Zinc Oxide Nanoparticles and Synthesized Pyrazolopyrimidine Alleviate Diabetic Effects in Rats Induced by Type II Diabetes

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ABSTRACT: Diabetes mellitus (DM) is a category of metabolic illness characterized by high blood sugar levels and insufficient pancreatic insulin production or activity within the body. The most common type of diabetes is type II diabetes, which is a metabolic condition characterized by insulin resistance and pancreatic islet β-cell failure, resulting in hyperglycemia. The goal of this study was to examine the anti-diabetic implications of zinc oxide nanoparticles (ZnO NPs) and/or pyrazolopyrimidine in type II diabetic rats. Rats with a weight of 150 ± 20 g were used. Animals were divided into five groups as follows: group 1: control, group 2: type II diabetic rats, group 3: diabetic rats received ZnO NPs (10 mg/kg/orally/day), group 4: diabetic rats received pyrazolopyrimidine (5 mg/kg/orally/day), and group 5: diabetic rats received ZnO NPs (10 mg/kg/orally/day) + pyrazolopyrimidine (5 mg/kg/orally/day), respectively, for 30 days. The results indicated that serum glucose, total cholesterol (TC), triacylglycerol (TG), low-density lipoprotein-cholesterol (LDL-c), very low-density lipoprotein-cholesterol (VLDL-c), malondialdehyde, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and peroxisome proliferator-activated receptor gamma coactivator 1-alpha PGC-1α mRNA expressions were increased in the diabetic group versus the control group, while serum insulin, high-density lipoprotein-cholesterol (HDL-c), superoxide dismutase (SOD), and carnitine palmitoyltransferase 1A (CPT1A) mRNA expression levels were decreased. These parameters were restored in the treated groups (ZnO NPs, pyrazolopyrimidine, and ZnO NPs + pyrazolopyrimidine). This study proved that ZnO NPs and pyrazolopyrimidine had an ameliorative effect on blood glucose levels, antioxidant status, lipid profile, liver function enzymes, and mRNA expression of hepatic genes.

INTRODUCTION

Diabetes mellitus (DM) is a term to describe a series of metabolic diseases that are characterized by excessive levels of blood glucose and insufficient pancreatic insulin production or activity within the body. According to the World Health Organization (WHO), more than 400 million people are reportedly suffering from diabetes worldwide, and by 2030, that number will reach 552 million. Type 2 DM (T2DM) is distinguished by progressive β-cell insulin secretion failure, which frequently manifests as insulin resistance. Individuals with DM, hypertension, and extreme obesity (BMI ≥ 40 kg/m²) are more likely to be compromised. COVID-19 exposes them to an increased risk of complications and death.

It has been shown that metals participate in the processing of glucose levels, and it has been reported that diabetes pathogenesis can be impaired by deficiencies in these metals. Vanadium, magnesium (Mg), and Zn are among the metals that play a significant part in regulating blood sugar levels and are successful in treating diabetes. Zn is an important pancreatic function micronutrient due to its impact on insulin sensitivity, and diabetes problems are linked to zinc metabolism defects. It is stated that Zn inhibits the secretion of glucagon.

Nanomedicine is one of the applications of nanotechnology, and zinc oxide nanoparticles (ZnO NPS) are one of the better-known NPs generally used for medical applications. Because of its remarkable biological properties and its low toxicity, it can resolve many challenges to the effective targeting of molecules and cells in many diseases. Numerous experiments have shown that in diabetic animals, ZnO NPs decrease blood glucose levels.

Pyrazolopyrimidine is a preferred type of fused heterocyclic compound containing nitrogen that contributes to a significant...
Figure 1. Experimental design and the induction of diabetes type II.

proportion of every molecule used in biological sciences. 1H-pyrazolo [3,4-d] pyrimidine, 1H-pyrazolo [3,4-d] pyrimidine, and pyrazolo [1,5-a] pyrimidine are all known pyrazolopyrimidine isomers. Furthermore, biological activities show that the presence of a nitro moiety on the phenyl group in pyrazolo [3,4-d] pyrimidines contributed significantly to their anti-diabetic activity. The anti-diabetic activity of pyrazolo [3,4-d] pyrimidine amide derivatives was excellent.

