Lipid-lowering efficacy and safety of *Monascus* biopigment beverage

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Abstract

The study aimed to test the efficacy of *Monascus* biopigment beverage, which was made from the extract of *Monascus*-fermented rice, to lower serum lipid levels of Sprague-Dawley rats consuming high cholesterol diet, and assess its safety through physical and physiological observations for toxicity evaluation and clinical blood chemistry examinations. Sixteen (16) adult male Sprague-Dawley rats were randomly divided into 2 groups (n=8 per group), negative control and treatment. The latter was administered with *Monascus* biopigment beverage, aside from commercial pellets and cholesterol diet (500 mg cholesterol/kg body weight) given to both groups daily in 30-day experimental period. Blood (0.5 mL) was drawn from the orbital sinus of each animal subject, which was performed at day 0, 15, and 30. The collected blood samples were then analyzed for lipid profile and toxicity evaluation. Results showed that *Monascus* biopigment beverage was effective in lowering lipid levels of Sprague-Dawley rats. Total cholesterol (TC) and low density lipoprotein-cholesterol (LDL-C) levels decreased significantly by 18.5% and 45.64%, respectively, while high density lipoprotein-cholesterol (HDL-C) significantly increased by 47.12%. However, triglyceride (TG) remained statistically unchanged but was reduced by 2.0%. Blood urea nitrogen (BUN), creatinine, and alanine aminotransferase (ALT) did not differ significantly between the two groups from day 0 to day 30. Moreover, *Monascus* beverage-treated rats did not develop any physical and physiological signs of toxicity.

Introduction

According to the World Health Organization (WHO) as of 2012, cardiovascular disease (CVD) is the number one cause of death, particularly in high- and middle-income countries (http://www.who.int). Nearly 50% of all deaths in the industrialized countries are result of CVD (Gaziano et al., 2006; Deng, 2009). In the United States, about 452,000 people die from heart disease each year (AHA and ASA, 2007). Of the CVDs, coronary heart disease (CHD) is the most prevalent cause of death, followed by stroke. In the Philippines, as of 2010, cardiovascular diseases, specifically diseases of the heart and vascular system, ranked 1st and 2nd respectively, among the ten leading causes of death per year (http://www.doh.gov.ph).

Clinical studies have shown that high serum cholesterol levels and high blood pressure are risk factors for the occurrence of CHD and stroke (Mahan and Escott-Stump, 2004). Based on the 8th Philippine National Nutrition Survey (NNS) (2013) conducted by the Food and Nutrition Research Institute (FNRI), though the prevalence of hypertension slightly decreased from 2008 to 2013, about 1 out of 4 adults (22.3%) is considered hypertensive (http://www.fnri.dost.gov.ph).

Therefore, cardiovascular diseases and its associated risk factors such as high blood cholesterol and hypertension continue to become a major public health problem which challenges humanity today necessitating research studies for solutions to prevent and minimize their occurrence. One of these alternatives maybe the use of the biopigment from *Monascus purpureus* as a means to lower total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG) while increasing the levels of high-density lipoprotein cholesterol (HDL-C) which is considered as good cholesterol in the body (Erdoğrul and Azirak, 2004). Research studies also showed that *Monascus*-fermented product, being a multifunctional food, was not only producing antihypercholesterolemic effect but also had beneficial effects against inflammation (Lee et al., 2006). In contrast, the safety of *Monascus*-fermented products has been an important issue due to its toxin, citrinin, content. Citrinin, a toxic secondary metabolite
of Monascus spp., is known to have adverse effects in liver and kidneys (Pattanagul et al., 2007). A limit of 20 ppb is advised in agricultural products on the current FDA action level of citrinin (Shi and Pan, 2011).

Although citrinin triggers the concerns for safety of Monascus-fermented products, several countries in Asia are still consuming Monascus-fermented rice as food additive and dietary supplement (Erdogru and Azirak, 2004). Incorporation of Monascus biopigment extract in beverages is one way to make it available for daily consumption. Nevertheless, the product should undergo experimental study to prove its efficacy and safety for those individuals with abnormal levels of blood lipids and to be eventually branded as a new genre of beverage with functional properties. Therefore, this study was done to test the lipid-lowering efficacy of the developed Monascus biopigment beverage on serum lipids of Sprague-Dawley rats consuming high cholesterol diet, and assess its safety through physical and physiological observations for toxicity evaluation and clinical blood chemistry examinations.

