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Antioxidant Activities and Inhibitory Effects of *Auricularia Auricular* and Its Functional Formula Diet against Vascular Smooth Muscle Cell *in Vitro*

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ABSTRACT

The functional formula diet AHP, containing polysaccharides from Auricularia auricular, polyphenolic compounds from Hawthorn (Crataegus) and Pueraria radix, has been recently developed as a dietary intervention against dyslipidemia in our previous study. In the present study, its antioxidant activities and protective effects against proliferation of vascular smooth muscle cells (VSMCs) were investigated. AHP possessed the potent radical-scavenging effects against hydroxyl and superoxide radicals, and also the inhibitory effects against peroxidation of low density lipoprotein induced by Cu^{2+} in vitro. The protective effects of AHP against proliferation of VSMCs were evaluated through the methodology of serum pharmacology. The serum containing AHP significantly inhibited the proliferation of VSMCs induced by oxidized low density lipoprotein in a time- and dose-dependent manner, and also promoted the nitric oxide production of VSMCs. Our study indicated that this functional formula diet would be a potent alternative as a functional diet to prevent atherosclerosis at early stage.

Keywords: *Functional Diet*, *Auricularia Auricula*, *Antioxidant Activity*, *Vascular Smooth Muscle Cells*, *Serum Pharmacology*

1. Introduction

Atherosclerosis is the major underlying cause of cardiovascular diseases (CVD), such as coronary artery disease, peripheral vascular disease and stroke, and has consequently become the principle entity responsible for morbidity and mortality in Western countries [1]. As a chronic inflammatory process of the arterial inner layer, the formation of atherosclerosis involves a complex of pathophysiological effects including apoptosis of foam cells and proliferation of vascular smooth muscle cells (VSMCs) at different stages of this disease [2]. VSMCs are one of the major constituents of blood vessel and vital for maintaining vascular structure and function. It is widely reported that oxidized low density lipoprotein (ox-LDL), hyperlipidemic serum, and many other factors stimulate proliferation of VSMCs [3,4]. Therefore, the antioxidants and hypolipdemic drugs are considered as the major therapeutic strategy for CVD through the inhibition of VSMCs proliferation.

Recently, the increasing attention has been attracted to the consumption of dietary antioxidants and functional ingredients to prevent CVD, instead of the intake of therapeutic drugs which would be inevitably associated with certain side effects that have been gradually uncovered [5,6]. In China, many traditional foods are recognized as well-documented medicinal plants and are formulated to develop functional foods as dietary intervention to prevent many chronic diseases. The combination of different bioactives from different source materials are reported to render synergistic effects and thus to target simultaneously on the complexity and redundancy of many chronic diseases [7]. Therefore, developing the formulae of functional foods/diets with the viability as a production is a major trend in current food and nutrition field, in order to provide adjuvant and alternative therapy for many chronic diseases without side effects.

The polysaccharides from *auricularia auricular* (AAP), the phenolic compounds from hawthorn (*Crataegus*) (HPC) and *Pueraria radix* (PPC) are the three functional components which are well-documented for their potent antioxidant and individual effects on chronic diseases.

Regarding the prevention or treatment for dyslipidemia or atherosclerosis, although they possess distinct pathways, the individual component does have certain limitations. Our previous study developed a novel dietary formulation of these three functional components, named as AHP, which has been suggested as a multifaceted dietary intervention against dyslipidemia *in vivo* through improving lipids profile and modulating activities of antioxidant enzymes [8]. The functional formula of AAP and HPC has also been investigated for its potent antioxidative property to inhibit LDL oxidation *in vitro* and hypolipidemic effect *in vivo* [9]. However, whether the dietary intervention of AHP is able to inhibit LDL oxidation as well as ox-LDL induced proliferation of VSMCs are still unknown. Hence, the present study was designed to probe its ability to inhibit LDL oxidation *in vitro* and prevent VSMCs proliferation with the method of Serum Pharmacology.

2. Materials and Methods

2.1. Materials

The sample of *A.auricula* fruit-bodies were provided by Daxing'anling region (Heilongjiang, China), and dry hawthorn fruits (*Crataegus pinnatifida*) and *Pueraria radix* were obtained from Tongrentang pharmacy chain store in Beijing, China. All plant materials were dried at ambient temperature and stored in a dry place prior to use.

