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RESEARCH ARTICLE

Hypoglycemic and Antioxidant Effects of Zinc oxide nanoparticals in alloxan-Induced Diabetes Rats.

Hasan F.Al-Azzawie[1] , Laith A.Yaaqoob[1] and Salah M.Muhsen[2].

1. Dept.of biotechnology, College of Science, Baghdad University, Iraq.
2. Biotechnology research center. Nahrain university, Iraq.

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Corresponding Author

Hasan F.Al-Azzawie.

Abstract

Zinc oxide nanoparticles (ZnONPs) of average diameter of 45 ± 5.0 nm were prepared using chemical reduction method and characterized by UV-Visible spectroscopy, Scanning electron microscopy (SEM), Atomic force microscopy (AFM), X-Ray diffraction (XRD) and Fourier Transmission Infrared spectroscopy (FTIR). To test the ability of ZnONPs to ameliorate antihyperglycemic and the oxidative stress status resulted in experimental diabetic rats induced by alloxan, sixty male albino rats with weight 220 ± 25 grams and age of 9 months were used in experimental design. Ten of them were served as control group and fifty rats were injected with alloxan at the single intraperitoneal dose of 150 mg/kg. Then, subdivided into, diabetic, diabetic rats + ZnONPs I, received single daily dose of 2.5 mg/kg b.w ZnONPs in suspension. Diabetic rats + ZnONPs II, received a single daily dose of 5.0mg/kg b.w ZnONPs in suspension , diabetic rats + ZnONPs III, received a single daily dose of 10 mg/kg b.w ZnONPs in suspension, diabetic rats + insulin; received a single daily subcutaneous dose of insulin 2U/kg b.w. At the end of experimental time(60 days) the blood glucose, serum insulin,glycoslated HbA1c, lipid peroxidation marker, malondialdehyde (MDA), reduced glutathione (GSH) and serum activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and Catalase (Cat) were determined. Results showed a significant alteration in the activities of SOD, GPx, CAT, MDA Insulin, HbA1c and FBS in animals treated of ZnONPs, compared with diabetic or diabetic + insulin group and their control group. The profound control of ZnONPs over the anti-oxidant enzymes in diabetic rats to normal, by inhibition of lipid peroxidation and reactive oxygen species generation during hyperglycemia evidence their antioxidant effect during diabetes. The administration of ZnONPs at 10 mg/kg b.w exhibited an insistent control over the blood glucose level, lipids and serum biochemical profiles in diabetic rats near to the control group provokes their effective role in controlling and increasing the organ functions for better utilization of blood glucose. Histopathological studies revealed the non-toxic and protective effect of the ZnONPs over the vital organs and can be used to ameliorate the hyperglycemia and oxidative stress status.

Introduction

Diabetes mellitus is a metabolic disorder characterized by high blood glucose. A large number of people suffer from diabetes all over the world[(1)]. These patients would require the development of several medications with multiple modes of actions. Many researches demonstrated the role of metals in glucose metabolism and the association of their deficiency with diabetes, such as Vanadium[(2)],chromium [(3)], magnesium [(4)],copper [(5)] and zinc [(6)] have been reported to play a role in blood sugar maintenance and have been included in diabetes therapy. Zinc, an essential metal, is an activator for more than three hundred enzymes in the body [(7)] and plays a key role in different metabolic

pathways including glucose metabolism. Zinc promotes hepatic glycogenesis through its actions on the insulin pathways and thus improves glucose utilization [8]. Zinc is also known to keep the structure of insulin and has a role in insulin biosynthesis, storage and secretion [9].Alkaladi, *et al*., (2014) ZnO NPs elucidated as anti diabetic agents[10]. They reported that ZnO NPs are more powerful in their effect than silver nanoparticles. ZnO NPs lead to reduction of blood glucose, increased insulin level and expression, increased GK activity and expression and improved expression level of *IRA*, *GLUT-2* in diabetic rats. Umrani et al reported that Oral administration of zinc oxide nanoparticles resulted in significant antidiabetic effects that is, improved glucose tolerance[11].

