



## The effect of insulin loaded nanoparticles on immuno-reactivity of beta cells in rats with diabetes type 1

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**Abstract:** The basic criteria of type 1 diabetes (T1D) are autoimmune destruction of beta cells that eventually leads to a full loss of insulin secretion capability, which is a crucial stage in the disease's pathophysiology. In recent investigations, it was shown that pancreatic tissues from long-term T1D patients included insulin-producing beta cells. These findings suggested the presence of working beta cells that had evaded apoptosis by lowering gene expression. The development of diabetic liver damage has been linked to oxidative stress, the formation of reactive oxygen species (ROS), and diabetes infections linked to beta-cell dysfunction. Other diabetes-related effects include immune-histochemical evaluation of the pancreas in male Wistar rats following oral treatment of insulin-loaded nanoparticles (INS) containing carboxymethyl chitosan nanoparticles (CMCNPs) and gold nanoparticles (AuNPs) independently. Because of their inhibitory and protective impact, beta cells' immunological reactivity was dramatically boosted as compared to the diabetic group, and a reduction in pancreatic inflammation was noted. In conclusion, promising effects of insulin-loaded carboxymethyl chitosan NPs (INS-CMCNPs) and insulin-loaded gold NPs (INS-AuNPs) in controlling and promoting organ functions when compared to subcutaneous insulin were observed. This may provide new evidence for the reversibility of beta-cell dysfunction and insulin insufficiency with precise glycaemic control.

**Keywords:** insulin, loaded nanoparticles, carboxymethyl chitosan nanoparticles, gold nanoparticles, beta-cell dysfunction.

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### 1. INTRODUCTION

The fundamental definition of T1D is a chronic illness in which the immune system destroys the insulin-producing pancreatic beta cells, resulting in hyperglycemia <sup>1</sup>. Genetics alone cannot account for the disease's development. In patients with risk factors, the death of these cells typically occurs before the clinical manifestations of diabetes. Apoptosis processes are caused by a variety of factors that are currently unknown <sup>2</sup>. Genetic and environmental variables have the most pivotal role. This multifaceted impact results in an autoimmune assault on the beta cells and hyperglycemia due to the loss of insulin from the beta cells. Despite significant

advances in the pathophysiology of the illness, the etiology of T1D development remains a mystery <sup>3</sup>. Type 1 diabetes is the most frequent type of diabetes in children, accounting for roughly 75 percent of new diabetes diagnoses in patients under the age of 19. According to epidemiological research, the incidence of autoimmune diabetes in people aged 30-49 years is now at least as high as in young adults aged 15 to 19 years <sup>4</sup>.

Because the age of diagnosis has a substantial influence on insulin levels at the time of diagnosis but may have less impact on the contour of decline, various longitudinal studies have been conducted to examine the gradual reduction in cell function following type 1 diabetes diagnosis <sup>5</sup>. Other studies, on the other hand, discovered that the loss of

cell function following diagnosis occurs more quickly in younger persons. It's becoming clear that the degree of beta-cell loss is related to age and the length of the disease. So even though the beta-cell loss is much more serious in the pancreas of younger children, 40 to 60 percent of islets in individuals identified in their teenage years or later tested positive for insulin<sup>6</sup>. The causes of beta-cell loss and malfunction vary from person to person and are dependent on a variety of circumstances. Functional studies of insulin production in live persons concur with these pathological results in a significant majority of instances. During the diagnostic stage, the degree of decreased insulin secretion is greater in younger individuals<sup>7</sup>.

Hyperglycemia and the production of reactive oxygen species (ROS) are known to disrupt beta-cell homeostasis, placing an increased demand on beta cells to produce insulin and antioxidant proteins, which can result in increased protein content and Endoplasmic reticulum stress. Furthermore, excessive ROS can damage proteins and organelles, seeking to make it more difficult for the cell to activate those kinds of adaptive stress-responsive pathways<sup>1</sup>. The toxicity of these compounds is dependent on their constant and endogenous antioxidant levels. In fact, research on exogenous antioxidants has shown that scavenging ROS is necessary for many years. This has led to the approval of numerous compounds derived from natural or synthetic sources for the treatment of various disorders. Most importantly, it has been demonstrated that a number of nanomaterials effectively boost endogenous antioxidants' activities by preventing them from degrading in harsh pH circumstances seen in the cellular environment. Additionally, nanomaterials can imitate different antioxidants or supply antioxidants to specific tissues to function as artificial redox systems, which can greatly reduce ROS-induced damage. With the innovative strategy of using insulin-loaded oral CMCNPs and AUNPs for type 1 diabetes treatment application, new hypotheses were generated<sup>8</sup>. CMCNPs and AuNPs have an antioxidant protective mechanism by reducing reactive oxygen species (ROS), protecting the pancreas beta cells from destruction by suppressing the free radical responsible for organ dysfunction and suppressing lipid peroxidation.