The purpose of this study was to examine the anti-diabetic implications of ZnO NPs and pyrazolopyrimidine in type II diabetic rats. We postulated that ZnO NPs and pyrazolopyrimidine had an ameliorative effect on blood glucose levels, antioxidant status, lipid profile, liver function enzymes, and mRNA expression of hepatic glucose metabolism-related genes.

## MATERIALS AND METHODS

**Animals.** Sixty healthy male adult albino rats weighing 150–170 g (7 weeks age) were obtained from the Faculty of Veterinary Medicine’s central animal house at Cairo University, Egypt. They had been conditioned for 2 weeks in standard laboratory conditions. They were maintained at a suitable temperature of 20–25 °C, had a 12-h light–dark cycle, and had free access to water and food.

**Chemical Substances.** Streptozotocin. A pale-yellow crystalline powder produced from Streptomyces chromogens was freshly prepared before injection by dissolving in citrate buffer (cold) (pH 4.5), and it was purchased from TOKU-E Company (Toku-E.com). Nicotinamide, a white powder with a molecular formula of C6H6N2O, is a component of the coenzyme nicotinamide adenine dinucleotide and the active form of vitamin B3 (NAD), and it was purchased from Sigma.

ZnO NPs were obtained from the Agricultural Research Center in Zagazig. It was synthesized by the precipitation method as mentioned in the experimental part.

**Synthesis of ZnO NPs.** The ZnO NPs were obtained from the Agricultural Research Center in Zagazig. They were synthesized by the precipitation method as mentioned in the experimental part; the reaction of potassium hydroxide and zinc acetate produces zinc hydroxide Zn(OH)2. The calcination process at 150 °C for 6 h was followed to convert the formed Zn(OH)2 to ZnO NPs.

The formation of ZnO NPs is represented by the following equations:

\[
\text{Zn(CH}_3\text{COO)}_2 + 2 \text{KOH} \rightarrow \text{Zn(OH)}_2 + 2\text{CH}_3\text{COOK}
\]

\[
\text{Zn(OH)}_2 + \text{heat (150}^\circ\text{C)} \rightarrow \text{ZnO} + \text{H}_2\text{O}
\]

**Characterization of Zinc Oxide Nanoparticles.** The synthesis of ZnO nanoparticles was characterized by different analytical techniques such as UV–Vis spectroscopy, FTIR, XRD, SEM studies, EDX, TEM precision, and DLS.

**Experimental Design and the Induction of Type II Diabetes in Rats.** According to Figure 1, 60 male adult albino weighing 150–170 g were derived from the Central Veterinary Medicine’s Animal House Faculty at Cairo Egypt University. They were prepared for 2 weeks under standard laboratory conditions. They were maintained at a suitable temperature of 20–25 °C, had a 12-h light–dark cycle, and had free access to water and food.

After 2 weeks of adaptation, 48 rats received an intraperitoneal injection of streptozotocin (STZ). Nicotinamide was dissolved in physiological saline and a citrate buffer (pH 4.5) solution. All rats were starved but given access to drinking water for 20 h before diabetes induction. The rat model of diabetes that was not insulin-dependent was caused by 110 mg/kg nicotinamide intraperitoneal administration 15 min before ip 65 mg/kg STZ injection.

Three days following STZ injection, whole-blood samples were drawn from overnight-fasted rats’ tail veins, and their glucose levels were measured with a suitable glucometer apparatus. The animals in that study had fasting blood glucose levels of more than 200 mg/dL.

