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Synergic Effects of Bitter Melon and β-Glucan Composition on STZ-Induced Rat Diabetes and Its Complications

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β-Glucan purified from oats (OG) and bitter melon, *Momordica charantia* Linn (MC), water extracts have shown favorable effects on diabetes and its complications. We investigated to find out the optimal composition showing hypoglycemic and antidiabetic complication effects in variable compositions (OG:MC = 1:1, 1:2, 1:4, 1:6, 1:8, 1:10, 2:1, 4:1, 6:1, 8:1, 10:1). Extracts were administered orally once a day for 28 days following 7 days post streptozotocin (STZ) dosing. Five rats per group (total 15 groups; Intact, STZ, OG, MC, and the variable composition groups) were selected according to the blood glucose and body weight at 6 days after STZ dosing. After 28 days of extracts dosing, the changes on the body weight, liver and kidney weight, blood glucose, blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), low-density lipoprotein (LDL), and total-cholesterol levels were observed. As the result of STZ-induced diabetes, decreases of body weight, increases of the liver and kidney weights, blood glucose, BUN, creatinine, AST, ALT, LDL, and total-cholesterol levels in STZ control were detected compared with intact control. However, these changes of hyperglycemia, diabetic nephropathy, hepatopathy, and hyperlipemia were dramatically decreased in the OG and MC single-dosing group, and all composition groups. In addition, there were more favorable effects in all composition groups compared with the OG and MC single-dosing groups. Among variable compositions, the OG:MC 1:2 mixed group showed the most synergic effects in this study.

Keywords: Beta-glucan, *Momordica charantia*, streptozotocin, diabetes, diabetic complication, rat

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Diabetes mellitus refers to a heterogeneous group of metabolic disorders characterized by hyperglycemia and glycosuria, and is a complex syndrome involving severe insulin dysfunction in conjugation with gross abnormalities in glucose homeostasis and lipid metabolism, and which has affected several millions of people all over the world [28]. The individual with diabetes has a 25-fold increase in the risk of blindness, a 20-fold increase in the risk of renal failure, a 20-fold increase in the risk of amputation as a result of gangrene, and a 2 to 6-fold increased risk of coronary heart disease and ischemic brain disease [36]. Other related events such as ketoacidosis, and loss of minerals, nitrogen, and body weight may occur. All of these events eventually lead to coma and death if treatment is not instituted. Two major categories of diabetes are recognized in humans: Insulin-dependent diabetes (Type 1: juvenile-onset) and non-insulin-dependent diabetes mellitus (Type 2: maturity-onset). Patients with type 1 have an absolute insulin deficiency, whereas individuals with type 2 often have below normal or above normal plasma insulin levels in fasting state, and may have an impaired insulin response to a glucose load.

A number of oral antidiabetic medicines are currently being used or developed for its treatment, including the thiazolidinediones and metformin, which improve insulin resistance. Metformin inhibits hepatic glucose production through reduced gluconeogenesis [30], and it effectively inhibits high-fat induced obesity in mice. Currently available pharmacological agents for diabetes or related obesity, however, have a number of limitations, such as various adverse effects and high rates of secondary failure [17]. Owing to these factors, diabetic patients and healthcare professions are increasingly considering complimentary and alternative approaches, including the use of medicinal herbs.

β-Glucan is a fiber-type complex sugar (polysaccharide) derived from the cell wall of yeast, oat, and barley fiber,

and mushrooms. The two primary uses of β-glucan are to enhance the immune system [12] and to lower blood cholesterol levels [3]. The evidences of hypoglycemic and antidiabetic complication effects of β-glucan extracts from some natural plants or a mushroom have been reported in animal experiments [10] and clinical trials [23] mediated by delay of carbohydrate digestion [5] or free radical scavenger activities [19, 29].

Momordica charantia (MC; family Cucurbitaceae) is a popular vegetable that is widely grow in tropical areas. In addition to culinary usage, MC is also used in folklore medicine. Although MC was found to possess antiviral, antibacterial, anti-HIV, anticancer, and immunomodulatory properties, attention has always been focused on its blood glucose-lowering effect [14]. Such an effect was demonstrated in STZ-induced diabetic [1] and diet-induced obese rats [7]. In addition, MC has shown promising effects in prevention as well as delay in progression of diabetic complications (nephropathy, neuropathy, gastroapresis, cataract, and insulin resistance) in animals [15, 26] mediated by insulin-like activity of a polypeptide-p (phyto-insulin) and enhancement of pancreatic beta cells of charantin or glucose utilization [34, 27].

