

## Chapter

# The Impact of Heavy Metals on Pancreatic $\beta$ -Cell Function: Mechanisms and Implications for Development of Diabetes Mellitus

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## Abstract

Environmental exposure to heavy metals is an increasingly recognized risk factor in the development of metabolic disorders, including diabetes mellitus (DM). Pancreatic  $\beta$ -cells are particularly vulnerable to oxidative and functional damage induced by toxicants such as cadmium (Cd), arsenic (As), lead (Pb), and manganese (Mn); however, the mechanisms of toxicity remain inadequately defined. This study aimed to evaluate the cytotoxic, oxidative, and functional effects of CdCl<sub>2</sub>, NaAsO<sub>2</sub>, MnCl<sub>2</sub>, and PbCl<sub>2</sub> on insulin-secreting MIN6 and INS-1 pancreatic  $\beta$ -cell lines. Cells were exposed to different concentrations of heavy metals for 24 h. Cell viability was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, while intracellular reactive oxygen species (ROS) and lipid peroxidation (measured as malondialdehyde, MDA), were quantified to determine oxidative stress. Glucose-stimulated insulin secretion (GSIS) was evaluated using a high glucose challenge and ELISA-based insulin quantification. Exposure to 2  $\mu$ M CdCl<sub>2</sub> and NaAsO<sub>2</sub>, and 5  $\mu$ M MnCl<sub>2</sub> and PbCl<sub>2</sub>, significantly reduced cell viability and insulin secretion in both cell lines. These treatments also induced a marked increase in ROS generation and malondialdehyde (MDA) levels, indicating enhanced oxidative stress and lipid peroxidation. Cd, As, Pb, and Mn exert dose-dependent cytotoxic effects on pancreatic  $\beta$ -cells by impairing viability, disrupting insulin secretion, and inducing oxidative stress. These findings support the hypothesis that chronic exposure to heavy metals contributes to  $\beta$ -cell dysfunction, and may play a role in diabetes pathogenesis. Gaining insight into these mechanisms is essential for guiding environmental health policies and creating targeted intervention strategies.

**Keywords:** heavy metals, pancreatic cell damage, diabetes mellitus, oxidative stress, environmental health

## 1. Introduction

Exposure to environmental pollutants is often underestimated; however, significant risk factors in the development and progression of various chronic diseases [1]. Despite increasing awareness, the role of these pollutants, particularly toxic heavy

metals, in metabolic disorders such as diabetes mellitus (DM) remains insufficiently explored. Epidemiological and experimental studies have drawn growing attention to the association between environmental toxicants and the global rise in diabetes prevalence [1, 2].

DM is a multifaceted metabolic disorder characterized by chronic hyperglycemia, resulting from impaired insulin resistance, insulin secretion, or a combination of both. It exists primarily in two forms: Type I (insulin-dependent) and type II (non-insulin-dependent) [3]. Emerging evidence suggests that exposure to environmental pollutants can overload pancreatic  $\beta$ -cells, induce cellular stress, and impair their functional capacity. In addition to promoting insulin resistance and disrupting glucose metabolism, these pollutants can directly damage pancreatic islets, contributing to diabetes pathogenesis [2]. Given the rising environmental burden of disease, there is a pressing need for comprehensive research into the mechanisms by which environmental contaminants contribute to diabetes.

Cadmium (Cd) is among the most harmful toxic metal pollutants, known for its ability to bioaccumulate over time and exert toxic effects on multiple organ systems, including the kidneys, bones, and pancreas [4–6]. Cd is commonly used in industrial processes, such as battery production, pigments, and plastic manufacturing, and is ranked seventh on the U.S. Agency for Toxic Substances and Disease Registry's Substance Priority List [7]. In nonoccupational environments, cadmium (Cd) exposure primarily happens through contaminated food, water, and tobacco smoke. Prolonged Cd exposure has been associated with nephrotoxicity, cancer, bone damage, and metabolic disturbances, including hyperglycemia and hypoinsulinemia [5, 8].

Arsenic (As), another widespread environmental pollutant, is commonly found in groundwater and industrial waste [9]. Chronic arsenic exposure is associated with oxidative stress, inflammation, and DNA damage, all of which can impair pancreatic  $\beta$ -cell function [10]. Epidemiological data link arsenic exposure to increased insulin resistance, glucose intolerance, and diabetes risk, particularly in populations with high levels of arsenic in drinking water [9, 10].

