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# Fucoidan from *Acaudina molpadioides* Protects Pancreatic Islet against Cell Apoptosis via Inhibition of Inflammation in Type 2 Diabetic Mice

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**Abstract** Inflammation induces pancreatic islet cell apoptosis. Effects of fucoidan from *Acaudina molpadioides* (*Am*-FUC) on inhibition of pancreatic islet cell apoptosis and inflammation in type 2 diabetic mice were investigated. *Am*-FUC repaired pancreatic islet cells, decreased serum C-reactive protein (CRP), macrophage inflammatory protein 1 (MIP-1), interleukin 1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α) levels, and increased the IL-10 level. Am-FUC also reduced TNF-α, CRP, MIP-1, IL-1β, and IL-6 mRNA expressions, and increased IL-10 mRNA expression in epididymal adipose tissues. Am-FUC reduced Bid, Bax, cytochrome *c*, caspase 9, and caspase 3 mRNA expressions, and increased Bcl-2 and Bcl-xL mRNA expressions. *Am*-FUC down-regulated t-Bid, Bax, cytochrome *c,* and caspase 9 activities, cleaved caspase 3 proteins, and up-regulated Bcl-2 and Bcl-xL proteins. Thus, an *Am*-FUC-blocked mitochondrial pathway was the suppression mechanism in pancreatic islet cell apoptosis *via* regulation of inflammatory cytokines providing dietary intervention in type 2 diabetes and inflammation-induced pancreatic islet apoptosis.

**Keywords:** fucoidan, apoptosis, inflammatory cytokine, pancreatic islet, *Acaudina molpadioides*

## Introduction

Pancreatic β-cell dysfunction contributing to reduction in insulin sensitivity is a characteristic of type 2 diabetes mellitus (1). Normal circulating concentrations of insulin, secreted by pancreatic β-cell, fail to regulate body glucose homeostasis and give rise to insulin resistance in the early phase of type 2 diabetes (2). Although the functional defect in insulin secretion toward the overall morbidity remains unclear, the pancreatic β-cell mass decreases by approximately 60% in the metabolic disorder compared to nondiabetic humans (3,4). However, in many multiple pharmacological therapies for type 2 diabetes, few of these therapies were reported to effectively keep off dysfunction and apoptosis of β-cell (5). Therefore, new drugs or agents with the curative effect on blocking β-cell apoptosis may create a revolution in type 2 diabetes therapy.

Dietary-induced inflammation plays a crucial role in development of type 2 diabetes (6). Involvement of inflammatory mediators, including tumor necrosis factor-α (TNF-α), macrophage inflammatory protein 1 (MIP-1), C-reactive protein (CRP), interleukin 1β (IL-1β), and IL-6, in pathogenesis of increased β-cell apoptosis has been widely documented in human and experimental diabetes models (7). A specific role has been proposed for TNF-α in impairment of insulin secretion and induction of β-cell apoptosis in pancreatic cells (8).

IL-10 is an anti-inflammatory protein that is negative for development of type 2 diabetes (9). IL-10 probably plays a key role in β-cell resistance to apoptosis (10). Therefore, inflammatory mediators play pivotal roles in development of β-cell apoptosis. On the other hand, other inflammation-related stressors, such as reactive oxygen species (ROS), nitric oxide (NO), and free fatty acids (FFA), are also related to type 2 diabetes and β-cell dysfunction (11). A recent study reported that chronic reduction of plasma FFA levels can improve mitochondrial function and insulin sensitivity in type 2 diabetic individuals (12).

The sea cucumber is a popular traditional marine food in China that exhibits many biological activities due to sulphated polysaccharide, saponin, and bioactive lipid compounds (13). Fucoidan (SC-FUC) is a polysaccharide component of the sea cucumber body wall. The structure of SC-FUC from *Acaudina molpadioides* (*Am*-FUC) has recently been clarified as [→3-α-L-Fuc*p*-1→3-α-L-Fuc*p*2,4($OS_3^-$)-1→3-α-L-Fuc*p*-1→3-α-L-Fuc*p*2 ($OS_3^-$)-1]$_n$ (14). SC-FUC contains a novel repeating unit distinctive of a sulphation pattern that was confirmed as a linear polysaccharide consisting of a tetrafucose repeating unit with 1→3 linkages (14). Current research on bioactivities of SC-FUC is mainly focused on anti-coagulation and anti-thrombotic activities,

promotion of neural stem/progenitor cell proliferation, inhibition of adipocyte differentiation, and osteoclastogenesis (15-18). *Am*-FUC can alleviate insulin resistance and regulate serum adipokines (including TNF-α) in type 2 diabetic mice (19). However, the effect of *Am*-FUC on pancreatic islet cells is not understood. In this study, effects of *Am*-FUC on inhibition of pancreatic islet cell apoptosis in type 2 diabetic mice was evaluated and mechanisms involved in apoptosis-related gene expression in islet cells and the whole-body inflammatory response were studied.

