Research Article **ISSN: 2347–8330**

Journal of Pharmaceutical and Biological Research
Online at ***www.pharmaresearchlibrary.com/jpbr***
JPBR, 2014, Vol.2(1): 79-84

Methanol Extract of *Phaleria macrocarpa* (Scheff.) Boerl improved renal and liver histological changes in fructose 10% induced rats

Aprilita Rina Yanti*[1], Maksum Radji[2], Abdul Mun'im[2], and FD Suyatna[3]

[1]*Faculty of Pharmacy, University of 17 Agustus 1945 Jakarta, Jakarta Indonesia*
[2]*Faculty of Pharmacy, University of Indonesia, Depok, Indonesia*
[3]*Dept. of Pharmacology and Therapeutic, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia*

Received: 6 February 2014, Accepted: 24 April 2014, Published Online: 21 June 2014

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***Corresponding author**
Aprilita Rina Yanti
Faculty of Pharmacy,
University of 17 Agustus 1945,
Jakarta, Indonesia
Manuscript ID: JPBR2103

PAPER QR-CODE

Abstract

Crown god [*Phaleria macrocarpa* (Scheff.) Boerl] is one of the medicinal plants that have been used empirically to treat various types of diseases such as cancer, heart disease, diabetes, gout, rheumatism, kidney, high blood pressure, eczema, acne, insect bites and wounds. Crown of god fruits contain flavonoids which suspected has the effect of hypoglycemic. This study investigated the effect of aethyl acetate extract crown god fruits [*Phaleria macrocarpa* (Scheff.) Boerl] against renal and heart damage induced by a high-fructose diet (10% v/v) in rats ad libitum for 8 weeks. The rats (n = 42) were divided into seven groups (of six each); normal control, negative control, captopril 10 mg/kg BW and exract methanolic *P. Marcocarpha* (PM) at the dose 0 start at week 6 until week 8 and 1 g/kg BW for 8 weeks. After completion of treatment schedule rats from each group were anesthetized with urethane (120 mg/100 gm, i. p.). Rats were sacrified to obain their kidney and liver for routine staining. Treatment with PM (methanolic extract) and captopril significantly reduced diameter of vena centralis and reduced the glomerular hypertrophy ($p<0,05$)

Keywords: Methanolic Extract, [*Phaleria macrocarpa* (Scheff.) Boerl], renal and liver damage, fructose

1. Introduction

The metabolic syndrome is an important public health concern that predisposes individuals to the development of cardiovascular disease and/or Type 2 diabetes. The metabolic syndrome is a cluster of clinical and biochemical features that includes abdominal obesity, insulin resistance, hypertension, accelerated atherosclerosis, and

dyslipidemia (Caglayan et al., 2005). It is well documented that each metabolic disturbance is an important risk factor for the development of cardiovascular disease. As a result, individuals afflicted with the metabolic syndrome are at an increased risk for developing Type 2 diabetes (Martinez et al., 1994) and/or cardiovascular disease. Fructose is a monosaccharide that is present in many fruits and vegetables. Although fructose has the same chemical formula ($C_6H_{12}O_6$) as glucose, it differs in its chemical structure. Fructose is a five-membered ring with a ketone functional group attached to the second carbon, whereas glucose is a six-membered ring with an aldehyde group at the first carbon. Fructose also exists in foods as a disaccharide in the form of sucrose, which is composed of one molecule of glucose linked to one molecule of fructose through a 1-4 glycoside bondof refined carbohydrates and fructose, is clear and correlates positively with an alarming increases in metabolic syndrome (Tran et al., 2009). The fructose-fed rat is an animal model of acquired systolic hypertension that displays numerous features of the metabolic syndrome. This animal model is used to study the relationship between insulin resistance/compensatory hyperinsulinemia and the development of hypertension (Tran et al., 2009; Johnson et al., 2007).

