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Enhancement of Lipid Metabolism and Antioxidant Defense Status in Mice Fed with High Fat Diet Supplemented with *Antheraea pernyi* Silk Fibroin Powder

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The effect of diet supplementation of *Antheraea pernyi* (*A. pernyi*) silk fibroin on the lipid metabolism and antioxidant defense status in high fat-fed mice was investigated. The animals were given normal control diet (NC group), high fat diet (HF group), or high fat diet supplemented with *A. pernyi* silk fibroin powder (HFS group) for 7 weeks. After the experimental period, the HF group showed significant increase in body weight, plasma and hepatic total cholesterol levels, and hepatic triglyceride concentration, and decreased activities of hepatic antioxidant enzymes relative to NC group. However, the HFS group exhibited marked reduction in body weight, plasma cholesterol and hepatic triglyceride levels, hepatic lipogenic enzyme activities, and lipid peroxidation rate, and higher high-density lipoprotein (HDL)-cholesterol level, fecal triglyceride content, and antioxidant enzymes activities compared with that of HF group. These findings demonstrate that dietary feeding of *A. pernyi* silk fibroin could improve the lipid metabolism and antioxidant defense system via regulation of hepatic antioxidant and lipogenic enzymes activities. Hence, this silk fibroin may be beneficial as a functional biomaterial for the development of therapeutic agent against high fat diet-induced hyperlipidemia and its related diseases.

Key words: *Antheraea pernyi* silk fibroin, Antioxidant enzyme, High fat diet, Lipid metabolism

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Introduction

High dietary fat intake has been associated with the development of obesity and hyperlipidemia (Bray and Popkin, 1998), which could then results in various metabolic syndromes, including type II diabetes mellitus and cardiovascular disease (Formiguera and Canton, 2004). Changes in the lifestyle patterns, particularly poor eating habit and sedentary lifestyle, have led to the rapid increase in the incidence of obesity and dyslipidemia in both developed and developing countries. With the rising trend in the global occurrence of obesity-associated health problems, the need for therapeutic measures against metabolic diseases has now become stronger and more urgent. Scientists and researchers are attempting to find natural drugs that could improve the lipid metabolism and promote the antioxidant defense system using animal models.

Silk proteins are natural polymers derived from the polyamino acid-based cocoons produced by silkworms to protect themselves during metamorphosis to moths (Hardy *et al*., 2008). The silk fiber, which contains 98% protein, is mainly composed of fibroin and sericin (Mondal *et al*., 2007), and has long been used in the production of textiles. In addition, various silk proteins have been studied and industrialized as a functional food due to its useful biological activities. It was reported that the *Bombyx mori* (*B. mori*) silkworm powders showed an excellent activity of lowering blood glucose levels (Ryu *et al*., 2002) and silk sericin exhibited a strong antioxidant activity (Fan *et al*., 2009). Hong *et al*. (2002) investigated the effect of the acid hydrolysate of silk fibroin on obesity using obese mice and found that the body weight was significantly reduced in mice fed with *B. mori* silk fibroin hydrolysate.

Among the various species of silkworms, *A. pernyi* and *B. mori* are the most commonly used in sericulture (Zhou and Han, 2006). However, in spite of the importance of *A. pernyi* silk in silk science and industry, the study on the biologically functional properties of *A. pernyi* silk has not been done in detail yet. Hence, this study was conducted to examine the physiological activity of *A. pernyi* silk fibroin using animal model. Specifically, the effect of dietary feeding of *A. pernyi* silk fibroin powder on the lipid metabolism and antioxidant defense status in high fat-fed C57BL/6N mice was investigated.

Materials and Methods

Preparation of *A. pernyi* silk fibroin powder

A. pernyi silkworm was cultivated in National Academy of Agricultural Science, Rural Development Administration, Korea and its cocoon was degummed using enzymatic degumming method as previously described (Kweon and Park, 2001; Kweon *et al*., 1999). Briefly, *A. pernyi* cocoon was cut into small pieces and treated with degumming solution (1 g/l Alcalase 2.5l, 5 g/l sodium bicarbonate, and 1 g/l nonionic surfactant) at 55°C for 60 min. The degummed cocoon was rinsed with a mixture solution containing 2 g/l nonionic surfactant and 5% sodium hydrosulfite and washed thoroughly with warm distilled water. The degummed cocoon was dried at room temperature and stored in a desiccator prior to use.

