Jundishapur J Health Sci. 2019 January; 11(1):e86041. doi: 10.5812/jjhs.86041.

Published online 2019 January 27. **Research Article**

Expression of VEGF and TGF-β Genes in Skin Wound Healing Process Induced Using Phenytoin in Male Rats

Roghaye Savari [1], Mohammad Shafiei [1,*], Hamid Galehdari [1] and Mahnaz Kesmati [2]

[1] Department of Genetics, Faculty of Sciences, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[2] Department of Biology, Faculty of Sciences, Shahid Chamran University of Ahvaz, Ahvaz, Iran

[*] *Corresponding author*: Department of Genetics, Faculty of Sciences, Shahid Chamran University of Ahvaz, Ahvaz, Iran. Email: m.shafiei@scu.ac.ir

Received 2018 November 03; **Revised** 2019 January 08; **Accepted** 2019 January 16.

Abstract

Background: Phenytoin is one of the most important chemical drugs used for the treatment of skin wound.
Objectives: In the present study, the molecular effect of phenytoin on the expression of VEGF and TGF-β genes was investigated.
Methods: This study was conducted on 30 male rats with approximately equal body mass. Skin wounds were generated with an area of 2 cm and a depth of 0.5 mm on the back of the necks. The rats were divided into two case and control groups (15 rats in each group). Topical phenytoin was administered to the two groups 2 times a day (1%) and Vaseline (as control group). Each of control and treated groups were divided into three subgroups and the rats were euthanized with chloroform on days 7, 14, and 21 of post-wounding. The wounds were harvested from control and treated rats. After homogenization of the tissues, RNA was extracted, purified, converted to cDNA, and the relative expression of VEGF and TGF-β genes in phenytoin and control groups was evaluated by real-time PCR. The gene expression was evaluated on days 7, 14, and 21.
Results: Increased expression of VEGF and TGF-β in the first week and decreased expression of both genes in the third week were observed in the phenytoin-treated group compared with the control group ($P < 0.001$).
Conclusions: Regarding the results of the expression of both TGF-β and VEGF genes, there is a significant relationship between the expression of these two genes and the rate of wound healing in rats.

Keywords: Topical Phenytoin, TGF-β, VEGF, Angiogenesis, Wound Healing

1. Background

Improvement of the wound healing and maximal restoration of tissue function remain a central clinical care concern (1). Wounds refer to the loss of epidermis and dermis. Wound healing is a complex physiological process in which damaged tissues are removed and new tissue is replaced (2). Wound healing is an active process consisting of the collaboration of various cells, including fibroblasts, leukocytes, monocytes, macrophages, endothelial cells, and epidermal cells (3). The process consists of four stages as follows: Homeostasis, inflammation, proliferation, and remodeling.

In hemostasis stage, the wound is closed by clotting. After leaking out of the blood, constriction of blood vessels occurs to restrict the blood flow. Then, platelets stick together and seal the break in the wall of the blood vessels (4). Inflammation is controlled with the infiltration of inflammatory cells such as neutrophils, macrophages, and lymphocytes to the site of the wound. The main objective of the inflammatory phase is the safety barriers against microorganisms and removal of damaged cells and pathogens from the wound (5).

In the proliferative phase collagen type III, hyaluronic acid, and fibronectin were produced to form a new extracellular matrix. Also, the new blood vessels are constructed and the new tissue can receive sufficient oxygen and nutrients (6).

In the remodeling phase, wound contraction and restructure formation were done by reorganization, degradation, and resynthesis of the extracellular matrix. At the start of the stage, the provisional wound matrix that predominantly includes fibronectin and collagen type III is replaced by collagen type I. In this final stage, the tensile strength of the wound matrix effectively is increased and the wound will start to close (7).

After the injury, the repair of the wound is initiated by the release of cytokines and various growth factors. Some of these factors include fibroblast growth factor (FGF), hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), transforming growth factor beta (TGF-β), epidermal growth factor (EGF), and platelet-derived growth factor (PDGF). Among

the growth factors, VEGF has been shown to play an important role in wound healing (8). VEGF is released by a variety of cells such as fibroblasts, smooth muscle cells, macrophages, endothelial cells, neutrophils, and platelets that participate in wound healing. This protein is secreted in response to ischemia and inflammation and resulted in endothelial migration, production of chemotactic agents, proliferation, granulation tissue formation, and angiogenesis (9). The increase of angiogenesis is an important property of VEGF. The main feature of the proliferative phase in wound healing is angiogenesis which is considerably increased by *VEGF*. In the angiogenesis process, the numbers of blood vessels are temporarily increased in the wound area (10).

