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Original Article

A randomized, double-blind clinical study to determine the effect of ANKASCIN 568 plus on blood glucose regulation

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ABSTRACT

Diabetes is the fourth major cause of death in Taiwan. High blood glucose can lead to macrovascular diseases, small vessel diseases (retinopathy, kidney disease), and neuropathy. This study aimed to investigate whether *Monascus*-fermented products (ANKASCIN 568 plus) can regulate blood glucose and blood lipids. This study enrolled 39 patients with a fasting blood glucose level between 100 mg/dL and 180 mg/dL, and a glycated hemoglobin (HbA1c) level of <9%. All patients were randomly divided into placebo ($n = 20$) and experimental ($n = 19$) groups. Each patient received two placebo capsules (maltodextrin) or ANKASCIN 568 plus capsules daily for 12 weeks. The patients were screened during follow-up 4 weeks after the administration of sample or placebo had been discontinued. Blood and urine samples were collected at the initial, 6th week, 12th week, and 16th week. The anthropometric indicators of blood pressure, fasting plasma glucose level, postprandial plasma glucose level, insulin level, insulin resistance, blood lipid changes, and liver, kidney, and thyroid function indices were measured. After 6 weeks, changes in fasting blood glucose, low-density lipoprotein cholesterol (LDL-C), and total cholesterol (TC) levels showed that ANKASCIN 568 plus had a more favorable effect than the placebo. Compared to baseline, a statistically significant decrease of 8.5%, 10.3%, and 7.5% was observed in fasting blood glucose, LDL-C and, TC levels, respectively ($p < 0.05$ for all pairs). Therefore, ANKASCIN 568 plus produced by *Monascus purpureus* NTU 568 fermentation may be a potentially useful agent for the regulation of blood glucose and blood lipids and for treatment of coronary artery diseases.

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1. Introduction

Diabetes mellitus (DM) is a common chronic disease and is associated with a high risk of major complications such as blindness, renal disease, foot ulcers, and cardiovascular disease, which account for ≥50% of all diabetes-related fatalities and lead to considerable disability [1]. The major site of impaired insulin-stimulated glucose utilization is the skeletal muscle; Type 2 DM (DM2) patients show reduced glucose uptake, glycogenesis, and glucose oxidation in the skeletal muscle [2,3].

Insulin-stimulated glucose uptake is impaired and suppression of lipolysis is decreased in the adipocytes of DM2 patients [4,5], although responsiveness to insulin might vary considerably between different adipose depots. The resulting elevated circulating free fatty acid levels disrupt the glucose–fatty acid (Randle) cycle, thereby aggravating insulin resistance (IR) in the muscle and liver cells and resulting in insulin-induced suppression of hepatic glycogenolysis. IR is a pathogenic condition that plays a key role in the development of metabolic and hemodynamic alterations and is thus responsible for the onset of the so-called cardiometabolic syndrome [6]. Given the differences among tissues with regard to insulin dependence and sensitivity, manifestations of IR syndrome are likely to reflect the composite effects of excess insulin and variable resistance to its actions [7,8]. Chronic physical conditions have been associated with an increased risk of depression in a range of cultural settings [9–12], and the risk has been shown to increase with the number of conditions [13].

At present, DM2 is often treated with pioglitazone. According to the Naranjo probability scale, pioglitazone might induce rhabdomyolysis, i.e., rapid breakdown of muscle cells leading to the release of intracellular contents into the circulation [14]. This conclusion was based on the exclusion of potential medical causes, such as hypothyroidism, infection, muscle trauma, alcoholism, patient drug exposure, and the observed resolution of signs and symptoms when pioglitazone was withdrawn [15]. Similarly, statins, which are the most widely prescribed class of cholesterol-lowering drugs [16], might induce rhabdomyolysis via various mechanisms [17].

Monascus fungal species 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), has antioxidative effects [18] and is useful for the treatment of Alzheimer's disease [19]. In our previous study, we investigated the effects of a combination of *Monascus*-fermented products and lovastatin on rhabdomyolysis in hyperlipidemic hamsters [20]. In addition, we also investigated *Monascus*-fermented products, a potentially functional food, with pioglitazone in the treatment of streptozotocin-induced diabetes rats [21]. *Monascus* species produce several kinds of pigments, which are functional secondary metabolites. These include the yellow pigments ankaflavin and monascin, the orange pigments monascorubrin and rubropunctanin, and the red pigments monascorubramine and rubropuctamine [22]. Monascin and ankaflavin have numerous biological effects such as inhibition of nonalcoholic fatty liver, amelioration of pancreatic damage and hyperglycemia in patients with diabetes, and antioxidant and antiinflammatory activities [23–25]. These compounds are peroxisome proliferator-activated receptor (PPAR)-γ agonists that subsequently initiate the transcription of downstream genes. Considering the reported health benefits of monascin and ankaflavin, which are the major constituents of *Monascus purpureus* NTU 568 fermented products, this clinical trial was conducted on the recommendation of the Food and Drug Administration, Taiwan, to evaluate the effects of oral administration of two capsules of ANKASCIN 568 plus (*M. purpureus* NTU 568 fermented product) daily on blood glucose and blood lipid regulation by evaluating the liver somatic index, kidney index, and muscle index.

