Moisturizing Efficacy of *Stichopus hermanii*-Derived Collagen in Balb/c Mice (*Mus musculus*)

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ABSTRACT

The gamma sea cucumber (*Stichopus hermanii*) is a marine invertebrate known for its high proteins and essential fatty acids, which may enhance collagen production and fibroblast proliferation, making it a valuable resource for health, particularly for skin and tissue regeneration and cosmetic applications. This study investigates the potential of a sea cucumber collagen extract formulated in a moisturizing cream to increase collagen levels and improve skin condition in *Mus musculus*. Collagen was extracted through sequential maceration with NaOH, CH_3COOH, and distilled water. The cream was tested for organoleptic properties, pH, washability, and homogeneity. Four groups of animals were treated with 10% sea cucumber collagen cream (P1), commercial cream (P2), base cream (vehicle control), and no treatment (untreated control) for 5 weeks. Collagen yield was 1.25% (w/w), and presented as a yellow, odorless solid. The P1 cream exhibited white color with brown granules, a waxy odor, and a semi-solid texture. Testing showed that P1 resulted in an 86.66±5.16% collagen increase, significantly higher than P2 (75.00±5.77%) and the vehicle control (73.33%±4.71%, $p<0.05$), without significantly differing from the untreated control (85.00±5.00%).

Keywords: Collagen, Cream, *Mus musculus*, *Stichopus hermanii*

INTRODUCTION

The gamma sea cucumber (*Stichopus hermanii*) is known for its rich nutritional content, including proteins, essential fatty acids, and various minerals. In addition to being a food source, this sea cucumber widely used in health and cosmetic products. In the study by Ahmed *et al*. (2017), the gamma sea cucumber is regarded in traditional medicine as possessing anti-inflammatory, antimicrobial, and effective wound-healing properties. Gamma sea cucumber extract has been shown to accelerate wound healing by increasing collagen production and fibroblast proliferation, both of which are essential for skin and tissue regeneration. Arundina *et al*. (2015) reported that this sea cucumber extract exhibits anti-inflammatory activity that can reduce inflammation, as well as antimicrobial properties that help prevent infection in wounds. Gamma sea cucumbers are also rich in nutrients such as protein and glycosaminoglycans, which contribute to tissue recovery and overall skin health.

Collagen plays an important role in repairing and regenerating skin cells damaged by UV exposure, pollution, and other environmental factors, helping keep the skin looking younger and healthier. Collagen also helps maintain skin hydration by forming a protective barrier that helps retain moisture, which is crucial in preventing skin dryness and stiffness. Healthy skin conditions are strongly supported by the collagen structure in the

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dermis layer. Collagen is the primary structural protein in the human body. Collagen production begins in the fifth week of the first trimester of pregnancy and continues during puberty (Aris *et al*., 2020). As age increases, collagen synthesis decreases by 1.0–1.5% per year, influenced by both internal and external factors. Maintaining optimal collagen production requires proper care and nutrition, including the use of moisturizers that preserve skin elasticity and strengthen its defense barrier.

Although extracts of *Stichopus hermanii* have been reported to exhibit wound-healing and anti-inflammatory activities, evidence regarding their application as topical, collagen-based cosmetic products remains limited. In particular, it is still unclear whether collagen derived from *S. hermanii*, when applied topically, can enhance dermal collagen levels or improve skin hydration and barrier function under *in vivo* conditions. To date, no studies have specifically evaluated a moisturizing cream formulation containing *S. hermanii* collagen using an *in vivo* model. Previous research by Arfani *et al*. (2021) demonstrated the wound-healing potential of collagen derived from *Actinopyga mauritiana*, suggesting that sea cucumber collagen can exert biological effects on skin tissue; however, differences in species composition and formulation type mean these findings cannot be directly extrapolated to *S. hermanii*. Therefore, further *in vivo* investigation is required to assess the efficacy and safety of *S. hermanii* collagen in moisturizing cream formulations and to clarify its role in supporting skin hydration and dermal collagen content.

The laboratory mouse (*Mus musculus*) has served as a widely used model for investigating human biology and disease. It provides a well-characterized and highly relevant model for evaluating skin-related outcomes, as its strong genetic similarity to humans and comparable skin structure enable controlled assessment of the safety and efficacy of topical treatments (Breschi *et al*., 2017). In another study, Qodriah *et al*. (2018) formulated a sheet mask with golden sea cucumber (*Stichopus variegatus*) extract at a concentration of 10% and demonstrated significant effectiveness in inhibiting protein denaturation. Furthermore, Stiani *et al*. (2021) formulated a serum using collagen extracted from *Stichopus horrens* and evaluated its antioxidant activity at concentrations of 0.5% and 1%. The findings showed that higher concentrations of extracts demonstrated significant antioxidant potential, emphasizing the effectiveness of sea cucumber-derived collagen in skin care products.

This study evaluates the moisturizing efficacy of collagen extracted from the gamma sea cucumber by analyzing its effects on skin collagen levels in Balb/c mice. Gamma sea cucumber collagen, rich in bioactive compounds such as proteins and essential fatty acids that promote fibroblast activity and collagen synthesis, is tested for its ability to improve skin health. The study compares a gamma sea cucumber-based collagen cream with a commercial cream, a base cream, and an untreated control to determine its effectiveness in enhancing skin hydration and stimulating collagen regeneration, thereby highlighting its promise for future dermatological and therapeutic applications.

MATERIALS AND METHODS

Sample collection and preparation

Dried gamma sea cucumber samples (200 g) were collected in the first week of February 2023 from sea cucumber farmers in the Karimunjawa Islands and transported to the Integrated Laboratory Unit at Diponegoro University, Semarang City. The samples were ground using a blender into a powder, then sieved through a 0.1 mm mesh. The samples were weighed and for the extraction process.

