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Protective Effects of Vescalagin from Pink Wax Apple [*Syzygium samarangense* (Blume) Merrill and Perry] Fruit against Methylglyoxal-Induced Inflammation and Carbohydrate Metabolic Disorder in Rats

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ABSTRACT: The unbalance of glucose metabolism in humans may cause the excessive formation of methylglyoxal (MG), which can react with various biomolecules to form the precursor of advanced glycation end products (AGEs). Vescalagin (VES) is an ellagitannin that alleviates insulin resistance in cell study. Results showed that VES reduced the value of oral glucose tolerance test, cardiovascular risk index, AGEs, and tumor necrosis factor-α contents while increasing C-peptide and D-lactate contents significantly in rats orally administered MG and VES together. The preventive effect of VES on MG-induced inflammation and carbohydrate metabolic disorder in rats was thus proved. On the basis of the experiment data, a mechanism, which involves the increase in D-lactate to retard AGE formation and the decrease in cytokine release to prevent β-cell damage, is proposed to explain the bioactivities of VES in antiglycation and in the alleviation of MG-induced carbohydrate metabolic disorder in rats.

KEYWORDS: *vescalagin, methylglyoxal, inflammation, antiglycation, carbohydrate metabolic disorder*

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease involving inflammation in the development. The World Health Organization estimated that more than 346 million people worldwide suffered from DM in 2011. This number is likely to more than double by 2030 in the case of no intervention.[1] The increased formation of methylglyoxal (MG) was found in the early stage of DM.[2] The prevalence of DM, obesity, and many other metabolic syndromes has been linked to the increased consumption of fructose-containing foods.[3] Reactive α-dicarbonyl compounds, including MG, are often formed in the processing of sugar products.[4] MG is also an endogenous metabolite formed in virtually all mammalian cells primarily from the triosephosphate intermediates of glucose metabolism. Other important precursors for MG generation include aminoacetone and ketone bodies from protein catabolism and fatty acid oxidation, respectively.[5] Pathogenesis of inflammation and diabetes may involve MG as a major precursor of advanced glycation end products (AGEs).[6] Studies have found that MG and AGEs can increase oxidative stress, promote the generation of inflammatory cytokines, and induce DM.[6,7]

Serum MG levels in healthy humans are <1 μM but can be elevated to 2−6 μM in diabetic patients, with a positive correlation to the degree of hyperglycemia.[8] Many foods, including steak, coffee, and beer, were found to be associated with high MG levels in the serum of human blood. For example, dietary exposure of the population in Spain to MG from cookies was estimated to be 216 μg/person/day.[9] The broiled steak has been reported to contain 12.73 μg/g of MG in a study in the United States.[10] Approximately 230 μM (17.7 mg/L) of MG was found in coffee.[11] Wine and beer were found to contain 21.59 and 13.88 μM MG, respectively. Long-term MG intake has been shown to result in protein glycation and insulin resistance in animals.[6]

MG is a potent glycating agent that may react with DNA, lipids, and proteins to produce AGEs, including argpyrimidine, $N\delta$-(5-hydro-5-methyl-4-imidazolon-2-yl)ornithine (MG-H1), $N\varepsilon$-(carboxyethyl)lysine (CEL), methylglyoxal−lysine dimer (MOLD), etc.[12] The reaction causes structural changes in various proteins, including insulin, hemoglobin, proteinaceous growth factors, extracellular matrix (ECM) proteins, etc.[7] Arginine and lysine residues are among the major sites in protein molecules to link with MG molecules. Arginine modification leads to the formation of imidazolones, mainly MG-H1 and argpyrimidine, whereas lysine modification leads to CEL and MOLD formation.[7] A good indirect method to assess the accumulation of MG in diabetic models is to determine the increases in MG-H1 and argpyrimidine contents in the tissue.[13,14]

Several therapeutic methods that involve the scavenging of MG have been tested to prevent protein glycation and AGEs formation.[15] Aminoguanidine (AG) is an antiglycation agent that was shown to retard the deposition of ECM and AGEs on the vessel wall. AG may also prevent morphologic alterations of renal glomerulum and alleviate proteinuria in streptozotocin (STZ)-induced diabetic rats.[15] Many antioxidant products, for example, AG, trolox, triterpenesare, soy isoflavones, and some other flavonoids, were found to be effective in trapping MG and alleviating protein glycation.[7,16]

