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Original Research

Therapeutic Effects of *Aloe vera* (L.) Burm.f. and *Vitis vinifera* L. Combination Cream on Wound Healing in Second-Degree Burn Model in Rats: Quantification of Compounds and VEGF & TGFβ Gene Expression

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Abstract

Finding more efficient agents with fewer side effects for the treatment of burns has been a concern for researchers. The current study aims to assess *Aloe vera* (L.) Burm.f. and *Vitis vinifera* L. extract combination (AVEC) effects on wound healing in rats compared with silver sulfadiazine (SSD). Identification of individual polyphenols of the plant extracts was performed by HPLC. The animals were randomly divided into twelve groups. Standard second-degree burn wounds were induced on the back. Groups were treated with *Aloe vera* (A.V) and *Vitis vinifera* (V.V) creams at concentrations of 0.5, 1, 1.5, and 2% with ED_{50} values of 1.5 ± 0.02 and 1.4 ± 0.08 %, respectively. The other group was treated with an AVEC cream (1.5%). The samples of burned skin tissue were collected from the rats for histopathological examination. To evaluate the expression of VEGF and TGFβ1, the real time-PCR method was used. Kaempferol was only detected in Aloe. This study revealed that AVEC cream exhibits significant wound healing activity. VEGF and TGFβ1 had a significant increase in the AVEC. Based on our findings, AVEC cream can be a therapy of choice for burn injuries.

Keywords: *Aloe vera*; *Vitis vinifera*; Second-degree burns; High performance liquid chromatography (HPLC); Vascular endothelial growth factor(VEGF)

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Introduction

Worldwide, each year burns are a major health problem because of their high incidence and chronic lesions they produce regardless of age groups [1]. The economic costs and expenditures incurred by the treatment of complications caused by incidents are extremely huge. Also, psychiatric disorders resulting from this injury, such as depression in patients, are sometimes irreversible [2]. When skin is damaged by burns, cuts, and/or ruptures, its tissue is damaged and ruptured losing its parallel regular shape. Subsequently, fibroblasts and some other cells migrate to the affected area, causing them to become edged and adhesion in that area [3]. The restoration of tissue integrity is the result of the interaction of cells such as neutrophils, endothelial cells, and fibroblasts as well as extracellular matrix components, such as fibronectin, glycosaminoglycans, or collagens [4]. Inflammation, re-epithelialization, and neovascularization are the stages of wound healing process. The use of topical medicines is important in healing and protecting the burn wound [5]. Silver sulfadiazine 1% cream (SSD) and mafenide acetate are the most important treatments for a burn. SSD may cause adverse effects such as erythema multiforme, neutropenia, methemoglobinemia, crystalluria, and delayed wound healing [6]. Active components of medicinal herbs such as heteroglycans, flavonoids, tannins, anthracene-derivatives, essential oils, and vitamins with emollient, astringent, antiseptic, anti-inflammatory, immunomodulatory, antioxidant, epithelizing, and cicatrizing effects contribute to wound healing [7]. Avicenna (980-1037 AD), an Iranian physician, has recommended medicinal herbs in his famous book (Canon of medicine) for the healing of burn wounds [8]. One of them is *Aloe vera* (L.) Burm.f. which was used to repair damaged skin cells by containing some amino acids. [9]. Traditional use of medicinal herb *Vitis vinifera* L. as wound healing is popular among people in Iran and in recent years anti-inflammatory effects of this plant have been reported [10]. Moreover, plant phenolic compounds have been implicated in diverse functional roles, including plant resistance against microbial pathogens, solar radiation protection, oxidative stress prevention, nutrition, and growth [11]. Presently, one acutely challenging medical issue is to manufacture an effective drug which can be applied to shorten the course of burn recovery, to reduce its complications, and to restore physical ability. Also, it has been shown that increasing the expression of vascular endothelial growth factor (VEGF) and transforming growth factors (TGF β-1) in fibroblast cells leads to their reconstruction of the extracellular matrix at wound area [12]. Therefore, the main objective of present study was to identify the components of *A. vera* and *V. vinifera*, the effects of combined cream and their mechanism of action on second degree burns wounds and compare it with SSD in rats.

