Nutritional Sciences 7(3), August, 2004, pp. 127-132

The Effects of Mulberry Fruit on the Antioxidative Defense Systems and Oxidative Stress in the Erythrocytes of Streptozotocin-Induced Diabetic Rats*

Jung-Hee Hong, Jung-Mo Ahn, Sang-Won Choi and Soon-Jae Rhee§

Department of Food Science and Nutrition, Catholic University of Daegu, Gyeongsan 712-702, Korea

The current study examined the effects of mulberry fruit on the antioxidative defense systems and oxidative stress in the erythrocytes of diabetes-induced rats. Sprague-Dawley male rats were randomly assigned to one normal and three streptozotocin (STZ)-induced diabetic groups. The diabetic groups were fed a mulberry fruit-free diet (DM-group), 0.3% mulberry fruit diet (DM-F group) or 0.6% mulberry fruit diet (DM-2F group). Diabetes was induced with STZ after three weeks of the experimental diets. The rats were sacrificed 9 days later for examination of the antioxidative defense systems and oxidative stress in the erythrocytes. Means of cy-3-*O*-glucopyranoside, cy-3-*O*-rutinoside, rutin, isoquercitrin, quercetin, morin and dehydroquercetin contents were 230.45, 131.5, 142.5, 10.3, 5.8, 1.6 and 3.83 mg per 100g dry weight, respectively, in the mulberry fruit. Mulberry fruit strengthened the antioxidative defense systems through increased activity of the antioxidant enzymes, such as glutathione peroxidase (GSH-px) and catalase (CAT), in the erythrocytes of the diabetes-induced rats. Accordingly, mulberry fruit was found to reduce the accumulation of thiobarbituric acid reactive substance (TBARS). Therefore, mulberry fruit was found to be excellent for strengthening the antioxidative defense system and reducing damaging oxidative substances in the erythrocytes of the diabetes-induced rats.

Keywords: Diabetes, Mulberry fruit, Antioxidative defense system, Oxidative stress

INTRODUCTION

Diabetes is a chronic disease that cannot be those suffering from the disease may develop complications if it is not properly controlled. Typical diabetic complications involve vascular complications, primarily categorized into macroangiopathy and microangiopathy. Recently, attentions has been drawn to the theory that oxidative stress is related to the cause of such chronic diabetic complications.[1] Diabetics and animal models exhibit high oxidative stress due to persistent and chronic hyperglycemia, thereby restricting the activity of the antioxidative defense system and instead promoting the generation of free radicals.[2] So to prevent diabetes and complications, it is essential control oxidative stress through the control of glycosemia. Recently, many investigators through the anti-diabetes studies and done anti-diabetes functional food material development using natural resources from silkworms,[3] mulberry leaves[4-6] and green tea catechin.[7-9] Attention has also been drawn to the study of mulberry fruit.[10,11]

Mulberries (*Morus sp.*) are a good source of sugars, acids and anthocyanin pigments, which are important constituents of juices, beverages and wines. Moreover, the crude drug "Sangsimja", fruit of *M. alba* (Moraceae), has been used in traditional Chinese medicine to cure and prevent diabetes, anemia, hypertension and arthritis.[12,13] Recently, mulberry fruit has been reported to have anti-diabetic,[14] anti-oxidative,[15-18] anti-inflammatory[17] and anti-hyperlipidemic[19] functions. *In vitro* studies of antioxidants in mulberry fruit, have found various ingredients such as flavonoids, silbenes, prenylflavonoids, coumarin and deoxynoijiri- mycine.[20,21] But, related research on antioxidants and oxidative stress in diabetic rats has not been thorough- going enough.

In this study we examined the effects of mulberry fruit on the antioxidative defense systems and oxidative stress in the erythrocytes of diabetes-induced experimental rats.

MATERIALS AND METHODS

Preparation of Plant Materials

The Cheongil mulberry fruit that was used in this experiment was grown in the fields of Youngcheon Silkworm Culture Agricultural Co-operative Association, Youngcheon, Korea and harvested in early June, 2003.

