DOI:10.1021/jf101194f

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Anti-diabetic Effects of *Monascus purpureus* NTU 568 Fermented Products on Streptozotocin-Induced Diabetic Rats

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Red-mold-fermented products have the unique ability to economically produce many secondary metabolites and are known to improve blood circulation. Diabetes mellitus is a chronic disease that is characterized by hyperglycemia caused by insufficient insulin action. In the current study, we examine the effect of *Monascus purpureus* NTU 568 fermented products on fasting blood glucose and oral glucose tolerance testing (OGTT) in streptozotocin-induced diabetic rats. After 8 weeks of being fed with red-mold-fermented products at a dose of 200 mg/kg, the experimental results indicate that oral administration of red-mold-fermented products can delay the development of the plasma glucose level in rats. A significant reduction was found in urine sugar and urine protein levels. The study scientifically validates the widely claimed use of red-mold-fermented products as an ethnomedicine to treat diabetes mellitus.

KEYWORDS: Anti-diabetic; diabetes; insulin resistance; *Monascus purpureus* NTU 568; streptozotocin

INTRODUCTION

In general, the genus *Monascus* includes five major species: *M. purpureus*, *M. ruber*, *M. aurantiacus*, *M. pilosus*, and *M. floridanus*, which belong to the class Ascomycetes and the family Monascaceae. The solid-state fermentation of rice by *Monascus* as a dietary staple and food additive has a long tradition in East Asia (*1*). Red mold rice is also mentioned in an ancient Chinese pharmacopoeia of medicinal food and herbs as improving the digestion and revitalizing the blood (*2*). *Monascus* spp. can produce several bioactive metabolites, such as pigments (red pigment, monascorubramine and rubropunctanin; orange pigment, monascorubrin and rubropunctanin; and yellow pigment, ankaflavin and monascin), isoflavones, polyketide monacolins, dimerumic acid, and γ-aminobutyric acid (GABA) (*3*−*6*). Monacolin K (lovastatin) is the worthy secondary metabolite found to inhibit the biosynthesis of cholesterol, an inhibitor of 3-hydroxy-3-methyglutaryl coenzyme A (HMG-CoA) reductase, for treating hyperlipidemia (*7*, *8*).

Sowers et al. (*9*) reviewed the utility of HMG-CoA reductase inhibitors (statins) in patients with metabolic syndrome, because statins may play a role in some modifiable clinical features of metabolic syndrome. It has been suggested that monascin is valuable as a potential cancer chemo-preventive agent in chemical and environmental carcinogenesis based on an evaluation of its inhibiting effect on 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation in mice (*10*). It has also been shown to be an anticancer agent in addition to being used as a colorant (*11*, *12*).

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GABA is the chief inhibiting neurotransmitter involved in the mammalian central nervous system. Okada et al. (*13*) have discovered that, within the pancreatic islets, insulin and GABA are secreted from β cells. Xu et al. (*14*) indicated that insulin induced the activation of $GABA_A$ receptors in the cells and suppressed glucagon secretion. GABA supplementation has been clinically proven to prevent and improve diabetic vision loss, as well as peripheral neuropathy, which can lead to amputation (*15*).

Metabolic syndrome (MS) is a multi-factorial condition that represents a risk factor in the development of type-2 diabetes mellitus (DMII) and cardiovascular disease (CVD) (*16*). Diabetes is a strong risk factor of cardiovascular disease, and end-stage renal disease begins before diabetes is diagnosed, during the preceding period of increased insulin resistance and impaired glucose tolerance (*17*). The prevalence of diabetes is rapidly increasing in industrialized nations, and this metabolic disease is one of the most frequent causes of death in developed countries. The introduction of new agents may be useful for the treatment of diabetic patients with blood glucose regulation and insulin resistance. In fact, there has been an enormous increase in the use of herbal and alternative medicines for this target.

Using a fermentation method to produce a higher quantity of monascin, ankaflavin, and GABA is the major concern of our study. According to the ancient Chinese medical book, Pen Ts'ao Kang Mu (*18*), dioscorea (*Dioscorea batatas* Dence) and adlay (*Coix lacryma-jobi* L. var. ma-yuen Stapf.) have been used as a traditional medical herb substance, health food supplement, and functional food for the treatment of diabetes in China (*19*). In a study by Hsu et al., the oral administration of dioscorea into streptozotocin-induced diabetic rats increased the response to exogenous insulin and decreased the plasma glucose in fructose-rich chow-fed rats (*20*). Adlay is a nourishing food with a high nutritional value and various functional effects on the human

 Published on Web 06/17/2010

Table 1. Production of Various Secondary Metabolites on Different Substrates by *M. purpureus* NTU 568[a]

substrate[b]	monascin (mg/kg)	ankaflavin (mg/kg)	GABA (mg/kg)	red pigment (A_{500}/g)	orange pigment (A_{470}/g)	yellow pigment (A_{400}/g)
rice	3099.7 ± 245.8	1048.8 ± 79.0	121.4 ± 10.5	75.62 ± 0.03	70.89 ± 0.13	85.17 ± 0.12
dioscorea	3572.7 ± 261.7	2444.3 ± 128.4	162.2 ± 6.9	63.53 ± 0.35	65.07 ± 0.10	89.55 ± 0.16
adlay	1098.7 ± 66.5	569.4 ± 89.6	87.3 ± 5.7	43.20 ± 0.13	32.55 ± 0.11	68.66 ± 0.14

[a] Data are presented as means ± SD (n = 5). [b] Culture conditions: 250 g of substrate was inoculated with 5% spore suspension and cultivated at 30 °C for 10 days.

body (*21*, *22*) and is considered to be a health food supplement in Asian countries. In this study, we investigated the effect of red-mold-fermented products on the development of diabetes in streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

Preparation of Red-Mold-Fermented Products. *M. purpureus* NTU 568 was maintained on potato dextrose agar (PDA) slant at 4 °C and transferred monthly. The preparation of red-mold-fermented products was carried out using the substrates of long-grain rice (*Ipomoea batatas*), dioscorea root (*D. batatas* Dence), and adlay (*C. lacryma-jobi* L. var. ma-yuen Stapf.). A solid-state culture method was used to prepare the fermented contents (*23*). Briefly, the 250 g substrates were soaked in distilled water for 4−8 h, and excess water were removed. The substrates were autoclaved for 20 min at 121 °C in a "koji dish" (the koji dish was made of wood, with the dimensions of 30 × 20 × 5 cm). After the substrates were cooled to room temperature, they were inoculated with a 5% (v/w) spore suspension and cultivated at 30 °C for 8−10 days.

