

Effect of Combination of Purple Sweet Potato (*Ipomoea batatas* L.) Ethanolic Extract and Ramipril Administration on Myocardial C-Reactive Protein Expression and Aortic Intima-Media Thickness in Hypertensive Rat Model

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ABSTRACT

Purposes: To determine the effect of giving a combination of purple sweet potato (*Ipomoea batatas* L.) ethanolic extract and ramipril in a rat model of hypertension. **Methods:** This is an experimental study with a post-test-only control group design and is a collaborative study of the effect of giving a combination of purple sweet potato (*Ipomoea batatas* L.) ethanolic extract and ramipril in a rat model of hypertension. Thirty male Wistar rats were given 2 mL/day of a 4% NaCl diet to induce hypertension. The rats were divided into three treatment groups, consists of NaCl 4% 2 mL/day + combination therapy with purple sweet potato (*Ipomoea batatas* L.) ethanolic extract 400 mg/kgBW/day and ramipril 1 mg/day (P1), NaCl 4% 2 mL/day + purple sweet potato (*Ipomoea batatas* L.) ethanolic extract 400 mg/kgBW/day (P2) and NaCl 4% 2 mL/day + ramipril 1 mg/day (P3). At week 5, the rat was executed to examine myocardial CRP expression and measure aortic IMT. All the data obtained were analyzed statistically. **Results:** There was a significant difference in myocardial CRP expression between the three treatment groups (p value <0.05). The combination of purple sweet potato (*Ipomoea batatas* L.) ethanolic extract 400 mg/kgBW/day + ramipril 1 mg/day (P1) showed a better effect on reducing myocardial CRP expression than administration of purple sweet potato (*Ipomoea batatas* L.) ethanolic extract 400 mg/kgBW/day (P2) (mean difference 216.44 ng/ml; 95% CI 133.56 – 299.32; p value <0.001) or Ramipril 1 mg/day (P3) (mean difference 144.44 ng/ml; 95% CI 29.40 – 199.47; p value=0.010). Administration of Ramipril 1 mg/day (P3) showed a better effect on reducing myocardial CRP expression than the group given purple sweet potato (*Ipomoea batatas* L.) ethanolic extract 400 mg/kg/day (P2) (mean difference 102.00 ng/ml; CI 95% 19.11 – 184.88; p value=0.018). There was a significant difference in aortic IMT between the three treatment groups (p value <0.05). The combination of purple sweet potato (*Ipomoea batatas* L.) ethanolic extract 400 mg/kgBW/day + ramipril 1 mg/day (P1) showed a better effect on reducing aortic IMT than administration of purple sweet potato (*Ipomoea batatas* L.) ethanolic extract 400 mg/kgBW/day (P2) (mean difference 10,16 μ m; 95% CI 6,63 – 13,69; p value <0.001) or Ramipril 1 mg/day (P3) (mean difference 5,10 μ m; 95% CI 1,40 – 8,70; p value=0.008). Administration of Ramipril 1 mg/day (P3) showed a better effect on reducing aortic IMT than the group given purple sweet potato (*Ipomoea batatas* L.) ethanolic extract 400 mg/kg/day (P2) (mean difference 5,05 μ m; CI 95% 1,50 – 8,50; p value=0.007). Combination of purple sweet potato (*Ipomoea batatas* L.) ethanolic extract and ramipril showed a higher potential effect on reducing myocardial CRP expression and aortic IMT compared to single therapy. **Conclusion:** The combination of purple sweet potato (*Ipomoea batatas* L.) ethanolic extract 400 mg/kgBW/day + ramipril 1 mg/day can provide the best decrease in the expression of myocardial CRP and aortic IMT compared to the administration of purple sweet potato (*Ipomoea batatas* L.) ethanolic extract 400 mg/kg/day or ramipril 1 mg/day.

Keywords: combination of purple sweet potato ethanolic extract and ramipril; myocardial CRP expression; aortic IMT; hypertension

INTRODUCTION

Cardiovascular disease still contributes to a third of death in Indonesia. The main cardiovascular risk factors, including obesity, diabetes mellitus, and hypertension, have experienced a significant increased in prevalence.[1] Hypertension is a disease that will cause changes in the shape of the myocardial core cells.[2]

The currently developed treatment for hypertension comes from chemical and herbal therapies. One of the medicinal uses that can be used is ramipril and a herbal proposed as an adjuvant for cardiac treatment is purple sweet potato (*Ipomoea batatas* L.) ethanolic extract, which are rich in antioxidants.[3]

Myocardial remodelling is a complex process driven by the response of cardiomyocytes, other cells of the myocardium (i.e., fibroblasts, endothelial cells, pericytes, and immune cells), and cells recruited from the circulation (e.g., immune and inflammatory cells and progenitor cells) to various dynamic stimuli, including mechanical and non-mechanical stimuli, present in cardiac injury conditions, which result in the volume, composition, and physiology of the cardiomyocyte, interstitial space, and coronary microvascular adapting to various interrelated changes, which have a detrimental impact on cardiac function and clinical outcomes of patients with hypertensive heart disease (HHD). Therefore, HHD is not simply a matter of the occurrence of left ventricle hypertrophy (LVH) but the result of complex myocardial, cellular, and tissue regulation that causes changes in the shape, size and function of the left ventricle (LV) and other heart chambers.[4]