Although β-glucan and MC have an antidiabetic effect, the effective dosages of complimentary or alternative drugs are much higher than that of reasonable dosage. The effective dosage of β-glucan or MC was also much higher than the ideal dosage to treat diabetes or related complications, and in spite of the good hypoglycemic effects of MC, combination therapies has been trialed [22]. The effective dosage of β-glucan is over 15 g/day in human subjects [5] and 1.2 g/day in animals [35]. In addition, the effective dosage of MC in rats is about 200 mg/kg/day [25] and it simply matched as 1 g/day in humans calculated based on the body surface (1/6 in rat against to human). We, thus, hypothesized that an appropriate mixture of β-glucan and MC should show synergic effects and reach an ideal dosage.

In this study, we evaluated the effects of 11 compositions of OG:MC on streptozotocin-induced diabetic rats to find out the optimal composition with hypoglycemic and antidiabetic complication effects.

Materials and Methods

Animals

Three hundred female SD rats (6-wk-old upon receipt, SLC, Japan) were used after acclimatization for 8 days. Animals were allocated three per polycarbonate cage in a temperature (20–25°C) and humidity (40–45%) controlled room. The light:dark cycle was 12 h:12 h and feed (Samyang, Korea) and water were supplied *ad libitum*. Two hundred ninety rats were dosed with STZ, and the other 10 rats were used as intact control. About half of STZ-dosed rats and intact control were selected (5 per group) based on the blood glucose level and body weight at 6 days after STZ dosing.

Preparations and Administration of Drugs

OG (Glucan Corp., Korea), β-glucan extracted from oats (17% dry substances), and water extracts of MC were used in this study. Briefly, 1,500 g powder of MC (fruit containing seed) was extracted in 15 l of water at 105°C, 12 h using a rotary vacuum evaporator (Lab. Camp, Korea) and lyophilized with a programmable freeze dryer (IlShin Lab., Korea) (yield 13.3%). The OG and MC were stored in a 4°C refrigerator to protect from light and prevent degeneration.

Table 1. Groups and mixed composition of OG and MC used in this study.

Group		Actual dosage (mg/kg)	
		Oat β-glucan (OG)	Water extracts of *M. charantia* (MC)
Control	Intact	0	0
	Diabetes	0	0
STZ[a] dosing	OG, single formulation	200	0
	MC, single formulation	0	200
	OG:MC, mixed formulation		
	= 1:1	100	100
	= 1:2	66.66	133.34
	= 1:4	40	160
	= 1:6	28.57	171.43
	= 1:8	22.22	177.78
	= 1:10	18.18	181.82
	= 2:1	133.34	66.66
	= 4:1	160	40
	= 6:1	171.43	28.57
	= 8:1	177.78	22.22
	= 10:1	181.82	18.18

[a]Streptozotocin (60 mg/kg) was dosed by single intraperitoneal injection; All extracts and vehicle were dosed by gastric gavage initiated 7 days after STZ dosing for 4 weeks at 200 mg/kg in distilled water.

OG, MC, and the variable compositions (OG:MC 1:1, 1:2, 1:4, 1:6, 1:8, 1:10, 2:1, 4:1, 6:1, 8:1, 10:1) were dissolved in distilled water, and orally administered at 200 mg/kg once a day for 28 days. The administration dose and compositions are listed in Table 1.

Diabetes Induction

For induction of diabetes, 60 mg/kg of STZ (Sigma, USA) in 50 mM citrate buffer (pH 4.5) was dosed once by intraperitoneal injection and the equal volume of vehicle (distilled water) was dosed in the intact control [28]. At 6 days after STZ dosing, animals showing over 220 mg/dl of blood glucose level were selected.

Body and Organ Weight Measurement

Changes of body weight were calculated at STZ-dosing, and at first extracts dosing, 7, 14, 21, 27, and 28 days (at sacrifice) using an automatic electronic balance (Sartorius Co., Ltd., USA). At blood collection, STZ-dosing and sacrifice days, animals were fasted during overnight (about 12 h; water was not restricted) to reduce the differences from feeding. Besides, body weight gains were calculated as following periods: (inducing period) between at STZ-dosing and 6 days after STZ-dosing, (processing period) between at first extracts dosing and at sacrifice day. At sacrifice, the weight of liver and kidney was calculated at g levels and to reduce the individual

Table 2. Changes of body weight and gain after STZ dosing and extracts administration.

