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Piceatannol attenuates streptozotocin-induced type 1 diabetes in mice

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Key words: Piceatannol, Type 1 diabetes, Streptozotocin, Oxidative stress, Inflammation, ER stress

Abstract: As a natural analog of resveratrol, piceatannol (Pic) exhibits good antioxidant and anti-inflammatory activities in different disease models. However, the role of Pic in type 1 diabetes mouse model has not been reported yet. In this study, we investigated the *in vivo* effect of Pic in streptozotocin (STZ)-induced type 1 diabetic mice. Mice were injected with STZ to establish the type 1 diabetes mellitus (T1DM) model. After stable hyperglycemia was achieved, mice were then orally treated with Pic (40 mg/kg b.w., i.g.) for 30 days. The results indicated that Pic supplementation efficiently alleviated the typical symptoms associated with T1DM, including body weight loss, polydipsia, hyperglycemia, and hypoinsulinemia. Pic treatment also improved the glucose tolerance of STZ-induced diabetic mice. In addition, Pic supplementation markedly decreased the expression of pro-inflammatory molecules TNF-α and IL-6, the expression of endoplasmic reticulum (ER) stress markers GRP78 and CHOP, and the level of oxidative stress in T1DM mice. Moreover, Pic administration also partly reversed the metabolic profiles of STZ-treated mice as detected by ^{1}H Nuclear Magnetic Resonance (NMR)-based metabolomics. Our study suggested that the therapeutic potential of Pic in type 1 diabetes and the anti-diabetic effects of Pic may be associated with its activities to suppress oxidative stress, inflammation, and ER stress.

Introduction

As one of the most common metabolic diseases, diabetes mellitus (DM) has become a global threat to public health. The World Health Organization (WHO) has estimated that more than 592 million people will suffer from diabetes in 2035 (Guariguata *et al.*, 2014). Moreover, the global incidence of type 1 diabetes mellitus (T1DM) is also increasing and will be more than 90 million in 2035 (Guariguata *et al.*, 2014). Extensive efforts have been devoted to investigating the underlying pathological mechanisms and promising therapeutics for diabetes (Dal Monte *et al.*, 2019; Gencoglu *et al.*, 2015; Ben Nasr *et al.*, 2017). Accumulative evidence suggests that T1DM exhibits as a chronic autoimmune metabolic disease and many factors including both genetic and environmental factors contribute to its development and the associated complications (Alberti and Zimmet, 1999). Among these different factors, oxidative stress and inflammation are two critical mediators responsible for the pathogenesis of T1DM and its secondary complications (Negi and Jena, 2019), suggesting that compounds or natural products that possess antioxidant and anti-inflammatory properties might exhibit potential effects in treating diabetes.

Piceatannol (Pic, 3,5,3',4'-trans-tetrahydroxystilbene) is a natural polyphenol found in many fruits, including grapes, blueberries, and passion fruits, etc. (Piotrowska *et al.*, 2012; Minakawa *et al.*, 2012). As a naturally occurring analog and a metabolite of resveratrol, Pic exerts improved bioavailability and metabolism properties than resveratrol (Zhang *et al.*, 2017). Hence it is more attractive to investigate the benefits of applying Pic in different disease models. Our previous study reported that Pic showed hepatoprotective effect against D-GalN/LPS-induced acute liver injury via inhibiting the generation of oxidative stress and pro-inflammatory cytokines (Wen *et al.*, 2018), suggesting that Pic might exhibit anti-oxidative and anti-inflammation properties *in vivo*. In addition, several studies also indicated that Pic was able to reduce the production of ROS and inflammation markers *in vitro* or *in vivo* (Minakawa *et al.*, 2012; Jin *et al.*, 2006). Therefore, the antioxidant and anti-inflammatory activities of Pic suggest its potential effect in diabetes models. However, to our knowledge, the effect of Pic on diabetes, especially in an animal model of T1DM, has not been well determined.

In this study, we investigated the effect of Pic on type 1 diabetes using an STZ-induced T1DM model. Our results

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indicated that Pic effectively lowered the blood glucose level, reversed the body weight loss and induction of polydipsia and hypoinsulinemia, improved the glucose tolerance in STZ-induced diabetic mice, suggesting that Pic supplementation could alleviate the symptoms associated with T1DM.

Materials and Methods

Reagents

Pic was obtained from Adamas Reagent Co., Ltd. (Switzerland). Pic was dissolved in 0.25% ethanol within 1 h before administration. Streptozotocin (STZ) was obtained from Sigma-Aldrich, St. Louis, MO (USA). All other chemicals were of analytical grade.

Mouse studies

C57BL/6 mice (males, 6-7 week-old) were obtained from the Model Animal Research Center of Nanjing University (Nanjing, China) and housed under standard laboratory conditions (22 ± 2°C, 12 h light/dark cycle) with free access to water and food. All mice experiments were performed in accordance with the protocols approved by the Animal Care and Use Committee at Nanjing University of Science and Technology.

All mice were randomly divided into four groups: (1) Control group (Con, n = 6), (2) Pic treatment group (Pic, n = 6), (3) diabetes mellitus group (DM, n = 8), and (4) diabetes plus Pic treatment group (DM + Pic, n = 8). The mice of DM and DM + Pic groups were injected with STZ (40 mg/kg b.w., i.p., dissolved in 10 mM citrate buffer, pH 4.4) for 5 consecutive days (Liu *et al.*, 2017; Bai *et al.*, 2016). Then mice with stable hyperglycemia (fasting blood glucose ≥11.1 mM) for at least 10 days were regarded as diabetic animals, and half of them (DM + Pic group) were treated with Pic (40 mg/kg b.w., i.g.) for 30 days. Mice from the DM group were treated by vehicle control accordingly. The daily food and water intake of mice were detected in the last week of Pic treatment. The dose of Pic was selected based on our previous in vivo study (Wen *et al.*, 2018) as well as our preliminary experiments. The rationale for the experimental design was illustrated in Fig. 1(A).

Oral glucose tolerance test (OGTT)

The mice were fasted for 12 h and then administrated with glucose (2 g/kg b.w.) by gavage on the 30th day after Pic treatment. The tail-vein blood was harvested at 0, 30, 60, 120 min after the administration of glucose, respectively. Then the glucose levels were measured by a blood glucose meter (ACCU-CHEK® Performa, Roche Diagnostics) according to the manufacturer's instruction.

Biochemical analysis

Collecting blood samples, centrifuged at 3000 *g* for 10 min, then the serum was harvested for insulin detection. Malondialdehyde (MDA) Assay kit (A003-1), the Glutathione Peroxidase (GSH-PX) Assay kit (A005), and the Total Superoxide Dismutase (SOD) Assay Kit (A001-1) were purchased from Jiancheng Bioengineering Institute (Nanjing, China). The levels of MDA, activities of GSH-PX and SOD were measured using the above kits following the manufacturer's instructions.

