



THE EFFECT OF SEA CUCUMBER (*Holothuria atra*) EXTRACT ON THE THICKNESS OF DERMAL AND CONNECTIVE TISSUE FIBERS IN WHITE RAT (*Rattus norvegicus*) SKIN

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Submitted: 19 September 2025/Accepted: 3 June 2026

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How to cite (APA Style 7th): Nursid, M., Halimatushadyah, E., Marraskuranto, E., Khatulistiani, T. S., Ugi, B., & Estuningsih, S. (2026). The effect of sea cucumber (*Holothuria atra*) extract on the thickness of dermal and connective tissue fibers in white rat (*Rattus norvegicus*) skin. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 29(9), 773-781. <http://dx.doi.org/10.17844/67fpzc62>

Abstract

Skin aging is a biological process characterized by increased covalent cross-linking between collagen units in the dermis, resulting in decreased skin tissue elasticity and structure of skin tissue. Sea cucumbers (*Holothuria atra*) contain bioactive compounds such as collagen, peptides, and antioxidants that can support skin regeneration and prevent premature aging. Thus, they are potentially used as active ingredients in cosmetics. This study aimed to evaluate the effect of the ethanol extract of *H. atra* on the number of connective tissues fibers and dermis thickness in white rats (*Rattus norvegicus*). A total of 18 male rats were divided into six groups: untreated control (A) and five treatment groups administered the extract orally for 90 days at doses of 25 (B), 50 (C), 100 (D), 200 (E), and 400 mg/kg body weight (BW) (F). Skin samples were histologically and quantitatively analyzed using ImageJ software to calculate the number of connective tissues fibers and dermal thickness. The results showed a significant increase in the number of connective tissues fibers and dermis thickness in the treatment group compared to the control group. These findings support the potential of *H. atra* as an anti-aging agent in natural cosmetic formulations.

Keywords: anti-aging, cosmetic, histology, *Holothuria atra* extract, *Rattus norvegicus*

Pengaruh Ekstrak Teripang Hitam (*Holothuria atra*) terhadap Ketebalan Dermis dan Serat Jaringan Ikat pada Kulit Tikus Putih (*Rattus norvegicus*)

Abstrak

Penuaan jaringan kulit adalah suatu proses biologis yang ditandai dengan peningkatan ikatan silang kovalen antara unit-unit kolagen di lapisan dermis, yang mengakibatkan penurunan

elastisitas dan struktur jaringan kulit. Teripang hitam (*Holothuria atra*) mengandung senyawa bioaktif yaitu kolagen, peptida, dan antioksidan yang berpotensi mendukung regenerasi kulit dan mencegah penuaan dini sehingga berpotensi digunakan sebagai bahan aktif dalam kosmetik. Penelitian ini bertujuan mengevaluasi pengaruh ekstrak etanol *H. atra* terhadap jumlah serat jaringan ikat dan ketebalan dermis pada tikus putih (*Rattus norvegicus*). Sebanyak 18 tikus jantan dibagi menjadi enam kelompok, yaitu (A) kontrol tanpa perlakuan dan lima kelompok perlakuan yang diberikan ekstrak secara oral selama 90 hari dengan dosis (B) 25, (C) 50, (D) 100, (E) 200, dan (F) 400 mg/kg berat badan (BB). Sampel kulit dianalisis secara histologis dan kuantitatif menggunakan perangkat lunak ImageJ untuk menghitung jumlah serat jaringan ikat dan ketebalan dermis. Hasil penelitian menunjukkan peningkatan signifikan dalam jumlah serat jaringan ikat dan ketebalan dermis pada kelompok perlakuan dibandingkan dengan kelompok kontrol. Temuan ini mendukung potensi *H. atra* sebagai agen anti-penuaan yang dapat diaplikasikan dalam formulasi kosmetik berbahan alami.

