Natural Product Sciences
15(1) : 37-43 (2009)

Phaleria macrocarpa Suppresses Oxidative Stress in Alloxan-induced Diabetic Rats by Enhancing Hepatic Antioxidant Enzyme Activity

Asih Triastuti[1], Hee-Juhn Park[2], and Jongwon Choi[3]*

[1]*Department of Pharmacy, UII University, Yogyakarta 55584, Indonesia*
[2]*Department of Industrial Pharmacy, Sangji University, Wonju 220-702, Korea*
[3]*College of Pharmacy, Kyungsung University, Busan 608-736, Korea*

Abstract – Oxidative stress is caused by an imbalance between the production of reactive oxygen and an ability of a biological system, to readily detoxify the reactive intermediates or easily repair the resulting damage. It has been suggested that developmental alloxan-induced liver damage is mediated through increases in oxidative stress. The anti-diabetic effect and antioxidant activity of *Phaleria macrocarpa* (PM) fractions were investigated in alloxan-induced diabetic rats. After two weeks administration of PM, the liver antioxidant enzyme and hyperglycemic state were evaluated. The results showed that oral administration of PM treatments reduced blood glucose levels in diabetic rats by oral administration ($P < 0.05$). Serum glutamic-oxaloacetic transaminase (sGOT) and serum glutamic-pyruvate-transaminase (sGPT) were also diminished by PM supplementation. The superoxide dismutase (SOD), catalase (CAT) and glutathione-peroxidase (GPx) activities, and glutathione (GSH) level in the alloxan-induced diabetic rats were significantly decreased ($P < 0.05$) compared to those in the normal rats but were restored by PM treatments. PM fractions also repressed the level of malondialdehyde (MDA) in the liver. Glutathione reductase (GR), glutathione-S-transferase (GST) and γ-glutamylcysteine synthase (GCS) were also reduced in alloxan-induced diabetic rats. PM fractions could restore the GR and GST activities, but the GCS activity was not affected in rat livers. From the results of the present study, the diabetic effect of the butanol fraction of PM against alloxan-induced diabetic rats was concluded to be mediated either by preventing the decline of hepatic antioxidant status or due to its indirect radical scavenging capacity.
Keywords - *Phaleria macrocarpa*, Diabetic, Free radicals, Antioxidant, Oxidative Stress

Introduction

Diabetes mellitus (DM) is a group of metabolic disorders characterized by hyperglycemia and abnormalities in the mechanism of carbohydrates, fats, and proteins resulting from defects in insulin secretion, insulin sensitivity, or both (Wells *et al.,* 2003) from which chronic microvascular, macrovascular and neurophathic complications may ensue due to its complications (Vincent *et al.*, 2004). According to WHO, the worldwide prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and is predicted to reach 4.4% by 2030. The total number of people with diabetes has been projected to rise from 171 million in 2000 to 366 million in 2030 (Wild *et al*., 2004).

There is growing evidence that the excess generation of highly reactive free radicals largely due to hyperglycemia (West, 2000; Johansen *et al.,* 2005), causes oxidative stress which further exacerbates the development and progression of diabetes and its complications (Baynes and Thorpe, 1999; Vinik and Vinik, 2003). The endogenous antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) as well as glutathione reductase (GR) are known to be inhibited in diabetes mellitus as a result of nonenzymatic glycosylation and oxidation (West, 2000; Valko *et al*., 2007). Reduced glutathione (GSH) synthesis inhibited by hyperglycaemia presumably due to glycation (Yoshida *et al*., 1995) were restored by the treatment with an anti-diabetic agent.

Many natural products are known to have effects in controlling diabetes, which represents a promising approach to the discovery of new diabetes drugs. *Phaleria macrocarpa* (Scheff) Boerl (PM), a medicinal plant from Papua, Indonesia has been used in traditional folk medicine as a treatment for several disease, including diabetes, as well as health foods such as tea and functional beverages (Harmanto, 2003). Since alloxan-

*Author for correspondence
Tel: +82-51-620-4804; E-mail: jwchoi@ks.ac.kr

induced diabetes results from the generation of free radicals in the liver, the possible protection by the butanol extract of PM was evaluated with the results are presented in this paper.

Materials and Methods

Animals – Male Sprague Dawley (200 ± 10 g) rats were provided by Hyochang Science, Daegu, Korea. The care and all procedures relating to the animals were approved by the pharmacology department of the Faculty of Pharmacy, Kyungsung University, Republic of Korea. Animals were kept for a week under environmentally controlled conditions (temperature (22 ± 3°C; 12 h light dark cycle) with free access to standard food and water ad libitum.

