

Comparing the Effects of Native and Standard Strains of *Monascus Purpureus* on Fat Metabolism in Rats

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Abstract

Background: *Monascus purpureus* (MP) is a microscopic fungus that belongs to the class of Ascomycetes. It has a wide range of use as in pigmentation, flavoring and producing preservative agents for food stuffs as well as cholesterol-lowering agents in medications. This study was designed to compare the effects of a native MP isolated from the microbial collection at the University of Isfahan in Iran and DSM1603, the standard strain on alterations in concentration levels of cholesterol (Chol), triglyceride (TG), low density lipoprotein (LDL) and high density lipoprotein (HDL) in treated rats sera.

Methods: Pigments from the two strains were produced through submerged fermentation. 25 Wistar rats with a mean body weight of 250 grams were distributed into 5 groups of 5 each. Group 1 and Group 2 received red native pigment with concentrations of either 25% or 100% respectively. Group 3 and Group 4 received red standard pigment with concentrations of either 25% or 100% respectively. Treated animals had only free access to pigment solutions as drinking while animals in the control group had only free access to regular drinking water.

Results: The results indicate that using pigment in the diet of rats routinely could decrease the concentration levels of Chol, TG and LDL but increase HDL. In this study by using optimization of the culture medium, no adverse effect was observed in the treated animal comparing with the control group.

Conclusion: Comparing the effects of the two strains, similar results were observed but the native strain was more effective in and the one were the same in increasing HDL concentration.

Keywords: *Monascus purpureus*; Pigment; Rat; Hyperlipidem

Abbreviations: MP (*Monascus purpureus*), Chol (cholesterol), LDL-C (Low Density Lipo Protein cholesterol), HDL-C (High Density Lipo Protein cholesterol), TG (Triglyceride), HMG-CoA reductase (3-hydroxy-3-methylglutaryl coenzyme A reductase), CVRF (cardiovascular risk factors), STD *Monascus purpureus* (Standard, DSM1603).

Introduction

Cholesterol (Chol) is a required blood lipid for the body maintenance in a reasonable amount. However, high cholesterol levels may cause atherosclerosis or other cardiovascular diseases which could lead to heart attack. All types of cardiovascular diseases are now among the main factors causing disabilities and mortalities in developed countries. There are many cardiovascular risk factors (CVRF) involved in cardiovascular diseases of which the most important one is unusual high level of blood cholesterol. The extensive epidemiological studies have revealed a direct correlation between atherosclerosis and total blood Chol levels particularly the cholesterol bound to low density lipoproteins (LDL).

Controlling Chol levels and the improved methods for increasing good cholesterol or lowering bad cholesterol levels could be an important step to lowering risks in people susceptible to cardiovascular diseases. Mostly, modifications in lifestyle may be good enough to normalize Chol levels. Such modifications can include dietary changes, moderate exercise, and regular use of antioxidants and other cholesterol lowering agents such as pigments, fibres, nicotinic acids and bile acid resins. There is an obvious need for further researches despite progresses in finding new compounds and developing methods to lower human blood TC and LDL to reduce CVRF. Comparing the effect of a native *Monascus purpureus* or more commonly known as

red mold rice isolated from the microbial collection at University of Isfahan in Iran with the effect of the standard strain, DSM1603 on alterations in concentration levels of cholesterol (Chol), triglyceride (TG), low density lipoprotein (LDL) and high density lipoprotein (HDL) in treated rats was the basic idea of designing this study.

The important characteristics of MP play significant roles in the human lifestyle as well as in the environment. This fungus produces pigments through its fermentation which have been traditionally used as additive, coloring or flavoring agents in foods and beverages in China [1,2]. The metabolites obtained from MP comprise six known azaphilone pigments with a color spectrum ranging from yellow to red [3]. These pigments have various biological properties including hypolipidemic [4], antitumor promotion [5], immunosuppressive [6] and plasma glucose-lowering [7]. In addition, the pigments contain some antioxidant compounds including dimeric acid [8], 3-hydroxy-4-methoxy-benzoic acid [9] and dihydromonacolin-MV [10].

