IJSCIA

International Journal of Scientific Advances
ISSN: 2708-7972
Volume: 4 | Issue: 5 | Sep – Oct 2023 Available Online: www.ijscia.com
DOI: 10.51542/ijscia.v4i5.4

Effect of Combination from Purple Sweet Potato (*Ipomoea batatas L.*) Ethanolic Extract and Ramipril Administration on Myocardial Platelet Derived Growth Factor an Expression and Myocardial Collagen Deposition in Hypertensive Rat Models

Komang Surya Bhuana*, I Nyoman Wiryawan, and I Made Putra Swi Antara

Department of Cardiology and Vascular Medicine,
Faculty of Medicine, Udayana University, Prof. dr. IGNG Ngoerah, Denpasar, Indonesia

E-mail: suryabhuana1303@gmail.com; dr_wiryawan@yahoo.com; Putra.antara@unud.ac.id

*Corresponding author details: Komang Surya Bhuana; suryabhuana1303@gmail.com

ABSTRACT

Objective: To determine the effect of administration of 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 ethanol extract of purple sweet potato tuber (*Ipomoea batatas L.*) and ramipril in a rat model of hypertension. Thirty male Wistar rats were given 2 mL/day of 4% NaCl diet to induce hypertension. The rats were divided into 3 treatment groups, consisting of NaCl 4% 2 mL/day+combination therapy with purple sweet potato tuber ethanol extract 400 mg/kgBW/day and ramipril 1 mg/day, NaCl 4% 2 mL/day+purple sweet potato tuber ethanol extract 400 mg/kgBW/day and NaCl 4% 2 mL/day+ramipril 1 mg/day. At week 5, rat was executed to examine myocardial PDGF-A expression and measure myocardial collagen deposition. All the obtained data were analyzed statistically. ***Results:*** Administration of ethanolic extract of purple sweet potato 400mg/kgBW/day + ramipril 1mg/day significantly reduced myocardial PDGF-A expression compared to ethanolic extract of purple sweet potato alone (mean difference 14.17 pg/mLCI95% 2.36 - 25.98, P value = 0.016) or ramipril 1mg/day (mean difference 25.58 pg/mL; CI95% 17.07 - 40.09l, P value < 0.001). Administration of ethanolic extract of purple sweet potato 400mg/kgBW/day + ramipril 1mg/day significantly reduced myocardial collagen deposition when compared to administration of purple sweet potato alone (mean difference: 3.30%; CI 95% 1.97 - 4.64; P value <0.001). Administration of ethanolic extract of purple sweet potato combined with ramipril has higher potential effect in reducing myocardial PDGF-A expression and myocardial collagen deposition, compared to single therapy. ***Conclusion:*** Administration of ethanolic extract of purple sweet potato combined with ramipril was able to provide the best reduction in myocardial PDGF-A expression and myocardial collagen deposition.

Keywords: combination of purple sweet potato ethanolic extract and ramipril; myocardial PDGF-A expression; myocardial collagen deposition; hypertension.

INTRODUCTION

Cardiovascular disease is the leading cause of death worldwide, and hypertension is known to be one of the major risk factors for cardiovascular disease progression. The results of a study conducted by the Global Burden of Disease in the United States stated that increased systolic blood pressure is the main cause of decreased quality of life [1]. The problem of hypertension in Indonesia tends to increase. Based on data from the 2018 Basic Health Research results in Indonesia, it was found that the prevalence of hypertension reached 34.11% in the population> 18 years. Hypertension is still a major risk factor for stroke, heart failure, kidney failure, atherosclerotic disease, and dementia, despite the many studies and therapies for hypertension. Surveys conducted by SKRT in 1995, 2001, and 2004 also showed that cardiovascular disease is the number one cause of death in Indonesia, where 20 - 35% of these deaths are caused by hypertension. This is due to a lack of patient awareness, and one-third of hypertensive patients are not adequately treated [2]

Uncontrolled hypertension can cause changes in the structure of the heart, leading to *hypertensive heart disease* (HHD). Anatomically, the effect of increased systemic blood pressure will cause cardiac hypertrophy which in the early phase is called compensatory hypertrophy. Furthermore, compensatory hypertrophy can progress to heart failure or decompensated hypertrophy due to systolic dysfunction and dilatation of the left ventricular muscle [3]. The *alteration/remodeling* of cardiomyocyte structure involves cellular and biochemically related pathophysiological processes such as impaired Ca2+ *handling*, utilization of myocyte anaerobic metabolism, increased angiogenesis, increased autophagy, inflammation and changes in extracellular matrix/fibrosis [4] .

Cardiac fibrosis is a process of pathological remodeling and excessive deposition of extracellular matrix, leading to abnormalities in the composition and quality of the extracellular matrix. The protein composition of the extracellular matrix includes collagen types I and III (and small amounts of collagen types IV, V and VI), fibronectin, laminin, elastin, fibrillin and proteoglycans.