2. Materials and methods

2.1. Materials

The study material consisted of ANKASCIN 568 plus product fermented by *M. purpureus* NTU 568, which was obtained from SunWay Biotech., Co., Ltd. (Taipei, Taiwan, R.O.C.). Two capsules (500 mg/capsule) of ANKASCIN 568 plus powder contained 6 mg of monascin and 3 mg ankaflavin. The same capsules containing maltodextrin were used as a placebo.

2.2. Patients

The study was conducted between September 2012 and November 2014 at Chung Shan Medical University, Taichung, Taiwan, after approval was received from the Institutional Review Board of the Taichung Chung Shan Medical University Hospital (Institutional Review Board proof document CHMUH No: CS12120). In total, 377 patients with diabetes in a series of stages were screened. In the first stage, 67 'subhealthy' patients with fasting glucose levels in the range of 100–125 mg/dL and glycated hemoglobin (HbA1c) 5.7–6.4% were included. Of these, 28 patients withdrew from the trial because of the development of some disease; thus, 39 patients completed this study. Written informed consent was obtained from all enrolled patients. The treatment and placebo groups included 19 patients and 20 patients, respectively.

During the trial, patients with hypertension were not allowed to use depressor drugs unless their blood pressure elevated suddenly. Patients with coronary heart disease were also not allowed to use analgesics unless they experienced symptoms of angina pectoris. All patients were on a low-cholesterol diet throughout the treatment period. Carotid ultrasound examinations and plasma biochemical assays were performed at the end of the treatment.

2.2.1. Inclusion and exclusion criteria

Inclusion criteria included the following: (A) age: 18–65 years; (B) fasting glucose range: 100–125 mg/dL; (C) HbA1C: 5.7–6.4%; (D) body mass index: 23–30 kg/m^2; and (E) administration of hypolipidemic, antihypertensive, or diabetes drugs stabilized for at least 3 months (if used). Exclusion criteria were as follows: (A) administration of antidiabetic drugs; (B) inconsistent or unstable administration of drugs that might interfere with

lipid or glucose metabolism; (C) chronic gastrointestinal diseases and administration of drugs for treatment of these diseases; (D) confirmation of thyroid, liver, renal, or muscular diseases; (E) known allergy or intolerance to a component of the test product; and (F) any medical or surgical condition that could lead to an inconsistent adherence to the study protocol.

2.3. Methods

2.3.1. Randomization, treatment, and follow-up

The patients were visited by investigators and informed about the rationale and main aims of the study. A written informed consent was obtained from the patients. Block randomization was used for treatment allocation. The patients were randomly assigned to groups. One group received ANKASCIN 568 plus standard (treatment group) and the other group received placebo (control group). The study was double-blind. The patients were also advised not to use antidiabetic drug regimen during the study.

2.3.2. Endpoints

The designated study endpoint of both trials was the incidence of adverse events. Additional safety endpoints included serious adverse events, adverse events leading to the discontinuation of the study health food (for patients in the ANKASCIN 568 plus group), and abnormalities in creatine kinase levels, liver and kidney function, and electrolyte balance. A prespecified exploratory outcome was defined as the incidence of confirmed cardiovascular events, which was ascertained over the course of the study.

2.4. Statistical analysis

Data are expressed as the mean ± standard deviation. The statistical significance of differences in the biochemical analyses was determined using one-way analysis of variance (ANOVA) by using the general linear model procedure of the Statistical Package for the Social Sciences (SPSS 16.0) software (SPSS Institute, Inc., Chicago, IL, USA). This was followed by an ANOVA with a paired *t*-test to evaluate the differences before and after sample and placebo administration, whereas the Student *t* test was used to compare the differences between test and placebo groups ($p < 0.05$).

3. Results

3.1. Anthropometric measurements

The body weight, body mass index, waistline, and blood pressure of the participants in this trial are shown in Table 1. There were no differences in these values among the groups during the course of the experiment. Therefore, we can conclude that the appearance and health condition of participants were maintained during the trial.

