Chapter 5
Effect of zinc oxide nanoparticles on pancreatic beta cell mass and function

Dynamics of beta cell mass
Chapter 5

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5.1. Introduction

Loss of beta cell mass and function is an ultimate consequence of diabetes, irrespective of its type. In type 1 diabetes, generation of antibodies against pancreatic beta cells results in severe loss of beta cell mass and insulin deficiency. In type 2 diabetes, pancreatic beta cells synthesize more insulin, known as compensatory hyperinsulinemia, to compensate for the hyperglycemia due to insulin resistance. However, beta cells eventually fail to compensate for the increased insulin demand rendering them vulnerable to death, this phenomenon often referred to as beta cell decompensation (Campos, 2012).

For the treatment of diabetes, besides increasing insulin sensitivity, preservation and/or replenishment of beta cell mass is also an effective therapeutic approach. However, increasing the proliferation of pancreatic beta cells is a challenging task, as they are quiescent cells and difficult to regenerate. The quest for developing newer anti-diabetic drugs is now increasingly focused on strategies / therapies for replenishing or conserving pancreatic beta cells (Vetere et al., 2014).

A major cause of pancreatic beta cell loss is oxidative stress. The results described in the present work (Chapter 4) confirmed the beneficiary role of ZON in the protection of rat pancreatic beta cells (RIN5f) from externally induced oxidative stress. Taking a step further, we have studied effects of ZON on pancreatic beta cell mass and function. Pancreatic beta cell mass is a function of beta cell loss and proliferation (Vetere et al., 2014). Therefore, effects of ZON on pancreatic cell death as well as proliferation were studied.
5.2. Cytotoxicity assay

Cytotoxicity of ZON to RIN5f cells was evaluated using MTT reagent as elaborated in section 3.3. Results of cytotoxicity assay revealed that ZON were safe up to the concentration of 10 µg/mL (~100% viability). At higher concentrations, i.e. 30 and 100 µg/mL, ZON showed cytotoxic effect, reducing the viability to 60 and 20% respectively (Figure 5.1). These results are in agreement with our previous results (in chapter 4 section 4.3) where higher concentrations of ZON (30 and 100 µg/mL) were found to exert oxidative stress and result in apoptotic death.

![Cytotoxicity assay in RIN5f cells. ***p<0.001 as analysed by One-way ANOVA followed by Dunnett’s multiple comparison test](image)

**Figure 5.1:** Cytotoxicity assay in RIN5f cells. ***p<0.001 as analysed by One-way ANOVA followed by Dunnett’s multiple comparison test

5.3. Cell death analysis

5.3.1. TUNEL assay

The apoptotic cell death was detected using In Situ Cell Death Detection Kit, Fluorescein (Roche Holding, AG, Switzerland). The assay is based on the detection of ss- and ds- DNA breaks that occur at the early stages of apoptosis. For experiments, RIN5f (4 x 10⁵) cells were seeded on coverslips in a 6 well plate and incubated for 72 h. Cells were then treated with saline or different concentrations of ZON (1, 3, 10, 30 and 100 µg/mL) for 24 h. Spent medium
was removed, and coverslips were stained using In Situ Cell Death Detection Kit as per manufacturer's instructions and visualized under fluorescence microscope (Eclipse E200, Nikon, Japan) at 400X magnification. Further, fluorescence intensity was measured (minimum 5 images per treatment) using ImageJ software (National Institute of Health, USA). The ratio of fluorescence intensity of apoptotic cells (green) to total cells (blue) was plotted in a graph using GraphPad Prism software (version 5, GraphPad Software Incorporation, USA). Statistical analyses were performed using One-way ANOVA followed by Dunnett's multiple comparison test.

