

Research Report

Anti-inflammation effects of *Sardinella longiceps* oil against paw oedema on *Rattus novergicus* induced by 1% carrageenan

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ABSTRACT

Background: People usually used non steroid anti-inflammation drugs (NSAID) such as aspirin in chronic inflammation treatment. However, using NSAID at long term therapy will cause many effects such as nausea and vomiting. *Sardinella longiceps* oil, on the other side, is reported as an alternative treatment for anti-inflammation since it is natural and also contained eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Thus, it may reduce paw oedema. **Purpose:** The aim of this study was to know anti-inflammation effects of *Sardinella longiceps* oil against paw oedema of Wistar rats induced by 1% carrageenan. **Methods:** The samples of this research were 32 Wistar rats which were divided into four groups, in group 1, the rats were given aquadest; in group 2, the rats were given aspirin; in group 3, the rats were given 1 ml *Sardinella longiceps* oil; in group 4, the rats were given 1.5 ml *Sardinella longiceps* oil. All of the rats, nevertheless, were given intraplantar induction of 1% carrageenan into the paw of rats to induce the inflammation condition. **Results:** All data were tested with normality test. The normal data were then analyzed with Homogeneity of Variances and also ANOVA test which result showed significant differences. The data which showed significant differences were tested again with LSD test. result then showed that group given 1 ml *Sardinella longiceps* oil and group given 1.5 ml *Sardinella longiceps* oil had no significant differences from group given aspirin, but there were significant differences between group given 1 ml *Sardinella longiceps* oil and group given 1.5 ml *Sardinella longiceps* oil, and also between group given 1.5 ml *Sardinella longiceps* oil and group given aquadest. **Conclusion:** *Sardinella longiceps* oil could reduce paw oedema in Wistar rats induced with 1% carrageenan.

Key words: *Sardinella longiceps* oil, aspirin, 1% carrageenan, anti-inflammation

ABSTRAK

Latar belakang: Penggunaan obat anti-inflamasi non steroid (OAINS) seperti aspirin sering digunakan dalam pengobatan inflamasi kronis. Namun penggunaan OAINS dalam jangka waktu panjang akan menyebabkan efek samping seperti mual dan muntah. Di sisi lain, minyak *Sardinella longiceps*, dilaporkan dapat digunakan sebagai alternatif anti-inflamasi karena selain alami, juga terdapat kandungan eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) yang dapat mengurangi edema pada telapak kaki. **Tujuan:** Tujuan dari penelitian ini adalah mengetahui efek anti-inflamasi minyak *Sardinella longiceps* pada edema telapak kaki tikus Wistar yang diinduksi karagenan 1%. **Metode:** Sampel penelitian ini adalah 32 tikus yang dibagi ke dalam empat kelompok. Kelompok 1, tikus diberi akuades; kelompok 2, tikus diberi aspirin; kelompok 3, tikus diberi minyak *Sardinella longiceps* 1 ml; kelompok 4, tikus diberi minyak *Sardinella longiceps* 1,5 ml. Semua tikus tidak terkecuali, diinduksi intraplantar karagenan 1% secara intraplantar pada telapak kaki untuk membuat kondisi inflamasi. **Hasil:** Semua data dilakukan uji normalitas. Selanjutnya data yang berdistribusi normal dilakukan uji homogenitas dan juga uji ANOVA yang hasilnya menunjukkan adanya perbedaan bermakna. Data yang menunjukkan perbedaan bermakna diuji lagi dengan LSD. Pada uji LSD menunjukkan bahwa tidak ada perbedaan bermakna antara kelompok minyak *Sardinella longiceps* 1 ml dengan kelompok aspirin dan kelompok minyak *Sardinella longiceps* 1,5 ml dengan kelompok aspirin, tetapi ada perbedaan bermakna antara kelompok minyak *Sardinella longiceps* 1 ml dan kelompok akuades dengan kelompok minyak

Sardinella longiceps 1.5 ml group given aquadest. **Kesimpulan:** *Sardinella longiceps* dapat mengurangi edema pada telapak kaki tikus Wistar yang diinduksi karagenan 1%.

Kata kunci: Minyak *Sardinella longiceps*, aspirin, karagenan 1%, anti-inflamasi

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INTRODUCTION

Inflammation is a living tissue reaction to invasion of pathogenic microorganisms, traumas, burns, or chemical materials.¹ Inflammation is often found in almost every case in dentistry. But, there are certain cases that are difficult to be treated since the inflammation is considered to be chronic and persistent, such as periodontitis.

