

The Effect of Sea Grapes (*Caulerpa cylindrica*) to Gastric Inflammatory Cell Infiltration Score and Catalase Activity in Indomethacin-induced Wistar Rats

Fitri Handajani*, Sulistiana Prabowo
Medical Faculty, Hang Tuah University, Surabaya - Indonesia

Correspondence Author: Fitri Handajani
Email: fitrihandajanidr@gmail.com

ABSTRACT

Introduction: Indomethacin works by inhibiting the cyclooxygenase enzyme resulting in the reduction of prostaglandins formation which negatively affecting the ability of the gastric mucosa to maintain its integrity. Damage of the gastric tissue and the subsequent inflammatory response in association with increase in oxidant compounds promote oxidative stress and oxidative damage of gastric tissue. This study aims to determine the protective effect of sea grape extract to indomethacin-induced Wistar rat gastric inflammation and catalase activity.

Methods: Four groups of rats (8 rats/group) received: 1) no treatment; 2) Indomethacin 30 mg/kg BW/day to day 7; 3) Indomethacin 30 mg/kg BW/day to day 7 + sea grape extract (*Caulerpa cylindrica*) 1g/100g BW/day from day 8 to 21; 4) Indomethacin 30 mg/kg BW/day to day 7 + sea grape extract 2g/100g BW/day from day 8 to 21. On day 22, all rats were sacrificed, and gastric tissue inflammatory scores and gastric tissue catalase activity were examined.

Results: The result of this study showed that indomethacin significantly increased ($p=0.001$) the inflammatory cell infiltration score (mean 1 vs 4). Both doses of sea grape extract significantly reduced ($p=0.004$ and $p=0.001$) the inflammatory cell infiltration score in indomethacin-induced gastric inflammation (mean 4 vs 2.875 and 4 vs 2.375 subsequently). Indomethacin significantly decreased ($p=0.001$) gastric tissue catalase activity (960.8 ± 8.32 U/ μ l vs 942 ± 2.03 U/ μ l). Both doses of sea grape extract significantly increased ($p=0.001$) indomethacin-induced catalase activity (942 ± 2.03 U/ μ l vs 957 ± 2.45 U/ μ l and 942 ± 2.03 U/ μ l vs 964 ± 2.17 U/ μ l subsequently).

Conclusion: Sea grape extract offers protective effect by significantly decreased gastric inflammatory cell infiltration score and increased the gastric tissue catalase activity in indomethacin treated rat.

Keywords: Indomethacin, sea grape, inflammatory cells, catalase

Correspondence:

Fitri Handajani
Medical Faculty, Hang Tuah University, Surabaya - Indonesia
Email: fitrihandajanidr@gmail.com

INTRODUCTION

Gastritis is a condition resulting from gastric mucosa damage in association with inflammatory reaction. If not corrected, it can progress to tissue damage which becomes a wound or peptic ulcer which can progress to gastric bleeding. Gastritis is caused by the action of destructive agents including bacterial infection, exposure to substances or compounds with a low pH (acid), alcohol and non-steroidal anti-inflammatory drugs (NSAIDs). Based on WHO data published in May 2014, peptic ulcers are the cause of 0.08% deaths in Indonesia comprising as many as 1,081 cases. Peptic ulcers often occur due to an imbalance between defensive and aggressive factors that play a role in maintaining the integrity of the gastric mucosa¹.

The use of NSAIDs in the community is often without the doctor's knowledge. Indomethacin is a class of NSAIDs that works by inhibiting the cyclooxygenase (COX) enzyme. This enzyme catalyzes arachidonic acid into prostaglandin. Inhibition of prostaglandin formation associated with reduced ability of gastric mucosa to maintain its integrity, resulted in irritation and cell damage. This condition cause inflammation which lead to the release of pro-inflammatory cytokines and increase of reactive oxygen species (ROS) formation that further resulted in oxidative stress and oxidative damage. Indomethacin also reduces blood flow to the gaster resulted in microcirculation disorders, decreases mucosal secretion, lipid peroxidation and activation of neutrophils. The increase in ROS will be suppressed by

endogenous antioxidants in the form of catalase, superoxide dismutase, glutathione and so on. If there is an imbalance between oxidants and antioxidants, oxidative stress will occurred which result in oxidative damage. The inflammatory process and oxidative damage are often associated with gastritis^{2,3,4,5}.

Gastrointestinal poisoning from NSAIDs occur around 4–8% annually and the complication is even higher in those with additional risk factors, such as a previous history of ulcer disease. A variety of synthetic antiulcer drugs are now available to treat gastric ulcers caused by NSAIDs, such as cimetidine, misoprostol, ranitidine, omeprazole and esomeprazole. But each of these drugs has side effects ranges from mild to severe. Research on phytotherapy of medicinal plants is very valuable and is widely used in traditional system medicine⁶. Indonesia is an archipelago that is rich in marine resources. Seaweed has been widely used as a source of food and traditional medicine. One of the green seaweed species is sea grape (*Caulerpa cylindrica*). *Caulerpa racemosa* variant *cylindracea* is a group of green algae that grows in several Indonesian waters. Sea grape is a type of algae that has been consumed as vegetables or fresh vegetables by people who live in tropical climates such as Indonesia. According to research conducted by Ridhowati and Asnani (2016) extracts of *Caulerpa sp.* contains a catechin (flavanol) and consists of 3 types, namely galocatechin, epicatechin and catechin gallate. Catechins are plant metabolites which are classified as flavonoids. Flavonoids

have pharmacological benefits as an anti-bacterial, anti-viral, anti-inflammatory and antioxidant treatment ^{7,8,9}. This study aims to determine the protective effect of sea grape extract (*Caulerpa cylindrica*) administered at doses of 1 and 2 g/100g BW/day for 14 days on gastric inflammation and gastric tissue catalase activity in indomethacin treated Wistar rats.

