

# Effect of Topical Phenytoin on Wound Healing

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Received 2015 December 13; Revised 2016 July 13; Accepted 2016 August 20.

## Abstract

**Background:** The effectiveness of topical phenytoin on wound healing has been cited in several research papers. However, methodological flaws and inappropriate controls, as well as the absence of randomization and double blinding devalue most of them.

**Objectives:** We attempted a more stringent assessment of topical phenytoin powder and its role in the enhancement of wound healing.

**Methods:** 76 rats were assigned and divided into two groups: phenytoin-treated and normal saline-treated. Phenytoin and saline were applied on incised open wounds in both groups. The efficacy of phenytoin and normal saline applications was assessed via morphological and histological evaluation over a 4-week period.

**Results:** The results showed considerable reeducation in epithelization in the phenytoin-treated group vs. the control group over the study period. Neovascularization and tensile strength were significantly higher in phenytoin-treated rats as well. There was an insignificant difference regarding wound contraction time.

**Conclusions:** Phenytoin application promoted wound healing. The healing properties of topical phenytoin powder were better in wounds.

**Keywords:** Phenytoin, Wound Healing, Neovascularization, Tensile Strength, Epithelization

## 1. Background

In 1958, Shapiro first observed the beneficial effect of phenytoin in wound healing in epileptic patients with gingival hyperplasia. He concluded that phenytoin increases collagen deposition needed for wound healing (1). Since then, the effectiveness of phenytoin in wound healing has been reported in several clinical trial studies for different types of wounds. Some investigators have demonstrated the effective role of phenytoin in neovascularization by stimulation of fibroblastic activities and other connective tissue components (2-4). Furthermore, other investigators have suggested that phenytoin accelerates the wound healing process by shortening the latent phase before maturation in wound healing, inhibiting glucocorticoid activity, and increasing alkaline phosphatase secretion in wounds (5). In addition, Shafer et al. (6) and Rovio (7) have reported other promoting effects of localized phenytoin administration, such as accelerated granulation formatting and an increase in wound tensile strength. In addition, in other studies conducted by Noriega (8) and El-Zayat (9), it has been shown that systemic and localized administration of phenytoin results in earlier control of

pain and overall enhanced healing in burn and warfare wounds. Likewise, other investigators have raised the efficacy of phenytoin powder in acceleration of healing time in complicated war wounds (10). In addition, these reports have mentioned the effectiveness of phenytoin in promotion of wound healing, but many have methodological flaws, such as inappropriate statistical analysis, inadequate control groups, and the absence of randomization and double blinding. Furthermore, the efficacy of topical phenytoin has not been confirmed by double blind, placebo-controlled trials in part due to lack of suitable placebo compounds and the fact that phenytoin is not approved by the FDA (11). We conducted this controlled study to evaluate the effectiveness of local phenytoin application in skin wound healing and contraction in a rat model.

## 2. Objectives

We attempted a more stringent assessment of topical phenytoin powder and its role in the enhancement of wound healing.

### 3. Methods

This study was conducted on 76 (Albino-N-Mari) rats, divided randomly into two groups: phenytoin-treated and control (normal saline). Over a 4-week study period, 38 rats were included in the phenytoin-treated group and 38 rats in the saline dressing group. After skin preparation, the anesthesia was administered with diluted of 2% Nesdonal. Dorsal incisional wounds were made having a  $1 \times 1$  cm square wound area. Next, wounds (in both groups) were cleansed with povidone iodine and alcohol (70%) and dried. In the phenytoin-treated group, incisional wounds were covered every day with a thin layer of phenytoin powder and sterilized gauze. In the control group, the therapy consisted of a normal saline application and dressed with sterilized gauze dressing every day. Morphological and histological evaluation was done on days 3, 7, and 28 by testing skin samples for evaluation of healing criteria.

The following criteria were considered to determine efficacy of treatment: 1) Epithelization, 2) Neovascularization, 3) Speed of wound contraction, and 4) Tensile strength.

Over the study period, evaluation of the healing process in both groups was performed with an investigator blinded to the study. The unpaired T-test was used to assess differences between the case and control groups.

