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Protective effects of *Allium stipitatum* Regel. extract against streptozotocin-induced cytotoxicity in pancreatic beta cells: Implications for diabetes management

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ABSTRACT

Introduction: *Allium stipitatum* is used against diabetes but its protective effects on beta cells remain unclear. This study investigated the cytoprotective and antioxidative effects of *A. stipitatum* extract in a streptozotocin (STZ)-induced beta-cell injury model.

Methods: *Allium stipitatum* methanol extract was prepared and tested at 10 µg/mL on cultured pancreatic beta cells, alone or in combination with STZ (100 µM). Cell viability (Trypan blue test), insulin secretion (ELISA), nitric oxide (NO) levels (Griess method), and total antioxidant capacity (FRAP assay) were evaluated. Data were analyzed using one-way analysis of variance (ANOVA) with Tukey's test ($P<0.05$).

Results: STZ significantly reduced insulin secretion (From 45.7±0.38 to 21.47 ± 0.78 mU/L) ($P<0.05$) in STZ group, while *A. stipitatum* extract increased it to 40.7 ± 0.41 mU/L ($P<0.05$) in the extract group. Additionally, in all four groups where the extract was applied (extract, extract + STZ (pre-treatment), STZ + extract (post-treatment), and extract – STZ (co-treatment), the extract increased insulin secretion levels. Beta-cell viability declined with STZ (From 33633±1537 to 11500 ± 577 cells) but improved with extract treatment to 26500 ± 1626 ($P<0.05$). Although the extract did not significantly enhance total antioxidant capacity, it markedly reduced NO levels, particularly in extract + STZ group ($P<0.05$).

Conclusion: *Allium stipitatum* extract supports beta-cell survival and insulin secretion in STZ-induced pancreatic damage, indicating potential therapeutic applications in diabetes management. Further studies are needed to identify active compounds and optimize its use.

Implication for health policy/practice/research/medical education:
The methanolic extract of *Allium stipitatum* showed significant antidiabetic effects in streptozotocin (STZ)-induced diabetic models, improving beta-cell function and reducing oxidative stress. It also enhanced insulin secretion and glucose regulation, highlighting its potential as a natural remedy for diabetes mellitus (DM).

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Introduction

Diabetes mellitus (DM) represents a significant metabolic disorder prevalent in both developing and developed countries, marked by hyperglycemia due to insulin deficiency or resistance (1). This condition leads to a range of complications, including retinopathy, nephropathy, and cardiovascular diseases, which can severely impact patients' quality of life. Globally, DM affects over 537 million adults, with projections rising to 783 million by 2045, making it one of the most pressing global health challenges (2). In Iran, the prevalence of DM has reached approximately 11.4%, further highlighting the need for urgent therapeutic interventions (3). The pathophysiology of DM varies based on its type. Type 1 diabetes (T1DM) is an autoimmune disorder in which immune-mediated destruction of pancreatic beta cells leads to absolute insulin deficiency. This process is primarily driven by autoreactive T-cells targeting beta-cell antigens, resulting in progressive loss of insulin production. In contrast, type 2 diabetes (T2DM) is characterized by insulin

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resistance, where peripheral tissues (such as muscle, liver, and adipose tissue) fail to respond effectively to insulin, leading to compensatory hyperinsulinemia. Over time, pancreatic beta cells become dysfunctional and fail to maintain adequate insulin secretion, causing sustained hyperglycemia. Additionally, oxidative stress and chronic low-grade inflammation contribute to beta-cell apoptosis and metabolic dysfunction in both types of diabetes (4,5). Streptozotocin (STZ), used in experimental models of T1DM, selectively induces beta-cell necrosis by generating reactive oxygen species (ROS) and DNA damage, mimicking the beta-cell destruction observed in autoimmune diabetes. STZ, a cytotoxic analog of glucose, was first isolated in 1960 from *Streptomyces achromogenes*. Its diabetogenic properties were not elucidated until 1963 (6-8).

STZ inhibits insulin secretion and induces insulin-dependent diabetes. Both effects can be attributed to its specific chemical properties, namely its alkylating power. The alteration in blood glucose and insulin levels following STZ injection is due to the abnormal functioning of pancreatic beta cells. STZ disrupts glucose oxidation and also reduces insulin synthesis and secretion. It is taken up by pancreatic beta cells via GLUT2, thereby entering these cells and causing tissue damage (7,9,10).

