Acanthopanax senticosus Extract Prepared from Cultured Cells Improves Lipid Parameters in Rats Fed with a High Fat Diet

Youn-Soo Cha[†], Ju-Ryoun Soh and Jae-Whune Kim[*]

*Department of Food Science and Human Nutrition, and Institute for Molecular Biology and Genetics,
Chonbuk National University, Chonju 561-756, Korea
Microplants Co., Ltd., Hanhwa R & D Center, Daejeon 305-804, korea

Abstract

Acanthopanax senticosus was grown by a novel, proprietary method, of culturing isolated cells in a bioreactor. An extract from the cells was evaluated for its effect on lipid metabolism in rats fed a high fat diet. Male Sprague-Dawley rats (n=6) were fed either an AIN-76 diet (control, NDCon), control diet plus *Acanthopanax senticosus* extract (ND+Ex), a modified AIN-76 diet supplemented with 20% beef tallow (high fat, HFCon), or a high fat diet plus *Acanthopanax senticosus* extract (HF+Ex), for 5weeks. Body weight gain was significantly higher in the HFCon group than the NDCon group. Feed consumption was significantly lower, but energy intake higher, in the groups fed high fat diets compared with the groups fed control diets. Serum HDL-cholesterol concentrations were significantly increased but serum LDL-cholesterol concentrations were decreased in the groups fed the *Acanthopanax senticosus* extract. Abdominal fat accumulation and serum leptin levels were significantly higher in the HFCon group than the other groups. Carnitine palmitoyltransferase-I (CPT-I) mRNA levels were increased in the groups fed *Acanthopanx senticosus* extract. These results suggest that supplementation of cell cultured *Acanthopanax senticosus* extract regulates CPT-I mRNA levels in liver and has an effect on the normalization of lipids in rats fed a high fat diet.

Key words: *Acanthopanax senticosus*, dietary fat, lipid parameter, carnitine palmitoyltransferase-I

INTRODUCTION

The etiology of obesity is associated with both genetic susceptibility (1) and environmental/life style factors (2). Obesity is a chronic disease which represents a major health problem in the Unitied States and is emerging as a major health problem in many other developed and developing countries (3). In the US, an estimated 33% of adults are obese (4), and an estimated 300,000 premature deaths annually (5) are attributed to obesity and physical inactivity. In addition to premature death, obese individuals have increased risks for coronary heart disease, hypertension, diabetes, arthritis, respiratory problems and certain types of cancer (6).

Acanthopanax senticosus is a frequently used, traditional oriental medicinal herb that has been traditionally used as a tonic, anti-rheumatic and prophylactic for chronic bronchitis, hypertention, anti-stress, ischemic heart disease, and gastric ulcer (7,8). A liquid extract from *Acanthopanax senticosus* roots inhibited the productive replication of the RNA viruses: human rhinovirus (HRV), respiratory syncytial virus (RSV), and influenza A virus in cultures of infected cells (9). In a recent study, it was reported that a methylene chloride extract of *Acanthopanax senticosus* exhibited a 97% inhibition of mouse leukemia L1210 cell replication, and thrombin activity by 82% (10). It was also able to decrease experimental hyperglycemia induced by injection of adrenaline, glucose and alloxan, without affecting the levels of blood sugar in mice (11). Hyperlipemia patients treated with a mixture containing wild *Acanthopanax senticosus* increased HDL cholesterol concentrations compared with an untreated control group (12).

Unfortunately, wild *Acanthopanax senticosus* is expensive, and the cultivated plants require many years to develop sufficient concentrations of the active components. As a result there have been many attempts to develop methods for cultivating fully developed plants more quickly (13-15). However, few studies have investigated the efficacy of cultured *Acanthopanax senticosus* cells. In this study we evaluated the effectiveness of *Acanthopanax senticosus* cells grown by a novel method proprietary method on lipid metabolism in animals fed a high fat diet.