Synthetic or chemical drugs have already become alternative medicines for those particular cases, especially acute inflammation. In chronic inflammation, those drugs, unfortunately, are not recommended since in the long-term treatment they can cause nausea, vomiting, and gastric bleeding since they can inhibit the cyclooxygenase-1 (COX-1) more than the cyclooxygenase-2 (COX-2).^{2,3} COX-1 is useful in physiological processes such as for maintaining and protecting stomach, kidneys and other organs, whereas COX-2 contributes to the production of pro-inflammatory prostaglandins and other inflammatory mediators in inflammatory process.³ For those reasons, in the treatment of chronic inflammation it is better to select drugs that have the fewest side effects since the treatment process requires long time.

Under these circumstances, it is necessary to choose another alternative treatment which use traditional medicines. Traditional medicine is a concoction of natural ingredients derived from plants, animals, minerals, whole preparation, or mixture of those materials which have traditionally been used for treatment based on empiric experience.⁴ Another advantage is that the traditional ingredients are easily available, and their benefit on certain diseases is quite large with low risk ratio and useful benefit for patients.⁴

In health sector, traditional medicines, have already been researched widely for their benefits, including traditional medicines that have anti-inflammatory effects. The result of recent researches even shows that fish oil containing omega-3 has anti-inflammatory effects that are good for long-term use. Omega-3 fatty acids are actually rich of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) that can inhibit the synthesis of arachidonic acid. The reason is because EPA and DHA can inhibit COX line and lipoxygenase (LOX) line.⁵ The inhibition of LOX line even can be more effective for treating chronic inflammation cases than NSAIDs that can only inhibit enzyme COX.⁶

In addition, fish mostly found in many of Indonesia's marine waters are *Sardinella longiceps*. The distribution of

Sardinella longiceps is mostly found around Muncar near Banyuwangi (East Java).⁷ Actually, *Sardinella longiceps* is a type of fish that has a significant economic value. This fish is one of the resources which has a large potential and good prospects. For instance, they can be processed to become canned food, cue, and salted fish, and their waste can also be processed to become fish meal. *Sardinella longiceps* can also be used as bait to catch bigger fish.⁸ The use of *Sardinella longiceps* as medicine is widely studied. It is also known that *Sardinella longiceps* contain much omega-3 that is good for health. The reason is because omega-3 widely contained in *Sardinella longiceps* is good for brain and chronic inflammatory medication.⁹

Therefore, the aim of this study was to investigate anti-inflammatory effects of *Sardinella longiceps* against the induction of 1% carrageenan suspension in rats' paw, compared to those induced with aspirin as anti-inflammatory drug. As a consequence, from this research, *Sardinella longiceps* can be widely cultivated in Indonesia.

MATERIALS AND METHODS

This research was an experimental laboratory using completely randomized design.¹⁰ The parameter of this research was the size of edema (mmHg) of rat paw that had been induced with 1% carrageenan suspension.¹¹ The samples of this research were 32 rats then randomly divided into 4 groups.¹² The criteria of those samples were male white Wistar rats (*Rattus Novergicus*) in the age of 2–3 months old and with the weight of 150–200 grams. Before the rats were treated, they had to adapt with the environment for one week under supervised condition. The research then was conducted in the Biochemistry Laboratory of Faculty of Medicine, Airlangga University Surabaya for 6 months.

Sardinella longiceps oil was created through intake process, cooking process, and separator process. Intake process was conducted to check the freshness of meat and oil of fish in order to obtain the expected good results. In the cooking process, raw fish was heated at the temperature of 90–95° C. The heating process was aimed to sterilize the fish, so their protein could be frozen and their cell membrane could be disrupted, as a consequence, the fat depot (thick and stiff) would be separated and the oil would be released. In the separator process, the liquid containing water and mostly oil was separated from fish, and then the protein and salt were dissolved in it. Afterwards, it was put in a decanter, and send to drying to be mixed with press