Vascular smooth muscle cells (VSMCs) were obtained from Cell Culture Center of Peking Union Medical College (Beijing, China). Fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), Penicillin-Streptomycin Solution, dimethyl sulphoxide (DMSO), and trypsin were purchased from Sigma (St. Louis, Mo., USA). The oxidized low density lipoprotein (ox-LDL) was provided by Peking Union Medical College (Beijing, China). Nitric oxide content in cell was measured by colorimetric assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). All other chemicals were of analytical grade.

2.2. Preparation of Functional Formula Diet

After rigorous screening experiments, this novel functional formula diet, named as AHP consisting of bioactive extracts from *A. auricular*, hawthorn and *Pueraria radix*, was first developed in our lab. The polysaccharides of *A.auricula* (AAP), polyphenolic components of hawthorn (HPC) and flavanoids components of *Pueraria radix* (PPC) were extracted and lyophilized according to our previous reported procedures [9,10]. Through our preliminary trials, the optimal formulation of weight ratio of three individual components in AHP was set as 5 (AAP): 3 (HPC): 2 (PPC). AHP was dissolved in distilled water before analysis.

2.3. Identification of Marker Compounds

The total carbohydrate content in AAP was 42.5%, consisting of rhamnose, xylose, glucose, uronic acids, and sulfate as main composition besides smaller amounts of mannose, galactose, and arabinose, as reported in our previous research [10]. The total phenolic compounds in HPC and PPC was 102 and 53.5 mg/g, respectively, expressed as gallic acid equivalent. Based on our previous HPLC analysis [8], the marker components in HPC contained chlorogenic acid, epicatechin, and hyperoside; and in PPC included 3'-methoxy puerarin, puerarin, daidzin, and daidzein.

2.4. Hydroxyl Radical-Scavenging Effect

The hydroxyl radical-scavenging effect of AHP was measured by the method of [11] with some modifications. In brief, 1,10-phenanthroline (1.865 mM) and $FeSO_4$ (1.865 mM) were dissolved in phosphate buffer (pH 7.4) and mixed thoroughly. H_2O_2 (0.025%) and samples (2 mg/ml) were then added into the mixture. After incubation at 37˚C at 60 min, the absorbance was measured at 536 nm. The HRSE was calculated as following:

$$\mathrm{HRSE}(\%) = \frac{A_S - A_1}{A_O - A_1} \times 100$$

where A_s, A_1 and A_o were the absorbance of the sample, control solution containing 1,10-phenanthroline, $FeSO_4$ and H_2O_2, blank control containing 1,10-phenanthroline and $FeSO_4$, respectively.

2.5. Superoxide Radical-Scavenging Effect

The SRSE was evaluated by using the method of photoreduction of NBT as reported before [9,12]. In brief, 0.5 ml test sample (2 mg/mL) was added to the 4.5 mL reaction mixture of phosphate buffer (pH 7.8). The final concentrations of reaction reagents were: 1×10^{-4} M methionine, 3×10^{-6} M riboflavin, 1×10^{-4} M NBT. The reaction was initialized by illumination of the mixture at 3000 Lux for 30 min and then the absorbance was measured at 560 nm. The control was prepared by replacing the test sample with deionized water. The SRSE was calculated by the following equation:

$$\mathrm{SRSE}(\%) = \frac{A_C - A_S}{A_C} \times 100$$

where A_s and A_c were the absorbance of the sample and control solution.

2.6. Inhibitory Effects against LDL Oxidation

LDL was separated from plasma sample obtained from healthy volunteers after overnight fast, according to the method of LEUNG *et al*. [13]. The LDL was dialyzed and diluted with PBS to 250 μg protein/ml, and then 200 μL of the test sample (0.5 mg/mL) was added. The oxidation of LDL was induced by adding freshly prepared $CuSO_4$ solution with the final concentration 2.5 μM. The final volume of reaction mixture was made up to 2.0 mL by PBS. The oxidation process was monitored by conjugated dienes formed at 234 nm, and the absorbance was recorded every 10 min up to 500 min. The lag phase of LDL oxidation was defined as the time interval (min) between the intercept of the slope of the curve with the initial absorbance axis [14].

