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Original Article

In vitro Investigation of the Anti-Diabetic Effects of Imperialine on Beta-TC6 Pancreatic and C2C12 Skeletal Muscle Cell Lines

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Abstract

Introduction: Imperialine (Imp) is a steroidal alkaloid present as the main active constituent of medicinal herb, *Fritillaria imperialis* with many biological and therapeutic effects. However, it has not been investigated in vitro for hypoglycemic effects. Herein, the effects of Imp on cell survival, carbohydrate-hydrolyzing enzymes (alpha-amylase and alpha-glucosidase), glucose uptake ability, insulin secretion levels, Advanced Glycation End products (AGEs) including pentosidine, methylglyoxal, and 3-deoxyglucosone levels and the activity of glyoxalase I as the main factor for degradation of AGEs were examined.

Materials and Methods: C2C12 skeletal muscle and beta-TC6 pancreatic cells were incubated with Imp at concentrations of 0, 25, 50, 75 and 10 microgram/ml and the cells were evaluated separately. The biological assays were based on ultraviolet-visible (UV/VIS) spectrophotometric and/or high-performance liquid chromatography (HPLC) methods.

Results: Imp had considerable and dose-dependent effects on glucose uptake and insulin secretion ($P<0.05$). The highest levels of glucose uptake were achieved at a concentration of 100 microgram/ml of Imp. Increased glycation index, cytotoxicity, and decreased glyoxalase I activity appeared mostly at concentrations of 75 microgram/ml and higher. The studied alkaloid demonstrated remarkable hypoglycemic effect by inhibition of alpha-amylase and alpha-glucosidase.

Conclusions: Consequently, the results of the present study revealed possible hypoglycemic effects of Imp and it could be suggested for future studies in the treatment of diabetes mellitus.

Keywords: Advanced Glycation End Products, Glucose Uptake, Imperialine, Insulin Secretion

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Introduction

Diabetes mellitus is a serious and chronic metabolic disorder which is distinguished by hyperglycemia resulting in defects in insulin action, inadequate secretion of insulin, or both.[1] Hyperglycemia is the main diabetes characteristic and manifestation that arise from failure in insulin production or resistance to the cellular function of insulin.[2] In this condition, to control post-prandial glucose levels, an efficient strategy focused on decreasing the glucose concentration by the inhibition of alpha-amylase and alpha-glucosidase, the responsible enzymes for the production of glucose in bloodstream from carbohydrate digestion.[3]

Under hyperglycemia circumstances, sugar aldehydes react non-enzymatically with free amino groups of proteins and form early glycation products, known as Amadori rearrangement products.[4] Propagation of glycation process through oxidation, dehydration and cyclization reaction leads to the generation of methylglyoxal, 3-deoxyglucosone (3-DG), and AGEs.[5,6] These compounds are associated with inflammation and several endothelial dysfunctions.[7]

Pentosidine is a well-characterized marker of AGEs that represents cumulative damage to protein structure in diabetes.[8] Five carbon sugars contributing to pentosidine formation originate from oxidative fragmentation of larger sugar.[9] Methylglyoxal, a small toxic alpha-ketoaldehyde is generated endogenously during the intermediate stage of the glycation process and is considered as a precursor of AGEs.[10] This precursor is a major substrate of glyoxalase I by which react with glutathione and convert to D-lactate that lowers its cytotoxicity within cells.[11,12]

Several studies have revealed that these bioactive and toxic compounds are formed and accumulate in plasma and tissue of diabetic patients.[13-15] In addition, direct glycation of enzymes and proteins can alter their normal molecular structure and physiological functions by which they implicate in diabetic complications.[16] Thus, their determination in clinical samples is relevant and monitoring would help to assess the risk of progression of diabetic complications.[17]

With regard to the consequences of glycation induce

damage, recently, more attention has been paid to plants bioactive compounds in pure and crud extract forms that are able to diminish or prevent the production of AGEs, particularly in diabetes.[18,19] Imp is a steroidal alkaloid that is presented as one of the main active constituents of the traditional herb, *Fritillaria imperialis*.[20] In vitro studies have shown that Imp suppresses the production of pro-inflammatory cytokines leading to anti-inflammatory and antitumor properties.[21,22] Imp is a potent antitussive and expectorant medicine and also possesses anticholinergic activity.[23]

Based on literature reviews, this compound has not yet been considered in vitro for hypoglycemic activity and in addition, its effects on the AGE levels have not been studied but some other steroidal alkaloids have shown promising effects in this regard.[24] Accordingly, our main goals were comparative determining the hypoglycemic effects of this compound by assessing alpha-amylase and alpha-glucosidase activities and insulin secretion status in beta-TC6 pancreatic cell line (mouse insulinoma) and mouse myoblast (skeletal muscle) C2C12 cell line. Moreover, we tried to clarify the possible role of AGEs within the treated cells in cell viability.