Fructose intake has been recently linked to the epidemic of metabolic syndrome and, in turn, the metabolic syndrome has been epidemiologically linked with renal progression. The renal hemodynamic effects of fructose intake are unknown, as well as the effects of different routes of administration. Fructose induced metabolic syndrome is associated with renal disturbances characterized by renal hypertrophy, arteriolopathy, glomerular hypertension, and cortical vasoconstriction (Sánchez et al., 2007). Fructose enriched diet in rat can induced fatty liver disesase, characterized changes in liver pathology, hepatic lipid composition, and hepatic iron concentration (Ackerman et al., 2005). Consumption of fructose is linked to the increased prevalence of fatty liver, associated with adverse alterations of plasma lipid profiles and metabolic changes in mice (Basaranoglu et al., 2013).

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 (Harmanto, 2003). The use of *Phaleria macrocarpa* traditional medicine is very likely due to leaf and fruit of *Phaleria macrocarpa* contains chemical compounds such as alkaloids, saponins, polyphenols, and fruit contained alkaloids and saponins also contain flavonoids (Sumastuti, 2003). *Phaleria macrocarpa* contains mahkotaside, mangiferin, kaempferol-3-O-D-glucoside, dodecanoat acid, palmitic acid, ethyl stearate and sucrose (Oshimi et al., 2008). The content of lignans in *Phaleria macrocarpa* (Scheff.) Boerl are pinoresinol, lariciresinol and matairesinol (Saufi et al., 2008). *Phaleria macrocarpa* (Scheff.) Boerl has antidiabetic effects that inhibit alpha-glukosidase and anti-diabetic effect in mice induced sterptozotosin. The in-vitro test results showed that the fruits of *Phaleria macrocarpa* has ACE inhibitory activity with IC_{50} values in the fruits was 162 µg/ml in Petroleum ether extract, 139 µg/ml in aethyl acetate extract and 122 µg/ml in metahanol extract (Rinayanti et al., 2013). The aim of the present study was to evaluate the effect of ethyl acetate extract of *Phalleria marcocarpha* against renal and hepatic damage induced by a high-fructose diet in rats.

2. 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 vacum evaporator at 40^{o}C until the concentrated methanol extract is obtained.

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 (Tran *et al*., 2009; Jadhav and Upasani, 2012; Jala et al., 2010). 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. Marcocarpha* 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). Body weight was measured at baseline and at every weeks for 8 week. After completion of treatment schedule rats from each group were anesthetized with urethane (120 mg/100 gm, i. p. Kidney and liver samples from each group were prepared for histopathological assessment and placed in 10% neutral buffered formalin. Each specimen fixed in 10% formalin solution was embedded in paraffin wax. Sections of 4 µm in thickness were stained with hematoxylin and eosin, and examined under a light microscope.

3. Results and Discussion

Body weight

Body weight significantly increased each week in the rats fed the high-fructose diet compared to the control rats ($P < 0.05$, Figure 1). The body weight of the *P. Marcocarpha* group were not significantly different from those of the control group. Increase in body weight occurs due to disturbance in the insulin signaling pathway. In experimental animals fructose feeding resulted in a significant increase in the body weight from 15 days till the end of experimental period. Insulin is involved in the regulation of body adiposity via its action on the central nervous system (CNS) to inhibit food intake and increase energy expenditure. Thus, reduced insulin delivery into the CNS or disruption of the insulin- signaling pathway in CNS may result in the weight gain and development of obesity (Rasineni and Desireddy, 2011).