Kata kunci: antipenuaan, ekstrak *Holothuria atra*, histologi, kosmetik, *Rattus norvegicus*

INTRODUCTION

Skin health plays a crucial role in supporting self-confidence and appearance, especially in women (Dreno *et al.*, 2021), while also serving as the body's primary defense against environmental exposures, such as pollution, ultraviolet (UV) rays, and microorganisms (Zieneldien *et al.*, 2025). As public awareness of the importance of natural and safe skin care increases, active ingredients derived from marine resources, such as algae, sea cucumbers, and seaweed, are increasingly being considered as potential alternatives in cosmetic formulations. These marine biotas contain various bioactive compounds, including antioxidants, polysaccharides, marine minerals, and peptides, which have been shown to maintain skin moisture, stimulate cell regeneration, and prevent premature aging (Lopez-Hortas *et al.*, 2021). The use of marine biota as raw materials for natural cosmetics not only provides functional benefits for overall skin health but also supports the trend of sustainable cosmetics that are environmentally friendly and free of synthetic chemicals (Raimundo *et al.*, 2018).

Sea cucumbers have long been used in traditional Asian medicine because of their high nutritional value and pharmacological properties. Sea cucumber extract contains important bioactive compounds, such as collagen, glycosaminoglycans, triterpenoids, saponins, and omega-3 fatty acids, which play roles in cell regeneration and skin tissue repair. The high collagen content of sea cucumbers

contributes to increased skin elasticity, reduced wrinkles, and accelerated wound healing (Wargasetia *et al.*, 2023). Furthermore, various studies have shown that sea cucumber extract has significant antioxidant and anti-inflammatory activity, which can protect the skin from damage caused by free radicals and ultraviolet (UV) light exposure (Bordbar, Anwar, & Saari, 2011).

The use of sea cucumber extract in skin care products is a growing trend in the cosmetic industry, particularly in anti-aging formulations and wound care products. *In vitro* studies have shown that the active compounds in sea cucumbers can stimulate fibroblast proliferation and collagen synthesis and accelerate the epithelialization process in burns and chronic wounds (Wen *et al.*, 2018). Furthermore, the antibacterial properties of sea cucumber extract are effective in inhibiting skin infections. With these diverse benefits, sea cucumbers have great potential as natural raw materials in the pharmaceutical and cosmetics industries, particularly for supporting comprehensive and sustainable skin health (Arundina *et al.*, 2015).

Holothuria atra (local name: *teripang hitam* or black sea cucumber) is a species of sea cucumber commonly found in Indonesian waters and has great potential in the health sector due to its bioactive compounds, such as saponins, phenols, flavonoids, and antimicrobial peptides (Arundina *et al.*, 2015). Various studies have shown that *H. atra* extract has significant pharmacological



activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer activities (Putram *et al.*, 2017), and can accelerate wound healing. The saponin content in *H. atra* inhibits the growth of cancer cells, whereas its phenolic compounds act as powerful antioxidants that protect cells from oxidative stress caused by free radical exposure (Halimatushadyah *et al.*, 2018; Hanifaturahmah *et al.*, 2024). In addition, *H. atra* extract has been shown to be effective in increasing tissue regeneration, making it a potential candidate as a natural raw material for pharmaceutical products and therapies for degenerative diseases (Dewi *et al.*, 2023).

Sea cucumbers are also known to have a high protein content, with approximately 70–80% collagen (Bordbar, Anwar, & Saari 2011). Collagen is the main structural protein in connective tissue and plays a vital role in the regeneration and renewal of tissues in both animals and humans. Type I collagen, the dominant type in human skin, is also found in large quantities in sea cucumbers (Cui *et al.*, 2007). Within the skin structure, collagen is located in the dermis layer and, together with elastin fibers, forms a woven network of fibers that provides tensile strength and the ability to stretch and contract (Tortora & Derrickson 2011). Therefore, collagen from sea cucumbers has been utilized in various health and cosmetic products, including tissue membranes, soaps, shampoos, moisturizing lotions, and burn therapy (Andririsnanti, 2012).

Although the biological benefits of *H. atra* have been extensively studied *in vitro*, *in vivo* studies on the effects of its extract on skin health remain limited. Based on this background, this study aimed to evaluate the effect of administering *H. atra* ethanol extract on dermis thickness and the number of connective tissue fibers in the skin of white mice (*Rattus norvegicus*).