The function of TGF-β was indicated in several processes of wound healing such as angiogenesis, inflammation, fibroblast proliferation, and collagen synthesis (11). The release of TGF-β at the early stage of the healing process prompts recruitment of inflammatory cells from circulation into the wounded area. These events lead to granular tissue formation, angiogenesis, and collagen synthesis (12). Also, TGF-β stimulates the cells to increase the synthesis of extracellular matrix proteins and simultaneously decreases the collagen proteases (13).

Diphenyl-hydantoin, also known as phenytoin, is a wound healing agent. At the first time in 1937, phenytoin was introduced as an oral anti-seizure drug. Phenytoin is administered for the treatment of wounds with different etiologies such as diabetic wounds, pressure ulcers, surgical wounds, epidermolysis bullosa, traumatic injuries, abscess, aphthous ulcers, venous stasis ulcers, and oral lichen planus (14). Phenytoin-induced gingival hyperplasia is characterized by enhanced proliferation of fibroblasts. The hyperplasia can be made by increased production of several growth factors such as FGF, EGF, PDGF, and TGF-β (15).

The mechanism of acceleration of wound healing related to phenytoin is unknown. However, studies suggest that phenytoin may decrease collagenase activity and enhance various processes such as the formation of granulation tissues, the proliferation of fibroblasts, and deposition of collagen (14, 15).

2. Objectives

Regarding the effective role of phenytoin in wound healing, the aim of this study is to examine the molecular effect of phenytoin on the expression of VEGF and TGF-β in the treatment of ulcers.

3. Methods

3.1. Wound Model

Thirty healthy male rats with a weight ranging from 200 to 300 grams were selected. The rats were divided into two case and control groups (15 rats in each group). Standard environments were kept for the rats. The animals were anesthetized by an intraperitoneal injection of ketamine at a dose of 80 mg/kg and xylazine at a dose of 10 mg/kg. The dorsal surface was shaved and cleaned with 70% ethanol. Two cm^2 open excision-type wound to the depth of loose subcutaneous tissues was created using a scalpel blade. After wounding, the wound was washed by normal saline to remove any additional fat and residual tissue fibers from the wound. The depth of the wounds is as thick as the skin so that the epidermis, dermal, and subcutaneous fat were present and the muscle was visible. Then, the rats were housed individually in sterile cages. After recovery from anesthesia, rats were kept under the standard environmental condition with a temperature of 22 ± 2°C and fed with a rodent diet and water. The case group received phenytoin and control group received Vaseline topically, twice in a day for three weeks. Rats were treated separately in a clean environment. The amount of used phenytoin and Vaseline for treatment was at the level that entire surface of the wound was covered.

3.2. Tissue Collection and Expression Analysis

Each group was divided into three subgroups and the rats were euthanized with chloroform on days 7, 14, and 21 post-wounding. The wound and an approximately 1 mm of surrounding skin were harvested from control and treated rats.

After homogenization of tissues, RNA was extracted and purified using the RNeasy mini kit (Qiagen, Valencia, CA). Then, quantitect reverse transcription kit was used to convert RNA to cDNA and cDNA was stored at -20°C until use.

In this study, the relative expression of VEGF and TGF-β genes in phenytoin-received and control groups were studied using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene expression as housekeeping gene. The gene expressions were determined by real-time PCR using real-time PCR cycler and platinum syber green supermix.

The primers for the selected genes were designed with GenScript software in https://www.genscript.com/ssl-bin/app/primer (Table 1). To evaluate the efficiency of the primers, standard curves encompassing from 8 - 10 to 102 copies were plotted using serial dilutions of each cDNA. The real-time PCR program was described as follows: 95°C for 2 minutes; 40 cycles of 95°C for 15 seconds, 60°C for 30 seconds, 72°C for 30 seconds; and then 72°C for 7 minutes. Melting curves were obtained at 80°C. Data acquisition was performed for every 0.2°C increase in temperature, with a 10 seconds step. Average CT was calculated by ABI StepOne devices and analyzed using relative expression software tool (REST) (version 2009) and SPSS software.