2.7. Preparation of Serum Containing Tested Diet

Sprague Dawley rats were 8-week of age, provided by Experimental Animal Center of Beijing, China. Rats were housed in plastic cages with wood shavings under controlled conditions (temperature 24 ± 0.5˚C, humidity 55 ± 5%, and 12 h of light from 08:00 to 20:00) and maintained according to the Guide for the Care and Use of Laboratory Animals established by China Agricultural University. After 1-week acclimation, they were randomly assigned to three groups (n = 3): one control group and two treatment groups. The two treatment groups were respectively provided with formulation diet AHP and AAP by oral administration through gastric infusion with capacity of 0.1 mL/10 g b.w. for 7 days. The control group was provided with equivalent amount of distilled water by gastric infusion. At 7th day, the blood was taken from abdominal aorta 1 h after the final gastric infusion, then incubated at 37˚C for 15 min and centrifuged at 3000 rpm/min for 10 min. Each sample of serum was used after inactivation for at 56˚C for 30 min, and then kept at –20˚C. The serum was filtered through 0.22 μm with bacterial removal.

2.8. Inhibition of Diet-Containing Serum on Proliferation of VSMCs

The VSMC were seeded into 96 multi-well plates in DMEM supplemented with 20% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. The cells were cultured in a humidified incubator (5% CO_2) at 37˚C, and were allowed to attach for 24 h. The proliferation of VSMC model was induced by incubation with 25 μg/mL ox-LDL for various time points (24, 48, and 72 h), according to the reported method with minor modification [15]. Control cells were incubated in an identical medium without addition of ox-LDL, and the model cells were incubated with ox-LDL but without addition of any treatment. In treatment cells, various percentage of FBS (2.5, 5, and 10%) were replaced with AHP and AAP drug serum. At each time point, the medium was removed and cells were washed with DMEM once, and then 20 μL of MTT (0.5 mg/mL) was added. After incubation for 4 h at 37˚C, 150 μL DMSO was subsequently added to dissolve the formazan formed. After shaking, the absorbance of each well was measured at 570 nm using a Labsystems Multiskan MK3 (Thermo Labsystems, Helsinki, Finland). At the time point of 72 h, the medium of each well was collected for NO measurement. All measurements were carried out in 8 replicates.

2.8. Statistical Analysis

All parameters were expressed as mean ± standard error (SE). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests. The significant level (p) was set at 0.05.

3. Results and Discussion

3.1. Radical-Scavenging Effects *in Vitro*

Recently, the abundant evidence suggests that the antioxidant plays important roles in the maintenance of human health and prevention and treatment of various disorder and diseases, especially the atherosclerosis and other CVDs [6]. Due to the inconsistency of many radical system used for antioxidant evaluation, it is widely suggested that at least two different methods should be applied to investigate the radical-scavenging capacities of selected antioxidant or a food system [16]. Because of the importance of antioxidant capacity in the prevention of atherosclerosis, the hydroxyl and superoxide anion radical systems were adopted to investigate the antioxidant effects of AHP functional diet. Both *in vivo* and *in vitro* antioxidant capacity of AAP has been widely reported [17-19], and AAP has been already formulated into breads as a functional ingredient to elevate antioxidant property and quality of bread products [20]. The antioxidant capacities of HPC and PPC have also been well-documented [21,22].

In present study, compared with AAP, the hydroxyl and superoxide radical scavenging effects of formula diet AHP were significantly elevated from 24% and 37% to 89% and 76%, respectively ($P < 0.01$, **Figure 1**). This might be explained by the introduction of polyphenolic compounds into the diet as well as the synergistic effects among components.

3.2. Inhibition of Cu^{2+}-Induced LDL Peroxidation *in Vitro*

According to free radical theory, LDL undergoes lipid

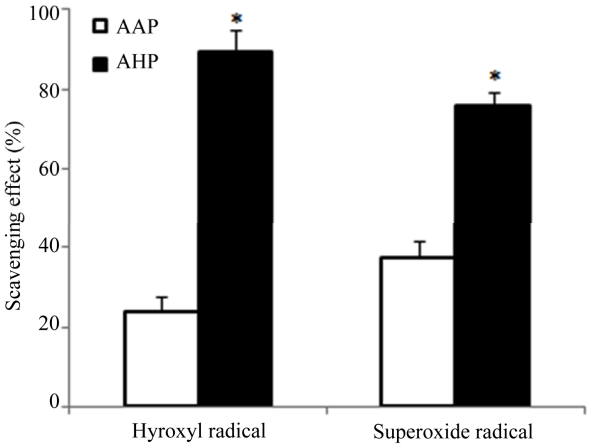

Figure 1. The scavenging effects of AAP and AHP against hydroxyl radical and superoxide radical. The values were expressed as mean ± SE, *P < 0.01. AAP, Auricularia auricula polysaccharide; AHP, the functional diet formula.