The main reasons people are reluctant to begin insulin therapy are anxiety from self-injection and discomfort from needles. Beginning insulin medication along with lifestyle adjustments significantly modifies and manages glucose levels. Numerous studies have shown that phobia of injections causes a two-year delay in the commencement of insulin therapy, even in those who eventually begin it because peripheral

hyperinsulinemia and other health issues are directly tied to SC insulin. A different strategy known as insulin pens helped some patients who were having trouble using needles, for an instance, but it also raised other issues. Due to the aforementioned factors and to improve diabetes adherence to insulin therapy, researchers started looking into a different new strategy known as oral insulin, which may increase the uptake of insulin therapy while avoiding the drawbacks of SC injection<sup>9</sup>.

Researchers preferred to use chitosan as nano-carriers because it could be used for both hydrophilic and hydrophobic medicines, as well as being non-toxic and target-specific, allowing for the supplementation of long-term lower doses of the drug in a controlled manner. Chitosan nanoparticles have different drug release mechanisms. Drug-loaded chitosan nanoparticles can improve patient adherence to treatment regimens<sup>10</sup>. Since chitosan depends on the mechanism of surface charge, which is sensitive to the pH of the surrounding environment and can dissolve at acidic pH due to its pKa of 6.5, it can open the epithelial junction and permit hydrophilic molecules like insulin to flow through. Chitosan depends on the protonation of amino groups, which is essential for the NPs' subsequent surface charge. Since it is uncommon in other nanoparticles and is considered helpful during medication delivery, this feature is perceived as a key benefit for chitosan nanoparticles. When scientists discovered that NPs could selectively distribute insulin throughout the intestinal medium in vivo, rather than just protecting the stomach, they created carboxymethyl chitosan to improve the physical and chemical properties of chitosan<sup>11</sup>.

Due to their unique physicochemical characteristics, gold nanoparticles are unquestionably the second most common nanoparticles. Since ancient times, gold has been utilized for both healing and decorative purposes. One of the materials that have undergone the most rigorous research to address both basic and practical issues in medicine is AuNPs, thanks to the explosive expansion of nanotechnology in the late 20th and early 21st centuries. Gold nanoparticles were produced by physical and chemical means. As was already indicated, nanoparticles have recently been used in nanomedicine as synthetic redox systems. In contrast to naturally occurring antioxidants, cerium oxide nanoparticles resemble SOD and catalase (CAT) and exhibit higher catalytic rates. The fact that AuNPs have mixed valence, which enables them to react with superoxide and hydrogen peroxide to detoxify ROS, may explain why they also exhibit CAT mimetic activity. The AuNPs coating on the insulin, however, prevents an increase in the antioxidants' stability<sup>12</sup>.

Therefore, this research proposed to determine, investigate and compare the effect of INS-CMCNPs and INS-AuNPs on the immuno-reactivity of beta cells in rats with diabetes type 1 and discover their protective effect on the pancreas.

## 2. METHODS

### 1.1. NP's preparation:

To make CMCNPs, ionic gelation of carboxymethyl chitosan (CM-chitosan) (**Fischer Co. Germany**) was implemented. Carboxymethyl chitosan (CMCs) was dissolved in deionized water at a predetermined concentration. Then 2 mL of Calcium chloride (CaCl<sub>2</sub>) (**Win Lab Co. India**) solution was added to 3 mL of CMCs solution under gentle magnetic stirring. The system then transformed spontaneously from a clear solution to an opalescent emulsion; the CMCs concentration was 500 mg/mL, and the CaCl<sub>2</sub> concentration was 1.0 mg/mL.