Materials and Methods

Materials

Monascus biopigment beverage was made from 7.2 mL Monascus extract, 0.3% citric acid (Kemrad), 9.7% sugar, and 0.05% natural identical flavor (International Flavors and Fragrances). The Sprague-Dawley rats, used in the animal study, were purchased at the Food and Drug Administration (FDA), Alabang, Muntinlupa City, Philippines. All biochemical analyses, total cholesterol (Chemplus Diagnostics, Texas, USA), triglycerides (Chema Diagnostica, Italy), HDL, (Chema Diagnostica, Italy), blood urea nitrogen (Chemplus Diagnostics, Texas, USA), creatinine (Chema Diagnostica, Italy), and ALT (Chema Diagnostica, Italy) were prepared based on the manufacturers’ protocols.

Preliminary animal experiment

A preliminary animal experiment was done to determine the possible effects on blood lipids of the different amounts of Monascus extract incorporated in the formulated beverage. Five (5) male Sprague-Dawley (SD) rats (Rattus norvegicus) were utilized, 1 model per treatment, and given with cholesterol (Ajax Finechem Pty Ltd., Australia) in a dose of 500 mg/kg body weight to induce hyperlipidemia. The Sprague-Dawley rats consuming high cholesterol diet, and assess its safety through physical and physiological observations for toxicity evaluation and clinical blood chemistry examinations.

Animal

Sixteen (16) adult male Sprague-Dawley rats, aged 8 to 10 weeks and weighing 200±30 g, were housed in the Laboratory Animal Room, College of Veterinary Medicine (CVM), University of the Philippines Los Baños. Each was put in a polycarbonate cage maintained at 26±2°C, 12h light: 12 hour day cycle. The rats were randomly divided into 2 groups (n= 8 per group) and were acclimatized one week prior to the test period. The first group was the negative control given with commercial pellets and cholesterol (dose of 500 mg/kg body weight) only. On the other hand, the second was the treatment group fed with commercial pellets, cholesterol (dose of 500 mg/kg body weight), and Monascus biopigment beverage. The animal experiment was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the College of Veterinary Medicine, University of the Philippines Los Baños.

Diet

Animals were provided with commercial pellets and distilled water ad libitum. The food and water intakes were recorded daily. The cholesterol diet consisted of 500 mg cholesterol per kg body weight dissolved in 1 mL coconut oil (Gupta and Jain, 2006a and 2009b). This was used to induce hyperlipidemia in rats and was administered daily, every morning, for 30 days through gavaging.

The amount of Monascus extract incorporated in the formulated beverage was based on the dose extrapolation according to body surface area according to Reagan-Shaw et al. (2007):

\[
\text{Animal dose (mg/kg) = Human equivalent dose (mg/kg) \times Human (K_a) \times Animal (K_a)}
\]
where:

Human equivalent dose = 2400 mg/60 kg  
Human ($K_m$) = 37  
Animal ($K_m$) = 6 (factor for rat)

The amount computed was mixed with 3 mL of combined water, sugar, acidulant, and flavorant. After giving high cholesterol diet, the formulated *Monascus* biopigment beverage was administered in the treatment group daily for 30 days through gavage. Body weights were also measured daily to determine the amount of beverage and the dose of cholesterol administration.

**Blood lipid determination**

Blood collection for the determination of TC, TG, LDL-C, and HDL-C was done at day 0, 15, and 30. Blood (0.5 mL) was drawn from the orbital sinus using heparinized capillary tubes and transferred to sterile test tubes. These were directly transported for analysis at the Majime Animal Diagnostic Laboratory, Cupang, Antipolo City, Philippines. The samples were immediately centrifuged, upon arrival, for 10 minutes (DIGI Systems) at 1500 x g to obtain serum and stored at refrigerated temperature after separating the plasma. The assays were carried out not more than 24 hours after the collection. Total blood cholesterol (mg/dL) and triglyceride (mg/dL) levels as well as HDL-C (mg/dL) were analyzed and measured as described in the earlier section.

The major analysis of efficacy was based on the percentage change in TC level of the *Monascus* beverage-treated group after 30-day beverage administration. Secondary measures of efficacy were the percentage changes in total TG, LDL-C, and HDL-C.

**Physical and physiological parameters for toxicity evaluation**

The assessment of safety was based on the physical and physiological observations for toxicity evaluation. Observations for signs of acute toxicity were performed daily in *Monascus* beverage-treated group during the 30-day experimentation period. The study made use of following procedures by Morgan et al. (1989): (a) animals were observed undisturbed in their cages, (b) animals were removed from their cages and given some examination, and (c) animals were observed after being returned to their cages. Physical and physiological parameters included the monitoring of body weights, food and water intakes, gastrointestinal signs such as inappetence, occurrence of diarrhea or constipation, dehydration, bloody feces and emaciation, over-all physical characteristics of rat particularly the hair coat and the nostril, and behavioral signs of irritability or inactivity.

