Biochemical and Biophysical Research Communications 460 (2015) 205–209

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Zerumbone protects INS-1 rat pancreatic beta cells from high glucose-induced apoptosis through generation of reactive oxygen species

Changyin Wang [1], Shibo Zou [1], Zhengjun Cui*, Pengfei Guo, Qingnan Meng, Xun Shi, Ya Gao, Gaoyuan Yang, Zhaofeng Han

The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China

ARTICLE INFO

Article history:
Received 27 February 2015
Available online 11 March 2015

Keywords:
Pancreatic β cells
Oxidative stress
Zerumbone
Survival
Hyperglycemia

ABSTRACT

The aim of this study is to explore the effect of zerumbone, a natural sesquiterpene isolated from *Zingiber zerumbet* Smith, on high glucose-induced cytotoxicity in pancreatic β cells. INS-1 rat pancreatic β cells were treated with 33 mM glucose with or without different concentrations of zerumbone and cell viability and apoptosis were assessed. The involvement of reactive oxygen species (ROS) and mitogen-activated protein kinase (MAPK) signaling in the action of zerumbone was examined. Notably, zerumbone significantly ($P < 0.05$) prevented the reduction of cell viability induced by high glucose. Such protection was in a concentration-dependent fashion up to 60 μM of zerumbone. Annexin-V/propidium iodide staining analysis showed that zerumbone impaired the apoptotic response of high glucose-treated INS-1 cells, which was coupled with a significant decline in cleaved caspase-3 and caspase-9. Pretreatment with the ROS inhibitor N-acetylcysteine abrogated the phosphorylation of p38 and JNK induced by high glucose. Zerumbone significantly ($P < 0.05$) decreased the generation of ROS and the phosphorylation of p38 and JNK MAPKs in high glucose-treated INS-1 cells. Pharmacological activation of p38 and JNK with anisomycin reversed the anti-apoptotic effect of zerumbone. Additionally, simultaneous inhibition of p38 and JNK significantly ($P < 0.05$) reduced the apoptotic response in high glucose-treated INS-1 cells. In conclusion, zerumbone confers protection against high glucose-induced apoptosis of INS-1 pancreatic β cells, largely through interfering with ROS production and p38 and JNK activation. Zerumbone may have potential therapeutic effects against hyperglycemia-induced β cell damage in diabetes.

1. Introduction

Irreversible damage and death of pancreatic β cells leads to a disruption in glucose homeostasis, which plays a critical role in the pathogenesis of diabetes [1,2]. Chronic exposure to high glucose concentration is an important factor resulting in pancreatic β cell dysfunction and loss [3]. Accumulating evidence suggests that high glucose-induced β-cell toxicity primarily results from oxidative stress [4]. It has been reported that intermittent high glucose can induce apoptosis in pancreatic β cells, which is linked to increased formation of reactive oxygen species (ROS) [5]. ROS has the ability to oxidize or nitrify proteins, lipids and DNA by direct chemical modification and activate signaling pathways that cause cell death [6]. Several molecular pathways such as mitogen-activated protein kinases (MAPKs) have been shown to mediate ROS-induced pancreatic β cell apoptosis [7,8]. There are three major MAPKs, i.e., JNK, ERK, and p38 MAPK, which play critical roles in cell survival and death [8,9]. Therefore, it is of significance to develop effective therapies against oxidative stress and apoptosis in pancreatic β cells induced by hyperglycemia.

Zerumbone is a natural sesquiterpene isolated from the herbal plant, *Zingiber zerumbet* Smith, which is widely distributed in Southeast Asia [10]. It shows numerous biological properties, including anticancer [11], anti-inflammation [12], and antioxidant [13] activities. Zerumbone yields cytotoxic or protective effects in different settings. It can trigger apoptotic death in many types of malignant cells such as breast cancer [11] and pancreatic cancer [14] cells. In contrary, dietary zerumbone has been shown to

* Corresponding author. Fax: +86 37166913114.
E-mail address: zhengjuncuichina@163.com (Z. Cui).
[1] Both authors equally contributed to this work.

http://dx.doi.org/10.1016/j.bbrc.2015.03.009
0006-291X/

protect ultraviolet B-treated mice from cataractogenesis [15] and photokeratitis [16].

