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EVALUATION OF DERMAL BURN HEALING ACTIVITY OF NIFEDIPINE IN WISTAR ALBINO RATS

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ABSTRACT

Improving the methods of burn wound healing and tissue repair offers tremendous opportunities to enhance the quality of life for burn patients. Nifedipine was found to enhance healing in regular and steroid suppressed wounds in albino rats. A partial thickness burn wound was employed using Wistar albino rats (180–250gm) under ketamine anesthesia. In batch 'A', nifedipine group showed significant improvement in burn wound contraction (80.0±0.1%) on 12th day and significant reduction in period of epithelialization (12.5±0.3days) when compared to control (60.8±0.4% and 21.8±0.1days) and SSD groups (68.8±0.3% and 16.6±0.3days). In batch 'B', the D+N group (82.8±0.8%) showed significant improvement in burn wound contraction on 16th day and significant reduction in period of epithelialization (18.1±0.3days) when compared to D+C (63.8±0.3% and 27.3±0.2days) and D+SSD groups (69.5±0.4% and 22.0±0.2days). Histopathological examination of the tissue specimens showed abundant bands of fibrocollagenous tissue with dilated blood vessels in nifedipine group when compared to control and SSD groups. D+N group showed moderate degree of fibrocollagenous tissue in comparison with D+C and D+SSD groups. Topical application of nifedipine was found to significantly improve healing in regular and steroid suppressed burn wounds. It could be used to enhance wound healing, especially if wound healing is suppressed by steroids.

Keywords: Burn wound, wound healing, nifedipine, dexamethasone, wound contraction, epithelialization.

INTRODUCTION

Burns are the fourth most common type of trauma worldwide, following traffic accidents, falls and interpersonal violence [1]. Few areas of medicine are as challenging medically and surgically as burn care. Burn injuries affect the very young and the very old of both sexes. According to World Health Organization (WHO), burn is an injury to the skin or other organic tissue primarily caused by heat or due to radiation, radioactivity, electricity, friction or contact with chemicals [2]. The most common are burns caused by scalds, building fires, and flammable liquids and gases. Thermal burn and related injuries have remained a major cause of death and disability [3].

In India, the estimated annual burn incidence is approximately 6-7 million per year, which is the second largest group of injuries after road accidents [4]. According to WHO over 1,000,000 people are moderately or severely burnt every year in India [5]. It is estimated that one-third of all burns in India are due to clothing catching fire from open flames [6].

Treatment of burn injuries is a significant healthcare problem, considering the factors like wound infection, scarring and wound contracture. Statistics prove that developing countries have a huge load of burn injuries with majority of thermally injured victims being primarily

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managed in the peripheral hospitals. The National Programme for Prevention of Burn Injuries is also not yet in place in India. Meagerly equipped government hospitals bear the brunt of this massive load [7]. Improving the methods of wound healing and tissue repair offers tremendous opportunities to enhance the quality of life for trauma and burn patients [3].

Reports suggest that cellular calcium metabolism appears to have regulatory action on extracellular matrix, collagen production and hence also on wound healing [8, 9]. Calcium channel blockers (CCBs) inhibit collagenase production and have a vasodilatatory effect. They also restrict the formation of ischemia-reperfusion induced free oxygen radicals. Nifedipine significantly increases collagen deposition and fibroblast ingrowth [10]. Nifedipine, is also shown to have antioxidant activity in in-vitro study [11]. In a study conducted by Bhaskar et al, nifedipine was found to enhance healing process in normal and steroid depressed wound in albino rats, as evidenced by increase in tensile strength of 10 days' old granulation tissue [10, 11].

Nifedipine could potentially be an attractive agent to study with regard to its influence on wound healing process. The literature available so far have investigated the wound healing activity of nifedipine using incision, excision and dead space wound models. Very little or no data is available regarding the study on wound healing activity of nifedipine using burn wound model. Hence the present study was taken up to assess the activity of nifedipine on experimentally-induced dermal burn wound healing, in both normal and steroid suppressed burn wounds.

METHODOLOGY

Materials

A total of 36 healthy Wistar albino rats of either sex, weighing around 180–250 gm were selected for the study. The animals were procured from an authorized breeder.