Preparation of the extract – Fruits of *Phaleria macrocarpa* (PM) were collected from the Laboratory of Biology and Pharmacy, Department of Pharmacy, Islamic University of Indonesia, Yogyakarta, Indonesia. The fruit pericarps were sliced, dried and ground into powder. Three kilogram of bulk fruit pericarps was dissolved three times in 3 liters of methanol for 3 days, filtered, and then evaporated to obtain the crude methanol extract (30.82 g). Half the methanol extract then was dissolved in 2 liters of ethyl acetate-water (1 : 1) to obtain the ethyl acetate and n-butanol soluble portions. One liter of water was added to the butanol soluble portion. The ethyl acetate, butanol and water portion were evaporated. All fractions and methanol extract then were freeze dried, and named the MeOH extract (MeOH ext., 13.71 g), ethyl acetate (EtOAc fr., 8.31 g), butanol (BuOH fr., 12.27 g) and aqueous (Aq. Fr., 20.12 g) fractions, respectively.

Experimental induction of diabetes mellitus – Diabetes was induced in fasted SD rats with alloxan (Sigma Chemical Co. St. Louis, MO, USA. 150 mg/kg) in saline by a single intraperitonial injection. Normal rats were injected with saline alone. Diabetes was confirmed after 72 h by measuring the blood glucose levels using glucose reagent strips (Glucometer 4 Ames, Bayer Diagnostics). The rats with fasted blood glucose levels above 250 mg/mL were considered to be diabetic and used in the experiment.

Determination of liver antioxidant activity of PM – Animals were divided into seven groups of five animals in each group as follows; Group I, normal rats treated with the vehicle only (distilled water); Group II, diabetic controls (treated with a single dose of alloxan 150 mg/kg); Group III, diabetic rats treated with the MeOH extract (250 mg/kg); Group IV, diabetic rats treated with the EtOAc fraction (250 mg/kg); Group V, diabetic rats treated with the BuOH fraction (250 mg/kg); Group VI, diabetic rats treated with the Aqueous fraction (250 mg/kg); and Group VII, diabetic rats treated with metformin (MFM, 150 mg/kg) as a reference drug. The PM fractions treatments were started on the fourth day after the alloxan injection and administered by oral gavage during the 2 weeks of treatments. After completion of the treatments, the animals were sacrificed using carbon dioxide as anesthesia. Blood was collected directly from the abdominal vein, with serum was separated for the estimations of the activities of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities. The livers were dissected for the determination of the antioxidant status.

Serum GOT and GPT were determined by kinetic method (Reitman and Frankel, 1957) using a the kit obtained from Asan Inc., Korea. Superoxide dismutase (SOD) was assayed according to the method described in Marklund and Marklund (1974). The assay procedure involved the inhibition of epinephrine auto-oxidation to adrenochrome in an alkaline medium (pH 10.2) which is markedly inhibited by the presence of SOD. Epinephrine was added to the assay mixture, containing tissue supernatant with the change in the extinction coefficient observed at 480 nm using a Spectrophotometer. Catalase (CAT) converts H_2O_2 into water. The CAT activity in plasma and tissue supernatant was measured spectrophotometrically at 240 nm by calculating the rate of degradation of H_2O_2, the substrate of the enzyme (Aebi, 1974). The glutathione peroxidase (GPx) activity was determined by measuring the decrease in the glutathione (GSH) content after incubating the sample in the presence of H_2O_2 and NaN_3 (Paglia and Valentine, 1967). The determination of total tissue sulfhydryl group was carried out according to the methods of Ellman (1959) and Mitchell *et al*., (1973) with minor modification. Tissue supernatant was taken up with the addition of 10 mM - DTNB (5,5'-dithio-bis-2-nitrobenzoic acid), pH 7.0, prepared in 10 mM - phosphate buffer (pH 7.0) was added. The mixture was maintained at room temperature for 20 min with the absorbance then measured at 412 nm. The glutathione reductase (GR) activity was measured by the oxidation of NADPH by measuring the absorbance at 340 nm (Mize and Langdon, 1962). The determination of γ-Glutamylcysteine synthetase (GCS) was performed according to the method of Richman and Meister (1975) with the absorbance measured at 600 nm. Glutathione S-transferase (GST) catalyzes the conjugation reaction with glutathione in the first step of mercapturic acid synthesis.