Quantification of Monascin and Ankaflavin. Red-mold-fermented product (0.5 g) was extracted with 5 mL of ethanol at 60 °C for 30 min (*24*). The suspension was then filtered with a 0.45 μm pore size filter and analyzed by high-performance liquid chromatography (HPLC) (model L-2450, Hitachi Co., Tokyo, Japan). Chromatographic separation was carried out by a C_{18} column (4.6 × 250 mm inner diameter, 5 mm, Agilent, Santa Clara, CA). The mobile phase consisting of 25% water, 75% acetonitrile, and 0.5% trifluoroacetate was eluted at a flow rate of 1.0 mL/min. UV detection was set at 387 nm.

Determination of the GABA Concentration. Red-mold-fermented product (0.5 g) was extracted with 5 mL of ethanol at 60 °C for 30 min (*24*). The suspension was then filtered with a 0.45 μm pore size filter and analyzed by HPLC (model L-2450, Hitachi Co.). The analysis method was described in a previous study (*25*). Chromatographic separation was carried out by a C_{18} column (4.6 × 250 mm inner diameter, 5 mm, Agilent). The fluorescence detector (FL-1, Rainin Co., Tokyo, Japan) was used in GABA (Sigma Chemical Co., St. Louis, MO) analysis.

Induction of Experimental Diabetes in Rats. Male Wistar rats aged 5 weeks were purchased from the Animal Centre of the National Taiwan University Medical College. The rats were housed in a temperature-controlled room (25 ± 1 °C) and kept on a 12:12 light/dark cycle (light on at 08:00). The experiments were carried out in a qualified animal breeding room in the animal center at our institute. The protocol complied with guidelines described in the Animal Protection Law, amended on Jan 17, 2001, Hua-Zong-(1)-Yi-Tzi-9000007530, Council of Agriculture, Executive Yuan, Taiwan, Republic of China. All rats were injected intraperitoneally with 65 mg/kg of streptozotocin (STZ, Sigma) in 0.1 M acetate buffer and 230 mg/kg nicotinamide after fasting for 12−24 h. Rats with a plasma glucose concentration of 200 mg/dL or greater were considered as diabetic animals.

Experimental Groups and Treatments. The animals were randomly divided into five treatment groups, and each group contained eight animals. The effects of different red-mold-fermented products on diabetic development were evaluated in STZ-induced diabetic rats receiving an oral administration of red mole rice (RMR, 200 mg/kg), red mold dioscorea (RMD, 200 mg/kg), and red mold adlay (RMA, 200 mg/kg). Rats were sacrificed at the end of the 8 week treatment. The kidneys were dissected, rinsed with saline, and then weighted. An index of renal hypertrophy was estimated by comparing the weight of two kidneys to the body weight.

Oral Glucose Tolerance Test (OGTT). The OGTT was performed at 2 weeks. The experiment was performed on animals after fasting for 12 h (free access to water). Animals were given glucose (2 g/kg of body weight) with an oral canula (*26*). Blood samples were collected from the tail vein at times 0, 30, 60, 90, 120, and 150 min after glucose administration. After centrifugation, plasma was divided into appropriate aliquots and stored at −20 °C until analysis.

Biochemical Index Analysis. Blood glucose, lipid, urea, and plasma insulin levels were measured at 8 weeks of red-mold-fermented product treatment. Blood glucose was immediately determined by the glucose oxidase method, using an analyzer (*27*). Plasma insulin was measured using an enzyme-linked immunosorbent assay (ELISA) insulin kit (Mercodia AB, Uppsala, Sweden). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated according to the formula HOMA-IR = fasting insulin (mIU/L) × fasting blood glucose (mmol/L)/22.5 (*28*). Plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatinine (CRE), and electrolyte levels were measured in triplicate using an automatic biochemical analyzer (Beckman-700, Fullerton, CA).

Histological Evaluation. Small pieces of kidney tissues taken from experimental animals were fixed in 10% neutral formalin, alcohol-dehydrated, paraffin-embedded, and sectioned to a mean thickness of 4 μm. The histological examination by the above conventional methods was evaluated for the index of diabetic-induced necrosis by assessing the morphological changes with hematoxylin and eosin (H&E) stain.

Statistical Analyses. Data are expressed as the mean ± standard deviation (SD). The statistical significance in the behavioral and biochemical effects was determined by one-way analysis of variance (ANOVA). A possibility of the p value less than 0.05 was considered as a significant difference between means.

RESULTS

The content of secondary metabolites in solid cultured products of RMR, RMD, and RMA are shown in **Table 1**. The RMR samples contained 3099.7 mg/kg of monascin (MS), 1048.8 mg/kg of ankaflavin (AK), 121.4 mg/kg of GABA, and 85.17 units/mL (A_{400}/g) of yellow pigment. The RMD samples contained 3572.7 mg/kg of MS, 2444.3 mg/kg of AK, 162.2 mg/kg of GABA, and 89.55 units/mL of yellow pigment. The RMA samples contained 1098.7 mg/kg of MS, 569.4 mg/kg of AK, 87.3 mg/kg of GABA, and 68.66 units/mL of yellow pigment. In comparison to RMR and RMA, RMD had high levels of anti-inflammatory yellow pigments, MS, and AK.

We investigated the effect of the intake of red-mold-fermented products on the development of diabetes and body weight of the same animal model for a long period (8 weeks). In this experiment, pioglitazone was used to prevent the progression of diabetes (positive control). In the eighth week of the diabetes induction, the body weight of the vehicle-treated STZ-induced rats was lower than other various groups (**Figure 1**). After 8 weeks of being fed with red-mold-fermented products, the daily food and water intakes of the vehicle-treated STZ-induced rats were higher than the other various groups. In addition, the exteriors and health of all of the experimental animals presented a normal expression.