Drugs and chemicals used

Pure form of nifedipine (obtained from Sigma labs)- as test drug, silver sulfadiazine cream 1% w/w- as standard drug, dexamethasone sodium phosphate injection 4 mg/ml, ketamine injection 50mg/ml- as an anaesthetic agent, normal saline- for dilution, yellow soft paraffin- for ointment preparation, hard paraffin- for inflicting wounds, surgical spirit- as an antiseptic, liquid petroleum jelly- as a base for control group, 10% formalin- to preserve tissue specimen.

METHODS

Preparation of animals

The animals were housed individually in polyethylene cages containing sterile paddy husk (procured locally) as bedding throughout the experiment and had free access to sterile food and water *ad libitum*. The animals were maintained in a well ventilated, temperature-controlled animal house under light and dark (12 hours) cycles for a period of 7 days prior to the experimental period to allow for acclimatization to the laboratory condition. The animals were kept under fasting for overnight and weighed before the experiment. Wound was inflicted aseptically under ketamine anaesthesia. The study was undertaken after obtaining approval of Institutional Animal Ethical Committee [12].

Procedure

The rats were randomly allocated into 2 main batches having 3 groups each, and each group containing 6 rats. In the batch 'A', group I served as plain control (petroleum base), group II and III received topical application of standard (1% silver sulfadiazine cream) [13] and test drug (0.5% nifedipine ointment) respectively. In the batch 'B', group I received dexamethasone (0.17mg/kg, i.m) [14] alone, group II and III received topical application of standard (1% silver sulfadiazine cream) and test drug (0.5% nifedipine ointment) respectively in addition to dexamethasone (0.17mg/kg, i.m). The above mentioned drugs were administered daily till the day of eschar falling [15].

Preparation of 0.5% (w/w) Nifedipine ointment

Nifedipine ointment was prepared by diluting 100mg of pure form of nifedipine in 20 gm of yellow soft paraffin to produce a preparation of 0.5%[(15)] by using trituration method [16].

Wound model

A partial thickness burn wound model was employed. The overnight starved rats were anaesthetized with ketamine (50mg/kg, i.m) and the dorsal surface (area below the nape of the neck) was shaved under sterile conditions. The burn wound was inflicted by pouring hot molten wax (2gm) at 80°C in a metal cylinder with 300 mm^2 circular opening which was placed on the shaven surface. The wax was allowed to remain on the skin till it got solidified [17]. After the procedure, rats were housed in individual cages and good animal care practices were followed.

Assessment of burn healing

After inflicting the wounds, animals were inspected on alternate days. Healing was assessed by parameters such as wound contraction, epithelialization and histopathological examination by a person who was masked to treatment allocation. The following parameters were observed:

Wound contraction

It helps to close the wound by decreasing the gap between its dermal edges and by reducing the wound surface area. It was monitored by measuring the progressive

changes in raw wound area, planimetrically on a transparent paper, every alternate day, excluding the day of wound infliction. The tracings were then transferred to 1 mm^2 graph paper from which the wound surface area was evaluated. This evaluated surface area was then employed to calculate the percentage of wound contraction, taking the initial size of wound (300mm^2) as 100% by using the following formula [18]

$$\text{Wound contraction (\%)} = \frac{\text{Initial wound size} - \text{specific day wound size}}{\text{Initial wound size}} \times 100$$

Wound epithelialization period

It is the time taken for full epithelialization of the wound surface. Fall of eschar leaving no raw wound behind was taken as end point of complete epithelialization and the days required for this was taken as period of epithelialization [13].

Histopathological examination

After complete epithelialization, the rats were sacrificed by cervical dislocation and the wound bed was dissected out. The specimens were preserved in 10% formalin and were sent for histopathological examination.

Statistical Analysis

Collected observations were subjected to one-way Analysis of Variance (ANOVA) followed by post Hoc Scheffe's test and were considered statistically significant when P<0.05. The results are expressed as mean±SEM.

RESULTS

In the present study, the influence of nifedipine on dermal burn wounds was investigated in Wistar albino rats.

Percentage of wound contraction

The percentage of burn wound contraction as described earlier was calculated on 4^{th}, 8^{th}, 12^{th}, 16^{th} and 20^{th} day after wound infliction.

