Nutritional Sciences 6(4), November, 2003, pp. 216 - 222

Preparation of Mulberry Leaf Extract by Adding Mugwort and Pine Needle and Effects on Lipid Composition in Rats Fed High Cholesterol Diets*

Jeong-Hwa Choi, Joo-Yeoung Chae* and Soon-Jae Rhee[1§]

Division of Food Science, Jinju International University, Jinju, 660-759, Korea

[1]Department of Food Science and Nutrition, Catholic University of Daegu, Gyungsan, 712-702, Korea

This study investigated the effects of feeding mulberry leaf extracts on lipid composition in rats fed high cholesterol diets. An initial 30-person sensory evaluation of preparations containing various concentrations of mulberry leaf extract showed that a preparation containing 9% mulberry leaf extracts was the most highly preferred. In addition, subsidiary materials of pine needle extracts and mugwort extracts were added to weaken the unpleasant smell of mulberry leaf extract. A preparation containing 9% mulberry leaf extract with 3% mugwort extract and 7% pine needle extract was given highest preference scores by the 30-person panel. When comparing the functional ingredients contents of the various preparations of mulberry leaf extracts, such as GABA, DNJ and flavonoids, no significant differences were found as a result of adding subsidiary materials (pine needle and mugwort extracts). Sprague-Dawley male rats weighing 100±10g were randomly assigned to one normal diet group, and to four high cholesterol diet groups containing 1% cholesterol, to elucidate the functionality of the mulberry leaf extract. The four high cholesterol diet groups were classified into : a mulberry leaf extract diet group free of subsidiary materials (EB group); a mulberry extract diet group with pine needle extracts (EP group); a mulberry leaf extract diet group with mugwort extracts (EM group); and a control group (HC group). The mulberry leaf extracts were provided as drinking water; the diet and water were fed *ad libitum*. Hepatic cholesterol and triglyceride levels were higher, by 279% to 475%, in the high cholesterol groups compared to the normal diet groups, but were significantly lower in the three groups supplied with mulberry leaf extracts, compared with the high cholesterol control. There were no changes in functionality of the mulberry leaf extract preparations due to the addition of subsidiary materials. In conclusion, preparations of mulberry leaf extracts were shown to improve lipid metabolism in rats fed a high cholesterol diet, by reducing hepatic and plasma triglyceride and cholesterol levels. Also human palatability of the mulberry leaf preparation was improved by adding subsidiary materials such as pine needle and mugwort extracts.

Key words : Mulberry Leaf Extract, Cholesterol, Triglyceride

INTRODUCTION

Convenient life patterns and affluent dietary habits in modern society have caused increases in the intakes of animal fats and of excess energy, resulting in a number of adult diseases such as diabetes, obesity, hyperlipidemia, heart disease, stroke, hypertension, and arteriosclerosis. Among these chronic degenerative diseases, the number of patients with cardiovascular disease has rapidly increased, such that this is currently the primary cause of death in Korea.[1] To address this problem, the general public and academics have shown great interest in the development of functional foods containing natural substances, which might reduce triglyceride or cholesterol levels in the blood.[2),3)] One such natural substance, that is attracting increasing interest, is the mulberry leaf.

The ingredients of mulberry leaves can be generally divided into volatile and non-volatile ingredients; the former includes guaiacol, eugenol, methyl salicylate, benzaldehyde and phenylacetaldehyde,[4)] while the latter mainly consists of a high number and diversity of flavonoids. Studies of the physiological effects of mulberry have demonstrated blood sugar repression in diabetes,[5),6)] lipid metabolism improvement,[7)] lowering of blood pressure,[8)] improvement of blood fluidity, the absorption and detoxification of heavy metals, suppression of cancer incidence,[8)] suppression of aging,[8)] and antioxidant effects.[9),10)]

However, to potentially raise the consumption of

*Acknowledgement : This work was supported by grant No. 20000065 from Agricultural Research & Development Promotion Cente
Accepted : November 18, 2003
§ To whom correspondence should be adressed.

functional foods based on mulberry leaves, it is necessary to provide better information on their physiological effects and to improve the palatability of specific products. In this regard, the present study attempts to prepare mulberry extracts with enhanced palatability and functionality, by adding functional natural substances such as mugwort and pine needles to mulberry extracts as subsidiary materials in order to remove the unpleasant smell. This study then goes on to examine the physiological effects on lipid composition of the prepared mulberry-based extracts, by feeding the extracts to rats fed high cholesterol diets.