Insulin loading NPs were created in the same way by adding CaCl<sub>2</sub> solution to a CMCs solution containing 3 mL of insulin, and the NPs were separated by centrifugation at 20,000 g (**3k30, Sigma, USA**) at 4°C for 30 minutes and lyophilized for future analysis.

Dextran (0.3 g) (**Fischer Co., Germany**) was dissolved in 50 mL of deionized water containing 0.001 g of Sodium Hydroxide (NaOH) (**Win Lab Co., India**) at room temperature under magnetic stirring using the Turkevich technique for the synthesis and loading of (AuNPs) with insulin. Following full dissolving, 3 mL chloroauric acid (0.017 M) (**Sigma-Aldrich Co., USA**) was added

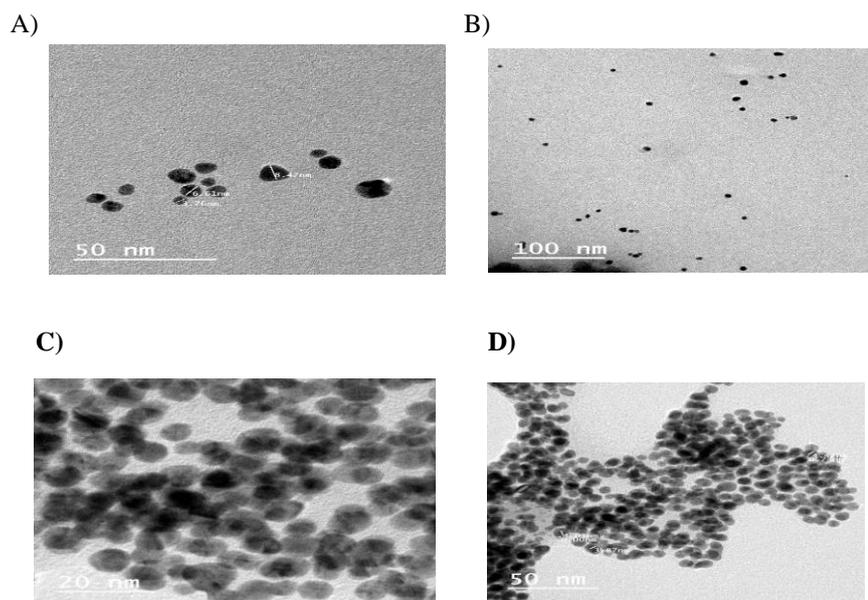
dropwise to the dissolved dextran in a lab microwave as the reaction continued for 30 seconds. After this period, the solution's appearance changed from colorless to a red-violet tint, indicating the creation of (AuNPs).

After allowing the solution to cool to room temperature, 3 mL of insulin was added to 3 mL of AuNPs and mechanically stirred for 15 minutes. The produced AuNPs loaded with insulin were then stored in the dark for subsequent investigation.

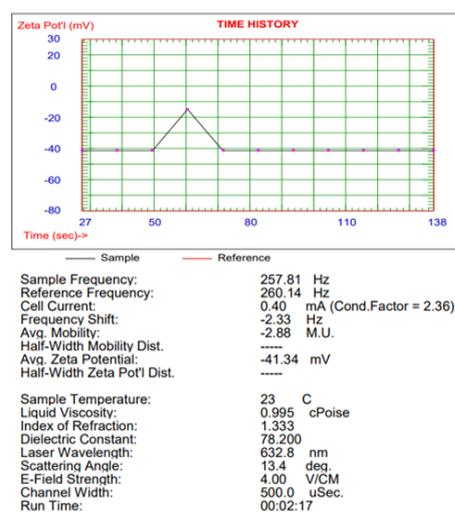
### 1.2. Characterization of NPs:

Transmission electron microscopy (TEM) and spectrophotometer were used to characterize the morphology, size, and form of the produced NPs. In the instance of Transmission electron microscopy, a drop of colloidal NPs solution adjusted to 20 mM was deposited onto a carbon-coated Transmission electron microscopy copper grid, the film dried for 5 minutes, and the particle size distribution was evaluated using enlarged pictures after blotting away the surplus solution with blotting paper. (**Figure 1, Figure 3**)