**BUN, creatinine, and ALT determination**

Aside from the physical and physiological observations, clinical blood chemistry examination, specifically, BUN, creatinine, and ALT were performed to further assess the safety of *Monascus* biopigment beverage in 30-day trial. Determination of the parameters was done in both studied groups at day 0, 15, and 30. The clinical examination results of the *Monascus* beverage-treated group were compared to those of the negative control group and to the obtained normal reference values to evaluate any significant changes on kidney and liver functions in 30-day experimental period. The same procedures as that of the blood lipid determination were employed for collection and transport of blood in the animal laboratory.

**Statistical analysis**

All data were presented as mean±standard deviation (SD). Student’s t-test was utilized to compare mean values of the two groups. One-way analysis of variance (ANOVA) with Least Significant Difference (LSD) was used to statistically analyze the variations of means of multiple groups. All statistical tests were performed with 1%, 5%, and 10% level of significance using Statistical Package for the Social Sciences (SPSS) version 10.0 (SPSS Inc., USA).

**Results and Discussion**

**Preliminary animal testing**

The lipid levels of rats, assigned in different treatments, after 30-day preliminary animal experiment are shown in Table 1. Administration of *Monascus* biopigment beverage at three different doses (7.2, 10.8, and 14.4 mL) showed decreased levels of serum total cholesterol, triglycerides, and LDL-C and slight increase in HDL-C. More than 10% of the baseline total cholesterol was reduced in all *Monascus* beverage-treated animals after 30 days of intake. Among the treatments, the rat given with 7.2 mL *Monascus* extract added (M1) in the beverage, had the lowest serum total cholesterol level. Likewise, it also had the highest decrease in the levels of TC, TG, and LDL-C. There were 27%, 25.2%, and 39.87% reductions in the levels of TC, TG, and LDL-C, respectively, of the animal given with high cholesterol and M1 diet after 30-day preliminary trial. On the other hand, increase in HDL-C levels was observed highest in the beverage with 14.4 mL *Monascus* extract added (25.77%
increase when compared to day 0 concentration). Normal values were obtained for BUN, creatinine, and ALT in rats based on the Research Animal Resources, University of Minnesota (2009) as follows: 10-21 mg/dL, 0.5-1 mg/dL and 35-80 U/L, respectively (Table 1). All Monascus beverage-treated animals had no sign of hepatotoxicity and nephrotoxicity as shown by the normal values of kidney and liver function tests obtained after 30 days of beverage intake.

Based on the results of preliminary animal testing, the formulation with 7.2 mL Monascus extract was chosen for the actual experiment due to its marked decrease in TC, TG, and LDL-C of the experimental rat after 30-day trial. Moreover, there was also a high possibility that the beverage was safe for consumption because of the normal levels of BUN, creatinine, and ALT observed.

**Actual animal experiment change in body weight and daily intake**

Administration of Monascus biopigment beverage for 30 days did not produce any significant gain in body weight of the treated rats when compared to negative control rats as shown in Table 2. Likewise, the levels of food intake between the studied groups had no significant difference. This means that the group given with Monascus beverage did not experience inappetence upon administration of the product. Unlike the above-mentioned parameters, the water intake values of the treatment and the negative control groups were significantly different from each other (p <0.10). This may be due to the contribution of formulated beverage to the fluid consumption of Monascus beverage-treated group. A percentage difference of 11.68% lower water intake of the rats given with Monascus beverage than the controlled group was recorded.

**Effect of Monascus biopigment beverage on serum lipid levels**

The changes in serum lipid levels of the negative controlled and the Monascus beverage-treated groups from day 0 to day 30 are shown in Table 3. Baseline levels of the two groups prior to cholesterol administration were statistically comparable. The concentrations of TC, TG, and LDL-C of the controlled group increased while HDL-C decreased from day 0 to day 30 upon administration of cholesterol powder (500 mg/kg body weight) although insignificant differences were obtained (p<0.05), except for TG. TC, TG, and LDL-C of the controlled group increased by 1.2%, 7.5%, and 1.15%, respectively, after 15 days of daily cholesterol gavaging. A higher percentage change was observed at day 30 in TC (2.7%) and TG (21.8%), while LDL-C had a slight increase of 0.3%. On the other hand, the levels of HDL-C had 3.28% and 4.29% reductions from day 0 to day 15 and day 30, respectively.