The chemical structures of the examined alkaloid and a summary of evaluations and results are presented in Figure 1.[21]

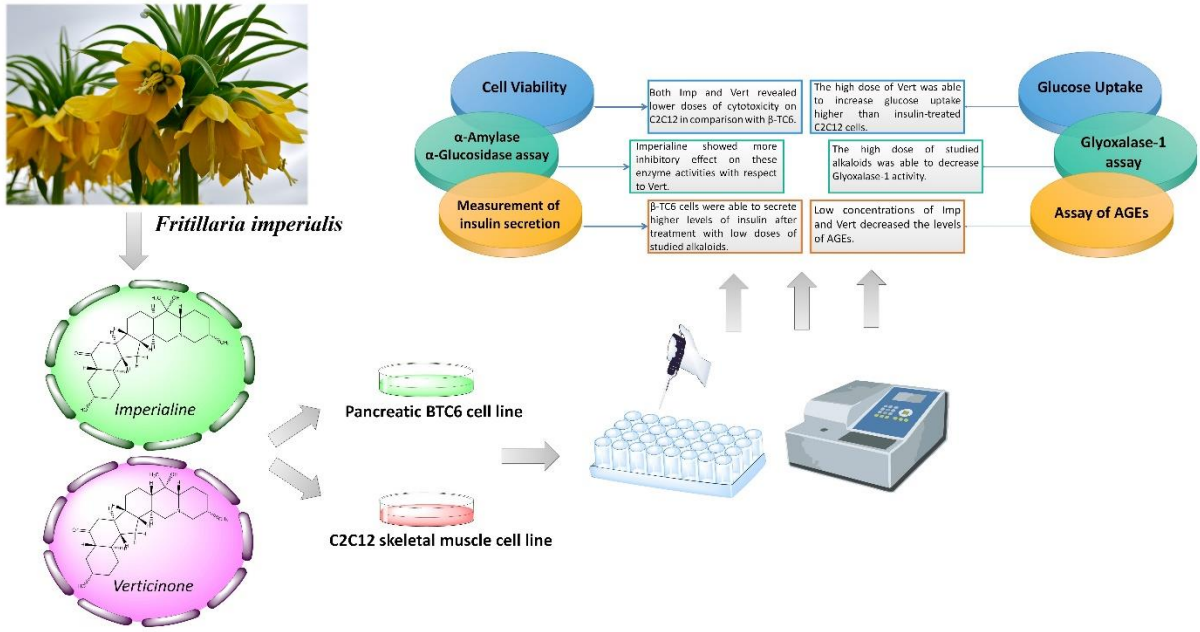

Figure 1. The Chemical Structures of the Assessed Steroidal Alkaloid: Imperialine ([1R, 2S, 6S, 9S, 10S, 11R, 14S, 15S, 18S, 20S, 23R, 24S]- 10, 20-dihydroxy- 6, 10, 23-trimethyl- 4- azahexacyclo [12.11.0.02, 11.04, 9.015, 24.018, 23] pentacosan- 17- one) and a summary of evaluations and results obtained from the current study.

Materials and Methods

Cell lines and Reagents

Chemicals reagents and Imp standard (with the purity of more than 98.0%) were obtained from Sigma-Aldrich (St. Louis, MO). Fetal Bovine Serum (FBS) inactivated with heat and Dulbecco's Modified Eagle's Medium (DMEM) containing 25 mmol/L D-glucose, 25 mmol/L 4-(2-hydroxy-ethyl)-1-piperazineethanesulfonic acid (HEPES), 44 mmol/L $NaHCO_3$, non-essential amino acid solution, L-glutamine, penicillin, and streptomycin were purchased from the Gibco Laboratory.

Mouse beta-TC6 pancreatic and myoblast C2C12 (with the origin of skeletal muscle) cell lines were purchased from the Institute of Pasteur Culture Collection of Iran. The C2C12 cells were grown in DMEM (Life Technologies, Inc) treated with 10% FBS (Sigma-Aldrich, St. Louis, MO), and 1% penicillin/streptomycin.[25,26] The cultures were kept in a damp incubator with 5% CO_2 at 37 °C and the growth medium was changed every three days.

Cell Viability Assay

The cytotoxic effects of the studied alkaloid were assessed by MTT cell viability assay as described previously.[27] 1.5×10^4 cells were incubated overnight in a 96-well plate at 37 °C in 5% CO_2.

