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Antioxidant effects of *Methanolic extract* of *Phaleria macrocarpa* (Scheff.) Boerl in fructose 10%-induced rats

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Abstract: The present study was carried out to investigate whether methanolic extract of *Phalleria macrocarpa* (Scheff) Boerl fruits (PM) mitigates oxidative stress in fructose fed rats. Male Sprague Dawley rats were fed with fructose 10% w/v ad libitum for 8 weeks. The rats (n = 36) were divided into six groups (of six each); normal control, negative control, captopril 10 mg/kg BW and extract methanolic *P. Marcocarpha* (PM)at the dose0.5, 1, 2 g/kg BW, were given start at week 6 until week 8 and 1 g/kg BW were given for 8 weeks. After completion of treatment schedule rats from each group were anesthetized with urethane (120 mg/100 gm, intraperitoneal). Blood was collected and then centrifugated to obtain plasma and later store at-70^{0}C. Plasma glucose, lipid peroxidation markers and antioxidant enzyme (MDA, GSH, and SOD) were analyzed in all the groups. Fructose consumption increased SOD and glucose plasma level and reduced GSH and SOD activity, whereas these levels were near-normal in the rats consuming PM and captopril.
Keywords: methanolic extract, Phalleria macrocarpa (Scheff) Boerl fruits, oxidative stress, fructose fed rats.

Introduction

Fructose is a simple sugar, a monosaccharidethat is present primarily in added dietarysugars, honey, and fruit. Dietary fructose intake is increasing primarily from added sugars,including sucrose and high fructose corn syrup, and correlates epidemiologically withthe rising prevalence of metabolic syndrome and hypertension worldwide. The administrationof fructose to animals and humans increases blood pressure and the developmentof metabolic syndrome. These changes occur independently of caloric intakebecause of the effect of fructose on ATP depletion and uric acid generation[1]. Fructose fed rat play a role in causing stress oxidative and imbalance between free radical production and antioxidant defense. Excessive fructose consumption may influence pathological conditions such as fatty liver and oxidative stress.High fructose feeding in rats has shown to increase oxidative stress that produces reactive oxygen species and plays a role in several cardiovascular disease and insulin resistance[2].

High fructose feeding can cause oxidative stress that can lead to cardiovascular abnormalities and insulin resistance. The concentration of free radicals three times higher than the control group in rats fed a high-fructose diet. Giving antioxidants such as vitamin E can prevent the development of insulin resistance and increased production of free radicals[3]. During membrane lipoprotein, protein and polyunsaturated fatty acids

(PUFA) was attacked by a number of free radicals it will produce a number of oxygen compounds.Level of malondialdehyde (MDA) was increasing which is one indicator of oxidative stress. Endogenous antioxidant systems allows the transformation of reactive oxygen species (ROS) becomes inactive. Natural antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) is the main defense against ROS[2].

Phaleria macrocarpa(Scheff.) Boerlis native plants from Indonesia, which has long been used as folkmedicines for treatments various types of diseases such as cancer, liver disorders, heart disease, diabetes, arthritis, kidney disorders, stroke, and high blood pressure[4].Qualitatively this plants showed the presence of alkaloids, saponins, polyphenols, and from fruit contained alkaloids, saponins and flavonoids[5].*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[6]. The content of lignans in *Phaleria macrocarpa* (Scheff.) Boerl are pinoresinol, lariciresinol and matairesinol[7]. *Phaleria macrocarpa* (Scheff.) Boerl has antidiabetic effects that inhibit alpha-glukosidase and anti-diabetic effect in mice induced sterptozotosin[8].Phenolics are one group of larger secondary metaboliteswhich are synthesized by plants and are utilized as UV,wounding and infection protectant in plants. Phenolicshave been indicated to have several biological activitiessuch as antioxidant, antimutagenic, anticarcinogenic,anti-inflammatory and antimicrobial activities in human[9].

Therefore, the purpose of this study was to determine beneficial effects of methanolic extract of *Phalleria macrocarpa* (Scheff) Boerl fruits (PM)in the prevention of fructose induced oxidative stress in rats.

Experimental

Material and Methods

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 vacuum evaporator at 40oC until the concentrated methanol extract is obtained.

Animal and study design

Forty-two adult male Sprague-Dawley rats (aged 3months), 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 waterad libitum during the study period. Stress oxidative was induced experimentally by fructose 10% W/V diet adlibitum 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 8weeks; 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 8weeks and received captopril 10 mg/kg BW start at week 6 until week 8 (CG); group IV, rats received10% 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 8weeks 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 8weeks and received methanol extract of PM 2 g/kgBW start at week 6 until week 8 (PM2); group VII ,rats received 10% fructose solution for drinking for 8weeks with methanol extract of PM 1 g/kg BW(PM3)[10].Blood was collected and then centrifugated to obtain plasma and later store at-700C for analysis MDA, GSH, SOD and glucose level. The antioxidant activity and glucose level were measured using spechrophotometer.