Oxidative stress plays a central role in the progression of DM, contributing to beta-cell dysfunction and insulin resistance. Pancreatic beta cells are highly susceptible to oxidative damage due to their low antioxidant enzyme expression. Excessive nitric oxide (NO) production, primarily through inducible nitric oxide synthase (iNOS), exacerbates oxidative stress, leading to beta-cell apoptosis and reduced insulin secretion. Studies have shown that elevated NO levels in diabetic conditions impair mitochondrial function, trigger inflammatory responses, and induce cytotoxicity in beta cells. Meanwhile, antioxidants help mitigate these effects by neutralizing ROS and preserving cellular integrity. The interplay between insulin, NO, and antioxidant capacity is crucial in DM progression, as oxidative stress-induced beta-cell damage diminishes insulin production, while increased NO levels further exacerbate this dysfunction. Targeting oxidative stress and NO regulation may therefore enhance beta-cell survival and improve insulin secretion, offering a potential therapeutic strategy for DM management (11,12).

Conventional DM therapies, such as insulin therapy, metformin, and SGLT2 inhibitors, help manage blood glucose but do not restore pancreatic function or prevent disease progression (13). Long-term use of synthetic drugs is associated with side effects, including hypoglycemia, weight gain, and cardiovascular risks (14). In response to these limitations, natural plant-derived compounds have gained attention due to their antioxidant, anti-inflammatory, and β-cell protective properties (15). Various studies have highlighted the role of antioxidants in counteracting the oxidative stress caused by STZ, suggesting that phytochemicals derived from ethnomedicinal plants can enhance the body's antioxidant defenses and offer cytoprotective effects (16,17).

The genus *Allium* is a large and diverse group of flowering plants within the Amaryllidaceae family, comprising approximately 700 species (18). Commonly known for their culinary and medicinal uses, *Allium* species include widely consumed vegetables such as garlic (*Allium sativum*), onions (*Allium cepa*), leeks (*Allium ampeloprasum*), and chives (*Allium schoenoprasum*). Characterized by their bulbous structures, these plants are notable for their aromatic qualities, primarily due to the presence of sulfur-containing compounds, including allicin, diallyl sulfide, and thiosulfates (19,20).

The health benefits of *Allium* species have been documented extensively, with research highlighting their antioxidant, anti-inflammatory, and antidiabetic properties (21). *Allium stipitatum*, commonly known as Persian shallot, is a species native to the mountainous regions of Central Asia, particularly in Turkey, Iraq and Iran. This species thrives in a variety of habitats, including rocky slopes, grasslands, and forest edges, adapting well to diverse soil types and climatic. *A. stipitatum* Regel, is a lesser-known member of *Allium* genus. It has been selected for this study due to its unique bioactive profile within the *Allium* genus. While many *Allium* species, such as garlic and onion, are renowned for their medicinal properties, *A. stipitatum* distinguishes itself with a distinct composition of sulfur-containing compounds and phenolic constituents. Research indicates that extracts from *A. stipitatum* exhibit significant antibacterial, antioxidant, and anti-inflammatory activities (22). Additionally, studies have highlighted the plant's potential in inhibiting α-glucosidase activity, suggesting possible antidiabetic effects (23). The alcoholic extract of *A. stipitatum* leaves exhibits various beneficial compounds such as allicin, polysulfides, flavonoids, and other distinctive properties, combined with its traditional use in regional medicine, underscore the rationale for selecting *A. stipitatum* over other *Allium* species to explore its therapeutic potential in diabetes management (24).

Given the promising profiles of *Allium* species in mitigating diabetes-related complications, this study aims to assess the efficacy of the alcoholic extract of *A. stipitatum* in alleviating STZ-induced cytotoxicity in pancreatic beta cells. With the increasing prevalence of T1DM and the lack of effective therapies to restore beta-cell function, exploring alternative treatments is crucial. By focusing on its potential to enhance cell viability and reduce oxidative stress, we seek to provide further insight into the therapeutic applications of this plant in diabetes type 1 management.

Material and Methods

Plant sample

Allium stipitatum samples were collected from the mountainous border region between Iran (Marivan city) and the Kurdistan region of Iraq in April 2022. The species was identified by the Faculty of Science Herbarium at Kurdistan University, Iran, and deposited there (Herbarium No. UOK 204).

