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Nile Tilapia Skin as Dermal Wound Healing Promoter in Cats

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ABSTRACT

Background: In cats major skin injuries need aggressive veterinary assistance for safe and speedy healing. These days Nile tilapia fish skin is gaining hype as wound healing promoter biological dressing in medical field. Keeping in view the successful findings on tilapia skin in human medical practice the present study was designed to evaluate the efficacy of Nile Tilapia fish skin as dermal wound healing promoterin cats.

Methods: The study was conducted on 10 clinical cases of cats received with different types of fresh dermal woundsat Department of Small Animal Clinical Sciences, University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan. Cats were divided into 2 groups *i.e.* A and B, 5 cats each group. Wounds in group A and B were treated with tilapia fish skin as a biological dressing and as open wounds, respectively. The parameter of study included wound size, physical characteristics of wounds and quality of wound healing and histo-pathological findings.

Result: On the basis of findings of this study it was concluded that Nile Tilapia fish skin derived biological dressing promotes the fast, safe and infection free healing of the dermal wounds in cats as compared to open wound healing.

Key words: Biological dressing, Cats, Tilapia fish skin, Wounds healing.

INTRODUCTION

Home escape, exposure to accidents, fights injuries or traumas are very commonly reported incidents particularly in male cats. Sometimes the skin injuries are massive and need aggressive medical assistance for safe and speedy healing of wounds and avoid the gangrene sepsis or any other systemic infection (Chalkowski *et al.,* 2019).

During recent years there is increasing trend to use tilapia fish for different medical purposes (Ibrahim *et al.,* 2020). Tilapia fish skin shows a number of unique characteristic and the most important of them is that it has thick slime coated skin that serves as a barrier for many bacterial infections. The simplicity in breeding tilapia is that it grows well in high density and this makes it suitable for farming (DeLong *et al*., 2009). Tilapia fish has great economic impact as it is in greater number in inland waters and can be cultivated easily (Yue *et al.,* 2016). The scientific name of Nile tilapia is *Oreochromis niloticus* and is commonly named “tilapia”. Tilapia (*Oreochromis niloticus*) is a fresh water most cultured fish in the world.

Skin of tilapia fish has unique property which separates it from other is that it contains gelatin that can be used in emulsions and in few recent years this property has made tilapia in medicine field a center of attraction for everyone. (Zhang *et al.,* 2020). Hydrolysates of gelatin obtained from tilapia fish skin proved to have dynamic antioxidant and anti-hypertensive effects (Choonpicharn *et al.,* 2015). Tilapia fish skin possesses antiviral and antibacterial properties. As it provides a barrier for bacteria for 24-48 hours and this antibacterial barrier activity of fish skin is tested by a 2-chamber model. Tilapia fish skin is preferred to use for wound healing because it prevents bacteria to invade the wound. Tilapia skin has greater ability to support the growth of cells on wound area in 3 dimensions (Magnusson *et al.,* 2017). Another use of Tilapia fish skin is as a biomaterial used for dressing in case of burn wounds because it is rich in collagen that promotes wound healing. High level of collagen in tilapia fish skin makes it more biocompatible to be used as graft. Collagen present in tilapia fish skin shows quick absorption on wound site as compared to bovine and procain collagen. It has gained hype in cosmetic industry too because COL-1 from tilapia fish skin is safest to use and has no adverse effects (Verde *et al*., 2021).

Widespread application of tilapia fish skin grafting has been reported in human medical practice following its approval by FDA. Tilapia fish skin has properties that enhance the process of wound healing. Due to its cost effectiveness these grafts are potential alternates of allograft and xenograft. Besides wound healing these grafts have shown encouraging results in treating diabetes foot ulcers, venous leg ulcers and other acute and chronic wounds. By using tilapia fish skin there are very less chances of

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transmission of any viral disease to other species and that is why preparation of fish skin as a graft requires less effort (Fiakos *et al*., 2020).

According to a report tilapia fish skin graft has resulted healing in 6.5 million patients in US by promoting wound healing to live a healthier life. Tilapia fish skin is also known for possessingvarious bioactive properties. It acts as bacterial barrier and promotes 3-dimensional (3-D) growth of cells on wound site. A random clinical trial study was conducted on 162 full, thickness wounds by using tilapia fish skin as it was noted that wound with tilapia fish skin healed within 14 days of application. After 14 days 13% of the fish skin treated group had healed as compared to the 6% procain SIS matrix treated group. After 21 days 72.5% of fish skin treated group had healed and 65% of the other group. Collectively the study showed that fish skin helps wound to heal faster. This study also showed that fish skin do not actives any autoimmune activity of body again the fish skin graft (Magnusson *et al.,* 2016).