Extraction of gamma sea cucumber samples

The extraction method was performed using three different solvents: NaOH, CH_3COOH, and distilled water (Safithri *et al*., 2018). A total of 200 g of gamma sea cucumber. Sample was placed in an Erlenmeyer flask and mixed with 2,000 mL

of 0.1 M NaOH (1:10, w/v) for deproteinization for 48 h at room temperature with solvent replacement every 6 h. The extract was then filtered using cheesecloth and neutralized with distilled water. The extraction process continued with 0.5 M CH_3COOH as a demineralization agent for collagen (Alhana *et al*., 2015). The extraction was performed for 48 h with solvent replacement every 12 h. After 48 h, the extract was filtered using cheesecloth and neutralized with distilled water (Stiani *et al*., 2021). After 2 h of neutralization, the extract was filtered again using cheesecloth and freeze-dried for 24 h. This stepwise maceration using NaOH, CH_3COOH, and distilled water was designed to target proteins and minerals separately to enhance the purity of the collagen extract. While the procedure effectively removes non-collagenous materials, the comparative efficiency of each solvent was not evaluated in this study.

The dried extract was weighed, and the yield was calculated using the formula by Wijaya *et al*. (2018) as follows:

$$\text{Extract yield (\%)} = \frac{\text{Extract weight (g)}}{\text{Initial sample weight (g)}} \times 100\%$$

Cream formulation and preparation

The cream preparation process was based on Mohamed *et al*. (2020), with ingredients including liquid paraffin, soft wax, cetostearyl alcohol, borax, and water. In this study, the formulation was modified by adding 10% sea cucumber collagen extract and replacing borax with nipagin as a preservative. The oil-in-water emulsion-based cream was prepared by combining liquid paraffin, soft wax, and cetostearyl alcohol in the oil phase, and nipagin in the water phase. Both oil and water phases were heated to 75 °C. After heating, the water phase was added to the oil phase while stirring continuously until a homogeneous cream formed. After emulsification was complete, 5 g of dried gamma sea cucumber extract (10% extract) was added when the temperature decreased to 55–60 °C. The use of 10% extract was based on the study by Wattimena *et al*. (2023). The 10% concentration was reported to be required to inhibit 50% of protein denaturation, which confirmed the bioactivity of the extract, supporting its potential use as an anti-inflammatory agent in cosmetic applications. For comparison, a moisturizing cream without sea cucumber collagen extract was also prepared. The ingredients used in the moisturizing cream are shown in Table 1.

The base cream, formulated with standard emulsion components and without active extracts, is expected to provide fundamental moisturization and barrier protection but lacks targeted bioactive properties. In contrast, the modified cream enriched with 10% sea cucumber collagen extract is anticipated to exhibit superior skin-regenerative, hydrating, and potential anti-aging effects due to the presence of bioactive marine-derived compounds. The commercial cream serves as a reference standard, offering consistent formulation and performance, though it may not possess the same level of natural bioactivity as the experimental preparation.

Table 1. Formulation of the moisturizing cream based on Mohamed *et al*. (2020).

Ingredients	Formula (%)		Function
	I	II	
Sea cucumber collagen	0	10	Active substance
Liquid paraffin	15	15	Emollient
Soft wax	10	10	Emollient
Cetostearyl alcohol	3	3	Emulsifier
Nipagin	15	15	Preservative
Water	57	47	Solvent

Cream characterization test

Organoleptic test procedure for cream

The organoleptic test assessed texture, color, and odor. Texture observation was conducted by sensory methods, which involve applying 1 g of cream to the skin. The observed texture was then categorized as solid, semi-solid, or non-solid (Nofriyanti and Wildan, 2019). Color observation was carried out visually under standard lighting conditions and described according to the visible color. Odor observation involved smelling the cream sample, which was then categorized as odorless, mildly scented, or having a specific smell. The product's odor should not be overpowering and should align with the ingredients used in the cream formulation (Wahid *et al*., 2022).

Physicochemical test procedure for cream

The physicochemical test included pH measurement, washability, and homogeneity tests. To measure pH, 1 g of cream was weighed, diluted with 10 mL of distilled water, and analyzed using a pH meter. The ideal pH for a cream is within the skin's pH range, which is 4.5–6.5 (Lumentut *et al*., 2020). The washability test was conducted by applying 1 g of cream to the palm, then washing it off with a measured volume of water using a burette (Nofriyanti and Wildani, 2019). The homogeneity test involved weighing 1 g of cream, placing it on a flat surface, and pressing it with a transparent glass plate. The cream was observed for the presence of coarse particles or lumps. A homogeneous cream indicates that all ingredients are well-mixed (Mawazi *et al*., 2022).

Cream testing on experimental animals

Preparation of experimental mice

The experimental animals used in this study were 20 male Balb/c mice strain, aged 6 weeks and weighing 20–30 g. The mice were distributed across four treatment groups. Prior to the experiment, the mice underwent a 7–day acclimatization period to adapt to the experimental environment and avoid stress, which could affect the study's results (Shinta and Sudyanto, 2016). During the experiment, the mice were fed B521 pellets and provided with water ad libitum. After acclimatization, the Balb/c mice were divided into four separate cages based on the treatment, with 5 mice per group. The mice were confirmed to be healthy, with no weight loss exceeding 10%.

Treatment of experimental animals

The treatment of the Balb/c mice was conducted based on Arfani *et al*. (2021), with differences in the extraction process, the use of collagen extract cream, and the fact that the skin was conditioned without injury. In this study, 20 male Balb/c mice were divided into four groups, each consisting of 5 mice. The group details are as follows:

P1: Treatment Group 1 (treated with cream containing 10% gamma sea cucumber collagen extract);
P2: Treatment Group 2 (treated with commercial cream);
K+: Vehicle control (treated with base cream);
K- : Untreated control (no treatment).