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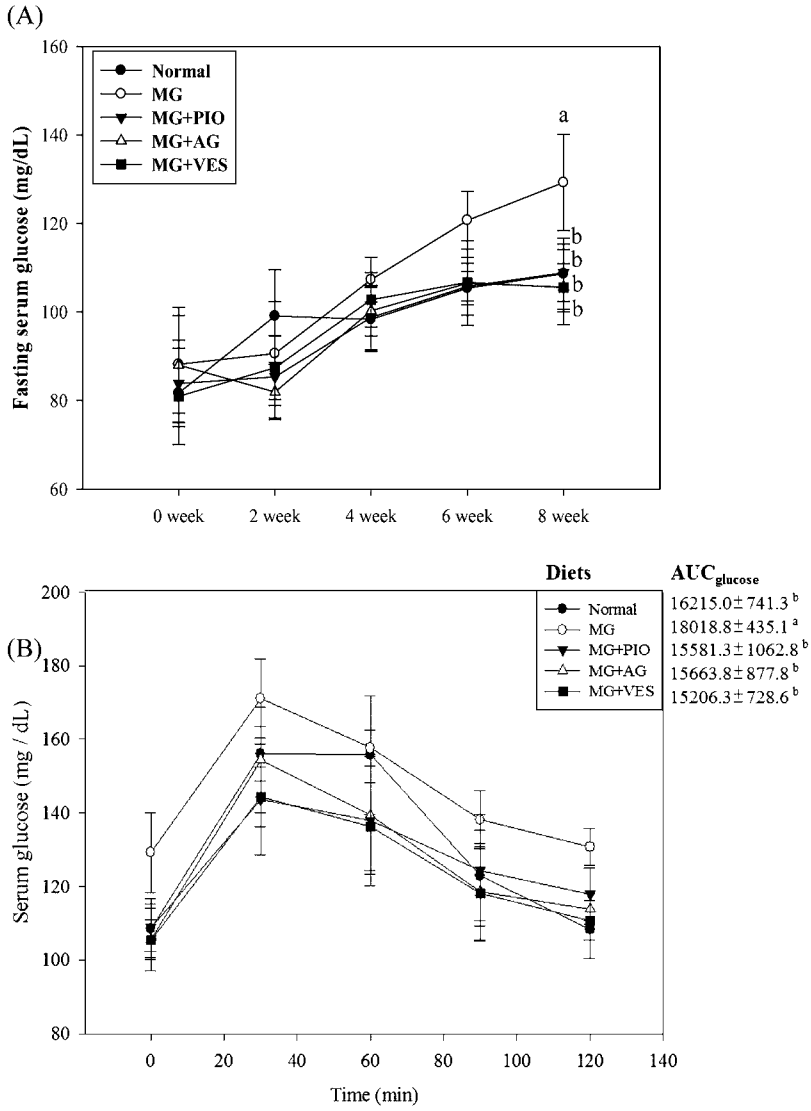

Figure 1. Serum glucose indicators of rats fed various diets: (A) fasting serum glucose levels; (B) $AUC_{glucose}$ values calculated from OGTT plot. Male Wistar rats (6 weeks old) were fed a normal diet and deionized water and orally administered or not a supplement for 8 weeks before sacrifice. Normal: rats fed a normal diet and deionized water. MG: rats fed a normal diet and deionized water and orally administered methylglyoxal (300 mg/kg body weight). MG+PIO: rats fed a normal diet and deionized water and orally administered methylglyoxal (300 mg/kg body weight) and pioglitazone (30 mg/kg body weight). MG+AG: rats fed a normal diet and deionized water and orally administered methylglyoxal (300 mg/kg body weight) and aminoguanidine (30 mg/kg body weight). MG+VES: rats fed a normal diet and deionized water and orally administered methylglyoxal (300 mg/kg body weight) and vescalagin (30 mg/kg body weight). Different letters (a, b) signify a statistically significant difference at $p < 0.01$. Results are from eight repetitions and expressed as the mean ± SD.

Ellagitannins are bioactive polyphenols with antioxidant and anti-inflammatory activities.[17] Wax apple fruit contains the ellagitannin vescalagin (VES). VES has been reported to be antitumor and cardiovascular disease preventive and to alleviative insulin resistance and dyslipidemia.[18,19] VES may also reduce serum glucose content with a simultaneous increase in serum insulin and C-peptide levels.[19] No literature with regard to the effects of VES on MG-induced carbohydrate metabolic disorder and inflammatory reactions has been reported yet. The aims of this study were to investigate the preventive effect of VES against MG-induced inflammation and carbohydrate metabolic disorder in rats and to elucidate its mechanism by analyzing the metabolites of MG in rats orally administered MG and VES.

MATERIALS AND METHODS

Chemicals. D-Glucose, MG, pioglitazone hydrochloride (PIO), AG, ethyl ether, and ethyl alcohol were purchased from Sigma (St. Louis, MO, USA). All chemicals were of analytical grade.

Preparation of VES. The method referred to Chang et al. with minor modifications.[20] Briefly, each 754 g aliquot of the reconstituted unripe wax apple fruit extract was run through a Diaion HP20 (Mitsubishi Chemical Industries, Tokyo, Japan) column, a Sephadex LH-20 (St. Louis, MO, USA) column, and an MCI-gel CHP 20P (Mitsubishi Chemical Industries) column in series with methanol/H_2O gradient elution. Each fraction was analyzed in thin-layer chromatography using Kieselgel 60 F254, 0.20 mm plates (Merck, Darmstadt, Germany), and a developing solvent of benzene/ethyl formate/formic acid = 1:5:2. Adjacent fractions of the eluate from a column were combined on the basis of the TLC profile, freeze-dried, and then redissolved in distilled water for further fractionation by the next column or for animal experiments for the eluate from the last