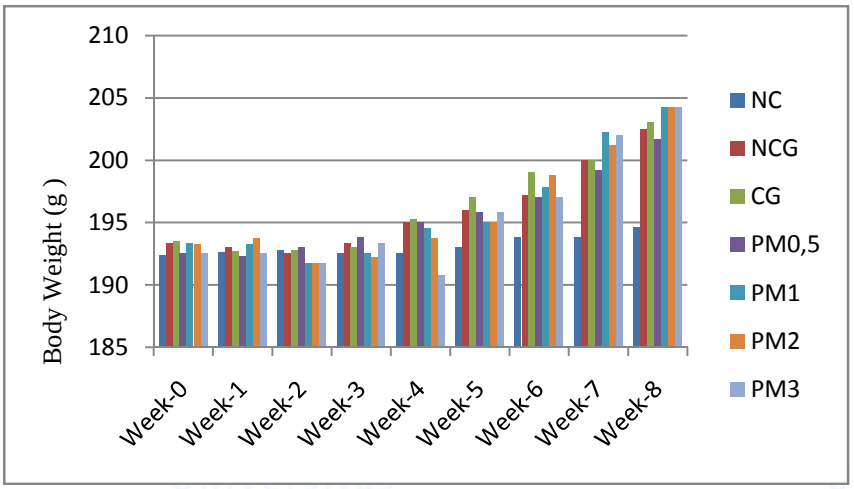

Figure 1. Body weight each rat group (week-o until week -8)

Liver Histopathology

Observation of liver damage can be seen from the change of central venous and liver cells. The central venous used as measurements because the area is the center of the liver lobules and at least receive oxygen, so that in the event of disruption it will first be damaged. If central venous damage the endothelial cells of the central vein will undergo lysis and result in enlargement of the diameter of central venous (Leeson and Thomas, 1998). Liver histology and diameter of central venous each treatment group are presented in Figure 2 and 3.

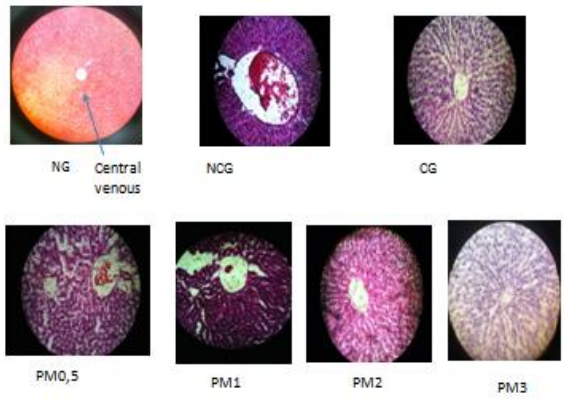

Figure 2. Liver histology in each group

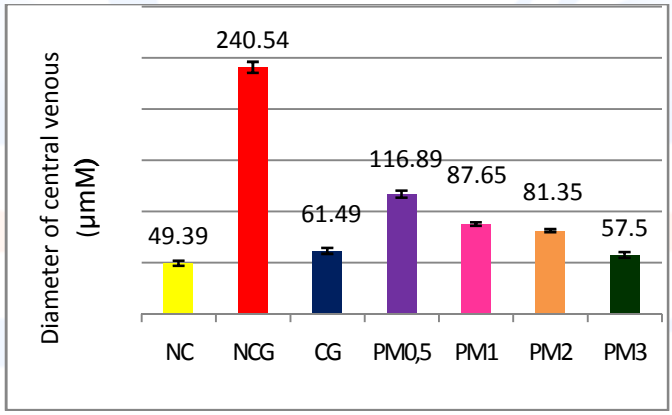

Figure 3. Diameter of central venous (µm)

The results of histological observation showed that the average size of central venous diameters significantly different between the NC with NCG, CG, PM0,5, PM1, PM2 and PM3 ($p < 0.05$).

Fructose administration for 5 weeks in the mice caused the occurrence of fatty liver and giving captopril for 2 weeks lowered plasma and liver trigliserida and lowers the score steatosis (Ackerman et al., 2005). Captopril, an angiotensin-converting enzyme inhibitor, has reported metabolic effects. Captopril improves insulin sensitivity, has antioxidant properties, and is able to scavenge reactive oxygen species and to attenuate the progression of hepatic fibrosis in rats. Captopril did not significantly improve insulin resistance; however, there was a significant reduction in plasma and hepatic triglycerides, with a decrease in the macrovesicular steatosis score (Ackerman et al., 2005; Benzie and Tomlinson, 1998).

Kidney Histopathology

The main parameter that are used to analyze the presence of kidney damage is the microscopic examination of tissue preparations, especially glomerular. The tests were by measuring glomerular diameter and the distance between the glomerulus and Bowman's capsule space. Kidney histology was presented in Figure 3 and glomerular diameter and the distance between the Glomerular and Bowman's capsular space were presented in Figure 4.

