frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 16 April 2025
DOI 10.3389/fphar.2025.1485952

OPEN ACCESS

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RECEIVED 25 August 2024
ACCEPTED 13 March 2025
PUBLISHED 16 April 2025

CITATION
Hoornenborg CW, Qomariyah N, González-Ponce HA, Van Beek AP, Moshage H and Van Dijk G (2025) *Dracaena trifasciata (Prain) Mabb* leaf extract protects MIN6 pancreas-derived beta cells against the diabetic toxin streptozotocin: role of the NF-κB pathway.
Front. Pharmacol. 16:1485952.
doi: 10.3389/fphar.2025.1485952

Dracaena trifasciata (Prain) Mabb leaf extract protects MIN6 pancreas-derived beta cells against the diabetic toxin streptozotocin: role of the NF-κB pathway

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Background: *Dracaena trifasciata (Prain) Mabb*. [*Asparagaceae*; also known as *Sansevieria trifasciata Prain* (*ST*)] may have health-promoting activities, including resolution of diabetes mellitus (DM). This *in vitro* study evaluated whether and how a leaf extract of *ST* could directly protect pancreas-derived MIN6 cells against the diabetogenic toxin streptozotocin (STZ).

Methods: Composition of the *ST* extract (by 100% methanol) was investigated using high resolution mass spectrometry, which revealed several compounds with beneficial bioactive efficacy. MIN6 cells were exposed to 50% lethal dose of STZ, with or without *ST* extract. Cell viability was assessed using the MTT method. Inflammatory activity of *ST* extract was assessed in MIN6 cells and macrophage-like RAW cells, and addition of TNF-α to combinations of *ST* and STZ were tested on MIN6 cell viability. The role of the NF-κB pathway in effects of STZ and *ST* were investigated using the proteosome inhibitor MG132.

Results: Exposure of MIN6 cells to the *ST* extract (in concentrations that did not notably affect MIN6 cells: 5-15 mg/mL) was indeed able to minimize STZ-induced toxicity in MIN6 cells. Exposing macrophage-like RAW cells to *ST* extract (at 10-15 mg/mL) increased TNF-α gene expression, and this response was highly augmented by co-exposure to lipopolysaccharide (LPS, 1.0 mg/mL), indicating that *ST* extract contained inflammatory compounds too. The implication of this finding was investigated by exposing MIN6 cells to a subthreshold dose (100 ng/mL) of TNF-α, which 1) prevented the protective effect of the *ST* extract (10-15 mg/mL) against STZ toxicity, and 2) caused *ST* to become toxic to MIN6 cells even without the presence of STZ. TNF-α is known to activate the NF-κB pathway leading to cell death, however, NF-κB is also known to stimulate cell proliferation and survival. To investigate the relevance of the NF-κB pathway in our findings, we treated MIN6 cells with the proteasome inhibitor MG132 (at doses ≥0.2 μM), and

observed that *ST* extract was no longer able to block STZ toxicity in MIN6 cells ($p < 0.05$), and to block CIAP2 expression, an anti-apoptotic target downstream from NF-κB.

Conclusion: These data suggest that a leaf extract of *ST* has anti-diabetogenic efficacy, which may depend on the integrity of the NF-κB pathway. This protective effect appears to be impeded in a pro-inflammatory environment.

KEYWORDS

***Sansevieria trifasciata*, diabetes, streptozotocin, cell viability, TNF-α, NF-κB**

1 Introduction

According to the IDF 537 million adults are living with diabetes. This number is predicted to rise to 634 million by 2030 and 783 million by 2045 (Cho et al., 2018). Loss of beta cell mass is a critical process in the etiology of type 1 (T1DM) and late-stage type 2 (T2DM) Diabetes Mellitus. While loss of beta cell mass in T1DM represents the key culprit of the disease (by autoimmune or inflammatory processes), progression of insulin resistance and hyperlipidemia leads to metabolic and inflammatory stress in pancreatic beta cells, ultimately leading to their death (by apoptosis) in T2DM (Rachdaoui, 2020; Xu et al., 2015). Diabetes can be experimentally induced with a beta cell toxin like alloxan or streptozotocin (Lenzen, 2008; Furman, 2015), and is often studied in rats or mice with representation of many characteristics also seen in humans with diabetes. Humans exposed to persistent metabolic and inflammatory stressors can experience a clinically relevant loss of beta cell mass, but some individuals appear to be less sensitive than others (Cerf, 2013; Chacón et al., 2007). It is possible that these individuals have a defense repertoire within their pancreatic beta cells capable of counteracting the damaging effects of metabolic and inflammatory stress (Bonora, 2008).

Diabetes is often accompanied by tissue infiltration of monocytes and macro-phages (Goldfine et al., 2011; Eguchi and Nagai, 2017) and recent advances have stimulated research targeting inflammatory pathways as part of the strategy to prevent or control the disease (Goldfine et al., 2011), but mixed results are found (Kolb and Eizirik, 2012; Sheehy et al., 2019). During metabolic and/or inflammatory stress, cells that express anti-apoptotic genes are able to counteract the pathways leading to cell death (Cerf, 2013; Kolb and Eizirik, 2012; Elmore, 2007). It is possible that failure of the defense response of pancreatic beta cells is due to the fact that the inflammatory pathways/cytokines "switch" from an anti-apoptotic phenotype towards a more pro-apoptotic one. TNF-α and the NF-κB pathway have been stated to regulate this "switch" between anti-apoptotic and pro-apoptotic pathways (Karin and Lin, 2002). Indeed, changes within the NF-κB pathway resulting in a shift towards apoptosis have been observed in rat hepatocytes and pancreatic beta cells during inflammatory stress (Hun et al., 2005; Schoemaker et al., 2002). Targeting this pathway may protect beta cells against metabolic and inflammatory stress and may curb the pathophysiology of diabetes.

Research on traditional medicine increased in recent years (Erejuwa et al., 2012; Shakib et al., 2019; Patel et al., 2012; Qomariyah et al., 2012; González-Ponce et al., 2018). Many bioactive ingredients in plants have diverse therapeutic niches, either as FDA-approved drug or as a supplement, and some have entered the market successfully (Li and Weng, 2017), with the prototypical example being metformin. Because of the great potential of plant compounds for clinical purposes (Erejuwa et al., 2012; Shakib et al., 2019; Patel et al., 2012; Qomariyah et al., 2012; González-Ponce et al., 2018; Li and Weng, 2017; World Health Organization WHO, 2013), the World Health Organization (WHO) has developed a strategy to implement traditional medicine as a way of coping with the relentless rise of non-communicable diseases (World Health Organization WHO, 2013).

The *Dracaena trifasciata (Prain) Mabb.* [*Asparagaceae;* better known under its synonym *Sansevieria trifasciata Prain* (*ST*), the name used in this paper], is used as a "traditional medicine" and has shown potential benefits as treatment against T2DM (Qomariyah et al., 2012; El-Din El-Hawary et al., 2021; Raslan et al., 2021). Oral administration of extracts of *ST* leaves to decrease circulating blood glucose levels and increase insulin-containing granule density in pancreatic beta cells in a diabetic rat model (Qomariyah et al., 2012). Under the assumption that some of the bioactive molecules within *ST* may actually be absorbed and affect key organs (such as the pancreas) involved in cardiometabolic health, the aim of the current *in vitro* study was to identify the phytochemical profile of *ST* leaf extract and evaluate whether *ST* leaf extract could directly protect cells from a pancreatic beta cell line (MIN6) against the pancreatic beta cell toxin streptozotocin (STZ), and – if so – to identify potential mechanisms underlying such a protective effect.

