Rice Husk Silica Liquid

Subjects: Biology

Contributor: Shih-Min Hsia

Type 2 diabetes mellitus is a complex multifactorial disease characterized by insulin resistance and dysfunction of pancreatic β-cells. Rice husk silica liquid (RHSL) is derived from rice husks and has not been explored in diabetes mellitus until now. Previous studies showed that rice husk is enriched with silica, and its silica nanoparticles are higher more biocompatible. To investigate the potential protective role of RHSL on pancreatic β cells, we utilized RIN-m5F pancreatic β cells and explored RHSL effect after streptozotocin (STZ)-stimulation. The recovery effects of RHSL were evaluated using flow cytometry, Western blotting, and immunofluorescence analysis. Results of our study showed that RHSL reversed the cell viability, insulin secretion, reactive oxygen species (ROS) production, and the change of mitochondria membrane potential (ΔΨm) in STZ-treated RIN-m5F cells. Moreover, the expression of phospho-receptor-interacting protein 3 (p-RIP3) and cleaved-poly (ADP-ribose) polymerase (PARP), phospho-mammalian target of rapamycin (p-mTOR), and sequestosome-1 (p62/SQSTM1) were significantly decreased, while the transition of light chain (LC)3-I to LC3-II was markedly increased after RHSL treatment in STZ-induced RIN-m5F cells. Interestingly, using autophagy inhibitors such as 3-methyladenine (3-MA) and chloroquine (CQ) both showed an increase in cleaved-PARP protein level, indicating apoptosis induction.

Keywords: rice husk silica liquid ; streptozotocin ; pancreatic β cell ; reactive oxygen species ; autophagy ; apoptosis

1. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder that has become a global concern [1]. The major pathogenesis involves the loss or destruction of pancreatic β cells, which in turn induces the state of imbalance between insulin secretion and blood sugar control in the body [2]. Streptozotocin (STZ) is a kind of glucosamine-nitrosourea compound and widely used as a broad-spectrum antibiotic and cytotoxic chemical [3]. Due to its structural similarity to 2-deoxy-D-glucose, it can enter pancreatic β cells through the GLUT2 glucose transporter and efficiently accumulates intracellularly. Intracellular accumulation induces DNA alkylation and exposes the cells to reactive oxygen species (ROS) and nitric oxide (NO) with subsequent damage, ultimately provoking diabetogenic action [4]. Diabetes induced by STZ is the most similar to the structural, functional and biochemical changes observed in human diabetes, so it is often used to simulate diabetes models in animal experiments [4][5] as well as to verify the mechanism of pancreatic β-cells in in vitro experiments [6], which involve mitochondrial dysfunction, ROS production, necrosis, and apoptosis.

Silicon (Si) is known as an essential component of collagen and glycosaminoglycan formed in bones and cartilage [7][8]. Several studies also demonstrated that dietary Si intake has benefits on bone mineral density [9][10]. In addition, Si showed an anti-diabetic effect by lowering blood glucose level, improving the tolerance to insulin and reducing the risk of glomerulopathy [11]. Previous studies have demonstrated rice husk is enriched with silica, and its silica nanoparticles are more biocompatible [12], thus expecting more applications in the food industry. However, food inspection and functional analysis of rice husk silica (RHS) remain insufficient. Furthermore, the potential anti-diabetic role of rice husk silica liquid (RHSL) on pancreatic β cell has not been explored by any research so far. Herein, we designed the present study to further investigate the mechanisms by which RHSL can protect against T2DM, aiming to shed new light on T2DM prevention by plant-derived silica.