Zinc, an essential metal, is an activator for more than three hundred enzymes in the body [7], and plays a key role in different metabolic pathways including glucose metabolism. Zinc promotes hepatic glycogenesis through its actions on the insulin pathways and thus improves glucose utilization. Zinc is also known to keep the structure of insulin [8] and has a role in insulin biosynthesis, storage and secretion [6]. There are several zinc transporters in pancreatic b cells [12] like zinc transporter 8 which has a potent role in insulin secretion [13] . In addition, zinc could improve insulin signaling by several mechanisms, including increased insulin receptor phosphorylation, enhancing PI3K activity and inhibition of glycogen synthase kinase [8]. The beneficial role of zinc in diabetes has been implicated by studies of the zinc supplies in diabetic rats [14]. Although zinc is an important metal in a huge number of metabolic processes; there is no data about the effective power of zinc oxide nanoparticles on the oxidative stress status in alloxan induced diabetic rats. This encourages us to study the effect of different concentration ZnONPs in comparison with insulin treatment.

Materials and methods

Synthesis of ZnONPs

The wet chemical method was used to prepare ZnO nanoparticals by using zinc acetate and sodium hydroxides precursors and soluble starch as an stabilizing agent. Soluble starch (0.5%) was dissolved in 500 ml of distilled water and treated to a microwave oven for complete solubilization. Zinc acetate, (0.1 mol), was added to the above solution. Then, the solution was kept under constant stirring at room temperature using a magnetic stirrer for 120 minutes. After complete dissolution of zinc acetate, 300ml (0.2 mol), of sodium hydroxide solution was added under constant stirring, drop by drop touching the walls of the vessel. The reaction was allowed to proceed for 120 minutes after complete addition of sodium hydroxide. After completing the reaction, the solution was allowed to settle for overnight and the supernatant solution was then discarded carefully. The remaining solution was centrifuged at 10,000×g for 10 min and the supernatant was discarded. Then, the produced nanoparticals were washed three times using distilled water. Washing was carried out to remove the byproducts and the excessive starch that were bound with the nanoparticals. After washing, the nanoparticals were dried at 80C for overnight. During drying, a complete conversion of Zn acetate into ZnO takes place.

Characterization of ZnO nanoparticals

Synthesis of ZnO nanoparticals by chemical method

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UV-Vis spectra analysis

The ZnONPs was confirmed by measuring the wavelength of reaction mixture in the UV-VIS spectrum of the PerkinElmer spectrophotometer at a resolution of 1 nm in 2 ml quartz Cuvette with 1 cm path length. Scanning range for the samples was 200-800 nm at a scan speed of 475nm/min. The spectrophotometer was equipped with "UVWinlab" software to record and analyze data. Base line correction of the spectrophotometer was carried out by

using a blank reference. The UV-Vis absorption spectra of all the samples were recorded and numerical data were plotted.

Scanning electron microscope (SEM)
In this research work, SEM machine was used to characterize the mean particle size and morphology of nanoparticals. The ZnO powder sample was sonicated with distilled water. A small drop of this sample was placed on glass slide allowed to dry. A thin layer of platinum was coated to make the samples conductive. Jeol JSM-6480 LV SEM machine was operated at a vacuum of the order of 10-5 torr. The accelerating voltage of the microscope was kept in the range 10-25 kV. A compositional analysis on the sample was carried out by the energy dispersive X-ray spectroscopy (EDS) attached with the SEM. The EDX analysis of ZnO sample was done by the SEM (JEOLJSM 5800) machine. The EDX normally reveals the presence of phases.

AFM analysis
The surface morphology of the ZnO was visualized by an atomic force microscope under normal atmospheric conditions. The examined samples were dispersed on small slide and explored on the contact mode of the instrument.

DLS Particle size and zeta potential analysis
The size distribution or average size of the synthesized ZnONPs was determined by dynamic light scattering (DLS) and zeta potential measurements. A laser diffraction method with a multiple scattering technique has been used to determine the particle size distribution of the powder. It was based on Mie scattering theory. In order to find out the particles size distribution the ZnO powder and Au dried sample were diluted 10 folds using 0.15M PBS (pH 7.4) and the measurements were taken in the range between 0.1 and 10,000 nm.The the experiment was carried out through computer controlled particle size analyzer [ZETA Sizers Nanoseries (Malvern Instruments Nano ZS)] to find out the particles size distribution.

X-RAY Diffraction method analysis
XRD measurements of the reduced ZnO perform was recorded on X-ray diffractometer instrument operating at a voltage of 40 kV and current of 30 mA with CuK(α) radiation to determine the crystalline phase and material identification. The samples were taken in lids and put under instrument for analysis. The generator voltage and current were set at 40 KV and 30 mA respectively. The ZnO NPs was scanned in the 2θ ranges 15 to 700C range in continuous scan mode. The scan rate was 0.04^{o}/sec. Phases present in the sample has been identified with the search match facility available with Philips X'pert high score software. The crystallite size of the prepared nanoparticals powders was determined from X-ray line broadening using the Scherer's equation.

