



## The Effects of Topical Sildenafil on Wound Healing in Rat

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### Abstract

Nitric oxide (NO) is an important molecule synthesized during wound repair. Studies have reported the use of NO donors on cutaneous wound repair, but their effects in different phases of healing are not elucidated. The aim of this work was to investigate the effects of topical sildenafil on wounds in rats. Sildenafil (25 mg) was topically applied once daily on wound in treatment groups. On days, 7, 14 and 21 of lesion, five animals in each group were randomly selected and sacrificed and histological properties were evaluated. Our results showed the wound area contracted to 15% of the original size by day 7 post-wounding in treated group. Whereas control rats showed significantly delayed wound healing. The wound area contracted to 26% of the original size by day 14 post-wounding and to 46% by day 21 post-wounding. Also sildenafil cream caused more fibroblast and macrophage migration, increased vascularization, collagen regeneration, and epithelialization. This study indicates that sildenafil cream augments the wound healing process and may be of clinical benefit.

*Key words:* Nitric oxide; Sildenafil cream; Wound healing.

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### 1. Introduction

Sildenafil citrate is a specific inhibitor of type 5 cyclic guanosine monophosphate (cGMP)-specific phosphodiesterases (PDE). It was used as an antianginal drug in the 1980's and due to an unexpected side effect caused erection in males. It increases the nitric oxide level and nitric oxide in turn increases the cGMP level within the cell. The accumulation of cGMP allows for an enhanced smooth

muscle relaxation and increased blood flow in target tissues [1]. Thus, sildenafil has been recognized as being effective for the treatment of erectile dysfunction.

Inhibiting cGMP degradation by sildenafil might be a rational approach to treat patients with diabetes, coronary artery disease or heart failure [2]. Sildenafil dilates epicardial coronary arteries, improves endothelial dysfunction and inhibits platelet activation in patients with coronary artery disease [3] and acutely enhances flow-mediated vasodilatation in patients with heart failure [4].

Similarly, PDE-5 inhibition with sildenafil prevents the smoking-induced decrease in

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flow-mediated vasodilatation and increases impaired NO bioactivity [5]. The relationship with endothelium dysfunction in diabetes patients and a low cGMP level has been also reported [6].

Nitric Oxide has been shown to play a major role in wound healing [7]. In addition to a role for endogenous NO in wound healing; improvement of wound healing following application of exogenous NO has also been reported. Enhancement of wound repair with topically applied NO releasing polymers has been reported for polyethyleneimine cellulose NONOate polymers [8] and for poly (vinyl alcohol) hydro gel dressings [9]. However, there has been no study on the topical use of sildenafil in different phases of wound healing. The process of healing a skin wound is complex and understanding the process of wound healing at the molecular level may lead to the design of better treatments [10].

Considering its actions as mentioned above, we hypothesis that topical application sildenafil may lead to an improvement in skin wound healing in an animal model.

## 2. Material and methods

### 2.1. Cream preparation

The needed ingredients for preparation of 5% sildenafil cream were accurately weighed. The required number of sildenafil tablets were roughly pulverized. Then propylene glycol and purified water were added to the sildenafil citrate powder to form a smooth paste. It was then incorporated into sufficient hydrophilic ointment (Dermabase, vanicream) to final weight and mixed well [11].

### 2.2. Surgical procedures and treatment protocol

Male Sprague-Dawley rats (200-250 g) were obtained from the Laboratory Animals Research Center of Shiraz University of Medical Sciences. All animals received humane care and the experimental protocol

was approved by the Committee of Laboratory Animals according to Shiraz University of Medical Sciences guidelines. Rats were housed in individual cages under controlled environmental conditions of temperature ( $22\pm 2$  °C) and a 12-h light/dark cycle, with normal food and water provided *ad libitum*. The rats were weighed and the extent of their intake of food and water consumption was monitored daily.

The surgical interventions were carried out under ketamine (5 mg/kg) and xylazine (2 mg/kg) anesthesia in sterile conditions. The back region of each animal was shaved and sharp punch (ID 300-mm<sup>2</sup>) used to remove the skin including panniculus carnosus and adherent tissues.

The animals were randomly divided into two groups of 15 rats each as follow: control groups and treatment groups. Based on Rockville *et al.* study [11], 0.5 ml of 5% sildenafil cream, equivalent to 25 mg of sildenafil, was topically applied once daily on the wound in treatment groups. The control group received the same cream base without sildenafil. On days, 7, 14 and 21 after the creation of lesion, five animals from each group were randomly selected and sacrificed with the same anesthetic solution.

### 2.3. Estimation of wound healing (wound closure)

The wound area was measured and wound closure rate was expressed as the percentage of the original wound area and was calculated by the following formula [12]:

$\% \text{ wound closure on day N} = \frac{(\text{area on day 0} - \text{open area on day N})}{\text{area on day 0}} \times 100$

The wound area as well as surrounding tissues was dissected in two parts, half for biochemical analysis and the other half for histopathological analysis.