## Materials and Methods

**Preparation of *Am*-FUC** Dried *Acaudina molpadioides* was purchased at a seafood market in Qingdao, China in July of 2013 and was identified by Professor Yulin Liao of the Chinese Academy of Sciences Institute of Oceanography in Qingdao, China. *Am*-FUC was prepared as reported previously (14,19). Obtained *Am*-FUC was composed of fucose alone with 26.3±2.7% sulphate and a Mw of 1,614.1 kDa.

**Animals and experimental design** Male C57BL/6J mice, 4-5 weeks old, were purchased from Vital River Laboratory Animal Center (Beijing, China) (Licensed ID: SCXK2011-0001). Mice were housed under 12-12 h light-dark conditions at 23±1°C daily. Use of animals in this study was approved by the Ethical Committee of Experimental Animal Care at Ocean University of China. An insulin resistant mouse model was established based on feeding of a high-fat, high-sucrose diet (HFSD) (Research Diets, New Brunswick, NJ, USA) (#D12331), consisting of 20% protein, 25% fat and 20% sucrose (13). Mice were randomly placed into 4 groups of 10 animals each as, 1) control, 2) HFSD-fed (HFSD), 3) 1 mg/kg/d rosiglitazone (RSG)-treated (RSG), and 4) 80 mg/kg/d *Am*-FUC-treated (*Am*-FUC). Mice in the control group were fed with a low-fat low-sucrose diet consisting of 20% protein, 25% fat, and no sucrose (LFSD) (#D12328). Other mice were fed with HFSD. Previous study has shown that a type 2 diabetic model can be successfully established (19). After 19 weeks of treatment, 8 h fasted animals were sacrificed through bloodletting from orbital plexus vein. Blood was collected for determination of NO, ROS, FFA, CRP, MIP-1, IL-1β, IL-6, and IL-10 levels. The tail of the pancreas was carefully removed to observe the histological structure of pancreatic islet cells and for detection of apoptosis-related gene expression. Epididymal adipose tissue was separated for detection of inflammatory gene mRNA expression.

**Serum NO, ROS, and FFA level assays** After 19 weeks of treatment, serum NO, ROS, and FFA levels were assessed using corresponding ELISA assay kits (Invitrogen, Carlsbad, CA, USA).

**Serum inflammatory cytokine level assays** Concentrations of the serum pro-inflammatory cytokines CRP, MIP-1, IL-1β, and IL-6, and the anti-inflammatory cytokine IL-10 were determined using corresponding ELISA assay kits (Invitrogen) according to manufacturer instructions.

**Microscopic structures of pancreatic islets** Pancreatic tails were fixed in 10% formalin, paraffin embedded, sectioned, and stained with hematoxylin and eosin (HE). Microscopic structures of pancreatic islet cells was observed and photographed using an optical microscope (BH-2; Olympus, Tokyo, Japan).

**Reverse transcriptase (RT) PCR analysis** Expression levels of the apoptosis-related genes Bid, Bax, Bcl-2, Bcl-xL, cytochrome *c*, caspase 9, and caspase 3 were examined using RT-PCR. Briefly, total RNA from the pancreas or adipose tissue was extracted using TRIzol reagent, and 1 μg of RNA was reverse transcribed to cDNA using M-MLV. PCR was carried out in a 30 μL system containing 0.5 μg of cDNA using an MJ Research Thermocycler (TC-96/G/H(b)A, Hangzhou, China). Thermocycling was performed via denaturation at 94°C for 45 s, annealing at the desirable temperature for 45 s, and extension at 72°C for 45 s for the appropriate cycling number for each primer pair, followed by additional extension at 72°C for 10 min. PCR products were separated on 1% agarose gel buffered with Tris-acetate-EDTA and visualized using ethidium bromide staining. The housekeeping gene β-actin was used as a control. The primer sequences used for amplification are listed in Table 1. Bands were quantified using the Image J program (Version 1.41o; NIH, Bethesda, MA, USA). mRNA relative expression levels were expressed as a ratio of signal intensity for target genes to the β-actin value.

