JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Monascin and Ankaflavin Have More Anti-atherosclerosis Effect and Less Side Effect Involving Increasing Creatinine Phosphokinase Activity than Monacolin K under the Same Dosages

Chun-Lin Lee,[†] Yu-Ping Hung,[†] Ya-Wen Hsu,[‡] and Tzu-Ming Pan*[,§]

[†]Department of Life Science, National Taitung University, Taitung, Taiwan, Republic of China

[‡]R&D Division, SunWay Biotechnology Company Limited, Taipei, Taiwan, Republic of China

[§]Department of Biochemical Science and Technology, National Taiwan University, 1, Sec. 4, Roosevelt Road, Taipei, Taiwan, Republic of China

ABSTRACT: Monacolin K has long been considered a major effective component in the hypolipidemic functions of *Monascus*. Monacolin K also serves as a well-known hypolipidemic medication, but its side effect myopathy is a concern. Monascin and ankaflavin, the yellow pigments produced by *Monascus* species, have been proven to possess hypolipidemic functions; however, no studies have compared the hypolipidemic effects of monascin, ankaflavin, and monacolin K under the same dosages. In this study, the equal dosages of monascin, ankaflavin, and monacolin K were oral administrated to hamsters fed a high cholesterol diet for 6 weeks. Comparison of the displayed hypolipidemic and anti-atherosclerosis effects was performed, in addition to an investigation into the inducement of side effect. The results indicated that monascin and ankaflavin were similar to monacolin K in significantly reducing total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C) levels in serum and lipid plaque ($p < 0.05$) in the heart aorta. In addition, ankaflavin achieved the effects of serum TC and TG reduction, with no significant difference as compared to those effects of monacolin K ($p > 0.05$). However, as compared to monacolin K, ankaflavin possessed more significant effects on the prevention of fatty liver and lipid plaque accumulation in heart aorta. More importantly, monascin significantly enhanced high-density lipoprotein cholesterol (HDL-C) concentrations, while monacolin K displayed the opposite effect. Regarding the side effect, monacolin K also raised elevated creatinine phosphokinase (CPK) activity, which was highly correlated with rhabdomyolysis development, while monascin and ankaflavin did not induce such a side effect. In conclusion, MS and AK had the potential to be developed as hypolipidemic agents without rhabdomyolysis development.

KEYWORDS: *monascin, ankaflavin, monacolin K, creatinine phosphokinase*

INTRODUCTION

Monascus-fermented product has long been thought to possess both medicinal and edible purposes. In the past, the usage of *Monascus* species focused mainly on pigment production and traditional food development. However, in recent years, studies on *Monascus* species have focused more on the prevention of cardiovascular diseases. *Monascus*-fermented products such as red mold rice (RMR) have been proven to significantly reduce total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C) concentrations in serum, in addition to raising apolipoprotein A (apo A), and reducing apolipoprotein (apo B) expressions.[1,2] Our previous studies have indicated that red mold dioscorea (RMD) displays tremendous capabilities for reducing TC, TG, and LDL-C in high cholesterol hamster models, and also significantly enhances high-density lipoprotein cholesterol (HDL-C) levels.[3] Such studies indicate that *Monascus*-fermented products possess significant hypolipidemic and anti-atherosclerosis effects. In the past, such hypolipidemic functions were considered to be contributed from monacolin K (lovastatin), as it is a HMG-CoA reductase (HMGR) inhibitor produced by *Monascus* species.[4] However, an increasing number of studies indicate that a long-term high monacolin K dosage may cause numerous side effects, including rhabdomyolysis and coenzyme Q10 (CoQ10) level reduction.[5−7] Therefore, the safety of *Monascus*-fermented product with high monacolin K concentration is also a concern. Moreover, even though monacolin K reduces TC, TG, and LDL-C levels in serum, it also significantly reduces HDL-C levels.[8,9]

However, recent studies have successively discovered that other metabolites of *Monascus* species also possess hypolipidemic functions. Our previous studies were the first to discover the hypolipidemic and anti-atherosclerosis functions of monascin and ankaflavin; these studies also discovered that monascin and ankaflavin significantly enhanced HDL-C levels, while imposing no damage on the liver or kidneys.[3] Monascin and ankaflavin also inhibited preadipocyte differentiation and stimulated the lipolysis of mature adipocytes.[10] Monascin has been shown to protect the liver from chemical damage via the anti-inflammatory effect.[11] Previous studies indicated that monascin significant inhibits peroxynitrite ($ONOO^-$; PN), and ultraviolet light B (UVB) induced skin carcinogenesis.[12] These studies have shown that monascin and ankaflavin possess

Received: October 10, 2012
Revised: December 13, 2012
Accepted: December 13, 2012
Published: December 13, 2012

dx.doi.org/10.1021/jf304346r | J. Agric. Food Chem. 2013, 61, 143−150

Table 1. Effect of Equal Dosage of Monascin, Ankaflavin, and Monacolin K on the Levels of TC, TG, HDL-C, and LDL-C and the LDL-C/HDL-C Ratio in Serum of Hyperlipidemic Hamsters Fed a High-Cholesterol Diet[a]

group	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C(mg/dL)	LDL-C/HDL-C ratio
NOR	102.4 ± 10.8 a	163.4 ± 30.7 ab	63.0 ± 4.3 a	19.0 ± 1.6 a	0.26 ± 0.03 a
HC	232.4 ± 25.6 d	268.6 ± 85.5 c	93.6 ± 17.2 bc	79.6 ± 9.2 d	0.70 ± 0.08 d
MK	143.5 ± 12.9 b	141.4 ± 28.2 a	84.7 ± 4.9 b	49.5 ± 5.5 bc	0.58 ± 0.10 c
MS	175.3 ± 17.8 c	202.3 ± 50.4 b	123.8 ± 20.9 d	56.4 ± 13.1 c	0.53 ± 0.11 bc
AK	154.5 ± 16.6 b	181.6 ± 36.6 ab	103.1 ± 10.1 c	44.5 ± 12.5 b	0.47 ± 0.07 b

[a]Two groups of the hamsters were fed a normal diet (NOR group) or a high cholesterol diet (HC group) without the administration of test materials, respectively. The other hyperlipidemic hamsters were administrated with monacolin K (0.624 mg/kg/day) (MK group), monascin (0.624 mg/kg/day) (MS group), and ankaflavin (0.624 mg/kg/day) (AK group). Data are presented as means ± SD (n = 8). Mean values with different letters are significantly different ($p < 0.05$). The statistical significance in the biochemical effects was determined by ANOVA with Duncan's multiple test.

