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In vivo hypolipidemic effects and safety of low dosage *Monascus* powder in a hamster model of hyperlipidemia

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Abstract *Monascus* or more commonly known as red mold rice is fermented rice on which *Monascus purpureus* has been grown. It has been a traditional Chinese food additive for thousands of years in China. Secondary metabolite product of *Monascus*, monacolin K, has been proven that it could be used as an antihypercholesterolemic agent. In this study, *M. purpureus* NTU568 mutated and selected from a monacolin K productivity strain—*M. purpureus* HM105 produced high quantities of monacolin K at a level of 9,500 mg kg^{-1}. This research focused on the effect of adding red mold rice powder of *M. purpureus* NTU568 to a hamster diet on total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol (LDL-C). In the results, the oral administration of *Monascus* powder in hyperlipidemia hamster was indeed proven to decrease TC, TG, and LDL-C levels. Plasma TC levels in hamster fed with *Monascus* powder at onefold dosage [10.78 mg (day 100 g bw)$^{-1}$] for 4 and 8 weeks were significantly lower (31.2 and 22.0%, respectively) than that in hyperlipidemia hamster. Plasma TG (30.1 and 17.9%) and LDL-C levels (36.0 and 20.7%) were also significantly lowered by feeding *Monascus* powder at onefold dosage for 4 and 8 weeks compared to hyperlipidemia hamster. In addition, examinations of liver TC and TG levels of hyperlipidemia hamster were also performed and showed similar effects on lipid-lowering action by oral administration of *Monascus* powder. Since citrinin is a mycotoxin that possesses nephrotoxic and hepatoxic effects, it has a negative impact on the safety of red mold rice for people. This study examined the liver somatic index [plasma glutamyl oxaloacetic transaminase (GOT) and glutamyl pyruvic transaminase (GPT) levels] and liver biopsy to investigate whether *Monascus* powder induced damage in liver. It was found that the plasma GOT and GPT levels were not significantly increased by feeding *Monascus* powder. There was no difference in the results of the liver biopsy between the *Monascus* powder-treated groups and the control group.

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Introduction

Monascus or red mold rice is fermented rice on which *Monascus purpureus*, a red mold species, has been grown. It has been a traditional Chinese food additive for thousands of years in China. Its special effects and application on food have been recorded in ancient Chinese records. The types of secondary metabolites produced from the *Monascus* species include (1) a group of pigments (yellow pigment, ankaflavin and monascin; orange pigment, monascorubrin and rubropunctanin; red pigment, monascorubramine and rubropuctamine (Wong and Koehler 1981), (2) a group of antihypercholesterolemic agents including monacolin K and hypotensive agent, γ-aminobutyric acid (GABA) (Su et al. 2003), (3) antioxidant compounds including dimerumic acid (Aniya et al. 1999) and 3-hydoxy-4-methoxy-benzoic acid (Wu and Wu 2000), and (4) an antibacterial activity compound including pigment and citrinin (also known as monascidin) (Blanc et al. 1995a,b).

Since the *Monascus* species contains multifunctional compounds, it becomes an important topic in the field of functional food. In recent years, cardiovascular disease has become one of the most difficult problems to handle in many countries. This is why prophylaxis and therapy are becoming more and more important subjects for many researches. Monacolin K (also known as lovastatin, mevinolin, mevacor) is a potent competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), the rate-limiting enzyme in cholesterol biosynthesis (Albert et al. 1980; Endo 1979). It cannot only

inhibit cholesterol biosynthesis, but also lower blood cholesterol level in both human and animals. Monacolin K and lovastatin are the same chemical compounds that inhibit HMG-CoA reductase in the cholesterol synthesis pathway (Endo 1979).

Red mold rice, a Chinese traditional food, has been used as a diet supplement or remedy in China and Japan for thousands of years. Red mold rice is still restricted and not accepted in many countries, and causes much controversy, because mycotoxin–citrinin is often secreted by *Monascus* spp. This fact, proven by Blanc et al. (1995a) indicates that *M. purpureus* and *Monascus ruber* produces an antibacterial compound, monascidin, which has been proven to be the same compound as citrinin. Citrinin is a potent renal toxin and hepatotoxin, which causes functional and structural kidney damage and alterations in liver metabolism (Da Lozzo et al. 1998). It inhibits several enzymes linked to the respiratory chain of the kidney cortex and liver mitochondria, as well as malate and glutamate dehydrogenases and the ATP-synthetase complex (Da Lozzo et al. 1998). Citrinin was often found in solid and submerged cultured products of *Monascus*, and it was detected from 0.2 to 122 mg kg^{-1} (Blanc et al. 1995a; Hsieh and Pan 2002). Many researchers suggest that *Monascus* products have been proven to contain multifunctional compounds and that, therefore, the function and application of *Monascus* species should not be annulled because it possesses citrinin. In addition, *Monascus* would be safe and harmless for the daily diet if the content of citrinin was less than the level that induces in vivo damage.