**Quantitative real time (qRT)-PCR analysis** The mRNA expression levels of the inflammatory cytokine genes TNF-α, CRP, MIP-1, IL-1β, IL-6, and IL-10 were examined using qRT-PCR. Briefly, total RNA from epididymal adipose tissues was extracted using TRIzol reagent, and 1 μg of RNA was reversed transcribed to cDNA. PCR was performed based on amplification of 15 ng of cDNA in a 25 μL system containing SYBR-Green mix in a quantitative real-time PCR thermocycler (iQ5; Bio-Rad, Hercules, CA, USA). Cycling conditions were pre-denaturation at 95°C for 10 min, denaturation at 95°C for 15 s, annealing at 60°C for 20 s, stretching at 72°C for 30 s with 45 cycles. Primer sequences used for amplification are listed in Table 2. PCR products were quantified using iCycler iQ5 software. The housekeeping gene β-actin was used as an internal reference.

**Isolation of the pancreatic cytosolic fraction** The cytosolic fraction was prepared following the method of Zhang *et al*. (8) with modification and used for measurement of cytochrome *c* release. Briefly, pancreatic tissues were disrupted using Western and IP lysis buffer (50 mM Tris (pH 7.4), 150 mM NaCl, 1% TritonX-100, 1% sodium deoxycholate, 0.1% SDS, 2 mM sodium pyrophosphate, 25 mM β-glycerophosphate, 1 mM EDTA, 1 mM $Na_3VO_4$, and 0.5 μg/mL leupeptin) in a glass homogenizer. The homogenate was centrifuged twice at 14,000×*g* for 30 min at 4°C, then the supernatant

**Table 1.** PCR primers used in apoptosis related gene mRNA expression

| Genes | Primer | Tm (°C) | No. of cycle |
|---|---|---|---|
| Bid | F 5′-CCAAATAGGCGATGAGAT-3′<br>R 3′-CACCTGGAAATAGGGAGA-5′ | 55 | 30 |
| Bax | F 5′-CTTCTTCCAGATGGTGAGTGAG-3′<br>R 3′-CCTTTTGCTTCAGGGTTTCA-5′ | 59 | 32 |
| Cytochrome *c* | F 5′-GTCCTCGTCCGTGGTCGTT-3′<br>R 3′-TCAAGGTGGGCTGGCAAA-5′ | 55 | 34 |
| Bcl-2 | F 5′-TGGGAGAACAGGGTACGATA-3′<br>R 3′-CCACCGAACTCAAAGAAGG-5′ | 59 | 32 |
| Bcl-xl | F 5′-ACCCCCTAAGAGCCATTT-3′<br>R 3′-GTCCCCTGAACCCACCTA-5′ | 58 | 32 |
| Caspase 9 | F 5′-ACAATAAATCTTCGGCAATAGG-3′<br>R 3′-TGGGGACCAGGCTCACTT-5′ | 52 | 34 |
| Caspase 3 | F 5′-TGACTGGAAAGCCGAAACT-3′<br>R 3′-CTGGATGAACCACGACCC-5′ | 52 | 36 |
| β-Actin | F 5′-GTTGGCGTTACACCCTTTCTTG-3′<br>R 3′-TGTCACCTTCACCGTTCCA-5′ | 60 | 27 |

was centrifuged at 100,000×*g* for 30 min at 4°C to yield a cytosolic supernatant used for assay of cytochrome *c* protein expression with Western blotting (WB).

**WB analysis** Pancreatic tails (0.1 g) were lysed in Western and IP lysis buffer to dissolve cellular protein, which were subsequently resolved using 10% SDS-PAGE, and transferred to polyvinylidene fluoride membranes. Members were then blotted with 5% non-fat milk. Protein was incubated with t-Bid, Bax, cytochrome *c*, Bcl-2, Bcl-xL, caspase 3, cleaved-caspase 3, caspase 9, or β-actin primary antibodies (Cell Signaling, Beverly, MA, USA) and HRP-IGG secondary antibodies (Cell Signaling), then subjected to detection using ECL (Applygen, Beijing, China). Normalization of protein expression was carried out using β-actin as a control.

**Statistical analysis** Data were expressed as mean±standard deviation (SD) with Graph Pad Prism version 3.0 for Windows. A one-way analysis of variance (ANOVA) followed by Tukey's test with SPSS version 17.0 software was used for multiple comparisons at $p<0.05$.

## Results and Discussion

***Am*-FUC repaired pancreatic islet cells** Pathologic manifestations of pancreatic islet cell atrophy, cell necrosis, uneven distribution of nuclear chromatin, and substantial inflammatory infiltration were found in type 2 diabetic mice (Fig. 1A). These histological changes were alleviated in *Am*-FUC-treated animals, indicating that *Am*-FUC can repair HFSD-injured pancreatic islets.