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Table 1. Masses of Selected Organs and Related Basal Serum Indices of Rats in Various Groups[a]

item	normal	MG	MG+PIO	MG+AG	MG+VES
kidney wt (g)	2.51 ± 0.16 b	2.95 ± 0.16 a	2.68 ± 0.13 b	2.65 ± 0.28 b	2.69 ± 0.14 b
liver wt (g)	11.4 ± 0.78b	13.2 ± 0.64 a	12.9 ± 0.74 b	10.8 ± 1.30 b	11.6 ± 0.63 b
pancreas wt (g)	1.29 ± 0.10 b	1.59 ± 0.12 a	1.25 ± 0.19 b	1.30 ± 0.27 ab	1.23 ± 0.12 b
adipose wt (g)	7.73 ± 0.89 a	8.43 ± 1.74 a	6.86 ± 1.75 a	6.80 ± 2.55 a	8.30 ± 1.96 a
AST (U/L)	188 ± 12.7 a	191 ± 24.0 a	136 ± 16.7 b	115 ± 24.1 c	99.6 ± 18.1 c
ALT (U/L)	52.1 ± 6.17 ab	59.0 ± 8.25 a	47.1 ± 7.20 bc	43.8 ± 6.27 c	43.6 ± 6.44 c
Alk-p (IU/L)	116 ± 8.57 a	113 ± 16.7 a	109 ± 12.3 ab	114 ± 20.7 a	97.3 ± 7.05 b
total protein (g/dL)	6.03 ± 0.12 a	5.95 ± 0.32 a	5.94 ± 0.48 a	5.35 ± 0.44 a	5.75 ± 0.38 a
albumin (g/dL)	4.24 ± 0.05 a	4.11 ± 0.19 ab	4.18 ± 0.25 a	3.81 ± 0.29 b	3.91 ± 0.25 ab
globulin (g/dL)	1.79 ± 0.06 a	1.70 ± 0.15 a	1.71 ± 0.24 a	1.48 ± 0.12 a	1.53 ± 0.19 a
Bili-total (g/dL)	0.08 ± 0.00 b	0.10 ± 0.01 a	0.09 ± 0.01 b	0.07 ± 0.01 c	0.06 ± 0.01 c
BUN (g/dL)	22.6 ± 0.85 a	21.7 ± 1.72 a	21.6 ± 1.16 a	21.6 ± 2.50 a	21.2 ± 2.75 a
creatinine (g/dL)	0.40 ± 0.00 a	0.41 ± 0.06 a	0.34 ± 0.12 ab	0.31 ± 0.06 b	0.34 ± 0.09 ab

[a]Normal: rats fed a normal diet and deionized water. MG: rats fed a normal diet and deionized water and orally administered methylglyoxal (300 mg/kg body weight). MG+PIO: rats fed a normal diet and deionized water and orally administered methylglyoxal (300 mg/kg body weight) and pioglitazone (30 mg/kg body weight). MG+AG: rats fed a normal diet and deionized water and orally administered methylglyoxal (300 mg/kg body weight) and aminoguanidine (30 mg/kg body weight). MG+VES: rats fed a normal diet and deionized water and orally administered methylglyoxal (300 mg/kg body weight) and vescalagin (30 mg/kg body weight). Different letters on the same line signify a statistically significant difference at $p < 0.01$. Results are from eight repetitions and expressed as the mean ± SD. AST, aspartate aminotransferase; ALT, alanine aminotransferase; Alk-p, alkaline phosphatase; Bili-total, total bilirubin; BUN, blood urea nitrogen.

column. The recovery of VES from the fruit was approximately 0.085% of the original weight of fruit.

Animals and Diets. Male Wistar rats (5 weeks old) were supplied by the National Laboratory Animal Breeding and Research Center, Taipei, Taiwan. The rats were maintained at standard laboratory conditions, at a temperature of 22 ± 1 °C with a 12 h light/12 h dark cycle, with free access to food and water for the entire duration of the study. The rats were fed a normal diet and deionized water for 1 week and then divided into five groups of eight rats each. One group was fed a normal diet and deionized water for 8 more weeks, and the other four groups were fed a normal diet and deionized water and orally administered 300 mg/kg bw/day of MG plus 30 mg/kg bw/day of deionized water, PIO, AG, or VES for 8 weeks. Each animal was fed MG first, followed by water or the chemical. Animals were sacrificed involving the use of ethyl ether asphyxia before the following analysis was performed.

Blood Sample Collection. Blood samples were taken from the venter vein of the sacrificed rat, allowed to clot for 30 min at room temperature, and then centrifuged at 3000*g* for 20 min to obtain the plasma, which was stored at −80 °C before use.

Oral Glucose Tolerance Test (OGTT). The OGTT was performed in overnight-fasted rats from all experimental groups in 2 days before sacrifice. All animals orally received a load of 1.5 g glucose/kg bw. Blood samples were taken from the tail veins of conscious animals before the oral administration of glucose ($t = 0$) and 30, 60, 90, and 120 min after. The samples were allowed to clot for 30 min and then centrifuged (4 °C, 3000*g*, 20 min) to collect the serum. Glucose content was determined with a glucose enzymatic kit (Crumlin, Antrim, UK). The mean area under curve of the oral glucose tolerance test ($AUC_{glucose}$) was calculated from the OGTT plot.

Biochemical Analyses. ELISA kits for rat aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (Alk-P), total bilirubin (Bili-total), total protein, albumin, globulin, blood urea nitrogen (BUN), creatinine, total cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), free fatty acid, insulin, C-peptide, fructosamine, AGEs, D-lactate, tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) were purchased from Randox Laboratories (Crumlin, Antrim, UK). Biochemical analyses were performed following the supplier's protocols.

Statistical Analysis. Results expressed as means ± SD were analyzed by one-way ANOVA and Duncan's new multiple-range tests. All p values <0.01 were considered to be significant.