Preparation of plant extracts

Allium stipitatum leaves were collected in the spring, dried, and ground into a fine powder. Methanol (10 mL) was added per gram of leaf powder, and the mixture was stirred for three days. The solution was then filtered using Whatman filter paper no. 42 (Millipore, USA). To remove the methanol, it was transferred to a rotary evaporator under vacuum (25). Then, the 1000 µg of dried extract was dissolved in DMSO (Brand: Sigma-Aldrich, CAS Number: 67-68-5), and from this stock, 10 µg/mL concentration of the extract were prepared.

Cell culture and treatment

Pancreatic beta cells (β-TC6) were purchased from the Pasteur Institute (Tehran, Iran) and cultured in T25 cell culture flasks (SPL, Cat No. 30025). The frozen cell vial was thawed in a 37 °C water bath, and 100 µL of fetal bovine serum (FBS, Sigma-Aldrich, Cat No. F2442) was added to stabilize the cells before transferring them into a T25 culture flask containing 5 mL of RPMI 1640 medium (Gibco, Cat No. 11875-093). The flask was placed in an incubator at 37 °C with 5% CO_2 and 95% humidity. After two passages, the cells were ready for treatment (26). The experimental groups included a control group without treatment, a group treated with STZ (100 mM) for 12 hours, a group treated with the plant extract (10 µg/mL) for 12 hours without STZ exposure, and three groups of extract and STZ. In the extract - STZ group (simultaneous treatment), the cells were exposed to STZ (100 mM) and the extract (10 µg/mL) at the same time for 12 hours. In the extract + STZ group (pre-treatment), the cells were first treated with the extract (10 µg/mL) for 12 hours, and subsequent treatment with STZ (100 mM) for another 12 hours. In the STZ + extract group (post-treatment), the cells were first treated with STZ (100 mM) for 12 hours and subsequent treatment with the extract (10 µg/mL) for another 12 hours. The concentration of the extract was chosen based on previous studies (8,17,19,25) and preliminary cytotoxicity testing, which showed that higher concentrations (>10 µg/mL) were cytotoxic to β-TC6 cells.

Medium replacement was performed for all treatment groups to ensure consistency in exposure conditions. In the STZ group, the cells were first incubated in the culture medium for 12 hours without any treatment. After this period, the medium was aspirated and replaced with fresh culture medium containing STZ (100 mM) for another 12 hours. Similarly, in the extract group, cells were first incubated for 12 hours without treatment, followed by medium replacement and subsequent exposure to the plant extract (10 µg/mL) for the next 12 hours. For the extract – STZ (simultaneous treatment) group, the cells were initially maintained in culture medium for 12 hours without treatment. After medium replacement, they were exposed to both STZ (100 mM) and the extract (10 µg/mL) simultaneously for the next 12 hours. Supernatant and cell samples for all study parameters were collected at specific time points following the final treatment, with a total experimental duration of 24 hours.

Measurement of NO using the griess method and a specific kit

To assess NO production, 1 mL of culture medium supernatant was collected from each treatment group at specific time points. NO levels were assessed using the Griess reagent method. Briefly, 50-100 µL of the culture supernatant was mixed with an equal volume of Griess reagent in a 96-well microplate (27). The reaction proceeded in two steps: first, sulfanilamide reacted with nitrite (NO_2^-) in the sample to form a diazonium ion; second, N-(1-naphthyl) ethylenediamine dihydrochloride (NED) reacted with the diazonium ion to produce a pink azo dye. After a 10-minute incubation at room temperature, the absorbance was measured at 540 nm using a microplate reader. The intensity of the color was directly proportional to the nitrite concentration, reflecting NO production. A standard curve was constructed using known concentrations of sodium nitrite as a reference (28).

Evaluation of total antioxidant capacity using the FRAP method

The ferric reducing antioxidant power (FRAP) method was used to evaluate total antioxidant capacity by measuring the ability of antioxidants in the samples (control, extract, STZ, extract + STZ, extract - STZ, and STZ + extract) to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). In this assay, the FRAP reagent, containing TPTZ (2, 4, 6-tripyridyl-s-triazine) and ferric chloride in an acidic medium, was added to the sample. Antioxidants present in the sample reduced the ferric-TPTZ complex to a ferrous form, producing a blue color. This color change was measured spectrophotometrically at 593 nm, with the intensity directly proportional to the sample's antioxidant power (29). A standard curve was constructed using various concentrations of an antioxidant standard (ascorbic acid), to quantify antioxidant capacity.

