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# ACUTE TOXICITY AND PHYSICOCHEMICAL EVALUATION OF MILKFISH (*Chanos chanos*) COLLAGEN SKIN LOTION

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**Abstract**

The continuous growth of milkfish (*Chanos chanos*) aquaculture in Indonesia has led to significant generation of scale waste. These scales are highly collagenous and may serve as active ingredients in skin care products. This study aimed to identify the optimal concentration of milkfish scale collagen lotion by evaluating its parameters, physicochemical properties, and acute dermal toxicity. Collagen extracted from milkfish scales was incorporated into lotion formulations at varying concentrations (0%, 1%, 3%, and 5% w/v) to evaluate its physicochemical effects. The physicochemical properties evaluated were spreadability, viscosity, and pH of the gel. Acute dermal toxicity was assessed in female mice (*Mus musculus*) by histopathological analysis of the skin. The resulting data were then analyzed using the One-Way ANOVA test and Fisher LSD test. The results showed that all the lotion formulations met the required physicochemical quality standards. Increasing collagen concentration decreased spreadability but increased viscosity, with all pH values remaining within the safe ranges. Acute dermal toxicity testing indicated no evidence of toxicity or mortality in the test subjects. Histopathological analysis indicated that higher collagen concentrations reduced epithelial thickness and the number of inflammatory cells while promoting angiogenesis in the skin tissue. In conclusion, milkfish scale collagen lotion is safe for topical use and has promising potential as a skincare product with favorable physicochemical properties.

Keywords: by product, dermal toxicity, histopathology, personal care, topical formulation

## Evaluasi Toksisitas Akut dan Karakteristik Fisikokimia Losion Kulit Berbasis Kolagen Sisik Ikan Bandeng (*Chanos chanos*)

**Abstract**

Industri pengolahan ikan bandeng (*Chanos chanos*) di Indonesia menghasilkan limbah berupa sisik dalam jumlah besar. Sisik kaya akan kolagen yang berpotensi dimanfaatkan sebagai bahan aktif dalam produk perawatan kulit. Penelitian ini bertujuan menentukan konsentrasi optimal kolagen sisik bandeng dalam formulasi losion berdasarkan parameter toksisitas akut dermal dan sifat fisikokimia. Kolagen diekstraksi dan diformulasikan ke dalam losion dengan konsentrasi 0%, 1%, 3%, dan 5%. Sifat fisikokimia yang diuji meliputi daya sebar, viskositas, dan pH. Sementara itu, toksisitas akut dermal dievaluasi pada mencit betina (*Mus musculus*) melalui analisis histopatologi kulit. Hasil penelitian menunjukkan bahwa seluruh formulasi losion memenuhi standar mutu fisikokimia yang dipersyaratkan. Peningkatan konsentrasi kolagen menurunkan daya sebar, tetapi meningkatkan viskositas, dengan nilai pH tetap berada dalam kisaran aman. Uji toksisitas dermal tidak menunjukkan tanda-tanda toksisitas maupun kematian pada hewan uji. Analisis histopatologi menunjukkan bahwa konsentrasi kolagen lebih tinggi menurunkan ketebalan epitel dan jumlah sel inflamasi, sekaligus meningkatkan angiogenesis pada jaringan kulit. Dengan demikian, losion kolagen sisik bandeng dinyatakan aman untuk penggunaan topikal serta berpotensi dikembangkan sebagai produk perawatan kulit dengan mutu fisikokimia yang baik.

Kata kunci: formulasi topikal, histopatologi, hasil samping, perawatan tubuh, toksisitas dermal

## INTRODUCTION

Aquaculture has become an essential sector for meeting the increasing demand for fishery products, particularly as marine capture fisheries show signs of stagnation in production (Ganesh *et al.*, 2020; Naylor *et al.*, 2021). This shift has drawn greater attention to species that are widely cultivated and highly preferred by consumers. One such species is milkfish (*Chanos chanos*), a marine fish that plays an important role in aquaculture development in Indonesia. Milkfish (*Chanos chanos*) is a marine species that is frequently consumed by the local community. Milkfish are widely distributed in the Pacific and Indian Ocean regions, including Indonesia. Milkfish have a silver, torpedo-shaped body with a length of up to 1.5 m and a weight of up to 20 kg. In Indonesia, milkfish have a high selling value owing to their fast growth cycle, good salinity tolerance, and high market demand (Djumanto *et al.*, 2017).