Monitoring skin conditions of experimental animals

Monitoring the skin condition of the experimental animals was part of the clinical testing in product development to ensure that the tested formulation does not cause irritation, allergies, or other adverse side effects in the test animals. It also fulfilled the ethical guidelines for research involving experimental animals (Cheluvappa *et al*., 2017). Observations were made daily and recorded in weekly tables using visual and sensory assessment. Aspects monitored included the presence or absence of shaving wounds, changes in skin color, texture, and skin odor. Changes in skin color or redness may indicate irritation. Skin texture observation recorded the presence or absence of rough areas and wrinkle lines, which could be reactions to the treatment, indicating possible skin irritation or dehydration (Valentina *et al*., 2020). Similarly, skin odor observations assessed whether there were any wounds or irritations that might have resulted from the treatment.

Histological data collection from mice skin

Histological data collection from the skin of the Balb/c mice was conducted following the preparation method of Afrida and Priyatno (2021), which began with the euthanasia of the test animals. The mice were anesthetized using chloroform, and the skin tissue was excised. The excised skin tissue was cut into 1×1×0.5 cm sections. The skin samples were then fixed in 10% buffered formalin. After fixation, the tissue underwent dehydration in alcohol concentrations of 30%, 40%, 50%, 70%, 80%, 90%, and 96%, each for 60 min. The remaining alcohol was cleared using a mixture of alcohol and xylol in a 1:1 ratio for 40 min. The samples were then immersed in xylol I, II, and III for 20 min each (Taufikurohmah *et al*., 2013).

The next step was embedding the skin tissue in a xylol-paraffin solution in a 1:1 ratio for 20 min, followed by immersion in paraffin I, II, and III for 20 min each. The paraffinized tissue was sectioned using a microtome into slices 4–8 µm thick (Taufikurohmah *et al*., 2013). After sectioning, the tissue was mounted on glass slides previously coated with an albumin-glycerin solution (1:1) and left to dry at room temperature. Once dried, the tissue was stained by immersing the slides in xylol I and II, followed by an alcohol-xylol solution (1:1) for 15 min. The slides were then dipped sequentially in 95%, 90%, 80%, 70%, 50%, 40%, and 30% alcohol, dipping 10 times in each concentration, and rinsed twice with water.

In the next step, the tissue was immersed in hematoxylin solution for 15 min, followed by rinsing with water three times, dipping the slides 10 times during each rinse. Subsequent staining was done using eosin by dipping the slides sequentially in 30%, 40%, 50%, 70%, 80%, 90%, and 96% alcohol, with 10 dips in each concentration. The slides were then immersed in 1% eosin solution for 5 min and rinsed with water three times, dipping the slides 10 times during each rinse (Sofyanita *et al*., 2024).

The final step was mounting and labeling the slides. The processed slides were covered with Canada balsam as a mounting medium, topped with a cover glass, and labeled with the sample name and other relevant details. The prepared slides were examined using a microscope at 100× magnification.

The collagen fibers, which appeared red in the histological images, were first processed using Adobe Photoshop for image enhancement, and then analyzed in ImageJ to quantify their percentage in the dermis. The percentage of collagen was calculated using the following formula by Soejanto (2017):

$$\text{Percentage of collagen} = \frac{\text{Collagen pixel area}}{\text{Total tissue pixel area}} \times 100\%$$

Data analysis

The research data were analyzed using both descriptive and inferential methods. Descriptive analysis was used to systematically, factually, and accurately presenting the characteristics of the phenomena under investigation and reporting the results according to established standards (Tino *et al*., 2021).

Prior to inferential analysis, data normality was assessed using the Shapiro–Wilk test. Homogeneity of variance was evaluated through visual inspection, and no additional formal homogeneity test was performed. This approach provided an approximate indication that the assumption for parametric testing was reasonably met. A significance level of $p<0.05$ was established as the threshold for statistical significance. If the assumptions were satisfied, the data were further analyzed using a one-way analysis of variance (ANOVA), followed by Duncan's New Multiple Range Test to determine differences between treatment groups.

RESULTS AND DISCUSSION

Characteristics of collagen extract from gamma sea cucumber

A total of 1,200 g of fresh sea cucumber flesh produced 200 g of gamma sea cucumber

powder, which was then extracted and freeze-dried, yielding 5 g of solid extract with a brown color and no odor. As mentioned in the Table 2, the collagen extract obtained using NaOH as the solvent weighed 150 g and had a granular appearance with a yellowish-white color and a fishy odor. Using CH_3COOH, 80 g of extract was obtained, also with a granular appearance, yellowish-white in color, and fishy in odor. Lastly, 30 g of collagen extract was produced using H_2O as the solvent, which appeared granular, yellowish-white, and had a less fishy odor than the previous extracts.

NaOH is a polar compound used for collagen deproteinization (Zaelani *et al*., 2019). In this study, 0.1 M NaOH was used to degrade non-collagen compounds such as fats, minerals, and pigments present in the sea cucumber flesh. The solvent was replaced every 6 h to maintain the extraction process and ensure that the NaOH retained its efficacy. This is consistent with the findings by Alhana *et al*. (2015), who reported that NaOH can become saturated, limiting the electrostatic interaction between polar or hydrogen bonds and non-polar or atomic bonds. The use of 0.1 M NaOH yielded 150 g of extract, a reduction from the initial sample weight of 200 g, which was attributed to electrostatic expansion. This expansion allowed water to penetrate, facilitating the release of non-collagen proteins and non-protein molecules trapped within the collagen (Safithri *et al*., 2018). The NaOH-extracted product appeared as yellowish-white granules with a fishy odor. The yellowish color and fishy smell likely originated from the sea cucumber sample itself, due to residual dissolved substances such as proteins, fats, or other organic matter that were not fully separated during the extraction process. This has also been reported by Wang and Wang (2022) who noted that collagen extracts often exhibit a fishy odor and a yellowish coloration due to compounds released during the extraction process.