***Am*-FUC decreased caspase 3 and caspase 9 expressions** Caspase 3 and caspase 9 gene expressions were studied to determine whether *Am*-FUC repaired pancreatic islet cells via inhibition of islets cell apoptosis. Caspases are important regulators of apoptosis, especially caspase 3, which is a marker of apoptosis (20). Caspase 9 is an initiator of caspase 3 activity (5). *Am*-FUC significantly ($p<0.01$) reduced caspase 3 and caspase 9 gene mRNA expressions in HFSD-induced type 2 diabetic mice pancreatic islet cells, compared with controls (Fig. 1B and 1C). Further verification using WB showed that *Am*-FUC significantly ($p<0.01$) decreased caspase 9 and cleaved-caspase 3 protein expressions, compared with controls. However, the caspase 3 protein level was unchanged (Fig. 1D and 1E). Therefore, HFSD induced changes in caspase 3 gene expression at the transcriptional level, but there was no change at the translational level. Thus, *Am*-FUC can inhibit pancreatic islet cell apoptosis.

***Am*-FUC regulated inflammatory cytokine and gene mRNA expressions** Pro-inflammatory mediators can induce apoptosis of pancreatic β-cells while anti-inflammatory cytokines can inhibit this process (21). Effects of *Am*-FUC on serum CRP, MIP-1, IL-1β, IL-6, and IL-10 levels are shown in Table 3. In HFSD group mice, significantly ($p<0.01$) higher levels of CRP, MIP-1, IL-1β, and IL-6, and a significantly ($p<0.01$) lower level of IL-10 were observed, compared with control group mice. *Am*-FUC consumption by HFSD-fed mice significantly ($p<0.05$) reduced serum CRP, MIP-1, IL-1β, and IL-6 concentrations by 17.6, 35.9, 29.0, and 20.6%, respectively, and significantly ($p<0.05$) increased the serum IL-10 concentration by 46.9%, compared with controls. A previous study showed that *Am*-FUC can decrease the serum TNF-α level (19). Thus, *Am*-FUC can suppress pancreatic islet cell apoptosis *via* regulation of inflammatory factors in type 2 diabetic mice.

**Table 2.** PCR primers used in inflammatory gene mRNA expression

| Genes | Forward primer | Reverse primer |
|---|---|---|
| TNF-α | 5′-CTGTGAAGGGAATGGGTGTT-3′ | 3′-CTGGAAAGGTCTAAGAAGGGAC-5′ |
| CRP | 5′-TCAAAGCCTTCACTGTGTGC-3′ | 3′-GGACACCTAGGTTGAGTGGA-5′ |
| MIP-1α | 5′-CATGACACTCTGCAACCAAGTCTT-3′ | 3′-ACGAAGAGGATGTCGGCCT-5′ |
| IL-1β | 5′-CTCGGCCAAGACAGGTCGCTC-3′ | 3′-GGATCGACAGTTGCACACCCCC-5′ |
| IL-6 | 5′-TTCTTGGGACTGATGCTG-3′ | 3′-TTGTTCTTTCTGTTTCGGTC-5′ |
| IL-10 | 5′-GGAGGGGTTCTTCCTTGGGA-3′ | 3′-CGGCGAAGTAGGGACTTTTG-5′ |
| β-Actin | 5′-CAAGGCATTGCTGACAGGATG-3′ | 3′-GGTCGTCTACACCTAGTCGT-5′ |