3.2. Effect of ANKASCIN 568 plus on glycemic index

In this trial, the first aim was to determine the effect of ANKASCIN 568 plus capsules on hyperglycemic patients. In our previous study, fermented products of *M. purpureus* NTU 568 were found to improve glucose metabolic syndrome-related indicators, including effective improvement of blood insulin levels, reduction of fasting blood glucose levels, and amelioration of IR and other effects. Animal test results suggested that streptozotocin-induced diabetic rats provided with RMR at 200 mg/kg daily for up to 8 weeks presented a significantly lower fasting blood glucose level than diabetic rats fed a normal diet [26].

After 6 weeks of administration of ANKASCIN 568 plus, fasting glucose levels were significantly reduced (8.5%, Table 2). Even 4 weeks after the test sample was discontinued, fasting glucose levels were lower than the initial level. No difference in the fasting glucose level was found in the placebo group. For glucose tolerance, postprandial blood glucose (post cibum) values remained unchanged. HbA1c is a reliable estimate of the mean plasma glucose levels over the previous 3–4 months for most individuals with diabetes, and as the basis for treatment adjustment. Higher blood glucose correlates to higher HbA1c; furthermore, since red blood cells have a lifespan of 120 days,

Table 1 – Effect of chronic administration of ANKASCIN 568 plus or placebo on anthropometric measurements of patients.

	Treatment				Placebo			
	0 (Initial)	6	12	16 (Follow-up)	0 (Initial)	6	12	16 (Follow-up)
	Week				Week			
Age (y)	60.9 ± 15.1				59.1 ± 14.5			
Weight (kg)	68.2 ± 9.0	68.8 ± 9.3	68.9 ± 9.7	69.4 ± 9.6*	70.5 ± 13.3	70.2 ± 12.9	70.2 ± 13.4	69.9 ± 12.8
Body fat (%)	30.6 ± 6.8	30.7 ± 6.9	30.9 ± 7.4	31.3 ± 6.3	32.2 ± 6.9	33.0 ± 6.5	31.4 ± 6.9	31.7 ± 6.9
BMI	25.2 ± 2.1	25.4 ± 2.0	25.5 ± 2.1	25.4 ± 2.5	25.2 ± 2.1	26.1 ± 4.3	26.2 ± 4.3	25.6 ± 3.8
Waist (cm)	86.2 ± 7.3	86.8 ± 7.5	86.2 ± 8.2	85.5 ± 8.3	90.9 ± 11.8	90.0 ± 11.7	90.1 ± 11.6	90.1 ± 11.6
Blood pressure								
SBP (mmHg)	137.5 ± 17.2	138.5 ± 15.7	142.0 ± 18.1	142.2 ± 18.4	132.5 ± 16.6	133.1 ± 16.3	137.5 ± 18.0	133.3 ± 14.7
DBP (mmHg)	79.3 ± 15.0	80.8 ± 12.5	79.6 ± 13.2	82.2 ± 12.1	84.4 ± 18.8	85.3 ± 16.7	87.4 ± 13.9	87.0 ± 18.1

Data are expressed as the mean ± standard deviation.
Student *t*-test showed no significant difference between placebo and treatment group at Week 0.
* $p < 0.05$ versus Week 0 for each group.
BMI = body mass index; DBP = diastolic blood pressure; SBP = systolic blood pressure.

Table 2 – Effect of chronic administration of ANKASCIN 568 plus or placebo on glycemic index of patients.

	Treatment				Placebo			
	0 (Initial)	6	12	16 (Follow-up)	0 (Initial)	6	12	16 (Follow-up)
	Week				Week			
FBG (mg/dL)	115.3 ± 12.0	105.5 ± 15.7*	104.6 ± 12.1*	110.2 ± 7.2*	118.8 ± 16.3	117.4 ± 22.1	114.6 ± 28.3	118.6 ± 22.1
PC (mg/dL)	143.5 ± 22.5	110.7 ± 31.7	165.3 ± 68.8	133.0 ± 16.0	138.4 ± 52.9	131.2 ± 45.9	128.8 ± 52.0	126.1 ± 47.6
HbA1c (%)	5.9 ± 0.7	5.9 ± 0.7	6.0 ± 0.7	6.0 ± 0.6	6.1 ± 0.7	6.1 ± 0.9	6.1 ± 1.1	6.2 ± 1.0
Insulin (mg/dL)	10.8 ± 5.4	11.9 ± 6.1	11.1 ± 4.9	11.6 ± 1.4	12.2 ± 7.5	12.3 ± 7.5	13.1 ± 10.0	12.6 ± 7.2
HOMA-IR	1.4 ± 0.7	1.6 ± 0.8	1.5 ± 0.6	1.5 ± 0.8	1.6 ± 1.0	1.7 ± 1.0	1.8 ± 1.3	1.9 ± 0.9

Data are expressed as the mean ± standard deviation.
Student *t*-test showed no significant difference between placebo and treatment group at Week 0.
* $p < 0.05$ versus Week 0 for each group.
FBG = fasting blood glucose; HbA1c = glycated hemoglobin; HOMA-IR = homeostasis model assessment of insulin resistance; PC = *post cibum*.

