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The effect of topical magnesium on healing of pre-clinical burn wounds

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ABSTRACT

Background: Magnesium (Mg) is an essential factor in the healing process. This study aimed to evaluate the effect of Mg creams on healing burn wounds in the rat model.

Methods: To induce burns under general anaesthesia, a 2 × 2 cm2, 100 °C plate was placed for 12 s between the scapulas in 100 male adult Sprague Dawley rats. Animals were divided into five groups (n = 20); positive control (induced burn without treatment); vehicle control (received daily Eucerin cream base topically); comparative control (induced burn and treated daily with Alpha burn cream topically); Treatment 1 and 2 (received daily Mg cream 2% and 4% topically, respectively). All animals were bled for hematological assessment of malondialdehyde (MDA) and TNF-α and sacrificed on days 0, 1, 7, 14, and 21 after interventions for biomechanical, histological, and stereological studies.

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Results: Stereologically speaking, in treatment groups an increase in dermal collagen volume and fibroblasts was noticed. In treatment groups, the length of vessels, angiogenesis, and skin stretch increased, but the wound area, MDA, and TNF-α level decreased.
Conclusion: Mg cream was effective in healing burns.

1. Introduction

Burns are still one of the most common types of injuries worldwide affecting both genders and all age groups in developed and developing countries. It is estimated that around 90% of burns happen in low- or middle-income countries and have an annual mortality rate of 180,000 cases [1].

The long-term burn wound healing process is complex and consists of several approaches associated with the body's immune system. Burn wound healing occurs in three consecutive stages: inflammation, formation of granulation tissue (proliferation), and remodeling; the last stage can lead to scarring [2]. The immediate reaction after burn includes a cascade of biological mediators of inflammation (interleukins (IL-1, IL-2, IL-4, IL-8, IL-10)), growth factors (fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF)), interferon-gamma (IFN- r), Tumor necrosis factor-α and β (TNF-α and β) and many immune system cells and extracellular matrix elements [3]. In tissue damage caused by burns, homeostasis is automatically broken; therefore, fluid accumulation, inflammation, and hypoxia occur. Such a condition is suitable for producing the angiogenic factor VEGF, which stimulates capillaries to develop new and immature vascular loops and branches [4]. A salient pathophysiological element of burns is oxidative stress. Perfusion of ischemic tissues after thermal injury leads to an imbalance between reactive oxygen species and the antioxidant defense system due to the excessive production of free radicals. ROS in burns increases the permeability of the vessels and lipid peroxidation of the plasma membrane (malondialdehyde (MDA) formation) and promotes local and systemic inflammation [3]. Previously, it was pointed out that lipid peroxidation in burns as an autocatalytic reaction initiates toxic metabolism and cell apoptosis. Accordingly, using antioxidants in burns is recommended [5,6].

In addition, inflammatory processes and the production of free radicals lead to the promotion of oxidative stress, and its suppression largely depends on the sufficient availability of mineral elements [7]. In burn wounds, a significant loss in elements such as selenium, copper, zinc, iron, magnesium, and phosphorus occurs in form of exudate from burn injuries [8,9]. Toppo et al. demonstrated hypomagnesemia in post-burn injuries [10]. It was suggested that Mg as a divalent cation has anti-inflammatory and wound-restorative properties with inflammation declining topical application that can prevent tissue necrosis [11]. Also, its role in dermal hydration and reduction of skin redness is demonstrated [12].

We wondered whether MgSO4 potentially meets the criteria for effective burn wound healing by anti-inflammatory, anti-necrosis, dermal hydration, vasodilation effect, and antioxidant properties. Therefore, we investigated the effect of topical Mgso4 (2% and 4%) on burn wound healing using an animal burn skin model. We also compared the histological, hematological, biomechanical, and stereomophological aspects between treatments and control groups.

2. Materials and methods

2.1. Ethical approval

All experiments in the current research were done in conformity with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were confirmed by the Medical and Research Ethics Committee of the Shiraz University of Medical Sciences, Shiraz, Iran (ethics code: 1396–01–65–14602).

2.2. Animals

The quality of the experimental studies was assessed using the *Animal Research: Reporting* of In *Vivo Experiments* (*ARRIVE) guidelines*. In the present study, 100 male 10–12 weeks Sprague Dawley rats (200 ± 20 g) were purchased from the laboratory animal center of Shiraz University of Medical Sciences, Shiraz, Iran. The animals were kept single in cages at 22 ± 2 °C and 12 h of light/dark cycles while having free access to water and food.

2.3. Experimental design

The animals were permitted at least one week to habituate to the new condition; then they were randomly divided into five equal groups (n = 20) including three control groups and two treatment groups.

1. Positive control (with burn wound without treatment);
2. Vehicle control (with burn wound and received daily Eucerin cream base topically)
3. Comparative control (with burn wound and treated daily with Alpha burn cream (Sina Daru, Tehran, Iran) topically)
4. Treatment group1 (with burn wound and received daily Mg cream 2% topically)
5. Treatment group 2 (with burn wound and received daily Mg cream 4% topically)

2.4. Induction of burn wounds

To induce burn wounds, a hot plate (2 ×2 cm^2, 100 °C) was placed between the scapulas for 12 s, as described before [13]. To anesthetize the rats during the study xylazine (20 mg/kg; Alphazyne) and ketamine (5 mg/ kg; Woerden, Netherlands) was administered intraperitoneally. Postburn, analgesia was induced utilizing 0.05 mg/kg buprenorphine (Produlab Pharma) three times daily (subcutaneous).