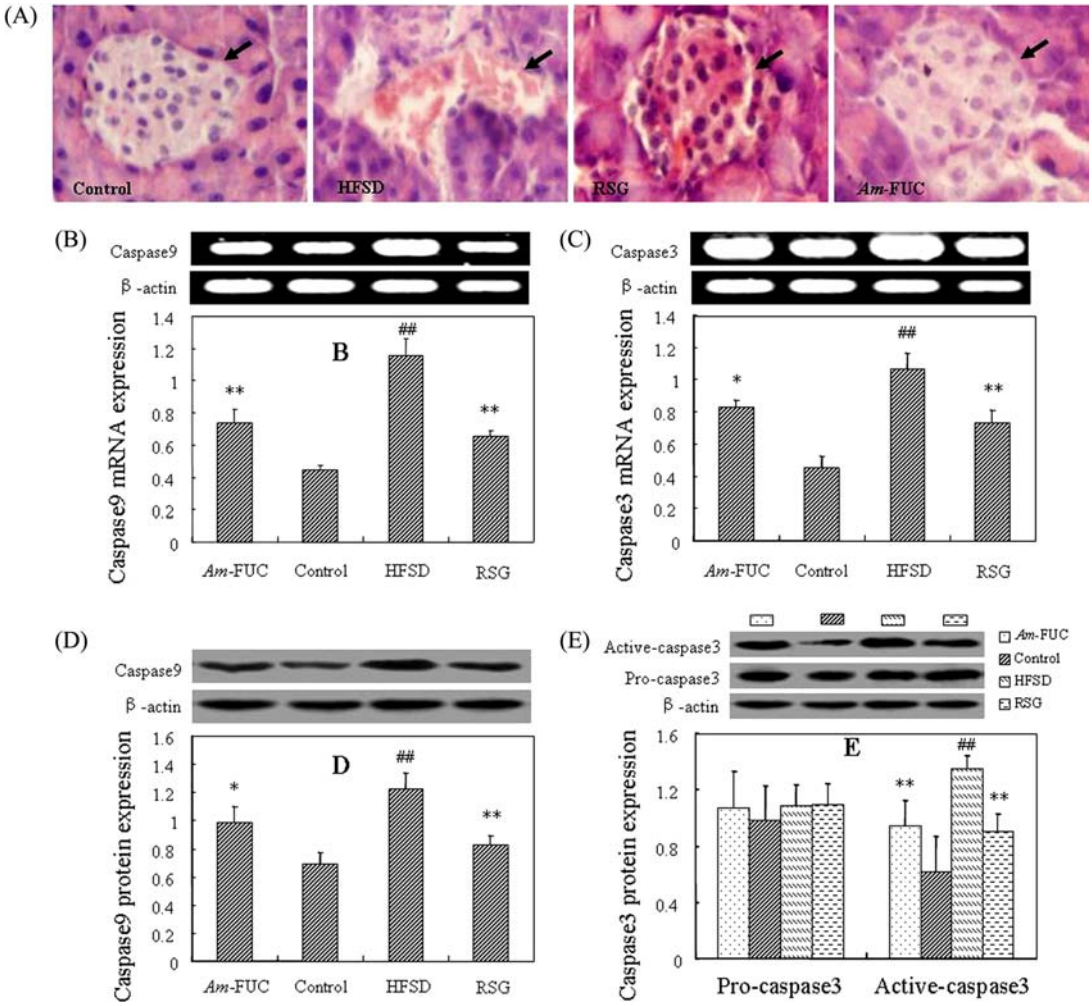


**Fig. 1.** Effect of *Am*-FUC on microstructure of pancreatic islet cells, and mRNA and protein expressions of caspase 9 and caspase 3 in type 2 diabetic mice. A, observation of histomorphology, × 400; arrow points to pancreatic islets, *n*=4/group; B: caspase 9 mRNA expression, C: caspase 3 mRNA expression, D: caspase 9 protein expression, E: caspase 3 protein expression, Data are expressed as mean±SD. (*n*=6/group). Multiple comparisons were done using a one-way ANOVA followed by Tukey's test. $^{\#\#}p<0.01$ versus control; $^{*}p<0.05$, $^{**}p<0.01$ vs. HFSD.

**Table 3.** Effects of *Am*-FUC on serum CRP, MIP-1, IL-1β, IL-6, and IL-10 levels in HFSD-induced insulin resistant mice

| Parameters | Control | HFSD | RSG | *Am*-FUC |
|---|---|---|---|---|
| CRP (ng/mL) | 98.9±8.0 | 142±15.0$^{\#\#}$ | 114±5.0** | 117±9.0** |
| MIP-1 (pg/mL) | 24.4±1.2 | 63.2±3.5$^{\#\#}$ | 35.3±3.0** | 40.5±3.3** |
| IL-1β (pg/mL) | 48.3±5.0 | 74.8±6.0$^{\#\#}$ | 57.4±5.5** | 53.1±4.9** |
| IL-6 (pg/mL) | 32.9±3.7 | 52.5±4.4$^{\#\#}$ | 38.7±3.1** | 41.7±2.7** |
| IL-10 (pg/mL) | 124±6.0 | 73.5±4.1$^{\#\#}$ | 113±2.0** | 108±6.0** |

Data are reported as mean±SD (*n*=10/group). Multiple comparisons were done using a one-way ANOVA followed by Tukey's test. $^{\#\#}p<0.01$ vs. control mice; $^{**}p<0.01$ vs. HFSD mice.

Serum inflammatory cytokines are regulated by *Am*-FUC. Therefore, mRNA expressions of these inflammatory genes were investigated in epididymal adipose tissues in which inflammatory cytokines were secreted. *Am*-FUC significantly ($p<0.05$, $p<0.01$) decreased TNF-α, CRP, MIP-1, IL-1β, and IL-6 mRNA expressions, and significantly ($p<0.01$) increased IL-10 mRNA expression, compared with controls (Fig. 2A). Thus, *Am*-FUC can regulate inflammatory cytokine gene expression at the transcriptional level.

