Journal of Research in Pharmacy

Research Article

www.jrespharm.com

Anti-inflammatory activity of *Stichopus variegatus* from Onogate capsule to treat joint pain

Syamsudin ABDILLAH [1 *], Deni RAHMAT [2], Ema HERMAWATI [2], Greesty Finotory SWANDINY [2], Sucipto KOKADIR [3], Edward BASILIANUS [3]

1 Department of Pharmacology, Faculty of Pharmacy, Universitas Pancasila, Jakarta 12630, Indonesia.
2 Department of Pharmaceutical Technology, Faculty of Pharmacy, Universitas Pancasila, Jakarta 12630, Indonesia.
3 PT. Natura Nuswantara Nirmala (Nucleus Farma), Jombang Raya, South Tangerang 15229, Banten, Indonesia.
* Corresponding Author. E-mail: syamsudin.abdillah@univpancasila.ac.id (S.A.); Tel. +62-21-727-0086.

Received: 22 May 2024 / Revised: 21 October 2024/ Accepted: 25 October 2024

ABSTRACT: Nonsteroidal anti-inflammation drugs (NSAIDs) are usually used to treat over-reaction of inflammatory reactions. However, the medicines have many side effects. On the other hand, a marine product like sea cucumbers (*Stichopus variegatus*) have to treat joint pain. This study aims to examine the anti-inflammatory effect of *Stichopus variegatus* extract powder from Onogate capsule produced by PT Nuswantara Nirmala Nusantara to treat joint pain. The research study employed 24 male white Wistar rats separated into six distinct categories: carrageenan control group, positive control group, healthy or normal group, group of *S. variegatus* extract test preparations at doses of 200 and 400 mg/Kg BW, and diclofenac Na as standard medicine. Microbial contamination was measured by total plate count (TPC) and total mold and yeast count (TMC). All data were analyzed using ANOVA with a significance level of $p < 0.05$. The results showed that amino acid profile of *S. variegatus* extracted from Onogate capsule contained high of glycine and the least of tryptophan. Microbial contamination tests such as *E. coli*, *Enterobacteriaceae*, *Shigella* sp., *Salmonella* sp., and *Clostridia* sp. showed negative results, although the TPC and TMC examinations were found to be 1.0×10^1, indicating that it is still under control since the value was below 1.0×10^5 colonies/g and 1.0×10^3 colonies/g, respectively. The lowest reduction of anti-inflammatory activity was seen in diclofenac Na 1.79 ± 0.03 (36.7%) and after treatment of *S. variegatus* extract 400 mg/kg 2.19 ± 0.01 (22.6%). *S. variegatus* extract 400 mg/kg from Onogate capsule has the potential to have an anti-inflammatory effect in treating joint pain.

KEYWORDS: Anti-inflammatory activity; joint pain; onogate capsule; *Stichopus variegatus*.

1. INTRODUCTION

Inflammation happens when inflammatory cells are activated and produce mediators such as cytokines and prostaglandins due to trauma, infection, external factors, or cellular alterations [1]. Furthermore, external factors frequently cause severe inflammation marked by swelling, rashes, fever, and discomfort. Endogenous factors, on the other hand, are chemicals generated as a result of tissue injury that connects to the innate immune system's PRR to cause persistent inflammation [2]. The majority of the hallmarks of acute inflammatory conditions will proceed to persistent inflammation, such as vascular enlargement (vasodilation), raised blood flow, permeability of capillary walls, and neutrophil recruitment into the infected area across the capillary wall (diapedesis) [3]. Inflammatory reactions can also result in losses caused by injury to normal tissues, for example, in inflammation with over-reaction (severe infection), prolonged, autoimmune, or abnormal allergies [4]. Modern drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used to treat over-reaction of inflammatory reactions. In the other hand, NSAIDs have many side effects, one of which is gastric ulcer [5]. Thus, researchers have explored natural medicine from natural resources that is low toxicity, abundant resources, ease of management [6], and one of them is marine products like sea cucumbers.

Sea cucumbers (*Stichopus variegatus* or *Stichopus herrmanni*), also known as gamat, are included in the phylum Echinoderms. They are one of the marine species that have been harvested and traded in more than 70 countries in the world, including Indonesia. *S. variegatus* are found in Indonesian waters because, geographically, Indonesian waters are located between the Pacific Ocean and the Indian Ocean, which is the

How to cite this article: Abdillah S, Rahmat D, Hermawati E, Swandiny GF, Kokadir S, Basilianus E. Anti-inflammatory activity of Stichopus variegatus from Onogate capsule to treat joint pain. J Res Pharm. 2025; 29(5): 1802-1810.