In human reconstructive surgical practice tilapia skin is now considered a great alternative for skin grafting. The structure of tilapia fish skin is highly porous, with high level of hydrophilicity, property to absorb water and greater permeability. All these properties make tilapia skin a good alternative of synthetic skin grafts as it promotes wound healing by enhancing angiogenesis and collagen deposition on wound site (Chen *et al*., 2021).

Currently, fish collagen is being used widely in different biomedical applications linked with wound healing, tissue engineering, regeneration, drug delivery, cell culture and other therapeutic procedures (Rodríguez *et al*., 2020). Nile tilapia fish skin is also proved to be effective when used as xenograft for the treatment of burn wounds (Dias *et al*., 2020).

Tilapia fish skin accelerates the wound healing by decreasing the effects of pro-inflammatory (TNF-α, IL-6 and IL-8) and increase the number of receptors on target cells for β-defense 14, pattern recognition receptor (NOD2), anti-inflammatory (IL-10), vascular endothelial growth factor (VEGF) and fibroblast growth factor (β-FGF). Excellent healing is obtained due to high content of collagen in tilapia fish skin and by its activity to increase the growth of bacteria that promotes wound healing and decrease the bacteria that disturbs the process of wound healing (Mei *et al*., 2020b).

So far no study data is available on wound healing properties of Nile tilapia skin in veterinary practice. The objective of this study was to evaluate the efficacy of Nile tilapia skin as wound healing promoter biological dressing in cats.

MATERIALS AND METHODS

Study area

The present study was performed at Department of Small Animal Clinical Sciences, University of Veterinary and Animal Sciences, Lahore, Pakistan in 2022. The study was conducted on randomly selected 10 vaccinated clinical cases of cats having minor or major losses of skin. Study included cats of different breed, age and sex.

Inclusion criteria

Fully vaccinated, fresh wounds with major or minor skin losses are included.

Exclusion criteria

Old necrosed infectious wounds, more than 7 days old dog/cat bite wound, wounds because of stray cat bites were not included, poor health condition with compromised immune system, underlying severe disease or zoonotic infection, pregnancy are not included.

Treatment plan

Selected cats were divided randomly into 2 groups *i.e*. A and B each with 5 cats and subjected to the treatment plan as follows (Table 1).

Fresh fish was collected from Tilapia hatchery and transported to UVAS Department of Small Animal Clinical Sciences in thermostat container having ice in it. Skin of fish was separated from muscles with the help of sharp scalpel blade. Harvested skin then washed with sterile normal saline (0.9% NaCl) to remove debris and impurities. Collected skin was placed in a sterile container filled with normal saline (Ibrahim *et al.,* 2020a). Collected fish skin strips were dipped in 10% povidone iodine solution for 10-15 minutes before application.

- Wound was washed with normal saline followed by disinfection with 10% povidone iodine.
- Skin edges of wound were trimmed in cosmetic appearance for regular pattern.
- Gas anesthesia was used by using isoflurane.
- Wound size was measured using vernier caliper.

Fish skin was trimmed and shaped according to the shape and size of recipient site. The dressing was applied on wound as per following technique.

Group A

Dressing was secured on wound site by applying simple interrupted sutures with non-absorbable suture material (silk 2-0) (Fig 1). The dressing was covered with gauze impregnated with paraffin to avoid adherence of tilapia skin with bandage. Gauze and wound were secured with simple adhesive tape.

Group B

Wound area was washed with 0.9% Normal Saline (NaCl) solution and treated as an open wound.

Parameters of study

Parameters of study includedwound size, physical characteristics of wound and Quality of healing (presence

Table 1: Treatment plan.

Group	Number of animals	Techniques	Follow up schedule
A	5	Sutured application of biological dressing	Dressing change: 2-14 days on alternate days or as required
B	5	Routine open wound management	Dressing change: 2-14 days on alternate days or as required

or absence of scar formation, speed of healing, sensitivity response to tilapia skin dressing, histo-pathological evidences) showing wound healing process and skin regeneration from day 0 to 14 and sterility of wound).

Histo-pathological findings

Biopsy sample was collected using 3mm punch biopsy on days 0, 3, 7 and 14 to evaluate the histo-pathological findings at various intervals during wound healing (Parente *et al*., 2012) Collected samples were preserved in formalin while tissue processing was performed in the Department of Pathology, University of Veterinary and Animal Sciences, Lahore, Pakistan.

Statistical analysis

The data thus obtained was statistically analyzed by using independent T test on SPSS version 20.

RESULTS AND DISCUSSION

In present study Nile tilapia fish skin was evaluated as biological dressing and skin wound healing promoter in cat. A total 10 clinical cases of cats received with massive skin damages were selected for this study and divided into two groups *i.e*. A and B.

Following were the findings of present study in group A and B on the basis of pre-selected parameters.