The degummed silk fiber cocoon was hydrolyzed using hydrochloric acid according to previously described method (Kweon and Park, 2001; Kweon *et al*., 1999). Briefly, the *A. pernyi* cocoon was treated with hydrochloric acid and the hydrolyzed solution was neutralized with sodium hydroxide and desalted using electro-dialysis system (Micro Acilyzer S3, Asahi Chemical Co., Japan). The desalted *A. pernyi* silk fibroin solution was then lyophilized to obtain the *A. pernyi* silk fibroin powder.

Animals and diet

Thirty male C57BL/6N mice of 4 weeks of age, weighing 12 g, were obtained from Orient Inc. (Seoul, Korea). They were individually housed in stainless steel cages in a room maintained at 25°C with 50% relative humidity and 12/12 h light/dark cycle and fed with a pelletized chow diet for 2 weeks after arrival. The mice were then randomly divided into 3 dietary groups (n=10). The first mice group was fed with a normal control diet (NC group), the second group was given a high fat (17%, w/w) diet (HF group), and the third group was fed with a high fat diet supplemented with 1% (w/w) *A. pernyi* silk fibroin (HFS group). The composition of the experimental diet (Table 1) was based on the AIN-76 semi-synthetic diet. The mice were fed for 7 weeks and allowed free access to food and water during the experimental period. The food consumption and weight gain were measured daily and weekly, respectively. Feces were collected during the final week to measure the level of fecal cholesterol and glyceride excretion. At the end of the experimental period, the mice were anaesthetized with Ketamine-HCl following a 12-h fast. Blood samples were drawn from the inferior vena cava into a heparin-coated tube and centrifuged at 1,000 x g for 15 min at 4°C to obtain the plasma and erythrocyte. The livers were removed, rinsed with physiological saline, and stored at −70°C until analysis. The current study protocol was approved by the Ethics Committee of Kyungpook National University for animal studies.

Table 1. Composition of the experimental diets

Component	Composition (%)		
	NC	HF	HFS
Casein	20.0	20.0	20.0
DL-Methionine	0.3	0.3	0.3
Sucrose	50.0	50.0	49.0
Corn starch	15.0	--	--
Cellulose	5.0	5.0	5.0
Corn oil	5.0	3.0	3.0
Cholinbitartrate	0.2	0.2	0.2
Mineral mixture[a]	3.5	3.5	3.5
Vitamin mixture[b]	1.0	1.0	1.0
Lard	--	17.0	17.0
A. pernyi silk fibroin	--	--	1.0
Total (%)	100.0	100.0	100.0

NC, normal control diet; HF, high fat diet; HFS, high fat diet + *A. pernyi* silk fibroin.
[a]AIN-76 mineral mixture.
[b]AIN-76 vitamin mixture.

Determination of lipid levels in plasma, liver, and feces

The plasma total cholesterol, triglyceride, and high-density lipoprotein (HDL) cholesterol levels were determined using a commercial kit (Asan Pharmaceutical, Seoul, Korea). The liver and fecal lipids for total cholesterol and triglyceride analyses were extracted and purified using the method described by Folch *et al*. (1957). The concentrations of cholesterol and triglycerides in liver and feces were measured using the same enzymatic kit used in the plasma analysis.

Measurement of lipid peroxidation

The erythrocyte and plasma thiobarbituric acid reactive

substances (TBARS) were measured according to the method of Ohkawa *et al*. (1979). Trichloroacetic acid (5%, v/v) and 0.06 M thiobarbituric acid were added to 50 µl of plasma and red blood cell preparation and incubated at 95°C for 90 min. After cooling at room temperature, the sample mixtures were centrifuged at 2,000 rpm for 25 min and the absorbance of the resulting supernatant was determined at 535 nm. A malondialdehyde (MDA) solution was used as standard. The results were calculated and expressed as nmol MDA/ml plasma or g hemoglobin (Hb).