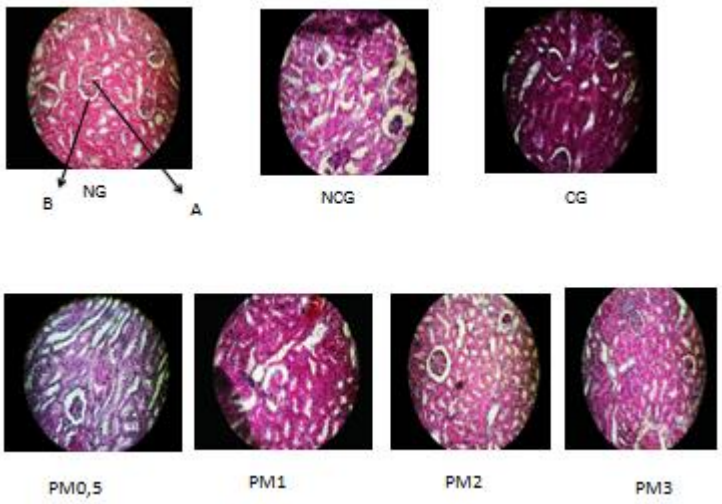

Figure 4. Kidney histology in each group
A : Glomerular diameter ;
B: the distance between the Glomerular and Bowman's capsular space

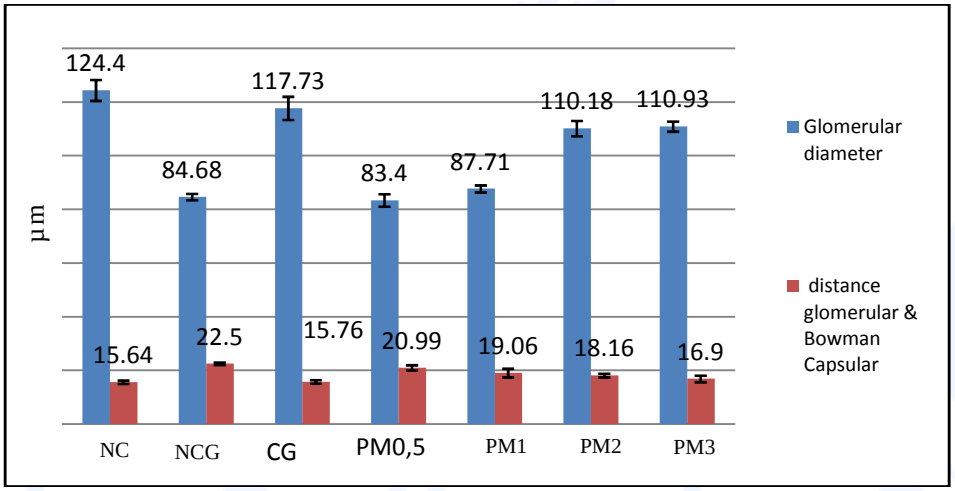

Figure 5. Glomerular diameter and the distance between the Glomerular and Bowman's capsular space

The results of histological observation showed that the average size glomerular diameters and the distance between the Glomerular and Bowman's capsular space significantly different between the NC with NCG, CG, PM1, PM2

and PM3 ($p < 0.05$) and between NC with NCG, CG and PM3, respectively. Fructose-induced metabolic syndrome is associated with glomerular hypertension and renal microvascular damage in rats (Sánchez et al., 2012). High fructose feeding has been demonstrated to induce renal hypertrophy with tubular cell proliferation and tubulointerstial injury, which may consequently impair renal function. Fructose feeding caused deterioration in both glomerular and tubular structures (Choi et al., 2011). The exact mechanisms for renal damage caused by fructose treatment have not been established, oxidative stress, the lipogenic effect, release of inflammatory cytokines, and endothelial dysfunction may be underlying mechanisms. Fructose consumption increases levels of lipid peroxides and decreases activities of antioxidant enzymes in the kidney (Johnson et al., 2010).

