The effects of ZnO-insulin nanoparticles on some oxidative stress biochemical and physiological markers in rats with alloxan-induced diabetes

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Abstract

In recent years, there has been a growing emphasis on nanomaterials, particularly in the realm of medical applications. Zinc oxide nanoparticles are widely recognized as a significant class of oxides of metals due to their inherent structural stability, little toxic effects, and ability to maintain cellular integrity. One of the most important medicinal uses of zinc oxide is medication administration. Twenty-four male albinos’ rats were randomly separated into four groups. Control group, diabetic group, diabetic group treated with insulin, and diabetic group treated with ZnO NPs loaded with insulin. The rats were given medication for 28 days. Diabetes mellitus was induced in the rats with blood sugar levels more than 250 mg/dL by administering alloxan intraperitoneally at a dose of 120 mg/kg. The nanoparticles' physicochemical properties were investigated using a variety of analytical techniques, including UV-Vis spectral analysis, X-ray diffraction (XRD), and Field Emission Scanning Electron Microscopy (FE-SEM). The data revealed significant increases in glucose, cholesterol, triglyceride and total oxidative stress (TOS) as well as a notable decrease in body weight and catalase levels, in the diabetic control group compared to the healthy control group (P < 0.05). Compared to control groups, ZnO NPs loaded with insulin lower total cholesterol, triglycerides and TOS on the other hand increase body weight and catalase levels. The results of our study indicate that the use of photo-synthesized ZnO-NPs in combination with insulin showed noteworthy antidiabetic benefits when compared to the control groups.
Keywords: ZnO NPs, Glucose, TOS, Hyperglycemia, Alloxan, Biochemical parameters, Diabetes, Rats.

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder that is distinguished by elevated levels of blood glucose, glycosylation of the erythrocyte membrane, insulin resistance, and impaired metabolism of carbohydrates, lipids, and proteins. The individual exhibits hyperuricemia, a loss in body mass, a rise in urine glucose levels, and many symptoms including impaired visual ability [1]. Diabetes is a global health crisis concerning incidence rates in the twenty-first century. Diabetes affects five-hundred million people globally. Moreover, the global population is expected to increase up to 578 million in 2030 and 700 million in 2045 [2]. Diabetes problems have been frequently linked to increased free radical production or weakened antioxidant defenses. This imbalance between oxidants and antioxidants upsets the physiological balance, resulting in oxidative stress and consequent injury for the living body [3]. Furthermore, a high free radical production may cause harm, especially in organs such as the liver, kidney, blood vessels, immune system, and gastrointestinal tract. Elevated blood glucose levels contribute to elevated blood cholesterol levels, which eventually leads to dyslipidemia. This syndrome, defined by aberrant lipid profiles, increases the risk of cardiovascular disease and stroke [4]. Bio nanotechnology is an interdisciplinary area that comes from the merging of biology and nanotechnology, with nanoparticles acting as the principal product. Nanoparticles are miniature entities with diameters ranging from 1 to 100 nm [5]. They are now used in a variety of applications, including medicines, diagnostics, medication delivery, medical imaging, and tumor suppression. The expanding discipline of nanotechnology has achieved great attention as a topic of scientific inquiry, and there has been a noticeable spike in excitement around the potential benefits of nanotechnology across many fields [6]. This phenomenon may be linked to its ability to stimulate scientific innovation while providing considerable societal advantages [5]. Zinc oxide nanoparticles have been the focus of intensive study in diabetes mellitus, displaying great potential and meriting additional inquiry [7]. Zinc's possible significance in metabolic control is generally acknowledged. Zinc oxide nanoparticles (ZnO-NP) have received substantial attention as a very promising and novel material.
among the numerous forms of zinc. This is due to their stimulating effects, specific antibacterial capabilities, cost-effectiveness, and flexible uses in a variety of disciplines [8]. Researchers utilize zinc oxide nanoparticles (ZnO NPs) because they are biocompatible and non-toxic. The safety of ZnO has been recognized by the US Food and Drug Administration. ZnO nanoparticles are employed in bioimaging because of their stability and photoluminescence. Nanocarriers carry medications in sick environments. Off-target toxicity is reduced by surface-modified zinc oxide nanoparticles (ZnO NPs) in isolation. However, zinc oxide nanoparticles (ZnO NPs) functioned well [9]. As drug delivery systems, zinc oxide nanoparticles (ZnO-NPs) can increase the absorption and distribution of therapeutic medicines or biomolecules, improving therapeutic efficacy. ZnO-NPs have also shown promise in treating diabetes and its complications by lowering blood glucose and increasing insulin levels. Finally extended research is required on these results [10].

