Iran. J. Ichthyol. (Special Issue 1, 2022): 104-109

P-ISSN: 2383-1561; E-ISSN: 2383-0964
http://www.ijichthyol.org

Research Article

The effects of common carp, *Cyprinus carpio* skin collagen extract on infected wounds

Zahraa M. AYAD[1], Zainab J. MALIK[*2], Ali I. ALAMEEDI[3], Maymunah ABDULAH[3]

[1]*Department of Nursing, Al-Mustaqbal University College, Al Hillah, Iraq.*
[2]*Department of Surgical and Obstetric, Veterinary Medicine College, Al- Qasim Green University, Babil, Iraq.*
[3]*Department of Physiology and Pharmacology, Veterinary Medicine College, Al-Qasim Green University, Babil, Iraq.*
[*]*Email: malikzai87@gmail.com*

Abstract

The current study was conducted to evaluate the healing aspect of skin fish collagen on the infected wound in local rabbits. A total of 16 mature animals were divided equally as control and treated groups. Lidocaine 2 % subcutaneously was used as a local anesthetic at the operative site, followed by surgical incision bout 3*4 cm induced full skin wound. Then, the wounds were contaminated with *Staphylococcus aureus*. After 24hr to 7 days of treatments, the results of temperature, respiration, and heart rates showed a significant difference between the two groups. Furthermore, the photographic scar aggregation in treated groups' wounds appears less than in the control one. The histological results of the skin biopsies on the seventh and 21st days postoperative in the control group revealed extensive tissue necrosis and hemorrhage, and highly cellular granulation tissue, respectively. Whereas in the treated group on the 7th-day and 21st days post-operation, the histopathological sections revealed good epithelization with early granulation tissue formation characterized, excellent epithelization with a small amount of keratin on the surface, respectively. It was concluded that common carp skin collagen has a good therapeutically antibacterial and anti-inflammatory effect in wound healing.

Keywords: Skin, Fish, Infection, Healing.

Citation: Ayad, Z.M.; Malik, Z.J.; Alameedi, A.I. & Abdulah, M. 2022. The effects of common carp, *Cyprinus carpio* skin collagen extract on infected wounds. Iranian Journal of Ichthyology 9(ICAB special issue 2022): 104-109.

Introduction

Skin is the main defense system against pathogens, toxins, and wounds, and damages to the skin can cause significant morbidity (Ekawati et al. 2020). The process of wound healing of the skin infection is a complex process, involving the deposition of collagen in response to tissue injury, and at the final stage produces scar formation. Such mechanisms include inflammation, fibroplasia, and maturation of scar tissue (Rosique et al. 2015). *Staphylococcus aureus* is a cosmopolitan and a growing problem in the community and hospitals. The infection caused by *S. aureus* is usually treated with antibiotics, but in some cases, it has been found that some strains of *S. aureus* are resistant to antibiotics (Tokajian 2014).

Fish collagen is a highly complex protein that supports the skin, ligaments, joints, bones, muscles, tendons, blood vessels, gums, eyes, nails, and hair to maintain their strength and flexibility. Collagen type I is the most abundant one in the human body and it is the best known for establishing the foundation for beautiful skin, strong connective tissues, and strong bones (Axe 2017). Fish skin has been used as an occlusive wound dressing for burn management in humans (Costa et al. 2019; Lima et al. 2019) and animals (Hicks 2018; Quinton 2018) showing a complete re-epithelialization in a brief time faster than expected. However, the treatment with the fish

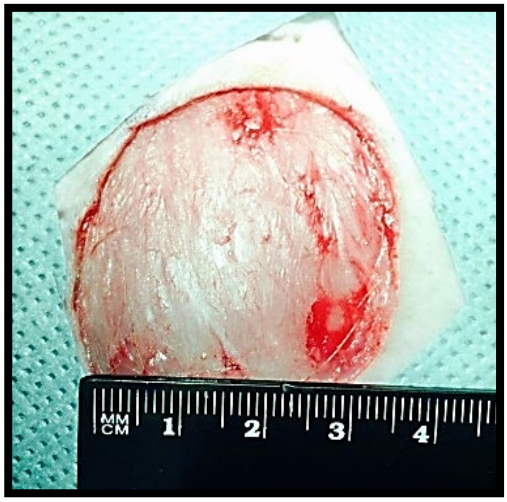

Fig.1. Length of the created injury 3*4.