ISSN: 2630-6344

https://doi.org/10.12991/jrespharm.1763400

best habitat for *S. variegatus*. Until now, Indonesia has been a major exporter of *S. variegatus* globally [7]. *S. variegatus* are reported in various Asian countries as a traditional medicine for wound therapy and several diseases. Information regarding the medicinal potential of *S. variegatus* extracts of various species shows efficacy as an antimicrobial, antitumor, antioxidant, antifungal, anticoagulant, antiviral, anti-arthritis, wound healing, immuno-modulatory, and antithrombotic [8], shown to suppress inflammation [9] and enhance the innate immune response [10]. Lin *et al*. have reported the peptide fraction of *S. variegatus* to attenuate oxidative stress and prolong the lifespan of fruit flies and mice [11]. In addition, the pigment isolated from *S. variegatus* has been proven to manage chronic inflammation [12]. However, there is limited data on *S. variegatus* for treating joint pain, especially species originating from Lampung, Indonesia.

In this research, Onogate, as a potential medicine containing 100% *S. variegatus* extract is available in the form of capsules produced by PT Natura Nuswantara Nirmala. Therefore, this study aims to examine the anti-inflammatory effect of *Stichopus variegatus* extract powder in Onogate capsule produced by PT Nuswantara Nirmala Nusantara to treat joint pain. The product will be assigned in-vitro and in-vivo analysis.

2. RESULTS

2.1. Amino acid content in dried *S. variegatus* extract

Table 1 shows the micronutrient and amino acid profile of *S. variegatus* extract from Onogate capsule. Glycine, an essential amino acid, was increased compared to other amino acids, whereas tryptophan was the least abundant one.

Table 1. Amino acid content and micronutrients in dried *S. variegatus* extract (Onogate capsules).

Composition of amino acids	Amino acids in mg/kg dry weight	Micronutrient	mg/kg dry weight
Alanine	504	Calcium (Ca)	66
Arginine	542	Zinc (Zn)	4
Aspartic acid and asparagine	650	Magnesium (Mg)	24
Glutamic acid glutamine	896	Phosphor (P)	18
Glycine	1256	Vitamin A	128
Histidine	96	Vitamin C	660
Cystine	216	Vitamin B1	320
Isoleucine	232	Vitamin B2	1.070
Lycine	406	Protein	36.800
Methionine	126	Fat	4.600
Phenylalanine	408	Collagen	28.814
Proline	576	Omega 3	1.000
Serine	498	Omega 6	2.334
Threonine	494	Omega 9	3.000
Tryptophane	34		
Tyrosine	186		
Valine	280		

2.2. Microbial contaminations test

Table 2. Microbiological examination of *S. variegatus* extract.

Microbiology assay	Results
Total plate count	1.0×10^1
Total mold count	1.0×10^1
Escherichia coli	Negative
Enterobacteriaceae	Negative
Shigella sp.	Negative
Salmonella sp.	Negative
Clostridia	Negative

Microbial contamination was carried out by TPC examination. It was found to be 1.0 × 101, at the tolerance threshold and under control because the value was below 1.0 × 105 colonies/g. In contrast, the result of the TMC examination was 1.0 × 10^1, at the tolerance threshold and still under control because the value was below 1.0 × 10^3 colonies/g. Furthermore, another microbial contamination test such as *E. coli*, *Enterobacteriaceae*, *Shigella* sp., *Salmonella* sp., and *Clostridia* sp. showed negative results (Table 2).

2.3. Assessment of in vitro anti-inflammatory activity

In testing this anti-inflammatory activity, the method was based on inhibiting the denaturation of bovine serum albumin (BSA) protein. BSA was chosen since it is an indicator of protein denaturation that is more sensitive than other albumin indicators. In this study, diclofenac sodium was the reference drug used because it is one of the most commonly used NSAIDs. The percentage inhibition rate was increased following the concentration of Onogate capsules and reference drugs (Table 3).

Table 3. The percentage inhibition rate of protein denaturation.