The second solvent, 0.5 M CH_3COOH, was used for collagen demineralization, i.e. the removal of minerals potentially bound to the collagen structure. Alhana *et al*. (2015) also reported that CH_3COOH in the demineralization process results in stable triple-helix collagen structures. After 48 h of extraction, 80 g of collagen extract was obtained. The lower yield is likely due to the time-intensive demineralization process, and the 0.5 M CH_3COOH solution may not have fully dissolved the sea cucumber collagen (Fawzya *et al*., 2020). The extract still exhibited a yellowish color and a fishy odor, which originated from the sea cucumber material.

After the extraction process, the extract was neutralized with H_2O to achieve a final pH suitable for making moisturizing cream (Stiani *et al*., 2021). The neutralization process yielded 30 g of extract, which had been separated from impurities. The extract appeared yellowish-white with a less fishy odor compared to the previous solvent extraction processes. The odor reduction indicates that H_2O may have contributed to reducing residual odorous compounds associated with the collagen extract. Saallah *et al*. (2021) noted that the use of H_2O helps ensure collagen purity by minimizing contamination and structural damage, thereby improving the final quality of the extracted collagen.

The extraction yield showed that the NaOH solvent produced the highest yield, with 150 g and a yield of 12.5%, compared to the acetic acid-extracted collagen, which yielded 80 g (6.67%), and the H_2O-extracted collagen, which yielded 30 g (2.5%).

Table 2. Characteristics of gamma sea cucumber (*Stichopus hermanii*) collagen extract.

Solvent	Extract weight (g)	Yield % (w/w)	Form	Color	Odor
Natrium hydroxide (NaOH)	150	12.5%	Granular	Yellowish-white	Fishy
Acetic acid (CH_3COOH)	80	6.67%	Granular	Yellowish-white	Fishy
Distilled water (H_2O)	30	2.5%	Granular	Yellowish-white	Less fishy
Final weight (Freeze drying)	15	1.25%	Solid	Yellow	Odorless

The yield percentage was calculated as the ratio of extracted weight to initial sample weight. Differences in yield may be attributed to variations in extraction methods, solvent concentrations, and the materials used in the collagen extraction process (Yang *et al*., 2022).

The final extraction results showed that NaOH, CH_3COOH, and H_2O yielded yellow granules. This final form is likely influenced by electrostatic expansion caused by the NaOH solvent (Siddiqui *et al*., 2013). The granular form was obtained again during the freeze-drying process, resulting from precipitation during the cooling and drying phase. The freeze-dried extract had a yield of 1.25%, which is comparable to the results of Safithri *et al*. (2018), who reported a 1.23% yield from 0.1% NaOH and CH_3COOH solvents.

While the collagen extraction yield of 1.25% may appear low, it is within the expected range for acid-alkali extraction methods, especially when working with certain raw materials or low-concentration solvents (Safithri *et al*., 2018). The yield of collagen can vary depending on the nature of the raw material and various factors related to the extraction process (Senadheera *et al*., 2020). Although this yield may limit large-scale production without optimization, it can still be considered viable for niche or high-value applications, such as biomedical or cosmetic products. Marine-derived collagen may exhibit inconsistent yields, but it possesses highly desirable attributes such as biocompatibility, antioxidant potential, and efficient absorption. As a result, even low-yield extractions can produce collagen of sufficient quality to be valuable in applications that prioritize specific bioactive properties (Prajaputra *et al*., 2024).

Organoleptic test of cream formulation

Gamma sea cucumber collagen extract was added to the cream formulation and tested for its organoleptic characteristics alongside the base cream and the commercial cream, focusing on color, odor, and texture.

The cream containing 10% sea cucumber collagen extract (P1) had a white color with brownish granules, giving it a semi-solid, granular texture that melts upon skin contact. The commercial cream (P2), containing green tea extract, was green with a waxy smell and semi-solid texture. The base cream (K+) was white with a wax-like smell and semi-solid texture (Figure 1). The organoleptic characteristics of the cream formulations are shown in Table 3.

Based on the results of the organoleptic tests, it can be concluded that the P1 cream (containing 10% sea cucumber extract) has a white color with brownish granules derived from the sea cucumber collagen extract. The white color and scent of the cream are attributed to the formulation containing liquid paraffin and soft wax, as also reported by Mohamed *et al*. (2020). Similarly, the base cream has a white color and a wax-like odor. Color and scent are essential factors that influence product acceptance. The results suggest that the color and scent of the moisturizing cream are acceptable. Meanwhile, the commercial cream containing green tea extract exhibited a green color with a wax-like scent. The green color is likely due to the chlorophyll from green tea leaves, with possible added artificial coloring.

Figure 1. Sample for organoleptic test: (a) cream base (K+), (b) cream containing 10% collagen extract (P1), and (c) commercial cream (P2).

In terms of texture, all the tested formulations, P1, P2, and K+, displayed a semi-solid texture. This texture is common in external cream formulations and provides the advantage of being easy to apply. Ramadhan *et al*. (2021) explained that ointments or creams with a semi-solid texture are frequently used for topical applications due to their ease of use. The semi-solid texture in this case is derived from the formulation containing emollients, such as liquid paraffin and soft wax, which are occlusive agents containing oils. Compounds containing oils or fats have emulsifying properties with relatively low viscosity, making them easily spreadable when applied to the skin (Mohiuddin, 2019).

Physicochemical test of cream formulation

Physicochemical characteristics were tested as a follow-up to the organoleptic test. All three cream formulations showed a pH of 6. The cream formulation P1 had a washability value of 23 mL, and the homogeneity test revealed broken collagen granules, resulting in a classification of low homogeneity. The cream formulation P2 had a washability value of 19 mL and exhibited a homogeneous composition. Meanwhile, the base cream (K+) had a washability value of 23 mL and demonstrated good homogeneity. The physicochemical characteristics of the cream formulations are shown in Table 4.

