Antidiabetic effects of curcumin/zinc oxide nanocomposite in streptozotocin-induced diabetic rats

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Abstract. Diabetes mellitus (DM) is a tremendously widespread endocrine disease that causes many complications risking patient's quality of life. The current study aims to evaluate the antidiabetic potential of curcumin nanoparticles (Curc-NPs), Zinc Oxide nanoparticles (ZnO-NPs), and Curcumin/Zinc oxide nanocomposite (Curc/ZnO-NC) on streptozotocin (STZ)-induced diabetic rats. Results are compared to rats treated by traditional anti-diabetic Diamicron and to normal non-diabetic rats. Adult Wistar albino rats with weight (180-200 g) were divided into 6 groups, each group contains 8 rats (4 males and 4 females). To induce type 2 DM, five groups were injected intraperitoneal with a single dose of 50 mg/kg b.w. freshly prepared STZ. Each group of diabetic rats were treated orally with a daily dose of 50 mg/kg b.w. of Curc-NPs, 10 mg/kg b.w. of both ZnO-NPs & Curc/ZnO-NC, and 5 mg/kg b.w. of Diamicron for 21 days. The antidiabetic potential of every treatment against diabetic rats was evaluated by investigating different biochemical parameters (glucose, insulin, urea, creatinine, HbA1-C, AST, ALT) and histopathological parameters as well as protein expression of Glucokinase (GK) and Glucose transporter protein 2 (GLUT-2) in the pancreas and livers of diabetic rats. All treated groups showed significant reduction in blood glucose, elevated insulin levels, regulated GLUT-2 and GK genes, however, Curc/ZnO-NC showed the most potent anti-diabetic activity compared to normal rats, the histopathological findings correlate with the achieved data.

1. Introduction
Diabetes mellitus (DM) is a metabolic disease distinguished by hyperglycaemia. It comes as a result of defects in insulin action, insulin secretion or both [1]. DM is regarded as one of the main chronic diseases all over the world. It reaches epidemic proportions in some cases. In the year 2030, it is estimated that the proportion of patients suffering from diabetes will increase by 69%. Serious diabetic problems and complications still face both the physicians and patients in spite of the increased understanding of the pathophysiological procedures involved in DM [2]. The epidemic of DM and its complications is currently considered as a major health threat on a global scale [3].

The field of Nanotechnology is one of the most rapidly growing fields. It has many applications in science and modern technology for the goal of producing new applications of different substances at
Nanoparticles are widely applied in many medical fields such as fluorescent biological labelling, imaging contrast agents, drug and gene delivery and in detection of bioactive molecules. The use of Nano drugs in treatment of diabetes was shown to be very effective [5]. Previous reports have indicated that selenium nanoparticles have a preserving ability towards the integrity of pancreatic $\beta$ cell. It can also amplify insulin secretion (thereby reducing glucose level), and restore oxidant/antioxidant homeostasis [6].

The remarkable function of metals in metabolism of glucose and the connection of diabetes with their deficiency was reported in numerous researches. It was reported that vanadium [7], chromium, magnesium, and zinc have been included in diabetes therapy and play a role in blood sugar maintenance [8]. Zinc has a role in insulin biosynthesis, storage and secretion, also known to keep the structure of insulin. Diverse of zinc transporters do exist in the $\beta$ cells of pancreas; such as zinc transporter 8. This transporter displayed a significant function in secretion of insulin. Moreover; zinc can enhance insulin signalling via various mechanisms; such as inhibition of glycogen synthase kinase-3, enhancing PI3K activity and increased insulin receptor phosphorylation [9]. Zinc is considered as an activator for more than three hundred enzymes in the body [10]; it is an essential metal in diabetes therapy. Zinc has an important role in enhancing hepatic glycogenesis by its actions on the insulin pathways; therefore it enhances glucose utilization [9].

Natural herbal products were used by people in the past for different therapeutic purposes. Thousands of secondary metabolites are produced in plants as a natural defence system [11]. Turmeric ($Curcuma longa$, family Zingiberaceae) is a medicinal rhizome well known for its major active constituent; curcumin. Chemically, curcumin is known as 7-bis (4-hydroxy-3-methoxyphenyl) - 1, 6-heptadiene-3, 5-dione, diferuloylmethane or (1E, 6E)-1, [12]. Curcumin, the major active polyphenolic constituent of turmeric rhizomes, is known for its pleiotropic effects on a wide spectrum of molecular targets including antioxidative, anti-inflammatory, chemo-preventive, chemotherapeutic, and antidiabetic activities with no considerable side effects [13]. Curcumin, when used as nano-micelle in DM treatment, caused an HbA1c lowering effect and partial decrease in serum LDL-C [14].

GK (also known as hexokinase IV) in the gut, hypothalamus, pancreas, and liver converts glucose to glucose-6-phosphate which may be used to determine the flux of glucose into these cells [15]. GK found in the $\beta$-cells serves as a “glucostat” by controlling glucose-stimulated insulin secretion (GSIS) [15]. GK, a strong marker for diabetic therapy, improves the glucose uptake by the liver, as well as the pancreatic insulin secretion [16].

GLUT-2 plays an important role in glucose homeostasis in living organisms and is mainly expressed in the $\beta$-cells of the pancreas, liver and the basolateral membrane of kidney proximal tubules [17]. Defective insulin secretion stimulated by glucose in diabetic lab animals was related to the reduced GLUT-2 gene expression [18].

No data were found about the effectiveness power of Curc/ZnO-NC on STZ-induced diabetic rats. Thus, a need emerged to study the biochemical, molecular and histopathological effect of Curc/ZnO-NC compared to ZnO-NPs, Curc-NPs and traditional anti-diabetic Diamicron.