A recent study has reported that the ethanol extract from the rhizome of *Z. zerumbet* Smith shows the ability to reduce hyperglycemia and renal dysfunction in diabetic rats [17]. This finding raises the possibility that zerumbone may confer protection against high glucose-induced cytotoxicity. We therefore explored the effect of zerumbone on β cell survival and apoptosis upon exposure to high glucose. Additionally, the involvement of the ROS/MAPK pathway was examined.

2. Materials and methods

2.1. Materials

RPMI-1640 medium, fetal bovine serum, streptomycin, and penicillin were purchased from Invitrogen (Carlsbad, CA, USA), zerumbone (≥98% in purity), anisomycin, glucose, dimethyl sulfoxide (DMSO), N-acetylcysteine (NAC), 3-(4,5-dimethyl-thiazol-2-yl) -2,5- diphenyltetrazolium (MTT), aprotinin, leupeptin, and benzamidine from Sigma (St. Louis, MO, USA). Phenylmethylsulfonyl fluoride (PMSF), SB203580, and SP60012 were obtained from Calbiochem (Gibbstown, NJ, USA) and 2′, 7′-dichlorofluorescin diacetate (DCFH-DA) from Molecular Probes (Eugene, OR, USA). Annexin V-FITC/PI Apoptosis Detection kit was purchased from Becton Dickinson Biosciences (San Jose, CA, USA). Anti-cleaved caspase-3, anti-cleaved caspase-9, anti-p38, anti-JNK, anti-ERK, anti-phospho-p38, anti-phospho-JNK, anti-phospho-ERK, and anti-β-actin antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). Horseradish peroxidase (HRP)-conjugated anti-mouse and anti-rabbit antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.2. Cell culture and treatment

INS-1 rat pancreatic β cells were purchased from the Shanghai Institute of Cell Biology (Shanghai, China) and maintained in RPMI-1640 medium containing 10% fetal bovine serum, 11 mM glucose, streptomycin (100 μg/ml), and penicillin (100 units/ml). For glucose-induced cell death, cells were incubated for 48 h in RPMI-1640 medium with various concentrations of zerumbone (10–100 μM) with 33 mM glucose, typically used as high-glucose stimulation [18]. Afterward, cells were tested for viability, apoptosis, and gene expression. For inhibitor experiments, cells were pretreated for 2 h with SB203580 (a selective p38 MAPK inhibitor; 10 μM), SP60012 (a specific JNK inhibitor; 10 μM), and NAC (a potent ROS inhibitor; 5 mM) before exposure to high glucose for additional 48 h. For rescue studies, cells were pretreated with anisomycin (a potent agonist of p38 and JNK [19,20]; 2 μM) for 4 h prior to treatment with high glucose and zerumbone. At this concentration, anisomycin is effective for JNK and p38 activation in INS-1 cells without inducing ERK1/2 phosphorylation (data not shown).

2.3. Cell viability assay

INS-1 cells were seeded onto 96-well plates at a density of 5×10^3 cells/well and treated with high glucose with or without zerumbone. After treatment, MTT at a final concentration of 0.5 mg/ml was added to the culture plates and incubated at 37 °C for 4 h. After medium removal, 100 mM DMSO was added to dissolve the formazan crystals. The absorbance at 570 nm was measured by a microplate reader.

2.4. Apoptosis detection by flow cytometry analysis

Cell apoptosis was detected by annexin V-FITC/PI double staining using the commercially available Annexin V-FITC/PI Apoptosis Detection kit. Briefly, after treatment, cells were trypsinized and resuspended in binding buffer. The single cell suspension was incubated with Annexin V-FITC and propidium iodide (PI) in the dark for 30 min. Apoptotic cells were examined using flow cytometry (Becton Dickinson Biosciences).

2.5. Measurement of ROS

Intracellular ROS levels were determined using the peroxide-sensitive fluorescent probe DCFH-DA, as described previously [21]. In brief, after treatment, cells were incubated with 20 μM of DCFH-DA for 15 min at 37 °C in the dark. After washing twice, cells were re-suspended in ice-cold phosphate buffered saline (PBS). Fluorescence was measured by flow cytometry (Becton Dickinson Biosciences).