The percentage of burn wound contraction was found to increase in a time-dependent manner in all groups

Percentage of burn wound contraction (Batch A) (Table- 2):

On 4^{th} day, the mean percentage of burn wound contraction in nifedipine group was statistically significant (P<0.05) showing 21.5±0.5, as compared to control and SSD groups showing 15.3±0.8 and 17.67±0.7 respectively.

The percentage of burn wound contraction on 8^{th} day, the mean percentage of wound contraction in nifedipine group was 63.0±0.2, which was statistically (P<0.05) from the control group and SSD group showing 40.6±0.6 and 44.6±0.4 respectively.

The percentage of burn wound contraction on 12^{th} day in nifedipine group was 80.0±0.1, which was statistically significant (P<0.05) compared to control and SSD group which were 60.8±0.4 and 68.8±0.3 respectively.

Fall of eschar was observed on 12.5±0.3 day in nifedipine treated group.

The difference between the mean percentage of burn wound contraction in nifedipine treated group as compared to control and SSD treated groups were statistically significant (P<0.05).

Percentage of burn wound contraction in the dexamethasone suppressed burn wound (Batch B) (Table- 3)

On 4^{th} day, the mean percentage of burn wound contraction in dexamethasone+nifedipine was statistically significant (P<0.05) showing 18.5±0.4, as compared to dexamethasone control and dexamethasone+SSD groups showing 10.3±0.2 and 16.6±0.4 respectively.

The percentage of burn wound contraction on 8^{th} day in dexamethasone+nifedipine was 52.6±0.4, which was statistically significant (P<0.05) as compared to dexamethasone control and dexamethasone+SSD groups showing 37.0±0.5 and 39.6±0.2 respectively.

The percentage of burn wound contraction on 12^{th} day in dexamethasone+nifedipine was 76.1±0.6, which was statistically significant (P<0.05) as compared to dexamethasone control and dexamethasone+SSD groups, which were 55.6±0.2 and 58.3±0.2 respectively.

On 16^{th} post wound day, the mean percentage of burn wound contraction in dexamethasone+nifedipine was 82.8±0.8, which was statistically significant (P<0.05) as compared to dexamethasone control and dexamethasone+SSD groups showing 63.8±0.3 and 69.5±0.4 respectively.

Fall of eschar was observed on 18.1±0.3 day in dexamethasone+nifedipine treated group.

The difference between the mean percentage of burn wound contraction in dexamethasone+nifedipine treated group as compared to dexamethasone control and dexamethasone+SSD were statistically significant.

Period of epithelialization

The fall of eschar without leaving a raw wound area behind was considered as complete epithelialization.

Time taken for complete epithelialization (Batch A) (Table- 4)

The mean time for complete epithelialization in nifedipine group was 12.5±0.3, which was statistically significant (P<0.05) as compared to control (21.8±0.1) and SSD groups (16.6±.03).

Time taken for complete epithelialization in the dexamethasone suppressed burn wound (Batch B) (Table- 5)

The mean time for complete epithelialization in dexamethasone+nifedipine group was 18.1±0.3, which was

statistically significant (P<0.05) as compared to dexamethasone control (27.3±0.2) and dexamethasone+SSD groups (22.0±0.2).

Histopathology

On histopathological examination of the tissue specimens, under H & E staining, the control group showed moderate amount of fibrocollagenous tissue with focal areas of moderate degree of chronic inflammatory cell infiltrates with granulation tissue. The SSD group showed irregular bands of collagen tissue but no inflammatory cell infiltrates were seen. Nifedipine group showed abundant amount of fibrocollagenous tissue with dilated blood vessels.

Dexamethasone+control group showed mild fibrocollagenous tissue with abundant fibroblasts but no inflammatory cells were seen. Dexamethasone+SSD group showed irregular pattern of collagen with mild to moderate granulation tissue and focal areas of chronic inflammatory cell aggregates. Dexamethasone+nifedipine group showed moderate degree of fibrocollagenous tissue with mild chronic inflammatory cell infiltrates.