Cell viability assessment

The cells (3×10^5 cells per well) were cultured in 6-well plates and treated with STZ (Brand: Sigma-Aldrich,

Cat No. S0130) (100 µM) and extract (10 µg/mL). After incubation, the cells were trypsinized and detached from the bottom of the wells, then transferred to Falcon tubes. The tubes were centrifuged at 1000 g for 5 minutes. The supernatant was discarded, and the remaining pellet was resuspended in 1 mL of culture medium. Ten microliters of the cell suspension were mixed with 10 µL of trypan blue, placed on a Neubauer chamber, and the dead and live cells were counted under a microscope (30). The treatment groups included control, extract, STZ, STZ + extract, extract - STZ, and extract + STZ. The following formulas were used to estimate the percentage of viability and the percentage of toxicity:

% Viability = Number of viable cells/Total number of cell counted × 100
%Cytotoxicity = 100 - % Viability

Measurement of insulin secretion by ELISA using a specific kit

For insulin quantification, an insulin ELISA kit (Mercodia AB, Uppsala, Sweden, Cat No. 10-1247-01) was used following the manufacturer's instructions. Standard solutions (0–1000 µU/mL) and sample supernatants were brought to room temperature, and 50 µL of each was added to the ELISA plate. Then, 50 µL of streptavidin-HRP conjugate was added, and the plate was incubated for 1 hour at room temperature. After four washes, 50 µL of TMB substrate was added, followed by a 30-minute incubation in the dark. The reaction was stopped with 50 µL of stop solution, and the absorbance was measured at 450 nm. Insulin concentration was determined using a standard curve, and appropriate positive and negative controls were included to ensure accuracy (31).

Statistical analysis

Data were analyzed using SPSS software (version 16; SPSS Inc., Chicago, IL, USA). The results were expressed as mean ± standard deviation (SD) from at least three independent experiments. Statistical differences among the groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to compare multiple groups. A P value of < 0.05 was considered statistically significant.

Results

Evaluation of NO levels affected by extract and STZ

STZ-induced diabetes significantly increased NO levels ($P<0.05$), inducing oxidative stress (Figure 1). Standard curve analysis (Figure 2A) was used to quantify NO levels, measured using the Griess method with sodium nitrite as the standard. While NO levels remained high in most extract-treated groups, a significant reduction was observed in the extract + STZ group, suggesting a potential role of the extract in mitigating STZ-induced oxidative stress.

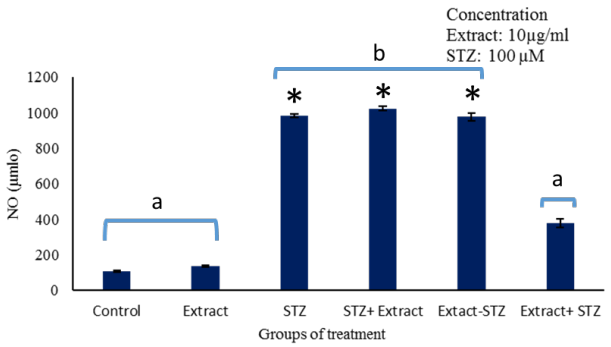

Figure 1. Nitric oxide (NO) levels in β-cells under different treatment conditions. The cells were treated with the plant extract (10 µg/mL), streptozotocin (STZ, 100 mM), or their combination for 12 hours. In the STZ + extract, extract – STZ, and extract + STZ groups the cells were post-treated, co-treated, and pre-treated with extract, respectively. Data are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way ANOVA followed by Tukey's test. Different letters (a, b, c, etc.) above the bars indicate significant difference ($P < 0.05$). *Significant difference compared to the control group.

Total antioxidant capacity assessment

The FRAP level of the control group was 400.5 ± 13.07 µmol, while the extract group showed a non-significant decrease at 372.9 ± 8.975 µmol ($P>0.05$). The STZ-treated group exhibited a significant increase in FRAP value measured using a standard curve with ascorbic acid as the standard (Figure 2B) from 400.5 ± 13.07 to 890.3±11.895 compared to the control group ($P<0.05$), indicating the induction of oxidative stress. The group treated with both the extract and STZ showed a similar FRAP level (889.6 ± 10.662) ($P<0.05$). The combination of extract and STZ also did not lead to a further significant increase in the FRAP level. The extract-STZ group showed a FRAP value of 976.3 ± 9.804, while the extract + STZ group was at 732 ± 8.211 ($P>0.05$) (Figure 3). STZ treatment significantly increased the FRAP value ($P<0.05$).