Although many *Monascus* species could synthesize monacolin K, the production of monacolin K was less than 500 mg kg^{-1} (Endo et al. 1985; Manzoni et al. 1999; Wang et al. 1998, 2003; Lai et al. 2003; Schneweis et al. 2001; Chang et al. 2002; Casas López et al. 2003). However, the recommended daily allowance of monacolin K for adults was suggested to be at least 10 mg per day (Havel et al. 1987). A dosage of general *Monascus* powder against cholesterol biosynthesis for a human adult therefore needed at least 10 g per day. Therefore, *Monascus* species with low monacolin K productivity would become more and more difficult to develop and apply on functional food with cholesterol-lowering effect, so it is important to increase the monacolin K production of the *Monascus* species. In our previous study, *M. purpureus* NTU568 was selected from the mutants of *M. purpureus* HM105 in view of the fact that it could render a high monacolin K production at 9,500 mg kg^{-1}. At the same time, the citrinin production was found to be decreased from 2,500 to 939 μg kg^{-1} [Lee et al. 2005, submitted to Journal of Agriculture and Food Chemistry (JAFC)].

Because red mold rice fermented by *M. purpureus* NTU568 has a high monacolin K production, we expect that the supplementation of a low dosage of *Monascus* powder will show a cholesterol-lowering effect. In this study, we used a low dosage of *Monascus* powder as an experimental sample to investigate the hypolipidemic effect in a hamster model of hyperlipidemia. Therefore, the reference dosage of *Monascus* powder for a human adult was chosen at 1 g a day in this study, which is at least ten times less than the efficient dosage of general *Monascus* powder. In addition, 1 g of *Monascus* powder fermented by *M. purpureus* NTU568 contains a monacolin K level of 9.5 mg, which is close to the recommended daily allowance of monacolin K for a human adult. However, the *Monascus* product has been categorized as a functional food according to the functional secondary metabolite monacolin K and GABA. Therefore, the purpose and design of this study should differ from that of an efficiency evaluation study for drugs. High-cholesterol (HChol) diet and *Monascus* powder were fed simultaneously to two groups of hamsters after an observed stage of 4 weeks to simulate an assessment model for functional food, similar to that for people supplementing a cholesterol-rich daily diet with a bit of *Monascus* powder for its hypolipidemic effects.

This research focused on the effects of oral administration of a small amount of *Monascus* powder fermented by *M. purpureus* NTU568 for hyperlipidemia hamsters on total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels in blood or liver. This study examined the liver somatic index [serum glutamyl oxaloacetic transaminase (GOT) and glutamyl pyruvic transaminase (GPT) levels] and liver biopsy to investigate whether *Monascus* powder induces damage in liver when the citrinin formation of *M. purpureus* NTU568 has been suppressed by mutation and, therefore, is much less than that of the wild type.

Materials and methods

Microorganism and seed cultures

The microorganism used in this study includes *M. purpureus* NTU568. This strain was mutated and selected from *M. purpureus* HM105 isolated from red mold rice. The culture strain was maintained on potato dextrose agar (PDA) slanted at 10°C and transferred monthly. Seed cultures were prepared by transferring a loopful of spore from the PDA agar slanted into a 500-ml Hinton flask containing 100 ml basal medium (100 g dextrose, 10 g peptone, 2 g KNO_3, 2 g $NH_4H_2PO_4$, 0.5 g $MgSO_4 \cdot 7\ H_2O$, 0.1 g $CaCl_2$ made up in 1,000 ml distilled water, pH 6.0). The cultures were incubated at 30°C for 3 days at 110 rpm. After that, 5% inoculum (volume/weight) was transferred for solid state fermentation.

Red mold rice preparation

The long-grain rice (*Ipomoea batatas*) was purchased from a local supermarket in Taiwan and was used for red mold metabolites production under solid state cultivation. The strains were incubated on moistured rice in a "koji dish" (the koji dish was made of wood and measured 30×20×5 cm high). The preparation of the rice medium for the solid culture was as follows: dehulled rice (500 g) was soaked in

distilled water for 8 h. After that, the excess water was removed with a sieve. The soaked rice was then autoclaved for 20 min at 121°C in a koji dish. After being cooled, the steamed rice (500 g) was inoculated with 25 ml spore suspension. The inoculated substrate was cultivated at 30°C for 10 days. After fermentation, *Monascus* fermented rice was dried by heating at 50°C for 24 h and then crushed to powder. The moisture content of *Monascus* powder is measured at 14 to 17%. The crushed and dried substrate containing the mold was used for the experiments (Su et al. 2003).

Animals and diets

One hundred and four male Golden Syrian hamsters weighing 100 to 120 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 4 weeks. Eight hamsters were killed and examined for plasma and liver lipid levels to establish a base line. The others were weighed and randomly assigned into six groups of 16 animals each before commencing the study.