2.6. Western blot analysis

After treatment, cells were collected and lysed in lysis buffer containing 50 mM Tris (pH 7.4), 150 mM NaCl, 3 mM $MgCl_2$, 0.1% sodium dodecyl sulfate (SDS), 1% Triton X-100, 0.1 mM Na_3VO_4, 1% NP-40, 1 mM PMSF, 1 μg/ml aprotinin, 1 μg/ml leupeptin, and 1 μg/ml benzamidine on ice. The protein samples (~50 μg) were separated by SDS-polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes. After blocking with 5% fat-free milk in Tris-buffered saline containing 0.05% Tween 20, the membranes were incubated overnight at 4 °C with the primary antibodies. After washing, the membranes were incubated with HRP-conjugated secondary antibodies. The proteins were detected using an enhanced chemiluminescence detection system (Amersham, Piscataway, NJ, USA), according to the manufacturer's recommendations.

2.7. Statistical analysis

Data are presented as means ± standard deviation (SD). Statistical analysis was performed using SPSS version 11.0 for Windows (SPSS Inc., Chicago, IL, USA). The significance of differences among groups was determined using one-way analysis of variance (ANOVA) followed by the Tukey's test. *P* values of less than 0.05 were considered to be statistically significant.

3. Results

3.1. Zerumbone promotes INS-1 cell survival upon exposure to high glucose

MTT assay revealed that exposure to high glucose (33 mM) for 48 h significantly reduced the viability of INS-1 cells, compared to

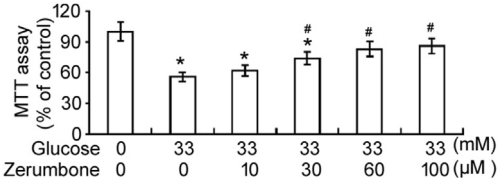

Fig. 1. Cell viability detected by the MTT assay. INS-1 cells were incubated for 48 h with glucose with or without various concentrations of zerumbone and tested for the viability. $^{*}P < 0.05$ vs. untreated cells; $^{\#}P < 0.05$ vs. cells treated with high glucose alone.

control cells in normal culture media ($P < 0.05$; Fig. 1). Up to the concentration of 60 μM, zerumbone protected INS-1 cells from high glucose-induced toxicity in a concentration-dependent manner (Fig. 1). Comparable protective effects were obtained when 60 and 100 μM of zerumbone was used.

3.2. Zerumbone protects INS-1 cells from high glucose-induced apoptosis

Next, we investigated the effect of zerumbone on high glucose-induced apoptosis of INS-1 cells. Flow cytometric analysis demonstrated that the percentage of early apoptotic cells (annexin-V-positive and PI-negative) increased from 4.2 ± 0.8% to 20.5 ± 1.4% after 48-h stimulation with high glucose (Fig. 2A). Notably, zerumbone at 60 μM significantly prevented the apoptotic response of high glucose-treated INS-1 cells, decreasing the percentage of apoptosis to 7.8 ± 1.1%.

Western blot analysis confirmed that high-glucose stimulation caused an increase in the levels of cleaved (active) caspase-3 and caspase-9, compared to control cells (Fig. 2B). Strikingly, such increase in caspase activation was abolished by the addition of zerumbone.

3.3. Zerumbone reduces ROS generation and p38 and JNK activation in high glucose-treated INS-1 cells

Next, we examined the effect of zerumbone on ROS formation in high glucose-treated INS-1 cells. As shown in Fig. 3A, zerumbone at 60 μM significantly ($P < 0.05$) decreased the generation of intracellular ROS in INS-1 cells after exposure to high glucose for 48 h. Substantial ROS has been found to activate MAPK signaling cascades [22]. Consistent with ROS reduction, zerumbone markedly prevented the phosphorylation of p38 and JNK in high glucose-treated INS-1 cells (Fig. 3B). However, zerumbone did not alter the phosphorylation level of ERK1/2. To confirm the role of ROS in MAPK activation, INS-1 cells were pretreated with the ROS inhibitor NAC for 2 h before high glucose exposure and the phosphorylation