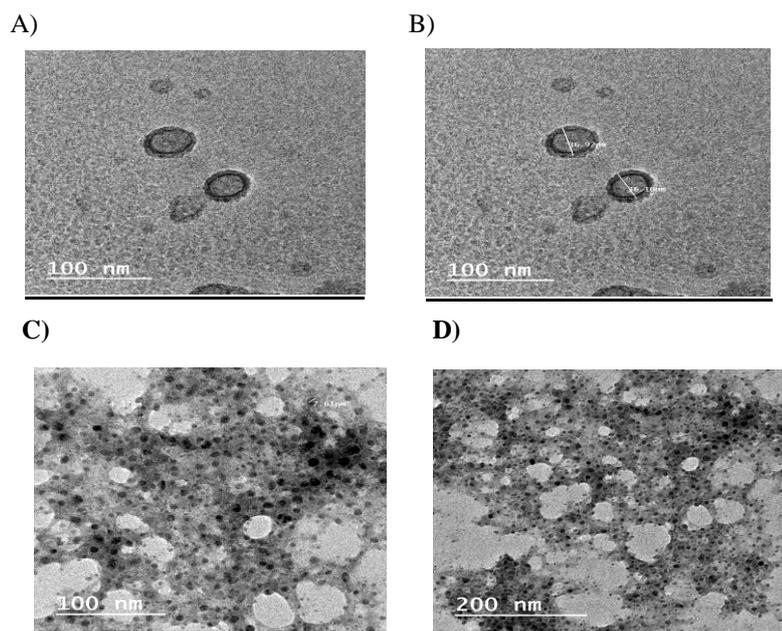
A solution with an NP concentration of 0.1 mg/ml was created for the spectrophotometer, as samples were newly prepared before usage by adding aliquots of NPs to 0.01 M Sodium Chloride (NaCl) buffer, which was then used for particle size measurement and surface charge analysis. Dynamic light scattering using the Zetasizer DTS1060 (**Malvern Instruments, MA**) based on photon correlation spectroscopy was used to measure the effectiveness of surface modification (particle size and charge) of NPs. (**Figure 2, Figure 4**)



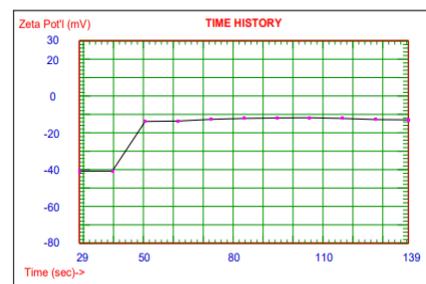
**Figure 1.** TEM images of AuNPs (A, B) and INS-AuNPs (C, D)



**Figure 2.** Zeta potential of AuNPs



**Figure 3.** TEM images of CMCNPs (A, B) and INS-CMCNPs (C, D)



Sample Frequency:	259.36 Hz
Reference Frequency:	260.08 Hz
Cell Current:	0.71 mA (Cond. Factor = 2.38)
Frequency Shift:	-0.72 Hz
Avg. Mobility:	-0.90 M.U.
Half-Width Mobility Dist.	---
Avg. Zeta Potential:	-12.97 mV
Half-Width Zeta Potl Dist.	---
Sample Temperature:	23 C
Liquid Viscosity:	0.995 cPoise
Index of Refraction:	1.333
Dielectric Constant:	78.200
Laser Wavelength:	632.8 nm
Scattering Angle:	13.4 deg
E-Field Strength:	4.00 V/CM
Channel Width:	500.0 uSec
Run Time:	00:02:18

**Figure 4.** Zeta potentials of CMCNPs

### 1.3. Experimental design:

The National Research Center (NRC), Cairo, Egypt, provided the 8 to 10-week-old male Wistar rats (n=70) weighing an average of 180-200 g. During the experiment, every five male Wistar rats in polypropylene cages (47cm × 34cm×20cm), were randomly assigned and housed under regulated temperature (25± 2 °C) and illumination (12-hour light/dark cycles). The rats were given a week to acclimate before the start of the experiment and were administered a commercially balanced rat chow diet with unlimited access to tap water. This study was carried out at the National Research Center (NRC) in Cairo, Egypt, with the permission of the resident ethics commission number 258 (Committee No. 25 on 27/9/2020) of Al-Azhar University's Faculty of Pharmacy and in conformity with the Recommendations of Laboratory Animal Care.