hypolipidemic, anti-atherosclerosis, antioxidative, and anti-inflammatory characteristics, which render them as substances that can potentially prevent cardiovascular diseases.[3,10−12]

Our previous studies have shown that in hyperlipidemic hamsters, RMD displayed better hypolipidemic and anti-atherosclerosis effects than RMR, as RMD consisted of other hypolipidemic components aside from monacolin K.[13] Studies have shown that yellow pigments monascin and ankaflavin in RMD were higher than those in RMR; these not only possessed hypolipidemic functions, but could also increase HDL-C levels.[3,13] These studies indicated that RMD including 2892 mg/kg monacolin K, 9822 mg/kg monascin, and 1428 mg/kg ankaflavin displayed better hypolipidemic and HDL-C-elevating effects in our previous study.[3] However, there is no study to discuss the comparison of hypolipidemic effects among monascin, ankaflavin, and monacolin K at the same dosage. This evidence may be useful to understand the potential of monascin and ankaflavin as innovative hypolipidemic compounds or medicines. In this study, the hamsters were fed with a high cholesterol diet and orally given monacolin K, monascin, and ankaflavin (0.624 mg/kg of BW/day), to compare the hypolipidemic and anti-atherosclerosis effect of these three compounds.

MATERIALS AND METHODS

Chemicals. Monascin and ankaflavin (99.9% purity) were provided from SunWay Biotechnology Co, (Taipei, Taiwan, ROC). Monacolin K (mevanolin, 99.9% purity), thiobarbituric acid (TBA), and malondialdehyde (MDA) were purchased from Sigma Chemical Co. (St. Louis, MO). LC grade chloroform, methanol, and dimethyl sulfoxide (DMSO) were purchased from Merck Co. (Darmstadt, Germany).

Animals and Diets. Forty male Golden Syrian hamsters weighing 90−110 g were housed in individual plastics cages and subjected to a 12 h light/dark cycle with a maintained relative humidity of 60% and a temperature at 25 °C. The animals were given free access to regular rodent chow and water for 2 weeks to adapt to the new environment. Hamsters were weighed and randomly assigned to five groups of eight animals each before the commencement of the animal experiment.

Dose and Grouping. The dose of monacolin K powder was calculated in accordance with Boyd's formula of body surface area as recommended by Boyd.[14] This study used 5.784 mg of monacolin K as the reference dose of an adult with a weight of 65 kg and a height of 170 cm to calculate the hamster dose according to our previous study.[13] After the prebreeding stage for 2 weeks, all test samples were respectively suspended in 1 mL of water and orally administered to the hamsters using a stomach tube for 6 weeks. Food intake was recorded daily, and animals were weighed weekly.

Experimental diets were provided in accordance with AIN-76 diet formulation with modification.[15] The control group was fed a normal diet via AIN-76 formulation, and the HC group was given a high-cholesterol diet including 0.2% cholesterol.[13] The MK (monacolin K feeding group), MS (monascin feeding group), and AK (ankaflavin feeding group) groups were fed the high-cholesterol diet and orally given monacolin K, monascin, and ankaflavin (0.624 mg/kg of BW/day), respectively.

Twenty-four hours before sacrifice, all food was removed. Animals were anesthetized and sacrificed by carbon dioxide inhalation, and whole blood, plasma, and serum samples were collected, prepared, and then stored at −80 °C. Liver tissue was lavaged and rinsed frequently with 0.8% sodium chloride solution for eliminating any blood. The biggest leaf of liver tissue was ground in ice-cold phosphate-buffered saline (PBS) and then centrifuged (8000 × g, 15 min). The supernatant was collected and stored at −80 °C for the assay of thiobarbituric acid reactive substances (TBARS). Part of the liver tissue was immersed in 10% formalin stock and then examined for pathology using H&E staining. The other liver tissue was immersed in liquid nitrogen and then stored at −80 °C.

Serum, Liver, and Fecal Lipid Analysis. Serum TC, TG, and HDL-C levels were measured in triplicate using commercial enzymatic kits. These kits were as follows: the TC assay kit (CH 200, Randox Laboratories Ltd., Antrim, U.K.), the TG assay kit (TR-210, Randox Laboratories Ltd.), and the HDL-C assay kit (CH-203, Randox Laboratories Ltd.). Serum LDL-C levels were gained via the following calculation: LDL-C (mg/dL) = TC − TG/5 − HDL-C. Liver tissue and feces (0.5 g) were ground in 10 mL of ice-cold Folch solution (chloroform/methanol = 2:1; v/v) and incubated for 30 min at room temperature.[16] The aqueous layer was aspirated and discarded, and the fixed volume of the organic layer was then evaporated to dryness. The dried lipid layer was dissolved with an equal volume of DMSO and then used to determine the TC and TG levels using commercial enzymatic kits (Randox Laboratories Ltd.).

Determination of Creatine Phosphokinase (CPK) Content. Blood samples (0.5 mL) were collected for measurement of CPK. Blood samples were immediately centrifuged at 3000 × g for 10 min. The serum was decanted and stored at 4 °C within 1 h after collection for biochemical analysis. Serum levels of CPK were determined with an autoanalyzer (COBAS C111, Roche Diagnostics, Basel, Switzerland) to obtain biochemical data.[17]

Determination of TBARS Content. The TBARS assay is also regarded as the accepted determination for in vivo lipid peroxidation.[18,19] According to the procedure of the previous study, the TBARS levels of serum and liver were determined by the method of thiobarbituric acid (TBA) colorimetric analysis, and the optical density (OD) value was measured at 532 nm.[20]

Stain of Aortic Plaque in Heart Aorta. The heart aorta was cut open longitudinally along the anterior side, and the lipid-rich lesions on the surface of the aorta were stained with 2% Sudan IV and then successively washed with a gradient concentration of methanol (100%, 90%, 80%, 70%, 60%) and PBS. The whole surface area of the thoracic aorta was stained by Sudan IV and photographed using a digital camera. The aortic surface area and its stained plaque area (red) were selected and quantitated by the Posterize program of Photoshop 7.0 software (Adobe Systems Inc., San Jose, CA). The selected pixel of the