The results of the physicochemical tests revealed that all three creams had a relatively normal pH of 6. Capitani *et al*. (2012) stated that the normal pH range for moisturizing creams is between 4.0 and 7.0. Within this range, products are unlikely to cause skin irritation and can support the natural function of the skin barrier. The pH results from this study also conform to the standards set for skin moisturizing products, as regulated by SNI 16-4399–1996, which requires the pH value to be between 4.5 and 8.0. Thus, the creams tested in this study are unlikely to cause skin irritation (Purwaningsih *et al*., 2020). Statistical analysis showed that the addition of collagen did not have a significant impact on the cream's pH. This is likely because the added collagen had a near-neutral pH, thus not significantly affecting the overall pH of the cream.

In the washability test, the results varied. The P1 cream had the highest washability value at 28 mL, compared to the P2 cream at 19 mL and the base cream (K+) at 23 mL. The high washability value of the P1 cream suggests that it is the most resistant to washing off. Compared to the base cream, the presence of the extract appeared to make the cream more resistant to washing. The low homogeneity observed in the P1 cream formulation, indicated by visible granules and uneven dispersion, raises concerns regarding the consistency and stability of the cream upon application. In topical

Table 3. Organoleptic characteristics of the cream formulations.

Parameter	P1	P2	K+
Color	White with brownish granules	Green	Milky white
Odor	Waxy	Waxy	Waxy
Texture	Semi-solid	Semi-solid	Semi-solid

Table 4. Physicochemical characteristics of the cream formulations.

Parameter	P1	P2	K+
pH test	6	6	6
Washability	28 mL	19 mL	23 mL
Homogeneity	Low homogeneity	Homogenous	Homogeneous

formulations, homogeneity is critical to ensure uniform delivery of the active ingredients and a pleasant user experience. The granularity could affect dosing accuracy and user satisfaction. The presence of undissolved collagen particles suggests incomplete solubilization or inadequate emulsification during formulation. To improve homogeneity, future formulations may benefit from micronizing the collagen extract prior to incorporation, using high-shear homogenization techniques, or encapsulating the collagen in liposomes or other carriers to facilitate better dispersion within the cream base (Nofriyanti and Wildani, 2019).

Monitoring results of Balb/c mice skin condition

During the first week, all treatment groups (P1, P2, K+, K-) experienced shaving wounds with rough and reddish skin texture. The K+ and K- groups showed a slightly fishy odor, while the P1 and P2 groups did not exhibit noticeable odor. In the second week, the skin condition of all groups showed no wounds or odor, and the skin had returned to its normal color. However, the skin texture in the P1 group had become smooth, while the other groups remained rough. From the third to the fifth week, all groups (P1, P2, K+, K-) showed significant improvements. There was no evidence of shaving wounds, no odor, the skin texture was smooth, and the skin color remained normal. The monitoring results of the mice's skin condition are shown in Table 5.

In the first week, all treatment groups (P1, P2, K+, K-) experienced shaving wounds, with rough and reddened skin. The K+ and K- groups exhibited a slightly fishy odor, while the P1 and P2 groups did not show any odor. The cream containing collagen extract appeared to

Table 5. Weekly monitoring of skin condition in/c mice (*Mus musculus*) treated with different cream formulations.

Week	Group subject	Shaving wounds	Skin odor	Skin texture	Skin color
1	P1	Present	No odor	Rough	Reddish
	P2	Present	No odor	Rough	Reddish
	K+	Present	Slightly fishy	Rough	Reddish
	K-	Present	Slightly fishy	Rough	Reddish
2	P1	Absent	No odor	Smooth	Normal
	P2	Absent	No odor	Rough	Normal
	K+	Absent	No odor	Rough	Normal
	K-	Absent	No odor	Rough	Normal
3	P1	Absent	No odor	Smooth	Normal
	P2	Absent	No odor	Smooth	Normal
	K+	Absent	No odor	Smooth	Normal
	K-	Absent	No odor	Smooth	Normal
4	P1	Absent	No odor	Smooth	Normal
	P2	Absent	No odor	Smooth	Normal
	K+	Absent	No odor	Smooth	Normal
	K-	Absent	No odor	Smooth	Normal
5	P1	Absent	No odor	Smooth	Normal
	P2	Absent	No odor	Smooth	Normal
	K+	Absent	No odor	Smooth	Normal
	K-	Absent	No odor	Smooth	Normal

Note: P1 = Treatment group 1 (cream with 10% gamma sea cucumber collagen extract (gamma sea cucumber); P2 = Treatment group 2 (commercial cream); K+ = Vehicle control (base cream); K- = Untreated control (no treatment).

demonstrate anti-inflammatory effects, such as minimizing the odor caused by wounds. The absence of odor in the P1 group suggests that collagen may have played a role in reducing the production of interleukin-8 during the inflammatory response (Thring *et al*., 2011). From the second to the fifth week, all treatment groups (P1, P2, K+, K-) showed significant improvement. No shaving wounds or additional injuries were observed, and all subjects had smooth skin with normal color and no odor.

The skin texture was rough in the first week and remained so until the second week, except in the P1 group. In the second week, the P1 group showed a change in skin texture, becoming smooth. This marked improvement compared to other groups indicates that the treatment was effective in improving skin texture. As noted in the study by Sudewi *et al*. (2020), collagen in cream formulations can help reduce the appearance of wrinkles and improve skin texture.

The redness observed in the first week gradually disappeared by the second week and remained absent throughout the fifth week, with all groups showing normal skin color. Redness is a sign of inflammation caused by capillary dilation (Mara, 2022).

Histological results of mice skin

The histological results of Balb/c mice back skin in all treatment groups showed the epidermis and dermis layers. Collagen fibers and fibroblast cells were visible in the dermis layer. The histological images included red arrows indicating collagen fibers and blue arrows indicating fibroblast cells. This histology figure was stained by Hematoxylin and Eosin staining method. The histological images were shown at 100× magnification (Figure 2).