MATERIALS AND METHODS

Collection of Mulberry Leaves and Subsidiary Materials

The YK-209 mulberry leaves used in this experiment were cultivated at Youngcheon Silkworm Culture Agricultural Co-operative and were harvested in May 2001. Collected mulberry leaves were used in the trial after cleaning. Mugwort was purchased in the market, and pine needles were gathered in Waegwan in August, 2003.

Production of mulberry leaves and subsidiary material extraction

Mulberry leaves were collected according to the method described in a previous report,[11] dried immediately using hot-air, extracted, filtered for 4 hours at temperatures of 85-90℃, and concentrated at the 52 vacuum level for 10 hours at 60-62℃; the resulting extracts contained 60% solid powder, and were used as the raw material for this experiment.

The mugwort and pine needles, the supplementary materials, were each added to distilled water, extracted for 4 hours at 65℃.

Production of Mulberry leaf extract

Mulberry leaf extract was produced by diluting mulberry concentrates into 3%, 6%, 9% and 12% solutions. The result of a sensory evaluation of the different concentrations of mulberry extracts showed 9% mulberry leaf extract to be the best dilution, as it obtained the highest preference score from a 30-person panel. Final mulberry leaves extract was made adding mugwort and pine needles by 3%, 5% and 7%, respectively.

Sensory Evaluation

For the sensory evaluation, at Daegu Catholic University were selected and educated to conduct a sensory evaluation of the various concentrations of mulberry extracts, and of the subsidiary materials (mugwort and pine needles). Fr30 persons in the 3rd year agrance, color, taste and overall preference were categorized into 7 items and measured using a 7-point scoring method.[12] The higher the preference, the closer the score is to 7 points.

Analysis of the functional components of mulberry leaf extracts

1) *Contents of γ - aminobutyric acid (GABA)*

Blood pressure and GABA contents were measured according to the method of Bang et.al.[13] Briefly, 20㎖ of 4% sulfosalicylic acid solution was added to 1 ml of mulberry extract and extracted for 1 hour at 30℃ by supersonic waves. The extracted sample was left at 4℃ for 60 minutes and centrifuged (12,000 rpm, 5℃, 15 min) to precipitate the protein. The supernatant was combined with an equal quantity of Uriprep (containing lithium ion) and left for 5 minutes at room temperature. The protein was removed through centrifuging (13,000 rpm, 4℃, 5 min). The supernatant obtained through centrifuging was filtered (at 0.45 ㎛). Then, the GABA contents were measured using an automatic analyzer of amino acids (Pharmacia Biotech Co., Biochrom 20, Switzerland) under the following column conditions : positive ion exchange column (Li-form) setting; retention time 110 minutes; mobile phase as 10 mM sodium phosphate (pH 2.5); 20 mM sodium phosphate buffer (pH 7.5); UV/Vis detector as 570 nm.

2) *1-deoxynojirimycin (DNJ)*

1-deoxynojirimycin (DNJ) was analyzed under the following column conditions : column; phenomenex Luna C18 column (4.6X250mm), flow rate; 1ml/min, detection; 264-314nm, mobile phase; 0.1% AcOH : CH3CN (50 : 50, v/v) using high performance liquid chromatography (HPLC : Spectra SYSTEM HPLC, ThermoQuest Co.), according to the Stead's method[14] and Cole's method.[15]

3) *Flavonoid analysis*

Flavonoid contents, the antioxidative ingredients of mulberry leaves, were measured by slight modification of the method of Yun and Lee.[16]

Physiological functions of prepared mulberry leaf extracts in rat fed high cholesterol diets

1) *Experimental animals and diet*

Sprague-Dawley male rats weighing 100 ± 10g were purchased from KRITC (Daejon, Korea). Rats were individually housed in stainless steel cages in a room with controlled temperature (20-23℃) and lighting (alternating 12h periods of light and dark). Rats were randomly assigned between five groups : one was given

a normal diet, and four were given a high cholesterol diet containing 1% cholesterol. Three of the four high cholesterol diet groups were given different mulberry extracts : mulberry extract without subsidiary materials (the EB group), mulberry extract with pine needle extract (the EP group), and mulberry leaf extract with mugwort extract (the EM group). The fourth high colesterol diet group provided a control. The rats were fed ad libitum for 4 weeks. The various extracts were provided as drinking water; the solid feed diet and water were freely provided. The experimental design was approved by the committee of the Catholic University of Daegu for care and use of laboratory animals (Tables 1 & 2)