C-reactive protein (CRP) is a sensitive marker of inflammation, which describes the condition of chronic low-grade inflammation of the arterial blood vessels and can be used to consistently predict cardiovascular outcomes.[5] A study by Cottone et al. (2007) found that CRP levels correlated with the incidence of LVH in a population of patients with moderate degrees of CKD.[6] Another study by Seyfeli et al. (2016) reported that high sensitivity C-reactive protein (hs-CRP) serum levels had a significant association with LV diastolic dysfunction in patients with hypertension. hs-CRP, a marker of systemic inflammation, is found at higher levels in patients with hypertension. Elevated baseline CRP levels are associated with future development of hypertension in healthy adults.[7]

Ramipril contains 2-aza-bicyclo[3.3.0]-octane-3-carboxylic acid derivative, which is an anti-hypertensive drug in the class of competitive inhibitors of Angiotensin Converting Enzyme (ACE), which converts Angiotensin I to Angiotensin II, besides that it can reduce Angiotensin II due to decreased plasma renin activity and decreased aldosterone secretion. Central nervous system (CNS) mechanisms may be involved in producing the hypotensive effect. ACE inhibitors convert vasoactive kallikreins into their active form (hormones) so that they will lower blood pressure. This drug also has effectiveness in myocardial remodelling, functions as a biomarker of endothelial dysfunction and reduces serum CRP, IL-6, and TNF- α . [8]

Herbal medicines based on plants have been developed and used empirically to treat and prevent various diseases, including cardiovascular disease. Purple sweet potato has antioxidant activity, which works as a free radical scavenger. Sweet potato has a high content of flavonoids (anthocyanins and polyphenols). It can also protect humans from diseases such as atherosclerosis, hypertension, low-density lipoprotein (LDL) oxidation and some cancers. Natural antioxidants can protect the body against cell damage caused by reactive oxygen species (ROS), which can inhibit the occurrence of degenerative diseases and inhibit lipid peroxidase. Anthocyanins and polyphenols are antioxidants that can prevent various types of damage due to oxidative stress to protect cells from free radicals.[9] Research results containing anthocyanins and polyphenols from various sources can reduce blood pressure by maintaining endothelial function through increasing endogenous nitric oxide synthase (eNOS) expression and subsequent nitric oxide (NO) bioavailability and reducing blood malondialdehyde (MDA) levels.[10]

Based on the above matters, this study was made to determine the effect of giving the combination of purple

sweet potato (*Ipomoea batatas L.*) ethanolic extract and ramipril on myocardial CRP expression as a marker of inflammation and aortic intima-media thickness (IMT) in a rat model of hypertension. Expected giving of the combination of purple sweet potato (*Ipomoea batatas L.*) ethanolic extract and ramipril can reduce myocardial CRP expression and decrease aortic IMT, thereby preventing cardiac hypertrophy and cardiac remodeling. If this hypothesis is proven, then the combination of purple sweet potato (*Ipomoea batatas L.*) ethanolic extract and ramipril can be considered as a cardioprotective therapy against cardiac hypertrophy and cardiac remodeling in hypertensive patients as a preventive effort to reduce the incidence of cardiovascular disease.

METHODS

This research was conducted purely experimentally with a post-test only control group design and is a collaborative study of the effect of giving a combination of purple sweet potato (*Ipomoea batatas L.*) ethanolic extract and ramipril in a hypertensive rat model that has received permission for ethical feasibility research with the number 1914/UN14.2.2.VII.14/LT/2023 Faculty of Medicine, Udayana University.

Raising research animals, providing a 4% NaCl diet, and measuring body weight were conducted at the Laboratory Animal Unit of the Pharmacology Section, Faculty of Medicine, Udayana University. CRP expression was performed at the Integrated Biomedical Laboratory Unit, Faculty of Medicine, Udayana University. Aortic IMT examination is carried out in the Laboratory Unit of Veterinary Pathology, Faculty of Veterinary Medicine, Udayana University.

This experimental study aims to determine the effect of giving a combination of purple sweet potato (*Ipomoea batatas L.*) ethanolic extract and ramipril on the expression of myocardial CRP and aortic IMT in a rat model of hypertension. The research sample used 30 rats, but there was one rat in group P1 (a combination of purple sweet potato (*Ipomoea batatas L.*) ethanolic extract and ramipril) and one rat in group P3 (ramipril) which died during the study. So, the final analysis in this study only used 28 rats. This study took male Wistar rats that met the inclusion criteria as samples. Inclusion Criteria: Wistar rat (*Rattus norvegicus*) male; Age 12-16 weeks; Body weight 150-200 grams; Condition of healthy rats (active and not disabled); Systolic rat blood pressure ≥ 140 mmHg or diastolic ≥ 90 mmHg. Exclusion Criteria: Wistar rat are not actively moving; Disabled mouse; Wistar rat died during the study; Decreased rat weight (rat weight becomes less than 200 grams).

Research procedure: 1. Chosen 30 male Wistar rats aged 12-16 weeks, weighing around 150-200 grams, and in good health; 2. Rats were kept in groups of as many as two rats per cage. Cages are made of plastic tubs measuring 30 x 20 x 20 cm with husk mats to absorb wetness and odours well and free from polluted chemicals to keep the cage clean. There is a top cover covered with woven wire. Cages are placed in a ventilated room and natural air, with temperatures ranging from 20 - 26°C and humidity from 40-70%; 3. Wistar rats are divided into three random groups: a. P1 group (treatment group), rats that received 4% NaCl 2 mL/day + combination of purple sweet potato (*Ipomoea batatas L.*) ethanolic extract dose of 400gr/kgBW/day and ramipril 1 mg/day for four weeks; b. P2 group (group control), Wistar rats that received 4% NaCl 2 mL/day + purple sweet potato (*Ipomoea batatas L.*) ethanolic extract dose of 400gr/kgBW/day for four weeks; c. P 3 group (group control), Wistar rats that received 4% NaCl 2 mL/day + ramipril 1 mg/day for four weeks; 4.

