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# Methanol Extracts Potential of Mas Ngur Shells (*Atactodea striata*) Against Protease Profile and Description of Histopathology of Jejunum Rats Exposed by Indomethacin

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## ABSTRACT

Inflammatory Bowel Disease (IBD) is a multi-factorial disorder caused by genetic factors, environmental and immune response. One of the causes of IBD is a side effect of non-steroidal of anti-inflammatory drugs (NSAIDs). Indomethacin is an NSAID that activates macrophages and triggers increasing of protease activity. Mas Ngur shells (*Atactodea striata*) contains an antioxidant that inhibits the protease activity. This research used rats (*Rattus norvegicus*) induced by Indomethacin and treated with methanol extract of Mas Ngur shells (*A. striata*) at doses of 100 and 400 mg/kg of body weight (BW). Therapy potency of the Mas Ngur shells methanol extracts were identified by measuring protease activity and observed the Jejenum histopathology. Statistical analysis showed that the Mas Ngur shells therapy showed significant differences ($p < 0.05$) by decreasing of protease activity and improve jejunum histopathology of IBD induced rats.

Key word: Inflammatory Bowel Disease, Protease Activity, Mas Ngur

## INTRODUCTION

Inflammatory bowel disease *(IBD)* is an idiopathic disease that can cause intestines inflammation. One of the causes of inflammatory bowel disease is the use of Non-steroidal Anti-Inflammatory Drugs (NSAIDs) as Indomethacin. Indomethacin will inhibit cyclooxygenase-1 which has a role in the inhibition of prostaglandin formation in the intestine, causing a decrease in protection against intestinal mucosal barrier that make pathogen bacterial invasion easily happens [1,2]. Invasion of pathogenic bacteria in the small intestine will activate neutrophil which can destroy microorganisms by phagocytosis so degraded by reactive oxygen species (ROS). The release of proteases causes tissue damage and inflammation [3]. Free radicals from various sources cause the body's defense mechanism. The defense mechanisms against oxidative stress of free radicals cause preventive mechanism, repair mechanisms, physical defense, and the antioxidant defense [4]. Antioxidant defense in the body can be done by providing exogenous antioxidants, through herbal therapies containing antioxidants such as quersetin, acacetin, vicenin-2 and apigenin.

Apigenin is a flavonoid compound. According to Skibola and Smith (2000) apigenin compound can be used as an antioxidant. Apigenin is a COX-2 inhibitors as anti-free radicals by stabilizing the free radicals and inhibiting the action of cyclooxygenase enzymes that

mediate the onset of pain and tinocrisposide substance functions as an anti-inflammatory [5]. The antioxidant activity functioning as free radical scavenger is able to reduce levels of free radicals due to the effects of Indomethacin [6,7]. The results of this research reported the methanol extract potential of Mas Ngur shells (*A. striata*) as a source of antioxidant as an alternative treatment of Inflammatory Bowel Disease based on protease activity and histopatology of jejenum.

## EXPERIMENT

### Bioactive Extraction of Mas Ngur Shells (*A. striata*)

Bioactive extraction, according to the procedure of Nurjanah (2011) in Darusman et al., (1995) extraction was done by maceration of 25 grams dry weight of Mas Ngur shells in methanol for 48 hours in orbital shaker. The filtrate obtained from the extraction was filtered with whatmann filter paper, and the resulting extract was evaporated to separate the solvent to extract using a rotary vacuum evaporator at 40 $^{o}$C [8].

### Preparation of Experimental Animals

Male white rats (*Rattus norvegicus*) of wistar strain aged 2 months with 175-200 grams weight as many as 20 rats; they were divided into 4 groups. In the first group, healthy rats (negative control) were *disonde* corn oil, second group of sick rats (positive control) were exposed to indomethacin at a dose of 15 mg /kg of body weight orally one time on first day and on the second day they were dissected, then the third and fourth groups of rats exposed to indomethacin on day 1 at a dose of 15 mg / kg of body weight orally one time and on a day-2 treated by methanol extract of Mas Ngur shells with dosage variation that is the third group was therapy with methanol extract of Mas Ngur shells at dose of 100 mg / kg of body weight and the fourth group was 400 mg /kg of rats body weight orally for fourteen days, then on the 16th day were dissected. The use of experimental animals has received approval of the ethics worthy from KEP-UB No. 337.