The levels of fasting serum glucose and insulin in the vehicle-treated STZ-induced diabetic rats were higher than other groups with red-mold-fermented product treatment (**Table 2**). The HOMA-IR for the glucose and insulin response in the STZ-induced diabetic rats treated with red-mold-fermented products was lower than that for their vehicle-treated counterparts. The fasting serum glucose, insulin, and HOMA-IR in the RMD group were similar to those of the pioglitazone control group.

Figure 2 shows the hypoglycemic effect of red-mold-fermented products on the blood glucose level of the STZ-induced diabetic

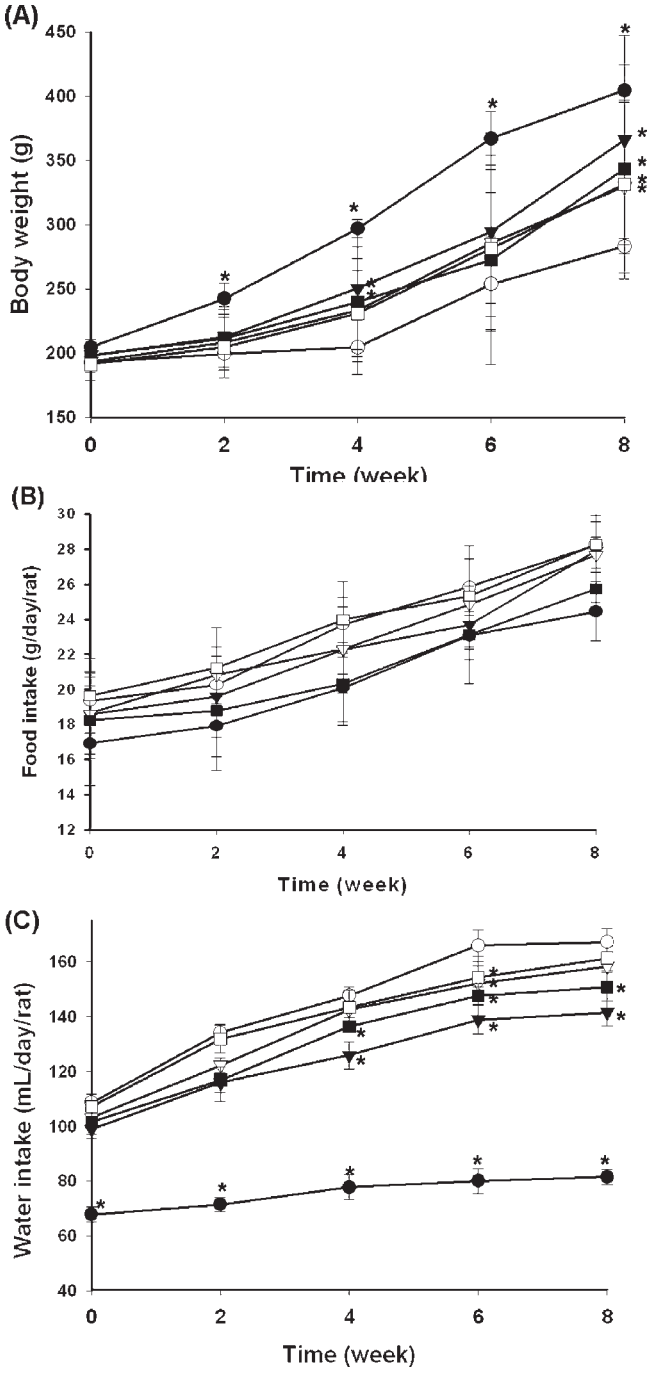

Figure 1. Red-mold-fermented products have a preventive effect on the progression of diabetes for long time periods without adverse effects. The body weight, food intake, and water intake of each group were measured every week. The standard vehicle was fed a normal diet without the administration of test materials (●). The other groups of STZ-induced diabetic rats were given pioglitazone (▼), red mold rice (▽), red mold dioscorea (■), red mold adlay (□), and vehicle (○). The data are presented as the mean ± SD of eight rats in each group. (∗) $p < 0.05$ is compared to data from standard vehicle and STZ-induced diabetic rats treated with vehicle.

rats during the later experiment period. The blood glucose level of the rats treated with red-mold-fermented products raised slowly at the sixth and eighth weeks. The RMD-treated group was similar to the pioglitazone control group. **Figure 3** depicts the hypoglycemic effect of a single oral administration with variable red-mold-fermented products on the OGTT of non-diabetic rats

Table 2. Effect of Red-Mold-Fermented Products of HOMA-IR by Levels of Serum Glucose and Insulin on STZ-Induced Diabetic Rats[a]

groups	blood glucose (mmol/L)	insulin (mIU/L)	HOMA-IR
standard vehicle	5.5 ± 0.8^b	9.9 ± 1.7^b	2.42 ± 0.7^b
STZ-induced vehicle	23.1 ± 1.2	36.2 ± 2.2	37.2 ± 2.2
pioglitazone (30 mg kg^{-1} day^{-1})	17.8 ± 6.0^b	21.6 ± 1.7^b	17.1 ± 1.2^b
RMR (200 mg kg^{-1} day^{-1})	20.1 ± 4.5^b	26.0 ± 5.8^b	23.2 ± 5.2^b
RMD (200 mg kg^{-1} day^{-1})	19.2 ± 4.1^b	25.1 ± 1.7^b	21.4 ± 1.4^b
RMA (200 mg kg^{-1} day^{-1})	21.2 ± 1.7^b	27.2 ± 2.7^b	25.6 ± 2.5^b

[a] Data are presented as the mean ± SE of eight rats in each group. [b] $p < 0.05$ is compared to data from standard vehicle and STZ-induced diabetic rats treated with vehicle.

