

Impact of Nanoparticles on The Binding of Insulin Hormone and Its Receptors in Sera of Patients with Type Two Diabetes

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Abstract

Nanotechnology is one of the most important modern technologies used in several areas. The most important of these fields are medical field, as it is used in the diagnosis, treatment, and prevention of many diseases due to its distinctive visual and engineering properties. This work has aimed to evaluate the impact of metal oxide nanoparticles on insulin binding to its receptor in sera of patients with type 2 diabetes at different stages of BMI. Zinc oxide (ZnO) nanoparticles were prepared by the precipitation method while copper oxide (CuO) nanoparticles were synthesized using the photo-irradiation method and their properties were studied using atomic force microscope (AFM), scanning electron microscope (SEM), X-ray diffraction (XRD) and transmission electron microscope (TEM). ZnO nanoparticles had a particle size of 21.8-36.6 nm while CuO nanoparticles had 20-28 nm. The effectiveness of ZnO and CuO nanoparticles on insulin and insulin receptor (INSR) levels was evaluated in obese and normal-weight diabetic patients. Both nanoparticles (NPs) showed an inhibitory effect on insulin and INSR levels, while contributing to a reduction in insulin resistance.

Keywords: Insulin hormone, Insulin receptor, ZnO NPs, and CuO NPs.

1. Introduction

Diabetes mellitus (DM) is the most common chronic disorder. It occurs when the pancreas is unable to produce the amount of insulin the body needs, or if the human body is unable to effectively manage

the insulin generated [1]. According to the reports of the international diabetes federation (IDF), there will be a significant increase in the number of people with diabetes in the coming years [2]. The most common symptoms are muscle soreness,

urination, increased thirst, and hunger. It is necessary to recognize the disease as soon as possible [3].

There are many pathological complications of DM that affect almost each body part, including the kidney, liver, nervous system, and other body parts [4]. Some medications that are administered based on various disease conditions are needed for people treated by this disease [5]. However, over the long term, these new drugs do not restore normal blood sugar balance and are not without side effects, including kidney damage, insulinoma, hypoglycaemia, gastrointestinal complications, cardiac risk issues, liver toxicity, and the need for lifelong medication [6].

Numerous studies have shown that trace minerals play a role in metabolism of glucose and its regulation and are linked to diabetes, such as copper, chromium, zinc and magnesium have been identified as having a role in blood sugar control and DM treatment [7]. There are many effects of diabetes-related Zn enhancement. In β cell, the insulin can create complexes and polymers with Zn [8].

It also stimulates more than 300 enzymes throughout the body and plays a key role in various metabolic pathways, such as the metabolism of glucose. In addition to that Zn is known to preserve the

insulin structure and plays a crucial role in the storage, biosynthesis, and production of insulin [7].

Studies have shown that several zinc transporters, such as zinc transporter 8, play a powerful role in insulin production in pancreatic beta cells [9-10]. Zinc may also enhance insulin signalling through several pathways, including increased INSR phosphorylation, inhibition of glycogen synthase kinase 3, and increased phosphoinositide 3-kinase activity [11].

Thus, a complex inter-relationship Zn, diabetes mellitus, and associated diabetic symptoms [12]. On another side, Cu NPs are an important element of NPs in the treatment of type 2 diabetes via inhibition of α -amylase and α -glucosidase activities consequent with radical-scavenging and antioxidant activity [13].

Furthermore, copper is now a crucial element for plant health and growth, it is very important for humans to be a basic nutrient and active redox metal. Oxidative stress plays a large function and is a main cause of complications with diabetes [14]. The purpose of this study was to evaluate the impact of ZnO and CuO nanoparticles on the binding of insulin with its receptor in of patients with T2DM.

2. Materials and Methods

2.1 Synthesis of Zinc Oxide (ZnO)

NPs

ZnO nanoparticles were synthesized using the method described by Gusatti et al. [15] with minor modifications. First, 0.5 M $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ solution and 1 M sodium hydroxide solution were prepared and heated to 70 °C while the alkaline solution was continuously stirred using a magnetic stirrer. The $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ solution was then slowly added dropwise into the alkaline solution under constant stirring.

A white ZnO nanoparticles suspension formed, indicating the distribution of ZnO nanoparticles within the mixture. After the addition of $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ was completed, the reaction mixture was stirred for an additional two hours and then allowed to cool to room temperature. The resulting ZnO nanoparticles were washed several times with deionized water and dried. Finally, the dried powder was calcined at 450 °C for 3 hours.