2. Materials and methods

2.1 Synthesis of ZnO-NPs

Photolysis was used to create zinc oxide nanoparticles (ZnO NPs). With a magnetic stirrer, 50 mL of 0.02 mole urea was slowly (one drop per second) added to 50 mL of 0.01 mole zinc acetate dihydrate Zn(CH$_3$COO)$_2$.2H$_2$O and stirred for fifteen minutes. The solution was then irradiated for thirty minutes with a photocell while chilled to five Celsius degrees. The irradiation solution was then treated with 25 mL of one normality sodium hydroxide solution. Then, the white powder precipitated, separated and washed multiple times with deionized water. The residue was oven-dried and calcined for three hours at 400 °C. Finally, the white powder of pure ZnO NPs resulted.

2.2 The characterization of ZnO nanoparticles (ZnO-NPs)

Several analytical methods were used to confirm the nanoscale size and form of the ZnO nanoparticles (NPs) powder. These techniques involved the UV-Vis spectral analysis using (Shimadzu UV-Vis 160V; Japan). The powder X-ray diffraction (XRD) using (XRD-6000; Japan). Besides, Field Emission Scanning Electron Microscopy (FE-SEM) using (SHIMADZU-6000 equipment).
2.3 Loaded insulin on the surface of nanoparticles

The insulin medication was placed onto the surface of nanoparticles (ZnO) as follows. One milligram of ZnO NPs were sequentially and individually added to 15 mL of 10 mg/mL insulin. The mixed solution was then shaken using water bath sonication at 37 °C for 0, 60, 120, 180, 240, and 300 minutes. The loading percentage was calculated using UV-Vis absorption spectroscopy using the following equation

\[
\text{Insulin loading percentage} = \left( \frac{A_0 - A_t}{A_0} \right) \times 100
\]

A0: Insulin absorption in water (without ZnO NPs) at 237 nm and insulin absorption in water with 1 mg ZnO NPs.

2.4 Experimental animals

The current research included twenty-four healthy male albino rats’ weight from 180 to 220 grams. The animals were acclimatized for fourteen days before the experiment was held. In addition, the bedding was changed every two days. The experiment was placed in a supervised animal housing facility with room temperature between 22-24 °C. Also, the experiment included twelve hours dark/light cycle, where animals had unlimited food and water access.

2.5 The experimental induction of diabetes in rats

A single intraperitoneal injection of 120 mg/kg alloxan monohydrate in normal saline caused diabetes. After 72 hours, rats' tail blood was drawn and tested for blood glucose using a "glucometer". One week following the injection, blood was drawn again to confirm diabetes. Diabetes was defined as 140-250 mg/dL blood glucose [11].

2.6 Animal grouping

The twenty-four experimental animals were divided into four groups where every group includes six animals. Also, every individual group was kept in a separate cage under the same conditions. The Control group of healthy rats received distilled water intraperitoneally for twenty-eight days. Moreover, group one diabetic rats received distilled water intraperitoneally for twenty-eight days. Furthermore, group two diabetic rats received two unite/100gm insulin subcutaneously every day for twenty-eight days. While group three diabetic rats treated intraperitoneally daily dose of ten mg/kg insulin, and two unites per100 gm of ZnO
NPs loaded with insulin for twenty-eight days.

2.7 Collection and processing of blood samples

After intramuscular anesthesia with ketamine (90 mg/kg) and xylazine (10 mg/kg), rats were killed twenty-four hours later. Sanitized vials held 5 mL blood samples from each treatment rat by heart puncture: In tubes without anticoagulants, 4 mL of blood was coagulated after thirty minutes at room temperature. Serum glucose, triglycerides, cholesterol, TOS and catalase were measured after centrifuging blood at 3000 rpm for fifteen minutes at 25 °C and storing them at -20 °C. Moreover, kits of glucose, triglycerides, cholesterol, TOS and catalase were supplied and measured by Cobas / Roche Instrument German.

2.8 Statistical analysis

Data were subjected to analysis using two software programs, Microsoft Excel and IBM SPSS V26. The findings presented in this research were represented as the mean + standard deviation. (M+SD between study groups was tested using a one-way analysis of variance [12].