Groups	At STZ[a]	At dosing	Weeks after dosing				At sacrifice[a]	BW[d] gain	
			1 week	2 weeks	3 weeks	4 weeks		Inducing period[e]	Processing period[f]
Controls									
Intact	168.00± 4.47	187.20± 2.59	207.80± 7.85	219.80± 4.44	225.60± 4.56	235.00± 5.10	213.40± 4.39	19.20± 2.28	47.80± 4.97
STZ	168.20± 2.95	162.40± 5.73*	158.60± 3.21*	153.20± 4.09*	151.60± 3.65*	149.40± 2.88*	139.80± 3.27*	-5.80± 5.72*	-13.00± 5.39*
OG[b]	167.40± 6.02	163.40± 2.07*	159.60± 3.36*	155.80± 4.76*	157.20± 2.17*,##	159.00± 1.56*,#	150.80± 1.30*,#	-4.00± 5.34*	-4.40± 2.88*,##
MC[b]	168.00± 2.35	162.60± 5.86*	158.80± 3.56*	155.60± 5.86*	157.60± 2.61*,##	159.40± 0.55*,#	151.60± 1.67*	-5.40± 6.69*	-3.20± 5.97*,##
OG:MC[c]									
= 1:1	168.40± 2.70	162.60± 1.67*	161.20± 1.30*	159.20± 0.84*	160.20± 1.92*,##	161.40± 1.14*,#	154.80± 3.27*,#	-5.80± 2.59*	-1.20± 1.30*,#
= 1:2	168.80± 4.21	163.80± 2.59*	163.80± 1.92*,#	163.40± 2.97*,##	162.40± 1.95*,#	165.20± 3.83*,#	157.80± 4.44*,#	-5.00± 4.42*	1.40± 2.70*,#
= 1:4	170.20± 1.92	164.00± 3.74*	162.80± 3.11*,##	162.00± 2.55*,##	161.60± 1.95*,#	159.80± 3.77*,#	152.80± 3.96*,#	-6.20± 2.77*	-4.20± 4.55*,##
= 1:6	167.60± 3.13	162.40± 2.30*	159.20± 3.77*	158.60± 3.78*	158.80± 2.17*,##	159.40± 3.65*,#	153.00± 3.54*,#	-5.20± 4.82*	-3.00± 2.74*,##
= 1:8	168.00± 3.16	162.00± 2.74*	160.00± 5.70*	157.40± 4.88*	157.80± 3.83*,##	158.20± 4.55*,##	150.60± 2.88*,#	-6.00± 2.92*	-3.80± 4.76*,##
= 1:10	168.00± 2.55	163.00± 2.74*	160.00± 6.48*	156.80± 4.78*	157.00± 3.08*,##	158.20± 2.49*,#	151.00± 1.56*,#	-5.00± 4.00*	-4.80± 2.17*,##
= 2:1	168.80± 3.35	163.60± 3.36*	161.00± 5.24*	161.00± 4.06*,##	161.20± 2.77*,##	161.40± 3.36*,#	155.40± 4.04*,#	-5.20± 1.92*	-2.20± 3.70*,##
= 4:1	166.80± 5.07	161.80± 1.30*	159.00± 3.00*	157.80± 3.56*	158.40± 2.19*,##	160.00± 0.71*,#	152.00± 2.00*,#	-5.00± 4.00*	-1.80± 1.64*,#
= 6:1	169.00± 3.39	161.60± 2.07*	160.60± 1.52*	156.80± 3.49*	157.40± 3.58*,##	158.40± 3.29*,##	152.20± 4.44*,##	-7.40± 3.51*	-3.20± 3.70*,##
= 8:1	169.20± 2.95	163.60± 3.51*	159.00± 2.55*	156.20± 4.49*	157.20± 4.44*,##	159.40± 2.97*,#	151.00± 2.12*,#	-5.60± 3.97*	-4.20± 5.81*,##
= 10:1	167.20± 3.63	162.80± 1.92*	160.60± 1.52*	157.20± 4.21*	157.80± 3.70*,##	158.40± 2.51*,#	150.20± 2.17*,#	-4.40± 2.51*	-4.40± 2.51*,##

n=5 (Mean ± SD, g); [a]Overnight fasted; [b]Single formulation; [c]Mixed formulation; [d]BW: body weight; [e]change 1: body weight change between at STZ dosing and 6 days after STZ dosing; [f]body weight change between at initiation of dosing and end of 28 day dosing; *$p<0.01$ compared with that of intact control by MW test; #$p<0.01$ and ##$p<0.05$ compared with that of STZ control by MW test.

difference, the relative weight (%) was calculated using body weight at sacrifice.