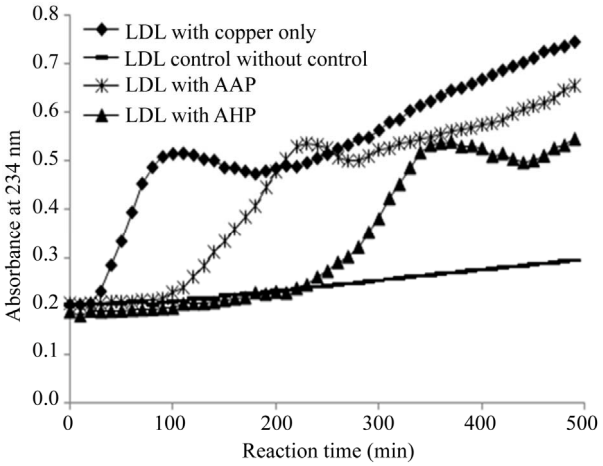

Figure 2. Effect of AHP and AAP on copper-induced formation of conjegated dienes in LDL. AAP, *Auricularia auricula* polysaccharide; AHP, the functional diet formula. AAP, *Auricularia auricula* polysaccharide; AHP, the functional diet formula.

peroxidation, induced by cells of arterial wall and metal ions, and the oxidized LDL (ox-LDL) could be easily taken up by macrophages leading to the formation of lipid-laden foam cells, a hallmark of early atherosclerotic lesions [23]. For the *in vitro* experimental model of LDL oxidation, several chemicals are reported to be able to induce the oxidative modification of normal LDL, including DDPH, AAPH, Cu^{2+}, etc [24].

In present study, Cu^{2+}-induced LDL peroxidation was adopted, because it has been reported to be more relevant to the *in vivo* situation than other chemical induced peroxidation model [25]. From **Figure 2**, and **Table 1**, the treatment of AAP showed a significant protection against LDL oxidation by prolonging the lag phase significantly from 30 min to 91 min ($P < 0.01$). However, the protection provided by AHP treatment was much better than AAP, by prolonging the lag phase further to 240 min ($P < 0.05$). The consumption of naturally occurring antioxidants has been discovered as a key approach to prevent atherosclerosis by inhibiting LDL oxidation and the oxidative lesion of endothelium [26]. The three compositions in AHP formula have all been proven to possess potent antioxidant individually, however, in present study the antioxidant activities of their combination was first assayed, showing dramatic improvement compared with the single treatment of AAP composition. The major contribution might be attributed to polyphenolic compounds from HPC composition, which has been reported to act as chelators to inactivate Cu^{2+} to quench the formation of free radicals in human LDL incubation [27].

Table 1. The lag phase of LDL peroxidation induced by Cu^{2+} in different treatments.

Treatment	Lag phase
LDL with copper only	30.37 ± 3.20^{a}
LDL with AAP	90.87 ± 7.60^{b}
LDL with AHP	240.17 ± 8.25^{c}

Values were expressed as mean ± SE. Values not sharing the same letter were significantly different with each other ($P < 0.05$). AAP, *Auricularia auricula* polysaccharide; AHP, the functional diet formula. AAP, *Auricularia auricula* polysaccharide; AHP, the functional diet formula.

3.3. Effect of Diet-Containing Serum on Ox-LDL Stimulated Proliferation of VSMCs

Serum pharmacology, first developed by Tashino in 1984 [28], is a method widely adopted for *in vitro* pharmacology studies of Traditional Chinese Medicine [29,30].

When the drug or diet consists of complicated compositions, it is believed that serum pharmacology is more scientific to demonstrate the exact effects of the interactions among those compositions after they undergo metabolism and biotransformation *in vivo*. In AHP functional diet formula, because the composition includes one polysaccharides extract and two polyphenolic extracts, the serum pharmacology was adopted to investigate its protective effects of serum containing AHP against proliferation of VSMCs induced by ox-LDL. As shown in **Table 2**, the proliferation of VSMCs was successfully induced by ox-LDL. The higher serum concentration or the longer time of incubation was, the more proliferation of VSMCs was observed. The inhibitive effect of AHP was both time and dose dependent. When the serum concentration was only 5%, both AHP and APP showed no significant inhibitive effects up to 72 h incubation. When the serum concentration increased to 10% and 20%, both

Table 2. Inhibitory effects of AHP and APP against proliferation of VSMC induced by Ox-LDL.