Rats were then allocated to one of five groups: group 1 (G1): control negative group (without treatment); group 2 (G2): control positive group (type II diabetic rats); group 3 (G3): type II diabetic rats received ZnO NPs orally (10 mg/kg/orally/day); group 4 (G4): type II diabetic rats received pyrazolopyrimidine orally (5 mg/kg/orally/day); and group 5 (G5): type II diabetic rats received ZnO NPs (10 mg/kg/orally/day) + pyrazolopyrimidine (5 mg/kg/orally/day) for 30 days. Fasting serum glucose and insulin levels in rats were measured every week. Blood samples were collected, and for 15 min, serum was separated by centrifugation at 3000 rpm before being sent for biochemical analysis. After sacrificing, 50 mg of liver tissue was wrapped up in aluminum foil and preserved in a container filled with liquid nitrogen at −80 °C until it was used in gene expression analysis.

**Biochemical Analysis.** Determination of Fasting Blood Glucose and Insulin Levels. Glucose was calculated in serum
by using the method of glucose oxidase by Spectrum Diagnostics Kits.\(^{18}\) Insulin was measured in serum following the procedure of the Insulin ELISA Kit (RayBiotech Inc., Norcross, Georgia).

**Estimation of the Level of a Lipid Profile.** TC was measured using the CHOD-POD enzymatic colorimetric method,\(^{19}\) TG using the GPO-POD-enzymatic colorimetric method,\(^{20}\) and HDL-c using the direct enzymatic colorimetric liquid method.\(^{21}\) The equation LDL-c = TC − HDL-c (TG/5) was used to calculate LDL-c, and the Friedewald formula was used to calculate VLDL-c.\(^{22}\)

**Levels of Alanine Transaminase (ALT) and Aspartate Transaminase (AST) in the Blood.** Egyptian Company for Biotechnology provided the kits, and the method was according to Tietz.\(^{23}\)

**Determination of Serum Antioxidant Enzymes (Superoxide Dismutase (SOD) and Catalase (CAT).** SOD was measured using the colorimetric activity kit. CAT was measured using the Catalase Assay (Colorimetric Method).  

**Estimation of Lipid Peroxidation (Serum Malondialdehyde (MDA)).** MDA was measured using the Rat Malondialdehyde ELISA Kit.

**Relative Quantitative RT-PCR Analysis.** The actual-time analysis has been previously reported;\(^{24}\) to summarize, total RNA was extracted from the liver using TRIzol (Invitrogen: Thermo Fisher Scientific, Inc.). Thirty milligrams of tissues was homogenized in 1 mL of TRIzol, and then 200 μL of chloroform was added to the homogenate. The expression levels of the target genes were normalized using the mRNA expression of a known housekeeping gene, B-actin. Following the \(2^{−ΔΔCT}\) method, the results are expressed as fold changes compared to the control group.\(^{25}\)

**Statistical Analysis.** The data were described as mean ± SEM. One-way ANOVA was used to compare the means of the five groups for the various parameters. \(P\)-value <0.05 was referred to as statistically significant. Duncan’s multiple-range test was used as a post hoc test after significant ANOVA results to determine group differences. Data analysis was carried out using IBM SPSS Statistics for Windows, version 21 (IBM Corp., Armonk, NY, USA).

## RESULTS

**Effect of ZnO NPs and/or Pyrazolopyrimidinide on Serum Glucose and Insulin Levels of Type II Diabetic Rats.** Figure 2A shows a significant \((P < 0.05)\) increase in serum glucose level in the diabetic group compared to the control group. Moreover, there was a significant decrease in the serum glucose levels in groups administered (ZnO NPs, pyrazolopyrimidine, and ZnO NPs + pyrazolopyrimidine) compared to the diabetic group. Figure 2B demonstrates a significant decrease \((P < 0.05)\) in serum insulin level of the diabetic group, when compared to the control group. Moreover, there was a marked increase in serum insulin levels in groups administered (ZnO NPs and ZnO NPs + pyrazolopyrimidine), while the pyrazolopyrimidine group was non-significant compared to the diabetic group.