Evaluation of cell viability affected by STZ and extract

Beta-cells were treated with *A. stipitatum* extract and STZ to assess their effects on cell viability. As shown in Figure 4, exposure to the extract (10 µg/mL) alone resulted in the highest cell viability (43417 ± 1141 cells), surpassing even the control group, with a statistically significant difference ($P<0.05$). Conversely, STZ (100 µM) caused a substantial reduction in cell viability, indicating significant cell death (11500 ± 577 cells). In the groups receiving both the extract and STZ, the extract demonstrated a protective effect by enhancing cell proliferation. Specifically, in the pretreatment group with extract, the cell count was significantly higher than in other groups exposed to STZ (26250 ± 1626 cells). However, there was no statistically significant difference in cell viability between the groups treated with the extract and STZ simultaneously (extract-STZ) and those treated with the STZ prior to extract (STZ + extract) compared to the STZ-only group.

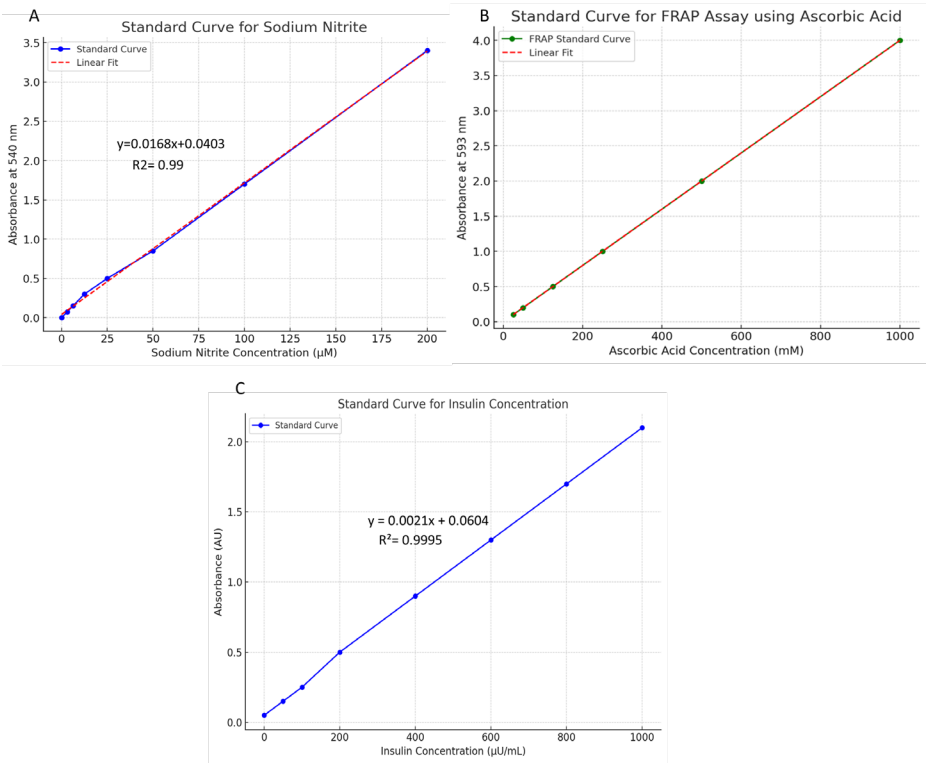

Figure 2. Standard curves for nitric oxide (NO) level (A), antioxidant activity (B), and insulin secretion (C). NO level was measured by Griess method using sodium nitrite as the standard. FRAP assay was used with ascorbic acid as the standard.

Assessment of insulin secretion levels

STZ treatment significantly reduced insulin secretion ($P < 0.05$). However, extract treatment in all groups co-exposed to STZ resulted in a significant increase in insulin levels ($P < 0.05$). The most pronounced effect was observed in the post treatment group (STZ+ extract) (41.4 ± 0.62 µU/mL), where insulin secretion remained stable despite STZ exposure. In the extract-STZ group, insulin secretion was elevated (31.5 ± 0.62 µU/mL), though less than in the pre-treatment group. The lowest insulin levels among treated groups were observed in the extract + STZ group (25.2 ± 1.3 µU/mL). These results indicate that while STZ impairs insulin production, *Allium stipitatum* extract enhances insulin secretion in viable cells (Figure 5). The standard

