



Cardioprotective Activity of Combination of Soursop Leaves (*Annona muricata* L.) and Avocado Seeds (*Persea americana* Mill.) on Isoproterenol-Induced Myocardial Infarction in Rats

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Abstract

The purpose of this study was to evaluate the protective role of soursop leaves and avocado seed extract, single or in combination against isoproterenol (ISO)-induced myocardial infarction (MI) in Male Wistar rats. The extracts were given orally for 15 days, while ISO 85 mg/kg were administered intraperitoneally twice at an interval 24h. The measured parameters were serum levels of biomarkers of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine kinase (CK), and creatine kinase-MB (CK-MB). Furthermore, histopathology was done to observe the integrity of the myocardial cell membrane. The results showed that pretreatment of single extract of soursop leaves (150 mg/kg) and avocado seeds (150 mg/kg) did not significantly inhibit the effects of ISO on ALT, AST, LDH, CK, and CK-MB. Meanwhile, pretreatment of the combination of soursop leaf ethanol extract and avocado seeds 75-75 mg/kg b.w. inhibited the increase of ALT, AST, LDH, CK, and CK-MB biomarkers level (5.4%; 27.08%; 35.68%; 14.29%; and 43.79%, respectively) significantly as compared to the ISO control group. The combination extract at doses of 75 mg/kg b.w. each could maintain the integrity of the myocardial cell membrane upon ISO administration. Overall, the cardioprotective activity of this extract combination of soursop leaf ethanol extract and avocado seeds at a dose of 75 mg/kg each was synergistic.

Keywords: myocardial infarction; isoproterenol; avocado seeds; soursop leaf; cardioprotective

1. Introduction

Cardiovascular disease (CVD) is a group of diseases that is leading cause of death in low to moderate income developing countries. Myocardial Infarction (MI) is one of the most common CVD diseases worldwide. According to World Health Organization (2018) report, Acute myocardial infarction (AMI) is prevalent in approximately 32.4 million people annually worldwide [1]. Myocardial infarction is the world's leading cause of death and results in approximately 17.7 million deaths per year [2]. In Indonesia, MI is one of the top five causes of death [3].

Myocardial infarction (MI) is also known as heart attack that causes irreversible damage or cardiac

muscle necrosis due to prolonged ischemia [4]. Myocardial infarction is caused by atherosclerosis (narrowing and thickening of the arteries by an accumulation of plaque) which blocks blood flow to cardiac muscle cells and causes the death of cardiac muscle cells [5]. Oxidative stress, which produces free radicals in cardiac cells, causes atherosclerosis [6]. Myocardial infarction is diagnosed by measuring the levels of biomarkers in the form of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine kinase (CK), and CK-MB and the increased levels of biomarkers determine the severity of cardiac muscle cell damage [7]. Isoproterenol is a compound that induces myocardial infarction, and it is a synthetic

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catecholamine compound that acts on β -adrenergic which stimulates the heart, especially cardiac muscle cells in animal testing [15].

Soursop (*Annona muricata* L.) is an Annonaceae group that originates from the tropical regions of the Caribbean and South America. Soursop grows and spreads in tropical and subtropical regions, such as Indonesia, Malaysia, South Africa, Angola, and Spain [8]. Soursop has been used to treat a variety of diseases as an antibacterial, antifungal, antinociceptive, and anti-inflammatory agent. According to Pulivarthi et. al., soursop leaves have a protective effect against oxidative stress in the liver and cardiac tissue by increasing antioxidant enzyme production [9]. Flavonoids, tannins, essential oils, and vitamins (Vitamins B1, B2, and C) are found in soursop [10].

Avocado (*Persea americana* Mill.) belongs to Lauraceae group that originates from Central America and is developing in tropical regions. Avocado is traditionally used to moisturize facial skin, relieve swelling, treat high blood pressure, diabetes mellitus, and as a cardioprotector. The flavonoids and antioxidants in avocados help ward off free radicals in the body [11]. Avocados also contain saponins, tannins, steroids, terpenoids, and cardiac glycosides [12]. The LD50 reported in Avocado seeds extract was 1200,75 mg/kg [13]. The presence of chemical compounds in avocados that have the potential to protect the heart, particularly cardiac muscle cells, prompted this study, because no research has been done on the cardioprotective effects of soursop leaves, avocado seeds, as well as when used in combination. As a result, the goal of this study was to see if ethanol extracts of soursop leaves and avocado seeds, as well as their combination, could prevent isoproterenol induced myocardial infarction in male Wistar rats.