2. Materials and Methods

2.1. Chemicals

Ethylene glycol pure P.A (lab – scan analytical sciences, catalogue number A00470X). Zinc nitrate (Hexahydrate) Purified (loba chemie, India), STZ was purchased from sigma – Aldrich. RQ1 RNAse-free DNase (Invitrogen, Germany). TRIzol® Reagent (Invitrogen, Germany). RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, Germany). TaqTM (TaKaRa, Biotech. Co. Ltd.) Sense primer, antisense primer.

2.2. Preparation of material nanoparticles
2.2.1. Preparation of (ZnO-NPs). ZnO-NPs were prepared using the Pechini method. Briefly, the required amount of Zn(NO$_3$)$_2$.6H$_2$O was dissolved in a similar amount of ethylene glycol. The solution was then heated on a hot plate until a viscous gel-like substance was formed. With continuous heating, autoignition took place resulting in a fluffy yellowish-white powder which was then washed and grinded. Obtained powder was annealed at 500°C for 2 hours in Lenton Furnace UAF 16/5 [19].

2.2.2. Preparation of (Curc-NPs). Commercial curcumin was suspended in acetone, and then subjected to ultrasonic waves in an ultrasonication bath for 30 minutes at 65°C. The suspension was then stirred using a magnetic stirrer with hot plate for 1 hour until drying. After 1 hour, the powder was dried in an oven for 30 minutes at 50°C to be ready for use [20].

2.2.3. Preparation of (Curc/ZnO-NC). Firstly, the required quantities of ZnO-NPs were suspended in distilled water and acetone. The suspension was then subjected to ultrasonic waves by an ultrasonic probe for 30 minutes. Simultaneously, Curc-NPs powders were suspended in acetone and were sonicated in an ultrasonic bath for 30 minutes. Secondly, the Curc-NPs solution was added slowly drop by drop to the ZnO-NPs suspension in the ultrasonic bath by graduated pipette. The mixture was resonicated thoroughly ultrasonic probe for 31 minutes, and then transferred to a hot plate magnetic stirrer for 2 hours until drying. Powder was dried in an oven at 40°C for 15 minutes [21]. Preparation and characterizations of the nanoparticles were performed in the materials science and nanotechnology Lab. at PSAS-BSU.

2.3. Characterization of nanoparticles
The size and shape of nanoparticles was observed using high resolution transmission electron microscope (HRTEM JEOL-JEM 2100, Japan) using an accelerating voltage of 200 kV. X-ray diffraction (XRD) patterns were recorded on a PANalytical (Empyrean) X-ray diffractometer using Cu K$_\alpha$ radiation (λ=1.5406 Å) at an acceleration voltage of 40 kV, with current of 30 mA, in the angle range from 5–80°, with step scan of 0.02°. The size distribution of particles was determined by Zetasizer nano-Zs90 (Malvern, UK).

2.4. Animals
The experiment was carried out on albino Wister rats (180-200 g) that were obtained from the National Research Centre (NRC), Egypt, after the approval from NRC animal ethical committee (Approval no.16-207), in polypropylene cages (47cm × 34cm×20cm) lined with husk, renewed each day. Purified drinking water, as well as the standard laboratory pellet feed were provided, room temperature was retained at 22 ± 3°C during a cycle of 12 hours of light/12 hours of dark.

2.5. Induction of diabetes mellitus
All experimental animals were fasting overnight and diabetes was induced by an intraperitoneal injected dose of STZ (50 mg/kg b.w) freshly dissolved in 0.1 M citrate buffer (pH 4.5). After 6 hours of injecting the STZ, glucose (20%) was provided to rule the fatal STZ-stimulated hypoglycaemia. Glucose levels were measured after 2 to 7 days. Animals with high glucose levels exceeding 15 mmol (250 mg/dl) were considered diabetic, and their glucose levels were measured daily using AquaChek device [22].

2.6. Experimental design
A total of 48 albino Wistar rats were categorized into 6 groups, 8 rats per group, each of 4 males and 4 females. All treatments were dissolved in de-ionized water and administered orally once daily for 21 days. Group 1: negative control, group 2: positive control diabetic rats without treatment, group 3: diabetic rats treated with ZnO-NPs 10 mg/kg b.w [23], group 4: diabetic rats treated with Curc-NPs 50 mg/kg b.w [24], group 5: diabetic rats treated with Curc/ZnO-NC 10 mg/kg b.w, group 6: diabetic rats treated with Diamicron 5 mg/kg b.w [25].
After one week, fasting blood samples were collected by the capillary to measure fasting glucose and insulin (initial glucose and insulin levels). Before ending the experiment, all animals were fasting during the night and the second collection of blood samples was done to measure liver and kidney function and insulin levels. Samples on sodium fluoride were collected to measure fasting glucose, and EDTA blood samples were collected to measure HBA1C. Also, different tissue samples from liver and pancreas were collected separately; for histopathological examination using 10% formalin, and for gene expression using liquid nitrogen and kept in -80°C.

2.7. Biochemical parameters
Serum blood glucose (mg/dL) was estimated by the glucose oxidase method using the kit supplied by SPINREACT. Kit purchased from DRG international, Inc. (NJ, USA) was used to assay serum insulin concentration by an enzyme-linked immunosorbant assay (ELISA) [26]. HBA1C was estimated by immunological assays for the invitro quantitative determination of hemoglobin A1C in whole blood [27]. Serum AST and ALT were detected by (NADH. Kinetic UV) [28]. Serum creatinine was measured based on the reaction of creatinine with sodium picrate as described by Jaffe method [29], and urea was measured using urease method [30].

2.8. