In vivo evaluation of Monascus-fermented durian seed for antidiabetic and antihypercholesterol agent

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In vivo evaluation of *Monascus*-fermented durian seed for antidiabetic and antihypercholesterol agent

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Abstract

Monascus-fermented durian seed (MFDS) had been developed previously. This research was aimed to evaluate the MFDS for antidiabetic and antihypercholesterol agent with *in vivo* method using *Wistar* rat. The MFDS was produced by inoculating the spore suspension of *Monascus* sp. KJR2 into durian seed cuts and then incubated at room temperature (30°C) for 14 days. The product was dried and ground to produce MFDS powder. The MFDS suspension was evaluated its lowering blood glucose and cholesterol concentration *in vivo* using male *Wistar* rats. Administration of 2 mL of product suspensions at the level of 0.05; 0.10; and 0.15 g/2 mL for 28 days decreased 12.89% of blood glucose and 49.3% serum cholesterol concentration in the rats. Higher level of MFDS suspension resulting higher effectiveness of the lowering blood glucose and total cholesterol. MFDS also improved lipid profile including HDL-cholesterol, LDL-cholesterol and Triglyceride levels.

1. Introduction

Monascus-fermented product, especially *Monascus*-fermented rice has been used by Asian people for centuries as natural food colorant, food supplement and traditional medicine. During solid state fermentation, *Monascus* produces various secondary metabolites primarily pigments and monacolines with various bioactivities, and the fermented products showed possitive health effects (Chang *et al*., 2006; Chen *et al*., 2006; Lin *et al*., 2008; Shi *et al*., 2010; Shi *et al*., 2012).

Monascus-fermented durian seed is a fermentation product of *Monascus* on durian seed as the substrate. Our previous finding showed that the Monascus-Fermented Durian Seeds (MFDS) contain pigments (yellow, orange and red pigments), monacolin K and phenolic (Srianta, Novita and Kusumawati, 2012; Srianta *et al*., 2012). Some researchers also reported that *Monascus*-fermented products contain monacolin K (Chairote *et al*., 2008). In vitro antidiabetic assay i.e. α-glucosidase inhibition activity of MFDS extract showed that the MFDS potent to be developed as antidiabetic product (Srianta *et al*., 2013). Monacolin K posses lowering blood cholesterol effect through inhibition of HMGCoA reductase activity, enzyme in cholesterol biosynthesis pathway (Endo, 1980; Endo, 1988). Li *et al*. (1998) reported the antihypercholesterol activity of *Monascus*-fermented rice. However, evaluation of antidiabetic and antihypercholesterol of MFDS have not been studied yet. Objective of this research was to evaluate *in vivo* antidiabetic and antihypercholesterol activities of MFDS.

2. Materials and methods

2.1 MFDS production

Durian seeds were boiled in a $CaCO_3$ solution of 5% w/v for 10 min to remove the mucus. After the seed coat were peeled off, the seeds were cut into small size. A 50 g of small cut durian seed was transferred into 300 mL flask, mixed thoroughly, autoclaved at 121°C for 15 min, then left to cool at room temperature, inoculated with the spore suspension of *Monascus* sp. KJR2 and incubated at room temperature (30°C) for 14 days in static conditions (with manual shaking daily). *Monascus*-fermented durian seed were dried in an oven at 45°C for 24 hours and ground.

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2.2 In vivo evaluation of MFDS antidiabetic and antihypercholesterol activities

The committee of ethical clearance of the Integrated Research and Testing Laboratory Gadjah Mada University approved this *in vivo* experiment with ethical clearance number of 142/KEC-FKPT/IV/2014. Animals were treated in accordance for the proper care and use of laboratory animals.

Twenty four (24) male *Wistar* rats about 2 months old (weight range of 250-300 g) were adapted for a week with feeding of standard feed (AIN-93M) (Table 1). At the end of the adaptation, blood glucose level and lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol, dan Triglyceride) of blood serum were analyzed. After that, the rats were fed with high cholesterol feed for a week to make hypercholesterol rats, with total cholesterol of > 130 mg/dL. The high cholesterol feed was formulated as standard feed with an addition of 1% of cholesterol (Matos *et al*., 2005). Then, the rats were induced to diabetic by injecting of streptozotocin (STZ) 65 mg/kg and Nicotineamide (NA) 230 mg/kg body weight (Szkudelski, 2012). The rats were fed with standard feed for 5 days until the rats got diabetic (Marsono *et al*., 2003). During the diabetic induction, to prevent the rats of having hypoglycemic condition they were given dextrose solution by forced feeding.