Results and Discussion

Antioxidant activity

Plasma antioxidant levels from each treatment group were measured on day 57 after the induction of fructose. Mean levels ofmalondialdehyde (MDA), superoxide dismutase (SOD), MDA and glutathione (GSH) in each treatment group can be seen in Figure 1, Figure 2 and Figure 3.

Oxidative stress occurs when the tissues and organs damaged by reactive oxygen species, causing an imbalance between prooxidant factors and the antioxidant defense system. Evidence indicates that reactive oxygen species play a role in the pathophysiology of metabolic syndrome and high intake of fructose can cause oxidative stress[2].

Renin angiotensin system plays a role in signal transduction and regulation of vascular tone. Excessive activation of the SRA will disrupt insulin transduction and cause insulin resistance. Studies was conducted by Shinozaki et.al prove that 60% fructose intake in the diet of animals for 8 weeks lead to insulin resistance and stimulate the production of superoxide anion. Superoxide anion increased two-fold in ratsfed a high fructose compared to the control group[11].

MDA is the end product peroxidation of unsaturated fatty acids that can react with tiobarbiturat acid (TBA) form a complex TBARS. Lipid peroxidation can be increased in rats fed high-fructose diet due to oxidative stress resulting in lower system antioxidant scavengers[12]. The results showed that MDA level increased in negative control group were given fructose for 8 weeks (Figure 1). Statistical analysis of each treatment groups showed significant difference between the normal control group (NC) with the negative control group (NCG), the experimental group 1 (PM0,5) and experimental group 2 (PM1) ($p < 0.05$), and no significant difference between NC with group positive control (Captopril), PM2 and PM3 ($p > 0.05$). There was no significant difference between the Captopril group with PM 2 and PM3 ($p > 0.05$).

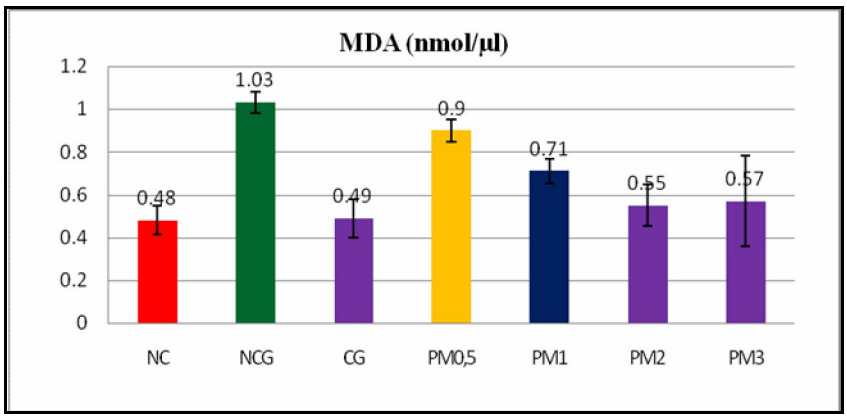

Figure I : mean plasma of MDA (nmo/µL)

Captopril is an ACE inhibitor has antioxidant activity in-vitro. The presence of thiol groups in the molecule captopril can donate one electron that can reduce free radicals. Captopril can inhibit the production of free radicals in the process of atherogenesis and have antioxidant properties. Captopril increase plasma MDA, MDA network and catalase[13].

GSH (glutathione) is a thiol group with low molecular weight, effective as scavengers of free radicals and other reactive oxygen species such as hydroxyl radicals, lipid peroxyl radicals and H_2O_2. GSH is synthesized from glutamate, cysteine and glycine are catalyzed by two enzymes, cytosolic glutamyl cysteine and glutathione synthase. Glutathione deficiency contributes to oxidative stress and can lead to various diseases such as Parkinson's, Alzheimer's, cystic fibrosis and diabetes mellitus[14].

The results showed that GSH level decreased in the negative control group were given a high-fructose diet for 8 weeks compared to the normal control group (Figure 2).Statistical analysis of each of the treatment groups showed significant difference between normal control with negative control,PM0,5, PM 1, and PM 2(p

> 0.05). There was no significant difference between normal control group with captopril group (CG) and PM 3 (8 weeks administration of extract) (p <0.05).