Materials and Methods

Animals

Male Wistar rats (300 ± 50 g) were purchased from Pasteur Institute (Tehran, Iran). The rats used as experimental animals were about 2 to

3 months old and divided into 12 groups of 6. The animals were kept in standard plastic cages (12 h of darkness and 12 h of light) at an optimum temperature of 24 ± 1 °C. Amid the course of the experiment, the rats have directed a standard pellet diet. The experiments were performed following the current ethical guidelines for the investigation of experimental pain in conscious animals from Zimmermann [13]. The committee on the use and care of laboratory animals at our University approved this study (No. EC/93/A/114).

Preparation of extracts

A. vera and *V. vinifera* leaves were collected in July in Ilam, Iran. Plant samples were identified by Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran (voucher specimen No. 6719 and 7583, respectively). Plant materials were washed with tap water and shade dried at room temperature for 15 days. An electrical blender powdered the dried plant materials (leaves). The coarse powder (30 g) were successively extracted with 250 ml of 30:70 methanol/water (at 40°C) using Soxhlet extractor for *A. vera* and *V. vinifera* for 8 h, respectively [14]. The extracts were filtered and evaporated at 40°C (IKA-RV10digital) and stored at -40°C until required. The extraction yield percentage of *A. vera* and *V. vinifera* leaves were 5.8 and 5.3, respectively. For the tests, the dry extracts or SSD were incorporated into cream basis (Eucerin) [15]. The pH value was determined using a calibrated potentiometer (JANEWAY, UK) (10%, w/v in water).

Quantification of phenolic compounds by HPLC-DAD Reverse phase chromatographic analyses (KNAUER, Germany) were carried out under gradient conditions using a C18 column (3 mm, 100 mm, Eurospher II) packed with 3 µm diameter particles. The mobile phase was water containing 2% acetic acid (Merck) (A) and methanol (Merck) (B), respectively [16]. The plant extract was dissolved in ethanol (12 mg/mL) and analyzed. The presence of five polyphenols including rutin, myricetin, quercetin, kaempferol, and apigenin (Sigma Chemical Co. USA) was assessed. Components were identified in comparison with the standard curve. The flow rate was 1.0 mL/min and the injection volume was 1µL. Stock solutions of the reference standards were prepared in the HPLC mobile phase at a concentration range of 0.025–0.250 mg/mL. The peaks were confirmed by comparing the retention times with those of the reference standards and by the DAD spectra (200–500 nm).

Design of experiment

A total of 72 rats was used with equal numbers being assigned to each of 12 groups, including group 1; control group (no topical agent was used). Group 2; treated group only with base cream (sham) (Eucerin). Group 3; were treated with SSD (1%, positive control). Group 4, 5, 6 and 7 were treated by *V. vinifera* 0.5%, 1%, 1.5% and 2%, respectively. Group 8, 9, 10, and 11 were treated with the cream of *A. vera* extract as 0.5%, 1%, 1.5%, and 2%, respectively. Group 12; received a combination of *V. vinifera* and *A. vera* leaves extract (1.5%:1.5%). Rats

were weighed and anesthetized by intraperitoneal injection of 50 mg/kg sodium thiopental. The hair on the backs of the rats was shaved by an electric clipper and 70% alcohol was used to disinfect the dorsal area. A deep second-degree burn wound was induced by a hot metal device (same size) in boiling water (5 minutes) and put on the back of rat skin (10 seconds) [17]. Burns was generated with a special metal device, with a diameter of 1 cm, equipped with a control thermometer. The mean wound size was reported as mm2. The half-maximal effective dose (ED_{50}) was calculated. In this investigation, approximately 8% of the total body surface was burned. Treatment started 24 h after the burn. Wounds were dealt with twice every day. The application of medications was carried out utilizing a sterile swab. The treatment was applied for 21 days after burn induction.

Histological study

21 days after wound induction, histological examinations were performed. After incision, samples (3 mm thickness) kept at formalin 10% overnight at 4°C. Samples were stained with hematoxylin and eosin (HE). Also, Masson Trichrome (MT) stain was used for the examination of the collagen fibers density and blood vessels [18]. The sections were examined with a light microscope under 40 and 100 x magnifications (Olympus, Japan). A histomorphological review was performed by a blind pathologist to evaluate the presence of inflammation, granulation, and fibrosis.

