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Full Length Research Paper

Protective effect of PM pericarp extract on renal histological changes of alloxan-induced diabetes

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Global prevalence of diabetes mellitus is increasing all over the world, resulting in higher prevalence of diabetic complication. Diabetic nephropathy is the most frequent complication suffered by diabetic patients. *Phaleria macrocarpa* (Scheff.) Boerl (PM) consist of active substance s which have antidiabetic properties. This study aims to evaluate the protective effect of PM pericarp extract on renal histological changes of alloxan-induced diabetes. Twenty five male albino (Sprague Dawley) rats were divided into five groups (of five each): normal control; diabetic; diabetic + metformin 100 mg/kgBW; diabetic + PM methanolic extract 250 mg/kgBW and diabetic + PM water extract 250 mg/kgBW. Diabetes was induced by alloxan monohydrate 150 mg/BW intraperitoneally. PM extract and metformin (as comparison group) was administered to diabetic rats for three weeks and at the end of the study, rats were sacrificed to obtain their kidneys for routine staining. Treatment with PM extracts (methanolic and water) and metformin significantly reduced glomerular hypertrophy, though the glomerular area of these groups was lower than that of the diabetic group ($p < 0.01$). Treatment with PM extract (methanolic and water) significantly reduced glomerulosclero sis, which showed a lower score of glomerulosclerosis degree ($p< 0.01$), and was still observed to be lower if compared with metformin ($p < 0.01$). It was concluded that PM extract re stored glomerular hypertrophy and improved glomerulosclerosis in alloxan -induced diabetes.

Key words: *Phaleria macrocarpa* (Scheff.) Boerl., glomerulosclerosis, glomerular hypertrophy .

INTRODUCTION

World Health Organization has estimated global prevalence of diabetes mellitus (DM) to increase from 2.8% in 2000 to 4.4% in 2030 (Wild et al., 2004). The increasing prevalence will be accompanied with diabetes complication in multiple organs. Diabetic nephropathy (DN) is a serious complication of diabetes that affect most patients (Kanwar et al., 2008). It is the cause of end-stage renal diseas e throughout the world (Schrijvers et al., 2004). Hyperglycemia in renal tissue induces mesangial expansion and changes in cellular and extracellular compartment of glomerulus characterized by glomerular hypertrophy (Malatiali et al., 2008), Glomerular Basement Membrane (GBM) thickening and glomerulosclerosis (Teoh et al., 2010). Diabetes induc ed by alloxan in rats results in development of nephropathy similar to early stage clinical DN (Rasch and Mogensen, 1980).

Recent studies discover antidiabetic and protective effect of some natural products, such as *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-glucosidas e (Sugiwati et al., 2006).

Previous studies suggested that PM can reduce renal hypertrophy and blood urea nitrogen level in

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Abbreviations: GBM: Glomerular Basement Membrane; **DN:** diabetic nephropathy; **DM:** diabetes mellitus; **PM:** *Phaleria macrocarpa* (Scheff.) Boerl; **PMM:** *Phaleria macrocarpa (Scheff.) Boerl* methanolic extract; **PMW:** *Phaleria macrocarpa (Scheff.) Boerl* water extract.

diabetic rats (Triastuti et al., 2009a) and also increase hepatic antioxidant enzymes (Triastuti et al., 2009b). In the present study, protection effect of the extract of PM's pericarp on the glomerular hypertrophy and glomerulosclerosis in the diabetes caused by alloxan was examined.

MATERIALS AND METHODS

Design of the study

A post-test only with control design was conducted from July-December 2012 in Pharmacology Laboratory of Medical Faculty and Health Sciences, Jenderal Soedirman University, Purwokerto, Indonesia.

Plant material collection and preparation of extracts

Ripe fruits of *P. macrocarpa (*Thymelaeaceae) were purchased from Merapi Herbal Farma, Yogyakarta, Indonesia. The plant species was identified by Plant Taxonomy Laboratory at Biology Faculty of Jenderal Soedirman University, Purwokerto, Indonesia. The pericarp of the fruits were sliced, dried in 70°C and ground into powder using a milling machine. To obtain methanolic extract, about 900 g of the grounded material was added with 5.5 L methanol for 24 h. The filtrate was collected using Whatman No. 1 filter paper and residue was re-extracted with methanol 5.5 L for 24 h and this step was repeated 2 times. The filtrate from 3 d ays methanol extraction was collected and evaporated by rotary evaporation. Water extracts as mimick of traditional use was obtained from 900 g of the grounded material was boiled in water for 30 min, filtrated with Whatman No. 1 filter paper and the filtrate was evaporated by rotary evaporation. The extracts were kept in the fridge (4°C) from where aliquots were withdrawn for the test procedures.