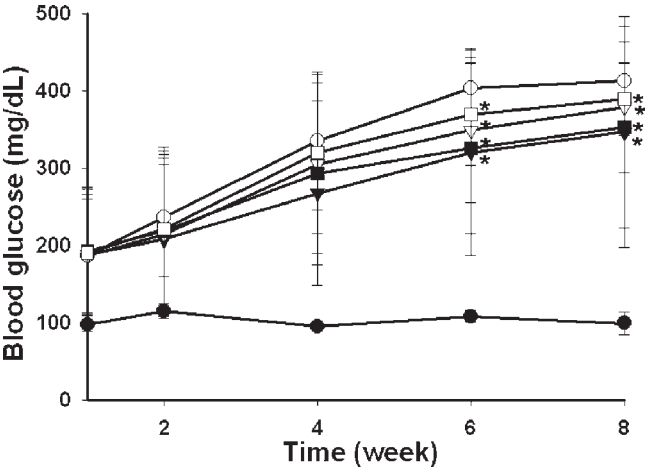

Figure 2. Effect of red-mold-fermented products on the blood glucose level of STZ-induced diabetic rats. Blood samples were collected to quantify the blood glucose levels every 2 weeks during the experimental periods. The standard vehicle was fed a normal diet without the administration of test materials (●). The other groups of STZ-induced diabetic rats were given pioglitazone (▼), red mold rice (▽), red mold dioscorea (■), red mold adlay (□), and vehicle (○). The data are presented as the mean ± SD of eight rats in each group. (∗) $p < 0.05$ is compared to data from standard vehicle and STZ-induced diabetic rats treated with vehicle.

and SZT-induced diabetic rats. The OGTT was impaired in the STZ-induced diabetic groups within 4 weeks. The slope of the oral glucose tolerance curve in RMD-, RMR-, and RMA-treated groups improved after 6 and 8 weeks, respectively (panels **C** and **D** of **Figure 3**). The slope of the OGTT curve in the RMD-treated rats was similar to that of the pioglitazone control group. With oral administration for 8 weeks, the group of RMD-treated rats showed a better effect of improving oral glucose tolerance and blood glucose regulation than the RMR and RMA groups.

During the animal experimental period, the levels of urea glucose and protein in vehicle-treated STZ-induced diabetic rats were higher than other groups (**Table 3**). Ketone bodies of urea were only detected in vehicle-treated STZ-induced diabetic rats. After 8 weeks of treatment, the value of urea glucose and protein in the STZ-induced diabetic rats administered with red-mold-fermented products (RMR, RMD, and RMA) were significantly lower than in the vehicle-treated STZ-induced diabetic rats (**Table 3**). When red-mold-fermented product treatment was compared, the RMD group had lower levels of urea glucose and protein than the RMR and RMA groups.

The ratio of the kidney weight/body weight in the vehicle-treated STZ-induced rats was significantly increased compared to other groups. Treating the STZ-induced rats with red-mold-fermented products (RMR/RMD/RMA) had reduced the degree

