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Gryllus bimaculatus extract protects against palmitate-induced β-cell death by inhibiting ceramide synthesis

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Abstract

Type I diabetes mellitus is an autoimmune disease characterized by the destruction of β-cells, leading to severe insulin deficiency. Environmental factors and genetic predisposition are implicated in β-cell destruction, which is the final step in a cascade of complex events. Possible triggers of β-cell destruction are activation of Fas, activation of perforin, increased generation of reactive oxygen species, increased production of inflammatory cytokines, and endoplasmic reticulum (ER) stress. In this study, we examined whether *Gryllus bimaculatus* (GB) extract could prevent palmitate-induced β-cell apoptosis. Exposure to GB extract prevented palmitate-induced death of MIN6 cells, a mouse pancreatic β-cell line. Palmitate increased total ceramide levels with the elevation of ceramide synthase (CerS)1, CerS4, and CerS6 expressions. Treatment with GB extract decreased the levels and expressions of ceramides related to insulin resistance. CerS4 and CerS6 overexpression, but not CerS1 overexpression, increased palmitate-induced MIN6 cell death by increasing ceramide synthesis. Oppositely, inhibition of ceramide synthesis by fumonisin B1 treatment partially recovered palmitate-induced MIN6 cell death. Furthermore, GB extract reduced ER stress (phosphorylation of PERK and eIF2α), NF-κB–iNOS signaling, and the phosphorylation of MAP kinase (JNK, p38). GB extract reduced pro-apoptotic Bax protein expression but increased anti-apoptotic Bcl2 expression. In addition, CerS4 and CerS6 overexpression aggravated impairment of insulin secretion by palmitate, but GB extract recovered it. In conclusion, GB could be a functional food that improves palmitate-induced β-cell death and insulin secretion.

Keywords: *Gryllus bimaculatus*, Ceramide, β-cell death, Ceramide synthase, Palmitate

Introduction

Type 1 diabetes mellitus (DM) accounts for 10% of all DM [1]. Type 1 DM is an autoimmune disease in genetically predisposed patients. Environmental factors can activate the immune system, which then attacks pancreatic β-cells, ultimately leading to insulin deficiency, which induces DM [1]. One of the pancreatic β-cell death mechanisms is endoplasmic reticulum (ER) stress, which is induced by free fatty acids (FFAs) and inflammatory cytokines [2]. FFAs induce β-cell toxicity and NF-κB activation [2]. TNF-α and IL-1β increase NF-κB and caspase-3 activation [3]. NF-κB activation induces several inflammation-related proteins, such as inducible nitric oxide synthase (iNOS) and inflammasome complex formation (NRLP3, ASC, caspase-1) [3, 4]. Palmitate, a C16 FFA, increases the generation of C16-ceramide [5, 6], which can induce inflammation and cell death.

Ceramide can be synthesized by several enzymes, and six ceramide synthases (CerS) can determine the acyl chain length of ceramide [7, 8]. For example, CerS1 generates C18-ceramide, CerS2 produces C22–C24-ceramides, CerS4 catalyzes the synthesis of C20-ceramide, and CerS5 and CerS6 produce C16-ceramide. Palmitate treatment increases the generation of C16-, C20-, and C24-ceramides, which reduces insulin secretion and

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induces β-cell death [9]. CerS4 is involved in glucolipotoxicity-induced apoptosis [10]. Therefore, ceramides and their regulatory enzymes are targets for therapeutic interventions for type 1 DM.

Tenebrio molitor, *A. diaperinus*, *L. migratoria*, and *G. bimaculatus* (GB) are some examples of edible insects. Most edible insects contain high amounts of protein, fat, vitamins, and minerals [11]. Therefore, edible insects are considered highly nutritional. In addition to nutritional benefits, some health benefits have been associated with specific edible insects due to their bioactive components. For example, *T. molitor* larvae and *A. dichotoma* larvae prevented high-fat diet-induced obesity [12, 13], and GB extract had a glucose-lowering effect in streptozotocin-induced diabetic mice [14] and attenuated alcohol-induced steatohepatitis, apoptotic responses in the liver, and intestinal permeability to bacterial endotoxin [15]. Furthermore, it had anti-inflammatory effects in chronic arthritis models [16], macrophage cells [4], and colon epithelial cells [17]. Therefore, GB extract has several benefits in the treatment of inflammatory and metabolic diseases. In this study, we examined the effects of GB extract on palmitate-induced β-cell death and the underlying mechanism.

Materials and methods

Materials

Palmitate, anti-CerS2 antibody (HPA027262), anti-CerS3 antibody (HPA006092), anti-CerS4 antibody (SAB4301210), anti-CerS5 antibody (SAB4301211), and anti-α-tubulin (T9026) antibody were obtained from Sigma-Aldrich (St. Louis, MO). Anti-p-PERK (Thr980) (3179), anti-p-eIF2α (Ser51) (3597), anti-p-SAPK/JNK (Thr183/Tyr185) (9255), anti-p-p38 (Thr180/Tyr182) (4511), anti-p-p65 (Ser536) (3033), anti-iNOS (13120), anti-cleaved caspase-3 (9664), anti-Bcl2 (3498), and anti-Bax (14796) antibodies were purchased from Cell Signaling Technology (Beverly, MA). Anti-LASS6 (CerS6) antibody (sc-100554) was sourced from Santa Cruz Biotechnology (Dallas, TX). Anti-CerS1 antibody (H00010715-M01) was purchased from Abnova (Taipei, Taiwan). Anti-mouse horseradish peroxidase (HRP)-conjugated (115-036-003) and anti-rabbit HRP-conjugated (111-035-003) antibodies were procured from Jackson Laboratory (Bar Harbor, ME).

GB extract

After fasting for 2 days, GB insects were washed, steamed, dried at 60℃, and pulverized as a powder form. Then, GB extract was prepared as previously described [4, 15]. Briefly, GB powder was extracted with 70% ethanol overnight. GB extract was filtered, dried, and kept at − 20 ℃ for further use.

MIN6 cell culture and palmitate treatment

MIN6 cells were grown in Dulbecco modified Eagle medium with 15% fetal bovine serum, 1% penicillin/streptomycin, and 55 mM β-mercaptoethanol and subcultured every 3 days. The cells were treated with 0.5 mM palmitate for 24 h to induce cell death.

Transfection

MIN6 cells were transfected with 3 μg of pcDNA3.1-CerS1-HA, pcDNA3.1-CerS4-HA, or pcDNA3.1-CerS6-HA using Metafectene (Biotex Laboratories, Munich, Germany) in 6-well plates. All plasmids were kindly provided by Professor Anthony H. Futerman (Weizmann

Table 1 Primers used for real-time PCR

Gene	Primer sequences
CerS1 (for mouse)	F: 5′- TCCATCTATGCCACCGTGTA -3′ R: 5′- GCGTAGGAAGAGGCAATGAG -3′
CerS2 (for mouse)	F: 5′- TCTGCATGACGCTTCTGACT -3′ R: 5′- GATGGCGAACACAATGAAGA -3′
CerS3 (for mouse)	F: 5′- AAGCATTCCACAAGCAAACC -3′ R: 5′- GCCGAATCCTAAGCCATCTT -3′
CerS4 (for mouse)	F: 5′- TGCGCATGCTCTACAGTTTC -3′ R: 5′- CAGAAACTGGCTCGTCATCA -3′
CerS5 (for mouse)	F: 5′- GCAATCTTCCCATTGTGGAT -3′ R: 5′- TCAGGAGAAGGGCATTGAAG -3′
CerS6 (for mouse)	F: 5′- TTAGCTACGGAGTCCGGTTC -3′ R: 5′- TGAAGGTCAGCTGTGAGTGG -3′
GAPDH (for mouse)	F: 5′- ACTCACGGCAAATTCAACGG -3′ R: 5′- ATGTTAGTGGGGTCTCGCTC -3′