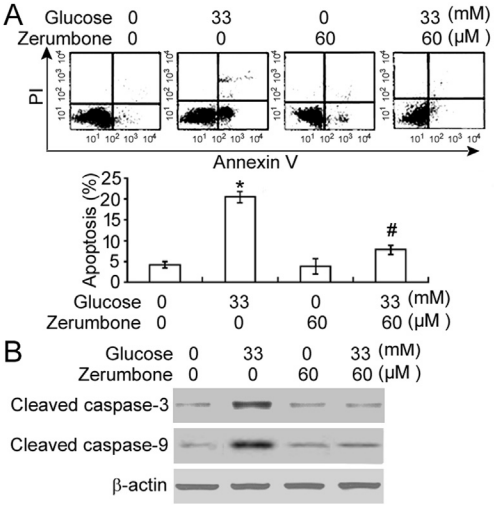

Fig. 2. Effect of zerumbone on the apoptosis of INS-1 cells induced by high glucose. Cells were treated with high glucose (33 mM) and zerumbone (60 μM), alone or in combination, and apoptosis was examined. (A) Apoptosis was detected by flow cytometry. Representative dot plots of apoptotic cells stained by annexin-V and PI are shown in top panels. The graph represents the mean percentage (±SD) of apoptosis determined from 3 independent experiments. $^{*}P < 0.05$ vs. untreated cells; $^{\#}P < 0.05$ vs. cells treated with high glucose alone. (B) Western blot analysis of active (cleaved) caspase-9 and -3. Representative blots of three independent experiments are shown.

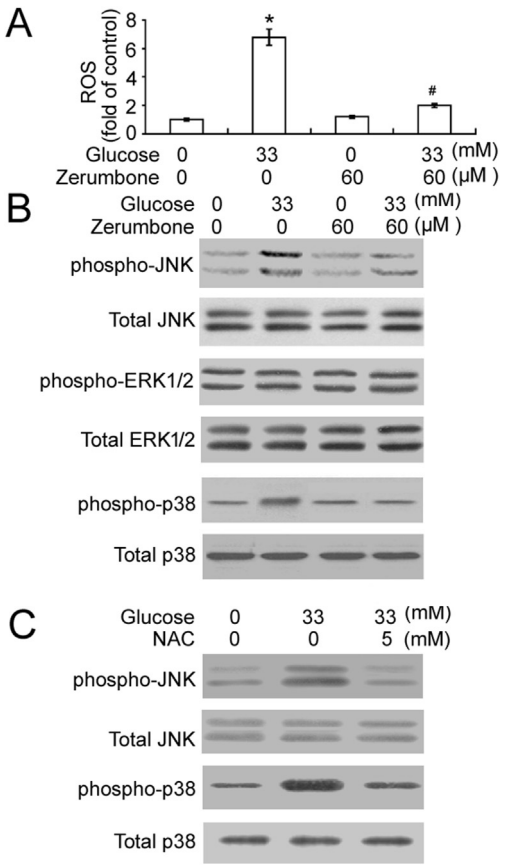

Fig. 3. Effect of zerumbone on ROS generation and MAPK activation in INS-1 cells after indicated treatments. (A) The graph represents fold change in ROS levels over untreated cells (assigned a value of 1). $^{*}P < 0.05$ vs. untreated cells; $^{\#}P < 0.05$ vs. cells treated with high glucose alone. (B) Western blot analysis of MAPK phosphorylation. Representative blots of three independent experiments are shown. (C) INS-1 cells were pretreated with NAC for 2 h before high glucose exposure and the phosphorylation of JNK and p38 was examined. Representative blots of three independent experiments are shown.

of MAPKs was examined. Pretreatment with NAC impaired the phosphorylation of p38 and JNK induced by high glucose (Fig. 3C).

3.4. p38 and JNK MAPKs mediate the protective effect of zerumbone against glucotoxicity

Finally, we checked the involvement of MAPK signaling in the anti-apoptotic activity of zerumbone in INS-1 cells. Pharmacological activation of p38 and JNK with anisomycin almost completely blocked the anti-apoptotic effect of zerumbone in high glucose-treated INS-1 cells, with an apoptosis percentage of 22.6 ± 2.2% (Fig. 4A). Furthermore, simultaneous inhibition of p38 and JNK significantly ($P < 0.05$) reduced the apoptotic response in high glucose-treated INS-1 cells (Fig. 4B), which phenocopied the protective effect of zerumbone.