The group administered daily with Monascus biopigment beverage had remarkable reductions in lipid levels, especially at day 30. From day 0 to day 15, TC and LDL-C levels of the Monascus beverage-treated group, declined by 1.5% and 9.0%, respectively; HDL-C levels increased by 18.92% and TG slightly increased by 0.7%. At day 30, significant changes in the concentrations of TC, LDL-C, and HDL-C were obtained (p <0.05). TC and LDL-C levels decreased significantly by 18.5% and 45.64%, respectively, while HDL-C significantly increased by 47.12%. Triglyceride levels remained statistically unchanged but were reduced by 2.0% from day 0.
to day 30. Moreover, all lipid concentrations of the treatment group obtained at day 30 were significantly different from the negative control group at the same day (p <0.01).

Another criterion for evaluating the lipid-lowering efficacy of *Monascus* biopigment beverage was by determining the ratio of LDL-C to HDL-C. Figure 1 shows a decreasing trend in LDL-C/HDL-C ratio of the negative control group from day 0 to day 30, but the values were not significantly different from each other (p <0.10). In contrast, in *Monascus* beverage-treated group, a significant reduction in the ratio of LDL-C to HDL-C was observed. The LDL-C to HDL-C ratio between the two studied groups at day 0 and day 15 were statistically comparable (p <0.01). However, at day 30, rats given with *Monascus* beverage had a significantly lower mean LDL-C to HDL-C ratio of 1.15 compared with 3.21 of the negative control group. Low ratio means that the content of HDL-C had a much higher percentage in TC levels, which was a good indication that the atherosclerotic risk factor, LDL-C, was reduced (Lee, 2006).

Figure 1. Comparison of LDL-C/HDL-C ratio of the negative control group and the *Monascus* beverage-treated group at different time periods.

Mean values with different letters in the same treatment group are significantly different from each other at p <0.01.

*Significantly different from control group at the same day, p <0.01.

### Table 2. Mean body weight, and food and water intakes of negative control and *Monascus* beverage-treated Sprague-Dawley rats

<table>
<thead>
<tr>
<th>GROUP</th>
<th>WEIGHT (g)</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>% GAIN IN BW (Gain in BW/day)</th>
<th>FOOD INTAKE (g/day)</th>
<th>WATER INTAKE (ml/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>173.85</td>
<td>±1.32</td>
<td>±11.32</td>
<td>±19.76</td>
<td>2.17±0.98</td>
<td>13.61±1.63</td>
<td>42.82±1.16</td>
</tr>
<tr>
<td>Monascus beverage</td>
<td>176.94</td>
<td>±7.02</td>
<td>±16.77</td>
<td>±19.65</td>
<td>2.08±0.93</td>
<td>13.76±1.67</td>
<td>37.82±5.63</td>
</tr>
</tbody>
</table>

Negative control group, fed with commercial pellets + cholesterol (500 mg/kg BW); *Monascus* beverage-treated group, fed with commercial pellets + cholesterol (500 mg/kg BW) + *Monascus* biopigment beverage containing 7.2 mL *Monascus* extract.

BW, body weight.

Mean values with different letters in the same treatment group are significantly different from each other at p <0.01.

*Significantly different from control group at the same parameter, p <0.10.

### Table 3. Mean total cholesterol (mg/dL), triglycerides (mg/dL), LDL-C (mg/dL), and HDL-C (mg/dL) of the negative control and *Monascus* beverage-treated Sprague-Dawley rats at different time periods

<table>
<thead>
<tr>
<th>GROUP</th>
<th>TC (mg/dL)</th>
<th>TG (mg/dL)</th>
<th>LDL-C (mg/dL)</th>
<th>HDL-C (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>176.2</td>
<td>151.7</td>
<td>147.7</td>
<td>130.7</td>
</tr>
<tr>
<td>Monascus beverage</td>
<td>176.2</td>
<td>145.2</td>
<td>151.7</td>
<td>147.7</td>
</tr>
</tbody>
</table>

TC, total cholesterol; TG, triglycerides; LDL-C, low density lipoprotein-cholesterol; HDL-C, high density lipoprotein-cholesterol.

*\(\text{LDL} = (\text{TG}/5) - \text{TC} - \text{HDL-C}\)

Mean values with different letters in the same treatment group are significantly different from each other at p <0.05.

*Significantly different from control group at the same day, p <0.01.

*\(\text{LDL} = (\text{TG}/5) - \text{TC} - \text{HDL-C}\)

Mean values with different letters in the same treatment group are significantly different from each other at p <0.01.
attributed to the components present in Monascus-fermented rice. The product was known to contain 14 monacolin-related active compounds which worked synergistically to lower the production of cholesterol in the liver (Gordon *et al*., 2010). Aside from monacolins, the product also contained sterols (β-sitosterol, campesterol, and stigmasterol), monounsaturated fatty acids, and isoflavones and isoflavone glycosides, all of which may also contribute to its lipid-lowering effects (Heber *et al*., 1999).