**Effect of ZnO NPs and/or Pyrazolopyrimidinide on Serum Lipid Profile of Type II Diabetic Rats.** Table 1 shows that the levels of TC, TG, LDL-c, and VLDL-c in the serum increased significantly \((P < 0.05)\) in the diabetic group, when compared to the control group. Moreover, there was a decrease in the levels of these parameters in groups administered (ZnO NPs, pyrazolopyrimidine, and ZnO NPs + pyrazolopyrimidine), when compared to the diabetic group. The serum HDL-c level in the diabetic group was significantly lower than in the control group. Furthermore, there was an increase in the level of HDL-c in groups administered (ZnO NPs, pyrazolopyrimidine, and ZnO NPs + pyrazolopyrimidine), when compared to the diabetic group (Table 2).

**The Influence of ZnO NPs and/or Pyrazolopyrimidinide on Serum Liver Function Enzymes (ALT and AST) of Type II Diabetic Rats.** In the diabetes group, serum ALT (Figure 3A) and AST (Figure 3B) levels were considerably higher \((P < 0.05)\) in the diabetic group. Furthermore, when compared to the diabetic group, these values were significantly lower in the groups administered (ZnO NPs, pyrazolopyrimidine, and ZnO NPs + pyrazolopyrimidine). Figure 4A shows a significant decrease \((P < 0.05)\) in MDA levels in the diabetic group in comparison to the control group. Furthermore, in comparison to the diabetic group, there was a significant decrease in serum MDA levels in the groups administered (ZnO NPs, pyrazolopyrimidine, and ZnO NPs + pyrazolopyrimidine).

**Effect of ZnO NPs and/or Pyrazolopyrimidinide on Serum Antioxidant Enzyme Level (CAT and SOD) and MDA in Type II Diabetics.** In the diabetic group, serum levels of SOD (Figure 4A) and CAT (Figure 4B) exhibits a substantial drop \((P < 0.05)\) when compared to the control group. In addition to the diabetic group, the groups supplied (ZnO NPs, pyrazolopyrimidine, and ZnO NPs + pyrazolopyrimidine) showed a significant rise in blood SOD and CAT levels. Figure 4C demonstrates a significant increase \((P < 0.05)\) in MDA levels in the diabetic group in comparison to the control group. Furthermore, in comparison to the diabetic group, there was a significant decrease in serum MDA levels in the groups administered (ZnO NPs, pyrazolopyrimidine, and ZnO NPs + pyrazolopyrimidine).

**Effect of ZnO NPs and/or Pyrazolopyrimidinide on the mRNA Expression Level of Hepatic CPT1A and PGC-1α in Type II Diabetic Rat.** Figure 5A demonstrates a significant decrease \((P < 0.05)\) in hepatic CPT1A mRNA expression levels in the diabetic group in comparison to the control group. Moreover, there was a marked increase in hepatic CPT1A mRNA expression levels in groups administered (ZnO NPs, pyrazolopyrimidine, and ZnO
NPs + pyrazolopyrimidine) in comparison to the diabetic group.

Figure 5B shows a significant increase (P < 0.05) in hepatic PGC-1α mRNA expression levels in the diabetic group in comparison to the control group. Moreover, there was a decrease in hepatic PGC-1α mRNA expression level in groups administered (ZnO NPs, pyrazolopyrimidine, and ZnO NPs + pyrazolopyrimidine) in comparison to the diabetic group.