* This study was supported by a special Grants Research Program (No. 102004-3) of the Problem-Oriented Technology Development Project for Agriculture and Forestry, Ministry of Agriculture and Forestry, Republic of Korea.
Accepted : July 28, 2004
§ To whom correspondence should be adressed.

All of the fruit used was reduced through abrasion to 100-mesh size after being freeze-dried for the experiment.

Analysis of Anthocyanin and Flavonoid Contents

The analysis of anthocyanin and flavonoid was done according to the method of Lee *et al.*.[22]

Experimental Animals and Diets

Male Sprague-Dawley rats weighing between 90 and 110 g were purchased from Bio genomics (Seoul, Korea). The rats were individually housed in stainless steel cages in a room with controlled temperature (20-23 ℃) and lighting (alternating 12 h periods of light and dark). They were fed a pellet commercial non-purified diet. (Samyang, Seoul, Korea) for 7 days after arrival. They were randomly divided into one normal group and three diabetic groups of 10 experimental rats each. The four groups were fed experimental diets for 3 weeks and the diabetic groups were given a mulberry fruit-free diet (DM group), 0.3% mulberry fruit diet (DM-F group) or 0.6% mulberry fruit diet (DM-2F group). The experimental design was approved by the committee for the care and use of laboratory animals at the Catholic University of Daegu.

Experimental Diabetes

Diabetes was induced by an intravenous injection of STZ (50 mg/kg body weight) in a citrate buffer (pH 4.3) via tail vein. Rats with a blood glucose concentration of 16.7 mmol/L after 9 days were used for the experiment.

Preparation of Erythrocytes

Blood samples were collected from the abdominal arota using 22-gauge injector and placed in heparin-coated tubes. After centrifugation at 1000×g for 15 min at 4℃, the plasma and buffy coat were carefully removed. The separated cells were then washed three times by resuspending in a 0.9% NaCl solution and repeating the centrifugation. The washed cells were lysed in an equal volume of water and mixed thoroughly.

Measurement of Antioxidative Enzyme Activity in Erythrocytes

To remove the hemoglobin by precipitation with chloroform ethanol, 0.4 mL of an ethanol chloroform (3:5, v/v) mixture was added to a 1 mL aliquot of the hemolysate cooled in ice. This mixture was stirred constantly for 15 min and then diluted with 0.2 mL of water. After centrifugation for 10 min at 1600×g, the pale yellow supernatant was separated from the protein precipitate and used to assay the superoxide dismutase. The hemolysate was diluted 20 times and used as the raw material for the SOD activity measurement. The activity of SOD was mearsured according to the method of Marklund and Marklund.[23] The hemolysate was diluted 5 times and used as the raw material for the GSHpx activity measurement. The activity of GSHpx

Table 1. Composition of experimental diets (g/kg diet)

Groups Ingredients	Normal[1]	DM[2]	DM-F[3]	DM-2F[4]
Corn starch[5]	698	698	695	692
Casein[6]	150	150	150	150
DL-methionine[7]	2	2	2	2
Mineral mix[8]	40	40	40	40
Vitamin mix[9]	10	10	10	10
Corn oil[10]	50	50	50	50
Cellulose[11]	50	50	50	50
Mulberry fruit[12]	-	-	3	6
Total(g)	1000	1000	1000	1000