HbA1c levels reflect glycemic control over the previous 3 months. A normal HbA1c value is about 5%, based on clinical findings. For adequate control of diabetes, HbA1c value should remain below 7% to reduce the risk of complications including cerebrovascular disease, cardiovascular disease, retinopathy, peripheral neuropathy, and renal dysfunction. The HbA1c values of the test group were lower than those of the placebo group were, although not statistically significant (Table 2). However, the data can be considered to exemplify a single case of downward trending HbA1c, and can be attributed to the fact that patients needed to adjust to the consumption of the RMR product to maintain blood glucose. Homeostasis model assessment of IR (HOMA-IR) studies have indicated that a level of ≤1.95 can be considered as IR [27]; however, in the present study, HOMA-IR values were not different between the two groups (Table 2). This could be because all patients had a normal status, with weak blood glucose control. Taken together, these results indicate that the administration of ANKASCIN 568 plus could effectively improve blood glucose regulation.

3.3. *Hypolipidemic effect of ANKASCIN 568 plus*

We used capsules containing ANKASCIN 568 plus fermented by *M. purpureus* NTU 568 as the study material. The total cholesterol (TC) levels at Week 0 were not significantly different between the placebo and treatment groups (Table 3). The TC levels for the treatment groups after 6 weeks of treatment with ANKASCIN 568 plus decreased significantly by 7.5% compared to that at Week 0 ($p < 0.05$). Serum triglyceride (TG) levels in the treatment and placebo groups were compared at Weeks 0, 6, 12, and 16 (Table 3). The results revealed that there were no differences between the treatment and placebo groups. However, the TG values did show considerable differences among patients in each group. This intragroup variability might have resulted in the lack of statistically significant differences between the treatment and placebo groups. However, the test substance-treated group showed a difference between the values obtained before (Week 0) and after 6 weeks of treatment. TG levels showed a downward trend, indicating that ANKASCIN 568 plus capsules effectively reduced the TG levels.

3.4. *Effect of ANKASCIN 568 plus on serum lipid profile*

Low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) levels can be used to determine the lipid metabolic status; in humans, the standards are <130 mg/dL and > 40 mg/dL, respectively. Extremely low HDL-C or high LDL-C levels are thought to have a considerable impact on cardiovascular health. In this study, the LDL-C level

Table 3 – Effect of chronic administration of ANKASCIN 568 plus or placebo on blood lipid profiles of patients.

	Treatment				Placebo			
	0 (Initial)	6	12	16 (Follow-up)	0 (Initial)	6	12	16 (Follow-up)
	Week				Week			
TG (mg/dL)	141.5 ± 84.0	133.3 ± 47.7	117.0 ± 55.5	130.3 ± 59.9	160.8 ± 92.4	168.4 ± 103.7	158.4 ± 85.7	165.0 ± 94.5
TC (mg/dL)	198.5 ± 38.7	183.6 ± 41.2*	194.5 ± 36.0	191.6 ± 33.0	199.6 ± 44.7	199.5 ± 46.5	202.6 ± 46.6	197.7 ± 23.0
HDL-C (mg/dL)	49.0 ± 6.9	46.1 ± 8.1	48.7 ± 6.7	46.6 ± 9.8	45.8 ± 8.7	44.1 ± 10.1	46.3 ± 10.1	41.5 ± 7.5
LDL-C (mg/dL)	125.9 ± 34.6	112.9 ± 38.5*	120.9 ± 32.7*	123.6 ± 37.8	122.9 ± 40.4	124.1 ± 42.2	123.6 ± 41.1	122.4 ± 40.3
LDL-C/HDL-C	2.6 ± 0.4	2.4 ± 0.3*	2.5 ± 0.5*	2.7 ± 0.3	2.7 ± 0.6	2.8 ± 0.6	2.7 ± 0.5	2.9 ± 0.5
TC/HDL-C	4.1 ± 0.3	4.0 ± 0.5	4.0 ± 0.4	4.1 ± 0.5	4.4 ± 0.3	4.5 ± 0.5	4.4 ± 0.3	4.8 ± 0.4

Data are expressed as the mean ± standard deviation.
Student *t*-test showed no significant difference between placebo and treatment group at Week 0.
* $p < 0.05$ versus Week 0 for each group.
HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; TC = total cholesterol; TG = triglyceride.

of the placebo group was not significantly different from that of the treatment group at Week 0.