**DISCUSSION**

T2DM is an increasing epidemic around the world. The disorder is associated with significant problems that decrease the quality of life and life expectancy. For T2DM therapy, there are a significant number of drug types, none of which has

Table 1. Lipid Profile Measurements (TC, TG, HDL-c, LDL-c, and VLDL-c)

| P              | control         | diabetic         | ZnO NPs         | pyrazolopyrimidine | pyrazolopyrimidine + ZnO NPs |
|----------------|-----------------|------------------|-----------------|--------------------|-------------------------------|
| TC (mg/dL)     | 126.99 ± 182d   | 172.42 ± 4.56d   | 124.65 ± 2.18d  | 153.44 ± 2.53b     | 140.07 ± 1.38d                |
| TG (mg/dL)     | 86.27 ± 2.44d   | 127.70 ± 2.75g   | 120.88 ± 1.65b  | 99.77 ± 3.67b      | 129.02 ± 1.21b                |
| HDL-c (mg/dL)  | 44.71 ± 1.69d   | 28.90 ± 0.81c    | 40.37 ± 1.37e   | 36.28 ± 1.83b      | 34.78 ± 1.08b                 |
| LDL-c (mg/dL)  | 65.12 ± 1.80d   | 126.04 ± 1.64d   | 55.15 ± 2.01b   | 93.38 ± 1.52c      | 79.49 ± 1.62c                 |
| VLDL-c (mg/dL) | 17.25 ± 0.49d   | 25.54 ± 0.55d    | 24.17 ± 0.33b   | 19.95 ± 0.73b      | 25.80 ± 0.24c                 |

The mean values ± SEM are shown. Different lowercase letters mean statistical differences according to Duncan’s multiple-range test at P < 0.05.

Table 2. The Primer Sequences of the Investigated Genes

| gene     | sequence                          | length | accession no. | reference |
|----------|-----------------------------------|--------|---------------|-----------|
| Cpt1a    | CGGTTCAGAATGGCATCATC TCACCAACCACCAGAT | 76     | NM_031559.2   | 26        |
| PGC1a    | ATGAAATGCAGGCTTCTAGC AACAATGCGAGGTTTGTTC | 74     | NM_031347.1   | 26        |
| GAPDH    | GGCACAGTCAGGCTGAGAATG ATGGTTGTAAGAGACCAGTA | 143    | NM_017008.4   | 27        |

CPT1A, carnitine palmitoyltransferase 1A; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

Figure 3. Serum liver function enzymes (ALT, AST) (U/L) in the control, diabetic, and treated groups. (A) Serum ALT level (U/L). (B) Serum AST level (U/L). P < 0.05 in the same column carrying different small letters indicates significance.

Figure 4. Serum antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT)) and MDA in the control, diabetic, and treated groups. (A) Serum SOD level (ng/mL). (B) Serum CAT level (ng/mL). (C) Demonstrated a significant increase (P < 0.05) in MDA levels in the diabetic group in comparison to the control group.

NPs + pyrazolopyrimidine) in comparison to the diabetic group.

Figure 5B shows a significant increase (P < 0.05) in hepatic PGC-1α mRNA expression levels in the diabetic group in comparison to the control group. Moreover, there was a decrease in hepatic PGC-1α mRNA expression level in groups administered (ZnO NPs, pyrazolopyrimidine, and ZnO NPs + pyrazolopyrimidine) in comparison to the diabetic group.
been convincingly shown to change the gradual deterioration in β-cell function over time. Zn is a necessary metal that stimulates over 300 enzymes in the human body.\(^1\) It promotes hepatic glycogenesis by acting on the insulin receptors, which improves glucose utilization.\(^2\) Nanoparticles of Zn, silver, iron, and gold, as well as their oxides, play an important function in medical and biological applications.\(^3\) ZnO NPs are among several kinds of the day.\(^4\)

In our study, results showed that fasting blood glucose levels after oral supplementation of ZnO NPs and/or pyrazolopyrimidine were significantly decreased compared with the type II diabetic group. This may be due to Zn-suppressing glucagon release, which reduces glycogenolysis and gluconeogenesis.\(^5\) In addition, ZnO NPs have been discovered to be a powerful metal that enhances glucose consumption and metabolism via its potent effect on hepatic glycogenesis via insulin signaling pathway actions.\(^6\) Moreover, pyrazole [3,4-d] pyrimidine-containing amide derivatives could be developed as oral hypoglycemic medications with a little more lead optimization.\(^7\)