1) Normal: no injection of streptozotocin + basal diet
2) DM: injection of streptozotocin + mulberry fruit free diet
3) DM-F: injection of streptozotocin + 0.3% mulberry fruit diet
4) DM-2F: injection of streptozotocin + 0.6% mulberry fruit diet
5) SamYang Co., Seoul, Korea
6) Lactic Casein, 30 mesh, New Zealand Dairy Board, Welligton, N. Z.
7) Sigma Chem. Co., St. Louis, Missouri, U.S.A
8) Mineral mix, AIN-76 (g/kg mixture) : Calcium Phosphate, dibasic ($CaHPO_4 \cdot 2H_2O$) 500, Sodium chloride (NaCl) 74, Potassium citrate, monohydrate ($K_3C_6H_5O_7 \cdot H_2O$) 220, Potassium sulfate (K_2SO_4) 52, Magnesium oxide (MgO) 24, Manganous carbonate (45-48% Mn) 3.5, Ferric citrate (16-17% Fe) 6, Zinc carbonate (70% ZnO) 1.6, Cupric carbonate (53-55% Cu) 0.3, Potasium iodate (KIO_3) 0.01, Sodium selenite ($Na_2SeO_3 \cdot 5H_2O$) 0.01, Chromium potassium sulfate [$CrK(SO_4)_2 \cdot 12H_2O$] 0.55, filled up to 1,000 with sucrose, Harlan TEKLAD Co.
9) Vitamin mix, AIN-76A (g/kg mixture): p-Aminobenzoic Acid 11.0132, Ascorbic Acid, coated (97.5%) 101.6604, Biotin 0.0441, Vitamin B_{12} (0.1% trituration in mannitol) 2.9736, Calcium Pantothenate 6.6079, Choline Dihydrogen Citrate 349.6916, Folic Acid 0.1982, Inositol 11.0132, Menadione 4.9559, Niacin 9.9119, Pyridoxine HCl 2.2026, Riboflavin 2.2026, Thiamin HCl 2.2026, Dry Vitamin A Palmitate (500,000 U/g) 3.9648, Dry Vitamin D3 (500,000 U/g) 0.4405, Dry Vitamin E Acetate (500 U/g) 24.2291, Corn Starch, Harlan TEKLAD Co.
10) Dong Bang oil Co., Seoul, Korea
11) Sigma Chem. Co. CMC (Sodium carboxyl methyl cellulose, non-nutritivefiber), St. Louis, Missouri, U.S.A
12) Mulberry fruit powder

and CAT were mearsured according to the method of Lawrence and Bulk[24] and Aebi.[25]

Measurement of Oxidative Damage in Erythrocytes

The content of TBARS was mearsured according to the method of Yagi.[26] That is, fluorescence in the excitation wave 515 nm, emission wave 553 nm extracting TBARS by *n*-butanol, and standard material used 1,1,3,3-tetra ethoxypropane. This was reacted for 5 min at room temperature after adding 4 mL 1/12 N H_2SO_4 solution and 0.05 mL 10% phosphotungstic acid solution for 0.05 mL serum. The supernatant liquid was centrifuged at 1,100×g for 10 minutes and reacted again after adding 2 mL 1/12 N H_2SO_4 solution and 0.3 mL 10% phosphotungstic acid solution to the sludge. It was then reacted for 60 minutes in a 95℃ constant-temperature water tank after adding 4 mL distilled water and 1 mL 0.67% TBA solution to sludge and removing the supernatant liquid after centrifuge for 10 minutes at 1,100×g. A measurement was made of the fluorescence material of *n*-butanol floor that had been centrifuged for 15 minutes in 1,100×g after mix adding 5.0 mL *n*-butanol and it was cooled immediately.

Determination of Hemoglobin

Hemoglobin concentration was estimated in an aliquot of the hemolysate.

Analysis was done of SOD, GSHpx, CAT enzyme activities and TBARS contents of the erythrocyte and colormetric fixed quantity to 540 nm was done using a commercial assay kit (AM 503 K, ASAN Pharmaceutical Co., Korea).

Statistical Analysis

All data were assessed by analysis of variance (ANOVA). If significance was found by ANOVA, comparisons among group means were made using Tukey's Honestly Significant Difference test.[27]

RESULTS

Means of Anthocyanins and Flavonoids

Table 2 shows the means of anthocyanins and flavonoids. Means of cy-3-*O*-glucopyranoside, cy-3-*O*-rutinoside, rutin, isoquercitrin, quercetin, morin and dehydroquercetin contents of the mulberry fruit were 230.45, 131.5, 142.5, 10.3, 5.8, 1.6 and 3.83 mg per 100 g dry weight, respectively.