Lead (Pb), although now less commonly used in industrial materials due to regulatory controls, remains an environmental hazard due to its persistence in soil and water [11]. Pb exposure has been shown to alter calcium signaling pathways, essential for insulin granule exocytosis. *In vitro*,  $PbCl_2$  has been reported to reduce insulin secretion and increase lipid peroxidation in INS-1 cells [12]. Moreover, Pb exposure enhances ROS production, promoting oxidative DNA damage and impairing  $\beta$ -cell function, as shown in studies using both  $\beta$ -cell lines and isolated islets [13, 14].

Manganese (Mn), though essential in small amounts for enzymatic and metabolic functions, can be neurotoxic and metabolically disruptive at high levels [15]. Excess Mn has been linked to impaired insulin signaling, mitochondrial dysfunction and  $\beta$ -cell apoptosis [16]. *In vitro* investigations using MIN6 cells revealed that high Mn concentrations cause a significant increase in ROS and a reduction in insulin secretion [14, 17]. Additionally, studies have shown that Mn disrupts the antioxidant defense system, including enzymes such as superoxide dismutase (SOD) and catalase, making  $\beta$ -cells more susceptible to oxidative injury [16, 18].

To investigate the cellular and molecular mechanisms underlying heavy metal-induced pancreatic damage, *in vitro* models such as INS-1 (rat insulinoma) and MIN6 (mouse insulinoma) cells are widely utilized [19, 20]. These  $\beta$ -cell lines provide a controlled system to evaluate the cytotoxic, oxidative, and metabolic effects of toxicants. Studies have demonstrated that exposure to Cd, As, Pb, and

Mn in INS-1 and MIN6 cells leads to decreased cell viability, reduced insulin secretion, altered gene expression, and increased oxidative stress. Such findings underscore the relevance of environmental toxicants in  $\beta$ -cell dysfunction and DM development [21].

This study aimed to investigate the cytotoxic and functional effects of four environmental heavy metals—Cd, As, Pb, and Mn—on pancreatic  $\beta$ -cell viability, oxidative stress, and insulin secretion using two *in vitro* models: MIN6 (mouse) and INS-1 (rat)  $\beta$ -cell lines. By comparing the responses of these two cell types to metal-induced toxicity, this study aimed to enhance the understanding of how environmental toxicants can contribute to  $\beta$ -cell dysfunction and the development of DM.

## 2. Materials and methods

### 2.1 Materials

Unless specified otherwise, all chemicals (including CdCl<sub>2</sub>, NaAsO<sub>2</sub>, MnCl<sub>2</sub>, PbCl<sub>2</sub>) and laboratory plastic wares were purchased from Sigma-Aldrich (Dorset, UK).

### 2.2 Cell culture

The mouse MIN6 cells, an insulin-secreting pancreatic  $\beta$ -cell line, were used in this study. The cells (<30 passages) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 15% heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin, and 0.1 mg/mL streptomycin. The cells were maintained at 37°C in a humidified incubator with 5% CO<sub>2</sub>. MIN6 cells were split when 80–90% confluent, then were used for experiments.

INS-1 cells, a rat insulin-secreting pancreatic  $\beta$ -cell line, were cultured in RPMI 1640 medium, supplemented with 50 U/mL penicillin, 2 mM L-glutamine, 10% FBS, 1 mM sodium pyruvate, 50  $\mu$ M  $\beta$ -mercaptoethanol, and 0.1 mg/mL streptomycin in a humidified atmosphere with 5% CO<sub>2</sub> at 37°C, based on the method of Asfari et al. [22]. INS-1 cells were split when 80–90% confluent, then were used for experiments.

### 2.3 Measurement of cell viability

MIN6 and INS-1 pancreatic  $\beta$ -cells were seeded in 96-well plates at a density of  $2 \times 10^4$  cells per well and allowed to adhere under standard culture conditions. Once confluent, the cells were treated with varying concentrations of Cd, As, Pb, or Mn for 24 h. After exposure, the treatment medium was removed and replaced with fresh medium with 0.2 mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). The cells were incubated for an additional 4 hours at 37°C to allow for the formation of formazan crystals by metabolically active cells. After incubation, the medium was carefully removed, and the resulting blue formazan crystals were solubilized by adding 100  $\mu$ L of dimethyl sulfoxide (DMSO) to each well. Absorbance was measured at 570 nm using a microplate reader (BioTek  $\mu$ Quant Monochromatic Microplate Spectrophotometer, MTX Lab Systems, Inc., USA). Cell viability was shown as a percentage relative to the untreated control group.

