Protective effects of aqueous extract from pepino (*Solanum muricatum* Ait.) in diabetic mice

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Running title: Anti-diabetic effects of pepino

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Abstract

BACKGROUND: This study analyzed the content of ascorbic acid, phenolic acids and flavonoids in aqueous and ethanol extracts of pepino (*Solanum muricatum* Ait.); and examined the protective effects of pepino aqueous extract (PAE) in diabetic mice. PAE at 1, 2, and 4% was supplied for 5 weeks.

RESULTS: Aqueous and ethanol extracts had similar level of total phenolic acids; but PAE had higher content of ascorbic acid and total flavonoids than ethanol extract. PAE treatments at 2 and 4% significantly lowered plasma glucose level ($P<0.05$); however, only at 4% significantly elevated plasma insulin level at wk 5 ($P<0.05$). PAE treatments significantly decreased malonyldialdehyde and reactive oxygen species levels in kidney ($P<0.05$); however, only 2 and 4% treatments significantly reduced oxidized glutathione formation, increased glutathione level, and retained renal glutathione peroxidase and catalase activities ($P<0.05$). PAE treatments at 2 and 4% significantly lowered renal interleukin (IL)-6 and tumor necrosis factor-α levels ($P<0.05$); however, only 4% treatments significantly diminished renal IL-1β and monocyte chemoattractant protein-1 levels ($P<0.05$). PAE treatments, at 4%, significantly decreased aldose reductase activity and sorbitol production in kidney ($P<0.05$).

CONCLUSION: These findings support that pepino aqueous extract could attenuate diabetic progression via its anti-oxidative, anti-inflammatory and anti-glycative effects.

Keywords: *Solanum muricatum* Ait; pepino; diabetes; oxidative stress; glycation; phytochemicals

INTRODUCTION

Pepino (*Solanum muricatum* Ait.) is a plant food with a sweet smell and yellow skin color with purple stripes. The original cultivation of pepino extended along the Andes, from southern Colombia to Bolivia and the Peruvian coast.[1] This plant food is considered as a fruit in Europe, and it has been cultivated as a new vegetable in Iran.[2] Pepino is a popular food in Penghu island, Taiwan. Local residents in that island always treat it as a vegetable. The volatile aroma constituents of pepino have been analyzed.[3] These authors reported that pepino contained terpenes and β-damascenone, which contributed to the exotic aromas of this food. So far, it remains unknown whether this plant food contains phenolic acids or flavonoids. If pepino is rich in these phytochemicals, this plant food may possess nutraceutical functions.

The anti-tumor effect of pepino has been reported.[4] These authors found that a lyophilized aqueous fraction extracted from pepino possessed cytotoxic activity against test tumor cell lines including prostate, stomach, liver, breast cancer cells, and concluded that this plant food could target various tumor cells by triggering apoptosis. Although the active compound(s) responsible for the anti-tumor effects of pepino remain unclear, this previous study implied that pepino was a potent medicinal food. Based on the safety and economic consideration, taking this plant food directly for consumers may be more practical than using its components. Therefore, the investigation and/or application of extracts from this plant food for preventing and alleviating the development of chronic diseases are reasonable and worthy.

Diabetes is a common chronic disease in Taiwan and other countries. Diabetic individuals are encouraged to consume more fresh vegetables and fruits in order to obtain phenolic compounds and flavonoids because most of these phytochemicals possess bioactivities, and may modify glucose homeostasis.[5] Thus, an animal study was designed to examine the effect of pepino extract on glycemic control in diabetic mice. Furthermore, it is well known that oxidative injury, inflammatory stress and activation of polyol pathway are interrelated, and

contributed to the diabetic pathological development or deterioration.[6-8] Therefore, the anti-oxidative, anti-inflammatory and anti-glycative effects from pepino extract were determined by measuring the variation of reactive oxygen species, glutathione, inflammatory cytokines, and activity of certain enzymes responsible for antioxidant defense and polyol pathway in diabetic mice.

In our present study, the content of phenolic acids and flavonoids in both aqueous and ethanol extracts of pepino was analyzed. The possible protective effects and actions from this plant food against diabetic progression were examined. These results could enhance understanding regarding the composition and application of pepino.