The histological results for the P1 group revealed a dense distribution of collagen fibers filling the dermis layer, as indicated by the red arrows in Figure 2. The collagen fibers were tightly packed, with minimal empty spaces between them. In addition to collagen, fibroblast cells were also observed, indicated by blue arrows, and they were evenly distributed in the dermis layer. The fibroblasts appeared active, indicating a high regenerative state.

In contrast, the P2 group showed collagen fibers in the dermis layer with moderate density and a sparse distribution, as shown in Figure 3. The collagen fibers were unevenly distributed and only occupied the upper part of the dermis layer. The lower part exhibited less dense collagen fibers

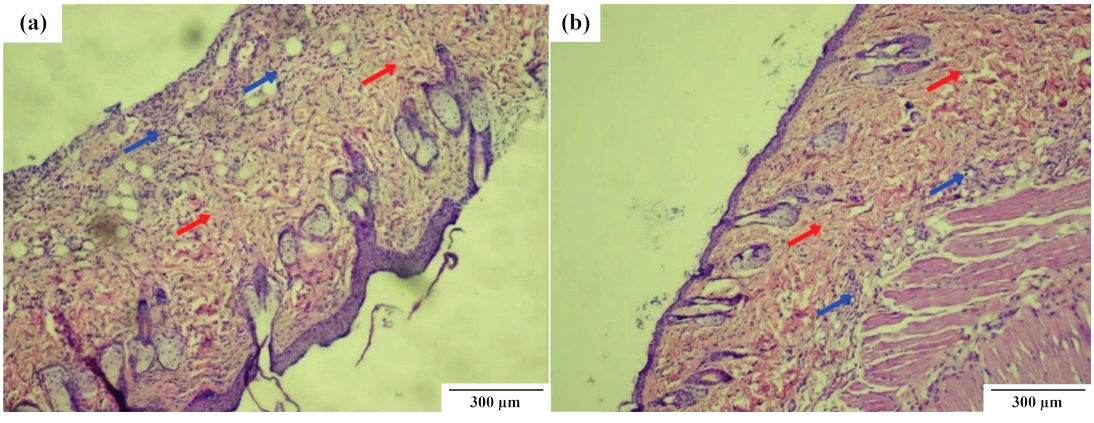

Figure 2. Hematoxylin and eosin (H&E)-stained histological sections of mouse dorsal skin treated with 10% sea cucumber collagen extract cream (P1). (a) and (b) Representative sections showing dermal structure after topical treatment, characterized by dense collagen fiber distribution (red arrows) and the presence of fibroblast cells (blue arrows) within the dermis. Scale bar = 300 µm.

with wider gaps. Fibroblast cells were scattered in the dermis layer, as seen in Figure 3a, with a low distribution. However, in the second section, collagen fibers with higher density and distribution were observed, as shown in Figure 3b. Fibroblasts were also present with a high distribution in the dermis.

The histological results for the K+ group revealed collagen in the dermis layer with high density and moderate distribution (less uniform), as indicated in Figure 4 a. The collagen fibers did not fill the dermis layer. Fibroblast cells, indicated by the blue arrows, were scattered throughout the dermis layer with high density and a wide distribution, spreading between the collagen fibers, as shown in figure 4a. The collagen fibers in the dermis were moderately dense but left some empty spaces, as shown in figure 4b. Fibroblast cells in this image were distributed throughout the dermis layer and scattered between the collagen fibers with high distribution.

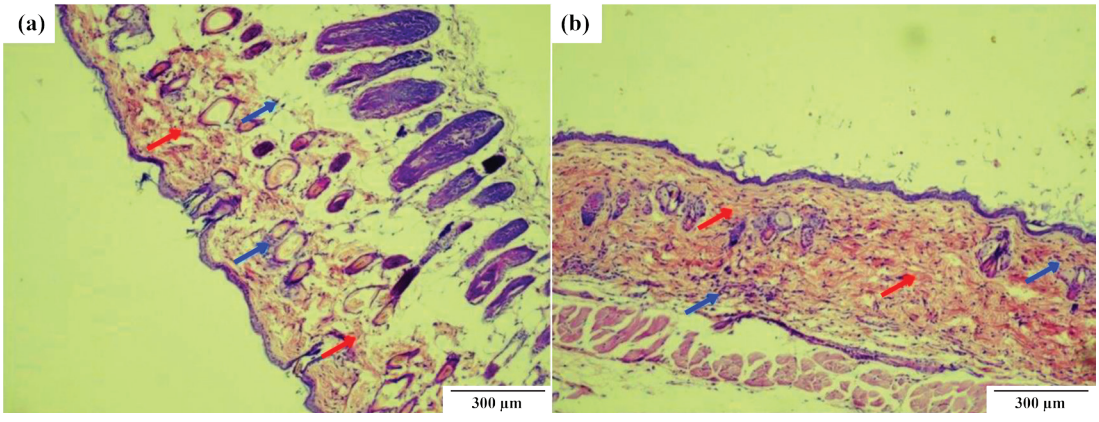

Figure 3. Hematoxylin and eosin (H&E)-stained histological sections of mouse dorsal skin treated with commercial cream (P2). (a) Collagen fibers (red arrows) exhibit moderate density and uneven distribution, primarily in the upper dermis, with scattered fibroblast cells (blue arrows). (b) Higher collagen density and fibroblast presence are observed. Scale bar = 300 µm.