## **2.4 Determination of intracellular reactive oxygen species (ROS)**

Intracellular ROS levels in MIN6 and INS-1 pancreatic  $\beta$ -cells were measured using the cell-permeant probe 2',7'-dichlorodihydrofluorescein diacetate (H<sub>2</sub>DCFDA), according to the manufacturer's instructions. Briefly, cells from different treatment groups were harvested and washed three times with sterile phosphate-buffered saline (PBS) to remove residual culture media and extracellular contaminants. Following washing, the cells were incubated in the dark at 37°C for 90 minutes in PBS containing 10  $\mu$ mol/L H<sub>2</sub>DCFDA. After incubation, excess extracellular dye was removed by washing the cells three additional times with sterile PBS. The oxidation of H<sub>2</sub>DCFDA to the fluorescent product 2',7'-dichlorofluorescein (DCF) within the cells was used as an indicator of intracellular ROS production. Fluorescence intensity was measured using flow cytometry with excitation at 485 nm and emission detection at 525 nm. ROS levels were presented as relative fluorescence units, normalized to those of the control group treated with 0.01% DMSO. All measurements were performed in triplicate to ensure reproducibility.

## **2.5 Lipid peroxidation (MDA)**

Lipid peroxidation in MIN6 and INS-1 pancreatic  $\beta$ -cells was measured by the detection of the endpoint product malondialdehyde (MDA), according to the manufacturer's instructions, using the MDA microplate assay kit from Cohesion Biosciences (London, UK). MDA levels in the cell culture supernatant were measured by colorimetric analysis at 532 and 600 nm. MDA concentrations were normalized to total protein content and expressed as nmol/mg protein.

## **2.6 Glucose-stimulated insulin secretion (GSIS)**

MIN6 and INS-1 pancreatic  $\beta$ -cells were plated and maintained under standard culture conditions until they achieved suitable confluency for the GSIS assay. The cells were then washed twice with PBS and subsequently treated with Cd, As, Pb, or Mn for 24 h at specified concentrations. After treatment, the culture medium was discarded, and the cells were rinsed with PBS to remove any remaining metal ions. Next, the cells were incubated at 37°C in glucose- and serum-free DMEM, supplemented with 2 mM L-glutamine and 25 mM HEPES (pH 7.4), and adjusted to a final glucose concentration of 25 mM to stimulate insulin secretion. Following a 15-minute incubation, the supernatants containing secreted insulin were collected, and the cells were subsequently lysed using the method outlined by Cheng et al. [23]. Cell lysis was performed using an acid/ethanol solution (1.5:70 v/v%) to extract intracellular insulin for determining total insulin content. Both media samples and cell lysates were analyzed using a commercial insulin ELISA kit, and absorbance was measured at 450 nm using a microplate reader. Insulin secretion was normalized to the total insulin content of the cells to account for variations in cell number or viability. Each experimental condition was conducted in at least four biological replicates per metal concentration.