Table 1. Composition of the diets (g/1000 g of diet)

Component	Standard *, (g)	High Cholesterol**, (g)
Corn starch	620.692	610.692
Casein	140	140
Sucrose	100	100
Soybean oil	40	40
Cellulosa	50	50
Cholesterol	-	10
Mineral mix	35	35
Vitamin mix	10	10
L-cystine	1.8	1.8
Cholin bitartrate	2.5	2.5
tert-butyl hydroquinone (TBHQ)	0.008	0.008
Total	1000	1000

Source: *) Reeves *et al.* (1993)

**) Hypercholesterolemic diets were suplemented with 10 g of cholesterol per kg of diet, at the expense of starch.

The rats were divided randomly into 4 groups of 6 rats i.e.: Control group fed with standard feed, and the other groups were fed with standard fed and given 2 mL of 0.05g MFDS suspension (MF0.05), 0.10g MFDS suspension (MF0.10) and 0.15g MFDS suspension (MF0.15), respectively.

The feed and water were given ad libitum and MFDS suspension was administered by forced feeding daily for 28 days. The rats were weighed at the early of the experiment and every week during the experiment. Analysis of blood glucose and lipid profile were carried out every week, after 12 hours fasting.

2.3 Data analysis

The obtained data were calculated for the average. The data obtained after 28 days treatment period were analyzed statistically using Analysis of varians with $\alpha = 5\%$ and Least Significant Difference with $\alpha = 5\%$.

3. Results and discussion

A high cholesterol diet and streptozotocin injection resulted in hypercholesterol (total cholesterol of blood serum were 211 and 225 mg/dL) and diabetic (glucose levels of blood serum were in a range of 216 and 220 mg/dL) rats. Figure 1(a) showed that the body weight of all the rats with MFDS treatments increase, while the control tend to decrease during the experiment. Figure 1(b) showed that the control feeding intake higher than those of the treatments. This rats weight decreasing during experiment seem was due to the effect of diabetes on rats. Diabetic rats tend to polyuria, polydipsia, polyfagia, and loose body weight. In a research by Akbarzadeh *et al*. (2007), it was shown that consumed food in normal rats was 10 ± 2 grams, while in streptozotocin induced diabetic rats was 45 ± 5 grams, the water consumed by normal rats was around 35 ml, while in streptozotcin induced diabetic rats was 150 ml, and the volume of urine in normal rats was 10 ± 1 ml and in the streptozotocin induced diabetic rats was 130 ± 5 ml.

Figure 2 is blood glucose levels during the experiment. Streptozotocin destroyed the B cells of the pancreas by DNA alkylation, which led to B cell necrosis (Szkudelski, 2001). From the figure, it was concluded that the glucose level for all groups were normal, and then the treatment using streptozotocin was successful to increasing the glucose level up to a constant level at the end of the period of induction. Administration of MFDS suspension at the level of 0.05; 0.10; and 0.15 g/2 mL for 28 days showed lowering blood glucose level effect in the rats. Higher level of MFDS suspension, higher effectiveness in lowering blood glucose of the rats. Chang *et al.* (2006) found similar result that oral administration of

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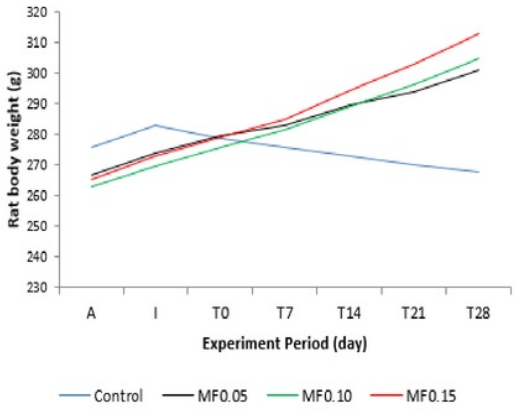
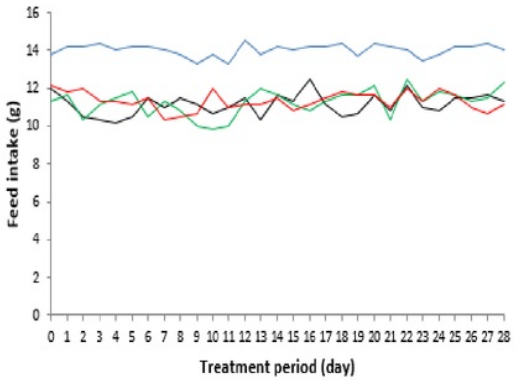


(a)



(b)

Figure 1. Rats body weight (a) and feed intake (b) during experiment. A (adaptation); I (induction); T0 (day 0 of treatment); T7 (7 days of treatment); T14 (14 days of treatment); T21 (21 days of treatment); T28 (28 days of treatment).

