Phaleria macrocarpa Suppress Nephropathy by Increasing Renal Antioxidant Enzyme Activity in Alloxan-Induced Diabetic Rats

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Abstract – The protective effects of Phaleria macrocarpa (PM) against oxidative stress in diabetic rats were investigated. Diabetes was induced in male Sprague Dawley rats using alloxan (150 mg/kg i.p). After the administration of PM fractions for two weeks the diabetic symptoms, nephropathy and renal antioxidant enzymes were evaluated. The results showed that the oral PM treatments reduced blood glucose levels in diabetic rats. The PM fractions decreased kidney hypertrophy and diminished blood urea nitrogen (BUN) in diabetic rats. Malondialdehyde (MDA), a lipid peroxidation marker, was increased in diabetic animals, but was suppressed by the PM treatments. In addition, the superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities, and glutathione (GSH) level in the alloxan-induced diabetic rats were significantly decreased compared with those in the normal rats, but were restored by PM treatments. The PM fractions also suppressed the level of MDA in the kidney. In conclusion, the anti hyperglycemic and anti-nephropathy of P. macrocarpa may be correlated to the increased renal antioxidant enzyme activity in the kidney.

Keywords – Phaleria macrocarpa, Hyperglycemia, Diabetic nephropathy, Oxidative stress

Introduction

In 2003 diabetes was the leading cause of end-stage renal disease worldwide. According to WHO, the global prevalence of diabetes for all age-groups has been estimated to increased from 171 million in 2000 to 366 million by 2030 (Wild et al., 2004) if no urgent action is taken.

There is growing evidence that the excess generation of highly reactive free radicals largely due to hyperglycemia (West, 2000; Evans et al., 2002), causes oxidative stress which further exacerbates the development and progression of diabetes and its associated complications (Baynes and Thorpe, 1999; Vinik and Vinik, 2003). It is commonly accepted that hyperglycemia-evoked oxidative stress plays a crucial role in the development of diabetic complications (Brownlee, 2001), including nephropathy (Stevens, 2005), which is throughout to result from the augmented generation of reactive oxygen species via NADPH oxidase (Gill and Wilcox, 2006). Present diabetes therapy approaches mainly involve drugs that enhance insulin secretion or signaling, as well as inhibitors of endogenous glucose production (Bryla et al., 2003), while the role of antioxidants, which act as important agents for restoring the redox balance of organisms, remains to be established.

Diabetes has been known can be controlled by natural products, therefore, discovery and development of novel drugs for DM is very important. Phaleria macrocarpa (Scheff) Boerl (Thymelaeaceae), a medicinal plant originally from Papua, Indonesia has been used in traditional medicine for treating several disease, including diabetes, rheumatism, high blood pressure, and acne, etc (Harmanto, 2003; Winarto, 2003). P. macrocarpa (PM) has been reported to contain phenolic glycosides, such as mahkotaside, mangiferin, and kaempferol-3-O-β-d-glucoside, as well as dodecanoic acid, palmitic acid, ethyl stearate, and sucrose (Zhang et al., 2006; Oshimi, et al., 2008), and the lignans pinoresinol, lariciresinol, and matairesinol (Saufi et al., 2008). In the in vitro experiments, extracts of PM have been analyzed for their hypoglycemic activities as an inhibitor of enzyme alpha-glucosidase (Sugiwati et al., 2006) and anticancer (Triastuti, et al., 2006; Faried et al., 2007). In a previous study, PM was found to possess anti-diabetic activity in streptozotocin-induced diabetic mice (Triastuti et al.,...
2008). In the present study, protection effect of the butanol extract of PM on the generation of free radicals in the kidney caused alloxan was examined

**Experimental**

**Chemicals** – Alloxan, thiobarbituric acid, reduced glutathione, 5,5-dithiobis (2-nitrobenzoic acid), Folin–Ciocalteu’s reagent, and bovine albumin were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The bioassay kit for blood urea nitrogen (BUN) was purchased from Asan Company, Korea. All other reagents purchased were of the highest commercial grade available from common commercial suppliers.

**Animals** – Male Sprague Dawley aged 6 weeks were purchased from Hyochang Science (Daegu, Korea), and maintained under a daily controlled 12 h-light: 12 h-dark lighting cycle at 22 ± 3 °C and 50% humidity on environmentally controlled cabinet with free access to standard food and water ad libitum.