Group	Rate of inhibition (%)
Onogate capsules (µg/ml)	
25	12.67 ± 0.98
50	16.21 ± 1.23
100	26.06 ± 0.98
200	48.55 ± 2.11
400	73.33 ± 1.26
800	85.51 ± 0.99
Diclofenac Na	
3,13	27.90 ± 1.98
6,25	37.12 ± 1.43
12,5	42.55 ± 0.98
25	49.57 ± 1.11
50	58.99 ± 2.26
100	73.92 ± 2.99

2.4. Assessment of *in vivo* anti-inflammatory activity

Anti-inflammatory activity was obtained with 0.1 mL of 1% freshly prepared carrageenan suspension to stimulate edema (Figure 1). Table 4 shows significant results at 300 and 360 min after treatment using *S. variegatus* extract at 200 mg/kg and 400 mg/kg. Anti-inflammatory activity significantly decreased at 360 min after Onogate capsule treatment compared with diclofenac Na. However, the lowest reduction of anti-inflammatory activity was seen in diclofenac Na 1.79 ± 0.03 (36.7%) and after treatment of *S. variegatus* extract 400 mg/kg 2.19 ± 0.01 (22.6%).

Table 4. Effect of extract of *S. variegatus* at 200 mg/kg and 400 mg/kg and diclofenac Na as compared to carrageenan control in carrageenan-induced paw edema model using vernier calipers.

Group	30 min	60 min	120 min	240 min	300 min	360 min
Carrageenan Carr + diclofenac Na	1.86 ± 0.05 1.79 ± 0.06	2.88 ± 0.03 1.91 ± 0.02 (33.7%)	2.93 ± 0.03 1.92 ± 0.03 (34.5%)	2.94 ± 0.02 1.93 ± 0.02 (34.4%)	2.85 ± 0.03 1.89 ± 0.03 (33.7%)	2.83 ± 0.02 1.79 ± 0.03[a] (36.7%)
Carr + *S. variegatus* 200 mg/kg	2.44 ± 0.05	2.44 ± 0.04 (13.8%)	2.53 ± 0.03 (13.7%)	2.54 ± 0.02 (13.6%)	2.43 ± 0.03[a] (14.7%)	2.42 ± 0.02[a] (15.9%)
Carr+ *S. variegatus* 400 mg/kg	1.98 ± 0.05	2.35 ± 0.02 (16.9%)	2.42 ± 0.03 (17.4%)	2.42 ± 0.02 (17.7%)	2.32 ± 0.02[a] (18.6%)	2.19 ± 0.01[a] (22.6%)

Values are expressed as mean ± SD.
a $P < 0.05$, significant compared to the carrageenan-treated group

Figure 1. Carrageenan assessment on rat (left), and Edema after treatment (right).

3. DISCUSSION

Sea cucumber has attracted researchers to evaluate its bioactive compounds as a natural medicine. The studies have been conducted both using molecular docking simulation and practical laboratory. In a previous study, the bioactive compounds contained in sea cucumber were molecular docking simulated for anti oxidant and anti inflammatory evaluation by inhibiting KEAP1 and iNOS proteins activities [13]. Another study revealed that the bioactive compounds of sea cucumber could attenuate inflammation and tissue damage in mouse ears [14]. Meanwhile, Ridhowati *et al.* [15] found that the safe consumption of *S. variegatus* in an aqueous extract was up to 2500 mg/kg/day in mice.

In this present study, glycine is the highest essential amino acid compared to other amino acids from *S. variegatus* (Table 1). These results are different from other studies conducted by Ridhowati *et al.* [16] that the essential amino acid lysine was the most essential amino acid, while methionine was the least essential amino acid found in *S. variegatus* taken from South Lampung. The various specific regions can cause this difference. As proven, the S. variegatus has an amino acid content of 33.32–54.13 g/100 g per dry weight [17]. The high-protein and low-fat content of the *S. variegatus* extract (Onogate capsule) was also higher than the dry extract of *S. variegatus* taken from South Lampung. *S. variegatus* are majorly consumed for its low-fat and high-protein content and known to possess some properties of tonic food [18]. Another study showed high-protein and low-fat values in this species [18, 19]. It means that there are bacterial and mold contaminations after examining them (Table 2).