cake. The liquid from the decanter, finally, was separated and stored.¹³

Before this research was conducted, nevertheless, those white rats fasted approximately for 18 hours, but they were still given water to drink. If there were any of them sick, those would be excluded from this research. Those white rats then were divided into four groups: group 1 was a control group orally given only 1 ml aquadest, group 2 was the treatment group orally given aspirin at the dose of 360 mg/kg, group 3 was orally given 1 ml *Sardinella longiceps* oil, while group 4 was orally given 1.5 ml *Sardinella longiceps* oil.¹⁴ Onset of action in each of those anti-inflammatory drugs was estimated around 30 minutes, so those drugs had already demonstrated anti-inflammatory effects before the induction of 1 % carrageenan. After 30 minutes, the left paw of each of those white rats was dipped into mercury (pletismometer) to be measured. Each left foot of those white rats was given intraplantar induction of 1 % carrageenan suspension, 0.05 ml, as illustrated by Vinegar *et al.*¹¹ Next, every 15 minutes, the volume of those white rats' left paw was measured again for 3 hours. All data obtained were then recorded, and the results of each group were also averaged.

Data obtained from the measurement of those white Wistar rats' paw every 15 minutes for 3 hours in each of those groups were tabulated. To analyze the effects of anti-inflammatory, the difference of the volume of inflammation of the rat paw between that at t minutes and that at initial volume ($t=0$) was calculated. Based on the calculation of the difference of the volume of inflammation in each group, the statistical calculations with ANOVA test followed by Least Significant Difference test (LSD) was then conducted for the significance analysis in each group.

RESULTS

Figure 1 shows the results of the mean volume of oedema in each group of treatment groups, starting from the largest to the smallest one; aquadest, 1 ml *Sardinella longiceps* oil, aspirin, and 1.5 ml *Sardinella longiceps* oil.

Based on ANOVA test, it is known that there were the mean differences of oedema volume for 180 minutes after the injection of 1% carrageenan ($p < 0.05$). Based on LSD test, it is also known that there was no significant difference ($p > 0.05$) not only between group given aquadest and group

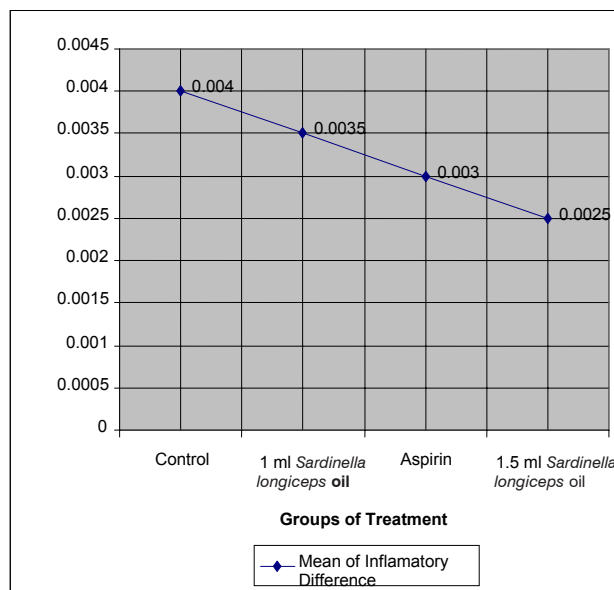


Figure 1. The mean differences of oedema volume for 180 minutes after the injection of 1% carrageenan.

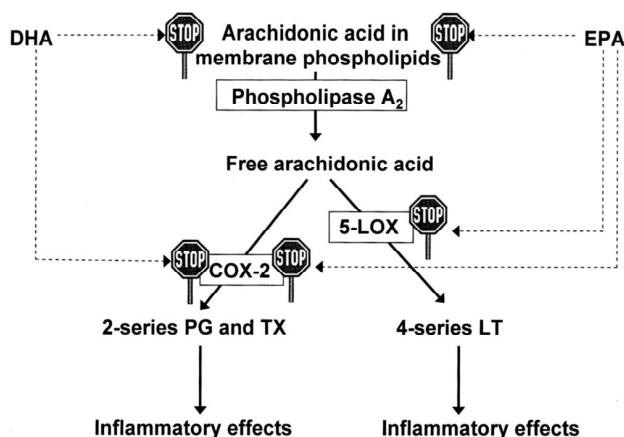


Figure 2. Classical mechanism of anti-inflammatory activity of omega-3 fatty acids.⁵

given 1 ml *Sardinella longiceps* oil ($p = 0.229$), between group given aspirin and group given 1 ml *Sardinella longiceps* oil ($p = 0.544$), but also between group given aspirin and group given 1.5 ml *Sardinella longiceps* oil ($p = 0.102$). Meanwhile, there were significant differences ($\text{sig} < 0.05$) not only between group given aquadest and group given aspirin ($p = 0.007$), between group given aquadest and group given 1.5 ml *Sardinella longiceps* oil