Body Weight Gains, Food Intake and Food Efficiency Ratio

Body weight gains, food intake and food efficiency ratio during the experimental period are shown in Table 3 and Figure 1. Body weights gain and food efficiency ratio significantly decreased after STZ injection. Food intake significantly increased in the diabetic groups, but it was not different among the diabetic groups.

Table 3. Effects of mulberry fruit on body weight gains, food intake and food efficiency ratio (FER) in streptozotocin-induced diabetic rats.

	Body weight gains (g)	Food intake (g)	FER
	During 4 weeks before STZ injection		
Normal	70.50±0.71^{NS}	300.97±0.00^{NS}	0.23±0.00^{NS}
DM	76.00±7.41	294.00±6.26	0.26±0.03
DM-F	77.70±33.01	327.22±18.48	0.24±0.10
DM-2F	73.50±11.63	324.50±5.87	0.23±0.04
	During 9 days after STZ injection		
Normal	15.10±6.36^{a}	154.51±0.00^{a}	0.10±0.04^{a}
DM	-58.10±22.77^{b}	175.13±0.29^{b}	-0.37±0.14^{b}
DM-F	-46.50±16.97^{b}	172.51±19.41^{b}	-0.27±0.10^{b}
DM-2F	-42.34±2.35^{b}	174.76±12.12^{b}	-0.24±0.03^{b}

All values are mean ± SE(n = 10).
Values with different superscript letters (a,b) in the same column are significantly different at $p<0.05$ by Tukey's test.
The experimental conditions were the same as those described in Table 1.
FER : Food effiency ratio

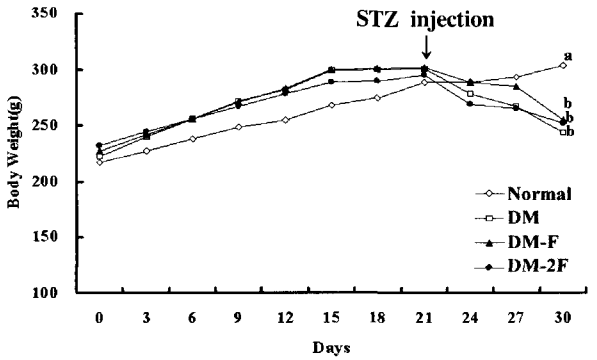

Fig 1. Changes in body weight in rat fed different experimental diets for 3 weeks and 9 days after streptozotocin-induced diabetic rats.
All values are mean ± SE (n = 10). Values with different superscript letters (a, b) in the same column are significantly different at $p<0.05$ by Tukey's test. The experimental conditions were the same as those described in Table 1.

Table 2. Means of anthocyanins and flavonoids from cheongil mulberry fruit.

Mulberry fruit	Anthocyanins (mg/100g, dry weight)		Flavonoids (mg/100g, dry weight)				
	Cy-3-*O*-glu[1]	Cy-3-*O*-rut[2]	Rutin	Isoquercitrin	Quercetin	Morin	Dehydroquercetin
Cheongil	230.45	131.5	142.5	10.3	5.8	1.6	3.83

1) Cyandidin 3-*O*-β-glucopyranoside. 2) Cyandidin 3-*O*-β-rutinoside.