2 Materials and methods

2.1 Plant material preparation

Freshly cultivated leaves of *Sansevieria trifasciata Prain (ST)* were collected from the Tropical Biopharmaca Research Center (IPB), Bogor Agricultural Institute, Bogor, Indonesia (March 2014; see Supplementary Material S1 for the letter of the head of the Tropical Biopharmaca Research Center). A botanist (Dr. J.S. Rahajoe) identified *ST* with the specimen code BMK0130092016 (Biopharmaka taxonomy database; Tropical Biopharmaca Research Center (IPB), Bogor Agricultural Institute, Bogor, Indonesia; see Supplementary Material S2). The leaves were dried at 38°C ± 2°C for 7 days, after which the leaves were powdered and kept at room temperature in dry and dark conditions followed by cold maceration extraction. In brief, the pulverized leaf material was dissolved in 100% methanol (5 mL/g material) and the leaf extract was shaken at room temperature for 5 days (Mimaki et al., 1996). The leaf extract

was filtered and evaporated using the rotary evaporator system at 60°C under vacuum for 3 h (Rotavapor, RTV-01, Buchi, Switzerland) to obtain a semi-solid extract (Qomariyah et al., 2012; Lontoc et al.). The product was kept in a closed flask and stored at −20°C until the start of the experiment. Before experiments, the semi-solid *ST* extract was dissolved in PBS, filtered (4.5 µm pore size), and shielded from light.

2.2 Chemical fingerprinting of the *Sansevieria trifasciata* extract

The full fingerprint following the standards established in the ConPhyMP statement (see Supplementary Materials S3, S4) of the *ST* extract was determined with liquid chromatography – high resolution mass spectrometry (LC-HRMS; Laboratorium Sentral Ilmu Hayati Universitas Brawijaya, Malang, Indonesia and Interfaculty Mass Spectrometry Center Department of Analytical Biochemistry University of Groningen, Groningen, the Netherlands). The LC-HRMS column (H5PMS, J&W Scientific) was a hypersil GOLD AQ Colum 50 × 1 mm and 1.9 µm particle size. The column and autosampler temperature were set at 50°C, respectively. The flow was set at 40 µL/min. Mobile phase A was water with 0.1% formic acid, and mobile phase B acetonitrile with 0.1% formic acid. A linear gradient was used: 0–2 min 5% B, 2–12 min linear increase to 40% B, 12–14 min linear increase to 60% B, 14–15.5 min linear increase to 100% B, 15.5–17 min held at 100% B, 17–17.1 min decrease to 5% B, and 17.1–20 min held at 5% B.

MS and MS/MS analyses were performed with electrospray ionization in positive mode at a spray voltage of 3.5 kV, and sheath and auxiliary gas flow set at 47.5 and 11.25, respectively. The ion transfer tube temperature was 256°C. Spectra were acquired in data-dependent mode with a survey scan at *m/z* 100–1,500 at a resolution of 70,000 followed by MS/MS fragmentation of the top 5 precursor ions at a resolution of 17,500. A normalized collision energy (NCE) of 30 was used for fragmentation and fragmented precursor ions were dynamically excluded for 10s. The data was analyzed by Compound Discoverer with MzCloud MS/MS Library (Adriani et al., 2022).

2.3 Cell lines

MIN6 cells (a mouse beta cell line originally established from an insulinoma developed in an IT6 transgenic C57BL/6 mouse line, which expresses the SV40 T antigen under control of the human insulin promoter (Miyazaki et al., 1990); passage 35–44) were cultured in Dulbecco's modified Eagle's (DMEM) high glucose medium, supplemented with 2 mM L-glutamine, 10% FCS, 0.2 µM β-mercapto-ethanol, 1% P/S, and humidified 5% CO_2 at 37°C. RAW cells (ATCC® TIB-71, a mouse macrophage cell line originally established from a tumor in male mice induced with the Abselon murine leukemia virus (Raschke et al., 1978); passage 28–32) were cultured in DMEM medium, supplemented with 2 mM L-glutamine, 10% FCS and 0.1% Gentamicin, and humidified 5% CO_2 at 37°C. The media of both cell types were changed 3 times a week (Ahn et al., 2020; Zhou et al., 2014).

2.4 Chemicals

Streptozotocin (STZ; Sigma-Aldrich, S0130-5G) was used due to its specific toxicity towards insulin-producing pancreatic beta cells (Eleazu et al., 2013). It has similarities with glucose and is transported into the cell by the glucose transport two protein (GLUT 2) (Eleazu et al., 2013; Saini et al., 1996). Before individual experiments, STZ was dissolved in 0.1 M citrate buffer (pH = 4.5), filtered (4.5 µm pore size), kept on ice, and shielded from light. The protease inhibitor MG132 (Sigma-Aldrich, CAS Number 133407-82-6) was used as blocker of the NF-κB pathway (Zanotto-Filho et al., 2012; Zanotto-Filho et al., 2010). TNF-α (Peprotech; catalog 315-01A) and lipopolysaccharide (LPS; Sigma-Aldrich CAS Number L2880) were used to induce an inflammatory response (Chen et al., 2018; Chang et al., 2003).

2.5 Measurement of cell viability

Cell viability of MIN6 cells was assessed using the MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay (Kumar et al., 2018). Before the experiments, cells were washed twice with phosphate buffered saline (PBS), and detached from the flasks with 0.5 mL Trypsin (incubation time: 5 min at 37°C and 5% CO_2). The cells were collected in medium and centrifuged for 5 min at 14,000 RPM. Cell count was performed with 50 µL of 0.4% Trypan Blue, 3.0 × 105 cells/mL (60.000 cells/well) were plated in a 96-wells plate and incubated overnight (37°C and 5% CO_2).

To validate if the methanol extract of *ST* could be safely used, different doses (2.5–20 mg/mL; 1.5%–10% v/v) of the *ST* extract were dissolved in different standard cell culture solvents (PBS, EtOH 0.5% and Saline) and applied to MIN6 cells. Viability of MIN6 cells was studied with several concentrations of streptozotocin (STZ: 0–20 mM) creating a dose-response curve (Eleazu et al., 2013; Gao et al., 2000), allowing the determination of the lethal dose of 50% (LD50%).

To investigate the protective effect of *ST* extract, cells were exposed to a LD 50% dose of STZ (10–15 mM) for 1 hour. Based on pilot experiments, doses of *ST* extract (2.5–20 mg/mL in PBS, 1.5%–10% v/v) were added 30 min before (prophylactic treatment) or directly after the addition of STZ (therapeutic treatment). To investigate whether the activity of *ST* extract was influenced by a pro-inflammatory environment, MIN6 cells were incubated with TNF-α (100 ng/mL (Chang et al., 2003)) for 6 hours (Start: 5 hours before the exposure to STZ). To investigate if the activity of *ST* extract was dependable on the NF-kB pathway, the proteasome inhibitor MG132 was administered for 3 hours (Start: 2 hours before the exposure to STZ) (Zanotto-Filho et al., 2010).