2.2 Synthesis of Copper Oxide (CuO) NPs

CuO nanoparticles were synthesized using a photo-irradiation method. Briefly, 50 mL of 0.02M $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ was mixed with 25 mL of

NaOH solution and irradiated in a photocell for 30 minutes while maintaining the temperature at 5 °C using a cooling system. A dark blue precipitate was formed, collected, and washed several times with deionized water and ethanol. The precipitate was then isolated by centrifugation for 20 minutes and subsequently dried in an oven at 90 °C for two hours. Finally, the dried product was calcined at 250 °C for three hours, yielding a deep black CuO nanoparticles powder.

2.3 Characterization

Several analytical instruments were used to characterize the synthesized ZnO and CuO nanoparticles. The morphology and particle size were examined using transmission electron microscopy (TEM), scanning electron microscopy (SEM), and atomic force microscopy (AFM). X-ray diffraction (XRD) was employed to determine the crystalline structure and phase composition of the nanoparticles.

2.4 Patients

A total of one hundred and twenty volunteers were classified into sixty patients with T2DM and sixty without T2DM, their ages ranged from between (33-57) years old. Patients group was classified into two groups. Thirty obese with T2DM (Group of OT2D) and thirty

normal weights with T2DM (Group of NWT2D). The control also was classified into two groups, thirty obese without T2DM (Group of OC) and thirty normal weights without T2DM (NWC) Samples were collected from Baghdad, Iraq. All patient samples were free of any physical abnormalities or chronic diseases.

2.5 Serum Insulin Hormone Measurement

Insulin levels were measured in both controls and patients using ELISA Kit (SunLong/China). This was performed in two steps, the first without nanoparticles and second with NPs present. The analysis was repeated twice, one with CuO nanoparticles and one with ZnO nanoparticles. They were incubated with the serum for 30 minutes. The volume of nanoparticles used was one-fifth (1/5) of the serum volume in the measurement.

2.6 Serum Insulin Receptors Measurement

Insulin receptor levels were measured in both controls and patients using ELISA Kit (SunLong/China). This was performed also in two steps, the first without nanoparticles and second with NPs present. The analysis was repeated twice, one with CuO nanoparticles and one with ZnO NPs. They were incubated with the

serum for 30 minutes. The volume of nanoparticles used was one-fifth (1/5) of the serum volume in the measurement.

3. Results and Discussion

3.1 X-ray diffraction (XRD) analysis

CuO and ZnO nanoparticles were analysed using XRD. The XRD measurements of ZnO nanoparticles showed diffraction peaks at 31.73° , 34.27° , 36.09° , 47.44° , 56.58° , 62.73° , 66.26° , 67.91° , 68.88° , 72.44° , and 76.77° as shown in (figure 1). Miller indices are 100, 002, 101, 102, 110, 103, 112, 201, and 202 according to the ASTM (American Society for Testing and Materials) card No. (00-36-1451).

The diffraction pattern showed well-defined peaks, indicating the hexagonal structure shape of the nano product. Also, Debye-Scherrer equation calculated the crystallite size of the hexagonal structure ZnO nanoparticles with an average crystallite size of 22.97 nm. Similarly, the XRD pattern of CuO nanoparticles showed diffraction peaks at 35.57° , 38.70° , 48.91° , 58.22° , 61.67° , 66.03° , 67.97° , and 75.02° as shown in (figure 2). While Miller indices are 110, 002, 111, 20^{-2} , 020, 202, 11^{-3} and 31^{-1} according to the card No. (00-48-1548).

The diffraction pattern exhibited distinct peaks within the pattern suggested a good monoclinic nano product and the average crystallite size was 19.97 nm which was calculated using the Debye–Scherrer equation.

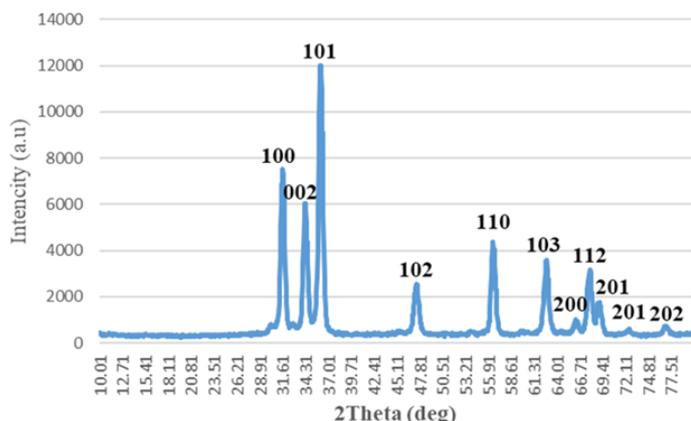


Figure 1: XRD pattern of ZnO nanoparticles.

