Eduvest – Journal of Universal Studies
Volume 3 Number 3, March, 2023
p- ISSN 2775-3735- e-ISSN 2775-3727

MALONDIALDEHYDE (MDA) CONCENTRATION AND HISTOPATHOLOGICAL IMAGE OF INDOMETHACINE-INDUCED WISTAR RAT, RATTUS NORVEGICUS, WITH INFLAMMATORY BOWEL DISEASE (IBD) AFTER MAS NGUR OYSTER (ATACTODEA STRIATA) EXTRACT THERAPY

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ABSTRACT

Inflammatory Bowel Disease (IBD) is an inflammatory disease occurring in gastirct tract, particularly colon, that could result from the side effect of anti-inflammatory non-steroid (AINS) drug utilization, such asindomethacine. Mas ngur oysters (Atactodea striata) have long been known by people of Kei-Southeast Mallucas as traditional medicine, but its use in reducing MDA level in rat with IBD has not been studied. This study was aimed at measuring the ability of the bioactive compounds contained in mas ngur oyster powder extract to reduce the MDA level in the ileum of indomethacine-induced rat with IBD and providing the histological image of the ileum after therapized with mas ngur oyster extract. Test animals were 8-12 week old-male rats (Rattus norvegicus) of 150 - 200 g BW. They were separated into 3 groups, healthy, sick (induced with 15 mg/kg BW indomethacine), therapy goups (15 mg/kg BW indomethacine oral induction then treated with mas ngur oyster powder of100, 400, 700 mg/kg BW). Indomethacine induction of 15 mg/kg BWand therapy of mas ngur oyster powder extract were administered orally. MDA level was measured usingThiobarbituric Acid (TBA)test and histological image of the ileum using Hematoxylin-Eosin staining. Results showed that mas ngur extract

How to cite: Celcius Waranmaselembun, Agustiana, Firlianty, Yuliana Anastasia Ngamel, Silvester B. Pratasik. (2023). Malondialdehyde (MDA) Concentration and Histopathological Image of Indomethacine-Induced Wistar Rat, Rattus Norvegicus, with Inflammatory Bowel Disease (IBD) after Mas Ngur Oyster (Atactodea Striata) Extract Therapy. Journal Eduvest. *3* (3): 544-551
E-ISSN: 2775-3727
Published by: https://greenpublisher.id/

therapy gave significant effectin MDA level (P<0.05) and difference between the treatments with effective dose of400 mg/kg BW

KEYWORDS *IBD, Indomethacin, Mas Ngur Oyster Extract, MDA Concentration, Histological Image*

INTRODUCTION

Indonesia, as an archipelagic country, possesses high marine biodiversity. In general, coastal villagers have long been using this biodiversity, especially for health care. One of the marine animals used as traditional medicines to cure hepatic disease is mas ngur (local name) oyster, Atactodea striata, particularly in Kei islands, Southeast Mallucas. This oyster holds active compounds, such as alkaloid, steroid, and saponin[1]pressumed to have anti-inflammatory agents and glutathione S-transferase (GST) enzyme functioning to reduce organic toxins, such as hydroperoxides (Edwards et al., 2000; Yang et al., 2003) .

Inflammation is one of the major responses of the body immune systemagainst infection or irritation (Erlina & Indah, 2017; Safrina et al., 2018). The disease resulting in inflammation of the gastric tract calledInflammatory Bowel Disease (IBD) (Krzystek-Korpacka et al., 2009) is generally caused by virusesand pathogenic bacteria. In inflammation medication, the drug groups mostly given are anti-inflammatory non steroid (AINS) drugs, and one of them is indomethacine (Farmakologi & Terapeutik, 2007; Hasanah et al., 2011). Nevertheless, several studies exhibited that IBD could be brought about by the side effect of indomethacine-like non steroidal anti-inflammatory drugs (NSAIDs) (Podolsky, 2002). The use of indonethacine can result in inflammation in the gastric tract, either in human or animals (Bures et al., 2011). Indomethacine will be fastly absorbed by the intestine when it enters the body through oral administration (Tanaka et al., 2001).

A dose of 15mg indonethacine/kg BB will be able to raise the activity and the productivity of ROS (Reactive Oxygen Species). It, then, reacts with phospolipid that produces lipid peroxidation and melondialdehyde (MDA) as free radical marker in the body. If the free radicals occur in excessive numbers, it will trigger or worsen the disease.

