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Therapeutic Effect of Non-Antibiotic Amalgamate on Full Thickness Skin Wounds in Rabbit Model

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ABSTRACT

Objective: The study aimed to evaluate the therapeutic effects of non-antibiotic amalgamate of, *Azadirachta indica* (Neem) combined with rhizome *Curcuma longa* (Turmeric), *Nigella sativa* (Black seed) combined with Acacia honey, and Oxoferin® on full-thickness skin wounds in rabbits.

Methods: Twenty-one rabbits were divided into 3 equal groups. Three equal size skin wounds approximately 2x2 cm induced on thoraco-lumber area of each rabbit. Group A and Group B were provided with non-antibiotic amalgamates, and Group C treated with Oxoferin®.

Results: The mean statistical values of wound contraction of Group A, B and C were (1.071±0.098), (1.129 ±0.093) and (1.127 ±0.091) respectively. The mean values of tensile strength were (92.37±4.02), (123.76±3.66) and (104.86±3.97). The mean values of wound healing were (21.14±0.404), (20.71± 0.286) and (22.43± 0.481) days respectively. Also, animals of Group B showed significant increase in skin thickness, collagen fiber deposition and tensile strength as compared to other treatment groups.

Conclusion: In Group A significant therapeutic effect was observed. Wound healing might be associated with therapeutic and nutritional activities of non -antibiotic amalgamates.

Keywords: Wound; Nigella sativa; *Curcuma longa*: *Azadirachta indica*; Oxoferin

Introduction

Delayed wound healing is disruption in the normal process of wound healing associated with infections leading to pain sensation and exudate production with life threatening consequences in some cases [1]. Wound healing constituted in three major phases are inflammation, proliferation and remodeling. Neutrophils, macrophages, and other inflammatory cells are attracted by growth factors released by platelets at the wound site. These cells are microbicidal in nature, remove wound debris, produce cytokines and ROS reactive oxygen species. Proliferation phase is characterized by re-epithelialization and angiogenesis. Fibroblasts number increase predominantly and is associated with production of collagen, proteoglycans, and glycosaminoglycans like extracellular matrix components. In the angiogenesis process activated by macrophages, tissue hypoxia and vascular endothelium growth factor (VEGF), new vasculature networks organized that provide oxygen and essential nutrient supplements required for wound healing. The wound area epithelialization is associated with keratinocytes. Remodeling, the final phase of wound healing when skin recovers 80% of its original strength [2]. Complex wound structure with wide range of available treatment modalities makes it difficult to select suitable product for a specific wound. Despite a wide range of conventional recommendations being made on scientific grounds for wound dressings. Non-antibiotic therapies have been addressed by the scientific community worldwide [3].

The extract and essential oil derived from *Nigella sativa* manifested anti-inflammatory, analgesic, hepato-protective, antipyretic and immunostimulatory effects [4,5]. Honey has anti-inflammatory, bactericidal and wound debridement effects [6,7]. Neem (*Azadirachta indica*) is reported to be useful therapy in several health disorders including fever, gastrointestinal parasites, bacterial, viral, fungal skin infections, and inflammatory conditions [8]. Turmeric, also known as Indian saffron used particularly as liniments and creams has shown anti-inflammatory, and bactericidal activity [9]. Oxoferin (Tetrachlorodecaoxide) is potential therapy in the healing of chronic wounds. The aqueous solution of tetrachloro deca oxide irrigates the wound. It contains a bio-activated oxygen carrier that interrupts the vicious cycle of hypoxia at wound site. It fulfills the increased demand for oxygen supply during phagocytic activation, without compromising the required physiological degree of local hypoxia for neo-angiogenesis. Therefore, the study aimed to compare therapeutic potential of two non-antibiotic amalgamates with tetrachloro deca oxide (Oxoferin®) on full-thickness skin wounds induced in rabbit model.

Materials and Methods

Study Design

Study was carried out on locally bred, clinically healthy adult rabbits weighing 1200–1500 g were purchased from the single vendor. Experimental rabbits were housed and acclimatized for fourteen days in Laboratory Animal Facility of the Department of Clinical Medicine and Surgery. Rabbits were kept at (~ 22-28°C) in a light cycle of 12 h light and 12 h darkness and provided green fodder (Berseem) and fresh tap water ad libitum. Twenty-one rabbits were divided into three experimental groups (n = 7) as follows: Group A (treatment with neem oil and turmeric), Group B (black seed oil and Acacia honey), and Group C (treatment with Oxoferin®).

Preparation of Non-Antibiotic Treatments

Amalgamate of non- antibiotics were prepared. Certified Acacia honey procured from National Agriculture Research Center, Islamabad, Pakistan. Oxoferin® (Brookes Pharmaceutical Lab, Pakistan) purchased from local market.