1. Experimental

1.1. Materials

Water bath, funnel, stirring rod, spatula, filter paper, evaporating dish, vial, reflux apparatus, rotary vacuum vaporizer, Erlenmeyer flask, analytical balance, oven, rat cage syringe, blender, oral gavage needle, centrifuge, distillation flask, pycnometer. Soursop leaves (*Annona muricata* L.), avocado seeds (*Persea americana* Mill.), 96% ethanol solution, 70% ethanol solution, isoproterenol powder, 0,9% sodium

chloride compound, Na-CMC compound, distilled aqua, 10% formalin buffer solution, toluene solution, 1% iron (III) chloride solution, gelatin, concentrated HCl solution, formaldehyde solution, sodium acetate solution, ether solution, acetic anhydride solution, concentrated sulfuric acid, 2 N hydrochloric acid solution, magnesium powder, amyl alcohol solution, 25% ammonia solution, chloroform solution, 10% hydrochloric acid solution, $\text{Bi}(\text{NO}_3)_3$ solution, potassium iodide solution, dan HgCl_2 solution.

1.2. Animals

The rats used in this test were male Wistar rats taken from the laboratory animals of Institut Teknologi Bandung – School of Pharmacy. During study, rats were housed in the animal chamber and fed a standard pellet with ad libitum access in food and water. All animals' procedures were done following the standard 102 ethical guidelines for animal use and care as described by the law 2010/63/EU of the European 103 Parliament and of the Council of 22 September 2010 on the protection of animals used for 104 scientific purposes (EEC 1986).

1.3. Determination of Raw Material

Determination of soursop (*Annona muricata* L.) and avocado (*Persea americana* Mill.) plant has been done at the Center for Biological Research and Development-LIPI, Raya Jakarta-Bogor street KM. 46, Bogor, West Java.

1.4. Extract preparation of Soursop leaves, Avocado seeds, and their Combination

Soursop leaves and avocado seeds were washed by running water and dried in an oven at 40-50 °C for two days. A blender was used to mill soursop leaves and avocado seeds into a simplicia powder. For two hours, the simplicia powders were refluxed in a 96% ethanol solvent. The extracts were concentrated by a rotating vacuum vaporizer at the temperature of 50 °C until thick extracts were obtained. The thick extracts were prepared in suspension preparation with CMC-Na 0.5% solution with the addition of 3% concentration of Tween 80 as a wetting agent. The addition of 3% concentration of Tween 80 was based on the result of the optimization in the manufacture of test preparations

1.5. Effect of Soursop leaves, Avocado Seeds, and their combination on Isoproterenol induced Myocardial Infarction

Male Wistar rats were used in this experiment with an average weight of 200-300 grams per group.

The male Wistar rats were divided into six groups consisting of (1) the negative control group was given CMC-Na solvent, (2) the positive control group was given CMC-Na solvent and was induced by isoproterenol, while group 3 to 6 were given tested extracts. This experiment was started with oral administration of the extracts for group 3 to 6, subsequently, ethanol extract of soursop leaves at a dose of 150 mg/kg, ethanol extract of avocado seed at a dose of 150 mg/kg, a combination of ethanol extract of soursop leaves and avocado seed at a dose of 150 mg/kg each and a combination of ethanol extract of soursop leaves and avocado seed at a dose of 75 mg/kg each for 14 days. Isoproterenol solutions at a dose of 85 mg/kg were injected intraperitoneally for group 2 to 6 on day 13 and 14. On day 15, the Wistar rats were sacrificed, and blood was drawn intracardially. Serum was obtained by centrifugation for 10 minutes at 12,000 rpm.

1.6. Determination of AST, ALT, LDH, CK, and CK-MB serum level

Serum was used to determine aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), lactate dehydrogenase (LDH), creatinine kinase (CK) and creatinine kinase MB (CK-MB). The measurement was done based on manufacturing instruction.

1.7. Statistical Analysis

The experimental parameters were analyzed using the One-way ANOVA statistical test method ($P < 0.05$) with the Graphpad Prism 8 application.

2. Results and Discussion

The concentrated ethanol extract of soursop leaves and avocado seeds was evaluated using yield, drying shrinkage, water, and ethanol soluble extract, special gravity, and phytochemical screening for alkaloid, flavonoid, saponin, steroid/triterpenoid, tannins, and quinone (Table 1).

Moreover, the result of the phytochemical screening showed that both ethanol extracts had flavonoid, tannin, and steroid/triterpenoid compounds (Table 2). In addition, the ethanol extracts from avocado seeds also contained saponin compounds.

