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Research Article

Effects of the Methanol extracts *Phaleria macrocarpa* (Scheff) Boerl fruits on Angiotensin Converting Enzyme (ACE) activity

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Abstract

The present study was carried out to investigate the antihypertensive effect and Angiotensin Converting Enzyme activity of methanolic extract of *Phaleria macrocarpa* (Scheff) Boerl fruits in rats . Hypertension was induced experimentally by 10% w/v fructose diet ad libitum for 8 weeks. *P. marcocarpa* was given at the dose 0.5, 1 and 2 g/kg BW start at week 6 until week 8 and 1 g/kg BW for 8 weeks. Blood pressure was measured at baseline and at every weeks for 8 weeks using tail-cuff plethysmography. After completion of treatment schedule rats from each group were anesthetized with urethane (120 mg/100 gm, i. p.). Blood was collected and then centrifugated to obtain plasma and later store at-70°C for analysis activity ACE plasma. The results suggests that methanol extract of *Phaleria marcocarpha* could prevent the development high blood pressure and normalized blood pressure in rat induced by diet rich in fructose. The results tend to suggest that methanol extracts of possesses antyhipertensive activity, through inhibition of angiotensin converting enzyme.

Keywords: fructose, hypertension, *P. macrocarpha,* angiotensin converting enzyme

1.1. INTRODUCTION

Angiotensin-converting enzyme (ACE) is a key enzyme in the renin–angiotensin aldosterone system (RAAS). ACE converts the inactive decapeptide angiotensin I, forming the active octapeptide angiotensin II. Angiotensin II is a main effector of the RAAS and seems to be involved in developing cardiovascular disease. Angiotensin II binds to the angiotensin receptors AT1 and AT2 [1]. Activation of AT1 receptors is associated with endothelial dysfunction, vasoconstriction, cell proliferation, platelet aggregation, inhibition of nitric oxide synthase, aldosterone release, and increases in reactive oxygen species [2].

High blood pressure is the leading preventable cause of cardiovascular diseases such as stroke, myocardial infarction, left ventricular hypertrophy, nephropathy, retinopathy and dementia [3]. In middle and old age groups, every 20 mmHg of systolic or 10 mmHg diastolic increase in blood pressure above 115 mmHg/75 mmHg results doubling of mortality from ischemic heart disease and stroke [4].

Fructose induced hypertension model gives clue about the role of dietary changes in hypertension. High fructose diets have been documented to increase blood pressure in experimental rats [5;6]. Hypertension, a component of metabolic/insulin resistance, impaired glucose tolerance syndrome is an important risk factor for cardiovascular diseases [7]. Insulin resistance is often linked to the macronutrient content in the diet. Oxidative stress, over lipid production plays an important role in development of hypertension and other cardiovascular complications [8]. Feeding of a high fructose diet to rats results in hyperglycemia, hypertriglyceridemia and

hyperinsulinemia. The hypertension is accompanied by several metabolic abnormalities. Insulin resistance and hyperinsulinemia are frequently associated with both clinical and experimental hypertension [9]. Fructose-induced hypertension has been related to volume overload, sodium retention by kidneys and increased sympathetic activity among other things [10]. Alterations in renal function may also play an important role in the development of hypertension in fructose-fed rats [11].

Several classes of pharmacological agents have been used in the treatment of hypertension. One class of antihypertensive drugs known as angiotensin I converting enzyme (ACE) inhibitors) are associated with a low rate of adverse side-effects and are the preferred class of anti-hypertensive agents for treating patients with concurrent secondary diseases [12]. The potent ACE inhibitors were frequently derived from food proteins [13].

Phaleria macrocarpa (Scheff.) Boerl is native plants from Indonesia, which has long been used as folk medicines for treatments various types of diseases such as cancer, liver disorders, heart disease, diabetes, arthritis, kidney disorders, stroke, and high blood pressure[14]. Qualitatively this plants showed the presence of alkaloids, saponins, polyphenols, and from fruit contained alkaloids, saponins and flavonoids [15]. *Phaleria marcocarpa* was reported to contain phenolic glycoside such as mahkotaside, mangiferin, kaempferol-3-O-β-dglucoside, dodecanoic acid, palmitic acid, ethyl stearate, and sucrose [16]. The content of lignans in *Phaleria macrocarpa* (Scheff.) Boerl are pinoresinol, lariciresinol and matairesinol[17]. *Phaleria macrocarpa* (Scheff.) Boerl has antidiabetic effects that inhibit alpha-glukosidase and anti-diabetic effect in mice induced sterptozotosin.

