JOURNAL OF FOOD AND DRUG ANALYSIS 23 (2015) 719–728

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jfda-online.com

Original Article

Effects of red mold dioscorea with pioglitazone, a potentially functional food, in the treatment of diabetes

Chien-Li Chen, Tzu-Ming Pan *

Department of Biochemical Science and Technology, College of Life Science, National Taiwan University, Taipei, Taiwan

ARTICLE INFO

Article history:
Received 28 November 2014
Received in revised form
16 April 2015
Accepted 5 May 2015
Available online 21 July 2015

Keywords:
diabetes
insulin resistance
Monascus purpureus NTU 568
red mold dioscorea
rhabdomyolysis

ABSTRACT

The prevalence of type 2 diabetes mellitus is increasing rapidly, and its treatment with pioglitazone is likely to induce rhabdomyolysis. We aimed to determine the effect of cotreatment with pioglitazone and red mold dioscorea (RMD) produced by *Monascus purpureus* NTU 568 on pancreas function in streptozotocin (STZ)-induced diabetic rats. In diabetic rats fed RMD, RMD with pioglitazone, and pioglitazone alone, insulin concentrations increased significantly by 18.6–40.4%, 64.0–100.0%, and 52.8%, respectively, compared with that in the diabetic group ($p < 0.05$). Oral glucose tolerance was impaired in the STZ-induced diabetic group within 4 weeks, however, oral glucose tolerance in rats treated with RMD or RMD with pioglitazone improved after 4 weeks, 6 weeks, and 8 weeks. Findings from this study might lend support to the use of RMD as a novel functional food for the prevention of diabetes.

1. Introduction

The prevalence of type 2 diabetes mellitus (DM2) is increasing rapidly in most parts of the world [1], which will result in an increase in the complications associated with this disease. Currently, this condition is often treated with pioglitazone. In individuals with DM2, insulin sensitivity is often less than half of that of average healthy individuals with normal glucose tolerance. Insulin resistance (IR) is the major finding for several metabolic disorders, including metabolic syndrome, DM2, dyslipidemia, hyperglycemia, and hypertension, and is an independent predictor of cardiovascular disease [2,3], although responsiveness to insulin may vary considerably between different adipose depots. The resulting elevated circulating free fatty acid levels disrupt the glucose–fatty acid (Randle) cycle, aggravating IR in the muscle and liver, and resulting in insulin-induced suppression of hepatic glycogenolysis. IR is indeed a pathogenetic element that plays a key role in the development of metabolic and hemodynamic alterations, and is responsible, in turn, for the onset of the so-called cardiometabolic syndrome [4]. Given tissue

* *Corresponding author*. Department of Biochemical Science and Technology, College of Life Science, National Taiwan University, Number 1, Section 4, Roosevelt Road, Taipei 10617, Taiwan.
E-mail address: tmpan@ntu.edu.tw (T.-M. Pan).

http://dx.doi.org/10.1016/j.jfda.2015.04.013

differences in insulin dependence and sensitivity, manifestations of IR syndrome are likely to reflect the composite effects of excess insulin and variable resistance to its actions [5,6].

The major site of impaired insulin-stimulated glucose utilization is skeletal muscle, which in DM2 shows reductions in glucose uptake, glycogenesis, and glucose oxidation [3,7]. According to the Naranjo probability scale, it is likely that pioglitazone will induce rhabdomyolysis—the rapid breakdown of muscle cells with the release of intracellular contents into the circulation [8]. This conclusion was based on the exclusion of potential medical causes, such as hypothyroidism, infection, muscle trauma, alcoholism, patient's drug exposure, and observed resolution of signs and symptoms when pioglitazone was withdrawn [9]. Similarly, statins, which form the most widely prescribed class of cholesterol-lowering drugs [10], may also lead to rhabdomyolysis via a variety of mechanisms [11]. Statins inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, thereby decreasing the conversion of HMG-CoA to mevalonate, the rate-limiting step of cholesterol biosynthesis [12]. Improved treatment of hyperglycemia, and other risk factors associated with DM2 and metabolic syndrome is thus required, because this would make it possible to markedly lower the risk of both micro- and macrovascular complications [13].

Monascus species of fungus have been used in preparing traditional foods in Eastern Asia for several centuries. In our previous studies, we showed that *Monascus*-fermented rice, known as red mold rice (RMR), demonstrates antioxidative effects [14] and is useful in the treatment of Alzheimer's disease [15]. Moreover, dioscorea, a traditional Chinese herb, is regarded as a functional food because it contains many beneficial ingredients that help in the prevention of various diseases [16]. Dioscorea is an ideal substrate for *Monascus* fermentation. It has been reported that red mold dioscorea (RMD) is a stronger HMG-CoA reductase inhibitor with a higher hypocholesterolemic activity than RMR [17]. Furthermore, we previously found that RMD exerts a greater antidiabetic effect than traditional RMR in streptozotocin (STZ)-induced rats [18].

In the present study, we used STZ-induced rats to investigate the effect of oral administration of a small amount of RMD or RMD along with pioglitazone on the liver somatic index, kidney index, and muscle index. Plasma electrolytes were also examined to investigate the safety of the combination of *Monascus*-fermented products and pioglitazone, and the risk of rhabdomyolysis.

2. Materials and methods

2.1. Preparation of RMD

Monascus purpureus NTU 568 was isolated in our laboratory. The strain culture was maintained on Potato Dextrose Agar (PDA) slant at 10°C and transferred monthly. The dioscorea root (*Dioscorea batatas* Dence) purchased from a local supermarket in Taiwan was used to produce RMD using the method of solid-state culture [14]. Briefly, a 500-g substrate was soaked in distilled water for 8 hours. After that, excess water was removed with a sieve. The substrate was autoclaved for 20 minutes at 121°C in a "koji-dish" (the koji-dish was made of wood with the dimensions of 30 cm × 20 cm × 5 cm). After having been cooled, the substrate was inoculated with 5% (v/w) spore suspension (1×10^5 spores/mL) of *M. purpureus* NTU 568. The inoculated substrate was cultivated at 30°C for 10 days. During the culturing stage, 100 mL of water was added daily to the substrate from the 2nd day to the 5th day. At the end of cultivation, the crushed and dried product with the mold was used for the experiments [19].

2.2. Animals and diets

Male Sprague-Dawley rats, weighing 140–160 g, aged 5–6 weeks, were used for this experiment. The experiments were carried out in a qualified animal breeding room in the Animal Center at our institute (protocol complied with guidelines described in the "Animal Protection Law," amended on June 29, 2011, Hua-Zong-(1)-Yi-Tzi-10000136211, Council of Agriculture, Executive Yuan, Taiwan, R.O.C.). Diabetes was induced by injecting the rats intraperitoneally with 65 mg/kg STZ (Sigma Chemical Co., St Louis, MO, USA) in 0.1M acetate buffer and 230 mg/kg nicotinamide after fasting for 12–24 hours. Rats with a plasma glucose concentration of ≥200 mg/dL were considered as diabetic animals and used in this study. The animals were randomly divided into nine groups and each group contained nine animals.