The activity of GST was measured according to the method of Habig *et al*. (1974) using p-nitrobenzylchloride as the substrate. The absorbance was measured spectrophotometrically at 310 nm using a UV-Double Beam spectrophotometer. The level of lipid peroxidation was measured as malondialdehyde (MDA), a thiobarbituric acid reacting substance (TBARS), using 1'1'3'3'-*tetra*-methoxypropane as the standard (Ohkawa *et al*., 1979). The tissue protein content in the tissue was determined using the method of Lowry *et al.* (1951) with bovine serum albumin (BSA) as the standard.

Statistical analysis – All data are represented as the means ± SD (n = 5). Significant differences between the mean values were statistically analyzed using a one-way analysis of variance. The normal, control group (alloxan alone treated group) and the extract plus alloxan treated groups were further analyzed using Duncan's multiple range tests. P values less than 0.05 were considered as significant.

Results

In the present study, the administration of alloxan, as expected, induced a diabetic state in the animals. As shown in Table 1, diabetic animals had significant responses after the 14 days treatment compared to the control group. On the initial day (day 0), there were differences between the normal and diabetic rats. On the day 7 of the PM treatments, the BuOH fraction showed reduced in blood glucose levels and the values are not significantly different from those of the metformin treated group (149.6 ± 4.16 and 151.2 ± 11.26 mg/mL respectively); also, there was no significantly differences between the MeOH extract (256.4 ± 5.13 mg/mL) and EtOAc fraction treated groups (247.0 ± 1.39 mg /mL). The blood glucose levels of the water fractions treated group showed no significant difference from that of the control groups ($p < 0.05$).

At the end of the treatment, the group treated with the BuOH fraction (157.4 ± 11.78 mg/mL) showed decreased in blood glucose levels and this was not significantly different to that of the group treated with metformin (150.2 ± 7.16 mg/mL); also, there was no significantly between the groups treated with the MeOH extract and EtOAc fractions (267.4 ± 8.59 and 258.0 ± 17.89 mg/mL, respectively). The blood glucose levels of the water fractions treated group were not significantly different from those of the control groups (365.0 ± 20.50 and 361.2 ± 17.81 mg/mL, respectively) ($p < 0.05$). The PM fractions also restored the body weight loss and water intake (data not shown), indicating the diabetic state was controlled in the animals treated with PM probably by improving the metabolism in the diabetic rats.

The liver function on diabetic animals can be described by the serum sGPT-sGOT levels. The control diabetic groups showed increase in the serum sGPT-sGOT levels (191.0 ± 6.48 IU/l and 111.5 ± 1.90 IU/l, respectively) compared to the normal group (sGPT was 61.3 ± 3.83 IU/l and sGOT was 20.7 ± 3.02 IU/l) (Table 2). The BuOH, MeOH, and EtOAc fractions significantly reduced the sGPT-sGOT levels compared with the control group but the water fraction did not reduce the sGPT-sGOT levels in diabetic rats and showed no difference to that of the control group (Table 2).

The antioxidant enzyme activities are summarized in Table 3. The SOD activity was decreased in the diabetic animals but was restored by the PM treatments. The PM treatments also showed improvements in the hepatic CAT and GPx activities.

Changes in the glutathione content and GR and GCS activities are summarized in Table 4. The GSH content in the liver of the diabetic rats decreased significantly

Table 1. Effect of *P. macrocarpa* fractions on the blood glucose levels in alloxan (ALX)-induced diabetic rats

Group	Dose (mg/kg)	Blood glucose (mg/mL)		
		0	7	14 (day)
Normal	Vehicle	83.8 ± 2.60^{b}	113.5 ± 4.04^{d}	108.3 ± 11.56^{d}
Control(ALX)	150	319.7 ± 33.15^{a}	349.5 ± 26.71^{a}	365.0 ± 20.50^{a}
MeOH ext. + ALX	250	295.8 ± 25.22^{a}	256.4 ± 5.13^{b}	267.4 ± 8.59^{b}
EtOAc fr. + ALX	250	294.4 ± 6.23^{a}	247.0 ± 1.39^{b}	258.0 ± 17.89^{b}
BuOH fr. + ALX	250	296.4 ± 8.50^{a}	149.6 ± 4.16^{c}	157.4 ± 11.78^{c}
Aq. Fr. + ALX	250	295.0 ± 11.77^{a}	342.0 ± 13.04^{a}	361.2 ± 17.81^{a}
Metformin + ALX	150	295.6 ± 5.68^{a}	151.2 ± 11.26^{c}	150.2 ± 7.16^{c}

Values are the means ± SD (n = 5). Values within a column with different superscripts are significantly different at < 0.05 by the Duncan's test.

