Anti-inflammatory Activities of Gel Extract Marine Sponges *(Axinella Carteri*) to White Mice Male

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ABSTRACT

Indonesia, especially the Kepulauan Riau, has very wide marine waters, and is rich in natural resources and marine biota diversity. One of the marine biota that has the potential to be developed is the marine sponge as an anti-inflammatory because it contains the flavonoid compound which is able to inhibit the cyclooxygenase enzyme that functions in the biosynthesis of prostaglandins. The purpose of this study was to determine whether marine sponge extract had anti-inflammatory activity against white male mice and to determine the optimal concentration of sea sponge extract gel was effective in inhibiting inflammationThe research method is experimental which uses carrageenan induction on the feet of mice. The thick extract of the marine sponge *(Axinella carteri)* will be formulated in the form of a gel with a concentration of 0.02%, 0.03%, 0.04% and a comparison (Voltaren emulgel) and a negative control in the form of (carbopol gel basis) gel. Parameters observed were inflammation caused by carrageenan and inhibition after treatment. Research data processing will be presented in the form of tables and graphs.This study showed that marine sponge extract gel preparations with a concentration of 0.02% to 0.04% had a healing effect on inflammation, and which had anti-inflammatory activity comparable to the positive control of Natrium diclofenac gel 1% is a marine sponge extract gel 0.04% with an inhibition percentage of 88.73%.The administration of marine sponge extract gel *(Axinella carteri)* gave a positive response to reducing the size of the leg edema of mice. From the percentage of inhibition that was calculated and the effective gel concentration was 0.04% because it had relatively the same effectiveness as the percentage of inhibition of the positive control. This can be used as a new alternative that utilizes natural ingredients, especially those from Natuna Regency, Riau Islands in the treatment of inflammation with fewer side effects.

Keywords: *Marine sponge, anti-inflammatory, gel, carrageenan.*

1. INTRODUCTION

Indonesia is an archipelagic country that has very wide marine waters, and is rich in natural resources and marine diversity. One of them is in Natuna Regency, Riau Islands. Marine sponges are one of the components of marine biota that make up coral reefs which have bioactive compounds that are still not widely used. Several studies have stated that marine sponges from the Haliclona genus have various secondary metabolite activities, including cytotoxic, antifungal, antimicrobial [1], and anti-inflammatory [2].

Inflammation is the body's response to injury or infection. When an injury occurs, the body will try to neutralize and eliminate harmful agents from the body and make preparations for tissue repair [3]. The presence of an inflammatory process is characterized by the appearance of redness, swelling in the area of inflammation, heat, and the onset of pain [4].

Several studies have found secondary metabolites contained in sea sponges, namely alkaloids, terpenoids, flavonoids, steroids, and others. One of the secondary metabolite compounds that can inhibit inflammation are flavonoids. One of the secondary metabolites that can inhibit inflammation is flavonoids. Flavonoids are able to inhibit cyclooxygenase enzymes that play a role in the biosynthesis of prostaglandinand inhibit lipookigenase enzymes that play a role in leukotriene biosynthesis in the formation of inflammation [5]. In Osman's research [6] it was stated that alkaloids also have anti-inflammatory abilities by preventing the synthesis or action of certain pro-inflammatory cytokines, suppressing histamine release, and nitric oxide production.

Many studies have been carried out on the anti-inflammatory activity of marine sponges,

namely methanol extract from the marine sponge *Hyrtios erectus*, showing that this species of sponge has a potential inhibitory anti-inflammatory effect on the denaturation of bovine serum albumin at a concentration of 25µg/ml of 91.22% [7]. This is in line with other studies on the anti-inflammatory activity of the sponge *Aaptos Sp.* which has an effective dose of 50 ppm [8]. Research conducted by Andriani [9] showed that the methanol extract of the marine sponge *Haliclona Amboinensis* could reduce NO production by >80%, as well as the anti-inflammatory in gel preparations of *Xestospongia Sp.* sponge ethanol extract. showed that the formula that had a high anti-inflammatory effect was at a concentration of 0.04% by 25% [10].