MP naturally forms 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) which is an inhibitor known as

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monacolin and commercially recognized as Mevacor, Cholestin, Lovastatin, Statin etcetera. Antioxidant effect of statin is quoted to be beyond atherosclerosis beneficent with the potential therapeutic advantages for atrial fibrillation and heart failure [11]. Monacolin K is a potent inhibitor of HMG-CoA reductase, the enzyme involved in the process of cholesterol biosynthesis. It has the potency to maintain a healthy blood lipid level by means of decreasing the production of cholesterol in human. MP pigments favorably influence lipid profiles in hypercholesterolemic patients. As the alternative treatments, they may be of great value in prevention and treatment of hypercholesterolemia and atherosclerosis [12-18].

Another application of MP is to extract and purify its pigments as food coloring substances. In order to enhance meat color, several authors [19-22] have used MP pigments as substitutes for nitrate/nitrate salts. It is reported that poultry and ham prepared with half the quantity of nitrite salting mixture of *Monascus purpureus* extract maintained the most desirable color, flavor and appearance as well as the best microbiological parameters along with the most suitable salt content [23]. A novel formulation approach to prepare nanoparticulate red mold rice is also reported with both in vitro and in vivo safety evaluations indicating no mutagenic or toxic responses [24].

Methods

Microorganism and pigment production

Submerged fermentation was the cultivation method applied in this study. The native MP and the standard one, DSM1603 were cultivated on slanted malt extract agar (MEA) at 25°C. Then, the seed cultures were prepared by transferring a loop full of spore from MEA agar slanted into a 500-ml flask containing 100 ml basal medium containing 5 g/l peptone, 3g/l malt extract and 10 g/l glucose with the pH set at 5.0. Each 500 ml Erlenmeyer flask containing 80 ml of the produced medium (semi-synthetic) was incubated with 20 ml of the inoculums culture and incubated at 30°C, 300 rpm for 64 to 72 hours [25]. The fermentation process was carried out under the conditions of pH, 6-7; agitation, 200-300 rpm; temperature, 25-30°C for a period of 5-7 days [26-27]. Pigments were released into the fermentation medium through mycelia [28]. The fermentation broth medium containing red pigment was separated from the mycelia using Watman filter No 1 [25]. The filtrated broth was used instead of drinking for the treated rats or it was diluted using clean tap water to prepare lower dose of the pigment (i.e. 25%).

Rats and pigment treatment

25 male Wistar rats, 8 weeks of age with an average body weight of 250 g, were obtained from the laboratory animal unit of Chamran University, Ahvaz, Iran. The animals were housed in standard plastic cages and kept in a room under standard conditions of temperature, 22 ± 2°C; hydrometry, 50 ± 5%; 12-hour reverse light-dark cycle and free access to regular rat chow pellets and water. After 2 weeks of accommodation, the animals were semi-randomly distributed into 5 groups of 5 each as indicated in Table 1. Group 1 and Group 2 received red native pigment with the concentrations of either 25% or 100% respectively. Group 3 and Group 4 received red standard pigment with concentrations of either 25% or 100% respectively. Treated animals had only free access to pigment solutions as drinking while animals in the control group had only free access to regular drinking water.

Sample preparation

At the end of the 14-day experimental period, the blood samples

were collected from eye medial canthus of each rat while the animal was anaesthetized with ether. Blood samples were collected in to the tubes containing no anticoagulant. Sera were separated via cold centrifugation at 3000×g for 10 minutes at 4°C. Chol, TG, LDL and HDL concentrations were determined using a commercial diagnostic kits provided by Pars Azmon Co., Iran.

Statistical analysis

The data obtained in this study were analyzed using ANOVA and LSD's test for multiple comparisons. Statistical significance was determined based on the p value being set at 0.05. SPSS, Version 15.0 was used to conduct statistical analysis.

Result

The Effect of MP pigments on serum Chol level

After 2-weeks period of treatment, blood factors were determined. Table 2 shows the alterations in sera Chol levels. Comparing the treated animals with the control ones, the result indicate that Chol level in rats sera reduced down to 61.6% and 35.5% influenced by use of 25% and 100% of Native MP pigments respectively. In rats treated with 25% and 100% standard pigment compare with the control animals, cholesterol level was also reduced down to 31% and 28.9% respectively. As demonstrated in Table 2 and Figure 1, the overall serum Chol level was significantly lower in the groups treated with both native and standard MP pigments, but the native pigment was more effective in reducing Chol level than the standard strain.

