Original Article

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Nanoparticle zinc oxide obviates oxidative stress of liver cells in induced-diabetes mellitus model

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Abstract

Background: Because of vital features including biocompatibility, high surface reactivity, and oxidation resistance, emerging nanomedicine is well-known for its potential therapeutic prospects. The goal of this study is to see if nanoparticle zinc oxide (ZnONPs) may reduce hepatic problems and oxidative stress in rats with diabetes mellitus induced by streptozotocin (STZ).

Materials and methods: This study included 39 rats separated into three groups, each consisting of 13 rats; control group, streptozotocin group, and streptozotocin + ZnONPs group. Malondialdehyde (MDA), nitric oxide (NO), glutathione (GSH), and reactive oxygen species (ROS) were measured as biochemical-specific oxidative stress measures. Fasting blood sugar (FBS), haemoglobin A1c (HbA1C), and tumour necrosis factor (TNF-β) were also measured, as well as total cholesterol and triglycerides. The percentage of P53 in the liver was determined. Inflammatory cell infiltration and fibrosis in tissues were also seen by histological investigation. **Results:** FBS, HbA1C, liver function tests, MDA, NO, ROS, P53, and TNF-β serum levels were increased in STZ-treated rats. Treatment with ZnONPs (5 mg/kg) in the STZ+ ZnONPs group significantly improved, FBS, HbA1C, MDA, NO, GSH, ROS, liver function, fasting blood sugar, HbA1C, and tumour necrosis factor. In addition, ZnONPs treatment banned inflammatory cell infiltration and P53 in STZ-administered rats. **Conclusion:** Our study provides evidence that ZnONPs may aid in controlling hepatic oxidative stress in STZ-induced diabetes mellitus in rats.

Introduction

Diabetes mellitus (DM) is a metabolic illness characterized by persistently high glucose levels in the human body [1]. Recurrent urination, increased thirst, and decreased appetite are all signs of elevated glucose.

Diabetes, if left untreated, can cause several problems [2]. Diabetes causes long-term damage, malfunction, and failure of multiple organs, including the eyes, kidneys, liver, heart, and blood vessels [3]. In diabetes, several pathogenic pathways are triggered, including reactive oxygen species (ROS) produced by elevated glucose levels, which are responsible for metabolic abnormalities and chronic problems [4].

Any instability in the synthesis and scavenging of reactive oxygen species (ROS) results in high amounts of either molecular oxygen or ROS. As a result, oxidative stress levels have increased [5]. Many studies have demonstrated that oxidative stress contributes to the development and progression of diabetes and its complications by causing the production of free radicals. Antioxidants will be an effective technique for improving oxidative stress treatments [6]. Because of crucial features including biocompatibility, high surface reactivity, oxidation resistance, and plasmon resonance, emerging nanomedicine is well-known for its potential therapeutic prospects [7].

Zinc, an important metal, acts as an activator for over 300 enzymes in the body [8] and is involved in a variety of metabolic processes, including glucose metabolism. Zinc enhances hepatic glycogenesis by acting on insulin pathways, improving glucose consumption, and maintaining insulin structure [9]. It has a function in insulin production, storage, and secretion, and it has the potential to enhance insulin signalling through a variety of pathways, including increased insulin receptor phosphorylation, increased PI3K activity, and inhibition of glycogen synthase kinase-3 [10]. Therefore, the goal of this research is to see how nanoparticle zinc oxide (ZnONPs) affects oxidative stress and hepatic problems in rats with streptozotocin-induced diabetes.

Keywords: Nano zinc oxide; oxidative stress, streptozotocin, hepatic disorders, and diabetes mellitus.
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Materials and methods

Experimental animals:

Healthy adult male Wister albino rats weighing 115-121 g were used in the experiment. Rats were kept in regular circumstances (12-hour light/dark cycle, 24°C, 35-60% humidity). The Ethical Committee at Zagazig University, Egypt, approved the study protocol for this experiment with the approved number ZU-IACUC/1/F/179/2022.

Experimental Design:

The experiment had 39 experimental animals that were kept together for 7 days before the start of the study. The rats were divided into three groups at random, each consisting of 13 rats. **Control group (C):** Throughout the study, the rats were fed a standard laboratory rat diet. **Streptozotocin group (STZ) (Diabetic group)**: Streptozotocin was administered subcutaneously (S.C) at a dosage of (35mg/kg b.w in 1ml saline solution) weekly for 8 weeks in rats given a standard rat diet. **STZ + ZnONPs**: For 8 weeks, rats are given 5 mg/kg b.w. of ZnONPs daily through oral gavage and Streptozotocin at a dosage of (35 mg/kg b.w. in 1ml saline) weekly subcutaneously.