Denaturation of protein generates an irregular pattern that induces a change in the hydrophobic, disulfide, and electrostatic hydrogen bonding [2]. When proteins are denatured, autoantigens are produced especially in clinical conditions such as rheumatic arthritis, diabetes, and cancer. This clinical condition is recognized to produce inflammation well. Therefore, inflammatory activity can be arrested by stopping or slowing the rate at which proteins are denatured [20, 21]. In this study, natrium diclofenac (NSAIDs) was used to represent other drugs with similar properties and effect (Tables 3 and 4). NSAIDs hinder inflammation by obstructing the activity of cyclooxygenase. Protein denaturation occurs due to an inflammatory process in some clinical conditions such as arthritis [20]. Mizushima [22] reported that the major function of NSAIDs is to prevent the activity of denaturation. The process of preventing protein denaturation is essential in the antirheumatic activity of NSAIDs.

Bovine serum albumin (BSA) undergoes denaturation (changes in primary and secondary structure) when heated, which is marked by a change in the test solution from clear to cloudy and thickened. It indicates that albumin is damage due to heating and is considered as a foreign material (antigen) by the body. Thus, the body fights through an inflammatory mechanism. The absorbance of the test solution was measured using a UV-visible spectrophotometric instrument at 660 nm. The absorption data obtained was calculated as the percentage inhibition. When the environmental temperature rises, the kinetic energy of the molecules that make up proteins rises, causing them to shift or migrate extremely quickly and destroying the structure of the molecule. Denaturation of proteins is an irreversible procedure that remains unchanged, and when a protein is denaturated, its fluid solubility decreases, allowing it to settle readily [23]. Table 3 shows that the positive control solution (diclofenac sodium) at 3.13 g/mL inhibited the denaturation of proteins by 27.90 ± 1.98, whereas the *S. variegatus* extract solution (*Stichopus variegatus*) at 100 g/mL inhibited protein denaturation by 26.06 ± 0.98.

The anti-inflammatory activity was analyzed using the Carr-induced edema method. It was carried out in the rats' subplantar region of the right hind paw [7]. The solution with anti-inflammatory activity has

a percent inhibition of more than 20% [24]. According to the rat paw edema analysis, the inhibitory action of *S. variegatus* extract (Onogate capsule) was highly significant in an inflammatory response when 400 mg/kg of Onogate capsule with carrageenan-induced was administered (Table 4). It was affirmed by the amount of leukocyte moving to the inflamed site. From the model used in the research, it was found that prostaglandin was normally implicated during an inflammatory response. The development of edema in the paw was as an effect of the link between different inflammatory intermediates and those that increase blood flow [24]. Furthermore, leukocytes initiate a defensive action when an inflammation occurs by releasing an enzyme called lysosome and proteases. However, this process subsequently leads to tissue damage with inflammatory activity [1].

A cell may become more prone to secondary damage when an injury to the cell membrane produces a free radical-induced lipid peroxidation [20]. The membrane proteins regulate the volume and water content present in a cell. They carry out this function by regulating the movement of potassium and sodium ions in and out of the cell. However, when damage to the cell membrane occurs, its functions are inhibited [1]. Inhibiting hemolysis in red blood can provide a better understanding of inflammatory activity due to the similarity of red blood cell membrane with that of the lysosomal membrane [1]. Stabilizing the cell membrane could prevent or slow the rate at which lysis occurs leading to the release of cytoplasmic material that helps prevent or reduce tissue damage and the inflammatory activity [1]. Therefore, any substances that help protect the cell membrane from damage can prevent inflammatory response. Recent studies have shown that *S. variegatus* (Onogate capsule) efficiently stabilises membrane activity and plays an important role in anti-inflammatory response.

The carrageenan test was used to determine the anti-inflammatory effect of various innate products due to its high reactivity to NSAIDs [8]. The alteration of Carr-induced inflammation has been shown to be more diagnostic in its actions as an anti-inflammatory drug in response to human inflammatory disease. Moreover, the quantity of NSAIDs used in this study was similar to the quantity given to the patient [25]. Carrageenan injections can stimulate biphasic events during an inflammatory condition through the prostaglandin hormone [26]. These hormones can initiate redness, secretions, and edema, especially in joint pain.