Table 2. Effect of Equal Dosage of Monascin, Ankaflavin, and Monacolin K on the Levels of TC and TG in Liver and Feces of Hyperlipidemic Hamsters Fed a High-Cholesterol Diet[a]

	liver		feces	
group	TC levels (mg/g)	TG levels (mg/g)	TC levels (mg/g)	TG levels (mg/g)
NOR	1.28 ± 0.04 a	2.16 ± 0.17 a	1.17 ± 0.09 b	0.88 ± 0.04 b
HC	4.50 ± 0.34 d	4.63 ± 0.60 c	1.92 ± 0.00 c	1.25 ± 0.04 c
MK	2.05 ± 0.08 b	2.96 ± 0.48 b	0.89 ± 0.13 ab	0.94 ± 0.04 b
MS	3.04 ± 0.31 c	2.24 ± 0.24 a	1.01 ± 0.22 b	0.50 ± 0.04 a
AK	2.24 ± 0.40 b	2.67 ± 0.35 b	0.67 ± 0.09 a	0.53 ± 0.00 a

[a]Two groups of the hamsters were fed a normal diet (NOR group) or a high cholesterol diet (HC group) without the administration of test materials, respectively. The other hyperlipidemic hamsters were administrated with monacolin K (0.624 mg/kg/day) (MK group), monascin (0.624 mg/kg/day) (MS group), and ankaflavin (0.624 mg/kg/day) (AK group). Data are presented as means ± SD (n = 8). Mean values with different letters are significantly different ($p < 0.05$). The statistical significance in the biochemical effects was determined by ANOVA with Duncan's multiple test.

plaque area and whole aorta was used to calculate the percent area of the aortic plaque as follows:[13]

$$\text{aortic plaque (\%)} = \text{pixel of stained plaque area} / \text{pixel of whole aorta} \times 100\%$$

Plasma Liver Index Analysis. Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were measured in triplicate using commercial enzymatic kits. The kits were as follow: AST assay kit (Part MP2-113, Johnson and Johnson, New Brunswick, NJ), ALT assay kit (Part MP2-36, Johnson and Johnson).

Histological Analysis. Liver tissue sections were cut at a thickness of 7 μm and mounted on silanized slides (Dako Japan, Tokyo, Japan). The sections were stained with hematoxylin−eosin (HE) to observe the histological features of the livers.[21]

Statistics. Data are expressed as the mean ± SD. The statistical significance in the biochemical effects was determined by one-way analysis of variance (ANOVA) with Duncan's multiple test.

RESULTS

Total Cholesterol and Triglyceride in Serum. This study applied equal dosages of monascin, ankaflavin, and monacolin K as test substances in the treatment of hyperlipidemic hamsters, and subsequently compared the hypolipidemic and anti-atherosclerosis effects of these three compounds.[3] The comparison of serum TC and TG levels is shown in Table 1. The results of this study showed that MS, AK, and MK all exhibited significant effects in decreasing TC and TG levels, as compared to the HC group ($p < 0.05$). The groups treated with MS, AK, and MK displayed significantly lower TC than the HC group (24.6% ($p < 0.05$), 33.5% ($p < 0.05$), and 39.3% ($p < 0.05$), respectively). As for TG, the MS, AK, and MK groups also exhibited significantly lower results than the HC group (24.7% ($p < 0.05$), 32.4% ($p < 0.05$), and 47.4% ($p < 0.05$), respectively). Monacolin K displayed better effects in reducing TC and TG levels than monascin; however, the results of comparison between monacolin K and ankaflavin did not achieve a significant difference ($p > 0.05$).

HDL-C, LDL-C, and LDL-C/HDL-C Ratios in Serum. LDL-C is the primary substance in causing cardiovascular diseases such as atherosclerosis. The results (as shown in Table 1) indicated that, as compared to the HC group, groups given identical dosages of MS, AK, and MK all significantly reduced LDL-C levels by 29.1% ($p < 0.05$), 44.1% ($p < 0.05$), and 37.8% ($p < 0.05$), respectively. Ankaflavin exhibited higher LDL-C-lowering effects than monacolin K, but without significant difference ($p > 0.05$). As compared to the HC group, MS and AK enhanced HDL-C levels by 32.3% ($p < 0.05$) and 10.1% ($p > 0.05$), respectively; on the contrary, MK treatment led to HDL-C reduction by 9.5%.

The LDL-C/HDL-C ratio is another critical indicator in hypolipidemic function assessment. A lower ratio indicates higher HDL-C levels in TC and naturally lower levels of LDL-C, which induces cardiovascular disease. The results in Table 1 indicate that after 6 weeks of a high cholesterol diet in the HC group, the LDL-C/HDL-C ratio was significantly increased as compared to the NOR group ($p < 0.05$). As for the groups treated with MS, AK, and MK, the LDL-C/HDL-C ratio was reduced, respectively, by 24.3% ($p < 0.05$), 32.9% ($p < 0.05$), and 17.1% ($p < 0.05$) as compared to the HC group. In conclusion, ankaflavin displayed the strongest effect in reducing the LDL-C/HDL-C ratio. The results indicated that monascin and ankaflavin possess higher suitability as functional compounds in cardiovascular disease prevention and anti-atherosclerosis.

Total Cholesterol and Triglycerides in Liver and Feces. As shown in Table 2, TC and TG levels in the HC group were significantly higher than those in the NOR group ($p < 0.05$), while in the groups treated with equal dosages of MS, AK, and MK, TC and TG levels were significantly reduced ($p < 0.05$). For TC in the liver, monacolin K displayed stronger reduction effects than monascin ($p < 0.05$); however, when compared to ankaflavin, no significant differences were observed ($p > 0.05$). As for liver TG, monascin displayed the strongest reducing effects, followed by ankaflavin and monacolin K. These results indicated that monascin, ankaflavin, and monacolin K all successfully reduced lipid levels in the liver.

TC levels reduction may be the result of cholesterol biosynthesis inhibition or cholesterol excretion enhancement. However, monacolin K is proven as the inhibitor of HMG-CoA reductase in the cholesterol biosynthesis. [4] As shown in Table 2, monascin, ankaflavin, and monacolin K all reduced TC excretion in feces. Their similar results indicated that monascin and ankaflavin possibly reduced TC in serum by inhibiting cholesterol biosynthesis, rather than by regulating TC excretion in feces.