It is evident from the Figure 5.2 A that ZON treatment at higher concentrations viz., 30, 100 µg/mL induced apoptotic cell death (increased green fluorescence), whereas, no apoptotic cell death was observed at lower concentrations of ZON (1, 3, 10 µg/mL). As can be seen in Figure 5.2 B, ratio of apoptotic cells to total cells was statistically higher after ZON treatment (30, 100 µg/mL) as compared to control. At lower concentrations of ZON treatment, apoptosis was not seen.

(A)
5.3.2. DNA fragmentation analysis

Fragmented DNA (180-200 basepairs) is a hallmark of apoptotic cell death which on gel electrophoresis produces a laddering pattern (Saadat et al., 2015). For experiments, RIN5f cells (5 x 10^6) were seeded in 25cm² tissue culture flasks and incubated for 72 h. Cells were then treated with saline or different concentrations of ZON (1, 3, 10, 30 and 100 µg/mL) for 12 h. Selective apoptotic DNA extraction was performed as per reported protocol (Darzynkiewicz and Juan, 1999). Briefly, cells were first collected by trypsinization and centrifugation. The cell pellet was resuspended in 1 mL PBS. Cells were then fixed in 9 mL of 70% ethanol on ice; and centrifuged at 1000 rpm for 5 min. Supernatant was decanted and the pellet was resuspended in 50 µl of extraction buffer (192 mL of 0.2 M Na₂HPO₄ and 8 mL of 0.1 M citric acid, pH 7.8) followed by incubation for 30 min. Further, RNase A treatment was given (5 µl of 2 mg/mL RNase A solution was added and incubated at 37°C for 30 min, Sigma-
Aldrich, USA) followed by proteinase K treatment (5 µl of 1 mg/mL proteinase K solution was added and incubated at 37°C for 30 min, Sigma-Aldrich, USA). DNA suspensions so obtained were then mixed with loading buffer, loaded on 0.8 % agarose gel (containing 5 µg/mL ethidium bromide in 1X Tris-acetate-EDTA buffer, pH 8.5) and electrophoresed at 90 V for 1.5 h. High range DNA ruler 100 bp - 10 kb (Banglore Genei, India) was used as a marker. Bands on the gel were visualized under UV light.

It was seen that saline control and low concentrations of ZON (1, 3, 10 µg/mL) did not show any DNA fragmentation (Figure 5.3). However, DNA laddering was evident at high concentrations of ZON (30, 100 µg/mL) indicating apoptotic cell death.

![Figure 5.3: DNA fragmentation analysis in ZON treated RIN5f cells.](image)
Taken together, results of TUNEL assay and DNA fragmentation confirm apoptotic cell death at cytotoxic concentrations of ZON.

5.4. Effect of ZON on pancreatic beta cell proliferation *in vitro*

5.4.1. Cell viability assay

Once the apoptotic cell death was confirmed at high concentrations of ZON treatment, it was important to study the effect of lower concentrations of ZON treatment on pancreatic beta cell mass. Cell viability was assessed by trypan blue dye exclusion method. Briefly, RIN5f cells (1x 10⁴, grown in 6 well plates) were exposed to saline or different concentrations of ZON (1, 3, 10 μg/mL) for different time intervals (0, 12, 24, 36, 48 and 60 h). After treatment, cells were collected by trypsinization, centrifuged and re-suspended in 1 mL fresh medium. Next, 10 μl of cell suspension was admixed with 10 μl of trypan blue (0.4% in PBS, HiMedia, India). The numbers of viable (unstained) cells were counted using a hemocytometer. Fold change in proliferation was calculated using the formula:

\[
\text{Fold change in proliferation} = \frac{\text{Cell viability treated} - \text{Cell viability control}}{\text{Cell viability control}}
\]

The graph of fold change in proliferation with respect to time was plotted using GraphPad Prism software (version 5, GraphPad Software Incorporation, USA). Statistical analyses were performed using Two-way ANOVA followed by Bonferroni post test.