Quantitative real-time PCR

Total RNA was extracted from the liver tissue using TRIzol (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. The reverse transcriptase kit (Invitrogen) was used for the reverse transcription reaction according to the manufacturer's protocol. Quantitative real-time PCR amplification was conducted using an ABI 7300 Detection System with SYBR Green dye (Kapa Biosystems Pty, Ltd.). Primers sequences were listed in Tab. 1.

^{1}H NMR sample preparation and analysis

All ^{1}H NMR samples were prepared with reference to the previous works (Sun *et al.*, 2017). Frozen liver tissue samples were homogenized in ice-cold acetonitrile solution (50% v/v, 5 mL/g tissue). After centrifugation (13000 *g*, 10 min at 4°C), the supernatant was collected, and the acetonitrile was removed by nitrogen blowing. Then the samples were frozen, lyophilized to dryness, and kept at −80°C. When subjected to NMR analysis, the dried extract samples were dissolved in 600 μL 99.8% D_2O phosphate buffer (0.2 M, pH = 7.0) containing 0.05% (w/v) sodium3-(trimethylsilyl) propionate-2,2,3,3-d4(TSP). D_2O was used for field frequency locking and TSP acted as a chemical shift reference (^{1}H, 0.00 ppm). After centrifugation (12000 *g*, 10 min), the transparent supernatant was pipetted into 5-mm NMR tubes for further detection. ^{1}H NMR spectra of all samples were recorded on Bruker AVANCE III 500 MHz NMR spectrometer at 25°C.

^{1}H NMR data analysis

The ^{1}H NMR spectra were pre-processed by MestReNova (version 11.0, Mestrelab Research SL). After phase and baseline correction and peak alignment, each spectrum was segmented into bins between 0.5 and 9.5 ppm with the excision of the regions from 4.7 to 5.2 ppm to remove the signals of water and its neighboring regions. The total spectral area of each spectrum was normalized to unity to facilitate the comparison among samples. The processed ^{1}H NMR data were exported to CSV files for further analysis. After mean-centered and Pareto-scaled by SIMCA (version 14.1, Umetrics), the software Chenomx NMR suite 7.7 (Chenomx Inc., Edmonton, AB, Canada) was utilized to identify the metabolites in the ^{1}H NMR spectra of the liver extracts. The data were also analyzed by orthogonal signal correction partial least squares discriminant analysis (OPLSDA). OPLSDA is an improved method to minimize the influence of unrelated variables between groups. Color-coded loading plots and Score plots of the OPLSDA model were obtained through MetaboAnalyst (www.metaboanalyst.ca) to show the separation between groups. The fold-change values of metabolites and their *p*-values were calculated and visualized in the table.

Statistical analysis

The statistical analysis of the results was performed using GraphPad Prism® 7.01 software (San Diego, CA, USA). All the data are expressed as the mean ± SE of the mean (SEM). $p < 0.05$ was considered to be a statistically significant difference.

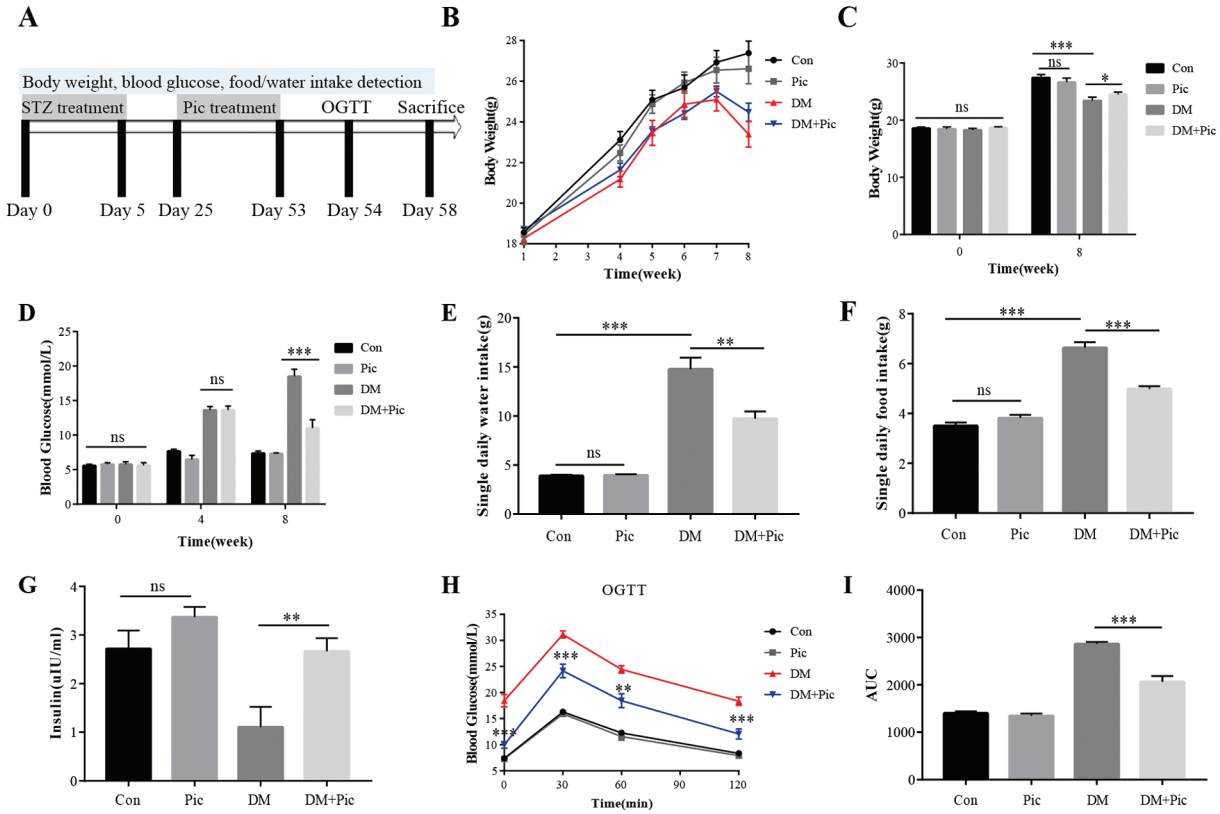

FIGURE 1. The anti-diabetic effects of Pic in STZ-induced type 1 diabetes mice.
Flow chart of the experiment design (A), the body weight (B and C), level of blood glucose (D), daily water intake (E), daily food intake (F), level of serum insulin (G), oral glucose tolerance test (OGTT) curves (H) and the calculated area under the curve (AUC) of OGTT curve (I) were detected or analyzed in all the groups of mice. Con: control group; Pic: control mice treated by Pic only; DM: diabetes mellitus group; DM + Pic: diabetic mice treated by Pic. Data are expressed as mean plus SEM. ns, not significant, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (unpaired Student's *t*-test).