Table 1. Sequence of Designed Primers for Each Gene is Shown as Forward and Reverse

Primer	Sequence	Primer Length	GC, %	Product Length, bp
GAPDH-F	5'-ATGACTCTACCCACGGCAAG-3'	20	55	89
GAPDH-R	5'-CTGGAAGATGGTGATGGGTT-3'	20	50	
VEGF -F	5'-ATGCCAAGTGGTCCCAG-3'	18	58.82	45
VEGF -R	5'-CAATAGCTGCGCTGGTAG-3'	18	55.56	
TGF-β-F	5'- CTGAACCAAGGAGACGGAAT-3'	20	50.7	142
TGF-β-R	5'-GGTTCATGTCATGGATGGTG-3'	20	9.50	

4. Results

The effect of phenytoin on the healing of the induced wounds was studied by assessment of the relative expression of VEGF and TGF-β gene in phenytoin and control groups. To evaluate the efficiency of the primers, standard curves were plotted using different dilutions of cDNA Changes in the genes expressions were investigated on day 7, day 14 and day 21 of wounding in the rats by real-time PCR.

4.1. GAPDH Gene

The differences in gene expression between treatment groups were evaluated by REST software and the expression of a target gene was normalized by GAPDH housekeeping gene a non-regulated reference-gene. The difference between the expressions of GAPDH gene in two groups was not observed. Then, this gene was used as a control gene.

4.2. VEGF and TGF-β Gene Expression Results

Results of the real-time PCR showed that expression of VEGF gene was increased during the first week and decreased on day 21 in phenytoin groups compared with control group in normal rats. This change expression was significant in level ($P < 0.001^{***}$) (Table 2). Also, results of the real-time PCR for TGF-β indicated the increase in expression level of the gene on day 7 and decrease of the gene expression on day 21 ($P < 0.001^{***}$) (Table 3).

5. Discussion

Wound and repair issues remain a central concern of clinical care. Researchers were always seeking a way to minimize complications in healing processes such as debilitating scarring, infection, and colloidal. In this regard, extensive researches and various methods were carried out, including the use of chemical drugs, herbal, homeopathic, and physical procedures such as laser therapy and other conventional methods (2). However, due to inadequate information about the wound healing molecular mechanisms as well as the lack of animal studies in this area of research, the treatment of chronic ulcers and their rate of recovery are still disappointing (16). Not treated open skin lesions may lead to local infections and even cancer. Considering this fact and the importance of wound healing, scientists put a lot of effort to introduce novel treatments with little or no side effects.

Phenytoin is a chemical drug that accelerates wound healing, but its mechanism is still unknown.

Recent developments in novel drug delivery systems (DDSs) focus on the use of effective drugs to release growth factors in healing and skin repair processes. In a microarray analysis of factors affecting wound healing, phenytoin was introduced as a new category of wound healing agents that induces growth factors, accelerates their activity and up-regulates the related receptors (17). Dill and Iacopino in 1997 reported an increase in muscle growth factors and the tissue fibroblasts as a result of phenytoin (18).

In order to evaluate the effect of phenytoin drug, the expression of VEGF gene in skin wounds treated with the drug was evaluated in the present study. Results showed an increase in VEGF gene expression on day 7 and a decrease in the gene expression on day 21 in phenytoin-treated group compare with control group. This change in VEGF gene expression was significant ($P < 0.001^{***}$). Therefore, phenytoin increases wound healing by the production of VEGF and its levels in wound fluids steadily raised through the first week after injury, while it was decreased in the third week because the mice were recovered completely.

Brem et al. demonstrated that local release of VEGF using adenovirus vector (ADV)-mediated gene transfer stimulates collagen deposition, angiogenesis, and the migration of fibroblasts (19).

Nissen et al. provides two lines of evidence to support a central role for VEGF in mediating angiogenesis in surgical wounds: First, during the initiation of new vessel growth, VEGF is released locally. Second, neutralization of VEGF greatly decreases the angiogenesis and endothelial cell chemotactic activity of surgical wound fluid (20).