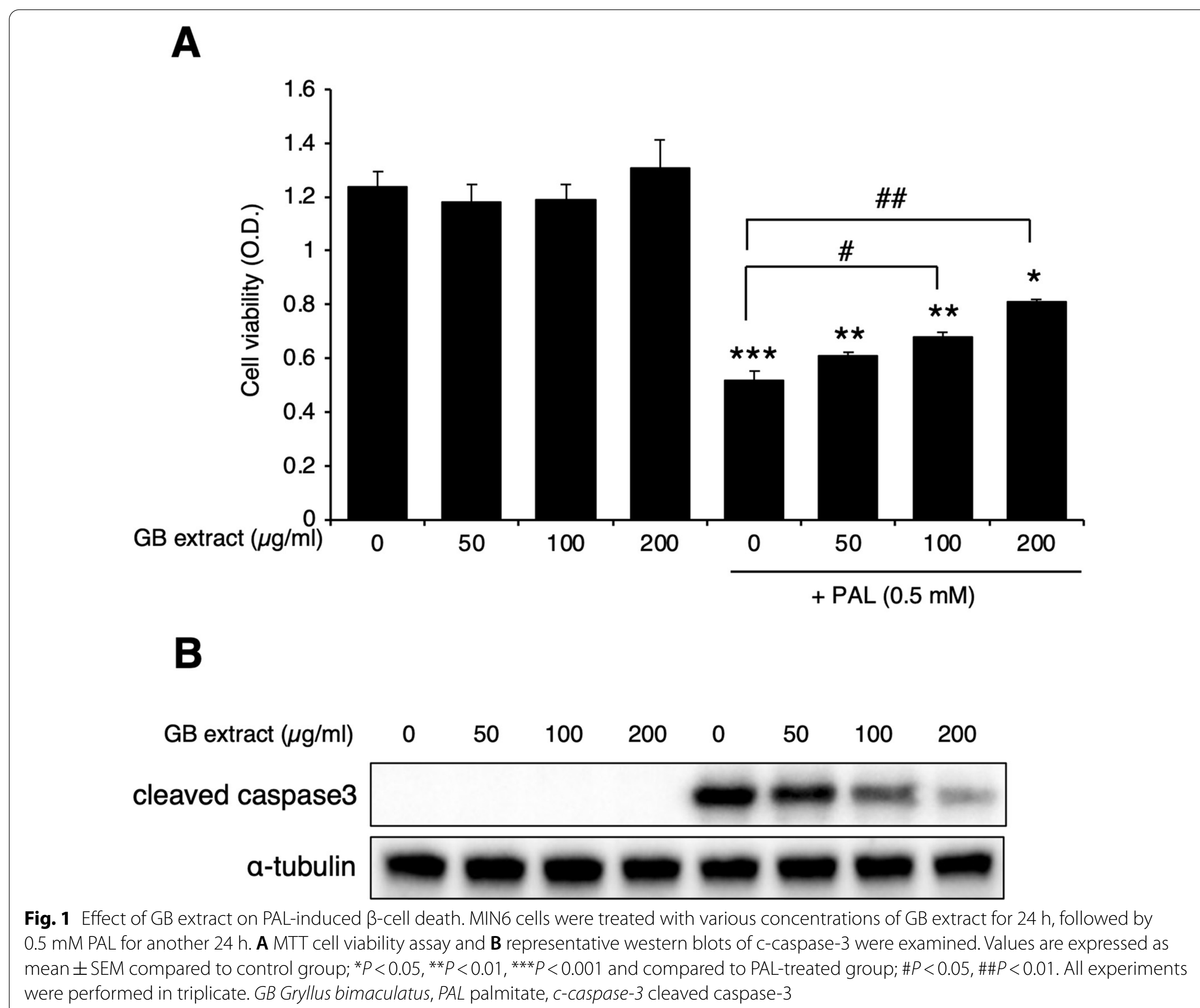

Fig. 1 Effect of GB extract on PAL-induced β-cell death. MIN6 cells were treated with various concentrations of GB extract for 24 h, followed by 0.5 mM PAL for another 24 h. **A** MTT cell viability assay and **B** representative western blots of c-caspase-3 were examined. Values are expressed as mean ± SEM compared to control group; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and compared to PAL-treated group; #$P < 0.05$, ##$P < 0.01$. All experiments were performed in triplicate. *GB Gryllus bimaculatus*, *PAL* palmitate, *c-caspase-3* cleaved caspase-3

Institute of Science, Israel). After incubation for 48 h, the transfected cells were treated with 0.5 mM palmitate.

Western blotting

MIN6 cells were lysed in RIPA buffer (50 mM Tris–Cl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% SDS) containing protease and phosphatase inhibitors (Sigma-Aldrich). Then, lysates were kept on ice for 30 min and centrifuged (10,000 ×*g*, 4 °C, 10 min). Protein levels in the supernatants were measured using the Bio-Rad Protein Assay dye reagent concentrate (Bio-Rad Laboratories, Hercules, CA). Proteins (50 μg) were loaded and separated on 10% SDS polyacrylamide gels and transferred to nitrocellulose membranes (Bio-Rad Laboratories). Membranes were incubated with blocking buffer [5% bovine serum albumin (BSA) in TBST (TBS with 0.1% Tween-20)] at 4 °C for 1 h. Primary antibodies (1:1000 dilutions) were attached by incubation overnight at 4 °C. This was followed by incubation with secondary antibodies at room temperature for 1 h. Protein bands were detected using the EzWestLumi Plus Reagent (ATTO Corp., Tokyo, Japan) and the ChemiDoc MP imaging system (Bio-Rad Laboratories).

MTT cell viability assay

MIN6 cells were seeded at 1×10^5 cells/well in a 96-well plate. The cells were pretreated with GB extract for 24 h and co-treated with 0.5 mM palmitate and GB extract for another 20 h. After treatment, MTT solution (0.5 mg/ml final concentration) was added, and the cells were further incubated for 4 h. The supernatant was discarded, and 200 μl dimethyl sulfoxide was added. After 24 h, the

