermertcan et al^[581] compared the effects of collagenase and *C. asiatica* in the rat model. Their immunohistochemical examinations showed strong inducible nitric oxide synthase (iNOS) and transforming growth factor-beta (TGF- β) immunoreactivities in *C. asiatica* group. Endothelial nitric oxide synthase (eNOS) immunoreactivity was moderate in this group.^[581] Zhang et al^[591] indicated that asiaticoside can downregulate TGF- β 1 mRNA and tissue inhibitors of metalloproteinases-1 (TIMP1) expressions and upregulate TGF- β 3 mRNA expression in postburn hypertrophic scars capable of

decomposing the products of type I collagen, contributing to the reduction of hypertrophic scars. Kimura et al^[601] suggested that the enhancement of burn wound healing by asiaticoside might be due to the promotion of angiogenesis during skin wound repair as a result of the stimulation of vascular endothelial growth factor (VEGF) production caused by the increase in monocyte chemoattractant protein-1 (MCP-1) expression in keratinocytes and the increase in IL-1 β expression in macrophages induced cooperatively by asiaticoside along with MCP-1. On the basis of clinical and experimental findings, our ointment originated from *C. asiatica* can promote the wound healing in burn wounds.

The use of *C. asiatica* is safe and some clinical trials in other topics were done. It was used clinically for periodontal treatment that showed adjunctive local delivery of extracts from *C. asiatica* in combination with significantly improved clinical signs of chronic periodontitis and IL-1beta level in maintenance patients.^[611] It was used for minor oral aphthous ulcers by Ruengprasertkit et al^[621] and they demonstrated that its oral paste was safe and effective in reducing pain, ulcer size, and erythema. Kuo et al^[631] evaluated the effects of a topical cream containing *P. amboinicus* (Lour.) Spreng. (Lamiaceae) and *C. asiatica* (L.) for diabetic foot ulcers and showed that treating diabetic foot ulcers with them is a safe alternative to hydrocolloid fiber dressing without a significant difference in effectiveness.

The pharmacological activity of *C. asiatica* is thought to be due to several saponin constituents, including asiaticoside, asiatic acid, and madecassic acid.^[641] In vitro, each of these compounds stimulated the production of human collagen I, a protein involved in wound healing.^[651] Stimulation of collagen synthesis in foreskin fibroblast monolayer cultures by an extract from *Herba Centellae* has also been reported.^[301] Asiaticoside quickened the healing of superficial postsurgical wounds and ulcers by speeding up cicatricial action.^[661] Asiaticoside stimulates the epidermis by activating t28he cells of the malpighian layer in porcine skin, and by keratinization in vitro.^[671] Topical application of asiaticoside promoted wound healing in rats and significantly increased the tensile strength of newly formed skin.^[66,681] Extracts of *C. asiatica*, and, in particular, its major triterpene ester glycoside, asiaticoside, are valuable in treating hypertrophic scars and keloids.^[661] Asiaticoside has been reported to decrease fibrosis in wounds, thus preventing new scar formation.^[661] The mechanism of action appears to be 2-fold: by increasing the synthesis of collagen and acidic mucopolysaccharides and by inhibiting the inflammatory phase of hypertrophic scars and keloids. It has further been proposed that asiaticoside interferes with scar formation by increasing the activity of myofibroblasts and immature collagen.^[661] In clinical trials, an extract of *C. asiatica* in a 1% salve or 2% powder accelerated healing of wounds.^[691] A formulation containing asiaticoside as the main ingredient healed 64% of soiled wounds and chronic or recurrent atony that was resistant to usual treatment.^[661] In an open clinical study, the treatment of 20 patients with soiled wounds and chronic or recurrent atony with a galenical formula containing 89.5% *C. asiatica* healed 64% and produced improvement in another 16% of the lesions studied. Local application of an extract of the drug to second- and third-degree burns speeded up healing, prevented the shrinking and swelling caused by infection, and further inhibited hypertrophic scar formation.^[661] Previous studies have focused on the effects of *Centella* ointment on the process of healing burn wounds or surgical in the laboratory and in vivo conditions. In this study, we showed that healing effects of Centiderm were significantly better than the routine drug _SSD_

for treating partial thickness burning wounds such as pain, objective and subjective signs, and also re-epithelialization and complete healing time. None of subjected patients had infection rather than SSD.

Previous studies displayed the positive antibacterial, anti-inflammatory effects, and positive results on in vitro and in vivo experiments of the drug on the wound healing and its useful and effective features. Our results showed that consumption of Centiderm ointment not only improved the objective and subjective signs but also improved the re-epithelialization and complete healing rather than SSD without any infection in the subjects with burning event. In our evaluation, patients who underwent Centiderm treatment showed their optimal effects in first 3 days that is clear in figures and detailed results. Centiderm is cheaper than SSD and it can be a suitable choice for treating partial thickness burning wounds. Performing a clinical trial for deeper thickness burning wounds for Centiderm is recommended. We also suggested using Centiderm in dressing form and comparing it with other dressing methods and also with other antiseptic and burn wound healer drugs.

Acknowledgements

We wish to thank Shahid Ali Es'hagh Moafi for moral support. We also thank Medicinal Plants research center of student Basij for scientific support and Dr. Morteza Fallah Karkan for helping in all of steps of the research. We thank Mr. Mahdi Nabaei and Mr. Mostafa Hasanpour for helping in formulation step, Mrs Fateme Keihanian and Mrs Motlagh, and other nurses of emergency ward of Velayat Hospital for helping us in patient gathering step. We also thank Mr. Asaadi for helping in identification and confirming herb, Mr. Mohammad Bagher Gholami for collecting herb from its ecology, Mr Hamid Morovati in extraction steps, and all other persons who helped us during this clinical trial. We also thank Guilan University of Medical Sciences for financial support.

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