MATERIAL AND METHODS

Preparation of ethanol extract of *Holothuria atra*

Samples of sea cucumber (*H. atra*) were obtained from the waters of Babua Island, Gueria, Halmahera, North Maluku, Indonesia

(1°01'06" N 127°27'53" E). The extraction process was carried out using the maceration method with 96% ethanol as the solvent. The detailed sampling procedure and extraction steps were based on a previous study (Nursid *et al.*, 2025).

In Vivo Study

This study used male white Sprague Dawley rats (*R. norvegicus*) aged 8-10 weeks weighing 220-250 g. The rats were obtained from the Food and Drug Monitoring Agency of Indonesia (BPOM RI). All animal research procedures were approved by the Animal Ethics Committee, Faculty of Veterinary Medicine, Bogor Agricultural University (IPB), Bogor, with the approval number 18-2016 IPB. The rats were divided into six treatment groups based on the dose of *H. atra* ethanol extract administered orally: 25 (Group B), 50 (Group C), 100 (Group D), 200 (Group E), and 400 mg/kg body weight (BW) (Group F). Group A served as a control and received no extract treatment but was administered 1 mL of carrier solution per rat. Animal care procedures, acclimatization, pre-treatment, extract administration, euthanasia, necropsy, and histological preparation and staining were performed according to the methods described in a previous study (Nursid *et al.*, 2025). For histological analysis of dermal tissue, Cason's Trichrome staining method (Alturkistani, Tashkandi & Mohammedsaleh, 2016; Cason, 1950) was used to identify and evaluate the number of connective tissues fibers more clearly.

Preparation Observation

Histopathological skin preparations were observed using a light microscope (Nikon Eclipse E200) with a digital eyepiece camera for micrograph documentation. The dermis layer and connective tissue fibers (collagen fiber density) were also observed. The dermis layer was captured at a 4× objective magnification, while the connective tissue fibers were captured at a 100× objective magnification. Dermis thickness was quantitatively observed by calculating the average thickness in one field of view, using five repetitions per sample. Representative

dermal micrographs (1.280×1.024 μm²) were analyzed to determine collagen fiber density based on the relative area of collagen-stained connective tissue. All measurements were performed using ImageJ software (National Institutes of Health, Bethesda, MD, USA) to obtain quantitative data.

Data Analysis

The observation data were analyzed statistically using SPSS software version 16, with one-way analysis of variance (ANOVA) to determine significant differences between treatment groups.

RESULTS AND DISCUSSION

Before discussing the histomorphological findings, body weight measurements before and after the 90-day experimental period were not included in the present study, as the primary focus was on evaluating dermal structural changes and connective tissue fiber deposition following administration of *H. atra* extract. This investigation was specifically designed to assess skin histological responses rather than systemic growth-related parameters. Furthermore, sub-chronic toxicity and systemic safety evaluations of *H. atra* extract, including assessments of major organs, have been reported in a previous study (Dewi *et al.*, 2025). Therefore, the present study emphasizes the localized histological effects of dermal

thickness and collagen-associated connective tissue development.

Dermis thickness and the number of connective tissue fibers were evaluated to determine the effectiveness of *H. atra* ethanol extract administration. The results showed that dermis layer thickness increased with increasing doses of *H. atra* ethanol extract. A significant difference ($p < 0.05$) was observed between the control and all treatment groups. The lowest dermis thickness was recorded in the control Group A (Group A), with an average of 281.54 μm, whereas the highest dermis thickness was found in Group F (dose 400 mg/kg BW) with an average of 517.25 μm (Table 1). Interestingly, the treatment group with a dose of 100 mg/kg BW (Group D) showed the most optimal increase in dermis thickness compared to the other dose groups, based on the difference value compared to the control. This finding indicates that a dose of 100 mg/kg BW was effective in stimulating an increase in connective tissue in the skin dermis. These results were supported by the results of Cason's trichrome staining, which was able to distinguish connective tissue (collagen) from smooth muscle tissue, cytoplasm, and adipose tissue, allowing for clearer visualization of the increase in connective tissue fibers. The increase in dermis thickness due to the administration of sea cucumber extract was also observed histologically through photomicrographs of rat skin, as shown in Figure 1.