The Effect of MP pigments on serum TG level

Blood TG level was decreased down to 56.2% and 32.5% under the influence of treating the animals with 25% and 100% of native MP pigment respectively. In rats treated with 25% and 100% standard pigment, TG levels were reduced to 48.8% and 21.4% respectively compared with the control animals. Thus, the standard pigment was more effective in decreasing blood TG levels (Table 3 and Figure 2).

Groups	Diet
Control	Tap water
1	25% red pigment concentration (Native)
2	100% red pigment concentration (Native)
3	25% red pigment concentration (STD)
4	100% red pigment concentration (STD)

Native (*M.purpureus*) isolated in the microbial Collection of the University of Isfahan), STD (*M.purpureus* DSM1603).

Table 1 : Drink Intake of Experimental Rats.

Groups	Dose of Pigment	MD ¹ Change %	MD ²	p
Control		+15.5%		
Native Pigment	25%	-61.6%	77.2%	0.000*
	100%	-35.5%	49.1%	0.000*
STD Pigment	25%	-31%	46.5%	*0.000
	100%	-28.9%	44.5%	*0.001

MD¹: Mean Deference compared to baseline

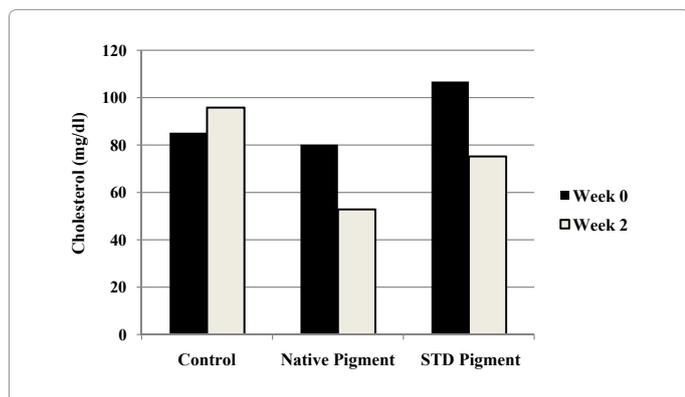
MD²: Mean Deference compared to the control group

* Significantly different from control group at the same week (p<0.05)

Table 2: The effect of Native and STD Pigment on Cholesterol Concentration of Rats serum compared to the control group.

Effect of MP pigments on serum LDL and HDL levels

As mentioned earlier, the results of this study indicated that MP pigment has a lowering effect on both serum Chol and TG levels. Considering the fact that LDL is one of the risk factors of atherosclerosis, it is remarkable that under the influence of treatment by MP pigment, blood LDL levels were decreased. It is so important that further investigation might be needed. However, the data indicate 40.6% and 24.6% reduction in LDL levels due to treatment doses of 25% and 100% of standard pigment respectively. Interestingly, the native pigment was more effective in this regard causing 43.2% and 25.4% in



Week 0, baseline; Week 2, post-treatment
Figure 1: The effect of Native and STD pigment on Cholesterol concentration of rat's serum in comparison with the control group.

Groups	Dose of Pigment	MD ¹ Change %	MD ²	p
Control		+23.2%		
Native Pigment	25%	-56.2%	79.4%	0.000*
	100%	-32.5%	55.7%	0.000*
STD Pigment	25%	-48.8%	72%	*0.000
	100%	-21.4%	44.6%	*0.000

MD¹: Mean Deference compared to baseline
 MD²: Mean Deference compared to the control group
 * Significantly different from control group at the same week (p<0.05)
Table 3: The effect of Native and STD Pigment on TG Concentration of Rats serum compared to the control group.

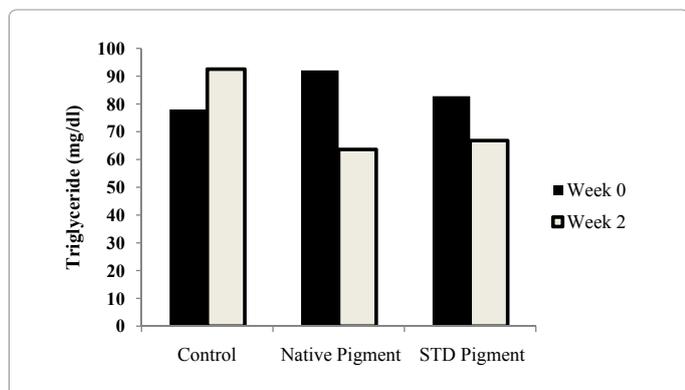


Figure 2: The effect of Native and STD pigment on Triglyceride concentration of rat's serum in comparison with the control group. Week 0, baseline; Week 2, post-treatment.