2. Current Researches

2.1. The Effect of RHSL and STZ on RIN-m5F Cells Viability

To investigate the effects of STZ on RIN-m5F cells, cell viability was quantified after exposing the RIN-m5F cell to toxin STZ. Our data indicated that STZ caused cytotoxicity in a dose and time-dependent manners (**Figure 1**A, $p < 0.001$). Briefly, STZ at 2 mM for 24 h markedly reduced the viability of RIN-m5F cells to ~60% (**Figure 1**A, $p < 0.001$). Based on these results, all subsequent experiments in this study were performed using 2 mM STZ as the induction model. On the other hand, we used different doses of RHSL from 400-fold dilution to 100-fold dilution and observed that RHSL treatment

can enhance cell viability, especially at 200-fold and 100-fold dilution, which both significantly increased cell viability compared to the control group for 24 and 48 h in a dose-dependent manner (**Figure 1**B,C, $p < 0.001$).
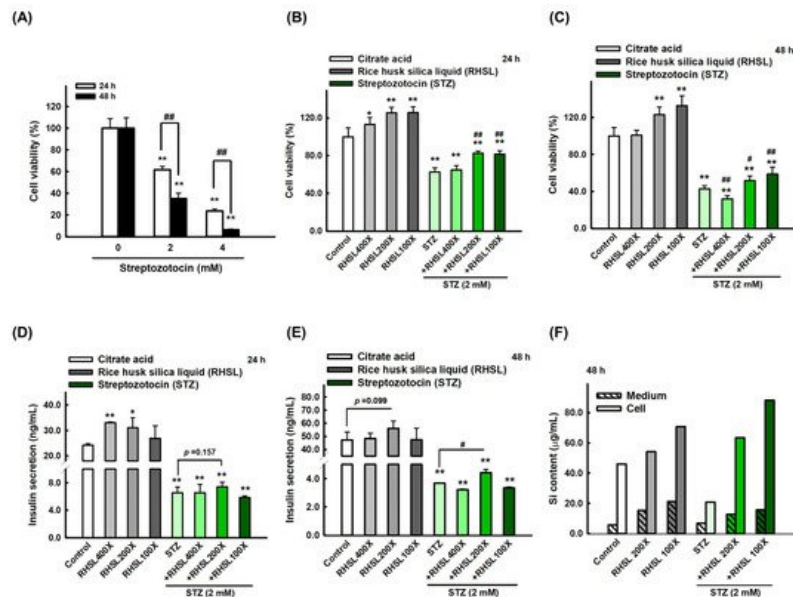

Figure 1. Rice husk silica liquid (RHSL) reverses streptozotocin (STZ)- induced cell viability and insulin secretion in RIN-m5F cells. (**A**) Screen the dosage of STZ (2 and 4 mM) for cell viability at 24 and 48 h. (**B**,**C**) The effect of RHSL and STZ treatment on cell viability for 24 and 48 h. (**D**,**E**) The amount of insulin (ng/mL) at 2 mM of STZ when treated with different dilutions of RHSL (400×, 200×, and 100×). (**F**) The level of silicon in cell pellet or culture medium at 2 mM of STZ when treated with different dilutions of RHSL (400×, 200×, and 100×). * $p < 0.05$; ** $p < 0.001$ compare with each control group (citrate acid). # $p < 0.05$; ## $p < 0.001$ compare with STZ group.

Considering the results of individual used doses, we exposed RIN-m5F cells to the combination treatment and found that adding RHSL caused a significant recovery in cell survival in STZ-treated cells. Briefly, STZ at 2 mM markedly reduced the viability of RIN-m5F cells to ~60% for 24 h (**Figure 1**B, $p < 0.001$), and to ~40% for 48 h (**Figure 1**C, $p < 0.001$), while the cell viability was significantly recovered after co-treatment with RHSL at 200-fold and 100-fold dilution (**Figure 1**B,C, $p < 0.001$). Therefore, these results suggest that RHSL possesses the potential ability to restore the damage resulted from STZ treatment.

2.2. STZ Inhibits Insulin Secretion and Silicon Content in RIN-m5F Cells Which Is Restored by RHSL Co-Treatment

Next, we measured the level of insulin secretion to explore the therapeutic efficacy of RHSL in STZ-treated RIN-m5F cells. Insulin secretion in the culture medium was significantly reduced after STZ treatment (**Figure 1**D,E, $p < 0.001$), while the 200-fold dilution of RHSL significantly restored the level of insulin after STZ treatment (**Figure 1**E, $p < 0.05$).

In the other hand, we accurately quantified the concentration of silicon (Si) in RIN-m5F cells and suspension after RHSL and STZ co-treatment. As expected, the RHSL group increased the Si content in the cells with or without STZ treatment (**Figure 1**F). Similar results were found in the suspension, that the RHSL group increased the Si content with or without STZ treatment (**Figure 1**F). Interestingly, compared to the control group, the treatment with STZ alone reduced the Si content in the cells (**Figure 1**F). Collectively, RHSL is shown to possess the potential ability to restore insulin secretion after STZ stimulation.

2.3. RHSL Protects RIN-m5F Cells Against STZ-induced ROS Production and Dysfunction of Mitochondria

To explore whether RHSL can suppress the cell damage provoked by ROS in STZ-treated RIN-m5F cells, a cellular ROS assay was performed (**Figure 2**A). As a positive control, H_2O_2 accelerating the process of beta cell destruction through significantly increasing the ROS production (**Figure 2**B, $p < 0.05$). Even though the peak value was too large and there was no significant difference between the two groups, we still observe that STZ increases the ROS production in RIN-m5F cells (**Figure 2**B, $p = 0.066$), while RHSL slightly suppresses the ROS production caused by STZ (**Figure 2**B, $p = 0.124$).
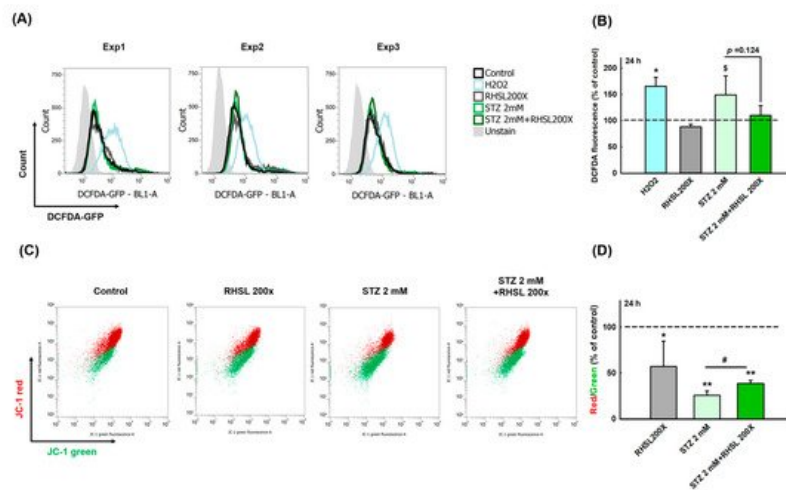
Figure 2. RHSL protects RIN-m5F cells against STZ-induced oxidant damage. Following treatment with RHSL in the absence or presence of STZ (2 mM) for 24 h. (**A**) The image of DCFDA fluorescence showed typical results of three independent experiments. (**B**) The DCFDA fluorescence intensity ratio represents the level of ROS production in RIN-m5F cells, and the dotted line represents the control group. (**C**) Distribution dot plot of mitochondrial depolarization stained by JC-1 (**D**) The red (~590 nm)/green (~529 nm) fluorescence intensity ratio represents depolarization of the mitochondrial membrane in RIN-m5F cells, and the dotted line represents the control group. $ $p = 0.066$; * $p < 0.05$; ** $p < 0.001$ compare with control group (citrate acid); # $p < 0.05$ compare with STZ group.

Next, to investigate the changes of mitochondria membrane potential (ΔΨm) in STZ-treated RIN-m5F cells after RHSL intervention, a JC-1 assay was utilized (**Figure 2**C). We observed that the red/green ratio significantly decreased after STZ stimulation (**Figure 2**D, $p < 0.001$), while RHSL significantly restored the red/green ratio in STZ-treated RIN-m5F cells (**Figure 2**D, $p < 0.05$). Therefore, these results have clearly indicated that RHSL possesses the potential ability to abrogate stress resulted from STZ stimulation.