Cell viability studies revealed an increase in the number of viable RIN5f cells in a time- and dose- dependent manner (Figure 5.4). ZON treatment (10 μg/mL) resulted in ~3 fold increase in cell proliferation as compared to saline control.
Figure 5.4: Cell viability assay of RIN5f cells exposed to ZON

**p<0.01 and ***p<0.001 as analysed by Two-way ANOVA followed by Bonferroni post test

5.4.2. Cell proliferation assay (Ki67 staining)

Ki67 is a cellular marker for proliferation which is present in all active phases of cell cycle viz., G1, S, G2, mitosis but is absent in resting state i.e. G0. An increase in the presence of Ki67 is indicative of actively proliferating cells. The ability of ZON to induce proliferation of pancreatic beta cells was evaluated by Ki67 immunocytochemistry.

For experiments, RIN5f cells (4 x 10^5, grown on lysine coated coverslips) were exposed to saline or different concentrations of ZON (1, 3, 10 μg/mL) for 24 h. Insulin (0.1 IU/mL) was used as positive control. Cells were washed with PBS, fixed with paraformaldehyde (4%, 15 min), permeabilized with Triton X-100 (0.25%, 15 min) and blocked using BSA (1% BSA in PBST) for 1 h. Cells were then incubated with anti- Ki67 antibody (1:300 dilution, 1 h, Thermo Fisher Scientific, USA). Endogenous peroxidases were blocked by H2O2 treatment.
(0.3% for 15 min). Next, cells were incubated with HRP labeled secondary antibody (1:500 dilution, 1 h, Thermo Fisher Scientific, USA). Brown color was developed by incubating with DAB substrate (7 min in the dark, Thermo Fisher Scientific, USA). Cells were counterstained using hematoxylin. Images were taken under an optical microscope (Eclipse E200, Nikon, Japan) at 400X magnification. Morphometric analysis was performed by counting the number of Ki67 positive cells and total cells from representative photomicrographs (10 images for each treatment). Cell proliferation (%) was calculated using the formula:

\[
\text{Proliferating cells (\%)} = \left( \frac{\text{Ki67 positive cells}}{\text{total number of cells}} \right) \times 100
\]

Representative photomicrographs of Ki67 immunostaining (brown spots) are presented in Figure 5.5 A. As can be seen from the figure, ZON treatment increased the proliferation of RIN5f cells, similar to insulin. Morphometric analysis revealed a dose-dependent increase in the number of proliferating cells (~30% at the highest concentration, Figure 5.5 B). Similarly, insulin, a known mitogenic agent, increased beta cell proliferation by ~25%.
Figure 5.5: Effect of ZON treatment on pancreatic beta cell proliferation. *p<0.05 and ***p<0.001 as analysed by One-way ANOVA followed by Dunnett's multiple comparison test.
5.5. Effect of ZON on pancreatic beta cell proliferation *in vivo*

One of the crucial findings of the present work was the proliferative effect of ZON on pancreatic beta cell line. However, it was important to assess if similar effects are observed *in vivo*. Therefore, the effect of ZON treatment on pancreatic beta islets was studied in type 1 diabetic rats.

All animal experiments were performed after approval from the institutional animal ethics committee (IAEC/CNB-01/2013). For induction of type 1 diabetes, 45 mg/Kg streptozotocin (Sigma-Aldrich, USA) was injected in the tail veins of 6-8 weeks old Wistar rats (Rodrigues et al., 1999). Animals showing hyperglycemia after a week were selected and grouped (n=6, uniformly distributed over glucose levels ~250-500 mg/dL). Diabetic rats were divided into four groups as, non-diabetic control, diabetic control, ZON treated and insulin treated. Insulin (4 IU/Kg, subcutaneous injection) was used as a positive treatment control. ZON (10 mg/Kg) was orally administered to the treatment group rats for 28 days. Non-diabetic control and diabetic control group rats received CMC (1 mL/Kg). On day 28, animals were sacrificed and pancreas were collected and stored in 10% formalin. Pancreas were later sectioned, and the tissue sections were stained using hematoxylin and eosin.