2.3. Determination of monascin and ankaflavin concentrations

The RMD powder (1 g) was extracted with 10 mL of methanol at 60°C for 30 minutes. The extract was further filtered with a 0.45-μm filter and analyzed using high-performance liquid chromatography. High-performance liquid chromatography analyses were performed on a LC-2000 series (Jasco, Tokyo, Japan) apparatus with a PU-2089 plus pump and an MD-2010 plus diode array detector, equipped with a LUNA C_{18} column (250 × 4.6 mm inner diameter, 5 μm particle size; Phenomenex, Torrance, CA, USA). Wavelength of the diode array detector was set at 238 nm. The analytical method was based on our previous study [14].

2.4. Dose and grouping

The dose of RMD powder and pioglitazone was calculated in accordance with Boyd's formula of body surface area, as recommended by the Food and Drug Administration [20]. The recommended and used daily dietary dose of commercial *Monascus*-fermented product is usually 1.0–2.0 g for an adult [21,22]. The use of 15 mg of monascin as the Department of Health, Executive Yuan, R.O.C. (Taiwan) maximum dosage of an adult per day to calculate the rat dose has been proved to exhibit a hypolipidemic effect [23]. Pioglitazone used as a positive hypoglycemic (antihyperglycemic, antidiabetic) drug also has a recommended dose of 30 mg/d for an adult. Therefore, RMD and pioglitazone are used as the reference dose for an adult with a weight of 65 kg and a height of 170 cm. These dosages were used as a frame of reference for the conversion of the dose into a type 2 diabetes animal model. All

test samples were respectively suspended in 1 mL of water and orally administered to the rats using a stomach tube for 8 weeks.

2.5. Experimental groups and treatments

The animals were randomly divided into eight treatment groups, and each group contained nine animals. The effects of different doses of RMD on diabetic development were evaluated in STZ-induced diabetic rats receiving an oral administration of RMD. Age-matched normal rats served as normal control (C). Diabetic rats were divided into eight groups: Group 1—diabetic control (DC); Group 2 received a onefold dose of RMD [176 mg/kg body weight (bw)/d including 1.2 mg monascin and 0.6 mg ankaflavin] (D1R); Group 3 received a twofold dose of RMD (352 mg/kg bw/d including 2.4 mg monascin and 1.2 mg ankaflavin) (D2R); Group 4 received a fivefold dose of RMD (880 mg/kg bw/d including 6.0 mg monascin and 3.0 mg ankaflavin) (D5R); Group 5 received pioglitazone 2.6 mg/kg (DM); Group 6 received one dose of RMD (176 mg/kg bw/d including 1.2 mg monascin and 0.6 mg ankaflavin) and pioglitazone 2.6 mg/kg (D1RM); Group 7 received a twofold dose of RMD (352 mg/kg bw/d including 2.4 mg monascin and 1.2 mg ankaflavin) and pioglitazone 2.6 mg/kg (D2RM); and Group 8 received a fivefold dose of RMD (880 mg/kg bw/d including 6.0 mg monascin and 3.0 mg ankaflavin) and pioglitazone 2.6 mg/kg (D5RM). Rats were anesthetized and sacrificed at the end of the 8-week treatment.

2.6. Oral glucose tolerance test

The oral glucose tolerance test (OGTT) was performed every 2 weeks. The experiment was performed on animals after fasting for 12 hours (free access to water). Animals were given glucose (2 g/kg bw) with an oral cannula [24]. Blood samples were collected from the tail vein at 0 minute, 30 minutes, 60 minutes, 90 minutes, 120 minutes, 150 minutes, and 180 minutes after glucose administration. Blood glucose, lipid, urea, and plasma insulin levels were measured at the end of 8 weeks of RMD treatment. Blood glucose was determined immediately using the glucose oxidase method, using an analyzer [25].

2.7. Serum lipid analysis

Serum total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) levels were measured in triplicate using commercial enzymatic kits. These kits were as follows: the TC assay kit (CH 200; Randox Laboratories Ltd, Antrim, UK), the TG assay kit (TR-210; Randox Laboratories Ltd), and the HDL-C assay kit (CH-203; Randox Laboratories Ltd). Serum low-density lipoprotein cholesterol (LDL-C) levels were estimated using the following equation [26]: LDL-C (mg/dL) = TC − TG/5 − HDL-C.

2.8. Plasma liver, kidney, electrolyte, and creatine phosphokinase analyses

Plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, blood urea nitrogen (BUN), creatinine, sodium, potassium, chloride, calcium, phosphorous, and creatine phosphokinase (CPK) levels were measured in triplicate using an automatic biochemical analyzer (Beckman-700; Beckman, Fullerton, CA, USA).

2.9. Statistical analysis

Data are expressed as the mean ± standard deviation. Statistical significance of the biochemical effects was determined by one-way analysis of variance using the general linear model procedure of SPSS software (SPSS Institute, Inc., Chicago, IL, USA), followed by analysis of variance using the Duncan's test. Differences with $p < 0.05$ were considered statistically significant.

3. Results

3.1. Changes in body weight and daily dietary intake of STZ-induced rats

We investigated the effect of RMD on the development of diabetes and body weight of the same animal model over an extended period (8 weeks). In this experiment, pioglitazone was used to prevent the progression of diabetes. Body weight of the vehicle-treated STZ-induced rats was lower than that in the other groups (Fig. 1A). After 8 weeks of being fed RMD, the daily food and water intake of the vehicle-treated STZ-induced rats was higher than that of the other groups (Fig. 1B and 1C). In addition, the exterior appearance and health of all experimental animals presented as normal.

3.2. Effect of RMD on the blood glucose level during OGTT

OGTT measures the body's ability to use glucose, which is the main source of energy, and can be used to diagnose prediabetes and diabetes. It is a screening test for diabetes that involves testing an individual's plasma glucose level after drinking a solution containing 2 g/kg bw of glucose. Individuals with a plasma glucose level of <200 mg/dL but ≥140 mg/dL are diagnosed as having impaired glucose tolerance or prediabetes. Fig. 2 depicts the hypoglycemic effect of a single oral administration of variable amounts of RMD on the OGTT results of STZ-induced diabetic rats. OGTT results indicated impaired glucose tolerance in the STZ-induced diabetic group within 4 weeks. The blood glucose concentrations in the groups treated with RMD or RMD with pioglitazone were improved after 4 weeks, 6 weeks, and 8 weeks (Fig. 2B–D). In the 8th week, we measured the blood glucose levels after 120 minutes. The blood glucose level in the DC group, which was up to 452 mg/dL, was significantly higher than that in the C group (120 mg/dL). After feeding with RMD, RMD–pioglitazone, or pioglitazone, blood glucose concentrations were significantly lowered by 8.0–13.1%, 14.3–19.9%, and 9.9%, respectively ($p < 0.05$; Fig. 2D). Rats treated with oral administration of RMD for 8 weeks showed enhanced oral glucose tolerance and blood glucose regulation than those receiving pioglitazone.