One potential method for developing large amounts of active herb is to culture isolated cells in bioreactors rather than growing plants from seeds. If successful, this technique could make possible the production of functional

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[†]Corresponding author. E-mail: cha8@moak.chonbuk.ac.kr
Phone: +82-63-270-3822, Fax: +82-63-270-3854

botanicals in days rather than years. This study evaluated the effect of an extract from cultured *Acanthopanax senticosus* cells on serum lipid and leptin levels, abdominal fat deposition, and hepatic CPT-I mRNA levels of mice fed a high fat diet, and assessed its possible protective effects against high fat diet-induced obesity.

MATERIALS AND METHODS

Materials

Cultured *Acanthopanax senticosus* cells with a torpedo shape were supplied by Microplants Co., Ltd (Yusung, Korea). The *Acanthopanax senticosus* cells were dried, ground to a fine powder, and extracted with deionized water, thirty times volume, for 9 hours at 80°C. The resulting extracts were filtered, concentrated under vacuum at 60°C and stored at 4°C until used.

Animal and diets

Twenty-four male Sprague-Dawley rats weighing about 130~160 g were purchased from Daehan Biolink Inc. (Daejon, Korea). They were housed individually in stainless steel cages with a randomized complete block design, at a temperature of 23 ± 1°C, humidity of 53 ± 2%, and a light controlled room with a 12-hr light-dark cycle. The animals were fed normal chow (Jeil-jedang, Suwon, Korea) for 1 week, then randomly divided into 4 groups: control diet (NDCon), high fat diet (HFCon), control diet plus *Acanthopanax senticosus* extract (ND + Ex), and high fat diet plus *Acanthopanax senticosus* extract (HF + Ex). The compositions of the experimental diets are shown in Table 1. The rats were allowed free access to the diets and water. The rats were orally administered *Acanthopanax senticosus* extract (0.5 g/kg body weight) once a day for 5 weeks in the *Acanthopanax senticosus*-treated groups, and distilled water of the same volume in *Acanthopanax senticosus*-non-treated groups.

Table 1. Composition of experimental diets[1]

Ingredient	Normal diet		High-fat diet	
	NDCon	ND + Ex[2]	HFCon	HF + Ex
Casein	20.0	20.0	20.0	20.0
Sucrose	50.0	50.0	50.0	50.0
Starch	15.0	15.0	-	-
Corn oil	5.0	5.0	-	-
Beef tallow	-	-	20.0	20.0
Cellulose	5.0	5.0	5.0	5.0
Mineral mixture	3.5	3.5	3.5	3.5
Vitamin mixture	1.0	1.0	1.0	1.0
Choline bitartrate	0.2	0.2	0.2	0.2
DL-methionine	0.3	0.3	0.3	0.3
Total	100.0	100.0	100.0	100.0

[1] All components are in units of g/100 g diet.
[2] In + Ex groups, *Acanthopanax senticosus* extract (0.5 g/kg body weight) was administered for 5 weeks, as described in Materials and Methods.

Before the rats were sacrificed, the diet was removed from the cages for 12 h. Blood samples were collected from each rat and incubated on ice water for 1 h. Serum was separated from the blood by centrifugation at 1,100 × g for 15 min at 4°C and kept at 80°C until analysis. The liver and abdominal fat were removed, rinsed with a phosphate buffered saline solution, wiped with a paper towel, weighed, quickly frozen in liquid nitrogen, and stored at -80°C until assayed.

Analysis of lipids and leptin

Triglyceride concentrations in serum were determined by the lipase-glycerol phosphate method (16) using a commercial kit (Asan Pharm. Co., Seoul, Korea). Serum total cholesterol was determined using a commercial kit from Asan Pharm. Co (Seoul, Korea), based on the cholesterol oxidase method (17). HDL-cholesterol was analyzed enzymatically using a commercial kit (Asan Pharm. Co., Seoul, Korea). The HDL-cholesterol fractions were prepared by the dextran sulfate-Mg^{++} method (18). LDL-cholesterol concentrations were calculated by the Friedwald method (19). Serum leptin levels were assayed by ^{125}I-labeled leptin RIA using a commercial kit (Linco Research, USA). Radioactivity of the samples was determined in a Gamma Scintillation counter.

