

Effect of Topical Application of Phenytoin and Extract *Malva Sylvestris* in Neutrophil Infiltration in Experimental Gingival Wound in Rabbit

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Abstract

Background: The aim of this study was to evaluate the effect of topical application of sodium phenytoin 1% ointment, 5% extract of *Malva sylvestris* on wound during inflammation, proliferation and remodeling phase.

Material & Methods: In this study, 15 male New Zealand white rabbits with a weight of about 2.5-3.5 kg, based on healthy clinical trials, will be used. The rabbits were then divided into three groups: the first, second and third groups received topical administration of *M. sylvestris* 5%, Phenytoin topical cream and control groups respectively. On days 3, 7 and 10 excisional biopsies were performed and inflammatory factors evaluated. The data were analyzed by SPSS.

Result: On days 3, 7 and 10, the numbers of inflammatory cells in phenytoin and *M. sylvestris* treated groups were significantly higher than the control group. On the tenth day of the study, the numbers of inflammatory cell in *M. sylvestris* showed better granulation tissue.

Conclusion: The present study showed that the use of ointment resulted in the acceleration of the function of inflammatory cells effective in the process of wound healing.

Keywords: *Malva sylvestris*; Rabbit; Phenytoin; Inflammatory factor

Introduction

Cutaneous wound occurs in a really complex process [1]. Wound healing takes place in three overlapping phases: inflammatory, proliferative and remodeling. In the inflammatory phase several types of inflammatory cells attend to the injury site and in addition

to their phagocytic and anti-microbial activity, they play an important role in helping the wound healing process [3]. Traditional clinical stages of wounding healing are still relevant, but more overlap between stages is likely a more accurate depiction

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of events. The role of cells such as platelets, macrophages, leukocytes, fibroblasts, endothelial cells, and keratinocytes is much better known, particularly during the inflammatory and proliferation stages of healing. [1,3]. The inflammatory phase is characterized by increased capillary permeability and cell migration into the wound site. Vasodilation that follows the early vasoconstriction is the result of capillary leaks that are mediated by histamine, leukotrienes, and prostaglandins [4]. Neutrophils are the first cells infiltrating the wound site, followed by monocytes and lymphocytes [5].

Reports about medicinal plants affecting various phases of the wound healing process, such as coagulation, inflammation, fibroplasia, epithelization, collagenation and wound contraction are abundant in the scientific literature [2]. *M. sylvestris*, locally known as "Panirak" is an herbaceous, perennial plant [7]. Fluid extracts of *M. sylvestris* flowers and leaves are used as a valuable remedy for cough and inflammatory diseases of mucous membranes [8]. Flowers are used for the treatment of various ailments, including cold, cough and burn and cut wound healing in rural areas of Iran [9]. This study compares the effect of topical application of sodium phenytoin 1% ointment, 5% extract of *Malva sylvestris* on wound in inflammatory phase of rabbits.

Materials and Methods

In this study, 15 male New Zealand white rabbits were used and weighed about 3 kg, based on healthy clinical trials. The rabbits were kept from animals for reproduction and maintenance and kept in special cages. In order to investigate the Institute of Pasteur Institute of Iran, an experiment was conducted to avoid stress and adaptation of animals to the environment. No experiments were performed on rabbits for two weeks and all animals under the same environmental and nutritional conditions (temperature, humidity, light, diet type and the contents of the same food were kept and stored in a 12 hour light/dark cycle).

Feeding rabbits were done using a pellet ready for experimental animals and water was provided free of charge to the animals. The protocol of this study was conducted in accordance with the ethical principles approved by the International Laboratories for the Protection of Animals.

Animals were stored 6 hours before surgery under NPO conditions and prevented from drinking water 2 hours before surgery. The

animals were divided into three groups of five. The studied groups included the first group control group (not treatment), the second group (phenytoin) and the third group (treated with 5% extract of *Malva sylvestris* ointment).

After preparing the area by aseptic technique, the size of all traumas in each of 15 rabbits was the same, first using punch biopsy number 5, the depth and area of the gum tissue were determined on the right side of the lower mandible, and then with a bolt number 11, a certain amount The gum tissue was cut in circular shape with a diameter of 5 mm Sampling days were 3, 7 and 10, and samples were transferred to 10% formalin stabilizer solution to the pathological laboratory and 24 hours later the formalin solution was replaced. The histopathology of the healing process was performed by staining with trichrome and H & E staining.