### 2.4. Histological grading

The tissue samples were fixed in 10% formalin and embedded in paraffin. Prepared

sections were stained with hematoxylin and eosin for histological examination and scoring. Morphological findings, including epithelialization, cellular content (neutrophils, macrophages, and fibroblasts), collagen regeneration, and vascularization were scored as follows: none, score 0; few, score 0.5; moderate, score 1; many, score 2; and considerable, score 3 [13].

The histological examination and scoring system were performed in a blinded fashion.

### 2.5. Statistical analysis

All data were expressed as means  $\pm$  SEM. Data were analyzed by one-way ANOVA. P value less than 0.05 were considered to indicate statistical significance (SPSS, version 15).

## 3. Result

### 3.1. Estimation of wound healing (wound closure)

The wound area contracted to 15% of the original size by day 7 post-wounding in treated group. The wound area contracted to 26% of the original size by day 14 post-wounding and to 46% by day 21 post-wounding ( $p < 0.001$ ). The control rats showed significantly delayed wound healing (Figure 1).

### 3.2. Histological evaluation

The histological evaluation, which included assessing the degree of neutrophil, macrophage and fibroblast infiltration, collagen regeneration, vascularization, and epithelialization in the wound area, was compared between the control and treatment groups (Table 1). As shown in Table 1, compared with the control group, rats in the treatment group tended to demonstrate more fibroblast and macrophage migration, increased vascularization, collagen regeneration, and re-epithelialization. A significant increase in the number of infiltrated fibroblasts in the subcutaneous tissue in the

treatment group was seen at 7 days post-wounding ( $p < 0.01$ ). Furthermore, a significant abundant collagen regeneration was observed

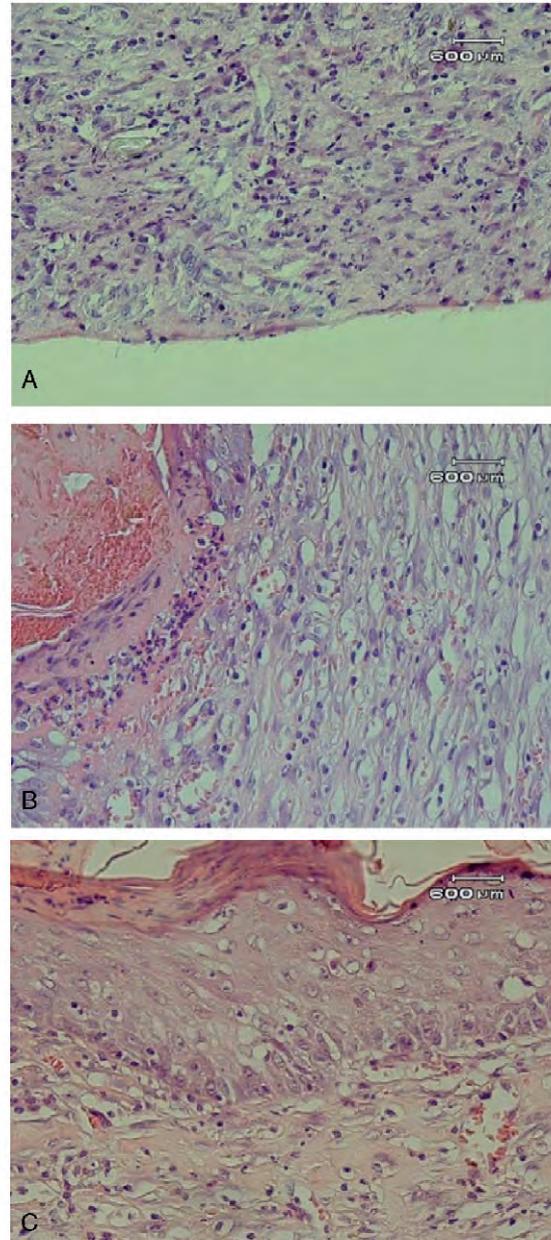


Figure 1. Effect of sildenafil on wound healing on the skin of rats. (A) Control group show a higher amount of inflammatory cells infiltration in granulation tissue compared with other treated groups (H&E $\times$ 400). (B) 7 days after exposition to sildenafil cream; granulation tissue formation and focal epithelialization (H&E $\times$ 400). (C) 21 days after exposition to sildenafil cream that show more fibroblast migration with collagen regeneration, increased vascularization and full wall thickness epithelialization in the subcutaneous tissue (H&E $\times$ 400).

**Table1.** Comparison of histological evaluation of wound healing in control and treated groups.

	Neutrophils	Fibroblasts	Macrophages	Vascularization	Collagen	Epithelialization
<b>Day 7</b>						
Control	3±0.5	0.5±0.0	0.5±0.0	1±0.0	0.5±0.0	0.5±0.0
Treated	2±0.2*	0.5±0.0	0.5±0.0	2±0.0*	0.5±0.0	1±0.0*
<b>Day 14</b>						
Control	3±0.5	1±0.1	1±0.0	2±0.5	1±0.5	1±0.0
Treated	1±0.2*	1±0.1	1±0.0	3±0.0*	1.5±0.0	2±0.0*
<b>Day 21</b>						
Control	2±0.1	2±0.1	2±0.5	2±0.5	2±0.5	1±0.0
Treated	0.5±0.0*	2±0.2	3±0.5*	3±0.5*	3±0.5*	3±0.1*

0: none, 0.5: few, 1: moderate, 2: many, 3: considerable, score3.; Rats received topical sildenafil (25 mg/daily) in treated group and the cream base without sildenafil in control group. Histopathological damages were assessed as explained under material and methods on days, 7, 14 and 21 of lesion; Results are mean±S.D. of 5 animals.; \*Significantly different from control group ( $p<0.05$ ).

in the treatment group at 14 days post-wounding ( $p<0.05$  at 7 and 14 days). Epithelialization was significantly greater in the treatment group than in the control group at 14 and 21 days ( $p<0.05$ ; Table 1).

