

ORIGINAL ARTICLE

**THE EFFECT OF CAULERPA CYLINDRACEA EXTRACT ON HISTOPATHOLOGY DEPICTION OF MALE RATTUS NORVEGICUS GASTER MUCOSA INDUCED BY INDOMETHACIN**

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ARTICLE INFO

**Article history:**

Received

July 20, 2020

Accepted

February 17, 2021

**Keywords:**

*Caulerpa cylindracea* extract, mucosal damage, indomethacin

ABSTRACT

**Background:** A lot of food can repair gastric mucosal damage. For example, sea grapes or the *Caulerpa cylindracea* with its flavonoid content. Anti-inflammatory and anti-ulcer effect can reduce gastric lesions due to ulcerogenic agents. Indomethacin is used to induce gastric ulcers in experimental animals. Based on this background, this study was conducted to analyze the effect of *Caulerpa cylindracea* extract, on the histopathological picture of gastric mucosal damage of *Rattus norvegicus* male rats.

**Method:** 32 male *Rattus norvegicus* rats were divided into 4 groups, (1) Group K (-), the untreated group, (2) Group K (+) induced by indomethacin 30 mg/kgBB per head (3) Group P1 induced by indomethacin 30 mg/kgBB and *Caulerpa cylindracea* extract 1 gr/100grBB per subject, (4) Group P2, induced by indomethacin 30 mg/kgBB and 2 gr/100grBB of *Caulerpa cylindracea* extract per subject for 7 days. At the end of the study, the experimental animals were sacrificed and their stomachs were histopathologically examined

**Result:** There was a significant decrease ( $p = 0.001$ ) in the degree of gastric mucosal damage between the P1 group (given Indomethacin and 1 gr/100grBB *Caulerpa cylindracea* extract) and K + groups (given only indomethacin). There was a significant decrease ( $p = 0.001$ ) in the degree of gastric mucosal damage between K (+) group and group P2 that given Indomethacin and 2 g/100grBB *Caulerpa cylindracea* extract. There was a significant decrease ( $p = 0.004$ ) in the degree of gastric mucosal damage between group P1 and group P2. Data revealed on group K (-) without any treatment did not obtain significant results ( $p = 0.060$ ) with group P2.

**Conclusion:** Giving *Caulerpa cylindracea* extract with 1 gr/100grBB dose and 2 gr/100grBB dose can repair mucosal damage in *Rattus norvegicus* male rats induced by indomethacin.

Medical and Health Science Journal.

INTRODUCTION

Gastritis is an inflammation that occurs in the mucosal layer of the stomach. The inflammation may be accompanied by bleeding into the mucosa and in more severe cases epithelial erosions of the mucosal surface may occur. Gastritis can arise due to infection with the *Helicobacter pylori* bacteria; use of non-steroidal anti-inflammatory drugs (NSAIDs) or severe stress. Usually characterized by mucosal edema

and neutrophil infiltration. If this mucosal damage has extended beyond the submucosal layer or deeper, peptic ulcers can appear<sup>1</sup>.

According to WHO data in May 2014, peptic ulcer or stomach ulcer has caused 1,081 deaths in Indonesia or 0.08% of total deaths. There are two types of peptic ulcers that are often found, that are gastric ulcer and duodenal ulcer. This naming is based on the location of the ulcer, gastric ulcer in the stomach, while duodenal ulcers in the

duodenum. Peptic ulcers caused by an imbalance between defensive and aggressive factors that maintain the integrity of the gastric mucosa <sup>2</sup>.

There are several food ingredients with anti-inflammatory properties that can maintain the integrity of the gastric mucosa, one of which is sea grapes. One type of sea grape is *Caulerpa racemosa*, a variant of *cyllindracea*, from a group of green algae that lives in several Indonesian waters. The algae variety type *C. cyllindracea* is a species commonly consumed as vegetables or fresh vegetables by people in tropical areas such as in Indonesia. Based on the research of Santoso (2004) extracts of *Caulerpa* sp. contains three kinds of catechins (flavanol), namely gallo catechin, epicatechin and catechin gallat. Catechins are the products of plant metabolites which are included in flavonoids. Flavonoids are one of the most effective ingredients that function pharmacologically as anti-bacterial, anti-viral, anti-inflammatory and antioxidant<sup>3,4</sup>.