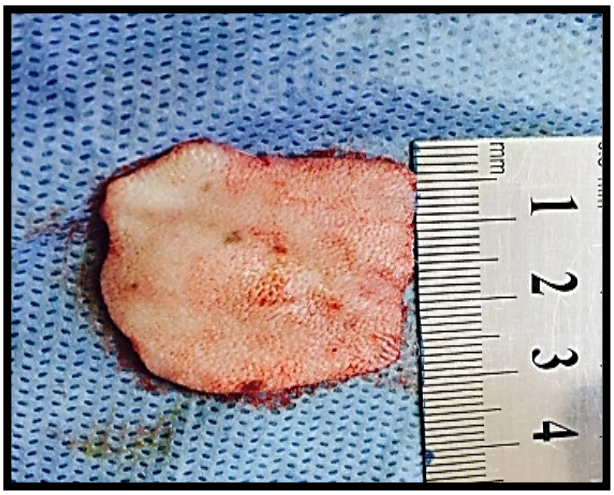

Fig.2. Pieces of skin that taken from animal.

skin is still in the experimental stages (Lima et al. 2019). Hence, the present study aimed to evaluate the skin collagen of common carp *Cyprinus carpio* as a biological antibacterial and anti-inflammatory against full-thickness cutaneous infected wounds in local rabbits.

Materials and methods

Skin preparation: Primary materials were the skins of four common carp obtained from the local market at Babil Province. The fresh skin was kept refrigerator at 4°C. Then, they washed with tap water before being sun-dried for 48 hours at a temperature of 30°C, which is regarded safe against deterioration. All of the chemical reagents utilized in this study were analytical grade.

Gelatin extraction: To eliminate any residual fat and pollutants, the skin was cleansed with hot water for 1min. The skins were chopped into small pieces and submerged for 10 hours at room temperature in a 0.05 M acetic acid solution with a 1:4 (w/v) ratio of skin fish to the acid solution. Then they were carefully cleaned with warm tap water until it was brought back to a neutral state. After that, the skins were immersed in distilled water at a 1:3 (w/v) ratio and heated to 80°C for 2 hours to extract the gelatin. The gelatin liquor was filtered using a filter paper-layered cloth to get gelatin filtrate. The filtrate was placed on a pan and dried in a dehydrator to make gelatin sheets. The filtrate was placed on a pan and dried in a cabinet drier at 55°C for 48 hours to yield gelatin sheets. Gelatin granules were made from the sheets and sealed in plastic and kept in the refrigerator until testing (Montero & GómezGuillén 2000).

Bacterial isolation: 1.5×10^7 CFU/ml of *S. aureus* in the physiological saline were obtained from the microbiology of the college of veterinary medicine Lab, at Al-Qasim green university.

Experimental animal: Sixteen adult rabbits were selected with 3 to 10 months old and weight of 1.5-2.5kg, kept in controlled conditions for observation and adaption during the study.

Design of experiments: The rabbits were separated into two groups, each including eight rabbits: Group A with numbers of 1 to 8, received a 3*4 cm skin wound contaminated with bacteria without being treated considered as the control group (Figs. 1, 2). Lidocaine 2% subcutaneously was used as a local anesthetic at the operative site, followed by surgical incision bout 3*4 cm induced full skin wound. Immediately after inducing a skin wound, these wounds were contaminated with *S. auraus* for the next 24 hours. Group B was numbered 9-16, and about 3*4 cm pf their skin wound was treated with extracted gelation of fish skin. In the first and third weeks, the two groups were randomly divided into two subgroups for histopathological studies.

Clinical examination: Temperature, respiration rate, heart rate, feces, and urination were all measured physically and clinically on the animals during one-week post-operation defecation and urination interval.

Histopathological examinations: Skin biopsies were taken on the 15th and 30th postoperative days. Biopsies were preserved in 10% neutral buffered formalin. Then, the samples were routinely implanted in the paraffin blocks and cut in 5-6 µm thickness and stained with Hematoxylin and Eosin, and examined under a light microscope (Luna 1968).

Fig.3. The scar tissue in the two studied groups at 7th days, (A) control group, and (B) treated with the skin of fish group.

Fig.4. The scar tissue in all groups at 21st days; (A) control, and (B) treated with the skin of fish groups.

Table 1. Effect of different treated on body temperature, heart rate, and respiratory rate (Mean±SE).