In this regard, several studies indicated 1,5-diarylpyrazole derivatives’ hypoglycemic effects.\(^8\) Furthermore, our results are consistent with the previously reported results, which state that fasting blood glucose levels were lower in the diabetic group while higher in the ZnO NP-treated group compared to the diabetic group.\(^9\) Furthermore, it was reported that the fasting blood glucose level was lower after oral ZnO NP supplementation compared to the diabetic group.\(^10\)

In our experiment, results showed that the serum insulin level after oral supplementation of ZnO NPs and/or pyrazolopyrimidine increased than in the diabetic group. Zn improves insulin signaling through a variety of pathways, including increased phosphorylation of insulin receptors, increased PI3K activity, and glycogen synthase kinase-3 inhibition.\(^11\) Zn is also regarded as preserving the structure of insulin.\(^12\) These results were matched with different experiments reported that insulin levels were increased in the group of ZnO NPs\(^13\) and also indicated that insulin levels were increased in the ZnO NP group.\(^14\)

In the current study, measurements of serum lipid profile levels (TC, TG, LDL-c, and VLDL-c) showed a significant decrease in the groups that were treated (ZnO NPs and/or pyrazolopyrimidine), when compared to the type II diabetic group. According to the literature, high blood glucose levels increased cholesterol, LDL-c, and triglyceride levels while lowering HDL-c levels. This is mostly due to lower insulin levels.\(^15\) Similarly, in another study, a rise in blood sugar levels was accompanied by an increase in levels of TG and TC, as well as a reduction in HDL-c. These negative effects could be caused by hormones that lead to cholesterol biosynthesis and/or a lower degree of lipolysis.\(^16\)

The findings were also consistent with the previous study, suggesting that Zn plays a major role in organizing a subset of metalloenzymes taking part in lipid digestion and absorption.\(^17\) The enhanced lipid profile levels after treatment with ZnO NPs, may be attributed to Zn’s important role in enzymatic processes, as Zn can serve as an α-blocker, as demonstrated in the temporal treatment of dyslipidemia.\(^18\) Our findings are in line with reported results,\(^19\) who reported that ZnO NP supplementation may also significantly reduce TC, TG, LDL-c, and VLDL-c and elevate HDL-c levels,\(^20\) which showed that the TG content decreases due to Zn exposure. Moreover, decreased TC and TG levels and elevated HDL-c levels were reported in the ZnO NP-treated diabetic group of mice.\(^21\)

In addition, serum enzyme ALT and AST levels decreased in the groups that were treated with (ZnO NPs and/or pyrazolopyrimidine), when compared to the type II diabetic group. Our findings are consistent with the reported results,\(^22\) which reported that ALT and AST levels in diabetic groups showed a dose-dependent decrease when receiving ZnO NPs. In addition, it was detected that in the STZ-induced diabetic group, both ZnO NPs and scoparia stem extract reduced the fasting blood glucose level was lower after oral ZnO NP administration (ZnO NPs and/or pyrazolopyrimidine). SOD and CAT, the measurement of MDA is a valid tool for assessing the peroxidation of lipids, which is a indicator of ROS-induced oxidative damage.\(^23\) A growing number of studies indicate that ZnO NPs can result in reactive oxygen species (ROS) and thus result in lipid peroxidation.\(^24\) Our results match with the reported results,\(^25\) which showed a decreased serum MDA level in type II diabetic rats after treatment with ZnO NPs.