Induction of Full Thickness Wound

Rabbits were anesthetized with ketamine (35mg/kg) and xylazine (5mg/kg) [10] and placed in sternal recumbency. Before surgical procedure skin sanitized with iodine followed by alcohol. The surgical site was marked. Three, 2×2 cm full-thick skin wounds were induced on the thoraco-lumber area of each rabbit. Sterilized wound tissues were immediately flushed with normal saline. Treatments were applied topically one time daily. To check any infection, wounds were evaluated daily for sepsis. All experimental protocols were adopted according to the prescribed ethical rules of standard surgery procedures.

Evaluation Parameters

To evaluate therapeutic effect of amalgamate of *Azadirachta indica* (Neem) oil and *Curcuma longa* (Turmeric), *Nigella sativa* (Black seed) oil and Acacia honey, with Oxoferin® following parameters were recorded.

Wound Contraction: Wound contraction rate was evaluated by tracing the edges of injured tissue on piece of trace paper at 3 days interval until complete recovery/epithelialization. These sketches were measured by graph paper (mm2) and values of contraction were estimated in percentile as described by [11].

Healing Time: Healing time defined as time from induction of tissue wound to the regeneration and epithelialization of the tissue. It was estimated and recorded on daily basis till scar fallen off [12].

Tensile Strength: At the end of the study, tensile strength of regenerated tissue was measured by using a tensiometer. The tensile strength of tissue at the time of tissue rupture was recorded as stated by [13].

Histopathological Studies: Microscopic studies of the tissue healing process were performed on tissue specimens from all treatment groups. The specimens were first fixed in buffered formalin. Specimens of full-thickness skin from each healed wound(5µm) were prepared and stained with Haematoxylin and Eosin. Wound epithelialization, deposition of collagen, skin layer thickness, fibroblasts proliferation and new vascularization in healed tissues were evaluated [14].

Statistical Analysis

Statistical analysis performed by using Analysis of Variance (ANOVA) technique and with help of SPSS software.

Results

All physiological parameters including temperature, heart rate, respiration were recorded within normal range. None of the rabbits developed anorexia, however, increase in water intake was observed in all experimental rabbits. Following the initial phase of expansion after surgical procedure, contraction in all induced wounds started and rate of contraction was measured with vernier's caliper from day1 onward until wound edges become united. Wound contraction in treatment groups is shown in (Figure 1). A significant rate of wound contraction was recorded after 18 days. Contraction rate was higher at a significant level in Group B (Figure 1). Statistically wound contraction rate between group B and group C were non-significant however, significant with group A. In rabbits the lowest mean wound healing time (20.7 days ± 0.286 and 21.1 ± 0.404) was noted in Group B and Group A followed by Group C (22.4 ± 0.481). Healing process was significant ($P< 0.05$) in Group B (Figure 2). Non-significant differences were observed between Group A and Group B, however, significant different was observed with Group C. Tensile strength was

low at significant level in Group A. A significant difference(P>0.05) in tensile strength was shown in three groups (Figure3). In the present study histopathological evaluation reveals that Group B showed significant development of epidermis and overall significant increase in thickness of the skin layer as compared to other two treatment groups. However the collagen fiber percentage was significantly higher (P>0.05) in Group B as compared to other groups (Figure 4).

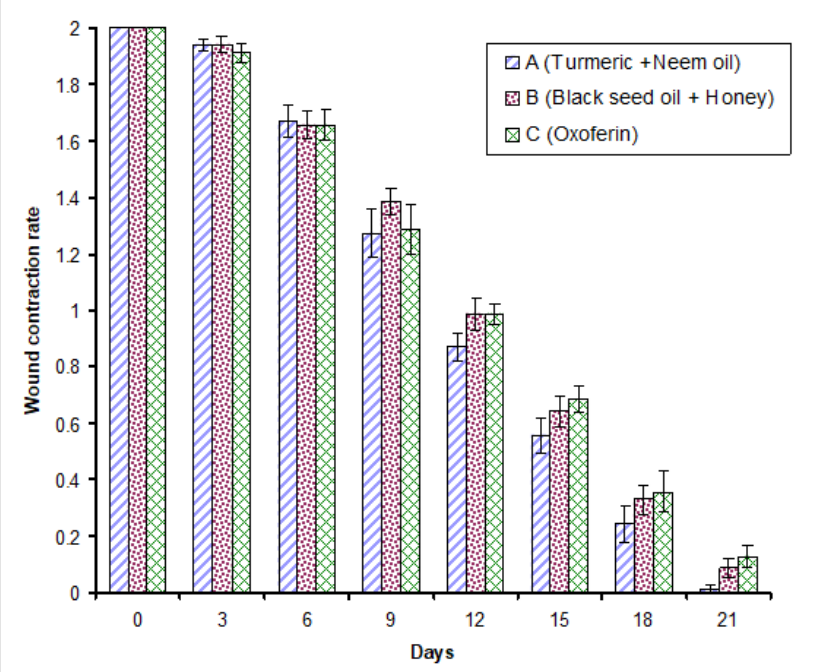

Figure 1: Wound contraction rate in the treatments A, B and C.