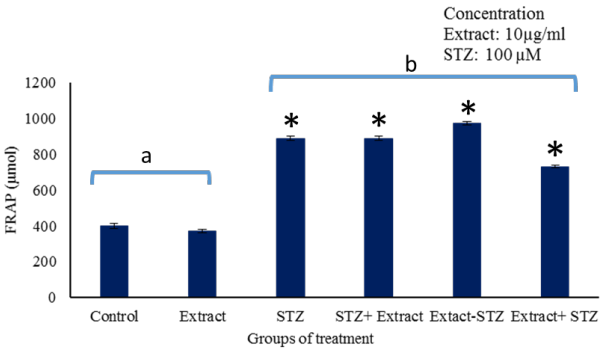

Figure 3. Total antioxidant capacity (TAC) in β-cells under different treatment conditions. The cells were treated with the plant extract (10 µg/mL), streptozotocin (STZ, 100 mM), or their combination for 12 hours. In the STZ + extract, extract – STZ, and extract + STZ groups, the cells were pre-treated, co-treated, and post-treated with extract, respectively. Data are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way ANOVA followed by Tukey's test. Different letters (a, b, c, etc.) above the bars indicate significant difference ($P < 0.05$). *Significant difference compared to the control group.

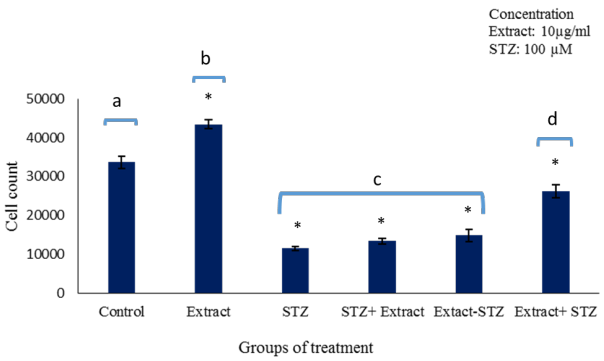

Figure 4. Cell viability in response to exposure to streptozotocin (STZ) and *A. stipitatum* extract under various conditions. The cells were treated with the plant extract (10 µg/mL), STZ (100 mM), or their combination for 12 hours. In the STZ + extract, extract – STZ, and extract + STZ groups the cells were pre-treated, co-treated, and post-treated with extract, respectively. Data are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way ANOVA followed by Tukey's test. Different letters (a, b, c, etc.) above the bars indicate significant difference ($P < 0.05$). *Significant difference compared to the control group.

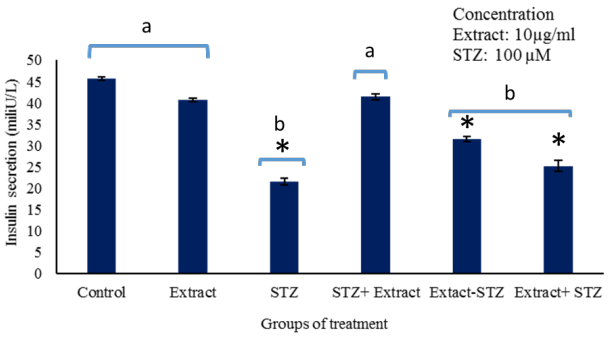

Figure 5. Insulin secretion levels in β-cells across different treatment groups. The cells were treated with the plant extract (10 µg/mL), streptozotocin (STZ, 100 mM), or their combination for 12 hours. In the STZ + extract, extract – STZ, and extract + STZ groups the cells were pre-treated, co-treated, and post-treated with extract, respectively. Data are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way ANOVA followed by Tukey's test. Different letters (a, b, c, etc.) above the bars indicate significant difference ($P < 0.05$). *Significant difference compared to the control group.

curve for NO evaluation (Figure 2C) was used to quantify NO levels in the culture medium, with concentrations correlated to absorbance measurements. The results were plotted based on the standard curve, allowing for accurate determination of NO levels in each treatment group.

Dissuasion

The study investigated the effects of *A. stipitatum* extract in mitigating cytotoxicity induced by STZ in β-cells. This model provides valuable insight into the potential of ethnomedicinal plants in supporting pancreatic β-cell function by improving insulin secretion, cell viability, and oxidative stress response. STZ, a potent diabetogenic agent, significantly reduced insulin secretion and induced cell death in β-cells. These results align with previous research that demonstrated STZ's toxic effect on β-cells by inducing oxidative stress and impairing insulin secretion (9). On the other hand, the *A. stipitatum* extract, when administered in conjunction with STZ, enhanced cell viability, although the increase was not statistically significant compared to the STZ group alone. This suggests that while the extract may aid in cell survival, it does not completely reverse the cytotoxic effects of STZ, which is consistent with other studies where plant extracts have shown partial protective effects on pancreatic cells (21,32,33).