Transmission Electron Microscopy (TEM) Measurements
Transmission Electron Microscopy measurements of the ZnONPs were performed on a JEOL Model 1200EX instrument operated at an accelerating voltage of 120 kV. Samples for TEM analysis were prepared by placing drops of the ZnO nanoparticals dispersions on carbon-coated TEM copper grids. The mixtures were allowed to dry for 1 min following which of the extra solution was taken off using a blotting paper.

Zeta Potential Measurements
The surface charge of ZnONPs was determined by the measurement of zeta potential. The zeta potential was determined using the Zetasizer 300 HAS based on photon correlation spectroscopy. The analysis time was 60 s and the average zeta potential were determined. The zeta potential of ZnONPs dispersion was determined as such without dilution

Animal Selection and Grouping
Sixty male albino rats, with average age and weight at the beginning of the experiment (9 months and 320 ± 20 g). All experimental animals were housed together for 7 days before the beginning of the experiment. Animals were randomly classified into six groups; the first group served as a control; these animals did not receive any type of treatment, The other group of animals which consist of 50 rats received a single intraperitoneal dose of alloxan with a dose equals 150 mg/kg for induction of diabetes. They were further classified into five groups; diabetic groups; they did not receive any type of treatment, diabetic + ZnONPs groups; received oral daily dose of ZnONPs of 2.5 mg/kg for 60 constitutive days, diabetic + ZnONPs group; received oral daily dose of ZnONPs of 5 mg/kg for 60

constitutive days, diabetic + ZnONPs group; received oral daily dose of ZnONPs of 10 mg/kg for 60 constitutive days and diabetic + insulin group; received subcutaneous dose of insulin 2U/kg.b.w for 60 constitutive days .

Diabetes Induction

The experimental induction of diabetes in male rats was induced by a single intraperetinoel (i.p) injected dose of 150 mg /kg body wt. of alloxan (Sigma Chemical Co. freshly dissolved in normal saline. After alloxan injection the animals were allowed to drink glucose solution (5%) w/v overnight to avoid hypoglycemia which might be induced by alloxan week later, alloxan–treated rats were fasted for 12 hours, and blood samples were collected from the orbital venous sinus for blood glucose determination. Rats in diabetic group with blood glucose levels higher than 250 mg /dl were considered diabetic and included for further studies.

Biochemical measurements-

Blood glucose was estimated by glucose oxidase method using the kit supplied by Randox(England). we measured blood glucose in all experimental animals before the beginning of the experimental procedures, after alloxan injection. For targeted-induced diabetic animals; blood glucose was routinely measured until diabetes was detected (animals with blood glucose >250 mg/dl are indicted as diabetics. After that, blood glucose was monitored in all experimental animals, and results were obtained at the end of the experimental period. Serum insulin level was estimated using a rat insulin ELISA kit(Abcam company,USA). Glycoslated hemoglobin was estimated spectrophotometrically using Stanbo kit(USA) .The level of malondialdehyde was determined by a modified procedure described by Ledwozyw's method [(15)].SOD was assayed by following the method of Misra and Frisovich [(16)].CAT activity was measured by the method of Aebi [(17)]. Activity of GPx was determined according to the method of Lawrence and Burk [(18)].method Reduced glutathione was determined by Beutler [(19)].

Histopathological Examinations

Specimens from the pancreas were collected and fixed in 10% buffered neutral formalin solution, dehydrated in gradual ethanol (70%–100%), cleared in xylene, and embedded in paraffin. Five-micron thick paraffin sections were prepared and then routinely stained with hematoxylin and eosin (HE) dyes [(20,21)]and then examined microscopically.

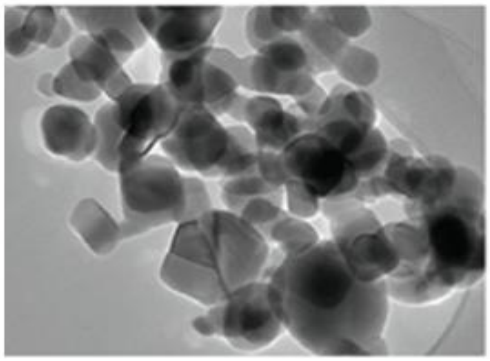
Statistical Analyses

All data are presented and analyzed using GraphPad Prism® software (GraphPad Software, Inc.,La Jolla, CA, USA). All results are expressed as mean ± standard error (SE). Comparison among groups was made by Student's *t*-test (unpaired), One-way analysis of variance (ANOVA). Duncan's test was used for testing the inter-grouping homogeneity. Statistical significance was set $p < 0.05$