Figure 5.6 shows the histological sections of pancreatic tissue of treated and untreated rats. As expected, diabetic control rats showed reduced pancreatic islet size as compared to non-diabetic rats. However, ZON and insulin-treated diabetic rats showed increased pancreatic islet size, indicating increased proliferation of pancreatic beta cells.
5.6. Effect of ZON treatment on pancreatic beta cell function

To study the effect of ZON treatment on the function of pancreatic beta cells, insulin secretion assay was performed. RIN5f cells (4 × 10⁵) were seeded in a 24 well plate. Cells were allowed to grow for 72 h, after which the medium was changed to serum-free medium, and ZON treatment was given (1, 3 and 10 μg/mL). After 4 h, supernatants were collected, centrifuged and stored at -20 °C until further assay. Insulin content in the supernatants was determined by the Rat/Mouse Insulin ELISA Kit (Millipore Corp., MA, USA). To study the glucose-dependent effect, insulin secretion was assessed in RIN5f cells maintained at 3
different glucose concentrations viz., 5.5 mM (100 mg/dL), 11.1 mM (200 mg/dL) and 25 mM (450 mg/dL).

Results obtained revealed that treatment of RIN5f cells with ZON led to glucose- and dose-dependent increase in insulin secretion (Figure 5.7). At high glucose concentrations (11.1 mM and 25 mM), ~4 fold increase in insulin secretion was observed. Interestingly, at low glucose concentration (5.5 mM, normoglycemic condition), no significant increase in insulin secretion was observed, suggesting no risk of hypoglycaemia.

![Insulin secretion assay](image)

**Figure 5.7:** Insulin secretion assay in ZON treated RIN5f cells

*p*<0.05, **p*<0.01 and ***p*<0.001 as analysed by One-way ANOVA followed by Dunnett’s multiple comparison test

### 5.7. Discussion

In a diabetic individual, there is an imbalance between pancreatic beta cell proliferation and death, leading to net loss of beta cell mass (Meier and Bonadonna, 2013). Restoration of pancreatic beta cell mass is crucial for
delaying the progression of the disease. Results of the previous chapter suggested that ZON protect pancreatic beta cells from oxidative stress induced death. In this chapter, we have studied the role of ZON on pancreatic beta cell mass and function.

Conflicting literature exists on the effects of ZON on eukaryotic cells. Several reports suggest in vitro as well as in vivo toxic effects of ZON viz., oxidative stress and cell death (Sharma et al., 2012a; Sharma et al., 2012b). However, the concentrations of ZON used in these studies are very high (20 µg/mL to 300 mg/Kg). On the other hand, several reports demonstrate protective effects of ZON via anti-oxidative and anti-inflammatory properties (Das et al., 2013; Nagajyothi et al., 2015).

Our results of cytotoxicity studies revealed that ZON reduce cell viability only at higher concentrations of 30 and 100 µg/mL. Cell death analysis by TUNEL assay and DNA laddering suggested an apoptotic mode of death at higher concentrations of ZON. Lower concentrations of ZON (1, 3, 10 µg/mL) were found to be non-cytotoxic. These results are in agreement with a report by Muthuraman et al. (2014) where ZON is shown to exert dose-dependent cytotoxicity in adipocytes.