Animals

A total of 25 male Sprague Dawley rats (*Rattus norvegicus*), aged 6-8 weeks and weighed 160-200 g, healthy were caged under conditions of 22-30°C temperature; 30% relative humidity; 12 h light and dark cycle. Animals had free access to standard pellet diet and mineral water. Rats were acclimatized for 10 days and then divided into five intervention groups (of five each): normal control group (received only distilled water orally); diabetic group; diabetic + metformin 100 mg/kgBW (orally), diabetic + Phaleria macrocarpa (Scheff.) Boerl pericarp methanolic extract (PMM) 250 mg/BW (orally) and diabetic + Phaleria macrocarpa (Scheff.) Boerl pericarp water extract (PMW) 250 mg/kgBW (orally). Diabetes was induced by single dose of allox an monohydrate intraperitoneally. The interventions were conducted for 21 days, after which animals were sacrificed to obtain their kidneys for routine histological staining. Ethical approval for this study was obtained from Research Ethics Committee, Faculty of Medicine and Health Sciences, Jenderal Soedirman University, Purwokerto.

Induction of diabetes

Induction of diabetic was done with a single dose intraperitoneal injection of 150 mg/kgBW of allox an monohydrate (Sigma, Germany) dissolved in distilled water after 8-12 h fasting (Triastuti et al., 2009a). After 72 h of alloxan injection, fasting blood glucose level was measured to confirm hyperglycemia. Animals were considered diabetic and employed for further studies if their fasting blood glucose level were above 130 mg/dl (Sedigheh et al., 2011).

Histological examination

Right kidney from each animal was removed and plac ed in 10% buffer formalin, cast in paraffin. Isolated organs were stained with Hematoxylin-Eosin and then examined for the microscopic morphology. Regarding glomerular histopathologic changes, glomerular sclerosis were scored semiquantitatively into four scales (0, no sclerosis of the glomerulus; 1, sclerosis of up to 25% of the glomerulus; 2, sclerosis of 25-50% of the glomerulus; 3, sclerosis of 50-75% of the glomerulus; 4, sclerosis of more than 75% of the glomerulus). About 60 glomeruli were analyzed in the kidney sections of each rat (Cha et al., 2004). Glomerular area were measured by Cellsense standard integrated with Olympus BX43.

Statistical analysis

Normally distributed data were expressed as mean ± SD and analyzed using one way ANOVA whose results were further subjected to LSD post hoc test for multiple comparis ons. If data were not distributed normally, nonparametric test was performed. All analyses were performed with SPSS 15.00 software for Windows and differenc es between means were accepted significant at $p<0.05$.

RESULTS

The glomerular area of diabetic rat was significantly wider than that of normal rat ($p< 0.01$) (Figure 1). The histological section showed that the diabetic group had wider glomerular area and minimal s pace of Bowm an's when compared with that of the normal group, indicating glomerular hypertrophy (Figure 2). Treatment with PM extract (methanolic and water) and metformin significantly reduced hypertrophy ($p < 0.01$), which was