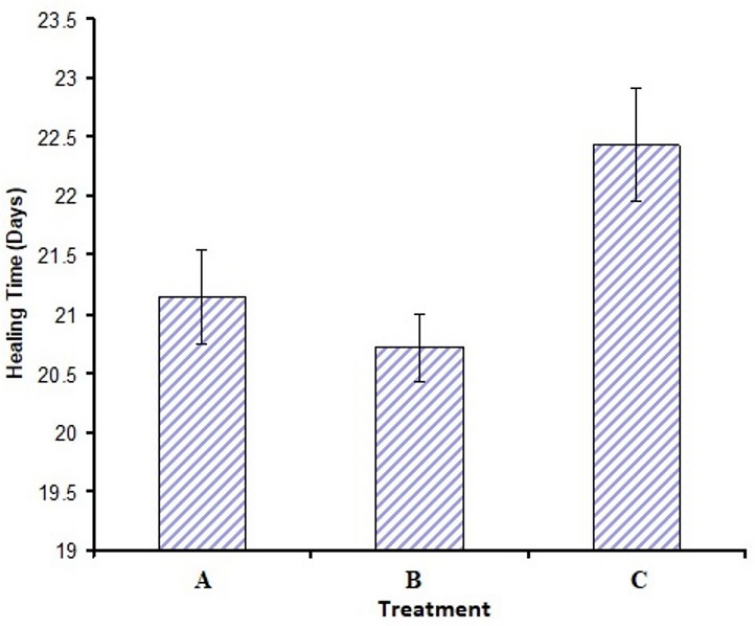

Figure 2: Wound healing time in different treatment groups.

Note: A=Turmeric+Neem oil, B=Black seed oil+Honey, C=Oxoferin.

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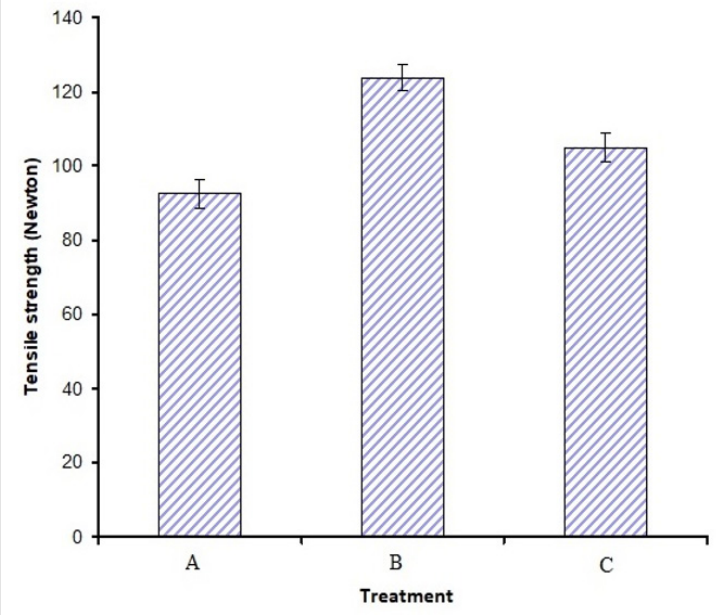

Figure 3: Tensile strength of wounds in different treatment groups.

Note: A=Turmeric+Neem oil, B=Black seed oil+Honey,C=Oxoferin.

