

## **A study on the effect of chitosan gel on the treatment of ornamental fish (*Carassius auratus*) externally infected with *Aeromonas hydrophila* bacteria**

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### **Abstract**

Chitosan is a polysaccharide extracted from chitin, which is taken from shrimp, crab and insects that acts as a gel after diluting it with dilute acetic acid 0.1, and the concentration was chosen 2% because it is the most inhibitory after trying it on the dishes, and the ulcers on the goldfish skin were wiped with it for a week and two weeks and gave clear healing results Significantly and tissueably, this is due to the efficiency of jelly in building muscle tissue, skin and skin, and researchers must pay attention to the use of extracted natural materials because of the lack of side effects and their low cost and ease of obtaining them.

**Keywords:** *Aeromonas Hydrophila*, *Carassius auratus*, Chitosan Gel

### **Introduction:**

Goldfish (*Carassius auratus*) is sensitive to bacterial infections, which may result in the external ulcer, erosion of the fin, and systemic disease [1]. These pathogens include the *A. hydrophila* that are hard to manage and subsequently lead to a high mortality rate in ornamental aquaculture [2]. Conventional antibiotics pose the threat of antimicrobial resistance, tissue residues, and the environment [2]. Thus, the aquaculture sector is actively finding natural, biodegradable, and safe alternatives [3]. The deacetylated form of chitin is chitosan, one of the most promising biomaterials because of its antimicrobial and biocompatible properties [1]. Chitosan, when shaped into a hydrogel, is a semi-permeable network that can attach itself to the tissues and provide an ideal environment in which the wound can heal [4]. Besides the physical security measure, chitosan hydrogel is a substance that allows the controlled release of active

molecules, enhancing the outcome of the therapeutic procedure [5]. Such a dual usage renders chitosan hydrogel especially effective at treating surface bacterial infections in fish [6]. Recent findings affirmed a positive antibacterial property of the chitosan and its nanoparticle against most common fish pathogens such as *A. hydrophila* and *Vibrio* species [7]. Chitosan also disrupts bacterial adhesion and biofilm formation and increases its therapeutic potential [6]. In addition to antimicrobial effect, chitosan increases immunity, antioxidant capacity, and regeneration of tissues in fish [7]. Wound dressing made of chitosan is faster in healing wounds because it promotes collagen formation, angiogenesis, and epithelial regeneration [8]. In addition, high technology or advanced chitosan the gel formulations like the magnetic chitosan hydrogels or composite gels demonstrate good mechanical stability and superior

antimicrobial effects [5]. Notably, the majority of the formulations follow a high level of biocompatibility and low toxicity in aquatic organisms [2]. Considering such characteristics, chitosan hydrogels can serve as a useful and safe alternative to antibiotics in the control of bacterial diseases among goldfish [6]. The current study aims to determine the therapeutic effectiveness of chitosan gel in the management of bacterial infections in goldfish through clinical healing, reduction of bacterial count, and tissue restoration.

## **Materials and Methods:**

### **Samples collection:**

There were also skin lesions and ulceration in several of the ornamental goldfish that were brought out of the tanks in a pet shop in Najaf province, where the owner complained that the lesions had increased and the response to the popular treatments had been poor. Since there were no applied studies in the area that investigated the ability of chitosan gel to treat such skin conditions, a comparative experiment was necessary to determine the role of chitosan gel in the treatment. In order to do this, the research was set as a controlled experiment where 18 goldfishes were separated into two equal categories; control group (9 fishes)

and a treatment group (9 fishes) to which chitosan gel was applied. The fish became confined in different plastic containers with a dimension of 30 × 20 × 25 cm with volume of 10 liters of water and fitted with an air pump. An acclimation period was given a period of one week upon which the therapeutic response of the two groups was observed under the standard environmental and nutritional conditions. Chitosan powder, diluted acidic solution (e.g., 1% v/v acetic acid), and distilled or sterilized water were used. Laboratory equipment included an analytical balance, magnetic stirrer, capped bottles or flasks, and a pH meter [9,10].

### **Preparation of Chitosan Gel**

The distilled water was used to prepare a 1% v/v solution of acetic acid. Chitosan powder (2 g to 100 mL solution to acquire a 2% w/v gel) was progressively put in under a steady mix until completely discharged. A homogeneous, bubble-free gel was obtained

either through mild heating (40 -50 C) or long stirring. The gel was either sterilized by filtration, heat or UV radiation and kept at 4 o C until it was used. The steps of the preparation are in accordance with the typical laboratory procedures [11].

## Experimental Procedure

Fish that had visible lesions of bacteria on the skin were used as ornamentation. The size, inflammation, and color of skin, and fish activity of pre-treatment wounds were noted.