Dosage and grouping

The dosage of *Monascus* powder was calculated in accordance with Boyd's formula of body surface area as recommended by the Food and Drug Administration (FDA) (Boyd 1935). *Monascus* powder and probucol are respectively recommended to supplement the daily diet at 1 g and 100 mg for an adult with a weight of 70 kg and a height of 170 cm. These dosages were used as a frame of reference for the conversion of the dosage into a hamster model. Therefore, feeding hamster with *Monascus* powder at a half-fold dosage per day corresponds to supplementing the daily diet with *Monascus* powder at 0.5 g for a human adult. *Monascus* powder or probucol were respectively suspended in 1 ml water and then fed daily to hamsters, using oral administration. The dosage of *Monascus* powder and probucol were adjusted weekly according to the average body weight of the hamster.

Experimental diets were provided in accordance with American Institute of Nutrition (AIN)-76 diet formulation (American Institute of Nutrition 1977), with modification. The control group was fed a normal diet via AIN-76 formulation, and the HChol group was given a high-cholesterol diet that contained 0.1% cholesterol (Table 1). The food intake was recorded daily, and animals were weighted weekly. HChol-M1/2, HChol-M1, and HChol-M5 were fed the high-cholesterol diet and were orally given a half-fold dosage (0.5× mg kg^{-1} bw per day) of *Monascus* powder (fermented by *M. purpureus* NTU568 and including monacolin K of 9,500 mg kg^{-1} and citrinin of 0.939 mg kg^{-1}), onefold dosage (1× mg kg^{-1} bw per day) of *Monascus* powder, and a fivefold dosage (5× mg kg^{-1} bw per day) of *Monascus* powder, respectively. In addition, the HChol–probucol group, which was regarded as the positive control group, was fed the high-cholesterol diet and given orally onefold dosage (1× mg kg^{-1} bw per day) of probucol. The hamsters were fed these experimental diets for 4 and 8 weeks, respectively.

Table 1 Composition of the experimental diet[a] (g kg^{-1} diet)

Composition	Normal diet[b]	High-cholesterol diet[c]
Casein	200	140
Corn starch	650	680
Cellulose	50	50
Soybean oil	50	80
Mineral	35	35
Vitamin	10	10
L-cysteine	3	1
Choline bitartrate	2	2
Cholesterol	–	1
Cholic acid	–	1

[a]Based on AIN-76 diet formula (American Institute of Nutrition 1977)
[b]The daily diet of the control group
[c]The daily diet of the HChol group, HChol–probucol group, HChol-M1/2 group, HChol-M1 group, and the HChol-M5 group

Twenty four hours before killing, all food was removed. Animals were anesthetized and killed by carbon dioxide inhalation, and blood and liver samples were collected for assay. Plasma and red blood cells were separated and stored at −20°C. Part of the liver was carefully removed and immersed in formalin stock, and the subsequently remaining liver was rinsed frequently with 0.8% sodium chloride solution for eliminating any blood. The liver tissue was examined for damage by microscopic examination of a liver biopsy. The experiment was reviewed and approved by the Animal Care and Research Ethics Committee of the National Taiwan University.

Plasma and liver lipid analysis

Plasma and liver TC and TG levels as well as serum HDL-C and LDL-C levels were measured in triplicate using commercial enzymatic kits. These kits were as follow: TC assay kit (Part no. MP2-35, Jonhson and Jonhson, NJ, USA), TG assay kit (Part No. MP2-19, Jonhson and Jonhson), LDL-C assay kit (Cat. no. 1.14992.0001, Merck Co., Darmstadat, Germany), and HDL-C assay kit (Cat. no. 1.14210.0001, Merck Co.).

Plasma liver index analysis

Plasma GOT and GPT levels were measured in triplicate using commercial enzymatic kits. The kits were as follow: GOT assay kit (Part No. MP2-113, Jonhson and Jonhson), GPT assay kit (Part No. MP2-36, Jonhson and Jonhson).

Table 2 The body weight and daily feed intake of experimental hamsters

Group	Body weight (g)			Daily feed intake (g day^{-1})	
	0th week	4th week	8th week	4th week	8th week
Control	82.1±8.12^{a}	93.6±9.5^{a}	107±8.48^{a}	6.78±0.50^{a}	7.73±0.78^{a}
HChol	79.5±8.15^{a}	87.0±7.43^{a}	99.4±9.32^{a}	6.64±0.45^{a}	7.23±0.92^{a}
HChol–probucol	82.3±7.52^{a}	87.1±7.50^{a}	99.2±7.80^{a}	6.75±0.69^{a}	7.52±1.03^{a}
HChol-M1/2	77.1±8.84^{a}	90.6±9.01^{a}	103±8.87^{a}	6.73±0.74^{a}	7.22±0.76^{a}
HChol-M1	79.9±9.57^{a}	87.3±8.40^{a}	105±10.0^{a}	6.84±0.75^{a}	7.23±0.60^{a}
HChol-M5	82.3±9.65^{a}	91.4±7.35^{a}	104±8.32^{a}	6.92±0.83^{a}	7.54±0.84^{a}