#### 3.1. Ethical Considerations

It should be noted that all surgical procedures and maintenance condition on laboratory animals were done in accordance with the ethics committee.

#### 3.2. Statistical Analysis

The non-parametric Friedman test was used to compare the groups. The level of statistical was considered significant difference ( $P = 0.032$ ). Measurement of treatment size of the two groups was done using Image J software.

### 4. Results

The results showed that there was considerable changes in epithelization in the control group on days 3, 7, and 28, compared with the phenytoin-treated group (Table 1); histological epithelization is shown in Figure 1. On the other hand, in the phenytoin-treated group, neovascularization was significantly higher than in the saline group on days 3, 7, and 28 (Table 2); its histological neovascularization is shown in Figure 2. There was an insignificant difference between the groups regarding the time of wound contraction on days 3, 7, 20, and 28 (Table 3). The average tensile strength was statistically higher in

the phenytoin-treated group (Table 4). Figure 3 and Table 5 show the treatment between the two groups on days 3, 7, and 28.

**Table 1.** Epithelization (Area Density %)<sup>a</sup>

	Day 3	Day 7	Day 28
Normal saline	47.5 ( $\pm 2.6$ )	67.2 ( $\pm 3.9$ )	78.3 ( $\pm 2.7$ )
Phenytoin	43.0 ( $\pm 2.1$ )	55.3 ( $\pm 5.3$ )	68.1 ( $\pm 2.9$ )

<sup>a</sup>The data in each column represents three independent experiments. Our results are based on these three experiments. Non-parametric Friedman test,  $P < 0.05$ .

**Table 2.** Neovascularization (Area Density %)<sup>a</sup>

	Day 3	Day 7	Day 28
Normal saline	3.5 ( $\pm 0.1$ )	9.1 ( $\pm 0.3$ )	4.6 ( $\pm 0.2$ )
Phenytoin	4.5 ( $\pm 0.5$ )	2.2 ( $\pm 0.7$ )	5.5 ( $\pm 0.5$ )

<sup>a</sup>The data in each column represents three independent experiments. Our results are based on these three experiments. Non-parametric Friedman test,  $P < 0.05$ .

**Table 3.** Wound Contraction ( $\text{mm}^2$ )<sup>a</sup>

	Day 3	Day 7	Day 28
Normal saline	326.8 ( $\pm 43.9$ )	298.3 ( $\pm 47.5$ )	4.1 ( $\pm 1.9$ )
Phenytoin	327.9 ( $\pm 34.9$ )	306.8 ( $\pm 73.9$ )	3.6 ( $\pm 0.9$ )

<sup>a</sup>The data in each column represents three independent experiments. Our results are based on these three experiments. Non-parametric Friedman test,  $P < 0.05$ .