Chitosan gel (2%) was either applied directly to the wounds or immersed for a

short time. Fish was observed on a daily basis or after every two days, and the gel was changed as needed. Healing of the wound, size change, inflammation, skin color, behavior, and indication of infection or death were noted. To study the regeneration of tissues and inflammation mitigation, optional skin biopsies were taken [12].

## Results and Discussion:

The histopathology of the skin tissues showed that there were definite differences between the control and the treated/infected specimen. In the normal control group, the skin section was to be found normal with no abnormal epidermal and muscular layers as illustrated in Figure (1). There were no signs of inflammation, necrosis or structural changes, and this is a healthy baseline architecture. Conversely, the mean of the samples of the infected group showed significant pathological alterations. The inflammatory cells intravenous infiltration in the dermal and subdermal layers is observed in Figure (2) and is accompanied by a significant number of adipocytes. The degeneration of the epidermal and muscular tissues was also related to these changes indicating the extent to which the bacteria had damaged it. The degeneration resulted in disturbance of the integrity of the tissues, expansion of intercellular spaces, and partial destruction of normal cell architecture. Such results prove that the infection caused major changes in tissues, and the control group had a normal histological structure. After one week of treatment with chitosan gel, the

results improved by approximately 50%, as the epidermal and dermal cells appeared normal, and the muscle cells also showed normal morphology (Figure 3). After two weeks of treatment, the outcomes were even better, with the epidermal and dermal cells appearing more restored compared to the one-week treatment, although edema was still present between the two layers (Figure 4). Figures 5, 6, and 7 illustrate the stages of ulcer healing following treatment with chitosan gel after one, two, and three weeks after treatment, and is accompanied by a significant number of adipocytes. The degeneration of the epidermal and muscular tissues was also related to these changes indicating the extent to which the bacteria had damaged it. The degeneration resulted in disturbance of the integrity of the tissues, expansion of intercellular spaces, and partial destruction of normal cell architecture. The current study determined the therapeutic effectiveness of chitosan gel in the treatment of bacterial skin lesions in ornamental goldfish. The results revealed that there were definite changes in the tissue structure, inflammatory reaction, and external wound

healing in the fish fed 2 percent chitosan gel as opposed to the control group. Histologic sections of treated fish demonstrated progressive recovery of epidermal and muscular layers, less infiltration of inflammatory cells and tissue structure reorganization throughout treatment. These findings are consistent with the past reports indicating antimicrobial, anti-inflammatory and wound-healing activity of chitosan in aquatic animals. Chitosan gel produced significant effects since week one of the treatment, whereby the clinically and microscopically observed reduction of lesion size and inflammation was observed. In the second and third weeks, the treated fish were almost fully cured, and epidermal tissues proliferated, and the necrotic changes vanished. These findings are in line with the results of [8], who have shown faster epithelial healing of fish wounds under chitosan-based dressings. On the same note, the reduction in inflammatory cell infiltrations is also associated with the immunomodulatory effect of chitosan as reported by [7], reporting improved immune response and antioxidant activity on treated fish. The unhealthy control group had evidence of chronic inflammation, fatty tissue build-up, epidermal tissue and muscular tissue degeneration. These pathological data are typical of bacterial infections in goldfish of *Aeromonas* spp which have been known to produce toxins that cause tissue necrosis. The delayed and partial recovery of the control group demonstrates the difficulties related to the presence of bacterial pathogens in the ornamental aquaculture, especially in the ecology with the recurrent stress or low water quality. The success of chitosan gel could be explained by a number of mechanisms. To begin with, its positive charge enables it to react with negatively charged bacterial cell walls leading to

membrane disruption and killing of bacteria. This mechanism of action, which [6] report, justifies the acute decrease in the severity of the infection that was recorded in this study. Second, the semi-permeable structure of the gel preserves the wound, keeps it moist, and helps in tissue repair an effect which is corroborated by [4]. Also, chitosan induces collagen deposition and angiogenesis, which are vital in the quick wound healing. The fact that chitosan is safe and biocompatible is also its strong point. The treatment group did not report any behavioral deviations and had no deaths during the experiment, supporting the above results by [13] who tested the toxicity of chitosan in aquatic life and proved that this compound is not very toxic. This renders chitosan a viable substitute to the antibiotics that tend to cause antimicrobial resistance, tissue residual and contamination of the environment. In spite of the positive outcomes, there are certain limitations. The sample size (18 fish) was also not very big and this can be a limitation to the statistical power of the results. Also, the concentration of chitosan gel (2) was considered only; further researches should be conducted on various concentrations and methods of use. The evaluation of the antimicrobial efficacy could be further enhanced with the help of microbiological tests, e.g. the number of bacteria before and after treatment. study conclusion proves that the chitosan gel is a good, safe and viable therapeutic tool in treating bacterial skin lesions in goldfish. It has a high potential as an alternative to antibiotics as a wound-healing agent in ornamental fish because it has antimicrobial properties and is biocompatible with the fish. Future studies are needed to build up on the optimization of dosage, long-term effects and applicability of the technology in other species of fish.