RESULTS

Induction of Carbohydrate Metabolic Disorder. Serum glucose indicators of the rats in the feeding experiment are shown in Figure 1. The fasting serum glucose level in rats fed a normal diet and deionized water and orally administered MG without other supplements (MG group) for 8 weeks reached 129.25 ± 10.9 mg/dL, which was significantly higher ($p < 0.01$) than the value of 108.6 ± 8 mg/dL found in the normal group (Figure 1A). The average $AUC_{glucose}$ value of rats in the MG group at end of the eighth week reached 18018.8 ± 435.1, which was also significantly higher than the value 16215 ± 741.3 in the normal group (Figure 1B). These results indicate the successful induction of carbohydrate metabolic disorder in rats by oral administration with MG.

Effect of VES on $AUC_{glucose}$. Figure 1B also shows the effect of VES on $AUC_{glucose}$ in the carbohydrate metabolic disordered rats. PIO is an enhancer of insulin sensitivity in diabetic patients.[6] AG is an effective scavenger of MG for preventing AGE formation.[21] Both PIO and AG are capable of alleviating the MG-induced carbohydrate metabolic disorder.[6,21] Significant differences in the initial ($t = 0$) serum glucose levels were found between the MG group and normal group, the rats fed a normal diet and deionized water and orally administered MG and PIO (MG+PIO group), or the rats fed a normal diet and deionized water and orally administered MG and AG (MG+AG group). In 30−120 min after the oral administration of glucose, the MG group showed a higher increment in $AUC_{glucose}$ as compared with the other groups ($p < 0.01$). The rats fed a normal diet and deionized water and orally administered MG and VES (MG+VES group) showed a significantly lower $AUC_{glucose}$ level ($p < 0.01$) in comparison with the MG group. No significant differences in $AUC_{glucose}$ level were found between the MG+VES group and the MG +PIO group or MG+AG group.

Effect of VES on Masses of Selected Organs and Related Basal Serum Indices. Table 1 shows the effect of VES on the masses of selected organs and related basal serum indices in rats. The weights of kidney, liver, and pancreas were significantly higher in the MG group at end of the eighth week

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Table 2. Selected Serum Biochemical Parameters of Rats in Various Groups[a]

item	normal	MG	MG+PIO	MG+AG	MG+VES
triglyceride (mg/dL)	51.0 ± 1.85 a	48.3 ± 9.11 ab	42.8 ± 4.74 bc	36.8 ± 8.61 c	43.4 ± 6.23 bc
cholesterol-T (mg/dL)	65.3 ± 7.28 b	70.1 ± 7.30 a	60.8 ± 6.96 b	52.5 ± 10.1 b	60.6 ± 10.2 b
free fatty acid (mmol/L)	1.13 ± 0.09 a	0.99 ± 0.06 b	0.65 ± 0.07 c	0.67 ± 0.14 c	0.66 ± 0.05 c
LDL-C (mg/dL)	8.38 ± 1.30 b	10.4 ± 2.67 a	7.86 ± 1.46 bc	7.88 ± 1.46 bc	6.13 ± 2.36 c
HDL-C (mg/dL)	60.3 ± 3.82 a	55.5 ± 5.40 a	56.4 ± 5.70 a	55.0 ± 9.57 a	55.7 ± 9.15 a
cardiovascular risk index	1.13 ± 0.05 b	1.23 ± 0.10 a	0.99 ± 0.07 c	1.03 ± 0.08 c	1.05 ± 0.08 c

[a]Normal: rats fed a normal diet and deionized water. MG: rats fed a normal diet and deionized water and orally administered methylglyoxal (300 mg/kg body weight). MG+PIO: rats fed a normal diet and deionized water and orally administered methylglyoxal (300 mg/kg body weight) and pioglitazone (30 mg/kg body weight). MG+AG: rats fed a normal diet and deionized water and orally administered methylglyoxal (300 mg/kg body weight) and aminoguanidine (30 mg/kg body weight). MG+VES: rats fed a normal diet and deionized water and orally administered methylglyoxal (300 mg/kg body weight) and vescalagin (30 mg/kg body weight). Different letters on the same line signify a statistically significant difference at $p < 0.01$. Results are from eight repetitions and expressed as the mean ± SD. LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; cardiovascular risk index, cholesterol-T/HDL-C ratio.

Table 3. Selected Pancreas Indices Related to Methylglyoxal Metabolism of Rats in Various Groups[a]

item	normal	MG	MG+PIO	MG+AG	MG+VES
insulin (μg/L)	0.25 ± 0.01 b	0.22 ± 0.00 c	0.26 ± 0.02 b	0.25 ± 0.04 bc	0.41 ± 0.11 a
C-peptide (pmol/L)	411.9 ± 106.1 ab	259.4 ± 71.8 c	396.6 ± 74.2 ab	357.9 ± 80.9 bc	470.5 ± 106.9 a
fructosamine (μmol/L)	86.38 ± 2.56 b	90.88 ± 5.38 a	78.88 ± 2.10 c	86.0 ± 2.83 b	83.13 ± 3.34 b
AGEs (μg/mL)	2.76 ± 0.54 a	2.50 ± 0.51 a	2.52 ± 0.78 ab	2.03 ± 0.66 b	1.81 ± 0.29 b
D-lactate (mg/dL)	78.0 ± 6.64 b	86.0 ± 20.7 b	125.0 ± 7.63 a	110.0 ± 19.1 a	114.0 ± 19.9 a
methylglyoxal (μg/mL)	215.2 ± 63.1 a	175.3 ± 37.4 ab	177.0 ± 70.8 ab	170.0 ± 75.8 ab	124.7 ± 20.6 b