After acclimation, all 70 rats were split into seven groups (each having ten rats) at random: Normal rats without therapy (**Group I**), diabetic rats without treatment (**Group II**), and diabetic rats treated with trade insulin (DM + INS, 8 IU/kg) sub continuously (SC) for 3 weeks/every morning dose (**Group III**). Diabetic rats of (**Group IV**) were given gold nanoparticles (DM+AuNPs, 1 ml by gavage) via oral injection every morning for three weeks. Diabetic rats of (**Group V**) were given an oral injection of 8 IU/kg insulin-loaded gold nanoparticles (DM+AuNPs, INS 1 ml by gavage) every morning for 3 weeks. Diabetic rats treated with chitosan nanoparticles (DM+CMCNPs, 1 ml by

gavage) oral injection for 3 weeks/every morning dose (**Group VI**) and diabetic rats treated with 8 IU/kg insulin-loaded Carboxymethyl chitosan nanoparticles (DM+ CMCNPs, INS 1 ml by gavage) oral injection for 3 weeks/every morning dose (**Group VII**)<sup>13,14</sup>. Mixtard® 30 (100 IU/ml) was the insulin administered.

After an overnight fast, diabetes mellitus type 1 was produced by injecting Streptozotocin (STZ) (**Sigma, USA**) (60 mg/kg, dissolved in 50 mM sodium citrate buffer pH=4.5 due to the instability of STZ in an aqueous medium)<sup>15</sup>. Following an overnight fast, blood glucose levels were analyzed using glucometer strips (**Accuchek; Roche, Germany**) as diabetes mellitus was confirmed three days later from the tail vein. Diabetic rats with blood glucose levels of more than 200 mg/dl were used in the experiment. Instead of STZ, rats in the control groups were given an identical amount of citrate buffer intraperitoneally. The rats were given the treatment for the last three weeks of the four weeks investigation.

At the conclusion of the experiment, chloroform was used to euthanize overnight fasting rats. The initial section of the pancreas was dissected and put in clean and dry tubes, as tissues were collected at the same time for all treatment groups to reduce variability due to circadian rhythm. Then preserved for 24 hours in neutral buffered formalin (10%). The samples were then rinsed in tap water before being dehydrated with various degrees of ethanol (70, 80, 90, 95, and 100%). Samples were then cleaned in two changes of xylene, impregnated with two changes of

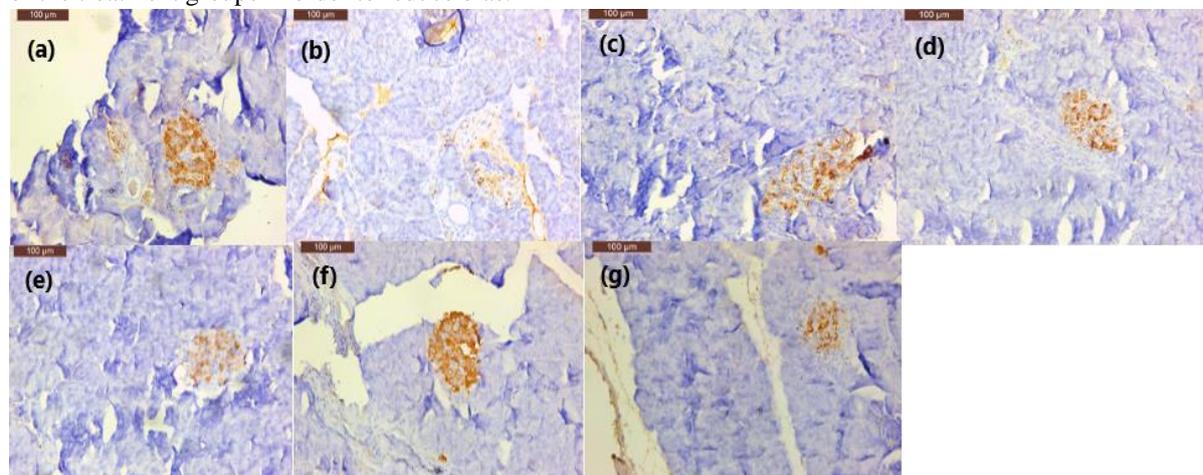
molten paraffin wax (melting point 58-60 °C), embedded, and blocked out for immunohistochemical analyses.

#### 1.4. Immunohistochemical Evaluation

In order to distinguish the integrity of the beta cells in the islets of Langerhans, immunohistochemical staining of insulin was performed according to the method described by **Missaoui et al** 16.