Control, normal diet (0% cholesterol); HChol, high-cholesterol diet; HChol–probucol, probucol and high-cholesterol diet; HChol-M1/2, *Monascus* powder [0.5×, 5.39 mg (day 100 g bw)$^{-1}$] and high-cholesterol diet; HChol-M1, *Monascus* powder [1×, 10.78 mg (day 100 g bw)$^{-1}$] and high-cholesterol diet; HChol-M5, *Monascus* powder [5×, 53.9 mg (day 100 g bw)$^{-1}$] and high-cholesterol diet
Data are presented as means±SD (n=8). Mean values within each column with different superscripts are significantly different (p<0.05)

Statistical analysis

Plasma and liver samples were measured in triplicate using commercial kits. The main treatment effects were analyzed by one-way analysis of variance (ANOVA) using the general linear model procedure of SAS software (SAS Institute Inc., Cary, NC, USA). Significant differences among dietary treatments were analyzed by the method of PDiff Matrix of least squares means after a significant main effect by one-way ANOVA. The significance level was set at p<0.05. Data were expressed as means±SD.

Results

The change of body weight and daily intake by hamsters

In this study, we selected hamster as an experimental animal model for hypolipidemic effects of *Monascus* powder. Experimental animals were given free access to regular rodent chow and water for the observation stage of 4 weeks, and then the 8-week study commenced. The average body weight and daily intake of hamsters are shown in Table 2. The results indicate that the body weight and daily intake of the hamsters increased normally and did not differ among the various groups during the period of the practical experiment. In addition, the externals and health of all experimental animals had a normal expression. Animals were anesthetized and killed by carbon dioxide inhalation at the 4th and the 8th week, respectively, and blood and liver samples were collected and examined for estimating the hypolipidemic effects of *Monascus* powder.

Effect of *Monascus* powder on plasma TC and TG levels

The red mold rice powder fermented by *M. purpureus* NTU568 was used as a hypolipidemic sample to be used in this study. Table 3 shows the change of plasma TC and TG levels. As is evident from the data of the plasma TC levels at the 4th week, the control group registered 91.8 mg dl^{-1}, and the HChol group fed with high-cholesterol diet had extremely high levels at 170 mg dl^{-1}. The plasma TC levels of the HChol group were maintained at over 170 mg dl^{-1} for 8 weeks by the feeding of the high-cholesterol diet. These results proved that the supplementation of a cholesterol diet results in a significant increase in the plasma TC levels in contrast with the control groups, and that it

Table 3 Effect of *Monascus* powder on experimental hamster performance plasma TC and TG levels

Group	0th week		4th week		8th week	
	Cholesterol (mg dl^{-1})	Triglyceride (mg dl^{-1})	Cholesterol (mg dl^{-1})	Triglyceride (mg dl^{-1})	Cholesterol (mg dl^{-1})	Triglyceride (mg dl^{-1})
Control	89.7±13.7	98.5±10.5	91.8±5.37^{d}	104±7.06^{e}	89±9.4^{c}	120±17.7^{c}
HChol	–	–	170±15.3^{a}	186±21.1^{a}	173±12.7^{a}	168±12.9^{a}
HChol–probucol	–	–	108±18.7^{bc}	165±15.3^{ab}	124±17.6^{b}	133±27.9^{bc}
HChol-M1/2	–	–	149±15.3^{b}	150±12.8^{b}	140±16.5^{b}	146±25.3^{b}
HChol-M1	–	–	117±10.6^{b}	130±14.4^{c}	135±19.4^{b}	138±12.4^{bc}
HChol-M5	–	–	104±16.1^{cd}	113±7.16^{d}	134±15.3^{b}	134±15.3^{bc}

Control, normal diet (0% cholesterol); HChol, high-cholesterol diet; HChol–probucol, probucol and high-cholesterol diet; HChol-M1/2, *Monascus* powder [0.5×, 5.39 mg (day 100 g bw)$^{-1}$] and high-cholesterol diet; HChol-M1, *Monascus* powder [1×, 10.78 mg (day 100 g bw)$^{-1}$] and high-cholesterol diet; HChol-M5, *Monascus* powder [5×, 53.9 mg (day 100 g bw)$^{-1}$] and high-cholesterol diet
Data are presented as means±SD (n=8). Mean values within each column with different superscripts are significantly different (p<0.05)

can be applied as an animal model for hyperlipidemia. Probucol, a cholesterol-lowering drug, was orally fed to the HChol–probucol group to establish a positive control group. The results after the 4th and the 8th week showed that probucol significantly lowered the plasma TC levels of hyperlipidemia hamster ($p<0.05$).