3. Discussion

The current study provides new insights regarding the potential utility of rice husk silica liquid (RHSL) in T2DM, RHSL abrogated the effect of streptozotocin (STZ) on the mass of RIN-m5F pancreatic β cells and the ability of insulin secretion, via reducing the reactive oxygen species (ROS) production, recovering the depolarization of mitochondrial membrane potential (ΔΨm), as well as reversing the expression of cleaved-PARP, p-RIP3, p-mTOR, p62/SQSTM1, and LC3B protein. The proposed mechanisms of RHSL action in the prevention of STZ-induced RIN-m5F cell death were summarized in **Figure 6**.
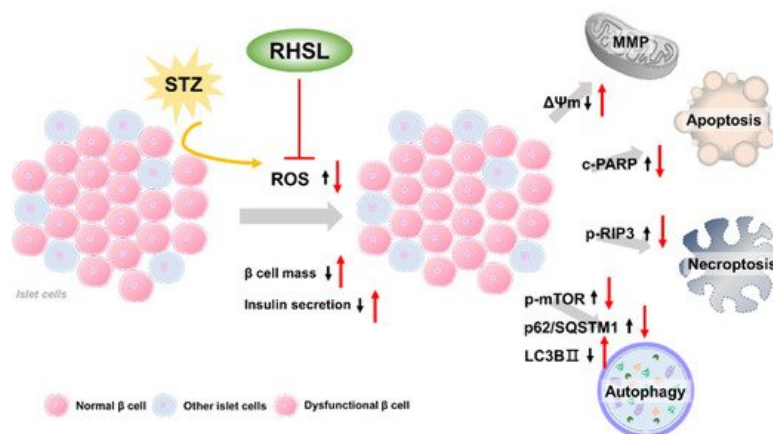

Figure 6. Schematic model of the potential mechanisms of RHSL protective effect on RIN-m5F cells from STZ-induced oxidative damage. Black arrows are the effects of STZ, red arrows are effects of RHSL. Abbreviation: RHSL, rice husk silica liquid; STZ, streptozotocin; ROS, reactive oxygen species; ΔΨm, mitochondria membrane potential; RIP3, receptor-interacting protein 3; PARP, poly (ADP-ribose) polymerase; mTOR, mammalian target of rapamycin; p62/SQSTM1, sequestosome-1; LC3, light chain 3.

The major pathways associated with STZ-induced pancreatic β cells death include DNA methylation and activation of DNA repair mechanisms [13], nitric oxide (NO) overproduction [14], and glucose auto-oxidation which in turn generates free

radicals [13]. Moreover, STZ induces pancreatic β cell toxicity through mitochondrial dysfunction, ROS production, necrosis, and apoptosis [4].

RIN-m5F cells are a widely used insulin-secreting cell line, mainly containing insulin and a small amount of glucagon and somatostatin [15]. STZ administration leads to the rapid destruction of pancreatic β cells, thus massive insulin was released from ruptured β cells to blood [4]. A previous study revealed that a considerable decrease in insulin secretion ability in STZ-treated INS-1 cell, whereas paeoniflorin (PF) pretreatment was capable of improving the insulin secretion ability of INS-1 cells [16]. In addition, $1,25(OH)_2D3$ enhances insulin secretion in STZ-treated d MIN6 cells at low and high glucose concentrations [17]. An in vivo experiment showed that, compared with the control group of diabetic mice, the plasma insulin of the Si treatment group was significantly reversed to near-normal levels [11]. Similar to the results of previous studies, the insulin levels of pancreatic β cells in the STZ treatment group were significantly reduced in our study, while RHSL significantly reversed the insulin level that was reduced by STZ.

Higher levels of ROS are found in the islets of T2DM patients, and their presence attenuates insulin secretion [4]. STZ that induces diabetes is also closely related to the production of ROS. Several studies have demonstrated that STZ induces damage predominantly by provoking the production of ROS and NO in islet cells [4]. In STZ-treated MIN6 β-cells, ROS generation was statistically significantly increased by $55.1 \pm 4.7\%$ ($p < 0.001$) [18]. Similarly, Al-Nahdi et al. observed a significant increase in intracellular ROS production in RIN-m5F cells treated with 1 mM STZ for 48 h [19]. Comparably, our study showed that ROS production was increased in RIN-m5F cells following treatment with 2 mM STZ for 24 h, while RHSL slightly reversed the effects caused by STZ. Therefore, we speculated that RHSL can reverse the cytotoxicity and damage induced by STZ by reducing the content of ROS in RIN-m5F cells.

Under the stimulation of oxidative stress, the mitochondrial membrane potential (ΔΨm) will be changed, which is characterized by the disordered membrane potential leads to the opening of mitochondrial pores, thus releases cytochrome C into the cytoplasm, which in turn triggers the downstream apoptotic cascade [20][21]. Al-Nahdi et al. observed a significant loss in the membrane potential after treatment with 10 mM STZ for 24 h [22]. Similarly, our results showed a significant mitochondrial membrane depolarization after treatment with STZ in RIN-m5F cells. Importantly, RHSL treatment showed a significant recovery of the membrane potential in STZ treated cells.

Most studies have shown that pancreatic β cell apoptosis is a key process in the pathogenesis of T1DM and T2DM, so it is important to alleviate or suppress the effect of STZ that induces diabetes. Fucoidan, a brown algae extract, which is used to be explored against diabetic nephropathy, has also been found to reduce the number of apoptotic cells in STZ-treated NIT-1 cell [23]. In addition, Lianga et al. showed that Fudan-Yueyang G. lucidum alleviated the apoptosis in STZ-treated INS-1 cell [24]. In our study, STZ induced the expression of apoptosis-related protein PARP, while RHSL reversed these results and significantly reduced the expression of the cleaved-PARP protein. The contradictory research revealed that the ability of nano-SiO2 to induce ROS and promote apoptosis in normal human hepatic L-02 cells [25], while no significant alteration of ROS levels and cell viability were seen in 48 and 86 nm of SiO2 exposed groups. Therefore, we believe that the size of silicon particles and cell viability are worthy of further research.

Necroptosis is considered to be an alternate cell death mechanism triggered when apoptosis is blocked. Besides the inhibition of caspase-8 prevents apoptosis induction [26], receptor-interacting protein 3 (RIP3) plays a critical role in the switch between apoptotic and necroptotic cell death triggered by tumor necrosis factor (TNF) [27]. In general, RIP1 mediated apoptosis does not require the presence of RIP3, while RIP1 is accompanied by high levels of RIP3 that promote the formation of necrosome complexes and switch cell apoptosis into necroptosis [26]. More specifically, RIP3 increases TNF-induced ROS production via activation of glutamate-ammonia ligase (GLUL), glutamate dehydrogenase 1 (GLUD1), and eventually induce necroptosis [27][28]. At present, there is no literature to investigate the level of RIP1/RIP3 for pancreatic β cells following induction by STZ, while a previous study indicated that STZ-induced renal injury tissue had significantly higher expression levels of RIP1 and RIP3 [29].

Autophagy is a catabolic process of the lysosomal degradation pathway under conditions of stress, which maintains metabolic turnover and homeostasis in pancreatic β cells [30]. The autophagy process involves the inhibition of the mammalian target of rapamycin complex 1 (mTORC1) and the deacetylation of autophagy components, which promotes the fusion and degradation of autophagosomes containing substances to be degraded and lysosomes containing acid hydrolases [30]. Once autophagy is dysregulated, it will also promote the loss of β cell mass and function [31]. Previous research confirms this argument, they found that autophagy-related (ATG) 7 knockout mice showed decreased β-cell mass through increased apoptosis, which ultimately lead to decreased insulin secretion and impaired glucose tolerance [32]. The most representative indicators of autophagic flux include light chain (LC)3 (autophagosome marker) and p62/SQSTM1 (autophagy adaptor), the latter being the link between LC3 and the ubiquitinated substrates [33]. In our

study, we found a significant increase in the transition of LC3-I to LC3-II, while p62/SQSTM1 was decreasing after RHSL treatment in STZ-induced RIN-m5F cells, which indicate that RHSL is indeed involved in the process of autophagy regulation.