The national milkfish production value reached 779,705.90 tons, with East Java contributing the highest production volume of 167,592.66 tons (KKP, 2024). For instance, the increase in milkfish production volume in East Java is supported by the growing market demand for processed deboned milkfish products. However, the increased production of deboned milkfish has led to the accumulation of milkfish scale by-products. The production of deboned milkfish generates by-products, specifically fish scales, which account for approximately 5% of the total raw materials used. An estimate indicates that East Java produces 8,379.63 tons of milkfish scale byproducts (Suseno *et al.*, 2022). Consequently, innovation is required to utilize this byproduct.

A recent study reported the presence of collagen peptides in milkfish scale by-products (Chuu *et al.*, 2023) and other fish (Widiyanto *et al.*, 2022; Pertiwi *et al.*, 2025). Collagen peptides isolated from milkfish scales had a good productivity value of 8.3% when dissolved in pepsin. Owing to their antioxidant and anti-inflammatory properties, milkfish collagen peptides can be further utilized in skin health products (Chen *et al.*, 2018). In addition, milkfish scales are known to contain type 1 collagen that can be utilized as an anti-aging agent (Hartati & Kurniasari, 2010; Wahyu & Widjanarko, 2018; Wahyu, 2018; Kusumaningtyas, 2021; Wahid *et al.*, 2022).

Research indicates that incorporating collagen from fish skin, specifically tilapia skin, into lotion or cream formulations markedly enhances skin moisture and improves spreadability and pH (Dewangga *et al.*, 2021). Notably, higher collagen concentrations led to a more acidic pH, which remained within the safe range for skin application. Additionally, topical products containing hydrolyzed fish skin collagen have demonstrated notable improvements in skin hydration, wrinkle reduction, and overall user satisfaction, indicating the strong potential of fish skin-based lotions as effective and sustainable cosmetic products (Pramesti *et al.*, 2025).

Collagen derived from milkfish scales and skin has the potential to hydrate, enhance elasticity, and improve skin texture with minimal side effects (Koizumi *et al.*, 2018; Mberato *et al.*, 2020; Lee *et al.*, 2024; Ragaza & Go, 2024). However, research on the use of milkfish collagen in topical preparations, particularly lotions, remains limited. Current research predominantly emphasizes the isolation, characterization, and processing of cream or nanoemulgel products (Nasyanka *et al.*, 2024; Pramesti *et al.*, 2025). Despite these advances, the actual application of milkfish collagen in lotions and comprehensive safety assessments for topical applications remain areas where research is still lacking. The utilization of milkfish scale collagen as an active ingredient for lotions not only has the potential to increase the added value of fishery waste but can also be used as an innovation for the local resource-based domestic industry that is both environmentally friendly and safe. This study aimed to determine the optimal concentration of milkfish scale collagen for producing skin lotions based on acute dermal toxicity and physicochemical characteristics. Hence, it serves as a foundation for developing effective, safe, and sustainable nature-based skin care products.

## MATERIALS AND METHODS

### Raw Material Preparation & Pre-treatment

This study utilized milkfish scales (*C. chanos*) obtained from the Teaching Factory (Tefa) at Politeknik Perikanan dan Kelautan Sidoarjo. Preparation and pre-treatment refer to Wahyu (2018). The procedure was carried out using 0.1 M NaOH for 8 h at 4°C, with a volume and weight ratio of 1:8. The immersion process involved replacing the solution every four hours. The milkfish scales obtained were then collected in a tub of water, filtered, and washed at a temperature of ±5°C. The scales were then stored in cold storage below 18°C before use. A pre-treatment process was conducted to remove the non-collagen proteins present in milkfish scales.

### Collagen Extraction

The milkfish scales obtained from pretreatment were washed with cold water to achieve a neutral pH. The freeze-drying technique for obtaining collagen from the extraction process was based on Wahyu (2018). Milkfish scales were demineralized using EDTA-2 Na 0.5 M at a ratio of 1:8 (b/v) at pH 7.5 for 24 h. Demineralization was performed by changing the bath every 12 h. The demineralized fish scales were then immersed in 0.2-0.8 M acetic acid at a ratio of 1:6 (b/v) and filtered. The collagen was then immersed in 0.9 mol/L NaCl for 24 h. The precipitate was obtained by centrifugation at 2,000 rpm for 20 min. The resulting solid was dissolved in 0.5 M acetic acid and dialyzed with 0.1 M acetic acid, followed by distilled water. The entire extraction process was performed at 4°C.