The indispensable role of zinc in cellular metabolic processes shows its importance in cell metabolism. Zinc is a known inhibitor of apoptosis, and depletion of zinc leads to programmed cell death in several cell types (Treves et al., 1994; Parat et al., 1997; Ahn et al., 1998; Chai et al., 2000). Interestingly, Shen et al. (2013) showed a strong correlation between the increased zinc levels and cell viability, where excessive zinc within cells leads to cell death in THP-1 immune cells. Excess of zinc becomes cytotoxic for cells leading to apoptotic cell death. Therefore, the intracellular concentration of zinc ions decides the fate of the cells for proliferation, differentiation or apoptosis (Chimienti et al., 2003).
Our cell viability studies showed ~3 fold increase in proliferation of RIN5f cells after ZON treatment at a lower concentration of 10 μg/mL. Immunocytochemistry studies of Ki67 (a proliferation marker) revealed that ZON treatment (10 μg/mL) increased beta cell proliferation up to ~30%, whereas, insulin (a known mitogenic hormone) increased beta cell proliferation by ~25%. Interestingly, oral administration of ZON to diabetic rats also resulted in increased pancreatic islet size, suggesting its role in preservation and/or restoration of pancreatic beta cell mass. Several reports are available on growth stimulatory effects of insulin (Straus, 1984). Ding et al. (2000) have reported that insulin induces glucose uptake as well as proliferation in pancreatic cancer cells such as CD11, CD18, HPAF, PANC-1, and MiaPaCa2. The role of zinc in cell proliferation is also well elucidated (Beyersman and Haase, 2001). However, the role of zinc oxide nanoparticles in cell proliferation is not entirely known.

The main function of pancreatic beta cells is to produce and secrete insulin. Physiologically, an increase in blood glucose levels is the primary signal for increasing insulin secretion. Besides, activation of phospholipases and protein kinase C; stimulation of adenyl cyclase activity and activation of beta-cell protein kinase A also enhance insulin secretion (Wilcox, 2005). Our results showed that treatment of pancreatic beta cells with ZON increases insulin secretion in a dose-dependent manner. These results are in agreement with a report by Nygaard et al. (2014) where, zinc supplementation (zinc chloride) within physiological range resulted in insulin secretion as well as proliferation of INS-E1 cells. Similar to our results, a recent report demonstrated protective effects of zinc oxide nanoparticles on pancreatic islets, where treatment with 70 ng/mL ZON increased cell viability as well as insulin secretion (Shoae- Hagh et al., 2014). Further, ZON mediated glucose-dependent insulin secretion in our study, suggesting no risk of hypoglcemia, unlike sulphonylureas.
The mechanism by which zinc oxide nanoparticles enhance insulin secretion is not yet clear. Richards-Williams et al. (2008) proposed that zinc acts as an autocrine molecule and activates P2X purinergic receptors (P2XR) channels thereby increasing glucose-stimulated insulin secretion (GSIS). Very recently a group of researchers has deduced a crystal structure of P2XR with a zinc binding site responsible for potentiation of the receptor (Kasuya et al., 2016). Together, these reports suggest a direct involvement of zinc ions in insulin secretion from pancreatic beta cells.

5.8. Salient findings

- Zinc oxide nanoparticles are safe up to 10 µg/mL and are cytotoxic at higher concentrations of 30 and 100 µg/mL.

- TUNEL and DNA laddering assay reveal apoptotic mode of cell death at higher concentrations of ZON (30 and 100 µg/mL).

- Cell viability assay showed that ZON at lower concentrations increases cell proliferation by 3 fold as compared to saline treated cells.

- Treatment of RIN5f cells with ZON resulted in 30% increase in Ki67 positive cells as compared to saline control, confirming proliferative effects.

- Increased pancreatic islet size in diabetic rats suggests that ZON treatment could delay disease progression and potentially reverse diabetes. Additional in vivo investigations on this aspect are warranted.

Taken together, results presented in this chapter elucidate the beneficial effects of ZON on pancreatic beta cell mass and function (summarized in Figure 5.8).
Figure 5.8: Effect of ZON on pancreatic beta cell mass and function
Chapter 6
Summary and Conclusions

Pleotropic anti-diabetic effects of ZON
Chapter 6

Summary and Conclusions

Diabetes is a group of metabolic disorders characterized by increased blood glucose levels. Current therapy for diabetes includes biguanides, sulphonylureas, thiazolidinediones, DPP-IV inhibitors and alpha glucosidase inhibitors. These agents are associated with several side effects. Further, these drugs are used either in combination with each other or with insulin, increasing treatment cost and patient non-compliance. Therefore, a single, cost-effective, oral antidiabetic therapy with multiple effects and fewer side effects is the need of the day.