Table 1. Classification of experimental groups

Groups	Cholesterol	Mulberry leaf extract	Additive
Normal[1)]	-	-	-
HC[2)]	+	-	-
EB[3)]	+	+	-
EP[4)]	+	+	+
EM[5)]	+	+	+

[1)] Normal : basal diet
[2)] HC : basal diet + 1% cholesterol
[3)] EB : basal diet + 1% cholesterol + YK-209 mulberry leaf extract
[4)] EP : basal diet + 1% cholesterol + YK-209 mulberry leaf extract with 7% pine needles extract
[5)] EM : basal diet + 1% cholesterol + YK-209 mulberry leaf extract with 3% mugwort extract

Table 2. Composition of experimental diet groups

groups / Ingredients	Normal	High cholesterol diet			
		HC	EB	EP	EM
Starch	70	69.0	69.0	69.0	69.0
Casein	15	15	15	15	15
Salt mixture	4	4	4	4	4
Vitamin mixture	1	1	1	1	1
Corn oil	5	5	5	5	5
Sucrose	5	5	5	5	5
Cholesterol	-	1	1	1	1
Total(%)	100	100	100	100	100

2) *Measurement of body weight, food intake and food efficiency ratio*

Body weights were measured regularly at the same time every other day throughout the experimental period. The food efficiency ratio was calculated by dividing the body weight by the food intake during the experimental period.

3) *Measurement of activity of GOT and GPT*

The levels of activity of GOT and GPT in the serum were measured by the method of Retiman and Frankel.[17]

4) *Measurement of triglyceride and cholesterol in serum and liver*

The levels of serum triglyceride and total cholesterol were measured with the Asan kit (Asan Co., Korea).

Hepatic triglyceride and cholesterol were extracted by the Folch's[18] method and measured by the Sale's method.[19]

5) *Protein determination*

Protein in the kidney tissues was determined using the method of Lowry et al,[20] with bovine serum albumin as the standard.

Statistical analysis

All the results were assessed by a variance analysis (ANOVA) to investigate the standard difference among the groups. If significance was found based on the variance analysis, the level of significance among the groups was analyzed by Tukey's HSD test.

RESULTS

Sensory Evaluation

Sensory evaluation was performed to determine the optimal concentration among the 3, 6, 9, and 12% mulberry leaf extracts; the results (Table 3) of the sensory evaluation showed that overall preference was

Table 3. Sensory evaluation of mulberry leaf extract according to concentration

Mulberry leaf extract[1)]	Flavor	Color	Sweet taste	Astringent taste	Delicate taste	Overall taste	Overall quality
3%	2.7	4.3	4.7	2.2	2.0	4.0	4.0
6%	3.7	5.0	4.7	4.0	4.0	6.2	6.0
9%	3.3	6.2	4.7	3.0	2.7	6.7	6.2
12%	3.8	3.7	3.7	4.3	4.3	4.0	3.8

[1)] Concentration of mulberry leaf extract

Table 4. Sensory evaluation of mulberry leaf extract with pine needles and mugwort

Mulberry leaf extract		Flavor	Color	Sweet taste	Astringent taste	Delicate taste	Overall taste	Overall quality
EB[1)]		4.0	4.0	4.0	4.0	4.0	4.0	4.0
EP[2)]	3%	4.2	4.3	3.8	4.3	3.3	4.2	4.3
	5%	4.7	4.8	4.2	4.7	3.5	6.0	4.8
	7%	5.4	6.0	5.8	4.3	3.0	6.3	6.3
EM[3)]	3%	5.3	3.7	4.5	3.8	4.5	6.0	6.0
	5%	4.3	3.7	4.8	4.7	4.5	5.7	5.0
	7%	4.2	3.7	4.3	4.7	4.8	4.2	4.7

[1)] EB : YK-209 mulberry leaf extract
[2)] EP : YK-209 mulberry leaf extract with pine needles
[3)] EM : YK-209 mulberry leaf extract with mugwort

highest for the 9% extract. The highest preference scores for the mulberry leaf extract were achieved with the addition of 3% mugwort extract and that of 7% pine needle extract. In terms of overall preference, mulberry extract supplemented with pine needle extracts achieved a higher score than supplementation with mugwort extract (Table 4).