3. Results and Discussion

3.1 Characterization of ZnO NPs

ZnO NPs UV-Vis absorption spectrum was tested from 200 to 400 nm. At 366 nm, individual spectra revealed a typical zinc oxide absorption peak. The method produced pure zinc oxide nanoparticles with no spectral peaks [13], as shown in figure 1.

Figure 1: Purified ZnO NPs undergo UV-Visible spectrum analysis.

Moreover, powder XRD utilized to spread powdered ZnO NPs widely. The XRD pattern of ZnO NPs displayed distinct peaks at 34.74o, 35.30o, 37.25o, 47.82o, 56.64o, 63.82o, 66.42o, 68.12o, and 69.13o with index values of 100, 002, 101, 102, 110, 103, 200, 114, and 202. These peaks are associated with the spherical and hexagonal (wurtzite) structure of ZnO, which favours the (101) plane in addition to powder alignment. The current work made use of zinc oxide (ZnO)
from an International Center of Diffraction Data card (JCPDS-36-1451), which had a crystalline monoclinic structure as shown in figure 2.

![Figure 2: The X-ray diffraction pattern of constructed ZnO nanoparticles.](image1)

Furthermore, the average crystallite size of the produced ZnO NPs was determined using the Debye-Scherrer equation, where $D$ is the crystallite size, $\lambda$ is the wavelength of the X-ray source (0.1541 nm) used in XRD, $\beta$ is the full width at half maximum of the diffraction peak, $K$ is the Scherrer constant with a value from 0.9 to 1, $\theta$ is the Bragg angle. The Scherrer constant is $k = 0.9$, the wavelength of the X-R ray source is $\lambda = 0.154$ nm, the peak width at half maximum is, and the Bragg diffraction peak angle. The average size of ZnO nanoparticles (NPs) was 30.7 nm, which was consistent with the TEM average. The absence of impure peaks in the XRD pattern indicated that the zinc oxide nanoparticles (ZnO NPs) were pure. This validates the purity of ZnO NPs and previous study [14]. Finally, field emission scanning electron microscopy (FE-SEM) was used to investigate the appearance and size distribution of oxide nanoparticles. The measurement photographs show porosity, which indicates amorphous ratios. Multiple resulted in samples are taken. According to FE-SEM, the spherical aggregates the outcome of a uniform sample distribution with equal-sized nanoparticles are crystalline, as demonstrated in figure 3.

![Figure 3: Field emission scanning electron microscopy (FE-SEM) images of ZnO NPs.](image2)
3.2 The effects of ZnO loaded with insulin nanoparticles on body weight

Results demonstrated a statistically significant reduction (p < 0.05) in the body weight of the group with diabetes G1 (180.3 ± 13.9 gm) compared with the control group (282 ± 44.9 gm). While a significant increase (p < 0.05) in the weight of treated groups G2 (268.4 ± 16.7 gm) and G3 (269 ± 30.5 gm) respectively. In comparison with group G1 (211.3 ± 13.9 gm), and no significant differences (p > 0.05) appeared between G2 and G3. Therefore, non-significant changes appeared with control group (282 ± 44.9 gm) as displayed in figure 4.

As a result, the body resorts to other energy sources, such as lipids and proteins, resulting in a significant reduction in body mass. The catabolism of muscle tissue to provide amino acids for hepatic gluconeogenesis also contributes to the reduction of body weight in unregulated diabetes [15]. Interestingly, animals receiving insulin treatment showed increasing weight. The weight increase implies that these medicines are effective. The group treated with insulin-loaded zinc oxide nanoparticles (ZnO NPs) achieved comparable body weights to the control group, indicating the possibility of these nanoparticles as effective hormone delivery systems [16]. The absence of a significant disparity in body weight between the groups G2, and G3 and the insulin group implies that the ZnO NPs effectively facilitated the transportation of insulin and had a discernible influence on metabolic processes [17]. Nanoparticles have the potential to safeguard insulin against premature breakdown and regulate its release. Potentially enhancing glycemic control over an extended period and mitigating the adverse effects associated with insulin administration, such as hypoglycemia [17,18].