Measurement of Serum Chemistry

Blood was collected from the orbital plexus and vena cava at 6 days after STZ dosing and at sacrifice. Collected blood (6 days after STZ dosing) was deposited into a NaF glucose vacuum tube (Becton Dickinson, USA) and then plasma was separated for blood glucose analyses. Blood glucose levels were detected using an automated blood analyzer (Toshiba 200 FR, Japan). Serum was separated from collected blood at sacrifice with general methods. Serum AST, ALT, BUN, creatinine, total-cholesterol, and LDL levels were detected using an automated blood analyzer.

Statistical Analyses

All data were calculated as mean ± standard deviation. Statistical analyses were conducted using the Mann–Whitney U-Wilcoxon Rank Sum W test (MW test) with SPSS for Windows (Release 6.1.3., SPSS Inc., USA) and $p < 0.05$ was considered statistically significant.

RESULTS

Changes in the Body Weight and Gain

Significant ($p<0.01$) decreases of body weights were detected in all STZ-dosing groups from 6 days after STZ dosing compared with intact control, and the body weight gains in inducing and progressing periods were also significantly ($p<0.01$) decreased. However, significant ($p<0.01$ or $p<0.05$) increases of body weight gains in the processing period were detected in all dosing groups compared with STZ control. The body weights were significantly ($p<0.01$ or $p<0.05$) increased from 7 days after dosing in OG:MC 1:2 and 1:4 mixed groups, from 14 days after dosing in the OG:MC 2:1 mixed group, and from 21 days after dosing in the other groups including OG and MC single-dosing groups compared with STZ control, respectively (Table 2).

Changes in the Organ Weight

Significant ($p<0.01$) increases of absolute and relative liver and kidney weights were detected in STZ control compared with intact control. However, significant ($p<0.01$ or $p<0.05$) decreases in organ weight were detected in all dosing groups compared with STZ control. More dramatic inhibitions of the increases of liver and kidney weights were detected in all composition groups compared with OG and MC single-dosing groups, respectively (Table 3).

Changes in the Blood Glucose Level

Significant ($p<0.01$) increases of blood glucose level and the changes between at 6 days after STZ dosing and at sacrifice were detected in the STZ control compared with intact control. However, significant ($p<0.01$ or $p<0.05$) decreases in blood glucose level and their changes after the end of the 28-day dosing were detected in all dosing groups compared with STZ control. More dramatic hypoglycemic effects were detected in all composition groups than in the OG single-dosing group, and more favorable hypoglycemic effects than in the MC single-dosing group were detected in OG:MC 1:1, 1:2, 1:4, 1:6, and 2:1 mixed groups, respectively (Table 4).

Changes in the Serum BUN and Creatinine Levels

Significant ($p<0.01$) increases of serum BUN and creatinine levels and the changes between at 6 days after STZ dosing and at sacrifice were detected in the STZ control compared with intact control. However, significant ($p<0.01$ or $p<0.05$) decreases in serum BUN and

Table 3. Changes on the organ weight after STZ dosing and extracts administration.

Groups	Kidney		Liver	
	Absolute weight (g)	Relative weight (%)	Absolute weight (g)	Relative weight (%)
Controls				
Intact	0.790±0.018	0.370±0.006	5.180±0.200	2.428±0.097
STZ	1.202±0.085*	0.861±0.073*	8.424±1.173*	6.021±0.788*
OG[a]	1.057±0.082*,##	0.701±0.057*,##	6.773±0.500*,##	4.493±0.355*,#
MC[a]	1.037±0.064*,#	0.684±0.045*,#	6.837±0.811*,##	4.512±0.553*,##
OG:MC[b]				
= 1:1	0.950±0.102*,#	0.614±0.065*,#	6.502±0.515*,##	4.200±0.311*,#
= 1:2	0.831±0.102#	0.528±0.079*,#	5.773±0.397#	3.638±0.321*,#
= 1:4	0.884±0.097#	0.578±0.059*,#	5.878±0.487**,#	3.846±0.294*,#
= 1:6	0.909±0.082**,#	0.594±0.043*,#	6.226±0.461*,##	4.075±0.372*,#
= 1:8	1.015±0.123*,##	0.673±0.073*,#	6.461±0.620*,##	4.295±0.457*,#
= 1:10	1.041±0.088*,##	0.689±0.057*,#	6.597±0.444*,##	4.368±0.270*,#
= 2:1	0.913±0.099*,#	0.589±0.077*,#	6.285±0.592**,##	4.052±0.464*,#
= 4:1	0.940±0.072*,#	0.618±0.046*,#	6.485±0.507*,##	4.266±0.324*,#
= 6:1	0.957±0.086**,#	0.628±0.040*,#	6.477±0.349*,##	4.260±0.290*,#
= 8:1	0.963±0.150**,##	0.638±0.103*,#	6.590±0.503*,##	4.368±0.380*,#
= 10:1	1.030±0.080*,##	0.686±0.062*,#	6.873±0.762*,##	4.575±0.490*,##