The functional components of extracts

The levels of functional ingredients of extracts (measurements of γ - aminobutyric acid (GABA) and of 1-deoxyinojirimycin (DNJ)) are shown in Tables 5 and 6. The levels of GABA were slightly higher in mulberry leaf extracts where subsidiary materials (pine needle extracts and/or mugwort extracts) had been added, compared to mulberry leaf extracts without subsidiary materials, but this difference was not significant. DNJ levels did not differ between the three leaf extract groups.

Flavonoids found in mulberry leaf extract (without subsidiary material) were rutin, isoquercitrin, kaempeferol-3-*O*-rutinoside and astragalin at the levels of 107, 1287, 8.5, and 40.5 mg%, respectively. The level of isoquercitrin was the highest, followed by rutin. Flavonoid contents were slightly higher when subsidiary materials, pine needle extracts and mugwort extracts, were added.

Table 5. Quantification of γ - aminobutyric acid (GABA) and 1-deoxyinojirimycin (DNJ) in mulberry leaf extract with pine needles and mugwort

Mulberry leaf extract	GABA (mg% wet base)	DNJ (mg% wet base)
EB	236±17.0^{NS}	192±11.4^{NS}
EP	260±18.2	208±12.4
EM	249±24.5	204±11.9

All values are mean±SE (n=10)
Values within a column with different superscripts are significantly different at p<0.05 by Tukey's test.
The experimental conditions are the same as Table 3 & 4
NS : not significant

Table 6. Contents of flavonoids in mulberry leaf extract with pine needles and mugwort

(mg% wet base)

Mulberry leaf extract	Rutin	Isoquercitrin	Kaempeferol-3-O-rutinoside	Astragalin	Total Flavonoid
EB	2.15±0.25^{NS}	25.74±1.25^{NS}	0.17±0.05^{NS}	0.81±0.02^{NS}	28.87±1.36^{NS}
EP	2.34±0.15	26.14±1.55	0.19±0.02	1.26±0.02	29.90±2.13
EM	2.28±0.25	27.44±1.84	0.23±0.04	1.03±0.04	30.98±1.87

All values are mean±SE (n=10)
Values within a column with different superscripts are significantly different at p<0.05 by Tukey's test.
The experimental conditions are the same as Table 3 & 4.
NS : not significant

Physiological functions in rats fed the mulberry leaf extract

Body weight gain, food intake and food efficiency ratio :

The results of body weight gain, food intake and food efficiency ratio during the experimental period are shown in Table 7. Both the body weight gain and food intake were higher in the groups fed high cholesterol diets than in the normal group, but there was no difference among the 4 different groups fed high cholesterol diets.

Table 7. Effects of mulberry leaf extracts on body weight gains, food intake, foode efficiency ratio (FER) in rats fed high cholesterol diets.

Groups	Body weight gains (g)	Food intake (g/day)	FER
Normal	168±7.0^{a}	24.5±0.61^{NS}	0.28±0.03^{NS}
HC	185±5.3^{b}	25.9±0.28	0.32±0.02
EB	171±8.1^{a}	26.7±0.37	0.29±0.01
EP	173±7.3^{a}	26.2±0.73	0.30±0.02
EM	170±6.4^{a}	25.8±0.67	0.29±0.01

All values are mean±SE (n=10)
Values within a column with different superscripts are significantly different at p<0.05 by Tukey's test.
The experimental conditions are the same as Table 1.
NS : not significant

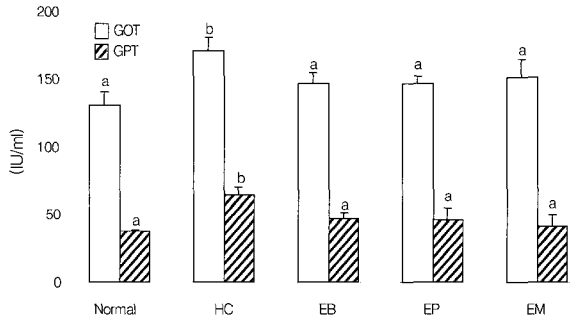

Fig 1. Effects of mulberry leaf extract on serum glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activities in rats fed high cholesterol diets.