In the fifth week, the Wistar rats were dissected for inspection of myocardial CRP expression and aortic IMT; 5. Wistar rats were euthanized with ketamine (dose 50 mg/kgBW) and xylazine (dose 10 mg/kgBW), neck dislocation was performed, and surgery was performed to remove the heart and aorta; 6. After all, the Wistar rats have been euthanized and properly buried (burial according to local customs is like burying humans where at least the rat bodies are given canning offerings and accessories) because they can no longer be used for other research.

All data collected on each group was then analyzed with the SPSS program version 26. Data analysis with the following steps: Descriptive analysis; The Blant-Altman test was used to screen for intra-observer variability with a significance level of CI 95%; Comparison test by using One-Way Anova followed by a post-hoc analysis with least significant difference (LSD) test to see the difference between each group; Correlation test using the Spearman correlation coefficient.

RESULTS

Differences in myocardial CRP expression in each group and post-hoc LSD analyses are presented in Table 1.

TABLE 1: ANOVA and post-hoc LSD Analysis of Myocardial CRP Expression Between Study Groups.

Variable	Mean ± SD (ng/ml)	CI 95%	F	ANOVA	LSD				
					Group	Mean difference (ng/ml)	CI 95%	p-value	
P1	455.56 ± 99.51	379.06-532.04	14,4	<0.001	P1	P2	216,44	133.56-299.32	<0.001
P2	672 ± 85.60	610.75-733,24				P3	144,44	29.40-199.47	0.010
P3	570 ± 76.32	511.33-628.66			P3 P2	102.00	19.11-184.88	0.018	

Combination of purple sweet potato (*Ipomoea batatas L.*) ethanolic extract 400 mg/kgBW/day + ramipril 1 mg/day (P1) had a better effect on reducing myocardial CRP expression than single therapy administration of purple sweet potato (*Ipomoea batatas L.*) ethanolic extract 400 mg/kgBW/day (P2) (mean difference 216.44 ng/ml; 95% CI 133.56 - 299.32; p-value <0.001) or Ramipril 1 mg/day (P3) (mean difference 144.44 ng/ml; 95% CI 29.40 - 199.47; p-value = 0.010). Compared groups administration of purple sweet potato (*Ipomoea batatas L.*) ethanolic extract 400 mg/kg/day (P2), administration of Ramipril 1 mg/day (P3) showed a better effect on reducing myocardial CRP expression (mean difference 102.00 ng/ml; CI 95% 19, 11 - 184.88; value p=0.018).

Comparison results of potential effect of group P1 (combination of purple sweet potato (*Ipomoea batatas L.*) ethanolic extract 400 mg/kg/day + ramipril 1 mg/day), the percentage of potential effect to reduce of myocardial CRP expression was 100%, group P2 (purple sweet potato (*Ipomoea batatas L.*) ethanolic extract 400 mg/kg/day) obtained by 60.23% and group P3 (ramipril 1 mg/day) obtained by 78.9%, so it can be concluded that giving combination of purple sweet potato (*Ipomoea batatas L.*) ethanolic extract 400 mg/kg BW/day + ramipril 1 mg/day has a higher potential effect in reducing myocardial CRP expression compared to the other groups in this study. A graphic of the potential effects between study groups on myocardial CRP expression is presented in Figure 1.

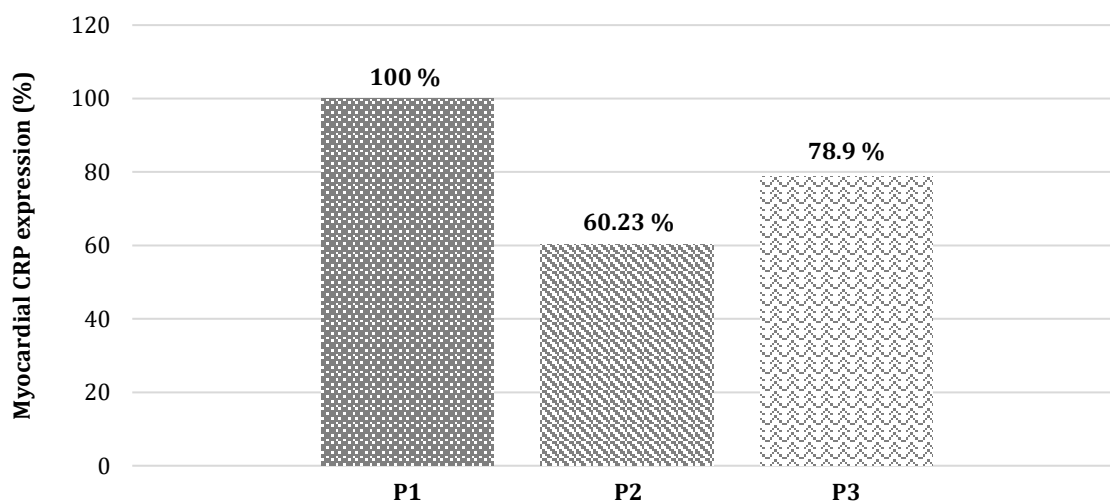


FIGURE 1: Potential effect graphic of group P1 (combination of purple sweet potato (*Ipomoea batatas L.*) ethanolic extract + ramipril) is better than the P2 (purple sweet potato (*Ipomoea batatas L.*) ethanolic extract) group and the P3 (ramipril monotherapy) group in reducing myocardial CRP expression.

