Research Article

Antidiabetic Efficacy of Zinc Oxide Nanoparticles and Empagliflozin Combinations

Saydat Saad Abd El-Megeed[1], Walaa Yehia El-Sayed[1*], Tarek Khamis[2]

[1]Department of Biochemistry, Faculty of Veterinary Medicine, Zagazig University, 44511 Zagazig, Egypt; [2]Department of Pharmacology, Faculty of Veterinary Medicine, Zagazig University, 44511 Zagazig, Egypt.

Abstract | Diabetes mellitus is a metabolic disorder characterized by elevated blood glucose levels. Because of the vast number of patients affected by the condition and the repercussions it has, many scientists are being forced to research multiple medicines, all of which are quite expensive. The antidiabetic activities of zinc oxide nanoparticles (ZnO NPs) were assessed in rats. ZnO NPs were characterized by transmission electron microscopy (TEM). Diabetes was induced by streptozotocin (STZ) -nicotinamide in rats, and some biochemical and molecular parameters were determined. The results revealed that ZnO NPs in combination with Empagliflozin suggestively declined the blood glucose levels, lipid profile, and liver and kidney functions. A reduction was recorded in malondialdehyde (MDA), as well as improved catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD). A reduction was found in the gene expression in the hepatic homogenate of Patched 1, hedgehog-interacting protein (Hhip-1), smoothened (SMO), glioma-associated oncogene homolog 1 (GLI1), and extracellular signal-regulated kinases 1 (ERK1). On the other hand, there was a significant upregulation in the mRNA expression of peroxisome proliferator-activated receptor (PPAR) –γ, insulin-like growth factor 1 (IGF1), PPAR-α, glucagon-like peptide 1 (GLP-1) in pancreatic homogenate, and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) in pancreatic homogenate which is also a dose- and time-dependent manner. Thus, the study has demonstrated that the combination of Empagliflozin and ZnO NPs could be a promising therapy to alleviate the progression of diabetes.

Keywords | ZnO nanoparticles; Diabetes mellitus; Glucagon-like peptide-1; Gene expression; Hedgehog signaling pathway.

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***Correspondence** | Walaa Yehia El-Sayed, Department of Biochemistry, Faculty of Veterinary Medicine, Zagazig University, 44511 Zagazig, Egypt; **Email:** walaarafeef97@gmail.com
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INTRODUCTION

High blood glucose levels are the outcome of a metabolic condition called diabetes mellitus, and the lack of insulin is the major cause of this disease. Diabetes is thought to result in part from a disruption in normal glucose metabolism, which can be caused by a lack of certain micronutrients such copper, chromium, manganese, iron, nickel, zinc, ascorbic acid, and vitamin E (Dubey et al., 2020). Insulin is produced by cells in the pancreas, and zinc is essential to this process. Numerous in-vitro and in-vivo investigations show that zinc has antidiabetic and insulin-like qualities; nevertheless, the molecular mechanisms behind these effects are still unclear (Bjørklund et al., 2020). Because of their similar size, nanoparticles and biomolecules such as proteins and poly-nucleic acids can be used in a wide range of applications in biology and medicine. Furthermore, because of their small size, they

are easily absorbed by the digestive system since they can quickly cross biological membranes (Cruz et al., 2020). In recent years, zinc oxide nanoparticles (ZnO NPs) have been produced and tested in streptozotocin-induced diabetic rats as a novel method for zinc delivery. There are numerous biomedical uses for ZnO NPs such as cancer prevention and treatment, antibacterial, anti-inflammatory, and bioimaging (Martínez-Esquivias et al., 2021).

It has been shown that many of the currently available diabetic medications are effective in treating cognitive impairment and have benefits against some of the hallmarks of Alzheimer's disease, such as the fact that empagliflozin, an experimental medication for treating type 2 diabetes (T2D), can reduce oxidative stress and slow the progression of cognitive decline in addition to improving blood sugar regulation (Chawla & Chaudhary, 2019).

Genes including pancreatic and duodenal homeobox 1 (pdx-1), glucose transporter (GLUT2), and glucokinase that are essential for glucose sensing, insulin secretion, and insulin transcription are all directly regulated by PPAR-γ in cells. Activation of PPAR-γ through pharmacological means has also been demonstrated to prevent stress pathway activation brought on by glucose, lipids, cytokines, and islet amyloid polypeptide (IAPP). Glucose-stimulated insulin secretion from pancreatic islets is positively correlated with PGC-1α expression, demonstrating that epigenetic mechanisms can control gene expression and, by extension, insulin secretion. Hedgehog signalling (Hh) activation in hepatocytes is associated with the progression of NAFLD. Smoothened (SMO) inhibits Hh signalling, while sonic hedgehog (Shh) binding to Ptch1 eliminates this inhibition, activating both Hh signalling and the transcription factor glioma-associated oncogene homolog (GLI). Ballooned hepatocyte-derived Shh increases Hh signaling in hepatocytes, leading to the production of osteopontin, which in turn induces the accumulation of liver macrophages and the progression of non-alcoholic fatty liver disease (NAFLD). NAFLD and insulin resistance are aided by the activation of Hh signalling in hematopoietic stem cells (HSCs), macrophages, and adipose tissues. Hh signalling blockade, achieved by taking SMO inhibitors orally, slows the development of NAFLD and insulin resistance (Seki, 2016).