As shown in Table 3, the amount of plasma TC was significantly less in the *Monasucs* powder-fed groups (HChol-M1/2, HChol-M1, and HChol-M5) than in the HChol group. From this result, we conclude that *Monascus* powder fermented by *M. purpureus* NTU568 has without doubt proven to reduce plasma TC levels. Feeding *Monascus* powder (onefold dosage) for 4 and 8 weeks will cause a 31.2 and 22.0% decrease in the plasma TC levels, respectively, compared to the HChol group. The *Monascus* powder dosage of HChol-M1/2, HChol-M1, and HChol-M5 were half-fold, onefold, and fivefold, respectively. A dose response was found between the feeding dosage of *Monascus* powder and the reduced rate of plasma TC levels, and there was a significant decrease in plasma TC levels compared to the HChol group by feeding *Monascus* powder at half-fold dosage.

As far as the plasma TG levels are concerned, the plasma TG levels of the HChol group at the 4th and the 8th week were measured at 186 and 168 mg dl^{-1}, respectively, which were both significantly higher than that of the control group. However, the results indicated that the plasma TG secretion of hamster was inhibited by feeding at least a half-fold dosage of *Monascus* powder daily for 4 and 8 weeks, respectively, although the hamsters were fed a high-cholesterol diet. The HChol-M1 group had a plasma TG levels at 130 and 138 mg dl^{-1}, which were also significantly less than that of the HChol group by 30.1 and 17.9%, respectively ($p<0.05$). The plasma TG-lowering effect depended on the dosage of *Monascus* powder, and there were remarkable differences in the plasma TG levels among the *Monascus* powder-treated groups. The results indicated that *Monascus* powder fermented by *M. purpureus* NTU568 positively proved to reduce plasma TG levels of hyperlipidemia hamster.

Effect of *Monascus* powder on serum HDL-C and LDL-C levels

The effects of *Monascus* powder on the serum HDL-C and LDL-C levels of hamster are shown in Table 4. The HDL-C levels were significantly higher in the HChol group than in the control group, because HDL-C and LDL-C levels are both typical of cholesterol, which will significantly increase by feeding of a high-cholesterol diet. The results obtained from Table 3 demonstrated the effect of *Monascus* powder of lowering plasma TC and TG levels. However, LDL-C is one of the risk factors of atherosclerosis, and, therefore, the influence of *Monascus* powder on the LDL-C-lowering effect needs to be further investigated. The results shown in Table 4 agreed approximately with the expectation. The major type of cholesterol decreased by *Monascus* powder was LDL-C. Fortunately, the HDL-C levels do not depend on the cholesterol-lowering effect as does the decrease in LDL-C level. As expected, the HChol group had higher LDL-C levels than the control group at the 4th and 8th week. Inhibition of LDL-C secretion depended on the dosage of the *Monascus* powder. *Monascus* powder feeding at a onefold dosage at the 4th and 8th week resulted in a significant decrease in the serum LDL-C levels, compared to those of the HChol group, by 36.0 and 20.7%, respectively ($p<0.05$). LDL-C has been proven to induce the risk of atherosclerosis. However, an efficient LCL-C-lowering effect by *Monascus* powder fermented by *M. purpureus* NTU568 will reverse this result.

The ratio of LDL-C to HDL-C was another criterion for evaluating the efficiency of hypolipidemic. If the ratio was low, then the content of HDL-C had a much higher percentage in TC levels, while on the contrary, the atherosclerotic risk factor LDL-C was lowered. The results in Table 4 indicate that feeding the cholesterol diet for 4 and 8 weeks led to a significant increase in the ratio of LDL-C to HDL-C compared to that of the control group. The results indicated that hamsters fed with *Monascus* powder would increase their HDL-C levels and significantly decrease their LDL-C level at the same time. Therefore, the results obtained by a statistical analysis shows that the ratio of

Table 4 Effect of *Monascus* powder on experimental hamster performance serum HDL and LDL levels

Group	0th week			4th week			8th week		
	HDL-C (mg dl^{-1})	LDL-C (mg dl^{-1})	LDL-C/ HDL-C	HDL-C (mg dl^{-1})	LDL-C (mg dl^{-1})	LDL-C/ HDL-C	HDL-C (mg dl^{-1})	LDL-C (mg dl^{-1})	LDL-C/ HDL-C
Control	58.8±6.76	30.15±5.61	0.51±0.084	$61.8±2.24^{d}$	$31.7±1.8^{b}$	$0.51±0.042^{b}$	$59.3±6.16^{d}$	$30.9±3.7^{c}$	$0.52±0.059^{b}$
HChol	–	–	–	$80.4±10.4^{ab}$	$59.2±8.6^{a}$	$0.74±0.096^{a}$	$81.0±8.47^{b}$	$61.4±5.8^{a}$	$0.77±0.114^{a}$
HChol–probucol	–	–	–	$67.7±5.78^{c}$	$36.0±8.1^{b}$	$0.53±0.107^{b}$	$79.0±10.3^{bc}$	$44.4±7.0^{b}$	$0.57±0.101^{b}$
HChol-M1/2	–	–	–	$74.3±6.74^{bc}$	$56.3±8.1^{a}$	$0.77±0.165^{a}$	$71.7±6.36^{c}$	$52.4±10.5^{ab}$	$0.74±0.172^{a}$
HChol-M1	–	–	–	$89.7±12.7^{a}$	$37.9±10.5^{b}$	$0.45±0.146^{bc}$	$89.2±11.0^{ab}$	$48.7±10.2^{b}$	$0.56±0.155^{b}$
HChol-M5	–	–	–	$89.5±12.8^{a}$	$32.6±8.1^{b}$	$0.37±0.116^{c}$	$96.8±14.9^{a}$	$45.0±9.4^{b}$	$0.47±0.095^{b}$