Lipid Peroxidation in Serum. MDA levels often serve as indicators of lipid peroxidation in the body. When MDA levels are excessive, the LDL in the blood is converted into oxidized LDL through lipid peroxidation. This oxidized LDL is highly correlated with the occurrence of atherosclerosis.[22] As shown in Figure 1, MDA levels in the HC group were significantly higher than in the NOR group ($p < 0.05$), indicating that feeding a high cholesterol diet stimulated an increase in MDA levels. As

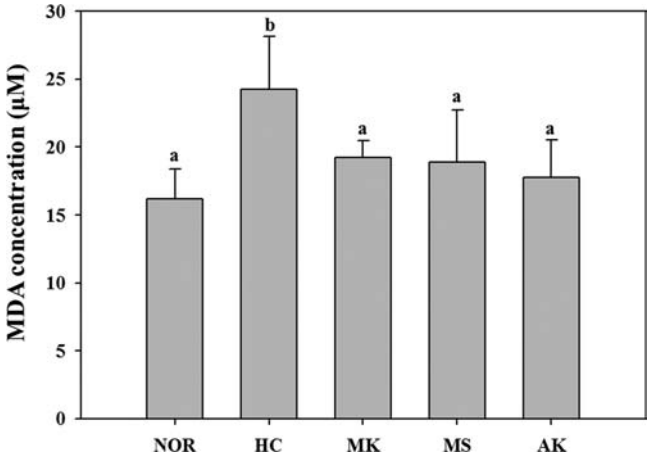

Figure 1. Effect of equal dosage of monascin, ankaflavin, and monacolin K on lipid peroxidation in serum of hyperlipidemic hamsters fed a high-cholesterol diet. Two groups of the hamsters were fed a normal diet (NOR group) or a high cholesterol diet (HC group) without the administration of test materials, respectively. The other hyperlipidemic hamsters were administrated with monacolin K (0.624 mg/kg/day) (MK group), monascin (0.624 mg/kg/day) (MS group), and ankaflavin (0.624 mg/kg/day) (AK group). Data are presented as means ± SD (n = 8). Mean values with different letters are significantly different (p < 0.05). The statistical significance in the biochemical effects was determined by ANOVA with Duncan's multiple test.

compared to the HC group, the groups treated with equal dosages of MS, AK, and MK displayed significantly lower MDA levels (22.1% (p < 0.05), 26.7% (p < 0.05), and 20.6% (p < 0.05), respectively). Ankaflavin exhibited the strongest effects in MDA reduction, followed by monascin and monacolin K.

Lipid Plaque in Heart Aorta. Lipid plaque accumulation is known to be correlated with the occurrence of atherosclerosis. The results for the lipid plaque accumulation of each group are shown in Figure 2. As compared to the NOR group, the HC group displayed larger amounts of plaque accumulation in the heart aorta. In contrast, the MS, AK, and MK groups displayed significantly lower plaque accumulation levels than the HC group (p < 0.05): reductions of 82.7% (p < 0.05), 89.7% (p < 0.05), and 57.5% (p < 0.05), respectively. The lipid plaque reduction in the MS and AK groups was significantly higher than in the MK group (p < 0.05); among these, the AK group exhibited stronger effects than the MS group, but did not achieve a level of significance (p > 0.05). This indicated that the monascin and ankaflavin displayed higher effectiveness in inhibiting atherosclerosis-related lipid plaque accumulation than the monacolin K.

Liver Function Test and Pathological Examination. ALT and AST activities in serum are considered the indicators of liver function. In this study, they were applied to assess the protective effects of monascin, ankaflavin, and monacolin K against fatty liver damage induced by a high cholesterol diet. As shown in Table 3, as a result of a high cholesterol diet, ALT and AST activity in the HC group was significantly higher than that in the NOR group (p < 0.05). However, ALT and AST activities in the MS, AK, and MK groups were significantly lower than those in the HC group (p < 0.05): 41.2% and 45.0% lower in the MS group; 65.7% and 57.2% lower in the AK group; and 60.1% and 40.8% lower in the MK group, respectively. These results indicate that monascin, ankaflavin, and monacolin K all successfully reduced ALT and AST activity, and displayed liver-protecting effects by decreasing liver damage induced by a high cholesterol diet. Among the three, ankaflavin displayed the strongest protective effects.

The results of the liver pathological examination are shown in Figure 3. The HC group, fed with a high-cholesterol diet, displayed vacuolar characteristics around the nucleus, in addition to a more complete cytoplasm condition than the NOR group. In the HC group, the cytoplasm in the liver was occupied by lipids, leading to the formation of vacuoles known as lipid vacuoles. The liver biopsy results indicated that an extremely fatty liver was observed in the HC group, due to the considerably large amounts of lipid vacuoles present in the liver. However, in the MS, AK, and MK groups, which were also fed with high cholesterol diets, the quantities of lipid vacuoles in the liver were significantly lower than in the HC group. These results indicate that monascin, ankaflavin, and monacolin K significantly prevented lipid accumulation in the liver and, consequently, the chance of fatty liver development. Among these three, ankaflavin displayed the strongest effects.

Creatinine Phosphokinase Activity in Serum. Excessive CPK activity in serum leads to muscle weakness or pain, even rhabdomyolysis.[23−25] As shown in Figure 4, the results indicated that CPK activity in the HC group did not display a significant difference as compared to the NOR group (p > 0.05). This indicated that a high cholesterol diet did not result in an increase in CPK activity. Previous studies have shown that long-term high monacolin K (lovastatin) dosages lead to rhabdomyolysis.[5,6] In this study, the MK group displayed significantly higher CPK activity as compared to the HC group (p < 0.05), indicating that monacolin K may significantly lead to the development of rhabdomyolysis. However, the MS and AK groups did not display any significant difference in CPK activity when compared to the HC group (p > 0.05). Monascin, ankaflavin, and monacolin K are all effective hypolipidemic substances, with the difference being that monacolin K increases CPK activity, while monascin and ankaflavin do not.

DISCUSSION

This study compared the hypolipidemic and anti-atherosclerosis effects of equal dosages of monascin, ankaflavin, and monacolin K. Monascin and ankaflavin are proven to have effective hypolipidemic, anti-inflammation, and antitumor components,[3,10,26] while monacolin K is an HMGR inhibitor, proven to effectively reduce TC in serum and the liver.[4,9] Our previous study focused on the contribution of MK, MS, and AK in the hypolipidemic effect of RMD, but their hypolipidemic effects cannot be compared among each other due to the different dosage use. Therefore, this study is designed to compare the hypolipidemic effect of MS, AK, with that of MK under the same dosage. This information is useful to evaluate the potential of MS and AK to develop as hypolipidemic agents or medicines in the future.