TABLE 1

Primers for q-PCR

Target gene	Primer sequences
Mouse β-Actin, Forward	5′-GTGACGTTGACATCCGTAAAGA-3′
Mouse β-Actin, Reverse	5′-GCCGGACTCATCGTACTCC-3′
Mouse TNF-α, Forward	5′-GCCTCTTCTCATTCCTGCTTGT-3′
Mouse TNF-α, Reverse	5′-GATGATCTGAGTGTGAGGGTCTG-3′
Mouse IL-6, Forward	5′-CTGCAAGAGACTTCCATCCAG-3′
Mouse IL-6, Reverse	5′-AGTGGTATAGACAGGTCTGTTGG-3′
Mouse GRP78, Forward	5′-CGAGGAGGAGGACAAGAAGG-3′
Mouse GRP78, Reverse	5′-TCAAGAACGGGCAAGTTCCAC-3′
Mouse CHOP, Forward	5′-CTGGAAGCCTGGTATGAGGAT-3′
Mouse CHOP, Reverse	5′-ATAGAGTAGGGGTCCTTTGC-3′
Mouse TXNIP, Forward	5′-CAGCCTACAGCAGGTGAGAAC-3′
Mouse TXNIP, Reverse	5′-CTCATCTCAGAGCTCGTCCG-3′

Results

Pic alleviates the symptoms and improves glucose tolerance in type 1 diabetes mice

To evaluate the effect of Pic on type 1 diabetic mice, the STZ-induced T1DM mouse model was used, and the experimental rationale was illustrated in Fig. 1(A). During the whole experiment process, we monitored the body weight, food and water intake in all groups of mice. As Figs. 1(B)–1(C) showed, firstly the body weight gain of the model group (DM) was lower than that of the control group. In the 7th week, the body weight of the diabetic mice started to decline in contrast to the control group. Moreover, the diabetic mice (DM group) took much more food and water than the

control group (Figs. 1(E)–1(F)). The body weight change and food/water intake of the model group were all consistent with the symptoms of type 1 diabetes. However, oral administration of diabetic mice with Pic not only attenuated the decline of body weight but also significantly reduced the daily food and water intake of diabetic mice (DM + Pic group, Figs. 1(B)–1(F)), suggesting that Pic could alleviate the typical symptoms of type 1 diabetes to a certain extent.

Fasting blood glucose levels in all groups of mice were also measured before and after STZ administration as well as Pic treatment. As Fig. 1(D) indicated, before STZ injection, the fasting blood glucose levels of all mice were in the normal range, around 5.5 mmol/L. However, at week 4 (3 weeks after STZ injection but prior to Pic treatment), the fasting blood glucose increased to 14 mmol/L in both DM and DM + Pic groups, indicating the successful establishment of the diabetes animal model. Pic supplementation significantly decreased the blood glucose level to 10 mmol/L, in contrast to the 17.5 mmol/L in the DM group. At the end of the experiment, the concentration of blood insulin was detected. The insulin levels in STZ-induced diabetic mice were much lower than that in the control group (Fig. 1(G)), demonstrating the hypoinsulinemia phenotype of type 1 diabetes. Pic treatment efficiently reversed the level of insulin of DM mice (Fig. 1(G)). Oral glucose tolerance test (OGTT) was performed to evaluate the glucose tolerance. As Fig. 1(H) demonstrated, the blood glucose of all mice reached the peak 30 min after glucose administration, and diabetic mice (DM group) showed obviously impaired glucose tolerance compared with the control group. The blood glucose of all time points, as well as the AUC value in the DM + Pic group, were significantly lower than that in the DM group (Fig. 1(I)), suggesting that Pic supplementation notably improved the glucose tolerance in STZ-induced diabetic mice. Taken together, these results indicated that Pic supplementation efficiently alleviates the diabetes phenotypes in STZ-induced type 1 diabetic mice, including body weight loss, polydipsia, hyperglycemia, hypoinsulinemia, and pancreatic injury.

Pic attenuates the oxidative stress in type 1 diabetic mice

To explore the potential mechanisms, we first measured the different parameters of oxidative stress since previous studies have suggested that oxidative stress contributes to the pathogenesis of type 1 diabetes (Sueishi *et al.*, 2017). In STZ-induced diabetic mice, malondialdehyde (MDA), which represents the level of lipid peroxidation and is often used as a marker of oxidative stress *in vivo*, was markedly increased in the liver tissue compared with the control group (Fig. 2(A)). Consistently, the activity of antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) were both significantly reduced in the DM group but not in the control mice (Figs. 2(B)–2(C)). Pic administration not only significantly down-regulated the MDA level but also increased the activity of SOD and GSH-PX in diabetic mice (Figs. 2(A)–2(C)). Thioredoxin interacting protein (TXNIP), which regulates the antioxidant functions of thioredoxin (Trx), plays a critical role in redox homeostasis and has been identified as an important component linking redox regulation and the pathogenesis of different diseases, including diabetes (Zhou and Chng, 2013; Yoshihara *et al.*, 2014). Our results indicated that the expression of TXNIP was markedly increased in STZ-induced diabetic mice, and Pic treatment completely inhibited the increase (Fig. 2(D)). These results suggest that Pic might exert the anti-diabetic effect via its anti-oxidative properties.

Pic reduces the inflammation and ER stress in type 1 diabetic mice

As chronic inflammation and ER stress have also been suggested to be closely involved in the pathogenesis of diabetes, we analyzed whether Pic treatment affected the status of inflammation and ER stress in STZ-induced diabetic mice. In agreement with previous studies, the

