

## **The effect of Celery Ethanol Extract (*Apium graveolans*) to Decrease Ulcer Index in Wistar Rats (*Rattus norvegicus*) with Gastric Ulcer Model**

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### **ABSTRACT**

#### **Introduction**

Celery (*Apium graveolans*) contains flavonoid called apigenin which has antioxidant activity. Flavonoid content, apigenin, has the ability as an antiulcer through the protection of gastric mucosa. The aim of this study was to determine the effect of celery ethanol extract to decrease ulcer index as preventive action of gastric ulcer.

#### **Methods**

This study was true experimental study with Post Control Only Control Group Design. Samples were divided into three groups treated by preventive therapy including celery ethanol extract with dose 200, 300, and 400 mg / kg of body weight for 5 days, then indomethacin induced. All data obtained from calculation formula of ulcer index and analyzed with One Way ANOVA statistical test with significance level 5%.

#### **Results**

Ethanol extract of celery in the three treatment groups had significant effect to decrease ulcer index. Based on Post Hoc test used Tukey HSD test, negative control group had significant difference compared to all groups. Between the treatment groups 1, 2, and 3 there was no significant difference in the results of the ulcer index.

#### **Conclusion**

It is indicated that celery ethanol extract has an effect to decrease ulcer index as preventive action of gastric ulcer.

#### **Keywords**

Celery; Ulcer Index; Gastric Ulcer

## BACKGROUND

Gastric ulcer is an excoriated area in the gastric that is principally caused by the digestive activity of gastric fluid, that damage the surface of the mucous membranes (1). Ulcers are also defined as mucosal damage with a diameter of approximately 3 mm, with or without tissue necrosis in the center of the lesion of the gastric (2). Gastric ulcers can be caused by various factors, such as stress, smoking, nutritional deficiencies and dangerous agents, including non-steroidal anti-inflammatory drugs (NSAIDs) (3). NSAID are the most common causes of gastric ulcer in the patient without H. Pylori infection (4). 85% of patient with gastric ulcer is associated with the use of non-steroidal anti-inflammatory drugs (NSAIDs) (5).

Non-steroidal anti-inflammatory drug (NSAID) is the most commonly prescribed in the world (6). Non-steroidal anti-inflammatory drugs (NSAIDs) extensively used for treating pain, fever, and inflammation, despite the fact that NSAIDs are carrying a significant risk for side effects, especially to gastrointestinal system. Mechanism NSAID in causing gastric mucosa injury and being an ulcer, mainly through the inhibition of *cyclooxygenase* (COX) enzymes and suppress the production of prostaglandin (PG), which is an agent for protecting mucosa (7). Reactive Oxygen Species (ROS) also contributes to the process of ulcer formation. ROS will react with other macromolecules and product lipid peroxidase in the membrane. This reaction causes a decreasing membrane permeability, enzymes activity, and cell activation (8). It can be minimized by defense system of antioxidants, such as antioxidant enzymes, food, and medicines. All of them are essential in preventing the negative effects of ROS (9).

Celery (*Apium graveolens*) contains flavonoid, apigenin, one of the antioxidant and anti-inflammatory agent. Compared with other flavonoids like quercetin, kaempferol, and luteolin, apigenin has the lowest level in toxicity (10). Apigenin has many pharmacological activity, including anti-inflammatory, antispasmodic, and antioxidant (11). Therefore, this study aimed to examine the effect of oral administration of celery ethanol extract (*Apium graveolens*) to decrease ulcer index as preventive action of gastric ulcer.

## METHODS

This study used a true experimental study with Posttest Only Controlled Group Design. The sample consisted of 30 male white rats divided into 5 groups, that is negative (KN) and positive control group (KP) and 3 treatment groups. The KN group was fed with standard diet and the KP group induced with indomethacin. The three treatment groups were given preventive action of celery ethanol extract with dose 200, 300, and 400 mg / kg of body weight for 5 days, then induced with indomethacin. All data obtained from calculation formula of ulcer index and processed with SPSS 20 for windows application.

### Population and Sample

The population that used in this study was white wistar rats (*Rattus norvegicus*). Samples were taken randomly using *simple random sampling* technique with this inclusion criteria:

- a. White wistar rat (*Rattus norvegicus* )
- b. Male
- c. 150-250 grams of body weight
- d. Healthy conditions, were characterized by active physical movement, no injuries
- e. Did not get treatment before

Animals were acclimatized and kept in well ventilated animal house with free access to food and water ad libitum within 7 days of period.