MATERIALS AND METHODS

Materials

Fresh pepino (*Solanum muricatum* Ait.), harvested in spring, 2008, was obtained from farms in Penghu island, Taiwan. A 50 g edible portion of pepino was chopped and mixed with 150 mL sterile distilled water, or 50% ethanol at 25 °C for 12 h, and followed by homogenizing in a Waring blender. After filtration through Whatman No. 1 filter paper, the filtrate was further freeze-dried to a fine powder. Pure standards of several phenolic acids and flavonoids were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Determination of ascorbic acid, phenolic acids and flavonoids content

Ascorbic acid content in aqueous and ethanol extracts of pepino was analyzed by the method of Zapata and Dufour.[9] Total phenolic acids content was determined by the Folin-Ciocalteau method.[10] Extract sample at 0.5 mL was mixed with 2.5 mL of 0.2 N Folin-Ciocalteau reagent for 5 min, and further mixed with 2 mL of 75 g L^{-1} sodium carbonate. After 2 h incubation, the absorbance was measured at 760 nm and result was expressed as gallic acid equivalents. Total flavonoids content was measured using the method of Zhishen *et al.*[11]

Sample at 0.5 mL was mixed with 0.5 mL of 2% $AlCl_3$ ethanol solution. After 1 h incubation, the absorbance was measured at 420 nm and result was expressed as quercetin equivalents. The content of caffeic acid, cinnamic acid, coumaric acid, ellagic acid, ferulic acid, rosmarinic acid, epicatechin, myricetin, naringenin, quercetin and rutin in aqueous and ethanol extracts of pepino was determined by HPLC methods described by Sellappan *et al*.[12] HPLC equipped with a diode array UV-visible detector and a Phenomenex Prodigy 5-μ, ODS-2, RP C18 column was used, and UV spectra were recorded from 220 to 450 nm. Quantification was performed based on external standards (6 phenolic acids and 5 flavonoids) with known concentrations. Calibration curves of these standards ranging from 10 to 200 ng mL^{-1} were used with good linearity and R^2 values exceeding 0.98 (peak areas vs concentration), and peak areas were used to quantify the content of each phenolic acid or flavonoid in the sample.

Animals and diets

Male Balb/cA mice, 3-4 wk old, were obtained from National Laboratory Animal Center (National Science Council, Taipei City, Taiwan). The use of mice was reviewed and approved by Chung Shan Medical University animal care committee. To induce diabetes, mice with body weight of 22.1 ± 1.2 g were treated with streptozotocin (50 mg kg^{-1} body weight in 0.1 mol L^{-1} citrate buffer, pH 4.5) i.p. for 3 consecutive days. The blood glucose level was monitored on d 10 from the tail vein using a one-touch blood glucose meter (Lifescan Inc. Milpitas, CA, USA). Mice with fasting blood glucose levels ≥ 14.0 mmol L^{-1} were used for this study. After diabetes was induced, mice were divided into several groups (10 mice per group).

Experimental design

Because pepino aqueous extract (PAE) contained more ascorbic acid and total flavonoids (as shown in Table 1), this extract was used for anti-diabetic study. Powder of PAE at 1, 2 or 4 g was mixed with 99, 98 or 96 g standard powder diet. After five weeks supplementation,

kidney from each mouse was collected and weighted. Blood was also collected, and plasma was separated from erythrocyte immediately. Kidney at 0.1 g was homogenized on ice in 2 mL phosphate buffer saline (PBS, pH 7.2). The protein concentration of plasma and kidney homogenate was determined by the method of Lowry *et al.*[13] using bovine serum albumin as a standard. In all experiments, sample was diluted to a final concentration of 1 g protein L^{-1}.

Blood glucose and insulin analyses

The plasma glucose level (mmol L^{-1}) was measured by a glucose HK kit (Sigma Chemical Co., St. Louis, MO, USA). Plasma insulin level (nmol L^{-1}) was measured by using a rat insulin kit (SRI-13K, Linco Research Inc., St. Charles, MO, USA).