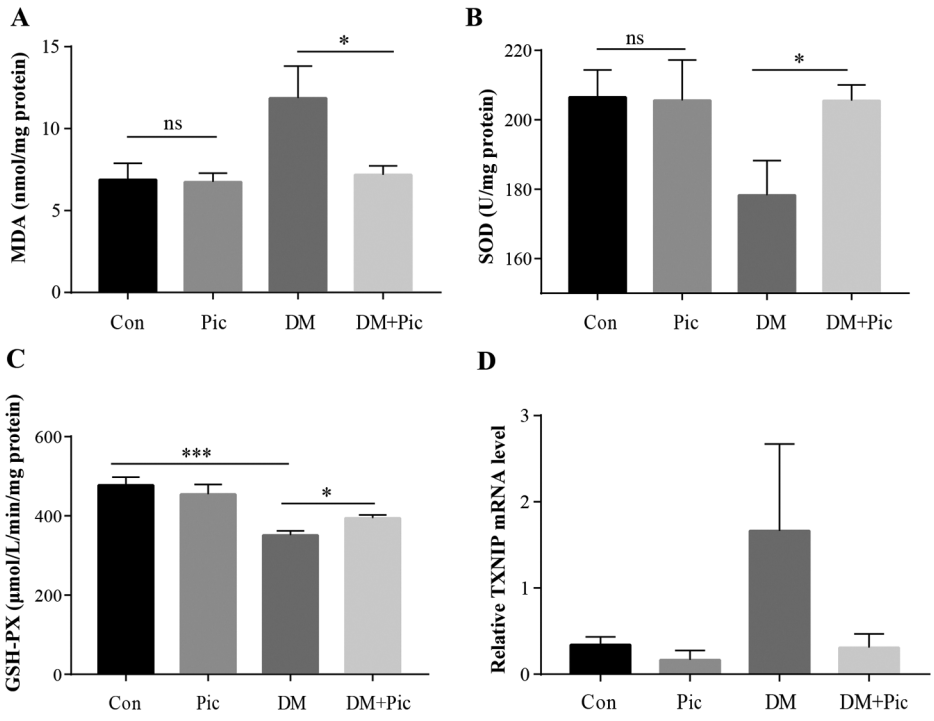

FIGURE 2. Pic supplementation attenuated the oxidative stress in the liver tissue of T1DM mice.
The levels of MDA (A), the activities of SOD (B) and GSH-PX (C), and the transcriptional expression level of TXNIP (D) in liver tissues of all groups of mice. Con: control group; Pic: control mice treated by Pic only; DM: diabetes mellitus group; DM + Pic: diabetic mice treated by Pic. Data are expressed as mean plus SEM. ns, not significant, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (unpaired Student's *t*-test).

transcriptional expression of pro-inflammatory cytokines including TNF-α and IL-6 and ER stress markers including GRP78 and CHOP were all significantly up-regulated in DM mice, while the increase in the expression of these genes were all inhibited by Pic supplementation (Figs. 3(A)–3(D)).

Pic alters the metabolic profiles of liver tissue in type 1 diabetic mice

The results suggested that Pic might affect the metabolism of diabetic mice by targeting oxidative stress, inflammation, and ER stress. We therefore investigated how Pic affected the metabolic homeostasis in mouse liver using NMR-based metabolomics. Typical ^{1}H NMR spectra of liver extracts from four groups of mice were showed in Fig. 4, and 30 metabolites were assigned (Fig. 4). The detailed information was presented in Tab. 2. The ^{1}H NMR data from four groups of mice were evaluated using OPLSDA analysis. The results indicated that the DM and DM + Pic groups were clearly separated in the OPLSDA score plot (Fig. 4A). According to the OPLSDA S-plot and color-coded loadings plots (Fig. 4), alanine, glutamate, taurine, inosine, histidine, oxypurinol were markedly increased in STZ-induced diabetic mice, while glucose was significantly decreased (Fig. 4, Tab. 2). Pic supplementation reversed the changes of alanine, taurine, inosine, histidine, and fumarate, but had no effect on the changes of succinate and creatine (Tab. 2).

Discussion

Pic is a natural analog and a metabolite of resveratrol. In recent years, the function and effect of resveratrol have caused intensive controversies. Although tons of studies have provided compelling evidence that resveratrol exhibits beneficial effects in various animal disease models, including the prevention of cardiovascular diseases, metabolic syndrome and diabetes, etc. (Cao *et al.*, 2018; Lee *et al.*, 2015; Simas *et al.*, 2017; Yang and Kang, 2018), the argument arises from the fact that it is difficult to translate these findings to clinical use for treating human diseases (Smoliga *et al.*, 2011). The failure in the clinical translation of resveratrol has been largely attributed to its poor bioavailability and metabolism. Pic, with improved bioavailability and metabolism properties than resveratrol (Piotrowska *et al.*, 2012; Minakawa *et al.*, 2012), might overcome this issue. Hence, it would be interesting to investigate the potential health-promoting and disease-limiting capabilities of Pic in different disease models. To the best of our knowledge, the effect of Pic in STZ-induced type 1 diabetic mice has not been reported yet. This study presents evidence indicating the beneficial effects of Pic in a mouse model of T1DM. Supplementation with Pic in T1DM mice efficiently preserved the body weight alleviated the symptoms of polydipsia and hyperglycemia and improved glucose tolerance. The decreased levels of serum insulin in diabetic mice caused by the destruction of pancreatic β-cells were also restored by Pic treatments. It is well known that insulin resistance is the main characteristic of type 2 diabetes mellitus (T2DM), while the destruction of the pancreatic β-cells is the main cause of T1DM, consequently leading to the impairment of insulin production. These results suggest that Pic might exhibit its anti-diabetic effect through protecting the pancreatic islets and thus reserving the function of the pancreas. More importantly, these beneficial effects were achieved when Pic was administered at the advanced diabetic stage with stable hyperglycemia, suggesting the therapeutic potential of Pic in treating T1DM patients.

Diabetes is a complicated chronic condition and mediated by multiple factors. Oxidative stress and inflammation are two critical factors mediating the pathogenesis of diabetes and its associated complications (Forbes *et al.*, 2008; Li *et al.*, 2013). During the progression of diabetes mellitus, persistent