Table 2. Effects of the *P. macrocarpa* extracts on serum GPT and GOT levels in alloxan (ALX)-induced diabetic rats

Group	Dose (mg/kg)	SGPT	SGOT
		IU/l	
Normal	Vehicle	61.3 ± 3.83[d]	20.7 ± 3.02[d]
Control(ALX)	150	191.0 ± 6.48[a]	111.5 ± 1.90[a]
MeOH	250	132.0 ± 4.67[b]	63.4 ± 7.54[b]
EtOAc	250	136.0 ± 6.69[b]	67.3 ± 6.63[b]
BuOH	250	85.1 ± 6.39[c]	32.4 ± 2.68[b]
H_2O	250	187.5 ± 4.30[a]	109.1 ± 2.42[a]
Metformin	250	78.5 ± 7.39[c]	28.5 ± 1.64[c]

Values are the means ± SD (n = 5). Values within a column with different superscripts are significantly different at $P < 0.05$ by the Duncan's test. SGPT, Serum Glutamate Pyruvate Transaminase; SGOT, Serum Glutamate Oxaloacetate Transaminase

Table 3. Effects of the *P. macrocarpa* extract on the hepatic SOD, GPx and CAT activities in alloxan (ALX)-induced diabetic rats

Group	Dose (mg/kg, orally)	SOD	GPx	CAT
Normal	Vehicle	30.0 ± 1.66[a]	2.8 ± 0.14[a]	3.3 ± 0.33[a]
Control(ALX)	150	3.9 ± 0.73[d]	1.6 ± 0.25[d]	1.9 ± 0.12[d]
MeOH	250	9.0 ± 0.73[c]	2.0 ± 0.09[c]	2.3 ± 0.09[c]
EtOAc	250	8.9 ± 0.55[c]	1.9 ± 0.12[c]	2.4 ± 0.13[c]
BuOH	250	10.9 ± 1.13[b]	2.3 ± 0.21[b]	2.8 ± 0.14[b]
H_2O	250	4.0 ± 0.65[d]	1.6 ± 0.16[d]	2.0 ± 0.13[d]
Metformin	250	11.1 ± 0.91[b]	2.4 ± 0.41[b]	2.9 ± 0.30[b]

Superoxide Dismutase (SOD, U/mg protein); Glutathione Peroxidase (GPx, NADPH oxidized/min/mg protein); and Catalase (CAT, nmol of H_2O_2 consumed/min/mg protein).
Values are the means ± SD (n = 5). Values within a column with different superscripts are significantly different at <0.05 by Duncan's test

Table 4. Effects of the *P. macrocarpa* extracts on the hepatic GSH content and GR, GCS activities in alloxan (ALX)-diabetic rats

Group	Dose (mg/kg, orally)	GSH	GR	GCS
Normal	Vehicle	29.0 ± 1.40[a]	2.1 ± 0.26[a]	7.6 ± 0.12[a]
Control (ALX)	150	15.8 ± 0.64[d]	0.3 ± 0.09[d]	4.9 ± 0.08[b]
MeOH	250	23.2 ± 0.48[c]	0.8 ± 0.27[c]	4.9 ± 0.26[b]
EtOAc	250	22.7 ± 0.31[c]	0.8 ± 0.08[c]	4.9 ± 0.06[b]
BuOH	250	25.2 ± 0.03[b]	1.1 ± 0.06[b]	5.0 ± 0.04[b]
H_2O	250	16.4 ± 0.72[d]	0.3 ± 0.04[d]	4.9 ± 0.05[b]
Metformin	250	25.9 ± 0.37[b]	1.2 ± 0.08[b]	4.9 ± 0.18[b]

Glutathione (GSH, nmol DTNB conjugated/mg protein); Glutathione Reductase (GR, nmol NADPH oxidized/min/mg protein); γ-GLutamylcystein-Syntethase (GCS, synthesis of GSH nmol/min/mg protein).
Values are the means ± SD (n = 5). Values within a column with different superscripts are significantly different at $P < 0.05$ by the Duncan's test

($p < 0.05$ vs. normal groups). Treatment with the PM fractions especially the BuOH fraction increased the GSH content increased the GR activity but did not increased the GCS activity in the liver of diabetic rats.