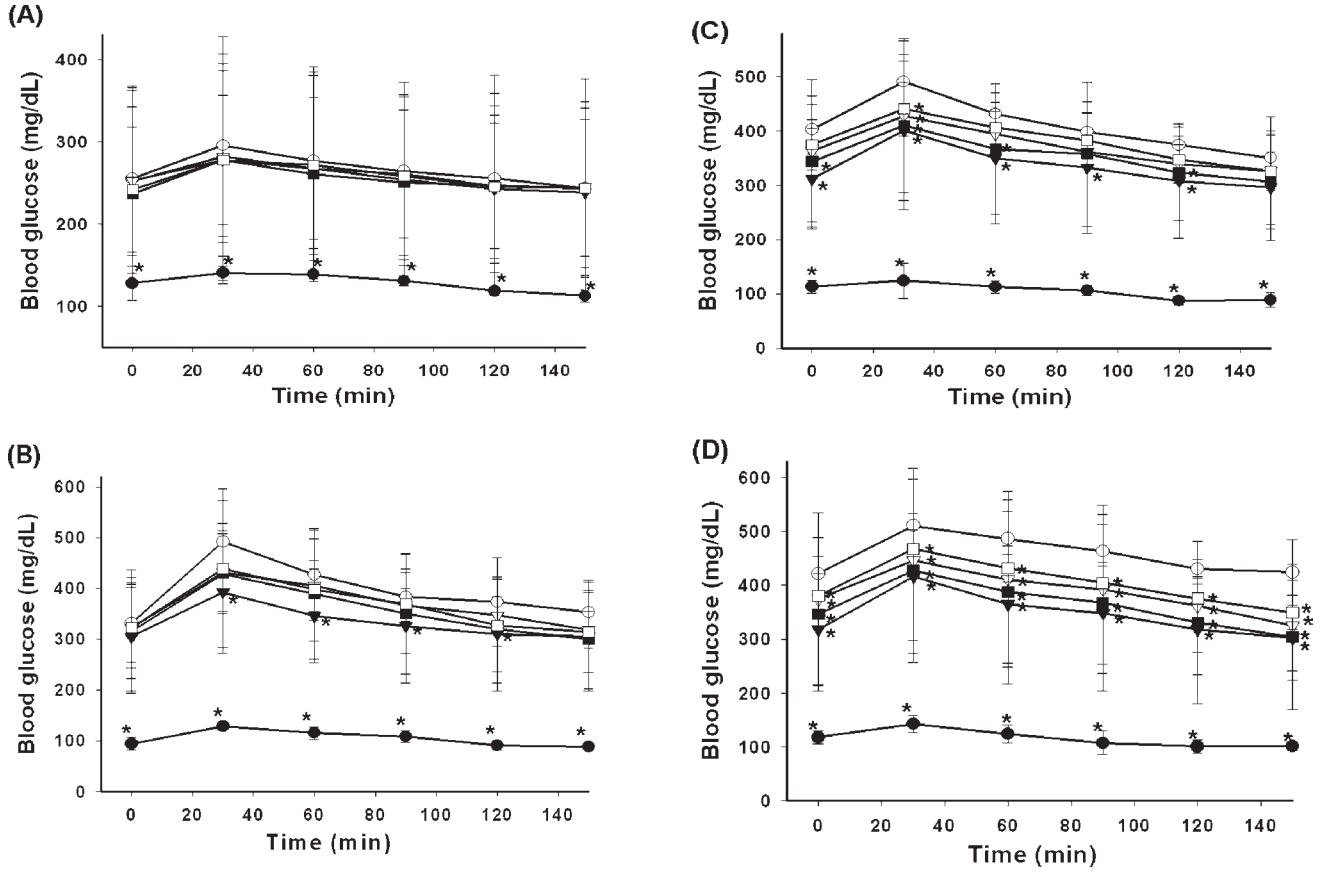

Figure 3. Hypoglycemic effect of red-mold-fermented products on the blood glucose level during the OGTT. Each group was given OGTT 4 times during the experimental periods: (A) second week, (B) fourth week, (C) sixth week, and (D) eighth week. The standard vehicle was fed a normal diet without the administration of test materials (●). The other groups of STZ-induced diabetic rats were given pioglitazone (▼), red mold rice (▽), red mold dioscorea (■), red mold adlay (□), and vehicle (○). The data are presented as the mean ± SD of eight rats in each group. (∗) $p < 0.05$ is compared to data from standard vehicle and STZ-induced diabetic rats treated with vehicle.

Table 3. Changes of the Renal Function Parameters in STZ-Induced Diabetic Rats Receiving 8 Weeks of Treatment with Red-Mold-Fermented Products[a]

groups	pH	glucose (mg/dL)	protein (mg/dL)	ketone bodies (mg/dL)	blood hemoglobin (mg/dL)	kidney weight (g)	kidney weight/body weight (%)
standard vehicle	6.7 ± 1.3^b	ND	23.2 ± 2.7^b	ND	ND	3.3 ± 0.3	0.82 ± 0.1^b
STZ-induced vehicle	9.0 ± 0.1	233.3 ± 0.1	58.3 ± 1.2	10.0 ± 0.9	ND	3.1 ± 0.4	1.35 ± 0.2
pioglitazone (30 mg kg^{-1} day^{-1})	8.6 ± 0.5	66.7 ± 1.4^b	41.7 ± 1.2^b	ND	ND	3.0 ± 0.3	0.87 ± 0.3^b
RMR (200 mg kg^{-1} day^{-1})	7.6 ± 1.0	75.0 ± 1.2^b	42.2 ± 1.8^b	ND	ND	3.2 ± 0.3	1.01 ± 0.2^b
RMD (200 mg kg^{-1} day^{-1})	8.4 ± 0.5	70.0 ± 0.7^b	39.5 ± 1.8^b	ND	ND	3.2 ± 0.5	0.98 ± 0.3^b
RMA (200 mg kg^{-1} day^{-1})	8.6 ± 0.5	87.5 ± 1.1^b	43.7 ± 1.5^b	2 ± 0.5	ND	3.4 ± 0.3	1.07 ± 0.2^b

[a] Data are presented as the mean ± SD of eight rats in each group. [b] $p < 0.05$ is compared to data from standard vehicle and STZ-induced diabetic rats treated with vehicle.

of renal hypertrophy at the termination of the eight weeks of treatment (**Table 3**). The cell of the kidney showed focal and moderate hydropic degeneration in the proximal tubules in the diabetic control group (**Figure 4B**). Therefore, animals with treatment had slight focal hydropic degeneration in the proximal tubules (panels **C**−**F** of **Figure 4**). The results of histopathology indicate that red-mold-fermented products may provide cytoprotective effects in kidney tissues.

There was no difference in the activity of creatinine in plasma between the vehicle diabetic, red-mold-fermented product treated group and the control group (**Table 4**). On the other hand, AST, ALT, and BUN levels were found to increase significantly in the plasma of the vehicle-treated STZ-induced rats, and these elevations were decreased to become close to the control level by all of the red-mold-fermented product treated groups. When the control and pioglitazone-, RMR-, and RMD-treated groups were compared, the rats in the vehicle-treated STZ-induced group had lower Na and Cl levels and a significantly higher K level (**Table 5**).

DISCUSSION

Red mold rice, a traditional Chinese food, has been used as a diet supplement in East Asia for several centuries, especially in China and Japan. The composition of incubation substrates is an important factor for the production of secondary metabolite and the growth of *Monascus* spp. In this study, *Monascus* fermentation using a dioscorea substrate results in increasing monascin, ankaflavin, and GABA compared to rice and adlay (**Table 1**). These results indicate that different substrates would lead to different secondary metabolite levels. Red mold dioscorea is also proven to possess a higher production of yellow pigments than red mold rice and red mold adlay. Yellow pigments produced by