Glutathione (GSH) and oxidized glutathione (GSSG) levels, catalase and glutathione peroxidase (GPX) activities assay

GSH and GSSG concentrations (nmol mg $protein^{-1}$) in kidney were determined by commercial colorimetric GSH and GSSG assay kits (OxisResearch, Portland, OR, USA). Catalase and GPX activities (U mg $protein^{-1}$) in kidney were determined by catalase and GPX assay kits (Calbiochem, EMD Biosciences, Inc., San Diego, CA, USA).

Determination of lipid oxidation and reactive oxygen species (ROS)

Lipid oxidation was determined by measuring the level of malondialdehyde (MDA, μmol L^{-1}) via an HPLC method[14] in kidney. The method described in Gupta *et al.*[15] was used to measure the amount of ROS in kidney.

Cytokines analyses

Kidney was homogenized in 10 mM Tris-HCl buffered solution (pH 7.4) containing 2 M NaCl, 1 mM ethylenediaminetetraacetic acid, 0.01% Tween 80, 1 mM phenylmethylsulfonyl fluoride, and centrifuged at 9000 xg for 30 min at 4°C. The resultant supernatant was used for cytokine determination. The levels of interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α and monocyte chemoattractant protein (MCP)-1 were measured by ELISA using cytoscreen

immunoassay kits (BioSource International, Camarillo, CA, USA).

Measurement of aldose reductase (AR) and sorbitol dehydrogenase (SDH) activities

Kidney homogenate was centrifuged and the supernatant was used for analysis. The method of Nishinaka and Yabe-Nishimura[16] was used to measure renal AR activity by monitoring the decrease in absorbance at 340 nm due to NADPH oxidation. SDH activity was assayed according to the method of Bergmeyer[17] by mixing 100 μL kidney homogenate, 200 μL NADH (12 mM) and 1.6 mL triethanolamine buffer (0.2 M, pH 7.4).

Determination of renal sorbitol and fructose content

Kidney was homogenized with PBS (pH 7.4) containing U-[^{13}C]-sorbitol as an internal standard. The supernatant was lyophilized, and the content of sorbitol and fructose in each lyophilized sample was determined by liquid chromatography with tandem mass spectrometry, according to the method of Guerrant and Moss.[18]

Statistical analyses

The effect of each treatment was analyzed from 10 mice (n=10) in each group. Data were subjected to analysis of variance (ANOVA) and computed using the SAS General Linear Model procedure.[17] Differences with $P<0.05$ were considered to be significant.

RESULTS

The content of ascorbic acid, total phenolic acids, total flavonoids, caffeic acid, cinnamic acid, coumaric acid, ellagic acid, ferulic acid, rosmarinic acid, epicatechin, myricetin, naringenin, quercetin and rutin in pepino extracts is shown in Table 1. Caffeic acid and epicatechin were not detectable in either aqueous or ethanol extract. Aqueous and ethanol extracts had similar content of total phenolic acids. Aqueous extract had more ascorbic acid, total flavonoids, cinnamic acid, ferulic acid, rosmarinic acid, quercetin and naringenin than ethanol extract. As shown in Table 2, compared with DM control groups, mice with 2 or 4% PAE treatments had

significantly lower water intake, lower feed intake and higher body weight at wk 5 (P<0.05). Plasma levels of glucose and insulin are presented in Figure 1. When compared with DM control group, PAE treatments at 2 and 4% significantly reduced plasma glucose level at wk 5 (P<0.05); however, PAE treatments only at 4% significantly elevated plasma insulin level at wk 5 (P<0.05).

As shown in Table 3, PAE treatments dose-dependently decreased MDA level in kidney (P<0.05). Renal ROS level was lowered by PAE treatments (P<0.05); but without dose-dependent effect. PAE treatments, at 2 and 4 %, significantly reduced GSSG formation, increased GSH level, and retained GPX and catalase activities in kidney (P<0.05). Renal levels of inflammatory cytokines are presented in Table 4. PAE treatments at 2 and 4% significantly declined IL-6 and TNF-α levels in kidney (P<0.05); however, PAE treatments at 4% only significantly decreased IL-1β and MCP-1 levels in kidney (P<0.05). The effects of PAE upon the renal levels of sorbitol and fructose, and activity of aldose reductase and sorbitol dehydrogenase are presented in Table 5. PAE treatments, only at 4%, significantly diminished aldose reductase activity, and decreased sorbitol and fructose production in kidney (P<0.05).