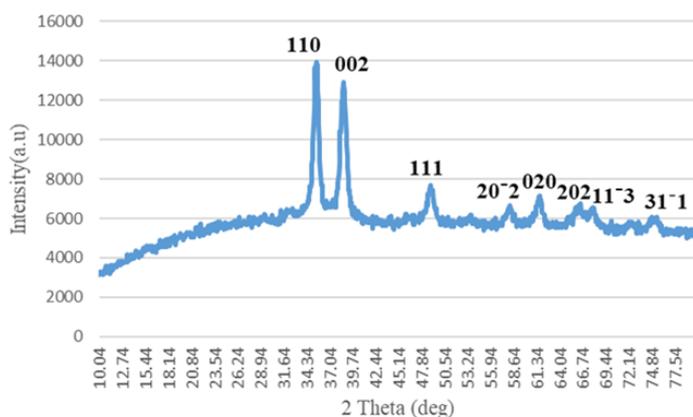


Figure 2: XRD pattern of CuO NPs.

3.2 Field Emission Scanning Electron Microscopy (FESEM)

Figure 3, and figure 4 show results of FESEM for both ZnO and CuO nanoparticles. Where the average diameter

for ZnO nanoparticles was 70.79 nm, and CuO nanoparticles was 20 nm.

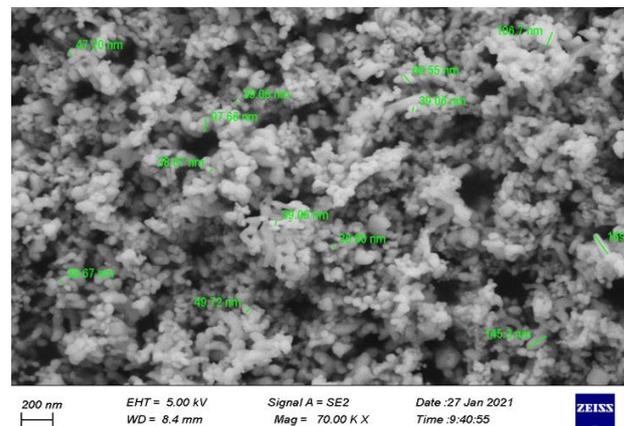


Figure 3: Field Emission Scanning Electron Microscope of ZnO nanoparticles.

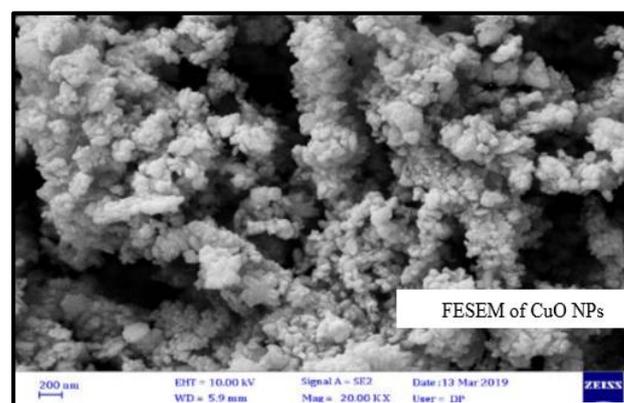


Figure 4: Field Emission Scanning Electron Microscope of the CuO nanoparticles.

3.3 Transmission Electron Microscope (TEM)

TEM results for ZnO nanoparticles are shown in (figure 5) and (figure 6). Images reveal that particles fall within the nanoscale range (<100 nm) and exhibit a morphology consistent with zero-dimensional nanoparticles. The average

particle size was calculated from randomly selected particles in figure 5.

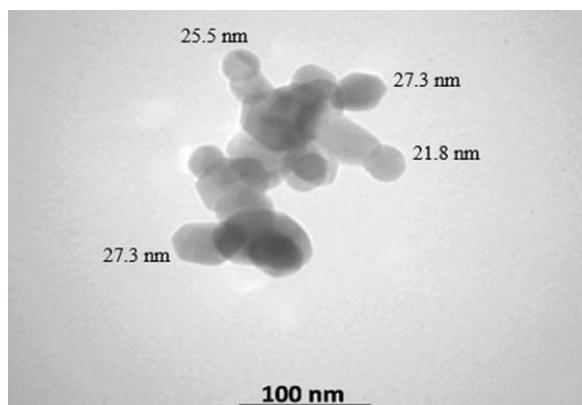


Figure 5: TEM of ZnO nanoparticles at 100 nm scale.