Cell culture & gene expression of VEGF and TGF-β1 analysis by Real-time PCR

In this study, the normal mouse skin fibroblast line (c147) was purchased from the Cell Bank of Iran, Pasteur Institute of Iran, Tehran, Iran. This cell line cultured based on the source's guidelines. Fibroblast cells were cultured in a complete culture medium RPMI 1640 (Biosera, France), containing bovine serum (FBS) 10% (Gibco, USA), 100 U/mL penicillin, and 100 µg/mL streptomycin (Bio Idea, Iran). Cells incubated under culture conditions at 37°C, 5% CO2, and 90% humidity. Passage-5 the normal mouse skin fibroblast line (c147) cultured in an RPMI medium containing 10% FBS, 100U/mL penicillin, 100 µg/mL streptomycin and 150 µg/mL *A. vera* (AV), 150 µg/ml *V. vinifera* (VV) and 75 µg/mL for 24 h and 48 h separately. Passage-5 the normal mouse skin fibroblast line (c147) untreated was used as a control group. Total RNA of these cells isolated after 24 h and 48 h. After total RNA extraction by RNA purification Kit (Jena Bioscience, Germany), cDNA was synthesized by the cDNA Synthesis Kit (Roche Diagnostics GmbH, Mannheim, Germany), then VEGF and TGF-β1 genes expression levels evaluated using Real-time PCR reaction, VEGF and TGF-β1 genes specific primers designed by the Oligo7 software. Real-time PCR reaction was performed by Rotor-Gene 6000 real-time PCR system and Real-time PCR reaction mixture prepared in 15 µl using SYBR-Green quantitative PCR (qPCR) kit (Jena Bioscience). Real-time thermal cyclic profile was: 95 ºC for 2 min followed by 40 cycles of 95 ºC for 20 seconds, 60 ºC for 40 seconds, and 72 ºC for 30 seconds. Data were standardized to the

GAPDH housekeeping gene expression level. Gene expression fold changes were calculated by the 2-ΔΔCT method [19].

Statistical analysis

The results are expressed as the means ± S.E.M. All data were analyzed by one-way or two-way analysis of variance (ANOVA). P values of less than 0.05 ($P < 0.05$) were considered significant. Statistical analysis was carried out using SPSS (version 16) software. Based on the dose-response model by using different doses and viewed response, doses were selected. According to the experimental design, ED_{50} was calculated following dose-response. Term half-maximal effective dose (ED_{50}) refers to the dose of a drug or extract of a plant that induces a response halfway between the baseline and maximum after a specified exposure time.

Results and Discussion

HPLC analysis

To investigate the effects of combination *V. vinifera* and *A. vera* extract on wound healing, it was formulated for the first time in this study as a topical cream (AVEC) that could be readily used. The results of the healing in this study indicated the preference of the group threated with AVEC in comparison to the control group. Burn-caused injuries are still an important cause of human mortality and disabilities and burn wound healing is considered one of the challenges facing modern medicine [20]. Nowadays, medical science is paying particular attention to making use of herbs for treating patients. The phenolic compound presence was confirmed by comparing retention times (RT) and overlapping UV spectra with standard compounds. The quantitative analysis of *A. vera* and *V. vinifera* extract revealed the presence of rutin (RT: 14.66 min), miricetin (RT: 12.76 min), quercetin (RT: 17.21 min), kaempferol (RT: 15.76 min), and apigenin (RT: 11.32 min). The accuracy was expressed as the recovery of standard compounds added to the pre-analyzed sample. The results are summarized in table 1. The proposed polyphenols were identified in both extracts, except for kaempferol, which was only detected in the *A. vera* extract. Rutin, miricetin, quercetin, and apigenin were the most abundant in the leaf extract of *A. vera*. *A. vera* and *V. vinifera* HPLC analysis indicated several peaks, revealing the great chemical diversity. Previous findings also detected phenols and flavonoids in leaf extracts of *A. vera* and *V. vinifera* [10]. Moreover, it has been illustrated that the polyphenols and phenolic compounds found in the genus Aloe and *V. vinifera* possess anti-inflammatory and antioxidant effects [21].