Therefore, the treatment of IBD case should be safe and use the natural material-based drugs without any deleterious effect on the ileum inflammation (Lanas & Scarpignato, 2006; Laudanno et al., 2006). One of those with active compound content (alkaloid, steroid, saponin, and flavonoid) potential as anti-inflammation in IBD case is mas ngur oyster (Atactodea striata). This study is expected to be able to show the possible dose of mas ngur (Atactodea striata) extract to cure theInflammatory Bowel Disease (IBD) through MDA decline shown in the histological image.

RESEARCH METHOD

This study used dry extract of mas ngur oyster (Atactodea striata)collected from Ohoililir, Kei Kecil district,Southeast Mallucas, white rat (Rattus norvegicus), indomethacine, corn oil, NaCl 0,9%, PFA 10%, PBS-azida, tyrosine standard solution, PBS-Tween, PSMF solution, aquadest, cool absolute ethanol, cool 20 mM of pH 6.8 -Tris-HCl, casein substrate, pH 7 – phosphate buffer solution, MDA kit, 400 µL ofTri Chloro Acetic Acid (TCA) 4% (b/v) solution,PFA, xylol, paraffin, hematoxyilin-eosin, aquadest, and alcohol.

Test animals were 8-10 weeks old male Wistar strained rats (Rattus norvegicus) of 150 - 200 g BW from Cell and Molecular Laboratory, Faculty of Basic Sciences, Brawijaya University, Malang,meeting ethical certificate, acclimated, and separated into 3 groups, healthy, sick (orally administeredwith 15 mg of indomethacine/kg BWonce), and therapy (oral administration with 15 mgof indomethacine/kg BWonce and continued with administration of 100, 400, 700 mg of dry mas gur oyster extract/kg BW for 14 successive days).

Mas Ngur Oyster (Atactodea striata) Extract Processing

Extraction of mas nguroyster was done using methods of (Harborne, 1987) through maceration in methanol solvent. The mas ngur oyster sample was weighed [in equivalence to the body weight of the treated rats and put into a beaker glasscontaining 50 mL of aquadest, then heated in a waterbath (70°C) up to 10 mL volume left. The therapy application volume was orally administered 2 mL/rat for 14 days.

Ileum Collection

Test animals (wistar rat) were firstly killed through neck dislocation. They were dissected on the abdomen part for ileum collection. It was then removed and washed in 0.9% NaCl and macerated in PBS for 5 minutes, then submerged in PBS-azidafor MDAconcentration measurement and HE-stained histophatolical preparat.

Malondialdehid Concentration Measurement

a. MDA Standard Curve Measurement. MDA standard curve was measured usingthe method of Aulanni'am et al (2012). MDA kit stock solution of 0, 1, 2, 3, 4, 5, 6. 7 and 8 µg/mL was taken 100 µL, inserted into different eppendorf, added 550 µL of aquadest, 100 µL TCA 100%, 250 µL HCl 1 N, 100 µL Na-Thio 10 % andthen homogenized. It was then incubated in water heater at 100oC for 30 minutes, and cooled at room temperature. MDA solution of 4 µg/mL was taken and its absorbance measured at the wavelength of 500-600 nm, then the MDA standard curve was made using the absorbance readings at the maximum wavelength.
b. Ileum MDA concentration measurement with Thiobarbituric Acid (TBA) test.MDA concentration was measured using the method applied by (Roosdiana & Rahmah, 2012). A much as 1 g of ileum was chopped into small pieces, and ground with a cool mortar laid on the ice cube, and then added 1 mL of 0.9% NaCl. The homogenat was moved to a microtube and centrifuged at 8,000 rpm for 20 minutes, then the supernatant was collected. One-hundred µL of ileum supernatant was added with 550 µL of aquadest, and then added 100 µL of TCA, 250 µL of 1N HCl, and 100 µL of Na-Thio. In each reagent addition, the solution was homogenized in a vortex, then centrifuged at 500 rpm for 10 minutes, and the supernatant was put into a new flask. The solution was incubated in a

waterbath at 100oC for 30 minutes and left at room temperature. For blank homogenat, this study used aquadest. The sample absorbance was measured at maximum MDA wavelength and plotted to a standard curve prepared to calculate the sample concentration.

c. Hematoxylin-Eosin Staining

Hematoxylin-Eosin staining was done by firstly putting the ileum preparat in the absolute xylol for 5 minutes 2 times,and then deparaffinized, in which the preparat was put into 1-3-level-xylol[xylol : absolute ethanol (3:1, 1:1, 1:3)]each for 5 minutes, dehydrated, and put into ethanol solution fromabsolute ethanol, 95%, 90%, 80% and 70% ethanol, respectively, each of which was done for 5 minutes, and then soaked in the aquadest for 5 minutes. The preparat was then stained with hematoxylin for 10 minutes until the best outcome was obtained. It was then washed in running water for 30 minutes, rinsed in aquadest, put into eosin stainer for 5 minutes, and soaked in aquadestto remove the excessive eosin. For dehydration phase, the preparat was put into multileveled ethanol from 80%, 90% and 95% up to absolute ethanol. Clearing was, then, conducted by putting the preparat into xylol for 5 minutes, air-dried, mounted on theentellanand covered withcover glass.