The LDL-C levels of the ANKASCIN 568 plus-treated and placebo groups were compared at Weeks 0, 6, and 12 (Table 3); the LDL-C level decreased by 10.3% and 4.0% ($p < 0.05$ for all pairs) at 6 weeks and 12 weeks, respectively. The HDL-C levels were not different between the ANKASCIN 568 plus-treated and placebo groups (Table 3; $p > 0.05$). These results indicated that the treatment group showed significant improvement in LDL-C but not HDL-C levels over time.

Ratios of LDL-C/HDL-C and TC/HDL-C that are >3.5 and >5.0, respectively, are considered risk factors for cardiovascular disease (including heart disease and stroke) and atherosclerosis. Therefore, the changes in LDL-C/HDL-C and TC/HDL-C ratios were determined in this study. The results showed that the LDL-C/HDL-C ratios decreased by 7.8% and 3.8% at 6 weeks and 12 weeks, respectively (Table 3; $p < 0.05$). In addition, the TC/HDL-C ratios for the treatment and placebo groups were not significantly different when comparing Weeks 0, 6, 12, and 16 (Table 3). This finding was contradictory to that for the TG outcome, which showed a lack of significant difference; this could be because the ratios are easily affected by various factors, including the patient's blood parameters and the diet consumed the day before testing. Control of these factors is typically more difficult in humans than in animals. Furthermore, the results suggest that ANKASCIN 568 plus capsules could effectively improve lipid metabolism and reduce the risk of hardening of the arteries and the probability of developing coronary atherosclerosis and heart disease. Increased LDL-C levels have been associated with atherosclerosis and other cardiovascular diseases. These results suggest that the functional components of ANKASCIN 568 plus can effectively reduce blood LDL-C levels and thus possibly reduce the incidence of cardiovascular diseases.

3.5. *Effects on liver function*

Most commercial red mold fermented products contain citrinin, which is toxic to the liver and kidney. Therefore, the food safety risks associated with red mold fermented products should be evaluated. The safety of the products was evaluated by assaying the liver levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

Different treatment weeks within the same group were compared, showing that there was no significant difference in the AST and ALT levels between the placebo and ANKASCIN 568 plus groups (Table 4). Gamma-glutamyltransferase (γ-GT), a membrane-bound heterodimeric glycoprotein, is abundant in many tissues, including the kidney, intestine, and liver. Under pathological conditions, when liver cells are damaged, γ-GT is released into the serum. The γ-GT levels between the treatment and placebo groups were not significantly different over the course of the experiment ($p > 0.05$, Table 4). Blood urea nitrogen (BUN) is a type of protein metabolite, and a high concentration is indicative of weak renal excretion. BUN and creatinine levels (Table 4) were not significantly different between the treatment and placebo groups ($p > 0.05$). Furthermore, there were no significant differences in the serum calcium, sodium, potassium, and chloride concentrations between the treatment and placebo groups ($p > 0.05$, Table 4). These results indicate that administration of the test substance had no significant effect on renal metabolism and physiological function.

3.6. *Effect of ANKASCIN 568 plus on serum thyroid function and creatine phosphokinase levels*

Thyroid-stimulating hormone (also known as thyrotropin, TSH, or human TSH) is a pituitary hormone that stimulates the thyroid gland to produce thyroxine (T4) and then

Table 4 – Effect of chronic administration of ANKASCIN 568 plus or placebo on the liver and kidney functions of patients.