The GST activities of diabetic animals are shown in Fig. 1. GST activities were significantly decreased in diabetic animals compared to the normal group. The GST activity in the control group was significantly decreased compared to that in the normal group (1.5 ± 0.04 U/mg protein) but were increased by the BuOH fr., MeOH ext., and EtOAc fr. treatments (1.1 ± 0.05, 0.9 ± 0.05, and 0.9 ± 0.05 U/mg protein, respectively). The water fraction did not increase the GST activity in the liver of diabetic rats and showed no significant difference with that of the control group ($p < 0.05$).

The level of lipid peroxidation levels in the liver were

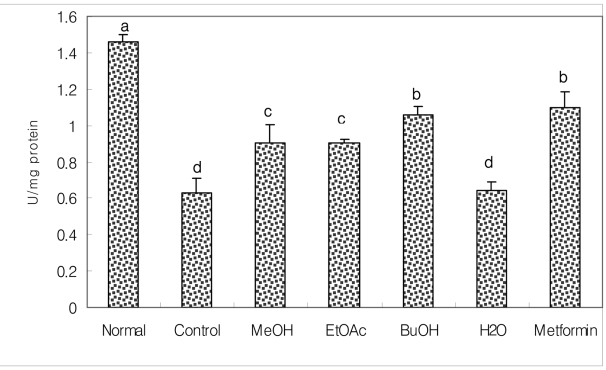

Fig. 1. Effects of the *P. macrocarpa* extracts on the Glutathione S-Transferase (GST) activities in the liver of alloxan-diabetic rats Values within a bar with different superscripts are significantly different at $p < 0.05$ by the Duncan's test.

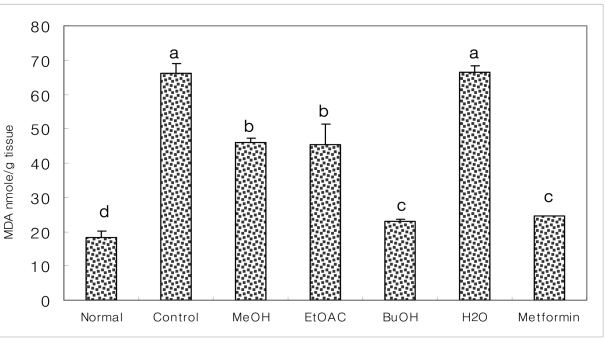

Fig. 2. Effects of the *P. macrocarpa* extracts on the level of lipid peroxidation in the liver of alloxan-diabetic rats. Values within a bar with different superscripts are significantly different at $p < 0.05$ by the Duncan's test.

increased in the diabetic animals groups compared to that of the normal group (Fig. 2). The malondialdehyde levels in the normal and control diabetic animals were 19.0 ± 2.01 and 66.6 ± 2.83 nmole/g tissue, respectively. Treatment with the PM fractions especially the BuOH fraction significantly decreased ($P < 0.05$) the lipid peroxidation levels compared to that of the control group, but this was not significantly different from that in the metformin treated group (MDA levels in BuOH fr. and metformin treated group were 22.8 ± 0.58 and 24.5 ± 0.12 nmole/g tissue, respectively). The aqueous fr. showed no significant difference in the level of lipid peroxidation in the liver compare to that of the control group ($p < 0.05$).

Discussion

P. macrocarpa has been proved empirically shown to have activity for the control of cancer, diabetes mellitus, rheumatism, high blood pressure, acne, and so forth (Harmanto, 2003; Winarto, 2003). It has been reported to contain phenolic glycosides such as mahkotaside, mangiferin, kaempferol-3-O-β-D-glucoside, dodecanoic acid, palmitic acid, ethyl stearate, and sucrose (Zhang *et al.*, 2006; Oshimi, *et al*., 2008) as well as the lignans pinoresinol, lariciresinol, and matairesinol (Saufi *et al.*, 2008). Extracts of *P. macrocarpa* have been analyzed for their hypoglycemic activity by *in vitro* experiments on the inhibition of enzyme α-glucosidase (Sugiwati *et al*., 2006) and as anti-cancer agents (Triastuti, *et al*., 2006; Faried *et al*., 2007). In a previous study, PM was found to have anti diabetic activity in streptozotocin-induced diabetic mice (Triastuti *et al*., 2008). Alloxan is widely used to induce experimental diabetes in animals. The mechanism of its action in B cells of the pancreas has been intensively investigated and is now reasonably well understood. The cytotoxic action of diabetogenic agents is mediated by reactive oxygen species, however, the source of their generation is different in the case of alloxan. Alloxan and the reduction product dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter highly reactive hydroxyl radicals are formed by the Fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in the cytosolic calcium concentration causes rapid destruction of B cells. (Lenzen and Panten, 1988, Szkudelski 2001, Elsner *et al*., 2002), involving a cyclic redox reaction, with the generation of reactive oxygen species (ROS) which are responsible for the death of pancreatic beta cells.