The ability of carrageenan to stimulate an acute inflammatory response has made it one of the most reliable tests used for screening various anti-inflammatory drugs. A biphasic curve was used to represent the development of edema in carrageenan-induced edema [9]. Carrageenan causes inflammation within an hour after being injected into the substance. This process is partly due to the trauma produced by the injection, histamine and serotonin components. Edema in paw induced by carrageenan was sensitive to the inhibitor called cyclooxygenase, and it was used to determine the action of other nonsteroidal anti-inflammatory agents that are known to block the action of cyclooxygenase that aids in synthesis of prostaglandin [10]. It stimulates the second phase in the anti-inflammatory reaction, which is observed in the third hour. From the study, the percentage inhibition of paw edema was significant ($p < 0.05$) at the fourth hour (22.6% at 400 mg/kg), while diclofenac Na, a major anti-inflammatory drug, was 36,7% (10 mg/kg) (Table 4). Diclofenac Na is a positive control and is acknowledged as an anti-inflammatory reference for paw edema in vivo [27]. Moreover, the previous studies by Aghazadeh-Habashi *et al*. stated that various doses of glucosamine (20, 40, 80, or 160 mg/kg/day) were present in the rats and it was discovered that the level of proinflammatory cytokines IL-1, IL-6, and TNF-α was depleted after a 6-day glucosamine supplementation [28]. It inhibited the increase in nitrite serum concentration, a reliable NO metabolite in rats with OA.

4. CONCLUSION

S. variegatus extract 400 mg/kg from Onogate capsule has an anti-inflammatory effect to treat joint pain. Due to the previous study about the evaluation of safe consumption of sea cucumber, this dosage was safe to be consumed. The limitation of this study was the use of *S. variegatus* extract that has been produced by an industry. This study will give an insight about the potential of *S. variegatus* as a natural resource for medicine, especially for treating joint pain.

5. MATERIALS AND METHODS

5.1. Sample preparation

An animal raw material called S. variegatus was retrieved from Lampung in August 2021. It was dried, crushed, and stored under the general Good Agricultural and Collection Practices requirements identified by Lembaga Ilmu Pengetahuan Indonesia (LIPI) [13].

5.2. Proximate analysis, amino acid, and fatty acid analysis

The assessment, verification, and validation service laboratory unit PT Saraswati in West Java, Indonesia, performed the proximate analysis of water, ash, fat, protein, crude fiber components, and amino and fatty acids, which were also assessed at the same location.

5.3. Microbial contamination

5.3.1. Total plate count (TPC)

Five tubes have been stuffed with 9 mL of peptone dilution fluid (PDF). The dry samples were diluted with 1 mL dilution and inserted into the tube containing PDF until a 10:2 dilution was generated, and the combined component was retrieved by shaking. It was then dissolved until a 10:6 dilution was achieved. Subsequently, 1 mL of each dilution was placed onto a sterilized petri dish and multiplied by two followed by 15-20 mL of medium plate count agar. The petri dish was shaken to distribute uniformly. A control test (blank) was performed to ensure both the medium and diluent sterility. After the medium hardened, the petri dish underwent incubation at 35°C-37°C for 24-48 hours inverted. The growth of colonies in the agar plate was noted and recorded [29].

5.3.2. Total mold and yeast count (TMC)

Three pieces of tube were filled with 9 mL of 0.05% distilled water agars (DWA). The dry sample was prepared using 1 mL dilution to obtain a 10:1 dilution that was placed in the first DWA tube. The sample was then diluted to 10:2 and agitated to obtain a homogeneous solution. Further dilution, up to 10:4, was conducted. A total of 0.5 mL of each dilution was collected and poured on the potato dextrose agar surface, then oscillated and rotated simultaneously around the suspension; thus, it spread evenly and produced a double. To ensure the media and diluent were sterile, a blank test was conducted by pouring a medium on one petri dish and then a diluent and medium on another, then leaving them to solidify. The petri dishes were incubated at 20°C–25°C for 5–7 days. The growth of different fungal colonies was observed after incubation for 5–7 days. Furthermore, 40–60 fungal colonies in the plates were observed [29].