3.3. The effects of ZnO loaded with insulin NPs on experimental animals' blood glucose levels

The blood glucose levels in the various experimental groups provide light on
the efficiency of the various therapies in treating hyperglycemia, a hallmark of diabetes. The results presented in figure 5 indicate a statistically significant (p < 0.05) elevation in the sugar levels of the diabetic group G1 (348.6 ± 34.4 mg/dL) compared to the control group (84.7 ± 6.6 mg/dL). Furthermore, a significant reduction in glucose levels (p < 0.05) was observed in the groups treated with insulin and insulin loaded with ZnO NPs G2 (93.2 ± 10.6 mg/dL) and G3 (134.8 ± 40.1 mg/dL) when compared to G1 (348.6 ± 34.4 mg/dL). The results also showed that the glucose level was within the normal range and did not show any significant differences (p > 0.05) between G2, G3.

Treatment of diabetic rats with insulin G2 effectively decreased blood glucose levels to near normal (93.2 ± 10.6 mg/dL), underscoring insulin's critical role in facilitating glucose uptake into cells and its efficacy as a standard treatment for diabetes [19]. The mechanism of action is mainly by promoting glucose transport into the muscle and adipose cells, inhibiting hepatic glucose production, and suppressing lipolysis [20]. Combining insulin with ZnO nanoparticles resulted in higher blood glucose levels (134.8 ± 40.1 mg/dL) relative to control and insulin-only groups, but much lower than the untreated diabetes group. This implies that ZnO nanoparticles may enhance insulin's glucose-lowering capabilities but may not equal the level [21]. Zinc oxide nanoparticles have been reported to exhibit beneficial effects in diabetes management, including the enhancement of insulin sensitivity and glucose utilization, due to their high surface area-to-volume ratio and unique physicochemical properties [22]. The protein entities, a minor crystallite distortion in ZnO might be helpful in creating ZnO activity for biological applications. In the current work, ZnO-insulin NPs medications were shown to have a hypoglycemic activity impact, as insulin. According to the findings, the medicine known as ZnO nanoparticles loaded

![Figure 5](image-url)
with insulin is the most effective hypoglycemic agent [23]. Differences in the duration, severity, or presence of comorbidities associated with diabetes can potentially impact the levels of liver enzymes and the effectiveness of treatment interventions. Moreover, further research is required to explore the specific impacts and potential synergistic interactions between ZnO and insulin [24].

### 3.4 Lipid profiles

Changes in lipid levels and other metabolic indicators are also observed in diabetic rats. Serum levels of cholesterol, and triglycerides G1 (98.42 + 7.10, 105.74 + 20.32) mg/dL were significantly increased (p < 0.05) in level for each parameter when compared with control group (63.52 + 5.32, 66.08 + 19.50) mg/dL consequently as shown in figure 6. Moreover, HDL level G1 (28.20 + 3.87) mg/dL significantly decreased in comparison with control group. On the other hand, the result indicate that treated diabetic rats showed significantly decreased (p < 0.05) in serum levels of cholesterol and triglycerides to G2 (64.14 + 13.18, 66.96 + 11.53) mg/dL and G3 (72.40 + 11.32, 70.20 + 16.45) mg/dL consequently when compared with G1. Serum levels of HDL G2 (37.26 + 2.89) and G3 (35.60 + 2.87) were significantly increased (p < 0.05) in comparison with G1.

The observed effect may be linked to disturbance in the regulatory mechanism of lipase, an enzyme that exhibits sensitivity to hormones, particularly insulin. The disruption in dispute may be attributed to a lack or insufficiency of insulin due to the loss of "β-cells inside Langerhans's islet", This damage may be triggered by the presence of alloxan [25]. Several explanations might be offered for the improvement in lipid level measures that were seen in diabetic rats. Fatty acid synthesis impairment is one of them, increased catabolism of very low-density lipoproteins (VLDL). Cholesterol acyltransferase activation of tissue lipases, inhibition of acetyl-CoA carboxylase, and the production of precursors for triglycerides such as acetyl-CoA and glycerol phosphate by zinc oxide nanoparticles ZnO and insulin NPs [26].
Figure 6: Zinc oxide nano particles loaded with insulin on cholesterol and triglyceride levels in experimental diabetic rats.