On the other hand, 3-methyladenine (3-MA) and chloroquine (CQ) are frequently used autophagy inhibitors that function in early autophagy through PI3K inhibition [34] and in late autophagy by blocking the fusion of autophagosomes and lysosomes [35], respectively. In our study, we found that RHSL significantly induced the expression of cleaved-PARP in 3-MA-treated RIN-m5F cells, and similar results were also observed in CQ-treated RIN-m5F cells, these results reveal that RHSL protects RIN-m5F cells by inducing autophagy. Similar results have also been observed in previous studies, vitamin D enhanced autophagy while inhibited apoptosis in STZ-treated MIN6 β-cells [17]. In addition, T. stricta extract was able to significantly reverse the autophagy markers in response to STZ to near normal level in RIN-m5F cells [36].

The factors that determine the bioavailability of Si element include concentration, type of food, and species in which silicon is present [37][38]. Si in serum and tissues exists in free form and can be absorbed and eliminated in urine quickly due to free diffusion across cell membranes [11]. This implies the advantage of long-term intake without excessive retention and accumulation in the body.

References

1. Zheng, Y.; Ley, S.H.; Hu, F.B. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nat. Rev. Endocrinol. 2018, 14, 88–98.

2. Galicia-Garcia, U.; Benito-Vicente, A.; Jebari, S.; Larrea-Sebal, A.; Siddiqi, H.; Uribe, K.B.; Ostolaza, H.; Martín, C. Pathophysiology of type 2 diabetes mellitus. Int. J. Mol. Sci. 2020, 21, 6275.

3. Bolzán, A.D.; Bianchi, M.S. Genotoxicity of streptozotocin. Mutat. Res. 2002, 512, 121–134.

4. Goyal, S.N.; Reddy, N.M.; Patil, K.R.; Nakhate, K.T.; Ojha, S.; Patil, C.R.; Agrawal, Y.O. Challenges and issues with streptozotocin-induced diabetes-a clinically relevant animal model to understand the diabetes pathogenesis and evaluate therapeutics. Chem. Biol. Interact. 2016, 244, 49–63.

5. Hayashi, K.; Kojima, R.; Ito, M. Strain differences in the diabetogenic activity of streptozotocin in mice. Biol. Pharm. Bull. 2006, 29, 1110–1119.

6. Sandler, S.; Andersson, A.K.; Larsson, J.; Makeeva, N.; Olsen, T.; Arkhammar, P.O.G.; Hansen, J.B.; Karlsson, F.A.; Welsh, N. Possible role of an ischemic preconditioning-like response mechanism in katp channel opener-mediated protection against streptozotocin-induced suppression of rat pancreatic islet function. Biochem. Pharmacol. 2008, 76, 1748–1756.

7. Reffitt, D.M.; Ogston, N.; Jugdaohsingh, R.; Cheung, H.F.; Evans, B.A.; Thompson, R.P.; Powell, J.J.; Hampson, G.N. Orthosilicic acid stimulates collagen type 1 synthesis and osteoblastic differentiation in human osteoblast-like cells in vitro. Bone 2003, 32, 127–135.

8. Nielsen, F.H. Update on the possible nutritional importance of silicon. J. Trace Elem. Med. Biol. (GMS) 2014, 28, 379–382.

9. Rico, H.; Gallego-Lago, J.L.; Hernández, E.R.; Villa, L.F.; Sanchez-Atrio, A.; Seco, C.; Gérvas, J.J. Effect of silicon supplement on osteopenia induced by ovariectomy in rats. Calcif. Tissue Int. 2000, 66, 53–55.