4. Discussion

Our data showed that zerumbone enhanced the viability of INS-1 β cells in the presence of high glucose. Furthermore, zerumbone significantly attenuated high glucose-induced apoptosis of INS-1 cells. Activation of caspase cascade plays an important role in apoptosis [23]. We found that zerumbone significantly abrogated the increase in active caspase-9 and -3 in high glucose-treated INS-

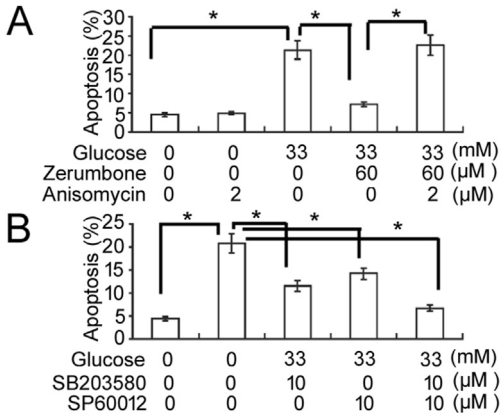

Fig. 4. p38 and JNK MAPKs mediate the protective effect of zerumbone against glucotoxicity. INS-1 cells were pretreated with anisomycin (A) or SB203580 and/or SP60012 (B) before treatment with high glucose and zerumbone. The graph represents the mean percentage (±SD) of apoptosis determined from 3 independent experiments. $^{*}P < 0.05$.

1 cells, confirming the anti-apoptotic activity of zerumbone. Zerumbone has shown anti-cancer and apoptosis-inducing properties in various tumor cells [11,14,24]. In contrast, zerumbone has mild genotoxicity to normal peripheral blood lymphocytes [25]. These studies suggest that zerumbone is selectively cytotoxic to malignant cells. Our data point toward an anti-apoptotic activity of zerumbone in INS-1 cells, suggesting a potentially protective effect on nonmalignant cells.

ROS plays an important role in apoptosis induction [26]. It has been suggested that high glucose-induced β cell apoptosis involves generation of ROS [27]. Inhibition of ROS is a promising strategy for blocking high glucose-triggered apoptosis in β cells [27]. Notably, our data revealed that zerumbone treatment significantly impaired ROS formation in high glucose-treated INS-1 cells. This finding suggests that the anti-apoptotic activity of zerumbone is associated with suppression of ROS generation in β cells. Zerumbone has shown the ability to modulate ROS formation in different cell types [13,14]. Zhang et al. [14] reported that zerumbone induces ROS production in pancreatic cancer cells. In contrast, Shin et al. [13] reported that zerumbone suppresses 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced ROS generation in JB6 mouse epidermal cells through upregulation of heme oxygenase-1. Alteration of ROS production may represent an important mechanism for zerumbone-mediated regulation of cellular behaviors.

Substantial ROS has been found to activate MAPK signaling cascades [28]. Consistent with suppression of ROS production, we found that zerumbone treatment resulted in a significant reduction in the phosphorylation of p38 and JNK MAPKs. However, zerumbone did not alter the phosphorylation level of ERK1/2 MAPK. To check the role of ROS production in MAPK activation, INS-1 cells were pretreated with the ROS inhibitor NAC before exposure to glucose and MAPK activation was examined. As expected, NAC pretreatment significantly abrogated the phosphorylation of p38 and JNK MAPKs induced by high glucose. Taken together, these results suggest that zerumbone impairs the activation of p38 and JNK MAPKs in high glucose-treated INS-1 cells via inhibition of ROS production. Activation of MAPK pathways may lead to either cell survival or apoptosis depending on cellular contexts and stimuli [29]. It has been reported that high glucose induces p53-dependent mitochondrial apoptosis of pancreatic RINm5F cells via activation of p38 MAPK [30]. Inhibition of p38 MAPK has been shown to prevent pancreatic β cell apoptosis after islet transplantation in a canine model [31]. Consistently, our data revealed that simultaneous inhibition of p38 and JNK significantly blocked the apoptotic response in high glucose-treated INS-1 cells. Moreover, the anti-apoptotic effect of zerumbone in high glucose-treated INS-1 cells was significantly reversed by pharmacological activation of p38 and JNK with anisomycin. These studies, combined with our findings, support that the protective effect of zerumbone on high glucose-treated INS-1 cells is largely mediated through inactivation of p38 and JNK MAPKs. The inhibition of p38 MAPK activation by zerumbone has also been described in a rat model of streptozotocin-induced diabetic nephropathy [32].

In conclusion, zerumbone has shown the ability to protect INS-1 pancreatic β cells from high glucose-induced apoptosis, which is associated with inhibition of ROS generation and JNK and p38 MAPK activation. These findings warrant further investigation of its therapeutic benefits for the treatment of diabetes.

Conflict of interest

The authors declare no conflict of interest.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.03.009.

References

[1] A.E. Butler, J. Janson, S. Bonner-Weir, et al., Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes, Diabetes 52 (2003) 102–110.
[2] J.N. Winnay, E. Dirice, C.W. Liew, et al., p85α deficiency protects β-cells from endoplasmic reticulum stress-induced apoptosis, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 1192–1197.
[3] M. Bensellam, D.R. Laybutt, J.C. Jonas, The molecular mechanisms of pancreatic β-cell glucotoxicity: recent findings and future research directions, Mol. Cell. Endocrinol. 364 (2012) 1–27.
[4] J.H. Lee, I.R. Jung, S.E. Choi, et al., Toxicity generated through inhibition of pyruvate carboxylase and carnitine palmitoyl transferase-1 is similar to high glucose/palmitate-induced glucolipotoxicity in INS-1 beta cells, Mol. Cell. Endocrinol. 383 (2014) 48–59.
[5] Z. Zhang, J. Li, L. Yang, et al., The cytotoxic role of intermittent high glucose on apoptosis and cell viability in pancreatic beta cells, J. Diabetes Res. 2014 (2014), 712781.
[6] C.P. Baines, R.A. Kaiser, N.H. Purcell, et al., Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death, Nature 434 (2005) 658–662.
[7] K.C. Chang, C.C. Hsu, S.H. Liu, et al., Cadmium induces apoptosis in pancreatic β-cells through a mitochondria-dependent pathway: the role of oxidative stress-mediated c-Jun N-terminal kinase activation, PLoS One 8 (2013) e54374.
[8] J.S. Lee, Y.R. Kim, I.G. Song, et al., Cyanidin-3-glucoside isolated from mulberry fruit protects pancreatic β-cells against oxidative stress-induced apoptosis, Int. J. Mol. Med. 35 (2) (2015) 405–412.
[9] M. Riopel, M. Krishnamurthy, J. Li, et al., Conditional β1-integrin-deficient mice display impaired pancreatic β cell function, J. Pathol. 224 (2011) 45–55.
[10] T. Kitayama, T. Okamoto, R.K. Hill, et al., Chemistry of zerumbone. 1. Simplified isolation, conjugate addition reactions, and a unique ring contracting transannular reaction of its dibromide, J. Org. Chem. 64 (1999) 2667–2672.
[11] A. Sehrawat, K. Sakao, S.V. Singh, Notch2 activation is protective against anticancer effects of zerumbone in human breast cancer cells, Breast Cancer Res. Treat. 146 (2014) 543–555.
[12] Y.H. Shieh, H.M. Huang, C.C. Wang, et al., Zerumbone enhances the Th1 response and ameliorates ovalbumin-induced Th2 responses and airway inflammation in mice, Int. Immunopharmacol. 24 (2015) 383–391.
[13] J.W. Shin, K. Ohnishi, A. Murakami, et al., Zerumbone induces heme oxygenase-1 expression in mouse skin and cultured murine epidermal cells through activation of Nrf2, Cancer Prev. Res. (Phila.) 4 (2011) 860–870.
[14] S. Zhang, Q. Liu, Y. Liu, et al., Zerumbone, a Southeast Asian ginger sesquiterpene, induced apoptosis of pancreatic carcinoma cells through p53 signaling pathway, Evid. Based Complement. Alternat. Med. 2012 (2012) 936030.
[15] B.Y. Chen, D.P. Lin, K.C. Su, et al., Dietary zerumbone prevents against ultraviolet B-induced cataractogenesis in the mouse, Mol. Vis. 17 (2011) 723–730.
[16] B.Y. Chen, D.P. Lin, C.Y. Wu, et al., Dietary zerumbone prevents mouse cornea from UVB-induced photokeratitis through inhibition of NF-κB, iNOS, and TNF-α expression and reduction of MDA accumulation, Mol. Vis. 17 (2011) 854–863.