### Treatment protocol

After 7 days of acclimatization, rats will be given treatment with this protocol (12):

- In negative control group, healthy rats were not giving any treatment
- In positive control group, rats induced with 30 mg/kg of body weight indomethacin on 6<sup>th</sup> day without giving any treatment
- In treatment group I, rats were given celery ethanol extract 200 mg/kg of body weight via oral for 5 days and induced indomethacin 30 mg/kg of body weight on 6<sup>th</sup> day
- In treatment group II, rats were given celery ethanol extract 300 mg/kg of body weight via oral for 5 days and induced indomethacin 30 mg/kg of body weight on 6<sup>th</sup> day
- In treatment group III, rats were given celery ethanol extract 400 mg/kg of body weight via oral for 5 days and induced indomethacin 30 mg/kg of body weight on 6<sup>th</sup> day

After 5 days of preventive therapy with celery ethanol extract, then on 6<sup>th</sup> day, indomethacin will be induced for making gastric ulcer conditions in 3 treatment group and positive control group. Induction procedure was performed as follows: rats were fasted 18 hours but free access to water ad libitum. Then, indomethacin was given to that groups orally. On 6<sup>th</sup> day, all rats were sacrificed by placing them inside a closed jar consist of cotton wetted with chloroform anesthesia (13). Then, the stomach was taken out and cut open along the greater curvature.

### Study outcome and measurement

This study was measuring the ulcer index of all groups. Ulcer index (UI) was calculated with this formula (14), as follows:

$$UI = UN + Us + Up \times 10^{-1}$$

where *UN* is the average of number of ulcers in each animal, *Us* is the mean severity of ulcer score and *Up* is the percentage of animals with ulcer incidence. Determine the mean severity of ulcer score was based with this scoring criteria (15):

- 0 – Normal colored stomach
- 0.5 – Red coloration
- 1 – Spot ulcers

- 1.5 – Hemorrhagic streaks
- 2 – Ulcers > 3 but < 5
- 3 – Ulcers > 5

**Statistical analysis**

The data was analyzed by *one way analysis of variant* (ANOVA) using SPSS 20 for Windows. One-Way ANOVA was performed at 95% confidence interval and 5% error level ( = 0.05). When the p- value < 0.05, it was considered significant. If there is significance differences, Posthoc Test was performed.

**RESULT**

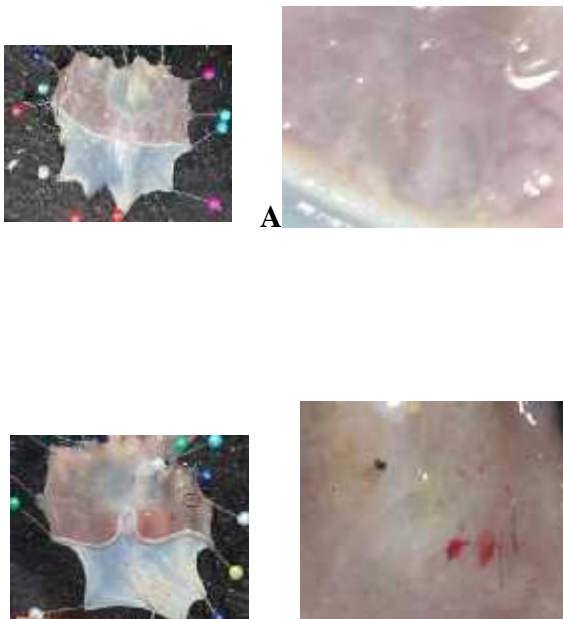
**Number of ulcers**

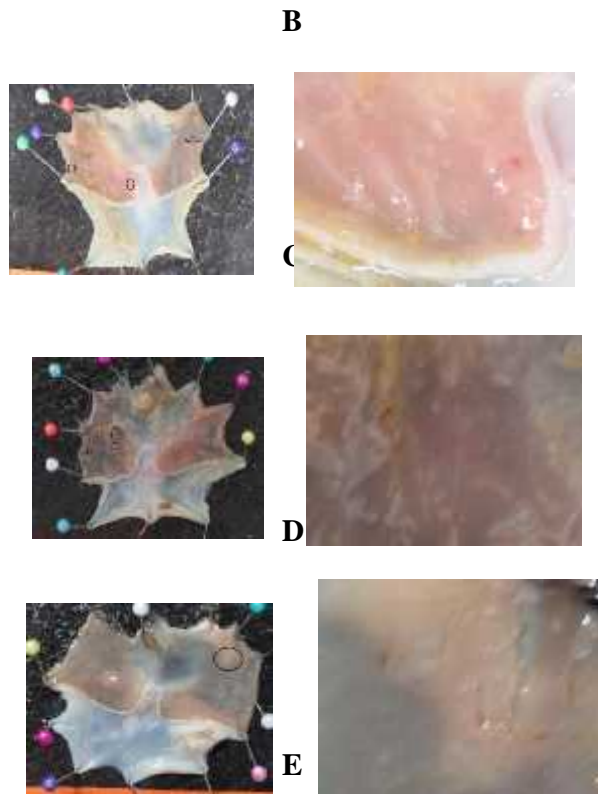
After the rats were given preventive therapy with celery ethanol extract in the dose 200, 300, and 400 mg / kg of body weight for 5 days, on the 6th day, 30 mg / kg of body weight indomethacin was induced. On the next day all rats were sacrificed. Based on the results of counting the number of ulcers, the average data on the number of ulcers are shown in Table 1, while the stomach conditions are shown in Figure 1.