## **2.7 Statistical analysis**

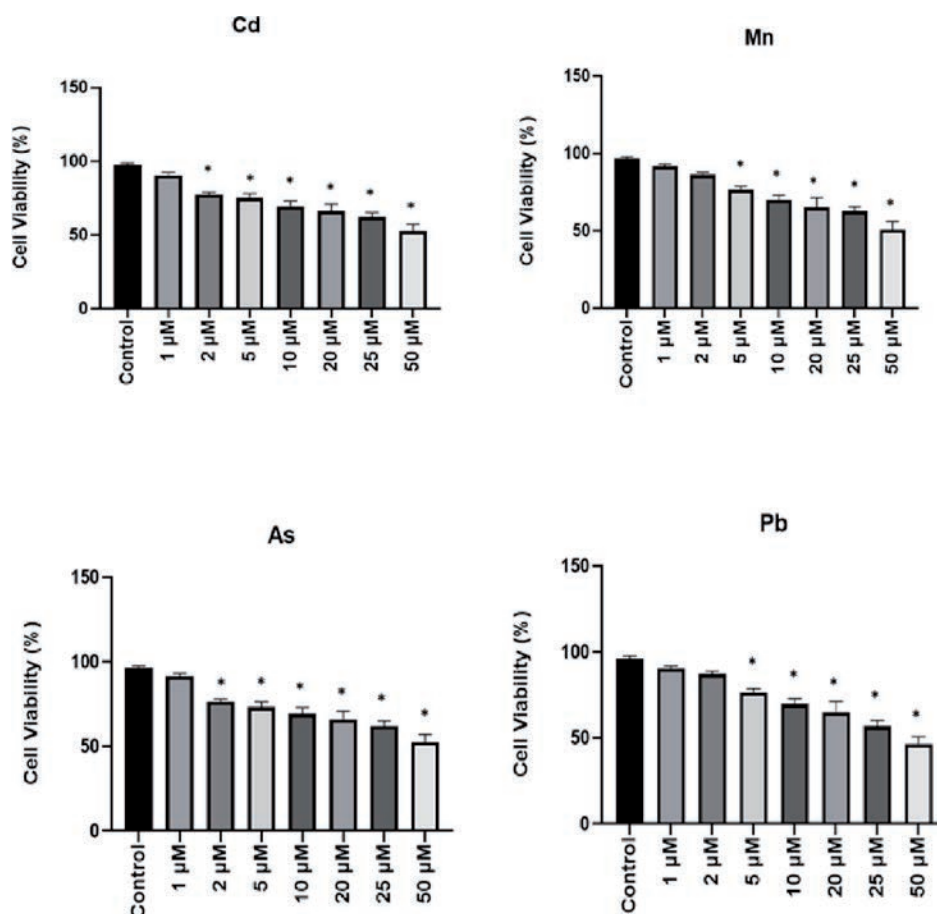
Data were expressed as means  $\pm$  SEM. Statistical analysis was performed by GraphPad Prism 10.0 (GraphPad Software, San Diego, CA, USA) using one-way analysis of variance (ANOVA) and Tukey's multiple comparisons post hoc test to

compare the means of the experimental groups. Data represents duplicate measurements from three independent experiments. Groups that do not share the same letters are significantly different ( $p < 0.05$ ).