In comparison to the diabetic group, a significant increase in serum levels of SOD and CAT was detected in groups administered (ZnO NPs and/or pyrazolopyrimidine). SOD and catalase are antioxidant enzymes that work together to remove active oxygen species and prevent oxidant compounds from damaging tissues and cells. Slight differences in these enzymes’ physiological concentrations may induce a breakdown in the body’s protection mechanism and render biomolecules susceptible to oxidative harm.\(^26\)
Since Zn is an important cofactor for SOD activity, supplementing with ZnO NPs during a hyperglycemic state may boost SOD activity. When ZnO NPs are present, CAT behavior often improves, and NADPH supply appears to improve.\textsuperscript{35} Diabetes has an inhibitory effect on antioxidant defense mechanisms.\textsuperscript{38} The administration of ZnO nanoparticles prevented the expression of oxidative stress parameters that are associated with diabetes.\textsuperscript{35} SOD is a zinc-binding enzyme that helps to neutralize ROS.\textsuperscript{60}

The current study showed a noticeable increase in the hepatic CPT1A mRNA expression level in groups administered ZnO NPs and/or pyrazolopyrimidine compared to the diabetic group. Zn increased the activity of the CPT-I lipolytic enzyme and fatty acid oxidation while decreasing the activities of the 6-phosphoglucuronate (6PGD), ME, ICDH, and FAS lipogenic enzymes.\textsuperscript{63} Increasing CPT1A expression could promote fatty acid oxidation, thus indirectly increasing insulin activity and enhancing the IR effect caused by consumption of a high-fat diet.\textsuperscript{51} Our result is consistent with the reported results\textsuperscript{55} that showed that the increases in NEFA concentration, CPT-I lipolytic enzyme activity, and fatty acid oxidation were all promoted by Zn, while the mRNA level of PGC-1\textsuperscript{α} significantly increased in the diabetic group in comparison to the control group. This mRNA expression level of PGC-1\textsuperscript{α} was significantly decreased in the treated groups (ZnO NPs and/or pyrazolopyrimidine), when compared to the diabetic group. Mitochondrial transcription factor a (mtTFA) is a protein that regulates mtDNA transcription and replication and is one of the factors that regulate mitochondrial biogenesis. Peroxisome proliferator activator receptor gamma-coactivator 1 (PGC-1) is mitochondrial biogenesis’s master regulator, which controls the expression of mtTFA.\textsuperscript{52}

PGC-1\textsuperscript{α} regulates a wide range of transcription factors, including the peroxisome proliferator-activated receptor (PPAR).\textsuperscript{53} ZnO NPs have been shown to impair mitochondrial biogenesis, as evidenced by a reduction in mitochondrial density and changes in mtDNA copy number and PGC-1\textsuperscript{α} inhibition.\textsuperscript{54} Our findings were matching with the reported results,\textsuperscript{55} where the PGC-1 expression of genes in the liver significant in the rats injected with Al\textsubscript{2}O\textsubscript{3}NPs, ZnO NPs, and their combination reduced the control value by about 23%, 51%, and 63%, respectively.

\section*{CONCLUSIONS}

We found that ZnO NPs and pyrazolopyrimidine alleviate the adverse effects of type II diabetes on blood glucose level, antioxidant status, lipid profile, liver function enzymes, and mRNA expression of hepatic glucose metabolism-related genes (CPT1A and PGC-1\textsuperscript{α} genes).

The dose of ZnO NPs that was used had a positive effect of reducing hyperglycemia and increasing insulinemia, which indicates an anti-diabetic effect of ZnO NPs. This is due to Zn, which has been shown to suppress glucagon release, which reduces glycogenolysis and gluconeogenesis. On the other hand, ZnO NPs have been discovered to be a powerful metal that enhances glucose consumption and metabolism via its potent effects on hepatic glycogenesis via insulin signaling pathway actions. The dose of pyrazolopyrimidinone that was used had a positive effect of reducing hyperglycemia, which indicates an anti-diabetic effect of pyrazolopyrimidinone. This is due to novel pyrazolo [3,4-d] pyrimidinones’ biological activity and docking investigations as DPP-IV inhibitors. The inhibition capacities of DPPIV for the developed molecules were checked. Our findings showed the agreement between the docking scores, the ability to block α-amylase, and the in vivo anti-diabetic activity, as well as favorable contacts with the amylase protein (1HNY).

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\section*{Notes}

The authors declare no competing financial interest.

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