ORIGINAL ARTICLE

The effect of bay leaf extract *Syzygium polyanthum* (Wight) Walp. on C-reactive protein (CRP) and myeloperoxidase (MPO) level in the heart of rat model of myocardial infarction

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ABSTRACT

Aim To study the anti-inflammatory effect of bay leaf on C-reactive protein (CRP) and myeloperoxidase (MPO) level in the heart of rat model with myocardial infarction.

Methods In the first phase, results of pathological examination and biochemical assay were compared between rats with and without induction of myocardial infarction. In the second phase, the effect of bay leaf extract on CRP and MPO of rats with myocardial infarction was studied.

Results C-reactive protein and MPO were higher in rats which had myocardial infarction. Administration of bay leaf extract reduced levels of CRP and MPO in the rats started from day 4 after the induction of myocardial infarction.

Conclusion Anti-inflammatory effect of bay leaf is confirmed, reflected by decreasing of CRP and MPO levels of rat model with myocardial infarction.

Keywords: bay leaf, myocardial infarction, inflammation

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Original submission:
13 August 2019;
Accepted:
25 September 2019
doi: 10.17392/1068-20

Med Glas (Zenica) 2020; 17(1):41-45

INTRODUCTION

The magnitude of cardiovascular disease (CVD) is increasing globally. World Health Organization (WHO) stated that 17.9 million people died due to cardiovascular disease every year (31% of all global deaths). Most of those deaths occurred in low- and middle-income countries (1). Indonesia, which is a middle-income country, is now facing an increase of non-communicable disease-related deaths. Of all deaths, coronary heart disease (CHD) is one of the highest causes of death in Indonesia (2).

Atherosclerosis plays an important part in pathogenesis of CHD. Most of the blockage in coronary arteries is caused by atherosclerosis (3). Inflammation takes part in every step of atherosclerosis, from the development until rupture of plaque that causes myocardial infarction (4). The inflammatory process can lead to endothelial dysfunction, leukocyte migration, extracellular matrix degradation, and platelet activation. Some inflammatory biomarkers can specifically describe cardiovascular inflammatory processes and can predict recurrent atherothrombosis (5).

Two inflammatory markers that increase in myocardial infarction are C-reactive protein (CRP) and myeloperoxidase (MPO). C-reactive protein is acute phase protein that is produced in response of IL-6 and other inflammatory cytokines. Although nonspecific, this high CRP level can indicate acute inflammation and severe tissue damage (6-8). Myeloperoxidase is a marker of inflammation and oxidative stress that increases in myocardial infarction. Plasma MPO level is high in people with acute myocardial infarction and has the potential as an early diagnostic marker in patients with chest pain (8-10).

Indonesia is an Asian country which is rich of natural plant biodiversity. Since long time ago, Indonesian inhabitants have consumed traditional herbs for health. Empirically, bay leaf (*Syzygium polyanthum*) or salam leaf (Indonesian term) stew was used for the treatment of increased hypercholesterolemia, diabetes mellitus, hypertension, gastritis and diarrhoea (11). Bay leaf contains flavonoid which was suspectedly responsible for those effects. Flavonoids are natural antioxidant polyphenol compounds, found in plants, fruits and tea, and wine drinks that can reduce cholesterol and triglyceride levels in the blood, protect from arterial damage, and reduce the amount of cholesterol deposited on the surface of the arterial endothelium. Research in rat showed that flavonoids can reduce lipid peroxidation (12,13). To date, no research has been conducted to assess effects of both bay leaf extract and compound contained in bay leaf on inflammation in myocardial infarction.

We conducted experimental study to demonstrate anti-inflammatory activity of bay leaf on rat model with myocardial infarction. C-reactive protein and MPO level in heart tissue were chosen as inflammatory markers in this study.

MATERIALS AND METHODS

Sample and study design

Male Wistar Rats (*Rattus novergicus*), 10-12 weeks old, around 200 grams were used as samples. Condition of the environment was set to 20-25 ^{o}C and 12 hour light/dark cycle. Access to food and drink were provided freely. Rats which got other disease or injury during this study or did not survive until the end of this study were excluded. All rats were housed in a steel cage in animal house laboratory of the School of Medicine, Universitas Sumatera Utara, Medan, Indonesia. Institutional Ethics Committee of Universitas Sumatera Utara, Medan, Indonesia approved all procedures conducted in this study.

Methods

To induce myocardial infarction, coronary artery ligation was performed. A rat was first anesthetised by ketamine. Thoracotomy was done and left anterior descending (LAD) artery was ligated permanently. A sign of myocardial infarction was pale light (blanching) area distal to the ligation (13). Thorax and skin were closed and the rat was left to recover by itself.

This study was conducted in two phases. In the first phase, the results of pathological examination and biochemical assay were compared between rats with and without induction of myocardial infarction. In the second phase, the results of biochemical assay between rats with and without administration of bay leaf extract were compared. All rats in the second phase study had already been induced to have myocardial infarction.