The extracellular matrix plays a role in mediating mechanical connections between cardiomyocytes, fibroblasts and blood vessels within the myocardium, transmitting extracellular mechanical signals, supporting cell migration and providing structural and functional integrity to the heart [5]. Excessive deposition of extracellular matrix, including collagen types I and III, is characteristic of cardiac fibrosis in patients with hypertension. Expression of *platelet derived growth factor receptor (*PDGF) is related to the degree of fibrosis. One study using a rat model of DOCA (*desoxy-corticosterone*) induced hypertension showed increased PDGF expression in myocardial fibroblasts and myofibroblasts, suggesting involvement of the PDGF/PDGFR pathway in myocardial fibrosis [6]. Fibrosis of the myocardium due to hypertension can trigger decompensation of cardiac function that can lead to heart failure syndrome, which begins with *preserved ejection fraction* (HfpEF), and in the *late stage,* systolic dysfunction can occur characterized by a decrease in left ventricular ejection fraction [7]

ACE inhibitors are one of the first-line and conventional therapies in patients with primary hypertension accompanied by left ventricular dysfunction. The efficacy of ACE blockers is supported by studies such as HOPE, where ramipril administration reduces the risk of death from cardiovascular causes, myocardial infarction and stroke [8]. The AIRE (*Acute Infarction Ramipril Efficacy*), and SAVE (*Survival and Ventricular Enlargement)* studies showed that ramipril also reduced the risk of death in patients with myocardial infarction, left ventricular dysfunction, and rehospitalization rates in patients with heart failure [9]. In addition to systemic effects, ACE blockers also have effects on cardiomyocyte tissue. ACE blockers can reduce fibrosis of the myocardium by reducing levels of the hormone angiotensin II, which stimulates collagen production in the heart. In addition, ACE blockers also reduce hypertrophy of cardiomyocytes, by decreasing hypertrophic response signaling in the heart [10].

Plant-based herbal medicines have been developed and used empirically to treat and prevent various diseases including cardiovascular diseases, with more affordable prices and minimal side effects. The effects of anthocyanins have been studied in hypertensive rat models where anthocyanins can reduce blood pressure by maintaining endothelial function through increased eNOS expression and bioavailability of NO subsequences [11] and reduce blood MDA levels [12] The antifibrotic effects of flavonoids from I. Batatas have been studied in a rat model with spontaneous hypertension. Flavonoids prevent fibrosis not only through antihypertensive effects, but also through anti-inflammatory effects such as suppression of interleukin IL-7 and suppression of *toll-lCIe receptor* 4 or TLR 4. [13,14]. One type of flavonoid that has been studied, delphinidin, has an antihypertrophic effect of the heart in mice with hypertrophy triggered by TAC (*Transverse Aortic Constriction*). Delphinidin decreased ROS accumulation through stimulation of Ang II via AMPK (*AMP-Activated Protein Kinase)* pathway and inhibition of Rac1 and P47 expression. Delphinidin was found to suppress cardiac hypertrophy through AMPK/NOX/MAPK signaling pathway [15]

With the availability of purple sweet potato tubers, the ease of processing into food, and the potential of the flavonoids in I. Batatas to lower blood pressure through antioxidant effects and reduce ROS accumulation and prevent cardiac fibrosis is expected to complement the anti-remodeling effects of ACE blockers in hypertensive populations.

Based on these things, this study was made to determine the effect of combined administration of ethanol extract of I. Batatas ethanol extract and ACE inhibitors on myocardial PGDF-A expression as a marker of fibrosis and myocardial collagen deposition as a sign of fibrosis in a rat model of hypertension. It is expected that the administration of ethanol extract of I. Batatas in combination with ACE blockers can reduce the expression of PDGF-A and myocardial collagen deposition to better prevent cardiac fibrosis. If this hypothesis is proven, then the administration of ethanol extract of I. Batatas ethanol extract combined with ACE blockers can be a consideration for cardioprotective therapy of cardiac fibrosis in patients with hypertension as a preventive effort in suppressing cardiovascular disease.

METHODS

This study was conducted in a pure experimental manner with a *post-test only control group* design and was a collaborative study of the effects of giving a combination of ethanol extract of purple sweet potato tubers (*Ipomoea batatas L.*) and ramipril on hypertension rat models. *Animal* husbandry, 4% NaCl diet, and blood pressure measurements were carried out at the *Laboratory Animal Unit of* the Pharmacology Section of the Faculty of Medicine, Udayana University. Measurement of myocardial PDGF-A expression was performed at the Integrated Biomedical Laboratory Unit, Faculty of Medicine, Udayana University. Examination of myocardial collagen deposition was conducted at the Veterinary Pathology Laboratory Unit, Faculty of Veterinary Medicine, Udayana University.

In this study, male Wistar rats were taken that met the inclusion criteria to be used as samples. Inclusion Criteria: a. Male Wistar rats (*Rattus norvegicus*); b. Age 12-16 weeks; c. Body weight 150-200 grams; d. Healthy rat condition (active and not disabled); e. Systolic blood pressure > 140 mmHg or diastolic > 90 mmHg or diastolic > 90 mmHg. Healthy rat condition (active and not disabled); e. Systolic rat blood pressure > 140 mmHg or diastolic > 90 mmHg. Exclusion Criteria: a. Rats did not move actively; b. Deformed rats; c. Rats died during the study; d. The weight of the rats decreased (the weight of the rats became less than 90 mmHg). Rat weight decreased (rat weight became less than 200 grams.

Research Procedure:

(1) 30 male Wistar rats aged 12-16 weeks, weighing approximately 150-200 grams, and in good health were selected.

(2) Rats were kept in cages in groups of 1 rat per cage. Cages were made of plastic tubs measuring 30 x 20 x 20 cm with a base of husks to absorb rat feces and a woven wire lid at the top. Cages were placed in a ventilated and natural air room, with temperatures ranging from 20 - 26^{o} C with air humidity ranging from 40 - 70%.

(3) The mice were randomly divided into three groups: a. Group P1 (treatment group), rats that received 4% NaCl, a combination of ramipril 1 mg/day and ethanol extract of purple sweet potato tubers at a dose of 400gr/kgBW/day for 4 weeks; b. Group P2 (control group), rats that received 4% NaCl and ethanol extract of purple sweet potato tubers at a dose of 400gr/kgBW/day for 4 weeks; c. Group P3 (control group), rats treated with 4% NaCl and ramipril 1 mg/day for 4 weeks.

(4) Body weight checks were conducted weekly.