**Table 1 - Average Data Number of Lesions (Mean ± SD)**

Group	N	Number of ulcers
		(Mean ± SD)
Negative	5	0 ± 0
Positive	5	4 ± 1
Treatment 1	5	3 ± 1
Treatment 2	5	2.2 ± 1.095
Treatment 3	5	1.6 ± 1.14

**Figure 1 - Gastric Conditions on Day 14**





Information: A (Negative control), B (Positive Control), C (Treatment 1), D (Treatment 2), E (Treatment 3)

### Ulcer Index

The measurement of the ulcer index was performed on day 14 after rats was sacrificed. The results were shown in Table 2.

**Table 2 - Average Data of Ulcer Index (Mean  $\pm$  SD)**

Group	N	Index of Ulcers
		(Mean $\pm$ SD)
Negative	5	0 $\pm$ 0
Positive	5	6.1 $\pm$ 1
Treatment 1	5	4.7 $\pm$ 1.51658
Treatment 2	5	3.3 $\pm$ 1.64317
Treatment 3	5	2,996 $\pm$ 1.34688

### One Way ANOVA Analysis (Analysis of Variance)

The ANOVA statistical test was used for data analysis to determine the existence of a significant difference effect on the negative control group, positive control group, and three treatment groups. *One Way ANOVA* analysis test results are shown in Table 3.

**Table 3 - One Way Analyze Analysis Results ANOVA Ulcer Index (Mean ± SD)**

Group	N	Index of Ulcers	df	F	p-value
		(Mean ± SD)			
Negative	5	0 ± 0	4	16,5	0,00
Positive	5	6.1 ± 1			
Treatment 1	5	4.7 ± 1.51658			
Treatment 2	5	3.3 ± 1.64317			
Treatment 3	5	2,996 ± 1.34688			

df: Degree of Freedom, p-value: Significance Value

Based on *One Way ANOVA* test result, it was obtained that *p-value* (0.000) < (0,005), which means that celery ethanol extract in three treatment groups were giving significant effect to decrease ulcer index. Based on the significant *One Way ANOVA* test results, then *Post Hoc test* was performed by using *Tukey HSD test* to determine the difference of ulcers index among groups. *Tukey HSD* test results were shown in Table 4.

**Table 4 - Tukey HSD Test Results**

Treatment	Mean difference	Sig
1 2	-6.1000*	0.000
1 3	-4.70000*	0.000
1 4	-3.30000*	0.004
1 5	-2.99600*	0.009
2 1	6.10000*	0.000
2 3	1.40000	0.417
2 4	2.80000*	0.016
2 5	3.10400*	0.007
3 1	4.70000*	0.000
3 2	-1.40000	0.417
3 4	1.40000	0.417
3 5	1.70400	0.237
4 1	3.30000*	0.004
4 2	-2.80000*	0.016
4 3	-1.40000	0.417
4 5	-.30400	0.995
5 1	2.99600*	0.009
5 2	-3.10400*	0.007
5 3	-1.70400	0.237
5 4	-0.30400	0.995

Table 4 showed that there was a significant difference between the negative control group and the positive control group with all of the three treatment groups. Similarly, there was significant

difference between the positive control group and the 1<sup>st</sup> treatment group. In contrast with the difference in the 1<sup>st</sup> treatment group with 2<sup>nd</sup> and 3<sup>rd</sup> treatment group, that was shown no significance difference. In line with the 2<sup>nd</sup> treatment group and 3<sup>rd</sup> treatment group that didn't has a significant difference. Based on that result, there was no significant difference between the three treatment groups in decreasing ulcer index, but all of that three treatment groups still give effect in decreasing the ulcer index.