METHOD

This research is an experimental laboratory research conducted in the Biochemistry Laboratory, Faculty of Medicine, Hang Tuah University, Surabaya, Indonesia. The research was carried out in accordance with the applicable research ethics at the Faculty of Medicine, Hang Tuah University (Certificate number of Ethical Clearance: I/027/UHT.KEPK.03/VII/2019). This study used male *Rattus norvegicus* Wistar strains aged 2-3 months with body weight between 150-200 grams. Experimental animals were divided into 4 groups, each group consisting of 8 rats.

Group 1: Negative control group received no treatment.

Group 2: The positive control group received 30 mg/kg BW/day indomethacin intragastrically before meal in the morning for the first 7 days ^{modified 10}.

Group 3: Treatment group 1 received 30 mg/kg BW/day indomethacin intragastrically for the first 7 days, then was given 1g/100g BW/day sea grape extract for 14 days on days 8-21¹¹.

Group 4: Treatment group 2 received 30 mg/kg BW/day indomethacin orally for the first 7 days, then was given 2g/100g BW/day sea grape extract for 14 days on days 8-21.

On day 22, all the rats were sacrificed and histological samples stained with Hematoxylin and Eosin were used to assess the inflammatory cell infiltration score and catalase activity were measured in gastric tissue.

Gastric inflammatory cell infiltration score that occurs in gastric inflammatory process due to indomethacin administration was assessed using the following score ^{modified 10,12}.

1 = Normal, no inflammation.

2 = Inflammation associated with low inflammatory cell infiltration.

3 = Inflammation associated with moderate inflammatory cell infiltration.

4 = Inflammation associated with high inflammatory cell infiltration.

Sea grape extraction procedures

The sea grape extract was prepared by maceration method using 60% ethanol as a solvent because ethanol can dissolve almost all substances, both polar, semi-polar and non-polar. Seven kilograms of fresh sea grapes washed clean then cut into small pieces and then put in a drying oven for 24 hours then grinded or blended, then sieved. Materials that have been refined are then macerated using ethanol solvent or soaked for at least 24 hours or a maximum of 5 days. After 24 hours or more then filtered using Whatman 42 filter paper using a vacuum pump. The resulting macerate is then put in the Rotary Vacuum Evaporator to evaporate the ethanol solvent so that only the sea grape extract remains.

Indomethacin Induction Treatment

The dose of indomethacin given to experimental animals is 30mg/kg BW/day. The dose for one rat with an average

weight of 200 grams was $0.2 \times 30 \text{ mg} = 6 \text{ mg}$. The indomethacin used was then dissolved in 1% CMC-Na and given orally. Indomethacin given to rats was dissolved in CMC-Na 1% and the volume given to rats for each treatment was 2 ml. Then 2 ml of 1% CMC-Na solution contains 6 mg of indomethacin.

Hematoxylin Eosin Staining

After immersion in xylol I, II and III for 5 minutes each, the gastric tissue histological preparations were then dehydrated with ethanol I and II for 5 minutes each. Washed with aquadest for 1 minute and soaked in hematoxylin solution for 15 minutes, then rinsed with running water, then washed with Lithium carbonate for 15-30 seconds, rinsed with aquadest for 1 minute. Then immersed in acid alcohol 4 times and rinsed with aquadest for 1 minute & 15 minutes. Then stained with Eosin for 4 minutes. The preparation was added to 70%, 80%, and 96% alcohol for 3 minutes, respectively. Then immersed in ethanol III and IV each for 3 minutes. Then in xylol IV and V 3 minutes each. The preparation is dried and dripped with permount adhesive and covered with a closing glass¹³. The histological preparations were viewed under a microscope with a magnification of 100 times and 400 times.

Gastric Tissue Catalase Examination

One hundred mg of gastric tissue was added with 900 l of phosphate buffer and homogenated. Centrifugation of 3000 rpm was performed for 10 minutes. Then the supernatant was taken and added with 1 ml of 60 mM H₂O₂. The results obtained were read with a spectrophotometer at a wavelength of 240 nm. The results obtained are interpolated on catalase standard curve (Figure 1).

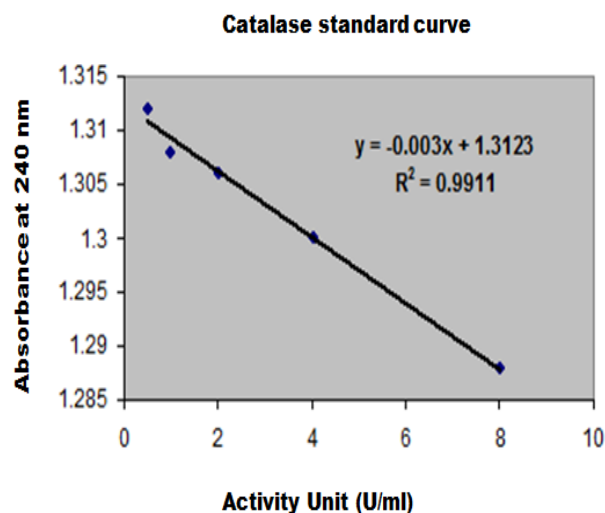


Figure 1. Catalase standard curve

RESULTS

Gastric inflammatory cell infiltration score

The results of gastric inflammatory cell infiltration score in the untreated group of rats, the group of rats induced with indomethacin 30 mg/kg BW/day on days 1-7, the group of rats induced with indomethacin 30 mg/kg BW/day on days 1-7 and received sea grape extract 1 g/100g BW/day on days 8-21 and the group of rats induced with indomethacin 30 mg/kg BW/day on days 1-7 and received sea grape extract 2 g/100g BW/days on days 8-21 can be seen in Table 1.