Based on the description above, the researchers are interested in conducting research on the anti-inflammatory activity of marine sponge extract gel (Axinella carteri) from Natuna Waters on male white mice. This is because no similar research has been found on the anti-inflammatory activity of marine sponges originating from the Riau Islands, even though these islands have a very wide sea with abundant marine natural resources.

2. MATERIALS AND METHOD

2.1. Research Time and Place

This research was conducted during March-May 2020 at the Pharmacology Laboratory of the Mitra Bunda Health Institute, Batam.

2.2. Tools and Materials

The tools used in this study were *rotary evaporator* (Heidolp), maceration bottle, analytical balance (Kenko), 1 mL syringe (Terumo), stopwatch, caliper (Tricle Brand), mouse cage, glass beaker (Pyrex), measuring cup (Pyrex), porcelain dish, pH meter (Milwaukee), brookfield viscometer, stove, stirring rod (Pyrex), measuring flask (Pyrex), dropper (Pyrex), glass object, filter paper, pycnometer (Pyrex), centrifuge (Hettich) , petri dish (Pyrex), mortar and pestle, analytical balance (Kenko).

The materials used were marine sponge (Axinella carteri) from Natuna Waters, methanol (Merck), carbopol (Merck), 1% carrageenan (Merck), diclofenac sodium as a positive control (Voltaren®), aquadest (Brataco), propylene glycol (Merck). , TEA (Merck), methyl paraben (Merck), 0.9% physiological NaCl (Merck), Mayer's reagent (Merck), concentrated HCl (Merck), chloroform (Merck), ammonia 10% (Merck), mg powder (Merck), 2N hydrochloric acid (Merck), 1% FeCl3 (Merck), acetic acid (Merck), concentrated H2SO4 (Merck), chloroform (Merck), mice.

2.3. Sampling

The research sample used was is marine sponges from Natuna waters and this collection is carried out in Kelarik Village, North Bunguran District, Natuna Island, Natuna Regency, Riau Islands. The marine sponge arrived at the Pharmaceutical Laboratory on Tuesday, February 11, 2020.

2.4 Sample Collection and Preparation of the Methanol Extract

4 kg of fresh sea sponge samples that have been washed, drained, chopped and then macerated with methanol until completely submerged in a brown glass bottle and stored in a light protected place for 3x5 days while occasionally stirring and filtered with filter paper. The methanol maserate was combined and concentrated with a Rotary Evaporator until a thick extract was formed and then weighed [11, 12].

2.5. Marine Sponge Extract Phytochemical Screening

2.5.1. Alkaloids

A total of 4 ml of marine sponge extract was put in a test tube, added 2 ml of chloroform and 5 ml of 10% ammonia, then added 10 drops of 2N sulfuric acid. The upper part of the formed phase is taken and 4-5 drops of Mayer reagent are added. If a precipitate is formed, it indicates that the sample contains alkaloids, with Mayer's reagent giving a yellow-red precipitate [13].

2.5.2. Flavonoids

As much as 1 ml of thick marine sponge extract add magnesium powder 0.05 mg. Then 10 drops of concentrated hydrochloric acid were added. The presence of flavonoids is characterized by the formation of a reddish black, yellow or orange color [13].

2.5.3. Saponins

A total of 5 drops of thick marine sponge extract then add hot aquadest then shaken for 15 minutes then add 2 drops of 2N hydrochloric acid, tested positive for saponins if a stable foam is formed ± 7 minutes [14].

2.5.4. Tanins

A total of 10 drops of thick marine sponge extract were added with methanol, then 1 ml of solution was taken into the test tube and 2-3 drops of 1% FeCl3 solution were added. It is declared positive if the formation of a dark blue or blackish green color [15].

2.5.5. Terpenoids and Sterois

A total of 10 drops of thick marine sponge extract were added 2-3 drops of acetic acid and then 2-3 drops of concentrated H2SO4 were added. The presence of triterpenoids was indicated by the occurrence of an orange opurple color change while steroids gave a blue or green color change [16].