5.4. Assessment of *in vitro* anti-inflammatory activity

The extract's anti-inflammatory properties from Onogate capsule were assessed using an improved version of the bovine serum albumin, or BSA assay. The solution of BSA (0.4%, w/v) has been made in Tris-buffered saline (one tablet was dissolved in 15 mL of deionized water and stirred to yield 0.05 M Tris and 0.15 M sodium chloride, pH 7.6 at 25 °C). The pH was lowered to 6.4 using glacial acetic acid. The extracted substances and reagent combinations were incubated in the water bath at 37°C ± 2°C for 15-20 minutes. The ambient temperature was raised to 70°C and held for 5 minutes. The mixture was allowed to settle at the ambient temperature for 15 minutes. The concentration was determined by measuring absorbance at 680 nm before and after denaturation (1000, 100, 10, 1, 0.1, and 0.01 µg/ml) using a colorimeter. The experiments were performed many times, and the average absorbance was taken into account. The inhibition percentage of protein was calculated concerning the control using the following calculation:

$$\text{Percentage inhibition (\%)} = \left\{ \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \right\} \text{X } 100$$

5.5. Ethical clearance

The in vivo research has been reviewed and declared ethically qualified by the Animal Ethics Commission of the Tropical Biopharmaca Research Center (Komisi Etik Hewan Pusat Studi Biofarmaka Tropika/KEH Trop BRC) with letter number 003-2021 KEH TROP BRC.

5.6. Assessment of *in vivo* anti-inflammatory activity

5.6.1. Animals

A total of 24 male white rats of the Wistar strain (200-250 g) were used in this study. They were divided into six groups: carrageenan control, positive control, normal or healthy rats, group of *S. variegatus* extract test preparations at a dose of 200 mg/Kg BW and 400 mg/Kg BW, and diclofenac Na as reference drug. This size sample was calculated using the Federer formula. Rats were acclimatized for one week in order to adapt to their surroundings. During acclimatization, the test animals were given adequate food and water. At this stage, observations were made on the general condition and weighed daily to obtain healthy test animals. Before giving the solution orally, the test animals were fasted for 8–10 h, and then the leg volume of the test animal was measured (V0). The experiment was conducted on a day the rats were given sample oral administration. Thirty minutes after oral administration of the solution (test preparation), the test animals were inflammation induced in rat paws by injection (subplantar) with 0.1 mL of 1% carrageenan solution.

A modified Winter method was used based on preliminary tests. Induction was carried out on the legs of experimental rats by injecting 0.4 ml of 2% carrageenan suspension subplantarly in the left foot behind the rat. The volume of the rat's paws was measured with a plethysmometer or other device designed according to Archimedes' law, along with rats in quarantine. The test drug was demonstrated by its ability to reduce the volume of edema foot resulting from induction.

5.6.2. Parameter measurement of rat paw edema

Any rise in volume can be measured using a plethysmometer or other equipment that follows Archimedes' law. The plethysmometer has immersion limitations, as indicated by the rats' feet. Foot edema was monitored every 30 minutes for the first 3 hours and every hour for the second 3 hours following carrageenan (Vt) administration.

5.6.3. Evaluation of edema volume

The data were obtained as a volume curve of the test animal's leg edema. Edema volume is the difference between the leg edema of the test animals before and after being induced, with the formula:

$$Ve = Vt - V0$$

Note:
Ve = rat paw edema volume
Vt = the volume of the legs of the test animals (rats) after being induced by carrageenan
V0 = initial leg volume of test animals (rats) before carrageenan induction

Calculation of percent inhibition by formula:

$$\text{Inhibition (\%)} = \frac{\Delta Vkt - \Delta Vt}{\Delta Vkt} \times 100\%$$

where:
$\Delta Vkt = Vkt - Vo$
$\Delta Vt = Vt - Vo$

Note:
Vt = leg volume of the test preparation group at time t
Vkt = negative control group leg volume at time t
V0 = initial leg volume before induction
ΔVt = volume of leg edema in the test preparation group
ΔVkt = volume of leg edema in the negative control group

The anti-inflammatory activity was calculated using the relation

$$\text{Percentage inhibition of edema} = \frac{T - T_0}{T} \times 100$$

Note:
T = thickness of the paw in control group
T_0 = thickness of the paw edema in the test compound of the treated group

5.7. Statistical analysis

One-way ANOVA was used to analyze the result of the anti-inflammatory activity, which was expressed as mean increase in the paw diameter ± SD. A $P < 0.05$ was considered statistically significant.

Author contributions: Concept – S.A., D.R.; Design – S.A., D.R.; Resources – S.A., D.R.; Materials – S.A., D.R.; Data Collection and/or Processing – E.H.,G.F.S., S.K., E.B.; Analysis and/or Interpretation – G.F.S., S.K., E.B.; Literature Search – G.F.S., S.K., E.B.; Writing – S.A., D.R.; Critical Reviews – E.H.,G.F.S., S.K., E.B.

Conflict of interest statement: The authors declared no conflict of interest.

REFERENCES

[1] Oronsky B, Caroen S, Reid T. What exactly is inflammation (and what is it not?). Int J Mol Sci. 2022; 23(23): 14905. https://doi.org/10.3390/ijms232314905
[2] Kotas ME, Medzhitov R. Homeostasis, inflammation, and disease susceptibility. Cell. 2015; 160(5): 816-827. https://doi.org/10.1016/j.cell.2015.02.010
[3] Pahwa R, Goyal A, Jialal I. Chronic inflammation. In: Statpearls. StatPearls Publishing, Treasure Island (FL), 2024.
[4] Suzuki K. Chronic inflammation as an immunological abnormality and effectiveness of exercise. Biomolecules. 2019; 9(6): 223. https://doi.org/10.3390/biom9060223
[5] Pangestuti R, Arifin Z. Medicinal and health benefit effects of functional sea cucumbers. J Tradit Complement Med. 2018; 8(3): 341-351. https://doi.org/10.1016/j.jtcme.2017.06.007
[6] Jo H-G, Baek CY, Lee J, Hwang Y, Baek E, Hwang JH, Lee D. Anti-inflammatory, analgesic, functional improvement, and chondroprotective effects of *Erigeron breviscapus* (Vant.) Hand.-Mazz. Extract in osteoarthritis: An in vivo and in vitro study. Nutrients. 2024; 16(7): 1035. https://doi.org/10.3390/nu16071035
[7] Huang G-J, Huang S-S, Lin S-S, Shao Y-Y, Chen C-C, Hou W-C, Kuo Y-H. Analgesic effects and the mechanisms of anti-inflammation of ergostatrien-3β-ol from *Antrodia camphorata* submerged whole broth in mice. J Agric Food Chem. 2010; 58(12): 7445-7452. https://doi.org/10.1021/jf1013764
[8] Kumar PP, Kuttan G. *Vernonia cinerea* L. Scavenges free radicals and regulates nitric oxide and proinflammatory cytokines profile in carrageenan induced paw edema model. Immunopharmacol Immunotoxicol. 2009; 31(1): 94-102. https://doi.org/10.1080/08923970802438391
[9] Vinegar R, Schreiber W, Hugo R. Biphasic development of carrageenin edema in rats. J Pharmacol Exp Ther. 1969; 166(1): 96-103.
[10] Seibert K, Masferrer JL. Role of inducible cyclooxygenase (COX-2) in inflammation. Receptor. 1994; 4(1): 17-23.
[11] Lin L, Zhu Q, Zheng L, Zhao M, Fan J, Liu S. Preparation of sea cucumber (*Stichopus variegates*) peptide fraction with desired organoleptic property and its anti-aging activity in fruit flies and d-galactose-induced aging mice. J Funct Foods. 2020; 69: 103954. https://doi.org/10.1016/j.jff.2020.103954
[12] Dolmatova LS, Dolmatov IY. Different macrophage type triggering as target of the action of biologically active substances from marine invertebrates. Mar Drugs. 2020; 18(1): 37. https://doi.org/10.3390/md18010037
[13] Wargasetia TL, Ratnawati H, Widodo N, Widyananda MH. Antioxidant and anti-inflammatory activity of sea cucumber (*Holothuria scabra*) active compounds against KEAP1 and INOS protein. Bioinforma Biol Insights. 2023; 17: 117793222211496. https://doi.org/10.1177/11779322221149613
[14] Olivera-Castillo L, Grant G, Kantún-Moreno N, Barrera-Pérez HA, Montero J, Olvera-Novoa MA, Carrillo-Cocom LM, Acevedo JJ, Puerto-Castillo C, May Solís V, Pérez-Vega JA, Gil-Zamorano J, Hernández-Garibay E, Fernández-Herrera MA, Pérez-Tapia M, Medina-Contreras O, Villanueva-Toledo JR, Rodriguez-Canul R, Dávalos A. A glycosaminoglycan-rich fraction from sea cucumber *Isostichopus badionotus* has potent anti-inflammatory properties in vitro and in vivo. Nutrients. 2020; 12(6): 1698. https://doi.org/10.3390/nu12061698
[15] Ridhowati S, Chasanah E, Syah D, Zakaria FR. Evaluation of the safe consumption of aqueous extract of flour from *Stichopus variegates*. Biomed Res Ther. 2019; 6(11): 3452-3459. https://doi.org/10.15419/bmrat.v6i11.574
[16] Ridhowati S, Chasanah E, Syah D, Zakaria F. A study on the nutrient substances of sea cucumber *Stichopus variegatus* flour using vacuum oven. Int Food Res J. 2018; 25(4):1419-1426.

[17] Xu X, Yin P, Wan C, Chong X, Liu M, Cheng P, Chen J, Liu F, Xu J. Punicalagin inhibits inflammation in LPS-induced RAW264.7 macrophages via the suppression of TLR4-mediated MAPKs and NF-KB activation. Inflammation. 2014; 37(3): 956-965. https://doi.org/10.1007/s10753-014-9816-2
[18] Haider MS, Sultana R, Jamil K, Tarar OM, Afzal W. A study on proximate composition, amino acid profile, fatty acid profile and some mineral contents in two species of sea cucumber. JAPS J Anim Plant Sci. 2015; 25(1): 168-175..
[19] Ridzwan BH, Hanita MH, Nurzafirah M, Norshuhadaa MPS, Hanis ZF. Free fatty acids composition in lipid extracts of several sea cucumbers species from Malaysia. Int J Biosci Biochem Bioinforma. 2014; 4(3): 204.
[20] Umapathy E, Ndebia EJ, Meeme A, Adam B, Menziwa P, Nkeh-Chungag BN, Iputo JE. An experimental evaluation of *Albuca setosa* aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation. J Med Plants Res. 2010; 4(9): 789-795.
[21] Raggi P, Genest J, Giles JT, Rayner KJ, Dwivedi G, Beanlands RS, Gupta M. Role of inflammation in the pathogenesis of atherosclerosis and therapeutic interventions. Atherosclerosis. 2018; 276: 98-108. https://doi.org/10.1016/j.atherosclerosis.2018.07.014
[22] Mizushima Y. Inhibition of protein denaturation by antirheumatic or antiphlogistic agents. Arch Int Pharmacodyn Ther. 1964; 149: 1-7.
[23] Paul S, Paul S. Molecular insights into the role of aqueous trehalose solution on temperature-induced protein denaturation. J Phys Chem B. 2015; 119(4): 1598-1610. https://doi.org/10.1021/jp510423n
[24] Sautebin L, Ialenti A, Ianaro A, Di Rosa M. Endogenous nitric oxide increases prostaglandin biosynthesis in carrageenin rat paw oedema. Eur J Pharmacol. 1995; 286(2): 219-222. https://doi.org/10.1016/0014-2999(95)00581-5
[25] Morris CJ. Carrageenan-induced paw edema in the rat and mouse. Methods Mol Biol (Clifton, NJ). 2003; 225: 115-121. https://doi.org/10.1385/1-59259-374-7:115
[26] Reanmongkol W, Noppapan T, Subhadhirasakul S. Antinociceptive, antipyretic, and anti-inflammatory activities of *Putranjiva roxburghii* Wall. leaf extract in experimental animals. J Nat Med. 2009; 63(3): 290-296. https://doi.org/10.1007/s11418-009-0336-6
[27] Fayez N, Khalil W, Abdel-Sattar E, Abdel-Fattah A-FM. In vitro and in vivo assessment of the anti-inflammatory activity of olive leaf extract in rats. Inflammopharmacology. 2023; 31(3): 1529-1538. https://doi.org/10.1007/s10787-023-01208-x
[28] Aghazadeh-Habashi A, Kohan MHG, Asghar W, Jamali F. Glucosamine dose/concentration-effect correlation in the rat with adjuvant arthritis. J Pharm Sci. 2014; 103(2): 760-767. https://doi.org/10.1002/jps.23819
[29] Lestantyo D, Husodo AH, Iravati S, Shaluhiyah Z. Safe food handling knowledge, attitude and practice of food handlers in hospital kitchen. Int J Public Health Sci. 2017; 6(4): 324-330. http://doi.org/10.11591/ijphs.v6i4.10778.