Results

Characterization of ZnONPs:

ZnO nanoparticles were synthesized by the chemical reduction method and the powder form nanoparticles looks white in colour as in figure (1).The white synthesized ZnONPs were characterized by different spectroscopic and analytical techniques such as XRD and SEM. X-ray diffraction and TEM analysis studies confirmed the formation of well-dispersed ZnONPs with average particle size to be in the range of 34 to 42nm as well as revealed their hexagonal structure. The UV-Vis spectra of ZnONPs prepared with 0.5% concentration of soluble starch was shown in figure (2). The absorption peak of the prepared ZnONPs was found at around 372 nm.The ZnO spectra obtained was compatible with the reported result published by Vigneswaran *et al.*, (2006).



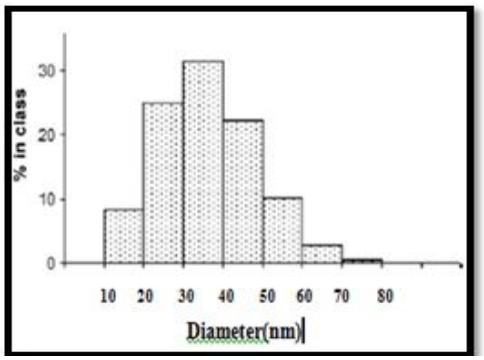


Figure 1: Characterization of ZnONPs (**A**) TEM image. (B) Size distribution and zeta potential of ZnONPs were determined using dynamic light scattering.

The XRD pattern of ZnONPs was presented in figure (3). All the peaks were hexagonal and approximately close to the reported information (jcpds-79-0206). Due to the crystal symmetry and related face velocities, the common crystal habit of ZnONPs is hexagonal in shape these ZnONPs are thermodynamically stable crystallographic phase. The width of the peaks in case of ZnONPs has increased due to the quantum size effect. The average particle size was estimated to be 42 nm using

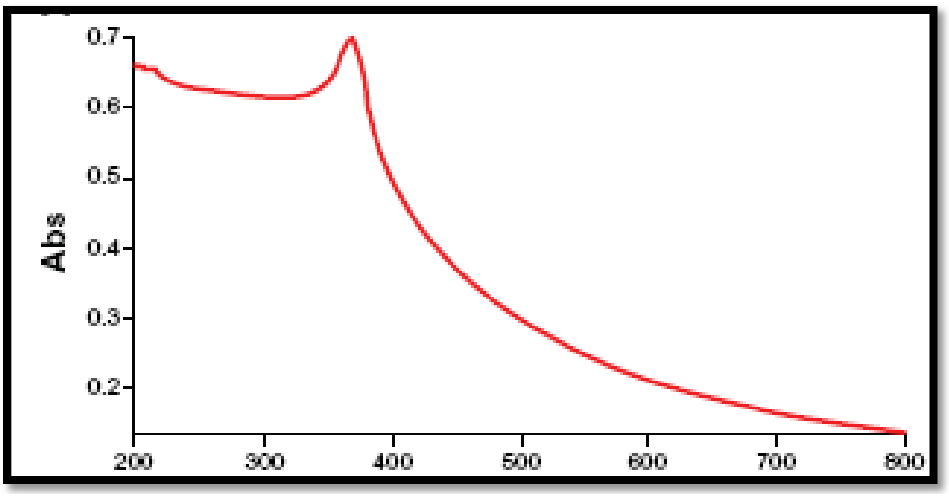
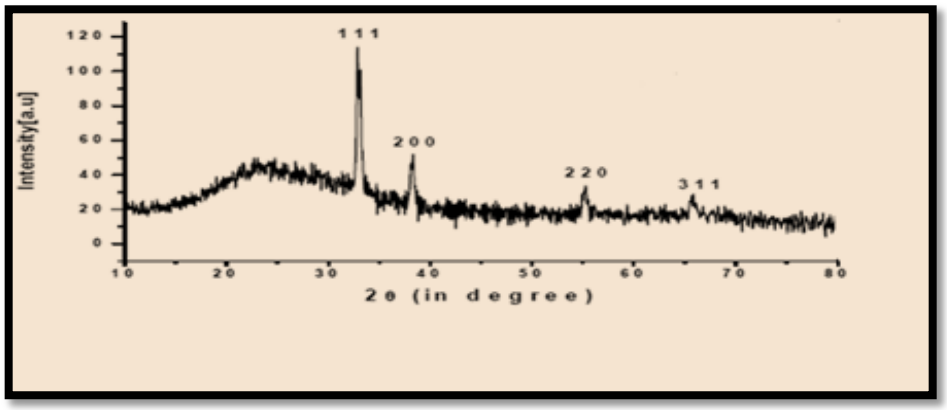