Our previous study demonstrated that the fruits of *Phaleria macrocarpa* has ACE inhibitory activity with IC_{50} values in the fruits of Petroleum ether extract, aethyl acetate extract and metahanol extract were 162 µg/mL, 139 µg/mL and 122 µg/mL respectively [18]. The aim of the present study was to evaluate the effect of metanolic extract on systolic and dyastolic blood pressure and plasma angiotensin converting enzyme levels in the fructose induced hypertensive rat.

1.2. Material and Methods

1.1.2. Extraction

1000 gram of *P. marcocarpha* fruits powder was put in 2 litre methanol 80% solvent for 7 days at a room temperature with 4 times replications, and then the mixture was filtered and evaporated by rotary vacum evaporator at 40^{o}C until the concentrated methanol extract is obtained.

1.1.3. Animal and study design

Forty-two adult male Sprague-Dawley rats (aged 3 months), weighing 200 - 280 g were obtained from the Animal Source Unit, Indonesia University. The rats were randomly assigned into seven dietary groups (two control and five experimental groups) comprising of six animals each. Prior study approval was obtained from the Ethics Committee Medical Faculty of Indonesia University. All animal management and procedures were performed in accordance with the recommended guidelines. The rats were kept in stainless-steel cages and maintained at room temperature of 27°C ± 2°C with a 12 h light-dark cycle. All rats had free access to food and water ad libitum during the study period. Hypertension was induced experimentally by fructose 10% W/V diet ad libitum for 8 weeks. Fructose solution was prepared every two days by dissolving the fructose in distilled water. After one week of acclimatization, each group of rats were fed on the following diets: group I (normal control/ NC), rats received no medication but were given distilled water for drinking for 8 weeks; group II (negative control/ NCG), rats received no medication but were given 10% fructose solution for drinking for 8 weeks; group III, rats received 10% fructose solution for drinking for 8 weeks and received captopril 10 mg/kg BW start at week 6 until week 8 (CG); group IV, rats received 10% fructose solution for drinking for 8 weeks and received methanol extract of *P. marcocarpa* 0,5 g/kg BW start at week 6 until week 8 (PM 0,5); Group V, rats received 10% fructose solution for drinking for 8 weeks and received methanol extract of PM 1 g/kg BW start at week 6 until week 8 (PM1); group VI, rats received 10% fructose solution for drinking for 8 weeks and received methanol extract of PM 2 g/kg BW start at week 6 until week 8 (PM2); group VII, rats received 10% fructose solution for drinking for 8 weeks with methanol extract of PM 1 g/kg BW (PM3). Blood pressure was measured at baseline and at every weeks for 8 weeks using tail-cuff plethysmography. After completion of treatment schedule rats from each group were anesthetized with urethane (120 mg/100 gm, i. p.). Blood was collected and then centrifugated to obtain plasma and later store at-70^{0}C for analysis activity ACE plasma. The activity of ACE was measured using commercially available kit (USCNLife, West Lake, Wuhan, China) following manufacturer's instruction. The intensity of coloured product was measured in a microplate reader 450 nm.

1.3. RESULTS AND DISSCUSSION

1.3.1. Blood pressure

Results of blood pressure measurement in all treatment groups showed that systolic blood increase

start at the second week of fructose administration, except in the normal controls and the group that received 10% fructose solution for drinking for 8 weeks with methanol extract of *P. marcocarpa* 1 g/kg BW (Figure 1). Statistical analysis showed a significant difference in systolic blood pressure at week 2 between the normal control group (NG) with the negative control group (NCG), captopril group (CG), PM group at dose 0.5 g / kg BW, PM group at dose 1 g / kg BW and PM group at dose 2 g / kg BW kg ($p < 0.05$). Systolic blood pressure of all treatment groups at week 0 was comparable , providing 10 % fructose in drinking water starting at week 0 to week 8 increased systolic blood pressure. Systolic blood pressure in group rats that given captopril, PM0,5, PM1 and PM 2 seen decreased and not significantly different with normal control. Systolic blood pressure in rats were given PM 3 was not increase, and showed that methanol extract *P. marcocarpa* can prevent increasing systolic blood pressure.