(5) If a rat dies during the study, it will be replaced with a spare rat.

(6) At week 5 mice were dissected and examined to measure PDGF-A expression levels and quantify myocardial collagen deposition.

(7) Mice were euthanized with ketamine and xylazine, then the neck was dislocated, then surgery was performed to remove the heart organ.

(8) After all rats were euthanized and properly buried (burial by following local customs such as burying humans where at least the rat body was given banten canang and its completeness) because it could not be used again for other studies.

All data collected in each group were then analyzed with the SPSS program. Data analysis included descriptive analysis, intra observer variability test performed to screen for variability in the reading of myocardial PDGF-A histopathology results that were read manually using a microscope. The Blant-Altman test was used to screen for intraobserver variability with a significance level of ±95%, Comparative test using *One Way Anova* followed by *post hoc least significant difference* (LSD) analysis to see the differences between each group. The correlation test aims to determine the correlation between myocardial PDGF-A expression and myocardial collagen deposition using the *Spearman correlation coefficient*. The direction of correlation is said to be unidirectional if the r value is positive. The correlation was assessed by looking at the correlation coefficient (r) value to determine very weak, weak, strong, and very strong correlations. The confidence level in this study is 95%. Ho is rejected if the p value is <0.05.

RESULTS

To determine the effect of the combination of ethanol extract of purple sweet potato tubers and ramipril on myocardial PDGF-A expression and myocardial collagen deposition in hypertensive rat models, an experimental study with *Post Test Only Group Design* was conducted.

The number of samples at the beginning of the study was 30, but 2 died during the study, 1 in group P1 (treatment group with a combination of ethanol extract of purple sweet potato tubers and ramipril) and 1 in group P3 (group with ramipril treatment).

The total sample at the end of the study was 28 which were divided into 3 groups. Group P1 (treatment group) was given a combination of ethanol extract of purple sweet potato tubers and ramipril, group P2 (control group) was given ethanol extract of purple sweet potato tubers alone and group P3 (control group) was given ramipril alone.

PDGF-A expression is the expression of PDGF-A protein from myocardial tissue samples, obtained through ELISA examination expressed as a numerical variable in units of pg/mL. Normality test with Saphiro-Wilk on PDGF-A expression found the data to be normally distributed with homogeneous variance ($p > 0.05$). Statistical tests with *One-Way ANOVA* found a value of $P < 0.001$ which means that there is a significant difference between study groups so that this must be continued to the next stage using the (*Least significant difference*) LSD test to determine differences between treatment groups in pairs. Based on analysis using LSD, it was found that the combination of purple sweet potato tuber ethanol extract 400mg/kgBW/day + ramipril 1mg/day (P1) had a better effect on reducing myocardial PDGFA expression compared to single therapy using purple sweet potato tuber ethanol extract 400mg/kgBW/day (P2) (mean difference 14.17 pg/mL; CI95% 2.36 - 25.98; p value = 0.016) or ramipril 1mg/day (p3) (mean difference 28.58 pg/mL; CI95% 17.07 - 40.09; p value <0.001). Compared to the group administered purple sweet potato tuber ethanol extract 400mg/kgBW/day (P2), Ramipril 1 mg/day (P3) showed a better effect on reducing myocardial PDGFA expression (mean difference 14.48 pg/mL; CI95% 2.98 - 25.98; p value = 0.012) (Table 1).

TABLE 1: ANOVA test and *post-hoc LSD of* myocardial PDGF-A expression between study groups.

Variables	Mean ± SB (pg/ml)	95% CI	F	ANOVA	LSD				
					Group		Mean Difference (pg/mL)	95% CI	P-value
P1	22,35 ± 3,27	19,84 - 24,88	19,1	<0,001	P1	P2	28,58	17,07 - 40,09	<0,001
P2	50,94 ± 14,43	39,90 - 61,98				P3	14,17	2,36 - 25,98	0,016
P3	36,53 ± 6,10	31,84 - 41,23			P3	P2	14,48	2,98 - 25,98	0,012

The results of the comparison of the potential effects of group P1 (combination of ethanol extract of purple sweet potato tubers 400 mg/kgBW/day + ramipril 1mg/day) on the percentage of myocardial PDGF-A expression were found to be 100%, group P2 (ethanol extract of purple sweet potato tubers 400mg/kgBW/day) was found to be 83,4% and group P3 (ramipril 1mg/day) obtained by 78.9% so it can be concluded that the administration of a combination of ethanol extract of purple sweet potato tubers 400mg / kgBW / day + ramipril 1mg / day has a higher potential effect in reducing myocardial PDGF-A expression compared to other groups in this study (Figure 1).

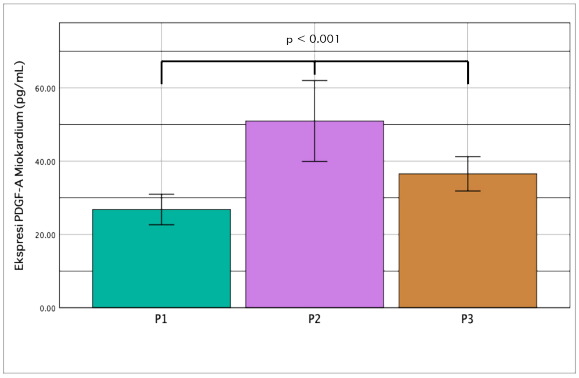

FIGURE 1: ANOVA graph of myocardial PDGF-A expression showing significant differences between study groups (*p-value One Way ANOVA* <0.05).

Myocardial collagen deposition was calculated based on the increase in the amount of collagen expression assessed by histopathological examination of myocardial tissue after staining with *Picro Sirius Red* staining. Heart organs were taken from rats, after euthanasia. Preparations were made from left ventricular myocardial tissue and the percentage of myocardial collagen deposition was calculated.