[a]Normal: rats fed a normal diet and deionized water. MG: rats fed a normal diet and deionized water and orally administered methylglyoxal (300 mg/kg body weight). MG+PIO: rats fed a normal diet and deionized water and orally administered methylglyoxal (300 mg/kg body weight) and pioglitazone (30 mg/kg body weight). MG+AG: rats fed a normal diet and deionized water and orally administered methylglyoxal (300 mg/kg body weight) and aminoguanidine (30 mg/kg body weight). MG+VES: rats fed a normal diet and deionized water and orally administered methylglyoxal (300 mg/kg body weight) and vescalagin (30 mg/kg body weight). Different letters on the same line signify a statistically significant difference at $p < 0.01$. Results are from eight repetitions and expressed as the mean ± SD. AGEs, advanced glycation end products.

as compared with the normal group. In comparison with the MG group, there are 9.15, 10.2, and 8.81% reductions in kidney weight and 2.27, 18.2, and 12.1% reductions in liver weight in MG+PIO, MG+AG, and MG+VES groups, respectively ($p < 0.01$). The weight of pancreas decreased by 21.4 and 22.6% in MG+PIO and MG+VES groups, respectively, as compared with MG group. There was no significant difference in adipose tissue weight among all groups.

Significant increases in serum AST (by 191 ± 24.0 U/L), ALT (by 59 ± 8.25 U/L), and Bili-T (by 0.1 ± 0.01 g/dL) were observed in the MG group as compared with the normal group ($p < 0.01$). In comparison with the MG group, those groups fed a normal diet and orally administered PIO, AG, or VES showed significant reductions of 28.8, 39.8, and 47.9% in serum AST, 20.2, 25.8, and 26.1% in serum ALT, and 10, 30, and 40% in serum Bili-T, respectively ($p < 0.01$). The serum Alk-p decreased by 13.4% in the MG+VES group as compared with the MG group. There were no significant differences in serum total protein, albumin, globulin, BUN, and creatinine contents among all groups.

Effect of VES on Serum Triglyceride, Total Cholesterol, Free Fatty Acid, LDL-C, HDL-C, and Cardiovascular Risk Index. The cardiovascular risk index of rats in the MG group at the end of the eighth week reached 1.23 ± 0.1, which was significantly higher than the value in the normal group, 1.13 ± 0.05 (Table 2). Meanwhile, the total cholesterol, free fatty acid, LDL-C contents, and cardiovascular risk index in the MG+VES group decreased by 13.6, 33.3, 41.1, and 14.6%, respectively, as compared with the MG group ($p < 0.01$). Total cholesterol, free fatty acid, LDL-C contents, and cardiovascular risk index decreased by 13.3, 34.3, 24.4, and 19.5% in the MG+PIO group and by 25.1, 32.3, 24.2, and 16.3% in the MG+AG group, respectively. No significant differences in serum triglyceride and HDL-C contents were found between the MG+VES group and the MG+PIO or MG+AG group.

Effect of VES on Serum Insulin, C-Peptide, and MG-Related Metabolites. Table 3 shows that serum insulin and C-peptide contents were significantly lower in the MG group at the end of the eighth week as compared with the normal group ($p < 0.01$). Contents of insulin and C-peptide in the MG+VES group were higher than those in the MG group ($p < 0.01$) by 86.3 and 81.4%, respectively. The fructosamine content in the MG group was significantly higher than those in the normal, MG+PIO, MG+AG, and MG+VES groups ($p < 0.01$). Fructosamine and AGE contents in the MG+VES group were lower than those in the MG group by 8.53 and 27.6%, respectively ($p < 0.01$). D-Lactate content in the MG+VES group was higher than that in the MG group by 32.6% ($p < 0.01$). There were no significant differences in serum MG content among MG, MG+PIO, MG+AG, and MG+VES groups.

Effect of VES on Serum TNF-α and IL-6. Figure 2 shows the effect of VES on TNF-α and IL-6 contents in rats. Significantly higher serum TNF-α and IL-6 contents were observed in the MG group as compared with the normal group, 851.5 ± 45.3 vs 139.4 ± 33.9 pg/mL and 516 ± 37 vs 60.1 ± 12.8 pg/mL, respectively ($p < 0.01$). MG+PIO, MG+AG, and MG+VES groups contained significantly lower amounts of serum TNF-α and IL-6 as compared with the MG group, by 59, 70.8, and 65.9% in TNF-α and 41.2, 58.4, and 51.7% in IL-6, respectively ($p < 0.01$).