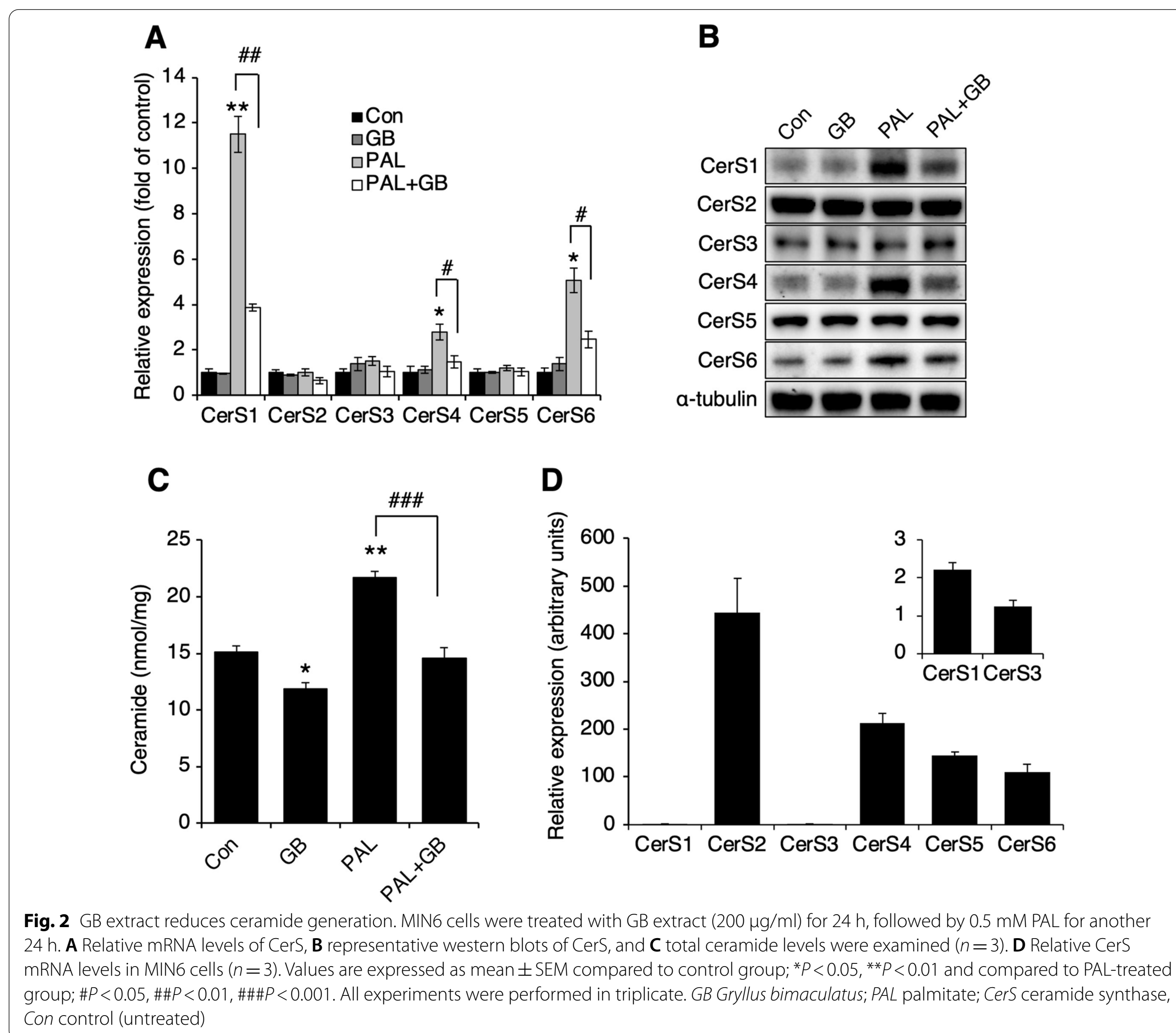

Fig. 2 GB extract reduces ceramide generation. MIN6 cells were treated with GB extract (200 μg/ml) for 24 h, followed by 0.5 mM PAL for another 24 h. **A** Relative mRNA levels of CerS, **B** representative western blots of CerS, and **C** total ceramide levels were examined ($n = 3$). **D** Relative CerS mRNA levels in MIN6 cells ($n = 3$). Values are expressed as mean ± SEM compared to control group; *$P < 0.05$, **$P < 0.01$ and compared to PAL-treated group; #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$. All experiments were performed in triplicate. *GB Gryllus bimaculatus*; *PAL* palmitate; *CerS* ceramide synthase, *Con* control (untreated)

solubilized purple formazan was quantified by spectrophotometric measurement at 540 nm. When MIN6 cells were transfected with pcDNA3.1-CerS1-HA, pcDNA3.1-CerS4-HA, or pcDNA3.1-CerS6-HA, followed by incubation for 48 h, the CerS1-, CerS4-, and CerS6-overexpressed cells were treated with 0.5 mM palmitate for 20 h, and then MTT solution was added, followed by incubation for another 4 h.

Real-time PCR

Total mRNA of MIN6 cells was collected using the RNeasy Kit (Qiagen, Valencia, CA), and cDNA was synthesized using the ReverTra Ace qPCR RT Master Mix (Toyobo, Tokyo, Japan). Quantitative PCR was carried out using the Thunderbird SYBR qPCR Mix (Toyobo) in a Bio-Rad CFX96 system (Bio-Rad Laboratories). Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method, as previously described [18]. The primers used are described in Table 1.

Insulin secretion assay

Insulin secretion was assayed as described elsewhere [19]. MIN6 cells were treated with 0.5 mM palmitate for 12 h and then washed with PBS and incubated in 5 mM Ca^{2+} containing HEPES-added Krebs–Ringer bicarbonate buffer (HKRB; 129 mM NaCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 4.7 mM KCl, 5 mM $NaHCO_3$, 2.5 mM $CaCl_2$, 10 mM HEPES [pH 7.4], containing 0.05% BSA) with 2.2 mM glucose. Insulin secretion in high-glucose