All the rats, that received an alloxan injection developed diabetes. The blood glucose levels in the diabetic animals treated with the MeOH extract, EtOAc fraction, and BuOH fraction were recovered after 14 days of treatments and were significantly different from that of the control diabetic group but the water fractions treated group showed no significance differences from that of the control diabetic group ($P < 0.05$).

The liver is an organ of central metabolic importance which is known to undergo oxygen free radicals mediated injury during diabetes. The liver function has been reported to be altered in diabetic animals (Zysset and Tlach, 1987). The levels of sGPT and sGOT have been reported to increase in diabetic rats but are lowered to aimal levels by PM treatments.

Glucose can undergo autooxidation with the generation ·OH radicals, which react with protein in a nonenzymatic manner leading to the development of advanced glycation end products (AGEs) and ROS are generated at multiple steps during this process (Turko *et al*., 2001; Giardino *et*

al., 1996). Since PM fractions can decrease the blood glucose levels, indicating that PM can block free radical production and prevent the production of ROS during DM.

SOD, CAT, and GPx are enzymes that destroy the peroxides and play a significant role in providing antioxidant defenses to an organism. SOD reduces superoxide to H_2O_2 that can be readily reduced to water principally by CAT and GPx (Robertson *et al*., 2003). The functions of all three enzymes are interconnected with the lowering of their activities resulting in the accumulation of lipid peroxides and an associated increase in oxidative stress in diabetic rats (Chaudhry *et al*., 2007). Treatment of *P. macrocarpa* especially the butanol fractions increased the activities of these enzymes and thus may help prevent the generation of free radicals generated during diabetes mellitus.

GR is the enzyme responsible for the conversion of oxidized from of glutathione back to the reduced state. Many observations stated that in diabetic patients have shown that the activities of SOD, CAT, GPx, GR, and GST activities are all significantly decreased (West, 2000). In our study, PM was observed to increase the activities of GR and GST but did not increased the activity of GCS in the livers of diabetic animals.

GSH is the most abundant low-molecular-weight thiol in animal cells, which effectively scavenges free radicals and other ROS (hydroxyl radical, lipid peroxyl radical, peroxynitrite, and H_2O_2). GSH is synthesized by GR from the oxidized from of glutathione (GSSG) by GR as well as from glutamate, cysteine, and glycine that catalyzed by GCS and glutathione synthetase (GS) (Wu *et al*., 2004). Our results showed that decreased levels glutathione levels were restored to normal when diabetic animals were treated with PM fractions. Since the availability of GSH is important for GSH dependent enzymes, the activities of GST and GPx are affected. Along with increased GR activities, the GSH content was also restored in the PM treatments groups.

The analyses of thiobarbituric acid reactive substances (TBARS) are widely used to evaluate lipid peroxidation (Ohkawa *et a*l., 1979). Malondialdehyde (MDA), an end product of unsaturated fatty acid peroxidation, can react with thiobarbituric acid (TBA) to form the pink colored complex TBARS (Chaudhry *et al*., 2007). Lipid peroxidation in diabetes mellitus can be increased due to increased oxidative stress in the cell as a result of persistent hyperglycemia which depletes the antioxidant scavenger systems (Mahboob *et al*., 2005; Akkus *et al*., 1996). In our study, the antioxidant enzymes activities were decreased hence the levels of lipid peroxidation were increased in diabetic animals. The MDA levels were significantly decreased in PM treated animals, compared to the control diabetic group. This may correlate with the data that showed increased antioxidant activities in PM treated animals.

The results of the present study concluded that the BuOH faction of the *P. macrocarpa* extract significantly prevented the alloxan-induced diabetic state by enhancing the hepatic antioxidant activity. However, further detailed studies should be required to establish its clinical application.

Acknowledgments

This research was supported by Kyungsung University Research Grants in 2009.