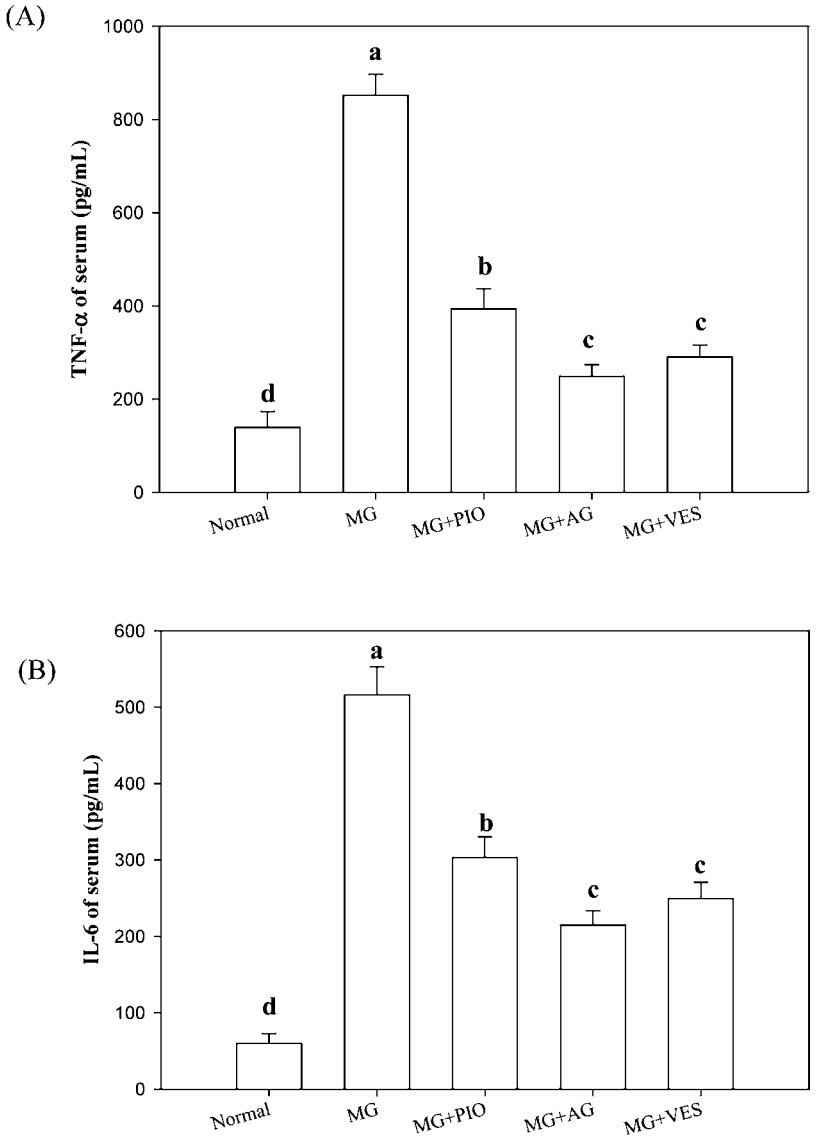

Figure 2. Effect of vescalagin on concentrations of cytokines, (A) TNF-α and (B) IL-6, in rat serum. Normal: rats fed a normal diet and deionized water. MG: rats fed a normal diet and deionized water and orally administered methylglyoxal (300 mg/kg body weight). MG+PIO: rats fed a normal diet and deionized water and orally administered methylglyoxal (300 mg/kg body weight) and pioglitazone (30 mg/kg body weight). MG+AG: rats fed a normal diet and deionized water and orally administered methylglyoxal (300 mg/kg body weight) and aminoguanidine (30 mg/kg body weight). MG+VES: rats fed a normal diet and deionized water and orally administered methylglyoxal (300 mg/kg body weight) and vescalagin (30 mg/kg body weight). Different letters (a–d) signify a statistically significant difference at $p < 0.01$. Results are from eight repetitions and expressed as the mean ± SD.

DISCUSSION

Dhar et al. demonstrated the induction of abnormality in glucose homeostasis by the chronic infusion of MG to rats.[8] The down-regulation of pro-inflammation cytokines and AGEs is among the effective mechanisms to ameliorate the abnormality.[8] VES has been proved to be an active compound in alleviating insulin resistance of mouse hepatocyte FL83B cells.[20] No animal studies with regard to changes in the expressions of pro-inflammation cytokines and AGEs by the intake of VES have been reported yet. The present study demonstrates the ability of VES to promote MG catabolism, to down-regulate pro-inflammation cytokines, and thereby to alleviate MG-caused carbohydrate metabolic disorder in rats.

MG and AGEs, such as fructosamine, are promoters of oxidative stress and insulin-resistance in vivo.[22,23] Elevated MG content and reactive oxygen species (ROS) concentration have been reported to induce insulin resistance in MG-cultured human hepatocellular carcinoma cells (HepG2 cells) and pancreas tissues of MG-infused Sprague–Dawley rats.[6,8] Results of the present study showed that the fasting serum glucose level and $AUC_{glucose}$ value in rats of the MG group are higher than those in the normal group (Figure 1). These results confirmed the effect of MG to induce insulin resistance in rats in feeding test and support the aforementioned studies.[6,8] Matafome et al. proposed the possible involvement of MG in mechanisms that induce insulin resistance, β-cell damage, and hyperglycemia.[7,8] PIO and VES are effective antioxidants for alleviating insulin resistance.[20,24] PIO is an insulin sensitizer and a peroxisome proliferator activated receptor γ (PPARγ) agonist as well.[25,26] The fasting serum glucose levels and $AUC_{glucose}$ values of the rats in the MG+PIO and MG+VES groups are lower than those in the MG group (Figure 1), suggesting the

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possibility of VES to be used as an insulin sensitizer and a PPARγ agonist in alleviating insulin resistance in diabetic conditions.