In the current research, many biomedical methodologies have been used to ascertain the therapeutic efficacy of ZnO NPs in streptozotocin (STZ) -nicotinamide-induced diabetic rats.

MATERIALS AND METHODS

Drugs

Zinc oxide nanoparticles (ZnO NPs) were purchased from NanoTech Egypt for photo-electronics (AlGiza, Egypt, Email;sales@nanotecheg.com). Empagliflozin (Jardiance from Boehringer Ingelheim Co) was purchased from a local pharmacy. STZ and nicotinamide were purchased from Sigma Chemical Co (USA).

Preparation of ZnO nanoparticles

The precursor zinc acetate dihydrate (0.1 M) was refluxed in diethylene glycol and tri-ethylene glycol at 180°C and 220°C, respectively, to produce ZnO nanoparticles. The solution was maintained at 80°C for 1.5-h on a magnetic stirrer before refluxing. The samples were centrifuged at 8000 rpm for 15 min after the reflux action was finished, and they were then three times rinsed with distilled water and ethanol. Additionally, it was overnight dried at 80°C.

Animals and study protocol

The experiment was conducted on 80 adult male Wistar rats. The animals were divided into eight groups (n=10), provided by the animal house of the Faculty of Veterinary Medicine, Zagazig University. The animals were maintained in microclimatic temperatures of 24°C, 12 h light, and 12 h dark with ad-libitum access to water and a standard chewable diet. Before beginning the experiments, the animals were acclimatized for one week. The experimental protocol was approved by ZU_ IACUC Committee approval number (ZU_IACUC/2/F/103/2022).

All animals were fasted overnight and type 2 diabetes was induced by intraperitoneal injection with a single dose of nicotinamide dissolved in 0.9% saline solution (110 mg/kg body weight).

and after fifteen minutes, a single dose of STZ was dissolved in citrate buffer (pH = 4.5) and, injected intraperitoneally (45 mg/kg body weight). Rats were kept on a glucose (10% w/v) solution for 3 days to avoid acute hypoglycemia. Seven days post-STZ injection, blood samples were collected from the tail vein, and post-fasting blood glucose concentration was measured by a digital glucometer (one touch, LifeScan, USA). Rats with fasting blood glucose levels of more than 250 mg/dl were enrolled in the study. All diabetic rats were transferred to metabolic cages and 24-h urine was collected at the end of the experiment (Lekshmi et al., 2015).

The treatments were classified as follows: G1 (control); the animals were injected with saline and served as controls and fed on a normal diet, G2 (diabetic non-treated); rats received no drug and served as the STZ-induced diabetic group, G3 (Diabetic+ZnO NPs); rats received ZnO NPs (0.25 mg/kg/d) in drinking water for 6 weeks (Amiri et al., 2018), G4 (Diabetic+ZnO NPs); rats received ZnO NPs (0.5 mg/kg/d) in drinking water for 6 weeks (Amiri

et al., 2018), G5 (Diabetic+Empagliflozin); rats received Empagliflozin (0.25 mg/kg/d) in drinking water for 6 weeks (Li et al., 2020), G6 (Diabetic+Empagliflozin); rats received Empagliflozin (0.5 mg/kg/d) in drinking water for 6 weeks (Li et al., 2020), G7; combination group between Empagliflozin (0.25 mg/kg/d) in drinking water and ZnO NPs (0.25 mg/kg) for 6 weeks, and G8; combination group between Empagliflozin (0.5 mg/kg/d) in drinking water and ZnO NPs (0.5 mg/kg) for 6 weeks.

Collection of samples and preparation of tissue homogenate

Blood samples were collected from the orbital venous plexus without anticoagulant and centrifuged at 3,000 rpm for 15 min (6 samples per group). The sera were preserved at -20 ˚C until used for further investigation. The liver, kidneys, and pancreas were immediately perfused in situ and rapidly removed (6 samples per group). The liver, kidneys, and pancreas were divided into two parts. The first part was collected in 10% formalin neutral buffer for histopathological examination, and the second part was collected in 1 ml thiazole and stored at – 80˚C until used for gene expression.

Biochemical and oxidant stress markers assay

The sera biochemical parameters were assisted using a commercial kit following manufacturing instructions, glucose (MyBioSource, USA), insulin (MyBioSource, USA), ALT (Abcam, USA), AST (Abcam, USA), creatinine (Abcam, USA), albumin (enzymatic colorimetric kit Spinreact, Girona, Spain), cholesterol (HDL and LDL/VLDL, USA), triglycerides (Quantification Abcam, USA), HDL (Fluorometric Abcam, USA), LDL (Crystal Chem's Rat LDL, USA), IgG (ELISA Kit, USA), IgM (ELISA Kit, USA), MDA (ELISA Kit MyBioSource, USA), CAT (ELISA Kit MyBioSource, USA), GPx (ELISA Kit Biodiagnostic, USA), and SOD (Abcam, USA).