References

Aebi, H., Catalase. In "Methods of enzymatic analysis" Vergmeyer, M.U., Academic Press, New York., **2**, 673 (1974).

Akkus, I., Kalak, S., Vural, H., Caglayan, O., Menekse, E., Can, G., and Durmus, B., Leukocyte lipid peroxidation, superoxide dismutase, glutathione peroxidase and serum and leukocyte vitamin C levels of patients with type II diabetes mellitus. *Clin. Chim. Acta*., **244**, 221-227 (1996).

Baynes, J.W. and Thorpe, S.R., Role of oxidative stress in diabetes vascular complications: a new perspective of an old paradigm. *Diabetes*, **48**, 1-9 (1999).

Chaudhry, J., Ghoxh, N.N., Roy, K., and Chandra, R., Anti hyperglycemic affect of a new thiazolidine analogue and its role in ameliorating oxidative stress in alloxan-induced dabetic rats. *Life Sci*., **80**, 1135-1142 (2007).

Ellman, G.L., Tissue sulfhydryl group. *Arch. Biochem. Biophys*., **237**, 1589-1595 (1959).

Elsner, M., Tiedge, M., Guldbakke, B., Munday, R., and Lenzen, S., Importance of the GLUT2 glucose transporter for pancreatic beta cell toxicity of alloxan. *Diabetologia,* **45,** 1542-1549 (2002).

Faried, A., Kurnia, D., Faried, L.S., Usman, N., Miyazaki, T., Kato, H., and Kuwano, H., Anticancer effects of gallic acid isolated from Indonesia herbal medicine, *Phaleria macrocarpa* (Scheff.) Boerl, on human cancer cell lines. *Int. J. Oncol.,* **30**, 605-613 (2007).

Giardino, I., Edelstein, D., and Brownlee, M., BCL-2 expression or antioxidants prevent hyperglycemia-induced formation of intracellular advanced glycation endproducts in bovine endothelial cells. *J. Clin. Invest*., **97**, 1422-1428 (1996).

Gorus, F.K., Malaisse, W.J., and Pipeleers, D.G., Selective uptake of alloxan by pancreatic B-cells. *Biochem. J.,* **208,** 513-515 (1982).

Habig, W.H., Pabst, M.J., and Jakoby, W.B., Glutathione s-transferase: the first enzymatic step in mercapturic acid formation. *J. Biol. Chem.,* **249,** 7130-7139 (1974).

Harmanto, N., *Conquering Disease in Unison with Mahkota Dewa*, Ir. Harmanto (Ed.), p.14 PT Mahkota Dewa Indonesia, North Jakarta (2003).

Johansen, J.S., Harris, A.K., Rychly, D.J., and Ergul. A., Oxidative stress and the use of antioxidants in diabetes: linking basic science to clinical practice. *Cardiovasc. Diabetol*., **4**, 5 (2005).

Lenzen, S. and Panten, U., Alloxan. History and mechanism of action. *Diabetologia*, **31**, 337-342 (1988).

Lowry, O., H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., Protein measurement with folin phenol reagent. *J. Biol. Chem*., **193**, 265-275 (1951).

Mahboob, M., Rahman, M.F., and Grover, P., Serum lipid peroxidation and antioxidant enzyme levels in male and female diabetic patients. *Singapore Med. J*., **46**, 322-324 (2005).

Marklund S. and Marklund, G., Involvement of the superoxide anion radical in the autooxidation of pyrogallol and convenient assay for superoxide dismutase. *Eur. J. Biochem*., **47**, 469-474 (1974).

Mitchell, J.R., Jollow, D.J., Potter, W.Z., Gillette, J.R., and Brodie, B.B., Acetaminophen-induced hepatic necrosis IV. Protective role of glutathione. *J. Pharmacol. Exp. Ther.,* **187**, 211-7 (1973).

Mize, C.E. and Langdon, R.G., Hepatic glutathione reductase I. Purification and general kinetic properties. *J. Biol. Chem*., **237**, 1962-1967 (1962).

Ohkawa, H., Ohishi, N., and Yaki, K., Assay for lipid peroxide in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.,* **95**, 351-358 (1979).

Oshimi, S., Zaima, K., Matsuno, Y., Hirasawa, Y., Iizuka, .T, Studiawan, H., Indrayanto, G., Zaini, N.C., and Morita, H., Studies on the constituents from the fruits of *Phaleria macrocarpa*. *Nat. Med* (Tokyo)., **62**, 207-210 (2008).