Table 1. The results of gastric inflammatory cell infiltration score

Gastric inflammatory cell infiltration score				
No.	K (-)	K (+)	P ₁	P ₂
1.	1	4	4	3
2.	1	4	3	2
3.	1	4	4	3
4.	1	4	3	3
5.	1	4	2	2
6.	1	4	2	2
7.	1	4	2	2
8.	1	4	3	2

Note:

K- = Group of rats without treatment

K+ = Group of rats treated with indomethacin 30 mg/kg BW on days 1-7

P1 = Group of rats treated with indomethacin 30 mg/kg BW on days 1-7 and sea grape extract 1g/100gBW/day on days 8-21

P2 = Group of rats treated with indomethacin 30mg/kg BW on days 1-7, and given sea grape extract 2g/100gBW/day on days 8-21

Score : 1 = Normal, no inflammation.
 2 = Inflammation associated with low inflammatory cell infiltration.
 3 = Inflammation associated with moderate inflammatory cell infiltration.
 4 = Inflammation associated with high inflammatory cell infiltration.

The pictures of gastric inflammatory cell infiltration score of 1,2,3,4 in the gaster induced by indomethacin can be seen in Figure 2.

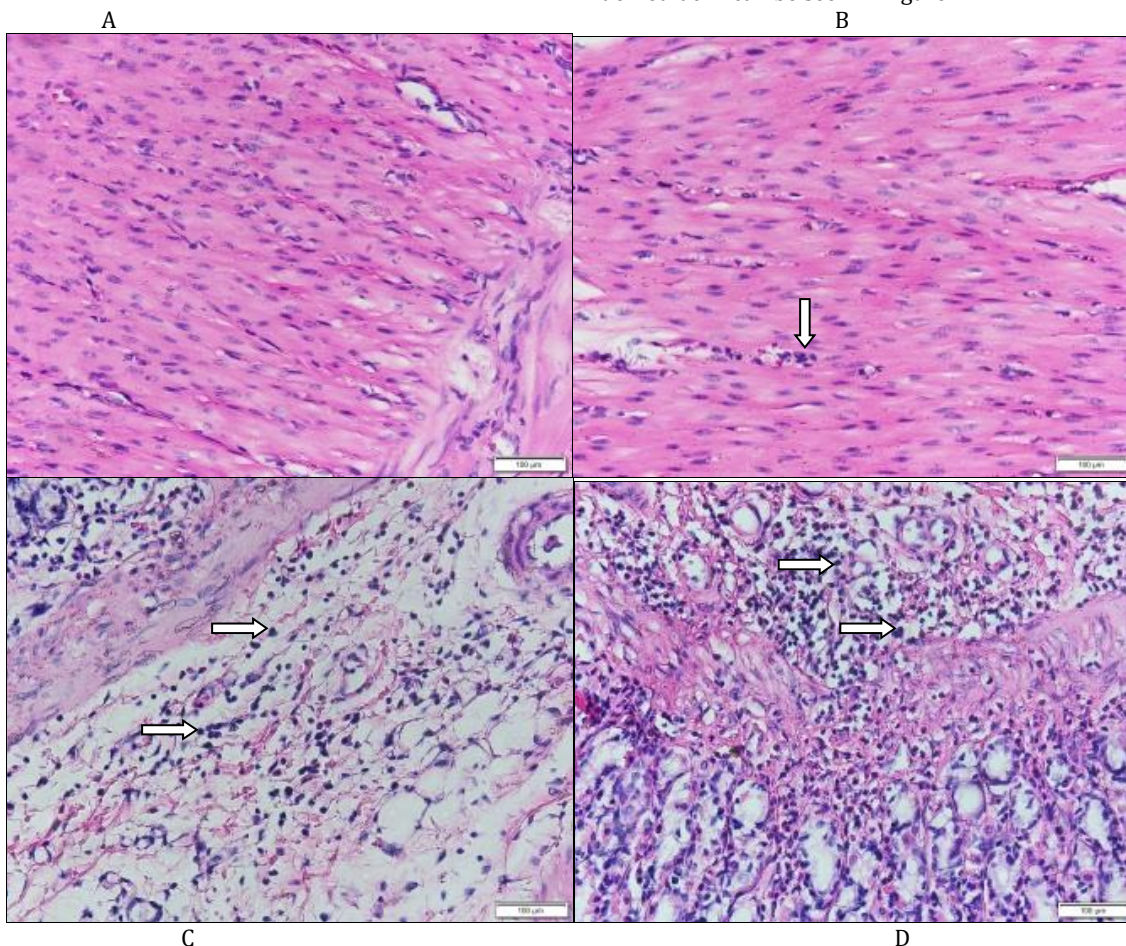


Figure 2. The pictures of gastric inflammatory cell infiltration score of 1,2,3,4 in the gaster. A. Score 1 = Normal, no inflammation; B. Score 2 = Inflammation occurs with a low infiltration of inflammatory cells; C. Score 3 = Inflammation occurs with moderate infiltration of inflammatory cells. D. Score 4 = Inflammation occurs with high infiltration of inflammatory cells.