	Temperature		Heart rate		Respiratory rate	
Day	control	T-fish collagen	control	T-fish collagen)	control	T-fish collagen
1	77.37±1.06a	73.00±1.83b	167.0±3.57a	173.6±3.12	73.00±3.2	67.25±2.7
2	77.00±0.82a	64.25±2.11b	159.7±5.25b	146.2±4.00	47.75±3.8ab	60.62±4.2a
3	73.37±2.37a	58.87±1.34b	149.3±3.40c	137.5±2.86 ab	46.62±2.7a	44.75±4.0 ab
4	64.12±2.24a	57.87±1.04b	133.1±2.53c	136.6±2.01a	39.87±1.3	43.12±2.5
5	59.00±1.70a	58.87±0.69a	135.3±1.85c	137.3±1.26	44.00±2.3	42.37±2.1b
6	57.62±2.17a	59.37±0.80a	134.8±1.91c	136.6±2.85	48.50±2.3	43.37±1.8b
7	56.87±1.10ab	60.12±1.23a	133.6±2.06cab	136.2±2.69a	44.25±1.9	44.62±3.0b
LSD	8.22	11.83	14.29	15.54	5.13	4.91

Statistical analysis: The results were recorded as means plus SD. Two strategies were used to look at the dataset. The least significant difference (L.S.D.) was employed in the analysis of variance (ANOVA), with $P>0.05$ being considered significant. SPSS (Statistics Package for Social Sciences) was used as the statistical program.

Results and discussion

The results of the physical and clinical examinations for temperature, respiration rate, heartbeat, defecation, and urination during the first-week post-operation revealed that all animals in the control group had a slightly elevated temperature, respiration rate, and heartbeat with normal

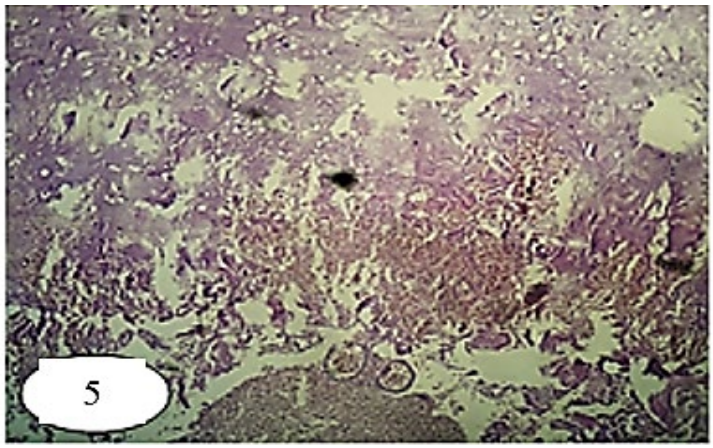

Fig.5. Histological section of rabbit skin for at 7th days showing extensive tissue necrosis and hemorrhage in epidermis and dermis (H and E) 100X.

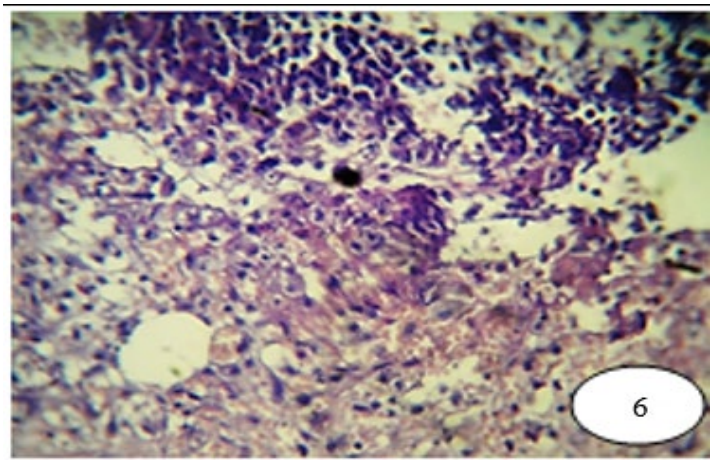

Fig.6. Histological section of rabbit skin for at 21th days showing highly cellular granulation tissue with neovascularsation.

defecation and urination. Significant convergence in the results between the treated and control groups began on the second postoperative day but quickly disappeared in the treated group on the fifth to seventh postoperative days, possibly due to increased blood flow in the operative area. Lu et al. (2017) also reported an increased dilatation of the blood vessels with increased capillary permeability, stating that there were no significant changes recorded in these clinical parameters before and after surgical operation. In the current study, the animal's activity (as represented by animal posture, motion, and alertness to its surroundings) and appetite were not altered. However, two animals in the control group had a normal appetite but limited activity three days after surgery, which could be attributed to visceral pain or adhesion formation. Previous studies have reported visceral pain during the first hours after surgical intervention or pain associated with adhesion formation postoperatively (Verma et al. 2006; Nakajima et al. 2009).

