Available online at www.elixirpublishers.com (Elixir International Journal)

Pharmacy

Elixir Pharmacy 69 (2014) 22953-22955

ISSN: 2229-712X

Healing effect of phenytoin and honey on excisional wound in experimental albino Rats

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ARTICLE INFO

Article history:
Received: 19 February 2014;
Received in revised form:
20 March 2014;
Accepted: 3 April 2014;

Keywords
2% Phenytoin,
Honey,
Wound healing,
Time for complete epithelisation.

ABSTRACT

Phenytoin stimulates connective tissue growth, while honey has been used in treating various wounds and ulcers. This study evaluates 2% phenytoin ointment and honey on excisional wound healing process in experimental albino rats in comparison to 5% Betadine ointment. Method: Wistar albino rats were divided into four groups (n=6). Excisional round full thickness skin wounds of diameter 15 mm were created on back of anaesthetized animals. Medications were applied topically twice daily up to 20 days. Group A – untreated (control), Group B – 5% betadine ointment, Group C - 2% phenytoin oinment and Group D received honey. Wound healing was measured on Days 0, 4, 8, 12, 16 and 20. Number of days taken for complete epithelisation of wound was noted. Statistical analysis was done using ANOVA followed by Tukey-Kramer test and $p<0.05$ was considered significant. Result: 5% Betadine, 2% phenytoin ointment and honey hastens healing and reduces time taken for complete epithelisation of excisional wound when compared to negative control ($p<0.05$). 2% phenytoin and honey were superior to 5% betadine. Conclusion: Both 2% phenytoin oinment and honey may be considered for wound healing but their role in healing of infected wound needs to be explored further.

Introduction

Widespread existence of unhealed wounds, ulcers, and burns has a great impact on public health and economy. Non-healing and chronic wounds are a significant healthcare problem in today's medical practice.[1] Appropriate treatment and wound care accelerate the healing process and prevent infection and chronicity of the wound.[2] Despite extensive efforts to improve wound healing, the outcomes of existing methods are far from optimal.[3] Many interventions, including new medications and technologies, are being used to help achieve significant wound healing and to eliminate infections.

Phenytoin (diphenylhydantoin) was introduced into therapy in 1937 for the effective control of convulsive disorders.[4] A common side effect with phenytoin is the development of fibrous overgrowth of gingiva.[5] This apparent stimulatory effect of phenytoin on connective tissue suggested an exciting possibility for its use in wound healing.[3] Studies have shown topical phenytoin to promote healing of decubitus ulcers, venous stasis ulcers, diabetic ulcers, traumatic wounds, burns, and leprosy trophic ulcers [6,7,8] but the efficacy of phenytoin in treatment of such conditions is still controversial. The present literature available indicates that topical phenytoin deserves further investigation as a wound healing agent in preclinical studies in a controlled environment.

Honey is a natural product that has been introduced in modern medical practice. It has been used both orally and topically to treat various ailments including gastric disturbances, ulcers, wounds, and burns. Honey has long been documented as having healing properties[4] Honey and sugar paste were associated with scar less healing in cavity wounds.[5] Furthermore, honey causes significantly greater wound contraction than controls, and it promotes the formation of granulation tissue and epithelialization of wound.[6] Antibacterial properties and its effects on wound healing have been thoroughly investigated. The present study was conducted to evaluate the effect of phenytoin and honey on the wound healing process as well as epithelisation of wound compared to standard therapy betadine on laboratory rats.