Table 1 Average dermis thickness (μm) and connective tissue fibers in the skin of rats treated with *Holothuria atra* extract for 90 days

Group	Dermis thickness (μm)	Connective tissue fibers
A	281.54±89.21 ^a	15.87±3.79 ^a
B	335.51±54.98 ^{ab}	20.80±7.62 ^b
C	379.34±57.78 ^b	25.73±3.55 ^{bc}
D	470.47±68.74 ^c	24.53±6.18 ^c
E	490.49±38.84 ^c	34.40±4.68 ^d
F	517.25±47.05 ^c	36.06±3.69 ^d

Description: A: Control group; B: 25 mg/kg BW group; C: 50 mg/kg BW group; D: 100 mg/kg BW group; E: 200 mg/kg BW group; F: 400 mg/kg BW group. Different superscript letters within the same column indicate statistically significant differences between treatments ($p < 0.05$).

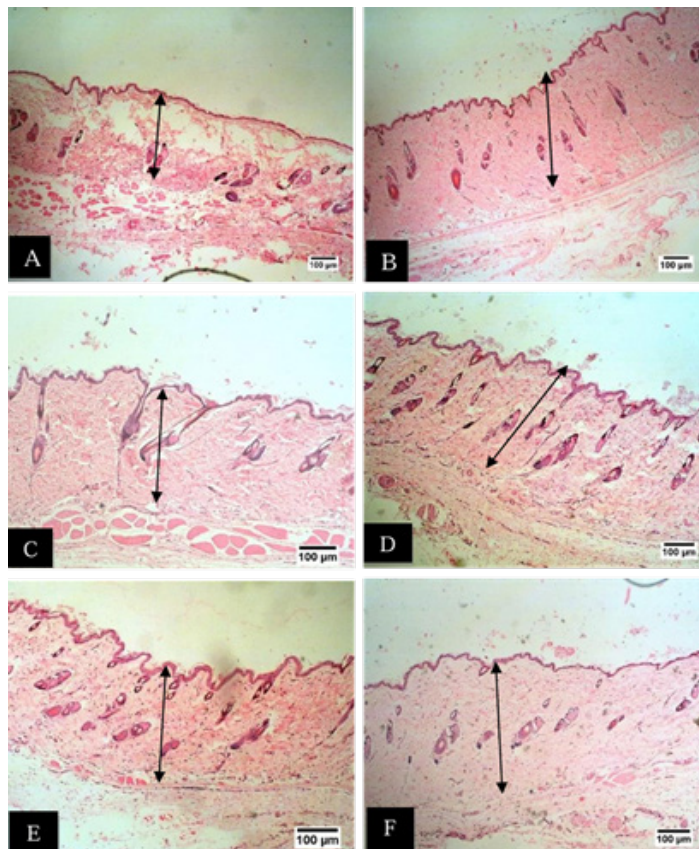


Figure 1 Photomicrograph of rat skin at 4× magnification. The arrow ($\Updownarrow$) indicates the dermis thickness. A: Control (distilled water); B: 25 mg/kg BW; C: 50 mg/kg BW; D: 100 mg/kg BW; E: 200 mg/kg BW; F: 400 mg/kg BW. Scale bar = 100 µm

The same pattern was also observed in the number of connective tissue fibers in the dermis layer of rat skin, where the number increased with increasing dose of *H. atra* ethanol extract given. Quantitative analysis showed that the number of connective tissue fibers in the control Group A (Group A) was 15.87 ± 3.79^a . This value was significantly lower than that in the treatment groups, including the group with a dose of 25 mg/kgBW (Group B), which had an average value of 20.80. The highest number of connective tissue fibers (36.06) was found in the group with a dose of 400 mg/kgBW (Group F) (Table 1). These results indicate a positive dose-response relationship between the of *H. atra* extract administration and an increase in the number of dermal connective tissue fibers. Histological visualization of the increase in connective tissue fibers was clearly observed in the micrograph of the rat skin histopathology preparation presented in Figure 2.