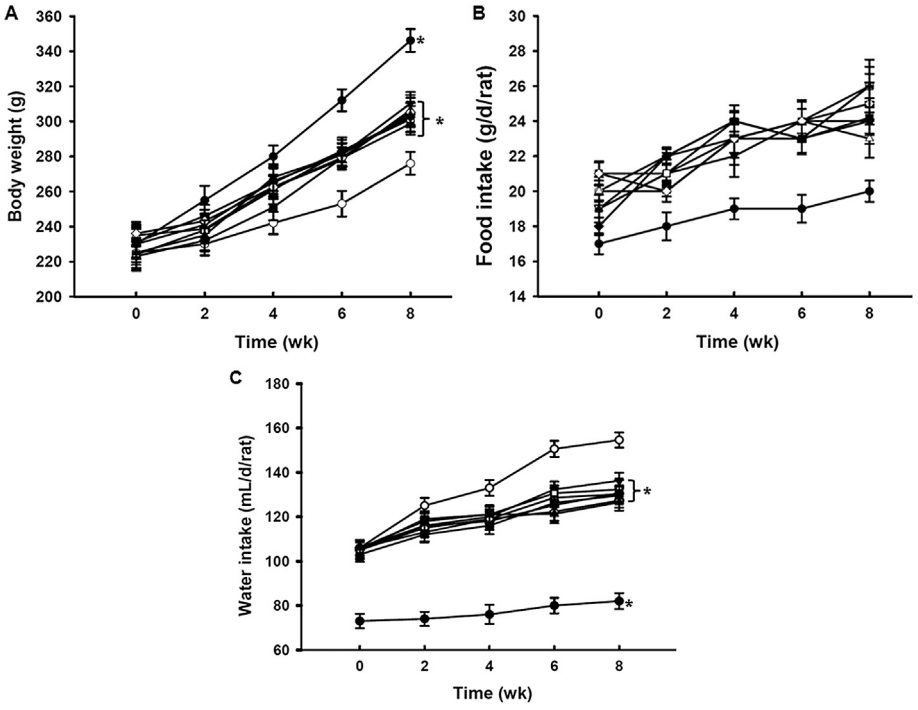

Fig. 1 – Effect of single oral administration of RMD on (A) body weight, (B) food intake, and (C) water intake in experimental STZ-induced diabetic rats. Sprague-Dawley rats fed a normal diet without the administration of RMD were used as control group (the C group; ●). The diabetic rats were fed a normal diet without the administration of RMD (the DC group; ○). The other rats with diabetes were administered a onefold dose of RMD [the D1R group; 176 mg/kg body weight (bw)/d including 1.2 mg monascin and 0.6 mg ankaflavin; ▼], a twofold dose of RMD (the D2R group; 352 mg/kg bw/d including 2.4 mg of monascin and 1.2 mg ankaflavin; △), and a fivefold dose of RMD (the D5R group; 880 mg/kg bw/d including 6.0 mg of monascin and 3.0 mg ankaflavin; ■). The DM group (□), a positive control group, was orally given pioglitazone 2.6 mg/kg bw/d. D1RM (◆), D2RM (◇), and D5RM (▲) were fed the normal diet and orally given pioglitazone (2.6 mg/kg bw/d) with a one-, two-, and fivefold dose of RMD, respectively. Each value is expressed as mean ± SD ($n = 9$). * Significantly different ($p < 0.05$) versus the DC group. bw = body weight; C = Sprague–Dawley rats fed normal diet; DC = streptozotocin-induced diabetic rats fed normal diet; DM = streptozotocin-induced diabetic rats fed normal diet and administered with pioglitazone; D1R = streptozotocin-induced diabetic rats fed normal diet and administered with red mold dioscorea (1×, 176 mg/kg bw/d including 1.2 mg monascin and 0.6 mg ankaflavin); D2R = streptozotocin-induced diabetic rats fed normal diet and administered with red mold dioscorea (2×, 352 mg/kg bw/d including 2.4 mg of monascin and 1.2 mg ankaflavin); D5R = streptozotocin-induced diabetic rats fed normal diet and administered with red mold dioscorea (5×, 880 mg/kg bw/d including 6.0 mg of monascin and 3.0 mg ankaflavin); D1RM = streptozotocin-induced diabetic rats fed normal diet and administered with red mold dioscorea (1×, 176 mg/kg bw/d including 1.2 mg monascin and 0.6 mg ankaflavin) and pioglitazone 2.6 mg/kg; D2RM = streptozotocin-induced diabetic rats fed normal diet and administered with red mold dioscorea (2×, 352 mg/kg bw/d including 2.4 mg monascin and 1.2 mg ankaflavin) and pioglitazone 2.6 mg/kg; D5RM = streptozotocin-induced diabetic rats fed normal diet and administered with red mold dioscorea (5×, 880 mg/kg bw/d including 6.0 mg of monascin and 3.0 mg ankaflavin) and pioglitazone 2.6 mg/kg; RMD = red mold dioscorea; SD = standard deviation; STZ = streptozotocin.

3.3. *Effect of RMD on the levels of serum insulin and glycated hemoglobin of STZ-induced diabetic rats*

In diabetic rats fed RMD (1-, 2-, or 5-fold dose), RMD (1-, 2-, or 5-fold dose) with pioglitazone, and pioglitazone alone, insulin concentrations increased significantly by 18.6–40.4%, 64.0–100.0%, and 52.8%, respectively, compared with that in the diabetic group ($p < 0.05$; Table 1). The levels of fasting serum insulin in the vehicle-treated STZ-induced diabetic rats were lower than those in other groups receiving RMD or RMD–pioglitazone treatment.