2.5. Preparation of magnesium sulfate cream

Magnesium sulfate (2–4%) (Avecina Shimi co., Tehran, Iran), methylparaben (0.2%), Eucerin (60%), beeswax (20%) (Pishgamanshimi co, Isfahan, Iran), and water to 100 cc were mixed at 70 °C.

2.6. Assessment of blood cell perfusion

A single-channel laser Doppler blood flowmeter was used with a specialized fiber optic probe (AD instrument) to measure blood cell perfusion in the microvasculature of tissues and organs before burn induction and on days 0, 1, 7, 14, and 21 post-burning.

2.7. Biomechanical study

To assess biomechanical skin properties in the burn, the cutometer MPA 580 (Biotek, USA) was used after 21 days. Measurements were undertaken by the same investigator in identical conditions. The prepared strips from the injured skin were 2–3 cm, while the wound was placed in the middle of the strips. The strips were kept in a wet condition and were wrapped in foil. They were placed between the two clips of the device to be stretched under a load of 100 kg and a speed of 20 mm/min.

2.8. MDA assay

MDA, an indicator of lipid peroxidation, is formed when free hydroxyl radicals react with fatty acid components and cause a chain reaction known as lipid peroxidation [14]. To measure MDA on day 21st based on the thiobarbituric acid assay [15], a 2 cm burned area was cut, chopped, and transferred into 10 mL of normal saline.

2.9. TNF-α measurement

To measure the TNF-α, 2 cm of the burned region was cut on day 21st and homogenized before the assay of its level with an ELISA kit (Diaclone, France). The absorbance of the samples was compared in a microplate reader (Biotek, USA) with a standard curve, and the concentrations were determined [16].

2.10. Stereological study

A digital image of the wound surface was provided on days 1, 7, 14, and 21 using a single-lens microscope that was calibrated for each photograph by a standard ruler [17]. The closure rate was determined using the point gride technique and the following formula:

Wound closure rate (%)= [(area at visit 1 - area at each visit)/area at visit 1]× 100.

2.10.1. Tissue collection and preparation

A circular piece (10 mm) was removed from the skin burn and was cut into slabs with 0.5 × 0.5 mm2; then 8–12 slabs were sampled by systematic random sampling. The slabs were processed, and embedded in cylindrical paraffin blocks, and sectioned by a microtome based on isotropic uniform random (IUR) sections. The sections were stained with hematoxylin-Eosin (H&E) and Masson's trichrome [17].

2.10.2. Estimation of the volumes of skin burn layers

In each section, the borders between the layers were identified and characterized (Fig. 1). The stereological tool is made of a Nikon E-200 microscope (Tokyo, Japan) with an oil objective lens, a Samsung video camera united to a computer, and software designed at the University's Histomorphometry and Stereology Research Center. The stereological probes were employed for the live figures and the parameters mentioned were estimated with the "point-counting method" [17].

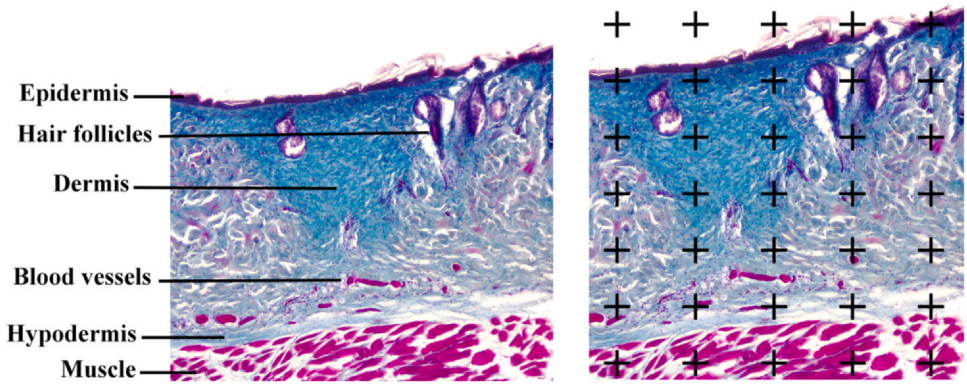

Fig. 1 – Histology of skin burn wound. Three layers of the skin (the epidermis, dermis, and hypodermis) illustrate in the histological section (a). The point-counting method was used to assess the volumes of the layers of the burn wounds (b).

The volume densities "Vv" of the skin burn layers were evaluated following formula:

V(structures)= [∑P(structures)/∑P(total)]

Where "∑P (structure)" and "∑P(total)" is the number of points hitting the epidermis, dermis and hypodermis, and the total skin burn tissue, respectively.

2.10.3. *Estimation of the volume density of the collagen bundles of the dermis layer*

The volume density of the collagen bundles of the dermis layer was estimated by the point-counting method as described previously by the following formula:

V(structures)= [∑P(structures)/∑P(total)]

Where "∑P (structure)" and "∑P(total)" is the number of points placed on the collagen bundles and the dermis layer, respectively [18].

2.10.4. *Estimating the length of the dermis and hypodermis blood vessels*

The length density of the vessels was evaluated by an unbiased counting frame to compare small vessels (less than 10μm) and larger vessels (more than 10μm). The unbiased counting frame with forbidden and acceptance lines was superimposed on the live image of the dermis and hypodermis layers. The blood vessels which were either completely or partially in the unbiased counting frame and did not contact the forbidden lines were counted [18]. The length of the vessels was assessed by the following formula:

LV (blood vessels / dermis and hypodermis) = 2 ∑Q/ (∑P × a/f)

where "∑Q" is the total number of the vessels, that their diameter was more than 10.1 µm and less than 10 µm, selected by the unbiased counting frame per dermis and hypodermis *layers*, "∑P" is the number of frame-related points hitting the dermis and hypodermis layers, and "a/f" is the counting frame area [19].