At the end of the mentioned days (day 3, 7, 10) a tissue sample was taken to carry out the test. A 6-mm punch was used to ensure complete tissue removal. In order to avoid any damage during the study, each rabbit was discarded from the study process after taking the sample.

After fixation and imaging of tissue samples in paraffin, micrometers were prepared with sections of 5 microns thicknesses and stained with the trichrome and hematoxylin-eosin method. Hematoxylin-Eosin stains method was applied to the cavities. Hematoxylin Harris (10-15 minutes) Hematoxylin Erlich color (45 minutes) Washing in flowing water (5 minutes) Eosin color (3-5 minutes) Alcohol dehydration, Transparent, staining result: Blue core and pink cytoplasm. In all treatment groups, the ulcers were measured on day 3, 7, and 10 with transparent millimeters paper.

Results and Discussion

In this study, the number of neutrophils in the three groups was counted on the 3rd, 7th, and 10th days in 20 fields. Then the mean of these numbers was calculated and used for statistical analysis and plotting of the graphs. Table 1 shows the extent of wound healing tissue histology in different groups and days of examination in the test. As indicated in the table, on the third day of the study, the number of neutrophil counts increased significantly in the *M. sylvestris* and Phenytoin group compared to the control group. On the seventh and tenth day of this study, the reduction in the number of neutrophils in the *M. sylvestris* group was statistically significant compared to the control group.

P_value	Neutrophil count (PMN)	Day/Group
		Third_day
0.002	6.16±0.16	Control
	25.33±0.44	M. sylvestris
	21.17±1.87	Phenytoin
		Seventh_day
0.05	19.33±1.16	Control
	8.5±0.57	M. sylvestris
	12.00±0.75	Phenytoin
		Tenth_day
0.005	16.5±0.28	Control
	5.5±0.57	M. sylvestris
	9.33±1.16	Phenytoin

Table 1

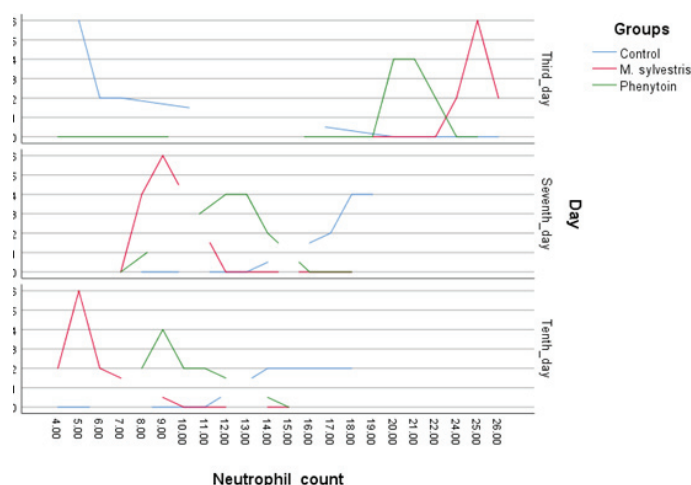


Figure 1

Wound healing, which is the result of simultaneously occurring processes, is classically described in three phases [15] the first phase is a state of acute inflammation. Within minutes an exudate clot, made up of neutrophils, red blood cells, fibrin and macrophages, can be formed. The second phase involves activation of wound macrophages and fibroblasts. The third phase is the remodeling phase [16,17] Using the *M. sylvestris* aqueous extract in our study caused improvement of the wound healing process, From the beginning of the study (on day 7 and 10) there was less inflammation in the *M. sylvestris*-treated than other groups. Several studies have shown the anti-inflammatory effects of *M. sylvestris* extract [10-14]. In the studies of Pirbalouti et al, the amount of inflammatory cell infiltration in the *M. sylvestris*-treated

group was lower than the other groups which is similar to our results [10]. Other studies also show the anti-inflammatory effect of the *M. sylvestris* [12]. In Zare., et al. study, all forms of *M. sylvestris* extracts had antimicrobial activity against *Staphylococcus aureus*. *Aureus* and *Pseudomonas aeruginosa* [18].

Conclusion

The present study showed that the use of ointment resulted in the acceleration of the function of inflammatory cells effective in the process of wound healing.

The authors of this study recommend the evaluation of all effective ingredients of this plant on wound healing process and using the most effective compounds in wound healing topical treatments.

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