Control, normal diet (0% cholesterol); HChol, high-cholesterol diet; HChol–probucol, probucol and high-cholesterol diet; HChol-M1/2, *Monascus* powder [0.5×, 5.39 mg (day 100 g bw)$^{-1}$] and high-cholesterol diet; HChol-M1, *Monascus* powder [1×, 10.78 mg (day 100 g bw)$^{-1}$] and high-cholesterol diet; HChol-M5, *Monascus* powder [5×, 53.9 mg (day 100 g bw)$^{-1}$] and high-cholesterol diet
Data are presented as means±SD ($n=8$). Mean values within each column with different superscripts are significantly different ($p<0.05$)

Table 5 Effect of *Monascus* powder on experimental hamster performance liver TC and TG levels

Group	0th week		4th week		8th week	
	Cholesterol (mg dl^{-1})	Triglyceride (mg dl^{-1})	Cholesterol (mg dl^{-1})	Triglyceride (mg dl^{-1})	Cholesterol (mg dl^{-1})	Triglyceride (mg dl^{-1})
Control	108±20.5	50.0±9.22	$103±12.8^{d}$	$52.7±5.27^{c}$	$96.3±15.1^{e}$	$55.1±7.18^{c}$
HChol	–	–	$227±27.7^{a}$	$66.8±5.25^{a}$	$222±12.8^{a}$	$67.7±5.80^{a}$
HChol–probucol	–	–	$148±29.2^{c}$	$51.7±6.52^{c}$	$189±28.3^{b}$	$58.6±10.1^{b}$
HChol-M1/2	–	–	$183±27.4^{b}$	$56.1±6.69^{bc}$	$163±19.5^{c}$	$60.3±5.83^{b}$
HChol-M1	–	–	$175±35.8^{bc}$	$57.4±6.20^{bc}$	$140±22.4^{d}$	$59.2±5.10^{b}$
HChol-M5	–	–	$150±30.9^{c}$	$59.6±4.27^{b}$	$156±31.2^{c}$	$57.8±4.32^{b}$

Control, normal diet (0% cholesterol); HChol, high-cholesterol diet; HChol–probucol, probucol and high-cholesterol diet; HChol-M1/2, *Monascus* powder [0.5×, 5.39 mg (day 100 g bw)$^{-1}$] and high-cholesterol diet; HChol-M1, *Monascus* powder [1×, 10.78 mg (day 100 g bw)$^{-1}$] and high-cholesterol diet; HChol-M5, *Monascus* powder [5×, 53.9 mg (day 100 g bw)$^{-1}$] and high-cholesterol diet
Data are presented as means±SD (n=8). Mean values within each column with different superscripts are significantly different (p<0.05)

LDL-C to HDL-C was less in the HChol-M1 and HChol-M5 groups than in the HChol group (p<0.05).

Effect of *Monascus* powder on liver TC and TG levels

The effect of *Monascus* powder on lowering TC and TG levels of liver is shown in Table 5. As expected, hamsters treated with high-cholesterol diet for 4 and 8 weeks showed a marked increase in liver TC and TG levels compared to the control group. Liver TC and TG levels in all hamsters fed with *Monascus* powder for 4 and 8 weeks were both significantly lower than those in hyperlipidemia hamsters (p<0.05).

Plasma liver index analysis and liver biopsy

Many commercial *Monascus* products contain high levels of citrinin, which is a type of mycotoxin causing liver and kidney damage. Although the mutation treatment in previous study showed a marked decrease from 2,500 to 939 μg kg^{-1} in citrinin production of *M. purpureus* NTU568 compared to the wild type strain (submitted to JAFC), the safety of *Monascus* powder needs to be evaluated. Plasma GOT and plasma GPT levels were assayed to evaluate whether or not the *Monascus* powder causes liver damage to the hamsters. Both GOT and GPT exists in the liver cells, which is released into the blood when a liver cell is damaged. As shown in Table 6, plasma GOT and GPT levels in hyperlipidemia hamsters were not increased by feeding them *Monascus* powder as compared with the HChol group. However, the HChol group had higher plasma GOT and GPT levels than the control group after the 4th week.

Sacrificing the hamsters after 8th week, liver tissue was removed, collected, and then a biopsy was done. The photo obtained by microscopic examination during the liver biopsy (Fig. 1) did not show any significant damage in liver tissue of *Monascus* powder groups compared with control group and HChol group.