Groups	Dose of Pigment	MD ¹ Change %	MD ²	p
Control		+20.8%		
Native Pigment	25%	-43.2%	64%	0.000*
	100%	-25.4%	46.2%	0.007*
*0.001	25%	-40.6%	61.4%	*0.001
	100%	-24.6%	45.4%	*0.008

MD¹: Mean Deference compared to baseline
 MD²: Mean Deference compared to the control group
 * Significantly different from control group at the same week (p<0.05)

Table 4: The effect of Native and STD Pigment on LDL Concentration of Rats serum compared to the control group.

Groups	Dose of Pigment	MD ¹ Change %	MD ²	p
Control		-32%		
Native Pigment	25%	+46.9	78.9%	0.000*
	100%	+35.7%	67.7%	0.001*
STD Pigment	25%	+40.2%	72.3%	*0.000
	100%	+26.1%	58.1%	*0.003

MD¹: Mean Deference compared to baseline
 MD²: Mean Deference compared to control group
 * Significantly different from control group at the same week (p<0.05)

Table 5: The effect of Native and STD Pigment on HDL Concentration of Rats serum compared to the control group.

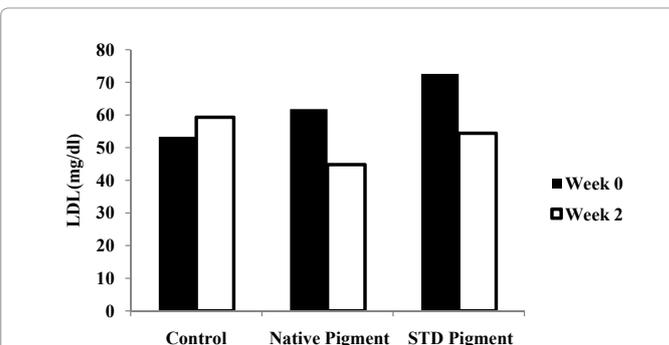


Figure 3: The effect of Native and STD pigment on LDL concentration of rat's serum in comparison with the control group. Week 0, baseline; Week 2, post-treatment.

blood LDL levels due to treatments with 25% and 100% of doses (Table 4). It is well established that increased blood HDL level is beneficial to human health through reducing the risk for developing cardiovascular diseases. Increases of 40.2% and 26.1% in HDL levels were observed under the influence of 25% and 100% doses of standard pigment respectively. Remarkably, the native pigment was more effective and increases of 46.9% and 35.7% were observed due to treatment of 25% and 100% doses respectively (Table 5 and Figure 4).

The ratio of HDL to LDL levels and the Atherogenic Index were other determined criteria (Table 6 and Table 7) which are commonly used to evaluate the efficiency of hypolipidemic medications. If the ratio was high, then the content of HDL had a much higher percentage in total Chol level. In other words, LDL level or the atherosclerotic risk factor was lowered. With this consideration, the statistical analysis of the data indicated that in rats fed with MP pigments blood HDL

Groups	Dose of Pigment	HDL-C/LDL-C		MD ¹	MD ²	p
		Week 0	Week 2	Change %		
Control		0.43	0.26	58.7%		
Native Pigment	25%	0.16	0.65	72%	130.8%	0.000*
	100%	0.49	1.08	51.8%	110.6%	0.000*
STD Pigment	25%	0.25	0.79	66.3%	125.1%	*0.000
	100%	0.28	0.60	44.3%	103%	*0.000

MD¹: Mean Deference compared to baseline

MD²: Mean Deference compared to control group

Week 0, baseline; Week 2, post-treatment

*Significantly different from control group at the same week (p<0.05)

Table 6: HDL-C/LDL-C changes in pigment treated groups compared to the control group.