Paglia, E.D., and Valentine, W.N., Studies on the quantitative and qualitayive charaterization of erythocytes glutathione peroxidase. *J. Lab. Clin. Med.,* **70**, 158-169 (1967).

Reitman, S., and Frankel, S., A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases, *Am. J. Clin. Pathol*., **28**, 56-63 (1957).

Richman, P.G. and Meister, A., Regulation of γ-Glutamyl-Cysteine Synthetase by nonallosteric feedback inhibition by glutathione*, J. Biol. Chem*., **40**, 1422-1426 (1975).

Robertson, R.P., Harmon, J., Tran, P.O., Tanaka, Y., and Takahashi, H., Glucose toxicity in â-cells: type 2 diabetes, good radicals gone bad, and the glutathione connection. *Diabetes*, **52**, 581-587 (2003).

Saufi, A., von Heimendahl. C.B., Alfermann, A.W., and Fuss, E., Stereochemistry of lignans in *Phaleria macrocarpa* (Scheff.) Boerl. *Z. Naturforsch*., **63**, 13-16 (2008).

Sugiwati, S., Kardono, L.B.S., and Bintang, M., Alpha-glucosidase inhibitory activity and hypoglycemic effect of *Phaleria macrocarpa* fruit pericarp extracts by oral administration to rats., *J. Applied Sci.,* **6**, 2312-2316 (2006).

Szkudelski, T., The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas, *Physiol. Res*., **50**, 536-546 (2001).

Triastuti, A., Bachri, M.S., and Choi, J.W., Protective effect of butanol fraction of *Phaleria macrocarpa* on oxidative stress associated with atreptozotocin induced diabetic mice, *International Pharmaceutical Symposium*, Jejudo-Korea (2008).

Triastuti, A., Tito, F. A., and Wibowo, A., *Antiangiogenic effect of the ethanolic extract from Phaleria macrocarpa Boerl. fruit on chick embryo chorio allantoic membrane (CAM) induced by bFGF*, National Symposium in Medicinal Plants of Indonesia, Solo-Indonesia (2006).

Turko, I.V., Marcondes, S., and Murad, F., Diabetes-associated nitration of tyrosine and activation of succinyl-CoA:3-oxoacid CoA transferase. *Am. J. Physiol. Heart Circ. Physiol*., **281**, H2289-2294 (2001).

Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T,, Mazur, M., and Telser, J., Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol.,* **39**, 44-84 (2007).

Vincent, A.M., Russell, J.W., Low, P., and Feldman, E.L., Oxidative stress in the pathogenesis of diabetic neuropathy, *Endocr. Rev.*, **25**, 612-628 (2004).

Vinik, A.I., and Vinik, E., Prevention of the complications of diabetes. *Am. J. Manag. Care*., **9**, S63-80 (2003).

Wells, B.G., Dipiro, J.T., Schwinghammer, T.L., and Hamilton, C.W., *Pharmacotherapy Handbook,* McGraw-Hill, pp. 170-181 (2003).

West, I.C., Radicals and oxidative stress in diabetes. *Diabet Med*., **17**, 171-180 (2000).

Wild, S., Roglic, G., Green, S., Sicree, R., and King, H., Global prevalence of diabetes, estimates for the year 2000 and projections for 2030, *Diabetes care*, **27**, 1047-1053 (2004).

Winarto, W.P., *Mahkota Dewa: Budidaya dan pemanfaatan Untuk Obat*. Penebar Swadaya, Indonesia (2003).

Wu, G., Fang Y.Z.,Yang, S., Lupton, J.R., and Turner, N.D., Glutathione metabolism and its implications for health. *J. Nutr.,* **134**, 489-92 (2004).

Zhang, Y.B., Xu, X.J., and Liu, H.M., Chemical constituents from Mahkota Dewa, *J. Asian Nat. Prod. Res.,* **8**, 119-123 (2006).

Yoshida, K., Hirokawa, J., Tagami, S., Kawakami, Y., Urata, Y., and Kondo, T., Weakened cellular scavenging activity against oxidative stress in diabetes mellitus: regulation of glutathione synthesis and efflux. *Diabetolgia,* **38,** 201-210 (1995).

Zysset, T. and Tlach C., Altered liver function in diabetes: model experiments with aminopyrine in the rat. *J. Pharmacol. Exp. Ther.*, **240**, 271-276 (1987).

(Accepted March 16, 2009)