Vessels were determined by their special characteristics (endothelial cells are squamous, polygonal, and elongated with the long axis in the direction of blood flow) and most of them had red blood cells.

2.10.5. *Estimation of the numerical density of the fibroblasts in the dermis layer*

The numerical density "Nv (fibroblasts/ dermis layer)" was estimated by the "optical disector" method. The optical disector contains an Eclipse microscope with a high Numerical Aperture (NA=1.30) ×40 oil-immersion objective lens joined to a video camera that transmits microscopic live figures to a computer monitor. Also, it has equipped with an electronic microcator with a digital readout to evaluate the number of fibroblasts by moving in the Z-direction [17]. The numerical density (NV) of the fibroblasts was assessed by the following formula:

Nv (fibroblasts / dermis layer) = ΣQ–/ (Σp× (a/f) × h)

Where "∑Q" was the number of selected fibroblasts; "∑P" was the number of dissectors; a/f was the area of the frame, and "h" was the height of the dissector.

2.11. *Statistical analysis*

SPSS statistical software (Version 18.0, SPSS Inc, Chicago, Illinois, USA) was used to conduct statistical tests. Data were presented as mean ± standard deviation (mean ± SD). Kruskal Wallis, One way ANOVA, and one-way repeated measures ANOVA were utilized for comparisons. $P < 0.05$ was considered statistically significant.

3. Results

3.1. *The wound area*

In the first to third days, we found no significant difference in the wounds created among the studied groups. We found a significant difference in wound area among groups during three weeks (F=19.201, $P < 0.001$). According to further analysis using a post hoc test (Tukey's), we found a significant difference between treatment groups and control groups (positive and vehicle) in 21 testing days ($P < 0.05$) (Fig. 2). Among the treatment groups, the wound area in treatment 2 was the lowest and significantly different from the comparative control group ($P < 0.05$) (Table 1).

3.2. *Stereology*

Stereological findings in treatment groups 1 and 2, receiving 2% and 4% Mg, illustrated an increase in fibroblast number compared with control groups (Positive and vehicle). Also, the number of fibroblasts increased significantly in treatment group 2 compared to treatment group 1 ($P < 0.001$). The length of the vessels with a diameter of < 10 µm in treatment group 2 was significantly more than other groups ($P < 0.001$). Lv in vessels with a diameter of > 10 µm showed a significant increase in treatment groups compared with the control groups (vehicle and positive) ($P < 0.001$). However, we found no significant difference in the results of the comparative control group and treatment groups (Fig. 3).

3.3. *Collagen volume*

There was no significant difference among groups regarding the collagen volume (Vv, mm^3/mm^3 ×100, $p = 0.200$) (Fig. 4).

3.4. *Epidermal, dermal, and hypodermal volumes*

We found no significant difference among the groups regarding the volume of epidermal, dermal, and hypodermal layers (Table 2).

3.5. *Biomechanical study*

Regarding skin strength, a significant increase was seen in treatment groups compared with control groups (Table 3).