Groups	Dose of Pigment	AI ¹	AI ²	MD ¹	MD ²	p
		Week 0	Week 2	Change %		
Control		4.36	5.70	82% Increase		
Native Pigment	25%	10	0.90	89.57% Decrease	171.60%	0.000*
	100%	2.33	0.37	94.65% Decrease	%176.68	0.000*
STD Pigment	25%	4	0.89	78.5% Decrease	160.55%	*0.000
	100%	4.77	2.31	54.94% Decrease	136.97%	*0.000

AI¹: Atherogenic Index at Week 0 (baseline)

AI²: Atherogenic Index at Week 2(post-treatment)

MD¹: Mean Deference compared to baseline

MD²: Mean Deference compared to the control group

*Significantly different from control group at the same week (p<0.05)

Table 7: Atherogenic Index in Pigment treated groups compared to the control group.

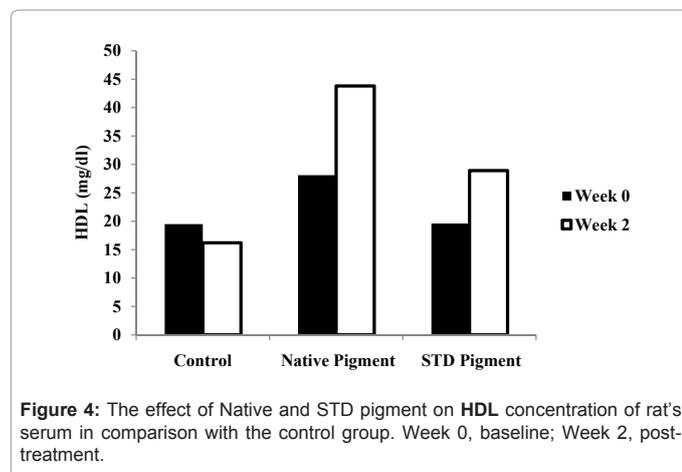


Figure 4: The effect of Native and STD pigment on HDL concentration of rat's serum in comparison with the control group. Week 0, baseline; Week 2, post-treatment.

levels were significantly increased while their blood LDL levels were decreased (p<0.05).

Discussion

It was earlier reported that that red mold rice caused significant decreases in LDL, Chol, TG levels [29]. In this study, two weeks of treatment with native MP pigment resulted in significant reductions in LDL (25.4%), Chol (35.5%) and TG (32.5%) levels from the baseline. As shown in the results, pigment of Native MP significantly reduced TC, TG, and LDL-C levels and increased the HDL-C level. The results of the present study showed that *Monascus* pigment significantly decreased TC and TG levels at the fourth-fold dosage.

Feeding rats with *Monascus* pigment at fourth -fold dosage administered at a lower dosage did indeed have a remarkable effect

of cholesterol-lowering action as compared with the higher dosage of *Monascus* pigment.

There was no statistically significant difference percentage of decrease in TG, LDL and HDL levels after treatment between Native and Standard pigment.

In addition, Rats taking pigment had an increase in HDL (good) cholesterol and a decrease in LDL (bad) cholesterol with the supplement.

The result of this study showed that adding red pigment containing broth medium fermented by *M. purpureus* (25% and 100% concentrations) into Rat' diet could significantly decrease TC, TG, and LDL levels. The particular dosage of pigment to be administered should be effective to reduce LDL-cholesterol concentration and to increase HDL-cholesterol concentration in the blood, and will depend on the route of administration. The addition of suspension produced through submerged fermentation of *Monascus* into Rat' diet not only increases HDL-C concentration but also, the ratio of HDL-C/LDL-C for the experimental groups was higher than that of the control group.

Since possesses the ability to lower cholesterol level. It has been developed into functional health food for human dietary supplement.

Previous studies were similar to this study in showing a marked effect of the pigment containing supplement on cholesterol concentrations. Pigment used in this study confirms *Monascus purpureus* red rice that it had positive effects on plasma lipids. However, there were differences in the route of pigment administration. Furthermore, pigment containing red yeast rice is only 0.3% [12] and less than pigment containing of suspension which used in this study. Red rice was produced through solid state fermentation but in the present study we used different natural pigment preparation through submerged fermentation.

Despite the advantages of using RYR, the presence of citrinin in the products has a negative effect on its acceptance by the people. Hsieh and Pan found that the citrinin content in red mold rice in a Taiwan market ranged from 0.1 to 122 ppm [30]. Therefore, the reduction of citrinin draws a lot of attention by researchers throughout the world. However, in this study by using optimization of the culture medium, no adverse effect was observed in the treated animal comparing with the control group. It is worthy to notify that a native strain was used in this experiment. In further studies we will try to use genetic engineering techniques to obtain mutants with lower citrinin producers.

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