CPT-1 mRNA levels

Total RNA from fresh rat livers was isolated with a commercial total RNA isolation kit (Sigma, St. Louis, USA) using the guanidine thiocyanate/silica-based system. Total RNA were loaded on 1.2% agarose-formaldehyde gel, electrophoresed and transferred to a nylon membrane (Ambion, Austin, USA). The CPT-I DNA fragment obtained form the CPT-I cDNA, and cDNAs were labeled with biotin using the non-isotopic Psoralen-biotin labeling kit (Ambion, Austin, USA). Hybridization of the probe to the membrane-bound mRNA was performed at 42°C for 22 hrs. The membrane was washed to remove the non-specifically bound probe and incubated in the blocking, conjugation, blocking and CDP-star solution of the BrightStart™ BioDetect™ kit (Ambion, Austin, USA). The membrane was then exposed to X-ray film (Fuji, Tokyo, Japan) for 90 min, at room temperature.

Statistical analysis

Data from individual experiments are expressed as the mean ± standard deviation. All statistical analyses were performed on SAS version 7 (SAS Institute, Cary, NC, USA). Significant differences between mean values were determined by Duncan's multiple range test (20); $p < 0.05$ was judged to be statistically significant.

RESULTS

Body weight gain and food intake

Feed consumption was significantly lower in the groups

fed high fat diets compared with the groups fed control diets (Table 2). The group fed the HF + Ex group consumed significantly less feed than the other groups, there was no difference between the two groups fed control diets in feed consumption. However, energy intake was increased in groups fed high fat diets, the highest energy intake was in the HFCon group (Table 2). Body weight gain was significantly higher in the high fat diet group (HFCon) compared with the other three groups. However, the *Acanthopanax senticosus* extract prevented the increased weight gain from the high fat diet (Table 2). Feed efficiency ratio was significantly lower in the ND + Ex group than in the HF + Ex group (Table 2).

Lipid levels

Serum HDL cholesterol was significantly increased in the ND + Ex group compared with the NDCon group (Table 3), conversely, serum LDL cholesterol concentrations were decreased in the ND + Ex group compared with the NDCon group (Table 3). In groups fed high fat diets, the extract supplement also increased HDL cholesterol while lowering LDL (Table 3).

Abdominal fat deposition and serum leptin levels

Abdominal fat deposits and serum leptin levels were the same in all groups except the HDCon group, in which they were both significantly higher than in the other groups (Fig. 1 and Fig. 2).
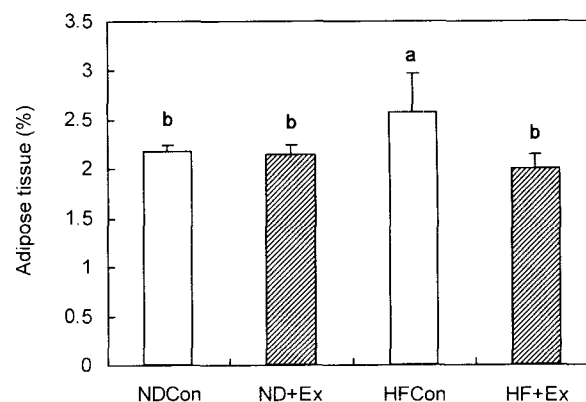
Fig. 1. Effects of the *Acanthopanax senticosus* extract on abdominal fat rate. The error bars show the standard deviation of the mean for 6 rats. Letters above the bars are indicate significantly differences (p < 0.05) by Tukey's test.

Hepatic CPT-I mRNA levels

In liver, CPT-1 mRNA levels, which are associated with fatty acid β-oxidation, were highest in the ND + Ex group lowest in the NDCon group, and intermediate in the HFCon and HF + Ex groups (Fig. 3).