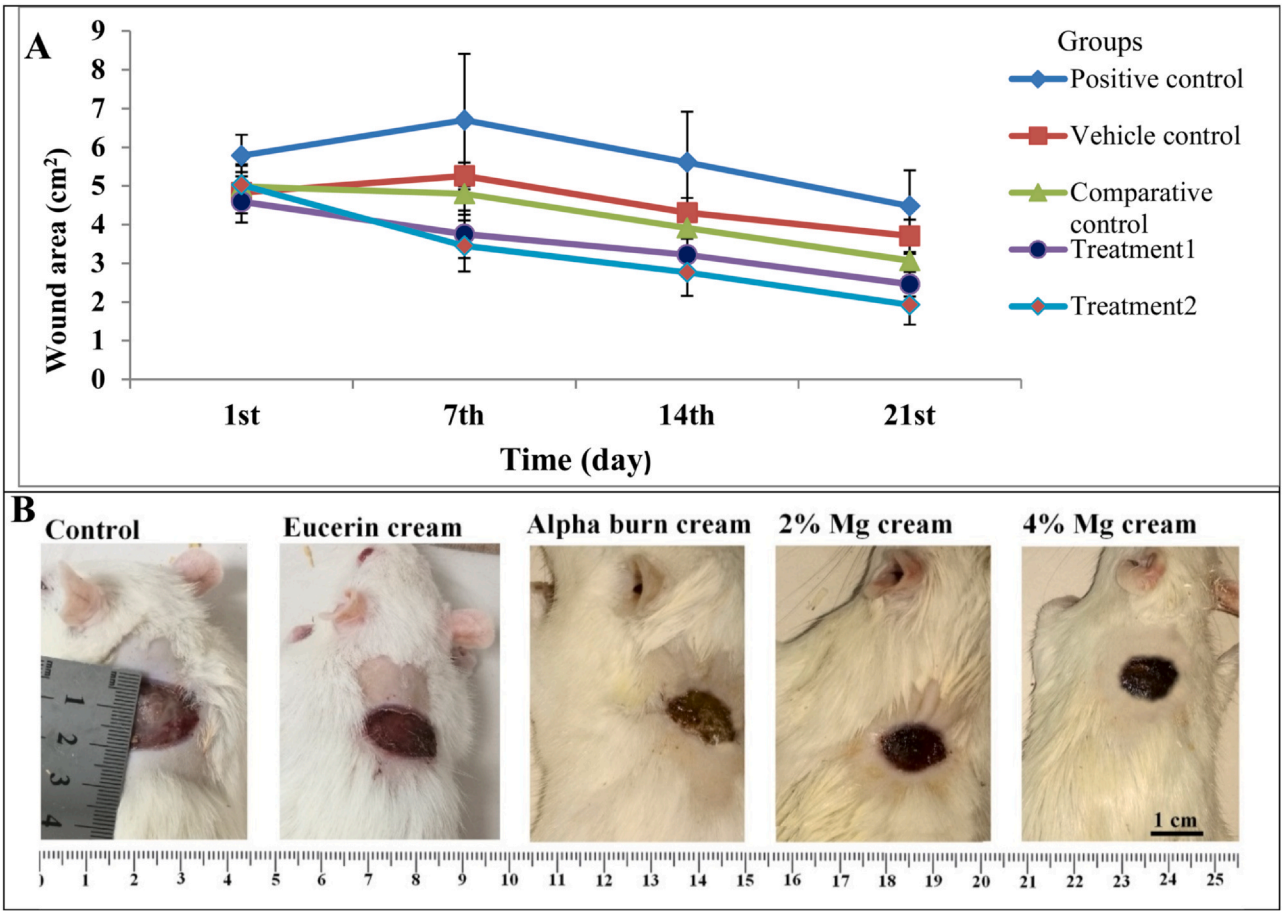

Fig. 2 – Comparison of the wound area in various groups in three consecutive weeks (A); Changes in the size of burn wounds in different groups after 21 days (B).

Table 1 – wound area among different groups during 21 days.

Groups	Wound area in 1st day (mean ± SD (Cm^2))	Wound area in 7th day (mean ± SD (Cm^2))	Wound area in14th day (mean ± SD (Cm^2))	Wound area in 21st day (mean ± SD (Cm^2))
Positive control	5.786 ± 0.534	6.70 ± 1.71	5.61 ± 1.31	4.48 ± 0.92
Vehicle control	4.824 ± 0.534	5.26 ± 0.34[a]	4.31 ± 0.38[a]	3.71 ± 0.42[a]
Comparative control	4.986 ± 0.534	4.8 ± 0.54[a]	3.92 ± 0.29[a]	3.07 ± 0.18[a]
Treatment1	4.593 ± 0.534	3.75 ± 0.61[a,b]	3.22 ± 0.51[a,b]	2.46 ± 0.32[a,b]
Treatment2	5.029 ± 0.534	3.45 ± 0.65[a,b,c]	2.77 ± 0.62[a,b,c]	1.93 ± 0.51[a,b,c]

[a,b,c]: statistical differences with the positive control group, vehicle control, and comparative control groups ($P < 0.05$), respectively

3.6. *Blood flow rate*

We found a significant increase in blood flow rate in different groups on days 1st, 7th, 14th, and 21st after induction of burns ($F=1.47$, $P < 0.0001$) (Fig. 5).

According to a post hoc test (Tukey's), no significant difference was found among groups regarding blood flow rate (Table 4).

3.7. *MDA and TNF-α*

Regarding the MDA level (M/L) and TNF-α level (μg/mL), we found a significant difference among groups. Regarding both parameters, a significant decrease was observed in the treatment groups and comparative control group compared to the positive and vehicle control groups (Table 5).

3.8. *Qualitative evaluation of the burn wound healing*

The micrograph of the effects of Eucerin cream, Alpha cream, 2%, and 4% Mg cream after 21 days on the collagen bundles and fibroblast in the skin tissues of rats are depicted in Fig. 6. An increase in the volume of collagen bundles and the number of fibroblast cells in the Eucerin cream (vehicle control group), Alpha cream (comparative control group), 2% Mg, and 4% Mg cream (treatment groups 1 and 2) are observed in