DISCUSSION

Pepino is consumed as a vegetable in Taiwan and Iran. The results of our present study revealed that both aqueous and ethanol extracts from pepino contained ascorbic acid, phenolic acids and flavonoids. These findings indicated that pepino, at least via the presence of these phytochemicals, could provide healthy benefits. Hyperglycemia, oxidative stress, inflammation and glycation are important factors responsible for the development of diabetic complications.[6-8] In our current study, intake of pepino aqueous extract, especially at 2 and 4%, markedly improved body weight loss, hyperglycemia, hypoinsulinemia, renal oxidative,

inflammatory and glycative stress in diabetic mice. These results suggest that the aqueous extract of pepino could attenuate diabetic progression via multiple actions, and also partially explained the possibility of pepino as a medicinal food.

Our present study found that the aqueous extract of pepino could mitigate renal oxidative stress via reducing the formation of MDA, ROS and GSSG; and enhance antioxidant defense via retaining GSH level and activity of GPX and catalase. The anti-oxidative effects of ascorbic acid, ferulic acid, rosmarinic acid, naringenin and rutin in human or diabetic animals have been reported.[20-22] Thus, the observed anti-oxidative protection in diabetic mice with pepino consumption could be partially ascribed to the presence of ascorbic acid, phenolic acids and flavonoids in this aqueous extract. In addition, it is notified that the intake of pepino effectively maintained renal activity of GPX and catalase. This finding implied that pepino might spare these antioxidant enzymes or be able to mediate these enzymes.

It has been documented that the excessive production of IL-6 and TNF-α in type I diabetes facilitated diabetic deterioration because IL-6 increased platelet sensitivity to thrombin activation, TNF-α impaired β-cell function, and both IL-6 and TNF-α increased intracellular ROS generation.[23] The results of our present study indicated that supplementation with pepino extract at 2 and 4 % declined the production of these two pro-inflammatory cytokines, which might in turn slow down the inflammatory response, inflammation-oriented coagulation and oxidative deterioration. The inhibitory effects of ellagic acid, rutin and naringenin upon IL-6 and TNF-α release in mast cell or mouse tissue have been reported.[24,25] Thus, the anti-inflammatory effects from pepino aqueous extract might be partially resulted from the contribution of these compounds. In addition, MCP-1 is a chemotactic factor for activating monocytes and macrophages, and could recruit monocytes to the sites of injury.[26] We found that renal MCP-1 level could be reduced by pepino aqueous extract only at 4%. Thus, 4% pepino extract might be able to suppress the activation of monocytes and macrophages, and

consequently diminished inflammatory stress. These results suggested that pepino extract at 4% might provide anti-inflammatory protection via both lowering pro-inflammatory cytokines production and deactivating monocytes and macrophages.

Hyperglycemia facilitates glucose metabolism via the polyol pathway and leads to the formation of advanced glycation endproducts and exacerbates diabetes-induced microvascular abnormalities.[27,28] Aldose reductase, the first and rate-limiting enzyme in this polyol pathway, reduces glucose to sorbitol, which could be further metabolized to fructose by sorbitol dehydrogenase, the second enzyme in the polyol pathway.[28] In our present study, the renal aldose reductase activity could be effectively reduced by 4% pepino extract, which consequently lowered renal sorbitol production. These findings suggest that pepino at 4% could suppress polyol pathway and alleviate diabetes associated glycative injury in kidney. The inhibitory effects of phytochemicals such as quercetin upon aldose reductase activity and sorbitol production in diabetic rats have been reported.[29] We also notified that pepino aqueous extract had marked quercetin content. Thus, the observed anti-aldose reductase effect from this extract could be partially ascribed to the presence of this phytochemical in this extract. Since aldose reductase activity had been diminished, the lower production of sorbitol in kidney could be explained. Pepino treatment failed to affect renal sorbitol dehydrogenase activity. It is possible that the decreased fructose production in kidney as we observed was simple due to the lower available sorbitol for sorbitol dehydrogenase. Since oxidative, inflammatory and glycative stress had been mitigated in pepino-treated diabetic mice, it was reasonable to observe the improved glycamic control and body weight loss in these diabetic mice.