Intima-media thickness (IMT) of the aorta was measured through histopathological examination of the ascending aorta using hematoxylin-Eosin (HE) staining. Aortic IMT measurements were measured by one observer with two measurements at different locations.

The Bland-Altman test did not find any significant intra-observer variability (no deviation from the difference in measurement of the data exceeding the 95% confidence limit). The results of the Bland Altman Aortic IMT test for all treatment groups are presented in Figure 2.

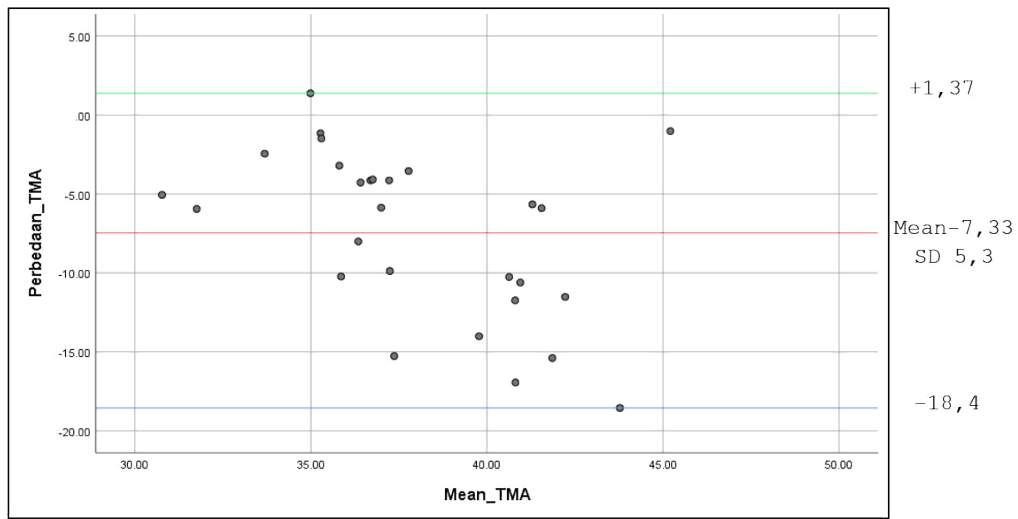


FIGURE 2: Bland Altman test of Aortic IMT Between Study Groups.

A comparative test using One Way ANOVA showed a significant difference in aortic IMT between the three treatment groups ($p < 0.05$).

The differences in aortic IMT for each group were then analyzed using the post-hoc LSD analysis. ANOVA and post-hoc LSD analysis of aortic IMT between study groups is presented in Table 2.

TABLE 2: ANOVA and post-hoc LSD analysis of Aortic IMT Between Study Groups.

Variable	Mean ± SD (ng/ml)	CI 95%	F	ANOVA	LSD				
					Group	Mean difference (ng/ml)	CI 95%	p-value	
P1	31,47 ± 2.61	29,73 - 33.75	17,6	<0.001	P1	P2	10,16	6,63-13,69	<0.001
P2	41.90 ± 5.09	38,26 - 45.55			P1	P3	5,10	1.4-8.7	0.008
P3	36,84 ± 2.71	34.75 - 38.93			P3	P2	5.05	1.5-8.5	0.007

Combination of purple sweet potato (*Ipomoea batatas L.*) ethanolic extract 400 mg/kgBW/day + ramipril 1 mg/day (P1) had a better effect on reducing aortic IMT than single therapy administration of purple sweet potato (*Ipomoea batatas L.*) ethanolic extract 400 mg/kgBW/day (P2) (mean difference 10.16 μm ; 95% CI 6.63 - 13.69; $p < 0.001$) or Ramipril 1 mg/day (P3) (mean difference 5.10 μm ; 95% CI 1.40 - 8.70; $p = 0.008$). Compared groups administration of purple sweet potato (*Ipomoea batatas L.*) ethanolic extract 400 mg/kg/day (P2), administration of Ramipril 1 mg/day (P3) showed a better effect on reducing aortic IMT (mean difference 5.05 μm ; CI 95% 1.50 - 8.50; value $p = 0.007$).

Comparison results of potential effect of group P1 (combination of purple sweet potato (*Ipomoea batatas L.*) ethanolic extract 400 mg/kg/day + ramipril 1 mg/day), the percentage of potential effect to reduce of aortic IMT was 100%, group P2 (purple sweet potato (*Ipomoea batatas L.*) ethanolic extract 400 mg/kg/day) obtained by 85.3% and group P3 (ramipril 1 mg/day) obtained by 92.5%, so it can be concluded that giving combination of purple sweet potato (*Ipomoea batatas L.*) ethanolic extract 400 mg/kg BW/day + ramipril 1 mg/day has a higher potential effect in reducing aortic IMT compared to the other groups in this study. A graphic of the potential effect between study groups on aortic IMT is presented in Figure 3.