From the combined results, this study investigates whether monascin and ankaflavin possess stronger hypolipidemic effects than monacolin K, and whether they exert side effects similar to those of monacolin K. Among monascin, ankaflavin, and monacolin K, monascin displayed the strongest effects in elevating serum HDL-C levels. However, in reducing LDL-C levels, AST and ALT activity, lipid plaque levels in aorta, antilipid peroxidation, and cholesterol accumulation in liver, ankaflavin displayed the strongest results. The cholesterol inhibitor monacolin K displayed better effects in TC and TG levels reduction, but did not achieve a significant difference

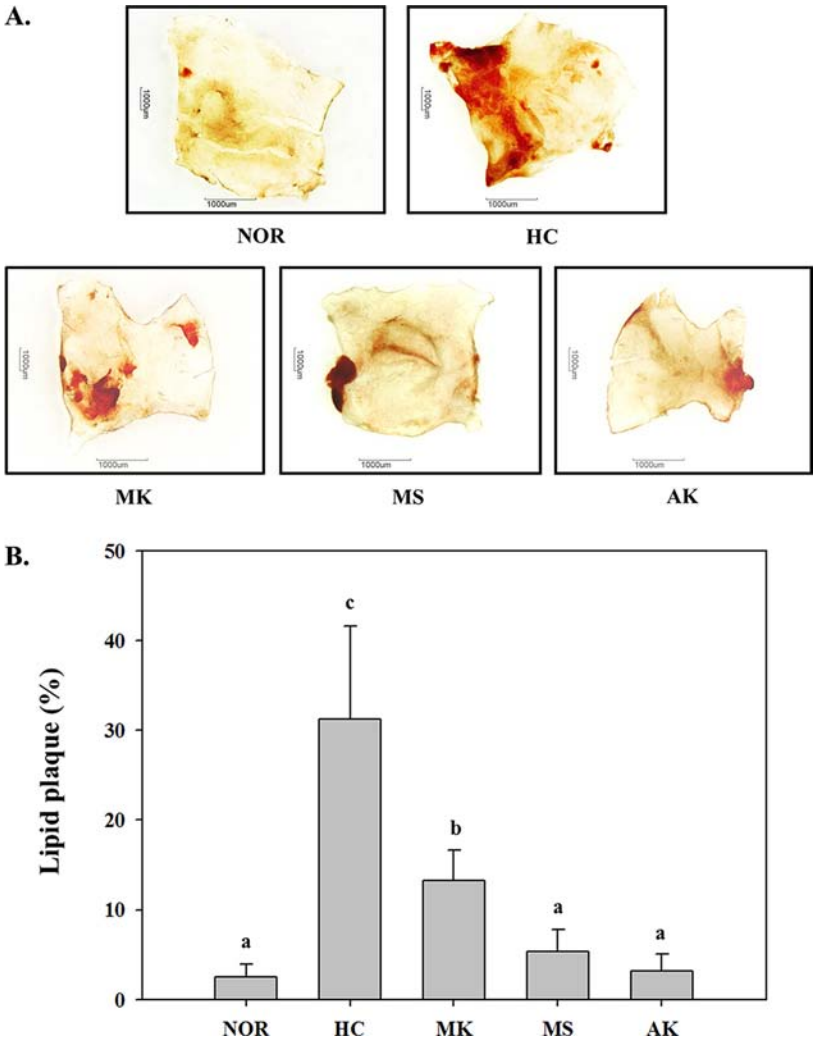

Figure 2. Effect of equal dosage of monascin, ankaflavin, and monacolin K on the atherosclerotic plaque in the heart aorta of hyperlipidemic hamsters: (A) atherosclerotic plaque presented as the red dye in the graph; (B) proportion of the area of the atherosclerotic plaque in the aorta. Two groups of the hamsters were fed a normal diet (NOR group) or a high cholesterol diet (HC group) without the administration of test materials, respectively. The other hyperlipidemic hamsters were administrated with monacolin K (0.624 mg/kg/day) (MK group), monascin (0.624 mg/kg/day) (MS group), and ankaflavin (0.624 mg/kg/day) (AK group). Data are presented as means ± SD (n = 8). Mean values with different letters are significantly different (p < 0.05). The statistical significance in the biochemical effects was determined by ANOVA with Duncan's multiple test.

when compared to ankaflavin (p > 0.05). Monascin displayed reduction effects similar to those of monacolin K. In addition to TC levels reduction, monacolin K also resulted in HDL-C levels reduction; on the contrary, monascin caused an elevation in HDL-C levels, as did ankaflavin. Although monascin induced the highest HDL-C levels, the MS group displayed higher TC levels than the AK group. Because HDL-C is a component of TC, TC levels increase with HDL-C levels. As the MS group exhibited higher TC levels in serum and liver, TC excretion in feces was also the highest in the MS group. Previous studies have shown that under a daily monascin dosage of 5.30 mg/kg and an ankaflavin dosage of 0.77 mg/kg, they performed no significant difference in TC reduction with each other.[3] These results indicate that 6 times more monascin is required to achieve effects similar to those of ankaflavin in TC reduction. However, this study discovered that ankaflavin possessed the strongest effects in reducing LDL-C levels, AST and ALT activity, lipid plaque levels in aorta, antilipid peroxidation, and cholesterol accumulation in liver in the three substances. Thus, ankaflavin possessed the most effective hypolipidemic and anti-atherosclerosis functions.

The effects of monascin and ankaflavin are likely correlated with the regulating mechanisms of lipid metabolism. In general, an increase in the TC results from a combination of food intake and cholesterol biosynthesis pathway; conversely, a decrease in the TC is a result of enhanced cholesterol excretion or inhibited cholesterol biosynthesis. The results of this study indicate that monascin and ankaflavin lead to reduced TC in feces, similar to the effects of monacolin K. Monacolin K is a typical competitive inhibitor, which inhibits HMGR activity in the TC biosynthesis pathway, resulting in TC reduction.[4] Therefore, reduction in TC levels in the serum, the liver, and feces is a result of inhibited TC biosynthesis, rather than enhanced TC excretion. Study results indicate that monascin and ankaflavin regulated lipid metabolism by reducing TC levels in serum, liver, and feces.