Turan et al. demonstrated that the production of VEGF and FGF was increased after treatment of a wound by phenytoin (21). Many other histological studies also proved phenytoin role in the angiogenesis which is probably due to VEGF (22, 23). In agreement with previous studies con-

Table 2. Results of VEGF Gene Expression

Gene	Type	Reaction Efficiency	Fold Change	Std. Error	Result
VEGF (first week)	Target	1.05	4.68	4.68	Up
VEGF (second week)	Target	1.05	1.36	1.36	
VEGF (third week)	Target	1.05	0.00001	0.00001	Down

Table 3. Results of TGF-β Gene Expression

Gene	Type	Reaction Efficiency	Fold Change	Std. Error	Result
TGF-β (first week)	Target	1.07	2.445	2.445 - 2.445	Up
TGF-β (second week)	Target	1.07	0.808	0.808 - 0.808	
TGF-β (third week)	Target	1.07	0.001	0.001 - 0.001	Down

ducted on the role of phenytoin in wound healing, our results proved that phenytoin accelerates the expression of VEGF growth factor that plays essential roles in the wound healing.

TGF-β is a well-known growth factor that involves in wound healing, which stimulates tissue renewal or repair. Also, it is an important fibrogenesis modifier, especially in the formation of connective tissues, and following an injury, it is immediately released as a key signal in the organization of wound healing by the platelets (24). In many studies, rapid expression of TGF-β1 has been introduced as a chemotactic agent for the recruitment of neutrophils, macrophages, and fibroblasts to the wound (25). In a study, the TGF-β1 gene was knocked out in embryonic stem cells of a mouse and it was observed that wound healing was almost normally progressed in the first day, but on day 10, the production of granulation tissue, collagen deposition, and angiogenesis, were significantly reduced (26). In another study, an increase in the production of collagen and extracellular matrix was observed with the injection of TGF-β in the wound (27). In our study, phenytoin increased the expression of TGF-β on day 7, but on day 21, the expression of TGF-β was decreased due to almost complete repair of the wound.

The differentiation of fibroblasts into myofibroblasts is an important step in wound healing and chronic ulcers repairing. Accordingly, TGF-β plays a key role in regulating fibroblast proliferation and the production of collagen and myofibroblasts, thus the presence of myofibroblasts in the phenytoin-treated gingival hyperplasia may be due to the presence of TGF-β (28).

In the present study, the results of the expression of VEGF and TGF-β genes in healthy male rats showed that the expression of both genes was increased in the first week and decreased in the third week. Regarding the results of the expression of both TGF-β and VEGF genes, there is probably a significant relationship between the expression of these two genes and the wound healing in rats. TGF-β is produced immediately after wounding by damaged platelets and is the stimulator of the inflammatory macrophages, which are the main source of production of the VEGF. The significant expression of these two factors is likely to be due to the fact that TGF-β affects the expression of VEGF and promotes the process of wound healing by producing granulation tissue and angiogenesis. Therefore, it can be suggested that phenytoin applies its wound healing functions by stimulating the production of these two essential growth factors.

Today, attention is focused on topical medications in the treatment of wounds and these drugs require costly clinical trials to prove their functions. Of note, phenytoin is a cheap and affordable drug and can be used easily.

Based on the molecular results obtained in this study and the molecular and tissue results obtained from other studies, it can be suggested that phenytoin therapy may be useful for the wounds treatment. Although the results are encouraging, the technical effectiveness of treatment with phenytoin still requires more controlled studies to demonstrate the other benefits of the topical drug.

Acknowledgments

The authors appreciate Shahid Chamran University of Ahvaz for financial support.

Footnotes

Authors' Contribution: Study concept and design: Roghaye Savari, Mohammad Shafiei, Hamid Galedari and Mahnaz Kesmati; analysis and interpretation of data: Roghaye Savari; drafting of the manuscript: Roghaye Savari, Mohammad Shafiei and Hamid Galedari; critical revision of the manuscript for important intellectual content: Mohammad Shafiei, Hamid Galedari and Mahnaz Kesmati; and statistical analysis: Roghaye Savari.

Conflict of Interests: The authors declare that there is no conflict of interests regarding the publication of this paper.

Ethical Approval: This study was approved by the Medical Ethics Committee of Shahid Chamran University of Ahvaz.

Funding/Support: The authors would like to thank research council of Shahid Chamran University of Ahvaz for the financial support.