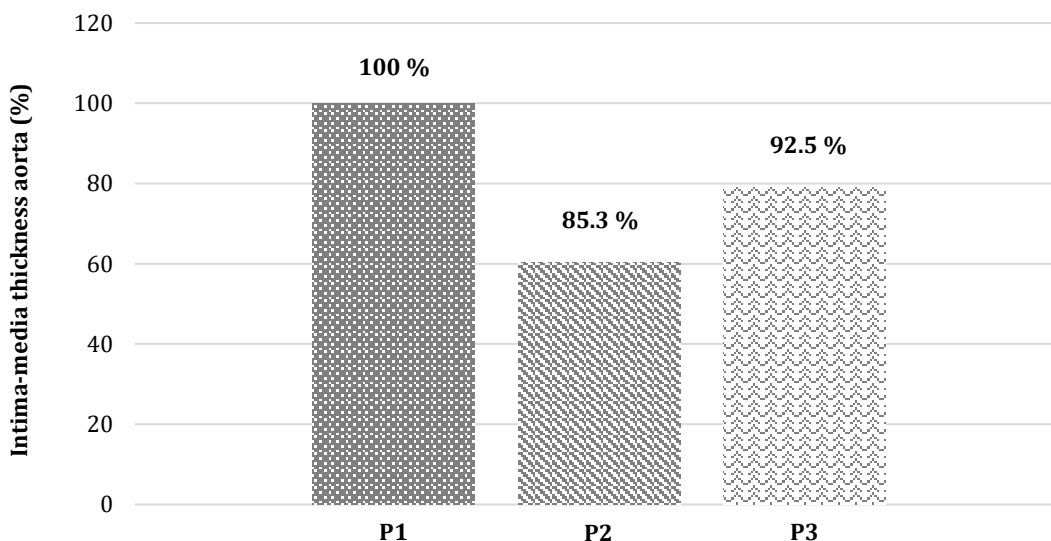


FIGURE 3: Potential effect graphic of group P1 (combination of purple sweet potato (*Ipomoea batatas L.*) ethanolic extract + ramipril) is better than the P2 (purple sweet potato (*Ipomoea batatas L.*) ethanolic extract) group and the P3 (ramipril monotherapy) group in reducing aortic IMT.

The relationship between expression of myocardial CRP and aortic IMT, when given a combination of purple sweet potato (*Ipomoea batatas L.*) ethanolic extract 400 mg/kgBW/day + ramipril 1 mg/day, showed a very strong correlation ($r = 0.857$), which was statistically significant with $p = 0.003$ (Figure 4).

The relationship between expression of myocardial CRP and aortic IMT in the administration of purple sweet potato

tuber ethanol extract 400 mg/kgBW/day showed a very strong correlation ($r = 0.902$), statistically significant with a p -value < 0.001 .

The relationship between expression of myocardial CRP and aortic IMT in the administration of ramipril 1 mg/day showed a very strong correlation ($r = 0.862$), which was statistically significant with $p = 0.003$.

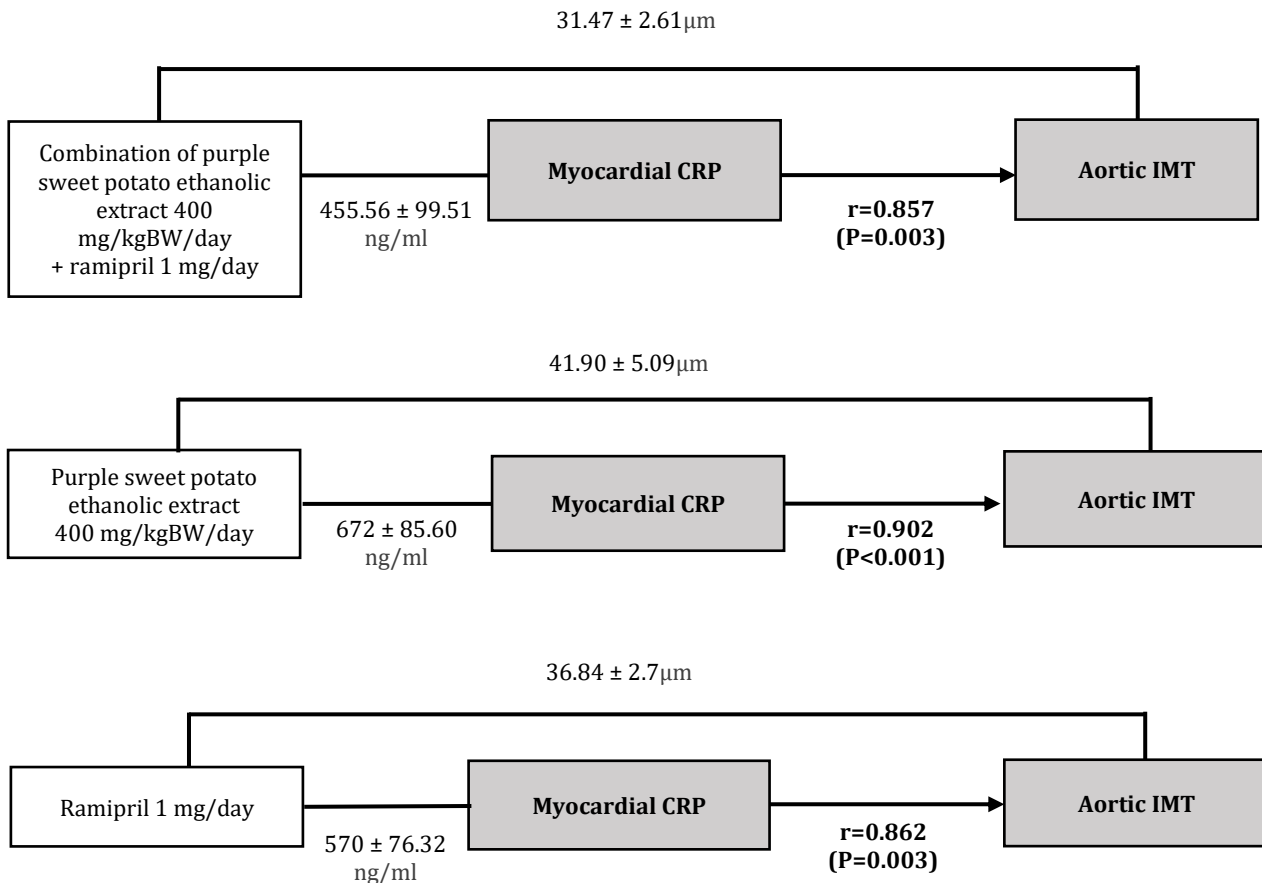


FIGURE 4: Analysis of the correlation between myocardial CRP expression and aortic IMT in each group showed statistically significant results ($p < 0.05$).