Gene expression

Briefly, total RNA was extracted from 30 mg of pancreatic tissue using Trizol (Invitrogen; Thermo Fisher Scientific, Inc.), analyzed with the NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, Delaware, USA), and cDNA was synthesized with a HiSenScriptTM RH (cDNA Synthesis Kit). All of these steps were performed in order (iNtRON Biotechnology Co., South Korea). Following the instructions provided by the manufacturer, the real-time RT-PCR was carried out using an MX3005P Real-Time PCR System (Agilent Stratagene, USA) with 5X HOT FIRE Pol EvaGreen qPCR Mix Plus (Solis BioDyne, Tartu, Estonia). The parameters for the PCR cycling were as follows: initial denaturation at 95oC for 12 min, 40 cycles of denaturation at 95oC for 20 sec, annealing at 60oC for 30 sec, and extension at 72oC for 30 sec, and followed by melting curve analysis. The level of expression of the target genes was standardized by comparing it to the mRNA level of a well-established housekeeping gene called GAPDH using the formula of 2-ΔΔct (Livak & Schmittgen, 2001). The primer sequences of the desired genes were designed according to Table 1.

Histopathological investigations

After 48 h in 10% neutral buffered formalin, samples of liver and kidney cells were washed overnight with running water. By employing an increased percentage of ethyl alcohol, the washed samples were dehydrated into ascending concentrations of ethyl alcohol. The samples were left in each concentration for 12 hours before being cleaned for 2 hours in xylol. After two hours in an oven at 48˚C, the samples were placed in a crucible with melting paraffin and held there for 3 hours. Tissue sections were then blocked in hard paraffin and sliced into 5 micron-thick pieces. For histopathological analysis, H&E-stained sections were mounted with Canada balsam and covered with cover slides for.

Statistical investigation

Results were reported as mean ± SEM (Standard Error of Mean). To assess the influence of the four treatment groups on the different biochemical parameters, one-way analysis of variance (ANOVA) by Duncan multiple tests as post-hoc tests was used. The value of $P < 0.05$ was used to indicate statistical significance. All analyses and charts were done using the Statistical Package for Social Sciences version 24.0 (SPSS, IBM Corp., Armonk, NY) and Graph Pad prism 8.0.2 (GraphPad Software, Inc).

RESULTS

ZnO NPs characterization

TEM was performed on a JEOL JEM-2100 high-resolution transmission electron microscope at an accelerating voltage of 200 kV, respectively. The average particle size of ZnO NP was 30 nm (Figure 1).

Effects of administration of Empagliflozin and ZnO NPs on some biochemical parameters in type 2 diabetic rats

The result of the present study in Figure 2 revealed that glucose, insulin, creatinine, GPT, and GOT levels were the highest ($p < 0.05$) in positive control groups. While their levels were significantly ($p < 0.05$) improved in the combination group between Empagliflozin and ZnO NPs (0.5 mg/kg/d). On the other hand, albumin was the lowest ($p < 0.05$) in the positive control group and increased ($p < 0.05$) in the combination group between Empagliflozin and

Table 1: Primer sequences for the real-time PCR.

Gene	Sequence	pb	Gene Bank accession number
Patched 1	F 5′-TCCCCTCCTCCTCCTCTTTC-3′	192	NM_053566.3
	R 5'-CTTGTTCTCCTCACCGACCC-3'		
Hhip-1	F 5′-GCTCTTTGGTCCTGATGGCT-3'	191	NM_001191817.1
	R 5'-GCTGGTTGGTGCTGTTGAAG-3'		
SMO	F 5′-TTCCTCATCCGAGGGGTCAT-3'	87	NM_012807.1
	R 5'-ATTGATCTTGCTGGCTGCCT-3'		
GLI1	F 5′-CCTCCACCCCAGTATCTCCA-3'	162	NM_001191910.1
	R 5'-ACAATTCCTGCTGCGACTGA-3'		
ERK1	F 5′-AACCCAAACAAGCGCATCAC-3'	87	NM_017347.3
	R 5'-AGCCACTGGTTCATCTGTCG-3'		
PPAR-ϒ	F 5′-TCATGACCAGGGAGTTCCTCA-3'	226	NM_25664
	R 5'-TCATCTAATTCCAGTGCATTGAACTT-3'		
IGF1	F 5′-AAGCCTACAAAGTCAGCTCG-3'	166	NM_001082477.2
	R 5'-GGTCTTGTTTCCTGCACTTC-3'		
GLP-1	F 5′-CACCTCCTCTCAGCTCAGTC-3'	128	NM_012707.2
	R 5'-CGTTCTCCTCCGTGTCTTGA-3'		
PPAR-α	F 5′-GTCCTCTGGTTGTCCCCTTG-3'	176	NM_013196.2
	R 5'-GTCAGTTCACAGGGAAGGCA-3'		
PCG-1α	F 5′-TTCAGGAGCTGGATGGCTTG-3'	70	NM_031347.1
	R 5'-GGGCAGCACACTCTATGTCA-3'		
GADPH	F 5′-GCATCTTCTTGTGCAGTGCC-3'	91	NM_017008.4
	R 5'-GGTAACCAGGCGTCCGATAC-3'		

ZnO NPs (0.5 mg/kg/d).