On the next day, the cells were treated with a two-fold dilution series of four dosages (25, 50, 75 and 100 µg/ml) of Imp separately, and they were then cultured for a day. The MTT solution (4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazoliumbromide) was added to the final concentration of 0.5 mg/ml and after 2 h of incubation at 37 °C in 5% CO_2, 100 µL of Dimethyl Sulfoxide (DMSO) was added to dissolve the generated formazan crystals. Then, the plates were read in Chameleon multi-label microplate reader (Hidex, Finland, Turku) at 570

nm absorbance. The absorbance ratio of the incubated cells to DMSO-treated cells (control group) was measured as the percentage of cell viability. The IC50 value was defined as the concentration of the compound required to attenuate the absorbance of investigated cells to 50% of the control group (DMSO-treated cells). The experiment was carried out in triplicates.[28,29]

Evaluation of Alpha-Amylase Inhibition

Sample and sodium phosphate buffer (equally) containing alpha-amylase solutions (0.5 mg/ml) were incubated at 25 °C for 10 min.[30] High purity alpha-amylase (*Bacillus licheniformis*) was procured from Sigma-Aldrich (USA). After pre-incubation, 500 µL of a starch solution (1%) in 0.02 M Na_2HPO_4 buffer (pH 6.9) was added to each sample at defined intervals and incubated for 10 min at 25 °C. Then, the reaction was stopped with 1.0 ml of dinitrosalicylic acid. In the next step, the test tubes were placed for 5 min in a boiling water bath and cooled to room temperature. Acarbose was considered as a standard positive. In the final step, after dilution by 10 ml of distilled water, the absorbance was measured at 540 nm by a multimode-reader. The results were expressed as % suppression of enzyme activity.

$$\%\ \text{inhibition} = \left(\left[\frac{A_{540}^{\text{Control}} - A_{540}^{\text{Extract}}}{[A_{540}^{\text{Control}}]} \right] \right) \times 100$$

Evaluation of Alpha-Glucosidase Inhibition

Saccharomyces cerevisiae alpha-glucosidase was obtained from Sigma-Aldrich (USA). Alpha-glucosidase (1.5 U/ml) was added to 200 µL of test compound at different concentrations mixed with phosphate buffer at (pH 6.8) and cultured at 37 °C. Afterward, para-nitrophenyl-alpha-D glucopyranoside (200 µL) in 50 mM of phosphate buffer was mixed with the prepared mixture and incubated at 37 °C. Acarbose was considered as a standard alpha-glucosidase inhibitor. The reaction was terminated by the addition of 500 µL of Na_2CO_3 (1M), and the final volume was made up to 1500 µL. The absorbance was determined at 405 nm.[31] Consequently, the alpha-glucosidase inhibitory activity was expressed as the percent of inhibition and was calculated as follows:

$$\%\ \text{inhibition} = \left(\left[\frac{\Delta A_{405}^{\text{Control}} - \Delta A_{405}^{\text{Extract}}}{[\Delta A_{405}^{\text{Control}}]} \right] \right) \times 100$$

Measurement of Insulin Secretion from Pancreatic Beta Cells

Briefly, the beta-TC6 pancreatic cells were cultured in RPMI 1640 media with 11.1 mM glucose, 10% FBS, 100 U/ml of penicillin and streptomycin. The cells were subcultured every 5-6 days and after treatment of beta-TC6 cells with Imp, their seeding was carried out into a 24-well plate. After incubation for 30 min in a buffer of Krebs-Ringers bicarbonate (pH 7.4) for 1 h at 37 °C, cells were centrifuged and supernatants were kept at -20 °C till insulin measurement. Insulin levels were determined by the mice insulin ELISA kit (Shibayagi Co.).[32]

Glucose Uptake

After overnight incubation of beta-TC6 or C2C12 cells at a seeding density of 1.5×10^4 viable cells/ml in a 96-well plate at 37 °C in 5% CO_2, the medium was removed and washed with PBS. Then glucose (2.5 mM) in a basal medium comprising DMEM and L-glutamine [15% (v/v)] and FBS was added to final serum glucose at a concentration of 0.25 mM. The cells were incubated again for 60 min and the medium was then replaced with 10 mM 2- [N- (7- nitrobenz- 2- oxa-1,3- diazol- 4- yl) amino]- 2- deoxy- D-glucose (2- NBDG) (Invitrogen) in the basal medium in the presence or absence of the studied alkaloid. After the entry of NBDG into the cells, the medium was removed and the investigated cells were stained with nucleic dye Hoechst 33342 independently. The mixtures were evaluated for fluorescence at excitation/emission of 350/461 nm and excitation/ emission of 475 nm/550 nm for dye Hoechst and 2- NBDG, respectively.[33]