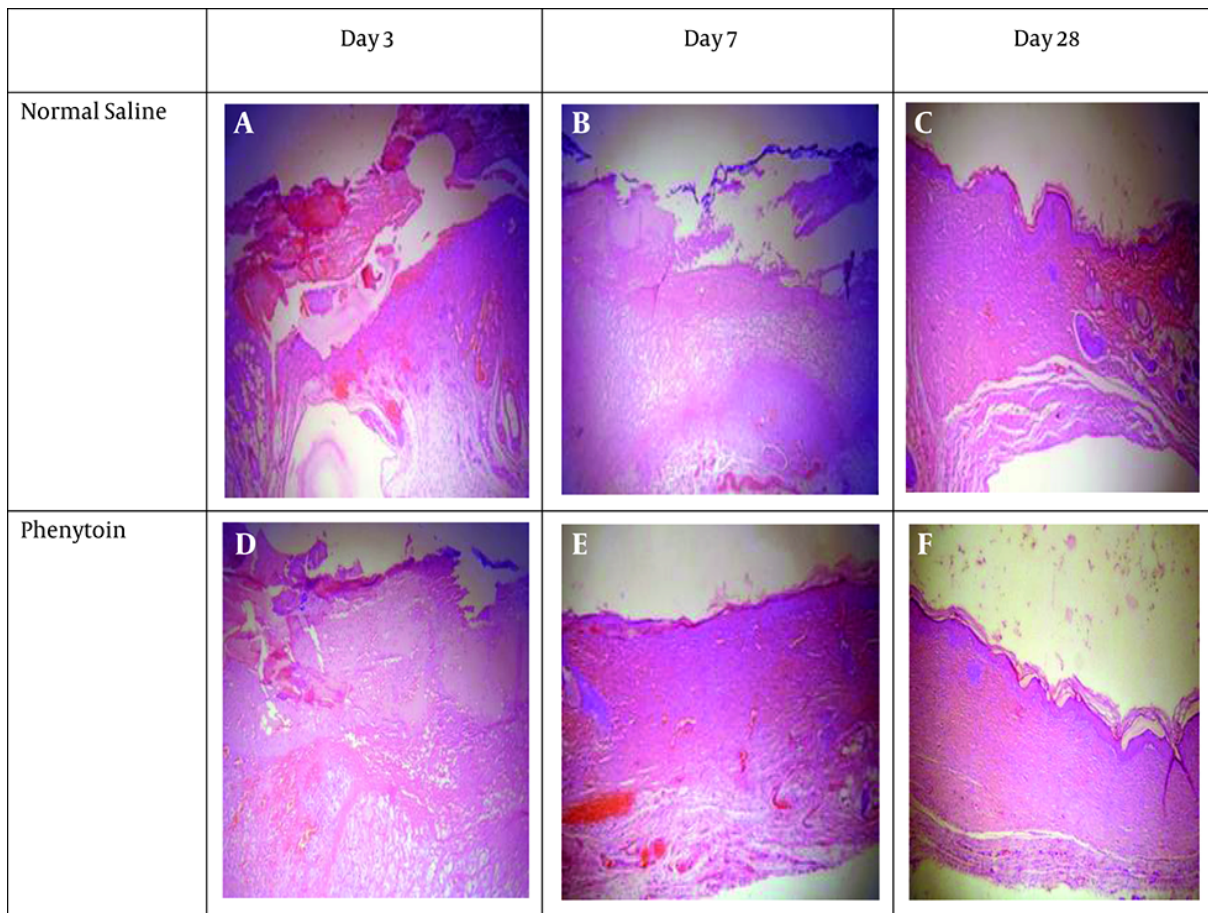
**Table 4.** Tensiometry ( $\text{gr}/\text{mm}^2$ )<sup>a</sup>

	Day 28
Normal saline	631.2 ( $\pm 32.7$ )
Phenytoin	855.0 ( $\pm 48.3$ )

<sup>a</sup>The data in each column represents three independent experiments. Our results are based on these three experiments. Non-parametric Friedman test,  $P < 0.05$ .

### 5. Discussion

Gingival hyperplasia, following administration of phenytoin in epileptic patients, has shown the efficacy of phenytoin in facilitating wound healing. Accordingly, many studies have been conducted to evaluate the promotion of wound healing through phenytoin usage. Based