10. Jugdaohsingh, R.; Tucker, K.L.; Qiao, N.; Cupples, L.A.; Kiel, D.P.; Powell, J.J. Dietary silicon intake is positively associated with bone mineral density in men and premenopausal women of the framingham offspring cohort. J. Bone Miner. Res. 2004, 19, 297–307.

11. Maehira, F.; Ishimine, N.; Miyagi, I.; Eguchi, Y.; Shimada, K.; Kawaguchi, D.; Oshiro, Y. Anti-diabetic effects including diabetic nephropathy of anti-osteoporotic trace minerals on diabetic mice. Nutrition 2011, 27, 488–495.

12. Ways, T.M.M.; Ng, K.W.; Lau, W.M.; Khutoryanskiy, V.V. Silica nanoparticles in transmucosal drug delivery. Pharmaceutics 2020, 12, 751.

13. Eleazu, C.O.; Eleazu, K.C.; Chukwuma, S.; Essien, U.N. Review of the mechanism of cell death resulting from streptozotocin challenge in experimental animals, its practical use and potential risk to humans. J. Diabetes Metab. Disord. 2013, 12, 60.

14. Turk, J.; Corbett, J.A.; Ramanadham, S.; Bohrer, A.; McDaniel, M.L. Biochemical evidence for nitric oxide formation from streptozotocin in isolated pancreatic islets. Biochem. Biophys. Res. Commun. 1993, 197, 1458–1464.

15. Skelin, M.; Rupnik, M.; Cencic, A. Pancreatic beta cell lines and their applications in diabetes mellitus research. Altex 2010, 27, 105–113.

16. Liu, Y.; Han, J.; Zhou, Z.; Li, D. Paeoniflorin protects pancreatic β cells from stz-induced damage through inhibition of the p38 mapk and jnk signaling pathways. Eur. J. Pharmacol. 2019, 853, 18–24.

17. Wang, Y.; He, D.; Ni, C.; Zhou, H.; Wu, S.; Xue, Z.; Zhou, Z. Vitamin D induces autophagy of pancreatic β-cells and enhances insulin secretion. Mol. Med. Rep. 2016, 14, 2644–2650.

18. Hu, S.; Kuwabara, R.; de Haan, B.J.; Smink, A.M.; de Vos, P. Acetate and butyrate improve β-cell metabolism and mitochondrial respiration under oxidative stress. Int. J. Mol. Sci. 2020, 21, 1542.

19. AI-Nahdi, A.M.T.; John, A.; Raza, H. Elucidation of molecular mechanisms of streptozotocin-induced oxidative stress, apoptosis, and mitochondrial dysfunction in rin-5f pancreatic β-cells. Oxidative Med. Cell. Longev. 2017, 2017, 7054272.

20. Sinha, K.; Das, J.; Pal, P.B.; Sil, P.C. Oxidative stress: The mitochondria-dependent and mitochondria-independent pathways of apoptosis. Arch. Toxicol. 2013, 87, 1157–1180.

21. Sha, W.; Hu, F.; Bu, S. Mitochondrial dysfunction and pancreatic islet β-cell failure (review). Exp. Ther. Med. 2020, 20, 266.

22. Al-Nahdi, A.M.T.; John, A.; Raza, H. Cytoprotective effects of n-acetylcysteine on streptozotocin-induced oxidative stress and apoptosis in rin-5f pancreatic β-cells. Cell Physiol. Biochem. 2018, 51, 201–216.

23. Yu, W.C.; Chen, Y.L.; Hwang, P.A.; Chen, T.H.; Chou, T.C. Fucoidan ameliorates pancreatic β-cell death and impaired insulin synthesis in streptozotocin-treated β cells and mice via a sirt-1-dependent manner. Mol. Nutr. Food Res. 2017, 61, 1700136.