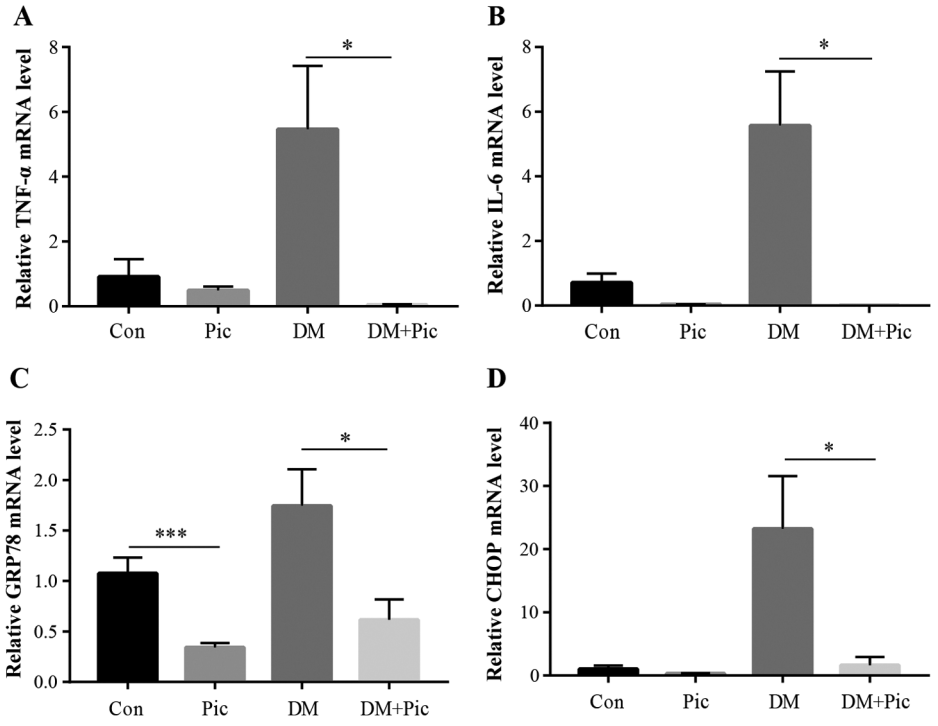

FIGURE 3. Pic treatment reduces the expression of pro-inflammatory cytokines and ER stress markers in type 1 diabetic mice.

The transcriptional expression levels of pro-inflammatory cytokines TNF-α (A) and IL-6 (B), ER stress markers GRP78 (C) and CHOP (D) in liver tissues of all groups of mice. Con: control group; Pic: control mice treated by Pic only; DM: diabetes mellitus group; DM + Pic: diabetic mice treated by Pic. Data are expressed as mean plus SEM. ns, not significant, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (unpaired Student's *t*-test).

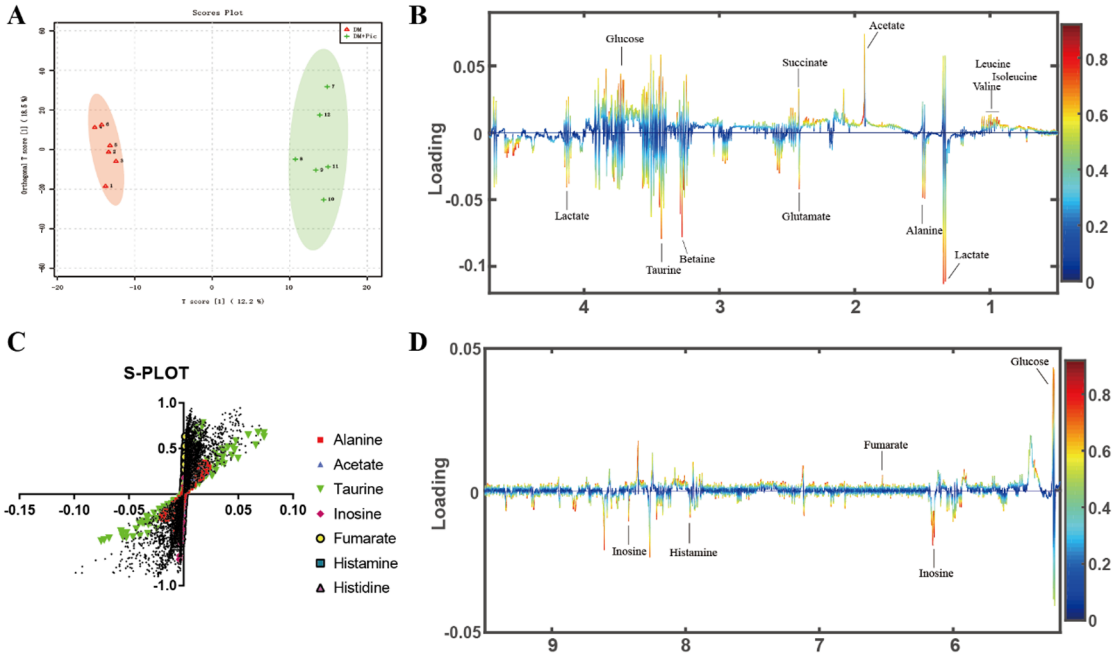

FIGURE 4. ^{1}H NMR analysis of the liver extracts from diabetic mice (DM group) and Pic-treated diabetic mice (DM + Pic group). (A) Score plot of OPLSDA analysis of ^{1}H NMR data between the liver samples of DM group (red) and DM + Pic group (green). (B and D) Color-coded loading plots for ^{1}H NMR data of the liver samples. The metabolites with significant differences were demonstrated in the loading plots. (C) S-plot of OPLSDA analysis with different metabolites distinguished by color and listed in the legend.

hyperglycemia induces the production of free oxygen radicals via several mechanisms, including autoxidation of glucose and non-enzymatic glycation between sugars and proteins to generate intracellular advanced glycation end products (AGEs). These free radicals induce peroxidation and damage to protein, DNA, and lipid, resulting in oxidative stress in different tissues. Regarding the underlying molecular mechanism, our recent study has investigated the interaction of Pic with bovine serum albumin (BSA) using fluorescence spectroscopy, ultraviolet-visible absorption spectroscopy, circular dichroism spectroscopy and molecular simulation (Xu *et al.*, 2019). Results indicated that Pic could inhibit the non-enzymatic glycosylation of BSA to a certain extent; Pic can effectively inhibit the formation of AGEs and protect BSA from undergoing structural changes induced by glycation, suggesting that Pic might exhibit its antidiabetic effect through inhibiting the glycosylation of protein and the formation of AGEs. Accumulating evidence from both experimental and clinical studies demonstrated that oxidative stress plays a critical role in the pathogenesis of T1DM through inducing cellular injury and chronic inflammation (Kobayashi and Schmid-Schönbein, 2006). Several studies have reported that hyperglycemia and hyperlipidemia contribute to the accumulation of ROS and antioxidants deficiency in diabetic animals (Ma *et al.*, 2018; Domingueti *et al.*, 2016).

Our results indicate that Pic could not only substantially alleviate the oxidative stress, but also markedly inhibit the expression of inflammatory molecules in STZ-induced diabetic mice, that is consistent with previous studies using different animal disease models by our and others' groups (Wen *et al.*, 2018). The health-promoting and disease-preventing ability of resveratrol and its analogs have previously been attributed to their antioxidant activity. However, Prysyazhna *et al.* (2019) recently reported that resveratrol exerts its blood pressure-lowering effect in hypertensive mice through directly inducing the oxidation of protein kinase 1α (PKG1α) instead of its antioxidant role (Prysyazhna *et al.*, 2019). Therefore, although the final outcome in our experimental model demonstrated that the liver oxidative stress in diabetic mice was suppressed by Pic supplementation, the underlying molecular action still needs to be further investigated.