Materials and Methods

The experiment was conducted on 24 albino rats of wistar strain of either sex weighing 150-200 g. The animals were caged individually (to avoid licking of wound) in a controlled environment (temperature 25°± 2°C) with a 12 hr light-dark cycle. Food and water were available ad libitum to the rats. Permission of Institutional Animal Ethics Committee was duly obtained. Prior to creating excisional wounds the rats were anesthetized by ether anaesthesia, shaved on the back and skin was disinfected using cotton and alcohol wipes. Using sterile surgical instrument round full thickness skin wounds of 15mm diameter were created in the paravertebral area, approx. 5 mm from midline on the back of rats.[2] The animals were randomly divided into four groups (n=6). Group A – served as negative control. Group B – 5% betadine ointment (positive control), Group C - 2% phenytoin ointment [9] and Group D honey were applied. Wound healing was measured on Days 0, 4, 8, 12, 16 and 20 of the experiment.[3]

To measure the contracture of the wound, a transparent plastic paper was placed on the wound and its shape was drawn on the same paper with a marker and then matched with the graph paper (mm^2) for finding the area of the wound to the nearest sq mm.[10] Time taken for complete epithelisation (fall of scab without any raw area) was also noted in all the groups.[11]

Statistical Analysis:

Statistical analysis was performed with Graph pad Instat 3 version. One way ANOVA followed by Tukey Kramer Multiple Comparisons Test was done. $p<0.05$ was considered significant and $p<0.001$ was considered very highly significant.

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Results:

Compared to Group A (control), groups B (5% betadine), C(2% Phenytoin) and D (honey) significantly reduced the wound surface area in all the animals over a period of 20 days (Table I). Contracture of wound surface area was noted as early as on day 4 with 2% Group C and D ($p<0.05$). This difference widened throughout with p value gradually becoming very highly significant ($p< 0.001$) in later part of the study.

Inter group comparison shows that from 12th day onwards, Group C and Group D was superior to Group B and ($p<0.01$) Groups C and D were comparable ($p>0.05$) **(Table I)**

Table I: Average area of wound measured in sq mm (n=6)

Days	Group A Negative control	Group B Betadine (5%)	Group C Phenytoin 2%	Group D Honey
O	178±2.1	178±2.3	178±2.2	178±2.1
4	144.6±6.6	137.2±4.5	133.1±6.3*	133.2±6.1*
8	124.1±4.8	115.3±3.6*	110.4±6.7**	113.4±6.1*
12	112.3±4.1	95.4±4.5***	84.5±5.4$^{***,++}$	83.5±5.7$^{***,++}$
16	99.2±4.5	67.5±5.6***	54.2±4.1$^{***,++}$	56.1±5.8$^{***,++}$
20	72.2±7.2	38.1±6.7***	22.7±5.2$^{***,++}$	20.1±4.8$^{***,++}$

Values (mean ± SD);

One way ANOVA followed by Tukey Kramer multiple comparison test done

* denotes comparison of Groups B, C, D vs A; * $p<0.05$ significant; **$p<0.01$ highly significant & ***$p<0.001$ very highly significant

$^{+}$ denotes comparison of Groups B vs. C &D; $^{+}$ $p<0.05$ significant; $^{++}p<0.01$ highly significant

$^{\#}$ denotes comparison of Group D vs. C; $^{\#}$ $p<0.05$ significant;

The average days required for complete epithelisation was significantly less($P< 0.05$) in all the treatment groups B, C & D compared to Group A. In between comparison of groups indicates that Group C and D were superior to group B ($p<0.05$) whereas group D was superior to group C ($p<0.05$). **(Table II)**

Table II: Time taken for complete epithelization of wound (n=6)

Groups	Average no. of Days required for complete epithelisation
Group A (negative control)	34.6±3.2
Group B (Betadine 5%)	27.9±2.2**
Group C (Phenytoin 2%)	23.6±2.7^{***+}
Group D (Honey)	18.8±2.3$^{***+++\#}$

Values (mean ± SD);

One way ANOVA followed by Tukey Kramer multiple comparison test done

* denotes comparison of Groups B, C, D vs. A; $^{**}p< 0.01$ highly significant & $^{***}p<0.001$ very highly significant

$^{+}$ denotes comparison of Groups C & D vs. B; $^{+}$ $p< 0.05$ significant & $p<0.001$ very highly significant

$^{\#}$ denotes comparison of Group D vs. C; $^{\#}$ $p<0.05$ significant;