Collagen is the main structural protein found in animal connective tissues, including the skin, bones, tendons, and cartilage. This protein constitutes approximately 30% of the total protein in the human body and plays a vital role in providing strength, elasticity, and structure to various body tissues. Collagen is primarily composed of the amino acids glycine, proline, and hydroxyproline and has a unique triple-helix structure that provides high stability (Ricard-Blum, 2011). Collagen is abundant in the dermis, tendons, ligaments, and bones (Kittiphattanabawon *et al.*, 2005).

Fibroblasts are the primary cells that synthesize collagen, beginning with the formation of procollagen molecules, which are helical polypeptide chains that are then secreted into the extracellular space and form collagen fibrils (Raed, 2013). These collagen fibrils are arranged in a hexagonal or quasi-hexagonal packing pattern and can be found as sheets or microfibrils. Collagen appears

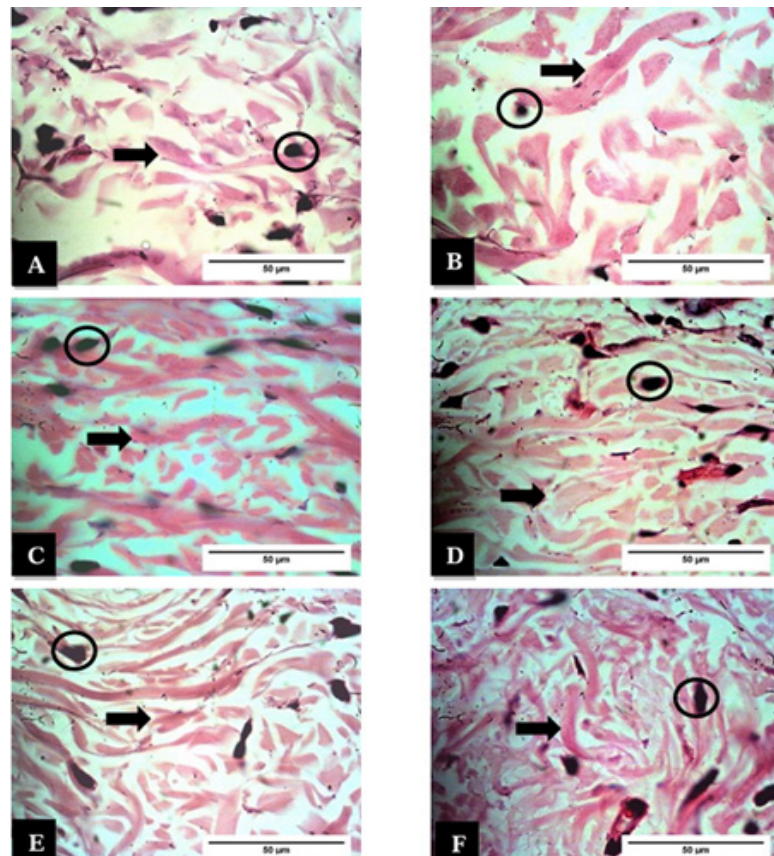


Figure 2 Photomicrograph of rat skin organs at 100× magnification. Circles (O) indicate fibroblast nuclei; arrows (→) indicate connective tissue fibers. A: Administration of distilled water (control); B: Administration of a 25 mg/kg BW dose; C: Administration of a 50 mg/kg BW dose; D: Administration of a 100 mg/kg BW dose; E: Administration of a 200 mg/kg BW dose; F: Administration of a 400 mg/kg BW dose. Scale bar = 50 µm.

as long fibrils microscopically (Szpak, 2011). In the dermis, collagen and elastin fibers form a woven fiber network with high tensile strength, allowing the skin to stretch and contract optimally (Tortora & Derrickson, 2011).

Sea cucumber extracts, particularly their components such as collagen and small peptides, have been extensively studied and shown to have skin rejuvenating effects via various mechanisms. The reported biological activities of these components include antioxidant activity, protection against DNA and mitochondrial oxidative damage, stimulation of skin cell migration and proliferation, and enhancement of skin barrier function (Dewi *et al.*, 2025). One study on peptide extracts from *Apostichopus japonicus*

showed that these compounds can protect skin cells from oxidative stress, accelerate cell migration and wound healing, and regulate the expression of proteins involved in cell repair and the response to oxidative stress (Mazumder *et al.*, 2024).