Table 1. Study design

Batch A			Batch B	
Groups	Treatment	No. of animals	Treatment	No. of animals
GI	Plain control (Petroleum base)	6	Dexa* control(Petroleum base)	6
GII	Silver sulfadiazine (1% cream w/w)	6	Dexa* + Silver sulfadiazine (1% cream w/w)	6
GIII	Nifedipine (0.5% ointment w/w)	6	Dexa* + Nifedipine (0.5% ointment w/w)	6

* Dexa → Dexamethasone (0.17mg/kg, i.m)

Table 2. Effect of nifedipine on the rate of burn wound contraction (Batch A)

Groups (n=6)	Burn wound contraction (%) (Mean±SEM)				
	Day 4	Day 8	Day 12	Day 16	Day 20
A-I Control	15.3±0.8	40.6±0.6	60.8±0.4	76.5±0.7	83.4±0.4
A-II SSD	17.67±0.7	44.6±0.4	68.8±0.3	82.0±0.1	
A-III Nifedipine	21.5±0.5^{ab*}	63.0±0.2^{ab*}	80.0±0.1^{ab*}		

*=P<0.05; a= Control, b= SSD.

Table 3. Effect of nifedipine on the rate of burn wound contraction in the dexamethasone suppressed burn wound (Batch B)

Groups (n=6)	Burn wound contraction (%) (Mean±SEM)				
	Day 4	Day 8	Day 12	Day 16	Day 20
B-I Dexa Control	10.3±0.2	37.0±0.5	55.6±0.2	63.8±0.3	73.5±0.2
B-II Dexa+SSD	16.6±0.4	39.6±0.2	58.3±0.2	69.5±0.4	82.5±0.4
B-III Dexa+Nifedipine	18.5±0.4^{de*}	52.6±0.4^{de*}	76.1±0.6^{de*}	82.8±0.8^{de*}	

*=P<0.05; d= Dexamethasone control, e= Dexamethasone+SSD.

Table 4. Effect of nifedipine on period of epithelialization in the burn wound (Batch A)

Groups (n=6)	Epithelialization period (Mean±SEM)
A-I Control	21.8±0.1
A-II SSD	16.6±.03
A-III Nifedipine	12.5±0.3^{ab*}

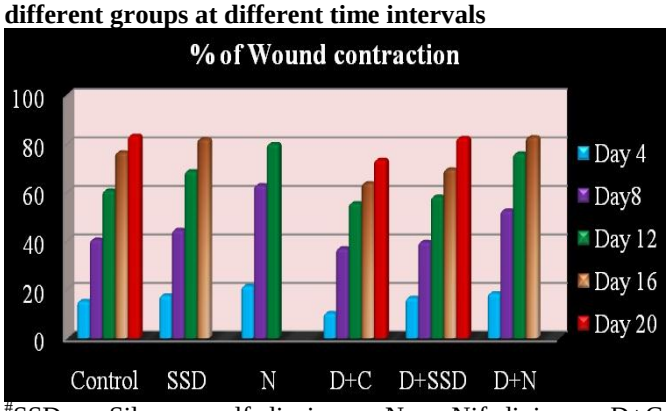
*=P<0.05; a= Control, b= SSD.

Table 5. Effect of nifedipine on period of epithelialization in the dexamethasone suppressed burn wound (Batch B)

Groups (n=6)	Epithelialization period (Mean±SEM)
B-I Dexa Control	27.3±0.2
B-II Dexa+SSD	22.0±0.2
B-III Dexa+Nifedipine	18.1±0.3^{de*}

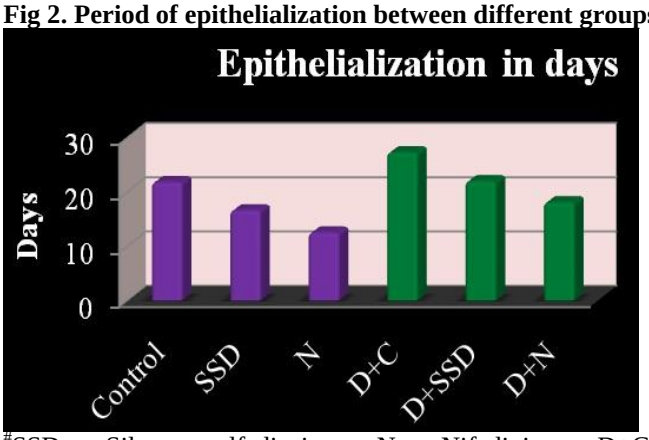
*=P<0.05; d= Dexamethasone control, e= Dexamethasone+SSD.