Discussion

Wound healing is the process of restoration of physical integrity of internal or external body structures and involves complex interactions between the cells and various other factors. The healing process consists of a sequence of overlapping events including inflammatory responses, regeneration of the epidermis, shrinkage of the wound and finally connective tissue formation, and remodeling.[3] By day 4 after injury, new granulation tissue begins to invade the wound gap and numerous new capillaries grow through the new stroma with its granular appearance.[12]

A common side effect with phenytoin is gingival hyperplasia. This apparent stimulatory effect of phenytoin on connective tissue suggested possibility for its use in wound healing. The mechanism by which phenytoin accelerates the wound healing process is unknown. Clinical studies suggest that stimulation of fibroblastic proliferation, enhancing the formation of granulation tissue, decreasing collagenase activity (by reducing its production or secretion or both), promoting deposition of collagen and other connective tissue components, decreasing bacterial contamination, and by decreasing the formation of wound exudate to be some of the ways by which topical phenytoin hastens the healing process.[3, 13, 14] Local pain relief due to membrane-stabilizing action of topical phenytoin therapy has been observed in several studies.[13, 15] Pendse *et al*. found antibacterial activity of phenytoin in the wound healing process.[16]

Topical application of phenytoin results in direct access of the drug to the target site and avoids the risk of getting systemic side effects.[15] The findings of the above-mentioned study were taken into consideration for selecting the route of administration of phenytoin for wound healing in the present study. Modaghegh et al.[9] compared four topical phenytoin formulations (gel, cream, powder) in a rat model of wound healing and concluded that the phenytoin powder showed the most favourable results thus we considered phenytoin as powder formulation in our study to avoid contamination .

Honey has proven antibacterial activity and medical literature on treating wounds with honey has been reviewed[17, 18] As a dressing on wounds, honey provides a moist healing environment, rapidly clears infection, deodorizes, reduces inflammation, edema, and exudation. It increases the rate of healing by stimulation of angiogenesis, granulation, and epithelialisation.[19] Honey stimulates tissue growth, synthesis of collagen, and development of new blood vessels in the bed of wounds.[20]

Honey's antibacterial constituents and mechanisms identified include hydrogen peroxide (H_2O_2), methylglyoxal, (MGO), bee defensin-1, osmotic effect, and pH effects.[21] When honey is used topically (example, a wound dressing), hydrogen peroxide is produced by dilution of honey with body fluids which is released slowly and acts as an antibacterial.[22] The acidic pH (3.2 - 4.5) of honey[23] prevents the growth of many bacteria. High osmolar solutions like honey inhibits the growth of microorganism because sugar molecule present in honey ties up water molecules so that bacteria have insufficient water to grow.[24] Topical honey possesses antimicrobial effects by promoting autolytic debridement and by stimulating growth of wound tissue reducing pain, edema and exudates production.[25]

Though both the agents have proven potential in healing of wounds, no available data of head to head comparison of the two were available. Further, honey being a readily available natural household remedy finds an edge over phenytoin.

In the present study, it is seen that both 2% phenytoin oinment and honey hastens both healing and time taken for complete epithelisation of excisional wound in albino rats when compared to negative control and 5% betadine. But honey proved to be superior in time taken for complete epithelisation of wound.

Conclusion:

Honey is a natural remedy without any known systemic side effects and has proven antibacterial activity too, but action of phenytoin on contaminated wounds and its systemic side effects needs further evaluation. However, there is still scope of further

detailed investigation of exact wound healing mechanism and action of both the agents.

Conflict of Interest: None

Funding Sources: Self

Author's contribution:

Name	Contribution
Dr Prithwijit Banerjee	Principal investigator
Dr Mohua Sengupta	Co investigator
Dr Suhrita Paul	Manuscript drafting and protocol deigning
Dr Musfikur Rahaman	Data interpretation and analysis
Dr Subhasish Dan	Manuscript drafting

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