Discussion

Endo (1979) demonstrated that *Monascus* sp. formed a cholesterol-lowering agent, monacolin K, which was proven to possess the identical structure with lovastatin. Conversion of HMG-CoA to mevalonate by HMG-CoA

Table 6 Effect of *Monascus* powder on experimental hamster performance plasma GPT and GOT levels

Group	0th week		4th week		8th week	
	GPT (U dl^{-1})	GOT (U dl^{-1})	GPT (U dl^{-1})	GOT (U dl^{-1})	GPT (U dl^{-1})	GOT (U dl^{-1})
Control	70.1±12.9	66.4±17.6	$68.3±13.6^{c}$	$64.2±18.4^{b}$	$112±12.6^{a}$	$70±18.3^{a}$
HChol	–	–	$109±10.8^{a}$	$86.6±21.7^{a}$	$104±18.9^{ab}$	$68±11.4^{a}$
HChol–probucol	–	–	$92.5±17.0^{b}$	$67.8±13.9^{b}$	$100±10.2^{ab}$	$64±17.3^{a}$
HChol-M1/2	–	–	$73.7±15.6^{c}$	$64.0±14.6^{b}$	$94±14.5^{bc}$	$61±6.2^{a}$
HChol-M1	–	–	$93.3±9.5^{b}$	$74.0±14.0^{ab}$	$86±11.3^{cd}$	$57±19.5^{a}$
HChol-M5	–	–	$76.5±13.1^{c}$	$66.3±17.9^{ab}$	$74±11.2^{d}$	$58±14.1^{a}$

Control, normal diet (0% cholesterol); HChol, high-cholesterol diet; HChol–probucol, probucol and high-cholesterol diet; HChol-M1/2, *Monascus* powder [0.5×, 5.39 mg (day 100 g bw)$^{-1}$] and high-cholesterol diet; HChol-M1, *Monascus* powder [1×, 10.78 mg (day 100 g bw)$^{-1}$] and high-cholesterol diet; HChol-M5, *Monascus* powder [5×, 53.9 mg (day 100 g bw)$^{-1}$] and high-cholesterol diet
Data are presented as means±SD (n=8). Mean values within each column with different superscripts are significantly different (p<0.05)

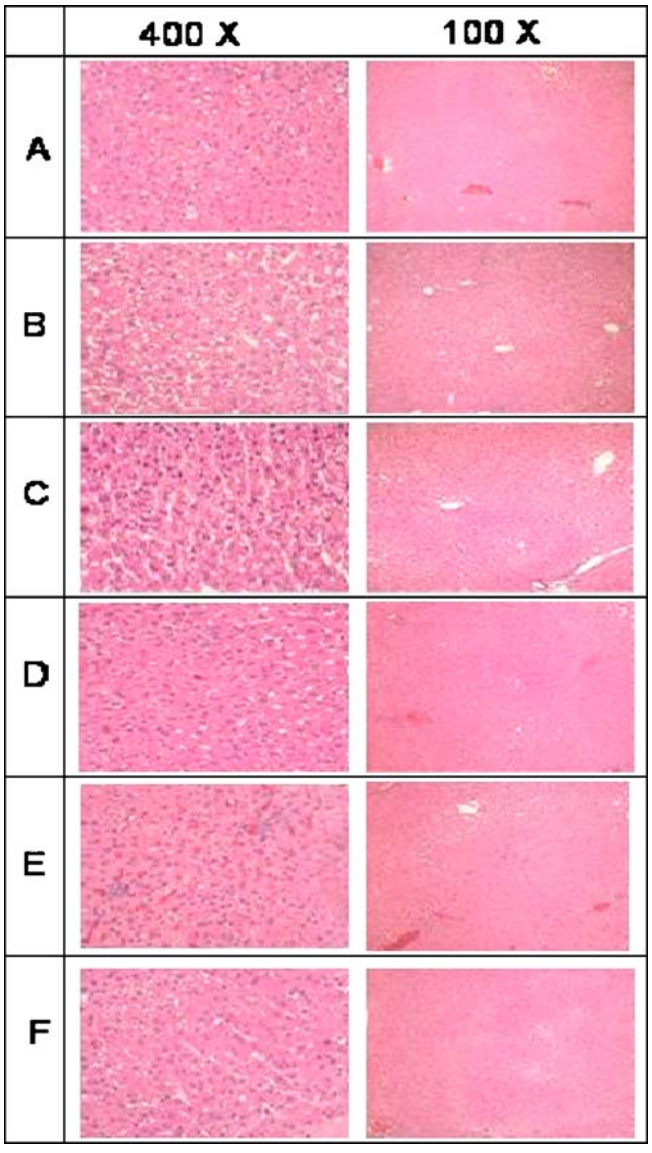

Fig. 1 The microscopy photos (400× and 100×) of liver biopsy of experimental hamster. **a** Control group **b** HChol group **c** HChol–probucol group **d** HChol-M1/2 group **e** HChol-M1 group **f** HChol-M5 group

reductase is a rate-limiting step in cholesterol biosynthesis. Lovastatin is a clinically used HMG-CoA reductase inhibitor known to lower serum cholesterol levels. In general, lovastatin is obtained via the purification of the crude fermented product by *Aspergillus terreus* (Manzoni and Rollini 2002). *A. terreus* is not a strain of GRAS (generally regarded as safe), and, furthermore, the purification of lovastatin has to be carried out through many chemical processes, so lovastatin is normally used as a clinical drug. Although red mold rice fermented by *Monascus* is regarded as a functional food having a cholesterol-lowering effect, since monacolin K is identical to lovastatin, monacolin K production is much lower in many *Monascus* species than in *A. terreus*, which makes it very difficult to use a *Monascus* product for cholesterol-lowering action.