DISCUSSION

Hypertension is an independent risk factor for cardiovascular disease. Cardiac remodeling is a complex process stimulated by the response of cardiomyocytes, other myocardial cells (i.e., fibroblasts, endothelial cells, pericytes, and immune cells), and cells recruited from the circulation (for example, immune cells, inflammatory cells and progenitor cells) to various stimuli. Dynamic, including mechanical and non-mechanical stimuli manifest in cardiac injury conditions. Abnormalities in this pathophysiology can lead to interrelated changes in volume, composition, cardiomyocyte physiology, interstitial space and coronary microvascular, which disturb the cardiac function, and one of them manifests clinically as hypertensive heart disease (HHD). [4]

Ramipril is an anti-hypertensive drug belonging to the ACE-inhibitor class, which has a mechanism to prevent stiffness in blood vessels (arterial stiffness) and can reduce blood pressure. Ramipril works by inhibiting the conversion of angiotensin I to angiotensin, causing vasodilation and decreased blood pressure.[11]

The cardioprotective effect of ACE-inhibitors has been demonstrated in several studies and is a first-line recommendation for pharmacological therapy in chronic heart failure (CHF) patients aimed for reducing afterload

and overload. The 2009 American College of Cardiology/American Heart Association (ACC/AHA) and the 2006 Canadian Consensus Guidelines strongly recommend using ACE-inhibitors in patients with heart failure with reduced ejection fraction (HFrEF).[12]

This research is a pre-clinical trial carried out purely experimentally with a post-test-only control group design and is a collaborative study to assess the effect of giving the combination of purple sweet potato (*Ipomoea batatas L.*) ethanolic extract and ramipril as cardio protector agent and anti-cardiac remodeling in a rat model of hypertension. The use of a rat model of hypertension induced by 4% NaCl 2 mL/day for one week and continued until the fifth week led to hypertension. It resembles the pathological process of hypertension in humans because it is associated with chronic hypertensive conditions. This model also meets other criteria as it allows the study of stable chronic diseases, produces predictable and controllable symptoms, and allows measurement of relevant cardiac, biochemical and hemodynamic parameters.[13] The results of an experimental acute toxicity study by Wei et al. (2018) reported that purple sweet potato tuber extract (*Ipomoea batatas L.*) was safe up to the highest dose of 5000 mg/day, and no lethal signs and symptoms or behavioural changes were observed.[9]

This study used two (2) quantitative parameters to assess cardiac remodelling, namely aortic IMT examination and myocardial CRP expression examination as a marker of inflammation.

In this study, it was found that a combination of purple sweet potato (*Ipomoea batatas L.*) ethanolic extract and ramipril has been proven have a cardioprotective effect in the form of decreased myocardial-CRP expression as a biomarker of inflammation related to the pathophysiology of myocardial remodeling and decreased aortic IMT which are associated with hypertension. This study shows that the effectiveness of adding purple sweet potato (*Ipomoea batatas L.*) ethanolic extract has a potential effect as cardioprotective agent and prevent the process of myocardial remodeling compared to monotherapy of ramipril or monotherapy of purple sweet potato (*Ipomoea batatas L.*) ethanolic extract.

Purple sweet potato has antioxidant activity, which works as a free radical scavenger. Sweet potatoes contain high levels of anthocyanins and flavonoids and have beneficial and protective effects against diseases such as atherosclerosis, hypertension and some cancers. The natural antioxidant content of purple sweet potato can protect the body against cell damage caused by ROS, can inhibit the occurrence of degenerative diseases and can inhibit lipid peroxidase. Anthocyanins and flavonoids are antioxidants that can prevent various types of damage due to oxidative stress to protect cells from free radicals.[13]

This study found that administration of purple sweet potato (*Ipomoea batatas L.*) ethanolic extract 400 mg/kgBW/day reduced myocardial CRP expression in a hypertensive rat model. This finding is supported by previous research by Salehi et al. (2020) that an inverse correlation was found between consumption of high doses of anthocyanins and reduced expression of CVD-related risk biomarkers, such as CRP and decreased overall inflammation score. The cardioprotective effect of dietary anthocyanins can be attributed to increased plasma antioxidant capacity and NO levels and decreased LDL oxidation and platelet aggregation levels.[14] In a rat study (n = 40 rats) by Mauray et al. (2010), it was reported that the anthocyanin content of the berries was reported to reduce serum CRP levels from the average CRP serum level before treatment of 679 ± 45 ng/ml decreased to 450 ± 25 ng/ml after administration of a high anthocyanin seed diet for 3 months[15].

The findings in this study are also supported by Xu et al. (2021) in the form of a meta-analysis study (44 randomized control trials (RCT) and 15 prospective study), which reported that feeding a berry diet (high in anthocyanins) resulted in a mean decrease in serum CRP levels of -0.046 mg/dL (95% CI; -0.070 to -0.022 mg/dL; $p < 0.001$)[16].