Allium stipitatum, contains several bioactive compounds that have been identified in previous studies. Notable among these are: Pyridine-N-oxide alkaloids, compounds such as 2- (methyldithio) pyridine-N-oxide, 2- [(methylthiomethyl) dithio] pyridine-N-oxide, and 2,2′-dithio-bis-pyridine-N-oxide have been isolated from *A. stipitatum*. These compounds possess disulfide functional groups and have demonstrated antibacterial activity against various strains, including methicillin-resistant *Staphylococcus aureus* (34). 2- (Methyldithio) pyridine-3-carbonitrile, has exhibited broad-spectrum antimicrobial activity, showing effectiveness against both bacterial and fungal pathogens (35). Pyrithione, identified in the bulbs of *A. stipitatum*, is known for its moderate cytotoxicity toward certain human tumor cell lines and its activity against fungi and Gram-negative bacteria (36). Additionally, *Allium* species, including *A. stipitatum*, are rich in organosulfur compounds and steroidal saponins. These compounds are believed to contribute to various biological activities, such as antioxidant, anti-inflammatory, and antimicrobial effects (37). Several studies also have investigated the intracellular ROS scavenging activity of various *Allium* species. For instance, *Allium roseum* L., a medicinal plant rich in flavonoids and organosulfur compounds, has demonstrated significant antioxidant properties. Research indicates that its ethanolic extract can inhibit amyloid beta protein toxicity by reducing oxidative stress and intracellular calcium levels in neuroblastoma cells, suggesting a neuroprotective effect against amyloid-induced cytotoxicity (38). Similarly, *Allium sativum* L. (garlic) contains bioactive compounds that act as potent antioxidants by scavenging ROS, promoting detoxification, and aiding in DNA repair. These properties contribute to its potential role in reducing oxidative stress and suppressing tumor-promoting agents (21).

The enhancement of insulin secretion observed in the groups treated with the extract in combination with STZ is noteworthy. This suggests that the extract supports insulin production in the remaining viable cells, indicating a potential cytoprotective effect. These findings align with previous studies on other *Allium* species, which have demonstrated insulin-enhancing and anti-inflammatory properties (19,39,40). Although the precise mechanism through which *A. stipitatum* modulates insulin secretion remains unclear, we propose that its protective effects against STZ-induced damage are mediated through multiple pathways. The organosulfur compounds and flavonoids in *A. stipitatum* are potent ROS scavengers, reducing oxidative stress and enhancing β-cell survival. Additionally, the extract may modulate inflammatory signaling pathways by inhibiting the overproduction of pro-inflammatory cytokines that contribute to β-cell dysfunction. The observed reduction in NO levels in the extract + STZ group further suggests that the extract regulates nitric oxide synthase (NOS) activity, preventing excessive NO production and associated oxidative damage. Furthermore, the insulinotropic effects of the extract may be linked to the activation of signaling pathways such as PI3K/Akt or the modulation of ATP-sensitive potassium channels in β-cells, which play a crucial role in insulin secretion. Further investigation is warranted to elucidate these mechanisms in greater detail. NO is a key signaling molecule in diabetes and its elevated levels are associated with oxidative stress and beta-cell dysfunction (4). In this

study, an increase in NO production was observed in STZ-treated cells, a common finding indicating the onset of oxidative stress and inflammation. Interestingly, while there were no significant differences in NO levels across the plant extract-treated groups, a significant reduction was noted in the group where the STZ was administered after extract treatment. This could suggest that the extract may help reduce NO-mediated oxidative damage, although the mechanism remains unclear. It is possible that the extract's phytochemicals, particularly its sulfur-containing compounds, act as scavengers of reactive nitrogen species, contributing to the observed reduction in NO levels (41).

The assessment of total antioxidant capacity using the FRAP method revealed a significant increase in the FRAP value in all groups treated with STZ, indicating an elevated antioxidant capacity in response to oxidative stress. This suggests that STZ treatment induced oxidative damage, leading to an increase in ROS in pancreatic cells, which subsequently activated antioxidant mechanisms. However, since no further experimental or statistical analysis was performed to directly measure ROS levels, the exact underlying processes cannot be conclusively determined from this result alone. These findings suggest that STZ-induced oxidative stress triggered an upregulation of antioxidant mechanisms in β-cells, a well-documented response to ROS generated by STZ toxicity (1, 42). In contrast, the group treated with the extract did not show any significant change in the FRAP level compared to the control, indicating that the extract may not have significantly influenced the overall antioxidant capacity in this experimental setup. This contrasts with some studies reporting enhanced antioxidant activity in response to plant extracts, suggesting that the effects of *A. stipitatum* may depend on factors such as dosage, method of administration, or cellular context (43).