After the experiments, medium was refreshed and cells were incubated for 24 hours (37°C and 5% CO_2). A volume of 22 µL MTT (0.5 mg/mL) was added to each well and shielded from light and the MIN6 cells were incubated for a final 2.5 h. The formazan crystals formed by the reduction of MTT under the action of mitochondrial dehydrogenase were dissolved with dimethyl sulfoxide (DMSO; 150 µL) and measured by a Micro-plate Reader (Bio-Tec

TABLE 1 Retention time (RT), identification and analysis of the compounds of *Sansevieria trifasciata* (ST). Only compounds with quality ≥90% are taken into account (full list available in the Supplementary Material S2).

RT (min)	Name compound	Formula	Percentage (%)	Quality
1.329	Adenosine	C_{10} H_{13} N_5 O_4	2.0	99.9
1.446	L-Norleucine	C6 H13 N O_2	3.9	99.7
0.894	D-(+)-Proline	C5 H9 N O2	5.9	99.7
1.714	L-Phenylalanine	C9 H11 N O2	2.6	99.6
20.369	α-Linolenic acid	C18 H30 O2	2.1	98.8
0.894	Isoleucine	C6 H13 N O2	1.1	98.4
0.942	Choline	C5 H13 N O	50.9	98.0
18.579	Diisobutylphthalate	C16 H22 O4	1.7	96.3
18.706	Dibutylphthalate	C16 H22 O4	0.5	95.6
1.061	Adenine	C5 H5 N5	0.7	92.6
1.289	L-Pyroglutamic acid	C5 H7 N O3	0.6	94.6
4.159	trans-3-Indoleacrylic acid	C11 H9 N O2	0.7	94.6
11.409	9S,13R-12-Oxophytodienoic acid	C18 H28 O3	0.6	94.4
13.089	2,2,6,6-Tetramethyl-1-piperidinol	C9 H19 N O	0.6	93.4

Instruments Inc.) between wavelengths 570–630 nm. The value was considered to reflect the activity of cell metabolism and is expressed as cell viability (% compared to the untreated cells: indicated as negative control).

2.6 Measurement of gene expression by real-time qPCR

To investigate whether *ST* extract had inflammatory properties, gene expression was determined in MIN6 and RAW cells. 3.0 × 105 cells/mL (6.0 × 105 cells/well) were plated in a 6-wells plate and incubated overnight. The MIN6 cells were exposed to MG132, STZ and *ST* extract as described before, and the RAW cells were exposed to different doses of *ST* extract (2.5–20 mg/mL in PBS, 1.5%–10% v/v) 30 min before (prophylactic treatment) or directly after (therapeutic treatment) the addition of a toxic dose of LPS (1.0 mg/mL).

RNA was isolated using Trizol Reagent (Sigma-Aldrich) and the RNA quality and quantity were determined using Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE, United States). Reverse transcription PCR (RT-PCR) was performed using the Moloney murine leukemia virus (M–MLV) reverse transcriptase system and random nanomers from Life Technologies (Breda, Netherlands). RT-PCR was performed in three steps: 10 min at 25°C, 1 h at 37°C and 5 min at 95°C with the GenAmp PCR system (Applied Biosystems, Niewekerk a/d IJssel, the Netherlands). Quantitative real-time PCR (qPCR) was performed using 4 µL 20-fold diluted cDNA in combination with 2x master mix (Eurogentec, Maastricht, Netherlands) in a total volume of 20 µL. Gene expression was expressed as fold induction and normalized for 18S as house-keeping gene (see Supplementary Material S7) (Wang et al., 2024).

2.7 Data analysis

All assays were repeated three times in duplicate or triplicate. Data is expressed as mean ± standard error of the mean (SEM). Differences between groups were tested for significance using one-way ANOVA with *post hoc* Tukey analysis. P-values <0.05 were considered statistically significant and were performed with IBM SPSS software 23.

3 Results

3.1 Chemical fingerprint of the *Sansevieria trifasciata* extract

LC-HRMS analysis showed the presence of 142 compounds in the *ST* extract. The full fingerprint of compound identification based on mass spectrometry is shown in Supplementary Material S5 (Heinrich et al., 2022). Only compounds with a quality ≥90% are stated in Table 1, the specific spectra and compound identification of Table 1 is shown in Supplementary Material S6.

3.2 Protective effect of *Sansevieria trifasciata* extracts against streptozotocin in MIN6 cells

MIN6 cells were incubated with methanol leaf extract of *ST* that was redissolved in different vehicles commonly used in cell culture research (PBS, Saline, EtOH 0.5%). The MTT-assay revealed no effect of vehicle (PBS, Saline or EtOH 0.5%) on cell viability compared to the negative control (NC: no *ST* was added to this group; Figure 1A). For this reason, PBS was used as vehicle for the *ST* extract for the remainder of the experiments. MIN6 cells incubated

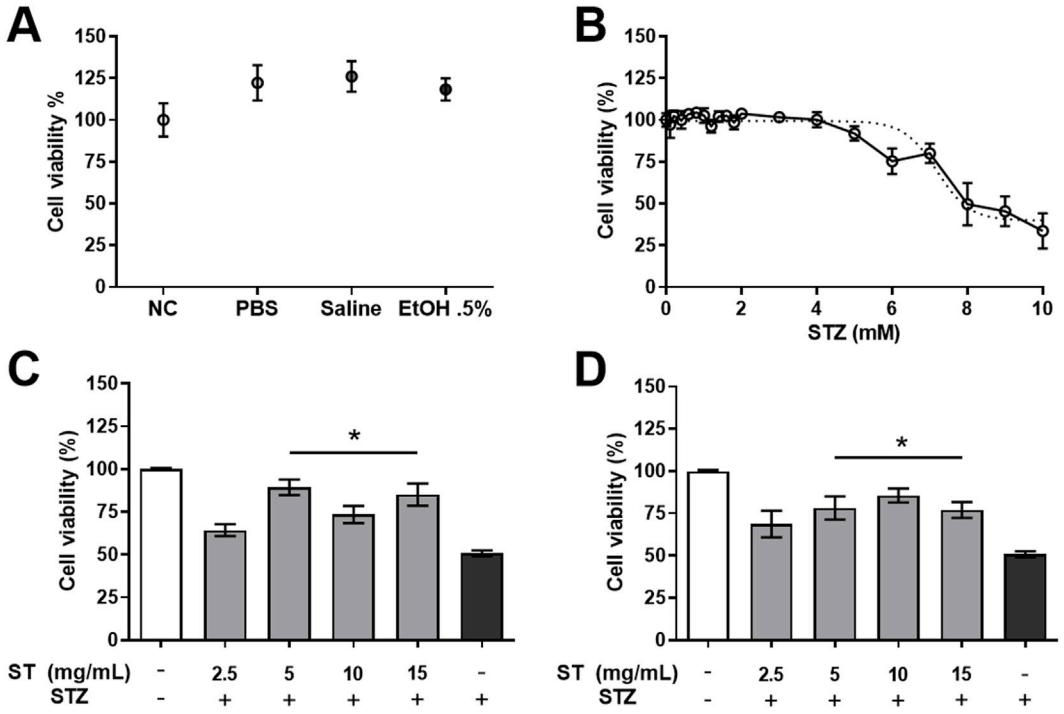

FIGURE 1
Cell viability (%) of MIN6 cells after administration of *Sansevieria trifasciata* leaf extract (ST) in different vehicles [**(A)**; NC = negative control (no *ST* was added to this group), PBS = phosphate buffered saline, EtOH 0.5% = ethanol 0.5%], **(B)** after exposure to increasing concentrations of streptozotocin (STZ). Cell viability (%) after prophylactic **(C)** and therapeutic **(D)** treatment of *ST* against STZ-induced reduction in cell viability (LD50%; 10 mM). Data is shown as mean ± SEM and compared to the STZ control group (indicated as; −/+), * $p < 0.05$ **(C, D)**.