Determination of hepatic lipid-regulating and antioxidant enzymes activities

The glucose-6-phosphate dehydrogenase (G6PD) activity was measured based on the reduction of 6 mM NADP+ by G6PD in the presence of glucose-6-phosphate (Beutler, 1971). The enzyme activity was determined by monitoring the increase in absorption of the reaction mixture at 340 nm at 37°C. The activity was expressed as the reduction of 1 nmol of NADP/min. The malic enzyme (ME) activity was determined using the method of Ochoa (1955). The reaction mixture contained 0.4 M triethanolamine (pH 7.4), 30 mM malic acid, 0.12 M $MgCl_2$, and 3.4 mM NADP. The absorbance was measured at 340 nm at 27°C and the activity was expressed as nmol reduced NADPH /min/mg protein. The superoxide dismutase (SOD) activity was spectrophotometrically measured according to the method developed by Marklund and Marklund (1974). The SOD was detected on the basis of its ability to inhibit superoxide-mediated reduction. The activity was expressed as unit/mg protein, wherein one unit represents the amount of enzyme that inhibited the oxidation of pyrogallol by 50%. The glutathione peroxidase (GSH-Px) activity was measured according to the method of Paglia and Valentine (1967) with slight modifications. The cytosolic supernatant was added to the reaction mixture (6 mM glutathione, 1.2 mM NADPH, and 1.25 µM H_2O_2 in 20 mM Tris-HCl, pH 7.0), which was pre-warmed at 25°C for 5 min. The mixture was further incubated at 25°C for 5 min and the absorbance was measured at 340 nm. A molar extinction coefficient of 6.22/mM/cm was used to determine the activity, which was expressed as nmol oxidized NADPH/min/mg protein. The catalase (CAT) activity was measured using the method of Aebi (1974). The disappearance of hydrogen peroxide was monitored spectrophotometrically at 240 nm for 5 min. A molar extinction coefficient of 0.041/mM/cm was used to determine the CAT activity. The activity was defined as the µmol decreased H_2O_2/min/mg protein. The glutathione reductase (GR) activity was determined using the method of Mize and Langdon (1952). The reaction mixture contained 1 mM EDTA and 1 mM GSSG in a 0.1 M potassium phosphate buffer (pH7.4). The oxidation of NADPH was monitored at 340 nm and the activity was expressed as nmol oxidized NADPH/min/mg protein. The paraoxonase (PON1) activity was determined based from the method described by Mackness *et al*. (1991). Briefly, 50 µl of serum was added to 1 ml Tris/HCl buffer (100 mM, pH 8.0) containing 2 mM $CaCl_2$ and 5.5 mM paraoxon. The absorbance of the mixture was measured at 412 nm at 25°C to determine the generation rate of 4-nitrophenol. The enzymatic activity was calculated using the molar extinction coefficient 17100/M/cm.

Statistical analysis

All data are presented as the mean ± S.E. The data was evaluated by one-way ANOVA using a Statistical Package for Social Sciences software program (SPSS Inc., Chicago, Illinois, USA) and the differences between the means were assessed using Duncan's multiple range test. Statistical significance was considered at $p<0.05$.

Results

Body weight gain

Prior to feeding with the experimental diets, the body weights of mice did not significantly differ (Table 2). Throughout the entire study, the daily food intake was similar and constant (3 g/day) in all the animal groups. At the end of the experimental period, a marked increase in the body weight of HF mice group was observed relative to that of the NC group. The HFS mice, on the other hand, exhibited significantly lower body weight gain than the HF mice.

Plasma, hepatic, and fecal lipids

The plasma triglyceride concentration was not significantly different among the animal groups (Table 3). The plasma total cholesterol content was substantially higher in HF mice than that of the NC and HFS groups. A

Table 2. Body weight gain of mice fed with high fat diet supplemented with *A. pernyi* silk fibroin powder.

Dietary group	Initial weight (g)	Final weight (g)	Weight gain (g)
NC	20.43 ± 0.20^a	23.34 ± 0.54^a	2.90 ± 0.50^a
HF	20.40 ± 0.19^a	31.02 ± 0.50^c	10.61 ± 0.19^c
HFS	20.18 ± 0.20^a	25.76 ± 0.40^b	5.58 ± 0.34^b

NC, normal control diet; HF, high fat diet; HFS, high fat diet + *A. pernyi* silk fibroin.

Values are means ± SE (n = 10). Means in the same column not sharing a common superscript are significantly different at $p< 0.05$.

Table 3. Plasma, liver, and fecal lipid profiles in mice fed with high fat diet supplemented with *A. pernyi* silk fibroin powder.