Table 1. Some of the polyphenol in *Aloe vera* and *Vitis vinifera* extracts presented as average values ± standard deviation (SD). Regression equation (r) and retention time (RT) were obtained for each polyphenolic compound.

Phenolic compound	Regression equation (r)		*Aloe vera* (mg/g)	*Vitis vinifera* (mg/g)
Quercetin	y = 24493x −26074	(0.9988)	39.4 ± 3.0	19.4 ± 2.0
Apigenin	y = 78702x −20316	(0.9976)	4.3 ± 0.4	1.3 ± 0.5
Miricetin	y = 12436x −28377	(0.9998)	17.6 ± 0.3	5.6 ± 0.6
Rutin	y = 19304x −64494	(0.9993)	29.3 ± 3.0	10.3 ± 2.0
Kaempferol	y = 30302x −57494	(0.9993)	2.2 ± 0.6	0

Wound measurement

The pH of base cream and AVEC cream were 5.87 and 4.85, respectively. On days 7, 14, and 21, the burn wound area was measured. Examination of the diagram of healing level and wound size's reduction measurements showed that these variables were significantly reduced in group 12 compared with control group and sham ($P < 0.005$), but was not significant in comparison to group 3. Also, the respective curve showed that group 12 exhibited a better trend of healing in the reduction of wound size than controls. Our results showed that healing in groups 2 and 3 were significantly different from the control group ($p < 0.05$; Figure 1). Also, burn measurement showed that over time, week-to-week recovery has been achieved. Treatment with *A. vera* and *V. vinifera* creams at concentrations of 0.5, 1, 1.5, and 2% led to a reduction in wound size with ED50 values of 1.5 ± 0.02 and 1.4 ± 0.08 %, respectively (Figure 1). Regarding the chemical structure of substances available in *A. vera* and *V. vinifera*, observed useful effects of these plants on day 21 could be attributed to their active components. Therefore, these activities could be responsible for the *A. vera* and *V. vinifera* effects observed in our study. To evaluate the wound healing activity of *A. vera* and *V. vinifera*, we used a thermal injury model [22]. In this study, our results indicated that the mixture of *A. vera* plus *V. vinifera* with a ratio of 1.5% showed a highly improved healing effect on second–degree superficial burn wound compared to each individual extract groups. This observed effect may be due to angiogenic properties and stimulating effects on epidermal cells proliferation. As evidenced by our study, it has been demonstrated that healing process of burn wounds by *A. vera* expedited the growth of cells in the healed area and limited scar formation [23]. In our study, rats with fully closed and healed wounds on day 21 were observed, showing a similar or better trend than that of SSD treatment (Figure 1, Table 2). A study showed that the wound healing process took place 50% faster with *A. vera* than with SSD [24]. According to our results, it has been also illustrated that the extract of 2% *V. vinifera* seed extract administered topically has a good potential to promote wound healing in wound models of rabbits [25].