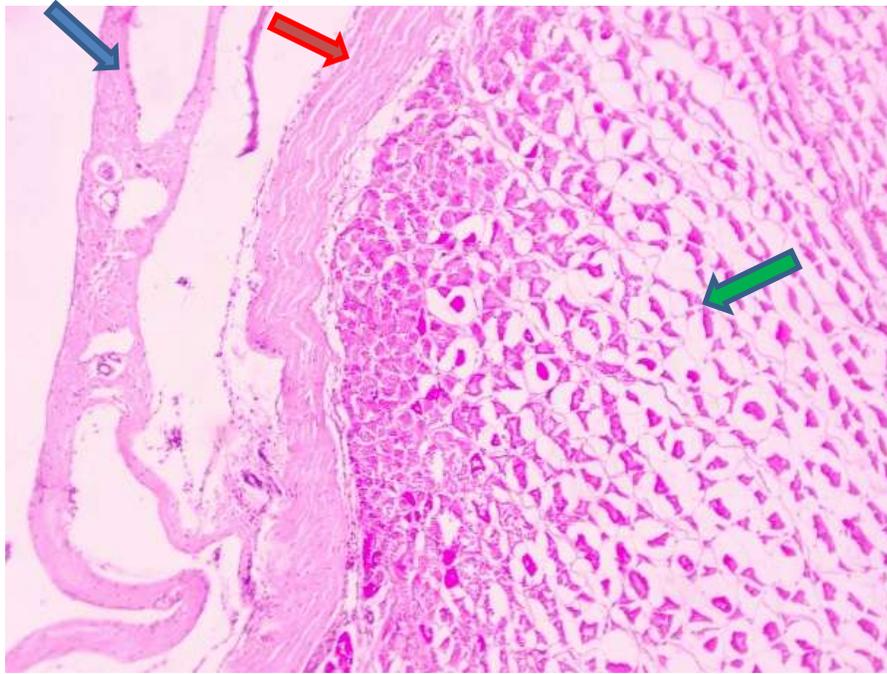


Figure 1: A section of skin for the uninfected control with 100x force

The epidermis is indicated by the blue arrow, while the red arrow marks the dermis, and the muscles are indicated by the green arrow

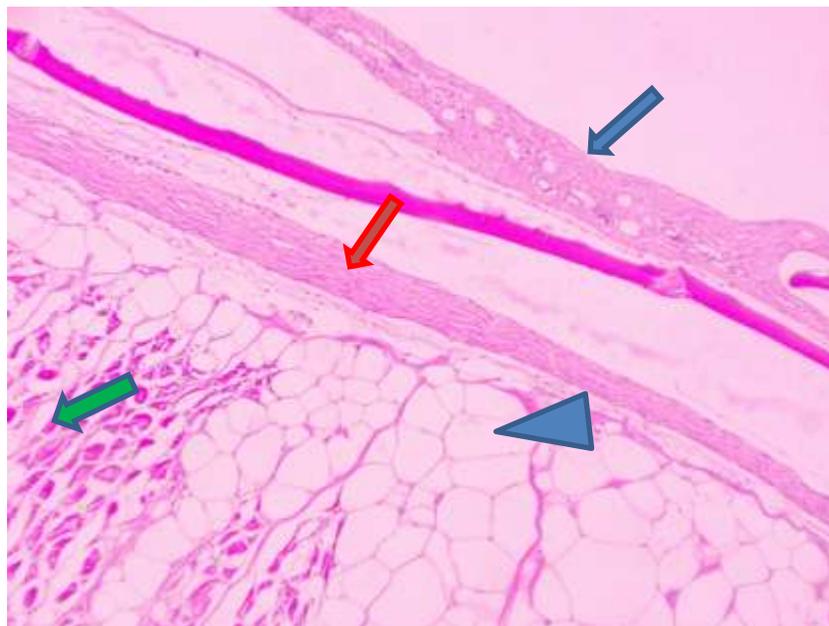


Figure 2: The presence of inflammatory cells and fat cells due to degeneration in the epidermal and muscular layers with 100x force.