Figure 2: Ultraviolet-visible spectroscopic analysis of zinc oxide nanoparticles



Figure(3): Representative XRD pattern of ZnO nanoparticles. The peaks were assigned to diffraction from various plans of ZnO

Effect of ZnONPs intake on glucose levels in diabetic rats:-

The once daily oral treatment with alloxan in a dose 150 mg/kg resulted in the development of diabetes after three days of administration in experimental rats. The effect was gradually increasing over a four week period to reach a rise of about two times % over initial values compared to the control rat. Rat administrated alloxan showed hyperglycemia ($p<0.001$) at week 4 and 8, a significant ($p<0.05$) weight loss by week 4 and both polydipsia by the end of the study period 8 week table 1. Administration with 10 mg/kg b.w daily ZnONPs reduced the weight loss and polydipsia significantly ($p<0.05$) after 8 weeks while associated with alloxan treatment. The concomitant use of the low dose of 5 mg/kg b.w was mild effective in suppressing the rise in blood sugar induced by alloxan, where in a dose of 10 mg/ kg b.w , it was capable of reducing the rise in blood sugar induced by alloxan from (287 ± 9.2 mg/dl) to (175 ±7.7mg/dl) by the end of 8 weeks .It was evident, that the use of the 10 mg/kg dose of ZnONPs had completely prevented the rise in blood sugar induced by alloxan **,** as shown by the lack of statistical significance at ($p < 0.05$) between that group and the normal control rats. The persistence of hyperglycemia in diabetic rats without treatment leads to an increase of oxidative stress by several mechanisms including glucose auto oxidation and non-

enzymatic protein glycation, so the positive effect of ZnONPs may be investigated with more details of more than one mechanism.

Effect of ZnONPs intake on Oxidant/antioxidant status in diabetic rats:-

Results from table 2 showed that induction of diabetes in rats by alloxan results in a threefold level of increase in MDA levels relative to the control rat. The diabetic rat treated with ZnONPs significantly lowered the high glucose-induced rise in ROS generation in comparison to diabetic control rat. This makes clear the inhibitory effect of ZnONPs over ROS generation during hyperglycemia which induced oxidative stress. Functional damage to cells under oxidative stress is not only by oxygen free radicals and unbalanced redox potential but also because of improved lipid peroxidation as reported by Zamboni, (2008). ZnONP inhibitory effect over the case of lipid peroxidation in the enzyme source is confirmed which is shown in table 2.A potent control effect of ZnONPs at10 mg/kg b.w treated to the diabetic treated rats revealed an important decrease in lipid peroxidation in comparison to diabetic control group rat. ROS generated by hyperglycemia condition play an essential role in the development of diabetic complications (Longmire *et al.*, 2008). It is the outcome of the oxidative stress developed because of the release of free radicals, thereby, reducing the level of antioxidant enzymes. Estimation of lipid peroxidation in the sera of diabetic rats treated with ZnONPs showed that gold nanoparticles blocked the high glucose induced increase in ROS generation to a maximum extent.

On the other hand results showed a significant alteration in oxidant/antioxidant status in diabetic rats induced by alloxan without treatment. GSH and all three enzymatic antioxidants (SOD, GPx, and Cat) were decreased significantly in diabetic rats compared to the healthy control rats due to increased ROS levels in diabetes which could be because of their increased production and/or decreased destruction by antioxidants like GSH, SOD, catalase and glutathione peroxidase, while administration either ZnONPs to diabetic rats improvement occurred and this improvement depends on the dose used. When the diabetic rats intake ZnONPs at 10 mg/kg.b.w once daily for 8 weeks the levels of all enzymatic and non-enzymatic antioxidants significantly ($P<0.001$) return to the normal values.