Time	Treatment	Serum concentration		
		5%	10%	20%
24 h	Control	0.138 ± 0.016	0.177 ± 0.014[a]	0.216 ± 0.013[a]
	Model	0.145 ± 0.018	0.210 ± 0.017[b]	0.263 ± 0.016[b]
	APP	0.147 ± 0.14	0.205 ± 0.015[b]	0.262 ± 0.012[b]
	AHP	0.139 ± 0.009	0.182 ± 0.013[a]	0.224 ± 0.014[a]
48 h	Control	0.185 ± 0.026[a]	0.294 ± 0.027[a]	0.413 ± 0.025[a]
	Model	0.241 ± 0.031[b]	0.366 ± 0.032[c]	0.542 ± 0.034[d]
	APP	0.234 ± 0.025[b]	0.341 ± 0.033[bc]	0.509 ± 0.026[c]
	AHP	0.214 ± 0.013[b]	0.317 ± 0.031[b]	0.474 ± 0.032[b]
72 h	Control	0.309 ± 0.021[a]	0.484 ± 0.027[a]	0.578 ± 0.029[a]
	Model	0.417 ± 0.033[b]	0.653 ± 0.031[c]	0.795 ± 0.036[d]
	APP	0.391 ± 0.017[b]	0.593 ± 0.021[b]	0.727 ± 0.021[c]
	AHP	0.397 ± 0.028[b]	0.587 ± 0.026[b]	0.654 ± 0.034[b]

Values were expressed as mean ± SE. Values in the same column not sharing the same letter were significantly different with each other (P < 0.05). AAP, *Auricularia auricula* polysaccharide; AHP, the functional diet formula. AAP, *Auricularia auricula* polysaccharide; AHP, the functional diet formula.

treatments showed dramatic effects at 48 h incubation, showing significant difference from Model group ($P < 0.05$). AHP treatment began to demonstrate significant effect at 24 h incubation, however, the AAP treatment had no significant effect until 72 h at 10% serum concentration and 48 h at 20% serum concentration. Therefore, it was suggested that the AHP formula diet could inhibit proliferation of VSMCs at earlier stage than AAP treatment dose. The proliferation of VSMCs is known as a potential progression in cardiovascular diseases including hypertension, atherosclerosis, and restenosis, consequently, the inhibition of proliferation of VSMCs at earlier stage plays an vital role to prevent these diseases. The present investigation suggests that the treatment of AHP formula diet might possess a better prevention effect than AAP treatment.

3.4. Effect of Diet-Containing Serum on NO Production of VSMCs Treated with Ox-LDL

NO, produced mainly by vascular endothelial synthase, has been proved to be a central anti-inflammatory and anti-atherosclerosis principle in the vasculature. The abnormality of NO production will lead to lipid peroxidation injury in vascular and consequently promote the progression of cardiovascular disease [31]. Recently, it has also been reported that serum pharmacology is a good method to investigate the effect of medicines containing complex compositions on NO production in VSMCs proliferation model [32]. The effects of AHP and AAP on the production of NO in VSMCs were shown in **Figure 3**. After 72 h incubation, the NO production in Model cells significantly decreased by 60% (P < 0.05), compared with Control cells. Both AAP and AHP could elevate the NO production in ox-LDL damaged VSMCs by 36% and 74%, respectively, compared with Model cell. The plant-derived products rich in polyphenols have been extensively studied for their potent capability to modulate endothelial NO recently [31]. The ethanolic extracts of *A. auricular* have also been proven to possess the antioxidant activities, NO synthase activation properties, as well as hypocholesterolemic effects