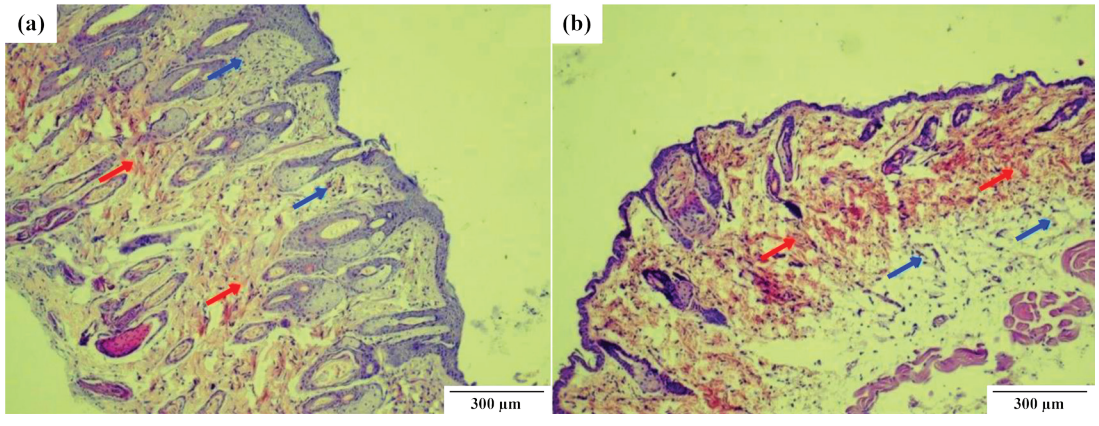

Figure 4. Hematoxylin and eosin (H&E)-stained histological sections of mouse dorsal skin from the vehicle control group (K+). (a) Collagen fibers (red arrows) show relatively high density but uneven distribution within the dermis, with fibroblast cells (blue arrows) widely scattered between collagen bundles. (b) Moderately dense collagen fibers with visible inter-fiber spaces and broadly distributed fibroblast cells are observed. Scale bar = 300 µm.

The histological results for the K- group showed collagen fibers scattered throughout the dermis layer with relatively high density and distribution, as indicated in Figure 5a. In some parts, the collagen fibers showed small gaps, preventing the fibers from being classified as highly dense. Fibroblast cells were located adjacent to the collagen fibers in the dermis layer, with high distribution, as indicated in Figure 5a. In the second section, collagen fibers were spread throughout the dermis layer with high density, but the distribution was only visible in the upper part of the dermis layer, as indicated in Figure 5b. The lower part of this layer showed uneven collagen distribution with visible gaps. Fibroblast cells were evenly distributed, positioned next to the collagen fibers.

The observation of the dermis layer in the back skin of test animals, as seen in the histological images, revealed differences between the P1 group and the other groups. Overall, the results of the P1 treatment showed the best outcome, marked by differences in the density and distribution of collagen fibers in the dermis layer. The noticeable difference in collagen density was most apparent between P1 and P2 and K+, with P1 having higher collagen density and distribution. Furthermore, the P1 group showed a high density and distribution of fibroblast cells, which also differed visibly from P2, K+, and K-.

The histological images of mice skin revealed that the group treated with P1 cream (containing 10% collagen extract) showed the highest collagen density and distribution compared to the P2, K+, and K- groups. The difference in collagen density suggests a potential stimulation of collagen production by the 10% sea cucumber collagen extract. Collagen is the primary structural protein in the skin, responsible for its strength and elasticity. Therefore, an increase in collagen density may indicate enhanced collagen production or retention in the skin tissue (Watson and Dawson, 2015). This can be interpreted as a positive response to the treatment with sea cucumber collagen extract. The increase in collagen content is also a positive indicator of skin health. As collagen production tends to decline with age, leading to sagging and loss of skin elasticity, Palmer and McCormick (2017) noted that an increase in collagen levels can have an anti-aging effect by improving the density and strength of the skin structure.

The collagen density and distribution in the P1 group appeared higher than in the K- group, but no significant difference was observed. This result may be due to the fact that during the treatment period of 5 weeks, the K- group underwent natural collagen synthesis by fibroblasts during the oxygenation process. Hamid *et al*. (2019) also stated that procollagen formation is supported by

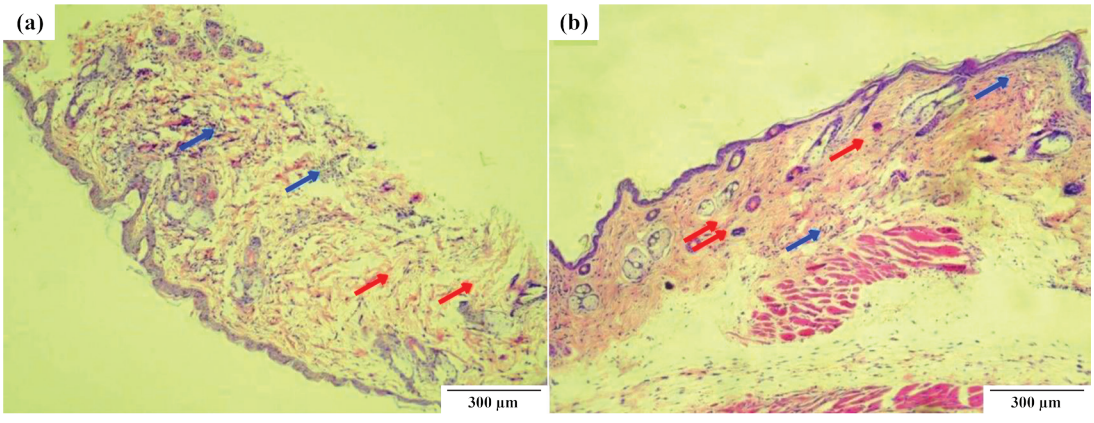

Figure 5. Hematoxylin and eosin-stained histological sections of mouse dorsal skin from the untreated control group (K−). (a) Collagen fibers (red arrows) are present throughout the dermis with small inter-fiber gaps, accompanied by fibroblast cells (blue arrows) adjacent to collagen bundles. (b) Collagen distribution is mainly confined to the upper dermis with uneven organization and visible gaps in deeper layers at 100× magnification. Scale bar = 300 µm.

the presence of oxygen, which acts as a cofactor in the hydroxylation of amino acids.