Evaluation of Glyoxalase-1

The activity assay of this enzyme was performed using a spectrophotometric procedure with evaluating the absorbance increment at 240 nm due to the formation of S-D-lactoylglutathione. The standard assay mixture contained glutathione, methylglyoxal, magnesium sulfate, and KH_2PO_4. After ensuring the equilibrium of hemithioacetal formation, the reaction started by adding the treated cells to the assay mixture. The formation of 1 mmol of S-D-lactoylglutathione (in a unit of time and cell extract protein) was defined as one unit of activity.[34]

Evaluation of 3-Deoxyglucosone (3-DG)

The level of 3-DG was measured by the High-Performance Liquid Chromatography (HPLC) method. Briefly, the cell extracts (density of 1.5×10^4 cells/ml) or 3-deoxyglucosone standard (500 µM) was added to the $HClO_4$ solution at a final protein concentration of 20 mg/ml and centrifuged at 3,000 rpm. Supernatant neutralized with Na_2CO_3 and then added to 2,3-diaminonaphthalene and 2,3-Pentanedione. After 24 h, ethyl acetate was used to extracting the reaction mixture followed by evaporation. The dried extracts contained 50% methanol and were later used for injection to reverse phase of HPLC system (column: TSKgel ODS-80Tm) that applied a linear gradient system of methanol/phosphate/ acetonitrile solvent and directly detected by UV detection (268 nm).[35]

Evaluation of Pentosidine

The measurement of pentosidine levels was done according to the samples dialyzing against phosphate buffer at a ratio of 1000: 1 for overnight and changing the dialysate every 8 h. The obtained solution was concentrated by evaporator (Jouan Speed Vac) and then hydrolyzed by adding 50 µL of HCl. After the re-evaporation of HCL and dilution by water, the mixture was neutralized by the addition of NaOH. The mixture was filtered and injected into the HPLC system with Nova-Pak C18 reversed-phase separation column. Trifluoroacetic acid (solvent A) and acetonitrile (solvent B) were used as elution solutions. Finally, after adjusting the volumes of solutions A and B, the pentosidine standard was determined by fluorescence at Ex 325/Em 385 nm and eluted within about 60 min.[36]

Evaluation of Methylglyoxal

For methylglyoxal assay, 150 µL of supernatant was mixed with 240 µL of water and 60 µL of phosphate buffer (500 mM, pH 7.4) and then 10 µL of 4-Methoxy-o-phenylenediamine (4MPD) (20 mg/ml) was added as a derivatizing reagent. The mixture was incubated at 40 ºC. In the next step, HCl (3 M), acetonitrile and NaCl were added to the samples and were then centrifuged (10.000 g). Finally, the acetonitrile part was mixed with mobile phases A and C, containing beta-mercaptoethanol (1:1). The injection volume to an HPLC with a fluorimetric detector system was 20 µL. After separation of methylglyoxal using three mobile phases (water, acetonitrile, and acetic acid with TEA), fluorimetric detection was accomplished based on excitation/emission wavelengths at 344/420 nm.[37]

Statistical Analysis

The experiments were performed separately in five groups (n = 3) in the range of 0-100 microgram/ml (0, 25, 50, 75 and 100) of the investigated compound. Data were expressed as mean ± SD and analyzed by one-way ANOVA, followed by Tukey's post hoc test. The software was SPSS (version 20.0) and for all analyses, p value < 0.05 was considered significant. The biological response of each concentration evaluated solely in separate cell lysate samples.

Results

Cell Viability

The cytotoxicity of Imp against beta-TC6 and C2C12 cells was evaluated with various levels of Imp and doxorubicin. In this evaluation, we determined 50% cell mortality (IC50) for the studied compounds (Table 1 and 2). The presented data showed IC50 of doxorubicin (as a positive control) at 4.1 and 3.5 for beta-TC6 and C2C12 respectively. However, this index was at very high doses for Imp with respect to doxorubicin (about 15 folds of doxorubicin). Our alkaloid revealed lower levels of cytotoxicity on C2C12 in comparison with beta-TC6.

Table 1. Evaluation of Cell Viability of C2C12 and Beta-TC6 Cells (% of Control) after 24 h Treatment with Various Levels of Imp Determined by the MTT Test

Cell line	Control groups	Imperialine (µg/ml)			
		25	50	75	100
C2C12	100±5.2	78.5 ± 6.5	66.7 ± 5.3	54.1 ± 4.3	24.1 ± 2.1
β-TC6	100±5.0	61.8 ± 4.8	48.5 ± 4.1	33.5 ± 2.7	21.8 ± 1.9

The cell survival after treatment with each concentration was assayed solely in separate cell lysate samples. Data are shown as relative survival to the untreated control (mean ± SD); n=3.