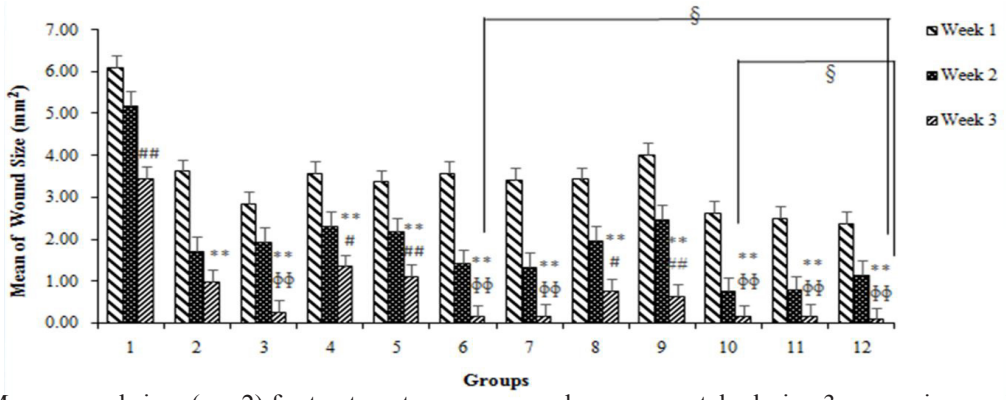

Figure 1. Mean wound sizes (mm2) for treatment groups were shown separately during 3 successive weeks graphically. Number 1 (control) and 2 (sham) groups have received no treatment. Number 3 group was treated with silver sulfadiazine (1%). Number 4 to 11 groups were treated with *Aloe vera* and *Vitis vinifera* creams at concentrations of 0.5, 1, 1.5, and 2%, respectively. The number 12 was treated with a combination of *Aloe vera* and *Vitis vinifera* cream (1.5%). Data are expressed as mean ± SEM. ** $P < 0.005$, compared to control. *$P < 0.01$, compared to control. §$P < 0.05$,compared to groups 6 and 10.# $P < 0.05$, compared to control. ## $P < 0.01$, compared to control. ϕ$P < 0.05$,compared to control. ϕϕ$P < 0.01$, compared to control.

Table 2. Descriptive indices of means and standard deviations of wound size (mm^2) among control, sham, SSD and 8 groups separately within 3 successive weeks. VV (*Vitis vinifera*), AV (*Aloe vera*)

Variable		Control	Sham	SSD	VV 0.5%	VV 1%	VV 1.5%	VV 2%	AV 0.5%	AV 1%	AV 1.5%	AV 2%	VV 1.5% + AV 1.5%
Week 1	N Mean S.D	6 6.09 0.982	6 6.09 0.982	6 2.84 0.315	6 3.59 0.397	6 3.38 0.333	6 3.57 0.852	6 3.42 0.509	6 3.44 0.472	6 4.02 0.604	6 2.63 0.460	6 2.50 0.617	6 2.38 0.895
Week 2	N Mean S.D.	6 5.20 1.448	6 1.72 0.621	6 1.94 0.554	6 2.32 0.808	6 2.18 0.207	6 1.79 0.391	6 1.34 0.169	6 1.97 0.711	6 2.48 0.646	6 0.60 0.888	6 0.80 0.917	6 1.16 0.617
Week 3	N Mean S.D.	6 3.46 0.546	6 0.99 0.452	6 0.27 0.320	6 1.35 0.864	6 1.12 0.183	6 0.38 0.439	6 0.24 0.227	6 0.77 0.442	6 0.99 0.359	6 0.13 0.194	6 0.07 0.120	6 0.19 0.292

Histological and morphometric findings

The histopathological changes of the combined cream, AVEC, and other groups are shown in figure 2 on the 21st day. Histological examination showed that re-epithelialization of wound area was much better when combination therapy (AVEC cream) was used. The specimens of the group 12 showed slight and moderate inflammation and slight, moderate, and advanced granulation. Specimens taken from the control group showed a lot of inflammation. Comparisons between the treatment groups and control groups indicate significant difference in the wound diameter scores, inflammation, and fibrosis. The extent of maturation, tissue organization, and re-epithelialization was higher in groups treated with AVEC cream compared with SSD and ointment base groups. According to table 3, the histological and morphometric results show that epithelium was formed completely in group 12, with high formation of hair follicles, sebaceous glands, and fibroblasts. Also, group 12 exhibited slight or mild infiltration of neutrophils, blood vessel formation, and macrophages, which were lower than those of other groups. For groups 6 and 10 histological findings indicated a slight amount of neutrophils, blood vessels, and macrophages on day 21 as well as slight formation of sebaceous glands and hair follicles, but a high number of fibroblasts. Besides, histological microscopic examinations, shown in figure 2, indicated that in group 1 (control), epidermis rupture, poor epidermis repair, fibrin exudates, and high infiltration of neutrophils were not observed concerning hair follicles; also, leukocytes were being repaired along with dispersed congested blood vessel margins on the surface of the collagenized dermis. Angiogenesis,