The results of the macroscopic assessment on the 7th postoperative day revealed that scar tissue formation was higher in the control group than the treated one, characterized by the small scar tissue that may be directly attributable to mechanisms that occur to varying degrees during the four types of healing (Figs. 3, 4). Primary healing occurs when a wound closes within hours of being created. When a wound is purposefully left open for an extended period of time prior to closure, delayed primary healing occurs. Secondary intention healing occurs when wounds are allowed to heal naturally, with or without topical therapy. Dressings are changed until the wound closes as a result of contraction and epithelialization. Finally, epithelialization heals partial-thickness wounds or wounds involving the epidermis and a portion of the dermis (Glat & Longaker 1997). Surgical trauma is among the most potent stimuli for the onset of an inflammatory

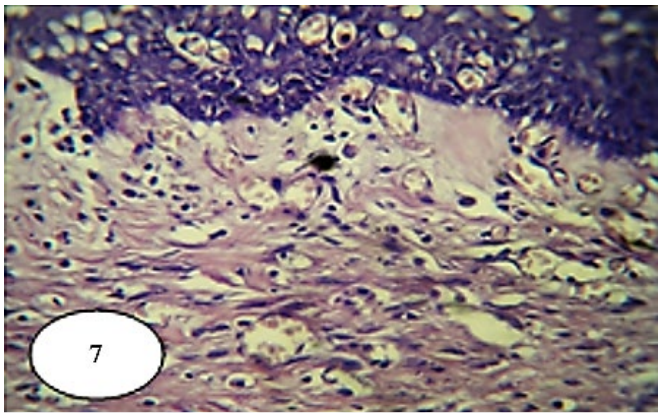

Fig.7. Histological section of rabbit skin for treated by natural skin fish for 7th days showing good epithelization with early granulation tissue formation characterized by highly cellular neovasculasation and myofibroblast also seen (200X).

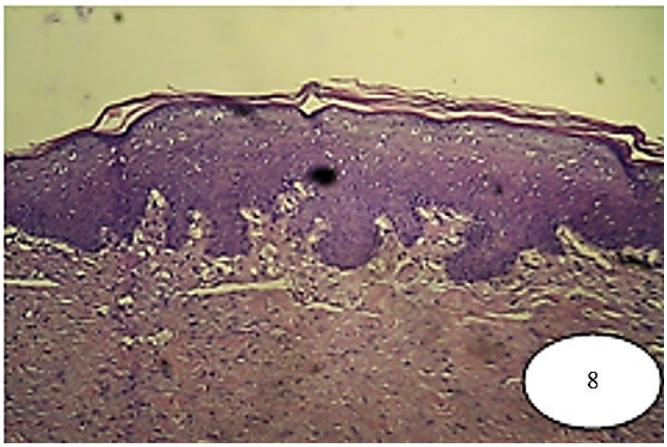

Fig.8. Histological section of rabbit skin for treated by natural skin fish 21th days showing excellent epithelization with small amount of keratin in the surface also good tissue remodeling 400X.

response and, as a result, the formation of adhesions. Various degrees of tissue handling is involved in routine surgical procedures, which can lead to tissue abrasion, desiccation, ischemia, bleeding, infection, and exposure to foreign materials. Any of these factors can cause inflammatory responses, which can lead to the formation of adhesions (Liakakos et al. 2001). During day's five to ten, fibroblasts become aligned with the adhesion, while collagen deposition and organization progress. The few cells present are primarily fibroblasts. Between the 7th and 21st days after injury, the collagen fibrils organize into discrete bundles interspersed by fibrocytes and a few macrophages. Extensive, well-defined adhesions that contain blood vessels and connective tissue fibers are frequently covered by mesothelium (Dizerega 1994; Alizzi 2005).