Phaleria macrocarpa is native to Indonesia efficacious as a medicine. Leaves and 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 (Harmanto, 2003). The use of *Phaleria macrocarpa* traditional medicine is very likely due to leaf and fruit of *Phaleria macrocarpa* contains chemical compounds such as alkaloids, saponins, polyphenols, and fruit contained alkaloids and saponins also contain flavonoids (Sumastuti.R, 2003). *Phaleria macrocarpa* contains mahkotaside, mangiferin, kaempferol-3-od glucoside, dodecanoat acid, palmitic acid, ethyl stearate and sucrose (Oshimi et al., 2008). The content of lignans in *Phaleria macrocarpa* (Scheff.) Boerl are pinoresinol, lariciresinol and matairesinol (Saufi et.al., 2008). PM 's pericarp has antioxidant activity. The antioxidant activity of P. macrocarpa fruit might be due to the presence of phenolic and flavonoid compounds. Hendra et al. reported the presence of kaempferol, myricetin, naringin, quercetin, and rutin as the major flavonoids present in P. macrocarpa fruit (Hendra et al., 2011). In hyperglycemic condition, flavonoid will be oxidized by free radicals resulting in a more stable structure (Nijveldt et al., 2001). PM was also reported to increase renal antioxidant enzymes such as superoxide dismutase, catalase, dan glutation peroxidase (Triastuti et al., 2009a).

Phaleria macrocarpa (Scheff.) Boerl has antidiabetic effects that inhibit the enzyme alfaglukosidase and have anti-diabetic effect in mice induced sterptozotosin. The leaf and fruit *Phaleria macrocarpa* (Scheff.) Boerl have ACE inhibitors activity with IC_{50} values in the leaves was 189.13 µg/ml in PEE, 157.74 µg/ml in EEA and 101.52 µg/ml ME. While the IC_{50} values in the fruit was 161.7 µg/ml in PEE, 139.11 µg/ml in the EEA and 122.38 µg/ml in ME (Rinayanti et al., 2013).

Animal and human studies suggest that a link between the renin - angiotensin system in the pathogenesis of insulin resistance. Angiotensin II causes an increase in glucose metabolism through their effects on insulin signaling pathway, decreased tissue blood flow, oxidative stress, increased sympathetic activity and adipogenesis. Increased sympathetic nerve activity causes an increase in catecholamines that cause endothelial dysfunction and increased blood pressure (Tran et al, 2009). Inhibition on the renin - angiotensin system (SRA) with ACE inhibitors or angiotensin receptor blockers may improve glucose metabolism by preventing the formation of angiotensin II or prevent the AII receptor activation (Kurtz and Pravenec, 2004). Insulin resistance also causes an increase in sympathetic nerve activity that causes sodium reabsorption in the kidney that contributes to hypertension (Corry and Tuck , 1999). Fructose cause of hypertension by reducing renal sodium excretion , increased sodium absorption in the intestine and kidney , and this process is mediated by a transporter Slc26a6 and Slc2a5 (Singh et al , 2008).

Phaleria macrocarpa (Scheff) Boerl (PM). PM has been investigated extensively in diabetes theraphy. PM's pericarp were reported to have hypoglycemic activities as an inhibitor of enzyme α-glucosidase (Sugiwati et al., 2006). PM can reduce renal hypertrophy and blood urea nitrogen level in diabetic rats (Triastuti et al., 2009a) and also increase hepatic antioxidant enzymes (Triastuti et al., 2009b). PM's pericarp has protection effect on the glomerular hypertrophy and glomerulosclerosis in the diabetes caused by alloxan (Sulistyoningrum and Ismaulidiya, 2013).

4. Conclusion

Methanol Extract o f *Phaleria macrocarpa* (Scheff.) Boerl improved renal and liver histological changes in fructose 10%-induced rats. Further studies are necessary to elucidate the underlying mechanisms of the renoprotective effects of *Phaleria macrocarpa* (Scheff.).

5. Acknowledgements

This research was supported by funds from the Excellent Research Grant University – Ministry of Education (DIKTI) 2013.

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