Table 1: The effect of different concentrations of ZnONPs (2.5, 5, and 10 mg/kg) on body weight, fluid intake and blood sugar in alloxan induced diabetic and normal rat. Values are expressed as mean (mg/dl ± S.E). (N=10)

Parameter	Time (wk)	Control G1	Diabetic G2	DM+ZnO G3	DM+ZnO G4	DM+ZnO G5	DM+insulin G6
Body weight (gm)	0	322 ± 20	324 ± 22	324±29	325 +23	323±32	325±27
	4	324 ±17	311 ± 19	322±21	318±24	324 ±24	324 ±29
	8	323 ± 18	300 ± 12	320 ±24	308 ±22	316 ±20	323 ±27
Fluid intake ml/day	0	44± 2.5	44 ± 2.4	44±7.2	40 ±2.6	43±1.5	4.1 ± 2.7
	4	46 ± 1.8	58 ± 2.1	55±1.8	50 ±2.3	47 ±2.9	4.2 ± 1.9
	8	47 ± 1.5	68 ± 4.6	49±1.5	45 ±4.4	44 ±4.3	4.3 ± 1.7
B.glucose mg/dl	0	88 ± 4.3	298 ±7.8	288±8.1	287±9.2	285±8.1	295±8.1
	4	92 ± 4.1	298 ± 9.5	233±7.9	212 ± 8.6	200 ±7.1	160 ±7.1
	8	94± 5.7	350 ±9.9	205±10	190 ±4.6	175 ±7.7	140 ±7.7

Table 2: The effects of intake different concentrations of ZnO nanoparticles (2.5, 5.0, and 10 mg/kg b.w) on oxidant/antioxidant status, Lipid profile , Insulin and glycoslated hemoglobin in alloxan induced diabetic and normal rats. Values are expressed as mean (mg/dl ± S.D).

Parameter	G1	G2	Diabetic rats treated with ZnONPs			G6
			G3	G4	G5	
MDA(µM)	0.86±0.11	2.8±0.34	1.82±0.11	1.42±0.11	0.98±0.11	0.86±0.11
GSH(µM)	6.55±0.18	3.55±0.18	4.25±0.18	5.45±0.18	5.85±0.18	6.15±0.18
SOD(U/L)	12.2±1.6	6.3±1.3	7.6±1.4	10.3±1.8	10.3±1.7	11.3±1.3
GPx (U/L)	10.7±1.8	7.7±1.4	8.3±1.8	9.1±1.8	9.9±1.8	10.3±1.8
CAT(U/L)	6.6±0.98	4.1±1.0	4.8±0.77	5.4±0.82	5.9±0.78	6.0±0.91
Chol(mg/dl)	93±10	115±13	104±10	97±10	93±10	93±10
Trig(mg/dl)	82±13	121±14	100±10	94±11	86±11	85±11
HDL(mg/dl)	20±2.2	17±2.7	18±2.4	19±2.4	20±2.2	20±2.2
LDL(mg/dl)	57±2.9	74±2.9	62±2.9	61±2.4	58±2.9	57±2.9
VLDL(mg/dl)	16±2.3	24±3.3	20±3.3	17±2.4	15±2.6	15±2.3
Insulin(pg/ml)	500±25	175±23	330±20	380±28	400±25	420±21
HbA1c%	4.5±0.4	8.0±0.5	6.93±0.4	6.5±0.4	6.0±0.4	5.1±0.3

Effect of ZnO nanoparticles intake on histomorphologic changes of pancreatic tissue in diabetic animals.
The cellular integrity and architecture were intact in the healthy animals group as shown in figure (4A). Islets of control animals had well defined boundaries. Most of the cells were of the β-type. B-cells were small polygonal arranged in groups & cords fine capillaries. Histological sections of endocrine regions of pancreas of alloxan induced diabetics(figure 4 B) revealed shrinkage of β-cells of islets of Langerhans in the diabetic animals and a significant reduction in the size of the islets when compared to that of the normal groups. Pancreatic sections stained with hematoxylin and eosin (H & E) showed that alloxan caused severe necrotic changes of pancreatic islets, especially in the centre of islets. Nuclear changes, karyolysis, disappearance of nucleus and in some places, residue of destroyed cells were visible; in addition the diabetic control rat revealed ground glass nuclei and lymphocytic infiltrations together with lobular inflammation with high fatty cells .

The possible mechanism for β-cell destruction by alloxan has been reported to implicate generation of some kinds of oxygen free radicals and alternation of endogenous scavengers of these reactive oxygen species .It has been suggested that ROS were a contributive element in the development of diabetes complications. Studying the pancreas of the treated diabetic groups with 10 mg/kg.b.w of ZnONPs showed an increased size of islets and hyperchromic nucleus in the sections stained with H & E.(figure:4C).The results of histopathological study of diabetic treated group indicated an increased volume density of islets and increased percentage of beta cells in the diabetics that received ZnO nanoparticles which may be a sign of regeneration. Signs of regeneration of ß-cells, potentiating of insulin secretion from surviving ß-cells of the islets of Langerhans and decrease of blood glucose have been reported following the consumption of some metal nanoparticles. On the other hand, the researcher observed that the diabetic induced rat treated with ZnO nanoparticles revealed an important decrease in fatty cells, normal central vein with no round glass nuclei with, stating the restorative effect of ZnONPs over the organ damage and its improvement like the alteration using insulin treatment as shown in figure(4F).