Table 2. Evaluation of Cytotoxicity (Presented as IC50) of Beta-TC6 and C2C12 Under the Treatment of Various Levels of Imp and Doxorubicin

Cell line	Doxorubicin (µg/ml)	Imperialine (µg/ml)
Beta-TC6	4.1	62.3
C2C12	3.5	55.1

Evaluation of Insulin Secretion

Insulin secretion levels in beta-TC6 cells after being treated with Imp have been presented in Figure 2 A. Beta-TC6 cells were able to secrete higher levels of insulin after treatment with low doses of studied alkaloid with respect to the control. On the contrary, insulin secretion was significantly inhibited by high levels of Imp in comparison to the control and in conditions in which low doses of alkaloid were used. At high treatment doses, the inhibition effect of Imp on insulin levels was more remarkable (insulin levels reached around 51% of control).

Glucose Uptake

The total amount of glucose uptake (as fluorescence intensity of ingested 2-NBDG) by studied cells in response to Imp is shown in Figure 2 B and C. Insulin was included as the positive control, by which the entrance of 2-NBDG was enhanced in the investigated cells, particularly in C2C12 cells. In both cells, Imp caused lower glucose uptake in comparison to the control group. The responses of C2C12 cells were more considerable than beta-TC6 at all treated concentrations of alkaloid. In this cell, glucose accumulation after exposure to high doses of Imp intensified significantly.

The Activities of Alpha-Amylase and Alpha-Glucosidase

In the presence of high doses of Imp, the enzyme activities decreased significantly with respect to low treatment concentrations. On the other hand, we determined the