Measurement of diastolic blood pressure in all treatment groups showed that blood pressure diastolic increase start the second week of fructose administration, except in the normal controls and in group received 10% fructose solution for drinking for 8 weeks with methanol extract of PM 1 g/kg BW (PM3) (Figure 2). Statistical analysis showed no difference in diastolic pressure in all treatment groups at week -0 and week 1. There was no significant difference in diastolic blood pressure between the NG with CG, PM 1, PM 2 and PM 3 ($p < 0.05$).

Fructose is a mediator of hypertension and hyperuricemia. Various studies have demonstrated administration of a high-fructose in rats leads to the onset of metabolic syndrome which includes hypertension, hyperglycemia, insulin resistance, obesity, and hyperuricemia [19]. The development of hypertension occurs in the second week after the administration of 10% fructose in drinks or 60% in the diet [20]. Increasing production or activity of vasoactive mediators such as endothelin-1 (ET1) [21], angiotensin II (ANGII) [22] and thromboxane A2 [23] is the mechanism underlying the relationship between resistance insulin and hypertension.

Studies in animals and humans suggest a link between the renin-angiotensin system to the pathogenesis of insulin resistance. Angiotensin II affects glucose metabolism through its effect on the insulin signaling pathway, decrease tissue blood flow, oxidative stress, increase sympathetic activity and adipogenesis. Increasing of sympathetic nerve activity causes increasing of catecholamine that leads to endothelial dysfunction and elevation in blood pressure [24]. Angiotensin receptor antagonists prevent Ang II from binding to angiotensin receptors and thereby inhibit AT1R-induced vasoconstriction, increasing blood flow through vasodilation. ACE inhibitors improve insulin sensitivity through two mechanisms. They reduce ACE-mediated generation of Ang II and they prevent the degradation of bradykinins, prolonging their half-life and allowing relaxation of blood vessels through endothelium-dependent mechanisms to occur [25]. Insulin resistance also causes increasing in sympathetic nerve activity that causes sodium reabsorption in the kidney and contributes to hypertension[26]. Fructose cause of hypertension by reducing renal sodium excretion, increasing of sodium absorption in the intestine and kidney, and this process is mediated by a transporter Slc26a6 and Slc2a5 [27].

SLc26ad in humans and in mice were also known as PAT1 (Putative Anion Transporter 1) or CFEX (Chloride / Formate Exchanger). There were major apical chloride/ base exchanger in the small intestine and proximal tubule, functioning in exchange Cl^- /$HCO3^-$ and Cl^-/oxalat and plays important role in the excretion of salt and bicarbonate in the small intestine. Fructose absorption in the small intestine and kidney proximal tubule is mediated primarily by Glut5 (Slc2a5) [27].

Another mechanism of fructose cause hypertension in rats through the formation of aldehyde conjugate levels is the result of the metabolism of fructose. Aldehyde binds to membrane protein sulfhydryl groups interfere calcium channels, thereby increasing the levels of free calcium, peripheral resistance and increased blood pressure[27].

In vitro studies showed that the methanol extract of mahkota dewa (*P. marcocarpha*) has effect as ACE inhibitors with IC_{50} value of 139.11 ppm [18]. Mahkota dewa contain mahkotaside, mangiferin, kaempferol-3-glucoside, dodekanoat acid, palmitic acid, ethyl stearate, sucrose and lignan. The lignans were pinoresinol, lariciresinol and matairesinol [16].

1.3.2. Activity ACE

Mean plasma ACE levels in each treatment group are summarized in Figure 3. Statistical analysis showed that the plasma ACE levels significant difference between the normal control group with the NCG, PM 0.5 , PM 1 and PM2 ($p < 0.005$). There was no significant difference between the normal control group and the CG with Group PM 1 (8 weeks delivery) ($p > 0.005$). There was a significant difference between the NCG with NC group, CG, PM 0,5, PM1 and PM2 AND PM3 ($p > 0.05$). There was no significant difference between CG with PM3 ($p < 0.005$) and no significant difference between the group PM1 and PM2 ($p>0,05$). Plasma ACE levels seen in the NCG 16 times larger than the CN.

The renin-angiotensin system (RAS) plays a pivotal role in the homeostatic control of arterial pressure, tissue perfusion, and extracellular volume leading to the development of hypertension. Dysregulation of the RAS can result in the pathogenesis of hypertension, cardiovascular and renal disorders. Owing to this, blockade of the RAS with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers has become important therapeutic approach in cardiovascular and renal medicine [1]. Pharmacological interruption of the RAAS is possible at five major site, renin release from the juxtagomerular cells, renin catalyzed`cleavage of angiotensinogen, ACE conversion of A-1 to A-II, AII action at angiotensin receptors and aldosteron action at the mineralcorticoid receptors [28].