n=5 (Mean ± SD); Relative liver weight (%) = [(Absolute organ weight/Body weight at sacrifice) × 100]; [a]Single formulation; [b]Mixed formulation; *$p<0.01$ and **$p<0.05$ compared with that of intact control by MW test; #$p<0.01$ and ##$p<0.05$ compared with that of STZ control by MW test.

Table 4. Changes in blood glucose levels after STZ dosing and drug administration.

Groups	At 6 days after STZ dosing	At end of 28-day dosing
Controls		
Intact	94.00±6.78	96.20±6.30
STZ	268.00±22.49*	464.40±42.12*
OG[a]	258.80±22.30*	400.20±10.94*,#
MC[a]	270.20±10.26*	317.40±18.42*,#
OG:MC[b]		
= 1:1	260.60±26.58*	301.00±13.66*,#
= 1:2	276.40±16.50*	256.20±25.90*,#
= 1:4	263.60±23.60*	299.20±28.41*,#
= 1:6	269.00±10.42*	307.20±12.58*,#
= 1:8	259.60±16.94*	308.00±7.58*,#
= 1:10	268.00±26.05*	318.40±23.71*,#
= 2:1	256.20±24.80*	287.60±22.38*,#
= 4:1	259.40±19.93*	334.80±25.75*,#
= 6:1	265.40±23.42*	338.80±40.93*,#
= 8:1	261.00±20.48*	381.20±56.53*,##
= 10:1	270.60±18.91*	392.80±21.09*,#

n=5 (Mean ± SD), mg/dl; [a]Single formulation; [b]Mixed formulation; *p<0.01 compared with that of intact control by MW test; #p<0.01 and ##p<0.05 compared with that of STZ control by MW test.

creatinine levels and their changes after the end of 28-day dosing were detected in all dosing groups compared with the STZ control. More dramatic inhibitions of the increases of serum BUN and creatinine levels were detected in all composition groups than in the OG and MC single-dosing groups except for the OG:MC 10:1 (BUN, creatinine) and 1:10 (creatinine) mixed groups in which quite similar effects were detected compared with the OG and MC single-dosing groups (Table 5).

Changes in the Serum AST and ALT Levels

Significant ($p<0.01$) increases of serum AST and ALT levels and the changes between at 6 days after STZ dosing and at sacrifice were detected in the STZ control compared with the intact control. However, significant decreases in serum AST ($p<0.01$ or $p<0.05$) and ALT ($p<0.01$) levels and their changes after the end of 28-day dosing were detected in all dosing groups compared with the STZ control. More dramatic inhibitions of the increases of serum AST levels were detected in all composition groups than in the OG and MC single-dosing groups, respectively (Table 6).

Changes in the Serum Total-Cholesterol and LDL Levels

Significant ($p<0.01$) increases of serum total-cholesterol and LDL levels and the changes between at 6 days after STZ dosing and at sacrifice were detected in the STZ control compared with the intact control. However, dramatic decreases in serum total-cholesterol and LDL levels and their changes after the end of 28-day dosing were detected in all dosing groups compared with the STZ control.

More dramatic inhibitions of the increases of serum total-cholesterol levels were detected in all composition groups than in the OG and MC single-dosing groups except for the OG:MC 10:1 mixed group in which quite similar effects were detected compared with the OG and MC single-dosing groups. All mixed formulation groups inhibited increases of LDL compared with the OG and MC single-dosing groups, respectively (Table 7).