In the first phase, eight rats were divided into two groups. The first group consisted of four healthy rats without induction of myocardial infarction,

and the second group consisted of four rats with surgical induction of myocardial infarction. Pathological examination of the heart and biochemical assay (CRP and MPO) were compared on day 1 after the induction of myocardial infarction.

In the second phase, 32 myocardial infarction-induced rats were divided equally into two groups: control group (16 rats) and treatment group (16 rats). For the treatment group, ethanol extract of bay leaf in sodium c*arboxymethyl cellulose* (3.6 mg/rats) was administered using orogastric tube (14). The extract was prepared by maceration in the research unit laboratory, School of Medicine, Universitas Brawijaya, Malang, Indonesia. The treatment was given since day 0 until termination day. Four rats of each group were sacrificed on day 1, day 4, day 7 and day 14. Only biochemical assay was done on every sacrificed rat.

Pathological examination included observation of the heart to find the sign of muscle blanching, especially near ligation area. Stereo microscope (Leica Microsystems, Singapore) was used to document pale light area of the heart. Histopathological examination of the blanching muscle was done using Masson's trichrome to witness deposition of collagen in heart muscle (15).

C-reactive protein and MPO were evaluated from blanching heart muscle of the sacrificed rats. Three-hundred μL extraction buffer (Tris 100 mM, pH 7.4, 150 mM NaCl, 1 mM ethylene glycol tetraacetic acid (EGTA), 1 mM ethylenediaminetetraacetic acid (EDTA). 1% Triton X-100, and sodium deoxycholate 0.5%) were added to 5 mg blanching heart tissue and homogenized. Homogenate was then stirred for 2 hours and centrifuged at 13,000 rpm for 20 minutes, both conducted at 4 ℃. Cell extraction was equipped with phosphatase, a protease inhibitor cocktail and PMSF up to 1 mM immediately before the use. Enzyme-linked immunosorbent assay (ELISA) commercial kit (MyBioSource, USA) was used to quantify CRP and MPO level. All assays were conducted in the Laboratory of Biochemistry and Molecular Biology of the School of Medicine, Universitas Brawijaya Malang.

Statistical analysis

CRP and MPO were expressed as mean±SD. Differences between groups were determined using independent t-test and ANOVA test.-Statistical significance was set as $p<0.05$.

RESULTS

On the first phase, both heart pathology and biochemical assay were compared. In the group of rats which had surgical ligation of LAD artery, area distal of the ligation appeared pale light and vessels were narrowed on day 1. When examined using Masson's trichrome, collagen deposition was also seen in the affected tissue (Table 1, Figure 1). Results of biochemical assay on CRP and MPO showed the elevation of CRP and MPO levels on the group with induction of myocardial infarction compared to those without induction ($p<0.001$).

Table 1. Results of first study phase (day 1 after induction of myocardial infarction)

Characteristics/ variable	Rats without induction	Rats with induction
Macroscopic pathology	normal vessel, normal heart muscle	narrowing of blood vessels and some pale light (blanching) area of the heart
Histopathology	normal heart tissue	increased collagen deposition
CRP (mg/L) (mean + SD)	0.44 + 0.05	3.80 + 0.15
MPO (ng/ml) (mean + SD)	6.40 + 1.90	15.72 + 3.05

CRP, C-reactive protein; MPO, myeloperoxidase;

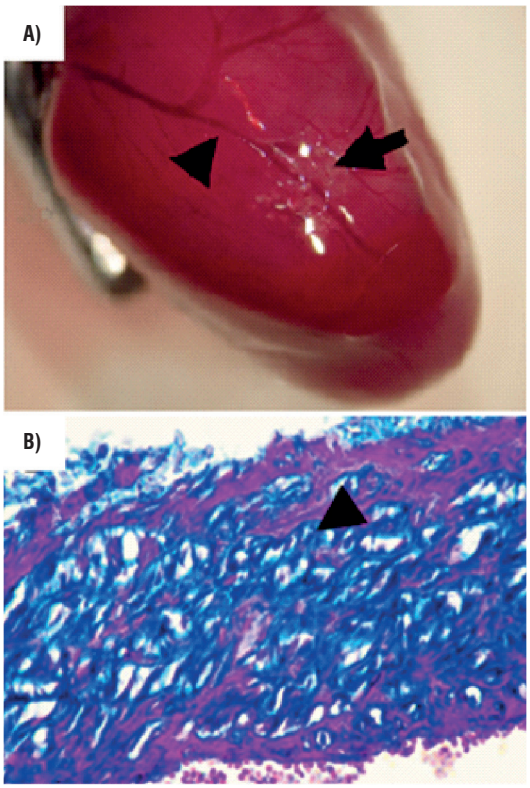

Figure 1. Pathology (A) and histopathology (B) of the heart on day 1 after ligation of the left anterior descending artery. Macroscopically, there was narrowing of coronary artery (A, arrow head) and pale light area distal to ligation area (A, arrow). On histopathological examination, collagen deposition was documented (B, arrow head) (Hasan R, School of Medicine, Universitas Sumatera Utara, Medan)