Table 2. Means of gastric inflammatory cell infiltration score

GROUP	MEAN
K-	1
K+	4
P1	2.875
P2	2.375

Note:

K- = Group of rats without treatment

K+ = Group of rats treated with indomethacin 30 mg/kgBW on days 1-7

P1 = Group of rats treated with indomethacin 30 mg/kgBW on days 1-7 and sea grape extract 1g/100gBW/day on days 8-21

P2 = Group of rats treated with indomethacin 30mg/kgBW on days 1-7, and given sea grape extract 2g/100gBW/day on days 8-21

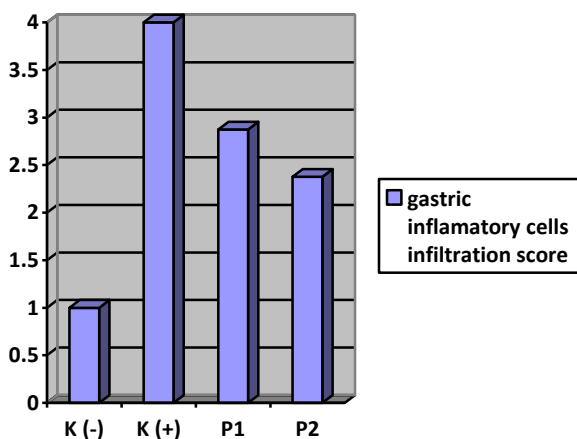


Figure 3. Means of gastric inflammatory cell infiltration score. K- = Group of rats without treatment. K+ = Group of rats treated with indomethacin 30 mg/kgBW on days 1-7. P1 = Group of rats treated with indomethacin 30 mg/kgBW on days 1-7 and sea grape extract 1g/100gBW/day on days 8-21. P2 = Group of rats treated with indomethacin 30mg/kgBW on days 1-7, and given sea grape extract 2g/100gBW/day on days 8-21.

The mean of gastric inflammatory cell infiltration score in the untreated group of rats was 1, in the group of rats induced by indomethacin 30 mg/kg BW/day on days 1-7 was 4, in the group of rats induced by indomethacin 30 mg/kg BW/day on days 1-7 and receiving sea grape extract 1 g/100g BW/day on days 8-21 was 2.875 and in the group of rats induced by indomethacin 30 mg/kg BW/day on days 1- 7 and got sea grape extract 2 g/100g BW on days 8-21 was 2.375 (Table 2).

The results of the Mann Whitney test showed that there was a significant difference in the gastric inflammatory cell infiltration score between untreated group of rats and the group of rats induced with indomethacin 30 mg/kgBW/day on days 1-7 ($p=0.001$). There was a significant difference in the gastric inflammatory cell infiltration score between the group of rats induced with indomethacin 30 mg/kg BW/day on days 1-7 and the group of rats induced by indomethacin 30 mg/kg BW on days 1-7 and received sea grape extract 1g/100g BW on days 8-21 ($p=0.004$). There was a significant difference in the gastric inflammatory cell infiltration score between group of rats induced with indomethacin 30mg/kg BW/day on days 1-7 and the group of rats induced with indomethacin 30 mg/kg BW/day on days 1-7 and received sea grape extract 2 g/100g BW on days 8-21 ($p=0.001$). There was no significant difference in the gastric inflammatory cell infiltration score between groups of rats induced with indomethacin 30 mg/kg BW/day on days 1-7 and received sea grape extract 1 g/100g BW on days 8-21 and the group of rats were induced with indomethacin 30mg/kgBW/day on days 1-7 and received sea grape extract 2 g/100g BW/day on days 8-21 ($p=0.203$). The results of the Mann Whitney test can be seen in Table 3.

Table 3. The results of Mann Whitney Test between groups

Groups		p Value
K-	K+	0.001
	P1	0.001
	P2	0.001
K+	P1	0.004
	P2	0.001
P1	P2	0.203

Note:

K- = Group of rats without treatment
 K+ = Group of rats treated with indomethacin 30 mg/kgBW on days 1-7
 P1 = Group of rats treated with indomethacin 30 mg/kgBW on days 1-7 and sea grape extract 1g/100gBW/day on days 8-21
 P2 = Group of rats treated with indomethacin 30mg/kgBW on days 1-7, and given sea grape extract 2g/100gBW/day on days 8-21

Indomethacin induction 30 mg/kg BW/day on days 1-7 significantly increased ($p=0.001$) the gastric inflammatory cell infiltration score (mean 1 vs 4). The administration of sea grape extract 1g/100g BW/day and 2g/100g BW/day on days 8-21 significantly reduced ($p=0.004$ and $p=0.001$) the gastric inflammatory cell infiltration score induced by indometacin 30 mg/kg BW/day on days 1-7 (mean 4 vs 2.875 and 4 vs 2.375 subsequently). The gastric inflammatory cell infiltration score in the administration of sea grape extract 1g/100g BW and 2g/100g BW/day on days 8-21 did not differ significantly ($p = 0.203$) on indometacin induction 30 mg/kg BW on days 1-7 (mean 2.875 vs 2.375).

Gastric Tissue Catalase Activity

The results of gastric tissue catalase activity in the untreated group of rats, the group of rats induced with indomethacin 30 mg/kg BW/day on days 1-7, the group of rats induced with indomethacin 30 mg/kg BW days 1-7 and received sea grape extract 1 g/100g BW/day on days 8-21 and the group of rats induced with indomethacin 30 mg/kg BW/day on days 1-7 and receiving sea grape extract 2 g/100g BW/day on days 8-21 can be seen in Table 4.