with streptozotocin (STZ) resulted in a dose-dependent decrease of cell viability compared to the untreated cells (Figure 1B; $R^2 = 0.8315$; $p < 0.001$). In order to determine if the *ST* extract had protective effects against STZ-induced reduction in cell viability (LD50%), the *ST* extract was used in a prophylactic and therapeutic regimen. Both the prophylactic (Figure 1C; $p < 0.05$) and therapeutic (Figure 1D; $p < 0.05$) treatment with *ST* extract (in the doses of 5–15 mg/mL) significantly increased viability of MIN6 cells (from 50% to ± 75% cell viability; $p < 0.05$) following exposure to STZ at a dose causing 50% reduction of cell viability.

3.3 Inflammatory properties of *Sansevieria trifasciata* leaf extract in RAW cells

Infiltration of reactive inflammatory macrophages into the pancreatic islets plays an important role in beta cell loss and the pathogenesis of diabetes (Goldfine et al., 2011; Eguchi and Nagai, 2017). To this end, we studied the effect of *ST* extract on the inflammatory properties of macrophage-like cells (RAW cells). *ST* extract caused an increase in gene expression of the inflammatory genes COX2 ($p < 0.001$; 10–15 mg/mL; Figure 2A), iNOS ($p < 0.05$, 15 mg/mL; Figure 2B) and TNF-α (10–5 mg/mL; Figure 2C) in inactive RAW cells. However, *ST* extract showed no effect on gene expression of IL-10 (Figure 2D).

Next, we evaluated the effect of *ST* extract on LPS-activated RAW cells. LPS significantly increased the expression of COX2, iNOS and TNF-α compared to the negative control ($p < 0.01$-$p<0.001$; Figure 3). Treatment with *ST* extract decreased gene expression of COX2 only when applied as therapeutic regimen in the dose of 10 mg/mL ($p < 0.05$; Figure 3B). In contrast, gene expression of TNF-α was increased, after prophylactic ($p < 0.05$–0.01; 5–10 mg/mL; Figure 3E) and therapeutic ($p < 0.001$; 10 mg/mL; Figure 3F) treatment with *ST* extract. Gene expression of iNOS (Figures 3A, B) and IL-10 (Figures 3G, H) were not influenced by the administration of *ST* extract after LPS challenge.

3.4 Protective effects of *Sansevieria trifasciata* leaf extract against TNF-α and streptozotocin in MIN6 cells

In this study, treatment of MIN6 cells with *ST* extract did not result in increased gene expression of TNF-α (Supplementary Material S7). Because *ST* extract did cause an increased expression of TNF-α in activated and non-activated RAW cells, and assuming that this would lead to increased production and release of TNF-α, we investigated whether addition of TNF-α alone or in combination with *ST* extract to the medium with MIN6 cells would influence STZ-induced reduction in cell viability of MIN6 cells.

TNF-α administration alone did not influence cell viability (Figure 4), however when combined with the highest dose of *ST* (15 mg/mL) it did attenuate cell viability. As shown before, *ST* extract alone could protect MIN6 cells against STZ-induced reduction in cell viability in the doses 5–15 mg/mL ($p <$

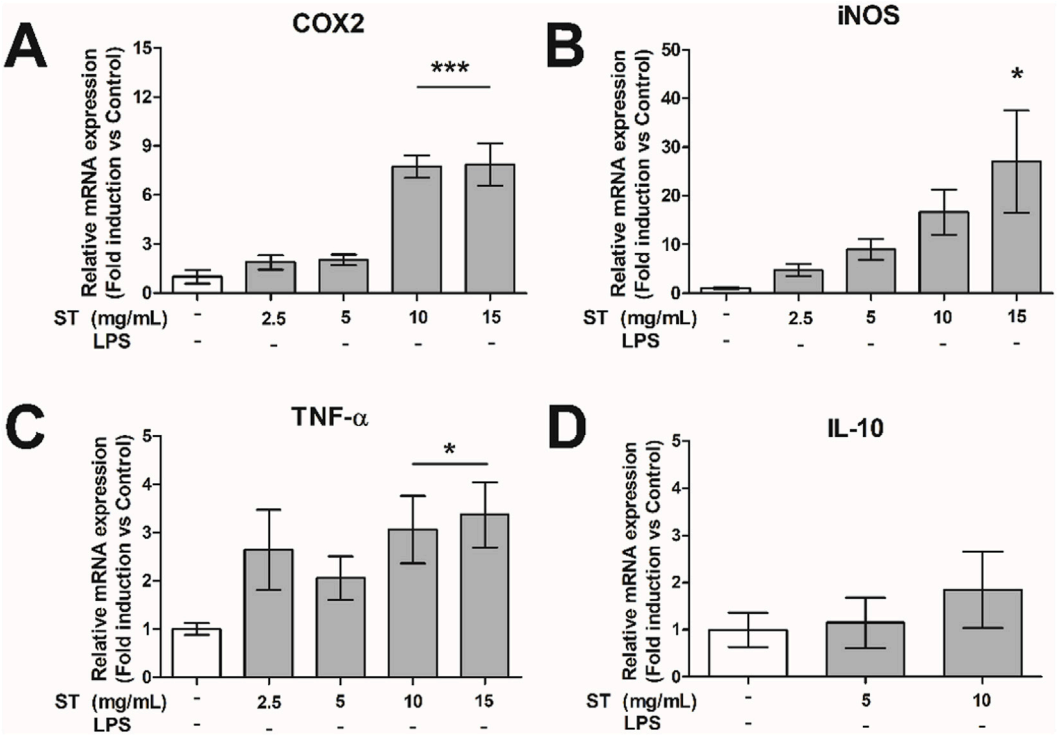

FIGURE 2
Relative gene expression of **(A)** COX2, **(B)** iNOS, **(C)** TNF-α and **(D)** IL10 in RAW-cells after administration of *Sansevieria trifasciata* leaf extract (*ST*). The experiment is repeated three times in du-plicate and data is shown as mean ± SEM and compared to the negative control group (indicated as; −/−), * $p < 0.05$, *** $p < 0.001$.

0.05–0.01), however addition of TNF-α rendered *ST* no longer protective against STZ-induced reduction in cell viability in MIN6 cells. Specifically, while a combined administration of TNF-α and 5 mg/mL *ST* extract was still beneficial against STZ-induced cell death ($p < 0.05$; Figure 4B), the higher doses of *ST* extract (10–15 mg/mL) that were found to be protective without TNF-α, lost their protective effects when TNF-α was added (Figures 4C, D). Furthermore, cell viability of MIN6 cells was already decreased when *ST* extract (15 mg/mL) was combined with TNF-α.