Under the same effective dosages, although monascin and ankaflavin displayed hypolipidemic mechanisms similar to those of monacolin K, these mechanisms were still different in nature.

dx.doi.org/10.1021/jf304346r | J. Agric. Food Chem. 2013, 61, 143−150

Table 3. Effect of Equal Dosage of Monascin, Ankaflavin, and Monacolin K on the Activities of Aspartate Aminotransferase (AST) and Alanine Transferase (ALT) in Serum of Hyperlipidemic Hamsters Fed a High-Cholesterol Diet[a]

group	AST activity (U/L)	ALT activity (U/L)
NOR	48.6 ± 6.5 a	61.9 ± 8.2 a
HC	120.5 ± 52.4 b	209.5 ± 38.8 c
MK	71.3 ± 8.3 a	83.5 ± 15.0 a
MS	66.3 ± 23.8 a	123.1 ± 28.5 b
AK	51.6 ± 10.7 a	71.9 ± 23.8 a

[a]Two groups of the hamsters were fed a normal diet (NOR group) or a high cholesterol diet (HC group) without the administration of test materials, respectively. The other hyperlipidemic hamsters were administrated with monacolin K (0.624 mg/kg/day) (MK group), monascin (0.624 mg/kg/day) (MS group), and ankaflavin (0.624 mg/kg/day) (AK group). Data are presented as means ± SD (n = 8). Mean values with different letters are significantly different ($p < 0.05$). The statistical significance in the biochemical effects was determined by ANOVA with Duncan's multiple test.

Importantly, monascin and ankaflavin resulted in increased HDL-C levels, whereas monacolin K did not. HDL-C is considered beneficial to atherosclerosis prevention, as it facilitates cholesterol transport from peripheral tissues to the liver. HDL prevents macrophages from forming foam cells, and also possesses atherosclerosis functions, as it is capable of reverse cholesterol transport (RCT), in other words, transporting excessive cholesterol from peripheral tissues to the liver for excretion.[27] Moreover, HDL possesses antioxidant, anti-inflammatory, antithrombotic, and antiapoptotic effects.[28−30] Previous studies have indicated that chitosan, young persimmon, gallic acid, linoleic acid, and an aqueous extract of the *Withania coagulans* fruit significantly reduce TC levels in mice fed a high fat diet, but meanwhile cause a reduction in HDL-C levels. [31] The results of this study indicate that monascin and ankaflavin significantly reduced TC levels; moreover, monascin significantly enhanced HDL-C levels, while ankaflavin also facilitated an HDL-C level increase. Monacolin K displayed fairly good effects in TC reduction, but had the undesired effect of causing a reduction in HDL-C levels.

However, the regulation of lipid metabolism is complex. The hypolipidemic and HDL-C raising mechanism of monascin and ankaflavin is currently unclear, but we try to discuss the possible regulation for lipid metabolism. Our previous studies proved that monascin and ankaflavin were able to inhibit PPARγ expression during the differentiation of preadipocyte involved in the obesity development.[10] Furthermore, ankaflavin was also proven as the PPARγ agonist to lower blood glucose and increase insulin sensibility.[32] Therefore, the two compounds are possible regulators of PPARs. PPARs family is highly associated with the regulation of lipid metabolism, adipogenesis, and diabetes control. PPARγ is related to the regulation of blood glucose, insulin resistance, adipogenesis, and anti-inflammation in adipose, muscle, and vessel wall.[33] The anti-adipoensis and anti-inflammation of monascin and ankaflavin may contribute to the antiobesity effect and anti-atherosclerosis. Furthermore, PPARα is proven to mediate lipid metabolism involving the decrease of TG levels, increase of HDL-C levels, and fatty acid oxidation in liver, muscle, and vessel wall. PPARα agonist such as fibrates exhibited the ability to decrease TG and increase HDL-C levels.[33] However, it is unclear whether monascin and ankaflavin decreased blood lipid and raised HDL-C levels via the regulation of PPARα. Detailed evidence remains to be discovered in the future.

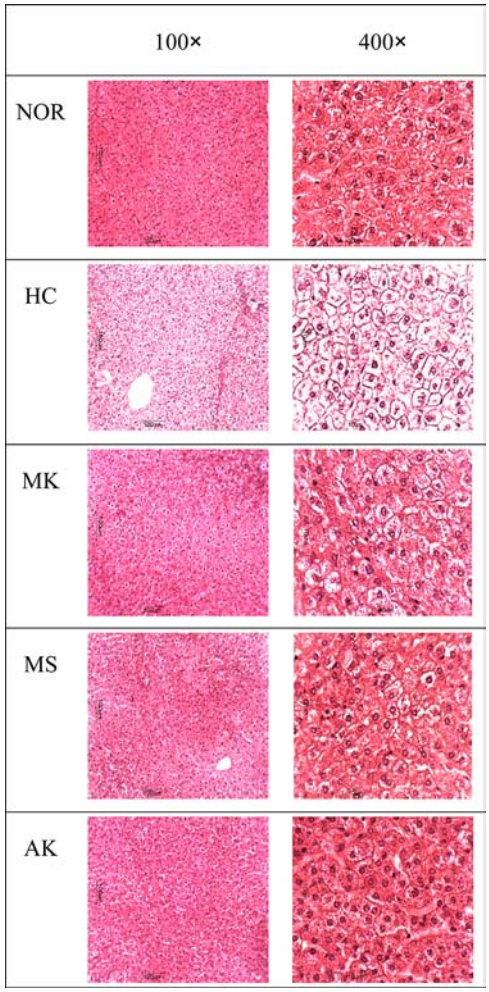

Figure 3. Pathological examination of liver of experimental hamsters in the 100× power field (A) and 400× power field (B). The liver sections were stained using H&E and observed in the light microscope. Two groups of the hamsters were fed a normal diet (NOR group) or a high cholesterol diet (HC group) without the administration of test materials, respectively. The other hyperlipidemic hamsters were administrated with monacolin K (0.624 mg/kg/day) (MK group), monascin (0.624 mg/kg/day) (MS group), and ankaflavin (0.624 mg/kg/day) (AK group).

Oxidative stress is regarded as the key risk factor for the development of atherosclerosis. Lipid peroxidation caused the ox-LDL formation and stimulated the deposition of lipid plaque, resulting in the occurrence of atherosclerosis.[18,19] Therefore, antioxidative ability is able to reverse the development of atherosclerosis. The results of this study have proven that monascin and ankaflavin expressed a significant effect in reducing high cholesterol diet-induced MDA levels and lipid plaque. However, these protections are probably contributed from the antioxidative ability of monascin and ankaflavin, because they were proven as the antioxidative and anti-inflammatory compounds.[12,26] Both monascin and ankaflavin are similar in structure, with ankaflavin possessing 2 additional carbon and 4 additional hydrogen atoms; the hydrogen atoms possibly contribute to the antioxidant ability of monascin and ankaflavin.