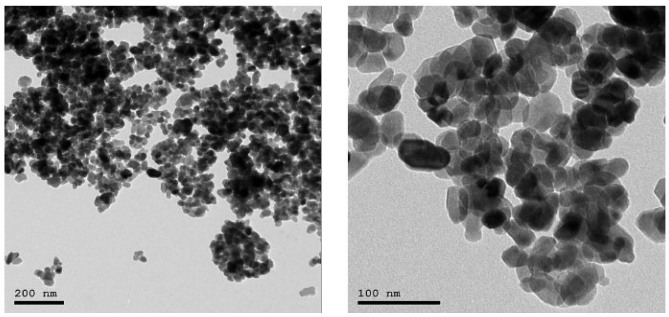

Figure 1: TEM image of the ZnO NP.

Figure 3 showed that triglycerides and LDL levels were the highest ($p < 0.05$) in positive control groups and significantly ($p < 0.05$) improved in Empagliflozin-treated animals (0.5 mg/kg/d), while LDL was improved in Empagliflozin-treated (0.25 mg/kg/d). On the other hand, HDL, IgG, and IgM levels were the lowest in the positive control group, while the cholesterol level was the lowest in ZnO NPs-treated (0.25 mg/kg/d) animals, and the cholesterol and HDL levels increased in the combination group (Empagliflozin and ZnO NPs, 0.25 mg/kg/d). On the other hand, IgG and IgM levels were increased in the combination group between Empagliflozin and ZnO NPs (0. 5 mg/kg/d).

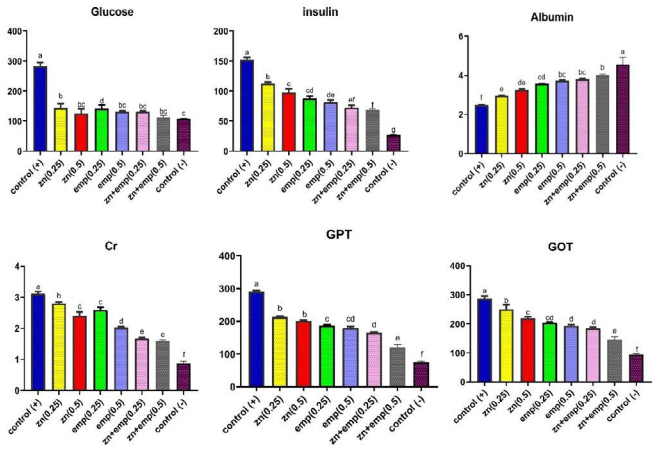

Figure 2: Effects of administration of Empagliflozin and ZnO NPs in the levels of some biochemical parameters in serum in type 2 diabetic rats.

Effects of administration of Empagliflozin and ZnO NPs on the levels of oxidant stress markers in serum in type 2 diabetic rats

Figure 4 demonstrated that GPx, CAT, and SOD activities were the lowest ($p < 0.05$) in positive control rats. While their levels were significantly ($p < 0.05$) improved in the combination group (Empagliflozin and ZnO NPs, 0. 5 mg/

kg/d). meanwhile, the MDA activity was the highest ($p < 0.05$) in positive control rats and decreased in the combination group (Empagliflozin and ZnO NPs, 0. 5 mg/kg/d).

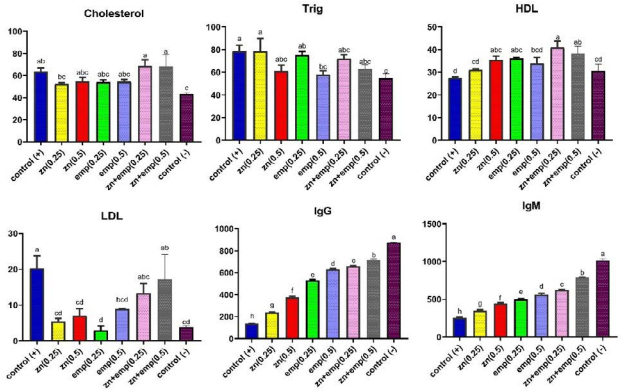

Figure 3: Effects of administration of Empagliflozin and ZnO NPs in the levels of some biochemical parameters in serum in type 2 diabetic rats.

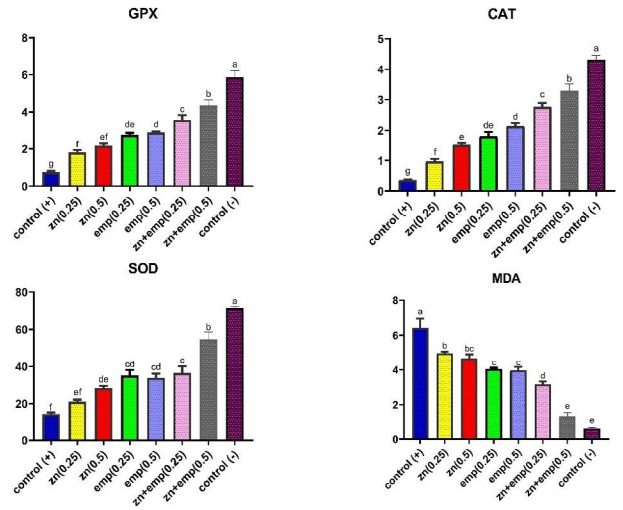

Figure 4: Effects of administration of Empagliflozin and ZnO NPs in the levels of oxidant stress markers in serum in type 2 diabetic rats.