The epidermis is indicated by the blue arrow, while the red arrow marks the dermis, and the muscles are indicated by the green arrow, the blue arrow head refers to adipose tissue.

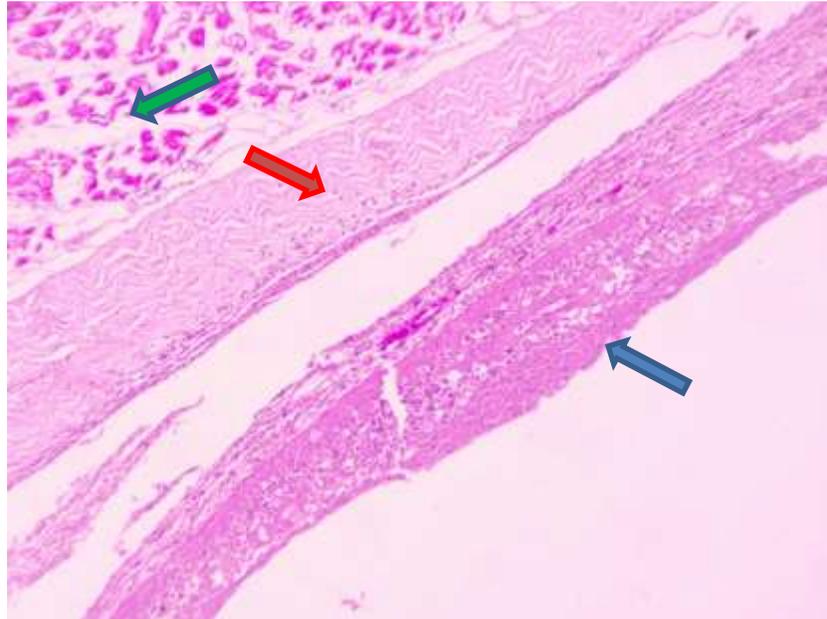


Figure 3: A skin section treated with 2% chitosan gel one week after treatment at 10x strength.

The epidermis is indicated by the blue arrow, while the red arrow marks the dermis, and the muscles are indicated by the green arrow

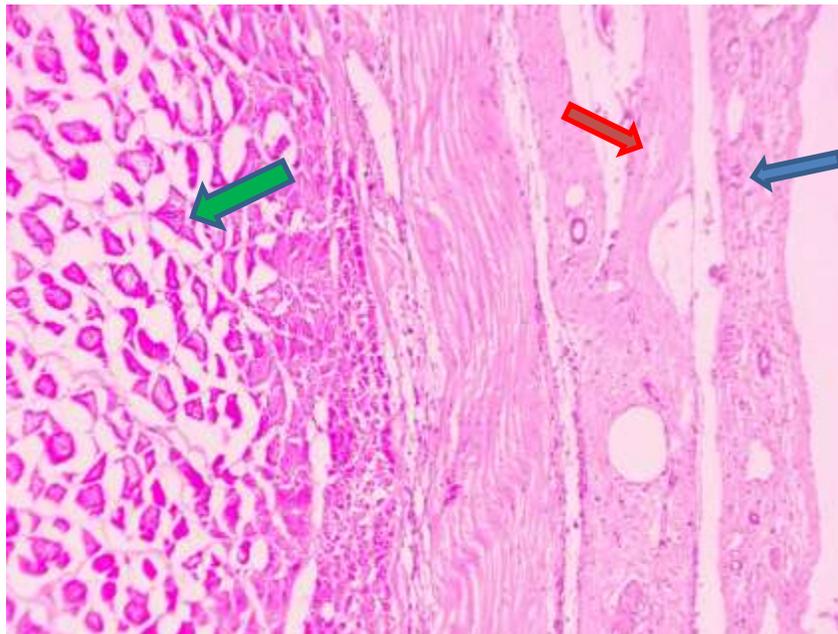


Figure 4: A skin section treated with chitosan gel two weeks after treatment with 10x strength.

The epidermis is indicated by the blue arrow, while the red arrow marks the dermis, and the muscles are indicated by the green arrow



Figure 5: Goldfish after one week of treatment with chitosan gel



Figure 6: Goldfish after two weeks of treatment with chitosan gel



Figure 7: A goldfish after three weeks of treatment with chitosan gel

### **Conclusion**

The use of chitosan gel is safe and effective in the treatment of ulcers resulting from bacterial diseases, Ornamental fish are more vulnerable to bacterial infections than the fish consumed.

**Acknowledgment:** I would like to thank the Faculty of Veterinary Medicine and the branch of poultry diseases and diseases and everyone who helped me in completing this scientific research, especially colleague Dr. Essam Aziz.

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