### Lotion Preparation

Lotion preparation referred to Pramesti *et al* (2025). The lotion preparation procedure consisted of two primary stages: preparation of the lotion base and incorporation of fish scale collagen into the lotion. The lotion base was prepared in two phases: oil and water. The oil phase comprised stearic acid, liquid paraffin, and cetyl alcohol. Stearic acid functions as an emulsifier, cetyl alcohol as a thickener, and liquid paraffin as an emollient to provide a moisturizing effect. The three components were heated and stirred at 70-75°C for approximately 10 min using a hotplate. The aqueous phase comprised glycerin, triethanolamine (TEA), distilled water, and methyl paraben. Glycerin served as a humectant, TEA as a pH stabilizer and emulsification agent, distilled water as a solvent, and methyl paraben as a preservative. The components were heated to 70-75°C and stirred for approximately 20 min.

The two phases were combined and heated at a constant temperature of 70°C. The mixture was stirred continuously to decrease the temperature to 40°C for 30 min, followed by 35°C for approximately 10 min until a thick and white lotion was obtained. The final preparation was tested for pH using a pH meter to confirm compliance with the safe skin pH range of 4.5–8.0 (Husni *et al.*, 2021). The measurement results showed a pH of 7,15, which was within the safe range and suitable for proceeding to the formulation stage with the addition of collagen. The lotion was formulated in four variants according to the concentration of fish scale collagen: control, 1%, 3%, and 5% (w/w). The four formulations were then tested using the *in vivo* method to evaluate the physical quality and conformity of the lotion characteristics with pharmaceutical standards. The formulation of the collagen lotion is presented in Table 1.

### Spreadability Analysis

The spreadability test assessed the capacity of the preparation to spread on the skin surface, which is crucial for its effectiveness and user convenience. The spreadability test was performed according to Ulaen *et al.* (2012). A total of 0.2 g of the preparation was placed on the surface of a glass slide and was subsequently covered with a second glass slide. In addition, a 50 g weight was applied for five minutes to produce uniform pressure. After removing the load, the spread diameter of

Table 1 Formulation of milkfish scale collagen lotion

| Ingredient | Concentration of milkfish scale collagen (%) | | | |
|---|---|---|---|---|
| | 0 | 1 | 3 | 5 |
| Stearic acid | 3.99 | 3.99 | 3.99 | 3.99 |
| Liquid paraffin | 3.99 | 3.99 | 3.99 | 3.99 |
| Cetyl alcohol | 3.99 | 3.99 | 3.99 | 3.99 |
| Glycerin | 2.00 | 2.00 | 2.00 | 2.00 |
| Triethanolamine | 2.00 | 2.00 | 2.00 | 2.00 |
| Distilled water | 83.83 | 83.83 | 83.83 | 83.83 |
| Methyl paraben | 0.20 | 0.20 | 0.20 | 0.20 |

the preparation was measured using a caliper. This evaluation was conducted following accelerated storage to assess the stability of the spreadability characteristics during storage.

## Viscosity Analysis

The viscosity of the formulation was evaluated using a Brookfield viscometer, as viscosity is a critical parameter influencing product stability and ease of application. Viscosity analysis in this study was performed according to Kamaruzaman & Yusop (2021). A total of 120 g of the preparation was placed into the test container, and spindle number 3 was installed and operated at 12 rpm. The viscosity was read from the digital display of the viscometer. According to the Indonesian National Standard (BSN 16-4399-1996), the recommended viscosity range for skin moisturizers is 2,000–50,000 cps. This test was conducted before and after accelerated storage to assess the physical stability of the formulation.

## pH Analysis

The pH was evaluated to ensure that the pH of the preparation matched the physiological pH range of the skin. It is essential to avoid irritation or disruption of the skin integrity. The pH meter electrode was immersed in the lotion to obtain direct pH measurements. The safe pH range for topical preparations is between 4.5 and 8.0 (BSN 1996). pH testing was conducted at two time points: before and after accelerated storage.