## DISCUSSION

### Ulcer Index on Control Group

Based on the means of the ulcer index, it is known that there is no incidence of gastric ulcer in negative control group. External *cytodestructive* factors that may contribute to gastric ulcers include alcohol, NSAID, eating habits, smoking, stress, and H. pylori infection (16). On the negative control group was not induced indomethacin and fed with standard diet, proof that there is no incidence of gastric ulcers. So, this indicates if the incidence of gastric ulcers are not influenced by the food that given to rats and where rats maintenance are evidenced by the average result of the ulcer index in the negative control group is  $0 \pm 0$ . So, in this case, cytodestructive factors were not contributing as the causes of gastric ulcer, not only in negative control group but also in other groups.

In contrast with the positive control group that was exposed to one of the *cytodestructive* factors, NSAID. So, the incidence of gastric ulcer in positive control group can be found, with an average of the number is  $6.1 \pm 1$ . Indomethacin is a one of the NSAID's class that can be giving the effect of gastric ulcers with mechanisms through the inhibition of enzymes *cyclooxygenase* (COX) and suppress the production of prostaglandin (PG), which is an agent for protecting mucosa (7). Decreasing production from prostaglandins may contribute to the pathogenesis of gastric ulcer by reducing the synthesis and secretions of mucus, inhibiting the secretions of bicarbonate, reducing mucous blood flow, change micro vascular structure, increasing acid secretion, and activating leukocytes (neutrophils) (17). Neutrophils on vascular endothelium will activate ROS, where *Reactive Oxygen Species* (ROS) also plays important role in the pathogenesis of ulcers in the gastric due to NSAID effects (18). This mechanism causes the formation of ulcers in the positive control group.

### Effect of Celery Ethanol Extract on Treatment Group

In this study there were 3 treatment groups (given the preventive therapy of celery ethanol extract with doses 200, 300, and 400 mg / kg of body weight and then induced with indomethacin). This preventive action has good effect on prevention of gastric ulcers through decreasing the number of ulcer index, sequentially  $4.7 \pm 1.51658$ ,  $3.3 \pm 1.64317$ , and  $2.996 \pm 1.34688$ . This is because celery leaves contain essential oils and flavonoids, where flavonoids are polyphenol compounds and known with the effect against some free radicals such as *Reactive Oxygen Species* (ROS) (19).

Flavonoids can act as antioxidants by inhibiting the reaction between macromolecule and ROS that is the causes of damage (19). Flavonoids can play role in several ways, including directly against ROS and regeneration antioxidant enzymes on the membrane, such as  $\alpha$ -tocopherol to provide protection on the gastric mucous membrane. Previous study showed that flavonoids have powerful antioxidant activity by increasing the levels of SOD and CAT in the gastric mucosa which are antioxidant enzymes and decreasing gastric acid volume, so it can be concluded that flavonoids had gastro-protective effects (20).

There are some limitations that may slightly affect the results in this study, like each sample has a different immunity level and can't be controlled by the researcher, so this situation can affect the prevention process of gastric ulcers in each sample. Other limitation is when counting process the number of ulcers to determine the outcome of the ulcer index, it is depend on the accuracy of the researcher.

## **CONCLUSION**

In general, this findings highlights the importance of preventing the ulcer index incidents in people that is using NSAID for long time period. Because, one of the side effect of NSAID is gastric ulcer formation. In summary, there is significant effect of celery ethanol extract in decreasing the ulcer index in all treatment group that is induced indomethacin. All doses of celery ethanol extract that given to the treatment group have an effect on the prevention of worse condition in gastric ulcers by decreasing the ulcer index, although there is no significant difference between all treatment groups. Further study is needed in other parameters to more derive recommendations, to proof celery as gastro-protective agents.

## **Declarations**

### **Authors' contributions**

In this article, each author has its own contibution. Nadia Oktiffany Putri as main author and researcher, Ns. Dina Dewi Sartika Lestari Ismail, S.Kep, M.Kep as first supervisor, and Ns. Heri Kristianto, S.Kep, M.Kep, Sp.KMB as second supervisor.

### **Ethics approval and consent to participate**

This study was approved by Medical Faculty Ethic Committee with this serial number: 154/EC/KEPK-S1-PSIK/02/2015

### **Consent for publication**

Not applicable

### **Availability of data and materials**

The original data in this article will not be shared because author thinks it is a research privacy. If need more information, author can be contacted through the information about corresponding author above.



### **Competing interests**

None of the authors have conflict of interest, relevant with this study.

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