Flavonoids are very effective and have low toxicity as a treatment for gastrointestinal diseases, especially peptic ulcers. Flavonoids act to protect the gastrointestinal mucosa from lesions formed from various ulcer models and can also protect the mucosa from necrotic agents. There are several mechanisms of action involved in the protective effects of flavonoids, the most important of which is their antioxidant properties. Apart from having gastroprotective properties, flavonoids can also improve the healing process of gastric ulcers <sup>5</sup>.

Indomethacin is one of the NSAIDs and a group of drugs that is most widely consumed worldwide for its analgesic, antipyretic, and anti-inflammatory effects. This group of drugs has side effects, one of which is aggressive factors that can cause damage or lesions to the gastric mucosa, both locally and systemically, in the form of gastritis and peptic ulcer. Indomethacin induction can be a method to make experimental animals experience gastritis and peptic ulcer. Based on the results of the exploration, it was found that the 30 mg / kgBB dose of Indomethacin is the optimal dose to cause bleeding and peptic ulcer in the stomach.<sup>6,7</sup>.

Based on the background, the study was designed to determine the effect of sea grape extract

(*Caulerpa cyllindracea*) on the histopathological picture of indomethacin-induced male rats (*Rattus norvegicus*) gastric mucosal damage.

## METHOD

This research was conducted in the Biochemistry laboratory of the Faculty of Medicine, Hang Tuah University, Surabaya for 29 days with the ethical clearance number I/027/UHT.KEPK.03/VII/2019. The design of this study was a laboratory experimental study using a post-test only control group design. The study used 32 male white rats (*Rattus norvegicus*) of the Wistar strain with a body weight of 150-200 grams, divided into Negative control group (K-) or normal/untreated group, positive control group (K+) : that group was given indomethacin induction at a dose of 30 mg / KgBW per day for 7 days, treatment group 1 (P1) : that group was given Indomethacin at a dose of 30 mg / KgBB and sea grape extract at a dose of 1 gr / 100grBB per day for 7 days, treatment group 2 (P2) : that group was given Indomethacin at a dose of 30 mg / KgBB and sea grape extract at a dose of 2 gr / 100grBB for 7 days.<sup>6</sup>

The sampling technique is simple random sampling. On the 29th day, all groups of rats were anesthetized to take their gastric organs and then made histological preparations with haematoxylin eosin as an addition. The preparation is then viewed using a light microscope with a 40-fold magnification and assessed by looking at the integrity of the gastric mucosal epithelial layer with the following scores:

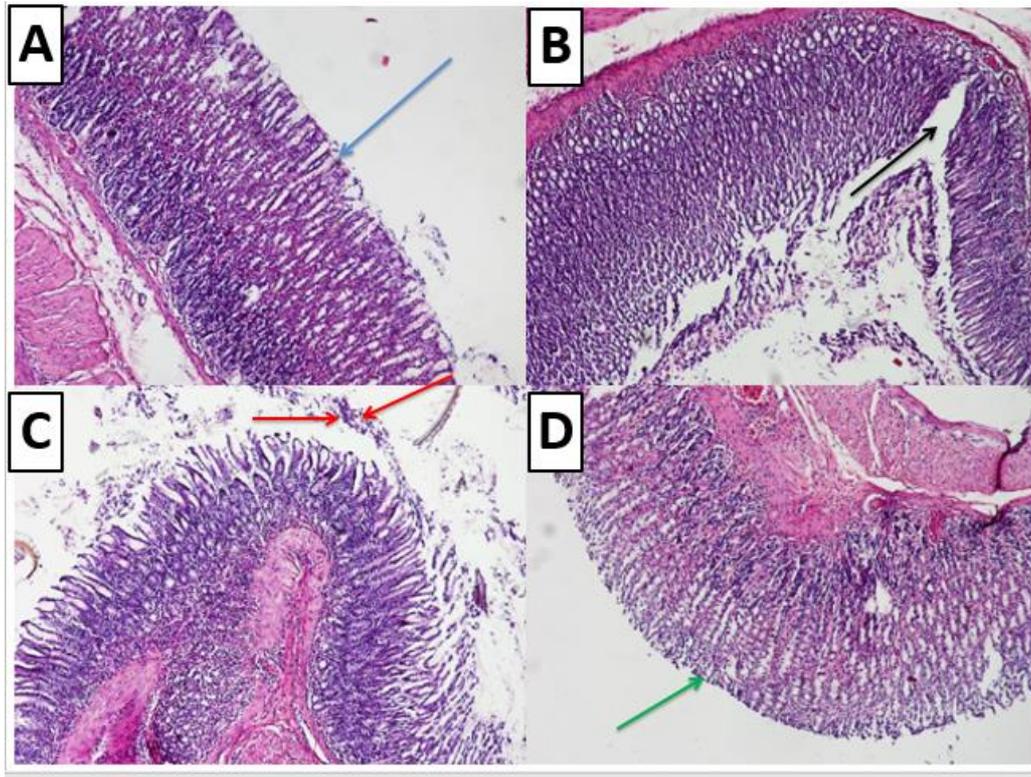
- normal / no pathological changes found = 0,
- there is desquamation of the gastric mucosal epithelium = 1,
- there is erosion of the epithelial surface of the gastric mucosa (the lesion in the epithelium appears to have peeled off almost half) = 2,
- ulceration of gastric mucosal epithelium (epithelial cell lesions reaching the muscularis lamina) = 3 <sup>8</sup>.