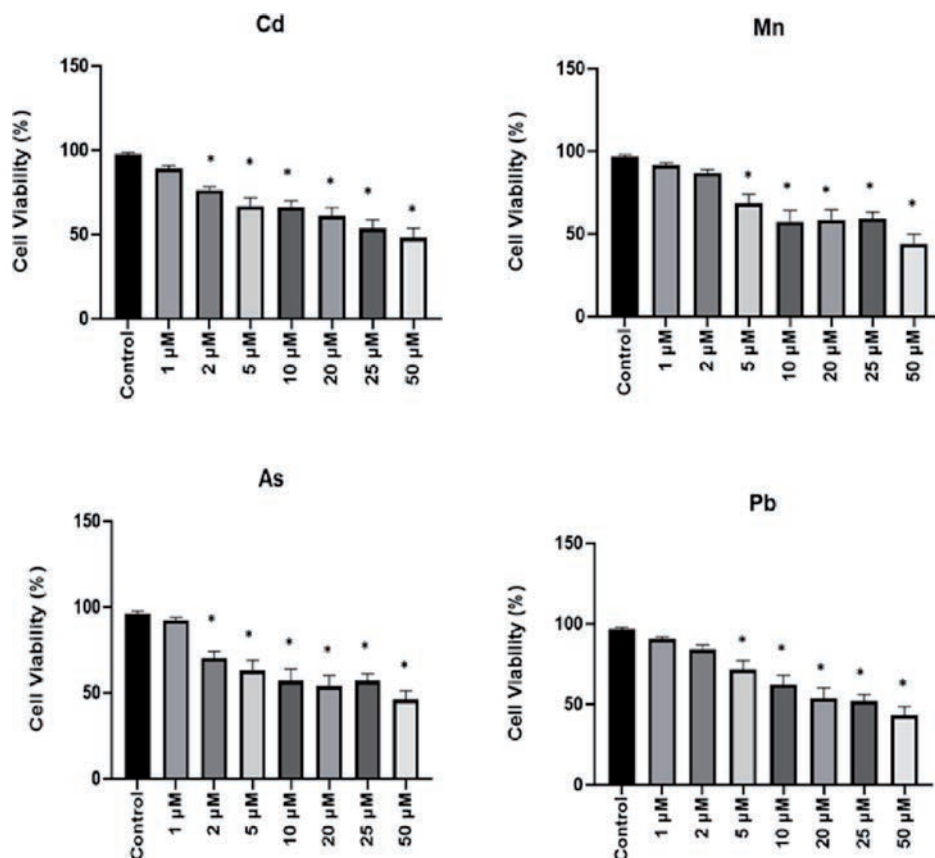
### 3. Results

#### 3.1 Cytotoxic effects of heavy metals on MIN6 and INS-1 pancreatic $\beta$ cells

The impact of various heavy metals on the viability of MIN6 and INS-1 pancreatic  $\beta$  cells was assessed using the MTT assay. To evaluate whether these metals could synergistically enhance cytotoxicity, the cells were exposed to  $\text{CdCl}_2$ ,  $\text{MnCl}_2$ ,  $\text{NaAsO}_2$ , and  $\text{PbCl}_2$  for 24 h at varying concentrations. A significant reduction in cell viability was observed at 2  $\mu\text{M}$  concentrations of  $\text{CdCl}_2$  and  $\text{NaAsO}_2$ , whereas  $\text{MnCl}_2$  and  $\text{PbCl}_2$  induced notable cytotoxicity at 5  $\mu\text{M}$  in both cell lines (Figures 1 and 2).



**Figure 1.**  
 Dose-dependent effect of heavy metals on MIN6 cell viability. Different doses of  $\text{CdCl}_2$ ,  $\text{MnCl}_2$ ,  $\text{NaAsO}_2$ , and  $\text{PbCl}_2$  were exposed to MIN6 for 24 h. Data are presented as mean  $\pm$  SEM, representing at least three independent experiments ( $n = 3$ ). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test, with  $p < 0.05$  indicating significant differences between control and treatment groups.



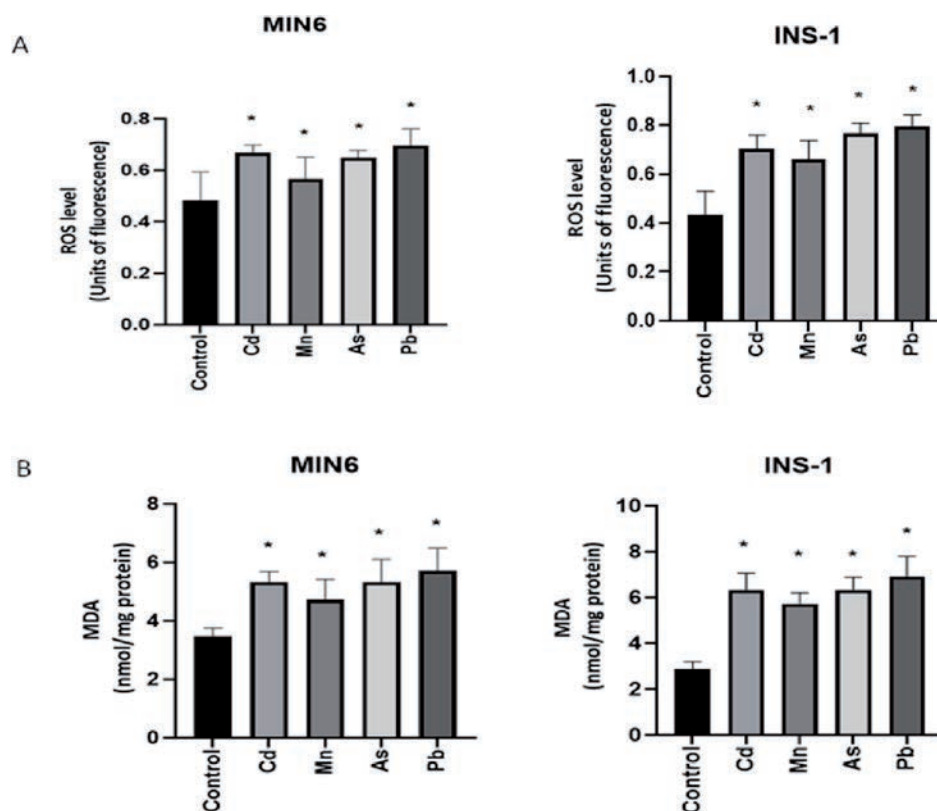
**Figure 2.** Dose-dependent effect of heavy metals on INS-1 cell viability. Different doses of  $\text{CdCl}_2$ ,  $\text{MnCl}_2$ ,  $\text{NaAsO}_2$ , and  $\text{PbCl}_2$  were exposed to MIN6 for 24 h. Data are presented as mean  $\pm$  SEM, representing at least three independent experiments ( $n = 3$ ). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test, with  $p < 0.05$  indicating significant differences between control and treatment groups.