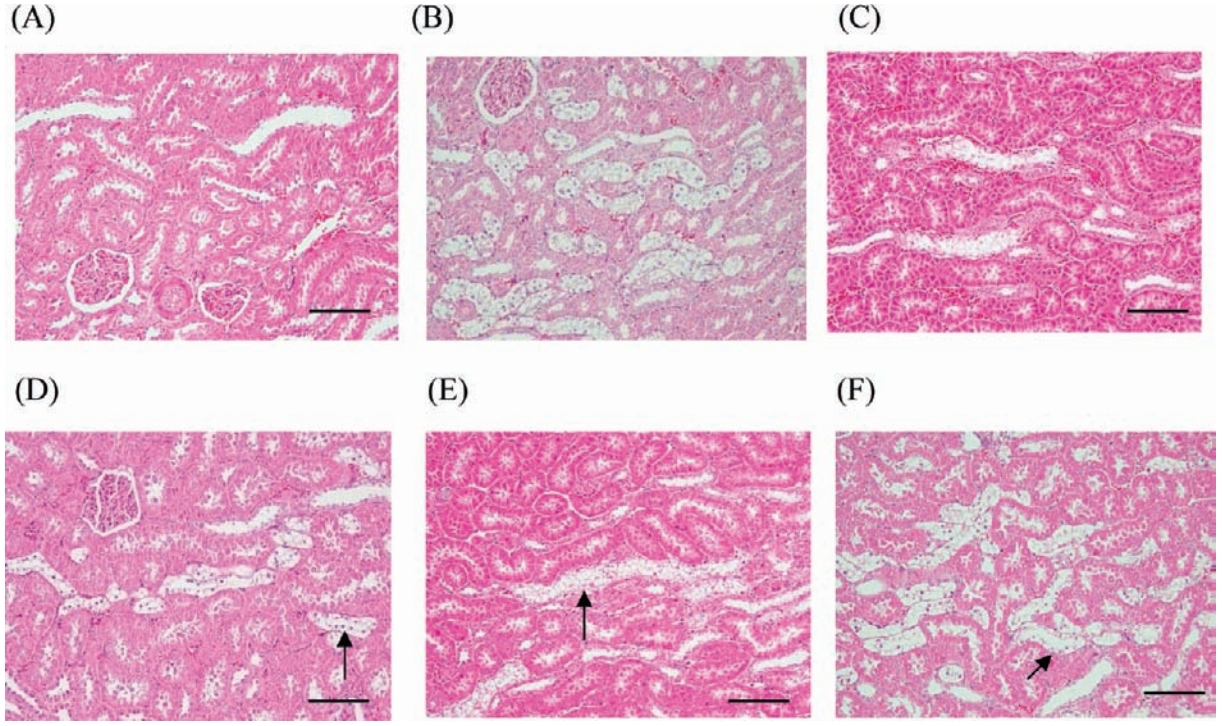

Figure 4. Histology of the kidney in experimental rats. (A) NC, (B) DC, (C) DM + piogiltazone, (D) DM + RMR, (E) DM + RMD, and (F) DM + RMA. The images were taken at 200× magnification with H&E staining. Scale bars indicate 150 μm.

Table 4. Effect of Red-Mold-Fermented Products on Performance Serum AST, ALT, BUN, and CRE Levels of STZ-Induced Diabetic Rats[a]

	liver		kidney	
groups	AST (units/L)	ALT (units/L)	BUN (mg/mL)	CRE (mg/mL)
standard vehicle	128.8 ± 15.0^b	62.0 ± 8.5^b	21.4 ± 3.4^b	0.5 ± 0.1
STZ-induced vehicle	457.0 ± 25.5	295.0 ± 21.2	35.1 ± 17.9	0.6 ± 0.1
pioglitazone (30 mg kg^{-1} day^{-1})	164.3 ± 71.8^b	119.0 ± 59.7^b	24.0 ± 4.9^b	0.5 ± 0.1
RMR (200 mg kg^{-1} day^{-1})	142.8 ± 57.6^b	91.8 ± 32.9^b	24.1 ± 3.3^b	0.4 ± 0.1
RMD (200 mg kg^{-1} day^{-1})	181.4 ± 70.4^b	106.9 ± 76.8^b	26.9 ± 9.8^b	0.6 ± 0.1
RMA (200 mg kg^{-1} day^{-1})	134.4 ± 61.2^b	100.8 ± 48.1^b	28.8 ± 3.9^b	0.5 ± 0.1

[a] Data are presented as the mean ± SD of eight rats in each group. AST, aspartate aminotransferase; ALT, alanine aminotransferase; BUN, blood urea nitrogen; CRE, creatinine. [b] $p < 0.05$ is compared to data from standard vehicle and STZ-induced diabetic rats treated with vehicle.

Table 5. Effect of Red-Mold-Fermented Products on the Performance Serum Electrolyte Levels of STZ-Induced Diabetic Rats[a]

groups	Na (mequiv/L)	K (mequiv/L)	Cl (mequiv/L)	Ca (mequiv/L)
standard vehicle	150.2 ± 1.9^b	8.1 ± 1.0^b	94.5 ± 1.8^b	12.3 ± 0.4
STZ-induced vehicle	140.8 ± 1.4	11.6 ± 0.9	83.0 ± 1.1	12.6 ± 0.2
pioglitazone (30 mg kg^{-1} day^{-1})	145.6 ± 4.7^b	7.5 ± 1.3^b	91.2 ± 4.9^b	12.8 ± 0.6
RMR (200 mg kg^{-1} day^{-1})	143.2 ± 3.8^b	7.8 ± 1.2^b	90.5 ± 1.9^b	12.0 ± 0.6
RMD (200 mg kg^{-1} day^{-1})	145.3 ± 3.7^b	8.4 ± 1.7^b	90.8 ± 4.3^b	12.6 ± 0.8
RMA (200 mg kg^{-1} day^{-1})	140.0 ± 2.3	8.9 ± 1.4^b	88.2 ± 2.8^b	12.9 ± 0.2

[a] Data are presented as the mean ± SD of eight rats in each group. [b] $p < 0.05$ is compared to data from standard vehicle and STZ-induced diabetic rats treated with vehicle.

Monascus include monascin and ankaflavin, which have been shown to be anticancer and anti-inflammatory agents (*11*, *12*).

The previous study was required to identify the mechanism of the effect of *Monascus*-fermented products on adipose, liver, and muscle insulin sensitivity, by means of modulating the insulin signal pathway to reverse the responsiveness of insulin (*29*). *Monascus*-fermented products can be used as a food-based adjuvant therapy for diabetic patients who have an urgent need to prevent insulin resistance and/or an impaired glucose metabolism (*30*). In our previous study, red mold rice supplement significantly reduced the serum insulin level in high-fat-induced rats (*31*). Hyperinsulinemia induced by a high fat diet would be improved by red mold rice treatment.

The current study shows a significant decrease in blood glucose levels of the STZ-induced diabetic rats with the red-mold-fermented product treatment at weeks 6 and 8 (**Figure 2**). This result provides direct proof of the antihyperglycemic function of red-mold-fermented products. A comparison of STZ-induced diabetic groups in this study has proven that red-mold-fermented products have antihyperglycemic effects. Insulin resistance is the major finding for several metabolic disorders, including metabolic syndrome, type-II diabetes, dyslipidemia, hyperglycemia, and hypertension (*32*). The degree of insulin resistance was estimated at the baseline by an insulin score (HOMA-IR) (*28*). Low HOMA-IR values indicated high insulin sensitivity, whereas high HOMA-IR values indicated low insulin sensitivity (insulin

resistance). In the present study, an oral administration of red-mold-fermented products was found to decrease the plasma glucose concentration of the STZ-induced diabetic rats, showing the beneficial action of red-mold-fermented products in rats with insulin resistance (**Table 2**). The OGTT was a screening method for acute antihyperglycemic activity, because the results give the overall effect of the tested sample on the handling of elevated blood glucose of the organism (*33*). We applied the glucose challenge test to the STZ-induced rats, and after 120 min of receiving oral treatment with red-mold-fermented products, a significant lowering of plasma glucose was observed in the STZ-induced rats (**Figure 3**), especially red mold dioscorea. This suggests the presence of compounds of red-mold-fermented products with different effects on glucose absorption and/or metabolism.