MG promotes the formation of peroxynitrite and pro-inflammatory cytokines in various cell types such as vascular smooth muscle cells, renal mesangial cells, and hepatocytes of rats.[7] It has been reported that inflammatory reactions stimulate the synthesis of collagen by fibroblasts and that antioxidants, such as α-tocopherol, can attenuate pancreas fibrosis by inhibiting inflammation.[27] In the present study, rats in the MG+VES group have lower weights of organs, including kidney, liver, and pancreas, as compared with those in the MG group (Table 1). Chao et al. reported that the animals treated with antioxidative substance showed significantly smaller increases in kidney and liver weights than the normal group.[28] We propose that the reduced weights of organs in the MG+VES group are a consequence of the alleviation of MG-induced inflammatory reactions by VES.

No information in regard to the anti-inflammation effect of VES in animals has been reported. The basal serum indices of liver and kidney in the sacrificed rats were evaluated in the present study. No differences in total protein, albumin, globulin, BUN, and creatinine contents were found among groups, whereas AST and ALT contents were significantly lower in the MG+PIO, MG+AG, and MG+VES groups in comparison with the MG group (Table 2). Sureshkumar and Mishra demonstrated that cytokine may alter cellular membranes in the state of liver lipid peroxidation, resulting in extracellular leakage of cytoplasmic enzymes such as AST and ALT.[29] Changes in serum levels of these enzymes are common indices for the evaluation of hepatic injury.[30] We propose that VES attenuates the leakage of AST and ALT through cellular membranes via the reduction in MG-induced cytokine release.

Serum Alk-p and Bili-T contents are significantly lower in the MG+VES group as compared with the MG group (Table 1). These contents are among key indices of liver condition. Previous study showed that fructose intake promotes lipid peroxidation, inflammatory reaction, and Alk-p and Bili-T formation.[29] Furthermore, excessive sugar intake causes tissue damage and serum protein disorders associated with oxidative and carbonyl stress cytotoxicity, as well as inflammation. These changes may lead to glucose homeostasis unbalance, artery calcification, and liver tissue cirrhosis in animals.[31] There is a positive correlation between MG content and glucose or fructose intake in animals.[4] We proved previously that VES reduces Alk-p and Bili-T contents in high-fructose diet rats via mediating dyslipidemia.[19] We now postulate that the lower contents of Alk-p and Bili-T in rats in the MG+VES group as compared with those in the MG group, as shown in Table 1, are also due to the mediation of dyslipidemia.

Dyslipidemia occurs when the contents of serum triglyceride, cholesterol, LDL, or reduced HDL in humans are abnormal. Insulin resistance at the adipocyte increases the release of free fatty acids into blood circulation. Increased free fatty acid flux to liver stimulates the assembly and secretion of very low density lipoprotein, which can be transformed to LDL by hepatic lipase or lipoprotein lipase.[32] There is a positive correlation between insulin resistance and free fatty acid content in insulin-resistant rats.[7] Our results show that cholesterol, free fatty acids, and LDL contents of the rats in the MG+PIO, MG+AG and MG+VES groups are lower than those in the MG group (Table 2), suggesting that VES mediates dyslipidemia via the alleviation of insulin resistance.

Previous studies showed that MG and AGEs can promote inflammatory reaction, metabolic disorder, and hyperglycemia.[7,33] Elevated levels of blood AGEs seem to be associated with diabetic microvascular complications. MG is more powerful in inducing vascular endothelial inflammatory injuries than AGEs, suggesting that MG may increase the risk of cardiovascular diseases in diabetic patients via the promotion of arterial atherogenicity.[34] AG has been recognized as an antiglycation agent that reacts rapidly with aldehydes, such as MG, thereby preventing AGE formation.[35] Our results show that cardiovascular risk was significantly lower in rats of the MG+VES and MG+AG groups in comparison with rats in the MG group (Table 2). Some ellagitannins other than VES have been reported to improve cardiovascular, metabolic, and liver functions in high-carbohydrate and high-fat diet rats.[36] We speculate that VES, which is a potent scavenger, reduces AGE production and thereby alleviates the cardiovascular complications of diabetes.

Exposure to MG may decrease insulin secretion from INS-1E (insulinoma) cells via redox-independent inhibition of the IRS-1/PI3K/Akt pathway.[37] MG was found to down-regulate pancreatic and duodenal homeobox 1 (PDX-1), the major transcription factor involved in insulin synthesis, and to inhibit insulin secretion from isolated β-cells in rats.[8] Rats in the MG+VES group showed significantly higher contents of plasma insulin and C-peptide as compared with those of the MG group (Table 3). C-peptide and insulin are the two products of the enzymatic cleavage of pro-insulin in equimolar concentrations. Both C-peptide and insulin levels have been reported to be good indices of insulin secretion.[19] Hotamisligil suggested that inflammation is associated with insulin resistance.[38] Cytokine TNF-α interferes with insulin signal transduction and subsequently influences carbohydrate metabolism in cells and tissues.[39] We speculate that VES alleviates inflammatory reaction and TNF-α release, reduces β-cell damage in MG-treated rats, and improves signal transduction for insulin secretion.