Table 4. The results of gastric tissue catalase activity

No	Catalase Activity Gastric Tissue (U/μl)			
	K(-)	K(+)	P1	P2
1.	966	939	953	961
2.	967	940	954	962
3.	968	941	955	963
4.	969	942	956	964
5.	950	943	957	965
6.	951	945	958	966
7.	952	943	959	967
8.	964	944	960	966

Note:

K- = Group of rats without treatment
 K+ = Group of rats treated with indomethacin 30 mg/kg BW on days 1-7
 P1 = Group of rats treated with indomethacin 30 mg/kg BW on days 1-7 and sea grape extract 1g/100g BW/day on days 8-21
 P2 = Group of rats treated with indomethacin 30mg/kg BW on days 1-7, and given sea grape extract 2g/100g BW/day on days 8-21

Table 5. Means and standard deviations of gastric catalase activity

GROUP	MEAN & STANDARD DEVIATION (U/ μ l)
K-	960.8 \pm 8.32
K+	942 \pm 2.03
P1	957 \pm 2.45
P2	964 \pm 2.17

Note:

K- = Group of rats without treatment
 K+ = Group of rats treated with indomethacin 30 mg/kg BW on days 1-7
 P1 = Group of rats treated with indomethacin 30 mg/kg BW on days 1-7 and sea grape extract 1g/100g BW/day on days 8-21
 P2 = Group of rats treated with indomethacin 30mg/kg BW on days 1-7, and given sea grape extract 2g/100g BW/day on days 8-21

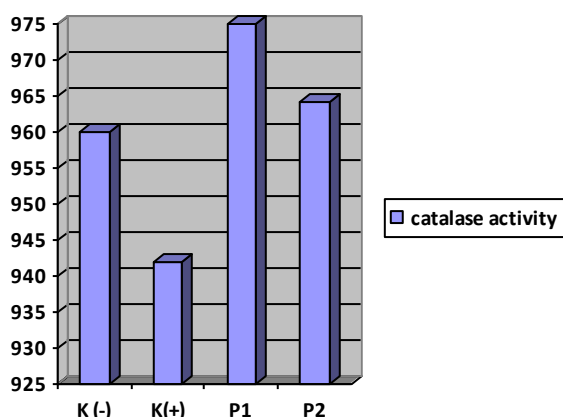


Figure 4. Means of gastric catalase activity. K- = Group of rats without treatment. K+ = Group of rats treated with indomethacin 30 mg/kg BW on days 1-7. P1 = Group of rats treated with indomethacin 30 mg/kg BW on days 1-7 and sea grape extract 1g/100g BW/day on days 8-21. P2 = Group of rats treated with indomethacin 30mg/kg BW on days 1-7, and given sea grape extract 2g/100g BW/day on days 8-21.

The mean and standard deviation of gastric tissue catalase activity in the untreated group of rats was 960.8 \pm 8.32 U/ μ l, in the group of rats induced with indomethacin 30 mg/kg BW/day on days 1-7 was 942 \pm 2.03 U/ μ l, in the group of rats induced with indomethacin 30 mg/kg BW/day on days 1-7 and receiving sea grape extract 1 g/100g BW/day on days 8-21 was 957 \pm 2.45 U/ μ l and in the group of rats induced with indomethacin 30 mg/kg BW/day on days 1-7 and got sea grape extract 2 g/100g BW/day on days 8-21 was 964 \pm 2.17 U/ μ l (Table 5).

Anova test results showed that there was a significant difference in gastric tissue catalase activity between the untreated group of rats and the group of rats induced with indomethacin 30 mg/kg BW/day on days 1-7 (p=0.001). There was a significant difference in gastric tissue catalase activity between the group of rats induced with indomethacin 30 mg/kg BW/day on days 1-7 and

the group of rats induced with indomethacin 30 mg/kg BW on days 1-7 and received sea grape extract 1 g/100g BW/day on days 8-21 (p=0.001). There was a significant difference in the gastric tissue catalase activity between the group of rats induced with indomethacin 30 mg/kg BW/day on days 1-7 and the group of rats induced with 30 mg/kg BW/day on days 1-7 and received sea grape extract 2 g/100g BW/day on day 8-21 (p=0.001). Furthermore, there was a significant difference in gastric tissue catalase activity between groups of rats induced with indomethacin 30 mg/kg BW/day on days 1-7 and received sea grape extract 1 g/100g BW/day on days 8-21 and groups of rats induced with indomethacin 30 mg/kg BW/day on days 1-7 and received sea grape extract 2 g/100g BW/day on days 8-21 (p=0.002). The results of Anova test for catalase activity between groups of rats can be seen in Table 6.

Table 6. Anova test results for gastric tissue catalase activity between groups

Groups	Groups	p Value
K (-)	K (+)	0.001
	P1	0.066
	P2	0.152
K (+)	P1	0.001
	P2	0.001
P1	P2	0.002

Note:

K- = Group of rats without treatment
 K+ = Group of rats treated with indomethacin 30 mg/kg BW on days 1-7
 P1 = Group of rats treated with indomethacin 30 mg/kg BW on days 1-7 and sea grape extract 1g/100gBW/day on days 8-21
 P2 = Group of rats treated with indomethacin 30mg/kg BW on days 1-7, and given sea grape extract 2g/100gBW/day on days 8-21

Indomethacin induction 30 mg/kg BW/day on days 1-7 significantly reduced (p=0.001) gastric tissue catalase activity (960.8 \pm 8.32 U/ μ l vs 942 \pm 2.03 U/ μ l subsequently). Administration of sea grape extract 1 g/100g BW/day and 2 g/100g BW/day on days 8-21 significantly increased (both p=0.001) gastric tissue catalase activity in indometacin-induced 30 mg/kg BW/day on days 1-7 (942 \pm 2.03 U/ μ l vs 957 \pm 2.45 U/ μ l and 942 \pm 2.03 U/ μ l vs 964 \pm 2.17 U/ μ l subsequently). Gastric tissue catalase activity in the administration of sea grape extract 2 g/100g BW/day on days 8-21 was significantly higher (p=0.002) compared to administration sea grape extract 1 g/100g BW/day on days 8-21 in indometacin induction 30 mg/kg BW/day on days 1-7 (964 \pm 2.17 U/ μ l vs 957 \pm 2.45 U/ μ l).