Antioxidative Defense Enzyme Activity of Erythrocytes

Table 4 shows the activity of SOD, an antioxidant enzyme that reduces superoxide radicals to H_2O_2, which in turn is excreted as H_2O based on the activity of GSH-px and catalase, thereby protecting the body from oxygen toxicity. The activity of erythrocytes SOD in the DM, DM-F and DM-2F groups decreased to 17%, 14% and 16%, respectively, compared with that of the normal group but there was no significant difference among the diabetes groups. The activity of GSH-px (Fig. 2) in the DM group decreased to 61% compared with that of the normal group. However, in the DM-F and DM-2F groups it increased to 33% and 69%, respectively, compared with that of DM group. The activity of CAT (Fig. 3) in the DM group decreased to 36% compared with that of the normal group. However, in the DM-F group it increased to 22% compared with that of the DM group and in the DM-2F group it remained at the normal level.

Table 4. Effects of mulberry fruit on erythrocyte superoxide dismutase (SOD) activities in streptozotocin-induced diabetic rats.

Groups	SOD *unit/min/g Hb*
Normal	122.65±2.56[a]
DM	101.95±3.39[b]
DM-F	105.77±1.02[b]
DM-2F	103.99±4.21[b]

All values are mean ± SE (n = 10)
Values with different superscript letters (a,b) in the same column are significantly different at p<0.05 by Tukey's test.
The experimental conditions were the same as those described in Table 1.

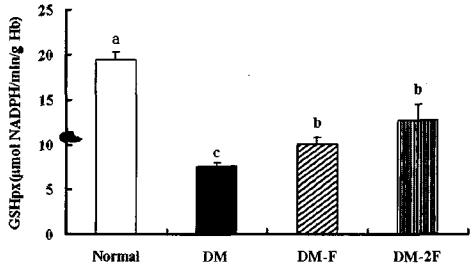

Fig 2. Effects of mulberry fruit on erythrocyte glutathione peroxidase (GSHpx) activities in streptozotocin-induced diabetic rats.

All values are mean ± SE (n = 10).
Values with different superscript letters (a, b, c) in the same column are significantly different at p<0.05 by Tukey's test.
The experimental conditions were the same as those described in Table 1.

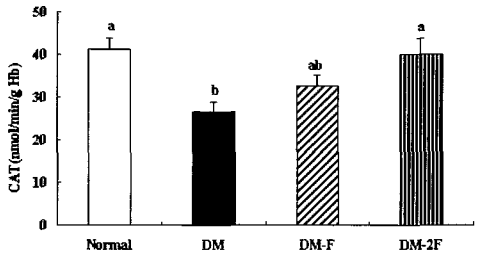

Fig 3. Effects of mulberry fruit on erythrocyte catalase (CAT) activities in streptozotocin-induced diabetic rats.

All values are mean ± SE (n = 10).
Values with different superscript letters (a, b) in the same column are significantly different at p<0.05 by Tukey's test.
The experimental conditions were the same as those described in Table 1.

Oxidative Damage of Erythrocytes

TBARS contents as an index oxidative damage is shown in Figure 4.

The concentration of TBARS in the DM group increased to 122% compared with that of the normal group. However, the concentration in the DM-F and DM-2F groups decreased to 17% and 28%, respectively, compared with that of the DM group.

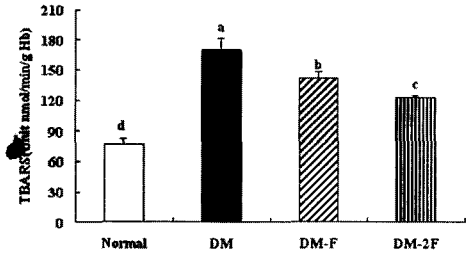

Fig 4. Effects of mulberry fruits on erythrocyte thiobarbituric reactive substances (TBARS) values in streptozotocin-induced diabetic rats.

All values are mean ± SE (n = 10)
Values with different superscript letters (a, b, c, d) in the same column are significantly different at p<0.05 by Tukey's test.
The experimental conditions were the same as those described in Table 1.

DISCUSSION

The current study examined the effects of mulberry fruit on the antioxidative defense system and oxidative stress in the erythrocytes of diabetes-induced experimental rats.