To avoid *intra-observer* interpretation variability, readings were taken twice on different days. After the Bland Altman test was performed, there was no deviation of data that exceeded 95% of the upper and lower limits of the Bland Altman diagram. The results of the Bland Altman test for Myocardial Collagen Deposition are shown in Figure 2.

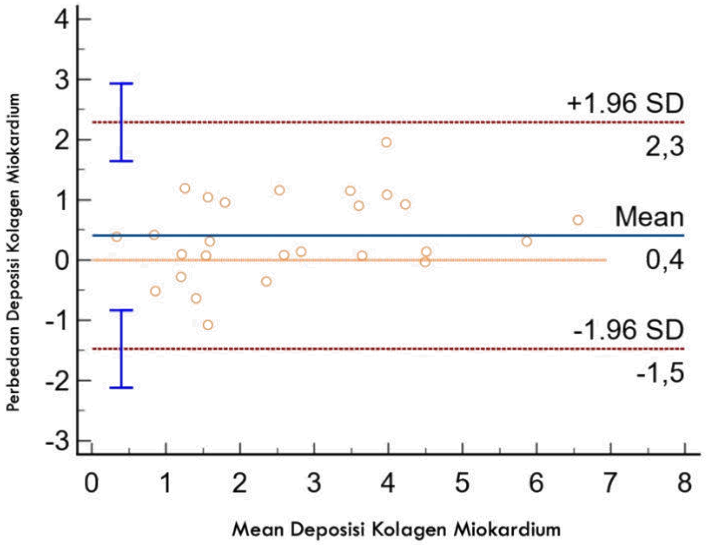

FIGURE 2: Bland Altman Test Diagram of Myocardial Collagen Deposition.

Normality test with Saphiro-Wilk on collagen obtained data normally distributed and with homogeneous variants (p>0.05). Statistical tests with *One-Way* ANOVA p value <0.001 which means there is a significant difference between the study groups, so proceed to the next stage using the (*Least significant difference*) LSD test to determine differences between treatment groups compared to the control. ANOVA and *post-hoc LSD* analysis are presented in Table 2 and ANOVA graph of Myocardial Collagen Deposition between study groups is presented in Figure 3.

TABLE 2: ANOVA and *LSD* test for percentage of myocardial collagen deposition between study groups.

Variables	Mean ± SB (%)	95% CI	F	ANOVA	LSD				
					Group		Average Difference (%)	95% CI	P-value
P1	1,29 ± 0,6	0,79 - 1,79	19,1	<0,001	P1	P2	3,30	1,97-4,64	<0,001
P2	4,60 ± 1,18	3,75 - 5,4				P3	1,60	0,23 - 2,97	0,020
P3	2,90 ± 1,50	1,74 - 44,05			P3	P2	1,70	3,04 - 0,36	0,011

Based on LSD analysis, the combination therapy of purple sweet potato tuber ethanol extract 400mg mg/kgBW/day + ramipril 1mg/day (P1) had a better effect on reducing collagen deposition than single therapy using purple sweet potato tuber ethanol extract 400mg/kgBW/day (mean difference: 3.30%; CI95% 1.97 - 4.64; p value <0.001).

Compared to the group administered with purple sweet potato tuber ethanol extract 400mg/kgBW/day (P2), Ramipril 1mg/day (P3) showed better effect on reducing myocardial collagen deposition (mean difference: 1.70; 95% CI 3.04 - 0.36; p value = 0.011) (Figure 3).

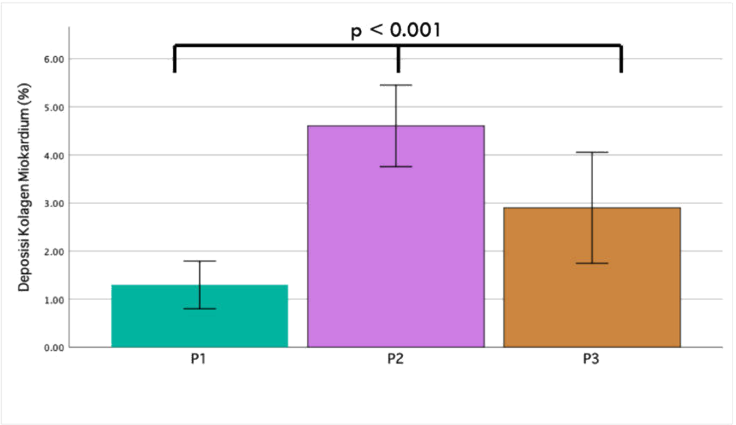

FIGURE 3: ANOVA graph of myocardial collagen deposition showing significant differences between study groups (One Way ANOVA p-value <0.05).

The results of the comparison of the potential effects of group P1 (combination of ethanol extract of purple sweet potato tubers 400mg / kgBW / day + ramipril 1mg / day) on the percentage of myocardial collagen deposition were found to be 100%, group P2 (ethanol extract of purple sweet potato tubers 400mg / kgBW / day) was found to be 85,3% and group P3 (ramipril 1mg/day) obtained by 92.5% so it can be concluded that the administration of a combination of ethanol extract of purple sweet potato tubers 400mg / kgBW / day + ramipril 1mg / day has a higher potential effect in reducing the presentation of myocardial collagen deposition than other groups in this study.

The relationship between myocardial PDGF-A expression and myocardial collagen deposition in the administration of a combination of ethanol extract of purple sweet potato tubers 400mg / kgBW / day and ramipril 1mg / day obtained a very strong correlation (r = 0.870) which is statistically significant with a value of p = 0.002.