As shown in the results, *Monascus* powder of *M. purpureus* NTU568 significantly reduced TC, TG, and LDL-C without changing the HDL-C levels. The results of the present study showed that *Monascus* powder significantly decreased TC and TG levels at the half-fold dose. Feeding hamster with *Monascus* powder at half-fold dosage corresponds to supplementing a daily diet with *Monascus* powder at 0.5 g for people with a body weight of 70 kg and a height of 170 cm. *Monascus* powder fermented by *M. purpureus* NTU568 and administered at a lower dosage did indeed have a remarkable effect of cholesterol-lowering action as compared with the effectual (and higher) dosage of other *Monascus* powder. In addition, the lipid levels were gradually reduced as the dosage of *Monascus* powder of *M. purpureus* NTU568 was increased.

As far as HDL-C levels was concerned, the serum HDL-C levels were increased compared with the HChol group and the control group, although not to a statistically significant level. The question of whether more *Monascus* powder can increase HDL-C levels more than less *Monascus* powder, or if probucol was more effective, was raised as well. It is well established that increased HDL-C levels are beneficial to human health by reducing the risk for developing cardiovascular disease. The elevated HDL-C levels obtained by the use of *Monascus* powder, especially when enhanced to a fivefold dosage, may benefit the cardiovascular system. However, more investigations are needed to ascertain this effect.

Probucol had been proven to reduce cholesterol levels in a previous study and is used as a clinical drug (Noto et al. 2003; Michihara et al. 2003). For this reason, it was used as the positive control on cholesterol-lowering action as a substitute for *Monascus* powder. As shown in the results, hamsters fed with probucol and high-cholesterol diet had a significant decrease in the plasma TC levels at the 4th and 8th week compared with the HChol group. The decrease in plasma TG levels were not significant in hyperlipidemia hamsters fed with probucol for 4 weeks as compared with the HChol group, but oral administration of probucol for 8 weeks resulted in a significant decrease in plasma TG levels. Probucol is used as a cholesterol-lowering medicine as a clinical remedy, although its mechanism of cholesterol-lowering action had not been demonstrated. Consequently, plasma TG levels might not be immediately decreased by oral administration of probucol as compared with hyperlipidemia hamsters.

Protection against liver damage by a *Monascus* product had been reported in previous study (Aniya et al. 1999). Aniya et al. demonstrated that some strains of *Monascus anka* would protect liver against chemical damage by leading to an increase in the activity of glutathione-*s*-transferase and aspartate aminotransferase (Aniya et al. 1999). In addition, feeding hypercholesterolemic rabbits with 0.2 to 0.8 g per day of *Monascus* powder would reduce not only the risk for developing cardiovascular disease but also the risk for developing hepatic fibrosis and hepatomegaly (Li et al. 1998). In this study, plasma GOT and GPT levels showed more variation among hamsters in

the same group, and, therefore, the significant effect among the various groups was hard to observe by statistical analysis. GOT and GPT both exist in the liver cell, which is released into the blood when the liver cell is damaged. The results indicated that feeding hamsters with a high-cholesterol diet may result in an increase in plasma GOT and GPT levels compared with control group, displaying a remarkable increase after the 4th week. It is highly probable that a massive amount of cholesterol supplement will lead to a burden on the liver metabolites and simultaneously increase the risk for developing hepatic fibrosis (Jeong et al. 2005; Aguilera et al. 2005; Papadia et al. 2004). However, there were tendencies toward lowering of the plasma GOT and GPT levels in hyperlipidemia hamsters when they were orally administered *Monascus* powder for 4 and 8 weeks, although it had no statistically significant effect. In addition, citrinin, formed from *Monascus*, is typical of the mycotoxin resulting in damage to the liver. Although *Monascus* powder fermented by *M. purpureus* NTU568 contained 939 μg kg^{-1} of citrinin, it did not lead to damage of the liver, as was evident when examining GOT and GPT levels as well as by the liver biopsy. It is worth noting that the product of *M. purpureus* NTU568 was proven to possess good antioxidant activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, conjugate dienes inhibition, and reducing power (Lee et al. 2005, submitted to JAFC). Therefore, we suppose that an antioxidant or another compound is formed by *M. purpureus* NTU568 not only to repair citrinin-induced liver damage but also to reduce the risk for developing hepatic fibrosis.

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