Our present study enhanced understanding about the composition of pepino, and we also notified that the sum of ascorbic acid, total phenolic acids and total flavonoids in this aqueous extract was about 2140 mg, only a small part in 100 g freeze-dried powder. Although the combination of ascorbic acid, phenolic acids and flavonoids might offer synergistic protective

effects toward these diabetic mice, it was hard to conclude that the observed anti-oxidative, anti-inflammatory and anti-glycative effects from pepino extract were only resulted from these components. The other possibility is that other component(s) in pepino also contributed to its anti-diabetic benefits. Further study is necessary to analyze and ensure the active compounds in pepino for its anti-diabetic protection. Oxidative, inflammatory and glycative injury is also involved in the pathological development of other chronic diseases such as cardiac and neurodegenerative diseases.[30,31] Since the aqueous extract of pepino is able to decrease these pathogenic stresses, the application of pepino might be helpful to attenuate the progression of other diseases.

In conclusion, our present study provided several novel findings to elucidate the composition and anti-diabetic effects of pepino (*Solanum muricatum* Ait.). This plant food contained ascorbic acid, phenolic acids and flavonoids. The aqueous extract of pepino exhibited anti-oxidative, anti-inflammatory and anti-glycative protection in diabetic mice. These findings suggest that pepino could be developed as a functional food for anti-diabetic prevention and/or alleviation.

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Table 1. Content of ascorbic acid, total phenolic acids, total flavonoids, caffeic acid, cinnamic acid, coumaric acid, ellagic acid, ferulic acid, rosmarinic acid, epicatechin, myricetin, naringenin, quercetin and rutin in pepino aqueous extract (PAE) or 50% ethanol extract (PEE). Data are expressed as mean ± SD (n = 9).

Compound (mg 100 g dry weight^{-1})	PAE	PEE
Ascorbic acid	43.8±8.3[b]	6.6±1.3[a]
Total phenolic acids	1217±188[a]	1073±245[a]
Total flavonoids	875±62[b]	461±53[a]
caffeic acid	-[c]	-
cinnamic acid	75.7±3.1[b]	23.0±1.5[a]
coumaric acid	14.5±2.3[a]	23.9±1.7[b]
ellagic acid	9.2±1.4[a]	6.8±2.1[a]
ferulic acid	82.3±2.6[b]	11.8±1.3[a]
rosmarinic acid	47.2±1.6[b]	8.4±0.7[a]
epicatechin	-	-
myricetin	31.7±3.8[a]	28.9±1.8[a]
naringenin	57.2±5.5[b]	14.7±4.2[a]
quercetin	126.5±10.7[b]	90.3±6.2[a]
rutin	30.8±7.1[a]	32.4±4.3[a]

[ab]Means in a row without a common letter differ, $P<0.05$.

