Effect of Omega-3 on Induced Cutaneous Wounds Healing in Rabbits

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Abstract

The present study was designed to evaluate the effects of omega3 on full-thickness cutaneous wounds healing in rabbits. All animals were created wound (1.5 cm2) full-thickness in dorsal back region in (16) male adult rabbits, clinically healthy weighing from (1.3- 1.8 kg). Under the effect of intramuscular administration of a mixture of xylazine hydrochloride 5mg/kg with ketamine hydrochloride 35mg/kg of and 1 mg/kg of Diazepam. The treatment group was given 300 mg of omega3 orally daily with single dose (group A), while in group (B), the rabbits were left without treatment, as a control group. For clinical and histopathological evaluation, each group was divided into four subgroups (two wounds/subgroup) on 3, 7, 14 and 21th days post-wound creation and treatment. The result revealed, Clinically, the rate of wound healing was same in all groups, no differences when section taken from the edge of the wounds. The results histopathologically, shown the treated groups have greater cellularity with improved vasculature with the superiority of omega 3 -treated groups than those in untreated groups. Conclusion; the histopathologically and clinically results confirmed that given effects of omega3 to the treated groups leads to enhance and develop of cutaneous wound healing.

Keywords: *omega3, wounds healing, rabbits,*

Introduction

A wound is a disruption of the normal continuity or contiguity of body structures caused by physical injury [(1)]. Wound healing is a biological process that happens after a physical, chemical, or biological causes led to breaken of epithelial barrier [(2-3)]. The wound care is older skill such as old human civilization, in the recent decades, available the advanced medical science, improve healing suffer from lack when get oral medication. Therefore, an active drugs as oral formulation to be immensely beneficial to the heal wound due to simple of use and enhance of healing time, Omega-3 is one of them [(4)]. Though, recently that adequate levels of essential nutrients is being recognized to prevent and treat some disturbances [(5)]. Omega-3 fatty acid is one of the essential fatty acids in human body that can be found in the sea products, especially fish and fish oil and in some of the seeds oils. omega-3 have many important benefits like they entered in a good feeding and treated for many diseases as they decrease rheumatoid arthritis [(6)]. Current modern studies demonstration that omega-3 can control the activity of the nuclear factor (NF-kß) [(7)], that acting a main role in the gene expression regulation in inflammatory reactions and associated in the pathogenesis of cardiovascular disease [(8)]. Therefore, The purpose of this study was to evaluate and compare the effects of omega3, on full-thickness cutaneous wounds healing.

Materials and Method

Experimental Animals

Sixteen adult male rabbits were used in the study that divided into two groups (eight rabbits for each group) weighing from (1.3- 1.7 kg). The animals were housed in the animal house of the Veterinary Medicine College, Karbala University, maintained in individual cages along the period of the experiment under controlled conditions including, management, environment and feeding.

Surgical operation

Food was hold for 6-12 hours and water 5 hours

before surgical operation. The area of dorsal back region should be shaved and cleaned, after that general anesthesia was induce with diazepam as a preanesthetic in dose 1mg/kg b.w. after 10 min. injected with xylazine in dose 10 mg/kg b.w. and ketamine in dose 50 mg/kg b.w. all this drugs injected intramuscularly [9]. The rabbits were hold in ventral recumbency and the dorsal aspect (back) of the animal was prepared for aseptic surgery. On each animal, one square (1.5×1.5) cm full-thickness skin wounds were created in dorsal back region. The rabbits was divided to two groups, group (A) as the treatment group was treated with single dose daily of 300 mg omega3 orally for 3 weeks, while, in group (B), the rabbits were left without treatment, as a control group.

Clinical Evaluations: In this study, all rabbits were exposed to complete clinical examination every three days during the experimental period. All wounds were taken by digital photographs after the area prepare with carefully shaved to see margin of the wound. The scab was removed carefully for each wound for well visualize of the granulation tissue and epithelization area.

Histopathological evaluation :The histopathological evaluation was performed on day (3, 7, 14 and 21) post-treatment with omega3 and the same period was depended for the control group (four rabbits / period). For histological analysis, a biopsy specimens were obtained (5-6) µm in thickness and they included about (3-4) µm of skin intact on both sides of the wound they were fixed in (10%) neutral formalin solution, and prepared routinely and staining by hematoxylin-eosin (H&E) [10].