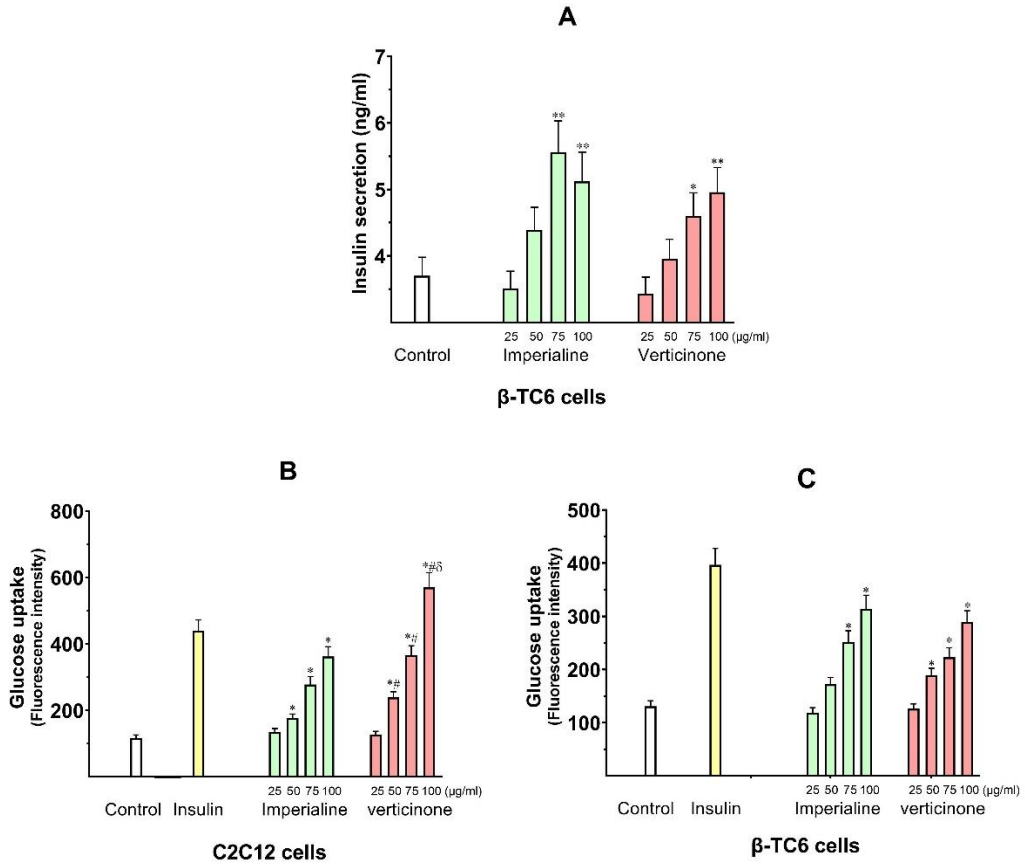

Figure 2. Insulin Secretion Levels in Beta-TC6 Cells after Treatment with Imp. The insulin levels of beta-TC6 pancreatic cells (A), the glucose uptake was presented as fluorescence intensity of 2-NBDG taken up by C2C12 (B) and beta-TC6 (C) after a day treatment with different levels of Imp. The biological response of each concentration of investigated alkaloid was determined separately in cell samples (triplicate). All the data have been demonstrated as mean ± SD.
* Significant difference in comparison to the control group according to one-way ANOVA, followed by Tukey's post hoc test ($p < 0.05$).
** Significant difference in comparison to the control group ($p < 0.001$).

concentration of the studied compound by which 50% inhibition (IC50) on enzyme activities were observed (Table 3). Acarbose, the standard compound for inhibition of these enzymes, showed IC50 values of 30 µg/ml and 171 µg/mL for alpha-amylase and alpha-glucosidase respectively.

The Activity of Glyoxalase I

Glyoxalase I activity assay in studied cells (control condition) confirmed that this index in beta-TC6 was significantly higher than C2C12 cells (Figure 3 A and B). Data revealed that at low levels of Imp, insignificantly elevated the enzyme activity in both cells. Also, the potentiating effect of Imp on this enzyme was notable at 50 µg/ml with respect to the control. On the contrary, enzyme activities decreased in the response of cells to high concentrations of Imp.

Evaluation of Advanced Glycation End Products (AGEs)

Evaluation of methylglyoxal levels are presented in Figure 4 A and B. Data showed low contents of this compound in beta-TC6 than C2C12 cells in control conditions. Low concentrations of Imp decreased methylglyoxal levels in both treated cells. In contrast, at high doses of Imp, the methylglyoxal content of both cells elevated markedly and significantly as compared to the untreated cells.

Table 3. The Inhibition of Alpha-Amylase and Alpha-Glucosidase in terms of IC50 (Dosage that Inhibited 50% of Enzyme Activity) Values. Acarbose was Considered as a Positive Control

Enzyme	Compound	IC50 (µg/ml)
α-Amylase	Imperialine	43
	Acarbose	30
α-Glucosidase	Imperialine	125
	Acarbose	170

The variations in pentosidine levels are also presented in Figure 4 (C and D). In the control group, the parameter was significantly lower in beta-TC6 than C2C12 cells. The treatment of both cells with Imp at low concentrations did not change pentosidine levels significantly in comparison to the untreated cells. However, at the highest doses of this alkaloid, the index elevated considerably in the treated cells.

Figure 4 (E and F) has demonstrated 3-DG content of cells in control and treated conditions. There was a significantly low concentration of 3-DG in beta-TC6 with respect to C2C12. Imp was able to decrease the parameter notably at low treated concentrations in comparison to the control group in beta-TC6 cells. Data also showed a slight but significant decrease in 3-DG level at 75 µg/ml of Imp in C2C12 cells. At higher doses of the studied alkaloid, Imp elevated 3-DG contents considerably in the treated cells.

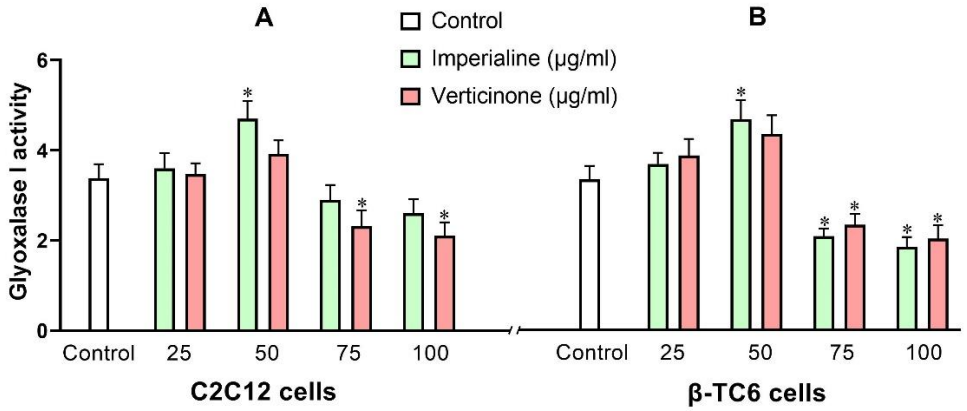

Figure 3. Evaluation of Glyoxalase I Activities on C2C12 (B) and Beta-TC6 (C) Cells after a Day Incubation with Various Concentrations of Imp (microgram/ml). The biological response of each concentration was determined independently in cell lysate samples (triplicate). All data have been presented as mean ± SD.
* Significant difference in comparison to the control group according to one-way ANOVA, followed by Tukey's post hoc test ($p < 0.05$).