Another study by Jawi et al. (2014 and 2016) found that the content of anthocyanins and flavonoids from various sources can reduce blood pressure by maintaining endothelial function through increasing eNOS expression and subsequent NO bioavailability and reducing levels of inflammatory markers MDA in the blood [3,10]

Purple sweet potato tubers contain high levels of anthocyanins and flavonoids, which are exogenous antioxidants that help the body overcome oxidative stress by binding to free radicals and increasing eNOS expression and increasing NO bioavailability. The effect of anthocyanins and flavonoids in purple sweet potato tubers on reducing blood pressure in rats given high doses of NaCl is possible through increasing antioxidant activity, which can prevent oxidative stress.

Research on the role of NO in the mechanism of vasodilation and lowering blood pressure has been widely studied. Oxidative stress or ROS with high levels, such as superoxide anion, can reduce the bioavailability of NO in the blood vessel walls so that the relaxation response of blood vessels decreases.[17]

Ramipril contains 2-aza-bicyclo [3.3.0]-octane-3-carboxylic acid derivative, which is an ACE-inhibitor anti-hypertensive drug that inhibits Angiotensin I to become Angiotensin II and can reduce Angiotensin II levels through a mechanism of decreasing plasma renin activity and decreased aldosterone secretion.[8]

This study found that administration of 1 mg/day of ramipril significantly reduced myocardial CRP expression levels in a rat model of hypertension. Previous study by Ateya et al. (2022), showed that ramipril has effectiveness in myocardial remodeling, functions as a biomarker of endothelial dysfunction and reduces serum levels of CRP, IL-6, ADMA, and TNF- α . [8] Another study by Mitrovic et al. (2005) on 24 patients with atherosclerosis, CRP serum levels were obtained before the administration of ramipril 10 mg/day with an average ± 1.61 mg/l. After being given ramipril 10 mg/day for four weeks, CRP serum levels decreased to 2.72 ± 1.19 mg/l.[5]

Study by Verma et al. (2009), intervention with ramipril 2.5 mg/day for 12 weeks to healthy middle-aged volunteers was reported not to reduce CRP serum levels compared to placebo. A total of 264 men and women with an average age of 53 ± 9 years, CRP levels > 2 mg/L and no cardiovascular history were randomly enrolled and divided into two groups, namely the treatment group that received a dose of 2.5 mg/day of ramipril (n = 132) for 12 weeks and the placebo group (n = 132) and CRP baseline mean results 3.84 mg/L (95% CI; 3.62 mg/L - 4.06 mg/L). The mean percentage change in CRP values over 12 weeks was -13.2% (95% CI; -22.3% to -3.2%) in the placebo group compared to -21.1% (95% CI; -29.9% to -11.2%) in the 2.5 mg/d ramipril group (P value not significant), which showed no significant reduction at the trial's main endpoint. [18]

This study found that the administration of purple sweet potato (*Ipomoea batatas L.*) ethanolic extract 400 mg/kgBW/day can reduced aortic IMT in a rat model of hypertension. This finding is supported by previous research by Salehi et al. (2020), who found that there is an inverse correlation between the consumption of high doses of anthocyanins and a decrease in CVD-related risk markers, such as decreased blood pressure, decreased carotid IMT (CIMT) and improvement of arterial stiffness.[14]

Research by Muninggar and Lestario (2019) reported on the effects of consuming a diet high in anthocyanins on Flaucortia for 14 days with lower CIMT results compared to the control group (placebo) with a mean difference $3,4-8,7 \mu\text{m}$. [19] Similar research results from Xu et al. (2021) by giving a berry diet, rich in anthocyanins, for three months, a decrease in CIMT was found to be $1.62 \mu\text{m}$ (95% CI; -2.76 to $-0.48 \mu\text{m}$; $p = 0.005$) compared to control (placebo)[16].

This study found that giving ramipril 1 mg/day effectively reduced aortic IMT, which is significantly different in the hypertensive rat model. Previous studies by Petrovic et al. (2005) performed on 75 people (age range 42-58 years) with moderate or severe hypertension (SBP 160-190 mmHg, DBP 90-110 mmHg). In the group of patients receiving ramipril 2.5 mg/day, there was a decrease in carotid IMT (CIMT) during the observation period of 1, 3,

and 6 months, namely 14.6%, 12.0% and 18.2% compared to the group of patients who did not receive therapy.[20].

Another study supports the findings of this study, by Lonn et al. (2001), as many as 244 patients aged ≥ 55 years with atherosclerosis. It was found that the group receiving ramipril 2.5 mg/day for three weeks was able to reduce CIMT by 0.12 ± 0.11 mm lower than the placebo group (n = 244 patients) (p = 0.004)[21]. Similar results were also reported by Napoli et al. (2008); a study of 48 patients receiving ramipril 10 mg/day was observed for 1, 3 and 5 years. It was reported that there was a decrease in CIMT by 12%, 24%, and 38% compared to before being given ramipril 10 mg/day[22].

This research is the first research to investigate the combined effect of giving purple sweet potato (*Ipomoea batatas L.*) ethanolic extract and ramipril, so no other evidence has been found from other studies that have combined administration of purple sweet potato (*Ipomoea batatas L.*) ethanolic extract and ramipril.

In this study, it was found that reducing in myocardial CRP expression (mean \pm SD of 455.56 ± 99.51 ng/ml) in the hypertensive rat model group given a combination of purple sweet potato (*Ipomoea batatas L.*) ethanolic extract 400 mg/kgBW/day + ramipril 1 mg/day compared to the hypertensive rat model group with ramipril 1 mg/day (mean \pm SD of 570 ± 76.32 ng/ml) and the hypertensive rat model group was given purple sweet potato (*Ipomoea batatas L.*) ethanolic extract 400 mg/kgBW/day (mean \pm SD of 672 ± 85.60 ng/ml).