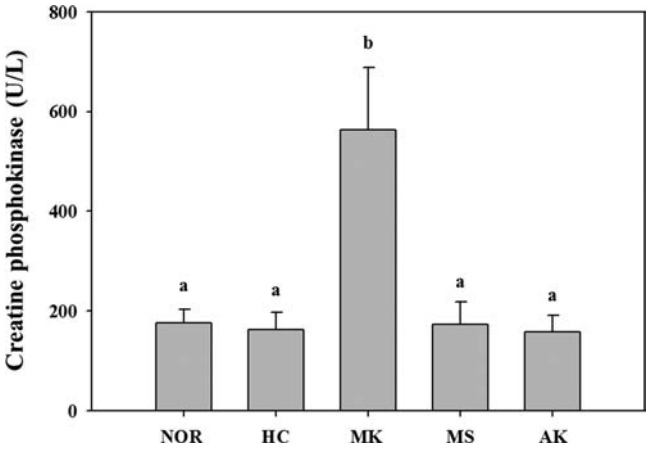

Figure 4. Effect of equal dosage of monascin, ankaflavin, and monacolin K on the activities of creatine phosphokinase (CPK) in serum of hyperlipidemic hamsters fed a high-cholesterol diet. Two groups of the hamsters were fed a normal diet (NOR group) or a high cholesterol diet (HC group) without the administration of test materials, respectively. The other hyperlipidemic hamsters were administrated with monacolin K (0.624 mg/kg/day) (MK group), monascin (0.624 mg/kg/day) (MS group), and ankaflavin (0.624 mg/kg/day) (AK group). Data are presented as means ± SD (n = 8). Mean values with different letters are significantly different ($p < 0.05$). The statistical significance in the biochemical effects was determined by ANOVA with Duncan's multiple test.

Studies have shown that a long-term high monacolin K dosage may lead to various side effects, including rhabdomyolysis and CoQ10 level reduction, while no studies have shown monascin and ankaflavin to possess similar side effects.[5−7] The results also indicate that monacolin K leads to a CPK increase, while monascin and ankaflavin do not exhibit such side effects. Monacolin K (lovastatin) is a competitive inhibitor of HMGR, thus inhibiting the synthesis of mevalonate, and in turn inhibiting cholesterol biosynthesis and preventing the synthesis of geranylgeranylated pyrophosphate (GGpp) downstream products, including CoQ10. CoQ10 is an important cofactor in the mitochondrial electron transport system. Insufficient CoQ10 can affect oxidative phosphorylation and synthesis of adenosine triphosphate (ATP) in mitochondria, resulting in mitochondrial ATP deficiency.[34] Insufficient mitochondrial ATP in turn decreases muscle aerobic capacity and causes muscle fatigue.[7,34] CoQ10 deficiency therefore possibly damages muscle energy metabolism and aggravates myopathy progression.[34] Muscle damage causes CPK to release into the blood, which results in muscle weakness or pain, and even in rhabdomyolysis.[23−25]

In our previous study, high concentration of monascin and low concentration of ankaflavin were found to contribute the hypolipidemic effect of RMD, but the result cannot be used to evaluate their hypolipidemic ability, as well as the potential for the medicine development. However, hypolipidemic ability is important for the development of a hypolipidemic medicine, which affects the cost, dosage, and acceptance of patient, etc. In this study, ankaflavin was found to express higher TC, TG, and LDL-C-lowering effect than monascin under the same dosage, but a higher HDL-C-raising effect was performed by monascin than by ankaflavin. Therefore, ankaflavin was suggested as a hypolipidemic agent, and monascin was potential to raise HDL-C levels according to this comparison study. Furthermore, monascin and ankaflavin do not cause a myopathy side effect. We hope this comparison study is useful to the mechanism study and development of hypolipidemic agents in the future.

AUTHOR INFORMATION

Corresponding Author

*Telephone: +886-2-33664519. Fax: +886-2-3366-3838. E-mail: tmpan@ntu.edu.tw.

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Lee, C. L.; Tsai, T. Y.; Wang, J. J.; Pan, T. M. In vivo hypolipidemic effects and safety of low dosage *Monascus* powder in a hamster model of hyperlipidemia. *Appl. Microbiol. Biotechnol.* **2006a**, *70*, 533−540.

(2) Wei, W.; Li, C.; Wang, Y.; Su, H.; Zhu, J.; Kritchevsky, D. Hypolipidemic and anti-atherogenic effects of long-term Cholestin (*Monascus purpureus*-fermented rice, red yeast rice) in cholesterol fed rabbits. *J. Nutr. Biochem.* **2003**, *14*, 314−318.

(3) Lee, C. L.; Kung, Y. H.; Wu, C. L.; Hsu, Y. W.; Pan, T. M. Monascin and ankaflavin act as novel hypolipidemic and high-density lipoprotein cholesterol-raising agents in red mold dioscorea. *J. Agric. Food Chem.* **2010**, *58*, 9013−9019.

(4) Endo, A.; Monacolin, K. A new hypocholesterolemic agent produced by a Monascus species. *J. Antibiot. (Tokyo)* **1979**, *32*, 852−854.

(5) Sylvain-Moore, H.; Worden, J. P., Jr. Lovastatin-associated rhabdomyolysis. *Heart Lung* **1991**, *20*, 464−466.

(6) Iskra, B.; Zivko, M.; Kes, P. Rhabdomyolysis as a side effect of simvastatin treatment. *Acta Med. Croat.* **2005**, *59*, 325−328.

(7) Yang, H. T.; Lin, S. H.; Huang, S. Y.; Chou, H. J. Acute administration of red yeast rice (*Monascus purpureus*) depletes tissue coenzyme Q(10) levels in ICR mice. *Br. J. Nutr.* **2005**, *93*, 131−135.

(8) Itskovitz, H. D.; Flamenbaum, W.; De Gaetano, C.; Pritchard, K. A., Jr.; Stemerman, M. B. Effect of lovastatin on serum lipids in patients with nonfamilial primary hypercholesterolemia. *Clin. Ther.* **1989**, *11*, 862−872.

(9) Kasiske, B. L.; Tortorice, K. L.; Heim-Duthoy, K. L.; Goryance, J. M.; Rao, K. V. Lovastatin treatment of hypercholesterolemia in renal transplant recipients. *Transplantation* **1990**, *49*, 95−100.

(10) Jou, P. C.; Ho, B. Y.; Hsu, Y. W.; Pan, T. M. The effect of *Monascus* secondary polyketide metabolites, monascin and ankaflavin, on adipogenesis and lipolysis activity in 3T3-L1. *J. Agric. Food Chem.* **2010**, *58*, 12703−12709.