3.5 Protective effect of *Sansevieria trifasciata* leaf extract against streptozotocin in MIN6 cells is dependent on the NF-κB pathway

To investigate whether the protective effect of *ST* extract depends on the NF-κB pathway, we used the proteasome inhibitor MG132 (in concentrations from 0.04 to 1.0 μM) as blocker of this pathway (Figure 5). As shown before, the *ST* extract did not result in reduced MIN6 cell viability per sé, and could protect substantially against STZ-induced reduction in cell viability ($p < 0.05$–0.01). Administration of MG132 alone (0.04 μM–1.0 μM), or in combination with *ST* (5 mg/mL) did not influence MIN6 cell viability. However, when *ST* extract was combined with MG132 (≥0.2 μM) as treatment against STZ-induced reduction in cell viability, the protective effect of the *ST* extract was lost. Furthermore, administration of MG132 (≥0.2 μM) in combination with STZ (15 mM) showed a slightly lower cell viability compared to that following STZ alone.

Protease inhibitors affect many pathways (amongst others NF-κB activity), therefore, to validate if the NF-κB pathway was blocked, we assessed expression of a NF-κB pathway dependent anti-apoptotic gene, the cellular inhibitor of apoptosis 2 (cIAP2). Administration of 0.04 μM MG132, which was not effective to decrease the protective effect of *ST* extract, also did not affect expression level of cIAP2. However, the dose of 0.5 μM MG132, which was able to decrease the protective effect of *ST* extract, also caused significant reductions in cIAP2 expression in MIN6 cells across all valid comparisons ($p < 0.05$–0.01; Figure 6). These data show the importance of the NF-κB pathway on cell survival and that the effect of *ST* extract may dependent on the NF-κB pathway.

4 Discussion

Plants are known to contain bioactive compounds with a potential for the treatment of diseases. Among these is *Sansevieria trifasciata (ST)*, native to eastern Africa and southern Asia, with presumed anti-diabetic activity (Qomariyah et al., 2012). Mass spectrometric characterization of the *ST* extract in this study revealed the presence of a variety of bioactive compounds, including a relative high abundance of choline, isoleucine, α-linolenic acid, which are known to lower risk of T2DM and/or reduced insulin resistance (Alencar et al., 2018; Ward et al., 2017; Nemecz et al., 2019; Virtanen et al., 2020). The lower abundance compounds – like

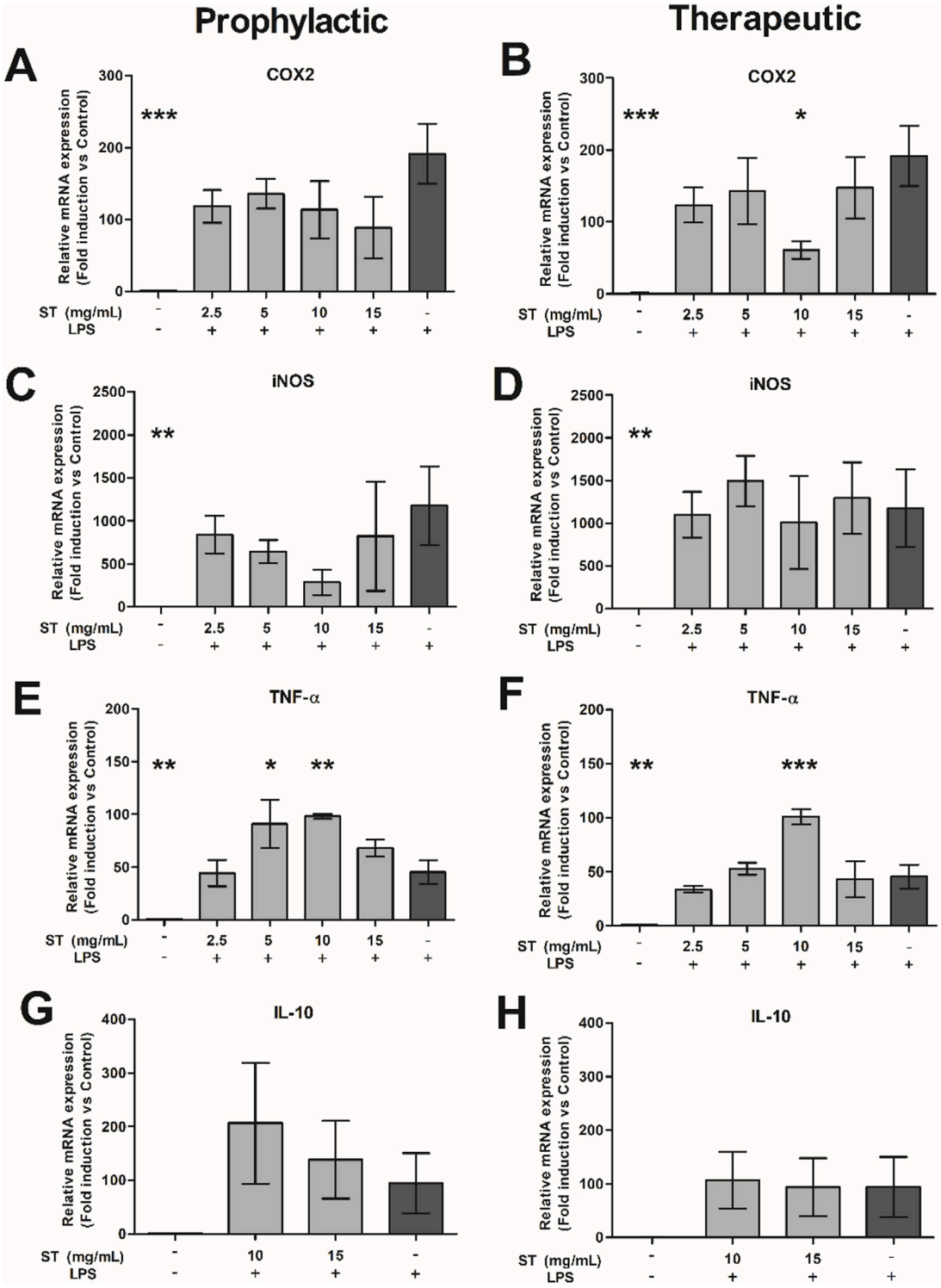

FIGURE 3
Relative gene expression of **(A, B)** COX2, **(C, D)** iNOS, **(E, F)** TNF-α and **(G, H)** IL-10 in RAW-cells after prophylactic **(A, C, E, G)** or therapeutic **(B, D, F, H)** treatment with *Sansevieria trifasciata* leaf extract (ST) either or not combined with lipopolysaccharide (LPS). The experiment was repeated three times in duplicate and data is shown as mean ± SEM and compared to the LPS control group (indicate as; −/+), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

pyroglutamic acids (Yoshinari and Igarashi, 2011) and oxophytodienoic acid (Zhang et al., 2022) – have several health improving effects in diabetic/inflammatory conditions too, although some others are also less helpful or even toxic (Al Za'Abi et al., 2018). In our experiments, however, the *ST* leaf extract (by methanol and redissolved in PBS) applied to a medium containing MIN6 cells (1.5%–10% v/v) was capable of protecting against streptozotocin (STZ)-induced toxicity. Important for consideration of these