Parameter	NC	HF	HFS
Plasma			
Triglyceride (mg/dl)	108.21 ± 1.78[a]	121.25 ± 1.80[a]	100.00 ± 2.41[a]
Total cholesterol (mg/dl)	134.89 ± 7.14[a]	170.18 ± 4.21[b]	136.19 ± 2.40[a]
HDL-cholesterol (mg/dl)	66.01 ± 2.88[a]	73.13 ± 5.47[ab]	81.93 ± 3.49[b]
HTR (%)*	49.71 ± 3.64[a]	43.09 ± 3.27[a]	60.03 ± 2.89[b]
Liver			
Triglyceride (mg/g)	22.59 ± 3.01[a]	51.88 ± 3.25[c]	38.52 ± 0.92[b]
Total cholesterol (mg/g)	3.26 ± 0.23[a]	5.39 ± 0.67[b]	4.47 ± 0.75[ab]
Fecal			
Triglyceride (mg/g)	5.91 ± 0.81[a]	7.20 ± 1.33[a]	62.12 ± 10.26[b]
Total cholesterol (mg/g)	4.60 ± 0.30[a]	6.53 ± 0.25[b]	4.09 ± 0.37[a]

NC, normal control diet; HF, high fat diet; HFS, high fat diet + *A. pernyi* silk fibroin.
Values are means ± SE (n = 10). Means in the same row not sharing a common superscript are significantly different at $p < 0.05$.
*HDL-cholesterol/total cholesterol ratio

marked increase in the HDL-cholesterol level was observed in HFS group. Accordingly, the HDL-cholesterol/total cholesterol ratio (HTR) was significantly higher in HFS mice than that of the NC and HF ones. The hepatic triglyceride and total cholesterol levels were considerably higher in HF mice compared with that of the NC mice. Supplementation of *A. pernyi* silk fibroin powder in the diet suppressed the increase in hepatic triglyceride concentration, but failed to elicit a significant effect on the hepatic total cholesterol level. Fecal excretion of triglyceride was higher in HFS group than that of the NC and HF groups. On the other hand, dietary feeding of *A. pernyi* silk fibroin powder did not significantly affect the fecal excretion of cholesterol in mice.

Table 4. Plasma and erythrocyte TBARS concentration in mice fed with high fat diet supplemented with *A. pernyi* silk fibroin powder.

Dietary group	Plasma TBARS (nmol/ml)	Erythrocyte TBARS (nmol/g Hb)
NC	6.81 ± 0.67[b]	2.81 ± 0.13[b]
HF	8.92 ± 1.21[b]	3.07 ± 0.13[b]
HFS	3.95 ± 0.33[a]	2.31 ± 0.09[a]

NC, normal control diet; HF, high fat diet; HFS, high fat diet + *A. pernyi* silk fibroin.
Values are means ± SE (n = 10). Means in the same column not sharing a common superscript are significantly different at $p < 0.05$.

Table 5. Hepatic lipid-regulating and antioxidant enzymes activities in mice fed with high fat diet supplemented with *A. pernyi* silk fibroin powder.

Enzyme	NC	HF	HFS
Hepatic lipid-regulating enzyme			
G6PD (nmol/min/mg protein)	26.35 ± 1.30[b]	14.45 ± 0.72[b]	9.78 ± 0.53[a]
ME (nmol/min/mg protein)	120.59 ± 8.63[c]	114.01 ± 11.68[b]	37.51 ± 8.55[a]
Hepatic antioxidant enzyme			
SOD (unit/mg protein)	1.93 ± 0.040[c]	1.64 ± 0.031[a]	1.77 ± 0.042[b]
GSH-Px (nmol/min/mg protein)	14.55 ± 0.25[b]	12.70 ± 0.29[a]	17.92 ± 0.68[c]
CAT (nmol/min/mg protein)	1.50 ± 0.056[b]	1.02 ± 0.054[a]	1.63 ± 0.05[b]
GR (nmol/min/mg protein)	9.59 ± 1.21[a]	12.27 ± 1.59[a]	25.23 ± 1.80[b]
PON1 (μmol/min/mg protein)	3.74 ± 0.28[b]	1.68 ± 0.50[a]	3.50 ± 0.41[b]

NC, normal control diet; HF, high fat diet; HFS, high fat diet + *A. pernyi* silk fibroin.
Values are means ± SE (n = 10). Means in the same row not sharing a common superscript are significantly different at $p < 0.05$.

Plasma and erythrocyte lipid peroxides

High fat feeding did not significantly affect the levels of plasma and erythrocyte TBARS in mice (Table 4). However, addition of *A. pernyi* silk fibroin in the high fat diet resulted in a significant decrease in the TBARS concentration.

Hepatic lipid-regulating and antioxidant enzymes activity

The HFS group exhibited significantly lower G6PD and ME activities than the NC and HF groups (Table 5). A marked increase in the activities of SOD, GSH-Px, CAT, and PON1 enzymes were found in HF mice, but dietary feeding of *A. pernyi* silk fibroin counteracted the decline in the enzyme activities. Although the high fat diet did not significantly change the GR activity in mice, the *A. pernyi* silk fibroin-fed mice showed a substantial increase in the enzyme activity.

