

## Evaluation of Wound Healing Activity of Topical Phenytoin in an Incision Wound Model in Rats

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### Abstract

**Background:** Wound healing is a significant healthcare problem in today's medical practice. Despite extensive treatment modalities that are supposed to hasten the wound healing process, the outcomes of existing methods are far from optimal. One such agent that has been tried previously and found controversial in wound healing is phenytoin. Therefore, this study was planned to evaluate and compare wound healing effect of topical phenytoin with povidone iodine ointment in rats.

**Materials and Methods:** Study was conducted after approval from Institutional Animal Ethics Committee (IAEC). Wound healing activity of topical phenytoin (1 gm% and 2gm%) was assessed in incision wound model in Sprague Dawley rats (n =8) which was compared with topical petroleum jelly and povidone iodine ointment. Parameters studied included measurement of wound tensile strength and estimation of hydroxyproline level in tissues obtained on the 10<sup>th</sup> post-operative day.

**Results:** The tensile strength was significantly more with both the preparations of phenytoin when compared to petroleum jelly and 10% povidone iodine. The hydroxyproline level was more in 1% and 2% phenytoin treated group as compared to other study groups. However, it showed significant difference only with petroleum jelly treated group and not with the povidone iodine treated group. There was no statistically significant difference between both the preparations of phenytoin

**Conclusion:** In this study, it was found that topical phenytoin accelerates wound healing process in an incision wound model.

**Keywords:** Phenytoin, Povidone iodine, Incision wound.

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### Introduction

Wound is defined as disruption of cellular and anatomical or functional continuity of living tissue.<sup>[1]</sup> In the process of wound healing, there is a restoration of physical integrity of internal or external body structures and involves complex interaction between the cells and various other factors. The wound healing process is a dynamic one which can be divided into three phases. The phases of wound healing are

- Inflammatory phase
- Proliferation phase
- Maturation phase

Wound healing is a significant healthcare problem in medical practice. Proper treatment and care of the wound accelerate the healing process and prevent chances of infection and chronic complications of wound.<sup>[2]</sup>

Oral phenytoin was introduced as a therapy for management of convulsions in 1938. On long term administration of phenytoin, it produces gingival hypertrophy and by virtue of this property, studies on its effect on wound healing were undertaken.<sup>[3]</sup> According to few studies conducted in the past,

phenytoin possesses wound healing activity, but there are few studies which show contradictory results. In view of these conflicting results, the present study was planned to evaluate the effect of topical phenytoin on wound healing in an incision wound model and to compare it with petroleum jelly and povidone iodine.

### Material and Methods

This study was carried out in Dr. D. Y. Patil Medical College, Hospital and Research Centre, Pimpri, Pune, Maharashtra, after approval from Institutional Animal Ethics Committee (IAEC).

**Animals:** A total of 32, healthy (150-200gm) Sprague-Dawley rats of either sex, aged 12weeks bred locally in the animal house of our college were used. For acclimatization, the animals were kept in the laboratory for 3-4 days, with free access to food and water. They were kept in standard polypropylene cages having husk bedding and wire mesh at the top.

Prior to the day of experimental procedure they were starved overnight and the area of skin where wound was to be made was depilated.

### Drugs used in the study

- Petroleum jelly
- 1% Phenytoin
- 2% Phenytoin
- 10% Povidone iodine

Pure powder form of phenytoin obtained from JPN Pharma Pvt. Ltd. Petroleum jelly and 10% povidone iodine required for the study were obtained from commercial resources.

**1% phenytoin cream:** 1gm of phenytoin powder was added to 99gm of petroleum jelly.<sup>[4]</sup>

**2% phenytoin cream:** 2gm of phenytoin powder was added to 98gm of petroleum jelly.

### Incision Model

**Procedure:** Sprague Dawley rats (150-250g) were used for the study. Rats were individually housed and were given standard diet and water ad libitum. The hair on the dorsal part of the animals was removed using a depilatory cream. After anesthetizing using intraperitoneal injection of ketamine (50mg/kg), animals were secured to operation table in prone position. A paravertebral straight incision of 6cms was made, at least 1.5cms lateral to the vertebral column, using blade as described by Ehrlich and Hunt. After complete haemostasis, wound was closed by interrupted sutures placed 1cm apart, mopped with cotton swabs soaked with 70% alcohol and then animals were caged individually. Removal of stitches was done on 8<sup>th</sup> day, while breaking strength was measured on 10<sup>th</sup> post wounding day by continuous, constant water flow technique of Lee.<sup>[4,5]</sup>