Zinc is an essential micronutrient with marked effects on glucose metabolism, and its deficiency is correlated with diabetes. Considerable research has been carried out to develop a zinc based anti-diabetic agent. However, poor bioavailability, toxicity, other lesser investigated effects of the added pharmacophore, and lack of clinical data are some of the limitations that have adversely affected the development of these agents as drugs. Earlier, in our laboratory, zinc oxide nanoparticles (ZON) were investigated for anti-diabetic activity. ZON treatment to type 1 and type 2 diabetic rats resulted in significant lowering of blood glucose levels. Further, cytotoxicity, hemolysis, genotoxicity, acute and sub-acute toxicity studies revealed that ZON were safe up to 100 times the anti-diabetic dose. Encouraged by these previous results, the current study was designed to identify the key mediators of anti-diabetic actions of ZON.

Earlier pharmacokinetic studies revealed increased zinc levels in blood up to 24 h and preferential tissue uptake. Further, in vitro dissolution study revealed that not all ZON gets ionized in aqueous media suggesting the presence of particulate zinc. Thus, it was essential to assess whether ZON get internalized and increase intracellular zinc ion levels.
In the present study, internalization experiments revealed rapid internalization of the ZON in RIN5f and HepG2 cells without inducing any alterations in cell morphology. Internalized particles could be observed up to 24 h in RIN5f cells, indicating sustained effects. Further studies revealed that ZON co-localized with lysosomal compartments in the cells. Zinquin staining experiments revealed that treatment with ZON resulted in a time- and concentration-dependent increase in intracellular zinc ions. Together, these results suggest an endocytotic mode of ZON uptake, lysosomal dissolution and subsequent release of zinc ions.

To study the mechanism of anti-diabetic effects of ZON (1, 3, 10 µg/mL), different parameters were evaluated as described below. Insulin (0.1 IU/mL) was used as a positive control in all these experiments.

Protein tyrosine phosphatase IB (PTP1B) is a negative regulator of insulin signaling. Treatment with ZON increased phosphorylated (S\textsuperscript{50}) PTP1B levels in HepG2 and L6 cell lines, suggesting its inhibition and improved insulin signaling.

Protein kinase B (PKB) is a key regulator of the several downstream events in the insulin signaling pathway. It was observed that ZON treatment to HepG2 and L6 cells lead to increased levels of phosphorylated PKB resulting in its activation, suggesting improved glucose metabolism in the liver as well as muscle.

GLUT4 is an insulin responsive glucose transporter found in muscles and fat tissue. Translocation of GLUT4 from vesicles to membrane surface increases glucose uptake. ZON treatment to L6 and differentiated adipocytes increased GLUT4 translocation and increased glucose uptake, similar to insulin.

Treatment with ZON also reduced G-6-Pase and PEPCK gene expression in a dose-dependent manner, comparable to insulin. These results suggested
Reduced gluconeogenesis and hepatic glucose output after ZON treatment. Expression of glucokinase gene was not altered after ZON treatment, suggesting no effects on glycolysis.

Hormone-sensitive lipase (HSL) is a key regulatory enzyme in lipolysis pathway. Insulin has an inhibitory effect on HSL resulting in inhibition of lipolysis. Reduction in levels of phosphorylated HSL after ZON treatment suggested inactivation of HSL, thereby inhibition of lipolysis.

Oxidative stress plays a major role in pathogenesis and progression of diabetes mellitus. In severe diabetic situations, uncontrolled hyperglycemia and elevated levels of free fatty acids leads to ROS generation and oxidative stress, adversely affecting pancreatic beta cell mass and function. Zinc is reported to be a structural component of Cu-Zn-Superoxide dismutase and enhance its activity. Hence, it was worthwhile to study anti-oxidative effects of ZON.