The data collected was tested using statistical analysis using the SPSS program in the

form of the Kruskal-Wallis test and then Post Hoc analysis, namely Mann-Whitney.

**RESULT**

Histopathological examination results with magnification 400X



**Figure 1.** gastric histopathological figure of each group

- Note:
- 1. Figure A : Control negative group;  
→ indicates a normal gastric mucosal layer
  - 2. Figure B : Control positive group;  
→ indicates gastric ulcer
  - 3. Figure C : Treatment group 1  
→ indicates gastric erosion
  - 4. Figure D : Treatment group 2;  
→ indicates an improved gastric mucosa

**Table 1.** Histopatological score the integrity of the gastric mucosal epithelial layer

No	K(-) or normal group	K(+)	P1	P2
1	0	3	2	0
2	0	3	2	0
3	0	3	2	1
4	0	3	2	0
5	0	3	2	1
6	0	3	1	1
7	0	3	1	0
8	0	3	1	0
Mean	0	3	1.71	0.43
SD	0	9	0.49	0.53

The results of the Kruskal Wallis test obtained a significance value of  $p (0.001 < \alpha (0.05))$  so it can be concluded that there is an effect of giving *Caulerpa cylindracea* extract on the histopathological picture of gastric mucosal

damage in indomethacin induced male *Rattus norvegicus*. Furthermore, the results of the Post Hoc test used Mann-Whitney to determine which groups were different.

**Table 2.** The results of the Mann Whitney test between groups

Group		P value
K-	K+	0.001
	P1	0.001
	P2	0.060
K+	P1	0.001
	P2	0.001
P1	P2	0.004

The results of data analysis, there is a significant difference between the degree of gastric mucosal damage in the experimental animal group given Indomethacin only (K +) and the experimental animal group given Indomethacin and sea grape extract at a dose of 1gr / 100grBB (P1). There was a significant difference between the degree of gastric mucosal damage in the experimental animal group given Indomethacin only (K +) and the experimental animal group given Indomethacin and sea grape extract at a dose of 2gr / 100grBB (P2). and sea grape extract dose of 1gr / 100grBB (P1) with a group of experimental animals given Indomethacin and sea grape extract with a dose of 2gr / 100grBB (P2). The results of data analysis on the experimental group K (-) without treatment with the P2 experimental group Indomethacin induced and given sea grape extract dose of 2gr / 100grBW did not show any significant results.

## DISCUSSION

In group K (-) experimental animals without treatment found no damage or changes in the gastric mucosal histopathological figure of the rats and were given a score of 0 (zero) according to Barthel Manja's criteria. In the K (+) group, experimental animals induced by Indomethacin showed a change in the gastric mucosal histopathological figure of the experimental animals, these changes were in the form of ulcers

with a score of 3 (three) according to Barthel Manja's criteria on the gastric mucosal layer of the experimental animals. The results of data analysis between the K (-) group of experimental animals without treatment with the K (+) group of experimental animals induced by indomethacin showed a significant difference with a significance level of  $p = 0.001$ .