which is an important factor in the 21 days of healing, was significantly higher in the group 12. On the 21st day epithelization higher than in groups 6, 10, 12 healing, and wound healing was preceded towards the chronic phase. During the wound healing process, epithelial cells on the epidermis surface proliferate to cover the wound site. To do this function, they need some dissolved oxygen, the amount of which depends on circulation in blood vessels [26]. Also, cell types including macrophages and neutrophils in burn wounds are susceptible to infiltration [27], and researchers believe that drugs such as SSD have harmful effects on the number of fibroblasts and collagen fibers [28]. The results of this study raise the possibility of the potential efficacy of *A. vera* and *V. vinifera* combination-AVEC- in accelerating cutaneous burn. It has been reported that *A. vera* increased re-epithelization in burn wounds as compared with SSD. Evidence indicated that by treatment with Aloe, a reduced number of inflammatory cells was seen in treated groups compared to untreated ones [29], which is in agreement with our findings on the number of neutrophils and other factors studied. On the other hand, neutrophil cells are an index of extreme inflammation, the intensity of which was minimal in groups 6, 10, and 12. According to some studies, *A. vera* treatment speeds up the healing of degenerated tissues because this plant provides micronutrients, anti-inflammatory effects, and stimulation of skin fibroblasts. Oral mucilage of *A. vera* has been shown to improve skin wounds caused by type-II diabetes in animals. The mechanisms of healing skin lesions are to increase expression in the gene of VEGF and TGF β-1[30]. Moreover, histological analysis was also shown that *V. vinifera* exhibits significant wound-healing potential. Increased rate of wound contraction, hydroxyproline content, and a decrease in epithelialization time in the treated animals support the use of grape-skin powder in the management of wound healing [31]. Given the results obtained, it is recommended that in future studies, other parts of *A. vera* along with *V. vinifera* to be tested for their healing effects on secondary burns, as well as their possible applications in the pharmaceutical industry.

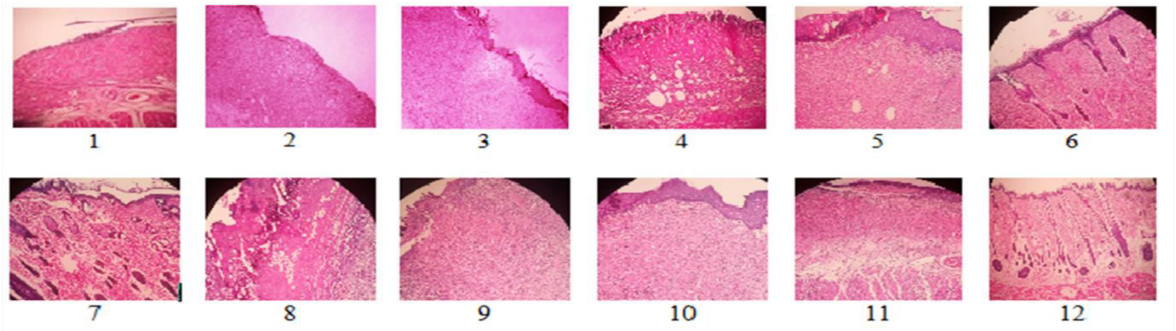

Figure 2. Photomicrograph of second-degree burn wounds in rats on 21st day after treatment (magnification, 100X). Number 1 (control) and 2 (sham) groups have received no treatment. Number 3 group was treated with silver sulfadiazine (1%). Number 4 to 11 groups were treated with *Aloe vera* and *Vitis vinifera* creams at concentrations of 0.5, 1, 1.5, and 2%, respectively. The other group was treated with a combination of *Aloe vera* and *Vitis vinifera* cream (1.5%). Macrophage infiltration, neovascularization, and fibroblast proliferation were more in groups 12, 6, and 10 compared to controls. Also, the thickness of the granular cell layer and epidermal thickness extent were prominent in groups 12, 6, and 10 in comparison with other groups on 21st day.

Table 3. Histological scoring criteria for burn wound in rats, Number 1 (control) and 2 (sham) groups have received no treatment. Number 3 group was treated with silver sulfadiazine (1%). Number 4 to 11 groups were treated with *Aloe vera* and *Vitis vinifera* creams at concentrations of 0.5, 1, 1.5, and 2%, respectively. The other group was treated with a combination of *Aloe vera* and *Vitis vinifera* cream (1.5%). Histological recovery indexes were prepared for each group. Samples were scored as follows: 0 = none and slight (+), slight or mild (+/-), moderate-severe (++), profound or advance (+++), or very severe.