***Am*-FUC decreased serum NO, ROS, and FFA levels** Other inflammatory stressors, such as NO, ROS, and FFA, also can induce apoptosis of pancreatic islet cells. Lin *et al*. (22) reported that ROS inhibition could ameliorate palmitate-induced INS-1 beta cell death. Serum NO, ROS, and FFA levels in type 2 diabetic mice are shown in Fig. 2B. Levels of these inflammatory stressors were significantly ($p<0.05$) higher in HFSD group mice than in control mice. *Am*-FUC treatment significantly ($p<0.05$) decreased serum NO, ROS, and FFA levels by 31.5, 23.5, and 23.6%, respectively, compared with controls. Thus, *Am*-FUC can protect pancreas cells from death via decreases in whole-body NO, ROS, and FFA levels in type 2 diabetic mice.

***Am*-FUC blocked the intrinsic mitochondrial pathway** The

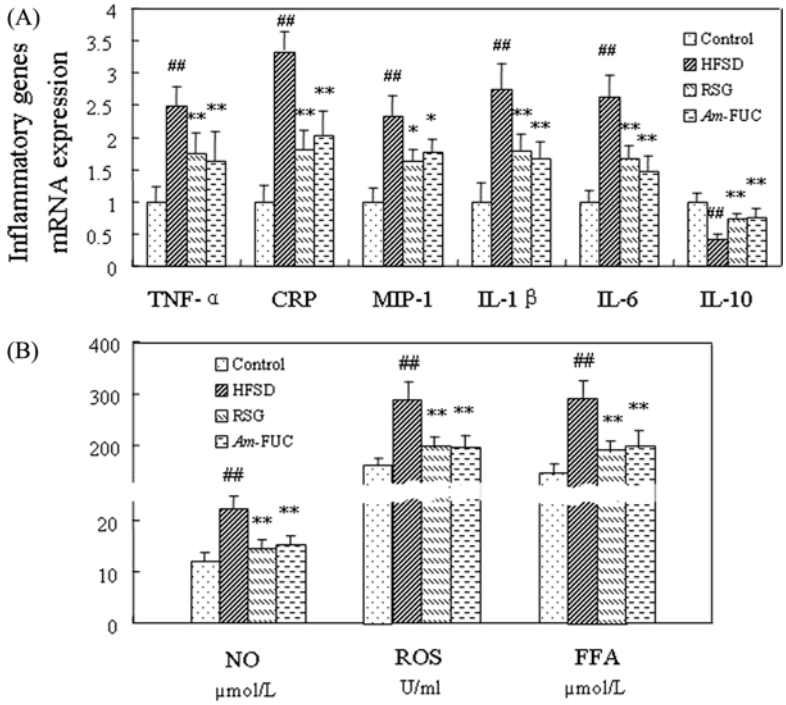


**Fig. 2.** Effects of *Am*-FUC on mRNA expression of TNF-α, CRP, MIP-1, IL-1β, IL-6, and IL-10 in epididymal adipose tissues, and serum NO, ROS, and FFA levels in type 2 diabetic mice. (*n*=10/group). A, mRNA expression of inflammatory cytokines; B, serum NO, ROS, and FFA levels. Multiple comparisons were done using a one-way ANOVA followed by Tukey's test. $^{\#\#}p<0.01$ versus control; $^{**}p<0.01$ vs. HFSD.

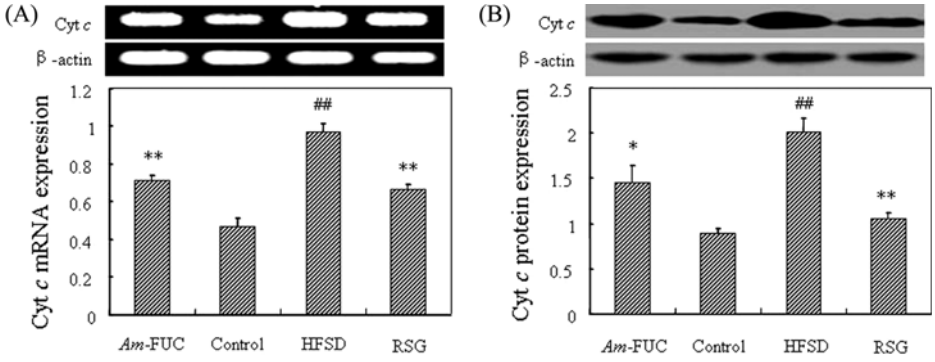


**Fig. 3.** Effects of *Am*-FUC on mRNA and protein expression of cytochrome *c* (Cyt *c*) in pancreatic islet cells of type 2 diabetic mice. Cytochrome *c* protein was detected using the cytosol fraction of pancreatic islet cells. A: cytochrome *c* mRNA expression, B: cytochrome *c* protein expression. Data are expressed as mean±SD. (*n*=6/group). Multiple comparisons were done using a one-way ANOVA followed by Tukey's test. $^{\#\#}p<0.01$ versus control; $^{*}p<0.05$, $^{**}p<0.01$ vs. HFSD.