Mulberry fruit includes several flavonols such as quercetin and its glycosides, rutin, isoquercitrin, and quercitrin and anthocyanins.[28-30] Many kinds of anthocyanins and flavonoids in mulberry fruit have been reported to play important roles as dietary antioxidants in the prevention of oxidative damage caused by active oxygen radicals in living systems.[31,32] Additionally, anthocyanins have been reported to possess several biological activities such as anti-convulsant, anti-carcinogenic, anti-atherosclerotic, and anti-inflammatory actions.[33-36] Flavonoids have various biochemical and pharmacological effects, including anti-cancer, anti-oxidative, anti-inflammatory and anti-mutagenic activities.[37,38] Means of cy-3-*O*-glucopyranoside, cy-3-*O*-

rutinoside, rutin, isoquercitrin, quercetin, morin and dehydroquercetin contents in the mulberry fruit were 230.45, 131.5, 142.5, 10.3, 5.8, 1.6 and 3.83 mg per 100 g dry weight, respectively.

Body weight gains and food efficiency ratio significantly decreased after STZ injection. Food intake significantly increased in diabetic groups, but it was not different among the groups.

Griesmacher and Kindhauser reported that an oxidative stress increase appeared with increase in nonenzymatic glycosylation, autooxidative glycosylation and a change in the antioxidative defense system in the diabetes state.[39] There is an oxidative defense system to prevent the accumulation of free radicals or lipid peroxidation *in vivo*.

SOD, an antioxidant enzyme, reduces superoxide radicals to H_2O_2, which in turn is excreted as H_2O based on the activity of GSH-px and catalase, thereby protecting the body from oxygen toxicity. Based on observation of the activity of the antioxidative defense system in the erythrocytes, the SOD activity seen in the diabetic groups significantly decreased compared to the normal group but there was no significant difference among the groups. GSH-px activity decreased considerably in the DM group compared to the normal group. However, there was a significant increase in the DM-F and DM-2F groups compared to the DM group. Catalase activity, which prevents peroxidative damage by removing hydrogen peroxide and organic peroxide, decreased significantly in the DM group compared to the normal group. However, in the DM-2F group it remained at the normal level. The inactivity of the antioxidant enzymes SOD, GSH-px and catalase in the diabetes groups was attributed to peroxidative damage to the tissues caused by diabetes,[40] while the feeding of mulberry fruit contributed to maintaining the optimum condition of the cell membrane organelles, essential for enzyme activity, by protecting them from peroxidation.

Lipid peroxidation is caused by the augmentation of oxidative stress and the decrease of an antioxidative defense system *in vivo*. TBARS content as an index oxidative damage in the DM group significantly increased compared to the normal group. However, the concentration in the DM-F and DM-2F groups were significantly decreased compared to the DM group. This results was suggest that the flavonoid compound of mulberry fruit acted as antioxidant.

In conclusion, mulberry fruit strengthened the antioxidative defense system thanks to the increased activity of antioxidant enzymes, such as GSH-px and catalase, in the erythrocytes of the diabetes-induced experimental rats. Accordingly, mulberry fruit was found to decrease the generation of oxidative damage-causing substances, such as lipid peroxidation lowered by oxidative damage.