Groups	Follicle hair	Sebaceous gland	Fibroblast	Macrophages	Blood vessels	Neutrophils	Layer of epithelium
1	0	0	+	++	-/+	+++	0
2	0	0	+	++	+	+++	0
3	+	+	++	+	++	+	++
4	0	0	+	++	+	+++	0
5	0	0	+	++	++	++	+
6	0	0	++	+	++	+	++
7	0	0	++	++	++	++	+
8	0	0	+	++	+	+++	0
9	0	0	+	++	++	++	+
10	+	+	++	+	++	+	++
11	+	+	++	+	++	+	+++

VEGF and TGFβ1 gene expression

Analysis of VEGF gene expression by real-time RT-PCR at 24 and 48 h, when there were 150 µg/mL of AV and VV, differences in gene expression were observed (Figure 3). VEGF gene expression was also observed when AV and VV were used each 75 µg/ml (total 150 µg/mL) in comparison to the isolated AV and VV ($P < 0.01$) (Figure 4). The results of the TGFβ1 gene expression showed that the expression of the gene was different at 24 and 48 h. There was no significant difference between 150 µg/mL AV and VV at a time point; however, there was a significant increase in this gene at different concentrations of AV and VV (75 and 75 µg/mL) at 48 h after treatment compared to the control ($P < 0.01$) (Figure 5). In confirmation of our results, it has already been shown that *A. vera* improves the wound by increasing the expression of VEGF and TGFβ1 genes in the regions affected by the skin of the rat in fibroblastic cells [22].

It has been demonstrated that *A. vera* was dose-dependent and time-dependent on the expression of bFGF and TGFβ1 in fibroblast cells in vitro [32].

Also, one study demonstrated that *A. vera* extract might be effective in wound healing, through the improvement in the migration of fibroblast cells and regulating the gene expression of VEGF and TGFβ1 genes in fibroblast cells [33].

Conclusion

According to the obtained results, the beneficial effects of AVEC cream in the healing of superficial burn wounds in rats are comparable with SSD. It seems that the effect of the combined cream of the two extracts on the burn wounds is partially due to the existing polyphenols. The

combination cream, at least in part, seems to act via increase in the expression of VEGF and TGFβ1 genes in the wound tissue. Thus, after clinical trials in humans, AVEC cream can be considered as a low cost, easily available, and potent topical agent in the improvement of superficial burn wound healing.

Gene	Primer sequences	Real-time PCR product
TGF-β1	Forward: CGTGGAAATCAATGGGATCAG Reverse: GGAAGGGTCGGTTCATGTCA	74 bp
VEGF	Forward: CAAGACAAGAAAATCCCTGTGG Reverse: CCTCGGCTTGTCACATCTG	162 bp

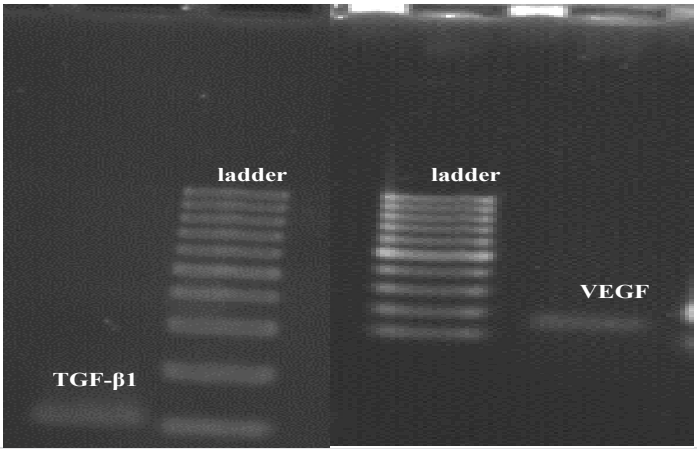

Figure 3. Sequences of primers for Real-time quantitative PCR and expression of VEGF and TGFβ1 genes in fibroblast cells as shown by RT-PCR.