The relationship between myocardial PDGF-A expression and myocardial collagen deposition in the administration of purple sweet potato tuber ethanol extract 400mg/kgBW/day obtained strong correlation results (r = 0.830) which is statistically significant with a p value = 0.003.

The relationship between myocardial PDGF-A expression and myocardial collagen deposition in ramipril 1mg/day administration was found to be a very strong correlation (r = 0.883) which was statistically significant with a p value of 0.002 (Figure 4).

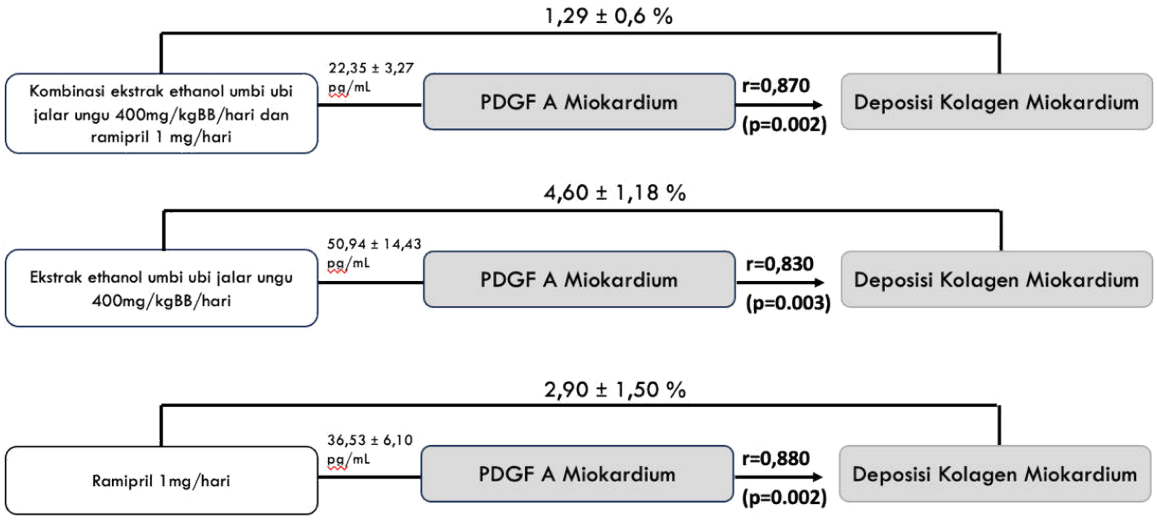

FIGURE 4: Analysis of the relationship between myocardial PDGFA expression and myocardial collagen deposition in each group obtained statistically significant results (p<0.05).

DISCUSSION

Hypertension-related cardiovascular complications are still one of the highest causes of morbidity and mortality. One of the causes of cardiovascular outcomes is related to changes in the cardiac myocardium called left ventricular remodeling. One of the changes that occur in the myocardium with increased blood pressure is fibrosis of the myocardium. Fibrosis in hypertension begins with disruption of molecular signaling and microstructural changes that manifest subclinically. Gradual cardiac fibrosis will lead to diastolic disturbances, which may manifest as heart failure with normal ejection fraction. In the absence of intervention, structural changes in the myocardium will continue, and in the final phase myocardial fibrosis will manifest as heart failure with a decreased ejection fraction.

Early phase structural changes in Hypertension patients can be evaluated using endomyocardial biopsy, but biopsy in humans is not routinely performed for screening metabolic and cellular changes of the heart, so with these considerations this study was conducted on a rat model. This study uses a rat model of hypertension through induction using *force feeding* of 4% NaCL as much as 2ml for 1 week, which is considered to reflect structural abnormalities in the course of hypertension.

In some literature, it is mentioned that in the myocardium of the heart that has fibrosis, there will be an increase in PDGF-A protein and type I collagen. Gallini and Lindblom showed that PDGF-A and PDGF-B can induce fibrosis in a mouse model with transgenic DNA injection. Overexpression of PDGF-A promoted a severe fibrosis reaction and increased heart size by 8-fold.

Expression of PDGF-A is mediated by interstitial mesenchymal cells, which are lCIely the source of matrix deposition and fibrosis reaction in the myocardium [16]

Ramipril is one of the antihypertensive drugs, an ACE blocker class that has a Carboxyl group in its chemical structure. Ramipril works by inhibiting the hydrolysis of Angiotensin I into Angiotensin II. The decrease in Angiotensin II causes cardioprotective effects such as vasodilation, decreased monocyte adhesion, decreased smooth muscle cell proliferation, release of Oxidan, decreased endothelial dysfunction. The cardioprotective effect of using ACE blockers has been widely demonstrated, and in some studies, it has become a recommendation for pharmacological therapy in heart failure patients with left ventricular dysfunction [17,18]

This study is a pre-clinical trial conducted in a purely experimental manner using a *post-test only control group* design and is a collaborative study to assess the effects of a combination of ethanol extract of purple sweet potato tubers (*Ipomoea batatas L.*) and ramipril as a cardioprotector and anti-cardiac remodeling in hypertensive rat models. The hypertensive rat model uses induction with 4% NaCl for 1 week to week 5, which causes hypertension and hypertension-induced pathological processes in the rat heart. Results from an experimental acute toxicity study by Ciumărnean et al (2020) reported that purple sweet potato tuber extract (*Ipomoea batatas L.*) was safe up to the highest dose of 5000mg/kgBW and no lethal signs and symptoms or behavioral changes were observed [17].