[17] T.F. Tzeng, S.S. Liou, C.J. Chang, et al., The ethanol extract of *Zingiber zerumbet* Attenuates streptozotocin-induced diabetic nephropathy in rats, Evid. Based Complement. Alternat. Med. 2013 (2013) 340645.
[18] C. Broca, E. Varin, M. Armanet, et al., Proteasome dysfunction mediates high glucose-induced apoptosis in rodent beta cells and human islets, PLoS One 9 (2014) e92066.
[19] J.F. Curtin, T.G. Cotter, Anisomycin activates JNK and sensitises DU 145 prostate carcinoma cells to Fas mediated apoptosis, Br. J. Cancer 87 (2002) 1188–1194.
[20] B. Törocsik, J. Szeberényi, Anisomycin affects both pro- and antiapoptotic mechanisms in PC12 cells, Biochem. Biophys. Res. Commun. 278 (2000) 550–556.
[21] W.H. Kim, J.W. Lee, Y.H. Suh, et al., AICAR potentiates ROS production induced by chronic high glucose: roles of AMPK in pancreatic beta-cell apoptosis, Cell. Signal. 19 (2007) 791–805.
[22] H. Shen, J. Liu, Y. Wang, et al., Aflatoxin G1-induced oxidative stress causes DNA damage and triggers apoptosis through MAPK signaling pathway in A549 cells, Food Chem. Toxicol. 62 (2013) 661–669.
[23] P. Palozza, S. Serini, A. Torsello, et al., Mechanism of activation of caspase cascade during beta-carotene-induced apoptosis in human tumor cells, Nutr. Cancer 47 (2003) 76–87.
[24] H.S. Rahman, A. Rasedee, A.B. Abdul, et al., Zerumbone-loaded nanostructured lipid carrier induces G2/M cell cycle arrest and apoptosis via mitochondrial pathway in a human lymphoblastic leukemia cell line, Int. J. Nanomed. 9 (2014) 527–538.
[25] A.S. Al-Zubairi, A.B. Abdul, M.M. Syam, Evaluation of the genotoxicity of zerumbone in cultured human peripheral blood lymphocytes, Toxicol. In Vitro 24 (2010) 707–712.
[26] H.U. Simon, A. Haj-Yehia, F. Levi-Schaffer, Role of reactive oxygen species (ROS) in apoptosis induction, Apoptosis 5 (2000) 415–418.
[27] M.H. Park, J.S. Han, Padina arborescens extract protects high glucose-induced apoptosis in pancreatic β cells by reducing oxidative stress, Nutr. Res. Pract. 8 (2014) 494–500.
[28] C. Huang, P. Zhang, W. Wang, et al., Long-term blue light exposure induces RGC-5 cell death in vitro: involvement of mitochondria-dependent apoptosis, oxidative stress, and MAPK signaling pathways, Apoptosis 19 (2014) 922–932.
[29] E.K. Kim, E.J. Choi, Pathological roles of MAPK signaling pathways in human diseases, Biochim. Biophys. Acta 1802 (2010) 396–405.
[30] L.A. Flores-López, M. Díaz-Flores, R. García-Macedo, et al., High glucose induces mitochondrial p53 phosphorylation by p38 MAPK in pancreatic RINm5F cells, Mol. Biol. Rep. 40 (2013) 4947–4958.
[31] T. Ito, K. Omori, J. Rawson, et al., Improvement of canine islet yield by donor pancreas infusion with a p38MAPK inhibitor, Transplantation 86 (2008) 321–329.
[32] T.F. Tzeng, S.S. Liou, C.J. Chang, et al., Zerumbone, a tropical ginger sesquiterpene, ameliorates streptozotocin-induced diabetic nephropathy in rats by reducing the hyperglycemia-induced inflammatory response, Nutr. Metab. (Lond.) 10 (2013) 64.