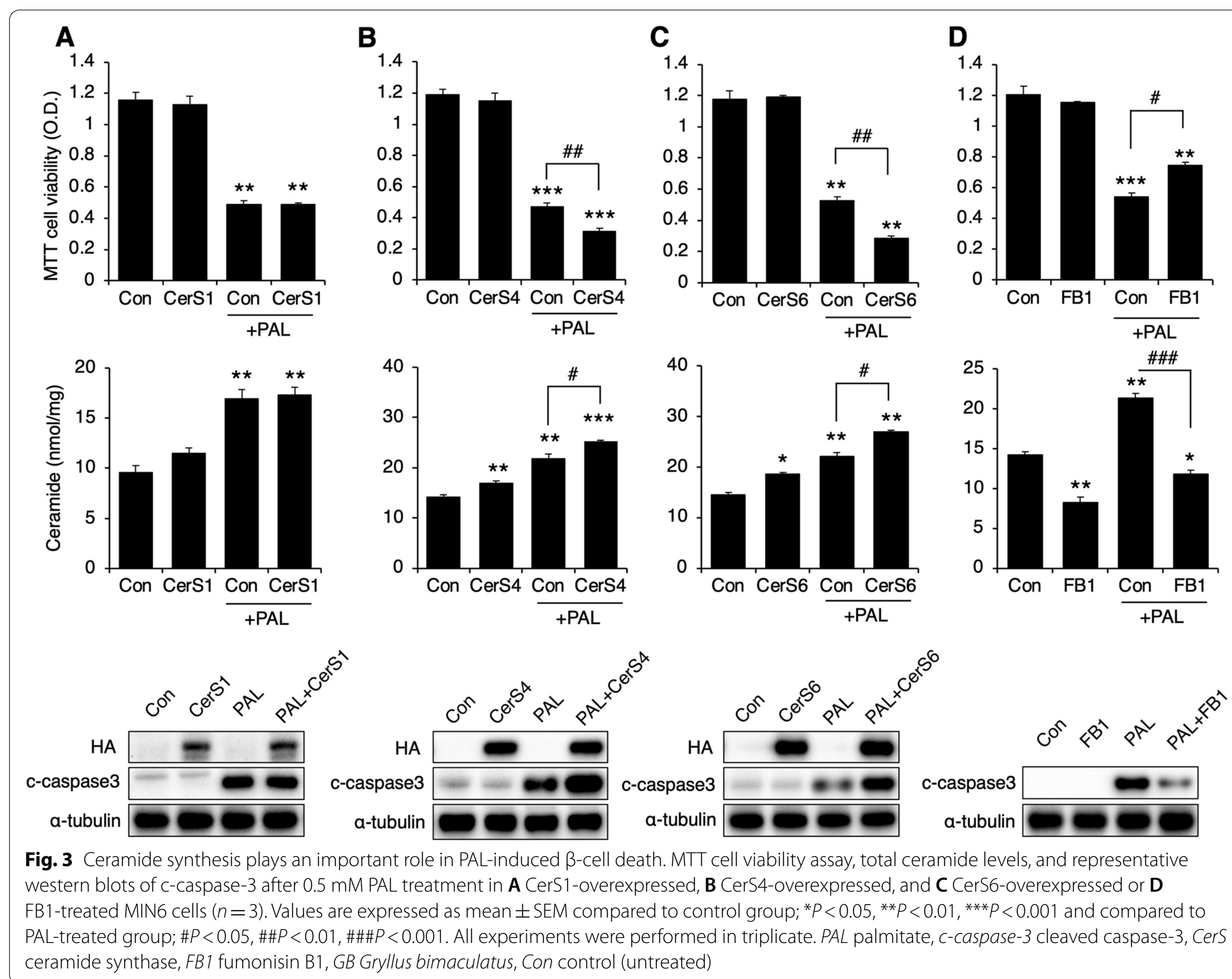

Fig. 3 Ceramide synthesis plays an important role in PAL-induced β-cell death. MTT cell viability assay, total ceramide levels, and representative western blots of c-caspase-3 after 0.5 mM PAL treatment in **A** CerS1-overexpressed, **B** CerS4-overexpressed, and **C** CerS6-overexpressed or **D** FB1-treated MIN6 cells ($n = 3$). Values are expressed as mean ± SEM compared to control group; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and compared to PAL-treated group; #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$. All experiments were performed in triplicate. *PAL* palmitate, *c-caspase-3* cleaved caspase-3, *CerS* ceramide synthase, *FB1* fumonisin B1, *GB Gryllus bimaculatus*, *Con* control (untreated)

conditions was performed by stimulation in HKRB containing 22 mM glucose for 1 h.

Enzyme-linked immunosorbent assay (ELISA)

TNF-α and nitrite levels were measured using TNF-α ELISA kits (Koma Biotech, Seoul, Korea) and the Nitrite Assay Kit (Griess Reagent) (Sigma-Aldrich), respectively. Insulin levels were examined using the Ultra-Sensitive Mouse Insulin ELISA Kit (Morinaga Institute of Biological Science, Inc., Yokohama, Japan). Total ceramide levels were measured using the Mouse Ceramide ELISA Kit (MyBioSource, San Diego, CA).

Statistical analyses

All experiments were repeated independently in triplicate, and the data were expressed as mean ± standard error of the mean (SEM). Statistical significance was calculated using Tukey's post hoc test (GraphPad Prism 6.0; GraphPad Software, San Diego, CA).

Results

GB extract prevents palmitate-induced death in MIN6 cells

To examine whether GB extract could prevent palmitate-induced cell death, we pretreated MIN6 cells with GB extract for 24 h, followed by treatment with palmitate for another 24 h. Palmitate treatment increased apoptosis, but GB extract pretreatment reduced palmitate-induced cell death (Fig. 1A) and caspase-3 cleavage (Fig. 1B).

GB extract reduces palmitate-induced ceramide generation in MIN6 cells

Some studies have shown that palmitate treatment can induce β-cell death by increasing the generation of C16-, C20-, and C24-ceramides and CerS4, CerS5, and CerS6 expressions [9, 10]. Therefore, we measured the changes

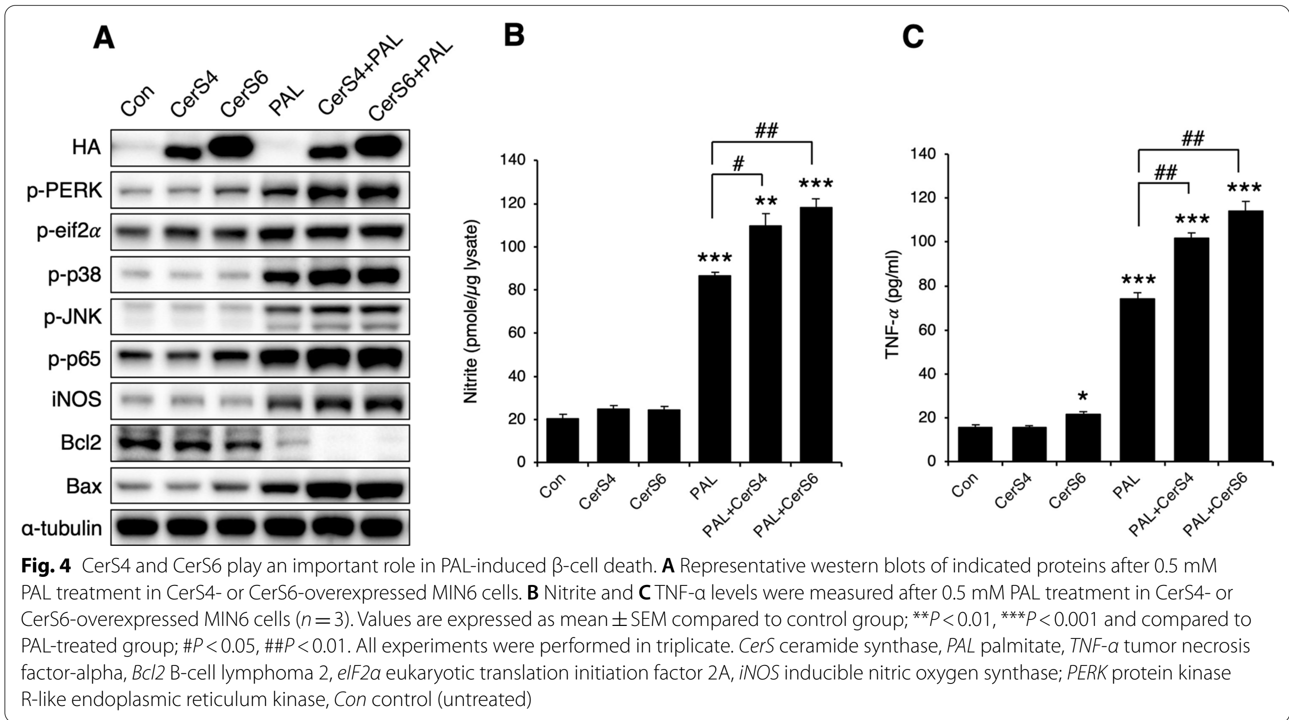

Fig. 4 CerS4 and CerS6 play an important role in PAL-induced β-cell death. **A** Representative western blots of indicated proteins after 0.5 mM PAL treatment in CerS4- or CerS6-overexpressed MIN6 cells. **B** Nitrite and **C** TNF-α levels were measured after 0.5 mM PAL treatment in CerS4- or CerS6-overexpressed MIN6 cells ($n = 3$). Values are expressed as mean ± SEM compared to control group; **$P < 0.01$, ***$P < 0.001$ and compared to PAL-treated group; #$P < 0.05$, ##$P < 0.01$. All experiments were performed in triplicate. *CerS* ceramide synthase, *PAL* palmitate, *TNF-α* tumor necrosis factor-alpha, *Bcl2* B-cell lymphoma 2, *eIF2α* eukaryotic translation initiation factor 2A, *iNOS* inducible nitric oxygen synthase; *PERK* protein kinase R-like endoplasmic reticulum kinase, *Con* control (untreated)