2.6. Marine Sponge Extract Gel Formula

The marine sponge extract gel formula was made in 100 grams with various concentrations of 0.02%, 0.03% and 0.04%. The formula can be seen in table 1 as follows: [10].

Table 1. Gel Formulation

Materials	Function	Formula % (b/b)			
		F0	FI	FII	FIII
Extract of Axinella carteri	Active substance	-	0.02	0.03	0.04
Carbopol	Gel base	1	1	1	1
TEA	Stabilizer	0.5	0.5	0.5	0.5
Propylene glycol	Humectant	10	10	10	10
Methyl paraben	Preservative	0.2	0.2	0.2	0.2
Aquadest	Solvent	Ad 100	Ad 100	Ad 100	Ad 100

Carbopol was developed in hot water at a temperature of 70-80ºC then stirred using a mortar and pestle so that it was completely dispersed and a gel base was formed, then triethanolamine was added little by little and stirred (mixture 1). Methyl paraben was dissolved in hot water to a temperature of 70°C until dissolved and then cooled, after that it was added little by little sea sponge extract and propylene glycol and then homogenized (mixture 2). Added mixture 2 into mixture 1 then ground again until homogeneous. Once homogeneous, put into a container.

2.7. Evaluation of Marine Sponge Extract Gel

2.7.1. Organoleptic Gel Test

Organoleptic testing was carried out on gel preparations made visually by observing changes in shape, smell, and color.

2.7.2. Gel Homogeneity Test

Preparations were taken at 3 different sampling points and smeared on a transparent glass. If there are no coarse grains, the test preparation is declared homogeny [17].

2.7.3. pH Test

The pH test was carried out using a pH meter by weighing 1gram of the gel preparation and then dissolving it with 10mL of distilled water and stirring until evenly distributed. After that the pH meter was dipped into the solution and the results were recorded. The pH that meets the requirements according to the pH of the skin is 4.5-6.5 [18].

2.7.4. Gel Spread Test

A total of 0.5 grams of the preparation is placed in the center of a scaled round glass, then covered with another round glass. Measurement of the diameter of the distribution of the preparations longitudinally and transversely, and each additional load of 50 grams to a total weight of 150 grams. The

dispersion that meets the requirements is 5-7 cm [19].

2.7.5. Gel Adhesion Test

The adhesion test was carried out by placing 0.5 grams of gel on a slide then covering it with another slide, and being given a load of 1 kg for 3 minutes. Determination of adhesion in the form of time required until the two slides are released. The requirement for adhesion is more than 1 second [19].

2.7.6. Gel Viscosity Test

Viscosity test was carried out using a viscometer on 25mL of gel preparation using spindle number 3 and a speed of 12 rpm. Good gel viscosity is 2000 - 4000 cPs.

2.7.7. Marine Sponge Extract Gel Stability Test

• Room Temperature (±25ºC)

The gel samples were stored at room temperature (±25ºC) for four weeks, then weekly observations were made, namely organoleptic examination (observed shape, color, and odor), homogeneity, pH measurement, viscosity measurement, adhesion and spreadability.

• Low Temperature (4±2ºC)

The gel samples were stored at low temperature (4±2ºC) for 4 weeks, then observed every week, namely organoleptic examination (observed shape, color, and odor), homogeneity, pH measurement, viscosity measurement, adhesion, and spreadability.

• High Temperature (40ºC)

The gel sample was stored at high temperature (40ºC) for 4 weeks, then observed weekly, namely organoleptic examination (observed shape, color, and odor), homogeneity, pH measurement, viscosity measurement, adhesion, and spreadability.

2.8. Grouping and Acclimatization of Test Animals

The test animals were grouped into 5 groups, each group consisting of three test animals. Fifteen healthy mice (20grams-30grams) were selected for this study. Mice were fed and watered and then acclimatized for one week before the experiment.

2.9. Preparation of 1% Carrageenan Solution

A total 0.1 grams of carrageenan, then gradually add 10 ml of physiological NaCl to the mark, then incubate at 37ºC for 24 hours [20].