All values are mean±SE (n=10).
Bars with different letters are significantly different at p<0.05 when determined by ANOVA followed by Turkey's test.
The experimental conditions are the same as described in Table 1

The activity of glutamic pyruvic transaminase(GPT) and glutamic oxaloacetic transaminase(GOT)

The levels of activity of GOT (for the extent of liver damage) and GPT (for hypertrophy) were measured to examine the physiological toxic response to the mulberry leaf extract (Figure 1). The activities of GPT and GOT appeared to decrease in the mulberry leaf extract group compared to HC groups, and there was no significant difference among mulberry leaf extract groups supple-

mented with different subsidiary materials.

Serum triglyceride and total cholesterol concentrations

The concentrations of serum triglyceride and total cholesterol are shown in Figure 2.

The serum triglyceride concentrations were significantly higher ($p<0.05$) in the HC group than in the normal group, whereas the mulberry leaf extract groups (EB, EP and EM) exhibited the same concentrations as the normal group. The serum cholesterol concentration was higher in the all groups fed the high cholesterol diet, compared to the normal group; however, mulberry leaf extract groups were significantly lower than the HC group. There was no significant difference among the three mulberry leaf extract groups.

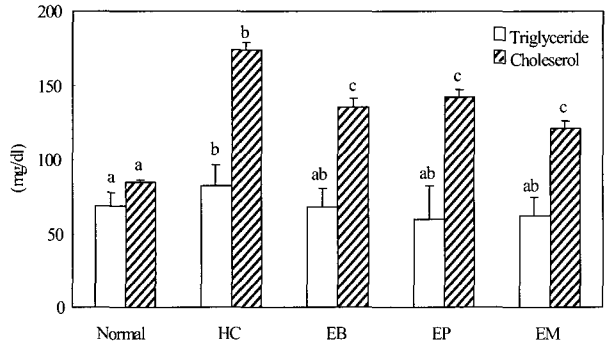

Fig 2. Effects of different mulberry leaf extract on serum triglyceride and cholesterol in rats fed high cholesterol diets

All values are mean±SE (n=10).
Bars with different letters are significantly different at $p<0.05$ when determined by ANOVA followed by Turkey's test.
The experimental conditions are the same as Table 1.

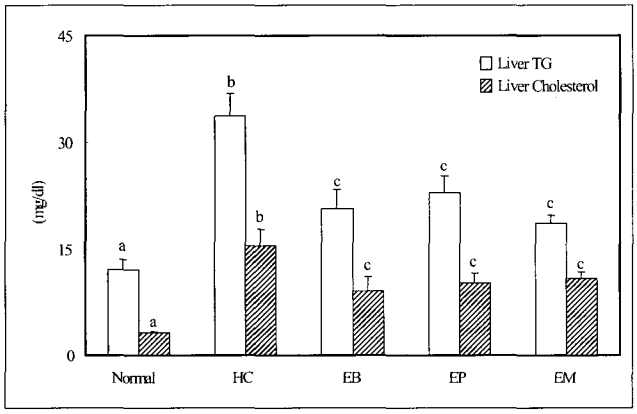

Fig 3. Effects of different mulberry leaf extract on hepatic triglyceride and cholesterol in rats fed high cholesterol diets.

All values are mean±SE (n=10).
Bars with different letters are significantly different at $p<0.05$ when determined by ANOVA followed by Turkey's test.
The experimental conditions are the same as Table 1.

Hepatic triglyceride and cholesterol concentrations

Hepatic triglyceride and cholesterol concentrations are shown in Figure 3.

The triglyceride concentration increased by 279% in the HC group compared to the normal group, while the EB, EP, and EM groups showed a decrease of 38%, 32%, and 45%, respectively, as compared to the HC group.

The hepatic cholestrol concentration was higher in the HC group than in the normal group, but concentrations were lower in the mulberry leaf extract supplemented groups compared to the HC group.

There was no significant difference among the various mulberry leaf extract groups.

DISCUSSION

This study was designed to prepare mulberry leaf extracts with acceptable human palatability using YK-209 mulberry leaves, to supply these mulberry leaf extracts to rats fed a high cholesterol diet, and then to measure the effects of mulberry leaf extracts on lipid composition in controlled conditions by observing the lipid composition of liver and blood.