Results

Clinical Evaluation

The clinical statement of wounds seemed actually that the treatment and control wounds were showed quickly decreased in size through the current study. The change was started at 3rd days in both control and treated wounds, which became clear at day 14th, especially in treated wounds. These differences continued to be present until day 21th post-treatment. In addition, the size of the treated wounds observed lesser than those in control wounds. The clear differences appeared in the in total wound healing between treatment & control wounds, mainly at the end of the study **(Fig.3.1**).

Histopathological Evaluation

The histopathological experimentally produced for tissue biopsies of the wound margins and beds shown the chief differences between control and treatment wounds were began after three days post-treatment. The histopathological sections, of treated group on day 3 post-wounding, shows aggregation of intensive of inflammatory cells. At the same period, the histopathological sections of control group were shown a little infiltration of inflammatory cells.

On 7 day post-treatment, the histopathological observation in control group was showed the new blood vessels and inflammatory cells. The histopathological sections of treatment group, 7 days post-treated, were showed presence of proliferation of more small blood vessels with proliferation of fibroblasts cells. On 14 day post-wounding, the histopathological section of control group, was showed the new blood vessels, inflammatory cells. the histopathological section of treated group on day 14 post-treated, was showed more vascularity with formation of keratin and thicken of epidermis. The histopathological section of control group, 21st days post-wounding, showed the presence of epidermis and presence of sebaceous glands. The histopathological section of treated group, 21st day post-treatment, was appeared thick epidermis layer with well development of hair follicles and presence of sebaceous gland.

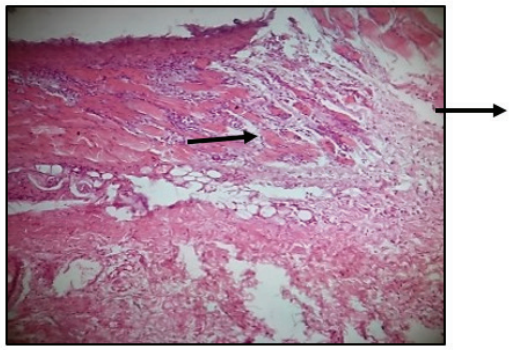

Fig.1: Histopathological section of treated group on day 3 post-wounding, shows aggregation of intensive of inflammatory cells (black arrow) (H&E X100).

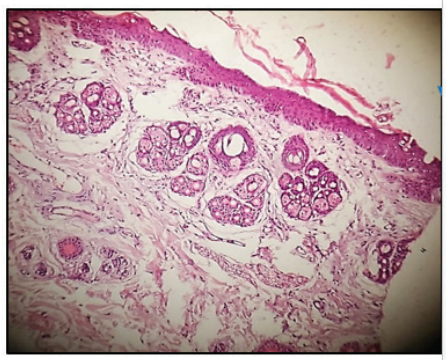

Fig 2: Histopathological section of control group on day 3 post-wounding, shows little infiltration of inflammatory cells (blue arrow) (H&E X100).

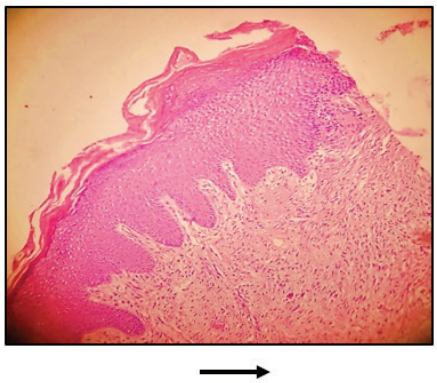

Fig 3: Histopathological section of control group on Day 7 post-wounding, shows the new blood vessels (black arrow), inflammatory cells (blue arrow) (H&E X100).