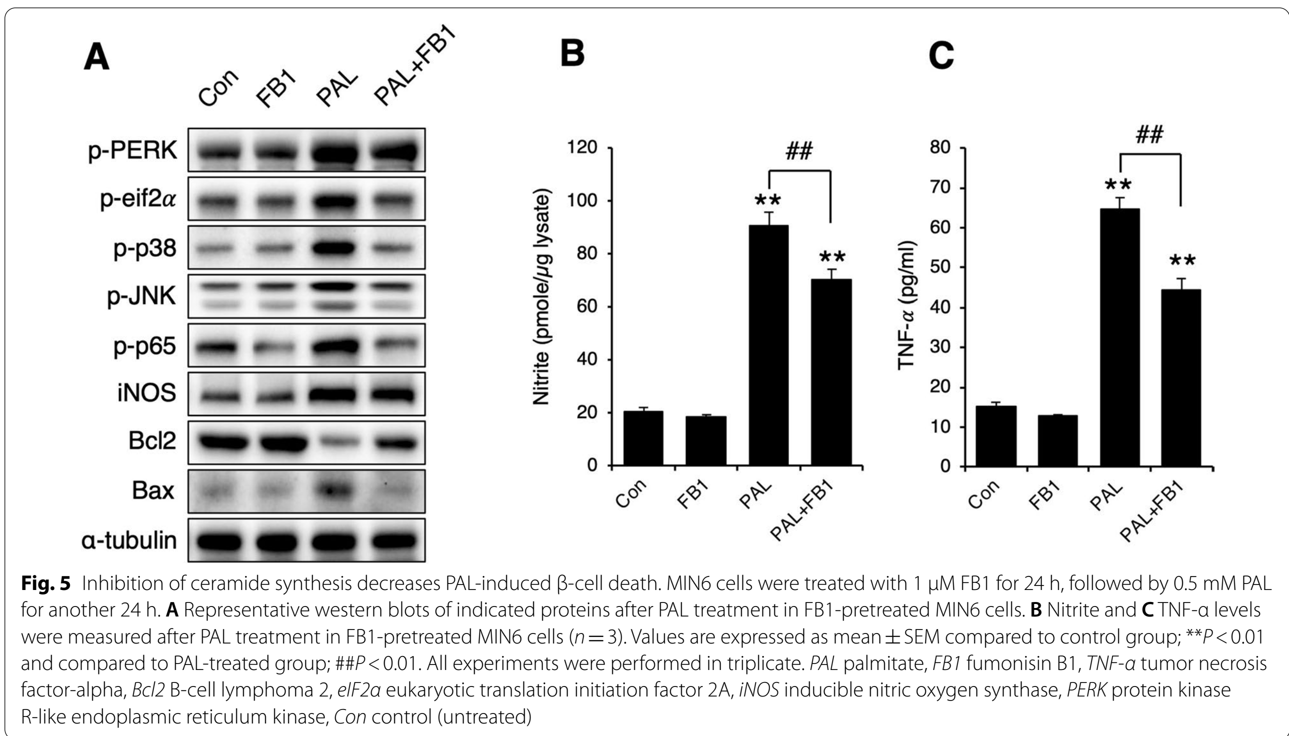

Fig. 5 Inhibition of ceramide synthesis decreases PAL-induced β-cell death. MIN6 cells were treated with 1 μM FB1 for 24 h, followed by 0.5 mM PAL for another 24 h. **A** Representative western blots of indicated proteins after PAL treatment in FB1-pretreated MIN6 cells. **B** Nitrite and **C** TNF-α levels were measured after PAL treatment in FB1-pretreated MIN6 cells ($n = 3$). Values are expressed as mean ± SEM compared to control group; **$P < 0.01$ and compared to PAL-treated group; ##$P < 0.01$. All experiments were performed in triplicate. *PAL* palmitate, *FB1* fumonisin B1, *TNF-α* tumor necrosis factor-alpha, *Bcl2* B-cell lymphoma 2, *eIF2α* eukaryotic translation initiation factor 2A, *iNOS* inducible nitric oxygen synthase, *PERK* protein kinase R-like endoplasmic reticulum kinase, *Con* control (untreated)

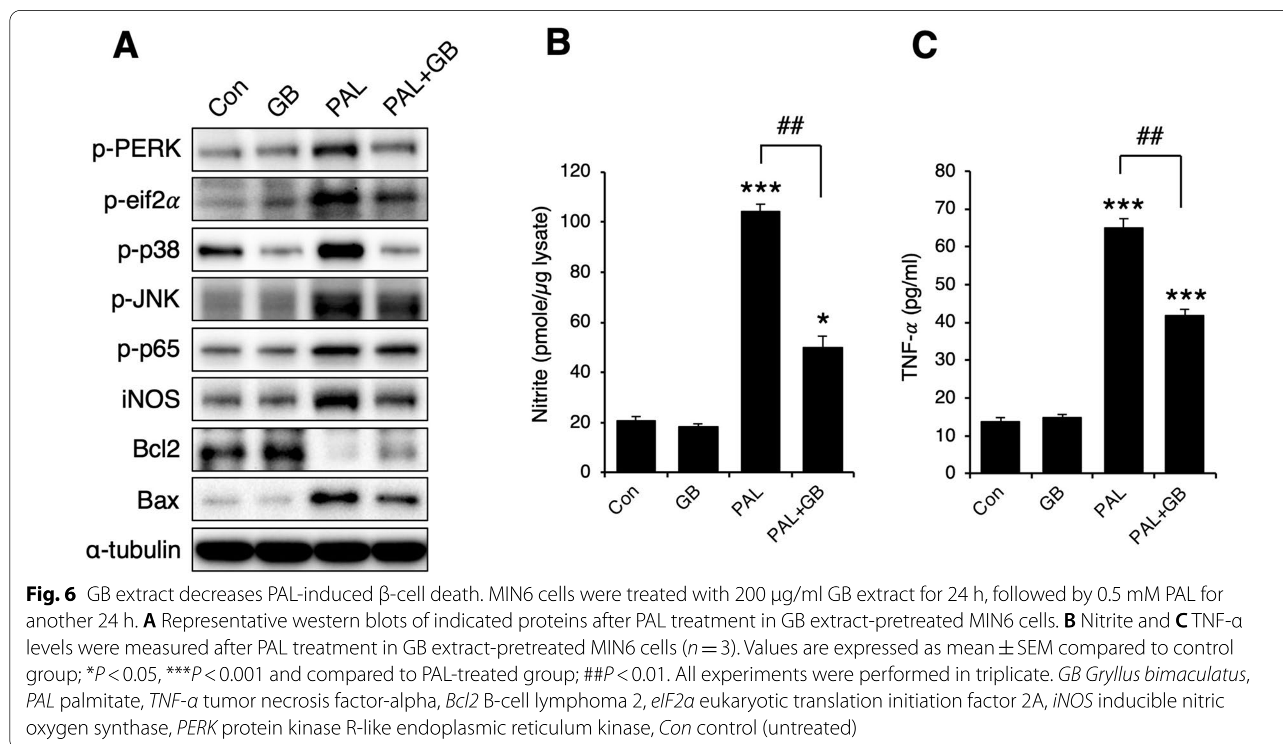

Fig. 6 GB extract decreases PAL-induced β-cell death. MIN6 cells were treated with 200 μg/ml GB extract for 24 h, followed by 0.5 mM PAL for another 24 h. **A** Representative western blots of indicated proteins after PAL treatment in GB extract-pretreated MIN6 cells. **B** Nitrite and **C** TNF-α levels were measured after PAL treatment in GB extract-pretreated MIN6 cells ($n = 3$). Values are expressed as mean ± SEM compared to control group; *$P < 0.05$, ***$P < 0.001$ and compared to PAL-treated group; ##$P < 0.01$. All experiments were performed in triplicate. *GB Gryllus bimaculatus*, *PAL* palmitate, *TNF-α* tumor necrosis factor-alpha, *Bcl2* B-cell lymphoma 2, *eIF2α* eukaryotic translation initiation factor 2A, *iNOS* inducible nitric oxygen synthase, *PERK* protein kinase R-like endoplasmic reticulum kinase, *Con* control (untreated)