Fig 1. Percentage of burn wound contraction between different groups at different time intervals



#SSD- Silver sulfadiazine, N- Nifedipine, D+C- Dexamethasone control, D+SSD- dexamethasone+SSD, D+N- Dexamethasone+Nifedipine

Fig 2. Period of epithelialization between different groups



#SSD- Silver sulfadiazine, N- Nifedipine, D+C- Dexamethasone control, D+SSD- Dexamethasone+SSD, D+N- Dexamethasone+Nifedipine

Fig 3. H & E: 40X: Control group showing chronic inflammatory cells and fibroblasts. (Granulation tissue)

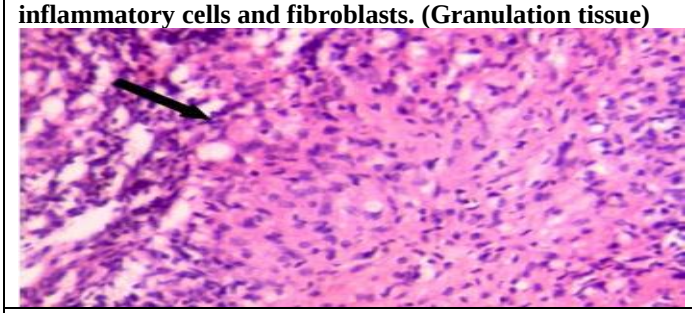


Fig 4. H & E: 40X: SSD group showing focal areas of irregular bands of collagen in subepidermis and dermis

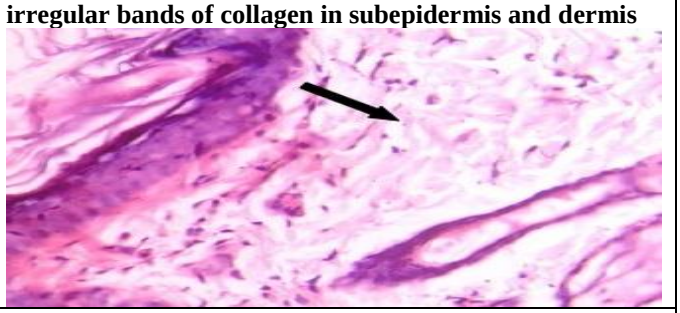


Fig 5. H & E: 40X: Nifedipine group showing abundant fibrillary collagen bundles.