Table 1. LSD test results

Dependent Variable	Groups of Treatment	Aquadest	Aspirin	1 ml <i>Sardinella longiceps</i> oil	1.5 ml <i>Sardinella longiceps</i> oil
The Mean Reduction of Oedema Volume	Aquadest	-	0.007*	0.229	0.001*
	Aspirin	-	-	0.544	0.102
	1 ml <i>Sardinella longiceps</i> oil	-	-	-	0.029*
	1.5 ml <i>Sardinella longiceps</i> oil	-	-	-	-

($p = 0.001$), but also between group given 1 ml *Sardinella longiceps* oil and group given 1.5 ml *Sardinella longiceps* oil ($p = 0.029$) (Table 1).

DISCUSSION

Inflammation is body's defense mechanism due to tissue response to the damaging effects that can be local or induced into the body.¹² Inflammatory reaction can be observed from clinical symptoms, such as oedema. Oedema is due to the release of various mediators of pain so that the liquid of blood plasma come out from blood vessels and come into the wound, as a result, tissue oedema then clinically occurs and make the condition of the acidosis area.¹⁵ Aspirin, on the other side, known as NSAIDs, has already been proven to have anti-inflammatory effects, but it has also side effects such as nausea, vomiting, and so on.¹⁶ In this study, therefore, anti-inflammatory effect test was conducted on white rats based on the ability of drugs to reduce the volume of oedema in the feet of white rats caused by the intraplantar induction of 1% carrageenan. In the analysis of the test then it is known that group given aspirin and group given 1.5 ml *Sardinella longiceps* oil had anti-inflammatory effects against carrageenan indicated by the decrease of the volume of oedema compared to group with aquadest and group with 1 ml *Sardinella longiceps* oil.

This result, can also be seen in figure 1 and table 1 describing there was no difference between group given 1.5 ml *Sardinella longiceps* oil and group given aspirin. This indicates that group given 1.5 ml *Sardinella longiceps* oil and group given aspirin had similar effects, so the potential of 1.5 ml *Sardinella longiceps* oil was the same as that of aspirin. However, group given 1.5 ml *Sardinella longiceps* oil had significant differences from group given 1 ml *Sardinella longiceps* oil, which means that the effects of 1 ml *Sardinella longiceps* oil were not as effective as those of 1.5 ml *Sardinella longiceps* oil. Similarly, group given aspirin also same at the group given aquadest. It indicates that group given 1.5 ml *Sardinella longiceps* oil had the strongest anti-inflammatory effects compared group given aspirin and 1 ml *Sardinella longiceps* oil. This could be due to omega 3 contained in 1 ml *Sardinella longiceps* oil is less than that in 1.5 ml *Sardinella longiceps* oil.

Sardinella longiceps is actually one of the most important fish resources because of its great potency and benefits. *Sardinella longiceps*, contains high omega-3 considered to be useful as anti-inflammatory since it contains EPA and DHA.⁹

EPA could inhibit 5- lox line and COX-2 line. Meanwhile, DHA could only inhibit COX-2 line.⁵ EPA and DHA from fish or fish oil could cause the decreasing of the metabolite PGE₂, the decreasing of thromboxane A₂ (TXA₂), the increasing of thromboxane A₃ (TXA₃) (weak platelet aggregator and weak vasoconstrictor), and the increasing of prostaglandine I₃ (PGI₃) triggering the increasing of the total

of prostacyclin by increasing PGI₃ without reducing PGI₂. Meanwhile, lipoxigenase (LOX) line could not only decrease the formation of leucotriene B₄ (LTB₄), an inflammatory inductor and a strong leukocytes chemotaxis agent, but can also increase leucotriene B₅ (LTB₅), an inflammatory inductor and a weak chemotaxis agent.¹⁷

The content of EPA and DHA in 1 ml *Sardinella longiceps* oil, however, is not as much as in 1.5 ml *Sardinella longiceps* oil. This is what causes barriers against COX and lox not as big as on 1.5 ml *Sardinella longiceps* oil. From these results, it can be concluded that *Sardinella longiceps* oil could reduce oedema paw in Wistar rats induced with 1% carrageenan.

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