Discussions

The current study investigated the effect of *A. pernyi* silk fibroin powder on the lipid metabolism and antioxidant defense system in C57BL/6N mouse model under a high fat diet condition. Results showed that high fat diet markedly increased the body weight, plasma and hepatic total cholesterol levels, and hepatic triglyceride concentrations. Previous studies have also revealed that high fat feeding caused a considerable increase in the body weight and cholesterol level of laboratory animals (Kim *et al*., 2002; Gallou-Kabani, 2007). Diet supplementation with *A. pernyi* silk fibroin powder, however, decreased the weight gain of the high fat diet-induced-obesity in mice and suppressed the increase in plasma cholesterol and hepatic triglyceride levels. Moreover, higher HDL-cholesterol level and HTR were found in mice fed with the silk fibroin compared with that of the normal diet-fed mice. Since HDL-cholesterol level and HTR have been shown to be inversely correlated with coronary heart disease due to the anti-atherogenic properties of HDL particles (Zuliani *et al*., 2007), results of the present study suggest that dietary feeding of silk protein powder could reduce the risk of cardiovascular disease. While it is generally believed that enhanced cholesterol metabolism is mediated by increased fecal excretion of lipids, there were also reports that there was no relevance found between the two (Anderson *et al*., 1990). In this study, it appears that the plasma cholesterol-lowering effect of *A. pernyi* silk fibroin was not mediated by increased fecal excretion of the steroid since no significant change was found in the fecal cholesterol content. On the other hand, the reduction in the hepatic triglyceride level in silk fibroin-fed mice may be partly due to the substantial increase in the fecal excretion of triglycerides. In addition, the considerable decrease in the activities of G6PD and ME enzymes in HFS mice relative to that of the NC and HF mice groups indicated that the *A. pernyi* silk fibroin powder reduced the plasma cholesterol and hepatic triglyceride levels through suppression of hepatic lipogenesis via regulation of the lipogenic enzyme activities. Hepatic lipogenic enzymes, such as G6PD and ME, are involved in the biosynthesis of fatty acids and cholesterol (Park *et al*., 2005). Hence, lower activities of these enzymes could restrict the availability of fatty acids required for the synthesis of hepatic triglycerides.

Excessive generation of free radicals and reactive oxygen species in the cellular system causes lipid peroxidatin and oxidative stress (Ibrahim *et al*., 1997), which in turn play a critical role in the pathogenesis of various metabolic diseases. A highly complex antioxidant protection system, including hepatic antioxidant enzymes, has been developed by the cells to control the destructive potential of free radicals and reactive oxygen species. In the present study, a significant decrease in the activities of SOD, GSH-Px, CAT, and PON1 enzymes were found in high fat-fed mice. This reduction in the antioxidant enzyme activities in mice indicates that high consumption of dietary fat could be detrimental to the intrinsic antioxidant defense system in animals. On the other hand, addition of *A. pernyi* silk fibroin in the high fat diet suppressed the decline in the enzyme activities. Furthermore, the TBARS concentration, an indicator of the lipid peroxidation and oxidative stress, was significantly lower in mice fed with the silk fibroin powder compared with that of the control- and high fat-fed animals. These findings suggest a marked improvement in the *in vivo* antioxidant status of the fibroin-fed mice, which made them less susceptible to peroxidative damage under the challenge of oxidative stress such as high fat diet. The *A. pernyi* silk fibroin powder exerted a protective effect against lipid peroxidation by elevating the activity of hepatic antioxidant enzymes. Among the enzymes analyzed in this study, GR appeared to be the most actively induced in HFS mice. The remarkable increase in the GR activity relative to that of the other antioxidant enzymes indicates that GR is an integral component in the antioxidant defense mechanism and could be the major hepatic antioxidant enzyme responsible for the decreased rate of lipid peroxidation in mice fed with *A. pernyi* silk fibroin.

In conclusion, the present study illustrates that *A. pernyi* silk fibroin powder could improve the lipid metabolism and enhance the antioxidant defense status in mice under a high fat diet condition. The hypolipidemic effect and the

antioxidant status-improving action were partly due to the inhibition of hepatic lipogenesis via regulation of lipogenic enzyme activities and activation of hepatic antioxidant enzymes. Thus, the *A. pernyi* silk fibroin may be useful as a functional biomaterial in the development of therapeutic agent against hyperlipidemia and its associated diseases. Further research is needed, however, to elucidate the exact mechanism by which this biopolymer reduces the lipid level and enhances the antioxidative status in order to have a better understanding of its therapeutic potential.