**Figure 1.** Histological Epithelization ( $\times 40$ )

**Table 5.** Measurement of Treatment Size Between Two Groups<sup>a</sup>

	Day 3	Day 7	Day 28
Normal saline	45.7	21.4	0.8
Phenytoin	44.5	5.8	0

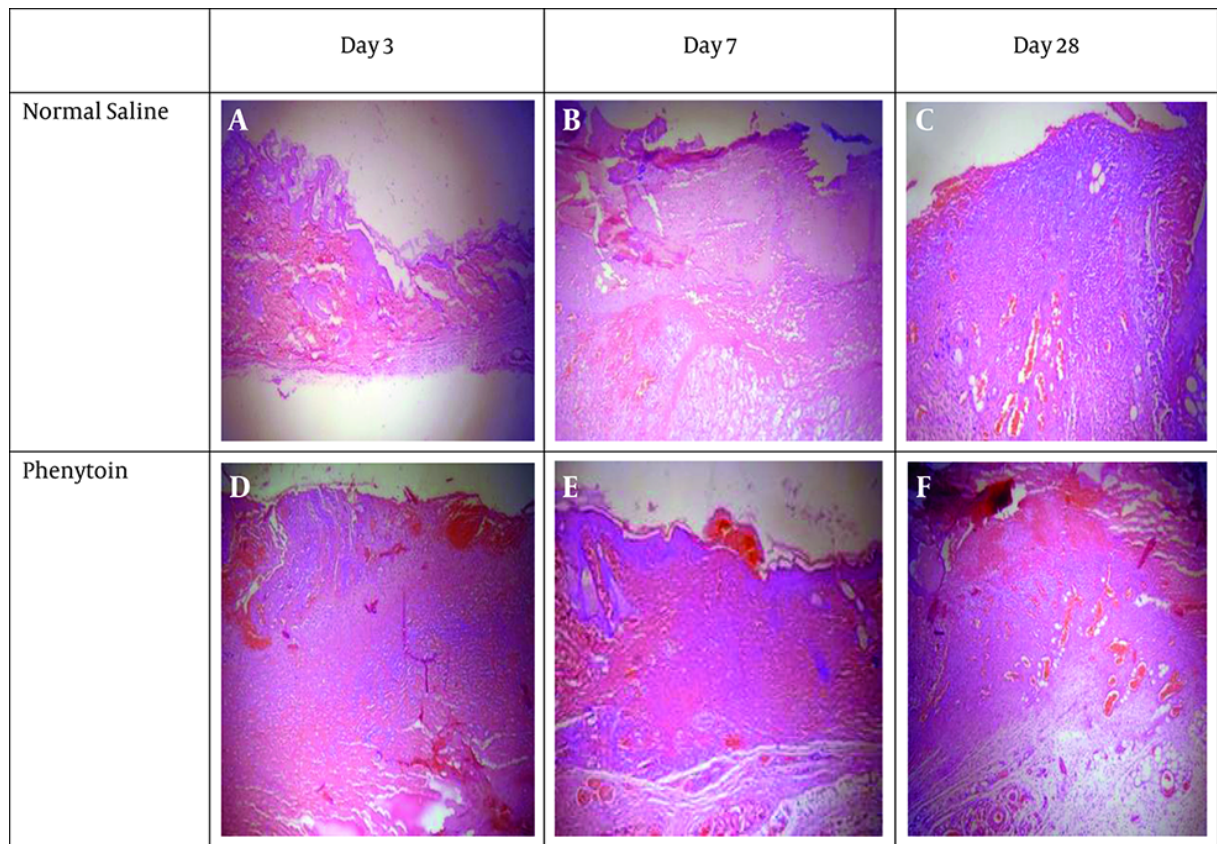
<sup>a</sup>The data in each column represents three independent experiments. Our results are based on these three experiments. Non-parametric Friedman test,  $P < 0.05$ .

on our microscopic assessment, there was less epithelial cell density, which is compatible with previous reports that point out the effect of phenytoin on epithelization, and epithelial cell migration in-vitro models (12). Presumably, this result can be justified by the differences in duration, dosage, and form of administered drug over the study period. Moreover, according to macroscopic and microscopic assessments, there were thicker scabs in the wound of the phenytoin-treated group, compared with

the normal saline group. This phenomenon presumably can reduce epithelial migration and follicle formation. The percentage in area density of neovascularization in the phenytoin-treated group was significantly higher than in the control group ( $P < 0.05$ ).

These findings are compatible with previous studies that reported the effectiveness of phenytoin in the promotion of wound healing. Mulkalwar et al. conducted research on evaluation of the wound healing activity of topical phenytoin in an excision wound model in rats and found that topical phenytoin accelerates wound healing process in that model (13). Hasamnis et al. did research on evaluation of the wound healing effect of topical phenytoin on excisional wounds in albino rats and showed that topical phenytoin accelerated healing of excisional wounds in albino rats (14). Aminifard et al. conducted research on evaluation of the therapeutic effects of topical phenytoin eye drop 1% on corneal alkali burns in the rabbit model and showed that topical phenytoin 1% can help in