Monascus-fermented rice at 50-350 mg/kg reduced plasma glucose of streptozotocin-diabetic rats. The decreasing plasma glucose was in a dose depedent manner. Shi and Pan (2010) also reported that feeding of different type of Monascus-fermented products i.e. Monascus-fermented rice, Monascus-fermented dioscorea and Monascus-fermented adlay reduced blood glucose levels of diabetic rats after after 8 weeks of feeding.

Those effects might be due to MFDS posses α-glucosidase inhibition activity, which related to the phenolic content of the product (Srianta *et al*., 2013). Moreover, during fermentation on durian seed substrate, the *Monascus* purpureus produce yellow, orange and red pigments (Srianta *et al*., 2012). The pigments might be contribute to the antidiabetic activity. Lee *et al*. (2011) reported that yellow pigment, Monascin, was able to improve insulin sensitivity through serine/threonine protein kinases pathway by stabilizing PPAR-γ structure, preventing its phosphorylation, and inhibiting c-Jun N-terminal kinase activation. Shi *et al*. (2012) suggest that the Monascin has a therapeutic potential on diabetes and diabetes-associated oxidative stress complications. Higher level of MFDS suspension has higher contents of phenolic and pigments which might be resulted higher effectiveness in lowering blood glucose. In this experiment, blood glucose level of the MF0.1 and MF0.15 group decreased by 5.76%, and 12.89% respectively when compared to those glucose levels after induction. The greatest effec of decrease in blood glucose level was achieved in 2 weeks after the treatment, followed by constant decrease until the end of experiment (1 month MFDS treatment).

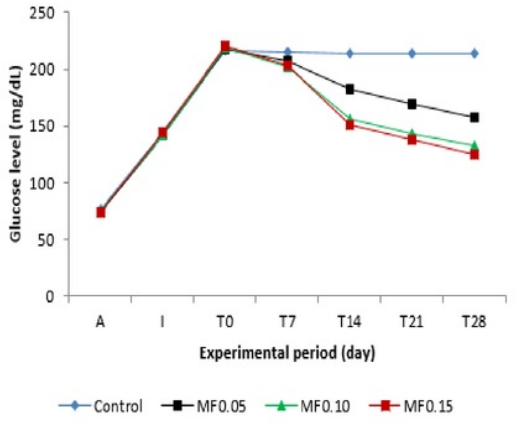


Figure 2. Blood glucose levels (mg/dL) during experiment. A (adaptation); I (induction); T0 (day 0 of treatment); T7 (7 days of treatment); T14 (14 days of treatment); T21 (21 days of treatment); T28 (28 days of treatment).

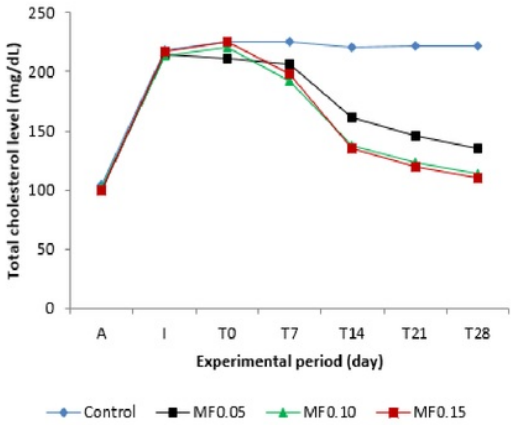


Figure 3. Blood cholesterol levels (mg/dL) during 28 days experiment. A (adaptation); I (induction); T0 (day 0 of treatment); T7 (7 days of treatment); T14 (14 days of treatment); T21 (21 days of treatment); T28 (28 days of treatment).

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Total cholesterol levels during experiment is shown in Figure 3. High cholesterol feeding resulted high blood total cholesterol level in all groups of rats. All treatment was succesful to lowering the blood cholesterol levels during 28 days experiments. Higher level MFDS resulted lower total cholesterol level. Total cholesterol level of the MF0.15 group decreased 49.3%. Yeap *et al*. (2014) found similar results that administration of *Monascus*-fermented rice water extracts (MARDI *Monascus*-fermented rice and Commercial *Monascus*-fermented rice) at 60 mg/kg reduced blood cholesterol levels signicantly.