in CerS expressions. Palmitate increased CerS1, CerS4, and CerS6 expressions, but treatment with GB extract reduced the expressions of CerS1, CerS4, and CerS6 (Fig. 2A, B). Furthermore, the total ceramide level was elevated upon palmitate treatment, but pretreatment with GB extract reduced it (Fig. 2C). CerS2, CerS4, CerS5, and CerS6 expressions were relatively high in MIN6 cells, contrary to the extremely low CerS1 and CerS3 expressions (Fig. 2D), suggesting minor roles for CerS1 and CerS3 in MIN6 cells. To examine whether each CerS affected palmitate-induced cell death, we overexpressed CerS1, CerS4, or CerS6 in MIN6 cells and then treated the cells with palmitate. Interestingly, CerS1 overexpression did not affect palmitate-induced cell death and ceramide generation (Fig. 3A), but CerS4 and CerS6 overexpression increased palmitate-induced cell death (caspase-3 cleavage) and ceramide generation (Fig. 3B, C). Fumonisin B1 (FB1), a CerS inhibitor [20], partially reduced palmitate-induced cell death (caspase-3 cleavage) and ceramide synthesis (Fig. 3D). We found that CerS1 overexpression did not affect palmitate-induced cell death, so we excluded CerS1 and focused on CerS4 and CerS6 in the next experiments.

CerS4 and CerS6 overexpression increases ER stress, cell death signaling, and inflammatory cytokine production

In consideration that palmitate may affect ER stress [21] and apoptotic signaling pathways, such as Bcl2 and Bax pathways [22], we examined these signaling pathways in CerS4- or CerS6-overexpressed MIN6 cells. CerS4 and CerS6 overexpression increased the phosphorylation of ER stress markers PERK and eIF2α [8], and the phosphorylation of the MAP kinases JNK and p38 [23] (Fig. 4A). It also increased NF-κB p65 phosphorylation, iNOS expression, and pro-apoptotic Bax protein expression, but reduced anti-apoptotic Bcl2 protein expression (Fig. 4A). CerS4 and CerS6 overexpression increased nitrite and TNF-α levels (Fig. 4B, C). However, FB1 reduced ER stress, MAP kinase phosphorylation (JNK, p38), NF-κB p65 phosphorylation, iNOS expression, and pro-apoptotic Bax protein expression, and increased Bcl2 expression (Fig. 5A). FB1 treatment also reduced nitrite and TNF-α levels (Fig. 5B, C).

GB extract reduces cell death signaling and improves insulin secretion

CerS4 and CerS6 regulate cell death and inflammatory cytokine production. Therefore, we pretreated MIN6 cells with GB extract for 24 h, followed by palmitate treatment for another 24 h. GB extract decreased ER stress (phosphorylation of PERK, eIF2α), MAP kinase signaling (JNK, p38 phosphorylation), NF-κB p65 phosphorylation, iNOS expression, and pro-apoptotic Bax protein expression, and increased anti-apoptotic Bcl2 protein expression (Fig. 6A). It also reduced the production of nitrite and TNF-α (Fig. 6B, C). Palmitate

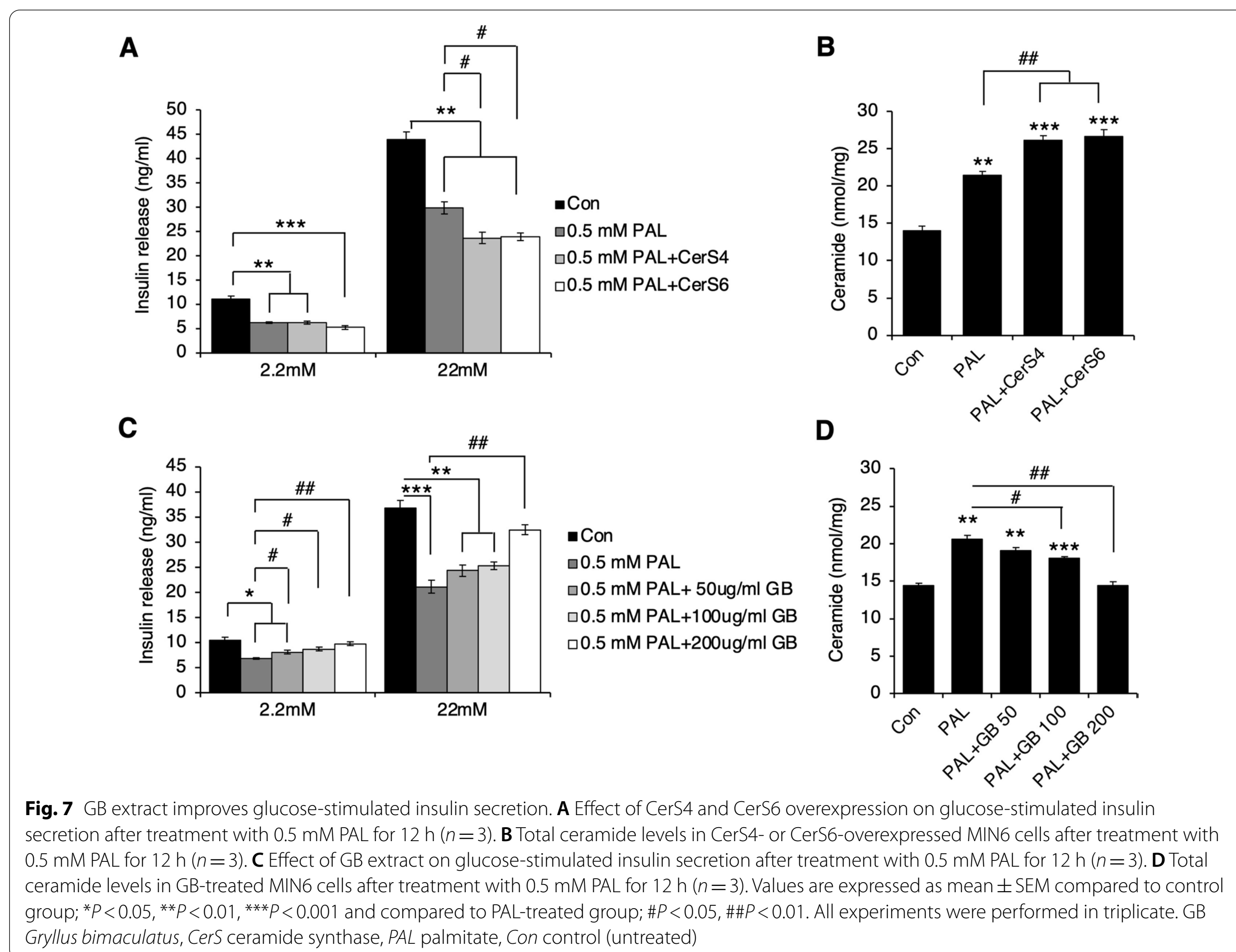

Fig. 7 GB extract improves glucose-stimulated insulin secretion. **A** Effect of CerS4 and CerS6 overexpression on glucose-stimulated insulin secretion after treatment with 0.5 mM PAL for 12 h ($n = 3$). **B** Total ceramide levels in CerS4- or CerS6-overexpressed MIN6 cells after treatment with 0.5 mM PAL for 12 h ($n = 3$). **C** Effect of GB extract on glucose-stimulated insulin secretion after treatment with 0.5 mM PAL for 12 h ($n = 3$). **D** Total ceramide levels in GB-treated MIN6 cells after treatment with 0.5 mM PAL for 12 h ($n = 3$). Values are expressed as mean ± SEM compared to control group; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and compared to PAL-treated group; #$P < 0.05$, ##$P < 0.01$. All experiments were performed in triplicate. GB *Gryllus bimaculatus*, *CerS* ceramide synthase, *PAL* palmitate, *Con* control (untreated)