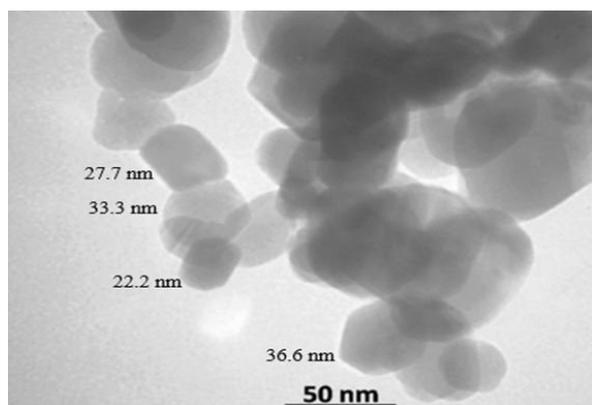


Figure 6: TEM of ZnO nanoparticles at 50 nm scale.

3.4 Atomic Force Microscopy

AFM results for CuO nanoparticles are shown in figure 7, with an average particle diameter of 28.21 nm. The AFM images reveal the surface morphology and size of analysed nanoparticles.

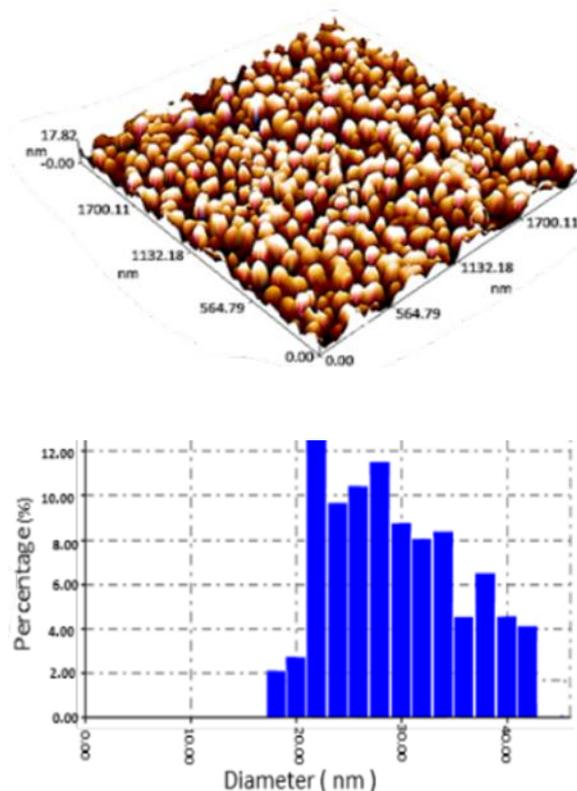


Figure 7: AFM of CuO nanoparticles.

3.5 Levels of Insulin in Sera of Patients with Type 2 Diabetes and Control Group

The level of insulin hormone was higher in the control group than in the T2DM patient group as listed in table 1. The mean \pm SD of insulin value of the obese patients with T2DM (OT2D) was $(1.1015 \pm 0.432 \text{ mU/L})$ lower than of obese control (OC) which was $(2.0769 \pm 1.32389 \text{ mU/L})$ and the mean \pm SD of normal weight patients with T2DM (NWT2D) was $(1.1512 \pm 0.37946 \text{ mU/L})$ lower than the normal weight control (NWC) which was $(1.6338 \pm 0.64388 \text{ mU/L})$.

Based on these data, the observed lower insulin levels in type 2 diabetes patients compared to the control group. Results contradict with results reported by Amr et al [16], that found that level of insulin hormone increases in the patients with type 2 diabetes and patients with obesity than the control group. However, some previous studies showed that insulin levels may be decreased in patients with type 2 diabetes compared to a control group [17].