DISCUSSION

Indomethacin induction 30 mg/kg BW/day on days 1-7 significantly increased (p=0.001) the gastric inflammatory cell infiltration score. Indomethacin is often used as an analgesic antipyretic drug which is known to have an impact on the gastric ulceration process. Indomethacin inhibits gastric mucosal defense systemically through inhibition of gastric mucosal COX activity. The COX-2 selective inhibitor has been

associated with minimal antiplatelet effect by COX-2 inhibitor, because it does not affect thromboxane A2 (TXA2). Thromboxane A2 is a platelet agonist and selective vasoconstrictor that suppresses endothelial prostacyclin. As a consequence of COX inhibitors, leukotriene synthesis will be increased by changing the metabolism of arachidonate to 5-lipoxygenase (5-LOX). Leukotrien plays a role in the process of damage to the gastric mucosa through inflammation and tissue ischemia. The expression of adhesion molecules such as intercellular adhesion molecule-1 will increase through proinflammatory mediators causing neutrophil-endothelial activation. Neutrophil adherence is associated with the pathogenesis of gastric mucosa damage. The main mechanism that occurs is microvascular occlusion of the gaster by microthrombus which causes a decrease in gastric blood flow and cell ischemia and an increase in the release of oxygen radicals. These free radicals react with unsaturated fatty acids in the gastric mucosa and cause a decrease in endogenous antioxidant activity and tissue damage in the gaster⁵.

The response to the inflammatory process involves a highly coordinated network of various cell types. Macrophages and active monocytes and other cells mediate local responses to tissue damage. At the site of tissue damage, epithelial cells and damaged endothelial cells release factors that stimulate the inflammatory cascade, along with chemokines and growth factors, which attract neutrophils and monocytes. The first to arrive at the damaged sites are neutrophils followed by monocytes, lymphocytes (natural killer cells, T cells and B cells), and mast cells. Monocytes can differentiate into macrophages and dendritic cells which accumulate through chemotaxis into damaged tissue¹⁴.

Neutrophils can also damage host cells and tissues. Neutrophils are key mediators of the inflammatory response and program antigen presenting cells to activate T cells and release local factors to attract monocytes and dendritic cells. Macrophages are an important part of the mononuclear phagocyte system, and are very important in the initiation of inflammation, inflammation continuation and resolution. During inflammation the macrophages present antigens, perform phagocytosis, and modulate the immune response by producing cytokines and growth factors. Mast cells, which are present on the connective tissue matrix and on the epithelial surface, are effectors that initiate the inflammatory response. Active mast cells release a variety of inflammatory mediators, including, cytokines, chemokines, histamines, proteases, prostaglandins, leukotrienes, and serglycin proteoglycans. The interaction of platelets with inflammatory cells mediates the pro-inflammatory response. The acute phase response (APR) is the earliest response to an injury and it is suspected that platelets induce APR. Immune cells after being collected by inflammatory stimuli, will increase and maintain APR by releasing local inflammatory mediators at the site where the cells accumulate¹⁴.

The inflammatory reaction is a defensive biological response to harmful stimuli and infections that initiate the production of inflammatory mediators. Oxidative stress plays an important role in the changes associated with the pathophysiology of inflammatory reactions. Damage to the gastric mucosa is directly related to the degradation of the extracellular matrix in which matrix metalloproteinases (MMPs) play an important role. Connective tissue remodeling and loss of tissue integrity

due to MMP action have been reported in several inflammatory diseases, including gastric ulcers. Indomethacin-induced gastric ulceration involves the formation of reactive oxygen species (ROS) and the reduction of MMP-2 transcription and translation. This situation triggers an increase in the synthesis of inflammatory cells in the injured area¹⁵.

So in indomethacin induce gastric damage through the formation of ROS, resulting in an inflammatory reaction and the occurrence of inflammatory cell infiltration in the gaster.

The administration of sea grape extract 1 g/100g BW/day and 2 g/100g BW/day on days 8-21 significantly reduced ($p= 0.004$ and $p=0.001$ subsequently) gastric inflammatory cell infiltration score induced by indometacin 30 mg/kg BW/ day on days 1-7. The gastric inflammatory cell infiltration score in administration of sea grape extract 1 g/100g BW and 2 g/100g BW/day on days 8-21 did not differ significantly ($p = 0.203$) in indometacin induction 30 mg/kg BW on days 1-7.

The results of a qualitative study of the extract from the seaweed *Caulerpa sp* from Noor and Nursandi (2014) show that the extract contains alkaloid, phenolic, flavonoid and triterpenoid compounds. Alkaloid, phenolic and flavonoid compounds have been widely reported to have antioxidant activity. Natural plant antioxidant compounds are generally phenolic or polyphenolic compounds which can be in the form of flavonoids, cinnamic acid derivatives, coumarin, tocoferol and polyphenolic organic acids. The flavonoids that have antioxidant activity include flavones, flavonols, isoflavones, catexins, and chalcones. Meanwhile cinnamic acid derivatives include caffeic acid, ferulic acid, chlorogenic acid¹⁶.

So because the content of sea grapes in the form of phenols, flavonoids and alkaloids which function as antioxidants can reduce oxidant compounds and free radicals caused by damage in gastric tissue due to indomethacin induction, so that oxidative stress can be inhibited and oxidative damage can be prevented. This will help to decrease the infiltration of inflammatory cells to the site of inflammation. Oxidative stress plays a key role in gastric mucosal damage caused by indomethacin, and the powerful antioxidants present in sea grapes can suppress the oxidative damage caused by indomethacin. The results of this study prove that sea grapes have a function as an antioxidant that reduced the inflammatory process by reducing oxidative damage of the gastric tissue so that gastric inflammatory cell infiltration score was reduced.