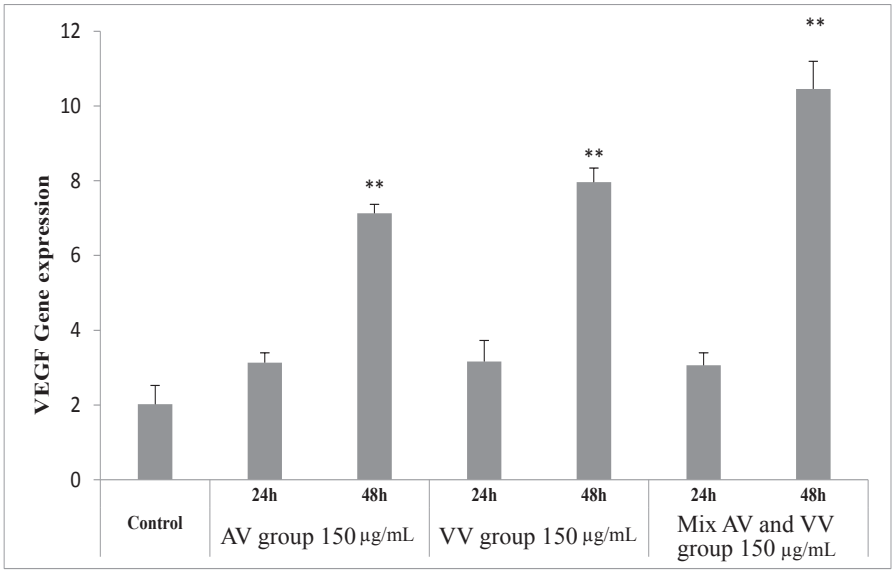

Figure 4. Expression of VEGF gene in mouse embryonic fibroblast cell, treated with various concentrations of AV(*Aloe vera*), VV(*Vitis vinifera*) and mixed (150 µg/mL, 150 µg/mL and 75 µg/mL AV+ 75 µg/mL VV) as compared with the control group at different time intervals (24 and 48 h). VEGF gene expression of mouse embryonic fibroblast cells was assessed by quantitative real-time PCR. All comparisons were analyzed with the control group. **P < 0.01.

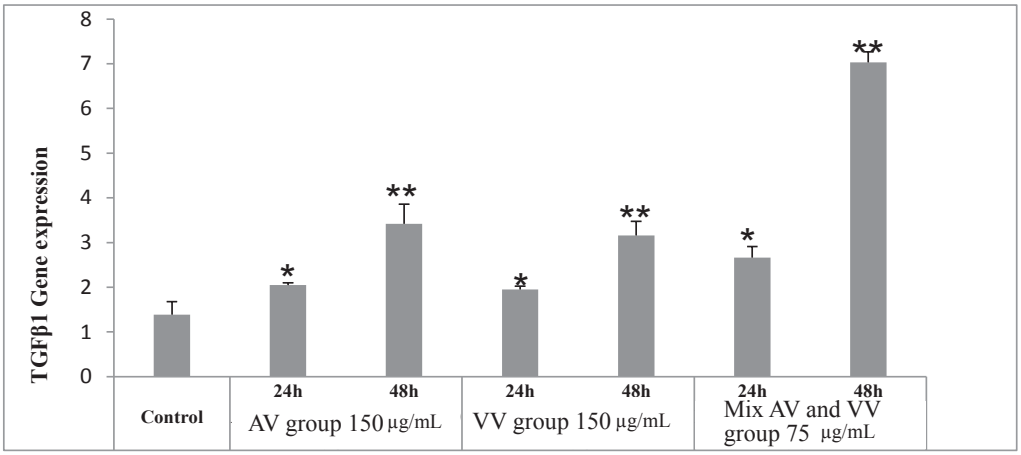

Figure 5. Expression of TGFβ1 gene in mouse embryonic fibroblast cell, treated with various concentrations of AV (*Aloe vera*), VV (*Vitis vinifera*) and mixed (150 µg/mL, 150 µg/mL and 75 µg/mL AV+ 75 µg/mL VV) as compared with the control group at different time intervals (24 and 48 h). TGFβ1 gene expression of mouse embryonic fibroblast cells was assessed by quantitative real-time PCR. All comparisons were analyzed with the control group. *P < 0.05, **P < 0.01.

Abbreviations

SSD Silver Sulfadiazine

HPLC High-Performance Liquid Chromatography

AVEC Aloe vera and Vitis Vinifera Extract Combination

ED50 The half-maximal effective dose

RT Retention Times

VEGF Vascular Endothelial Growth Factor

TGFβ1 Transforming growth factor β1

Conflict of Interests

The author declared no competing interests.

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