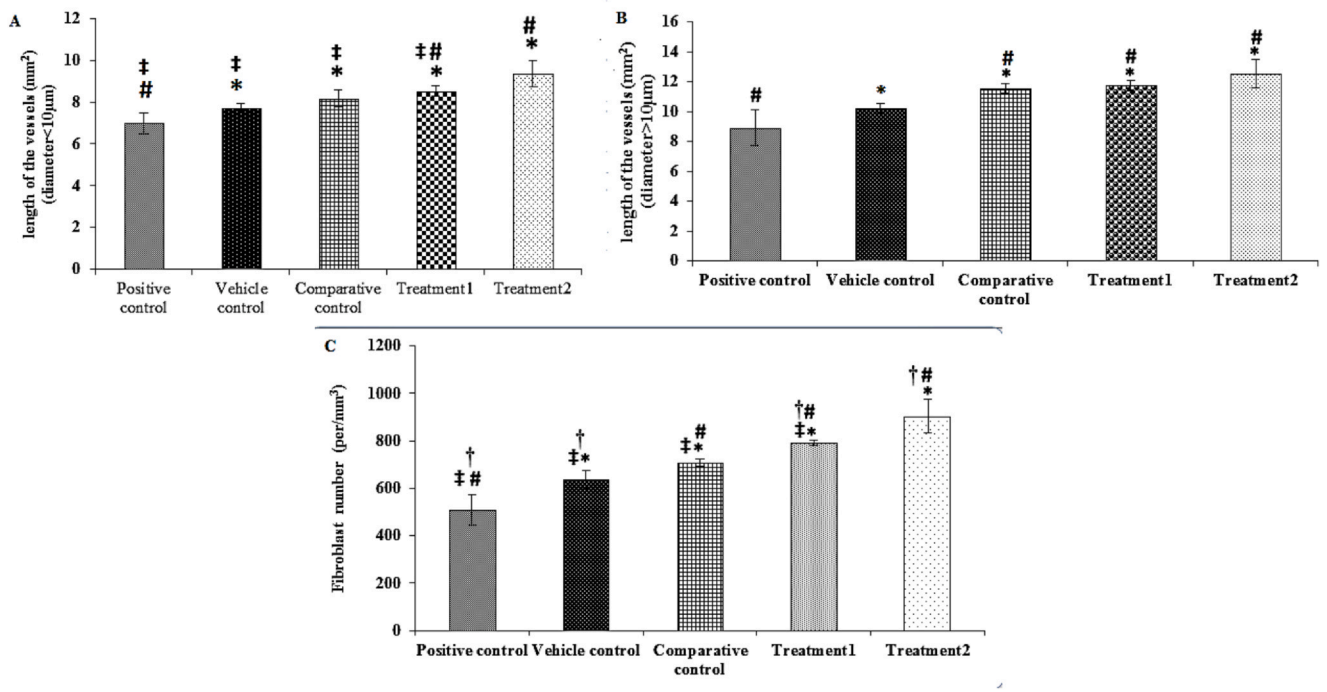

Fig. 3 – Stereological study on numerical density of the fibroblasts in different layers of the skin (A); The length of the vessels with diameter < 10 µm in different groups (B); The length of the vessels with diameters > 10 µm in different groups (C). *Significant difference with positive control (P < 0.0001); #Significant difference with vehicle control (P < 0.001); † Significant difference with comparative control (P < 0.001); ‡ Significant difference with treatment 2 (P < 0.001).

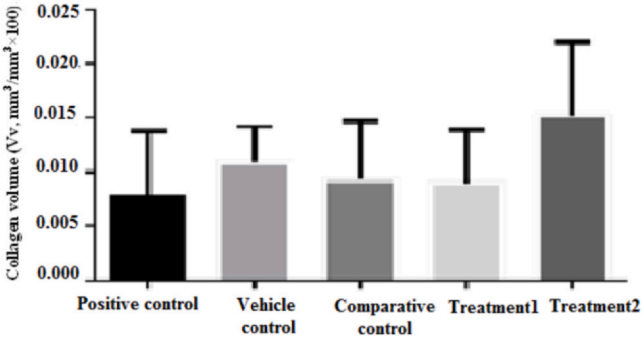

Fig. 4 – Stereological study of collagen volume in the skin of various groups (Vv, mm^3/mm^3 ×100, $p = 0.200$).

the burn wound dermis compared to the positive control group.

4. Discussion

Treatment of burn wounds is one of the most clinical problems and the application of the most effective burn repair methods is still a continuous challenge in medicine [20]. Despite several chemical products utilized to treat burn wounds, worldwide interest is growing in natural products and traditional medicine [21]. The importance of nutritional interventions in wound care has always been a research topic. Although the impact of macronutrient supplementation was previously illustrated, there is no official recommendation to apply minerals to promote wound healing [22]. Lipkin et al. displayed that the Mg level decreases in skin injuries and can affect tissue repair [23]. According to previous reports, Mg has anti-inflammatory, wound-restorative, and inflammation-declining topical properties [11]. Also, it has been successfully applied as an antidote for hydrofluoric acid dermal burns in the rat model [24].

We illustrated Mg increases dermal collagen volume, fibroblast number, length of vessels, angiogenesis, and skin's stretch; while it decreases the wound area, TNF-α, and MDA. It was shown that a network of hydrogel consisting of Mg ion accelerates burn wound healing due to promote the proliferation of fibroblast cells [22,25]. Several researchers have also shown the efficacy of Mg ointment as an antidote to repair hydrofluoric acid dermal burns in the rat model [24]. Harris et al. demonstrated that treatment with subcutaneous Mg salts leads to fewer injuries in severe burns [26].

Fibroblast count is an index to evaluate the healing process and assess the quality of the repair. In line with this index, we indicated the positive effect of Mg on the number of fibroblasts and in burn wound repair [27]. During the process of angiogenesis in wound healing, the vascular basement membrane and fibrin or interstitial matrix are destroyed by endothelial cells, according to which endothelial cells begin to migrate into the matrix and proliferate by forming new capillary-like tubes. Fibroblasts are important for the production of new extracellular matrix, which is necessary to support additional cell growth. Blood vessels that are placed in such a matrix play an important

Table 2 – Volume of epidermal, dermal, and hypodermal layers in different groups after 21st day.

Volume of differen layers of Skin	Groups	Mean ± SD* (mm^3)	P-value
Epidermal layer	Positive control	0.04 ± 0.017	0.962
	Vehicle control	0.044 ± 0.015	
	Comparative control	0.04 ± 0.027	
	Treatment1	0.048 ± 0.021	
	Treatment2	0.046 ± 0.020	
Dermal layer	Positive control	0.624 ± 0.15	0.974
	Vehicle control	0.61 ± 0.16	
	Comparative control	0.58 ± 0.18	
	Treatment1	0.612 ± 0.12	
	Treatment2	0.648 ± 0.16	
Hypodermal layer	Positive control	0.334 ± 0.15	0.957
	Vehicle control	0.334 ± 0.15	
	Comparative control	0.384 ± 0.18	
	Treatment1	0.31 ± 0.16	
	Treatment2	0.352 ± 0.11	

* SD (Standard deviation)

Table 3 – Skin strength in different groups after 21st day.