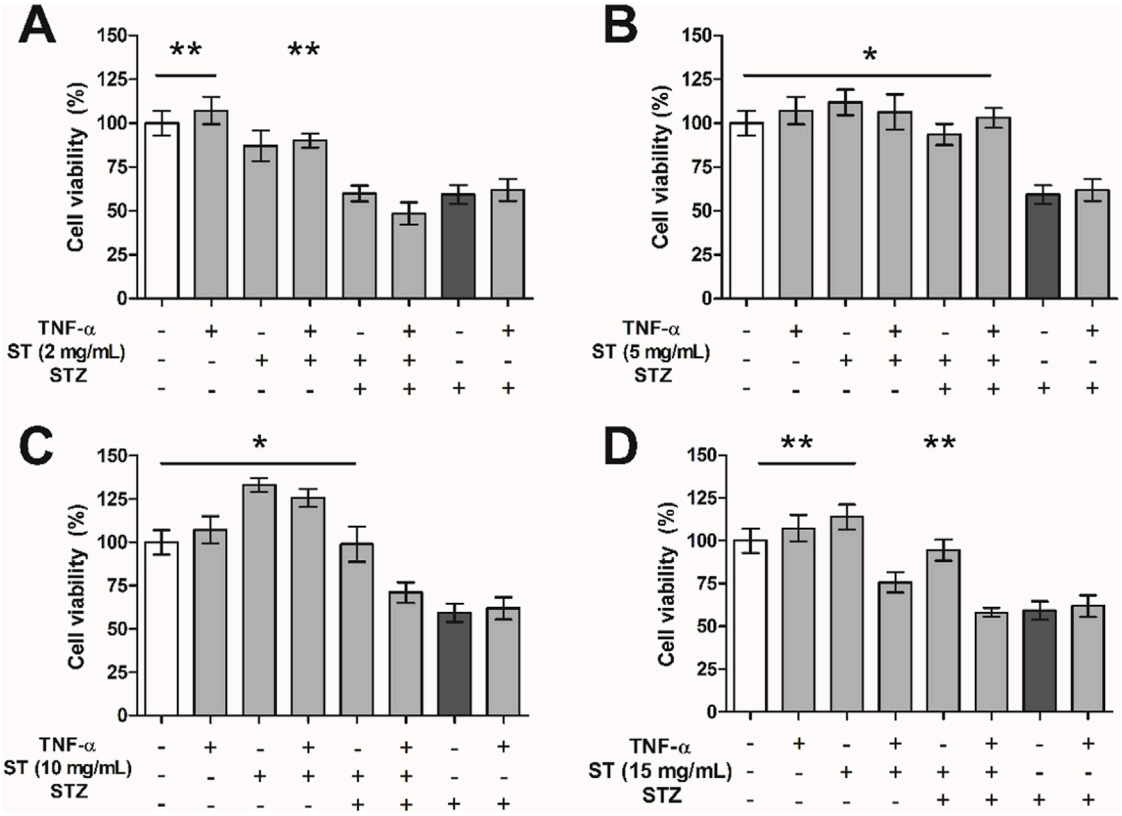

FIGURE 4
The effect of *Sansevieria trifasciata* leaf extract (ST), TNF-α (100 ng/mL) or a combination of both on streptozotocin (STZ)-reduced MIN6 viability. **(A)** 2 mg/mL, **(B)** 5 mg/mL, **(C)** 10 mg/mL and **(D)** 15 mg/mL of *ST* leaf extract. All groups are compared with the STZ control (indicated as; −/−/+) and differences are indicated with * $p < 0.05$, ** $p < 0.01$.

findings was that addition of the *ST* extract alone did not affect viability/toxicity of MIN6 cells in any way. Although much work needs to be done to pinpoint these effects to the specific bioactive compounds within *ST* that can actually be absorbed and circulate in the blood stream, our results are in line with an *in vivo* study showing that *ST* extract has anti-diabetic effects in a rodent model for T2DM induced by alloxan (Qomariyah et al., 2012).

Diabetes is often accompanied by activation of the inflammatory system, which a.o. includes pancreatic tissue infiltration of monocytes and macrophages (Goldfine et al., 2011; Eguchi and Nagai, 2017) that locally secrete inflammatory cytokines like TNF-α. For this reason, we also investigated the effect of the *ST* extract on RAW cells, a macrophage-like cell line. To our surprise, we observed that *ST* extract itself induced a low-grade inflammatory response in inactive RAW cells as indicated by increasing gene expression of COX2, iNOS and TNF-α, especially with the higher doses of *ST* extract (10–15 mg/mL). Gene expression of the anti-apoptotic cytokine IL-10 was not affected by addition of *ST* extract, although it needs to be noted that anti-apoptotic cytokines have slower kinetics, and we simply could have missed a response of IL-10 within the time frame of this experiment (Kolb and Eizirik, 2012). Activation of RAW cells by LPS profoundly increased the expression of TNF-α, which appeared to be further enhanced by prophylactic as well as therapeutic addition of *ST* leaf extract. Expression of other pro-inflammatory markers, like iNOS and COX2, were not enhanced by *ST* extract. In fact, *ST* leaf extract reduced LPS-enhanced COX2 gene in the range of 10 mg/mL, which could be interesting in light of relief of insulin resistance (Hsieh et al., 2009). Although the *ST* extract did not induce gene expression of TNF-α in MIN6 cells, these data suggest that the *ST* extract does have pro-inflammatory activity, which may be relevant for the macrophage-pancreas interactions that underlie progression of diabetes.

To mimic (at least part of) the condition of infiltrated macrophages that secrete TNF-α, we administered TNF-α directly in the medium containing MIN6 cells, and assessed their viability following STZ administration, with and without *ST* leaf extract. While the used dose of TNF-α was too low to induce apoptosis of MIN6 cells (Stephens et al., 1999) either alone or in combination with STZ, the dose of TNF-α did limit the efficacy of *ST* extract at higher doses to counter the STZ toxicity in MIN6 cells. While more work needs to be done on this front (e.g., by applying different doses of TNF-α in this experimental setting), these data may be interpreted to indicate that *ST* leaf extract could sensitize TNF-α for its well-known apoptotic effects in the context of STZ treatment. TNF-α mediates its effects via nuclear factor (NF)-κB pathway, leading to deregulated processes involving activation of inhibitory κB proteins (IκB) kinase complex, and its activation has deleterious effects in pancreatic beta cells (Meyerovich et al., 2016).