In the K (+) group, they were given Indomethacin induction at a dose of 30mg / kgBW so that it could cause ulcers to form. The way indomethacin works on the stomach is inhibition of Cox-I and Cox-II. Cox-I in the stomach plays a role in keeping the surface of the stomach healthy by preventing the formation of stomach acid, increasing bicarbonate and mucus production, besides that Cox-I is responsible for maintaining homeostasis. Cox-II is responsible for PG (prostaglandin) formation in acute inflammatory conditions. Inhibition against Cox-II can relieve signs and symptoms of inflammation while inhibition against Cox-I can cause damage or exfoliation (desquamation) of the gastric mucosal layer. Indomethacin is more effective in inhibiting Cox-I than Cox-II, causing erosion of the gastric mucosa which results in peptic ulcers.<sup>9, 10</sup>

There was a significant difference between the K (+) group of experimental animals induced by indomethacin, namely  $p = 0.001$  with the P1 group of experimental animals induced by indomethacin and given *Caulerpa* extract. *cylindracea* dose of 1gr

/ 100grBB. On the histopathological examination of the gastric mucosa in group P1, it was found that the gastric mucosal layer of the experimental animals was erosion with a score of 2 (two) according to Barthel Manja's criteria and a small proportion only experienced desquamation with a score of 1 (one) according to Barthel Manja's criteria.

The histopathological examination of gastric mucosa in group P2 experimental animals induced by indomethacin and given 2gr / 100grBB of *Caulerpa cylindracea* sea grape extract, it was found that changes in the gastric mucosa layer were even better, namely only in the form of desquamation with a score of 1 (one), even some preparations showed a score of 0 (zero.) according to Barthel Manja's criteria. The comparison between the K (+) group of experimental animals induced by indomethacin and the P2 group of experimental animals induced by indomethacin and given *Caulerpa cylindracea* extract at a dose of 2gr / 100grBB resulted in a significant difference with a significance level of  $p = 0.001$ .

This research used *Caulerpa cylindracea* extract. *Caulerpa* sp contains three kinds of catechins (flavanol), namely gallo catechin, epicatechin and catechin gallat. Catechins are the products of plant metabolites which are included in flavonoids. Flavonoids are one of the ingredients of sea grapes which are pharmacologically most effective as anti-bacterial, anti-viral, anti-inflammatory and antioxidant.<sup>10,11, 12</sup>

Flavonoid compounds can also prevent or reduce gastric lesions induced by ulcerogenic agents with an important mechanism that flavonoids have is anti-ulcer activity. The protective effect through its anti-histamine properties can reduce histamine levels and thus prevent the release of histamine from gastric mast cells and inhibit gastric H + / K + proton pump, stimulate mucosubstance synthesis in gastric mucosa and increase prostaglandin levels thereby reducing gastric acid secretion.<sup>5, 10, 13</sup>

The results of the analysis test between the P1 experimental animal groups that were induced by indomethacin and given *Caulerpa* extract.

*Cylindracea* dose of 1gr / 100grBB with the experimental group P2 indomethacin induced and given *Caulerpa cylindracea* extract at a dose of 2gr / 100grBB showed significant differences with a significance level of  $p = 0.004$ . This shows that there is a significant difference with the addition of the dose of sea grape extract. The results of the data analysis test in the experimental group K (-) without treatment with the P2 experimental group that was induced by indomethacin and given *Caulerpa cylindracea* extract at a dose of 2gr / 100grBB did not get any significant results. ( $p = 0.060$ ) so it can be interpreted that the induction in the P2 group was able to improve with results that were close to normal.<sup>12, 13</sup>

The results of this research, the giving *Caulerpa cylindracea* extract can significantly reduce / repair lesions in the gastric mucosal layer in male rats (*Rattus norvegicus*) indomethacin induced.

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## CONCLUSION

Indomethacin induction in male *Rattus norvegicus* caused gastric ulcers. *Caulerpa cylindracea* extract at a dose of 1gr / 100grBB and 2gr / 100grBB can repair indomethacin-induced damage to the gastric mucosal layer of male *Rattus norvegicus*. 2gr / 100grBB dose of *Caulerpa cylindracea* extract can repair indomethacin-induced damage to the gastric mucosal layer of male *Rattus norvegicus* better than *Caulerpa cylindracea* extract at a dose of 1gr / 100grBB.

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