DISCUSSION

High fat diets are known to lead to increased body weight (21). High fat diets result in increases in energy intake and efficiency of energy storage (22). In this study,

Table 2. Effects of *Acanthopanax senticosus* administration and/or a high fat diet on feed consumption and body weight gain in rats

	Normal diet		High-fat diet		ANOVA[1]		
	NDCon	ND + Ex	HFCon	HF + Ex	D	A	DxA
Feed consumption (g)	$19.29 \pm 0.43^{2)a3)}$	19.96 ± 1.29^a	18.13 ± 0.25^b	16.14 ± 0.63^c	0.0001	0.0423	0.001
Energy intake (kcal/d)	74.26 ± 1.66^c	76.85 ± 4.99^c	94.27 ± 1.31^a	83.93 ± 3.25^b	0.0001	0.0405	0.0001
Initial body weight (g)	190.86 ± 3.62	190.56 ± 4.25	184.70 ± 5.64	186.44 ± 4.20	NS	NS	NS
Weight gain (g)	119.19 ± 9.45^b	122.31 ± 10.20^b	139.89 ± 3.92^a	120.49 ± 13.01^b	NS	NS	NS
Feed efficiency ratio[4]	1.65 ± 0.16^{ab}	1.72 ± 0.17^a	1.49 ± 0.15^{ab}	1.43 ± 0.17^b	0.0066	NS	NS

[1]The degree of significance resulting from the 2-way ANOVA are shown with effects of diet (D), administration of *Acanthopanax senticosus* extract (ASEx), and the interaction of diet and administration of ASEx (DxA) being expressed as the numerical value or as not significant (NS) when p < 0.05.
[2]All values are means ± SD (n=6).
[3]Values with different superscripts are significantly different (p < 0.05).
[4]Feed efficiency ratio was calculated as weight gain (day)/dietary intake (day).
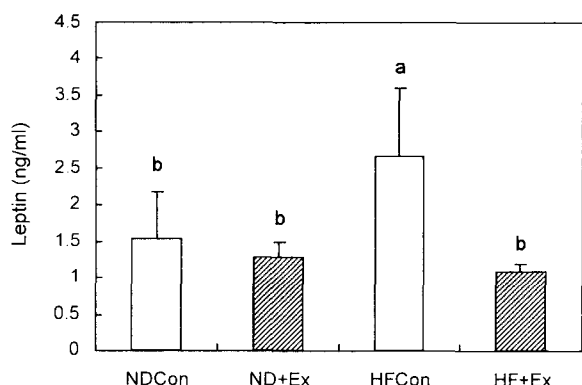
Table 3. Effects of *Acanthopanax senticosus* extract on serum lipid concentrations   (mg/dL)

|	Normal diet		High-fat diet		ANOVA[1]		
	NDCon	ND + Ex	HFCon	HF + Ex	D	A	DxA
HDL-cholesterol	$40.13 \pm 5.75^{2)b3)}$	53.11 ± 8.36^a	30.20 ± 7.08^c	42.96 ± 7.03^b	0.0138	0.0017	NS
LDL-cholesterol	32.44 ± 7.26^b	21.72 ± 9.12^b	46.40 ± 11.90^a	24.83 ± 3.37^b	NS	0.0015	NS

[1]The degree of significance resulting from the 2-way ANOVA are shown with effects of diet (D), administration of *Acanthopanax senticosus* extract (ASEx), and the interaction of diet and administration of ASEx (DxA) being expressed as the numerical value or as not significant (NS) when p < 0.05.
[2]All values are means ± SD (n=6).
[3]Values with different superscripts are significantly different (p < 0.05).