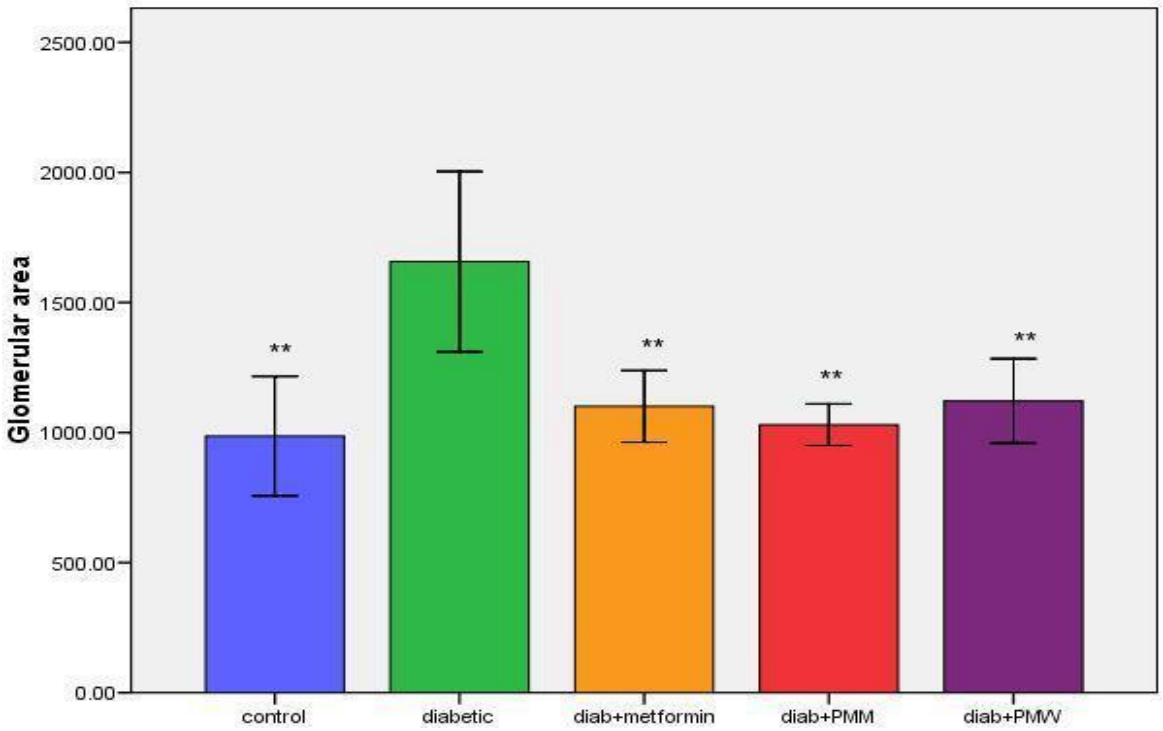

Figure 1. Analysis of glomerular capillary area (μm^2), n = 5, values are mean ± standard deviation, ANOVA follow ed by LSD test, ** p < 0.01 compared w ith diabetic group; diab + PMM = diabetic + methanolic extract of Phaleria macrocarpa; diab + PMW = diabetic + w ater extract of Phaleria macrocarpa.

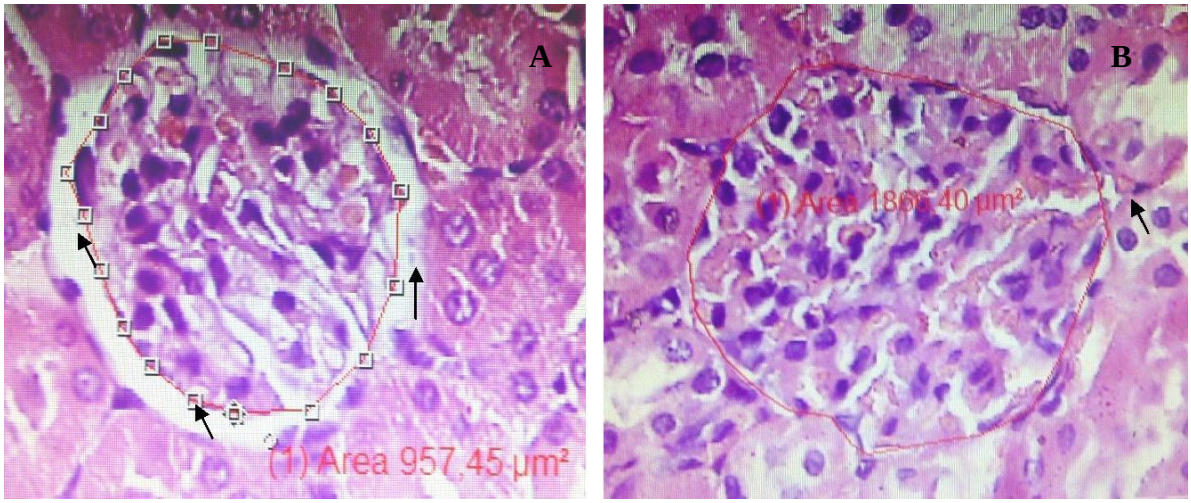

Figure 2. Comparative glomerular capillary area. (A) Nor mal glomerulus show s smaller area of glomerulus and w ider Bow man's space, (B) w hile diabetic rat shows glomerular hypertrophy and minimal Bow man's space. Arrow s indicate Bow man's space.