This study used two (2) quantitative parameters to assess cardiac fibrosis, namely examination of myocardial PDGF-A expression and examination of myocardial collagen deposition as a marker of fibrosis. Of the 30 rats at the beginning of the study, 2 rats died during the study. The cause of death in rats is not known for certain because autopsies were not performed on rats, but several risk factors that cause the death of experimental animals include: sudden cardiac death, anoxia, heat shock, pain shock, anaphylaxis, anemia, severe infection, and arrhythmias [19]. In addition, administration via sonde also has possible complications that can cause death, namely aspiration pneumonia, esophageal perforation, and even gastric perforation [20]

This study found that the combination of ethanol extract of purple sweet potato tubers (*Ipomoea batatas L*) and ramipril has been shown to provide cardioprotective effects, in the form of decreased myocardial PDGF-A expression as one of the biomarkers of fibrosis related to the pathophysiology of myocardial fibrosis, and also decreased myocardial collagen deposition which is the final evidence of fibrosis [21]

PDGFA is a protein involved in the process of fibrosis in cardiac myocardium. Gallini and Lindblom showed that PDGF-A and PDGF-B can induce fibrosis in mouse models by transgenic DNA injection. Overexpression of PDGF-A promoted a severe fibrosis reaction and increased heart size by 8-fold. Expression of PDGF-A is mediated by interstitial mesenchymal cells, which are the most source of matrix deposition and fibrosis reaction in the myocardium [22]

To date, no studies have examined specific PDGFA expression in subjects given a combination of ethanol extract of purple sweet potato tubers (*Ipomoea batatas L*) and ramipril. Some of the reasons why this study uses PDGF-A expression is that PDGF-A examination is one of the early indicators of fibrosis, and PDGF-A ELISA examination can be done.

In this study, myocardial PDGF-A expression decreased significantly in the group of rats that received a combination of ethanol extract of purple sweet potato tubers (*Ipomoea batatas L*) and ramipril (P1) when compared with the group of rats with purple sweet potato tubers (P2) and with the group with ramipril administration (P3). In the group given ethanol extract of purple sweet potato tubers (*Ipomoea batatas L*) and ramipril alone also experienced a significant decrease, but it appears that the group with combination therapy is superior compared to single therapy. This result is a new finding because no one has studied the potential of this combination therapy before. Purple sweet potato has antioxidant activity that works as a free radical scavenger. Sweet potatoes are high in anthocyanins and flavonoids and have benefits and protective effects against various 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 *reactive oxygen species* (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 so that they can protect cells from free radicals.

This study found that the administration of ethanol extract of purple sweet potato tubers (*Ipomoea batatas L.*) 400mg/kgBW/day can reduce PDGF expression in hypertensive rat models. This finding is supported by research from Oak et al (2006), where the administration of flavonoid and polyphenol compounds in red wine (Delphinidin and Cyanidin) can reduce the expression of VEGF induced by a decrease in PDGF A in human vascular cell culture models.

Administration of flavonoids and polyphenols decreased the activation of the PDGF A fibrosis pathway by decreasing the phosphorylation of the ERK1/2 pathway [15]. Another in vitro study conducted by Brodwaska (2017) found that flavonoid compounds genin, quercetin, genistein, daidzin have an inhibitory effect on PDGFA which inhibits the proliferation of myofibroblast cells which are the main cells in the formation of collagen deposition and other matrix proteins, in a sample of induced rat liver myofibroblast cells [23]

Ramipril also has an effect in reducing PDGF-A levels which play a role in the fibrosis process. An *in vitro* study conducted by G Grandaliano et al in 1999, used ramipril in a mesangial cell culture model. This study found that ramipril administration decreased the expression of PDGF A and B, with a mechanism that is not directly related to the inhibitory mechanism of the ACE enzyme. Sandor Koszegi et al in 2019 examined the effect of inhibition of the RAAS system on increasing *growth factor* levels that cause interstitial fibrosis in diabetic rat models. This study found that inhibition of the RAAS effect decreased the expression of PDGF and CTGF, thereby reducing the production of extracellular matrix that causes fibrosis [15]

This study is the first to examine the combined effect of purple sweet potato (*Ipomoea batatas L*) ethanol extract and ramipril, so there is no other evidence from other studies that combine purple sweet potato ethanol extract and ramipril. In this study, the expression of myocardial PDGFA was lower in the hypertensive rat model with the combination of purple sweet potato tuber ethanol extract and ramipril (mean ± SB of 22.3 ± 2.2 pg/mL), compared with the hypertensive rat model group with ramipril 1mg/day (mean ± SB of 36.53 ± 6.10 pg/mL) and compared with the hypertensive rat model group with purple sweet potato tuber ethanol extract 400mg/kgBW/day (mean ± SB of 50.94 ± 14.43 pg/mL).

Based on this, it can be postulated that the combination of the administration of ethanol extract of purple sweet potato tubers (*Ipomoea batatas L.*) and ramipril has an effect in reducing PDGF A expression better than other treatment groups. This is most lCIely due to the inhibition of both fibrosis pathways either through the pathway mediated by purple sweet potato tuber, or by ramipril. However, the exact mechanism of this postulation requires further research.

In histopathological examination, the combination of ethanol extract of purple sweet potato tubers (*Ipomoea batatas L.*) and ramipril was also found to significantly reduce the presentation of myocardial collagen deposition when compared with hypertensive rat models that were only given ethanol extract of purple sweet potato tubers (*Ipomoea batatas L.*) and hypertensive rat models that were only given ramipril. Ramipril therapy also had a better effect on reducing myocardial collagen deposition when compared with ethanol extract of purple sweet potato tubers (*Ipomoea batatas L)* alone.

The effect of flavonoid compounds has been known to reduce the synthesis of collagen. Studies conducted by Tamara Stipcevic et al, found that flavonoid compounds (mainly quercetin, pentahydroxyflavone, and hydroxyflavone) significantly decreased the total collagen concentration due to a direct effect on fibroblasts, which are the main cells involved in collagen and extracellular matrix synthesis [24]

In a study with a sample of hypertensive rats conducted by Ebenezer KC Kong et al using that flavonoid compound baicalein, extracted through the Scutellaria baicalensis Georgi plant, which was given to rats with spontaneous hypertension for 4-12 weeks.