Compounds found on each extract tested might have cardioprotective activity. It has been reported that flavonoids contain antioxidants that can counteract free radicals by Reactive Oxidative Stress (ROS). In addition, flavonoids also inhibit the activity of cyclooxygenase, lipoxygenase, and nitric oxide enzymes [14]. Tannin contains antioxidants used to

inhibit the activity of free radicals and lipid peroxide [15]. Moreover, steroids and triterpenoids work as anti-inflammatory and also inhibit the activity of cyclooxygenase and lipoxygenase [16]. Furthermore, saponin, as an anti-inflammatory and antioxidant compound, can be an agent against diabetes mellitus, chronic inflammation, oxidative stress, and heart disease (as a cardioprotective agent) [17]. An active component of Soursop leaves, Roseoside, has been reported to have a variety of functional activities such as antihypertensive, antidiabetic and anti-inflammatory which contributes to cardioprotective mechanisms.

Induction of isoproterenol at a dose of 85 mg/kg b.w. two times with a delay of 24 hours can produce myocardial infarction [18]. This induction significantly increased serum biomarker levels compared to the negative control group (the group without isoproterenol induction). Previous studies showed that increased of serum biomarker levels was an important indicator of tissue damage [19], [20]. Pathophysiological changes myocardium were significantly found well in accordance with the increase in cardiac biomarker enzyme activities in the serum of rats with MI [20].

The present study shows that pretreatment with combination extract of soursop leaves and avocado seeds significantly prevent the increase of AST, CK, CK-MB, and LDH serum levels. In Table 3, the results showed that isoproterenol injection could significantly increase ALT, AST, LDH, CK, and CK-MB mice serum levels compared to negative control group. Pre-treatment with single soursop leaves or avocado seeds extract could attenuate the increase of those markers. Interestingly, extract combination at a dose of 75 mg/kg each significantly attenuated all levels of cardiac damage marker enzymes compared to the isoproterenol-treated group. In this study, cTnI was not determined. Although cTnI is known as one of important marker for cardiac injury, CK-MB also can be used as it has similar increase pattern for acute myocardial infarction [20].

Histopathological examination of the myocardium of a normal rat showed clear integrity of the myocardial cell membrane (Figure 1). The changes in integrity of myocardial cell membrane were observed in ISO-treated rats. All extract-treated groups showed similar results as ISO-treated rats, except for extract combinations at a dose of 75 mg/kg each. Infiltration

leukocyte as inflammation minimal in extract combination at dose 75 mg/kg each.

This study implies a pharmacological knowledge of combination of soursop leaf and avocado seed extracts for the protection of the heart with myocardial infarction. The therapeutic use of this combination extract can be explored further for other types of cardiovascular diseases. Limitations of this study are no electrocardiography and infarction size data were determined due to limited laboratory facilities. Therefore, for further study, more parameter measured to understand the effect of combination for myocardial infarction treatment will be very interested to be done. Moreover, clinical study needs

to be done in humans to affirm the cardioprotective potential.

3. Conclusion

Combined pretreatment of extract of soursop leaves at a dose of 75 mg/kg and avocado seeds at a dose of 75 mg/kg was highly effective than single pretreatment. The combination extracts significantly attenuated the increase of heart biomarker serum upon ISO-induced myocardial infarction injury in rats, and also could protect myocardial heart tissue. The results of this study suggest that the combination may have potential for the prevention of myocardial infarction.

Table 1: Characterization of the Extract Sample

Characterization	Extract Sample	
	Soursop Leaves	Avocado Seeds
Yield (% b/b)	24.418	13.961
Water content (% b/v)	9.804	4.931
Water soluble extract content (% b/v)	18.288	17.265
Ethanol soluble extract content (% b/v)	12.565	11.563
1% specific gravity (g/mL)	0.808	0.806
Drying shrinkage (% b/b)	11.497	10.315

Table 2: Result of Phytochemical Screening of Extract Sample

Phytochemical Screening	Simplicia	
	Ethanol extracts of Soursop Leaves	Ethanol extracts of Avocado Seeds
Alkaloid	-	-
Flavonoid	+	+
Saponin	-	+
Steroid/Triterpenoid	+	+
Tannin	+	+
Quinone	-	-

Description: (+) = detected. (-) = not detected

Table 3: Effect of Soursop leaves (*Annona muricata L.*), avocado seeds (*Persea americana Mill.*) and its combination on Serum Cardiac Marker Enzymes on isoproterenol induced Myocardial Infarction in Rats