Fig. 2. Effects of the *Acanthopanax senticosus* extract on serum leptin levels. The error bars show the standard deviation of the mean for 6 rats. Letters above the bars are indicate significant differences ($p < 0.05$) by Tukey's test.
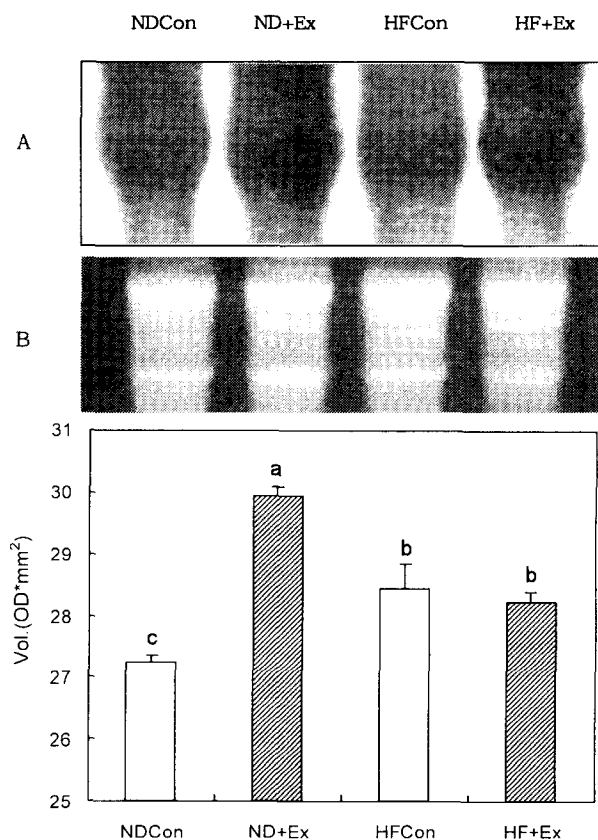
Fig. 3. Hepatic CPT-I mRNA levels. Total RNA was separated by 1.2% agarose gel electrophoresis and transferred to a Nylon membrane. Hybridization and visualization were conducted as described in Materials and Methods. A, Northern blot of mRNA; B, ethidium bromide stain of RNAs; NDCon, normal diet control HFCon, high fat diet control; ND + Ex, Normal diet plus *Acanthopanax senticosus* extract; HF + Ex, high fat diet plus *Acanthopanax senticosus* extract. The expression level in each animal was quantified by densitometer. Value with superscript above the bar is significantly different ($p < 0.05$, n=6).

body weight was increased by high fat diet in the HFCon group, but remained normal in the HF + Ex group. Therefore, we demonstrated that cell cultured *Acanthopanax*

senticosus extract reduces the effect of high fat diets in increasing body fat stores in rats.

There is a substantial body of evidence that high serum LDL cholesterol levels are a reliable indicator of abnormal lipoprotein metabolism and are positively correlated with the risks for both coronary heart disease and atherosclerosis (23,24).

In a study of the effect of wild *Acanthopanax senticosus* on lipid profiles, the water extracts administered orally for 5 consecutive days significantly reduced serum total cholesterol and triglyceride concentrations in rats fed a lipid rich diet (15% cholesterol, 1% sodium cholate and 84% corn oil). HDL-cholesterol, however, was increased significantly (25). In this study we demonstrated that serum HDL-cholesterol levels are also significantly increased by *Acanthopanax senticosus* extract from isolated cell cultures, suggesting that the cultured cells of *Acanthopanax senticosus* also have sufficient concentrations of the active component for regulation lipid metabolism, and provides evidence that cultured cells of *Acanthopanax senticosus* are a viable alternative to wild or cultivated plants.

Ainslie et al. (26) reported that feeding rats a high fat diet for 4 weeks resulted in reduced leptin secretion in adipose tissue. Adiposity located centrally in the abdominal region is distinctly associated with hyperlipidemia, compared with generalized distributions of body fat, and is also associated with lipoprotein abnormalities characterized by elevated VLDL and LDL concentrations. An increase in abdominal fat is a precursor to increased lipolysis, elevated free fatty acid flux, and in part promotes aberrations in insulin actions. The results of the impaired insulin action include changes in glucose/insulin homeostasis and lipoprotein metabolism, leading to metabolic disorders such as glucose intolerance, and increased risk for coronary heart disease (27). In this study that abdominal fat accumulation was decreased in the HF + Ex group compared with HFCon group, suggesting that cell cultured *Acanthopanax senticosus* extract can decrease the risk for coronary heart disease.