The study found that baicalein-treated rats had reduced heart weight to body weight ratio, decreased plasma levels of BNP, cardiac interventricular thickness, and decreased myocardial collagen deposition. This study concluded the antifibrosis effect of administering the flavonoid compound baicalein [25]

Ramipril is an antihypertensive agent with an anti-remodeling effect, leading to improved cardiovascular outcomes, especially in populations with left ventricular dysfunction. One of the effects of inhibiting the hydrolysis of Angiotensin I to Angiotensin II is the improvement or reversal of myocardial remodeling that occurs through AT1 receptor inhibition. Studies of ACE blockers on myocardial collagen deposition have been investigated. One of them was conducted by Brilla et al. This study used oral Lisinopril at 20 mg/kg in a spontaneously hypertensive rat model. From this study, it was found that after administration for 8 months, there was a decrease in blood pressure, and reversal of myocardial fibrosis in spontaneously hypertensive rats given intervension ($p<0.25$).

A study conducted by Ute Seeland et al, in 2002 using a rat model of hypertension and myocardial infarction rats, found that the administration of ramipril 1mg/kg/day combined with furosemide 2mg/kg/day BID, could reduce the expression of myocardial collagen deposition, when compared with vehicle/placebo. This study also explained that ramipril administration could prevent dilatation of the left ventricle, which directly improved the mortality rate of rats, but furosemide administration had no direct effect on LV function and remodeling [26]

In this study, the reduction in myocardial collagen deposition from the combined administration of purple sweet potato tuber ethanol extract with ramipril was greater than the administration of purple sweet potato tuber ethanol extract. This is in line with studies that have been conducted on ACE inhibitor drugs, which have been shown to have both clinical and subclinical benefits against changes in the pathological structure of the heart. Some of the mechanisms explaining this effect may be the inhibition of different fibrosis pathways by ACE blockers and ethanol extract of purple sweet potato tuber.

Currently, there are not many studies that examine the benefits of a combination of ethanol extract of purple sweet potato tubers (*Ipomoea batatas L.*) and ramipril that specifically look at changes in myocardial structure due to hypertension, especially in humans. This is because examining fibrosis through invasive and non-invasive modalities is still difficult to do. Based on the results of this study, the combination of ethanol extract of purple sweet potato tuber and ramipril as antifibrosis is promising to be studied further. This study is expected to be a preliminary study that can then be extrapolated to humans so as to benefit the survival of patients with hypertension in the prevention of cardiovascular disease.

The limitation of this study is that blood pressure was not checked during treatment and after treatment, due to the limitations of the examination equipment. The second is not measuring the weight of the left ventricle, the size of the heart chamber dimensions, cardiac hemodynamic parameters, and other markers such as TGF-Beta, AT 1, which is another marker of fibrosis, due to limited costs and facilities. Finally, other histopathological markers such as CSA, MMP immunohistochemistry were not examined due to cost and facility constraints.

CONCLUSION

(1) The combination of purple sweet potato tuber ethanol extract (*Ipomoea batatas L.*) 400 mg/kgBW/day + ramipril 1 mg/day was able to provide the best reduction in myocardial PDGF-A expression compared to the administration of purple sweet potato tuber ethanol extract (*Ipomoea batatas L.*) 400mg/kgBW/day and ramipril monotherapy 1mg/day in this study.

(2) The combination of purple sweet potato tuber ethanol extract (*Ipomoea batatas L.*) 400 mg/kgBW/day + ramipril 1 mg/day was able to provide the best reduction in myocardial collagen deposition compared to the administration of purple sweet potato tuber ethanol extract (*Ipomoea batatas L.*) 400mg/kgBW/day and ramipril monotherapy 1mg/day in this study.

CONFLICT OF INTEREST

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

FUNDING

This research did not receive funding from the government or other private sectors.

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. 1907/UN14.2.2.VII.14/LT/2023

REFERENCES

[1] Murray CJL, Aravkin AY, Zheng P, Abbafati C, Abbas KM, Abbasi-Kangevari M, et al. Global burden of 87 risk factors in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. The Lancet 2020; 396:1223–49. https://doi.org/10.1016/S0140-6736(20)30752-2.

[2] Mills KT, Bundy JD, Kelly TN, Reed JE, Kearney PM, Reynolds K, et al. Global Disparities of Hypertension Prevalence and Control. Circulation 2016; 134:441–50. https://doi.org/10.1161/CIRCULATIONAHA.115.018912.

[3] Sorrentino MJ. The Evolution from Hypertension to Heart Failure. Heart Fail Clin 2019; 15:447–53. https://doi.org/10.1016/j.hfc.2019.06.005.

[4] Tham YK, Bernardo BC, Ooi JYY, Weeks KL, McMullen JR. Pathophysiology of cardiac hypertrophy and heart failure: signaling pathways and novel therapeutic targets. Arch Toxicol 2015; 89:1401–38. https://doi.org/10.1007/s00204-015-1477-x.

[5] Martins D, Garcia LR, Queiroz DAR, Lazzarin T, Tonon CR, Balin P da S, et al. Oxidative Stress as a Therapeutic Target of Cardiac Remodeling. Antioxidants 2022; 11:2371. https://doi.org/10.3390/antiox11122371.

[6] Kamareddine L, Ghantous CM, Allouch S, Al-Ashmar SA, Anlar G, Kannan S, et al. Between inflammation and autophagy: The role of leptin-adiponectin axis in cardiac remodeling. J Inflamm Res 2021;14:5349–65. https://doi.org/10.2147/JIR.S322231.