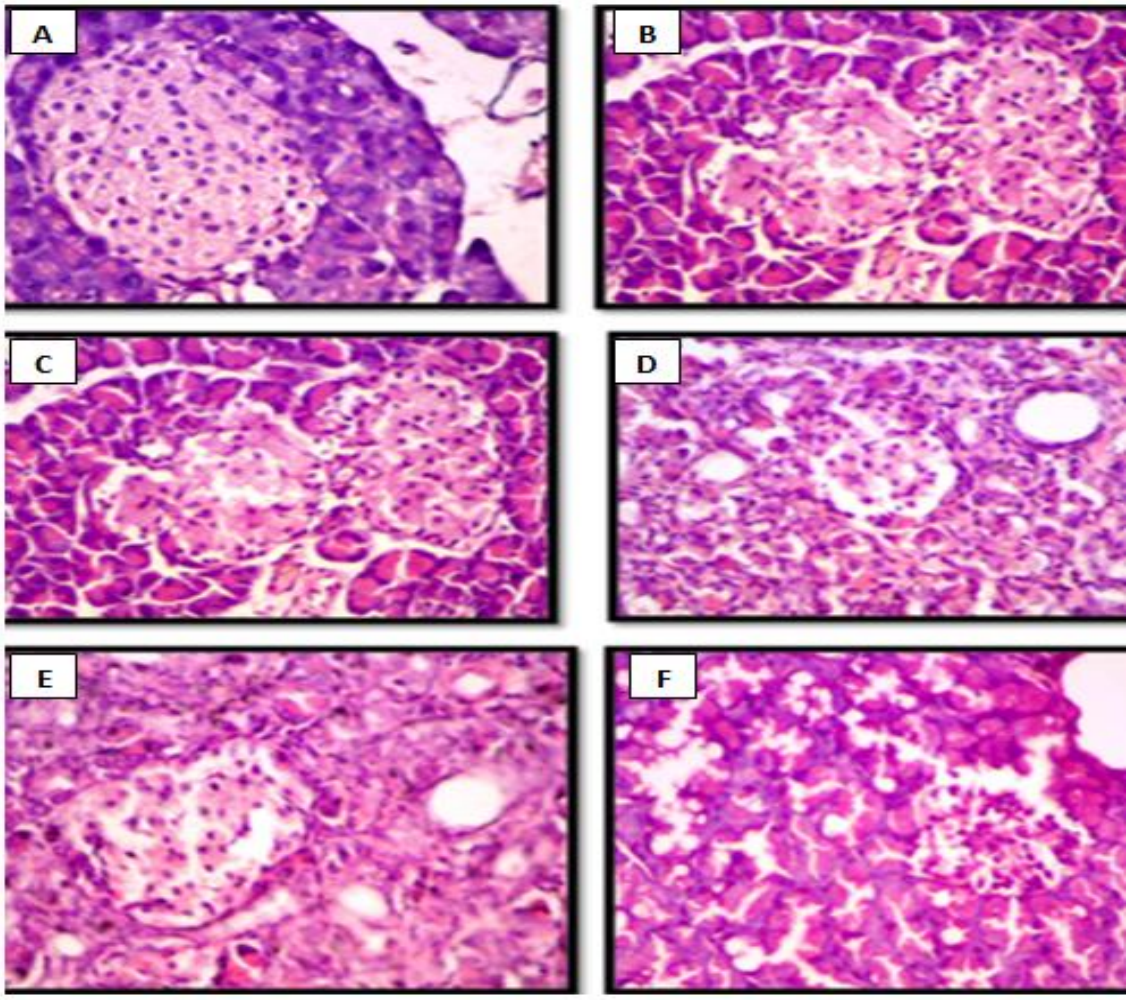


Figure 4: Histopathology of rat pancreatic tissue (A) Healthy non diabetic. (B) Diabetic rat without treatment. (C) Diabetic rat treated with ZnONPs (10 mg/kg b.w). (D) Diabetic rat treated with ZnONPs (5 mg/kg b.w). (E) Diabetic rat treated with ZnONPs (2.5 mg/kg b.w). (F) Diabetic rat treated with insulin (1.6 U/kg).