**Animal Groups:** 32 Sprague Dawley rats were randomly divided into 4 groups, 8 rats in each group.

Group I: Topical petroleum jelly

Group II: Topical 1% phenytoin

Group III: Topical 2% phenytoin

Group IV: Topical 10 % povidone iodine

**Treatment:** All formulations were applied topically on the wound surface once a day for a period of 10 days.

Parameters evaluated were

**A. Measurement of tensile strength using tensiometer:** The measurement of tensile strength of the healed wounds was carried out on the 10<sup>th</sup> day under ketamine anaesthesia. After giving anaesthesia to the animal, it was secured to operation table in prone position and lines were drawn on both the side of the incision wound, 3mm away from the wound margin on adjacent normal skin leaving about 5mm wound towards both ends. Two allis forceps were firmly applied on the lines, facing each other, the forceps on one side was hooked to a metal rod, fixed firmly to the operation table, while the other to a light polythene container through a string run over a pulley. Water was allowed to flow at a constant rate into the polythene container so as to create pulling force necessary to disrupt the wound. Water flow was regulated by means of an occlusion clamp on rubber tubing which was then connected to water reservoir, kept at a suitable height. As soon as the gaping of wound was observed, the water flow was cut-off. Further opening of the wound was avoided by releasing the pulling force on the wound

immediately by lifting up the polythene container. The volume of water in the polythene container was measured and was converted to the corresponding weight. The breaking strength is expressed as minimum weight of water necessary to bring about gaping of wound.<sup>[6]</sup>

**B. Estimation of Hydroxyproline levels:** Hydroxyproline (4-hydroxyproline-2-carboxylic acid), an amino-acid, is a major component of collagen by forming hydrogen bonds.

This procedure includes the extraction of hydroxyproline from the tissue using hydrochloric acid and heat. It is followed by oxidation of hydroxyproline with sodium peroxide and colour formation with copper sulphate and p-dimethylaminobenzaldehyde. The procedure followed for estimation was standardized by Neuman and Logan.<sup>[7]</sup>

### Statistical analysis

- The data for incision wound model was analyzed using one-way ANOVA followed by post hoc tukey's test.
- GraphPad Prism 5.0 software was used for data analysis. P value <0.05 was considered statistically significant.

### Result

Table 1 and Fig. 1 depicts wound tensile strength measured on the 10<sup>th</sup> post operative day in incision model.

Tensile strength represents how much the repaired tissue resists breaking under tension and may indicate in part the quality of repaired tissue. The wound tensile strength measured in 1% and 2% phenytoin treated groups was significantly more than control as well as 10% povidone iodine treated groups. However, there was no statistical difference between both the preparations of phenytoin.

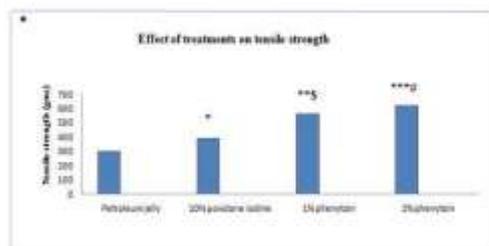
Table - 1

Measurement of wound tensile strength (Mean ± SEM)

Study groups	Tensile strength (gms)
Petroleum jelly	295.63 ± 13.42
Povidone iodine	388.8 ± 45.98 <sup>*</sup>
1% phenytoin	564.4 ± 17.46 <sup>***</sup>
2% phenytoin	622 ± 28.71 <sup>***</sup>

(\*p<0.05, \*\*p<0.001, \*\*\*p<0.001 when compared with control. <sup>†</sup>p<0.01, <sup>††</sup>p<0.001 when compared with povidone iodine)

Figure - 1: Effect of treatments on tensile strength



(\*p<0.05, \*\*p<0.001, \*\*\*p<0.001 when compared with control, †p<0.01, \*p<0.001 when compared with povidone iodine)

**Table 2 and Fig. 2** depicts estimation of hydroxyproline level in the tissues of the study groups. The measurement of hydroxyproline can be used as an index for collagen turnover. Increase in hydroxyproline content indicates increased collagen synthesis which in turn facilitate wound healing. The hydroxyproline level was more in both the preparations of phenytoin treated groups as compared to other groups, however, the results showed statistically significant difference only in comparison with control.

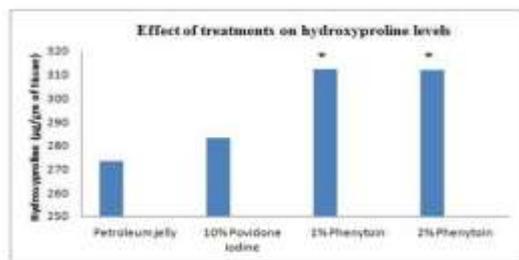
Table - 2

Estimation of hydroxyproline levels in the tissues of the study groups (Mean ± SEM)

Study group	Hydroxyproline level (µg/gm of tissue)
Petroleum jelly	273.3 ± 4.92
Povidone iodine	283.1 ± 8.70
1% phenytoin	312.4 ± 5.84*
2% phenytoin	311.8 ± 10.41*

(\*p<0.05, as compared to petroleum jelly)

Figure-2: Effect of treatments on hydroxyproline levels



(\*p<0.05, as compared to petroleum jelly)

## Discussion

Wound healing is a response to tissue injury resulting in restoration of tissue integrity. It involves a complex process which includes vascular and inflammatory phase, re-epithelisation and granulation tissue formation with matrix and collagen remodelling. Any agent that promotes any of the above processes is a promoter of wound healing.