Obtaining blood samples:

Overnight fasting rats were slaughtered at the end of the experiment. The blood samples were collected in centrifuge-clean glass tubes after the last treatment, allowed to clot, and then centrifuged at 4000 rpm for 15 minutes. The non-hemolyzed sera were rapidly extracted and placed in labelled Eppendorf's tubes; the sera were then frozen at -20°C for various biochemical investigations.

Biochemical parameter evaluation:

All biochemical parameters were measured using a commercial kit provided by Human (Germany) following the manufacturer's instructions.

Estimation of biomarkers of oxidative stress

The clear supernatant obtained was utilized to test for lipid peroxidation (MDA), endogenous antiperoxidative enzymes nitric oxide (NO), hepatic glutathione (GSH), and reactive oxygen species (ROS) to assess oxidative stress indicators. The apoptotic marker P53 gene was assessed through qRT-PCR as described in [11].

Examination of the histopathology

Liver tissues were fixed in a neutral formalin solution (10%), dehydrated in ethanol, cleared in xylene, embedded in paraffin wax, and inspected under a light microscope as described in [12].

Statistical analysis

SPSS software was used to code, tabulate, and statistically analyze the obtained data. The mean and standard error of the mean were calculated using descriptive statistics for parametric quantitative data (SEM). The one-way ANOVA test was used to compare the groups in non-parametric quantitative data. The significance threshold was set at (P-value <0.05).

Results

In comparison to control rats, STZ treatment significantly elevated ALT, AST, and alkaline phosphatase levels while decreasing albumin and total protein. STZ + ZnONPs considerably reduced ALT, AST, and ALP while significantly increasing serum albumin and total protein (all P 0.001) as publicized in **(Table 1)**.

In the STZ group, there was a substantial rise in RBS, HbA1C %, fructosamine concentrations, total cholesterol, triglyceride, and TNF-β compared to the control group, as revealed in **(Table 2)**. While giving Zinc to rats (STZ+ ZnONPs) caused a substantial drop in all of these markers.

In comparison to control rats, STZ administration increased MDA and ROS in liver tissues while decreasing NO and hepatic GSH. In the STZ+ZnONPs group, however, ZnONPs therapy greatly enhanced these indicators. From the resulting data, there was a significant increase in P53 in the STZ group compared to the control group. However, the administration of Zinc to rat groups (STZ+ZnONPs) led to a significant decrease in P53 compared to the STZ group as presented in **(Table 3)**.

Figure 1(A) depicts a photomicrograph of the liver in the control group, which reveals normal hepatic architecture, including the central vein (CV), portal region (P), polyhedral-shaped hepatocytes, and blood. **Figure 1(B)** demonstrates pleomorphic, hyperchromatic, and darkly basophilic cell aggregations in the perivascular region around the dilated and congested central vein (CV) and hepatocellular necrosis in STZ-induced diabetic rats' livers. **Figure 1 (C)** shows a photomicrograph of the STZ+ ZnONPs group's liver, which shows slight central vein (CV) congestion, as well as mild congestion of certain blood sinusoids and a modest increase in Kupffer cell activity.

Table 1. Comparison of liver function tests in different groups

Group	Control N=13 mean±SD	STZ N=13 mean±SD	STZ+ ZnONPs N=13 mean±SD	P-Value
ALT (U/L)	13.72±0.42	48.30±0.52	36.79±1.44	<0.001
AST (U/L)	43.55±0.34	75.98±0.87	49.54±1.47	0.00
Albumin (g/dl)	3.86±0.155	2.73±0.08	3.03±0.05	0.00
ALP (mg/dl)	144.75±0.513	288.14±3.63	234.67±13.5	<.001
TP(g/dL)	4.83±0.27	3.25±0.11	3.77±0.14	0.00

Table 2. Diabetic markers, Lipid profile, and TNF-β in studied rats.