The hypoglycemic effect of RMD on the blood glucose levels of the STZ-induced diabetic rats was particularly noticeable during the latter part of the experiment. The glycated hemoglobin test is an important blood test used to determine how well diabetes is being controlled. Glycated hemoglobin provides an average of blood sugar control over a 4–12-week period, and is used in conjunction with a home blood sugar monitoring device to guide adjustments to a diabetes medicine regime. In our study, diabetic rats showed elevated glycated hemoglobin levels (Table 1). Feeding these rats RMD or RMD with pioglitazone resulted in stabilization of

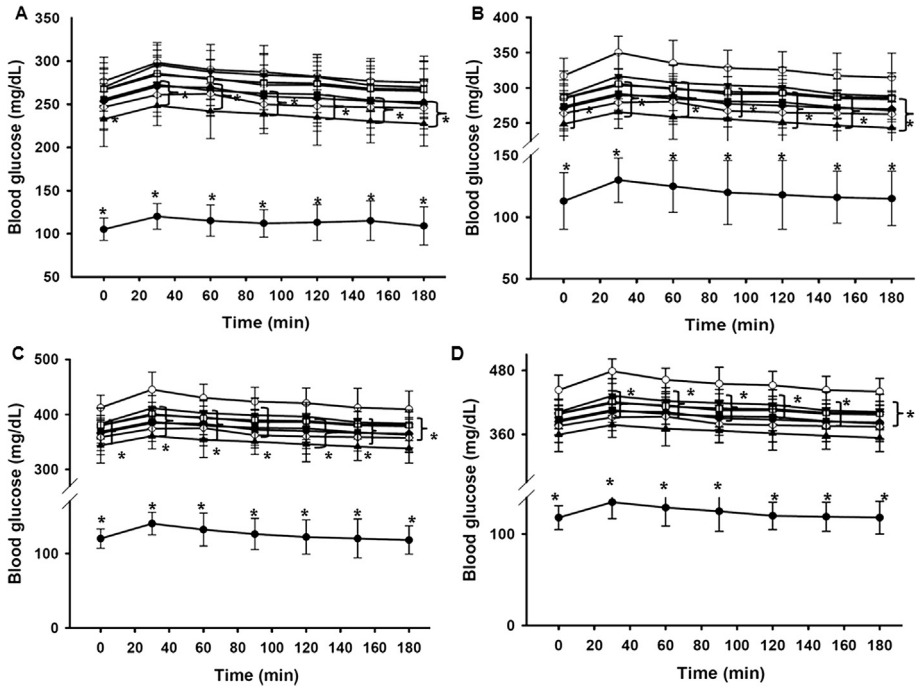

Fig. 2 – Effect of RMD on the blood glucose level of STZ-induced diabetic rats. During the experimental period, blood samples were collected to quantify the blood glucose levels at (A) 2 weeks, (B) 4 weeks, (C) 6 weeks, and (D) 8 weeks. The various symbols used in this figure represent the same groups as those shown in Fig. 1. Each value is expressed as mean ± SD ($n = 9$). *Significantly different ($p < 0.05$) versus the DC group. RMD = red mold dioscorea; SD = standard deviation; STZ = streptozotocin.

Table 1 – Effects of RMD powder on experimental STZ-induced diabetic rats, performance serum BUN, creatinine, glycated hemoglobin, insulin, and CPK.

Group	BUN	Creatinine	Glycated hemoglobin	Insulin	CPK
	(mg/dL)		(U/L)	(IU/mL)	(U/L)
C	26.9 ± 0.5*	0.66 ± 0.11	4.13 ± 0.07*	42.6 ± 1.3*	467.5 ± 12.4
DC	47.1 ± 0.8	0.51 ± 0.07	7.73 ± 0.71	16.1 ± 1.3	576.6 ± 21.3
D1R	39.1 ± 2.2*	0.55 ± 0.06	6.23 ± 0.49*	19.1 ± 1.1*	491.3 ± 24.5
D2R	37.1 ± 4.2*	0.51 ± 0.08	5.96 ± 0.43*	20.3 ± 1.8*	489.2 ± 10.0
D5R	26.9 ± 9.9*	0.55 ± 0.05	5.80 ± 0.59*	22.6 ± 1.6*	487.2 ± 11.5
DM	30.4 ± 1.5*	0.52 ± 0.05	6.18 ± 0.64*	24.6 ± 1.7*	479.7 ± 23.3
D1RM	34.1 ± 5.2*	0.58 ± 0.08	5.96 ± 0.31*	26.4 ± 2.5*	487.0 ± 12.2
D2RM	32.1 ± 2.2*	0.57 ± 0.09	5.93 ± 0.23*	28.9 ± 1.5*	490.5 ± 12.6
D5RM	31.1 ± 2.6*	0.58 ± 0.07	5.76 ± 0.13*	32.2 ± 2.4*	489.6 ± 12.7

Data are presented as mean ± SD ($n = 9$).
*Significantly different ($p < 0.05$) versus the DC group.
BUN = blood urea nitrogen; C = Sprague–Dawley rats fed normal diet; CPK = creatinine phosphokinase; DC = streptozotocin-induced diabetic rats fed normal diet; DM = streptozotocin-induced diabetic rats fed normal diet and administered with pioglitazone; D1R = streptozotocin-induced diabetic rats fed normal diet and administered with red mold dioscorea (1×, 176 mg/kg body weight(bw)/d including 1.2 mg monascin and 0.6 mg ankaflavin); D2R = streptozotocin-induced diabetic rats fed normal diet and administered with red mold dioscorea (2×, 352 mg/kg bw/d including 2.4 mg of monascin and 1.2 mg ankaflavin); D5R = streptozotocin-induced diabetic rats fed normal diet and administered with red mold dioscorea (5×, 880 mg/kg bw/d including 6.0 mg of monascin and 3.0 mg ankaflavin); D1RM = streptozotocin-induced diabetic rats fed normal diet and administered with red mold dioscorea (1×, 176 mg/kg bw/d including 1.2 mg monascin and 0.6 mg ankaflavin) and pioglitazone 2.6 mg/kg; D2RM = streptozotocin-induced diabetic rats fed normal diet and administered with red mold dioscorea (2×, 352 mg/kg bw/d including 2.4 mg monascin and 1.2 mg ankaflavin) and pioglitazone 2.6 mg/kg; D5RM = streptozotocin-induced diabetic rats fed normal diet and administered with red mold dioscorea (5×, 880 mg/kg bw/d including 6.0 mg of monascin and 3.0 mg ankaflavin) and pioglitazone 2.6 mg/kg; RMD = red mold dioscorea; SD = standard deviation; STZ = streptozotocin.

their diabetes, which was defined as glycated hemoglobin levels lower than that in the DC group.

3.4. Effect of RMD on blood lipid levels

TGs play an important role as transporters of lipids in the blood. However, excessive TG levels are associated with predisposition to cardiovascular disease. As shown in Table 2, the RMD-treated or RMD with pioglitazone cotreated groups of STZ-induced diabetic rats had lower TG levels (26.1–42.4%; $p < 0.05$) compared with the diabetic group. High TC levels may lead to occluded blood vessels and accelerated atherosclerosis. The RMD-treated and RMD with pioglitazone cotreated groups of diabetic rats displayed statistically significantly lower TC values than the DC groups (15.7–23.6%; $p < 0.05$).

HDL-C enables mobilization of cholesterol back to the liver for breakdown and has vascular protective effects, and a higher content of this lipoprotein may reduce the incidence of cardiovascular disease. Diabetic rats that had been treated with RMD displayed dose-dependently higher HDL-C levels than the rats of the DC group. High levels of LDL-C accumulate in the walls of blood vessels and contribute to atherosclerosis. Therefore, LDL-C is an important indicator for the prediction of coronary heart disease. No significant differences were observed in the LDL-C levels after concomitant administration of RMD or RMD with pioglitazone to STZ-induced diabetic rats. We also calculated the ratio of TC/HDL-C, which are indices of ischemic heart disease risk in humans. In diabetic rats fed RMD (1-, 2-, or 5-fold dose), RMD (1-, 2-, or 5-fold dose) with pioglitazone, and pioglitazone alone, the TC/HDL-C ratio decreased significantly compared with that in the diabetic group ($p < 0.05$; Table 2).

3.5. Safety assessment: plasma liver function tests

Because the majority of commercially available red mold fermented products contain citrinin, a hepato- and nephrotoxin, we assessed the safety of products by measuring the levels of AST and ALT in blood (plasma). As shown in Table 3, AST and ALT levels were found to increase significantly in the plasma of the vehicle-treated STZ-induced rats; these elevations were decreased virtually to control levels in all the RMD-treated groups. We also determined the concentrations of ALP, which is abundant in the kidneys, intestine, and liver, as well as total protein. Serum ALP activity is frequently requested in clinical routine, mostly to estimate skeletal and hepatobiliary status. The RMD-treated or RMD with pioglitazone cotreated groups of STZ-induced diabetic rats had lower ALP levels ($p < 0.05$) than the diabetic group. This finding suggests that RMD or RMD with pioglitazone could improve liver function by decreasing AST, ALT and ALP enzymes activities in STZ-induced diabetic rats.

3.6. Safety assessment: examination of renal function, electrolyte balance, and CPK concentrations in STZ-induced diabetic rats

BUN is a protein metabolite; its high concentrations indicate impaired renal excretion. The first clinical manifestations of diabetes involve increased proteinuria, followed by gradual increases in serum urea nitrogen and creatinine, and finally uremia. BUN levels in the STZ-induced diabetic rats in the RMD-treated groups decreased significantly after dosing (Table 1). In the normal group, BUN was not abnormal, whereas in the diabetes (DC) group, BUN concentrations were

Table 2 – Effects of RMD powder on the levels of serum TG, TC, HDL-C, and LDL-C in experimental STZ-induced diabetic rats.

Groups	TG	TC	HDL-C	LDL-C	TC/HDL-C
	(mg/dL)				
C	86.3 ± 3.1*	86.7 ± 2.1*	59.2 ± 6.8	10.7 ± 3.6	1.5 ± 0.05
DC	229.5 ± 6.3	94.5 ± 4.9	56.5 ± 1.3	9.0 ± 1.4	1.7 ± 0.21
D1R	169.7 ± 2.2*	77.7 ± 1.8*	60.8 ± 3.3	14.5 ± 4.1	1.3 ± 0.26*
D2R	156.7 ± 2.9*	76.7 ± 1.8*	63.8 ± 3.3	12.5 ± 4.1	1.2 ± 0.26*
D5R	136.1 ± 3.9*	72.2 ± 2.1*	62.6 ± 6.2	10.0 ± 1.8	1.2 ± 0.07*
DM	198.1 ± 6.4*	86.8 ± 2.4*	65.6 ± 3.1	20.6 ± 1.9	1.3 ± 0.27*
D1RM	168.5 ± 3.6*	79.7 ± 1.6*	63.7 ± 8.7	12.3 ± 2.5	1.3 ± 0.17*
D2RM	151.5 ± 4.3*	75.7 ± 2.3*	64.7 ± 8.7	10.3 ± 2.5	1.2 ± 0.17*
D5RM	132.1 ± 4.9*	74.8 ± 2.8*	66.5 ± 9.7	8.5 ± 3.5	1.1 ± 0.18*

Data are presented as mean ± SD ($n = 9$).
*Significantly different ($p < 0.05$) versus the DC group.
C = Sprague–Dawley rats fed normal diet; DC = streptozotocin-induced diabetic rats fed normal diet; DM = streptozotocin-induced diabetic rats fed normal diet and administered with pioglitazone; D1R = streptozotocin-induced diabetic rats fed normal diet and administered with red mold dioscorea [1×, 176 mg/kg body weight(bw)/d including 1.2 mg monascin and 0.6 mg ankaflavin]; D2R = streptozotocin-induced diabetic rats fed normal diet and administered with red mold dioscorea (2×, 352 mg/kg bw/d including 2.4 mg of monascin and 1.2 mg ankaflavin); D5R = streptozotocin-induced diabetic rats fed normal diet and administered with red mold dioscorea (5×, 880 mg/kg bw/d including 6.0 mg of monascin and 3.0 mg ankaflavin); D1RM = streptozotocin-induced diabetic rats fed normal diet and administered with red mold dioscorea (1×, 176 mg/kg bw/d including 1.2 mg monascin and 0.6 mg ankaflavin) and pioglitazone 2.6 mg/kg; D2RM = streptozotocin-induced diabetic rats fed normal diet and administered with red mold dioscorea (2×, 352 mg/kg bw/d including 2.4 mg monascin and 1.2 mg ankaflavin) and pioglitazone 2.6 mg/kg; D5RM = streptozotocin-induced diabetic rats fed normal diet and administered with red mold dioscorea (5×, 880 mg/kg bw/d including 6.0 mg of monascin and 3.0 mg ankaflavin) and pioglitazone 2.6 mg/kg; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; RMD = red mold dioscorea; SD = standard deviation; STZ = streptozotocin; TC = total cholesterol; TG = triglyceride.

significantly increased ($p < 0.05$), indicating poor renal function in diabetic rats. Regardless of whether the rats were treated with RMD or RMD and pioglitazone, treatment resulted in a significant reduction in the concentration of BUN; thus, RMD protects kidney function. During rhabdomyolysis, damaged muscle cells release CPK. CPK levels are often used in the diagnosis and monitoring of clinical myocardial infarction and muscle diseases. Table 1 shows that STZ-induced diabetic rats administered a one-, two-, or fivefold dose of RMD or RMD with pioglitazone did not exhibit any significant increase in their CPK levels.

Serum electrolytes were also compared between different rat groups (Table 4); the rats in the vehicle-treated STZ-induced group had lower sodium and chloride levels, and

Table 3 – Effects of RMD powder on the levels of plasma AST, ALT, ALP, and total proteins in experimental STZ-induced diabetic rats.