Literature cited

1) Baynes JW. Role of oxidative stress in the development of complications in diabetes. *Diabetes* 40: 405-12, 1991
2) Hammers HD, Martin S, Fedesrlin K, Geisen K, Brownlle M. Aminoguanidine treatment inhibit the developement of experimental diabetic retinopathy. *Proc Nat Acad Sci USA* 88:11555-58, 1991
3) Cho MR, Choue RW, Chung SH, Whan RJ. Effects of Silkworm Powder on Blood Glucose and Lipid Levels in NIDDM (Type 2) Patients. *Korean J Nutrition* 31:1139-50, 1998
4) Yoo SK, Kim MJ, Kim JW, Rhee SJ. Effects of YK-209 mulberry leaves on disaccharidase activites of small intestine and blood glucose-lowering in streptozotocin-induced diabetic rats. *J Korean Soc Food Sci Nutr* 31:1071-77, 2002
5) Yoo SK, Rhee SJ. Effects of YK-209 mulberry leaves on antioxidative defense system of liver in streptozotocin-induced diabetic rats. *J Korean Soc Food Sci Nutr* 31: 1065-70, 2002
6) Hong JH, Park MR, Rhee SJ. Effects of YK-209 mulberry leaves on HMG-CoA reductase and lipid composition of liver in streptozotocin-induced diabetic rats. *J Korean Soc Food Sci Nutr* 31:826-33, 2002
7) Rhee SJ. Kim MJ, Kwag OG. Effects of green tea catechin on prostaglandin synthesis of renal glomerular and renal dysfunction in streptozotocin-induced diabetic rats. *Asia Pacific J Clin Nutr* 11: 232-36, 2002
8) Rhee SJ, Choi JH, Park MR. Green tea catechin improves microsomal phospholipase A_2 activity and the arachidonic acid cascade synstem in the kidney of diabetic rats. *Asia Pacific J Clin Nutr* 11: 226-31, 2002
9) Yang JA, Choi JH, Rhee SJ. Effects of green tea catechin on phospholipase A_2 activity and antithrombus in streptozotocin diabetis rats. *J Nutr Sci Vitaminol* 45:337-46, 1999
10) Kim CJ, Hahn DR. The biological activity of a new glycoside, chiisanoside from acanthopanax chiisanensis nakai leaves. *Yakhak Hoeji* 24:123-34, 1980
11) Kim LH, Han SS, Choi YS. Antioxidant effects of the extracts of Acanthopanax senticosus. *Kor J Pharmacon* 33:359-63, 2002
12) Kim SK. Bonchohak, Chapter 17, Beneficial medicine, Mulberry fruits. pp.598, Younglimsa, Seoul, 1991
13) Kangjoshineuihakwon, Jungyakdaesajon (2nd), Sohakkwyan, pp.3717, Sanghai, 1985
14) Kim TY, Kwon YB. A study on the antidiabetic effect of mulberry fruits. *Kor J Seri Sci*, 38:100, 1996
15) Kim SY, Park KJ, Lee WC. Antiinflammatory and antioxidative effects of *Morus* spp. fruit extract. *Kor J Med Crop Sci* 6:204, 1998