Effects of administration of Empagliflozin and ZnO NPs in the levels of mRNA expression of some genes in type 2 diabetic rats

The mRNA expression of Patched 1, Hhip-1, SMO, GLI1, and ERK1 in Figure 5 demonstrated their activities in tissue homogenate were at the highest ($p < 0.05$) levels in positive control rats and significantly decreased in the combination group (Empagliflozin and ZnO NPs, 0. 5 mg/kg/d). The lowest ($p < 0.05$) levels were revealed in control negative groups.

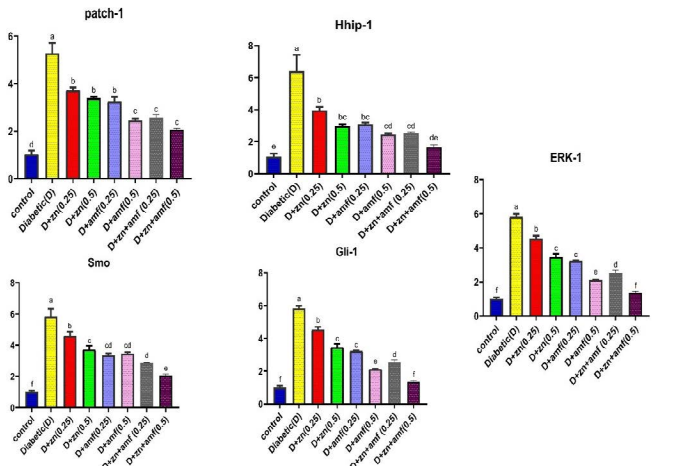

Figure 5: Effects of administration of Empagliflozin and ZnO NPs in the mRNA expression of some genes in type 2 diabetic rats.

Figure 6 showed that the mRNA expression of PPAR-Y, IGF1, GLP-1, PPAR-α, and PCG-1α in tissue homogenate were at the lowest ($p < 0.05$) levels in control positive rats, and their levels significantly ($p < 0.05$) improved in the combination group (Empagliflozin and ZnO NPs, 0. 5 mg/kg/d).

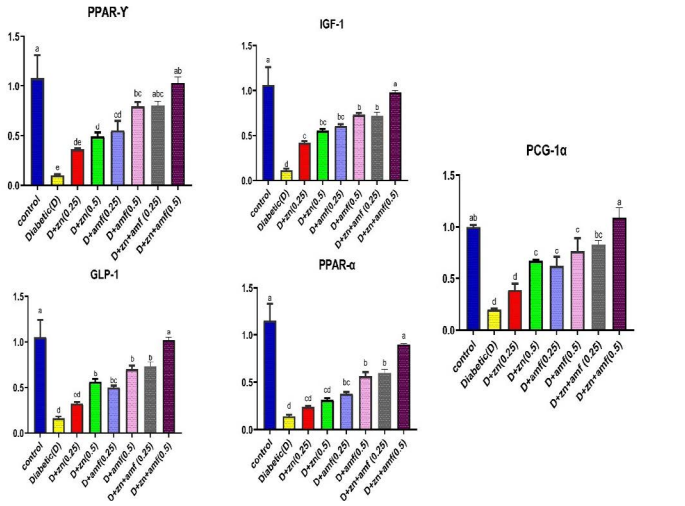

Figure 6: Effects of administration of Empagliflozin and ZnO NPs in the mRNA expression of some genes in type 2 diabetic rats.

Histopathological results

The liver of the control rat showed a normal histological picture (Figure 7A). The liver of diabetic rats showed acute cellular swelling with marked vacuolations, single-cell necrosis, and endothelial hypertrophy (Figure 7B). The liver of a 0.25 mg ZnO NPs-treated rat showed vacuolations of the hepatocytes, portal congestion, and mononuclear cell infiltration (Figure 7C). The liver of 0.5 mg ZnO NPs-treated rats showed vacuolations of the hepatocytes and portal infiltration with few numbers of mononuclear cells (Figure 7D). The liver of a 0.25 mg empag-

liflozin-treated rat showed notable sinusoidal dilatation with vacuolation of the hepatocytes and a few single-cell necroses (Figure 7E). The liver of a 0.5 mg empagliflozin-treated rat shows portal congestion with the presence of few numbers of inflammatory cell infiltrate (Figure 7F). The liver of 0.25 mg ZnO NPs + 0.25 mg empagliflozin-treated rats showed mild sinusoidal dilatation and vascular congestion (Figure 7G). The liver of 0.5 mg ZnO NPs + 0.5 mg empagliflozin-treated rats showed an almost normal histological picture except for mild vascular congestion (Figure 7H).