1. Tissue sections showing the maximum surface area of the islets of Langerhans were selected for comparisons between the groups.
2. The sections were then deparaffinized, hydrated by a decreasing series of ethanol. They were subsequently incubated with a guinea pig anti-insulin polyclonal antibody diluted in Tris 50 mM HCl pH = 7.6 (DAKO).
3. After washing, the sections were incubated with an anti-guinea pig antibody conjugated with phosphatase. For the revelation, a TechMate 500 machine was used.
4. The obtained sections were stained with hematoxylin/eosin and ten pancreatic islets per rat were examined under the microscope.

The readings of the slides were conducted in another lab under the supervision of the microscope lab head at the National Research Center. The reviewer in a manner that blinded from the identity of the treatment groups in order to reduce bias.



**Figure 5.** Immuno-reactivity for insulin, Scale Bar: 100 µm

(a) A segment of the pancreas from the control group demonstrating high insulin immune-reactivity in beta cells, which make up the majority of the islet. (b) A segment of the pancreas from diabetic rats demonstrated a significant drop in insulin immunohistochemistry expression in beta cells (arrow). (c) A segment of the pancreas from a diabetic rat administered AuNPs showed an increase in the number of beta-cells when compared to diabetes groups. (d) A segment of the pancreas from a diabetic rat was given AuNPs and insulin, demonstrating a considerable increase in the number of beta-cells when compared to diabetic groups. (e) Compared to diabetic rats given CMCNPs and insulin, a segment of the pancreas from diabetic rats given CMCNPs and insulin exhibited a slight increase in the number of beta-cells. (f) When diabetic rats were administered CMCNPs, a segment of the pancreas showed a significant increase in insulin immune positive beta-cells when compared to diabetic rats. (g) In comparison to the diabetic group, a segment of the pancreas from a diabetic rat administered insulin reveals a little increase in insulin immune positive beta-cells.

### 3. RESULTS

**3.1.** Transmission electron microscopy (TEM) was performed to confirm the relative size of the nanoparticles. The TEM images showed spherical morphology with a narrow particle size distribution when dispersed in the solution. Quantitative analysis of NPs size was performed by measuring the core diameter of 100 individual particles from multiple micrographs as shown in are illustrated in (**Figure 1, 3**).

**3.2.** Zeta potentials of nanoparticles were measured in disposable capillary cells using a Zetasizer as seen in (**Figure 2, 4**).

#### 3.3. Immuno-histochemical Results (**Figure 5**):

**Group I:** The segment of the pancreas from the Normal rats without therapy (control group) demonstrated high insulin immune reactivity in beta cells, which make up the majority of the islet. In the microscopic examination of the pancreas from the control group, beta cells represented the main cell population of the islets, occupying mostly the central zone. Positive insulin expression was seen in the form of dark brown granules present in the cytoplasm of beta-cells.

**Group II:** The segment of the pancreas from diabetic rats without treatment demonstrated a significant drop in insulin immunohistochemistry expression in beta cells. Immunohistochemical investigation of the diabetic group showed a marked reduction in immune-stained beta-cells compared to the control group.

**Group III:** In comparison to the diabetic group, a segment of the pancreas from a diabetic rat administered subcutaneous insulin reveals a little increase in insulin immune positive beta-cells.

**Group IV:** Immuno-reactivity for insulin in beta-cells in Langerhans islets of the pancreas from diabetic rats given AuNPs presenting an apparent increase compared with the diabetic group.

**Group V:** Diabetic rats that were given INS-AuNPs showed that immune-reactivity of beta-cells presented a significant increase compared with the diabetic group. Presenting an apparent increase in the number of beta cells compared with diabetic groups

**Group VI:** Diabetic rats given CMCNPs showed a marked increase in the insulin immune positive beta-cells, compared with the diabetic group.

**Group VII:** In the case, diabetic rats were given INS-CMCNPs, sections of the pancreas showed a mild increase in the immunoreactivity of beta-cells as compared with the diabetic group. Presenting a mild increase in the number of beta cells compared with the diabetic group.