Wound size

Wound size was measured in each cat of both groups on days 0, 3, 7 and 14. In group A, on days 0, 3, 7 and 14 mean wound size was 73.92±30.20, 41.24±15.26, 25.36±13.61 and 12.2±9.23, respectively. Statistical analysis revealed significant difference (P<0.05) among means of wound size changes during healing from days 0-14. In group B, on days 0, 3, 7 and 14 mean wound size was 75.40±29.91, 69.40±27.55, 61.52±26.61 and 54.44±23.04, respectively. Statistical analysis showed a significant difference (P<0.05) among means of wound healing area in group B from days 0-14. According to Guo and DiPietro (2010) skin wound healing is a naturally complicated biological mechanism that involves highly programed stages *i.e.* inflammation, proliferation and remodeling.

Physical characteristics of wound

Both groups A and B included cats with different characteristics of skin wound in terms of shape, size and healing status (Table 2). In all cases wounds were not more than 24 hours old. In group A out of total 5 cats 3 cats were wounded due to cat fight cases where as other 2 cats were accidental injuries. In group B, out of total 5 cases 3 were the cat fight cases where as two cases involved accidental injuries (Table 3).

Quality of wound healing

Quality of wound healing was evaluated on the basis of following factors.

a. Scar formation

In both groups A and B, dressing was changed after every 48 hours to avoid contamination of site. In group A, out of total 5 cats no cat showed scar formation. In group B, out of total 5 cats 3 cats showed scar formation and in rest of the 2 cats no scar formation took place mainly because Tilapia fish skin accelerates wound healing by decreasing the effects of pro-inflammatory mediators (TNF-α, InterLeukin-6 and InterLeukin-8) and it increases the number of receptors on target cells for β-defense 14, pattern recognition receptor (NOD2), anti-inflammatory (IL-10), vascular endothelial growth factor and fibroblast growth factor (β-FGF) as observed by Mei *et al.* (2020a).

b. Sensitivity raction

In both groups A and B, during the entire course of study, no cat showed any sign of sensitivity reaction to tilapia skin dressing. According to Magnusson *et al.* (2016) tilapia fish skin does not active any autoimmune activity of body again the fish skin graft that is why no sensitivity reaction is noted. These findings are also in line with the findings of Ibrahim *et al.* (2020). They reported that Tilapia fish skin is a rich source of gelatin as well as collagen. It possesses biocompatible type 1 collagen which has potential for using as clinical regenerative procedure to treat massive dermatological losses smoothly.

c. Speed of healing

According to statistical analysis in group A, the data on the relation between mean wound size with respect to days of complete healing showed a significant difference (P<0.05)

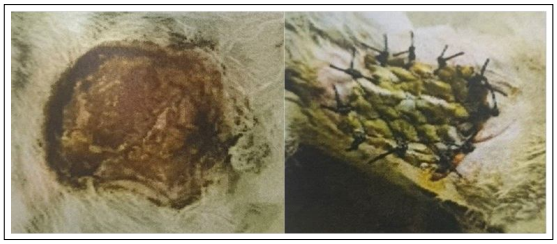


Fig 1: Showing wound before and after Nile tilapia skin application.

Table 2: Physical characteristics of wound (group A).

No. of cats	Mean wound size (mm)	Shape of wound	Infected/Non-infected
Cat I	72.9	Irregular	Non infected
Cat II	87	Irregular	Non infected
Cat III	122.5	Circular	Non infected
Cat IV	67.5	Irregular	Non infected
Cat V	39.2	Oval	Non infected

Table 3: Physical characteristics of wound (group B).

No. of cats	Mean wound size (mm)	Shape of wound	Infected/Non-infected
Cat I	50	Irregular	Non infected
Cat II	87	Oval	Non infected
Cat III	122.5	Circular	Non infected
Cat IV	55	Irregular	Non infected
Cat V	62.5	Elliptical	Non infected

that indicates speedy wound healing process. Graphical presentation with respect to statistical data also demonstrated that during in most of the wounds maximum healing was achieved by day 14. In group B, according to statistical analysis, data on relation between mean wound size of this group with respect to days of complete healing showed a significant difference ($P<0.05$) that indicate wound healing process. Graphical presentation with respect to statistical data showed that during trial in most of wounds delayed healing was achieved as compared to group A. Same observations are documented by Mei *et al.* (2020a). They documented that Tilapia fish skin accelerates wound healing by decreasing the effects of pro-inflammatory mediators (TNF-α, InterLeukin-6 and Inter Leukin-8) and it increases the number of receptors on target cells for β-defense 14, pattern recognition receptor (NOD2), anti-inflammatory (IL-10), vascular endothelial growth factor and fibroblast growth factor (β-FGF). It increases the bacterial growth responsible to promote wound healing and decreases that bacterial growth which disturbs wound healing.