ACE converts inactive AI to potent vasoconstrictor AII and raising blood pressure. AII increases generation of superoxide free radicals via NADPH/NADH oxidase system. AII has dual role in elevating blood pressure, direct vasoconstrictor effects and increasing production of free radicals which reduce of bioavailability of NO and indirectly attenuating endothelium dependent relaxation responses [29] .

The mechanisms underlying fructose-induced hypertension are not completely clarified, it has been proposed that elevation of blood pressure in fructose-fed rats is secondary to the development of insulin resistance and hyperinsulinemia. That is, compensatory hyperinsulinemia has been thought to be a cause of hypertension because insulin could cause sodium retention, sympathetic nerve activation, and vascular smooth muscle cell proliferation [7].

Hypertension after chronic fructose administration is due to the interaction between the ET - 1 system and the renin angiotensin system. ET - 1 is a vasoactive peptide produced by endothelial cells , are working on various tissues endocrine , paracrine or autocrine. ET-1 acts as an endogenous vasoconstrictor and growth factor for vascular smooth muscle cells. On the condition of hyperinsulinemia and insulin resistance , insulin can induce the release of ET-1 and causes an increase in blood pressure and vascular dysfunction [30]. Studies conducted by Tran et.al in rats fed fructose and given ET-1 receptor antagonists can normalize levels of chronic angiotensin II [31]. Renin-angiotensin system is a mediator in maintaining the blood pressure. Excessive activation of this system will lead to an increase in angiotensin II that causes hypertension and insulin resistance. Ang II may mediate the development of insulin resistance through its vasoconstrictor actions, thereby reducing blood flow and glucose uptake into insulin-sensitive tissues and the findings that Ang II infusion induced endothelial dysfunction, hepatic insulin resistance and elevations in blood pressure [19,31]. ACE inhibitors improve insulin sensistivitas by reducing the formation of angiotensin II and prevent the degradation of bradykinin, prolonging the half-life and cause relaxation of blood vessels through endothelial -dependent mechanism [32].

Phaleria macrocarpa is native to Indonesia, efficacious as a medicine. Fruits of *Phaleria macrocarpa* empirically been used to treat various types of diseases such as cancer, liver disorders, heart disease, diabetes, arthritis, kidney disorders, stroke, and high blood pressure [14]. Qualitatively this plants showed the presence of alkaloids, saponins, polyphenols, and from fruit contained alkaloids, saponins and flavonoids [15]. *Phaleria marcocarpa* was reported to contain phenolic glycoside such as mahkotaside, mangiferin, kaempferol-3-O-β-dglucoside, dodecanoic acid, palmitic acid, ethyl stearate, and sucrose [16]. *Phaleria macrocarpa* (Scheff.) Boerl also contain lignat, that are pinoresinol, lariciresinol and matairesinol [17]. The results showed that *Phaleria macrocarpa* (Scheff.) Boerl has antidiabetic effects that inhibit the enzyme alpha-glukosidase and have anti-diabetic effect in mice induced sterptozotosin. The fruits of *Phaleria macrocarpa* has ACE inhibitory activity with IC_{50} values was 122 µg/ml in methanol extracts.

Elevations in Ang II and increased oxidative stress or impaired nitric oxide (NO) levels may also contribute to the pathogenesis of fructose fed rats. Some of evidence suggests that vascular oxidative stress is involved in the pathogenesis of many cardiovascular disorders in the insulin-resistant states, including diabetes, hypertension, and atherosclerosis. Fructose-fed rats had increased vascular production of superoxide anions that was accompanied by increased nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity [3]. Pericarp and mesocarp *P. macrocarpa* fruit show good antioxidant and anti-inflammatory activities. These activities might be due to the presence of phenolic and flavonoid compounds [33].

The results suggests that methanol extract of *Phalleria marcocarpha* could prevent the development high blood pressure and normalized blood pressure in rat induced by diet rich in fructose. The results tend to suggest that methanol extracts of possesses antyhipertensive activity, through inhibition I of angiotensin converting enzyme AII .