MG is both a potent inducer of oxidative stress and a major precursor of AGEs, which also promote oxidative stress.[7] Oxidative stress plays an important role in the pathophysiology of protein glycation, inflammation, insulin resistance, atherogenesis, and diabetes.[8] We observed the increase in the concentrations of serum fructosamine and AGEs in MG group (Table 3). MG infusion in rats causes protein damage via the formation of glycation products, such as fructosamine and AGEs.[22] Frustosamine is a good indicator for prediabetes in mammals and also an early product of advanced glycation.[5,19] Several therapeutic methods have been tested to scavenge dicarbonyls, including MG, for preventing protein modification and AGE formation.[7] AG is known for its action in blocking glycation and preventing arterial protein cross-linking in diabetes patients.[40] The present study demonstrated that fructosamine and AGE contents are highest in the MG group, followed by the MG+AG group, and lowest in the MG+VES group (Table 3), indicating that VES is better than AG in glycation inhibition.

Quantification of D-lactate in serum and urine in rats is an effective method to evaluate the activity of the glyoxalase system in MG metabolism.[7] The content of D-lactate in rats of the MG+VES group is higher than that of those in the MG group (Table 3). MG can be eliminated either by scavengers or by enzymes, such as glyoxalases.[7,33] Glyoxalases I and II in cytosol may hydrolyze MG to D-lactoylglutathione and D-lactate

and thereby retard the hyperglycemia-induced formation of AGEs.[33,41] On the basis of the data in Table 3, we propose that VES promotes MG hydrolysis, increases the amount of metabolites, such as D-lactate, and thereby alleviates the formation of AGEs.

MG and AGEs may promote the release of pro-inflammatory cytokines, increase the activity of many pro-oxidant enzymes such as NADPH oxidase and c-Jun N-terminal kinases (JNK), and up-regulate the expression of advanced glycation end product receptor (RAGE) in inflammation.[7] RAGE can promote ROS formation, activate protein kinase-C, extracellular-regulated kinase (ERK)-1/2, and JNK, and lead to NF-κB translocation to the nucleus as a consequence. NF-κB in the nucleus in turn up-regulates cytokines (TNF-α, IL-1, and IL-6) release.[7] The reduction in AGE formation in rats in the MG +VES group resulted in lower TNF-α and IL-6 contents as compared with those in the MG group (Figure 2). The mechanism for VES to prevent inflammation in rats in the presence of MG may presumably involve a reduction in TNF-α and IL-6 contents.

On the bais of all the experimental data in the present study, we propose a mechanism, as shown in Figure 3, for VES to alleviate hyperglycemia and metabolic disorder in rats orally administered MG, which involves the reduction in MG and AGE contents, followed by the consequential reduction in cytokine contents.

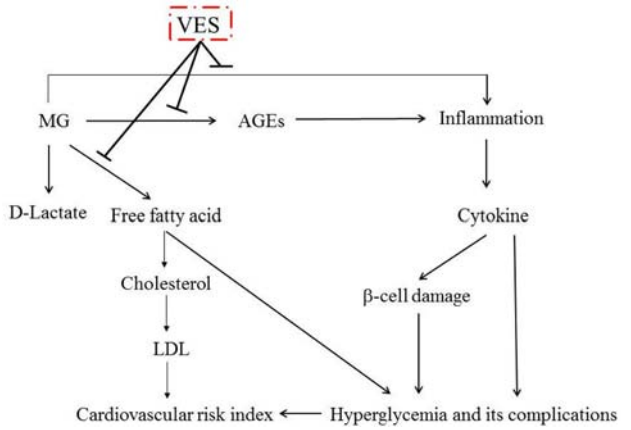

Figure 3. Postulated mechanism for VES to prevent MG-induced inflammation and carbohydrate metabolic disorders in the rats orally administered MG and VES.

In the present study we demonstrated that VES promotes anti-inflammatory and antiglycation bioactivities and alleviates hyperglycemia in rats orally administrated MG. We also found that VES down-regulates the expressions of pro-inflammatory factors involved in MG metabolism and increases insulin secretion in β-cells in the rats. The protective effects of VES against MG-induced inflammation and carbohydrate metabolic disorder were thus confirmed in vivo. These findings suggest the potential for VES to become an ingredient of food supplements in the prevention of diabetes and its complications. The effect of VES on signal pathways of MG metabolism is currently being investigated in our laboratory.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

MG, methylglyoxal; AGEs, advanced glycation end products; VES, vescalagin; DM, diabetes mellitus; MG-H1, Nδ-(5-hydro-5-methyl-4-imidazolon-2-yl)ornithine; CEL, Nε-(carboxyethyl)lysine; MOLD, methylglyoxal−lysine dimer; ECM, extracellular matrix; AG, aminoguanidine; STZ, streptozotocin; PIO, pioglitazone hydrochloride; OGTT, oral glucose tolerance test; $AUC_{glucose}$, area under curve of oral glucose tolerance test; AST, aspartate aminotransferase; ALT, alanine aminotransferase; Alk-P, alkaline phosphatase; Bili-total, total bilirubin; BUN, blood urea nitrogen; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; ROS, reactive oxygen species; PPARγ, peroxisome proliferator activated receptor γ; PDX-1, duodenal homeobox 1; JNK, c-Jun N-terminal kinases; RAGE, advanced glycation end products receptor

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