### 3.2 Induction of oxidative stress by heavy metals

The influence of heavy metals on intracellular ROS levels and lipid peroxidation (MDA) was evaluated. As shown in **Figure 3A** and **B**, exposure to cytotoxic concentrations of the metals significantly elevated ROS levels in both cell lines compared to untreated controls. Lipid peroxidation, assessed by measuring malondialdehyde (MDA) levels, also increased markedly following treatment with 2  $\mu\text{M}$   $\text{CdCl}_2$ , 2  $\mu\text{M}$   $\text{NaAsO}_2$ , and 5  $\mu\text{M}$   $\text{MnCl}_2$  or  $\text{PbCl}_2$  (**Figure 3B**).

### 3.3 Effect of heavy metals on insulin secretion

To assess insulin secretion, MIN6 and INS-1 cells were incubated with 2.5 mM glucose (low glucose, negative control) or 16.7 mM glucose (high glucose, positive control). Cells treated with 2  $\mu\text{M}$   $\text{CdCl}_2$ , 2  $\mu\text{M}$   $\text{NaAsO}_2$ , 5  $\mu\text{M}$   $\text{MnCl}_2$ , or 5  $\mu\text{M}$   $\text{PbCl}_2$  showed a significant decline in insulin release compared to the high glucose control (**Figure 4**), indicating a disruption in  $\beta$ -cell function.

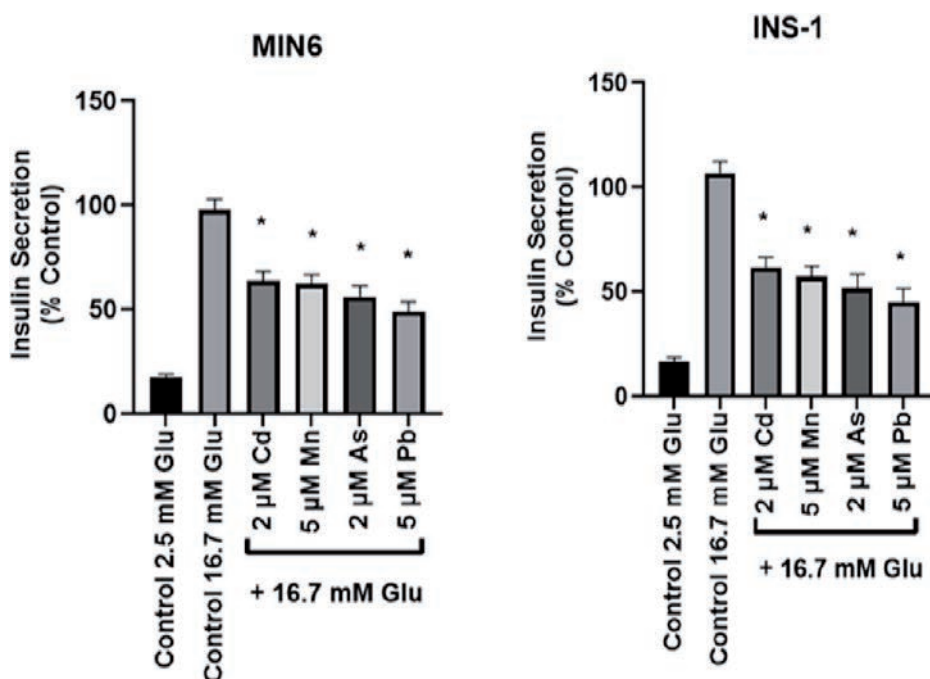


**Figure 3.** Heavy metals increased intracellular ROS (A) and MDA (B) levels in MIN6 and INS-1 cells. The effect of heavy metals on intracellular levels of ROS (A) and (B) MDA in MIN6 and INS-1 cells was determined by assays. Data are presented as mean  $\pm$  SEM, representing at least three independent experiments ( $n = 3$ ). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test, with  $p < 0.05$  indicating significant differences between control and treatment groups.