24. Liang, H.; Pan, Y.; Teng, Y.; Yuan, S.; Wu, X.; Yang, H.; Zhou, P. A proteoglycan extract from ganoderma lucidum protects pancreatic beta-cells against stz-induced apoptosis. Biosci. Biotechnol. Biochem. 2020, 84, 2491–2498.

25. Ye, Y.; Liu, J.; Xu, J.; Sun, L.; Chen, M.; Lan, M. Nano-sio2 induces apoptosis via activation of p53 and bax mediated by oxidative stress in human hepatic cell line. Toxicol. Vitr. Int. J. Publ. Assoc. BIBRA 2010, 24, 751–758.

26. Kaczmarek, A.; Vandenabeele, P.; Krysko, D.V. Necroptosis: The release of damage-associated molecular patterns and its physiological relevance. Immunity 2013, 38, 209–223.

27. Zhang, D.W.; Shao, J.; Lin, J.; Zhang, N.; Lu, B.J.; Lin, S.C.; Dong, M.Q.; Han, J. Rip3, an energy metabolism regulator that switches tnf-induced cell death from apoptosis to necrosis. Science 2009, 325, 332–336.

28. Hsu, S.-K.; Chang, W.-T.; Lin, I.L.; Chen, Y.-F.; Padalwar, N.B.; Cheng, K.-C.; Teng, Y.-N.; Wang, C.-H.; Chiu, C.-C. The role of necroptosis in ros-mediated cancer therapies and its promising applications. Cancers 2020, 12, 2185.

29. Yi, W.; OuYang, Q. Adiponectin improves diabetic nephropathy by inhibiting necrotic apoptosis. Arch. Med. Sci. AMS 2019, 15, 1321–1328.

30. Marasco, M.R.; Linnemann, A.K. B-cell autophagy in diabetes pathogenesis. Endocrinology 2018, 159, 2127–2141.

31. Mazza, S.; Maffucci, T. Autophagy and pancreatic β-cells. Vitam. Horm. 2014, 95, 145–164.

32. Jung, H.S.; Chung, K.W.; Won Kim, J.; Kim, J.; Komatsu, M.; Tanaka, K.; Nguyen, Y.H.; Kang, T.M.; Yoon, K.H.; Kim, J.W.; et al. Loss of autophagy diminishes pancreatic beta cell mass and function with resultant hyperglycemia. Cell Metab. 2008, 8, 318–324.

33. Klionsky, D.J.; Abdelmohsen, K.; Abe, A.; Abedin, M.J.; Abeliovich, H.; Acevedo Arozena, A.; Adachi, H.; Adams, C.M.; Adams, P.D.; Adeli, K.; et al. Guidelines for the use and interpretation of assays for monitoring autophagy. Autophagy 2016, 12, 1–222.

34. Yang, Y.P.; Hu, L.F.; Zheng, H.F.; Mao, C.J.; Hu, W.D.; Xiong, K.P.; Wang, F.; Liu, C.F. Application and interpretation of current autophagy inhibitors and activators. Acta Pharmacol. Sin. 2013, 34, 625–635.

35. Pasquier, B. Autophagy inhibitors. Cell. Mol. Life Sci. CMLS 2016, 73, 985–1001.

36. Bahramsoltani, R.; Farzaei, M.H.; Sajadimajd, S.; Iranpanah, A.; Khazaei, M.; Pourjabar, Z.; Hajimahmoodi, M.; Rahimi, R. In vitro and in vivo antidiabetic activity of tamarix stricta boiss.: Role of autophagy. J. Ethnopharmacol. 2021, 269, 113692.

37. Jugdaohsingh, R.; Anderson, S.H.; Tucker, K.L.; Elliott, H.; Kiel, D.P.; Thompson, R.P.; Powell, J.J. Dietary silicon intake and absorption. Am. J. Clin. Nutr. 2002, 75, 887–893.

38. Robberecht, H.; Van Cauwenbergh, R.; Van Vlaslaer, V.; Hermans, N. Dietary silicon intake in belgium: Sources, availability from foods, and human serum levels. Sci. Total Environ. 2009, 407, 4777–4782.