Phenytoin was introduced into therapy in 1938 for the effective control of convulsion disorders. In 1939, Kimball observed gingival hyperplasia occurring in some patients treated with phenytoin<sup>[9]</sup> and Shapiro

carried out first clinical trial in 1958, showing less inflammation, less pain and accelerated healing in patients with surgical wounds who were pre-treated with oral phenytoin.<sup>[10]</sup> Goebel et al<sup>[11]</sup> showed that phenytoin promoted the healing of dental extraction sockets and Shafer et al<sup>[12]</sup> concluded that phenytoin increased the tensile strength of skin wounds. In spite of all these studies showing positive effect on wound healing, there are few studies showing that topical application of phenytoin doesn't have any effect on dermal or epidermal growth, suggesting that it may not possess wound healing property.<sup>[3]</sup>

The mechanism by which phenytoin stimulates wound healing process is not yet known. Clinical, animal and in vitro studies suggest different mechanism of phenytoin in the healing process such as increasing fibroblast proliferation, formation of granulation tissue, deposition of collagen and other connective tissue components. Phenytoin also contributes by decreasing collagenase activity, bacterial contamination and wound exudates. Biopsies of phenytoin treated open wounds show neovascularisation, collagenisation, decreased polymorphonuclear cells infiltrate and eosinophils.<sup>[13]</sup>

A number of clinical studies suggest that phenytoin reduces the bacterial load of wounds. Topical phenytoin application was reported to eradicate *Staphylococcus aureus*, *E. coli*, *Klebsiellaspp.* and *Pseudomonas spp.* from wounds within a period of 7-9 days. In a guinea pig model of wound healing, it was found that phenytoin cleared gram negative organisms from the wounds more readily than gram positive bacteria. It is unknown if phenytoin has intrinsic antibacterial activity, or this effect on bacterial load of wounds is due to its effect on inflammatory cells and neovascularisation.<sup>[14-18]</sup>

Various clinical studies have shown decrease in bacterial load of wounds after using phenytoin. Topical phenytoin eliminates *Staphylococcus aureus*, *E. coli*, *Klebsiella spp.* and *Pseudomonas spp.* from wounds within 7-9 days. Its effect is found to be more prominent on gram negative organisms than gram positive. It is unknown if phenytoin has intrinsic antibacterial activity, or if the effect of phenytoin on the bacterial load of wounds may be mediated indirectly by effects on inflammatory cells and neovascularisation.<sup>[14-18]</sup>

Hasamnis et al<sup>[12]</sup> showed that topical phenytoin accelerated the healing of excision wounds when compared with control (no treatment).

Deshmukh et al<sup>[3]</sup> studied and found out that there was no benefit with topical application of phenytoin as compared to petroleum jelly on wound surface area and percentage wound healing in an incision wound model.

Jarrah et al<sup>[19]</sup> showed no effect of phenytoin on the rate of wound healing in incision wound model when compared to topical application of cold cream.

Chan FC et al<sup>[20]</sup> showed significant increase in wound breaking strength and hydroxyproline levels and

concluded that topical diphenylhydantoin sodium accelerates wound healing in an incision wound model in diabetic rats when compared to control.

The results of the present study are in accordance with the above studies suggesting that phenytoin accelerates wound healing process in incision wound model.

Adverse effects of topical phenytoin are rare. Previous studies show some patients have a transient burning sensation when the powder is initially applied, but this can be prevented by using pure phenytoin powder which has been used in our study instead of phenytoin sodium. Systemic absorption of topical phenytoin is not significant. Most studies that have monitored serum phenytoin levels during topical phenytoin studies have shown the levels to be undetectable.<sup>[21]</sup>

### Conclusion

The results of our study showed comparable results in phenytoin treated group as compared to the povidone iodine treated group. This signifies not only an experimental use but also a possibility of use of topical phenytoin clinically in treatment of wounds.

In a therapeutic area of wound healing, at present the treatment of wounds include more of supportive measures like maintenance of hygiene, proper wound dressing and prophylactic use of antibiotics and not the agents which inherently improve the healing process. Ideally the drugs for wound healing should be designed in a way that they should be effective, promise fast results to reduce the morbidity and sufferings of the patients and most importantly cost effective. Formulation of phenytoin shows great promise by accelerating wound healing process and is cost effective too. Studies which have been conducted till now are limited and comparative studies using different formulations, route of administration and dosing intervals are lacking. Considering these findings, large number of animal studies should be conducted and extensive clinical trials should be carried out to support the animal study based evidence.

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**Conflict of Interest:** None declared

**Ethical approval:** Obtained from Institutional Animal Ethics Committee.

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