In diabetic rats administered INS-AuNPs, immune-reactivity of beta cells increased considerably when compared to a diabetic group. In rats administered INS-CMCNPs, however, regions of the pancreas revealed a mild increase in beta cell immunological reactivity as compared to diabetic rats. In comparison to the diabetic group, sections of the pancreas from diabetic rats administered insulin revealed a little increase in insulin immune positive beta cells. While also results from diabetic rats that were given AuNPs presented an apparent increase in the number of beta-cells compared with the diabetic group and diabetic rats given CMCNPs showed also a marked increase in the insulin immune positive beta-cells, compared with the diabetic group.

#### **4. DISCUSSION**

One electron is removed from a form of oxygen in the body to produce reactive oxygen species. Proteins, lipids, and DNA can be harmed by a high concentration of reactive oxygen species, which causes oxidative stress, an unbalanced state in the body. Numerous diseases, including cancer, Parkinson's disease, atherosclerosis, neurodegeneration, destruction of beta-cells in diabetes, and cardiovascular diseases, are brought on and progressed as a result of oxidative stress.

Antioxidants can stop the progression of many chronic diseases, including lipid peroxidation, or scavenge free radicals. The most prevalent antioxidants are phenolic substances like propyl gallate, tert-butyl hydroquinone, and butylated hydroxyanisole. However, some of these antioxidants are hazardous, thus nowadays individuals are more likely to search for natural, effective antioxidants that are not poisonous. The main reasons for the popularity of biological synthesis are the use of non-toxic substances without extra stabilisers and reducing agents, renewable resources, low energy consumption, and ecological safety<sup>8</sup>.

The results of an immune-histochemical study of the pancreas in diabetic rats administered INS-AuNPs revealed that the immune-reactivity of beta cells increased dramatically in the diabetic group, with an apparent increase in the number of beta cells when compared to diabetic groups. On the other hand, sections of the pancreas from diabetic rats given INS-CMCNPs showed a mild increase in beta-reactivity, and sections of the pancreas from diabetic rats given insulin showed a mild increase in insulin immune positive beta-cells compared to the diabetic group. In addition, diabetic rats that were given AuNPs and CMCNPs separately, presented a marked increase in the insulin immune positive beta-cells when compared with the diabetic group. This supports the data that AuNPs and CMCNPs have a protective mechanism by reducing reactive oxygen species (ROS).

Researchers hypothesized that nanomaterials needed to stop ROS from causing harm in addition to breaking the free radical cycle. Although studies have shown that nanomaterials molecules can influence ROS scavenging capacity in vitro, the low biocompatibility, biodegradability, hydrophobicity, and hydrophilicity of loading molecules have prevented the application of these findings to clinical trials. In order to make choosing nanomaterials with loading molecules for the treatment of diseases caused by ROS easier, the following aspects should be taken into account. In this regard, it has been suggested that the use of gold materials, like AuNPs, is successful in the treatment of a number of disorders. However, the most accurate measure of these materials' advantages is their capacity to influence ROS reactions. AuNPs drive ROS generation or prevent ROS formation depending on their size, shape, and oxidation state. Additionally, by promoting the regulated release of ROS, AuNPs change a number of redox-signaling pathways. For instance, in human umbilical vein endothelial cells, bioconjugated AuNPs control ROS production and associated signaling pathways to induce angiogenesis without affecting inflammatory

cascades. The usage of gold-coated nanoparticles upregulates the cluster of differentiation 163 (CD163), which is essential for detoxifying ROS and reducing oxidative stress. Gold nanoparticles (AuNPs) have received a lot of attention in the last ten years as instruments with potential uses in the medical industry. Due to their electrical and optical characteristics as well as their capacity to form robust complexes with biomolecules, gold nanoparticles are used in a variety of applications. By adjusting variables like pH, temperature, and gold content, it is possible to alter the particle size and the pace of AuNPs creation <sup>12</sup>.

According to earlier pancreatic immunohistochemistry data, INS-AuNPs produced greater outcomes, whereas INS-CMCNPs and subcutaneous insulin produced similar results. This supports the idea <sup>17</sup> that long-standing beta cells may develop the intrinsic capability of surviving beta cells to overcome the immune destruction in long-term type 1 diabetes. Because there was less insulin in the surviving beta cells, the expression of genes related to beta-cell identity was reduced, and insulin release in response to glucose stimulation was reduced <sup>18</sup>. Similarly, genes linked to immune regulation were upregulated. The fourth hypothesis is that a de-differentiated phenotype protects against autoimmunity. A histological study of gold NPs were used by **BarathManiKanth et al** <sup>19</sup> to see if they might protect the liver and pancreas from damage. In the instance of hyperglycemia, AuNPs were found to have inhibitory and protective effects in the face of organ damage, according to the findings. The livers of control mice exhibited normal hepatic architecture, portal veins, and central veins, according to the results. As an anti-oxidant, AuNPs work by inhibiting the production of reactive oxygen species and scavenging free radicals, boosting antioxidant defense enzymes, and preserving hyperglycemic control <sup>19</sup>.