References

1. Esmaeili H, Dehghan MM, Rabbani M, Haririan E. [Effect of bovine colostrum on open wound healing in guinea pigs]. *Razi J Med Sci*. 2014;**21**(125):66–74. Persian.
2. Dovi JV, He LK, DiPietro LA. Accelerated wound closure in neutrophil-depleted mice. *J Leukoc Biol*. 2003;**73**(4):448–55. doi: 10.1189/jlb.0802406. [PubMed: 12660219].
3. Bainbridge P. Wound healing and the role of fibroblasts. *J Wound Care*. 2013;**22**(8):407–8. 410-12. doi: 10.12968/jowc.2013.22.8.407. [PubMed: 23924840].
4. Ghatak S, Maytin EV, Mack JA, Hascall VC, Atanelishvili I, Moreno Rodriguez R, et al. Roles of proteoglycans and glycosaminoglycans in wound healing and fibrosis. *Int J Cell Biol*. 2015;**2015**:834893. doi: 10.1155/2015/834893. [PubMed: 26448760]. [PubMed Central: PMC4581578].
5. Pettet GJ, Byrne HM, McElwain DL, Norbury J. A model of wound-healing angiogenesis in soft tissue. *Math Biosci*. 1996;**136**(1):35–63. doi: 10.1016/0025-5564(96)00044-2. [PubMed: 8755336].
6. Deonarine K, Panelli MC, Stashower ME, Jin P, Smith K, Slade HB, et al. Gene expression profiling of cutaneous wound healing. *J Transl Med*. 2007;**5**:11. doi: 10.1186/1479-5876-5-11. [PubMed: 17313672]. [PubMed Central: PMC1804259].
7. Lobmann R, Schultz G, Lehnert H. Proteases and the diabetic foot syndrome: mechanisms and therapeutic implications. *Diabetes Care*. 2005;**28**(2):461–71. doi: 10.2337/diacare.28.2.461. [PubMed: 15677818].
8. Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. *Physiol Rev*. 2003;**83**(3):835–70. doi: 10.1152/physrev.2003.83.3.835. [PubMed: 12843410].
9. Bao P, Kodra A, Tomic-Canic M, Golinko MS, Ehrlich HP, Brem H. The role of vascular endothelial growth factor in wound healing. *J Surg Res*. 2009;**153**(2):347–58. doi: 10.1016/j.jss.2008.04.023. [PubMed: 19027922]. [PubMed Central: PMC2728016].
10. Johnson KE, Wilgus TA. Vascular endothelial growth factor and angiogenesis in the regulation of cutaneous wound repair. *Adv Wound Care (New Rochelle)*. 2014;**3**(10):647–61. doi: 10.1089/wound.2013.0517. [PubMed: 25302139]. [PubMed Central: PMC4183920].
11. Nall AV, Brownlee RE, Colvin CP, Schultz G, Fein D, Cassisi NJ, et al. Transforming growth factor beta 1 improves wound healing and random flap survival in normal and irradiated rats. *Arch Otolaryngol Head Neck Surg*. 1996;**122**(2):171–7. doi: 10.1001/archotol.1996.01890140057011. [PubMed: 8630211].
12. Barrientos S, Stojadinovic O, Golinko MS, Brem H, Tomic-Canic M. Growth factors and cytokines in wound healing. *Wound Repair Regen*. 2008;**16**(5):585–601. doi: 10.1111/j.1524-475X.2008.00410.x. [PubMed: 19128254].
13. El Gazaerly H, Elbardisey DM, Eltokhy HM, Teaama D. Effect of transforming growth factor Beta 1 on wound healing in induced diabetic rats. *Int J Health Sci (Qassim)*. 2013;**7**(2):160–72. doi: 10.12816/0006040. [PubMed: 24421745]. [PubMed Central: PMC3883606].
14. Kuru L, Yilmaz S, Kuru B, Kose KN, Noyan U. Expression of growth factors in the gingival crevice fluid of patients with phenytoin-induced gingival enlargement. *Arch Oral Biol*. 2004;**49**(11):945–50. doi: 10.1016/j.archoralbio.2004.04.010. [PubMed: 15353252].
15. Anstead GM, Hart LM, Sunahara JF, Liter ME. Phenytoin in wound healing. *Ann Pharmacother*. 1996;**30**(7-8):768–75. doi: 10.1177/106002809603000712. [PubMed: 8826558].