Indomethacin induction 30 mg/kg BW/day on days 1-7 significantly reduced ($p = 0.001$) gastric tissue catalase activity. Catalase is an endogenous enzymatic antioxidant. This enzyme works by converting oxidants in the form of hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2) compounds which are not toxic. Antioxidants are a major element in the prevention of oxidative stress. Oxidative stress is the impact of an increase in oxidants which anti-oxidants cannot suppress. This situation will result in oxidative damage to the tissue^{4,17}.

The decrease in catalase activity in the indomethacin-induced group of rats compared to the untreated group of rats was due to indomethacin induction stimulating defenses in the gastric mucosa causing gastric mucosal damage and gastric tissue damage followed by inflammation. Krisnendhu's research reported that indomethacin administration resulted in an increase in

the production of hydrogen peroxide and mediated the regulation of expression of matrix metalloproteinase-2 (MMP-2) formation which led to inflammation and ulceration of the stomach. The inflammatory process triggers the secretion of pro-inflammatory cytokines and free radicals^{5,18,19,20}. This increase in free radicals forces the body to use antioxidants in the body to reduce them, so that the antioxidant activity of gastric tissue catalase decreases in the indomethacin-induced group of rats.

Giving sea grape extract 1 g/100g BW/day and 2 g/100g BW/day on days 8-21 significantly increased (both $p=0.001$) indomethacin-induced gastric tissue catalase activity 30 mg/kg BW/day on days 1-7. Gastric tissue catalase activity in the administration of sea grape extract 2 g/100g BW/day on days 8-21 was significantly higher ($p=0.002$) compared to giving sea grape extract 1 g/100g BW/day on days 8-21 in indomethacin induction 30 mg/kg BW/day on days 1-7.

The antioxidants contained in sea grape extract in the form of gallic catechin, epicatechin and catechin have been shown to be able to reduce oxidants and free radicals formed due to indomethacin induction, thereby helping to increase endogenous anti-oxidant activity, one of which is catalase. Antioxidants provide protection against H₂O₂-mediated inactivation and downregulation of MMP-2 expression during the onset of indomethacin-induced ulceration^{20,21,22,23}.

Furthermore, sea grapes contain high levels of antioxidants in the form of phenols, flavonoids and alkaloids, proven to be able to reduce the increase in oxidants that occur in indomethacin induction, this will reduce the oxidative stress that occurs, so that it can increase the catalase activity of experimental animals due to indomethacin induction^{4,8,17,23}.

If the dose of sea grapes is doubled, the catalase activity will increase significantly, this is because the amount of antioxidant content in it will also increase so that the catalase activity of the gastric tissue will also increase.

CONCLUSION

Indomethacin significantly increased the inflammatory cell infiltration score in the inflammatory process in the gaster and significantly decreased the gastric tissue catalase activity. The administration of sea grape extract significantly decreased the gastric inflammatory cell infiltration score and increased significantly gastric tissue catalase activity.

REFERENCES

1. Irramah M, 2017. Pengaruh uncaria gambir roxb terhadap ulkus gaster dan kadar malondialdehid hewan coba yang diinduksi etanol. [The effect of uncaria gambir roxb on gastric ulcers and ethanol-induced malondialdehyde levels in experimental animals *Majalah Kedokteran Andalas*; 40(1): 1-10. doi: 10.22338/mka.v40.i1.p1-10.
2. Abdelfattah MS, Elyamalah MY, Ebrahim HY, Almeer RS, Eltanany RMA, Moneim AEA, 2019. Prodigiosins from a marine sponge associated actinomycete attenuate HCl/ethanol-induced gastric lesion via antioxidant and anti-inflammatory mechanisms. *PLoS One*; 14(6):e0216737, doi: 10.1371/journal.pone.0216737.
3. Salis KM, Ramabhimaiah, 2013. Beneficial Effects of Vegetable Oils (Rice Bran and Mustard Oils) on Anti-inflammatory and Gastrointestinal Profiles of

Indomethacin in Rats. *Biomedical & Pharmacology Journal*; 6(2), 375-379. DOI: <http://dx.doi.org/10.13005/bpj/429>.