RESULT AND DISCUSSION

MDA concentration in rat's, *Rattus norvegicus,*ileum treated with dry extract powder of mas ngur oyster (*Atactodea striata*) induced with indomethacine, based on statistical test of SPSS 21 *for windows* ($p < 0.05$) revealed significant effect between treatments. Further test using Honest Significant Difference showed inter-treatment significant difference. The MDA concentration of the rat's,*Rattus norvegicus,*ileum is presented in Table 1. This test was carried out to know the severity level of an inflammation from indomethacine induction of 15 mg/kg BW and after mas ngur oyster flesh extract powder application, in which the MDA level will rise from normal condition when inflammation occurs.

Table 1 MDA level ofrat's, *Rattus norvegicus,* ileum

Treatment Group	Mean MDA (µmol/ml.min) ± SD	MDA (%)	
		Increment	Reduction
Healthy group	0.6747 ± 0.0864^{a}	0	0
Sick group	3.6040 ± 0.2746^{e}	534.1317	-
Therapy of 100 mg/kg BB	2.6970 ± 0.1895^{d}	-	25.1682
Therapy of 400 mg/kg BB	0.9838 ± 0.1588^{b}	-	72.7018
Therapy of 700 mg/kg BB	2.9354 ± 0.1718^{c}	-	18.5538

Note: a,b,c,d,e indicate significant difference between treatment groups at $p < 0.05$

Table 1 demonstrates that in negative control group,mean MDA level was0.6747 ± 0.0864 µmol/ml.min. This value is used as a standard to detect the MDA level increment or decline after therapized with mas ngur oyster extract. Positive control group induced with indomethacine (*e*) had significant difference from the negative control group (*a*). The extract doses of 100 mg/kg BW (*d*), 400 mg/kg BW (*b*), and 700 mg/kg BW (*c*) had also significantly different effect from the negative control group(*a*). Homogenity (Lavene) test and One-way ANOVA

revealed significant different effect between the rats of positive control and negative control groups ($p<0.05$) meaning that indomethacine could increase the MDA level.

Indomethacine induction (Table 1) of 15 mg/kg BW evidently causes increment in MDA level of rat's ileum to3.6040 ± 0.2746 µmol/ml.min or 534.1317% of the negative controlwith mean MDA of0.6747 ± 0.0864 µmol/ml.min. According to Bures et al. (2011), indomethacine induction of 15 mg/kg BW could result in acute Inflammatory Bowel Disease (IBD) in colon. After therapized with mas ngur oyster extract of 100 mg/kg BW, the MDA level could be reduced as much as 25.1682 % of the IBD group. The extract therapy of 400 mg/kg BW could reduce the MDA level as much as72.7018 %, while the extract therapy of 700 mg/kg BB could only reduce the MDA level as much as18.5538 %. Even though overall mas ngur extract therapy could have reduce the MDA level**,** the use of mas ngur oyster extract higher than 400 mg/kg BW will be able to produce toxins as proved in previous finding (Mutaqin, 2009).

One of the free radicals in the body is Reactive Oxygen Spesies (ROS). High ROS production in the cell will disturb cell activities, especially in cell membrane**,** and the radicals will interact with lipid bilayer called lipid peroxidation, one of the oxidative stresses measurable in *malondialdehyde* (MDA) level**.** MDA compounds are known toxic to cells, and thus, MDA amount could be used as an indicator of cell or tissue damages fromincrement of lipid peroxidation activity (Utari & Riyadi, 2011).