In a different study, hepatic CPT-I mRNA expression was increased by exercise and high fat diet (28), suggesting that control of CPT-I gene expression is a key feature in the regulation of fatty acid oxidation during exercise (28,29). Fat feeding, fasting, induced diabetes or treatment of rats with peroxisomal/mitochondrial proliferating agents, all enhance the capacity for hepatic fatty acid oxidation, and increase the mRNA and activity levels of CPT-I (30, 31). Under these conditions, the expression of CPT-I is required to obtain energy from fatty acids, the primary energy substrate.

In this study, cell cultured *Acanthopanax senticosus* extract significantly increased CPT-I expression at the tran-

scription level in rats fed a normal diet but not rats fed a high fat diet, although the high fat diet did marginally increase CPT-1 expression with or without the extract. It is reasonable to assume that increased availability of fatty acid substrate from the high fat diets induced the increased hepatic CPT-1 mRNA expression that was seen in this study. It is interesting that both high fat diet and administration of cell cultured *Acanthopanax senticosus* extract increased hepatic CPT-I expression, but there was not a cumulative effect when the two were combined. Rather, a high fat diet appeared to partially reduce the effect of the extract on CPT-1 expression; it is possible that a higher dose of the extract could restore CPT-1 expression to the same level as the extract combined with a normal diet.

In conclusion, we have demonstrated that cell cultured *Acanthopanax senticosus* extract has beneficial effects, and high fat diet negative effects, on abdominal fat accumulation, and on serum HDL-cholesterol and LDL cholesterol concentrations. Accordingly, this research demonstrates that culturing isolated cells is a viable method for producing *Acnathopanax senticosus* with active components; and suggests that this novel technology may be useful for producing large quantities of functional ingredients rapidly and inexpensively.