The *Allium* genus is widely recognized for its antioxidant potential due to its diverse bioactive compounds, including organosulfur compounds, flavonoids, and phenolics, which contribute to its ability to neutralize free radicals and reduce oxidative stress. Numerous studies have assessed the antioxidant activity of *Allium* species using chemical assays such as DPPH, ABTS, and FRAP, demonstrating their strong potential in oxidative stress mitigation (44). Among these, *Allium stipitatum*, the target species of the present manuscript, has been studied alongside other *Allium* species. According to the recent findings of Hosseini et al, while *A. hooshidaryae* exhibited the highest antioxidant capacity with a DPPH IC50 value of 724.4 ± 0.31 µg/mL and a FRAP value of 36.87 mg ascorbic acid equivalent per gram of extract, *A. stipitatum* showed comparatively lower antioxidant activity. However, *A. stipitatum* demonstrated notable antibacterial effects, particularly against *Staphylococcus aureus*, with a MIC of 12.5 µg/mL (23). These findings suggest that while *Allium* species are generally rich in antioxidants, the extent of their activity varies, and *A. stipitatum* may exert its bioactive effects through mechanisms beyond direct antioxidant potential.

The timing of extract administration significantly influenced its effects on various parameters. Administering STZ before the extract (STZ + extract) resulted in greater enhancement of insulin secretion, suggesting that the extract may play a reparative role in restoring β-cell function after STZ-induced damage. In contrast, administering the extract before STZ (extract + STZ) was more effective in preserving cell viability and reducing NO levels, indicating a protective effect that helps mitigate STZ-induced toxicity. These findings suggest that early administration of the extract may offer protective benefits, while post-STZ treatment may aid in cellular recovery. This aligns with diabetes treatment strategies that focus on both β-cell protection and regeneration.

The findings of this study are consistent with previous research on the effects of STZ on β-cells and the protective potential of plant-derived antioxidants. The impact of STZ on insulin secretion and cell viability, particularly through oxidative stress, is well-documented in the literature (5,45). Additionally, the role of *Allium* species, including *A. sativum*, in mitigating oxidative stress and diabetes-related complications has been highlighted in various studies (19,21).

This study had certain limitations that should be acknowledged. First, different doses of the extract were not evaluated, which could provide more insight into dose-dependent effects on β-cell function and oxidative stress parameters. Future studies should investigate multiple concentrations to determine the optimal dose for protective effects. Second, a positive control was not included in the experimental design, which could strengthen the interpretation of the extract's efficacy. Third, the study only examined a single time point for analysis, and the effects of shorter and longer treatment durations were not evaluated. Future research should explore the time-dependent impact of *A. stipitatum* extract on β-cell function and oxidative stress to determine the optimal treatment duration. Lastly, while the in vitro model used in this study provides valuable insights, in vivo studies are necessary to confirm the therapeutic potential of *A. stipitatum* in diabetic conditions.

Conclusion

This study investigated the effects of *A. stipitatum* extract in mitigating STZ-induced cytotoxicity in β-cells, focusing on insulin secretion, cell viability, and oxidative stress response. While the extract demonstrated some protective effects, particularly in enhancing insulin secretion and reducing NO levels in the STZ + extract and extract + STZ groups respectively, its impact on overall antioxidant capacity was not significant. These findings suggest that

A. stipitatum may exert its effects through mechanisms beyond direct antioxidant activity, potentially involving inflammatory modulation or insulinotropic pathways. Given these results, further research is necessary to fully elucidate the therapeutic potential of *A. stipitatum* as an adjunct treatment for diabetes. Future studies should focus on isolating and characterizing its active compounds to determine their specific roles in β-cell protection. Additionally, in vivo investigations are essential to validate the extract's efficacy and explore its pharmacokinetics, bioavailability, and long-term effects in diabetic models. Clarifying these mechanisms will provide a stronger foundation for considering *A. stipitatum* in diabetes management strategies.

Conflict of interests

The author declares that there is no conflict of interest.

Data availability statement

All data are presented in this manuscript; however, further details can be requested from the corresponding author.

Ethical considerations

This study did not include the use of animal or human models. The author meticulously followed ethical standards, ensuring the absence of plagiarism, misconduct, data fabrication, falsification, duplicate publication, submission, or redundancy.

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