Table 1: ANOVA test results of the insulin levels

Parameter s	OT2D (mean ± SD)	OC (mean ± SD)	NWT2 D (mean ± SD)	NWC (mean ± SD)
Insulin (mU/L)	1.1015 ± 0.432	2.0769 ± 1.32389	1.1512 ± 0.37946	1.6338 ± 0.64388
P value	0.0001**			

(OT2D): Obese type 2 diabetes, (OC): Obese controls, (NWT2D): Normal weight type 2 diabetes, (NWC): Normal weight controls. (*): Significant P< 0.05, (**) highly significant P<0.01

3.6 Levels of Insulin Receptor in Sera of Patients with Type 2 Diabetes and Control Group

A clear difference in the level of the insulin receptor between the control group and the diabetic group is listed in table 2. As observed through this study there is a decrease in the insulin receptor level of the

T2DM group compared to the control group. The mean ± SD for OT2D was (1.5026 ± 0.36728 ng/ml) less than OC which was (1.8296 ± 0.443337 ng/ml). In addition, observed the mean ± SD for the NWT2D was (1.5442 ± 0.34175 ng/ml) less than the NWC which was (1.8338 ± 0.69272 ng/ml).

Table 2: ANOVA results of the insulin receptor of the patients with type 2 diabetes Parameters OT2D

parameters	OT2D (mean ± SD)	OC (mean ± SD)	NWT2 D (mean ± SD)	NWC (mean ± SD)
INSR (ng/ml)	1.5026 ± 0.36728	1.8296 ± 0.443337	1.5442 ± 0.34175	1.8338 ± 0.69272
p-value	0.048*			

(OT2D): Obese type 2 diabetes, (OC): Obese controls, (NWT2D): Normal weight type 2 diabetes, (NWC): Normal weight controls, Insulin receptor (INSR). (*): significant P< 0.05.

3.7 Effect of Nanoparticles on Insulin Hormone Levels and Receptors in Patients Groups

Effect of NPs on insulin hormone and INSR in both groups of patients (OT2D) and (NWT2D) listed in tables 3 and 4. From the data of table 3, ZnO nanoparticles have inhibition effect on the insulin hormone levels and INSR in both concentrations (2 ppm, 20 ppm). Before adding NPs, the mean of insulin hormone was (mean = 2.63

mU/L for OT2D) and (mean = 2.18 mU/L for NWT2D). After adding ZnO nanoparticles (2 ppm) the mean of insulin hormone was (mean = 1.74 mU/L for OT2D) and (mean = 1.07 mU/L for NWT2D), with a percentage of inhibition equal to (33.84 % for OT2D), and (50.91 % for NWT2D).

Also adding ZnO nanoparticles (20 ppm) the mean of insulin hormone was (mean = 1.33 mU/L for OT2D) and (mean = 1.09 mU/L for NWT2D). With a percentage of inhibition equal to (49.42 % for OT2D), and (50 % for NWT2D). The INSR before adding the NPs (mean = 2.25 ng/ml for OT2D) and (mean = 2.13 ng/ml for NWT2D). However, after adding ZnO nanoparticles (2 ppm) the mean was (mean = 1.69 ng/ml for OT2D) and (mean = 1.80 ng/ml for NWT2D), with a percentage of inhibition equal to (24.88 % for OT2D), and (15.49 % for NWT2D) and adding ZnO nanoparticles (20 ppm) the mean of INSR was (mean = 2.06 ng/ml for OT2D) and (mean = 1.53 ng/ml for NWT2D), with a percentage of inhibition equal to (8.44 % for OT2D), and (28.16 % for NWT2D). ZnO nanoparticles has an inhibition effect on insulin hormone and its receptor levels in sera of patients with T2DM.

Table 3: Distribution effect of ZnO nanoparticles on Insulin levels in patient groups

Groups	Concentration	Mean of Insulin	% Inhibition	Mean of INSR	% Inhibition
OT2D	Without NPs	2.63	-	2.25	-
	2 ppm	1.74	33.84 %	1.69	24.88 %
	20 ppm	1.33	49.42 %	2.06	8.44 %
NWT2D	Without NPs	2.18	-	2.13	-
	2 ppm	1.07	50.91 %	1.80	15.49 %
	20 ppm	1.09	50 %	1.53	28.16 %

(OT2D): Obese type 2 diabetes, (INSR): Insulin receptor, (NWT2D): Normal weight type 2 diabetes.

CuO nanoparticles also has an inhibition effect on the level of insulin hormone and insulin receptor. After adding CuO nanoparticles (2 ppm) the mean of insulin hormone was (mean = 1.66 mU/L for OT2D) and (mean = 1.70mU/L for NWT2D), with a percentage of inhibition equal to (36.88 % for OT2D), and (22.01 % for NWT2D) and after adding CuO nanoparticles (20ppm) the mean was (mean = 1.54 ng/ml for OT2D) and (mean = 1.18 ng/ml for NWT2D) with a percentage of inhibition equal to (41.44 % for OT2D), and (45.87 % for NWT2D).