ACKNOWLEDGMENTS

REFERENCES

1. Pusse L, Chagnon YC, Weisnagel SJ, Rankinen T, Snyder E, Sands J, Bouchard C. 2001. The human obesity gene map: The 2000 update. *Obes Res* 9: 135-169.
2. Popin BM, Doak CM. 1998. The obesity epidemic is a worldwide phenomenon. *Nutr Rev* 56: 106-114.
3. Bjorntorp P. 1997. Obesity. *Lancet* 350: 423-426.
4. Kuczmarski RJ, Flegal KM, Campbell SM, Johnson CL. 1994. Increaseing prevalence of overweight among US adults: The National Health and Nutrition Examination Surveys, 1960 to 1991. *J Amer Med Assoc* 272: 205-211.
5. McGinnis JM, Foege WH. 1993. Actual causes of death in the United States. *J Amer Med Assoc* 270: 2207-2212.
6. Pi-Sunyer FX. 1993. Medical hazards of obesity. *Ann Intern Med* 110: 655-660.
7. Nishibe S, Kinoshita H, Takeda H, Okano G. 1990. Phenolic compounds form stem bark of *Acanthopanax senticosus* and their pharmacological effect in chronic swimming stressed rats. *Chem Pharm bull* 38: 1763-1765.
8. Fujikawa T, Yamaguchi A, Morita I, Takeda H, Nishibe S. 1996. Protective effects of *Acanthopanax senticosus* harms from Hokkaido and its components on gastric ulcer in restrained cold water stressed rats. *Biol Pharm Bull* 19: 1227-1230.
9. Bernadette GS, Fritz S, Anke E. 2001. Antiviral activity of an extract derived from roots of *Eleutherococcus senticosus*. *Antiviral Research* 50: 223-228.
10. Elena AC, Petrichenko VM, Solodnikov SU, Suhinina TV, Kline MA, Cunningham G, Nguyen C, Miles H. 2002. Anticancer and antithrombin activity of Russian plants. *J Ethnopharm* 81: 337-342.
11. Sui DY, Lu ZZ, Li SH, Cai Y. 1994. Hypoglycemic effect of saponin isolated from leves of *Acanthopanax senticosus*. *Chinese Materia Medica* 19: 683-685.
12. Shi Z, Liu C, Li R. 1990. Effect of a mixture of *Acanthopanax senticosus* and *Elsholtzia splendens* on serum-lipids in patients with hyperlipemia. *Chinese Materia Medica* 10: 155-156.
13. Choi YE, Kim JW, Yoon ES. 1990. High frequency of plant production via somatic embryogenesis from callus or cell suspension cultures in *Eleutherococcus senticosus*. *Ann Botany* 83: 309-314.
14. Kim JW, Kim HS. 2001. Mass production of Siberian Ginseng (*Eleutherococcus senticosus*) somatic embryos by cell culturing. *Oriental Pharm Exper Med* 1: 34-38.
15. Jeong HJ, Koo HN, Myung NI, Shin MK, Kim JW, Kim DK, Kim KS, Kim HM, LeoYM. 2001. Inhibitory effects of mast cell-mediated allergic reactions by cell cultured Siberian Ginseng. *Immunopharm Immunotoxicol* 23: 107-117.
16. McGowan MW, Artiss JD, Strandbergh DR, Zak B. 1983. A peroxidase-couple method for the colormetric determination of serum triglycerides. *Clin Chem* 29: 538-542.
17. Allain CC, Poon LS, Chan CS, Richman W, Fu PC. 1974. Enzymatic determination of total serum cholesterol. *Clin Chem* 20: 470-475.
18. Warnick JB, Benderson J, Albers JJ. 1978. HDL precipitation by dextran sulfate-MgCl$_2$ method. *Clin Chem* 28: 1379-1383.
19. Friedwald WT, Levy RL, Fredrickso DS. 1972. Estimation of the concentration of low density lipoprotein cholesterol without use of the preparation ultracentrifuge. *Clin Chem* 18: 499-502.
20. Duncan DB. 1993. Multiple range test for correlated and heteroscedastic means. *Biometerics* 13: 164-176.
21. Choi JS, Lee JH, Young HS. 1995. Anti-hyperlipidemic effect of Phragmites communis and its active priniciples. *J Korean Soc Food Nutr* 24: 523-529.
22. Hill JO, Melanso EL, Wyatt HT. 2000. Dietary fat intake and regulation of energy balance: Implications for obesity. *J Nutr* 130: 284S-288S.
23. Goldstein JL, Brown MS. 1983. Lipoprotein receptors: Genetic defense gainst atherosclerosis. *Clin Res* 30: 417-423.
24. Steinberg D, Witztum JL. 1990. Lipoproteins and atherogenesis. *J Am A* 264: 3047-3052.
25. Lee YS, Jung SH, Lim SS, Ji J, Lee SH, Shin KH. 2001. Effects of the water extract from the stem bark of *Acnathopanax senticosus* on hyperlipidemia in rats. *Kor J Pharmacogn* 32: 103-107.
26. Ainslie DA, Proietto J, Fam BC, Thorburn AW. 2000. Short-term, hight-fat diets lower circulationg leptin concentration in rats. *Am J Clin Nutr* 71: 438-422.
27. Kissebah AH. 1996. Intra-abdominal fat: is it a major factor in developing diabetes and coronary artery disease? *Diabetes Res Clin Pract* 30: 25-30.
28. Shon HS, Oh SH, Cha YS. 1999. Effects of exercise and/or high fat diet on carnitine and carnitine palmitoyltransferase-I mRNA levels in rats. *J Korean Soc Food Sci Nutr* 28: 670-676.
29. Cha YS, Kim HY, Soh JR, Oh SH. 2001. Effects of regular

endurance exercise or acute-exercise and rest on the levels of lipids, carnitines and carnitine palmitoyltransferase-I in rats. *J Biochem Mole Biol* 34: 434-439.

30. McGarry JD, Brown NF. 1997. The mitochondrial carnitine-palmitoyltransferase system: from concept to molecular anal-ysis. *Eur J Biochem* 244: 1-12.

31. Bonnefont JP, Demaugre F, Prip-buus C, Saudubray JM, Brivet M, Abadi, Thuiller L. 1999. Carnitine palmitoyltransferase deficiencies. *Mole Gene Metabol* 68: 424-440.