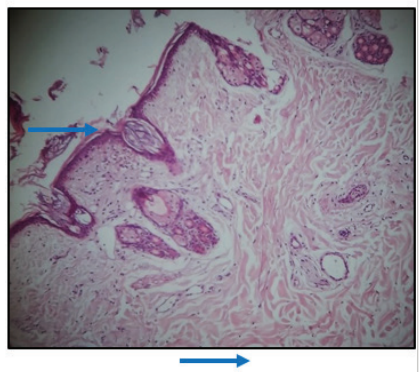

Fig. 4: Histopathological section of treated group on day 7 post-wounding, shows more small blood vessels (angiogenesis) (yellow arrow) with proliferation of fibroblasts cells (black arrow)) (H&E X100).

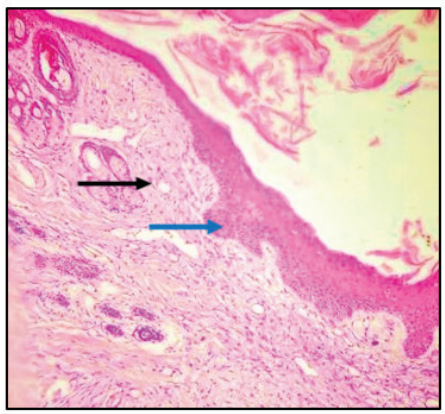

Fig 5: Histopathological section of control group on 14 day post-wounding, shows the new blood vessels (black arrow), inflammatory cells (blue arrow) (H&E X100)

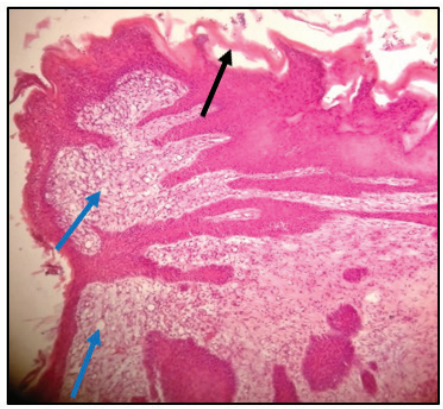

Fig 6: Histopathological section of treated group on14 day post-wounding, shows more vascularity (blue arrow) with formation of keratin (black arrow) and thicken of epidermis (H&E X100).

Discussion

Clinical Evaluation

Wound healing process is a biological, complex, progressive that occurs directly because of disruption in epithelial barrier consequently after chemical, physical, or biological causes. In this process activation occurs for inflammatory cells such as platelets, neutrophils, macrophages and fibroblasts [2-3]. In the current study, the assess and compare the effects of omega3 on the healing of full-thickness cutaneous wounds in the rabbits supply improved blood vessels and better epithelization, to all post-treated groups compared with the control groups. The clinical observations of wounds in the present study appeared that the level of development of healing process was started rapidly in both control and treatment wounds, but the progression was high in the treated groups & than those in control wounds. The clinical following-up appeared that all wounds of treatment and control groups were decreased rapidly in size along the study, but the clinical inspection revealed that the rate of wound closure (contraction) in treated groups were significantly more along the period of the study as compared to control groups. These differences were continued to be present until day 21 post-treatment. These results are close to the results obtained by [11] who used topical and systemic effects of omega-3 on oral mucosal wound healing in albino rats. They investigated the omega3 have greatly enhanced ulcer healing with superior effects when using topical application. In the other research that shown increase cell membranes fluidity when used omega-3 [12]. Cell membranes provide a significant function into and out of cells by regulating crossing of chemical signals, hormones, and nutrients; this led to different effects, from increasing glycogen storage to enhancing muscle protein synthesis [13]. On the other hand, [14] Animals before surgical incision for 21 days get a food containing fish oil was made and for 10 or 30 days after wounding, showed that content of collagen was similar at the same period.

In the study of [15] revealed that omega-3 enhanced closing of open wounds at first (5 days), not as other fatty acids moreover may have significant effects on healing of the wound. Treated the wounds with oleic acid healing faster than the wounds that treated with linoleic acid.

Histopathological Evaluation

The valuation histopathologically of tissue biopsies from the wound peripheries and beds in the current study discovered that the chief differences between control and treatment wounds were happening at day 3 post-treatment and continuous until the end of the study. The histopathological sections showed that the proliferation of blood capillaries and intensive of inflammatory cells infiltration in treated group were more developed than that in control wounds through 3 and 7 day of the study. Also in the histopathological section of treated group on day 14 and 21st days post-treated, was showed more vascularity with formation of keratin and thicken of epidermis with well development of hair follicles and presence of sebaceous gland than control group.