Group	Control N=13 Mean±SEM	STZ N=13 Mean±SEM	STZ+ ZnONPs N=13 Mean±SEM	P-Value
FBS (mg/dl)	96.02±0.155	194.77±0.08	125.91±0.74	< 0.001
HbA1C (%)	4.76±0.15	15.68±3.15	9.94±3.22	< 0.0001
Fructosamine (mmol/L)	26.23±0.513	136.19±3.63	99.15±13.5	0.0001
TC (mg/dL)	134.94±4.27	167.57±13.11	142.20±9.14	0.01
TG (mg/dL)	134.89±2.33	395.90±31.87	145.91±14.20	< 0.001
TNF-β (pg/ml)	4.52±0.130	8.78±0.138	6.26±0.193	< 0.001
P53	27.17±0.60	67.17±3.5	48.37±1.01	0.001

Table 3. Comparison of oxidative stress markers in liver tissues studied groups.

Group	Control N=13 Mean±SEM	STZ N=13 Mean±SEM	STZ+ ZnONPs N=13 Mean±SEM	P-Value
MDA (nmol/g)	382.52±0.12	705.49±0.26	418.92±21.2	<0.0001
NO (µmol/g)	43.53±0.17	28.39±0.10	40.46±1.49	< 0.0001
GSH (mmol/g)	5.35±0.11	3.51±0.12	4.62±0.16	0.002
ROS (nmol/g)	0.75±0.07	1.79±0.06	0.90±0.03	0.001

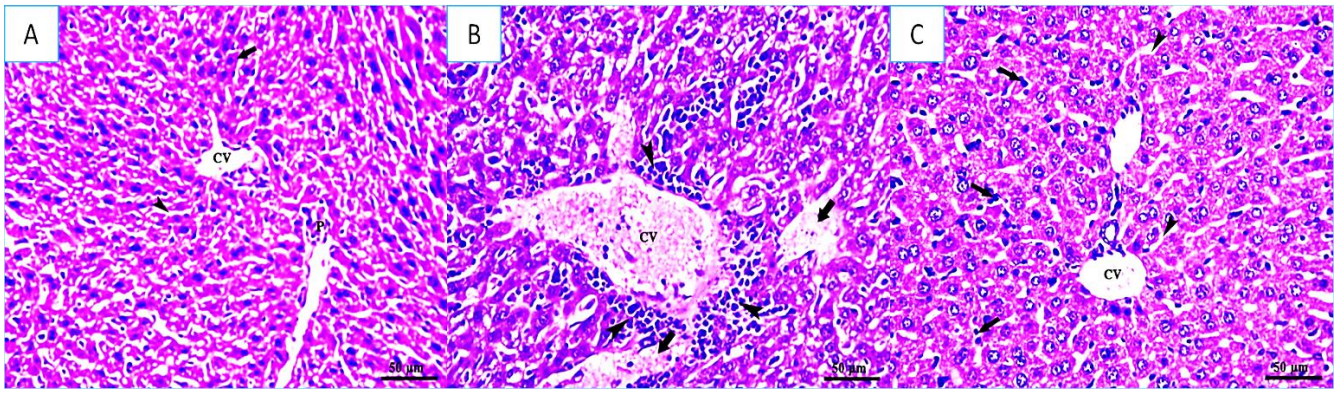

Figure 1: (A) Photomicrograph of liver of control group screening normal hepatic architecture; central vein (CV), portal area (P), polyhedral-shaped hepatocytes (arrow), and blood sinusoids (arrowhead) H&E, bar=50 µm. **(B)** Photomicrograph of liver of STZ group displaying aggregations of pleomorphic, hyperchromatic, and darkly basophilic cells (arrowheads) in the perivascular area around the dilated and congested central vein (CV). **(C)** Photomicrographs of the paraffin section in the liver in Zinc and STZ-treated rats revealed alike to the control group which showed localized pleomorphic darkly basophilic cells (arrows) in the perivascular area around the mild dilated central vein (CV) besides the less degeneration of hepatocytes (arrowheads).

Discussion

In this study, the animal group given STZ had a considerable rise in blood levels of ALT, AST, and alkaline phosphatase (ALP), as well as a significant decrease in serum albumin and total protein. The same results were found by Hariri et al., who interpreted the elevated levels of ALT, AST, and ALP as a result of STZ's cytotoxic action, which caused harm to hepatocytes and canaliculi, followed by the release of these enzymes, which led to their rise in the liver and circulation [13]. In our investigation, however, giving zinc oxide nanoparticles together with streptozotocin to the STZ/ ZnONPs group prevented streptozotocin from having this impact. The restoration of AST and ALT activity in STZ rats after zinc supplementation highlighted the hepatoprotective effect of zinc, as zinc therapy assisted in maintaining homeostasis through protein synthesis control. Furthermore, zinc's antioxidant properties can help to maintain the hepatic cell membrane and protect

hepatocytes from the harmful effects of STZ, reducing enzyme leakage into the bloodstream [14].