2.10. In Vivo Antiinflammatory Test on Male White Mice

Animals were grouped into 5 groups, each consisting of 3 tails, namely; the control group was negative (not containing sea sponge extract), the comparison group used diclofenac sodium emulgel, the F1 group was 0.02%, the F2 group was 0.03% and the F3 group was 0.04%. Test animals were fasted for 18 hours before testing and were still given water. On the day of testing, each test animal was weighed and marked on its left ankle and the foot diameter of the mice was measured as the initial diameter (D0). Injected 0.1 mL of 1% carrageenan suspension on the soles of the test animal's feet suplantar. After 1 hour, each group was treated topically according to the treatment group. The diameter of the test animal's left leg was measured again after 1 hour. Changes were recorded as the diameter of the left leg of the test animal after treatment (Dt) and measurements were made every 60 minutes for 6 hours.

2.11. Data Analysis

The diameter of the mice was expressed in cm. The percentage of inflammation was calculated by comparing it to the initial diameter (Do) before the injection of carrageenan. The formula used is[5]:

$$\% \text{ inflamation} = \frac{Dt-Do}{Do} \times 100\%$$

Where:

Do = Diameter of the feet of mice at zero time (before any treatment)

Dt = Diameter of the feet of mice at time t

Furthermore, to see the anti-inflammatory effect, the % inhibition power can be calculated using the following formula:

$$\% \text{ Inflammation inhibition} = \frac{a-b}{a} \times 100\%$$

Where:
a = % edema in the negative control group
b = % edema in the treatment group

3. RESULT AND DISCUSSION

3.1. Extraction

Axinella carteri which was used was obtained from the sea waters of out in Kelarik Village, North Bunguran District, Natuna Island, Natuna Regency, Riau Islands which is used as much as 4 kg. The results of the maceration process using 96% methanol solvent, obtained as much as 99.74 g thick extract with the yield of 2.5 %.

Figure 1. Marine sponge *Axinella carteri*

3.2. Secondary Metabolite Screening

Marine sponge extract positive contains bioactive compounds that play a role in providing biological properties or effects, including alkaloids, flavonoids, terpenoids and saponins.

3.3. Organoleptic Gel Test

Organoleptic tests were carried out on gel preparations made visually by observing changes in shape, smell, and color. Organoleptic testing aims to determine the organoleptic preparations which include color, shape and aroma. The results showed that the four marine sponge extract gel formulas produced a clear white color on a gel basis (negative control), yellowish white at a concentration of 0.02% (FI), faded yellow at a concentration of 0.03% (FII), to yellow at a concentration of 0.04% (FIII).), this corresponds to the concentration of the addition of the extract in the gel preparation where the color of the marine sponge extract is brownish yellow. The more concentration of extract added, the more yellow the gel preparation color will be. All formulas obtained are semisolid thick and have a characteristic carbopol odor.

Table 2. Observations of organoleptic gel preparations

Concentration of extract	Temperature Storage	Observation	Week Of				
			0	1	2	3	4
0	Low temperature	Form Color Smell	Semi-Solid Gel White Karbopol's Typical Smell				
	Room temperatue						
	High temperature						
0.02%	Low temperature	Form Color Smell	Semi-Solid Gel Yellowish White Karbopol's Typical Smell				
	Room temperature						
	High temperature						
0.03%	Low temperature	Form Color Smell	Semi-Solid Gel Fade Yellow Karbopol's Typical Smell				
	Room temperature						
	High temperature						
0.04%	Low temperture	Form Color Smell	Semi-Solid Gel Yellow Karbopol's Typical Smell				
	Room temperature						
	High temperature						

3.4. Gel Homogeneity Test

The homogeneity test was carried out to see the mixing of the ingredients contained in the gel preparation so that when using the gel preparation it could be absorbed and enter the skin. This homogeneity is characterized by the absence of grains or fine particles that are visible on the object glass cross section

[17].The homogeneity examination of the four gel formulations showed homogeneous results because there were no granules in the prepared preparations. This shows that the preparation of the active substance in the marine sponge extract is expected to be delivered evenly at each application of the gel.

Table 3. The observation of gel homogeneity

Concentration of extract	Temperature Storage	Week Of				
		0	1	2	3	4
0	Low temperature	Homogeneous				
	Room temperatue					
	High temperature					
0.02%	Low temperature	Homogeneous				
	Room temperature					
	High temperature					
0.03%	Low temperature	Homogeneous				
	Room temperature					
	High temperature					
0.04%	Low temperature	Homogeneous				
	Room temperature					
	High temperature					

3.5. pH Gel Test

Testing the pH value of the gel preparation aims to ensure that the gel preparation made has a pH that is in accordance with the physiological pH of the skin. As shown in Table 4, showed no increase or decrease in pH. The range of pH values is still within the safe limits for topical preparations, namely 4.5-6.5 [18].

Table 4. Observation pH gel test

Concentration	Temperature Storage	Weeks of				
		0	1	2	3	4
0	Room temperature	4.50	4.82	5.00	5.30	5.45
	Low temperture	4.65	5.01	5.25	5.12	6.00
	High temperature	4.65	4.60	4.55	5.67	5.55
0.02%	Room temperature	5.04	5.00	5.03	5.10	5.04
	Low temperature	5.71	5.03	5.11	5.20	5.50
	High temperature	5.16	5.23	5.00	5.45	5.67
0.03%	Room temperature	5.03	5.00	5.21	5.11	5.15
	Low temperature	5.35	5.44	5.60	5.25	5.26
	High temperature	5.02	5.00	4.59	5.06	5.12
0.04%	Room temperature	5.10	5.00	5.00	5.00	5.05
	Low temperature	5.07	5.09	5.27	5.01	5.01
	High temperature	5.00	5.13	5.44	5.78	5.67

3.6. Gel Viscosity Test

The results of the marine sponge extract gel viscosity test are as shown in table 5. It shows that there is no increase in viscosity. It can be concluded that the moisture contained in the gel preparations made is maintained.

Table 5. Observation of gel viscosity

Concentration	Temperature Storage	Weeks of				
		0	1	2	3	4
0	Room temperature	3800	4000	3900	3850	3800
	Low temperture	3850	3750	3700	3800	3850
	High temperature	3700	3850	3250	3450	3400
0.02%	Room temperatura	3850	3750	3700	3800	3850
	Low temperature	3900	3950	3900	3850	3800
	High temperature	3400	3450	3300	3200	3100
0.03%	Room temperature	3400	3500	3600	3550	3700
	Low temperature	3500	3400	3550	3600	3700
	High temperatura	3700	3600	3800	3900	3700
0.04%	Room temperature	3750	3700	3800	3750	3700
	Low temperature	3650	3600	3700	3750	3700
	High temperature	3200	3400	3500	3200	3100

3.7. Gel Spread Test

The results of the dispersion test shown in table 6 show that the gel dispersion did not change significantly, this is in line with the resulting viscosity results. Based on the results obtained, the dispersion power is still within

Table 6. Observation of gel spreadibility

Concentration	Temperature Storage	Weeks of				
		0	1	2	3	4
0	Room temperature	6.25	6.09	5.55	6.00	5.30
	Low temperature	5.05	5.05	5.06	5.10	5.25
	High temperature	6.35	5.20	5.45	5.15	6.00
0.02%	Room temperature	6.14	6.15	5.90	6.10	5.10
	Low temperature	5.07	5.12	5.35	5.15	5.32
	High temperature	6.50	5.45	6.00	5.25	5.50
0.03%	Room temperature	6.86	6.60	6.00	6.25	5.28
	Low temperature	5.00	6.20	5.20	5.65	5.05
	High temperature	6.15	5.30	5.10	5.20	5.10
0.04%	Room temperature	6.50	6.10	5.85	6.20	5.29
	Low temperature	5.02	5.05	5.03	5.13	5.20
	High temperature	6.70	5.70	5.15	5.20	5.45

the allowable limit, which is in accordance with the parameters of 5-7 cm [19].