In addition to oxidative stress and inflammation, ER stress has been identified as another important factor involved in the pathogenesis of diabetes. The disruption of ER homeostasis may contribute to β-cell dysfunction and diabetes. Many studies have detected the elevated ER stress markers in different animal models of diabetes as well as in diabetes patients (Özcan *et al.*, 2004; Bhatta *et al.*, 2015; Zhong *et al.*, 2012). Hyperglycemia has also been shown to prompt the induction of ER stress (Özcan *et al.*, 2004; Zhong *et al.*, 2012). Treatment with ER stress-inhibiting chemicals could efficiently lead to the normalization of hyperglycemia, improved glucose and insulin tolerance, and enhancement of insulin action in diabetic mice (Liu *et al.*, 2015; Hosoi and Ozawa, 2016). Moreover, increasing evidence demonstrated that ER stress is able to induce oxidative stress and inflammatory responses (Grootjans *et al.*, 2016; Cao *et al.*, 2016; Ochoa *et al.*, 2018), suggesting that ER stress, oxidative stress, and inflammation are closely interconnected, the interventions that regulate the ER stress response offer other opportunities for preventing and treating diabetes. Our previous study found that Pic reduced the expression of ER stress markers in D-GalN/LPS-induced acute liver injury model and could also inhibit the inflammation induced by ER stress-inducing drugs *in vitro* (Wen *et al.*, 2018). Combined with the results in this current study that Pic markedly down-regulated the

TABLE 2

Assignments of ^{1}H NMR spectra signals for the corresponding metabolites in the liver tissues of all groups of mice, their changes, and the associated *p*-values.

No.	Metabolite	Assignments	ppm	Pic/Con		DM/Con		DM + Pic/Con		DM + Pic/DM	
				FC	*p*-value	FC	*p*-value	FC	*p*-value	FC	*p*-value
1	Isoleucine	δCH3, γCH3, αCH	0.93(t), 1.0(d), 1.46(m)	1.04		0.85		0.58	*	0.68	
2	Leucine	δCH3, δCH3, γCH, αCH	0.94(d), 0.96(d), 1.71(m), 3.74(m)	1.06		0.81		0.61		0.75	
3	Valine	γCH3, γCH3	0.98(d), 1.04(d), 2.26(m), 3.61(d)	0.98		1.01		0.82		0.81	
4	Propionate	CH3	1.12(t)	0.93		0.99		0.81		0.82	*
5	Lactate	CH3, CH	1.33(d), 4.11(q)	1.09		1.09		1.21	*	1.1	
6	Alanine	βCH3, αCH	1.48(d), 3.78(q)	0.97		0.71	*	0.76	*	1.06	
7	Acetate	CH3	1.92(s)	0.62		1.51		0.76		0.5	
8	Glutamate	βCH2, βCH2, γCH2, αCH	2.14(m), 2.36(m), 2.50(m), 3.77(t)	1.13		0.8	*	0.66	**	0.82	
9	Glutamine	βCH2, γCH2, αCH	2.46(m), 3.77(t)	1.11		1.01		0.93		0.92	
10	Glutathione	S-CH2, N-CH, N-CH2, CH2	2.14(m), 2.55(m), 2.95(m)	1.14	*	1.07		0.99		0.92	
11	Succinate	CH2	2.4(s)	1.08		0.84		0.82	*	0.98	
12	Creatine	CH3, CH2	3.04(s), 3.93(s)	1.09		0.82		0.73		0.89	
13	Betaine	CH2	3.22(s)	1.11		0.78		0.77		0.99	
14	Taurine	NH2-CH2, SO3-CH2	3.28(t), 3.42(t)	1.03		0.87	**	0.94		1.07	*
15	Glycine	CH2	3.57(s)	0.89		1.03		1.02		0.99	
16	Maltose	CH, CH2	3.56-3.92 (m), 5.23 (d), 5.40 (m)	0.79		0.85		0.62	*	0.72	
17	Glucose	2H, 3H, 4H, 5H, 6H, 6'H	3.4-3.95 (m), 5.24(d)	0.98		1.08	*	1.08	*	1	
18	Tyrosine	CH, CH	6.89(d), 7.18 (d)	0.86		1.03		1.07		1.06	
19	Inosine	O-CH-N, N-CH=N, N-CH=N	6.10 (d), 8.23 (s), 8.34 (s)	0.86		0.75	***	0.69	***	0.93	
20	Fumarate	CH=CH	6.53(s)	1.39		0.63		0.69		1.09	
21	Phenylalanine	CH=CH	7.38(m), 7.43(m)	1		1.22	*	0.99		0.82	
22	Histamine	NH2-CH2, CH=C-CH2, CH=N-CH	3.00(t), 3.29(t), 7.12(s), 7.93(s)	1.07		0.93		0.98		1.11	
23	Histidine	CH, CH	7.14(s), 8.03(s)	0.95		0.66	*	0.76		1.14	
24	Hypoxanthine	CH, CH	8.18(s), 8.20(s)	1.11		0.91		1.1		1.22	
25	Oxypurinol	CH	8.22(s)	1		0.72		0.85		1.17	
26	Nicotinate	2H, 3H, 4H, 5H	7.53(dd), 8.26(dt), 8.62(dd), 8.95(d)	1.04		0.86	*	1.07		1.25	**
27	Formate	CH	8.46(s)	1.08		1.07		1.32		1.23	
28	ATP		8.5(s)	0.99		0.94		1.12		1.19	
29	AMP	N-CH=N	8.595(S)	1.14		1.26		1.41		1.13	
30	NAD+	NH=CH-H	8.17(m), 8.43(s),	1		0.95		1.19		1.25	

[a]fold change (FC) = A group/B group; Color-coded according to the $\log_2$(FC), red means "increased" and blue means "decreased" concentrations in the former group when two groups were compared.
Color bar -1 -0.75 -0.5 -0.25 0 0.25 0.5 0.75 1
[b]*p*-values were calculated by unpaired Student's t-test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

expression of ER stress markers in diabetic mice, it suggests that Pic could alleviate ER stress in different disease models and it should take this mechanism into account when investigating the effect of Pic.