## Acute Dermal Toxicity Test

The research procedure was approved by the Animal Care and Use Committee of Brawijaya University (No: 211-KEP-UB-2024). This study used female mice (*Mus musculus*), 3-4 months old, weighing 200-250 g, nulliparous, and not pregnant. Animals were acclimatized for seven days under controlled environmental conditions (temperature 25°C, humidity 60-70%, light/dark cycle 12 h). Standard pelleted feed (20 g/head/day) and drinking water were provided, ad libitum. Identification and randomization were performed before treatment. This procedure followed the animal welfare guidelines of the National Research Council (2011). The hair on the dorsal area was shaved (6×8 cm) the day before the treatment. Shearing was performed after intraperitoneal anesthesia (Ket-A-Xyl® 0.1 mL/head) to prevent skin injury. Only animals with intact skin were used in this study (OECD, 2017).

The test was conducted according to the OECD 402 guidelines (OECD, 2017). The preliminary test used one mouse at a dose of 1, 000 mg/kgBB. Since no mortality or toxicity symptoms were observed, the main test was conducted at 2,000 mg/kgBB in the treatment and control groups (n=3). The lotion was applied topically once, whereas the control group received a placebo with the same volume. Observations were conducted hourly for the first 6 h, then daily until day 12. The parameters included toxicity symptoms (tremors, convulsions, diarrhea, and lethargy), skin changes, behavior, and mortality. Body weight was measured daily before treatment throughout the observation period. Animals exhibiting terminal conditions were euthanized.

Skin samples were analyzed histologically to assess the epithelial thickness, angiogenesis, and inflammatory cell count. Staining was performed using hematoxylin and eosin and immunohistochemistry. Image analysis was performed using Image-J on five random fields of view, and readings were performed using a light microscope (Nikon Eclipse Ei) connected to an Optilab device and Optilab Viewer 4.0 software.

## Data Analysis

The statistical analysis commenced with a normality test using the Kolmogorov-Smirnov method, followed by an assessment of the homogeneity of variance using Levene's test. If parametric assumptions were met, One-Way ANOVA was used, followed by Fisher's LSD test if the significance value was <0.05 (Agbangba *et al.*, 2024). If no assumptions were fulfilled, non-parametric Kruskal-Wallis and Mann-Whitney tests were used. All analyses were performed using the Minitab 17 software.

## RESULTS AND DISCUSSION

### Visual Characteristic of Lotion

The milkfish scale collagen lotions produced in this study appeared homogeneous, white, and smooth in texture across all treatments (Figure 1). The control formulation exhibited a lighter and softer consistency than the other formulations. With the addition of collagen, the lotions displayed a progressively denser appearance, with the 1% formulation appearing relatively light, the 3% formulation appearing creamier and more compact, and the 5% formulation appearing the thickest with the most solid texture. All formulations showed no signs of phase separation or instability during observation, indicating that the addition of collagen produced visually stable lotions suitable for further evaluations.

### Physicochemical Evaluation

#### Spreadibility

The findings of this study indicate that all collagen lotion formulations demonstrated standard spreadability, measuring between 5 and 7 cm (Dominica & Handayani, 2019). Different concentrations of milkfish scale collagen affect the spreadability of skin lotion. The 0% and 1% were not significantly different, but 3% and 5% were significantly different ($p<0.05$). However, increasing the collagen concentration in the lotion decreased its spreadability (Table 2). This finding indicates that the viscosity of the lotion increases in parallel with increasing collagen concentration (Dwi *et al.*, 2019).

Spreadability tests using various weights showed that spreadability decreased as the collagen concentration in the formulation increased. The lotion without collagen (0%) exhibited a spreadability value of 6.13 cm, whereas the lotion with 5% collagen showed a reduced spreadability of 5.06 cm. Previous studies have reported that adding active ingredients, such as collagen and protein,