16. Eming SA, Martin P, Tomic-Canic M. Wound repair and regeneration: Mechanisms, signaling, and translation. *Sci Transl Med*. 2014;**6**(265):265sr6. doi: 10.1126/scitranslmed.3009337. [PubMed: 25473038]. [PubMed Central: PMC4973620].
17. Swamy SM, Tan P, Zhu YZ, Lu J, Achuth HN, Moochhala S. Role of phenytoin in wound healing: Microarray analysis of early transcriptional responses in human dermal fibroblasts. *Biochem Biophys Res Commun*. 2004;**314**(3):661–6. doi: 10.1016/j.bbrc.2003.12.146. [PubMed: 14741686].
18. Dill RE, Iacopino AM. Myofibroblasts in phenytoin-induced hyperplastic connective tissue in the rat and in human gingival overgrowth. *J Periodontol*. 1997;**68**(4):375–80. doi: 10.1902/jop.1997.68.4.375. [PubMed: 9150043].
19. Brem H, Kodra A, Golinko MS, Entero H, Stojadinovic O, Wang VM, et al. Mechanism of sustained release of vascular endothelial growth factor in accelerating experimental diabetic healing. *J Invest Dermatol*. 2009;**129**(9):2275–87. doi: 10.1038/jid.2009.26. [PubMed: 19282838].
20. Nissen NN, Polverini PJ, Koch AE, Volin MV, Gamelli RL, DiPietro LA. Vascular endothelial growth factor mediates angiogenic activity during the proliferative phase of wound healing. *Am J Pathol*. 1998;**152**(6):1445–52. [PubMed: 9626049]. [PubMed Central: PMC1858442].
21. Turan M, Saraydyn SU, Bulut HE, Elagoz S, Cetinkaya O, Karadayi K, et al. Do vascular endothelial growth factor and basic fibroblast growth factor promote phenytoin's wound healing effect in rat? An immunohistochemical and histopathologic study. *Dermatol Surg*. 2004;**30**(10):1303–9. doi: 10.1111/j.1524-4725.2004.30404.x. [PubMed: 15458527].
22. Pitiakoudis M, Giatromanolaki A, Iliopoulos I, Tsaroucha AK, Simopoulos C, Piperidou C. Phenytoin-induced lymphocytic chemotaxis, angiogenesis and accelerated healing of decubitus ulcer in a patient with stroke. *J Int Med Res*. 2004;**32**(2):201–5. doi: 10.1177/147323000403200213. [PubMed: 15080024].
23. DaCosta ML, Regan MC, al Sader M, Leader M, Bouchier-Hayes D. Diphenylhydantoin sodium promotes early and marked angiogenesis and results in increased collagen deposition and tensile strength in healing wounds. *Surgery*. 1998;**123**(3):287–93. doi: 10.1016/S0039-6060(98)70181-3. [PubMed: 9526520].
24. Ruhrberg C. Endogenous inhibitors of angiogenesis. *J Cell Sci*. 2001;**114**(Pt 18):3215–6. [PubMed: 11591810].
25. O'Kane S, Ferguson MW. Transforming growth factor beta s and wound healing. *Int J Biochem Cell Biol*. 1997;**29**(1):63–78. doi: 10.1016/S1357-2725(96)00120-3. [PubMed: 9076942].
26. Brown RL, Ormsby I, Doetschman TC, Greenhalgh DG. Wound healing in the transforming growth factor-beta-deficient mouse. *Wound Repair Regen*. 1995;**3**(1):25–36. doi: 10.1046/j.1524-475X.1995.30108.x. [PubMed: 17168860].
27. Lawrence WT, Norton JA, Sporn MB, Gorschboth C, Grotendorst GR. The reversal of an Adriamycin induced healing impairment with chemoattractants and growth factors. *Ann Surg*. 1986;**203**(2):142–7. doi: 10.1097/00000658-198602000-00006. [PubMed: 3511865]. [PubMed Central: PMC1251061].
28. Abdolahi Fakhim S, Babaei H, Zarringhalam M, Ashrafi J. Wound healing effect of topical phenytoin on rat palatal mucosa. *Int J Curr Res Aca Rev*. 2015;**3**(6):463–76.