Based on statistical analysis total antioxidant capacity (T-AOC), indicate a significant difference of serum total oxidative status (TOS) levels as shown in figure [7]. The highest significant (p < 0.05) increase value of serum TOS was observed in experimental diabetic group G1 (75.53 ± 10.7 mol equivalent/l) compared to the healthy rat group (35.61 ± 5.67 mol equivalent/l) as the oxidative stress burden for the diabetic status. While, during administration of ZnO NPs loaded with insulin therapy showed a significant (p < 0.05) reducing in serum TOS in all other experimental diabetic groups G2 and G3 (36.02 ± 4.87, 38.94 ± 7.24 mol equivalent/l) respectively when compared to diabetic rats’ group G1 (75.53 ± 10.7 mol equivalent/l).

Figure 7: Serum TOS (mol equivalent/l) of control and experimental diabetic groups treated by ZnO NPs for 28 days.
Current studies showed that experimental diabetics increased serum TOS. High TOS indicates an imbalance between oxidants and antioxidants, that can damage cells and tissues due to excessive reactive oxygen species (ROS) production. When, ROS levels rise, antioxidant protection weakens, causing oxidative stress [27]. ROS reactive oxygen ions and peroxides are created in all biological systems' metabolisms. ROS damage biomolecules including DNA, RNA, protein, and lipids, causing most human illnesses [28]. Studies on animals with genetically modified antioxidant enzymes show that oxidative stress disrupts insulin-mediated intracellular signaling pathways [29]. Oxide nanoparticles have inherent physicochemical properties that allow them to scavenge reactive nitrogen and oxygen species and mimic an antioxidant molecule that has been shown to be effective in treating a variety of ailments brought on by oxidative stress [30]. As a result, the NPs prevent their damaging effects because the nanoparticles composites can be incorporated into composite materials, where they act as antioxidant [31].

Furthermore, serum catalase levels (mol/ml) were depicted in the accompanying as displayed in figure 8. The statistical data showed a significant (p < 0.05) decrease in serum catalase levels in experimental diabetic rats G1 (3.01 ± 109 mol/ml) compared to healthy control rats (7.26 ± 0.89 mol/ml). While serum catalase levels were increased significantly (p > 0.05) in treated by ZnO NPs. After 28 days G2 and G3 (7.02 ± 1.26, 16 ± 1.43 mol/ml) respectively than to experimental diabetic rats G1 (3.01 ± 109 mol/ml) and no significant differences (p > 0.05) were observed in the serum catalase levels of these treated groups between them compared to the control group (7.26 ± 0.89 mol/ml).

![Figure 8](image_url)

**Figure 8:** Serum catalase (mol/ml) of control and experimental diabetic groups treated by ZnO NPs for 28 days.
Catalase biological marker is a cellular antioxidant defense. The much-reduced cellular catalase level in hyperglycemic rats in our research may be attributed to elevated risk of diabetes and weakened cellular defense against oxidative damage [31]. Diabetics increased oxidative stress may exceed their antioxidant capability. ROS causes cellular changes by increasing oxidative stress. High glucose levels in diabetes may cause ROS production and β-cell death [32]. In the current study, insulin, or in combination with ZnO increased serum catalase activity in diabetic experimental rats aligning more closely with healthy rats. This may benefit the antioxidant defense system by reducing oxidative stress caused by diabetes. This suggests that ZnO and insulin synergistically increase catalase expression and activity to strengthen antioxidant defenses [33]. However, recent studies suggest that nanotechnology can treat some diabetic complications through the application of nanotechnology in the repair of diabetic segmental bone injury. The healing of diabetic skin ulcers, the therapeutic effect, and improvement strategies and deficiencies of nanotechnology in diabetic complications [34].

4. Conclusion

The current study investigates effectively assessed the observed and prospective antidiabetic properties of insulin-loaded ZnO nanoparticles. The utilization of nanoparticles as a delivery exhibits a noteworthy (p < 0.05) reduction in blood sugar, plasma cholesterol and triglyceride concentrations levels in rats with alloxan-induced diabetes after 28 days treatment duration. The exceptional crystal structure and the considerable surface area shown by nanoparticles of zinc oxide (ZnO NPs), in conjunction with inulin, contribute to their notable antidiabetic characteristics by promoting improved absorption and distribution. No observable alterations in total oxidative status (TOS) and antioxidants catalase level in serum, in rats subjected to insulin therapy in conjunction with exposure to ZnO-NPs. Therefore, it is important to conduct more research on the toxicity, safety and drug delivery of these nanoparticles in animal models to evaluate the results of this research and determine their appropriateness and effective dosage.
5. References


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