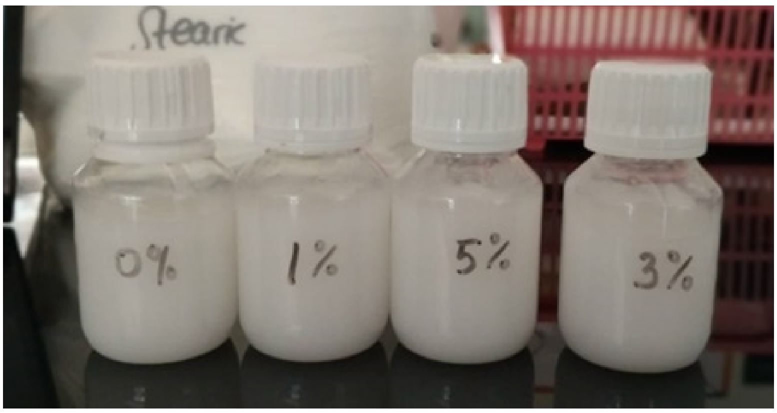


Figure 1 Visual appearance of skin lotion from milkfish scale collagen with different concentrations

can affect the consistency of formulations, influencing their spreadability and ability to penetrate the skin (Kim *et al*., 2018; Zhang *et al*., 2020).

Lotion spreadability tests evaluate the ability of a product to spread uniformly over the skin surface. Evaluating the spreadability of a cosmetic product is crucial, as it directly impacts the product effectiveness upon application (Rahman, 2008; Purwasari, 2013). These findings highlight the importance of selecting the ideal collagen concentration for formulating collagen-based lotions. Formulating an appropriate collagen concentration is essential to ensure that the final product maintains optimal viscosity and spreadability, thereby enhancing stability and user comfort (Purwasari, 2013; Lee *et al*., 2017).

## Viscosity

Different concentrations of milkfish scale collagen affect the viscosity of the skin lotion. All treatments were significantly different (p<0.05). This study demonstrated that an increase in the concentration of milkfish scale collagen in the lotion formulation resulted in a proportional increase in viscosity. The lotion containing 0% milkfish scale collagen exhibited a viscosity of 6,059 cP. Incorporating milkfish scale collagen at concentrations of 1%, 3%, and 5% yielded viscosity values of 6,159, 6,200, and 6,299 cPs, respectively (Table 2).

The viscosity values obtained in this study align with the standards outlined in BSN No. 16-4399-1966 for sunscreen preparations. A lotion formulation should exhibit a viscosity ranging from 2,000 to 50,000 cPs (Hendrawan *et al*., 2020). The observed increase in viscosity is attributed to collagen's properties as a macromolecular protein capable of forming hydrogen bonds and helical structures that create a three-dimensional network. These interactions enhance the viscosity of the dispersion system (Kawamata *et al*., 2017). Structural stability is enhanced by collagen's significant water retention capacity, which limits molecular motion within the formulation and consequently increases viscosity. An appropriate viscosity ensures user comfort during lotion application (Calienni *et al*., 2023). Viscosity is a key parameter in the physical characterization of topical preparations, including lotions. This is an important consideration in cosmetic formulations, as it affects product stability, user comfort, spreadability, and ease of application (Isaac *et al*., 2013).

## pH

The results indicated that the pH values across the four concentrations of preparations ranged from 7.15 to 7.60 (Table 2). Different concentrations of milkfish scale collagen affected the pH of the skin lotion. The 0% and 1% not significantly difference but with 3% and 5% significantly difference (p<0.05). The results of this study align with the pH quality requirements for sunscreen standards outlined in BSN 16-4399-1996, which specifies an optimal pH range of 4.0-8.0 for skin lotion preparations (Husni*et al*., 2021). The formulations developed with the four variations of milkfish scale collagen lotion concentrations complied with and met the standard quality criteria for lotion pH.

Maintaining the lotion pH within this range supports enzymatic activities essential for skin barrier repair and ceramide synthesis,

Table 2 Spreadibility, viscosity, and pH of skin lotion from milkfish scale collagen

| Concentration of milkfish scale collagen (%) | Spreadibility (cm) | Viscosity (cPs) | pH |
|---|---|---|---|
| 0 | 6.54±0.41[b] | 6,059±3.21[a] | 7.15±0.02[a] |
| 1 | 6.39±0.39[b] | 6,159±3.78[b] | 7.15±0.03[a] |
| 3 | 5.44±0.16[a] | 6,200±1.00[c] | 7.40±0.02[b] |
| 5 | 5.14±0.09[a] | 6,299±1.00[d] | 7.60±0.02[c] |

Different superscript letters under the same column indicate significant differences ($p$<0.05) which optimally function at a pH of 4.5 to 5.6. Formulations beyond this range may disrupt the acid mantle, impair barrier function, increase susceptibility to irritation, and cause microbial imbalance. The adherence of milkfish scale collagen lotion formulations to this pH standard indicates their potential effectiveness in supporting skin homeostasis and hydration (Shi *et al.*, 2012).

In contrast, other studies emphasize the benefits of slightly acidic skincare products (pH ≤4.5) in acidifying and maintaining physiological skin pH, particularly in aged or compromised skin, which often exhibits elevated pH levels. Although the lotion formulations in this study fell within a broader acceptable range, their compliance with the pH standard ensured that they would not adversely affect the skin's natural acidity. This is consistent with the findings that products formulated near physiological pH help preserve the skin's protective barrier and promote optimal skin function (Brinke *et al.*, 2021; Lukić et *al.*, 2021).

The pH test is an important parameter in the formulation of cosmetic products, as it plays a crucial role in maintaining stratum corneum homeostasis and skin barrier permeability. Furthermore, pH plays a crucial role, as it affects keratinocyte differentiation, epidermal lipid formation, and the balance of the skin microbiome (Lukić *et al.*, 2021). The application of lotion with a pH value exceeding that of the skin's natural pH can lead to considerable skin damage, resulting in dryness, irritation, and increased sensitivity (Tan & Lio, 2025).

## Acute Dermal Toxicity

Skin histopathology was evaluated by examining various parameters, such as epithelial thickness (µm), inflammatory cells, and angiogenesis cells (Figure 2). The results indicated a significant difference in the lotion treatment group across various doses compared to the control group without exposure. The healthy control group exhibited the highest epithelial thickness, measuring 107.20±9.91 µm. Treatment with collagen lotion prepared from milkfish scales resulted in decreased epithelial thickness. Concentrations of 1%, 3%, and 5% correspond to epithelial thicknesses of 97.57±9.77µm, 92.39±9.75µm, and 84.20±8.07µm, respectively (Table 3).

The decrease in epithelial thickness observed in this study may be attributed to the role of collagen content in regulating epidermal cell proliferation. COL17 type collagen, localized in the basal layer of the epidermis, functions as an inhibitor of excessive proliferation in the epidermis. This is the body's mechanism for maintaining the normal condition of healthy skin (Watanabe *et al.*, 2017).

The angiogenesis cell parameters indicated an increase in both cell count and collagen concentration in the lotion preparation. The healthy control group exhibited a mean cell angiogenesis of 5.04±0.99. The treatment of the lotion at 0%, 1%, 3%, and 5% yielded results of 3.04±0.68, 6.12±0.81, 8.20±0.55, and 15.04±1.99, respectively. Increased angiogenesis indicates massive improvement in the skin tissue (Figure 2). Collagen, especially COL-1, supports

Table 3 Skin histopathology after apply skin lotion from milkfish scale collagen

| Sample treatments (%) | Histopathology assessment | | |
|---|---|---|---|
| | Epithelial thickness (µm) | Angiogenesis | Inflammatory cells |
| Normal skin without lotion | $107.20±9.91^{bc}$ | $5.04±0.99^{b}$ | $40.36±8.77^{c}$ |
| 0 | $116.75±8.12^{c}$ | $3.04±0.68^{a}$ | $51.28±7.44^{d}$ |
| 1 | $97.57±9.77^{ab}$ | $6.12±0.81^{b}$ | $33.04±2.43^{b}$ |
| 3 | $92.39±9.75^{ab}$ | $8.20±0.55^{c}$ | $27.84±2.46^{b}$ |
| 5 | $84.20±8.07^{a}$ | $15.04±1.99^{d}$ | $9.84±2.98^{a}$ |

Different superscript letters under the same column indicate significant differences ($p$<0.05)

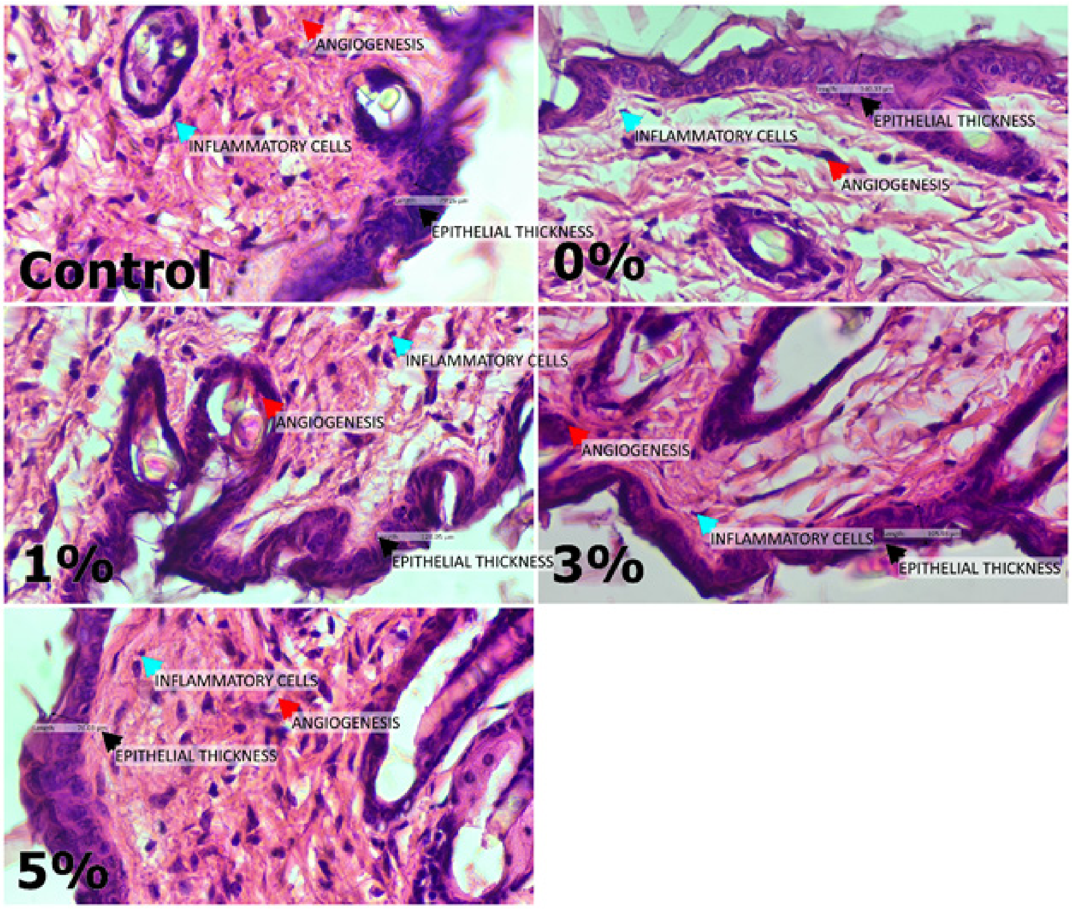


Figure 2 Skin histopathology assessment

skin structure and induces the formation of new blood vessels, commonly referred to as angiogenesis (Minor & Colulombe, 2021; Zhang *et al*., 2024). Therefore, increasing the collagen concentration can induce angiogenesis.

The increase in angiogenesis with increasing collagen concentration is also in line with the results of the histopathological assessment of inflammatory cell parameters. Based on the results of this study, there was a decrease in the number of inflammatory cells as the concentration of collagen increased. The healthy control group had an average number of inflammatory cells of 40.36±8.77. In the treatment group, the concentrations of 0%, 1%, 3%, and 5% yielded average values of 51.28±7.44, 33.04±2.43, 27.84±2.46, and 9.84±2.98, respectively. The decrease in inflammatory cell numbers may be attributed to the capacity of collagen to modulate proinflammatory cytokines, inhibit inflammatory pathways, and diminish oxidative stress, thereby facilitating skin tissue repair (Schwarz *et al*., 2022; Kim *et al*., 2023; Xin *et al*., 2025).

Assessment of skin histopathology in experimental animals treated with varying concentrations of milkfish scale collagen lotion is a key component of the acute dermal toxicity test. This procedure was performed to determine the microscopic interpretation related to the biological effects of the preparation on the structure and tissue of the exposed skin. Histological assessment can be used as a reference to detect tissue changes after the administration of this preparation. In this way, conclusions related to the safety and biocompatibility of a particular product can be drawn (Zahi *et al*., 2017; Meza & Hermawati, 2024).

## CONCLUSION

The 5% milkfish scale collagen lotion exhibited the greatest therapeutic potential and physicochemical characteristics. It had the closest epithelial thickness to normal skin, promoted angiogenesis the most, and had the fewest number of inflammatory cells. This indicates superior wound healing and anti-inflammatory properties.

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