Groups	Serum Cardiac Marker Enzymes				
	ALT (U/I)	AST (U/I)	LDH (U/I)	CK (mg/dl)	CK-MB (U/I)
Negative control	36.72±2.48	67.18±10.94	829.82±208.33	0.96±0.02	137.84±27.73
Positive control	47.41±2.06*	159.14±25.22*	2575.49±206.29*	1.12±0.05*	356.99±30.55
Soursop leaves 150 mg/kg b.w.	43.13±0.33	129.17±10.07*	2156.57±190.06*	1.08±0.05*	317.57±29.82 *
Avocado seeds 150 mg/kg b.w.	40.03±4.60	130.82±16.49*	2004.55±79.02*#	1.14±0.08*	273.63±86.44*
Combination of Soursop leaves 150 mg/kg and Avocado seeds 150 mg/kg b.w.	41.79±1.99	125.06±9.28*	1965.28±197.99*#	1.00±0.02	64.11±19.95#
Combination of Soursop leaves 75 mg/kg and Avocado seeds 75 mg/kg b.w.	44.85 ±6.02	116.04±15.69*#	1656.43±240.56*#	0.96±0.03#	200.65±42.90#

Note:

*=P<0.05 vs negative control

*=P<0.05 vs positive control

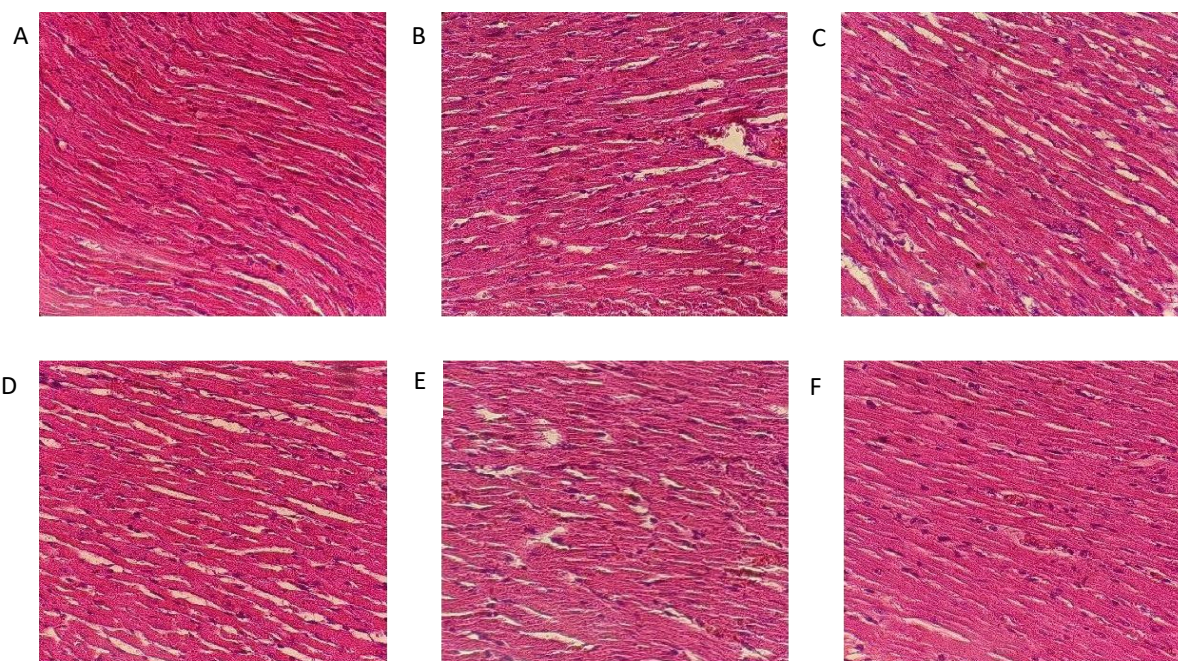


Fig.-1: Histopathological observation of the heart using H&E staining. (A) negative control, (B) positive control, (C) Soursop leaves ethanol extract 150 mg/kg b.w., (D) Avocado seeds ethanol extract 150 mg/kg b.w., (E) Combination of Soursop leaves 150 mg/kg and Avocado seeds 150 mg/kg b.w., and (F) Combination of Soursop leaves 75 mg/kg and Avocado seeds 75 mg/kg b.w.

4. Conflicts of interest

There are no conflicts of interest.

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