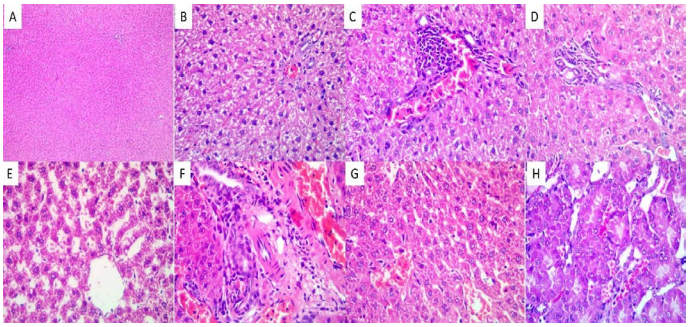

Figure 7: Histopathological results in the liver. (A) The liver of the control rat shows a normal histological picture, H&E, X 40. (B) The liver of diabetic rats showed acute cellular swelling with marked vacuolations, single-cell necrosis, and endothelial hypertrophy, H&E, X 40. (C) The liver of 0.25 mg ZnO NPs treated rat showed vacuolations of the hepatocytes, portal congestion, and mononuclear cell infiltration, H&E, X 40. (D) The liver of 0.5 mg ZnO NPs-treated rats showed vacuolations of the hepatocytes and portal infiltration with few numbers of mononuclear cells, H&E, X 40. (E) The liver of 0.25 mg empagliflozin-treated rats showed notable sinusoidal dilatation with vacuolation of the hepatocytes and few single-cell necroses, H&E, X 40. (F) The liver of a 0.5 mg empagliflozin treated rat shows portal congestion with the presence of few numbers of inflammatory cell infiltrates, H&E, X 40. (G) the liver of 0.25 mg ZnO NPs + 0.25 mg empagliflozin-treated rats showed mild sinusoidal dilatation and vascular congestion, H&E, X 40. (H) The liver of 0.5 mg ZnO NPs + 0.5 mg-empagliflozin-treated rats showed an almost normal histological picture except for mild vascular congestion, H&E, X 40.

The kidney of the control rats showed a normal histological picture (Figure 8A). The kidney of diabetic rats showed notable vascular congestion with endothelial hypertrophy, interstitial edema, and tubular necrosis (Figure 8B). The kidney of 0.25 mg ZnO NPs-treated rats showed interstitial mononuclear cell infiltration and vacuolation of the tubular epithelium with single-cell necrosis (Figure 8C). The kidney of 0.5 mg ZnO NPs-treated rats showed tubular attenuation, hyaline cast formation, congestion of the peritubular capillaries, regenerative tubular epithelium, and endothelial hypertrophy (Figure 8D). The kidney of 0.25 mg empagliflozin-treated rats showed cast formation, tubular attenuation, vascular congestion, endothelial hypertrophy, and the presence of few numbers of mononuclear cells in the interstitial tissue (Figure 8E). The kidney of a 0.5 mg empagliflozin-treated rat shows vascular congestion, and endothelial hypertrophy (Figure 8F). The kidney of 0.25 mg ZnO NPs + 0.25 mg empagliflozin-treated rat shows mild vascular congestion, and luminal debris (Figure 8G). The kidney of 0.5 mg ZnO NPs + 0.5 mg empagliflozin-treated rats showed an almost normal histological picture except for mild glomerular congestion (Figure 8H).

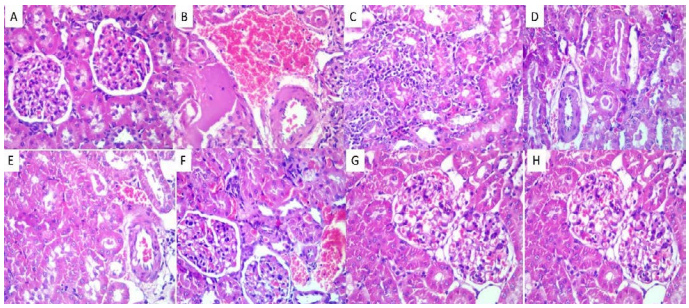

Figure 8: Histopathological results in the kidney. (A) The kidney of control rats showed a normal histological picture, H&E, X 40. (B) The kidney of diabetic rats showed notable vascular congestion with endothelial hypertrophy, interstitial edema, and tubular necrosis, H&E, X 40. (C) The kidney of 0.25 mg ZnO NPs-treated rats showed interstitial mononuclear cell infiltration, and vacuolation of the tubular epithelium with single-cell necrosis, H&E, X 40. (D) The kidney of 0.5 mg ZnO NPs-treated rats showed tubular attenuation, hyaline cast formation, congestion of the peritubular capillaries, regenerative tubular epithelium, and endothelial hypertrophy, H&E, X 40. (E) The kidney of 0.25 mg empagliflozin-treated rats showed cast formation, tubular attenuation, vascular congestion, endothelial hypertrophy, and the presence of few numbers of mononuclear cells in the interstitial tissue, H&E, X 40. (F) The kidney of 0.5 mg empagliflozin-treated rats showed vascular congestion, and endothelial hypertrophy, H&E, X 40. (G) The kidney of 0.25 mg ZnO NPs + 0.25 mg empagliflozin treated rats showed mild vascular congestion, and luminal debris, H&E, X 40. (H) The kidney of 0.5 mg ZnO NPs + 0.5 mg empagliflozin-treated rats showed an almost normal histological picture except for mild glomerular congestion, H&E, X 40.