Besides the above mentioned deleterious role of the NF-κB pathway, there are however also studies showing that NF-kB

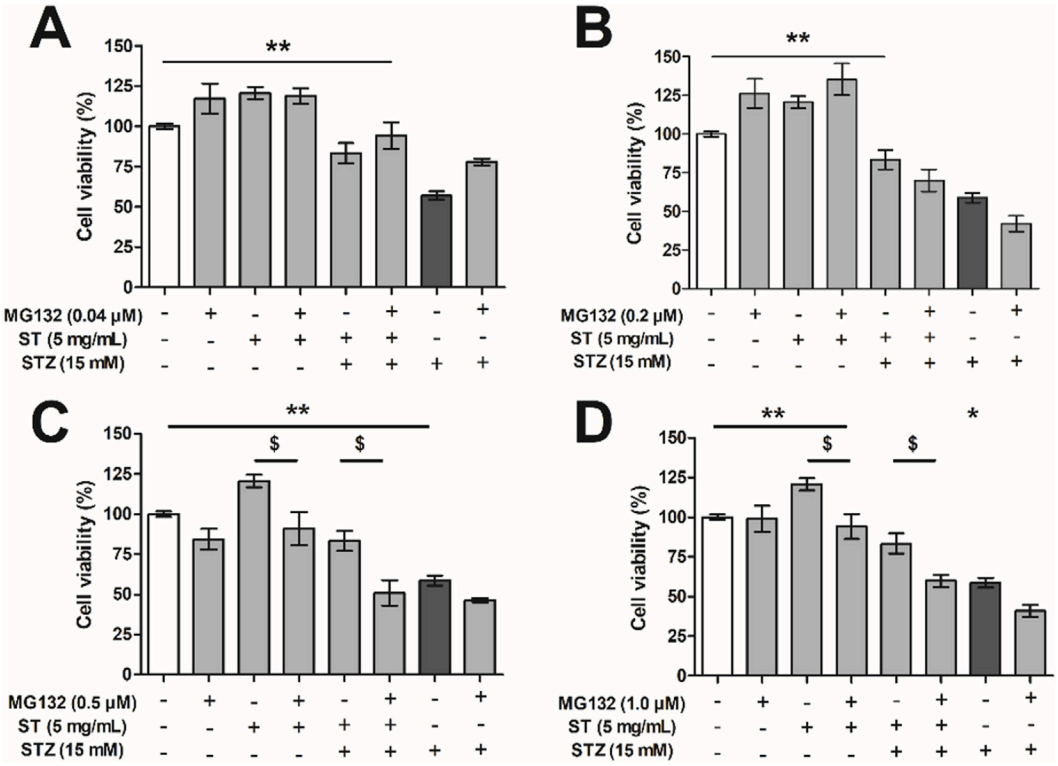

FIGURE 5
The effect of *Sansevieria trifasciata* leaf extract (*ST*), the proteasome inhibitor MG132 or a combination of both on streptozotocin (STZ)-reduced MIN6 viability. **(A)** 0.04 μM, **(B)** 0.2 μM, **(C)** 0.5 μM and **(D)** 1.0 μM of MG132. All groups are compared with the STZ control (indicated as; −/−/+) and differences are indicated with * $p < 0.05$, ** $p < 0.01$ (or $ $p < 0.05$ when the addition of MG132 had an effect).

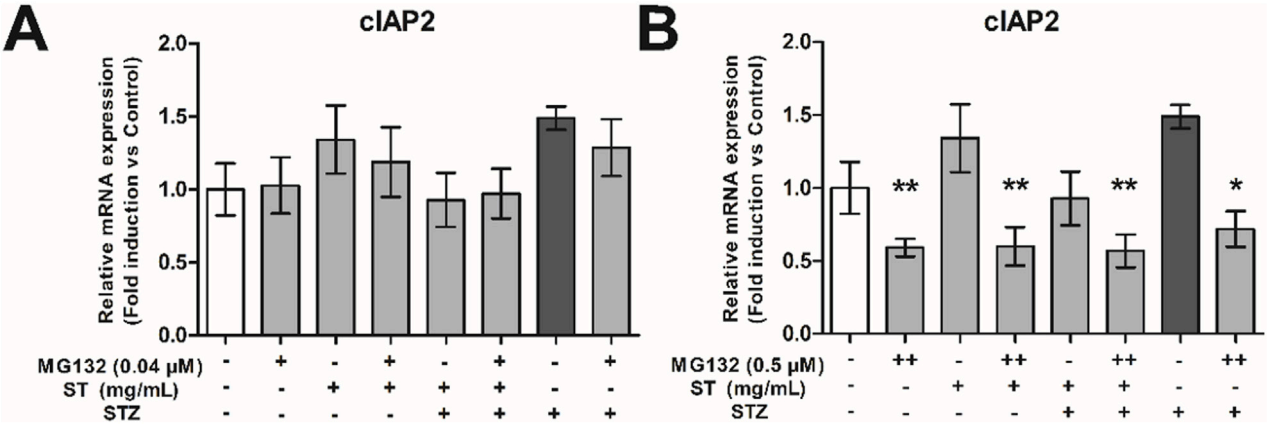

FIGURE 6
The effect of *Sansevieria trifasciata* leaf extract (*ST*), the proteasome inhibitor MG132 or a combination of the two on cIAP2 expression in MIN6 cells. **(A)** 0.04 μM, **(B)** 0.5 μM MG132. All groups are compared with the STZ control (indicated as; −/−/+) and differences are indicated with * $p < 0.05$, ** $p < 0.01$.

pathway is required for protection against cell death *in vitro* in pancreatic beta cells (Chang et al., 2003) as well as *in vivo* in diabetic animal models (Kim et al., 2007; Hofmeister-Brix et al., 2013). We have previously shown that inhibition of NF-κB activity leads to TNF-α-induced apoptosis of hepatocytes via inhibition of the induction of NF-κB dependent anti-apoptotic genes (Schoemaker et al., 2002). The protective effects of the *ST* extract against STZ-induced toxicity could also very well be mediated too via an NF-κB dependent anti-apoptotic pathway. To put this idea to the test, we investigated the protective effects of the *ST* extract against STZ-induced toxicity and employed 1) different doses of MG132, a compound that blocks a. o. the NF-kB pathway, and 2)

measurement of expression levels of inhibitor of apoptosis protein 2 (cIAP2) (Hun et al., 2005; Stehlik et al., 1998), a direct target gene of NF-κB needed for its anti-apoptotic properties (Schoemaker et al., 2002; Zanotto-Filho et al., 2012). With the lowest dose of MG132, we did not influence the protective effect of the *ST* extract against STZ-induced toxicity, nor did it alter the expression level of cIAP2 in any of the conditions, implying that this dose of MG132 was simply too low to block an anti-apoptotic pathway potentially induced by *ST*. The same low dose of MG132, however, was able to markedly attenuate STZ-induced toxicity, implying that MG132 blocked an apoptotic pathway responsible for the STZ-induced toxicity. At this moment, we do not know whether or to what extent MG132 blocked specifically an apoptotic pathway *via* NF-kB activation or any alternative apoptotic pathway involving the proteasome (Zanotto-Filho et al., 2010; Stehlik et al., 1998), but it certainly is in line with the involvement of NF-kB in beta cell apoptosis following STZ treatment (Eldor et al., 2006). When increasing the dose of MG132, however, it was observed that the protective effects of *ST* extract to alleviate STZ-induced toxicity was lost, and this was associated with a strong decline in cIAP2 expression, indeed suggestive of *ST*'s dependency on the NF-kB pathway involving cIAP2 to engage its anti-apoptotic effect. Meanwhile, the higher doses of MG132 were no longer capable of blocking the STZ-induced toxicity itself, and in fact STZ became more toxic with increasing MG132 concentrations. Our data may be interpreted to indicate that STZ is inducing apoptotic pathways, but beta cells react to this by promoting anti-apoptotic responses, with the latter ones potentially aimed at counterbalancing the apoptotic ones.