	Treatment				Placebo			
	0 (Initial)	6	12	16 (Follow-up)	0 (Initial)	6	12	16 (Follow-up)
	Week				Week			
Liver function								
AST (IU/L)	22.4 ± 6.1	24.2 ± 6.5	19.9 ± 4.1*	20.1 ± 3.6	33.7 ± 18.7	30.5 ± 18.3	30.2 ± 15.1	27.2 ± 11.5*
ALT (IU/L)	23.8 ± 10.7	24.1 ± 9.7	19.9 ± 6.0*	20.8 ± 6.3	37.4 ± 29.2	33.2 ± 25.2	31.8 ± 19.8	29.9 ± 15.0
γ-GT (IU/L)	23.5 ± 17.7	26.3 ± 27.4	20.6 ± 15.6	21.4 ± 18.5	43.8 ± 33.0	39.4 ± 32.9	40.8 ± 37.7	37.7 ± 34.6
Albumin (g/dL)	4.2 ± 0.3	4.1 ± 0.3	4.1 ± 0.3	4.2 ± 0.3	4.2 ± 0.3	4.1 ± 0.3	4.1 ± 0.3	4.1 ± 0.4
Kidney function								
Creatinine (mg/dL)	0.9 ± 0.3	0.9 ± 0.3	0.9 ± 0.3	0.8 ± 0.2	1.0 ± 0.3	0.9 ± 0.3	0.9 ± 0.2	1.2 ± 0.4
BUN (mg/dL)	14.5 ± 6.1	13.8 ± 4.6	14.7 ± 5.0	14.4 ± 4.2	16.3 ± 6.9	15.0 ± 6.0	15.8 ± 6.3	16.1 ± 5.9
Electrolyte balance								
Ca (mg/dL)	9.4 ± 0.3	9.3 ± 0.3	9.1 ± 0.2	9.0 ± 0.2	9.1 ± 0.6	9.3 ± 0.5	9.2 ± 0.5	8.9 ± 0.6
Na (mmol/L)	139.0 ± 2.8	138.9 ± 2.3	138.2 ± 2.0	137.2 ± 2.9	139.1 ± 2.0	139.3 ± 2.2	137.5 ± 2.0	136.9 ± 2.9
K (mmol/L)	4.5 ± 0.7	4.6 ± 0.7	5.1 ± 1.2	4.2 ± 0.5	4.6 ± 1.1	4.4 ± 0.8	4.3 ± 0.8	4.3 ± 0.9
Cl (mmol/L)	104.6 ± 3.2	104.7 ± 2.1	104.8 ± 2.7	105.7 ± 1.7	104.7 ± 2.8	105.2 ± 2.3	104.5 ± 2.5	104.1 ± 3.6

Data are expressed as the mean ± standard deviation.
Student *t*-test showed no significant difference between placebo and treatment group at Week 0.
* $p < 0.05$ versus Week 0 for each group,
ALT = alanine aminotransferase; AST = aspartate aminotransferase; γ-GTP = γ-glutamyl transpeptidase.

Table 5 – Effect of chronic administration of ANKASCIN 568 plus or placebo on the thyroid function and creatine phosphokinase level of patients.

	Treatment				Placebo			
	0 (Initial)	6	12	16 (Follow-up)	0 (Initial)	6	12	16 (Follow-up)
	Week				Week			
Free T4 (ng/dL)	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.2	0.9 ± 0.1	1.0 ± 0.2	0.9 ± 0.2	1.0 ± 0.2	1.1 ± 0.3
TSH (μIU/mL)	2.1 ± 1.1	2.4 ± 1.4	2.5 ± 1.6	2.3 ± 1.1	2.0 ± 1.5	2.0 ± 1.1	3.0 ± 5.3	2.1 ± 1.3
CPK (IU/L)	99.0 ± 41.9	120.6 ± 59.5	92.2 ± 39.0	99.3 ± 43.6	104.7 ± 52.9	98.2 ± 70.3	95.4 ± 36.0	94.5 ± 36.0

Data are expressed as the mean ± standard deviation.
Student *t*-test showed no significant difference between placebo and treatment group at Week 0.
* $p < 0.05$ versus Week 0 for each group.
CPK = creatine phosphokinase; TSH = thyroid stimulating hormone.

triiodothyronine (T3), which stimulates metabolism in almost every body tissue. TSH is a glycoprotein hormone synthesized and secreted by thyrotrope cells in the anterior pituitary gland, which regulates the endocrine function of the thyroid. The serum TSH level is often interpreted in conjunction with free T4. TSH levels decrease and increase in hyperthyroidism and hypothyroidism, respectively, and high levels of free TSH (free T4) are indicative of hyperthyroidism and low organic energy. In this study, no significant differences in free T4 and TSH levels were found between the groups, with values remaining in the normal range, indicating that administration of ANKASCIN 568 plus did not affect thyroid function (Table 5). During rhabdomyolysis, damaged muscle cells release creatine phosphokinase (CPK), which is often used in the diagnosis and monitoring of clinical myocardial infarction and muscle diseases. As shown in Table 5, the CPK of the treatment group at Week 0 and Week 6 was not statistically different, owing to large differences in standard deviation. Administration of ANKASCIN 568 plus did not increase the CPK levels of the treated patients (Table 5). Lipid peroxidation and oxidative modification of LDL have been implicated as causal factors in the pathogenesis of atherosclerosis. Therefore, prevention of LDL oxidation by antioxidants might be an effective strategy for inhibiting disease progression. Furthermore, oxygen-derived radicals impair endothelial function and have been implicated as mediators of this process.