Table 5. Changes of serum BUN and creatinine levels after STZ dosing and drug administration.

Groups	BUN		Creatinine	
	At 6 days after STZ dosing	At end of 28-day dosing	At 6 days after STZ dosing	At end of 28-day dosing
Controls				
Intact	20.70±0.75	21.46±1.46	0.65±0.06	0.77±0.08
STZ	50.36±8.11*	98.36±11.57*	1.19±0.27*	2.02±0.15*
OG[a]	52.74±6.37*	82.60±5.65*,##	1.25±0.34*	1.62±0.14*,#
MC[a]	48.84±6.40*	81.66±6.05*,##	1.16±0.23*	1.58±0.13*,#
OG:MC[b]				
= 1:1	48.86±5.40*	72.18±8.46*,##	1.14±0.28*	1.36±0.12*,#
= 1:2	51.64±7.21*	59.08±3.38*,#	1.15±0.09*	1.32±0.10*,#
= 1:4	52.10±6.11*	69.80±8.34*,#	1.23±0.22*	1.45±0.21*,#
= 1:6	49.70±3.71*	73.30±5.59*,#	1.17±0.10*	1.46±0.15*,#
= 1:8	50.88±1.88*	78.66±9.17*,##	1.23±0.21*	1.54±0.06*,#
= 1:10	50.30±7.97*	79.98±6.24*,##	1.21±0.25*	1.60±0.10*,#
= 2:1	51.18±2.47*	70.90±4.88*,#	1.14±0.25*	1.38±0.11*,#
= 4:1	52.90±6.45*	67.04±6.30*,#	1.20±0.29*	1.43±0.13*,#
= 6:1	49.52±3.28*	74.08±7.61*,#	1.17±0.13*	1.50±0.09*,#
= 8:1	51.62±6.59*	79.90±9.94*,##	1.20±0.12*	1.57±0.14*,#
= 10:1	49.22±0.68*	82.94±5.99*,##	1.20±0.32*	1.58±0.10*,#

n=5 (Mean ± SD), mg/dl; [a]Single formulation; [b]Mixed formulation; *p<0.01 compared with that of intact control by MW test; #p<0.01 and ##p<0.05 compared with that of STZ control by MW test.

Table 6. Changes of serum AST and ALT levels after STZ dosing and drug administration.

Groups	AST		ALT	
	At 6 days after STZ dosing	At end of 28-day dosing	At 6 days after STZ dosing	At end of 28-day dosing
Controls				
Intact	125.60±9.66	128.00±4.47	32.80±4.44	34.60±4.93
STZ	371.80±38.05*	778.60±47.71*	150.20±13.59*	320.80±14.18*
OG[a]	378.40±51.43*	570.80±50.55*,#	149.80±7.56*	266.40±22.84*,#
MC[a]	368.00±20.95*	572.60±50.17*,#	150.60±13.20*	264.60±23.64*,#
OG:MC[b]				
= 1:1	374.20±45.09*	525.60±25.87*,#	147.00±10.22*	227.60±17.21*,#
= 1:2	370.00±21.66*	458.20±82.59*,#	149.00±2.29*	190.80±48.47*,#
= 1:4	371.40±60.29*	489.00±38.10*,#	148.00±6.16*	216.80±25.38*,#
= 1:6	373.80±29.64*	528.00±24.83*,#	151.60±8.08*	229.80±14.31*,#
= 1:8	376.40±36.60*	545.60±33.57*,#	150.40±5.94*	245.60±15.09*,#
= 1:10	362.80±43.33*	553.40±29.97*,#	147.00±11.25*	254.00±9.06*,#
= 2:1	370.60±27.57*	498.00±60.74*,#	149.60±3.65*	225.60±37.12*,#
= 4:1	368.20±25.05*	512.00±55.00*,#	150.20±3.96*	230.80±8.44*,#
= 6:1	365.80±35.15*	550.40±33.07*,#	147.20±5.81*	246.20±14.65*,#
= 8:1	377.80±38.28*	558.80±70.19*,#	149.40±14.10*	255.00±14.78*,#
= 10:1	369.20±38.26*	556.80±27.21*,#	150.80±9.44*	257.00±12.31*,#

n=5 (Mean ± SD), IU/l; [a]Single formulation; [b]Mixed formulation; *p<0.01 compared with that of intact control by MW test; #p<0.01 compared with that of STZ control by MW test.