[7] Besse S, Nadaud S, Balse E, Pavoine C. Early Protective Role of Inflammation in Cardiac Remodeling and Heart Failure: Focus on TNFα and Resident Macrophages. Cells 2022;11. https://doi.org/10.3390/cells11071249.

[8] Sleight P. The HOPE Study (Heart Outcomes Prevention Evaluation). Journal of the Renin-Angiotensin-Aldosterone System 2000; 1:18–20. https://doi.org/10.3317/jraas.2000.002.

[9] Pfeffer MA, Braunwald E, Moyé LA, Basta L, Brown EJ, Cuddy TE, et al. Effect of Captopril on Mortality and Morbidity in Patients with Left Ventricular Dysfunction after Myocardial Infarction. New England Journal of Medicine 1992; 327:669–77. https://doi.org/10.1056/NEJM199209033271001.

[10] Silverman DN, Shah SJ. Treatment of Heart Failure with Preserved Ejection Fraction (HFpEF): The Phenotype-Guided Approach. Curr Treat Options Cardiovasc Med 2019; 21:20. https://doi.org/10.1007/s11936-019-0709-4.

[11] Jawi IM, Wita IW, Suprapta DN. Aqueous Extract of Purple Sweet Potato Tuber Increases Sod And Decreases VCAM-1 Expression By Increasing Nrf2 Expression In The Aortic Endothelia Of Hypercholesterolemic Rabbits 2014;4:3–4.

[12] Jawi IM, Yasa IWPS, Mahendra AN. Antihypertensive and Antioxidant Potential of Purple Sweet Potato Tuber Dry Extract in Hypertensive Rats. Bali Medical Journal 2016; 5:65. https://doi.org/10.15562/bmj.v5i2.217.

[13] Hsieh SW, Huang LC, Hsieh TJ, Lin CF, Hsu CC, Yang YH. Behavioral and psychological symptoms in institutional residents with dementia in Taiwan. Geriatr Gerontol Int 2021; 21:718–24. https://doi.org/10.1111/ggi.14220.

[14] Yue E, Yu Y, Wang X, Liu B, Bai Y, Yang B. Anthocyanin Protects Cardiac Function and Cardiac Fibroblasts from High-Glucose Induced Inflammation and Myocardial Fibrosis by Inhibiting IL-17. Front Pharmacol 2021;11. https://doi.org/10.3389/fphar.2020.593633.

[15] Oak M-H, Bedoui JE, Madeira SVF, Chalupsky K, Schini-Kerth VB. Delphinidin and cyanidin inhibit PDGF $_{AB}$ -induced VEGF release in vascular smooth muscle cells by preventing activation of p38 MAPK and JNK. Br J Pharmacol 2006; 149:283–90. https://doi.org/10.1038/sj.bjp.0706843.

[16] Iurciuc S. Vascular aging and subclinical atherosclerosis: why such a “never ending” and challenging story in cardiology? Clin Interv Aging 2017; 12:1339–45.

[17] Ciumărnean L, Milaciu MV, Runcan O, Vesa Ştefan C, Răchişan AL, Negrean V, et al. The Effects of Flavonoids in Cardiovascular Diseases. Molecules 2020; 25:4320. https://doi.org/10.3390/molecules25184320.

[18] Ateya AM, El Hakim I, Shahin SM, El Borolossy R, Kreutz R, Sabri NA. Effects of Ramipril on Biomarkers of Endothelial Dysfunction and Inflammation in Hypertensive Children on Maintenance Hemodialysis: the SEARCH Randomized Placebo-Controlled Trial. Hypertension 2022; 79:1856–65. https://doi.org/10.1161/HYPERTENSIONAHA.122.19312.

[19] Chen QM, Maltagliati AJ. Nrf2 at the heart of oxidative stress and cardiac protection. Physiol Genomics 2018; 50:77–97. https://doi.org/10.1152/physiolgenomics.00041.2017.

[20] McNally JD, Menon K. Vitamin D deficiency in surgical congenital heart disease: prevalence and relevance. Transl Pediatr 2013; 2:99–111. https://doi.org/10.3978/j.issn.2224-4336.2013.07.03.

[21] Travers JG, Tharp CA, Rubino M, McKinsey TA. Therapeutic targets for cardiac fibrosis: From old school to next-gen. Journal of Clinical Investigation 2022;132. https://doi.org/10.1172/JCI148554.

[22] Gallini R, Lindblom P, Bondjers C, Betsholtz C, Andrae J. PDGF-A and PDGF-B induces cardiac fibrosis in transgenic mice. Exp Cell Res 2016; 349:282–90. https://doi.org/10.1016/j.yexcr.2016.10.022.

[23] Brodowska KM. Natural flavonoids: classification, potential role, and application of flavonoid analogues. Eur J Biol Res 2017; 7:108–23. https://doi.org/10.5281/zenodo.545778.

[24] STIPCEVIC T, PILJAC J, BERGHE D VANDEN. Effect of Different Flavonoids on Collagen Synthesis in Human Fibroblasts. Plant Foods for Human Nutrition 2006; 61:27–32. https://doi.org/10.1007/s11130-006-0006-8.

[25] Ebenezer GJ, Scollard DM. Treatment and Evaluation Advances in Leprosy Neuropathy. Neurotherapeutics 2021. https://doi.org/10.1007/s13311-021-01153-z.

[26] Seeland U, Kouchi I, Zolk O, Itter G, Linz W, Böhm M. Effect of Ramipril and Furosemide Treatment on Interstitial Remodeling in Post-Infarction Heart Failure Rat Hearts. J Mol Cell Cardiol 2002; 34:151–63. https://doi.org/10.1006/jmcc.2001.1497.