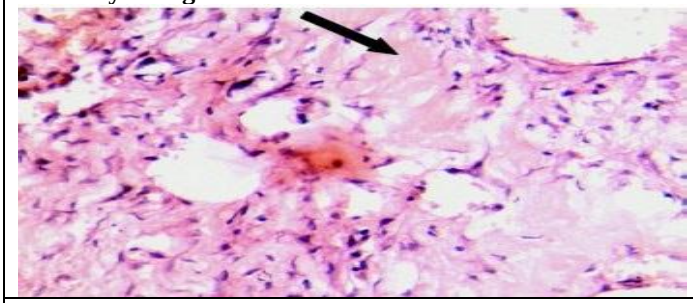


Fig 6. H & E: 40X: D+C group showing fibrous tissue

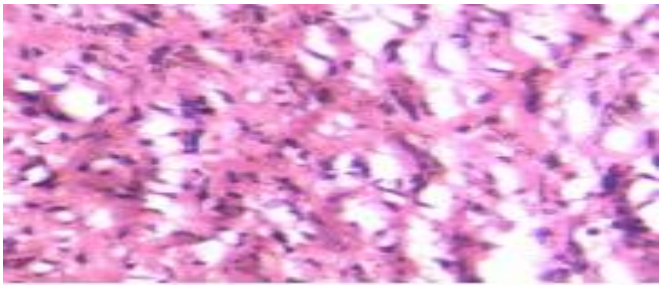


Fig 7. H & E: 40X: D+SSD group showing mild inflammatory cells and fibroblasts

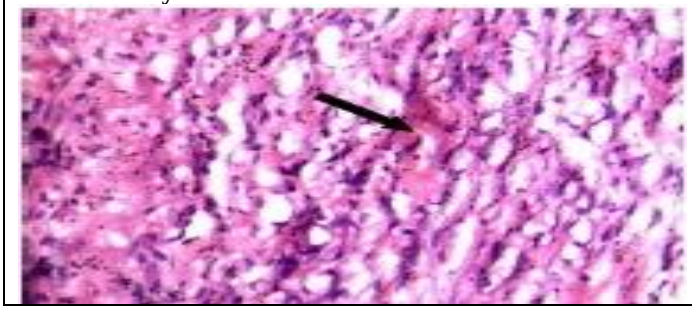
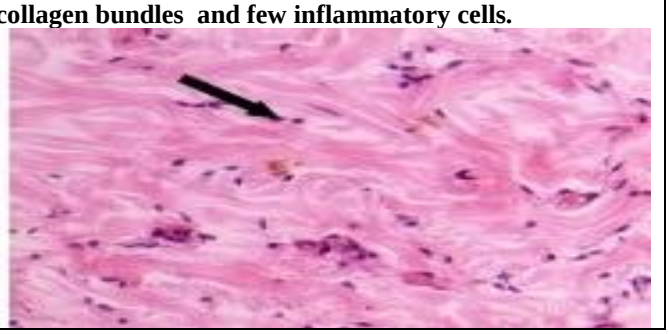


Fig 8. H & E: 40X: D+N group showing abundant collagen bundles and few inflammatory cells.



DISCUSSION

Currently, much attention is being placed on the development of expensive topical agents for wound healing. The efficacy of such agents still needs to be proven in clinical trials and the cost factor should be kept in mind. To help promote closure of dermal wounds, off-label prescribing of customized medicines has been employed by wound care specialists. The concept of off-label prescribing

of approved pharmaceuticals to treat cutaneous wounds has generated substantial attention [19].

Collagenation, wound contraction and epithelialization are crucial phases of wound healing [20]. Wound contraction is the process of mobilizing healthy skin surrounding the wound to cover the denuded area. This is believed to be due to the activity of myofibroblast [17]. The phases of inflammation, macrophasia, fibroplasia and collagenation are intimately interlinked. Thus an intervention into any one of these phases by drugs could eventually effect one or all the phases of healing [20].

It may be recalled that cellular calcium metabolism appears to have regulatory action on extracellular matrix, collagen production and hence also on wound healing. This leads to the inference that nifedipine could potentially be an attractive agent with regard to its influence on the wound healing process [8,9].

The findings of the present study indicates that topical application of nifedipine significantly enhanced regular and steroid suppressed burn wound healing process as assessed by rate of wound contraction, time taken for complete epithelialization and histopathological examination.

Topical application of nifedipine was found to hasten the rate of burn wound contraction significantly (80.0±0.1 on 12th day) as compared to that of control and SSD. Nifedipine would have either enhanced contractile property of fibroblasts or increased the number of fibroblasts recruited into the wound area. Also nifedipine significantly hastened the period of epithelialization (12.5±0.3 day) in comparison to that of control and SSD.

Topical application of nifedipine in dexamethasone suppressed burn wound was also found to enhance wound healing has evidenced by significant improvement in rate of wound contraction (82.8±0.8 on 16th day) and period of epithelialization (18.1±0.3) as compared to dexamethasone control and dexamethasone+SSD groups.

On histopathological examination of the tissue specimens, it was found that the topical application of nifedipine significantly improved the quality of granulation tissue in comparison with control and SSD groups, possibly by increasing fibroblast proliferation, maturation of collagen content on one hand decreasing collagenase activity on the other. Topical application of nifedipine in dexamethasone suppressed wound showed moderate amount of fibrocollagenous tissue in comparison with dexamethasone control and dexamethasone+SSD groups.

CONCLUSION

Treatment of burn injuries is a significant healthcare problem. Improving the methods of burn wound healing and tissue repair offers tremendous opportunities to enhance the quality of life for trauma and burn patients.

The findings of the present study indicates that topical application of nifedipine exhibits significant healing of burn wounds and also significantly reverse the steroid suppressed burn wound healing process. The healing activity of nifedipine could be attributed to its ability to alter the intracellular calcium levels, inhibition of collagenase enzyme production and/or increasing maturation or cross-linking of collagen along with its antioxidant property and vasodilatatory effect.

Having considering the above data, it could be possible that topical application of nifedipine could be useful for the treatment of burn wounds in patients who are receiving steroids. Nifedipine is cheap, easy to use, and readily available. The efficacy of topical nifedipine therapy should be confirmed by further well designed large scale clinical studies.

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CONFLICT OF INTEREST:
The authors declare that they have no conflict of interest.

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