inflammatory stressors TNF-α and FFA can indirectly activate mitochondrial apoptotic pathways. Increased circulating concentrations of TNF-α and FFA induce caspases and c-Jun N-terminal protein kinase proteins activation (23), and the latters trigger cytochrome *c* into the cytoplasm from mitochondria (24). Cytochrome *c* release is crucial for the activation of the mitochondrial apoptotic pathway (25,26). FHSD induced cytochrome *c* mRNA increased ($p<0.01$) and the elevation significantly reversed by *Am*-FUC (Fig. 3). Furthermore, *Am*-FUC significantly ($p<0.01$) decreased cytochrome *c* protein level in the cytoplasm. These indicated that *Am*-FUC inhibited cytochrome *c* release from mitochondria to cytoplasm. Cytochrome *c* in the cytoplasm can cause β-cell apoptosis via activation caspase 3 and caspase 9 (27,28). In the present study, *Am*-FUC treatment decreased the mRNA expressions of caspase 3 and caspase 9, as well as the protein expressions of activated-caspase 3 and caspase 9. Thus, the mitochondrial apoptotic pathway was finally blocked by *Am*-FUC treatment.

Bax exerts an essential effect in activation of the execution phase of apoptosis (29). Upon activation, Bax protein assembles on the surface of mitochondria and forms homodimers. These homodimers changes the potential between inside and outside of the mitochondrial outer membrane (30). A pore was formed because the changes. Cytochrome *c* in between inner and outer membrane of mitochondria releases into the cytoplasm from the pore (31). Bax protein

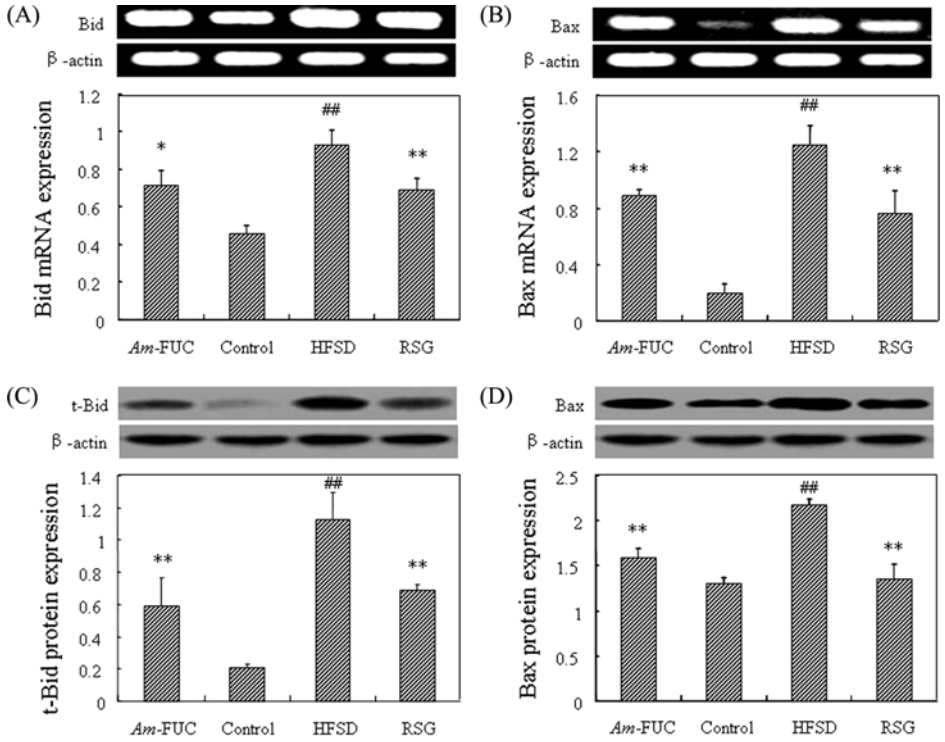


**Fig. 4.** Effects of *Am*-FUC on mRNA and protein expression of Bax and Bid in pancreatic islet cells of type 2 diabetic mice. A: Bid mRNA expression, B: Bax mRNA expression, C: Bax protein expression, D: t-Bid protein expression. Data are expressed as mean±SD. (*n*=6/group). Multiple comparisons were done using a one-way ANOVA followed by Tukey's test. $^{\#\#}p<0.01$ versus control; $^{*}p<0.05$, $^{**}p< 0.01$ vs. HFSD.