4. Handajani, 2019. Oksidan dan antioksidan pada beberapa penyakit dan proses penuaan. Penerbit: Zifatama Jawara. p 5-19
5. Fang Y, Wen Li Xu, LanWang, Qing Wu Lian, Li Feng Qiu, Hui Zhou, and Shu Jie Chen, 2019. Effect of Hydrotalcite on Indometacin-Induced Gastric Injury in Rats. *BioMed Research International Volume 2019*
6. Zhou YX, Xin HL, Rahman K, Wang SJ, Peng C, Zhang H, 2015. *Portulaca oleracea L.: A Review of Phytochemistry and Pharmacological Effects*. *BioMed Research International*; 2015:1-11. doi: 10.1155/2015/925631.
7. Sabiu S, Garuba T, Sunmonu T, Ajani E, Sulyman A, Nurain I, Balogun A, 2015. Indomethacin-induced gastric ulceration in rats: Protective roles of Spondias mombin and Ficus exasperate. *Toxicology Report 2015*; 2: 261-267.
8. Ridhowati S, Asnani, 2016. Potensi Anggur Laut Kelompok *Caulerpa racemosa* Sebagai Kandidat Sumber Pangan Fungsional Indonesia. [Potential of Sea Grapes Group of *Caulerpa racemosa* as Candidates for Indonesian Functional Food Sources]. *Oseana*; XLI(4):50-62.
9. Yudasmaras GA, 2017. Budidaya Anggur Laut (*Caulerpa racemosa*) melalui Media Tanam Rigid Quadrant Nets Berbahan Bambu. [Cultivation of Sea Grapes (*Caulerpa racemosa*) through Bamboo-Made Rigid Quadrant Nets]. *Jurnal Sains dan Teknologi*; 3(2): 468-473. doi: 10.23887/jst-undiksha.v3i2.4481.
10. Tastekin E, Ayvaz S, Usta U, Aydogdu N, Ekrem E, Puyan FO, 2018. Indomethacin-induced gastric damage in rats and the protective effect of donkey milk. *Arch Med Sci*; 14(3):671-678. doi: 10.5114/aoms.2016.59645.
11. Lau R, Prabowo S, Riemi, 2020. Peran Ekstrak Anggur Laut Terhadap Penurunan Kadar Kolesterol LDL *Rattus norvegicus* Jantan yang Mendapat Diet Tinggi lemak. [The Role of Sea Grape Extract in Decreasing LDL Cholesterol Levels of *Rattus norvegicus* in Males Who Get a High-Fat Diet]. *Hang Tuah Medical Journal*; 17(2):192-201.
12. Hehi KH, Loho L, Durry MF, 2013. Gambaran Histopatologi Lambung Tikus Wistar pasca Pemberian Metanol. [Stomach Histopathological Features of Wistar Rats after Methanol induced]. *Jurnal e-Biomedik (eBM)*; 1 (2): 890-895.
13. Berata I K, 2013. Teknik Pembuatan Preparat Histopatologi. Seminar Nasional, Workshop dan Demo Illegal Wild Life Trade. [Making Histopathological Slide Techniques. National Seminar, Workshop and Demo]. Denpasar 23-25 February 2018.
14. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X, Zhao L, 2018. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*; 9(6):7204-7218.
15. Akanda MR, Kim IS, Ahn D, Tae HJ, Nam HH, Choo HK, Kim K, Park BY, 2018. Anti-inflammatory and Gastroprotective Roles of *Rabdosia inflexa* through Downregulation of Pro-inflammatory Cytokines and MAPK/NF- κ B Signaling Pathways. *Int. J. Mol. Sci.*; 19(2):584. doi: 10.3390/ijms19020584.

16. Noor NM, Nursandi J, 2014. Karakteristik Kimiawi Rumput Laut Lokal (*Caulerpa* sp.) Dan Potensinya Sebagai Sumber Antioksidan. Prosiding Seminar Nasional Pengembangan Teknologi Pertanian Politeknik Negeri Lampung, [Chemical Characteristics of Local Seaweed (*Caulerpa* sp.) And Its Potential As A Source Of Antioxidants. Proceedings of the National Seminar on Agricultural Technology Development at State Lampung Polytechnic], 24 May 2014. ISBN 978-602-70530-0-7. Halaman 577-584.
17. Karthikeyan K, Ganapathi J, Kolanthasamy P, 2015. Antimicrobial and antioxidant potential of selected seaweeds from Kodinar, Southern Coast of Saurashtra, Gujarat, India. *Journal of Applied Pharmaceutical Science*; 5(7):035-040. doi: [10.7324/JAPS.2015.50706](https://doi.org/10.7324/JAPS.2015.50706).
18. Song H, Zeng M, Chen X, Chen X, Peng J, Lin Y, Yu R, Cai X, Peng Q, 2020. Antiulcerogenic Activity of Li-Zhong Decoction on Duodenal Ulcers Induced by Indomethacin in Rats: Involvement of TLR-2/MyD88 Signaling Pathway. *Evidence-Based Complementary and Alternative Medicine*; 2020 : 1-14. <https://doi.org/10.1155/2020/6538156>.
19. Yang J, Zhou W, Gu Y, Dai J, Li X, Tai P, Li Y, Ma X, Zhang Y, 2018. Protective effect of Pu erh tea extracts against ethanol induced gastric mucosal damage in rats. *Biomedical Reports*; 8(4):335-342. doi: [10.3892/br.2018.1068](https://doi.org/10.3892/br.2018.1068).
20. Krisnendhu G, ParagK, Aditi B, Russel JR, Snehasikta S, 2006. Hydrogen peroxide-mediated downregulation of matrix metalloproteinase-2 in indometacine-induced acute gastritisulceration is blocked by melatonin and other antioxidants. *Free Rad Biol Med*; 41:911-925.
21. Indraswari CI, Kalsum U, Sudjari, 2017. Pengaruh Pemberian Temulawak Pada Lambung Tikus Yang Mengalami Ulkus Peptikum Akibat Induksi Indomethacin [The influence of temulawak (*Curcuma xanthorrhiza*) to the rat gaster with peptic ulcer caused by the indomethacine induced]. *Jurnal Kedokteran Brawijaya*; 20(2):96-99. doi: [10.21776/ub.jkb.2004.020.02.8](https://doi.org/10.21776/ub.jkb.2004.020.02.8).
22. Artika F, Sandhika, Yuliawati, 2020. Tulsi (*Ocimum sanctum*) Leaf Ethanol Extract Reduces Inflammatory Cell Infiltration in Aspirin-Induced Gastritis Rats. *Jurnal Kedokteran Brawijaya* Vol. 31, No. 1, pp. 49-52.
23. Kan J, Hood M, Burns C, Scholten J, Chuang J, Tian F, Xingchang Pan, Jun Du, and Min Gui, 2017. A Novel Combination of Wheat Peptides and Fucoidan Attenuates Ethanol-Induced Gastric Mucosal Damage through Anti-Oxidant, Anti-Inflammatory, and Pro-Survival Mechanisms. *Nutrients*, 9, 978; doi:[10.3390/nu9090978](https://doi.org/10.3390/nu9090978).