Discussion

The possible therapeutic effect of ZnONPs in alloxan-induced diabetic rats was evaluated compared with insulin treatment. Concerning to ZnONPs and glucose homeostasis, our results pointed out blood glucose levels were improved in diabetic rats with ZnONPs and/or insulin treatment and this improvement was a dose dependent and our results agree well with the previous studies in the same line [10,11] whom reported the ability of ZnONPs for improve the blood glucose in STZ-diabetic rats. Glucose is one of the body's main sources of energy. In normal physiology, the body maintains blood glucose levels within a narrow range (75-130mg/dl).The body regulates the processes that control the production and storage of glucose by secreting, insulin, from the pancreatic β-cells. Insulin facilitates anabolic metabolism throughout the body .An increase in insulin above basal concentrations (2-12 U/l) will decrease the release of glucose from the liver and increase glucose uptake into insulin-receptive tissues.

Our results showed a great reduction in blood glucose level in diabetic groups treated with ZnONPs and insulin (76.1% and 80.2%) respectively. This showed a great antidiabetic activity of ZnONPs as zinc has been elucidated to be a potent metal that improves glucose utilization and metabolism through its potent influence on enhancement of hepatic glycogenesis through actions on the insulin signaling pathway [8]. As well as. Improved glucose could be as a result of several possible mechanisms. Firstly, ZnONPs treatment might result in inhibition of intestinal alpha-glucosidase enzyme and thereby reduce glucose absorption. Secondly, blood glucose levels might be lowered as a result of increased glucose uptake in the liver and its subsequent storage (glycogenesis). Thirdly, enhanced glycolysis by ZnONPs could result in improved glucose disposal. Also, the antidiabetic effects of ZnONPs may be due to that zinc is closely involved in general metabolism of protein, carbohydrate, and lipids .In the case of glucose

metabolism, zinc is a cofactor of key enzymes. It is an activator of fructose 1-6 diphosphate aldolase, and an inhibitor of fructose 1-6 diphosphatase [(23).]

ZnONPs treatment indicates inhibitory effects on glycogenolysis and gluconeogenesis, mechanisms that are active during the fasted state. Interestingly, zinc is reported to regulate glucagon secretion from pancreatic alpha cells [(24)]. As a result, glucagon-stimulated hepatic pathways (i.e., glycogenesis and gluconeogenesis) would be suppressed in the fasting state [(25)] contributing to a reduction of fasted glucose levels.Concerning to The increasing effect of ZnONPs on serum insulin level, Insulin is synthesized and stored in the secretory granules of the pancreatic p-cells, from where it is continually secreted into the pond circulation. Insulin secretion is controlled by multiple signals which include substrate availability, hormone concentrations, and autonomice nervous system activity [(26).]

Our experiments revealed that ZnONPs could increase serum insulin level in diabetic groups treated with ZnONPs (79.4%) if compared with diabetic groups treated with insulin (97.3%). There are few studies that have investigated the therapeutic effect of ZnONPs on insulin levels or secretion. However, others have demonstrated that zinc could enhance the glucose stimulated insulin secretion from rat isolated pancreatic islets [(27).] Interestingly, on the basis of ZnONPs did not possess the risk of hypoglycemia in living organisms so it can act as an insulin secretagog. Also, increase serum insulin level in diabetic groups treated with ZnONPs this may be due to accumulation of zinc in the secretory vesicle of β-cells using transporter 8 [(11,13).] Zinc transporters are also identified in adipose tissues and liver [(28)] Such organs are the major regulator of glucose metabolism. Many studies, agree with our finding of a higher serum insulin concentration in the zinc treated groups[(29)] demonstrated that diet induced zinc deficiency in rats resulted in a decrease in the ability of the pancreas to secrete insulin in response to a glucose load. Another study showed that decreased zinc in the pancreas may reduce the ability of the islet b-cells to produce and secrete insulin[(30)] and zinc deficiency is positively correlated with diabetes and may also affect the progress of Type 2 diabetes [(9)] .

Conclusion

It is evident from the findings of the present systematic review, that ZNONPs plays an important role in β-cell function, insulin action, glucose homeostasis and a depressant effects of lipids and protein oxidation and modulation of oxidant antioxidant balance . However further randomized double-blinded placebo-controlled clinical trials conducted for an adequate duration, are required to establish therapeutic efficacy and safety in humans.

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