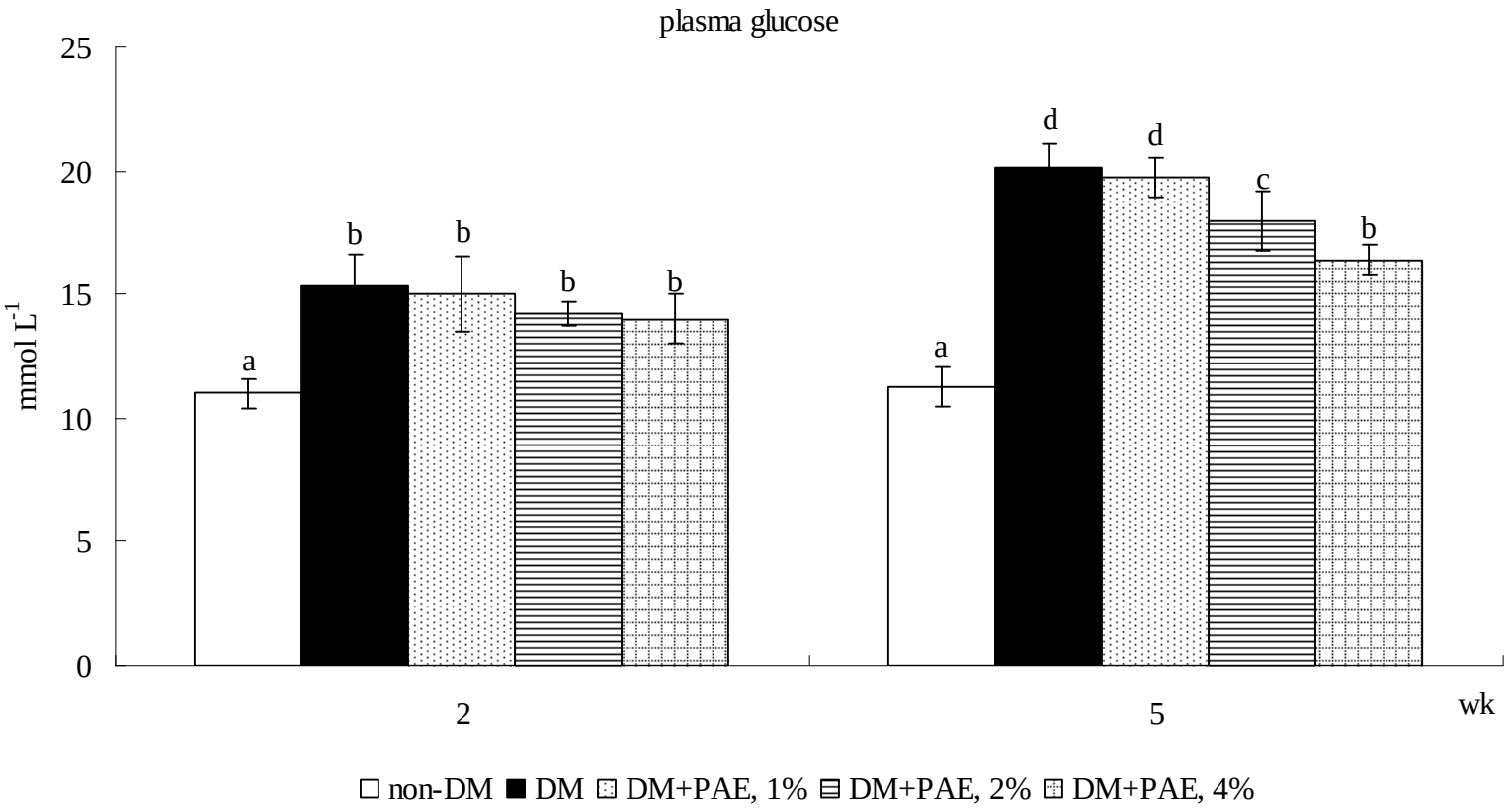
[c]Means too low to be detected.

Table 2. Water intake (WI, mL mouse^{-1} d^{-1}), Feed intake (FI, g mouse^{-1} d^{-1}) and body weight (BW, g mouse^{-1}) of non-diabetic (non-DM), diabetic mice consumed normal diet (DM), or 1, 2, 4% pepino aqueous extract (PAE) at wks 2 and 5. Data are expressed as mean ± SD, n=10.

	non-DM	DM	DM+PAE, 1%	DM+PAE, 2%	DM+PAE, 4%
WI					
2	2.1±0.6^{a}	3.7±1.0^{b}	3.4±1.3^{b}	3.6±0.7^{b}	3.2±1.0^{b}
5	2.3±1.1^{a}	5.4±1.5^{c}	5.2±1.2^{c}	4.3±0.6^{b}	4.0±0.9^{b}
FI					
2	1.9±0.7^{a}	2.7±1.0^{a}	2.8±0.8^{a}	2.5±1.0^{a}	2.6±1.1^{a}
5	2.2±1.0^{a}	4.8±1.2^{c}	4.5±1.4^{c}	3.3±0.9^{b}	3.1±0.7^{b}
BW					
2	23.0±1.2^{b}	19.5±0.9^{a}	20.2±1.3^{a}	19.1±0.6^{a}	20.4±1.4^{a}
5	26.7±2.3^{c}	15.0±1.4^{a}	15.3±0.9^{a}	17.0±1.0^{b}	17.5±1.3^{b}

$^{a-c}$Means in a row without a common letter differ, $P<0.05$.