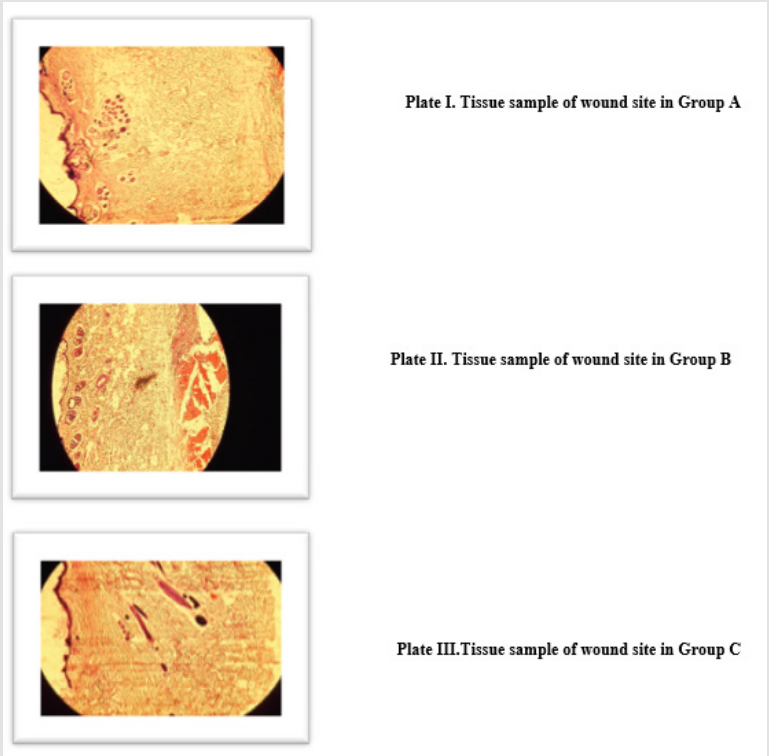

Figure 4: Histopathological evaluation wound tissues in three treatment groups (H&Ex100).

Discussion

Advancement in research revealed wide information about the complexity of wound and innumerable therapies for management. Present findings revealed non-significant (P>0.05) difference in the vital physiological parameter including temperature, heart rate and no. of per minute respiration in rabbits. A slight increase in all physiological parameters were observed on day 1 post-surgery in rabbits which resumed normal range after day 3 post surgery. A slight increase in body temperature was associated with mild inflammatory reaction and sepsis after surgical trauma. Present findings are also supported by literature [13,15] considering the fluctuation in physiological parameters as a consequence of stress response to the induced surgical trauma. Wounded tissue contraction is defined as movement of wounded tissue edges inward to close the site. In the present study, the wound contraction rate was high in Group B as compared to other groups. The improvement in wound contraction with non-antibiotic amalgamate of black seed oil and acacia honey was also supported by scientific literature [16-18]. The debridement process is accelerated by prolonged neutrophil activity and proteolytic enzymes reaction and is associated with moisture content and nutritional enrichment of black seed oil. Moreover, fibrin degeneration and its products are main drivers to activate macrophages and release growth factors at wound site. It might be assumed that therapeutic effect of black seed in wound healing either at local or systemic level of administration is associated with its scientifically proved antioxidant activity [19]. Tensile strength was at significant level (P>0.05) in non-antibiotic amalgamate of honey with black seed oil which might be associated with high copper and zinc contents in honey and synergistic therapeutic effect in presence of growth hormone and fatty acids derived from black seed oil.

Copper is associated with collagen production at the site of skin wounds. Another reason for significant increase in tensile strength in Group B might be associated with increased density, keratin production, granulation of tissue and increased fibroblasts activity ultimately increasing collagen production at site of surgical trauma. Development in tissue granulation process and contraction of tissue edges are interdependent as granulation and myofibrils are main driver for the tissue edges contraction. Present study revealed that tissue edges contraction rates were significantly high in Group B [16]. The early and fast healing trend in honey treated wounds might be associated with high contents of collagen producing amino acids predominantly proline, glycine, and methionine .The high sugar content in honey might also be associated with prompt granitization of damaged tissue. Furthermore, the bactericidal activity of honey is associated with excessive production of hydrogen peroxide during glucose oxidation process catalyzed by enzyme glucose oxidase. Honey also contains inhibin like factor which is associated with bacteriostatic activity. Honey is said to be associated with the production of moist environment a driving factor enhancing the wound debridement process [20]. Honey therapeutic effect in wound healing is associated with decrease in pH and deposition of free acid content to cause hindrance in microbial growth. The findings of early healing with black seed oil and acacia honey were also supported by literature [20,21]. Neem oil and turmeric amalgamate also showed better results in wound healing and these results of improved wound healing were supported by [22,23]. Neem oil is claimed to be potential therapy for skin ailments. The neem oil is rich in fatty acids associated with buildup of collagen, promoting healing and maintaining the skin elasticity. The fatty acids in neem oil also enhance tissue granulation and ultimately promote the production of elastin and collagen. These fatty acids also required for maintenance of moisture and softening the texture of the injured skin during the process of healing. The bactericidal property of neem oil also protects injured tissue from sepsis. Neem oil also manifests beneficial effect on wound healing due to its fly repellent activity [22]. Turmeric (*Curcuma longa* Linn) possesses anti-inflammatory properties and contain of Vit A and proteins promoting early synthesis of collagen fibers by enhancing fibroblast activity [24]. These turmeric rhizomes contain bioactive compounds including curcumin (Di feruloyl methane), turmeric oil or turmerol & 1, 7-bis, 6- hepta-diene-3, 5- Dione. Curcumin has strong anti-inflammatory and analgesic properties. C. longa also contains volatile oils manifesting bactericidal and anti-inflammatory properties. The results of histopathological examination of current study were supported by [20,21].

Conclusion

The findings conclude that topical application of non-antibiotic amalgamate of black seed oil and Acacia honey manifested potential therapeutic effect in full thickness wounds in rabbit model.

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