Groups	Mean of skin strength ± SD
Positive control	0.53 ± 0.15[a,b]
Vehicle control	0.46 ± 0.21[a,b]
Comparative control	0.67 ± 0.23[a,b]
Treatment1	1.86 ± 0.40
Treatment2	1.84 ± 0.71

[a,b] Statistically significant difference with treatment groups 1 and 2 ($P < 0.0001$)
*SD: Standard Deviation

role in the stability of cell metabolism by providing oxygen and nutrients. The migration of fibroblasts to the wound bed begins about 5 days after wound formation. Growth factors, especially Platelet-derived growth factor (PDGF) and TGF-B, along with fibrin and fibronectin make fibroblasts suitable for the proliferation and expression of integrin receptors and migration to the wound. When fibroblasts enter the wound, they replace fibrin by producing type I collagen and other extracellular matrix proteins. A few days after the migration of fibroblasts to the extracellular matrix, they change to the myofibroblast phenotype and are completed with actin bundles in the cytoplasmic region near the plasma membrane [28]. Increasing the proliferation rate of the fibroblasts, production of the extracellular matrix, and tissue contractile reduce the wound area and increase the repair process in the tissue epithelium [29]. The rate of wound shrinkage is a criterion for evaluating the extent of wound healing because the wound area decreases simultaneously with the healing process and scab formation. Fibroblasts flow to the wound and release substantial factors that participate in tissue repair and wound healing [30]. Yang et al. (2023) showed that the

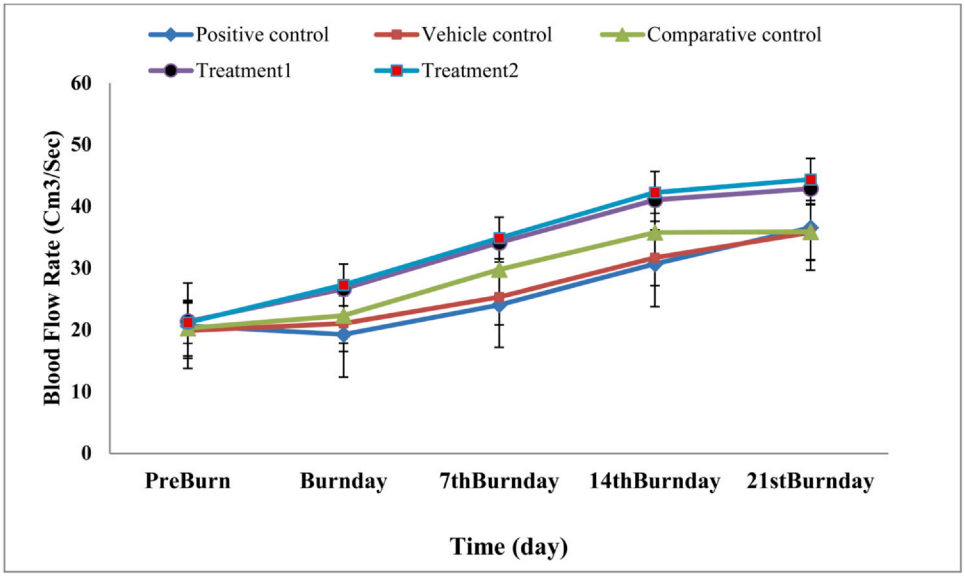

Fig. 5 – Comparison of the blood flow rate in various groups in different days.

Table 4 – The mean Blood flow rate among groups on different days.

	Groups	Mean ± SD*	P-value
Pre-Burns	Positive control	20.7 ± 6.91	0.418
	Vehicle control	19.87 ± 4.5	
	Comparative control	20.28 ± 4.5	
	Treatment1	21.37 ± 4.51	
	Treatment2	21.23 ± 3.39	
BurnDay	Positive control	19.30 ± 6.91	0.320
	Vehicle control	21.08 ± 4.48	
	Comparative control	22.37 ± 4.49	
	Treatment1	26.68 ± 4.51	
	Treatment2	27.33 ± 3.4	
BurnDay7th	Positive control	24.1 ± 6.85	0.42
	Vehicle control	25.37 ± 4.53	
	Comparative control	29.78 ± 4.49	
	Treatment1	34.17 ± 4.52	
	Treatment2	34.93 ± 3.38	
BurnDay14th	Positive control	30.70 ± 5.59	0.09
	Vehicle control	31.67 ± 4.52	
	Comparative control	35.68 ± 4.5	
	Treatment1	40.07 ± 4.6	
	Treatment2	42.33 ± 4.0	
BurnDay21st	Positive control	36.6 ± 6.92	0.07
	Vehicle control	35.77 ± 4.51	
	Comparative control	35.97 ± 4.49	
	Treatment1	42.97 ± 4.5	
	Treatment2	44.43 ± 3.41	

* SD Standard deviation

Table 5 – The mean of the level of Malondialdehyde and TNF-α in tissue samples on day 21st.