3.8. Gel adhesion test

The test of gel adhesion was carried out to see the ability of the prepared preparation to adhere to the skin. The general property of the gel is that it is able to adhere to the surface where it is applied for a long time before the preparation is washed or cleaned. The longer the adhesion to the gel preparation, the better the gel preparation. The preparation is still within the permissible limits, namely the requirement for the adhesion of topical preparations to be more than 1 second [19].

3.9. In Vivo Antiinflammatory Test on Male White Mice

Figure 2. Comparison of the feet of mice that have been injected with carrageenan and those that have not been injected with carrageenan

Figure 3. Before and after the feet of the mice

Inflammation is a localized protective response caused by tissue damage caused by physical trauma, damaging chemical substances, or microbiological substances. Anti-inflammatory functions to destroy, reduce, or localize (sekuster) both damaging agents and damaged tissues. Before the test day, the test animals were acclimatized for a week with the aim of adapting the test animals to the new environment. Furthermore, each mouse that will be used is fasted for 18 hours and only given to drink, this is intended to avoid the possible influence of food on the effect of the gel to be tested so that the gel given can give a good effect. Testing the anti-inflammatory activity in this study by inducing 1% carrageenan on the left foot of mice. Based on research that has been done that after giving carrageenan, the feet of mice experience inflammation. Induction of carrageenan resulted in the formation of inflammation consisting of two phases, namely 1-2 hours after injection of carrageenan, causing trauma due to inflammation caused by carrageenan. In the first phase, there is a release of serotonin and histamine to the site of inflammation and an increase in prostaglandin synthesis in the damaged tissue. In the second phase, prostaglandin release occurs and is mediated by bradykinin and leukotrienes

The anti-inflammatory effect was tested using a caliper. The use of a caliper with the aim of measuring the diameter or thickness of inflammation on the soles of mice. This caliper measurement method is one method that is often used in anti-inflammatory tests, it is relatively simple, both from the required instruments, treatment processes, observations, measurements to data processing.

Figure 4 shows the average diameter of edema measured using a caliper in each treatment

group. Figure 5 shows a different decrease in inflammation in each treatment group. The administration of gel base (negative control group) did not decrease inflammation, this is due to the absence of active substances that can reduce inflammation in the soles of mice. Giving gel formula I (0.02%) decreased inflammation by 74.79% at the 6th hour, while the FII (0.03%) and FIII (0.04%) groups were 77.1% and 88, respectively. 73% and in the positive control group there was a significant decrease in edema, which was 91.59% at the 6th hour. This significant decrease occurred because the positive control was treated with Voltaren® emulgel, where votaren contains diclofenac sodium which inhibits the action of the cyclooxygenase enzyme which functions to help the formation of prostaglandins during injury and causes pain and inflammation. The positive control group also showed that edema inhibition occurred at the 3rd hour. The graphic data above shows that the FI (0.02%) group, FII (0.03%) and FII (0.04%) took slightly longer time than the positive control group.

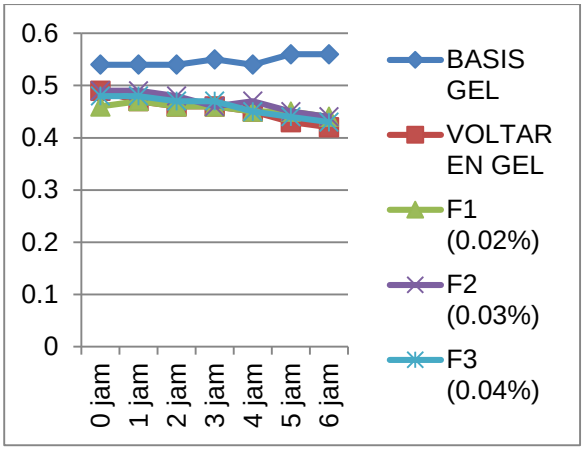

Figure 4. Average Diameter of inflammation Against Time Using Calipers

Figure 5 also shows that all treatment groups experienced a decrease in edema except for the negative control group which did not contain thick extract of sea sponge which is the active substance. This is because the negative control induced by carrageenan was only treated in the form of a gel base so that there was no healing process to reduce inflammation and the inflammatory response only depended on the natural immunity of mice. This causes the edema to continue so that the percentage reduction in edema is 0%.