Potassium and calcium are the most important electrolytes in humans (*34*, *35*). The serum glucose concentration and total carbon dioxide content correlated significantly with the presenting serum potassium concentration, and the most common cause of hyperkalemia (potassium overload) is kidney disease (*36*). The mean serum sodium in the STZ-induced diabetic rats was considerably lower than in the non-diabetic rats. Milanov et al. ascribed this drop in serum sodium to dieresis, which followed the diabetic state (*37*). The serum electrolyte changes were different. There was no consistent or marked decrease in the concentration of sodium and chloride in rats with diabetes insipidus, but there was the usual increase in the concentration of potassium (*38*). In comparison to the non-diabetic rats in our experiment, pioglitazone-, RMR-, and RMD-treated groups, the rats in the vehicle-treated STZ-induced group had lower Na and Cl levels and a significantly higher K level (**Table 5**). Levels of serum CRE and BUN are generally considered as markers of the renal function. The STZ-induced diabetic rats showed high urea glucose and protein levels compared to the non-diabetic rats (**Table 3**). After 8 weeks of treatment with red-mold-fermented products, attenuated albuminuria and the ameliorated loss of renal function were present in the STZ-induced diabetic rats. Diabetic nephropathy in human diabetes usually occurs in experimental diabetes. Renal hypertrophy is an important early manifestation of both experimental and human diabetes, although the metabolic events responsible for its development are not yet completely understood. The index of renal hypertrophy by the kidney weight/body weight ratio (**Table 3**) was increased in all of the STZ-induced diabetic rats, although the increases tended to be less in the rats treated with red-mold-fermented products.

Our present study shows that red-mold-fermented products attenuated the development of diabetes and alleviated hyperglycemia and the resistance of the STZ-induced diabetic rats. In comparison to anti-diabetic drugs, red-mold-fermented products have the advantage of being a common food supplement. Using dioscorea as a fermented substrate is considered as having a more beneficial effect on diabetic development. In conclusion, our results suggest that red-mold-fermented products may have beneficial effects on reducing plasma glucose and improving insulin resistance, which may partly explain the reduction of the development of diabetes by treatment with red-mold-fermented products.

LITERATURE CITED

(1) Meyer, H. G. Die wirkung von stickstoff und phosphat auf die pigmentbildung bei *Monascus purpureus* went DSM 1379. Diplomarbeit, Fachrichtung Microbiologie, Fachbereich Biologie, Universitat Des Saariandes, Saarbrucken, Germany, 1990.

(2) Heber, D.; Yip, I.; Ashley, J. M.; Elashoff, D. A.; Elashoff, R. M.; Go, V. L. Cholesterol-lowering effects of proprietary Chinese red-yeast-rice dietary supplement. *Am. J. Clin. Nutr*. **1999**, *69*, 231–236.

(3) Endo, A. Monacolin K, a new hypocholesterolemic agent produced by a *Monascus* species. *J. Antibiot*. **1979**, *32*, 852–854.

(4) Juslova, P.; Martinkova, L.; Kren, V. Secondary metabolites of the fungus *Monascus*: A review. *J. Ind. Microbiol*. **1996**, *16*, 163–170.

(5) Ma, J.; Li, Y.; Ye, Q.; Li, J.; Hua, Y.; Ju, D.; Zhang, D.; Cooper, R.; Chang, M. Constituents of red yeast rice, a traditional Chinese food and medicine. *J. Agric. Food Chem*. **2000**, *48*, 5220–5225.

(6) Ahn, J.; Jung, J.; Hyung, W.; Haam, S.; Shin, C. Enhancement of *Monascus* pigment production by the culture of *Monascus* sp. J101 at low temperature. *Biotechnol. Prog*. **2006**, *22*, 338–340.

(7) Shepherd, J.; Cobbe, S. M.; Ford, I. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention Study Group. *New Engl. J. Med*. **1995**, *333*, 1301–1307.

(8) Vaughan, C. J.; Gotto, A. M.; Basson, C. T. The evolving role of statins in the management of atherosclerosis. *J. Am. Coll. Cardiol*. **2000**, *35*, 1–10.

(9) Sowers, J. R.; Epstein, M.; Frohlich, E. D. Diabetes, hypertension, and cardiovascular disease: An update. *Hypertension* **2001**, *37*, 1053–1059.

(10) Akihisa, T.; Tokuda, H.; Ukiya, M.; Kiyota, A.; Yasukawa, K.; Sakamoto, N.; Kimura, Y.; Suzuki, T.; Takayasu, J.; Nishino, H. Anti-tumor-initiating effects of monascin, an azaphilonoid pigment from the extract of *Monascus pilosus* fermented rice (red-mold rice). *Chem. Biodiversity* **2005**, *2*, 1305–1307.

(11) Su, N. W.; Lin, Y. L.; Lee, M. H.; Ho, C. Y. Ankaflavin from *Monascus*-fermented red rice exhibits selective cytotoxic effect and induces cell death on HepG2 cells. *J. Agric. Food Chem*. **2005**, *53*, 1949–1954.

(12) Ho, B. Y.; Pan, T. M. The *Monascus* metabolite monacolin K reduces tumor progression and metastasis of Lewis lung carcinoma cells. *J. Agric. Food Chem*. **2009**, *57*, 8258–8265.

(13) Okada, Y.; Taniguchi, H.; Baba, S. High concentration of GABA in the pancreatic islets with special emphasis on β cells. In *Problems in GABA Research from Brain to Bacteria*; Okada, Y., Robert, E., Eds.; Excerpta Medica: Amsterdam, The Netherlands, 1982; pp 379−386.