shown by lower glomerular area width. However, reduction in the glomerular area of PM extracts-treated diabetic rats was not different with metformin. There is no significant difference in glomerular area among those diabetic groups that received methanolic PM extract and water PM extract.

Semiquantitave analysis of glomerulosclerosis showed that diabetic rat significantly had the most severe glomerulosclerosis degree, when compared with normal and other groups ($p < 0.01$) (Figure 3). Treatment with

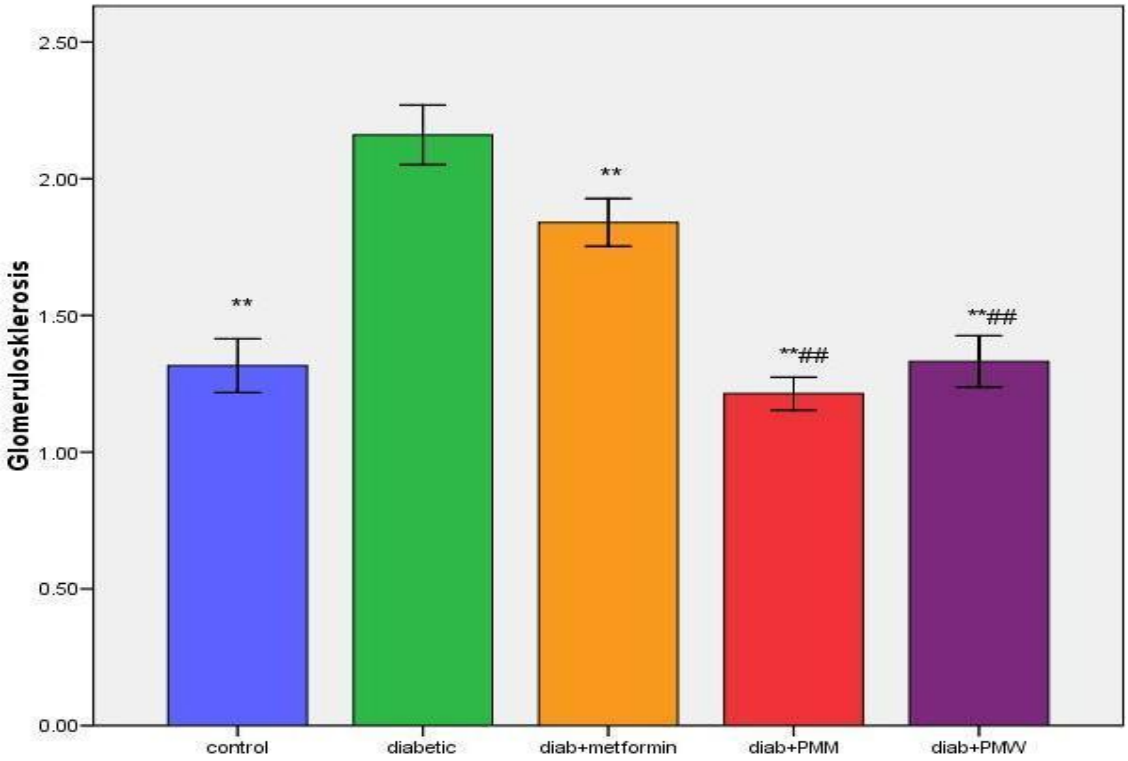

Figure 3. Semiquantitative analysis of glomerulosclerosis; n = 5; values as mean ± standard deviation, ANOVA follow ed by LSD test, ** p < 0.01 compared w ith diabetic group, ## p < 0.01 compared w ith metfor min group; diab + PMM = diabetic + methanolic extract of Phaleria macrocarpa; diab + PMW = diabetic + w ater extract of Phaleria macrocarpa.