Research by Jesús Romero-Prado et al. (2015), that support the findings in this study, who reported that the combination of ramipril 10 mg/day with dietary flavonoids (containing green tea, red apple and dark chocolate) in 37 men and 42 women compared to the group given 10 mg/day ramipril monotherapy, the results showed a greater reduction in CRP levels. Statistically significant after three months of administration (p<0.001). CRP levels in the combination treatment group were previously obtained with an average of 4.8 ± 3.7 mg/dL and decreased to 2.4 ± 2.7 mg/dL (p < 0.0001), whereas in the group that was only given ramipril 10 mg/day the initial CRP level was 4.8 ± 3.4 mg/dL and decreased to 3.2 ± 2.8 mg/dL (p=0.005). These results indicate that giving flavonoids as a dietary supplement for three months can reduce CRP levels better than those given ramipril monotherapy.[23].

Results of other combination therapies that support the findings in this study, namely research by Nuruszewicz et al. (2007) in 11 women and 33 men, the mean age was 66 years, with the administration of chokeberry extract (*Aronia melanocarpa E*), which is rich in flavonoids combined with ramipril 10 mg/day for six weeks. It was found that CRP levels decreased 23-29% compared to those given ramipril monotherapy (p=0.007)[24].

Other research by Mozos et al. (2021), who reported that anthocyanins in purple sweet potato provide anti-inflammatory effects by inhibiting nuclear factor kappa beta (NF- κ), impairing the expression of adhesion molecules, interfering with the release of monocyte chemoattractant protein (MCP-1), reducing COX 2 expression in cells. Vascular smooth muscle (VSMC) reduces immune cell migration and circulating CRP levels.[25] Other study by Ciurănean et al. (2020), who reported that a diet high in flavonoids could reduce the production of inflammatory cytokines such as TNF- α , IL-6, and CRP and reduce signalling through the NF- κ pathway which plays a role in the inflammatory process.[26]

Research by Siti et al. (2020), reported that flavonoids have potential effects on reverse cardiac remodelling mechanisms, including causing changes at the cellular and molecular levels. The potential effects of flavonoids that play a role in the process of reverse cardiac remodelling through the inflammatory pathway are decreased expression levels of pro-inflammatory factors (CRP, TNF- α , IL-6 and NF- κ), decreased areas of cardiac inflammation and increased expression levels of NF κ B inhibitor- α (I κ B- α).[27]

This study found lower aortic IMT (mean \pm SD of 31.47 ± 2.61 μ m) in the hypertensive rat model group given a combination of purple sweet potato (*Ipomoea batatas L.*) ethanolic extract 400 mg/kg/day and ramipril 1 mg/day compared to the hypertensive rat model group given ramipril 1 mg/day (mean \pm SD of 36.84 ± 2.71 μ m) and the hypertensive rat model group by administering purple sweet potato (*Ipomoea batatas L.*) ethanolic extract 400 mg/kgBW/day (mean \pm SD of 41.90 ± 5.09 μ m).

This finding is supported by previous research by Aviram et al. (2004), who reported that giving a combination of ramipril 10 mg/day and pomegranate juice, which is rich in flavonoids, was found a decrease in CIMT of 19% in 10 patients with atherosclerosis during a 1-year follow-up period [28].

Previous research by Sun et al. (2014) and Mattioli et al. (2020) also reported that anthocyanins in purple sweet potato tubers have benefits as antioxidants by reducing superoxide anions so that they can lower blood pressure, prevent cardiac hypertrophy and improve vascular endothelial function in a rat model of hypertension.[29,30] Other studies by Su et al. (2012), reported that a diet high in flavonoids directly affected vascular tone. In vitro studies show that flavonoids can improve vascular function by increasing NO availability, a potent vasodilator synthesized from the vascular endothelium. Blackcurrant fruit with high anthocyanin content has strong vasorelaxant activity on blood vessels from human and animal models.[31] Other previous study by Ateya et al. (2022), who showed that ramipril has effectiveness in preventing myocardial remodeling and reducing CIMT, through the mechanism of inhibiting Angiotensin I to become Angiotensin II and modulating the kallikrein-kinin system into its active form (hormone) to reduce blood pressure and reduce arterial stiffness.[8].

In this study, there were several limitations. First, in this study, the rats were not checked for blood pressure during and after treatment. Second, this study did not measure left ventricular weight as a parameter of hypertrophy and did not examine other hypertrophy biomarkers, such as b-MHC, BNP mRNA, ANP mRNA, and cardiotrophin-1, due to limited costs and facilities. Third, this study did not examine other inflammatory biomarkers, such as IL-6 and GDF-15, due to limited costs and facilities.

CONCLUSION

The combination of purple sweet potato (*Ipomoea batatas L.*) ethanolic extract 400 mg/kgBW/day + ramipril 1 mg/day was able to provide the best decrease in the expression of myocardial CRP and aortic IMT compared to administration of purple sweet potato (*Ipomoea batatas L.*) ethanolic extract 400 mg/kg/day or ramipril 1 mg/day.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest related to the publication of this research article.

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ETHICS IN RESEARCH

This research has received approval from the research ethics committee of the Prof. Dr. IGNG Ngoerah Hospital/Faculty of Medicine, Udayana University with No. 1914/UN14.2.2.VII.14/LT/2023

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