(14) Xu, E.; Kumar, M.; Zhang, Y.; Ju, W.; Obata, T.; Zhang, N.; Liu, S.; Wendt, A.; Deng, S.; Ebina, Y.; Wheeler, M. B.; Braun, M.; Wang, Q. Intra-islet insulin suppresses glucagon release via GABA-$GABA_A$ receptor system. *Cell Metab*. **2006**, *3*, 47–58.

(15) Reetz, A.; Solimena, M.; Matteoli, M.; Folli, F.; Takei, K.; De Camilli, P. GABA and pancreatic β-cells: Colocalization of glutamic acid decarboxylase (GAD) and GABA with synaptic-like microvesicles suggests their role in GABA storage and secretion. *EMBO J*. **1991**, *10*, 1275–1284.

(16) Averna, M.; Verde, A. L. Statins and metabolic syndrome. *Int. Congr. Ser*. **2003**, *1253*, 243–246.

(17) Sowers, J. R. Effects of statins on the vasculature: Implications for aggressive lipid management in the cardiovascular metabolic syndrome. *Am. J. Cardiol*. **2003**, *91*, 14–22.

(18) Li, S. Z. *Pen Ts'ao Kang Mu* (*Compendium of Materia Medica, Systematic Pharmacopoeia*); People's Health: Beijing, China, 1596.

(19) Yeh, P. H.; Chiang, W.; Chiang, M. T. Effects of dehulled adlay on plasma glucose and lipid concentrations in streptozotocin-induced diabetic rats fed a diet enriched in cholesterol. *Int. J. Vitam. Nutr. Res*. **2006**, *76*, 299–305.

(20) Hsu, J. H.; Wu, Y. C.; Liu, I. M.; Cheng, J. T. Dioscorea as the principal herb of Die-Huang-Wan, a widely used herbal mixture in China, for improvement of insulin resistance in fructose-rich chow-fed rats. *J. Ethnopharmacol*. **2007**, *112*, 577–584.

(21) Chiang, W. C.; Cheng, C. Y.; Chiang, M. T.; Chung, K. Effects of dehulled adlay on the culture count of some microbiota and their metabolism in the gastrointestinal tract of rats. *J. Agric. Food Chem*. **2000**, *48*, 829–832.

(22) Otsuka, H.; Hirai, Y.; Nagao, T.; Yamasaki, K. Anti-inflammatory activity of benzoxazinoids from roots of *Coix lachryma-jobi* var. ma-yuen. *J. Nat. Prod*. **1988**, *51*, 74–79.

(23) Su, Y. C.; Wang, J. J.; Lin, T. T.; Pan, T. M. Production of the secondary metabolites γ-aminobutyric acid and monacolin K by *Monascus*. *J. Ind. Microbiol. Biotechnol*. **2003**, *30*, 41–46.

(24) Lee, C. L.; Wang, J. J.; Pan, T. M. Synchronous analysis method for detection of citrinin and the lactone and acid forms of monacolin K in red mold rice. *J. AOAC Int*. **2006**, *89*, 669–677.
(25) Herbert, P.; Barros, P.; Ratola, N; Alves, A. HPLC determination of amino acids in musts and port wine using OPA/FMOC derivatives. *J. Food Sci*. **2000**, *65*, 1130–1133.
(26) Gniuli, D.; Libera, L. D.; Caristo, M. E.; Calvani, R.; Castagneto, M.; Mingrone, G. High saturated-fat diet induces apoptosis in rat enterocytes and blunts GIP and insulin-secretive response to oral glucose load. *Int. J. Obes*. **2008**, *32*, 871–874.
(27) Jalal, R.; Bagheri, S. M.; Moghimi, A.; Rasuli, M. B. Hypoglycemic effect of aqueous shallot and garlic extracts in rats with fructose-induced insulin resistance. *J. Clin. Biochem. Nutr*. **2007**, *41*, 218–223.
(28) Matthews, D. R.; Hosker, J. P.; Rudenski, A. S.; Naylor, B. A.; Traecher, D. F.; Turnre, R. C. Homeostasis model assessment: Insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **1985**, *28*, 412–419.
(29) Su, C. F.; Liu, I. M.; Cheng, J. T. Improvement of insulin resistance by Hon-Chi in fructose-rich chow-fed rats. *Food Chem*. **2007**, *104*, 45–52.
(30) Younis, N.; Soran, H.; Farook, S. The prevention of type 2 diabetes mellitus: recent advances. *Q. J. Med*. **2004**, *97*, 451–455.
(31) Chen, W. P.; Ho, B. Y.; Lee, C. L.; Lee, C. H.; Pan, T. M. Red mold rice prevents the development of obesity, dyslipidemia and hyperinsulinemia induced by high-fat diet. *Int. J. Obes*. **2008**, *32*, 1694–1704.
(32) Reaven, G. M. Role of insulin resistance in human disease. *Diabetes* **1988**, *37*, 1595–1607.
(33) Verspohl, E. J. Recommended testing in diabetes research. *Planta Med*. **2002**, *68*, 581–590.
(34) Guerra, S. M. O.; Kitabchi, A. E. Comparison of the effectiveness of various routes of insulin injection: Insulin levels and glucose response in normal subjects. *J. Clin. Endocrinol. Metab*. **1976**, *42*, 869–874.
(35) Schade, D. S.; Eaton, R. P. Dose response to insulin in man: Differential effects on glucose and ketone body regulation. *J. Clin. Endocrinol. Metab*. **1977**, *44*, 1038–1053.
(36) Antonios, H.; Tzamaloukas, M. D.; Pratap, S.; Avasthi, M. D. Serum potassium concentration in hyperglycemia of diabetes mellitus with long-term dialysis. *West J. Med*. **1987**, *146*, 571–575.
(37) Milanov, S.; Marinov, C.; Dimitrov, D. Changes of potassium and sodium metabolism in experimental diabetes. *Nauchni. Tr. Vissh. Med. Inst., Sofia* **1968**, *47*, 51–55.
(38) Winter, C. A.; Gross, E. G.; Ingram, W. R. Serum sodium, potassium and chloride after suprarenalectomy in cats with diabetes insipidus. *J. Exp. Med*. **1938**, *67*, 251–265.

Received for review March 29, 2010. Revised manuscript received May 31, 2010. Accepted June 8, 2010.