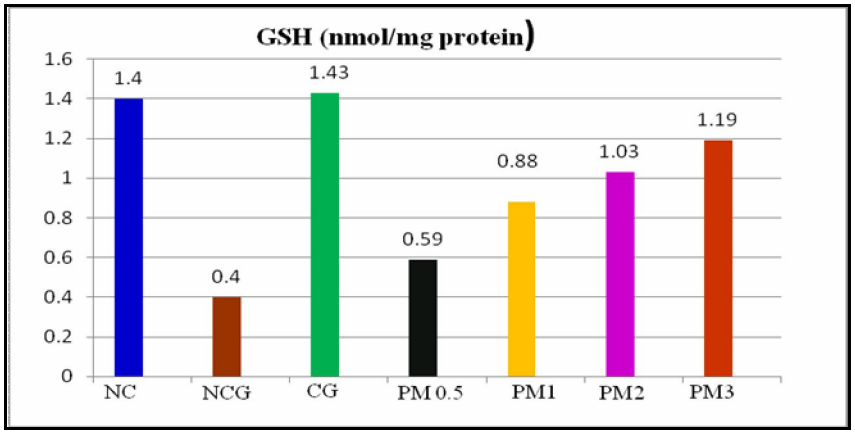

Figure 2.Mean Plasma of GSH (nmol/mg protein)

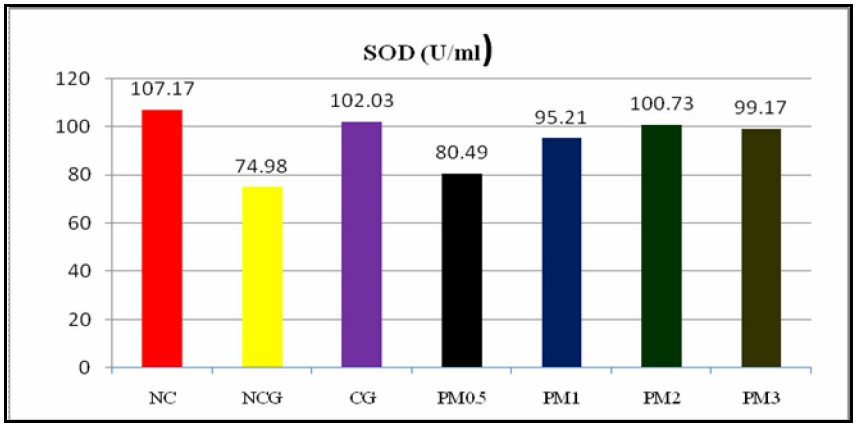

Figure 3.Mean Plasma of SOD (U/mL)

High fructose feeding in rats can cause oxidative stress and decrease dendogenous antioxidant activity. Activity of Superoxide dismutase (SOD) as an endogenous antioxidant will increased by changing the superoxide anion (O_2^*) into hydrogen peroxide (H_2O_2) and O_2. SOD is an enzyme that stimulates the superoxide radical (O2*) into H_2O_2. This enzyme inhibits the simultaneous presence of O_2 and H_2O_2 derived from radical formation of hydroxyl radicals (OH)[15].The results showed that SOD increased in Captopril group, MD 1 g / kg BW group, MD 2 g / kg BW group and MD 1 g/kg BW group (8 weeks) were not significantly different than normal control group (P<0,05). There were no significant differences between CG,PM 1, Pm 2and PM 3 (8 weeks) (p <0.05) (Figure 3).

Study conducted by Hendra, et.al showed that part of pericarp and mesocarp *P marcocarpa* have high antioxidant activity, respectively 71.97% and 62.41%. The antioxidant power to reduce the iron compounds is 92.5% in the pericarp and 78.78% in the mesocarp. Part of pericarp and mesocarp able to induce the synthesis of nitric oxide respectively by 63.4% and 69.5%. The antioxidant effects suspected because of the phenolic compounds and flavonoids. Kaempferol, miristin, quersetin and routine as an antioxidant effect. The relationship between the flavonoids and antioxidant activity due to three hydroxyl groups on the heterocyclic cicncin and the addition of 3,4-dihydroxy group in ring B[16].

Results of phytochemical screening to extract *P marcocarpa* shows that it contains tannins, terpenoids and flavonoids.Hendra et al. have proven that the flavonoid effect as an antioxidant and antimicrobial[17].Triastuti and Choi proved that the crown of the gods able to suppress oxidative stress in diabetic rats[12].