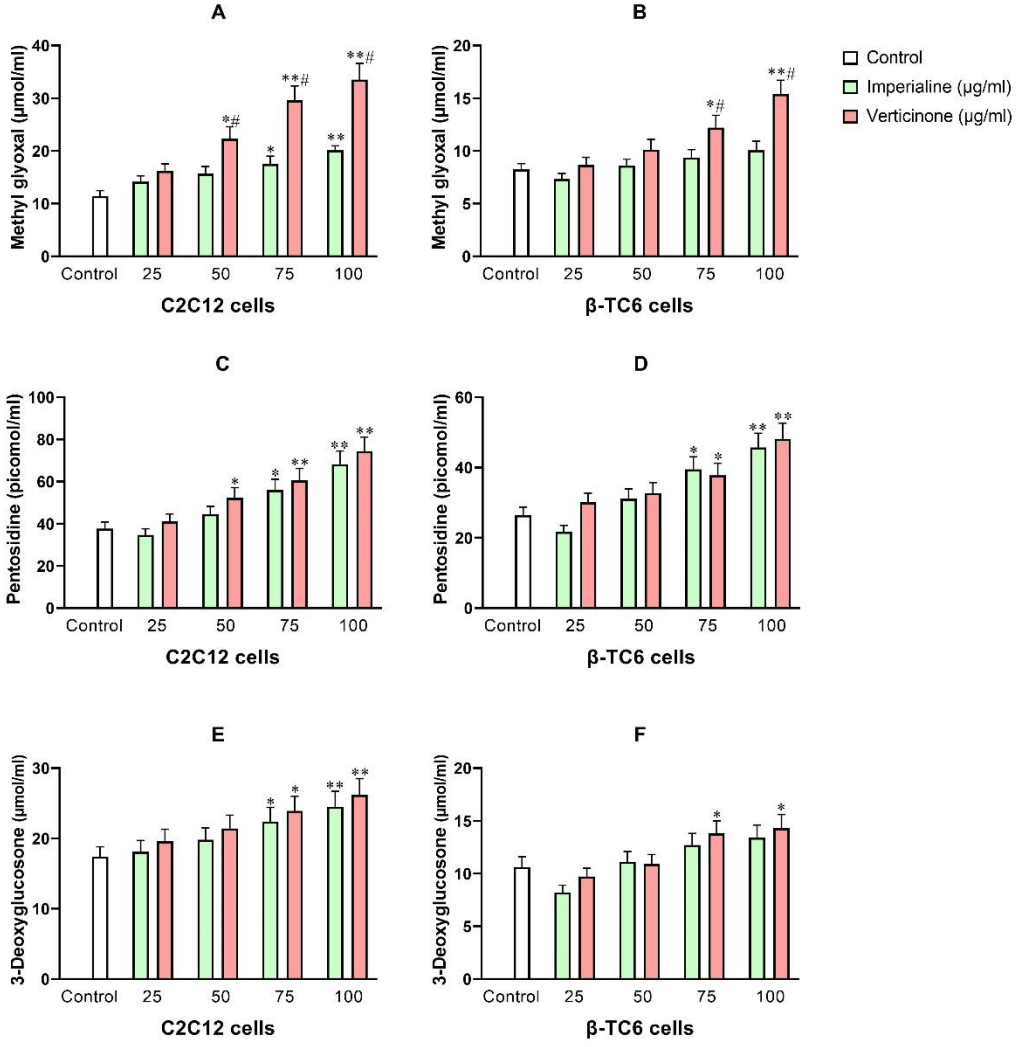

Figure 4. The Content of Methylglyoxal (micromol/ml), pentosidine (picomol/ml) and 3-deoxyglucosone (micromol/ml) in C2C12 and beta-TC6 Cells Exposed to Different Concentrations of Investigated Alkaloid after a Day of Incubation (A-F). Each mediator content determined independently in cell lysate samples (triplicate). All data have been presented as mean±SD.
* Significant difference in comparison to the control group according to one-way ANOVA, followed by Tukey's post hoc test ($p < 0.05$).
** Significant difference in comparison to the control group ($p < 0.001$).