Table 2. Blood LDL-c, HDL-c and triglyceride levels (mg/dl) during 28 days experiment

Group	Period (week)				
	0	1	2	3	4
	LDL-c level (mg/dL)				
Control	100.3	102.86	103.35	102.42	103.2^{d}
MF0.05	96.15	99.69	93.70	70.00	63.26^{c}
MF0.10	95.24	99.29	98.48	57.58	51.15^{b}
MF0.15	99.7	104.19	103.15	57.98	48.05^{a}
	HDL-c level (mg/dL)				
Control	23.29	22.47	21.40	23.42	22.22^{a}
MF0.05	28.38	27.31	29.55	39.94	47.48^{b}
MF0.10	28.68	27.46	34.79	53.45	60.90^{c}
MF0.15	25.91	24.82	34.50	61.93	70.56^{d}
	Triglyceride level (mg/dL)				
Control	141.33	146.94	147.79	145.40	148.28^{c}
MF0.05	135.90	142.05	131.20	100.30	93.12^{b}
MF0.10	133.64	140.06	120.85	84.01	79.37^{a}
MF0.15	139.22	145.57	127.70	80.09	74.44^{a}

Note: different character in the same column indicated a significantly different at α = 5%

Table 2 is blood LDL-c, HDL-c and triglyceride levels data during the experiment. HDL-c ("good cholesterol") and LDL-c ("bad cholesterol") are the forms of cholesterol circulated in the blood. HDL is called good cholesterol as it has little cholesterol and high protein content, while LDL is the opposite way. LDL is the source of artery clogging plaque, while HDL actually works to clear cholesterol from the blood (Ma, 2006).

As seen in Table 2, HDL in the MF0.15 group was highest, while the LDL level was the lowest. Triglycerides are another form of fat in the bloodstream. High level of triglycerides indicated high risk of cardiac disease. A high triglycerides level with high LDL level or lower HDL level increases the speed of atheroschlerosis, which increases the risk of heart attack and stroke (Ma, 2006). In this experiment, triglycerides of the MF.015 group decreased by 54.88% from the hypercholesterol level, the highest decrement compared to the other treatment level, while the control group triglycerides level increased by 5.46%.

It is concluded that MFDS improved lipid profile including lowering total cholesterol, LDL-cholesterol, triglyceride and increasing HDL-cholesterol. Yeap *et al*. (2014) found similar results that administration of *Monascus*-fermented rice water extracts (MARDI *Monascus*-fermented rice and Commercial *Monascus*-fermented rice) at 60 mg/kg decreased blood cholesterol, triglyceride, LDL-cholesterol levels and increase HDL-cholesterol significantly.

The mechanism was might be due to combination effect of bioactive compounds in the MFDS. The MFDS contain monacolin K and pigments (Srianta *et al*., 2012). Monacolin K is well known cholesterol lowering agent and it has been used for hypocholesterol drug widely. Its action is to inhibit HMG-CoA reductase, the first enzyme in cholesterol biosynthesis pathway, activity. Jou *et al*. (2010) reported that *monascus* yellow pigments, Monascin and Ankaflavin, might reduce triglyceride accumulation and suppress expression of adipocyte-specific transcription factors to decrease proliferation and differentiation preadipocyte. Both of them might also promote mature adipocyte delipidation by releasing glycerol and downregulating the HR-LPL activities. According to Li *et al*. (1998), *Monascus*-fermented product contain not only inhibitor of HMG-CoA reductase, but also monosaturated fatty acids, sterols, proteins, saccharide, isoflavone and its glycoside, saponin and sapogenin, and trace elements such as selenium and zinc. They suggest that combination of its total constituents might effect on the improving cholesterol status. However, the precise contribution need further study.

Drugs lowering cholesterol are clofibrate, gemfibrozil, nicotinic acid (niacin), resin, statin, bile acid sequestrants and fibrates. MFDS was similar to statin as it lowers the LDL and triglyceride and has mild effect in increasing HDL (Yee *et al*., 2004; Zhang *et al*., 2008). Statins blocks the production of cholesterol in the liver itself (Ma, 2006). However, there are side effects of these drugs, such as muscle aches, abnormal liver function, allergic reaction (skin rashes), heartburn, dizziness, abdominal pain, constipation and decreased sexual desire) (Ma, 2006). In this experiment, side effects of MFDS both in physical form and in molecular form in short and long term was not observed yet.

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Conclusion

Administration of MFDS suspension at the level of 0.05; 0.10; and 0.15 g/2 mL for 28 days showed lowering blood glucose and cholesterol effect. Higher level of MFDS suspension, higher effectiveness in lowering blood glucose and cholesterol levels of the rats. Moreover the treatments also improved blood lipid profile including HDL cholesterol, LDL cholesterol and triglyceride levels. Further study on side effects of MFDS need to be carried out.

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