Variable	Groups	Mean ± SD
MDA (M/L)	Positive control	15.44 ± 5.98
	Vehicle control	15.13 ± 5.98
	Comparative control	10.94 ± 1.96^{a,b}
	Treatment1	8.69 ± 2.25^{a,b}
	Treatment2	9.32 ± 2.27^{a,b}
TNF-α (μg/mL)	Positive control	1.61 ± 0.53
	Vehicle control	1.74 ± 0.68
	Comparative control	1.30 ± 0.53^{a,b}
	Treatment1	1.06 ± 0.26^{a,b}
	Treatment2	1.28 ± 0.22^{a,b}

^{a,b} Statistically significant difference with positive and vehicle control groups ($P < 0.001$)
MDA: Malondialdehyde; SD: standard deviation

combination of ZN and Mg particles in wound dressings enhanced the migration of human skin fibroblasts and human immortal keratinocytes. On the other hand, it caused the transformation of skin fibroblasts into myofibroblasts by activating the STAT3 signaling pathway and then accelerated the production and remodeling of the extracellular matrix [31]. In this study we used a rat model to induce burn wounds, previous research found that skin of rodents has a panniculus carnosus layer and this thin muscle layer is found only in the platysma of the neck in humans. This layer causes rapid wound contraction after injury. On the other hand, human wounds heal through re-epithelialization and granulation tissue formation [32]. In our study, probably wound closure was caused by increasing the number of fibroblasts inside the wound under the influence of magnesium. A possible mechanism in the literature for this finding is that fibroblasts were converted into myofibroblasts due to phenotypic changes after the burn and the wound was closed through α-smooth muscle actin (α-SMA) contraction [33].

In our study, Doppler laser sonography and stereological findings denoted an increase in angiogenesis in treatment groups. The stereological findings on changes in the vessel length reveal an increased rate of angiogenesis which affects wound healing [34]. The rate of angiogenesis and the development of blood vessels in burn injuries were previously described. Mg stimulates and intensifies the formation of new blood vessels; therefore, supplying the blood flow to the injured tissue and accelerating the repair process [35]. A reduction in Mg level leads to an increase in intracellular calcium and results in vascular contraction and blood pressure which negatively impact wound healing [36]. Mg through localization of calcium ions in smooth muscles of vascular tissue, modulation of vascular endothelium causes rising of vasodilation in the injured tissues [37]. Mg ion ($[Mg^{2+}]$) acts in an enzymatic reaction for cell proliferation [38], cell differentiation [39], and collagen formation [40].

Although there are many reports of the healing effect of minerals in wound healing [8,9], there are conflicting accounts of their chelates. Mohammadpour et al. (2012) applied the local iron chelators (Kojic acid and deferiprone) to accelerate wound healing; their argument, in this case, was the antioxidant effect of these compounds and their ability to chelate iron [41]. Ayadi et al. (2020) used a topical lotion containing EDTA, methylsulfonylmethane, and Livionex formulation lotion (all are chelators for redox-active metal ions) to reduce the progression of burn wounds by lowering the accumulation of active aldehydes and decreasing the expression of some proteins such as IL-6, TNF-α and TNF-α converting enzyme [42] In addition, some compounds such as dimethyl aminoethyl methacrylate: methacrylic acid hydrogel in tissue-engineered skin models, cause increased $[Mg^{2+}]$ and decreased $[Ca^{2+}]$ to potentiate keratinocyte migration and reduce wound contraction [43]. It seems that the reason for the reported contradictions in the role of metal ions in wound healing is the importance of their base concentrations during different stages of wound healing, which should be considered in studies. Grzesiak and Pierschbacher (1995) showed that at the beginning of the wound process, cell migration is accompanied by an increase in the concentration of magnesium and a decrease in the concentration of calcium at the site. With the progress of wound healing, the concentration of these ions returns to the normal level. They showed that changes in magnesium and calcium levels in wound fluid occur at the same time that inflammatory cells, keratinocytes, fibroblasts, and new vasculature migrate in vivo during wound healing [44]. Coger et al., 2019, established the kinetics of metal concentrations during the healing of wounds in a rat model. According to their results, the