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References

Aebi H (1974) Catalase. In: Bergmeyer HU (Ed.), Method of Enzymatic Analysis; Vol. 2; New York, Academic Press. pp. 673-684.

Anderson JW, Ahn J, Nathani S, Reeves RD (1990) Soluble fiber: hypocholesterolemic effects and proposed mechanisms. In: Krichevsky D, Bonfield C, Anderson J (Eds.), Dietary Fiber: Chemistry, Physiology, and Health Effects. New York, Plenum Press. pp. 339-363.

Beutler E (1971) Red Cell Metabolism: A Manual of Biochemical Methods. Grune and Stratton: New York.

Bray GA, Popkin BM (1998) Dietary fat intake does affect obesity. Am J Clin Nutr 68, 1157-1173.

Fan JB, Wu LP, Chen LS, Mao XY, Ren FZ (2009) Antioxidant activities of silk sericin from silkworm Bombyx mori. J Food Biochem 33, 74-88.

Folch JM, Lee M, Stanely GHS (1957) A sample method for the isolation and purification of total lipids from animal tissue. J Biol Chem 26, 497-509.

Formiguera X, Canton A (2004) Obesity: epidemiology and clinical aspects. Best Pract Res Clin Gastroenterol 18, 1125-1146.

Gallou-Kabani C, Vige A, Gross MS, Rabes JP, Boileau C, Larue-Achagiotis C, Tome D, Jais JP, Junien C (2007) C57BL/6J and A/J mice fed a high-fat diet delineate components of metabolic syndrome. Obesity 15, 1996-2005.

Hardy JG, Romer LM, Scheibel TR (2008) Polymeric materials based on silk proteins. Polymer 49, 4309-4327.

Hong S, Park K, Suh B, Do M, Hyun C (2002) Effect of silk fibroin hydrolysate on adipocyte metabolism in db/db mice. Korean J Pharmocogn 33, 312-318.

Ibrahim W, Lee US, Yeh CC, Szabo K, Bruckner G, Chow CK (1997) Oxidative stress and antioxidant status in mouse liver: effects of dietary lipid, vitamin E and iron. J Nutr 127, 1401-1406.

Kim MR, Park Y, Albright KJ, Pariza MW (2002) Differential responses of hamsters and rats fed high-fat or low-fat diets supplemented with conjugated linoleic acid. Nutr Res 22, 715-722.

Kweon HY, Lee KG, Lee YW (1999) Characterization of tussah (*Antheraea pernyi*) silk fibroin powder prepared by HCl and NaOH. Korean J Seric Sci 41, 54-60.

Kweon HY, Park YH (2001) Dissolution and characterization of regenerated *Antheraea pernyi* silk fibroin. J Appl Polym Sci 82, 750-758.

Mackness MI, Arrol S, Durrington PN (1991) Paraoxonase prevents accumulation of lipoperoxides in low-density lipoprotein. FEBS Lett 286, 152-154.

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

Mize CE, Langdon RG (1952) Hepatic glutathione reductase, purification and general kinetic properties. J Biol Chem 237, 1589-1595.

Mondal M, Trivedy K, Kumar SN (2007) The silk proteins, sericin and fibroin in silkworm, *Bombyx mori* Linn., - a review. Caspian J Env Sci 5, 63-76.

Ochoa S (1955) Malic dehydrogenase from pig heart. In: Colowick SP, Kaplan NO (Eds.), Methods in Enzymology; Vol. 1; New York, Academic Press. pp. 735-739.

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

Paglia ED, Valentine WN (1967) Studies on quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 70, 158-169.

Park J, Rho HK, Kim KH, Choi SS, Lee YS, Kim JB (2005) Overexpression of glucose-6-phosphate dehydrogenase is associated with lipid dysregulation and insulin resistance in obesity. Mol Cell Biol 25, 5146-5157.

Ryu KS, Lee HA, Kim I (2002) Effects and mechanisms of silkworm powder as a blood glucose-lowering agent. Int J Indust Entomol 4, 93-100.

Zhou J, Han D (2006) Safety evaluation of protein of silkworm (*Antheraea pernyi*) pupae. Food Chem Toxicol 44, 1123-1130.

Zuliani G, Vigna GB, Fellin R (2007) The anti-atherogenic properties of HDL particles. Int Congr Ser 1303, 103-110.