As one of the metabolic diseases, T1DM affects the metabolism homeostasis significantly (Abu Bakar Sajak *et al.*, 2017), and this was reflected by our results obtained from NMR-based metabolomics analysis. In STZ-induced

diabetic mice, the metabolic profiles that were involved in energy metabolic pathway (glucose, succinate, acetate, creatine), amino acid metabolism pathway (isoleucine, leucine, valine, alanine, tyrosine, phenylalanine) and oxidative stress pathway (glutamate, glutamine, betaine, taurine), were markedly disturbed. The increased levels of glucose in STZ-treated mice suggested a lowered glycolysis, while the decreased level of succinate and fumarate, which are the intermediates of the tricarboxylic acid (TCA) cycle, suggested an inhibited TCA cycle, indicating that STZ disturbed the energy homeostasis *in vivo* (Sun *et al.*, 2017). Betaine and taurine have been found to have anti-oxidative potential and betaine plays an important role in fatty acid metabolism. Hence, the decrease of betaine and taurine in STZ-treated mice suggests that STZ may affect lipid metabolism by regulating the choline metabolic pathway (Nam *et al.*, 2017). However, Pic supplementation attenuated these changes, suggesting that Pic might target on the energy metabolism pathway and redox regulation pathway to restore the metabolism homeostasis disturbed by STZ injection. However, it also has several drawbacks as it did not improve the metabolite levels of leucine and valine, which are involved in protein synthesis pathways.

In summary, oral treatment of STZ-induced type 1 diabetic mice with Pic effectively attenuated the symptoms associated with T1DM. The anti-diabetic effects of Pic might be associated with its activities in inhibiting ER stress, inflammation, and oxidative stress. Our study provides evidence that treatment with Pic is a potentially useful strategy for the treatment of diabetes. Considering the improved bioavailability properties of Pic than resveratrol, it would be interesting to investigate the potential use of Pic for treating TIDM, although further studies regarding the underlying mechanisms are still required.

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References

Abu Bakar Sajak A, Mediani A, Maulidiani, Mohd Dom NS, Machap C, Hamid M, Ismail A, Khatib A, Abas F (2017). Effect of *Ipomoea aquatica* ethanolic extract in streptozotocin (STZ) induced diabetic rats *via* 1H NMR-based metabolomics approach. *Phytomedicine* **36**: 201–209. DOI 10.1016/j.phymed.2017.10.011.

Alberti KG, Zimmet PZ (1999). Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabetic Medicine* **15**: 539–553. DOI 10.1002/(SICI)1096-9136(199807)15:7<539::AID-DIA668>3.0.CO;2-S.

Bai D, Zhang Y, Shen M, Sun Y, Xia Q, Zhang Y, Liu X, Wang H, Yuan L (2016). Hyperglycemia and hyperlipidemia blunts the Insulin-Inpp5f negative feedback loop in the diabetic heart. *Scientific Reports* **6**: 22068. DOI 10.1038/srep22068.

Ben Nasr M, Tezza S, D'Addio F, Mameli C, Usuelli V, Maestroni A, Corradi D, Belletti S, Albarello L, Becchi G, Fadini GP, Schuetz C, Markmann J, Wasserfall C, Zon L, Zuccotti GV, Fiorina P (2017). PD-L1 genetic overexpression or pharmacological restoration in hematopoietic stem and progenitor cells reverses autoimmune diabetes. *Science Translational Medicine* **9**: eaam7543. DOI 10.1126/scitranslmed.aam7543.

Bhatta M, Ma JH, Wang JJ, Sakowski J, Zhang SX (2015). Enhanced endoplasmic reticulum stress in bone marrow angiogenic progenitor cells in a mouse model of long-term experimental type 2 diabetes. *Diabetologia* **58**: 2181–2190. DOI 10.1007/s00125-015-3643-3.

Cao MM, Lu X, Liu GD, Su Y, Li YB, Zhou J (2018). Resveratrol attenuates type 2 diabetes mellitus by mediating mitochondrial biogenesis and lipid metabolism via Sirtuin type 1. *Experimental and Therapeutic Medicine* **15**: 576–584.

Cao SS, Luo KL, Shi L (2016). Endoplasmic reticulum stress interacts with inflammation in human diseases. *Journal of Cellular Physiology* **231**: 288–294. DOI 10.1002/jcp.25098.

Dal Monte M, Cammalleri M, Pecci V, Carmosino M, Procino G, Pini A, De Rosa M, Pavone V, Svelto M, Bagnoli P (2019). Inhibiting the urokinase-type plasminogen activator receptor system recovers STZ-induced diabetic nephropathy. *Journal of Cellular and Molecular Medicine* **23**: 1034–1049. DOI 10.1111/jcmm.14004.

Domingueti CP, Dusse LMSA, Carvalho MDG, De Sousa LP, Gomes KB, Fernandes AP (2016). Diabetes mellitus: the linkage between oxidative stress, inflammation, hypercoagulability and vascular complications. *Journal of Diabetes and its Complications* **30**: 738–745. DOI 10.1016/j.jdiacomp.2015.12.018.

Forbes JM, Coughlan MT, Cooper ME (2008). Oxidative stress as a major culprit in kidney disease in diabetes. *Diabetes* **57**: 1446–1454. DOI 10.2337/db08-0057.

Gencoglu H, Tuzcu M, Hayirli A, Sahin K (2015). Protective effects of resveratrol against streptozotocin-induced diabetes in rats by modulation of visfatin/sirtuin-1 pathway and glucose transporters. *International Journal of Food Sciences and Nutrition* **66**: 314–320. DOI 10.3109/09637486.2014.1003534.

Grootjans J, Kaser A, Kaufman RJ, Blumberg RS (2016). The unfolded protein response in immunity and inflammation. *Nature Reviews Immunology* **16**: 469–484. DOI 10.1038/nri.2016.62.

Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE (2014). Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Research and Clinical Practice* **103**: 137–149. DOI 10.1016/j.diabres.2013.11.002.

Hosoi T, Ozawa K (2016). Possible pharmacological approach targeting endoplasmic reticulum stress to ameliorate leptin resistance in obesity. *Frontiers in Endocrinology* **7**: 59. DOI 10.3389/fendo.2016.00059.

Jin CY, Moon DO, Lee KJ, Kim MO, Lee JD, Choi YH, Park YM, Kim GY (2006). Piceatannol attenuates lipopolysaccharide-induced NF-κB activation and NF-κB-related proinflammatory mediators in BV2 microglia. *Pharmacological Research* **54**: 461–467. DOI 10.1016/j.phrs.2006.09.005.

Kobayashi N, Schmid-Schönbein GW (2006). Intrinsic disturbance of cellular redox balance enhances blood lymphocyte apoptosis in the spontaneously hypertensive rat. *Free Radical Biology and Medicine* **41**: 484–492. DOI 10.1016/j.freeradbiomed.2006.04.016.

Lee HN, Jang HY, Kim HJ, Shin SA, Choo GS, Park BK, Kim BS, Jung JY (2015). Induction of apoptosis by piceatannol in YD-15 human oral cancer cells. *Journal of the Korean Society of Food Science and Nutrition* **44**: 975–982. DOI 10.3746/jkfn.2015.44.7.975.