Plasma Glucose Level

Plasma glucose levels from each treatment group were measured on day 57 after the induction of fructose. The mean plasma glucose levels in each treatment group is shown in Figure 4

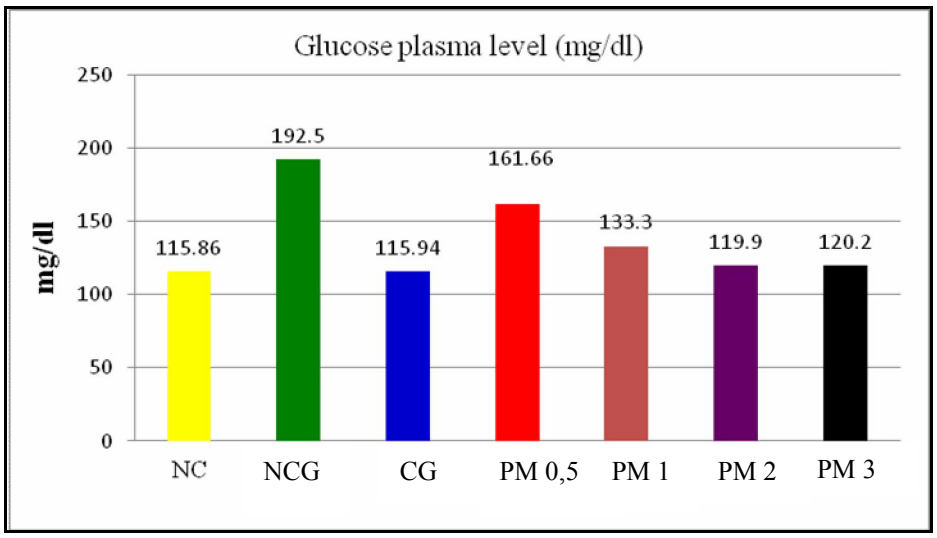

Figure 4Mean Plasma Glucose Level (mg/dL)

Results of statistical analysis on plasma glucose levels from each treatment group showed significant difference between NC group and PM 1. There were no significant differences between NC group with PM 1, PM 2 and PM 3 (for 8 weeks) and CG (captopril group). There were no significant differences between CG with NC group, PM 1, PM 2 and PM 3 (for 8 weeks). There was no significant difference between PM 2 and PM 33 (for 8 weeks). This shows that *P marcocarpa* extract at dose of 1 g / kg BW and 2 g / kg BW given for 2 weeks and at dose of 1 g / kg given for 8 weeks can reduce plasma glucose levels.

Study conducted by Ali et.al, 2012 in diabetic rats has proven that butanol extract of *P marcocarpa had* antidiabetic effect. The extract able to reduce plasma glucose levels by 66.67% ($p < 0.05$) is comparable to metformin (51.1%), glibenclamide (66.67%) and insulin (71.43%) after 12 days of treatment[18].

Insulin resistance is a condition in which insulin is unable to produce its responses, such as increasing glycogen synthesis and decreasing gluconeogenesis. An impaired ability of insulin to suppress hepatic glucose occurs in fructose-fed rats. Livers from fructose-fed rats showed enhanced glucose outflow that was suppressed by insulin to a lesser degree as compared to control rats. the insulin resistance resulting from chronic fructose feeding is due to the diminished ability of insulin to suppress hepatic glucose output, and not to a decrease in insulin-stimulated glucose uptake by muscle[19].

ACE inhibitor therapy can improve insulin sensitivity and also delay the development of diabetes in patients at high risk for the development of this disease. The mechanism whereby ACE inhibitors improve glucose metabolism and protect against the development of clinical diabetes may involve the improvement of blood flow through the microcirculation tofat and skeletal muscle tissue and/or the improvement of insulin action at the cellular level (by interfering with the angiotensin II antagonism of insulin signaling). ACE inhibitors improve insulin resistance and their action on glucose metabolism may be mediated via bradykinin metabolism. ACE inhibitor improves insulin responsiveness through increasing blood flow and improved protein glucose transporter (GLUT4) and hexokinase activity, an enzyme that plays a role in glucose metabolism[20]. Our study previous demonstrated 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 ofpossesses antyhipertensive activity, through inhibition I of angiotensin converting enzyme AII [10].

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[4]. Qualitatively this plants showed the presence of

alkaloids, saponins, polyphenols, and from fruit contained alkaloids, saponins and flavonoids [5].*Phaleria marcocarpa* was reported to contain phenolic glycoside such as mahkotaside, mangiferin, kaempferol-3-O-β-dglucoside, dodecanoic acid, palmitic acid, ethylstearate, and sucrose[6].*Phaleriamacrocarpa* (Scheff.) Boerl also contain lignan, that are pinoresinol, lariciresinol and matairesinol[7]. The results showed that *Phaleria macrocarpa* (Scheff.)Boerl has antidiabetic effects that inhibit the enzymealpha-glukosidase and have anti-diabetic effect inmice induced sterptozotosin. The fruits of *Phaleria macrocarpa*has ACE inhibitory activity with IC_{50}values was 122 µg/ml in methanol extracts(Rinayanti, Radji, Mun'im, & Suyatna, 2013).

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