DISCUSSION

Diabetes mellitus is associated with several complications such as atherosclerosis, myocardial infaction, neuropathy, nephropathy, and so on. These complications have long been assumed to be related to chronically elevated glucose levels and subsequent hyperlipidemia in streptozotocin-induced diabetic rats. Streptozotocin, the insulin-producing

Table 7. Changes of serum total-cholesterol and LDL levels after STZ dosing and drug administration.

Groups	Total-cholesterol		LDL	
	At 6 days after STZ dosing	At end of 28-day dosing	At 6 days after STZ dosing	At end of 28-day dosing
Controls				
Intact	68.40±3.97	73.20±4.49	6.60±0.71	6.40±1.14
STZ	117.80±10.66*	141.40±7.13*	10.80±1.30*	15.60±1.14*
OG[a]	116.00±12.27*	123.40±4.62*,#	11.20±0.84*	13.80±0.84*,##
MC[a]	118.00±9.03*	124.10±8.65*,##	10.60±1.52*	13.60±1.14*,##
OG:MC[b]				
= 1:1	118.00±6.04*	115.60±13.48*,##	10.80±1.48*	12.60±1.52*,##
= 1:2	118.00±9.95*	89.20±6.76*,#	10.80±1.10*	9.40±1.14**,#
= 1:4	119.80±1.79*	111.40±11.10*,#	11.40±1.14*	10.20±1.30*,#
= 1:6	113.60±5.08*	119.20±10.40*,##	10.60±2.07*	12.00±1.58*,##
= 1:8	112.80±7.56*	118.40±7.13*,#	10.20±1.48*	12.40±1.14*,##
= 1:10	121.60±5.64*	121.60±8.59*,##	10.80±1.79*	13.40±0.89*,##
= 2:1	117.20±4.92*	105.80±10.76*,#	10.60±1.14*	11.80±0.84*,#
= 4:1	114.80±7.82*	113.20±14.53*,##	10.80±1.10*	11.00±1.87*,#
= 6:1	119.40±1.52*	118.60±11.19*,##	10.60±1.52*	12.60±1.52*,##
= 8:1	120.00±8.46*	120.60±2.70*,#	10.80±1.79*	12.80±1.30*,##
= 10:1	115.60±7.89*	124.40±13.22*,#	11.20±2.49*	13.40±1.14*,##

n=5 (Mean ± SD), mg/dl; [a]Single formulation; [b]Mixed formulation; *p<0.01 and **p<0.05 compared with that of intact control by MW test; #p<0.01 and ##p<0.05 compared with that of STZ control by MW test.

beta cells cytotoxin, induces "chemical diabetes" in a wide variety of animal species and induces its diabeteogenic activity mainly by inducing oxygen free radicals [13, 18] and damaging the insulin-secreting cells of the pancreas [9], leading to hyperglycemia [24, 28]. In this study, we have found the synergetic effect of OG and MC extract mixture in the STZ-induced diabetes and its complication. To find out the optimum composition of OG and MC extract mixture, each extract mixture was administered orally once a day for 28 days from 7 days after STZ dosing. Blood glucose, body and organ weights, serum BUN, creatinine, AST, ALT, LDL, and total-cholesterol were observed, respectively. Generally the hypoglycemic effects of extract would be based on the blood glucose levels, and the diabetic nephropathy has been tested based on the BUN and creatinine levels with AST and ALT levels for diabetic hepatopathy [2, 24, 28]. In addition, the effects of extract on the diabetic hyperlipemia has been evaluated base on the LDL, HDL, triglyceride, and total-cholesterol levels [20, 33].

As the result of STZ-induced diabetes, dramatic decrease of body weight, increase of the liver and kidney weights, and increase of blood glucose, serum BUN, creatinine, AST, ALT, LDL, and total-cholesterol levels in the STZ control were detected compared with intact control, respectively. However, these changes of hyperglycemia, diabetic nephropathy, hepatopathy, and hyperlipemia were dramatically decreased in OG and MC single-dosing and all composition groups, and more favorable effects in all composition groups were detected compared with OG and MC single-dosing groups. Among 11 types of mixed formula, the OG:MC 1:2 mixed group showed the most dramatic effects in this study.