DISCUSSION

Diabetes mellitus has reached pandemic proportions worldwide and is only expected to increase.

The prevalence of diabetes and its complications—cardiomyopathy, nephropathy, retinopathy, neuropathy, and an

increased risk of limb amputation—is rising in tandem with the prevalence of obesity, inactivity, and high-calorie diets. Controlling diabetes mellitus necessitates a combination of approaches, the two most important being maintaining lower blood sugar levels and lowering oxidative stress. The relationship between diabetes and zinc homeostasis suggests that zinc-based therapy may be an effective treatment option (Wahba et al., 2016).

Several studies found that administration of ZnO NPs significantly decreased lipid peroxidation marker (MDA) and improved the levels of antioxidant enzymes, which could be attributed to the antioxidant effect of zinc as the main trace element needed for the ROS and RNS scavenging system (Afifi et al., 2015; Hussein et al., 2018; Kabel et al., 2020; Nazarizadeh & Asri-Rezaie, 2016; Siddiqui et al., 2020; Umrani & Paknikar, 2014). Others found that docosahexaenoic acid-loaded ZnO NPs had a stronger antioxidative effect, as seen by a reduction in DNA damage, MDA, AOPP, and SOD levels. ZnO NPs may have antioxidant effects via negatively regulating inflammatory cytokine gene expression, which generates ROS, and by stabilizing cell membranes against oxidative damage. All biochemical tests, including blood glucose, insulin, insulin resistance, oxidants and antioxidants, cholesterol and triglycerides, fatty acid parameters, and PI3K levels, showed the successful development of diabetes in experimental rats. Biocompatibility and physicochemical characteristics of zinc oxide nanoparticles helped treat diabetes (Hussein et al., 2019).

On the other hand, empagliflozin is considered one of the PPAR coactivator (Ala et al., 2022) that potentially improve cellular energy balance, enhancing insulin secretion and sensitivity as they are modulators for PPAR-α and PPAR-γ which play a crucial role in glucose utilization in the peripheral tissues and improve insulin secretion as the major elements of glucose-stimulated insulin secretion (GSIS) that finally restored the oxidant scavenging system thus the empagliflozin play antioxidant role (Oshima et al., 2019).

The previous finding could be owed to the stimulation of empagliflozin and ZnO NPs for the PPAR nuclear receptor superfamily as a potent coactivator, besides the antioxidant effect of zinc and empagliflozin (Kabel et al., 2020). Individuals who have type 2 diabetes often have abnormally low amounts of the hormone GLP-1, which the body typically produces. The GLP-1 pathway's ability to modulate blood sugar levels improves insulin synthesis and secretion (de Lima Ávila et al., 2013). The prevalence and severity of type II diabetes are major global public health concerns. Proper glucose control has been linked to the signalling and transcriptional pathways involving the PGC-1 family of peroxisome proliferator-activated receptor gamma coactivators. This category of transcriptional coactivators exerts substantial control over mitochondrial and metabolic activities. PGC-1s have been demonstrated to have distinct roles across tissues, as evidenced by tissue-specific overexpression and deletion models (Rowe & Arany, 2014).

The results of the current work showed significant downregulation in GLP-1 and PCG-1α in the diabetic group, while their levels were restored in groups administrated empagliflozin and/or ZnO NPs. These data are in line with those previously reported that the anti-diabetic effect of ZnO NPs was examined by increasing insulin and its receptors in glucose-metabolizing enzymes. According to several studies, ZnO NPs decreased blood glucose in diabetic rats. Type 2 DM causes insulin resistance. Zinc is insulin-mimetic and antioxidant, and deficiency is linked to insulin resistance (Umrani & Paknikar, 2014).

ZnO NPs have been shown to lower glucose levels in clinical experiments with diabetics. Rats with diabetes had lower blood sugar levels after receiving oral doses of ZnO NPs (1-10 mg/kg/day). When given orally to diabetic rats for a month, ZnO NPs alone or in combination with vildagliptin significantly improved insulin sensitivity and decreased pancreatic histological abnormalities (El-Gharbawy et al., 2016).

For the management of type 2 diabetes, the sodium-glucose cotransporter 2 inhibitor empagliflozin has been authorised. Glycated haemoglobin levels have been lowered in patients with type 2 diabetes, including those with chronic renal impairment, who have received the medication as monotherapy or as an adjunct therapy. Empagliflozin has been associated with a reduction in body weight and hypotension without an increase in heart rate. Empagliflozin lowers levels of indicators for arterial stiffness and vascular resistance, including visceral obesity, albuminuria, and plasma urate. A rise in both low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol has been associated with the use of empagliflozin (Zinman et al., 2015).

IGF1 is involved in glucose metabolism. Low-IGF1 is linked to glucose intolerance, and people with pathologically low- or high-IGF1 risk diabetes. Enterocyte proliferation and differentiation are stimulated by IGF-1, a hormone generated in the GI tract. In weaned piglet mucosa, 3000 mg ZnO/kg increased mRNA expression of IGF-1 and IGF-1R (Li et al., 2006). Another study showed that increases in IGF-1 mRNA expression in the mucosa were observed with both high and low doses of zinc oxide, whereas feeding coated zinc oxide improved small intestine shape in part through increasing IGF-1 expression in

the GI tract (Shen et al., 2014) and this comes in agreement with our results in the diabetic group IGF-1 level was downregulated while its level was restored in groups administered empagliflozin and/or ZnO NPs.