**Figure 2.** Histological Neovascularization ( $\times 40$ )

improvement of epithelial defects caused by alkali burns as a supplementary treatment (15). Şimşek et al. researched the effects of topical phenytoin on nasal wound healing after mechanical trauma: an experimental study (16). Jayala et al. (2015) researched the efficiency of topical phenytoin in healing diabetic foot ulcer; they concluded that the topical application of phenytoin sodium powder on diabetic foot ulcer promotes early wound healing (17). Our results showed that topical phenytoin is effective in healing wounds: these results were similar to the other studies mentioned above. According to our results, the high-tensile strength of wounds in the phenytoin-treated group on day 28 of the study indicated substantial facilitation of neovascularization and higher stimulation of fibroblastic proliferation, compared with the saline group ( $P < 0.05$ ). Similar results were shown by Habibpour et al., who examined the effect of phenytoin on skin wound healing in a rat model. Their study indicated a significant increase in neovascularization, which resulted in increased wound tensile strength and accelerated the healing process (18).

### 5.1. Conclusion

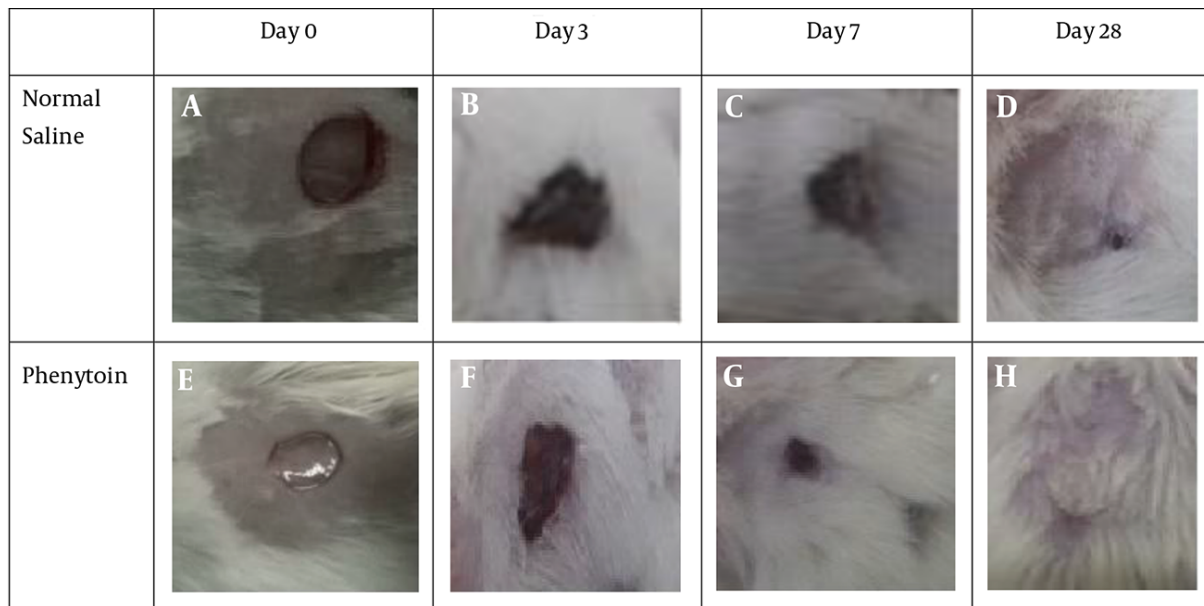
The conclusion can be drawn that local phenytoin usage has a considerable effect on neovascularization in healing wounds, especially under ischemic conditions. Because of its considerable effect on tensile strength, its usage is recommended for skin and mucosal wound healing in patients with connective tissue disorders. Nonetheless administration of phenytoin is not recommended for routine wound care or for increasing epithelial cells or for hair follicles.

### Acknowledgments

This research was financially supported by the Chemical Injuries Research Center, Baqiyatallah University of Medical Sciences, Tehran, IR Iran.

### Footnotes

**Authors' Contribution:** All the authors contributed equally.



**Figure 3.** Treatment Between Two Groups

**Funding/Support:** This research was financially supported by the chemical injuries research center, Baqiyatalah University of Medical Sciences, Tehran, IR Iran.

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