The distribution and density of fibroblast cells in the histological results showed that the P1 group had the highest levels compared to P2, K+, and K-. This study indicates that the presence of fibroblasts suggests tissue repair and regeneration in damaged skin during the preparation of test animals and as a response to the application of moisturizing cream. The distribution of fibroblast cells in the P1 group was the highest among the groups, resulting in the highest collagen density and distribution in this group. This aligns with Nanda *et al*. (2017), who stated that collagen is derived from fibroblast cells, and accelerated fibroblast growth promotes collagen production. Fibroblast migration and proliferation stimulate the synthesis of collagen fibers and fibronectin, which enhance the extracellular matrix (Djatumurti *et al*., 2021). Collagen synthesis by fibroblasts in the formation of new tissue is stimulated by macrophages migrating to the wound area and releasing growth factors.

While histological images show qualitative differences in collagen density and fibroblast distribution between groups, the interpretation currently relies primarily on visual assessment. Incorporating more robust quantitative metrics—such as collagen area fraction, fibroblast count per unit area, or histomorphometric analysis using image analysis software like ImageJ—would strengthen the conclusions. For example, collagen fiber density could be calculated as the percentage of stained areas in a standardized field of view, allowing for statistical comparison across groups. These enhancements would provide objective evidence for the observed effects and help eliminate bias from visual interpretation.

Percentage of collagen content in Balb/c mice skin.

The percentage of collagen in the dermal layer was obtained by analyzing histological sections of the dorsal skin of Balb/c mice. The group of mice treated with P1 cream had a relatively higher percentage of collagen compared to other groups. The average collagen percentage for this group was 86%, which was higher than the group treated with P2 cream, with an average of 75%, the untreated control group with an average of 85%, and the vehicle control group with an average of 73.3% (Table 6).

Through histological images of the samples, the percentage of collagen in each group was determined to assess the impact of the moisturizing cream application. This study focused on the percentage of collagen in the dermal layer, which is influenced by the application of moisturizing cream with 10% gamma sea cucumber collagen extract.

Statistical analysis revealed a significant difference in collagen percentages between the P1 group, with 86.66±5.16%, and the P2 group, with 75.00±5.77%, as well as the K+ group, with 73.33±4.71% ($p<0.05$). This indicates that the use of cream with 10% collagen extract (P1) affected the percentage of collagen in the dermal layer, and that these differences were associated with changes in the skin condition of the mice. The higher presence of collagen in the dermis layer was observed in the P1 treatment group. According to Damaiyanti *et al*. (2019), sea cucumber extract contains omega-3, which induces collagen production. Omega-3 influences prostaglandin E2 (PGE2) production by upregulating collagen synthesis.

The same statistical data also showed no significant difference between the P1 group, with 86.66±5.16%, and the K- group, with 85.00±5.00%. This indicates that the collagen percentage in the skin of both groups did not show a significant difference. In the K- treatment, the skin was not subjected to any intervention, allowing natural skin regeneration processes to occur, including collagen synthesis. In wounded skin, collagen synthesis begins three days after trauma and occurs rapidly, reaching its peak 14 days post-trauma, marked by collagen fiber thickening (Djatumurti *et al*., 2021). A study by Pringgenies *et al*. (2012) also demonstrated collagen fiber formation in the dermis as a result of the application of *E. cottoni* seaweed powder and *Loligo* sp. shell powder for 5 weeks. Therefore, it can be inferred that during the 5-week cream application period in the test animals, natural

Table 6. Percentage of dermal collagen in Balb/c mice (*Mus musculus*) following treatment with different cream formulations.

Subject	Collagen percentage
P1 (n = 6)	86.67±5.16^{c}
P2 (n = 4)	75.00 ±5.77^{ab}
K+ (n = 3)	73.33 ±4.71^{a}
K- (n = 2)	85.00 ±5.00^{bc}

Note: Values are mean±SD. n indicates the number of independent experimental units. Mean±SD in the same column with different superscript letters are significantly different ($p<0.05$).

collagen synthesis occurred, contributing to the increased collagen percentage in the dermal layer.

Although the P1 group showed a higher average collagen percentage (86.66±5.16%) compared to the untreated control group (85.00± 5.00%), the difference was not statistically significant ($p\geq0.05$). This result suggests that natural skin regeneration processes in mice may restore collagen levels to a comparable degree over a 5-week period without treatment. While it could be argued that the cream offers a marginal benefit, particularly in accelerating early improvements in skin texture and reducing signs of inflammation, the clinical relevance of this benefit remains uncertain and warrants further investigation. In future studies, earlier time-point analyses or models of impaired healing could clarify whether the collagen cream provides a substantial therapeutic advantage beyond natural healing.

CONCLUSIONS

The collagen extract from gamma sea cucumber demonstrates potential for use in moisturizing cream products, as evidenced by improved skin texture, reduced signs of inflammation, and increased collagen deposition in Balb/c mice over a 5-week period. However, the study also found no statistically significant difference in collagen levels between the treatment and untreated control groups, suggesting that the benefit may be limited under normal healing conditions. This study is subject to several limitations. The small sample size (n = 5 per group) and short observation period (5 weeks) may have reduced the statistical power and obscured longer-term effects. Additionally, reliance on visual histological interpretation without rigorous quantification may limit the objectivity of the findings.

Future research should expand sample sizes, include longer treatment durations, and incorporate quantitative histomorphometric analyses. Exploring the efficacy of this collagen cream in models of impaired wound healing (e.g., aged or diabetic mice) could clarify its therapeutic relevance. Moreover, potential applications beyond cosmetics, such as biomedical wound dressings or tissue engineering scaffolds, should be explored to leverage the bioactivity of gamma sea cucumber-derived collagen more fully.

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