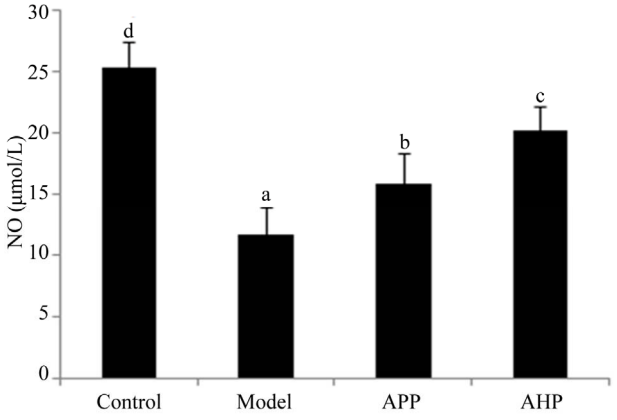

Figure 3. Effects of AAP and AHP on production of NO by VSMC induced by Ox-LDL. Values were expressed as mean ± SE. Values not sharing the same letter were significantly different with each other (P < 0.05). AAP, *Auricularia auricula* polysaccharide; AHP, the functional diet formula.

[33]. The polysaccharides from *A. auricular* in our study also demonstrated significant promoting effect on NO production of VSMCs, and this effect was greatly enhanced in AHP formula functional diet. Therefore, it was suggested that the improvement of NO modulation effect in AHP might be partially attributed to the synergistic effects between polyphenolic compounds and poly- saccharides compositions in the diet formula.

4. Conclusions

In conclusion, our results suggested that the functional diet formula AHP possessed potent antioxidant activity to scavenge free radicals and inhibit LDL peroxidation induced by Cu^{2+} *in vitro*. The serum containing AHP could provide protection against ox-LD stimulated proliferation of VSMCs as well as promote NO production. The present study showed that all of these health-promoting effects were greatly improved by formulating two polyphenolic compositions into AAP, compared with the effects of individual treatment of AAP composition. There- fore, the functional diet formula AHP developed might be further developed as an alternative dietary strategy to prevent atherosclerosis in the near future.

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REFERENCES

[1] C. K. Glass and J. L. Witztum, "Atherosclerosis. The Road Ahead," *Cell*, Vol. 104, No. 4, 2001, pp. 503-516. doi:10.1016/S0092-8674(01)00238-0

[2] Y. H. Zhang, J. T. Liu, B. Y. Wen and N. Liu, "Mechanisms of Inhibiting Proliferation of Vascular Smooth Muscle Cells by Serum of Rats Treated with Dahuang Zhechong Pill," *Journal of Ethnopharmacology*, Vol. 124, No. 1, 2009, pp. 125-129. doi:10.1016/j.jep.2009.04.012

[3] Y. H. Zhang, J. T. Liu, B. Y. Wen and X. H. Xiao, "*In Vitro* Inhibition of Proliferation of Vascular Smooth Muscle Cells by Serum of Rats Treated with Dahuang Zhechong Pill," *Journal of Ethnopharmacology*, Vol. 112, No. 2, 2007, pp. 375-379. doi:10.1016/j.jep.2007.03.018

[4] D. Giugliano, "Dietary Antioxidants for Cardiovascular Prevention," *Nutrition Metabolism and Cardiovascular Diseases*, Vol. 10, No. 1, 2000, pp. 38-44.

[5] H. E. Seifried, D. E. Anderson, E. I. Fisher and J. A. Milner, "A Review of the Interaction among Dietary Antioxidants and Reactive Oxygen Species," *Journal of Nutritional Biochemistry*, Vol. 18, No. 9, 2007, pp. 567-579. doi:10.1016/j.jnutbio.2006.10.007

[6] F. Shahidi, "Functional Foods: Their Role in Health Promotion and Disease Prevention," *Journal of Food Science*, Vol. 69, No. 5, 2004, pp. 146-149. doi:10.1111/j.1365-2621.2004.tb10727.x

[7] Y. Luo, G. Chen, B. Li, B. Ji, Z. Xiao and F. Tian, "Dietary Intervention with AHP, a Functional Formula Diet, Improves Both Serum and Hepatic Lipids Profile in Dyslipidemia Mice," *Journal Of Food Science*, Vol. 74, No. 6, 2009, pp. H189-H195. doi:10.1111/j.1750-3841.2009.01241.x

[8] Y. Luo, G. Chen, B. Li, B. Ji, Y. Guo and F. Tian, "Evaluation of Antioxidative and Hypolipidemic Properties of a Novel Functional Diet Formulation of *Auricularia Auricula* and Hawthorn," *Innovative Food Science and Emerging Technologies*, Vol. 10, No. 2, 2009, pp. 215-221. doi:10.1016/j.ifset.2008.06.004