treatment has been shown to reduce insulin secretion [22]. Hence, we measured insulin secretion in CerS4- and CerS6-overexpressed MIN6 cells and GB extract-treated MIN6 cells. Palmitate treatment attenuated glucose-stimulated insulin secretion, and CerS4 and CerS6 overexpression reduced insulin secretion compared to the palmitate-treated group (Fig. 7A). GB extract treatment dose-dependently recovered insulin secretion and palmitate-induced ceramide generation (Fig. 7B), indicating that GB extract reduced ceramide generation and improved insulin secretion.

Discussion

Many mechanisms, such as ER stress, apoptosis, inflammation, oxidative stress, and mitochondrial dysfunction, are involved in pancreatic β-cell death. Palmitate induces ER stress, disrupts ER-to-Golgi protein trafficking [21], increases oxidative stress, and causes mitochondrial dysfunction [24], resulting in lipotoxicity. It can also induce β-cell death and impair insulin secretion [21]. In our study, GB extract reduced palmitate-induced cell death by regulating several mechanisms, including the inhibition of ER stress, the NF-κB signaling pathway, MAP kinase, inflammatory cytokines, and nitrite production. It seemed that all these inhibitory mechanisms might be due to ceramide synthesis.

Ceramide plays a major role in many diseases. In MIN6 cells, ceramide has inhibitory effects on β-cell insulin secretion [25, 26]. Palmitate increases C16-, C20-, and C24-ceramides, which impair insulin secretion [9]. Treatment with GB extract reduced the total ceramide generation and CerS1, CerS4, and CerS6 expressions. Furthermore, GB extract recovered insulin secretion by reducing ceramide generation. Even though GB extract reduced CerS1, CerS4, and CerS6 expressions, the role of CerS1 might be limited. CerS1 overexpression did not affect palmitate-induced pancreatic β-cell death and caspase-3 activation in contrast to overexpression of CerS4 and CerS6, which increased palmitate-induced pancreatic β-cell death and caspase-3 activation in MIN6 cells.

CerS1 expression is extremely low in MIN6 cells, but CerS4 and CerS6 expressions are relatively high, suggesting that CerS1 plays a minor role in MIN6 cells. Moreover, FB1 treatment prevented palmitate-induced cell death. In detail, palmitate increased various apoptotic signaling pathways, such as ER stress and MAP kinase, NF-κB–iNOS, Bax, and Bcl2 expressions. Overexpression of CerS4 and CerS6 aggravated all these pathways. However, inhibition of ceramide generation by FB1 treatment attenuated all cell death pathways. In addition, FB1 treatment reduced pro-apoptotic Bax protein and increased anti-apoptotic Bcl2 protein. Therefore, palmitate increases ceramide biosynthesis, and ceramide plays a critical role in various cell death signaling pathways. GB extract inhibited ceramide generation, attenuated both β-cell death, and increased insulin secretion.

In a previous study, we found that GB extract has high amounts of unsaturated fatty acids, such as oleic acid and linoleic acid [4]. Both acids increase insulin production [27, 28]. Oleic acid protects against palmitate-induced ER stress [29, 30] and reverses the impairment of insulin secretion by palmitate [29]. Furthermore, oleic acid has proliferative effects on β-cells [31]. Inflammatory cytokines, such as TNF-α and IL-1β, inhibit insulin secretion via nitric oxide generation [32, 33]. Palmitate increased inflammation and inflammatory cytokine production, whereas GB extract reduced the nitrite levels and TNF-α generation. Therefore, unsaturated fatty acids in GB extract and its anti-inflammatory effects might protect against palmitate-induced cell death and impairment of insulin secretion.

There is a large body of evidence on the beneficial effects of some edible insects in modulating palmitate-induced lipotoxicity and blood glucose levels. For example, *A. dichotoma* larva extract reduced palmitate-induced β-cell death [34], and *Oxya chinensis sinuosa*, *Protaetia brevitarsis*, and Korean grain larvae reduced fasting blood glucose [35]. Edible insects have a high content of unsaturated fatty acids, some minerals, and various bioactive compounds [11]. Therefore, edible insects might have considerable benefits in patients with diabetes and metabolic diseases. Dietary intervention trials are needed to confirm the efficacy of edible insects as functional foods to regulate blood glucose and insulin secretion.

Abbreviations

BSA: Bovine serum albumin; CerS: Ceramide synthase; DM: Diabetes mellitus; ELISA: Enzyme-linked immunosorbent assay; ER: Endoplasmic reticulum; FB1: Fumonisin B1; FFA: Free fatty acid; GB: *Gryllus bimaculatus*; iNOS: Inducible nitric oxide synthase.

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Not applicable.

Author contributions

WJP contributed to the conception and design of the study. IBP, MHK, JSH, and WJP performed the experiments and shared raw data for legitimacy. IBP and WJP contributed to the acquisition of data and wrote the manuscript. MHK edited figures and WJP and JSH reviewed and edited the manuscript. All authors read and approved the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests

The authors declare that they have no competing interests.

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References

1. Pirot P, Cardozo AK, Eizirik DL (2008) Mediators and mechanisms of pancreatic beta-cell death in type 1 diabetes. Arq Bras Endocrinol Metabol 52:156–165
2. Kharroubi I, Ladrière L, Cardozo AK, Dogusan Z, Cnop M, Eizirik DL (2004) Free fatty acids and cytokines induce pancreatic beta-cell apoptosis by different mechanisms: role of nuclear factor-kappaB and endoplasmic reticulum stress. Endocrinology 145:5087–5096
3. Souza KL, Gurgul-Convey E, Elsner M, Lenzen S (2008) Interaction between pro-inflammatory and anti-inflammatory cytokines in insulin-producing cells. J Endocrinol 197:139–150
4. Park WJ, Han JS (2021) *Gryllus bimaculatus* extract protects against lipopolysaccharide and palmitate-induced production of proinflammatory cytokines and inflammasome formation. Mol Med Rep 23:206
5. Schilling JD, Machkovech HM, He L, Sidhu R, Fujiwara H, Weber K, Ory DS, Schaffer JE (2013) Palmitate and lipopolysaccharide trigger synergistic ceramide production in primary macrophages. J Biol Chem 288:2923–2932
6. Alsahli A, Kiefhaber K, Gold T, Muluke M, Jiang H, Cremers S, Schulze-Späte U (2016) Palmitic acid reduces circulating bone formation markers in obese animals and impairs osteoblast activity via c16-ceramide accumulation. Calcif Tissue Int 98:511–519
7. Park WJ, Park JW (2015) The effect of altered sphingolipid acyl chain length on various disease models. Biol Chem 396:693–705
8. Park WJ, Park JW (2020) The role of sphingolipids in endoplasmic reticulum stress. FEBS Lett 594:3632–3651
9. Manukyan L, Ubhayasekera SJ, Bergquist J, Sargsyan E, Bergsten P (2015) Palmitate-induced impairments of β-cell function are linked with generation of specific ceramide species via acylation of sphingosine. Endocrinology 156:802–812
10. Véret J, Coant N, Berdyshev EV, Skobeleva A, Therville N, Bailbé D, Gorshkova I, Natarajan V, Portha B, Le Stunff H (2011) Ceramide synthase 4 and de novo production of ceramides with specific N-acyl chain lengths are involved in glucolipotoxicity-induced apoptosis of INS-1 β-cells. Biochem J 438:177–189