These results are in agreement with the results obtained by [11] when use local and systemic omega 3 application, there was a gradual reduction in inflammation, increase in epithelial and connective tissue regeneration, appearance of keratin layer, and finally high vasculature, all occurring gradually throughout the period of 2, 4, and 8 days, giving the best repair effect at the end of the treatment period. In the study of [16] showed that omega-3 fatty acids augmented closure of open wounds first 5 days, Also mention that omega-3 in fish oil may have a support effect on primary wound epithelialization, then reducing scar formation through inhibit later collagen deposition.

Conclusions

Omega3 can be used for acceleration and enhancement of cutaneous wounds healing. The healing in the treated group appear an effective when compared with control group.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Surgery and Obstetrics, College of Veterinary Medicine and all experiments were carried out in accordance with approved guidelines.

References

1. Weledji E P. Perspectives on Wound Healing. Austin Journal of Surgery, 2017;4(3): 1104.
2. Tonnesen M G, Feng X, Clark R A. Angiogenesis in wound healing. The journal of investigative dermatology.Symposium proceedings / the Society for Investigative Dermatology, Inc.[and] European Society for Dermatological Research, 2000;5(1): 40-46.
3. Clark R A. Fibrin and wound healing. Annals of the New York Academy of Sciences, 2001;936: 355-367.
4. Jeffrey M L. The Effect of Oral Medication on Wound Healing. Advances Skin Wound Care, 2017;30 (3):137–42.
5. Silva J R, Burger B, Kühl C M C, Candreva T, dos Anjos M B P, Rodrigues H G. Wound Healing and Omega-6 Fatty Acids: From Inflammation to Repair, Mediators of Inflammation, 2018; Article ID 2503950: 17.
6. Kremer J M. N-3 fatty acid supplements in rheumatoid arthritis. Am J. clinical. Nutrition, 2000;71(1): 3495- 3515.
7. Adlins Y K. Mechanisms underlying the cardioprotective effects of omega-3 polyunsaturated fatty acids. J. Nutr. Biochem, 2010;21:781- 792.
8. Winther M P, Kanters E, Krall G. Hofker M H. NF-kß singling in atherogensis. Aterioscler Thromb Vasc. Biol. 2005; 25: 904-914.
9. Albozachri J M K, Hameed F M, AL-Tomah H M, Muhammid H A. Evaluation of tow general anesthetic regime by use xylazine and ketamine with atropine and diazepam in rabbits. J. University of Kerbala. 2017;15: 21-30.
10. Luna L G. Histopathologic methods and color atlas of special stains and tissue artifacts. Maryland: American Histolabs. Inc. 1992.
11. Soha B, Sahar M E, Mervet M. Assessment of the Topical and Systemic Effects of Omega-3 on Oral mucosal wound healing in albino rats: A Histopathological and Biochemical study. Madridge J. Case Rep. Stud. 2018;2 (1): 26-31.
12. Alexander J W. Immunonutrition: The role of omega-3 fatty acids. Nutrition, 1998;14(7–8):627–633.
13. Lovejoy J C. The influence of dietary fat on insulin resistance. Curr. Diab. Rep. 2002;2(5):435–440.
14. Albina J E, Gladden P, Walsh W R. Detrimental effects of an omega-3 fatty acid-enriched diet on wound healing. J. Parenter Entearl Nutr. 1993;17: 519.
15. Cardoso C R B, Souza M A, Ferro E A V, Favoreto S, Pena J D O. Influence of topical administration of n-3 and n-6 essential and n-9 nonessential fatty acids on the healing of cutaneous wounds. Wound Repair Regen. 2004;12: 235.
16. Cardoso C R, Favoreto S, Oliveira L L, Vancim J O, Barban G B, Ferraz D B, Silva J S. Oleic acid modulation of the immune response in wound healing: a new approach for skin repair. Immunobiology, 2011;216: 409.