In the control group on the 7th day postoperation, the histopathological examinations revealed extensive tissue necrosis and hemorrhage in the epidermis and dermis, and a marked inflammatory zone (Fig. 5) and on 21st day postoperation, a highly cellular granulation tissue, and invasion of the area with new blood vessels were observed (Fig. 6). Another section revealed a large area of the abscess. In the treated group by the skin of fish on the 7th-day post-operation, the histopathological sections revealed good epithelization with early granulation tissue formation characterized by highly cellular neovascularisation and myofibroblast (Fig. 7). On the 21st postoperative day, the histopathological sections of the treated group revealed excellent epithelization with a small amount of keratin on the surface, as well as good tissue remodeling (Fig. 8). Collagen has many benefits such as preventing and improving signs of skin aging. Consuming collagen may provide skin benefits such as improved smoothness, improved moisture retention, increased suppleness, and prevention of deep wrinkle formation. Hydrolyzed fish collagen is composed of small, low molecular weight peptides that are easily

digested, absorbed, and distributed by the human body. Fish collagen peptides have been studied for their effects on collagen synthesis, quality, and mineralization. Our results are inconsistent with the findings of Axe (2017) who showed how collagen can aid in bone repair and regeneration. Fatty acids influence the production of proteins known as proinflammatory cytokines, which signal biological processes during the inflammatory stage of wound healing. Interleukin-1 beta (IL-1b), interleukin-6 (IL-6), and tumor necrosis factor-alpha are the primary cytokines involved in the process (TNF-a) (Axe 2017).

References

Alizzi, A. 2005. *Reduction of post-surgical adhesions using a pig model*. Doctoral dissertation, James Cook University. 241 p.

Axe, J. 2021. *Fish collagen: The Anti-Aging Protein with the Best Bioavailability*, Ancient Remedies. Little Brown Spark; 1st edition.

Costa, B.A.; Junior, E.M.L.; Filho, M.O.D.; Fechine, F.V.; De Moraes, M.E.A.; Junior, F.R.S.; Soares, M.F.A.D. & Rocha, M.B.S. 2019. Use of tilapia skin as a xenograft for pediatric burn treatment: a case report. Journal of Burn Care and Research 40: 714-7.

Dizerega G.S. 1994. Contemporary adhesion prevention. Fertility and Sterility 61: 219-235.

Ekawati, E.R.; Darmanto, W. & Wahyuningsih, S.P.A. 2020. Detection of Staphylococcus aureus in wound infection on the skin surface. IOP Conf. Series: Earth and Environmental Science 456: 12-21.

Glat, P.M. & Longaker, M.T. 1997. Wound healing. In: Aston SJ, Beasley RW, Thorne CHM eds. Grabb and Smith's Plastic Surgery, 5th Ed. Philadelphia: Lippincott-Raven 3-12.

Hicks, J. 2018. Fish skin healed bears' paws burned in the California fires. 133 p.

Laikakos, T.; Thornakos, N. & Fine, P.M. 2001. Peritoneal adhesions: Etiology, pathophysiology, and clinical significance. Recent advances in prevention and management. Digestive Surgery 18: 260-273.

Lima-Junior, E.M.; Filho, M.O.D.; Costa, B.A.; Fechine, F.V.; De Moraes, M.E.A.; Silva-Junior, F.R.; Soares, M.F.A.D.; Rocha, M.B.S. & Leontsinis, C.M.P. 2019. Innovative treatment using tilapia skin as a xenograft for partial thickness burns after a gunpowder explosion. Journal of Surgical Case Reports 6: 1-4.

Lu, Y.; Liu, P.; Fu, P.; Chen, Y.; Nan, D. & Yang, X. 2017. Comparison of the DWI and Gd-EOB-DTPA-enhanced MRI on assessing the hepatic ischemia and reperfusion injury after partial hepatectomy. Biomedicine & Pharmacotherapy 86: 118-126.

Luna, L.G. 1968. *Manual of Histological Staining Methods of the Armed Forces Institute of Pathology*, 3rd ed., Mc Graw Hill Book Company. 59 p.

Montero, P. & Gómez-Guillén, M.C. 2000. Extracting conditions for megrim (*Lepidorhombus boscii*) skin collagen affect functional properties of the resultant gelatin. Journal of Food Science 65: 434-438.

Nakajima, J.; Sasaki, A.; Toru-obuchi, T.; Baba, S.; Nittaand, H. & Wakabayashi, C. 2009. Laparoscopic Subtotal Cholecystactomy for severe Cholecystitis. Surgery Today 39(10): 870-875

Tokajian, S. 2014. New epidemiology of Staphylococcus aureus infections in the Middle East. Clinical Microbiology and Infection 20(7): 624-628.

Verma, G.R.; Lyngdoh, T.S. & Kaman, L. 2006. Placement of 0.5% bupivacaine-soaked surgical in the gallbladder bed is effective for pain after laparoscopic cholecystectomy. Surgical Endoscopy 20: 1560-1564.