[9] G. Chen, Y. Luo, B. Ji, *et al.*, "Effect of Polysaccharide from *Auricularia Auricula* on Blood Lipid Metabolism and Lipoprotein Lipase Activity of ICR Mice Fed a Cholesterol-Enriched Diet," *Journal of Food Science*, Vol. 73, No. 6, 2008, pp. H103-H108. doi:10.1111/j.1750-3841.2008.00821.x

[10] I. G. De Avellar, M. M. Magalhaes, A. B. Silva, L. L. Souza, A. C. Leitao and M. Hermes-Lima, "Reevaluating the Role of 1,10-Phenanthroline in Oxidative Reactions Involving Ferrous Ions and Dna Damage," *Biochimica et Biophysica Acta*, Vol. 1675, No. 1-3, 2004, pp. 46-53.

[11] R. C. Stewar and J. D. Beewley, "Lipid Peroxidation Associated with Accelerated Aging of Soybean Areas," *Plant Physiology*, Vol. 65, No. 2, 1980, pp. 245-248. doi:10.1104/pp.65.2.245

[12] L. K. Leung, Y. L. Su, R. Y. Chen, Z. S. Zhang, Y. Huang and Z. Y. Chen, "Theaflavins in Black Tea and Catechins in Green Tea are Equally Effective Antioxidants," *Journal of Nutrition*, Vol. 131, No. 9, 2001, pp. 2248-2251.

[13] H. M. Liu, Y. Cheng, J. F. Lu and R. C. Li, "The Mechanism of Kinetic Inhibition of Cu (Ii)-Induced Oxidation of Low Density Lipoprotein by Lanthanide Ions," *Journal of Inorganic Biochemistry*, Vol. 100, No. 7, 2006, pp. 1280-1289. doi:10.1016/j.jinorgbio.2006.03.002

[14] M. N. Chahine, D. P. Blackwood, E. Dibrov, M. N. Richard and G. N. Pierce, "Oxidized LDL Affects Smooth Muscle Cell Growth through MAPK-Mediated Actions on Nuclear Protein Import," *Journal of Molecular and Cellular Cardiology*, Vol. 46, No. 3, 2009, pp. 431-441. doi:10.1016/j.yjmcc.2008.10.009

[15] E. Niki, "Assessment of Antioxidant Capacity *in Vitro* and *in Vivo*," *Free Radical Biology and Medicine*, Vol. 49, No. 4, 2010, pp. 503-515. doi:10.1016/j.freeradbiomed.2010.04.016

[16] Y. S. Kho, S. Vikineswary, N. Abdulah, U. R. Kuppusamy and H. I. Oh, "Antioxidant Capacity of Fresh and Processed Fruit Bodies and Mycelium of *Auricularia Auricula-Judae* (*Fr.*) *Quél*," *Journal of Medicinal Food*, Vol. 12, No. 1, 2009, pp. 167-174. doi:10.1089/jmf.2007.0568

[17] Q. Wu, Z. Tan, H. Liu, L. Gao, S. Wu, J. Luo, W. Zhang, T. Zhao, J. Yu and X. Xu, "Chemical Characterization of

Auricularia Auricula Polysaccharides and Its Pharmacological Effect on Heart Antioxidant Enzyme Activities and Left Ventricular Function in Aged Mice," *International Journal of Biological Macromolecules*, Vol. 46, No. 3, 2010, pp. 284-288. doi:10.1016/j.ijbiomac.2010.01.016

[18] H. Zhang, Z. Y. Wang, Z. Zhang and X. Wang, "Purified *Auricularia Auricular-Judae* Polysaccharide (AAP I-a) Prevents Oxidative Stress in an Ageing Mouse Model," *Carbohydrate Polymers*, Vol. 84, No. 1, 2011, pp. 638-648. doi:10.1016/j.carbpol.2010.12.044

[19] L. S. Fan, S. H. Zhang, L. Yu and L. Ma, "Evaluation of Antioxidant Property and Quality of Breads Containing *Auricularia Auricula* Polysaccharide Flour," Food Chemistry, Vol. 101, No. 3, 2006, pp. 1158-1163. doi:10.1016/j.foodchem.2006.03.017

[20] J. L. Liu, J. F. Yuan and Z. Q. Zhang, "Microwave-Assisted Extraction Optimised with Response Surface Methodology and Antioxidant Activity of Polyphenols from Hawthorn (*Crataegus Pinnatifida Bge.*) Fruit," *International Journal of Food Science and Technology*, Vol. 45, No. 11, 2010, pp. 2400-2406. doi:10.1111/j.1365-2621.2010.02416.x