## 4. Discussion

The present study investigated the cytotoxic effects of four environmentally relevant heavy metals: Cd, Mn, As, and Pb, on pancreatic  $\beta$ -cell function using two widely accepted *in vitro* models: MIN6 and INS-1 cells. Our findings demonstrate that exposure to these metals significantly impairs  $\beta$ -cell viability, disrupts GSIS, and elevates intracellular ROS, all of which are indicative of  $\beta$ -cell dysfunction and stress.

Cd was found to be the most toxic among the tested metals, consistent with previous reports highlighting its high affinity for sulfhydryl groups in proteins and its ability to disrupt cellular redox homeostasis [7]. *In vitro* studies using MIN6 and INS-1 cell lines have demonstrated that Cd reduces cell viability, disrupts insulin gene expression, and impairs insulin secretion through mechanisms involving mitochondrial dysfunction and the generation of reactive oxygen species [8, 24]. For example, Hong et al. [7] reported that Cd exposure at low micromolar concentrations significantly decreased insulin secretion in MIN6 cells by interfering with calcium homeostasis and mitochondrial potential.



**Figure 4.**

The effect of heavy metals on insulin secretion in high glucose-treated MIN6 and INS-1 cells. Insulin secretion in MIN6 and INS-1 cells treated with high glucose (16.7 mM) was significantly reduced following exposure to heavy metals. Data are presented as mean  $\pm$  SEM, representing at least three independent experiments ( $n = 3$ ). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test, with  $p < 0.05$  indicating significant differences between control and treatment groups.

As, commonly present in contaminated drinking water, it has also been shown to damage  $\beta$ -cells. Chronic exposure to inorganic arsenic compounds such as  $\text{NaAsO}_2$  has been linked to impaired insulin signaling and  $\beta$ -cell death. *In vitro*, exposure in MIN6 cells has been shown to reduce GSIS, increase oxidative stress markers, and alter  $\beta$ -cell gene expression, including key regulators such as Pdx1 and Ins1 [25]. Similarly, Sanchez-Soria et al. [26] demonstrated that arsenic trioxide induced mitochondrial fragmentation and suppressed insulin secretion in INS-1 cells, emphasizing the role of mitochondrial integrity in  $\beta$ -cell survival. Those results aligned with this study, supporting the hypothesis that chronic arsenic exposure contributes to insulin resistance and the development of DM.

Pb exposure led to moderate but significant reductions in both viability and insulin output. Pb is known to interfere with calcium signaling and disrupt mitochondrial activity, both of which are essential for insulin granule exocytosis. Furthermore, Pb-induced ROS production likely contributes to lipid peroxidation and membrane damage in  $\beta$ -cells. Our results align with previous studies, reporting an association between elevated blood lead levels and impaired glucose tolerance [11, 14].

Mn as an essential trace element, exerted cytotoxic effects at elevated concentrations. Mn-induced  $\beta$ -cell dysfunction is likely mediated through oxidative stress and mitochondrial dysregulation, consistent with earlier studies showing Mn overexposure impairs insulin signaling and alters antioxidant enzyme activities [15, 25]. Interestingly, the biphasic nature of Mn's biological activity—beneficial at physiological levels but harmful at toxic concentrations—highlights the need for precise regulation of metal exposure in maintaining  $\beta$ -cell integrity.

Comparative analysis of MIN6 and INS-1 cells revealed generally consistent responses across both models, though some variation in sensitivity was noted [27, 28]. This may reflect species-specific differences in metal uptake, detoxification pathways, or antioxidant capacity. Nonetheless, the use of both cell lines strengthens the translational relevance of our findings, suggesting that heavy metal exposure poses a substantial risk to  $\beta$ -cell function across mammalian systems [19, 27].

Overall, our study reinforces the emerging paradigm that environmental pollutants—particularly heavy metals—are important, but often overlooked, contributors to  $\beta$ -cell dysfunction and the pathogenesis of DM. The observed impairments in cell viability, insulin secretion, and redox balance underscore the need for stricter environmental controls and increased public health efforts to mitigate exposure to these toxic elements. Future research should aim to elucidate the precise molecular mechanisms underlying metal-induced  $\beta$ -cell damage and explore potential protective strategies, such as antioxidant supplementation or pharmacological intervention.

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## Conflict of interest

The authors declare no conflict of interest.

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