As a result of a human sensory evaluation that was conducted to determine the optimal concentrates of mulberry leaf extract in the preparation, overall preference was found to be highest for a solution with a concentration of 9%. In addition, when conducting the sensory evaluation with the addition of various levels of pine needle extracts and mugwort extracts (for weakening the unpleasant smell of the mulberry leaves), the addition of a 7% solution of pine needle extracts was most preferred, with next best preference shown for the addition of a 3% solution of mugwort, with the 9% unadulterated mulberry solution being least preferred.

The γ - aminobutyric acid (GABA) contents of the preparations were relatively higher in preparations where the mulberry leaf extracts were supplemented with pine needle extracts and/or mugwort extracts, compared to the mulberry leaf preparations without the supplementation. In addition, the contents of flavonoids (sulfur oxidizing ingredients) and 1-deoxyinojirimycin (DNJ) (a blood sugar reducing ingredient) were no different among the preparations, whether or not the mulberry leaf extracts were supplemented. Consequently, the increased preference arising from supplementation did not result in any difference in the contents of functional ingredients.

There were no significant differences in food intake and food efficiency ratio between the experimental groups. Although body weight increased at a significantly higher rate in the experiment groups fed the high cholesterol diet than in the normal group, body weight

was significantly lower in those groups supplied with mulberry leaf extracts compared with the high cholesterol control group. This result is consistent with the study of Kim, et al.[21] who reported that the weight of the high cholesterol group decreased when rats were supplemented with mulberry leaf extracts.

The activities of GPT and GOT appeared to increase in the HC group compared to the mulberry leaf extract supplemented groups, and mulberry leaf extract groups has same the normal group. Therefore, the mulberry leaf extract preparation not only has no toxicity, but also can detoxify the damage inflicted on liver due to high cholesterol intakes. This result is consistent with the study of Hong, et al[22] where GOT activity was significantly reduced in the mulberry leaf extracts groups.

The results of serum lipid composition measurements, designed to test any improvements in lipid metabolism due to the mulberry leaf extracts, showed that serum triglyceride contents increased significantly in the high cholesterol groups compared to the normal group, but were lower in the mulberry leaf extract groups than in the high cholesterol control group. There were no significant differences in these results whether or not the mulberry leaf extract preparations were supplemented with pine needle or mugwort extracts. Total cholesterol concentrations in serum were higher in the HC groups than in the normal group ($p<0.05$), but were significantly lower in the groups supplied with mulberry leaf extracts compared with the HC control group. The triglyceride contents of liver increased by 279% in the high cholesterol control group compared to the normal group, but were significantly lower in those groups provided with mulberry leaf extracts compared to the HC control group. There were no significant differences in measured lipid metabolism between those groups fed the mulberry leaf extracts. The levels of cholesterol in liver tissue showed a similar trend between treatments as the change of triglyceride lipid contents. These improvements in the lipid composition of blood and liver tissue are consistent with the reports of Kim et al.[21] which concluded that rats with hyperlipidemia showed decreases in total cholesterol and lipid levels due to the supplementation of diets with mulberry leaf extracts. In addition, reports of Doi et al[23] showed that triglyceride levels were lowered as a result of supplementing a 1% cholesterol diet with a 10 % dried mulberry powder, and that a 1% cholesterol diet group fed with 2.5% of dried mulberry leaf powder recorded effective results in improving triglyceride levels.

These results indicate that cholesterol levels in the serum were lower when mulberry leaf extracts were fed to rats with serum high cholesterol,[21] due to the reduced activity of HMG-CoA reductase in the initial stage of combining cholesterol. Given the fact that dosage with mulberry leaf extracts reduces the level of serum cholesterol and the concentration of serum triglyceride, it is expected to have potential for the effective treatment and prevention of arteriosclerosis as well as for the prevention of hyperlipemia.

In summary, mulberry leaf extracts prepared by adding subsidiary materials such as pine needle and mugwort extracts were found to reduce the unpleasant smell of mulberry leaf extracts, thereby increasing human palatability, and mulberry leaf extracts were found to improve the lipid composition of liver and serum in rats.