CuO nanoparticles in INSR, after adding CuO nanoparticles (2ppm) the mean of INSR was (mean = 2.09 ng/ml for

OT2D) and (mean = 2.01 ng/ml for NWT2D), with a percentage of inhibition equal to (7.11 % for OT2D), and (5.63 % for NWT2D). After adding CuO nanoparticles (20 ppm) the mean was (mean = 2.10 ng/ml for OT2D) and (mean = 1.47 ng/ml for NWT2D), with a percentage of inhibition equal to (6.66 % for OT2D), and (30.98 % for NWT2D), as shown in table 4.

Table 4: Distribution of the effect of CuO nanoparticles on Insulin levels in patient groups

Gro ups	Concent ration	Me an of insulin	% Inhib ition	Me an of IN SR	% Inhib ition
OT2 D	Without NPs	2.63	-	2.25	-
	2 ppm	1.66	36.88 %	2.09	7.11 %
	20 ppm	1.54	41.44 %	2.10	6.66 %
NW T2D	Without NPs	2.18	-	2.13	-
	2 ppm	1.70	22.01 %	2.01	5.63 %
	20 ppm	1.18	45.87 %	1.47	30.98 %

(OT2D): Obese type 2 diabetes, (INSR): Insulin receptor, (NWT2D): Normal weight type 2 diabetes.

The addition of ZnO and CuO nanoparticles exerts an inhibitory effect on insulin levels in both patient groups, independent of concentration. This inhibitory effect may lead to a decrease in

the binding of insulin to its receptor. These findings are not consistent with previous studies conducted on animal models. For instance, Shafayet et al. [7] Also, another study investigated ZnO nanoparticles increases the level of serum insulin in rats [18].

A Study by Arun et al [13], showed that CuO nanoparticles have a role in lowering the level of glucose in diabetic mice. The effect of nanoparticles is still uncertain, more research is required broadly and with greater research capabilities, as well as analysing more samples.

3.8 Effect of nanoparticles on insulin resistance in patient groups

Effect of NPs on insulin resistance in both groups (OT2D and NWT2D) before and after adding NPs shown in table 5. Data in table 5 shows that the mean of IR before the addition of nanoparticles was (1.3011 for OT2D), and (0.8737 for NWT2D). After adding ZnO nanoparticles 2 ppm was (0.8608 for OT2D) and (0.4288 for NWT2D).

Also adding ZnO nanoparticles 20 ppm was (0.6579 for OT2D) and (0.4368 for NWT2D). As for CuO nanoparticles after adding NPs (2 ppm ,20 ppm) was (0.82125,0.76188) for OT2D and (0.6813,0.4729) for NWT2D.

Table 5: The effect of NPs on the insulin resistance

Concentration	Mean different	
	OT2D	NWT2D
IR without NPs	1.3011	0.8737
ZnO NPs (2 ppm)	0.8608	0.4288
ZnO NPs (20 ppm)	0.6579	0.4368
CuO NPs (2 ppm)	0.82125	0.6813
CuO NPs (20 ppm)	0.76188	0.4729

(OT2D): Obese type 2 diabetes, (IR) Insulin resistance, (NWT2D): Normal weight type 2 diabetes.

From the data that there is a clear effect of NPs on improving T2DM by reducing insulin resistance, as by increasing the concentration of NPs, insulin resistance decreases in obese people with T2DM. As for non-obese people, a ZnO nanoparticles 2 ppm was the best.

4. Conclusion

Results of the research showed that nano metal oxides were successfully prepared with high purity. The FESEM showed that the average size of ZnO nanoparticles was 70.79 nm and CuO nanoparticles were 20 nm. The average crystallite size from the X-ray diffraction

for ZnO nanoparticles was 22.97 nm and 19.97 nm for CuO nanoparticles.

In addition, depending on the TEM analysis indicated the size of ZnO nanoparticles was (21-36.6) nm, whereas AFM analysis showed of the size of CuO nanoparticles was 28.21 nm. Both nanoparticles ZnO or CuO had an inhibition effect on the insulin hormone and its receptor in sera of patients with type 2 diabetes and which may reduce the binding between insulin and its receptor, on the other hand, it had a decrease on the insulin resistance level.

5. References

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