(11) Akihisa, T.; Tokuda, H.; Yasukawa, K.; Ukiya, M.; Kiyota, A.; Sakamoto, N.; Suzuki, T.; Tanabe, N.; Nishino, H. Azaphilones, furanoisophthalides, and amino acids from the extracts of *Monascus pilosus*-fermented rice (red-mold rice) and their chemopreventive effects. *J. Agric. Food Chem.* **2005**, *53*, 562−565.

(12) Akihisa, T.; Tokuda, H.; Ukiya, M.; Kiyota, A.; Yasukawa, K.; Sakamoto, N.; Kimura, Y.; Suzuki, T.; Takayasu, J.; Nishino, H. Anti-tumor-initiating effects of monascin, an azaphilonoid pigment from the extract of *Monascus pilosus* fermented rice (red-mold rice). *Chem. Biodiversity* **2005**, *2*, 1305−1309.

(13) Lee, C. L.; Hung, H. K.; Wang, J. J.; Pan, T. M. Red mold dioscorea has greater hypolipidemic and antiatherosclerotic effect than traditional red mold rice and unfermented dioscorea in hamsters. *J. Agric. Food Chem.* **2007**, *55*, 7162−7169.

(14) Boyd, E. *The Growth of the Surface Area of Human Body*; University of Minnesota Press: Minneapolis, MN, 1935.

(15) American Institute of Nutrition. Report of the American Institute of Nutrition ad hoc committee on standards for nutritional studies. *J. Nutr.* **1977**, *107*, 1340-1348.

(16) Friedewald, W. T.; Levy, R. I.; Fredrickson, D. S. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* **1972**, *18*, 499−502.

(17) Wu, W. T.; Lee, C. C.; Lee, C. J.; Subeq, Y. M.; Lee, R. P.; Hsu, B. G. Rosiglitazone ameliorates endotoxin-induced organ damage in conscious rats. *Biol. Res. Nurs.* **2011**, *13*, 38−43.
(18) Mabile, L.; Fitoussi, G.; Periquet, B.; Schmitt, A.; Salvayre, R.; Negre-Salvayre, A. α-Tocopherol and trolox block the early intracellular events (TBARS and calcium rises) elicited by oxidized low-density lipoproteins in cultured endothelial cells. *Free Radical Biol. Med.* **1995**, *19*, 177−187.
(19) Schimke, I.; Romaniuk, P.; Schimke, E.; Papies, B. Concentration of thiobarbituric acid-reactive substances (TBARS) in the plasma of patients with atherosclerosis with different localizations and different degrees of severity. *Z. Med. Laboratoriumsdiagn.* **1990**, *31*, 176−180.
(20) Ohkawa, H.; Ohishi, N.; Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **1979**, *95*, 351−358.
(21) Aoi, W.; Naito, Y.; Hung, L. P.; Uchiyama, K.; Akagiri, S.; Mizushima, K.; Yoshikawa, T. Regular exercise prevents high-sucrose diet-induced fatty liver via improvement of hepatic lipid metabolism. *Biochem. Biophys. Res. Commun.* **2011**, *413*, 330−335.
(22) Lee, C. L.; Kung, Y. H.; Wang, J. J.; Lung, T. Y.; Pan, T. M. Enhanced hypolipidemic effect and safety of red mold dioscorea cultured in deep ocean water. *J. Agric. Food Chem.* **2011**, *59*, 8199−8207.
(23) Hodel, C. Myopathy and rhabdomyolysis with lipid-lowering drugs. *Toxicol. Lett.* **2002**, *128*, 68−159.
(24) Aboulafia, D. M.; Johnston, R. Simvastatin-induced rhabdomyolysis in an HIV-infected patient with coronary artery disease. *AIDS Patient Care STDS* **2000**, *14*, 8−13.
(25) Stolcpart, R. S.; Olson, K. L.; Delate, T.; Rasmussen, J.; Rehring, T. F.; Merenich, J. A. The risk for significant creatine kinase elevation with statins. *Am. J. Cardiovasc. Drugs* **2010**, *10*, 92−187.
(26) Su, N. W.; Lin, Y. L.; Lee, M. H.; Ho, C. Y. Ankaflavin from *Monascus*-fermented red rice exhibits selective cytotoxic effect and induces cell death on Hep G2 cells. *J. Agric. Food Chem.* **2005**, *53*, 1949−1954.
(27) Meurs, I.; Van Eck, M.; Van Berkel, T. J. High-density lipoprotein: key molecule in cholesterol efflux and the prevention of atherosclerosis. *Curr. Pharm. Des.* **2010**, *16*, 67−1445.
(28) Zhang, H. L.; Tao, Y.; Guo, J.; Hu, Y. M.; Su, Z. Q. Hypolipidemic effects of chitosan nanoparticles in hyperlipidemia rats induced by high fat diet. *Int. Immunopharmacol.* **2011**, *11*, 457−461.
(29) Matsumoto, K.; Yokoyama, S.; Gato, N. Bile acid-binding activity of young persimmon (*Diospyros kaki*) fruit and its hypolipidemic effect in mice. *Phytother. Res.* **2010**, *24*, 205−210.
(30) Jang, A.; Srinivasan, P.; Lee, N. Y.; Song, H. P.; Lee, J. W.; Lee, M.; Jo, C. Comparison of hypolipidemic activity of synthetic gallic acid-linoleic acid ester with mixture of gallic acid and linoleic acid, gallic acid, and linoleic acid on high-fat diet induced obesity in C57BL/6 Cr Slc mice. *Chem.-Biol. Interact.* **2008**, *174*, 109−117.
(31) Hemalatha, S.; Wahi, A. K.; Singh, P. N.; Chansouria, J. P. Hypolipidemic activity of aqueous extract of *Withania coagulan*s Dunal in albino rats. *Phytother. Res.* **2006**, *20*, 614−617.
(32) Lee, B. H.; Hsu, W. H.; Chang, Y. Y.; Kuo, H. F.; Hsu, Y. W.; Pan, T. M. Ankaflavin: a natural novel PPARγ agonist upregulates Nrf2 to attenuate methylglyoxal-induced diabetes *in vivo*. *Free Radical Biol. Med.* **2012**, *53*, 2008−2016.
(33) Tenenbaum, A.; Fisman, E. Z. Balanced pan-PPAR activator bezafibrate in combination with statin: comprehensive lipids control and diabetes prevention? *Cardiovasc. Diabetol.* **2012**, *11*, 140−148.
(34) Caso, G.; Kelly, P.; McNurlan, M. A.; Lawson, W. E. Effect of coenzyme Q10 on myopathic symptoms in patients treated with statins. *Am. J. Cardiol.* **2007**, *99*, 1409−1412.