Table 2. Results of the second study phase

Day	CRP (mg/L)			MPO (ng/mL)		
	Control group	Treatment group	P	Control group	Treatment group	P
1	3.80 + 0.15	3.37+0.25	1.000	15.72+3.05	14.54+0.37	1.000
4	3.95+0.07*	1.48+0.17*	0.002	21.36+1.99*	7.36+1.46*	0.002
7	4.57 + 0.28†	0.64 + 0.38†	0.001	24.36+2.41†	6.20+2.20†	0.001
14	6.04 + 0.45‡	0.26 + 0.10‡	0.001	30.18+1.27‡	4.80+1.19‡	0.001

CRP, C-reactive protein; MPO, myeloperoxidase; *day 1 vs day 4; †day 4 vs day 7; ‡day 7 vs day 14;

Mean CRP in the control group was 3.80 mg/L on day 1. This number kept increasing until day 14 (mean 6.04 mg/L: $p<0.001$). In the treatment group, mean CRP was 3.37 mg/L on day 1, which kept decreasing and reached its lowest level on day 14 (mean 0.26 mg/L, $p<0.001$). Between the control group and the treatment group, CRP differed significantly from day 4 until day 14. Similar pattern was shown also by MPO. Myeloperoxidase increased from day 1 (mean 15.72 ng/mL) to day 14 (mean 30.18 ng/mL; $p<0.001$). In the treatment group, a decreasing level from day 1 (mean 14.54 ng/mL) to day 14 (mean 4.80 ng/mL; $p<0.001$) was also observed (Table 2).

DISCUSSION

Ligation of coronary artery is a procedure known to induce myocardial infarction. Successful procedure in inducing myocardial infarction model is evidenced blanching (pale light) on myocardium distal to ligation area (13,16). This is caused by lack of blood perfusion that enters the tissue. In the first phase of this study, we demonstrated successful myocardial infarction induction, which was marked by blanching heart muscle. Additional information was obtained by histopathology test, which showed collagen deposition of affected area. Since collagen deposition is the sign of infarction of tissue, we confirmed that these rats had myocardial infarction (15).

Further, we assessed inflammation process on heart tissue measuring CRP and MPO. C-reactive protein and MPO are common proteins released after acute myocardial infarction. Both proteins could be detected in early myocardial infarction and changes could reflect inflammation status (7,8). In the current study (first phase), we found rats with myocardial infarction had higher CRP and MPO that already been detected since first 24 hours. It means that inflammation occurred in this group of rats. In second phase we evaluated the effect of bay leaf extract on rats with myocardial infarction. In the control group of myocardial infarction, increasing CRP and MPO were observed until day 14. In the treatment group, we administered ethanol extract of bay leaf in sodium c*arboxymethyl cellulose at the dose of* 3.6 mg per rat. This dose was chosen based on Syahreza et al. who investigated the effect of this extract on patients with dyslipidaemia (14). We observed a reduction of CRP and MPO in the treatment group, and the difference was significant from day 4 to day 14. On day 14, these levels were even lower than baseline CRP and MPO levels that were observed on rats without myocardial infarction on the first phase of this study. It showed that bay leaf extract might have strong anti-inflammatory effects that could benefit even in early myocardial infarction. In this study we did not assess the optimal dose of bay leaf extract to reduce heart inflammation. It should be addressed in further studies.

Bay leaf, which is usually used as traditional medicine in Indonesia, has been studied for various effects on cardiovascular disease. The same results were also shown by Agustina et al. who reported that anti-inflammatory effect of bay leaf extract could be observed as early as 4 hours after administration (17). Flavonoid and tannin in bay leaf were two compounds that could have anti-inflammatory effect (18). Quercetin and phloretin were identified flavonoids in bay leaf (19). Flavonoid is abundant in plants and has been long studied for its anti-inflammatory, antiplatelet, anticholesterol, antidiabetes and antioxidant effect (12). Anti-inflammatory effects of bay leaf are caused by inhibition of cyclooxygenase in arachidonic acid metabolism (17). Furthermore, quercetin in bay leaf could also benefit in cardiovascular disease by inhibiting activity and oxidation of HMG CoA reductase and inhibiting the secretion of Apo-B 100, therefore, decreasing total cholesterol and low-density lipoprotein. Moreover tannin could inhibit cholesterol absorption in gut (20).

In conclusion, we confirmed anti-inflammatory potential of bay leaf. Bay leaf extract could reduce inflammation of the heart caused by myocar-

dial infarction in rat model, reflected by decrease in of CRP and MPO levels. Flavonoid maybe responsible for this effect, so that this compound should be further researched for its potential in myocardial infarction.

ACKNOWLEDGMENTS

Authors thank the Department of Biochemistry and Molecular Biology Laboratory, School of Medicine, Universitas Brawijaya Malang, Indonesia, for their support to the research.

FUNDING

No specific funding was received for this study.

TRANSPARENCY DECLARATION

Conflicts of interest: none to declare.

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