The decreases of body weight and gain are observed along with progression of diabetes, and inhibitions of these decreases have been regarded as evidence of treatment of diabetes and its complications [16]. The body weight decrease induced by STZ was effectively inhibited by treatment of OG and MC single-dosing and all 11 types of composition, respectively. Among the 11 types of composition, OG:MC 1:1, 1:2, 2:1, and 4:1 mixed groups showed somewhat synergic effects for reducing the body weight decrease compared with OG and MC single-dosing, and the most favorable efficacy was detected in the OG:MC 1:2 mixed group in this study.

Hyperglycemia is the main sign of diabetes, and hyperglycemia should be controlled to treat the diabetes [28]. In this study, OG and MC single-extract and 11 types of composition showed favorable hypoglycemic effects, respectively. Among the 11 types of composition, OG:MC 1:1, 1:2, 1:4, 1:6, and 2:1 mixed groups showed somewhat synergic hypoglycemic effects compared with OG and MC single-dosing groups. The most favorable hypoglycemic efficacy was detected in the OG:MC 1:2 mixed group in this study.

As a progression of diabetes, increase of kidney weight due to the swelling, inflammation, and necrotic processes was observed with elevation of serum BUN and creatinine levels [32]. Improvement of these abnormal changes have been considered as direct evidence of inhibition or treating the diabetic nephropathy [8]. Diabetic nephropathy induced by STZ dosing was effectively inhibited by treatment of OG and MC single-dosing and all 11 types of composition, respectively. They inhibited the increases of kidney weights, and serum BUN and creatinine levels. All 11 types of composition showed somewhat synergic effects for reducing diabetic nephropathy compared with OG and MC single-dosing groups, except for the OG:MC 1:10 or 10:1 mixed groups in which quite similar nephroprotective effects were detected compared with each single extract. The most favorable nephroprotective efficacy was detected in the OG:MC 1:2 mixed group in this study.

As a progression of diabetes, increase of liver weight due to the fibrosis or abnormal glycosylations was observed with elevation of serum AST and BUN levels [6, 28]. Improvement of these abnormal changes has been considered as direct evidence of improved diabetic hepatopathy [24]. AST is found in several body tissues but is especially high in the liver and striated muscle. Serum AST activity is elevated with skeletal muscle necrosis and hepatocellular necrosis. Elevated serum AST activity with no ALT elevation indicates muscle necrosis, but AST activity rising more slowly than ALT indicates more complete cellular disruption in liver damage because it leaks from the cell only with necrosis, not membrane instability [31]. ALT is present in large quantities in the cytoplasm of hepatocytes. This enzyme enters the blood when liver cells are damaged or destroyed, and circulates for a few days. This enzyme is a sensitive indicator of active liver damage but does not indicate the cause or reversibility of the damage [31]. Diabetic hepatopathy induced by STZ was effectively inhibited by treatment of OG and MC single-extract and all 11 types of composition, respectively. They inhibited the increases of liver weight, and serum BUN and creatinine levels. All 11 types of mixed formulation showed somewhat synergic effects for reducing diabetic hepatopathy compared with the OG and MC single-extract. The most favorable hepatoprotective efficacy was detected in the OG:MC 1:2 mixed group in this study.

Hyperlipemia has been regarded as another diabetic complication [11]. Generally, the most critical problems in hyperlipemia are the decrease of serum HDL levels, and increase of LDL, triglyceride, and total-cholesterol levels [21]. Improvement of these abnormal changes has been considered as direct evidence of improved diabetic hyperlipemia [4, 20, 33]. In this study, the diabetic hyperlipemia induced

by STZ dosing was effectively inhibited by treatment of OG and MC single-extract and all 11 types of composition, respectively. They inhibited the increases of serum total-cholesterol and LDL levels. All 11 types of composition showed somewhat synergic hypolipemic effects compared with OG and MC single-dosing groups, except for the OG:MC 10:1 mixed group in which quite similar hypolipemic effects were detected compared with each single-extract. The most favorable hypolipemic efficacy was detected in the OG:MC 1:2 mixed group in this study.

Based on these results, the OG:MC 1:2 mixture showed the most dramatic anti diabetic effect among the 11 types of composition tested. Therefore, we selected OG:MC 1:2 as the optimum composition to improve diabetes and its complications. Although the exact synergic mechanisms were unknown in the present study, it is considered that favorable synergic effects of OG and MC were complicatedly mediated by various different action mechanisms: to delay the carbohydrate digestion, glucose utilization, free radical scavenger activity, insulin-like activity, and enhancement of pancreatic beta cells. However, more detailed dosage studies should be tested to detect the efficacy of this formula.

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