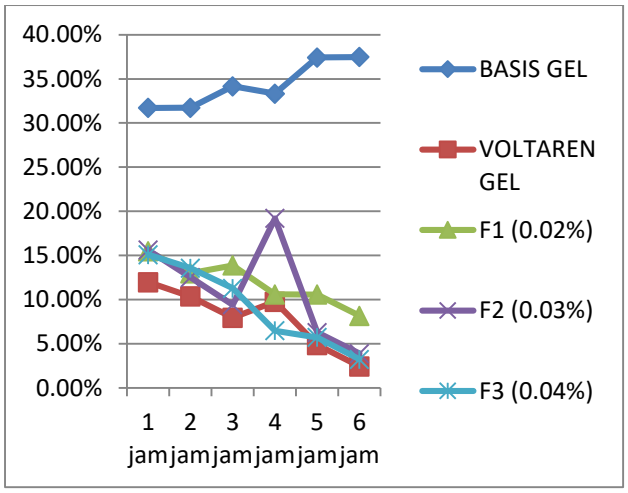

Figure 5. Average Percentage of inflammation Against Time

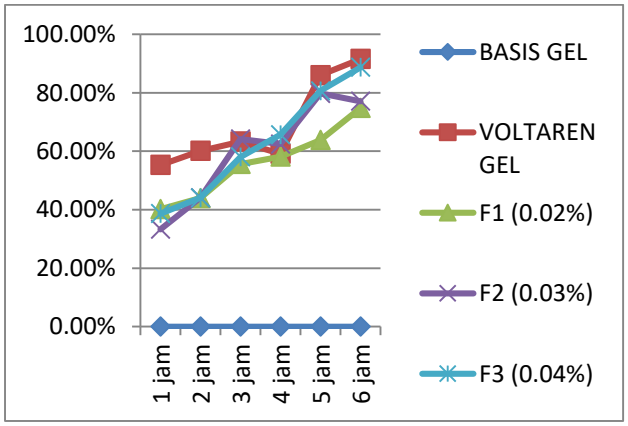

Figure 6. Results of the Percentage of inflammation Inhibition in Each Treatment

From the data on the percentage decrease in the diameter of the mice's paw edema for 6 hours, it shows that there is an anti-inflammatory potential produced. A material is said to have anti-inflammatory activity if the carrageenan-induced test animals experienced a reduction in swelling of up to 50% or more [21]. From this study, it was found that the sea sponge extract gel had great potential in inhibiting inflammation as indicated by the percentage of overall edema inhibition of up to 50% or more and not much different from the positive control results, namely Voltaren® emulgel.

Based on the average percentage of inhibition produced, the FI (0.02%) group, FII (0.03%) and FIII (0.04%) had effectiveness and activity as anti-inflammatory, this was because the average value of inhibition or inhibition showed great results. And the one who has the greatest activity is the FIII group (0.04%) at 88.73%, this is in line with the results obtained from the positive control group, which is 91.59%. The existence of anti-inflammatory activity because sea sponge extract contains secondary metabolites, namely alkaloids and flavonoids. Alkaloids have anti-inflammatory properties by preventing the synthesis or action of certain proinflammatory cytokines, suppressing histamine release, and nitric oxide production[6]and flavonoids can inhibit cyclooxygenase enzymes that play a role in prostaglandin biosynthesis and inhibit lipookigenase enzymes that play a role in leukotriene biosynthesis in the formation of inflammation [5].

4. CONCLUSION

Based on the research that has been done, it can be concluded that the sea sponge extract gel has anti-inflammatory activity on the soles of mice induced by carrageenan 1% at various concentrations of FI (0.02%) of 74.79%, FII (0.03%) of 71 .10%, and FIII (0.04%) of 88.73%. And the sea sponge extract gel treatment group with a concentration of 0.04% which showed the best inhibition compared to other treatment groups. compared to other treatment groups.

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