1.4. ACKNOWLEDGEMENT

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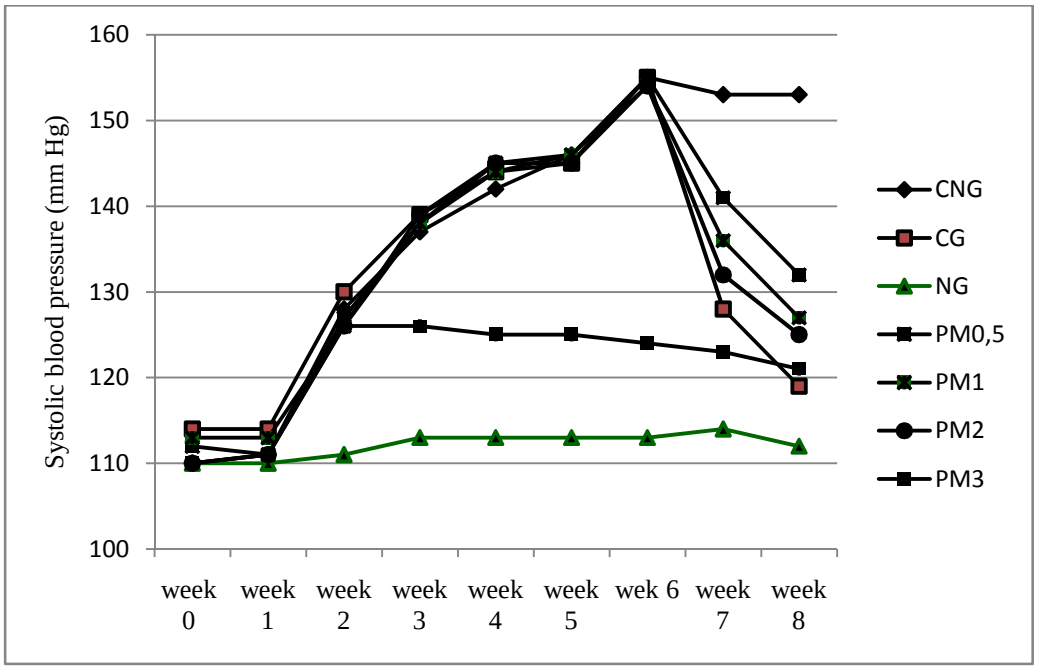

Figure 1
Effect of methanol extract *P. marcocarpa* in mean change systolic blood pressure in fructose induced hypertensive in rats

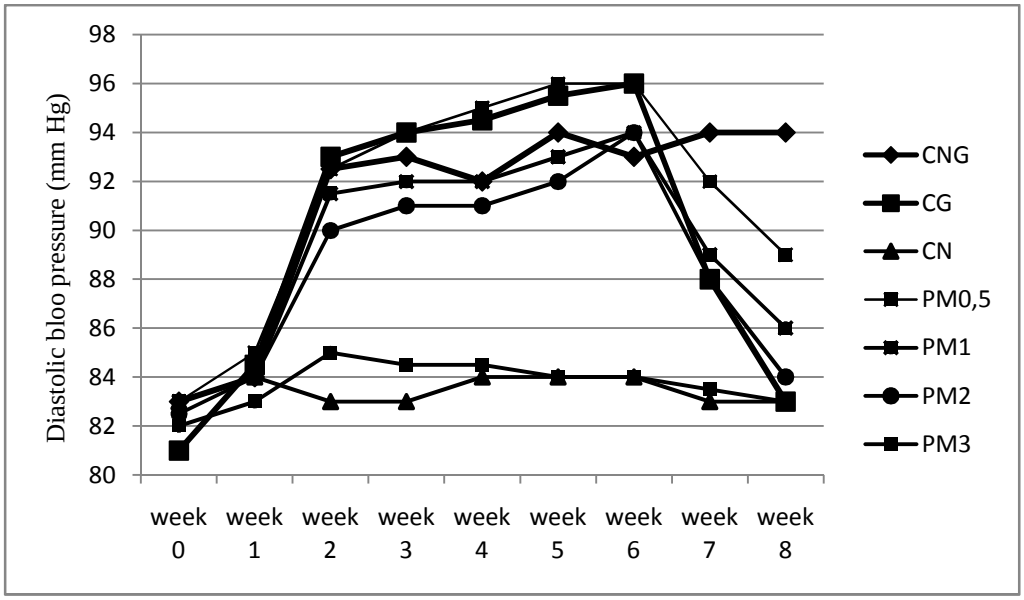

Figure 2
Effect of methanol extract *P. marcocarpa* in mean change diastolic blood pressure in fructose induced hypertensive in rats

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