PM extract (methanolic and water) significantly reduc ed glomerulosclerosis, which was shown by lower score of degree ($p < 0.01$) and the degree of glomerulosclerosis was not different with the normal control group. This result showed better improvement than in metformin-treated group, which had higher degree of glomerulosclerosis when compared with PM extract-treated groups ($p < 0.01$). Among those with PM's pericarp extract, there was no different degree of glomerulosclerosis. Diabetic rats showed that sclerosis affect more than 75% area of glomerulus (Figure 4B), while diabetic rats treated with PM and metformin showed lower grade of glomerulosclerosis as well as normal rats (Figure 4A, C, D).

DISCUSSION

Diabetes mellitus has become one of the top ten disorders which cause mortality throughout the world. Chronic hyperglycemic condition can lead to various diabetes complication in multiple organs. Diabetic nephropathy is a major cause of ESRD throughout the world and has become the most serious c omplication of diabetes. Appropriate management of diabetes is needed to prevent these conditions. The present antidiabetic agents possess several side effects and their properties for complication prevention is not studied extensively yet (Obineche and Adem, 2005). The treatment of diabetes with medicines of plant origin that proved much safer than synthetic drugs is an integral part of many cultures throughout the world and has gained importance in recent years.

Histological study of the normal kidney of the nondiabetic rats revealed that normal glomerulus is surrounded by the Bowman's capsule. Hyperglycemia induced by alloxan increased glomerular histological changes. The kidneys of untreated diabetic rats showed hypertrophy characterized by increase of capillary area and narrow Bowman's space. This result is consistent with that of Malatiali et al. (2008) and Yamamoto et al. (2004) which reported that diabetic rats had higher area of glomerular capilary. Diabetes produces several changes in the structure of glomerulus (Vishnawathan, 2004) and inc reasing expression of various growth factors (Brosius, 2003). Growth factors may contribute to both the cellular hypertrophy and enhanced collagen synthesis is observed in diabetic nephropathy as nodular or diffus eosinophilic mass accumulation in the glomerulus known as glomerulosclerosis (Teoh et al., 2010). Glomerulus of diabetic rats also show mesangial expansion, accumulation of extrac ellular matrix, and thickening of the basement membrane. Hyperglycemia