Monacolin K, the primary monacolin in Monascus-fermented rice, has been found to have cholesterol-lowering effect due to its competitive inhibition on HMG-CoA reductase, an enzyme needed to form mevalonate and other subsequent products in cholesterol synthesis (Heber *et al*., 1999; Endo, 2004). After absorption, the lactone form monacolin K is immediately converted into β-hydroxyacid form (Ganrong *et al*., 2005), the active form of monacolin K in the body. The structure of the β-hydroxyacid form has a strong similarity with the substrate, HMG-CoA, (Seenivasan *et al*., 2008) and it binds at the same site where the substrate attaches the HMG-CoA reductase enzyme, resulting in reduced synthesis of cholesterol.

Safety evaluation of Monascus biopigment beverage

Physical and physiological evaluation of animals

The insignificant difference in food intake between the Monascus beverage-treated group and the negative control group was an indication that the former showed no sign of inappetence. Even, after 30 days of beverage administration, there was no sign of emaciation due to insignificant mean percentage gain in body weight of the treated animals when compared to the controlled group. The only significant difference observed between the two studied groups was the amount of water intake which may be accounted for by the daily administration of the developed beverage. Other gastrointestinal signs such as occurrence of diarrhea or constipation, dehydration, and bloody feces were not observed. The stools of both animal groups during the 30-day experiment were frequent, non-sticky and pelleted, which may indicate that there was no incidence of gastrointestinal disturbances. Over-all physical appearance of the Monascus beverage-treated group was also comparable to that of the controlled group. There was no visible sign of abnormal behaviors recorded. Throughout the study, the rats given with Monascus beverage were observed active but not irritable, had smooth and shiny hair coat, and bright clear eyes.

Serum BUN, creatinine, and ALT levels

BUN, creatinine, and ALT levels obtained from day 0 to day 30 in Monascus beverage-treated group were insignificantly different from that of the negative control group, as shown in Table 4 (p <0.10). The high BUN levels at day 0 and day 15 can be associated with the commercial feeds given to the animal models. A protein-rich meal may result in increased blood or serum urea (Gilor and Gilor, 2011). Nevertheless, at day 30, BUN levels returned to normal limits of 10-21 mg/dL in both treatment groups.

Mean creatinine levels of both animal groups also showed no significant change at day 15 and day 30 as compared to day 0 values. Same result as what was obtained in creatinine levels, no significant change was observed in ALT levels of the treatment group from day 0 to day 30. Furthermore, all creatinine and ALT values of both groups were within the normal levels.

Table 4. Mean BUN (mg/dL), creatinine (mg/dL), and ALT (U/L) of the negative control and Monascus beverage-treated Sprague-Dawley rats at different time periods

<table>
<thead>
<tr>
<th>GROUP</th>
<th>BUN (mg/dL)</th>
<th>CREA (m/dL)</th>
<th>ALT (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>Negative Control</td>
<td>26.3</td>
<td>24.7</td>
<td>20.8</td>
</tr>
<tr>
<td>±3.1*</td>
<td>±7.5*</td>
<td>±5.3*</td>
<td>±0.38*</td>
</tr>
<tr>
<td>Monascus Beverage</td>
<td>25.7</td>
<td>22.1</td>
<td>20.9</td>
</tr>
<tr>
<td>±4.0*</td>
<td>±3.8*</td>
<td>±4.0*</td>
<td>±0.35*</td>
</tr>
</tbody>
</table>

Negative control group, fed with commercial pellet + cholesterol (500 mg/kg BW); Monascus beverage group, fed with commercial pellet + cholesterol (500 mg/kg BW) + Monascus beverage containing 7.2 mL Monascus extract.
BUN, blood urea nitrogen; CREA, creatinine; ALT, alanine aminotransferase.
Mean values with different letters in the same treatment group are significantly different from each other at p<0.05.
Conclusion

This study showed that the beverage incorporated with 7.2 mL of Monascus-fermented rice extract from Monascus purpureus was effective in lowering lipid levels of Sprague-Dawley rats in 30-day experimental period. Moreover, based on the daily observations and results of clinical blood examinations, the group treated with Monascus biopigment beverage did not develop any physical, physiological, and clinical signs of toxicity. Thus, the beverage was neither nephrotoxic nor hepatotoxic and may be considered safe within 30-day consumption using rats as animal model.

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