Groups	AST	ALT	ALP	Total protein
	(U/L)		(IU/L)	(g/dL)
C	127.5 ± 3.3*	72.7 ± 3.8*	132.1 ± 2.4*	7.6 ± 0.2
DC	457.5 ± 2.1	297.5 ± 2.1	144.3 ± 2.1	8.5 ± 0.4
D1R	185.0 ± 3.4*	121.3 ± 3.6*	135.8 ± 2.7*	7.9 ± 0.2
D2R	173.0 ± 1.4*	119.3 ± 3.6*	133.5 ± 2.8*	8.0 ± 0.3
D5R	152.6 ± 8.4*	108.3 ± 4.1*	131.5 ± 2.7*	7.8 ± 0.5
DM	184.0 ± 8.4*	120.6 ± 5.9*	134.8 ± 3.1*	7.6 ± 0.4
D1RM	169.3 ± 3.2*	113.8 ± 5.9*	132.3 ± 3.4*	8.2 ± 0.2
D2RM	164.3 ± 3.2*	103.8 ± 5.9*	132.1 ± 2.6*	7.8 ± 0.3
D5RM	142.6 ± 3.5*	91.2 ± 2.8*	131.6 ± 3.4*	7.5 ± 0.2

Data are presented as mean ± SD ($n = 9$).
*Significantly different ($p < 0.05$) versus the DC group.
ALP = alkaline phosphatase; ALT = alanine transaminase; AST = aspartate transaminase; C = Sprague–Dawley rats fed normal diet; DC = streptozotocin-induced diabetic rats fed normal diet; DM = streptozotocin-induced diabetic rats fed normal diet and administered with pioglitazone; D1R = streptozotocin-induced diabetic rats fed normal diet and administered with red mold dioscorea [1×, 176 mg/kg body weight (bw)/d including 1.2 mg monascin and 0.6 mg ankaflavin]; D2R = streptozotocin-induced diabetic rats fed normal diet and administered with red mold dioscorea (2×, 352 mg/kg (bw)/d including 2.4 mg of monascin and 1.2 mg ankaflavin); D5R = streptozotocin-induced diabetic rats fed normal diet and administered with red mold dioscorea (5×, 880 mg/kg (bw)/d including 6.0 mg of monascin and 3.0 mg ankaflavin); D1RM = streptozotocin-induced diabetic rats fed normal diet and administered with red mold dioscorea (1×, 176 mg/kg (bw)/d including 1.2 mg monascin and 0.6 mg ankaflavin) and pioglitazone 2.6 mg/kg; D2RM = streptozotocin-induced diabetic rats fed normal diet and administered with red mold dioscorea (2×, 352 mg/kg (bw)/d including 2.4 mg monascin and 1.2 mg ankaflavin) and pioglitazone 2.6 mg/kg; D5RM = streptozotocin-induced diabetic rats fed normal diet and administered with red mold dioscorea (5×, 880 mg/kg (bw)/d including 6.0 mg of monascin and 3.0 mg ankaflavin) and pioglitazone 2.6 mg/kg; RMD = red mold dioscorea; SD = standard deviation; STZ = streptozotocin.

Table 4 – Effects of RMD powder on the levels of serum sodium, potassium, chloride, calcium, and phosphorus in experimental STZ-induced diabetic rats.

Groups	Sodium	Potassium	Chloride	Calcium	Phosphorus
	(mEq/L)			(mg/dL)	
C	152.6 ± 0.2*	7.9 ± 0.5*	94.5 ± 2.1*	12.4 ± 0.7	14.3 ± 1.3
DC	142.4 ± 0.8	12.1 ± 1.6	83.2 ± 1.4	12.1 ± 0.4	14.2 ± 2.5
D1R	152.3 ± 0.7*	7.6 ± 0.5*	89.5 ± 1.5*	12.3 ± 0.3	14.1 ± 1.2
D2R	149.1 ± 1.1*	8.2 ± 0.5*	90.1 ± 1.3*	12.2 ± 0.5	14.5 ± 1.7
D5R	149.9 ± 1.2*	8.1 ± 0.4*	92.3 ± 1.4*	12.4 ± 0.2	14.7 ± 1.6
DM	151.1 ± 1.4*	8.6 ± 0.6*	91.9 ± 2.3*	12.6 ± 0.8	13.9 ± 2.3
D1RM	152.4 ± 0.9*	8.8 ± 0.4*	92.6 ± 1.2*	12.4 ± 0.5	14.6 ± 2.6
D2RM	150.5 ± 0.7*	8.6 ± 0.6*	92.5 ± 1.4*	12.5 ± 0.7	14.4 ± 3.6
D5RM	149.1 ± 1.1*	8.5 ± 0.8*	93.3 ± 1.3*	12.4 ± 0.6	14.5 ± 2.4

Data are presented as mean ± SD ($n = 9$).
*Significantly different ($p < 0.05$) versus the DC group.
C = Sprague-Dawley rats fed normal diet; DC = streptozotocin-induced diabetic rats fed normal diet; DM = streptozotocin-induced diabetic rats fed normal diet and administered with pioglitazone; D1R = streptozotocin-induced diabetic rats fed normal diet and administered with red mold dioscorea [1×, 176 mg/kg body weight (bw)/d including 1.2 mg monascin and 0.6 mg ankaflavin]; D2R = streptozotocin-induced diabetic rats fed normal diet and administered with red mold dioscorea (2×, 352 mg/kg (bw)/d including 2.4 mg of monascin and 1.2 mg ankaflavin); D5R = streptozotocin-induced diabetic rats fed normal diet and administered with red mold dioscorea (5×, 880 mg/kg (bw)/d including 6.0 mg of monascin and 3.0 mg ankaflavin); D1RM = streptozotocin-induced diabetic rats fed normal diet and administered with red mold dioscorea (1×, 176 mg/kg (bw)/d including 1.2 mg monascin and 0.6 mg ankaflavin) and pioglitazone 2.6 mg/kg; D2RM = streptozotocin-induced diabetic rats fed normal diet and administered with red mold dioscorea (2×, 352 mg/kg (bw)/d including 2.4 mg monascin and 1.2 mg ankaflavin) and pioglitazone 2.6 mg/kg; D5RM = streptozotocin-induced diabetic rats fed normal diet and administered with red mold dioscorea (5×, 880 mg/kg (bw)/d including 6.0 mg of monascin and 3.0 mg ankaflavin) and pioglitazone 2.6 mg/kg; RMD = red mold dioscorea; SD = standard deviation; STZ = streptozotocin.

significantly higher potassium levels than those in the RMD-treated groups. Calcium and phosphorus are essential minerals found in the bone, blood, and soft tissue of the body, and have a role in numerous body functions. Similar levels of serum calcium and phosphorus were noted among groups, indicating that administration of RMD or RMD with pioglitazone has no significant effect on electrolyte balance at any of the doses studied. Electrolyte levels in the RMD-treated groups and control group showed no significant difference; thus, it can be inferred that RMD can improve sodium, potassium, and chloride levels in the body.