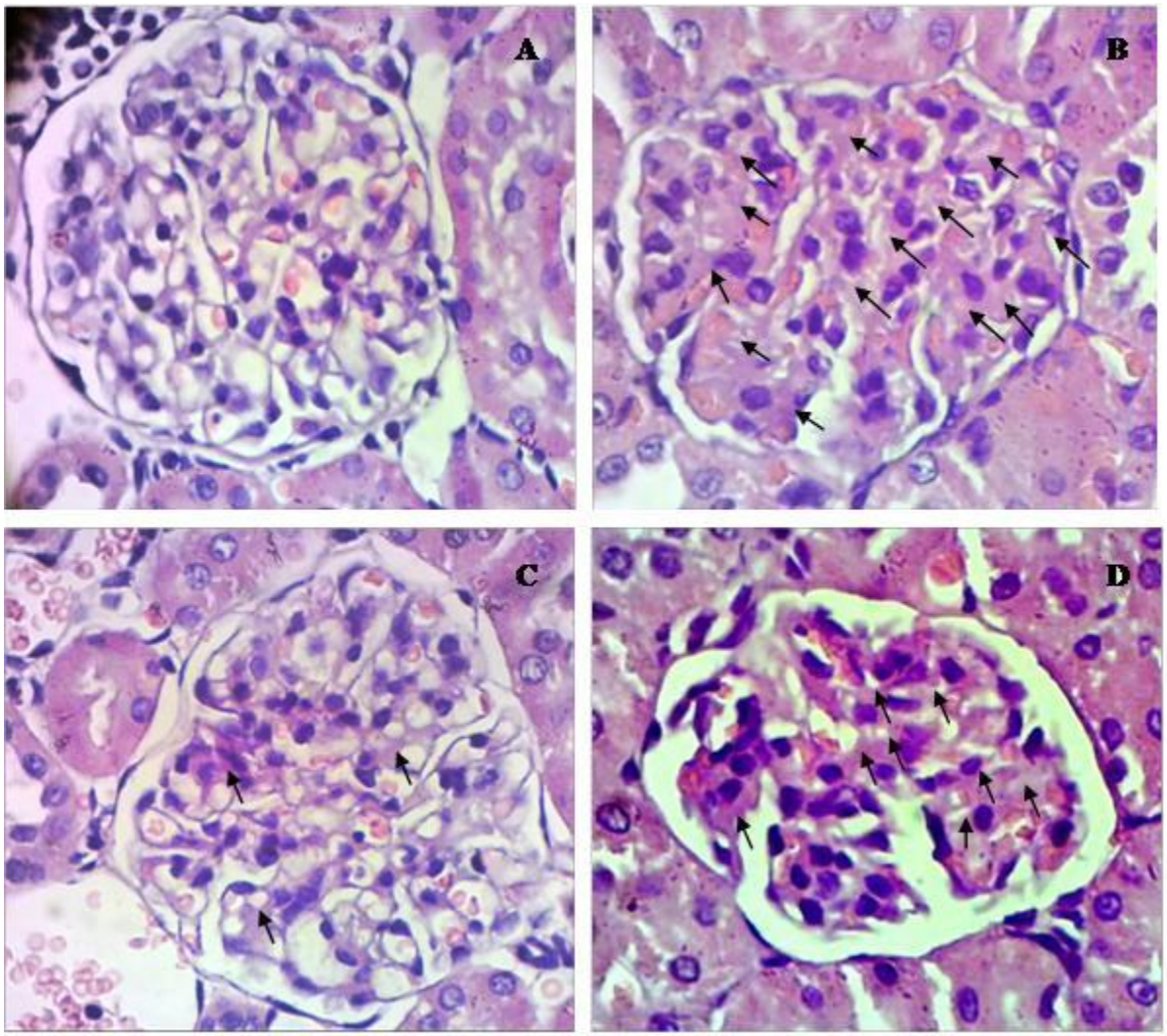

Figure 4. Various degrees of glomerulosclerosis. (A) Nor mal glomerulus of the control group show ed no sclerosis of the glomerulus (score 0), and the glomerular capilarry lumen w as opened ; (B) Glomerulus of the diabetic group show ed sclerosis of more than 75% of the glomerulus (score 4); (C) Glo merulus of the diabetic group treated w ith Phaleria macrocarpa show ed smaller area of sclerosis up to 25% of the glomerulus (score 1); (D) Glomerulus of the diabetic group treated w ith metformin show ed sclerosis of 50-75% of the glomerulus (score 3) (arrow s indicate area of sclerosis).

also induce Reactive Oxygen Species production which stimulate proinflammatory cytokines and reduction of Nitrit Oxide (NO) formation in endothelial cells. Reduction in NO formation will activate increasing Matrix Metalloproteinase (MMP)-2 and MMP-9 that will promote glomerulosclerosis followed by glomerular hypertrophy (Min et al., 2005).

In the present study, diabetic animal treated PM showed reduction in glomerular area and lower grade level of glomerulosclerosis. This result is consistent with that of Triastuti et al. (2009a) who reported that PM extract reduced hypertrophy and blood urea nitrogen via enhancement of renal antioxidant enzymes. This nephroprotective effect is presummably explained via the mechanism of PM action as antihyperglycemic and antioxidant.

Sugiwati et al. (2006) reported that pericarp of PM contains flavonoids that reduce blood gluc ose level via intrapancreatic and extrapancreatic mechanism. PM works intrapancreatically by regenerate pancreatic β cell and prevent it from oxidative stress-induced cellular damage. Alcaloid also increases glucose uptake in peripheral tissues and inhibits glucose absorbtion, thus it works extrapancreatically in reducing blood glucose level (Li et al., 2001).

Antioxidant activity of PM's pericarp was reported by Hendra et al. (2011) whose research showed that the scavenging activity of PM's pericarp was 71.97%. Active

constituvies that had antioxidant properties are alcaloids, saponin and flavonoids. 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).

Conclusions

Alloxan-induc ed diabetic condition results in glomerular hypertrophy and glomerulosclerosis, treatment with *Phaleria mac rocarpa* (Scheff.) Boerl., restored hypertrophy and improved glomerulosclerosis.

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