### Protease Activity Measurement of Isolation Results from Jejunum

The protease enzymes of jejunum (200 µL) were added by 500 µL casein of 1% pH 6.5, incubated for 10 minutes at 37 °C. The reaction was stopped by the addition of TCA (trichloroacetic acid) of 5% (w/v) 500 µL and incubated at room temperature for 30 minutes. After centrifuged at 3000 rpm for 5 minutes, the supernatant (300 µL) were added by 1500 µL distilled and the absorbance values were measured by UV-Vis spectrophotometer at 275 nm.

One unit of protease activity is the number of µmol tyrosine produced per 1 mL enzyme per minute. The measurement of enzyme activity is done by converting the absorption value into tyrosine concentrations (µg/mL) using a standard curve of tyrosine. The value of enzyme activity measured from tyrosine levels obtained from the plot of the tyrosine standard curve with the following formula:

$$\text{Protease activity} = \frac{[\text{Tyrosine}]}{\text{Mr Tyrosine}} \text{ x } \frac{\text{v}}{\text{p x q}} \text{ x fp}$$

Where, v = volume of total sample (mL), p = number of enzyme (mL), q = incubation time (min), fp = dilution factor, and Mr tyrosine = 181 µg/µmol.

**Hematoxylin-Eosin staining**

Hematoxylin-Eosin staining initially was done by inserting the jejunum equipment in absolute xilol for 5 minutes by 2 times. Then it was conducted de-paraffination stage, in which equipment put into graded xilol 1-3 [xilol: absolute ethanol (3: 1, 1: 1, 1: 3)] respectively for 5 minutes. Next, it was rehydrated; the equipment put into graded ethanol starting from absolute ethanol, ethanol 95%, 90%, 80% and 70% respectively for 5 minutes. Then it was soaked in distilled water for 5 minutes. Furthermore, the equipment was stained with hematoxylin dye for 10 minutes to obtain the best results. Wash with running water for 30 minutes, rinsed with distilled water and put in the dye eosin for 5 minutes. Equipment was soaked in distilled water to eliminate the excess of eosin. Then to conduct the stages of dehydration, the equipment was included in the graded ethanol of 80%, 90% and 95% to absolute ethanol. Next, clearing was conducted by inserting equipment into xilol for 5 minutes, dried-wind and mounting with entellan. Finally, it was covered with a cover glass.

## RESULT AND DISCUSSION

Protease activity of jejenum was measured based on tyrosine product formed from the hydrolysis of casein. The results showed that Mas Ngur shells (*A. striata*) therapy reduce protease activity (table 1**)**. Results of statistical analysis (One Way ANOVA) showed that therapy of methanol extract of Mas Ngur shell reduce protease activity significantly ($P<0.05$).

**Table 1.** Protease activity in Jejunum of Rats Exposed by Indomethacin and Post-Treated with Methanol Extract of Mas Ngur Shells (*A. striata*)

| Group | Average of Protease Activity (unit) | Increasing of Protease Activity against control (%) | Decreasing of Protease Activity against IBD (%) |
|---|---|---|---|
| Negative Control | $0.081 \pm 0.004^{a}$ | - | - |
| IBD | $0.193 \pm 0.006^{d}$ | 138.27 | - |
| Therapy 100 mg/kg BW | $0.145 \pm 0.004^{c}$ | - | 24.87 |
| Therapy 400 mg/kg BW | $0.096 \pm 0.002^{b}$ | - | 50.26 |

Note: Different notations indicate a significantly different effect ($p<0.05$)

Research results of protease activity in rats exposed to indomethacin had greater activity than the healthy rats and rats were treated with the methanol extract of Mas Ngur shells (*A. striata*). Mucosa of the small intestine in normal conditions will protect the serine protease activity of the pancreas, while at the IBD condition, the mucosa damaged and the function weakened which triggered the secretion of protease. The more protease secreted, it trigger of cell destructions, and if it constantly undertaken then initiate tissue damage. The release of proteases into the cells can be pressured by giving antioxidant that can inhibit the activity of protease (protease inhibitors). In this study, it used methanol extract of Mas Ngur shells (*A. striata*) as an antioxidant and anti-inflammatory. Methanol extract of Mas Ngur shell (*A. striata*) contains apigenin and vicenin-2. Apigenin and vicenin-2 is a class of flavonoids as COX-2 inhibitors and work as anti-free radical by stabilizing free radicals and inhibiting the action of cyclooxygenase enzymes. This mediates the onset of pain and tinocrisposide substance that functions as an anti-inflammatory agent. Flavonoids itself is a polyphenols function as anti-inflammatory and antioxidant [9]. Mas Ngur shells contains polyphenols i.e.

apigenin and vicenin-2. These two flavonoids also as a free radical scavenger and are able to reduce free radicals level [6].