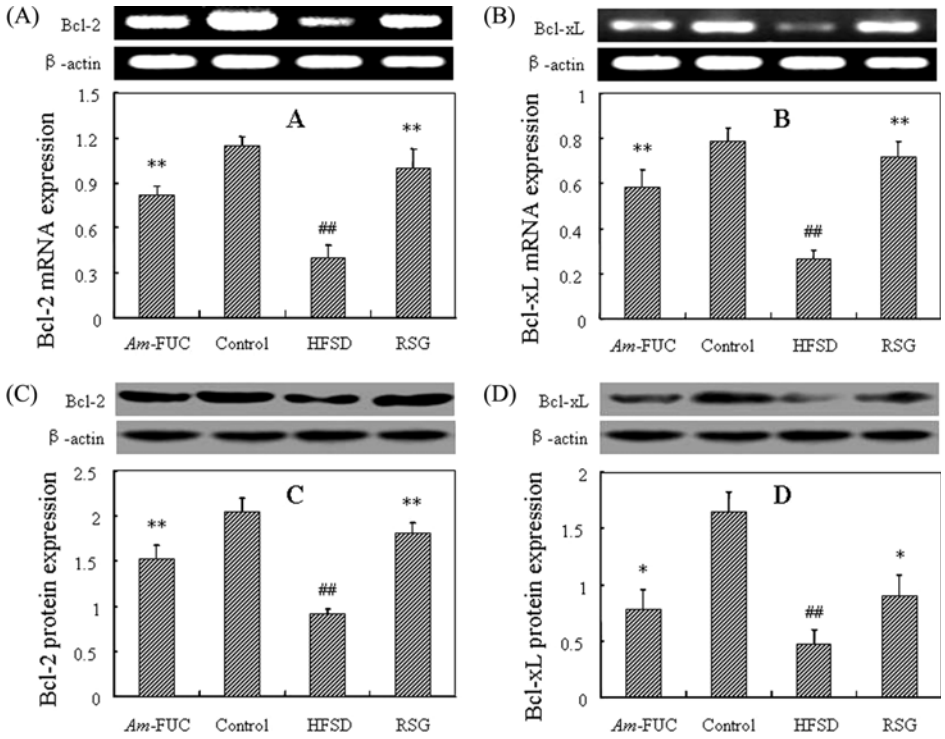


**Fig. 5.** Effects of *Am*-FUC on mRNA and protein expression of Bcl-2 and Bcl-xL in pancreatic islet cells of type 2 diabetic mice. A: Bcl-2 mRNA expression, B: Bcl-xL mRNA expression, C: Bcl-2 protein expression, D: Bcl-xL protein expression. Data are expressed as mean±SD. (*n*=6/group). Multiple comparisons were done using a one-way ANOVA followed by Tukey's test. $^{\#\#}p<0.01$ versus control; $^{*}p<0.05$, $^{**}p<0.01$ vs. HFSD.

translocation to mitochondrial outer membrane was motivated by a BH3-only protein, Bid (32). Only truncated Bid (t-Bid) exhibits its bioactivity when Bid was split by some stimulation of apoptotic factors, such as caspase 8 (26,29). In our study, the mRNA expressions of Bax and Bid were significantly ($p$<0.01) reduced by *Am*-FUC treatment. Meanwhile, Bax and t-Bid protein expressions in pancreatic islet cells were also significantly ($p$<0.01) decreased after treatment with Am-FUC.

Bcl-2 family of proteins controls the mitochondria apoptotic pathway (25). In the family, Bcl-2 and Bcl-xL are the proteins which play a role in inhibition of apoptosis. Bcl-2 and Bcl-xL can block Bax protein translocation to mitochondrial outer membrane (33). They also suppress cleavage of Bid protein (34). mRNA expressions of Bcl-2 and Bcl-xL were significantly ($p$<0.01) higher in *Am*-FUC-treated mice than in control mice (Fig. 5). Furthermore, WB assays showed that *Am*-FUC also significantly increased gene protein expressions of Bcl-2 ($p$<0.05) and Bcl-xL ($p$<0.01), compared with controls. Thus, *Am*-FUC inhibited pancreatic islet cell apoptosis via inactivation of the BH3-only protein Bid and consequent inactivation of the intrinsic mitochondrial pathway. Reduction of Bax activity, and activation of Bcl-2 and Bcl-xL by *Am*-FUC inhibited cytochrome *c* release into the cytoplasm and, subsequently, inactivated the apoptotic effectors caspase 9 and caspase 3.

In conclusion, this study revealed that *Am*-FUC can repair HFSD-induced pancreatic islet cell apoptosis. Inhibition in inflammatory stressors leading to blocking of the intrinsic mitochondrial pathway was the underlying mechanism for the beneficial effects of *Am*-FUC. Therefore, supplementation with *Am*-FUC provides a dietary intervention against inflammation-induced pancreatic islet cell apoptosis, providing an alternative therapy for type 2 diabetes.

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**Disclosure** The authors declare no conflict of interest.

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