4. Discussion

RMR has been used as a dietary supplement in East Asia for several centuries, especially in China and Japan. Our previous study showed that *Monascus* fermentation using a dioscorea substrate resulted in higher levels of monascin, ankaflavin, and γ-aminobutyric acid [28]. *Monascus*-fermented products can be used as food-based adjuvants for diabetic patients to ameliorate IR and/or impaired glucose metabolism [29]. This study showed that *Monascus*-fermented products of ANKASCIN 568 plus significantly decreased blood glucose levels at Week 6 and Week 12, and after discontinuation of the treatment (Table 2), providing direct evidence for the anti-hyperglycemic effects of ANKASCIN 568 plus. Additionally, the treated group showed changes in TC and LDL-C levels, as well as LDL-C/HDL-C ratios (Table 3) and a significant hyperglycemic effect. Furthermore, the LDL-C/HDL-C ratios were significantly lower in the ANKASCIN 568 plus-treated group than in the placebo group. LDL-C is a key indicator for assessing coronary heart disease and is an important human lipoprotein cholesterol that can be transported to body cells for use. However, high blood levels of LDL-C result in its accumulation in vessel walls, leading to atherosclerosis, which blocks blood vessels. Therefore, high LDL-C is considered a risk factor for vascular obstruction. In contrast, HDL-C is an important component for the *in vivo* prevention of arteriosclerosis. Low levels of HDL-C are an important predictor of coronary atherosclerosis and coronary heart disease. High TG level is the main cause of low HDL-C. Therefore, the positive effects of ANKASCIN 568 plus on HDL-C suggest potential benefits for coronary heart disease.

The levels of both AST and ALT, which are enzymes found in liver cells, increase in response to inflammation and damage to liver cells. However, destruction and injury to red blood cells, heart, muscle, and other types of cells cause an increase in AST but not ALT levels. Thus, ALT levels are more accurate indicators of liver disease. Although changes in serum protein composition in response to different diseases vary, an excessively low total serum protein level generally indicates the presence of chronic disease or liver damage. Because most commercially available red mold products contain citrinin, a hepatotoxin and nephrotoxin, we assessed the safety of our products by measuring the levels of AST and ALT in the liver. The AST, ALT, and γ-GTP levels in our clinical trial were not significantly different from those of the control (Table 4).

Potassium and calcium are the most important electrolytes in the human body [30,31]. 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 [32]. Previously, no consistent or marked decrease in the concentration of sodium and chloride was reported in rats with diabetes insipidus, but a typical increase in potassium levels was noted [33]. BUN is a type of protein metabolite, and a high concentration of BUN indicates weak renal excretion. In the present study, the minor changes in this marker (Table 4) suggested a lack of ANKASCIN 568 plus-induced toxic effects on the liver and renal function as well as electrolyte balance, also rhabdomyolysis was not induced following 12 weeks of treatment. Deterioration of renal function leads to an increase

in BUN concentrations. Therefore, urea nitrogen concentration is an important indicator of kidney function. Furthermore, creatinine levels are also an important indicator of renal function, and increased creatinine levels are indicative of abnormalities. The *in vivo* extracellular cation sodium plays an important role in maintaining osmotic pressure by regulating the body fluid balance, whereas potassium prevents muscle contraction and nerve conduction. The kidneys excrete excess potassium in the plasma. Administration of ANKASCIN 568 plus resulted in no significant effect on the metabolic or physiological functions of the kidneys.

In addition, the anthropometric measurements were not different after 12 weeks of intervention in the test and placebo groups. Similar results were obtained with regard to blood pressure (Tables 1 and 5). This result clearly indicates that the risk of cardiovascular diseases could be greatly reduced by the administration of ANKASCIN 568 plus. In this study, no effect was observed on liver and kidney function (Table 4).

5. Conclusion

This study suggests that patients who were administered two capsules (500 mg/capsule) of ANKASCIN 568 plus daily for more than 6 weeks exhibited a significant reduction in serum blood glucose, TC, and LDL-C levels. The blood glucose was well regulated in the treated patients. Therefore, ANKASCIN 568 plus produced by *M. purpureus* NTU 568 fermentation might be a potentially useful agent for regulation of blood glucose and blood lipids and treatment of DM2 and coronary artery diseases.

Conflicts of interest

All authors declare no conflicts of interest.

REFERENCES

[1] van Dieren S, Beulens JW, van der Schouw YT, Grobbee DE, Neal B. The global burden of diabetes and its complications: an emerging pandemic. Eur J Cardiovasc Prev Rehabil 2010;17:3–8.

[2] 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.

[3] 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. Diabetologia 1980;19:205–10.

[4] Olefsky JM. Insulin resistance and insulin action: an *in vitro* and *in vivo* perspective. Diabetes 1981;30:148–62.

[5] Groop LC, Bonadonna RC, DelPrato S, Ratheiser K, Zyck K, Ferrannini E, DeFronzo RA. Glucose and free fatty acid metabolism in non-insulin-dependent diabetes mellitus. Evidence for multiple sites of insulin resistance. J Clin Invest 1989;84:205–13.