Literature Cited

1) Korean Statistical Association. Korea Death Satistical, 2002
2) Trowell H. Crude fiber, dietary fiber and atherosclerosis. *Atherosclerosis* 16(1) : 138-140, 1972
3) Kohomoto T, Fukui F, Takaku H, Machida Y, Mitsuoka T. Effect of isomaltooligosaccharide on human fecal flora. *Biofidobactria Microflora* 7 : 61-67,1988
4) Tang W, Eisenbrand G. Chinese drugs of plant origin. *Springer-Verlag,* 669-696,1992
5) Purusotam Basnet, Shigetoshi Kadota, Satoshi Terashima, Mineo Simazu and Tsuneo Namba. Two new 2-arylbenzofuran derivatives from hypoglycemic activity-bearing fractions of Morus insignis. *Chem Pharm* Bull 41(7) : 1238-1243, 1993
6) Massayasu Kimura, Fu-jun Chen, Noboru Nakashimqa, Ikuho Kimura, Naoki Asano, Sakuji Koya. Anti Hyperglycemic effect of N-containing sugars delived from Mulberry leaves in streptozotocin-induced diabetic mice. J *Traditional Medicine* 12 : 214-219,1995
7) Lee JS. Blood Glucose Lowering Effect of SangYup (Mori Folium). Kyung Hee University Master course in Pharmacy, 1994
8) Kim SY, Lee WC, Kim HB, Kim SK. Antihyperlipidemic effects og methanol extracts from mulberry leaves in cholesterol induced hyperlipidemia in rats. *J Kor Soc Food Sci Nutr* 27 : 1217-1222, 1998
9) Shin DH. Antioxidative substances in mulberry leaves, *Journal of Korean Oil Chemists' Soc.* 16(3) : 27-31, 1998
10) Yun SJ, Lee WC. Characteristics of superoxide dismutases of mulberry leaf. *Korean J. Breed.* 26(4) : 389-393, 1994
11) Chae JY, Lee JY, Hoang IS, Whangbo D, Choi PW, Lee WC, Kim JW, Kim SY, Choi SW, Rhee J. Analysis of functional components of leaves of different mulberry cultivars. *J. korean Soc. Food Sci. Nutr.* 32(1) : 15-21, 2003
12) Mailgaard M, Civille GV, Carr BT. Sensory evaluation techniques. 2nd ed. *CRC Press* 231-232, 1990
13) Bang HS, Lee WC, Shon HR, Choi YC, Kim HB. Varietal comparison of γ - aminobutyric acid content in mulberry root bark. *Kor J Seric Sci.* 40 : 13-16, 1998
14) Stead DA, Richards RM. Sensitive fluorimetric determination

of gentamicin sulfate in biological matrices using solid-phase extracction, pre-column derivatization with 9-fluorenylmethyl chloroformate and reversed-phase high performance liquid chromatography. *J Chromatogr B Biomed Appl* 26 : 675(2) : 295-302, 1996

15) Cole MD, Fellows LE, Romeo JT. Separation of hydroxy and polyhydroxy derivatives of 2-carboxy and 2-hydroxy-methyl piperidine and pyrrolidine by high-performance liquid chromatography. *J Chromatogr* 445 : 295-298, 1998.
16) Yun SJ, Lee WC. Studies on the utilization of pharmacologically active constituents in mulberry : 1. varietal and seasonal variations of flavonol glydoside content in leaves. *RDA J Agr Sci.* 37 : 201-205, 1995
17) Reitman S, Frankel S. A colocrimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. *Am J Clin Pathol,* 28 : 56,1957
18) Folch JM, Lee M, Stanley GHS. A simple method for the isolation and purification of total lipids from animal tissue. *J Biol Chem* 26 : 497-509, 1957
19) Sale FD, Marchesini S, Fishman PH, Berra B. A sensitive enzymatic assay for determination of cholesterol in lipid extracts. *Academic Press Inc,* p347-350, 1984
20) Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with thefolin phenol reagent. *J Biol Chem* 193 : 265-275, 1951
21) Kim SY, Lee WC, Kim HB, Kim AJ, Kim SK. Antihyperlipid emic effects of methanol extracts from muberry leaves in cholesterol -induced hyperlipidemia in rats. *J Korean Soc Food Sci Nutr* 27 : 1217-1222, 1998
22) Hong YJ. Effects of Mori Radicis Cortex on lipidperoxidation and hepatotoxicity. Sook Myung Women's University in Pharmacy, 1995
23) Doi K, Kojima T, Harada M, Horiguchi Y. Effect of mulberry leaves on lipid metabolism in rabbits fed a cholesterol diet. *J Jpn Soc Nutr Food Sci* 47 : 15, 1994