Discussion

Imp is a steroidal alkaloid with several pharmacological effects.[38,39] In order to assess the possible therapeutic potential for diabetes, we evaluated the cytotoxicity effects of Imp on beta-TC6 and C2C12 cells as the candidates of two important organs that play crucial roles in blood glucose balance by MTT assay.[40] Data based on the IC50 of cell viability confirmed that Imp was at least 15 folds less toxic toward target cells in comparison to doxorubicin as a potent cytotoxic drug used in chemotherapy. To carry out the following study, we examined antidiabetic parameters at different concentrations of Imp.

The main function of beta cells is insulin secretion and insulin itself has a key role in maintaining normoglycemia.[1] Our evaluation revealed that Imp had efficient protection in a dose-dependent manner on insulin biosynthesis and apparent stimulation on the secretion of this hormone. By improving insulin secretion, Imp may be used for glucose-lowering goals in hyperglycemic conditions. We also observed that fewer IC50 caused less inhibitory effects on insulin secretion. This finding may rise from the cytotoxicity effect of this compound that not only lowered cell viability but may also have damaged insulin synthesis or secretion.[41]

To clarify the mechanism underlying the observed cytotoxicity, our main target was precursors of AGEs in studied cells. These precursors derived from glucose, thus at first we evaluated glucose uptake by cells in the absence and presence of Imp. In the current study, C2C12 had higher glucose uptake with respect to beta-TC6 cells even in the control condition. This may be due to the fact that C2C12 is a skeletal muscle cell and normally in the body, it is responsible for more than 70% of glucose uptake and oxidation.[42] Predictably, in both pancreatic and muscle cells, glucose uptake increased in a dose-dependent manner with the studied compound. It should be noted that as much as glucose accumulation is harmful in blood circulation, glucose overload within cells can generate toxic compounds.[43-45]

Starch and disaccharides are the main sources of carbohydrates that their hydrolysis by alpha-amylase and alpha-glucosidase can lead to glucose generation.[46] Thus inhibition of these enzymes is an efficient hypoglycemic strategy against diabetes.[47] To check whether our compound can affect these hydrolyzing enzymes, their activities were monitored. We observed that Imp moderately lowered the activity of these enzymes. In this way, Imp can possibly be used to change hyperglycemic to normoglycemic conditions. In accordance with our findings, other alkaloids, particularly in indole-type, had inhibitory effects on alpha-amylase and alpha-glucosidase. Researchers have suggested them to be used in therapeutic issues in patients with type 2 diabetes in order to lower their glucose levels.[48-50] The study of Jan et al. revealed that steroid alkaloids isolated from *Sarcococca saligna* possess hypoglycemic effect and improve other diabetes-associated complications.[24]

To better understand the therapeutic potential of Imp, we focused on the status of precursor and metabolites of AGEs in the treated cells. These reactive intermediates or end products are causative factors of apoptosis, cell dysfunction, and progress or exacerbating of many degenerative disorders, such as diabetic condition.[51,52] Hence, the targeting of AGEs could be a therapeutical approach to reduce diabetic complications.[53] Methylglyoxal as an intermediate of AGEs is derived from the decomposition of triosephosphate and fragmentation of aldehyde sugars.[54,55] Also, 3-DG as a highly reactive carbonyl intermediate in glycation reactions and pentosidine as a cross-linking reagent have been considered effective factors for cumulative damage to proteins in diabetes.[56-58] Our evaluation confirmed that low concentrations of Imp did not have any remarkable effect in the generation of AGEs and even moderate to high concentrations of the studied alkaloid can increase the level of end glycation products. It seems that this effect can arise slightly from the potentiating effect of Imp on glyoxalase I activity and greatly cause the glucose accumulating effect of this alkaloid.

In accordance with our findings, other isoquinoline alkaloids such as jatrorrhizine, magnoflorine, and palmatine have exhibited hypoglycemic effects via beneficial effects on increasing insulin release or improving insulin response.[59] Also, berberine has revealed the promising potential to decrease fasting serum glucose levels in mice in a dose-dependent manner.[60] It seems that these effects appear due to the inducing effects of berberine on glycolytic enzymes and inhibition of gluconeogenesis.[61,62]

Conclusion

The results of the present study showed that moderate to high concentrations of Imp in pancreatic and skeletal muscle cells exhibit potential alpha-amylase and alpha-glucosidase inhibitory properties and increases glucose uptake and insulin secretion significantly. However, the use of this compound at high doses was associated with increased glycation end products, which may be due to their increased glucose uptake. These findings and related signaling pathways involved in hypoglycemia should be investigated in future studies.

Authors' Contributions

All the authors equally contributed to the present study.

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Conflict of Interest Disclosures

The authors confirm that there are no known conflicts of interest associated with the current publication.

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