#### 4. Discussion

Nitric oxide activates angiogenesis, acts as chemotactic to fibroblasts and promotes fibroplasias. It is known for its antioxidant potential [14]. Nitric oxide is produced by nitric oxide synthase which exists in 3 different isoforms. Neuronal (nNOS) and epithelial (eNOS) forms are 2 isoforms that are constitutively expressed and produce nitric oxide from L-arginine. The third form, the inducible isozyme (iNOS), is found only in certain circumstances. iNOS is induced by stimuli, one of which is wound healing [15].

Conditions associated with impaired wound healing, such as protein calorie malnutrition, diabetes, and steroid use, have all been shown to be associated with reduced NO expression [16].

Wound repair is subdivided into three complementary and overlapping phases: inflammatory, granulation tissue formation (proliferative), and remodeling. The regulation of inflammation, immunomodulation and oxidation are important roles in the process of wound healing [17].

Another important parameter that should be analyzed in wound healing process is the re-epithelialization. Several studies reported

that wound re-epithelialization is NO dependent and may be regulated by several growth factors and cytokines, including TGF- $\beta$ 1, VEGF, EGF, and IL-1 and IL-8. In proliferative phase, synthesis and deposition of collagen as well as angiogenesis occur. The increase in collagen content during wound repair may be attributed to an increase of collagen synthesis and/or proliferation of fibroblasts. In all phases, several cell types in the wound site are able to produce and release NO [18].

Beneficial effects of NO on wound repair may be attributed to its functional influence on angiogenesis, inflammation, cell proliferation, matrix deposition and modulation of cytokine cascade [13]. Various potential sources of the NO ligand should be considered: (1) endogenous from NOS enzymes, (2) exogenous from the gas flow, (3) released from an internal storage pool of nitrosyle-iron complex or S- nitrosothiols [19].

Sildenafil citrate is a specific inhibitor of type 5 cyclic guanosine monophosphate (cGMP)-specific phosphodiesterases (PDE). It increases the nitric oxide level and nitric oxide in turn increases the cGMP level within the cell. The vasodilatory properties of sildenafil are largely responsible for unwanted effects. The most common adverse effects of sildenafil are mainly symptoms of vasodilatation; such as headache, flushing, and nasal congestion. So sildenafil may

change the platelet aggregation and can cause peptic ulceration [20].

In another study, it has been shown in vivo that sildenafil citrate causes the dilation of peripheral arteries and veins and prevents the formation of thrombi by platelets [1]. Sildenafil is contraindicated in patients taking organic nitrates or nitric oxide donors [21].

In the present study, an enhancement of wound contraction 14 and 21 days after wounding was shown. The enhancement of wound contraction is probably due to an increase in myofibroblastic differentiation during granulation tissue formation, since studies reported that NO stimulates inflammatory cells to secrete a larger amount of growth factors, such as the TGF- $\beta$ 1 [46], which is known to stimulate myofibroblastic differentiation [22].

The effect of sildenafil on digital ulcer healing and related clinical symptoms evaluated by Brueckner et al. This study indicated an effect of sildenafil on digital ulcer healing in patients with systemic sclerosis and an improvement of Raynaud's phenomenon and associated symptoms [23]. Derici et al., 2010 also showed the effect of sildenafil on the healing process of abdominal wall wound in rats. They applied sildenafil orally 10 mg/kg once a day for 10 days. Breaking strength for the midline incision, acute inflammation score on postoperative day 14, and neovascularization on postoperative days 7, 14, 21, and 35 were significantly higher in the sildenafil group [24].

Our results showed an increase in the amount of fibroblast as well as an increase of collagen deposition, due to the topical sildenafil application. The results also showed a more organized granulation tissue in the group where sildenafil was applied compared with the other groups. In remodeling phase, the complete re-epithelialization determines the remodeling of vessels as well as apoptosis of myofibroblasts.

Results of the study showed that, use of topical sildenafil tended to more fibroblast and macrophage migration, increased vascularization, collagen regeneration, and epithelialization. Also, it caused a significant increase in the number of infiltrated fibroblasts in the subcutaneous tissue at 7 days post-wounding. Furthermore, a significant abundant collagen regeneration was observed at 14 days post-wounding. Epithelialization was significantly greater after 14 and 21 days. Therefore, we feel that the NO-therapy may give a valuable contribution to patient care in modern human medicine.

Taking into account the present findings and some previously reported studies we may conclude that topical sildenafil is effective in skin wound healing.

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