[21] W. Cherdshewasart and W. Sutjit, "Correlation of Antioxidant Activity and Major Isoflavonoid Contents of the Phytoestrogen-Rich *Pueraria Mirifica* and *Pueraria Lobata Tubers*," *Phytomedicine*, Vol. 15, No. 1-2, 2008, pp. 38-43. doi:10.1016/j.phymed.2007.07.058

[22] R. Stocker and J. F. Keaney, "Role of Oxidative Modifications in Atherosclerosis," *Physiological Review*, Vol. 84, No. 4, 2004, pp. 1381-1478. doi:10.1152/physrev.00047.2003

[23] D. Steinberg, "Low Density Lipoprotein Oxidation and Its Pathobiological Significance," *Journal of Biological Chemistry*, Vol. 272, No. 34, 1997, pp. 20963-20966. doi:10.1074/jbc.272.34.20963

[24] H. Esterbauer and P. Ramos, "Chemistry and Pathophysiology of Oxidation of LDL," *Review of Physiology Biochemistry Pharmacology*, Vol. 127, 1995, pp. 31-64. doi:10.1007/BFb0048264

[25] A. C. Kaliora, G. V. Z. Dedoussis and H. L. Schmidt, "Dietary Antioxidant in Preventing Atherogenesis," *Atherosclerosis*, Vol. 187, No. 1, 2006, pp. 1-17. doi:10.1016/j.atherosclerosis.2005.11.001

[26] Z. S. Zhang, Q. Chang, M. Zhu, Y. Huang, W. K. K. Ho and Z. Y. Chen, "Characterization of Antioxidants Present in Hawthorn Fruits," *Journal of Nutritional Biochemistry*, Vol. 12, No. 3, 2001, pp. 144-152. doi:10.1016/S0955-2863(00)00137-6

[27] S. Tashino, "'Serum Pharmacology' and 'Serum Pharmaceutical Chemistry': From Pharmacology of Chinese Traditional Medicines to Start a New Measurement of Drug Concentration in Blood," *Therapeutic Drug Monitoring Research*, Vol. 5, 1988, pp. 54-64.

[28] D. Tang, Z. Zhang, Y. Gao, Y. Wei and L. Han, "Protective Effects of Serum Containing Ginkgo Biloba Extract on Glomerulosclerosis in Rat Mesangial Cells," *Journal of Ethnopharmacology*, Vol. 124, No. 1, 2009, pp. 26-33. doi:10.1016/j.jep.2009.04.017

[29] H. Zhang, B. Ji, G. Chen, F. Zhou, Y. Luo, H. Yu, F. Gao, Z. Zhang and H. Li, "A Combination of Grape Seed-Derived Procyanidins and Gypenosides Alleviates Insulin Resistance in Mice and HepG2 Cells," *Journal of Food Science*, Vol. 74, No. 1, 2009, pp. H1-H7. doi:10.1111/j.1750-3841.2008.00976.x

[30] C. A. Schmitt and V. M. Dirsch, "Modulation of Endothelial Nitric Oxide by Plant-Derived Products," *Nitric Oxide*, Vol. 21, No. 2, 2009, pp. 77-91. doi:10.1016/j.niox.2009.05.006

[31] W. Bochu, Z. Liancai and C. Qi, "Primary Study on the Application of Serum Pharmacology in Chinese Traditional Medicine," *Colloids and Surfaces B*: *Biointerfaces*, Vol. 43, No. 3-4, 2005, pp. 194-197. doi:10.1016/j.colsurfb.2005.04.013

[32] K. Acharya, K. Samui, M. Rai, B. B. Dutta and R. Acharya, "Antioxidant and Nitric Oxide Synthase Activation Properties of *Auricularia Auricular*," *Indian Journal of Experimental Biology*, Vol. 42, No. 5, 2004, pp. 538-540.

[33] G. Chen, Y. Luo, B. Ji, B. Li, W. Su, Z. Xiao and G. Zhang, "Hypocholesterolemic Effects of *Auricularia Auricula* Ethanol Extract in ICR Mice Fed a Cholesterol-Enriched Diet," 2010. http://www.springerlink.com/content/b156t26171935104/