11. da Silva J, Lucas A, Menegon de Oliveira L, da Rocha M, Prentice C (2020) Edible insects: an alternative of nutritional, functional and bioactive compounds. Food Chem 311:126022
12. Seo M, Goo TW, Chung MY, Baek M, Hwang JS, Kim MA, Yun EY (2017) *Tenebrio molitor* larvae Inhibit adipogenesis through AMPK and MAPKs signaling in 3T3-L1 adipocytes and obesity in high-fat diet-induced obese mice. Int J Mol Sci 18:518
13. Yoon YI, Chung MY, Hwang JS, Han MS, Goo TW, Yun EY (2015) *Allomyrina dichotoma* (Arthropoda: Insecta) larvae confer resistance to obesity in mice fed a high-fat diet. Nutrients 7:1978–1991
14. Park SA, Lee GH, Lee HY, Hoang TH, Chae HJ (2020) Glucose-lowering effect of *Gryllus bimaculatus* powder on streptozotocin-induced diabetes through the AKT/mTOR pathway. Food Sci Nutr 8:402–409
15. Hwang BB, Chang MH, Lee JH, Heo W, Kim JK, Pan JH, Kim YJ, Kim JH (2019) The edible insect *Gryllus bimaculatus* protects against gut-derived inflammatory responses and liver damage in mice after acute alcohol exposure. Nutrients 11:857
16. Ahn MY, Han JW, Hwang JS, Yun EY, Lee BM (2014) Anti-inflammatory effect of glycosaminoglycan derived from *Gryllus bimaculatus* (a type of cricket, insect) on adjuvant-treated chronic arthritis rat model. J Toxicol Environ Health A 77:1332–1345
17. Kim K, Park EY, Baek DJ, Jang SE, Oh YS (2021) *Gryllus bimaculatus* extract protects against lipopolysaccharide-derived inflammatory response in human colon epithelial caco-2 cells. Insects 12:873
18. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25:402–408
19. Kim SY, Kim YR, Park WJ, Kim HS, Jung SC, Woo SY, Jo I, Ryu KH, Park JW (2015) Characterisation of insulin-producing cells differentiated from tonsil derived mesenchymal stem cells. Differentiation 90:27–39
20. Park JW, Park WJ, Futerman AH (2014) Ceramide synthases as potential targets for therapeutic intervention in human diseases. Biochim Biophys Acta 1841:671–681
21. Boslem E, MacIntosh G, Preston AM, Bartley C, Busch AK, Fuller M, Laybutt DR, Meikle PJ, Biden TJ (2011) A lipidomic screen of palmitate-treated MIN6 β-cells links sphingolipid metabolites with endoplasmic reticulum (ER) stress and impaired protein trafficking. Biochem J 435:267–276
22. Hao F, Kang J, Cao Y, Fan S, Yang H, An Y, Pan Y, Tie L, Li X (2015) Curcumin attenuates palmitate-induced apoptosis in MIN6 pancreatic β-cells through PI3K/Akt/FoxO1 and mitochondrial survival pathways. Apoptosis 20:1420–1432
23. Song Z, Ma J, Lu Y, Zhou C, Zhao T, Ai X, Wei X, Lin J, Wang W, Yan W, Jiao P (2019) The protective role of the MKP-5-JNK/P38 pathway in glucolipotoxicity-induced islet β-cell dysfunction and apoptosis. Exp Cell Res 382:111467
24. Wehinger S, Ortiz R, Díaz MI, Aguirre A, Valenzuela M, Llanos P, Mc Master C, Leyton L, Quest AF (2015) Phosphorylation of caveolin-1 on tyrosine-14 induced by ROS enhances palmitate-induced death of beta-pancreatic cells. Biochim Biophys Acta 1852:693–708
25. Sjöholm A (1995) Ceramide inhibits pancreatic beta-cell insulin production and mitogenesis and mimics the actions of interleukin-1 beta. FEBS Lett 367:283–286
26. Guo J, Qian Y, Xi X, Hu X, Zhu J, Han X (2010) Blockage of ceramide metabolism exacerbates palmitate inhibition of pro-insulin gene expression in pancreatic beta-cells. Mol Cell Biochem 338:283–290
27. Vassiliou EK, Gonzalez A, Garcia C, Tadros JH, Chakraborty G, Toney JH (2009) Oleic acid and peanut oil high in oleic acid reverse the inhibitory effect of insulin production of the inflammatory cytokine TNF-alpha both in vitro and in vivo systems. Lipids Health Dis 8:25
28. Briscoe CP, Peat AJ, McKeown SC, Corbett DF, Goetz AS, Littleton TR, McCoy DC, Kenakin TP, Andrews JL, Ammala C, Fornwald JA, Ignar DM, Jenkinson S (2006) Pharmacological regulation of insulin secretion in MIN6 cells through the fatty acid receptor GPR40: identification of agonist and antagonist small molecules. Br J Pharmacol 148:619–628
29. Sargsyan E, Artemenko K, Manukyan L, Bergquist J, Bergsten P (2016) Oleate protects beta-cells from the toxic effect of palmitate by activating pro-survival pathways of the ER stress response. Biochim Biophys Acta 1861:1151–1160
30. Sommerweiss D, Gorski T, Richter S, Garten A, Kiess W (2013) Oleate rescues INS-1E β-cells from palmitate-induced apoptosis by preventing activation of the unfolded protein response. Biochem Biophys Res Commun 441:770–776
31. Castell AL, Vivoli A, Tippetts TS, Frayne IR, Angeles ZE, Moullé VS, Campbell SA, Ruiz M, Ghislain J, Des Rosiers C, Holland WL, Summers SA, Poitout V (2022) Very-long-chain unsaturated sphingolipids mediate oleate-induced rat β-cell proliferation. Diabetes 71:1218–1232
32. Zhang S, Kim KH (1995) TNF-alpha inhibits glucose-induced insulin secretion in a pancreatic beta-cell line (INS-1). FEBS Lett 377:237–239
33. Southern C, Schulster D, Green IC (1990) Inhibition of insulin secretion by interleukin-1 beta and tumour necrosis factor-alpha via an L-arginine-dependent nitric oxide generating mechanism. FEBS Lett 276:42–44
34. Kim K, Kwak M-K, Bae G-D, Park E-Y, Baek D-J, Kim C-Y, Jang S-E, Jun H-S, Oh YS (2021) *Allomyrina dichotoma* larva extract attenuates free fatty acid-induced lipotoxicity in pancreatic beta cells. *Allomyrina dichotoma* larva extract attenuates free fatty acid-induced lipotoxicity in pancreatic beta cells. Nutr Res Pract 15:294–308
35. D'Antonio V, Battista N, Sacchetti G, Di Mattia C, Serafini M (2021) Functional properties of edible insects: a systematic review. Nutr Res Rev. https://doi.org/10.1017/S0954422421000366

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