16) Park JC, Choi JS, Choi JW. Effects of the fractions from the leaves, fruits, stems and roots of *Cudrania tricuspidata* and flavonoids on lipid peroxidation. *Kor J Pharmacogn* 26:377, 1995
17) Cha JY, Kim HJ, Chung CH, Cho YS. Antioxidative activities and contents of polyphenolic compound of *Cudrania tricuspidata*. *J Kor Soc Food Sci Nutr* 28:1310, 1999
18) Kim HJ, Cha JY, Choi ML, Cho YS. Antioxidative activities by water-soluble extracts of *Morus alba* and *Cudrania tricuspidata*. *J Kor Soc Agric Chem Biotechnol* 43:148, 2000
19) Kim HB, Kim SY, Ryu KS, Lee WC, Moon JY. Effect of methanol extract from mulberry fruit on the lipid metabolism and liver function in cholesterol induced hyperlipidemia rats. *Kor J Seri Sci* 43:104, 2001
20) Oh H, Ko EK, Jun JY, Oh MH, Park SU, Kang KH, Lee HS, Kim YC. Hepatoprotective and free radical scavenging activities of prenylflavonoids, coumarin, and stibene from *Morus alba*. *Planta Med* 68:932, 2002
21) Asano N, Yamashita T, Yasuda K, Ikeda K, Kizu H, Kameda Y, Kato A, Nash RJ, Lee HS, Ryu KS. Polyhydroxylated alkaloids isolated from mulberry trees (*Morus alba* L.) and silkworms (*Bombyx mori* L.). *J Agric Food Chem* 49:4208, 2001
22) Lee JY, Moon SO, Kwon YJ, Rhee SJ, Choi SW. Identification and quantification of anthocyanins and flavonoids in mulberry (Morus spp.) cultivars. *Food Sci Biotechnol* 13:176-84, 2004
23) Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem* 47:467-74, 1974
24) Lawrence RA, Burk RF. Glutathione peroxidase activity in selenium-deficient rat liver. *Biochem Biophys Res Commun* 71:952-58, 1976
25) Aebi H, Wyss SR, Scherz B, Skvaril F. Heterogeneity of erythrocyte catalase II. Isolation and characterixation of normal and variant erythrocyte catalase and their subunits. *Eur J Biochem* 48:137-45, 1974
26) Yagi K. A simple fluorometric assay for lipoperoxide in blood plasma. *Biochem Med* 15:212-16, 1976
27) Sreel RGD, Torrie JH. Principles and procedures of statistics. McGrow Hill, New York, USA, 1990
28) Kim HB, Bang HS, Lee HW, Seuk YS, Sung GB. Chemical characteristics of mulberry syncarp. *Korean J Seri Sci.* 41:123-8, 1999
29) Lee HW, Shin DH, Lee WC. Morphological and chemical characteristics of mulberry (*Morus*) fruit with varieties. *Korean J Seric Sci* 40:1-7, 1998
30) Gerasopoulos D, Stavroulakis G. Quality characteristics of four mulberry (*Morus spp*) cultivars in the area of chania, greece. *J Sci Food Agric* 73:261-4, 1997
31) Tsuda T, Shiga K, Ohshima K, Kawakishi S, Osawa T. Inhibition of lipid peroxidation and the active oxygen radical scavenging effect of anthocyanin pigments isolated from *Phaseolus vulgaris* L. *Biochem Pharmacol* 20:933-56, 1996
32) Wang H, Cao G, Prior RL. Oxygen radical absorbing capacity of anthocyanins. *J Agric Food Chem* 45:304-9, 1997
33) Drenska D, Bantutova I, Ovcharov R. Anticonvulsant effect of anthocyanins and antioxidants. *Fomatsiya (Sofia)* 39:33-40, 1989
34) Kamei H, Kojima T, Hasegawa M, Koide T, Umeda T, Yukawa T, Terabe K. Suppression of tumor cell growth by anthocyanins *in vitro*. *Cancer Invest* 13:590-4, 1995
35) Satue-Gracia MT, Heinonen M, Frankel EN. Anthocyanins as antioxidants on human low-density lipoprotein and lecithin-liposome systems. *J Agric Food Chem* 45:3362-7, 1997
36) Wang H, Nair MG, Strasburg GM, Chang YC, Booren AM, Gray JI, Dewitt DL. Antioxidant and anti-inflammatory activities of anthocyanidins and their aglycone, cyanidine, from tart cherries. *J Nat Prod* 62:294-6, 1999
37) Havsteen B. Flavonoids, a class of natural products of high pharmacological potency. *Biochem Pharmacol* 32:1141-8, 1983
38) Kim JH, Kang BH, Jeong JM. Antioxidant, antimutagenic and chemopreventive of a phyto-extract mixture derived from various vegetables, fruits, and oriental herbs. *Food Sci Biotechnol* 12:631-8, 2003
39) Griesmacher A, Kindhauser M, Andert SE, Schreiner W, Toma C, Knoebl P, Pietschmann P, Prager R, Schnack C, Schernthaner G. Enhanced serum levels of thiobarbituric-acid-reactive substances in diabetes mellitus. *Am J Med* 98:469-75, 1995
40) Kwag OG, Kim SO, Choi JH, Rhee IK, Choi MS, Rhee SJ. Vitamin E improves microsomal phospholipase A_2 activity and the arachidonic acid cascade in kidney of diabetic rats. *J Nutr* 131:1297-301, 2001