Li R, Liang T, Xu L, Li Y, Zhang S, Duan X (2013). Protective effect of cinnamon polyphenols against STZ-diabetic mice fed high-sugar, high-fat diet and its underlying mechanism. *Food and Chemical Toxicology* **51**: 419–425. DOI 10.1016/j.fct.2012.10.024.

Liu HJ, Fan YL, Liao HH, Liu Y, Chen S, Ma ZG, Zhang N, Yang Z, Deng W, Tang QZ (2017). Apigenin alleviates STZ-induced diabetic cardiomyopathy. *Molecular and Cellular Biochemistry* **428**: 9–21. DOI 10.1007/s11010-016-2913-9.

Liu J, Lee J, Salazar Hernandez MA, Mazitschek R, Özcan U (2015). Treatment of obesity with celastrol. *Cell* **161**: 999–1011. DOI 10.1016/j.cell.2015.05.011.

Ma X, Chen Z, Wang L, Wang G, Wang Z, Dong X, Wen B, Zhang Z (2018). The pathogenesis of diabetes mellitus by oxidative stress and inflammation: its inhibition by berberine. *Frontiers in Pharmacology* **9**: 782. DOI 10.3389/fphar.2018.00782.

Minakawa M, Miura Y, Yagasaki K (2012). Piceatannol, a resveratrol derivative, promotes glucose uptake through glucose transporter 4 translocation to plasma membrane in L6 myocytes and suppresses blood glucose levels in type 2 diabetic model db/db mice. *Biochemical and Biophysical Research Communications* **422**: 469–475. DOI 10.1016/j.bbrc.2012.05.017.

Nam J, Greenwald E, Jack-Roberts C, Ajeeb TT, Malysheva OV, Caudill MA, Axen K, Saxena A, Semernina E, Nanobashvili K, Jiang X (2017). Choline prevents fetal overgrowth and normalizes placental fatty acid and glucose metabolism in a mouse model of maternal obesity. *Journal of Nutritional Biochemistry* **49**: 80–88. DOI 10.1016/j.jnutbio.2017.08.004.

Negi CK, Jena G (2019). Nrf2, a novel molecular target to reduce type 1 diabetes associated secondary complications: the basic considerations. *European Journal of Pharmacology* **843**: 12–26. DOI 10.1016/j.ejphar.2018.10.026.

Ochoa CD, Wu RF, Terada LS (2018). ROS signaling and ER stress in cardiovascular disease. *Molecular Aspects of Medicine* **63**: 18–29. DOI 10.1016/j.mam.2018.03.002.

Özcan U, Cao Q, Yilmaz E, Yilmaz E, Lee AH, Iwakoshi NN, Özdelen E, Tuncman G, Görgün C, Glimcher LH, Hotamisligil GS (2004). Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* **306**: 457–461. DOI 10.1126/science.1103160.

Piotrowska H, Kucinska M, Murias M (2012). Biological activity of piceatannol: leaving the shadow of resveratrol. *Mutation Research/Reviews in Mutation Research* **50**: 60–82. DOI 10.1016/j.mrrev.2011.11.001.

Prysyazhna O, Wolhuter K, Switzer C, Santos C, Yang X, Lynham S, Shah AM, Eaton P, Burgoyne JR (2019). Blood pressure-lowering by the antioxidant resveratrol is counterintuitively mediated by oxidation of cGMP-dependent protein kinase. *Circulation* **140**: 126–137. DOI 10.1161/CIRCULATIONAHA.118.037398.

Simas JN, Mendes TB, Paccola CC, Vendramini V, Miraglia SM (2017). Resveratrol attenuates reproductive alterations in type 1 diabetes-induced rats. *International Journal of Experimental Pathology* **98**: 312–328. DOI 10.1111/iep.12251.

Smoliga JM, Baur JA, Hausenblas HA (2011). Resveratrol and health—a comprehensive review of human clinical trials. *Molecular Nutrition & Food Research* **55**: 1129–1141. DOI 10.1002/mnfr.201100143.

Sueishi Y, Nii R, Kakizaki N (2017). Resveratrol analogues like piceatannol are potent antioxidants as quantitatively demonstrated through the high scavenging ability against reactive oxygen species and methyl radical. *Bioorganic & Medicinal Chemistry Letters* **27**: 5203–5206. DOI 10.1016/j.bmcl.2017.10.045.

Sun Q, Xu X, Yang X, Weng D, Wang J, Zhang J (2017). Salecan protected against concanavalin A-induced acute liver injury by modulating T cell immune responses and NMR-based metabolic profiles. *Toxicology and Applied Pharmacology* **317**: 63–72. DOI 10.1016/j.taap.2017.01.007.

Wen J, Lin H, Zhao M, Tao L, Yang Y, Xu X, Jia A, Zhang J, Weng D (2018). Piceatannol attenuates D-GalN/LPS-induced hepatoxicity in mice: involvement of ER stress, inflammation and oxidative stress. *International Immunopharmacology* **64**: 131–139. DOI 10.1016/j.intimp.2018.08.037.

Xu X, Zhao M, Han Q, Wang H, Zhang H, Wang Y (2019). Effects of piceatannol on the structure and activities of bovine serum albumin: a multi-spectral and molecular modeling studies. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* **228**: 117706. DOI 10.1016/j.saa.2019.117706.

Yang DK, Kang HS (2018). Anti-diabetic effect of cotreatment with quercetin and resveratrol in streptozotocin-induced diabetic rats. *Biomolecules and Therapeutics* **26**: 130–138. DOI 10.4062/biomolther.2017.254.

Yoshihara E, Masaki S, Matsuo Y, Chen Z, Tian H, Yodoi J (2014). Thioredoxin/Txnip: redoxisome, as a redox switch for the pathogenesis of diseases. *Frontiers in Immunology* **4**: 514. DOI 10.3389/fimmu.2013.00514.

Zhang AJ, Rimando AM, Mizuno CS, Mathews ST (2017). α-Glucosidase inhibitory effect of resveratrol and piceatannol. *Journal of Nutritional Biochemistry* **47**: 86–93. DOI 10.1016/j.jnutbio.2017.05.008.

Zhong Y, Li J, Chen Y, Wang JJ, Ratan R, Zhang SX (2012). Activation of endoplasmic reticulum stress by hyperglycemia is essential for Müller cell-derived inflammatory cytokine production in diabetes. *Diabetes* **61**: 492–504. DOI 10.2337/db11-0315.

Zhou J, Chng WJ (2013). Roles of thioredoxin binding protein (TXNIP) in oxidative stress, apoptosis and cancer. *Mitochondrion* **13**: 163–169. DOI 10.1016/j.mito.2012.06.004.