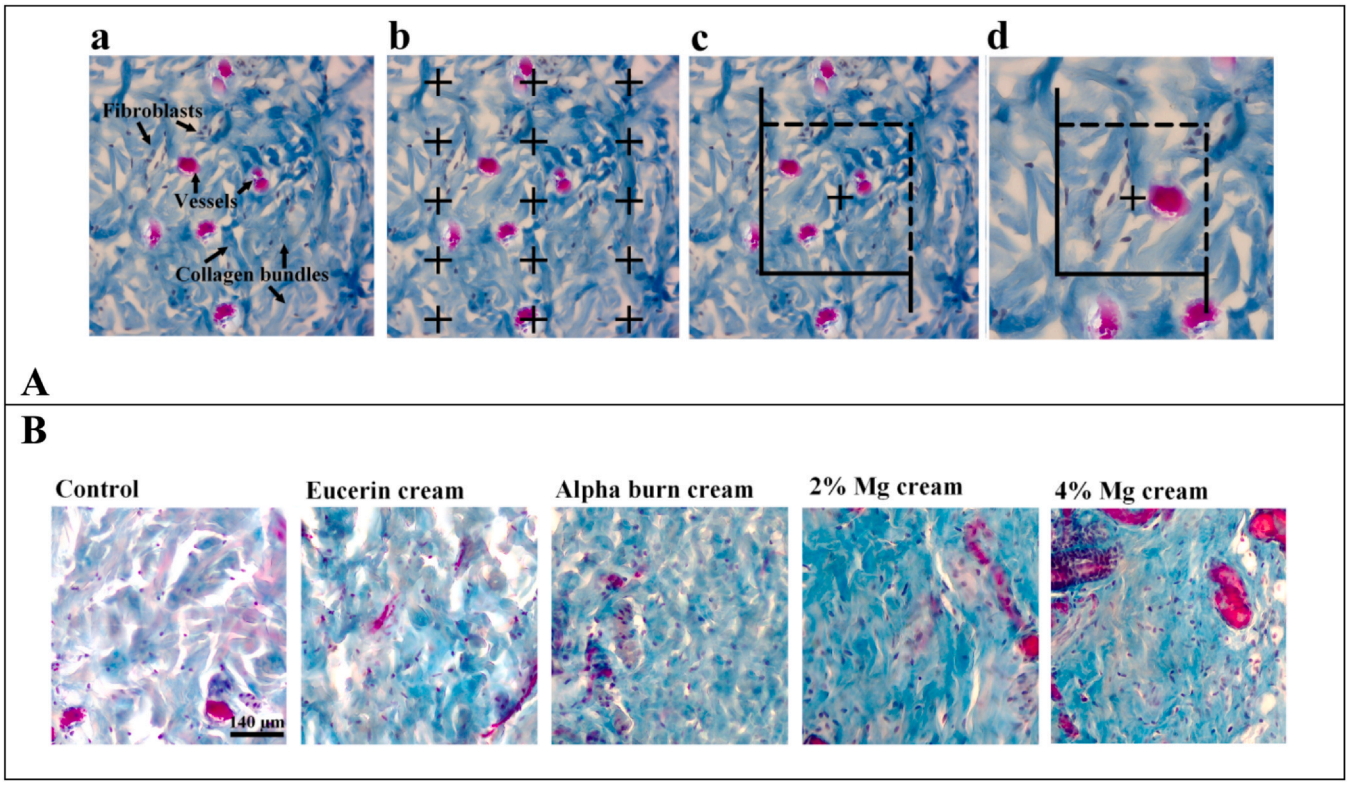

Fig. 6 – The dermal component (collagen bundle, fibroblast, and blood vessels, etc.) are demonstrated on the histological section (a). The point-counting technique was utilized to estimate the volume density of the collagen bundles (b). An unbiased counting frame was employed to estimate the length of the blood vessels (c). The optical disector technique was used to assess the numerical density of the fibroblasts (d) (A). The Effects of Eucerin cream,Alpha cream, 2% and 4% Mg cream after 21 days on the collagen bundles and fibroblast of the burn wound healing in rats (B).

supplementation of zinc, iron, and magnesium in the wound healing process is considered from the late inflammation until the mid-proliferation phase, and Copper supplementation is restricted to the early stage of wound healing [40].

We demonstrated a decrease in MDA level after administration of Mg cream when compared to control groups. MDA has been shown to increase in the damaged and inflamed tissue as the final product of lipid peroxidation and is considered one of the oxidative damage indexes [45]. Regarding TNF-α level, we determined a significant decrease in treatment groups compared to control groups revealing the efficacy of Mg in wound healing. TNF-α is an inflammatory cytokine that plays a main role against the causative agent of inflammatory processes [46]. Sun et al. studied the topical application of antibodies targeting TNF-α to reduce the extension of necrosis by modulating inflammation locally in a partial-thickness rat burn model. According to their assumption, necrotic expansion is driven by elevated levels of pro-inflammatory mediators. Decreasing the necrotic tissue and diminishing the macrophage infiltration in anti-TNF-α treated tissues suggest inhibition of a downstream mediator of inflammation and reduction in overall inflammation [47]. In our study, magnesium is likely to have an impact on accelerating wound healing by adjusting inflammatory intermediaries. On the other hand, Mg ions by controlling the calcium entry into the wound site lead to the shortening of the inflammation phase, thus accelerating the recovery in the absence of scarring [48]. One of the most important aspects of burns to evaluate the needs of care and treatment of burns is to pay attention to the depth of the burn, which requires more challenges to achieve satisfactory results in the case of a deeper burn. Therefore, addressing the depth of the burn is vital when describing the physiological responses in the wound environment after the burn [49]. However, one of the limitations of the present study was the lack of assessment of the depth of the burn, which should be considered in future studies.

5. Conclusion

Regarding the stereological findings for the increase in the number of fibroblasts and the length of the vessels, the reduction in the wound area, the changes in blood flow as an angiogenesis index, and the decrease in the TNF-α level in the improvement of inflammation and wound healing, it was shown that 2% and 4% Mg cream is effective in the treatment of burn wounds and acceleration of wound healing. Also, it may prevent early scar formation by balancing collagen production. Probably Mg cream (2% and 4%) is efficient for healing burn wounds.

CRediT authorship contribution statement

Rahmanian E: conception and design of the study; **Namazi M:** conception and design of the study; **Tanideh N:** acquisition of data; **Karbaleidust S:** acquisition of data **EskandariRoozbahani N:** analysis and interpretation of data; **Ketabchi D:** analysis and interpretation of data; **Rezazadeh D:** analysis and interpretation of data; **Hamidzadeh N:** drafting the article; and **Rahmanian F:** drafting the article; **Rahmanian E:** revising the critically for important intellectual content; **Namazi R:** revising the critically for important intellectual content;**Karbalaeidust S:** revising the critically for important intellectual content; all authors approved the final version to submit.

Conflict of Interest

None declared.

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