Decrease in MDA level from mas ngur oyster extract treatment could results from bioactive compounds contained in the oyster, such as, alkaloid, steroid, saponin, and flavonoid that can prevent lipid peroxidation formation. In general, alkaloid is often used in medication (Harborne, 1987), since it could function as antioxidant (Hanani, 2005). Working mechanism of the alkaloid is to accelerate wound healing and to increase stomach mucus production after injury from induced material (Tan et al., 2000). Working mechanisms of flavonoid as anti-inflammatory agent occurs by pressing netrophyl/cytokine formation in the gastric tract (Kim et al., 2004), triggering tissue improvement through expression of various growth factors, anti-oxidant activity, and reacting with **reactive oxyhen** species (Pastrana-Bonilla et al., 2003) (Liu et al., 2002).

Destruction level and body organ improvement could be known through one of measured parameters, organ histology. All histological images of the ileum of negative control (healthy), positive control positif (sick), andmas ngur extract treatment groups are presented in Fig. 1.

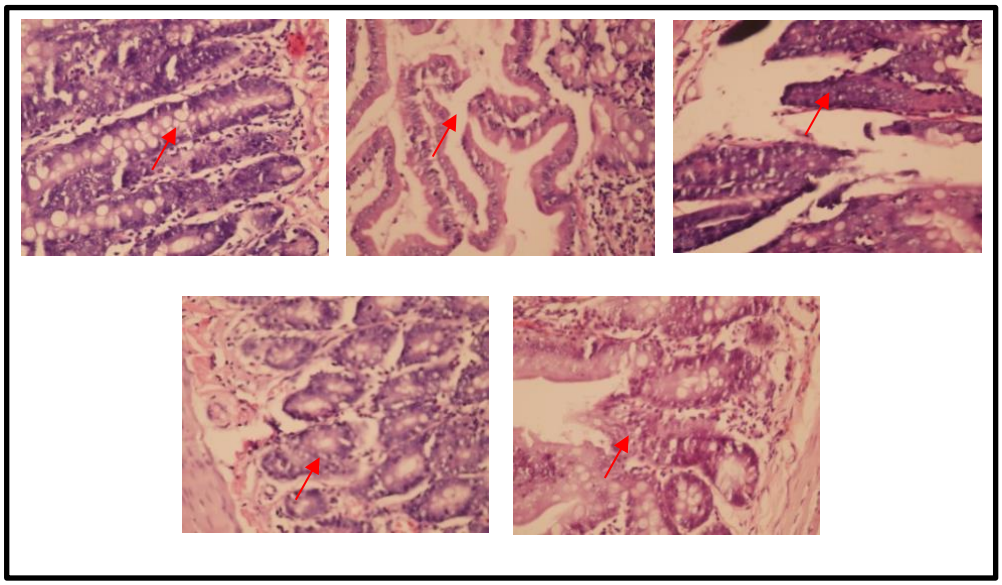

Figure 1. Histoligical image of rat ileum. 400x enlargement

Note:
A. healthy group rat
B. sick group rat induced with indomethacine
C. Therapized rat (indomethacine induction + 100 mg of mas ngur oyster extract/kg BW)
D. Therapized rat (indomethacine induction + 400 mg of mas ngur oyster extract/kg BW)
E. Therapized rat (indomethacine induction + 700 mg of mas ngur oyster extract/kg BW)

In Fig.1, it is apparent that vili of the negative control (A) ileum be good and have more compact matrix, while in sick group induced with indomethacine (B) the vili look damaged. It could result from that induced indomethacine will cause immune response that eases the pathogenic bacteria invasion into the small intestine, and it will activate the macrophages through cytokine secretion, such as TNF-αand ROS.

Fig. 1 also shows that the sick rats induced with indomethacine then treated with mas ngur oyster extract show improvement of small intestinal tissues with the highest improvement recorded in 400 mg/kg BW (D) treatment and the lowest in 700 mg/BW (E) treatment. This improvement is indicated with more compact intestinal vili than those in sick group rats.

CONCLUSION

Tissue repair of small instestine revealed that mas ngur oyster extract containing bioactive compounds, such as alkaloid, steroid, and saponin,had ability to suppress ROS formation as tissue damage cause stimulated by indomethacine induction. Thus, this study could conclude that the bioactive compound of mas ngur oyster extract possessed an ability to repair tissue damage caused byROS, through regeneration mechanism, new absorbing epithelial cells will be produced to replace

the damage cells, and therefore, the ileum tissue of small intestine could be recovered. Moreover, Indomethacine induction of 15 mg/kg BWand therapy of mas ngur oyster powder extract were administered orally. MDA level was measured usingThiobarbituric Acid (TBA)test and histological image of the ileum using Hematoxylin-Eosin staining. Results showed that mas ngur extract therapy gave significant effectin MDA level ($P<0.05$) and difference between the treatments with effective dose of400 mg/kg BW

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