[6] 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.

[7] Sowers JR, Frohlich ED. Insulin and insulin resistance: impact on blood pressure and cardiovascular disease. Med Clin North Am 2004;88:63–82.

[8] Wilcox G. Insulin and insulin resistance. Clin Biochem Rev 2005;26:19–39.

[9] Moussavi S, Chatterji S, Verdes E, Tandon A, Patel V, Ustun B. Depression, chronic diseases, and decrements in health: results from the world health surveys. Lancet 2007;370:851–8.

[10] Lee S, Ling Y, Tsang A. Community-based co-morbidity of depression and chronic physical illnesses in Hong Kong. Int J Psychiatry Med 2010;40:339–48.

[11] Li Y, Chen C, Tu H, Cao W, Fan S, Ma Y, Xu Y, Hua Q. Prevalence and risk factors for depression in older people in Xi'an China: a community-based study. Int J Geriatr Psychiatry 2012;27:31–9.

[12] Luppa M, Sikorski C, Luck T, Weyerer S, Villringer A, Konig HH, Riedel-Heller SG. Prevalence and risk factors of depressive symptoms in latest life–results of the Leipzig Longitudinal Study of the Aged (LEILA 75+). Int J Geriatr Psychiatry 2012;27:286–95.

[13] Kessler RC, Birnbaum HG, Shahly V, Bromet E, Hwang I, McLaughlin KA, Sampson N, Andrade LH, De Girolamo G, Demyttenaere K. Age differences in the prevalence and co-morbidity of DSM-IV major depressive episodes: results from the WHO world mental health survey initiative. Depress Anxiety 2010;27:351–64.

[14] 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.

[15] Slim R, Ben SC, Zamy M, Biour M. Pioglitazone-induced acute rhabdomyolysis. Diabetes Care 2009;32:e84.

[16] 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.

[17] 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.

[18] Lee CL, Wang JJ, Kuo SL, Pan TM. *Monascus* fermentation of dioscorea for increasing the production of cholesterol-lowering agent-monacolin K and antiinflammation agent-monascin. Appl Microbiol Biotechnol 2006;72:1254–62.

[19] 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.

[20] Chen CL, Pan TM. Red mold dioscorea: A potentially safe traditional function food for the treatment of hyperlipidemia. Food Chem 2012;134:1074–80.

[21] Chen CL, Pan TM. Effects of red mold dioscorea with pioglitazone, a potentially functional food, in the treatment of diabetes. J Food Drug Anal 2015;23:719–28.

[22] Shi YC, Pan TM. Characterization of a multifunctional *Monascus* isolate NTU 568 with high azaphilone pigments production. Food Biotechnol 2010;24:349–63.

[23] Hsu WH, Lee BH, Liao TH, Hsu YW, Pan TM. *Monascus*-fermented metabolite monascin suppresses inflammation via PPAR-c regulation and JNK inactivation in THP-1 monocytes. Food Chem Toxicol 2012;50:1178–86.

[24] Hsu WH, Liao TH, Lee BH, Hsu YW, Pan TM. Ankaflavin regulates adipocyte function and attenuates hyperglycemia caused by high-fat diet via PPAR-γ activation. J Funct Foods 2013;5:124–32.

[25] Lee BH, Hsu WH, Hsu YW, Pan TM. Dimerumic acid attenuates receptor for advanced glycation endproduct (RAGE) signal to inhibit inflammation and diabetes mediated by Nrf2 activation and promoted methylglyoxal metabolism into D-lactate acid. Free Radic Biol Med 2013;60:7–16.
[26] 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.
[27] Jennings CL, Lambert EV, Collins M, Joffe Y, Levitt NS, Goedecke JH. Determinants of insulin-resistant phenotypes in normal-weight and obese black African women. Obesity 2008;16:1602–9.
[28] 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.
[29] Younis N, Soran H, Farook S. The prevention of type 2 diabetes mellitus: recent advances. Q J Med 2004;97:451–5.
[30] Guerra SMO, Kitabchi AE. Comparison of the effectiveness of various routes of insulin injection: insulin levels and glucose response in normal subjects. J Clin Endocrinol Metab 1976;42:869–74.
[31] Schade DS, Eaton RP. Dose response to insulin in man: differential effects on glucose and ketone body regulation. J Clin Endocrinol Metab 1977;44:1038–53.
[32] 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.
[33] Winter CA, Gross EG, Ingram WR. Serum sodium, potassium and chloride after suprarenalectomy in cats with diabetes insipidus. J Exp Med 1938;6:251–65.