Furthermore, oral administration of hydrolyzed sea cucumber collagen to a rat model exposed to UV-A light reduced wrinkle formation, maintained skin moisture, and inhibited the expression of inflammatory molecules such as TNF- α and IL-6 (Liu *et al.*, 2022). Other studies have shown that *H. atra* protein hydrolysate stimulates collagen production in human dermal fibroblast (HDF) cells, indicating its role in skin tissue regeneration and repair (Dewi *et al.* 2023; 2025).



In this study, administration of *H. atra* ethanol extract significantly increased dermis layer thickness and the number of connective tissue fibers in the skin of *R. norvegicus* rats. This effect is thought to be closely related to the presence of various bioactive compounds in the extract (Khatulistiani, Dewi, & Patantis 2021). Saponin is a major component of *H. atra*, with levels reaching 121.82 µg/mg of extract, phenol content of 4.13 µg/mg, and collagen content of 0.52 mg/mg (Dewi *et al.*, 2023). Furthermore, the total amino acid content in *H. atra* was reported to be 8.0 g per 100 g of sample.

Saponins from *H. atra* are known to possess potent anticancer activity (Halimatushadyah *et al.*, 2018) and have potential as immunostimulatory agents (Nursid *et al.*, 2021). Collagen and essential amino acid content also support tissue regeneration and dermal structure formation. This study is the first to report the effects of oral administration of *H. atra* ethanol extract for 90 days on dermal thickness and the number of connective tissue fibers in an *in vivo* animal model of skin aging.

The present study demonstrated that oral administration of *H. atra* extract for 90 days produced a dose-dependent increase in dermal thickness and collagen fiber content in the skin of *R. norvegicus*, suggesting a stimulatory effect on skin structural regeneration in rats. These findings may indicate enhanced fibroblast activity and remodeling of the extracellular matrix, particularly through increased collagen deposition in the dermal layer. Sea cucumber-derived bioactive compounds, including collagen peptides, triterpene glycosides, saponins, and antioxidant molecules, are widely associated with tissue repair, anti-inflammatory activity, and improved skin integrity. Low-molecular-weight peptides from *H. atra* significantly enhance collagen production in human dermal fibroblasts while suppressing matrix metalloproteinase-1 (MMP-1), thereby reducing collagen degradation and supporting dermal regeneration (Dewi *et al.*, 2025). These mechanisms may explain the histological improvements observed in this study, where

higher extract doses corresponded with greater connective tissue development.

These results highlight the promising potential of *H. atra* extract as a marine-derived nutraceutical, dermatological therapeutic, and cosmeceutical ingredient for skin health maintenance. Enhanced dermal thickness and collagen density are directly relevant to anti-aging, wound healing, skin elasticity, and tissue repair. Sea cucumber collagen and associated metabolites possess moisturizing, antioxidant, and regenerative properties, making them attractive for the development of oral supplements, topical formulations, and functional skincare products (Li *et al.*, 2019). Therefore, *H. atra* may be a valuable natural resource for promoting skin rejuvenation and preventing dermal degeneration. However, further molecular studies are needed to confirm the specific signaling pathways involved, such as TGF-β, fibroblast proliferation, and collagen synthesis regulation, before translational or clinical applications can be fully established.

CONCLUSIONS

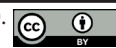
Oral administration of *H. atra* ethanol extract for 90 days increased the thickness of the dermis and the number of connective tissue fibers in the skin of white rats (*R. norvegicus*). These findings indicate that *H. atra* has the potential to be used as a regenerative agent that supports the repair of skin tissue structure. Therefore, *H. atra* extract has the potential to be further developed as a natural active ingredient in cosmetics and skin care products, particularly for anti-aging and tissue regeneration applications.

ACKNOWLEDGMENTS

This research was supported by the RIIM LPDP Grant (Grant No. B-4038/III.4/FR.06.00/11/2023, dated November 27, 2023), awarded to Muhammad Nursid. The authors gratefully acknowledge the Research Center for Marine and Inland Water Bioindustry, Research Organization for Earth Sciences and Maritime (ORKM), National Research and Innovation Agency (BRIN), Republic of Indonesia for providing research facilities and institutional support.

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