One caution that needs to be raised here is that MG132 affects many pathways, and may be not very specific. For example, MG132 could also activate apoptosis via the JNK/p38 pathway (Zanotto-Filho et al., 2012), however administration of MG132 in a dose of 0.04 μM protected against STZ-induced toxicity, implying anti-apoptotic activation instead. In conditions where *ST* extract was no longer effective in protecting STZ-induced toxicity (i.e., when MG132 was dosed at 0.5 μM), we found that expression of cIAP was inhibited, indicative of blockade of an anti-apoptotic pathway downstream form NF-κB (Schoemaker et al., 2002; Zanotto-Filho et al., 2012). cIAP2 is probably not driving this anti-apoptotic effect of *ST* extract, as the gene expression of cIAP2 was not increased in *ST*-treated beta cells on top of the STZ-treatment, but a constitutive level of cIAP2 expression could be regarded permissive to these anti-apoptotic effects. We also cannot exclude other mechanisms involving the proteasome, which are blocked by MG132 too. There are several publications discussing the balance of both the NF-κB pathway and TNF-α in pro- and anti-apoptotic programming (Chang et al., 2003; Xu et al., 1998; Wang et al., 2008; Wang et al., 1998; Webster and Vucic, 2020). This balance is not only dependent on NF-κB, but also on caspase signaling (Wang et al., 1998). In this study, emphasis is put on downstream (anti- and pro-apoptotic) pathways from NF-κB, however NF-κB protein levels or changes in localization of NF-κB after treatment with *ST* leaf extract should further be investigated. In addition, to refine the therapeutic application of *ST* leaf extract, we also aim to further explore the interaction between the NF-κB pathway with other pathways (e.g., caspase signaling and JNK/p38 pathways) that could potentially be involved in the effects of *ST* leaf extract. Finally, another important factor by which the *ST* extract could have influenced cell number is that it stimulates proliferation. While also more work needs to be done on that front (e.g., by assessing the effect of the *ST* extract over a longer-period than 24 h in MIN6 cells), *ST* extract indeed caused a slight increase in beta-cell number when added alone, and any proliferative effect on top of its protective effects should be kept in mind too.

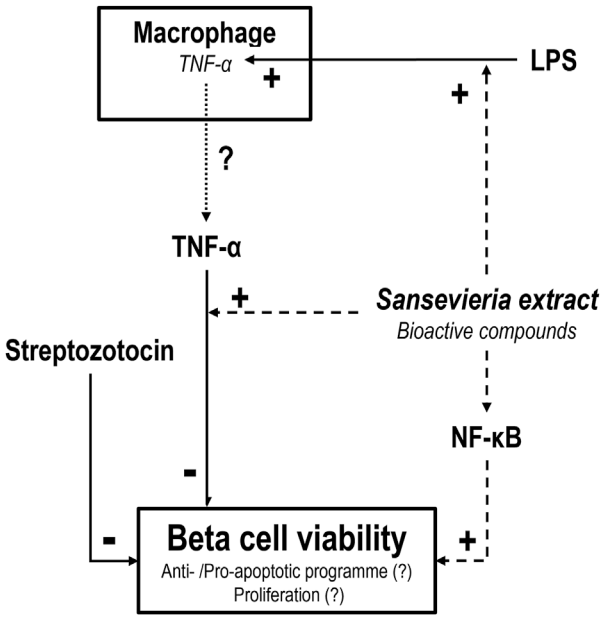

FIGURE 7
Schematic representation of the effect of *Sansevieria trifasciata* leaf extract (*ST*) on pancreatic beta (MIN6) cells and macrophage-like (RAW) cells. *ST* extract protects pancreatic beta cells (dashed line) against streptozotocin (STZ)-induced toxicity (solid line). This positive effect of ST extract is dependent on the NF-κB pathway, as the addition of MG132 (blocker of the NF-κB pathway) counters the effect of *ST* extract. This could be the result of a "switch" from an anti-apoptotic program towards a pro-apoptotic program or a decrease in proliferation. Furthermore, *ST* extract increases expression of TNF-α potentially increasing TNF-α release (dotted line) in LPS-activated macrophages. However, TNF-α in combination with *ST* extract results in a loss of the effect of *ST* extract against STZ-induced toxicity. This may be explained by a sensitizing effect of bioactive compounds in *ST* that sensitize MIN6 cells to a sub-threshold dose of TNF-α that overpowers the positive effects of *ST* to counter STZ-induced toxicity.

In summary, we show that *ST* extract (in part) protects MIN6 cells against STZ-induced cytotoxicity. This positive effect of *ST* extract is likely dependent on the integrity of the NF-κB pathway. Furthermore, *ST* extract could activate a low-grade inflammatory response by increasing the mRNA expression of COX2, iNOS and TNF-α in non-activated RAW cells in the dose range of 10–15 mg/mL. Finally, *ST* extract could increase expression of TNF-α and decrease COX2 in LPS-activated RAW cells. However, *ST* extract loses its protective effect against STZ-induced cytotoxicity when combined with TNF-α, which may be explained by a sensitizing effect of bioactive compounds in *ST* that render MIN6 cells sensitivity to canonical and non-canonical pathways involved in STZ-induced cell death, which apparently overpower the protective pathways induced by *ST*. Future studies should shed light on the bioactive compounds present in *ST* leaf extract that show their

efficacy in pancreatic beta cell survival via potentially anti-apoptotic and/or proliferative actions (Figure 7).

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical approval was not required for the studies on animals in accordance with the local legislation and institutional requirements because only commercially available established cell lines were used.

Author contributions

CH: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing–original draft, Writing–review and editing. NQ: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing–original draft, Writing–review and editing. HG-P: Conceptualization, Data curation, Formal Analysis, Methodology, Visualization, Writing–original draft, Writing–review and editing. APV: Conceptualization, Funding acquisition, Resources, Supervision, Writing–original draft, Writing–review and editing. HM: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Visualization, Writing–original draft, Writing–review and editing. GVD: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Validation, Visualization, Writing–original draft, Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. CH received a grant from Stichting de Cock – Hadders 2020-66 and the Ph. D scholarship of NQ was supported by the Directorate General of Higher Education from the Ministry of Education, Culture, Research and Technology, Republic of Indonesia and personal funding of GVD.

Acknowledgments

We thank dr. Janine Kruit of the Department of Pediatrics, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands, for providing us the MIN6 cells, who received them from the Department of Pathology and Laboratory Medicine, Faculty of Medicine, the University of British Colombia, Vancouver, Canada; Manon Buist-Homan of the Department of Gastroenterology and Hepatology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands and Wanda Douwenga of the Department of Molecular Neuroscience; Groningen institute for Evolutionary Life Sciences; University of Groningen, Groningen, the Netherlands for technical support.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2025.1485952/full#supplementary-material

SUPPLEMENTARY FILE S1
Letter from the head of the Tropical Biopharmaca Research Center.

SUPPLEMENTARY FILE S2
Identification of the *Sansevieria trifasciata* plant.

SUPPLEMENTARY FILE S3
ConPhyMP checklists Table 1.

SUPPLEMENTARY FILE S4
ConPhyMP checklist Table 2a.

SUPPLEMENTARY FILE S5
Full fingerprint of compound identification based on mass spectrometry.

SUPPLEMENTARY FILE S6
Specific spectra and compound identification of compounds listed in Table 1.

SUPPLEMENTARY FILE S7
Sequences used for real-time qPCR analysis.

SUPPLEMENTARY FILE S8
The effect of *Sansevieria* extract on TNF-α expression in MIN6 cells.

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