Figure 1. Plasma levels of glucose (mmol L^{-1}) and insulin (nmol L^{-1}) of non-diabetic (non-DM), diabetic mice consumed normal diet (DM), or 1, 2, 4% pepino aqueous extract (PAE) at wks 2 and 5. Data are expressed as mean ± SD, n=10. $^{a-d}$Means among bars without a common letter differ, $P<0.05$.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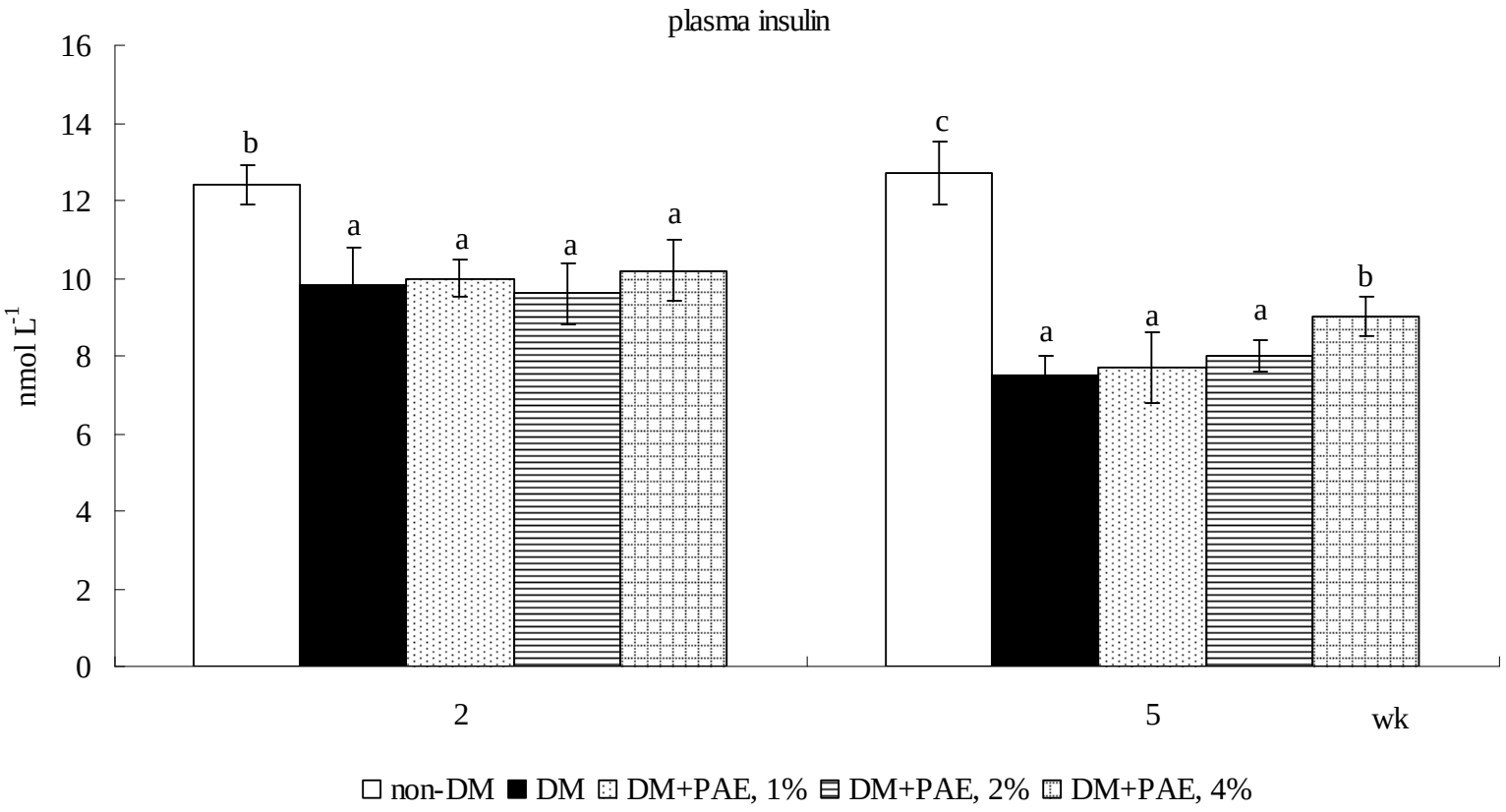
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DM+PAE, 4%