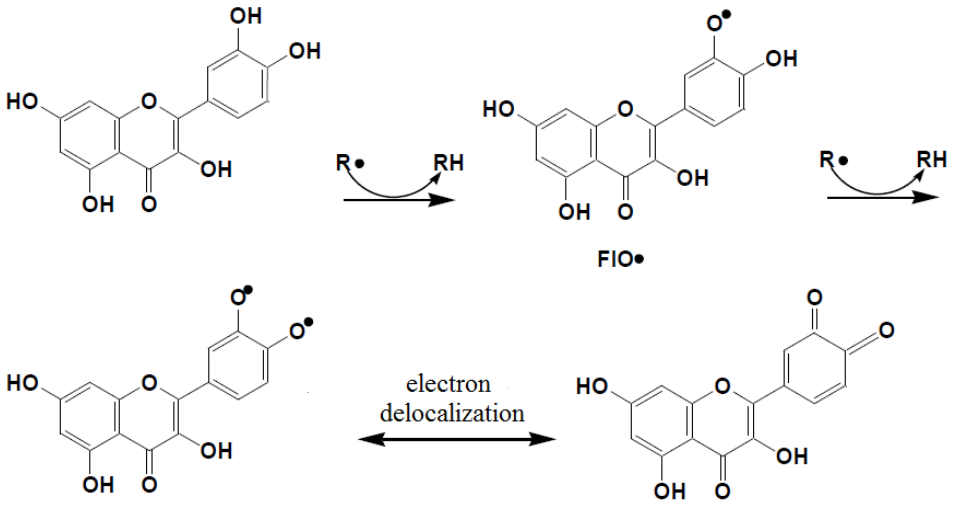


**Figure 1.** The reaction of free radical scavenging by flavonoids [6]

**The Potency of Methanol Extracts of Mas Ngur Shells against Histopathology Repairing with Hematoxylin-Eosin Staining**

Indomethacin exposure cause mucosal damage through exfoliation of the surface epithelial cell and reduce mucus secretion which is the protection of the mucosal barrier and barriers prostaglandin synthesis (Figure 2).

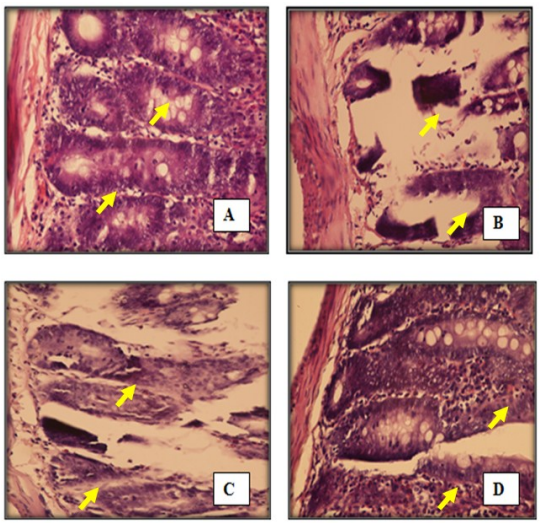


**Figure 2.** Histopathology overview of rat small intestine jejunum with hematoxylin-eosin staining, magnification 400x. Description: (A) negative control rats; (B) IBD Rats (exposed to *Indomethacin* 1 times, incubated 24 hours); (C) IBD Rats treated with methanol extract of 100 mg / kg Mas Ngur shells, and (D) IBD Rats treated with methanol extract of 400 mg/ kg BW Mas Ngur shells. Shows vili differences among treatments (➔)

Prostaglandins increases intestinal motility, inhibit secretion of gastric hydrochloric acid, prevents ulceration caused by prostaglandin inhibitors and cytoprotection. Indomethacin inhibits (irreversible) the enzyme cyclooxygenase work functions as the synthesis of prostaglandins that will decrease mucosal protection. Intestinal damage caused by Indomethacin which led to an increasing of mucosal permeability, microvascular damage, focal intravascular thrombus formation, fibrin deposition and neutrophils infiltration [10]. The activation of phagocytic cells in rat jejunum tissue of Inflammatory Bowel Disease induced by Indomethacin produces large amounts of reactive oxygen. In inflammatory Bowel

Disease rats, the antioxidant granting of Mas Ngur shells (*A. striata*) immersed the oxidant compounds resulting from the inflammatory process that reduce the inflammation and repair tissue destruction observed by the improvement of the jejunum histological structure.

## CONCLUSION

The potential of the methanol extract of Mas Ngur shells with dosage variation of 100 and 400 mg/kg BW in IBD rats induced by indomethacin 15 mg/kg of body weight decreases the activity of protease and repair the histopathology figure of jejunum tissue.

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