4. Discussion

Our study shows that *Monascus* fermentation using a dioscorea substrate results in higher levels of monascin and ankaflavin [27]. *Monascus*-fermented products can be used as food-based adjuvant therapy for diabetic patients to ameliorate IR and/or impaired glucose metabolism [18]. In our previous study, we showed that RMR supplements significantly reduced serum insulin levels in high-fat-induced rats [28]. Our current study showed a significant decrease in blood glucose levels of STZ-induced diabetic rats fed RMD at Week 6 and Week 8 (Fig. 2C and 2D), providing direct evidence of the antihyperglycemic effects of RMD.

OGTT is a screening method used to assess acute antihyperglycemic activity [29]. We applied this glucose challenge test to STZ-induced rats, and by 120 minutes after receiving oral treatment with RMD, we observed a significant lowering of plasma glucose levels (Fig. 2). This suggests that compounds present in RMD have effects on glucose absorption and/or metabolism.

Levels of serum creatinine and BUN are generally considered as markers of renal function. The diabetic nephropathy observed in human diabetes usually also occurs in experimental diabetes models. BUN is a type of protein metabolite, and a high concentration of BUN indicates weak renal excretion. In particular, increased BUN levels would indicate renal dysfunction. However, administration of RMD or RMD along with pioglitazone improved the metabolic and physiological functions of the kidneys of rats in our study. The major components of RMD (e.g., monascin) may have antidiabetic effects in STZ-induced diabetic rats [30]. Glycated hemoglobin (HbA1c) was a useful indicator of mean blood glucose concentrations over the preceding 2–3 months. Epidemiological studies have reported that higher HbA1c values were strongly associated with microvascular diabetic complications, renal function, chronic kidney disease, and all-cause mortality in populations with and without diabetes [31–33].

In 2007, the Food and Drug Administration warned consumers to avoid cholesterol-lowering RMR supplements (RMR, RMR–policosanol complex, and Cholestrix) due to the possibility of developing myopathy and kidney dysfunction. Rhabdomyolysis is characterized by necrosis of skeletal muscle and the subsequent release of toxic intracellular components into the systemic circulation [34]. Approximately 26% of patients with rhabdomyolysis cannot be diagnosed on the basis of urine myoglobin concentrations because of the low sensitivity of this assay [35]. Elevated CPK levels, which are used in the diagnosis and monitoring of clinical myocardial infarction and muscle diseases, are also good diagnostic indicators of rhabdomyolysis. CPK levels increase as early as 2–12 hours after muscle injury, and blood CPK levels remain high for longer than blood myoglobin levels [36]. Moreover, abnormally high CPK values of up to 5000 U/L have been reported in cases of rhabdomyolysis [37]. However, we found that treatment of STZ-induced rats with RMD or RMD with pioglitazone did not significantly affect CPK values (Table 1).

Potassium and calcium are the most important electrolytes in the human body [38,39]. The serum glucose concentration and total carbon dioxide content correlate significantly with the serum potassium concentration, and the most common cause of hyperkalemia (potassium overload) is kidney disease [40]. Previously, it was reported that there was no consistent or marked decrease in the concentration of sodium and chloride in rats with diabetes insipidus, however, there was a typical increase in potassium levels [41]. In comparison with the nondiabetic rats and the RMD and RMD and pioglitazone groups, the rats in the vehicle-treated STZ-induced group had lower sodium and chloride levels and a significantly higher potassium level (Table 4). In particular, the mean serum sodium in the STZ-induced diabetic rats was considerably lower than that in the nondiabetic rats. Milanov et al [42] ascribed this drop in serum sodium to diuresis, which ensues from the diabetic state. In this study, we investigated a number of indicators of kidney function, including plasma electrolytes (i.e., sodium and potassium), BUN, and creatine. In particular, increased BUN levels would indicate renal dysfunction. However, administration of RMD or RMD along with pioglitazone improved the metabolic and physiological functions of the kidneys of rats in our study.

5. Conclusion

The present study showed that RMD attenuated the development of diabetes, and alleviated hyperglycemia and IR in STZ-induced diabetic rats. In comparison with antidiabetic drugs, RMD has the advantage of being a common food supplement. Findings from this study might lend support to the use of RMD as a novel functional food for the prevention of diabetes. However, we found that treatment with RMD improved the indices of diabetes in these rats. In addition, we did not find any evidence implicating RMD products in rhabdomyolysis. We showed that administration of five times the recommended dose of RMD did not cause any adverse effects.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

The authors would like to express their gratitude to the Department of Health, R.O.C. (DOH98-TD-F-113-098001) for supporting this research work and subsidiary.