Table 3. Level of MDA (μmol L^{-1}), ROS (nmol mg $protein^{-1}$), GSSG (nmol mg $protein^{-1}$), GSH (nmol mg $protein^{-1}$) and activity (U mg $protein^{-1}$) of catalase and GPX in kidney from non-diabetic (non-DM), diabetic mice consumed normal diet (DM), or 1, 2, 4% pepino aqueous extract (PAE) at 5 week. Data are expressed as mean ± SD, n=10.

	non-DM	DM	DM+PAE, 1%	DM+PAE, 2%	DM+PAE, 4%
MDA	1.03 ± 0.10^{a}	3.89 ± 0.40^{e}	3.14 ± 0.27^{d}	2.67 ± 0.20^{c}	2.10 ± 0.23^{b}
ROS	0.42 ± 0.06^{a}	1.19 ± 0.16^{c}	0.81 ± 0.13^{b}	0.76 ± 0.08^{b}	0.74 ± 0.05^{b}
GSSG	0.31 ± 0.08^{a}	1.23 ± 0.16^{c}	1.20 ± 0.17^{c}	0.82 ± 0.13^{b}	0.73 ± 0.11^{b}
GSH	10.9 ± 1.1^{c}	5.2 ± 1.2^{a}	5.5 ± 0.9^{a}	7.1 ± 1.2^{b}	7.4 ± 1.0^{b}
Catalase	17.4 ± 1.4^{c}	9.2 ± 1.0^{a}	8.9 ± 1.3^{a}	11.3 ± 0.8^{b}	12.0 ± 1.2^{b}
GPX	20.1 ± 2.2^{c}	12.9 ± 1.5^{a}	13.2 ± 1.0^{a}	14.9 ± 1.3^{b}	15.4 ± 1.8^{b}

[a-e]Means in a row without a common letter differ, $P<0.05$.

Table 4. Renal level (pg mg protein^{-1}) of inflammatory cytokines (IL-6, TNF-α, IL-1β and MCP-1) in non-diabetic (non-DM), diabetic mice consumed normal diet (DM), or 1, 2, 4% pepino aqueous extract (PAE) at 5 week. Data are expressed as mean ± SD, n=10.

	non-DM	DM	DM+PAE, 1%	DM+PAE, 2%	DM+PAE, 4%
IL-6	19±5^{a}	257±24^{d}	239±21^{d}	187±16^{c}	149±10^{b}
TNF-α	14±4^{a}	230±28^{d}	219±13^{d}	174±17^{c}	141±11^{b}
IL-1β	15±3^{a}	221±21^{c}	217±18^{c}	208±16^{c}	178±10^{b}
MCP-1	18±5^{a}	213±18^{c}	209±20^{c}	201±16^{c}	165±15^{b}

$^{a-d}$Means in a row without a common letter differ, $P<0.05$.

Table 5. Activity of aldose reductase (AR), sorbitol dehydrogenase (SDH), and level of sorbitol and fructose in kidney from non-diabetic (non-DM), diabetic mice consumed normal diet (DM), or 1, 2, 4% pepino aqueous extract (PAE) at 5 week. Data are expressed as mean ± SD, n=10.

	non-DM	DM	DM+PAE, 1%	DM+PAE, 2%	DM+PAE, 4%
AR, nmol min^{-1} mg $protein^{-1}$	1.09 ± 0.19^{a}	2.48 ± 0.30^{c}	2.51 ± 0.22^{c}	2.33 ± 0.16^{c}	1.83 ± 0.18^{b}
SDH, U g $protein^{-1}$	3.9 ± 0.5^{a}	8.2 ± 1.2^{b}	8.4 ± 1.5^{b}	8.0 ± 1.0^{b}	7.7 ± 0.8^{b}
Sorbitol, nmol mg $protein^{-1}$	2.8 ± 0.5^{a}	21.4 ± 1.6^{c}	22.0 ± 1.2^{c}	20.3 ± 1.4^{c}	15.1 ± 1.0^{b}
Fructose, nmol mg $protein^{-1}$	10.2 ± 1.4^{a}	78.5 ± 4.2^{c}	75.2 ± 5.2^{c}	76.9 ± 3.6^{c}	64.5 ± 4.9^{b}

[a-c]Means in a row without a common letter differ, $P<0.05$.