**Preparation of the *Phaleria macrocarpa* Fractions** – Old (red color) *Phaleria macrocarpa* fruits were processed into extract in the Laboratory of Biology and Pharmacy, Department of Pharmacy, Islamic University of Indonesia, Yogyakarta, Indonesia. The pericarps of the fruits were sliced, dried, and ground into powder. To obtain crude methanol extracts, 1 kg of Powder of PM were dissolved 3 times in 2 L of methanol for three days, filtered, and then evaporated. To obtain the ethyl acetate soluble and n-butanol soluble parts, half of the methanol extract was then dissolved in 1 L ethyl acetate-water (1 : 1) One liter of water was added to the soluble butanol part, the ethyl acetate, butanol, and water parts were then evaporated. Preparation of the PM fractions yielded methanol (MeOH; 139.54 g), ethyl acetate (EtOAc; 11.07 g, butanol (BuOH; 28.07 g), and water (69.74 g) fractions. All fractions then were lyophilized and used for the experiment.

**Induction of Experimental Diabetes** – Fasted rat was induced by alloxant (150 mg/kg, i.p) in saline to make Diabetes rats. Normal rats were injected with saline alone. Diabetes was confirmed 72 h after the alloxan injection. Rats with a fasting blood glucose levels above 250 mg/dl were considered diabetic and used in the experiment.

**Experimental Groups** – Normal and hyperglycemic rats were randomly allocated and similarly grouped into seven groups (five in each): non – diabetic control (normal) group; diabetic control (control) group; diabetic + MeOH fraction 250 mg/kg group; diabetic + EtOAc fraction 250 mg/kg group; diabetic + BuOH fraction 250 mg/kg group; diabetic + water fraction 250 mg/kg group, and diabetic + metformin 150 mg/kg as reference drug. Both normal and control groups were administered an equivalent volume of the vehicle (double distilled water) for the two weeks of the treatment. The PM fractions treatments were started on the fourth day after the alloxan injection and administered orally during the 2 weeks of the treatments. After completion of the treatments, the animals were decapitated after fasting for 18h, with the blood and kidneys collected.

**Assay of serum enzymes and components** – For the blood glucose analysis, a drop of blood was collected from the tail vein of animals. The blood glucose level was determined using a one touch glucometer (Roche). Serum was extracted from the blood collected directly from the abdominal vein after the rats had been subjected to anesthesia. Serum was separated for the estimation of the blood urea nitrogen (BUN). At the end of the experiment, the kidneys were excised, and then washed thoroughly in ice-cold saline to remove the blood. They were then gently blotted between the folds of a filter paper and weighed. The kidney/body weight ratio was calculated, with the data expressed as an organ index (relative organ weight of one kidney to 100 g of total body weight). Ten percent of homogenate was prepared on 0.1 M phosphate buffer (pH 7.4) using a Teflon homogenizer at 4 °C. The homogenate was centrifuged at 3000 g for 20 min to remove the cell debris, unbroken cells, nucleus, erythrocytes, and mitochondria. The cytosolic fraction was used to estimate the superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPOs). The Kidney homogenate was analyzed for the reduced glutathione (GSH), lipid peroxidation, and total protein levels.

**Assays of renal enzymes** – The SOD activity was determined via the ability of the tissue homogonate to scavenge the superoxide anion generated from the photo-illumination of riboflavin according to the method of Marklund and Marklund (1974). The GPx activity was determined by measuring the decrease in the GSH content after incubation of the sample in the presence of H2O2 and NaN3 (Paglia and Valentine, 1967). Renal CAT activity was determined from the rate of decomposition of H2O2 (Aebi, 1974). The reduced GSH was determined according to the method of Ellman (1959) and Mitchell et al., (1973), based on the formation of the yellow colored complex with DTNB (5, 5′-dithiobis (2-nitrobenzoic acid). The level of lipid peroxidation was measured as malondialdehyde (MDA), a thiobarbituric acid reacting substance (TBARS), using 1′ 1′ 3′ 3′-tetra-methoxypropane as standard (Ohkawa et al., 1979). The protein