Studies have linked diabetes to abnormalities that increase serum lipids in the bloodstream, which in turn increases the risk of cardiovascular disease. Diabetes was associated with increased total cholesterol, triglyceride, and LDL-cholesterol levels as well as decreased HDL-cholesterol levels in the study population. The results of this study agree with those of the current inquiry. High-density lipoprotein (HDL-C) levels were shown to be reduced in diabetic rats. Diabetic rats given Vildagliptin alone or in combination with varying concentrations of ZnO NPs showed a significant decrease in nonesterified fatty acid, total cholesterol, triglyceride, and LDL-cholesterol concentrations (El-Gharbawy et al., 2016).

High levels of insulin resistance in the liver and other tissues are associated with type 2 diabetes. This resistance is caused in part by overexpression of SMO and Patched 1, two genes involved in the upstream regulation of the Hh pathway (Lin et al., 2019).

Our results were in line with the previous studies as the gene expression of Patched 1, Hhip-1, SMO, GLI1, and ERK1 was significantly downregulated. On the other hand, there was a significant upregulation in the mRNA expression of PPAR–γ, IGF1, GLP-1, PPAR-α, and PGC-1α which is also a dose- and time-dependent manner.

Our results are in agreement with the study that suggested that ERK is linked to insulin resistance in obesity and type 2 diabetes and also during the gastro-intestinal epithelial repair. In a study, a diosmectite–zinc oxide composite increased ERK activation over acetic acid (Song et al., 2015). It was found that zinc stimulated ERK signalling pathways to promote osteogenic activity in mouse MC3T3-E1 osteoblasts (Liang et al., 2012). ZnO NPs were cytotoxic to breast cancer cells, at which the nanoparticles downregulated Bcl-2, AKT1, and ERK1/2 dose-dependently (Boroumand Moghaddam et al., 2017).

Hepatic conditions can be very harmful to zinc because the majority of zinc metabolism takes place there. The opposite is true for zinc deficiency in inflammatory liver illnesses, which may influence hepatocyte activity and immunological responses. Hepatitis susceptibility, a weakened acute-phase response, and lipid peroxidation are all consequences of oxidative stress, which can be caused by a zinc deficiency. Loss of zinc disrupts oxidative-sensitive transcription factors' ability to regulate cell function, proliferation, and survival by altering the redox state. Our findings that ZnO NPs and/or empagliflozin reduced the liver function enzymes are consistent with previous research showing that zinc therapy increased AST and ALT levels in patients (Gruengreiff et al., 2016).

Zinc overconsumption leads to an increase in systemic blood pressure and a decline in renal function due to the oxidative stress induced by superoxide radicals. Low zinc levels are common among those with chronic renal disease because to dietary restrictions and poor zinc absorption by the GUT. Zinc supplementation enhanced zinc levels in the blood and enhanced the sense of taste. ZnO NPs and/or empagliflozin decreased the kidney function enzymes, and this is consistent with our research showing that it increased calorie consumption in patients with renal insufficiency that wasn't too severe (serum creatinine 5.0 mg/dl) (Neto et al., 2016).

The current study confirmed the presence of histological abnormalities of ZnO NPs in liver tissue, which is in line with the findings of Muzammil et al. (2013), who discovered that zinc-oxide oral therapy generated severe pathological consequences on the liver and heart. Variable degrees of chromatin condensation and fragmentation were seen in the nuclei of different liver hepatocytes as a result of apoptosis. In the group that received the body weight-based dose of ZnO NPs (400 mg/kg), liver tissue showed signs of haemorrhaging, localized necrosis, and lymphocyte infiltration.

CONCLUSION

ZnO NPs showed a promising antidiabetic effect and the potential to be used to build an antidiabetic medication. ZnO NPs contribute to a reduction in blood glucose, insulin levels, lipid profile, and liver and kidney functions in diabetic rats when combined with empagliflozin. In addition, they reduce the inflammation, oxidative stress, and insulin resistance associated with diabetes, as well as the structural damage to the kidney, by inhibiting the Hh pathway. Possibly, this is the first study to show the influence of ZnO NPs and empagliflozin on Hh signalling pathways in diabetes. According to the current and earlier studies, nanoparticle inclusion in therapeutic strategies could provide new avenues for increasing their effectiveness against chronic illnesses like T2DM.

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ETHICAL STATEMENT

The experimental work was approved by the Insti-

tutional Animal Care and Use Committees Zagazig University (ZU-IACUC) with approval No. ZU_IACUC/2/F/103/2022.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

NOVELTY STATEMENT

However, some work has been carried out to ZnO NPs effective in treating diabetes and reducing its consequences and previous works have not comprehensively considered the combination between Empagliflozin and ZnO NPs. Therefore, further investigations and clinical trials must be carried out in order to utilize it to the full potential of this combination.

AUTHOR'S CONTRIBUTION

All authors contributed equally.

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