Due to their availability and nontoxicity, chitosan and its derivatives have recently garnered the greatest attention for their antioxidant activities. These studies revealed a relationship between the amount of active hydroxyl and amino groups in the polymer chains and the antioxidant activity of chitosan and its derivatives. Additionally, as molecular weights drop. Many researchers proposed that the superoxide anion's ability to combine with the active hydrogen atoms in chitosan to generate the most stable macromolecular radical is the basis for the scavenging mechanism of chitosan. The amino and hydroxyl groups are responsible for the antioxidant activity of chitosan and carboxymethyl chitosan. Even at large concentrations, there is essentially no action of scavenging free radicals when there are no free amino groups in the disaccharide. In addition, the location of the amino

groups, which is just as critical as their existence for neutralizing free radicals, is another crucial factor <sup>20</sup>.

According to the findings of **Jamshidi, et al** <sup>15</sup>, the antioxidant protective mechanism of CMCNPs reduces reactive oxygen species (ROS), inactivates the free radical responsible for organ dysfunction, and so suppresses lipid peroxidation. **Jamshidi, et al** <sup>15</sup> study approved that CMCNPs alleviate oxidative stress by lowering lipid peroxidation. These findings revealed that CMCNPs might have a dose-dependent protective impact.

## 5. CONCLUSIONS

This study revealed diabetic rats that were given AuNPs and CMCNPs separately, presented a marked increase in the insulin immune positive beta-cells as immune-reactivity of beta cells increased when compared with the diabetic group. This supports the data that AuNPs and CMCNPs have a protective mechanism by reducing reactive oxygen species (ROS), protecting the pancreas beta cells from destruction by suppressing the free radical responsible for organ dysfunction. Also, AuNPs and CMCNPs when loaded with insulin and given to the diabetic rats, presented an increase in the number of beta-cells. In type 1 diabetic Wister rats, this study highlights the promising effects of INS-CMCNPs and INS-AuNPs in controlling and promoting organ functions when compared to SC insulin and may provide new evidence for the reversibility of beta-cell dysfunction and insulin insufficiency with precise glycaemic control. We further emphasize that the oral insulin strategy has a promising impact that warrants further investigation to develop a more suitable technique for type 1 diabetes patients.

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**Conflicts of Interest:** The authors declare no conflicts of interest.

**Ethical Statement:** The protocol for animal treatment was taken under the ethical standards of animal facilities, National Research Center, Cairo, Egypt, with the approval of the Animal Ethics Institutional Committee.

**Author Contribution:** Eman Mahmoud Shaaban was involved in the practical work, methodology, data collection analysis, and paper writing. Khaldia Sayed Amr contributed to the methodology and the supervision and paper reviewing. Ahmed Mahmoud Mohamadin contributed to supervision and English polishing. Doha Ellakwa contributed to data collection, the supervision of paper writing, and editing. All authors read and approved the final manuscript.

**List of Abbreviations:** T1D: Type 1 Diabetes; ROS: Reactive Oxygen Species; CMCs: Carboxymethyl chitosan; CMCNPs: CarboxyMethyl Chitosan Nanoparticles; AuNPs : Gold Nanoparticles; INS: Insulin; INS-CMCNPs: Insulin-loaded CarboxyMethyl Chitosan NPs; INS-AuNPs: Insulin-loaded Gold NPs; SOD: Superoxide Dismutase; CAT: Catalase; CaCl<sub>2</sub>: Calcium chloride; NaOH: Sodium Hydroxide; TEM: Transmission electron microscopy; NaCl: Sodium Chloride; NRC: National Research Center; DM: Diabetes Mellitus; SC: Sub-continuously; STZ: Streptozotocin; HCL: Hydrochloride acid; DNA: Deoxyribonucleic Acid; CD163: Cluster of Differentiation 163

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