REFERENCES

[1] Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27:1047–153.
[2] Reaven GM. Role of insulin resistance in human disease. Diabetes 1988;37:1595–607.
[3] Despres JP, Lamarche B, Mauriege P, Cantin B, Dagenais GR, Moorjani S, Lupien PJ. Hyperinsulinemia as an independent risk factor for ischemic heart disease. N Engl J Med 1996;334:952–7.
[4] Reaven G. The metabolic syndrome or the insulin resistance syndrome? Different names, different concepts, and different goals. Endocrinol Metab Clin North Am 2004;33:283–303.
[5] Sowers JR, Frohlich ED. Insulin and insulin resistance: impact on blood pressure and cardiovascular disease. Med Clin North Am 2004;88:63–82.
[6] Wilcox G. Insulin and insulin resistance. Clin Biochem Rev 2005;26:19–39.
[7] Ducimetiere P, Eschwege E, Papoz L, Richard JL, Claude JR, Rosselin G. Relationship of plasma insulin levels to the incidence of myocardial infarction and coronary heart disease mortality in a middle-aged population. Diabetology 1980;19:205–10.
[8] Naranjo CA, Busto U, Sellers EM, Sandor P, Ruiz I, Roberts EA, Janecek E, Domecq C, Greenblatt DJ. A method for estimating the probability of adverse drug reactions. Clin Pharmacol Ther 1981;30:239–45.
[9] Slim R, Salem BC, Zamy M, Biour M. Pioglitazone-induced acute rhabdomyolysis. Diabetes Care 2009;32:e84.
[10] Shepherd J, Cobbe SM, Ford I, Isles CG, Lorimer AR, MacFarlane PW, McKillop JH, Packard CJ. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. N Engl J Med 1995;333:1301–7.
[11] Tokinaga K, Oeda T, Suzuki Y, Matsushima Y. HMG-CoA reductase inhibitors (statins) might cause high elevations of creatine phosphokinase (CK) in patients with unnoticed hypothyroidism. Endocr J 2006;53:401–5.
[12] Mauro VF, MacDonald JL. Simvastatin: a review of its pharmacology and clinical use. DICP 1991;25:257–64.
[13] Gaede P, Vedel P, Larsen N, Jensen GV, Parving HH, Pedersen O. Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. N Engl J Med 2003;348:383–93.
[14] Lee CL, Wang JJ, Kuo SL, Pan TM. *Monascus* fermentation of dioscorea for increasing the production of cholesterol-lowering agent-monacolin K and anti-inflammation agent-monascin. Appl Microbiol Biotechnol 2006;72:1254–62.
[15] Lee CL, Kuo TF, Wang JJ, Pan TM. Red mold rice ameliorates impairment of memory and learning ability in intracerebroventricular amyloid beta-infused rat via repressing amyloid beta accumulation. J Neurosci Res 2007;85:3171–82.
[16] Chang WC, Yu YM, Wu CH, Tseng YH, Wu KY. Reduction of oxidative stress and atherosclerosis in hyperlipidemic rabbits by *Dioscorea rhizome*. Can J Physiol Pharmacol 2005;83:423–30.
[17] Lee CL, Hung HK, Wang JJ, Pan TM. Red mold dioscorea has greater hypolipidemic and antiatherosclerotic effect than traditional red mold rice and unfermented dioscorea in hamsters. J Agric Food Chem 2007;55:7162–9.
[18] Shi YC, Pan TM. Anti-diabetic effects of *Monascus purpureus* NTU 568 fermented products on streptozotocin-induced diabetic rats. J Agric Food Chem 2010;58:7634–40.
[19] Su YC, Wang JJ, Lin TT, Pan TM. Production of the secondary metabolites gamma-aminobutyric acid and monacolin K by *Monascus*. J Ind Microbiol Biotechnol 2003;30:41–6.
[20] Boyd E. The growth of the surface area of human body. Minneapolis: University of Minnesota Press; 1935.
[21] Heber D, Yip I, Ashley JM, Elashoff DA, Elashoff RM, Go VL. Cholesterol-lowering effects of a proprietary Chinese red-yeast-rice dietary supplement. Am J Clin Nutr 1999;69:231–6.
[22] Wang WH, Zhang H, Yu YL, Ge Z, Xue C, Zhang P. Intervention of xuezhikang on patients of acute coronary syndrome with different levels of blood lipids. Chin J Integr Tradit Western Med 2004;24:1073–6.
[23] Chen CL, Pan TM. Red mold dioscorea: a potentially safe traditional function food for the treatment of hyperlipidemia. Food Chem 2012;134:1074–80.
[24] Gniuli D, Libera LD, Caristo ME, 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;32:871–4.
[25] Jalal R, Bagheri SM, Moghimi A, Rasuli MB. Hypoglycemic effect of aqueous shallot and garlic extracts in rats with fructose induced insulin resistance. J Clin Biochem Nutr 2007;41:218–23.
[26] Usman HA. Hypocholesterolemic effect of *Lactobacillus gasseri* SBT0270 in rats fed a cholesterol-enriched diet. J Dairy Res 2001;68:617–24.
[27] Wu CL, Lee CL, Pan TM. Red mold dioscorea has greater anti-hypertensive effect than traditional red mold rice in spontaneously hypertensive rats. J Agric Food Chem 2009;57:5035–41.
[28] Chen WP, Ho BY, Lee CL, Lee CH, Pan TM. Red mold rice prevents the development of obesity, dyslipidemia and hyperinsulinemia induced by high-fat diet. Int J Obes 2008;32:1694–704.
[29] Verspohl EJ. Recommended testing in diabetes research. Planta Med 2002;68:581–90.
[30] Shi YC, Pan TM. Red mold, diabetes, and oxidative stress: a review. Appl Microbiol Biotechnol 2012;94:47–55.
[31] Plantinga LC, Crews DC, Coresh J, Miller ER, Saran R, Yee J, Hedgeman E, Pavkov M, Eberhardt MS, Williams DE, Powe NR. Prevalence of chronic kidney disease in US adults with undiagnosed diabetes or prediabetes. Clin J Am Soc Nephrol 2010;5:673–82.
[32] Whaley-Connell A, Pavey BS, McCullough PA, Saab G, Li S, McFarlane SI, Chen SC, Vassalotti JA, Collins AJ, Bakris G, Sowers JR. Dysglycemia predicts cardiovascular and kidney disease in the Kidney Early Evaluation Program. J Clin Hypertens (Greenwich) 2010;12:51–8.
[33] Sundström J, Risérus U, Byberg L, Zethelius B, Lithell H, Lind L. Clinical value of the metabolic syndrome for long term prediction of total and cardiovascular mortality: prospective, population based cohort study. BMJ 2006;332:78–82.
[34] Sauret JM, Marinides G, Wang GK. Rhabdomyolysis. Am Fam Physician 2002;65:907–12.
[35] Moore KP, Holt SG, Patel RP, Svistunenko DA, Zackert W, Goodier D, Reeder BJ, Clozel M, Anand R, Cooper CE, Morrow JD, Wilson MT, Darley-Usmar V, Roberts LJ. A causative role for redox cycling of myoglobin and its inhibition by alkalinization in the pathogenesis and treatment of rhabdomyolysis-induced renal failure. J Biol Chem 1998;273:31731–7.
[36] Brown CV, Rhee P, Chan L, Evans K, Demetriades D, Velmahos GC. Preventing renal failure in patients with rhabdomyolysis: do bicarbonate and mannitol make a difference? J Trauma 2004;56:1191–6.

[37] Zager RA. Rhabdomyolysis and myohemoglobinuric acute renal failure. Kidney Int 1996;49:314–26.
[38] Antonios H, Tzamaloukas MD, Pratap S, Avasthi MD. Serum potassium concentration in hyperglycemia of diabetes mellitus with long-term dialysis. West J Med 1987;146:571–5.
[39] Winter CA, Gross EG, Ingram WR. Serum sodium, potassium and chloride after suprarenalectomy in cats with diabetes insipidus. J Exp Med 1938;67:251–65.
[40] Sharma A, Hirulkar NB, Ranka P. Effect of hyperglycemia on electrolytes imbalance. Int J Pharm Biol Arch 2011;2:526–33.
[41] Tzamaloukas AH, Gardner KD. Plasma potassium changes in anuric hyperglycemia treated with insulin. Am J Med Sci 1984;287:27–30.
[42] Milanov S, Marinov C, Dimitrov D. Changes of potassium and sodium metabolism in experimental diabetes. Nauchni Tr Vissh Med Inst Sofiia 1968;47:51–5.