d. Histo-pathological findings

Histo-pathological evaluation in group A, revealed a fast healing process followed by the complete wound healing

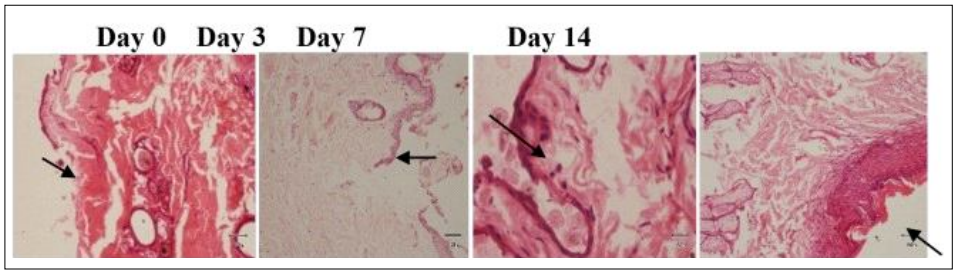


Fig 2: Histo-pathological findings in group A: An absence of epidermis observed on day 0. On day 3, no changes observed in the dermal area of the section. No epidermis seen except in the middle of the section. In the peripheral area the epidermal layer was thin and regenerating. Small amount of dead tissue mass was observed over the affected area. On day 7, active cells of stratum basale were seen dividing. Layers of stratum spinosum had formed. Epithelialization was in progress. On day 14, Epithelialization was at its peak. Multiple layers of squamous epithelial cells had deposited. Mature keratin was visible in the stratum corneum. Kerato-hyalin granules in the stratum granulosum were large and numerous.

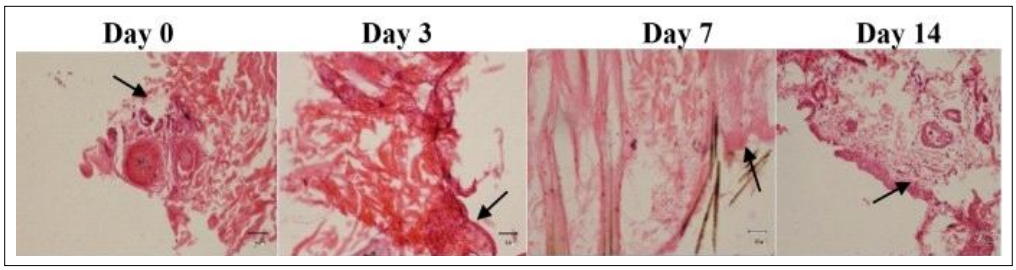


Fig 3: Histo-pathological findings in group B: A complete absence of epidermis on day 0. On day 3, dead tissue mass observed along with the dead and dying neutrophils present in the section. On day 7, epidermal regeneration was noted. On day 14, the process of epithelialization was still incomplete and minimal keratin was seen on the upper layer.

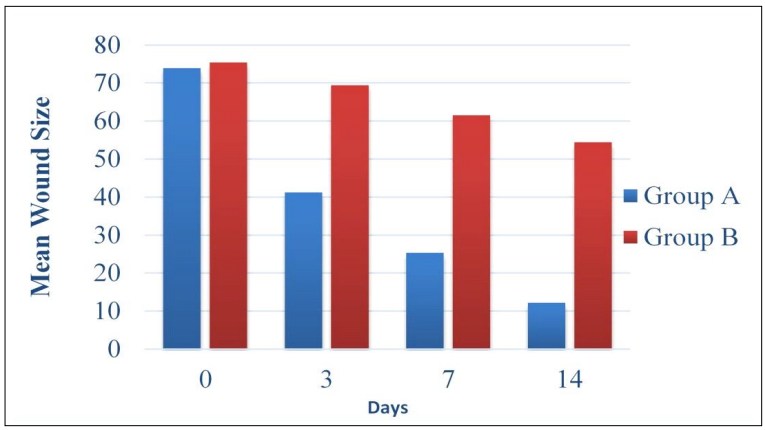


Fig 4: Wound size comparison between groups A and B.

by day 14 in all cats of this group (Fig 2). Based on the histo-pathological evaluation in group B, the healing process was slow and wound healing was incomplete in all cats on day 14 (Fig 3).

Wound size comparison between Group A and B

Statistical analysis based comparison of wound size on days 0-14 in group A and B revealed a significant difference ($P<0.05$) both groups with respect to wound size at different intervals (Fig 4).

CONCLUSION

According to findings of this study it was concluded that sutured biological dressing of Nile tilapia fish skin ishighly supportive for fast, safe and aseptic healing of wounds as compared to the open wound healing for major or minor dermal losses in cats. Tilapia fish skin was also proved to be an effective and economic source to cover large area dermal losses in cats as compared to other sources available in market.

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Conflict of interest

All authors declare that they have no conflict of interest.

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