ROS staining suggested that treatment with ZON did not induce ROS production at lower concentrations (1, 3, 10 μg/mL) whereas, mild increase in ROS was observed at higher concentration (30 μg/mL). Superoxide dismutase enzyme activity was increased after ZON treatment, suggesting anti-oxidative effects. ZON treatment also resulted in an increase of reduced glutathione content in cells, indicating enhanced ability of cells to counter oxidative stress. Acridine orange/ Ethidium bromide (AO/EB) staining showed no significant alteration in cell morphology up to 10 μg/mL concentration whereas, at higher concentrations of ZON (30, 100 μg/mL) cells showed apoptotic death. Taken together, these results suggested that ZON per se do not induce oxidative stress.

Further, to study protective effects of ZON, RIN5f cells were challenged with H₂O₂ induced oxidative stress. ROS staining revealed that pre-treatment with ZON reduced H₂O₂ induced ROS generation. Further, ZON pre-treatment
increased SOD activity and enhanced reduced glutathione levels, indicating a protective effect of ZON against H₂O₂ induced oxidative stress. AO/EB assay suggested that treatment with ZON protected RIN5f cells from H₂O₂ induced apoptotic cell death.

Progressive pancreatic beta cell loss is a characteristic feature of type 1 and type 2 diabetes. In a diabetic individual, there is an imbalance between pancreatic beta cell proliferation and death, leading to net loss of beta cell mass. Replenishment of pancreatic beta cell mass can be a useful strategy for treatment of diabetes. In the present work, the effect of zinc oxide nanoparticles on pancreatic beta cell mass was evaluated by studying cell death as well as cell proliferation.

Cytotoxicity studies in RIN5f cells suggested that ZON were safe up to 10 μg/mL, beyond which they were cytotoxic. The mechanism of cell death at high concentrations of ZON was found to be apoptotic as evidenced by TUNEL assay and DNA fragmentation studies.

Immunocytochemistry studies of Ki67 (a proliferation marker) revealed that treatment with ZON increased cell proliferation, similar to insulin. Further, cell viability studies revealed ~3 fold increase in cell proliferation after ZON treatment. Increased pancreatic beta islets size in diabetic rats confirmed proliferative effects of ZON. Further, ZON treatment increased insulin secretion from RIN5f cells in glucose- and dose- dependent manner. Glucose-dependent insulin secretion suggested no risk of hypoglycemia. These results suggested beneficial effects of ZON on pancreatic beta cell mass and function.

Thus, zinc oxide nanoparticles exert pleiotropic anti-diabetic effects summarized in Figure 6.1.
Conclusions

In the present work, the key mediators of anti-diabetic effects exerted by zinc oxide nanoparticles (ZON) have been identified. This study has thus provided incremental data on the mechanism of action of ZON and added to the scientific knowledge. Further, the current study has laid the foundation for basic research to elucidate the detailed mechanism of action, especially with respect to glucose dependent insulin secretion and pancreatic beta cell proliferation.

Although, the cytotoxicity of zinc oxide nanoparticles is well reported, these reports are conflicting. Further, there is a contradiction related to effects of ZON on oxidative stress. A comprehensive investigation was therefore initiated on the cytotoxicity and oxidative stress parameters across a wide concentration range.
range of ZON. Results of the present investigation revealed differential dose dependent effects of ZON on cytotoxicity and oxidative stress; a significant addition to the existing knowledge base. Furthermore, it was observed that ZON at non-cytotoxic concentrations in fact protected pancreatic beta cells from externally induced oxidative stress. The novelty of the present work therefore lies in elucidation of the differential effects of ZON on pancreatic beta cells and its implications in diabetes treatment.

In the light of above findings, the present work tends to the advancement of knowledge and paves the way for future research.