In addition, as compared to the control group, the induction of rats by STZ therapy resulted in a substantial reduction in albumin and total protein. These findings are consistent with previous research. Zn's function in the induction of metallothionein (Zn binding protein), which in turn induces the amino acid precursors for protein synthesis, might be linked to its regulation of protein synthesis [15-16].

In this work, streptozotocin administered subcutaneously in diabetic rats increased fasting blood sugar and glycated haemoglobin (HbA1C), but zinc oxide nanoparticles ingested with streptozotocin prevented this diabetogenic impact and showed a significant anti-diabetic effect. Ohly et al discovered that in mice with diabetes induced by multiple low doses of streptozotocin (MLD-STZ), treatment with zinc-enriched drinking water reduced blood glucose concentrations and kept them below the euglycemic level, whereas non-Zn treated MLD-STZ mice had blood glucose concentrations much higher than the euglycemic level. This anti-diabetic activity of zinc was explained by the fact that it dramatically increased metallothionein synthesis (cytosolic proteins that are produced by zinc ions (Zn^{2+}) and scavenged hydroxyl radicals (OH)) in mouse pancreatic islets, preventing diabetes caused by MLD-STZ [17].

Tobia et al. found that high Zn supplementation significantly delayed the development and decreased the severity of diabetes in BioBreed Wistar rats [18], which supports the anti-diabetic benefits of Zn. Zinc's significant impact on the development of hepatic glycogenesis via activities on the insulin signalling pathway has been demonstrated to promote glucose consumption and metabolism [17]. Zinc's insulin mimic and hypoglycemic characteristics have also been proven in vitro and in vivo investigations [19]. Zinc ions are known to be a target of the protein tyrosine phosphatase 1B (PTP 1B), a critical regulator of the phosphorylation state of the insulin receptor [20]. Zinc has been proven in studies to improve peripheral insulin sensitivity by potentiating insulin-stimulated glucose transport [21].

STZ caused a significant increase in serum lipids and TNF-β in rats in our investigation, although nanoparticle zinc oxide treatment mitigated this impact. According to Wang et al., zinc supplementation reduces diabetic symptoms such as polydipsia and increases blood levels of high-density lipoprotein cholesterol in STZ-induced diabetic Wistar rats, showing that zinc supplementation has a potential therapeutic impact on diabetic conditions [22]. In addition, Jayawardena et al. found that diabetics had a better lipid profile, lower lipid peroxidation, and a better antioxidant profile [23].

Diabetes is known to cause oxidative stress and oxidative tissue damage, which may be linked to its consequences. STZ-produced DM in rats was linked to altered indicators of oxidative stress in this study, with STZ increasing malondialdehyde and reactive oxygen species (ROS) in liver tissues while decreasing nitric oxide and glutathione (GSH) in comparison to control rats. The oxidative stress of STZ in zinc-treated mice was alleviated by the administration of nanoparticle zinc oxide. In line with our findings, Marreiro et al [24] discovered that zinc protects cells from oxidative damage, acts in membrane stabilization, and inhibits the enzyme nicotinamide adenine dinucleotide phosphate oxidase (NADPH-Oxidase), as well as inducing the synthesis of metallothioneins, which are proteins effective in reducing hydroxyl radicals and sequestering reactive oxygen species (ROS); Zinc can also stimulate the enzyme glutamyltranspeptidase (-GT), which replenishes intracellular glutathione on the sinusoidal surface of liver cells, hence enhancing its detoxifying ability [13-25].

In addition, in STZ-treated rats, ZnONPs therapy prevented inflammatory cell infiltration and decreased P53. The p53 tumour suppressor is a transcription factor that comprises a single zinc ion adjacent to its DNA binding interface. $Zn^{(2+)}$ is essential for effective transcriptional activation and site-specific DNA binding [26]. P53 is activated in response to genotoxic and non-genotoxic stress and coordinates many anti-proliferative mechanisms to permanently remove cells with damaged DNA from the pool of actively proliferating cells. Intracellular free Zn modifies the P53 protein structure and inhibits P53's DNA binding [26]. Intracellular free Zn influences P53 activity and stability, and excess Zn alters the P53 protein structure and inhibits P53's DNA binding.

Conclusion

This study demonstrates that administering ZnONPs to STZ-treated rats reduces inflammation in the liver. Improved liver functions as a result of ZnONPs administration may be related to a reduction in oxidative stress and restoration of antioxidant defence in tissues.

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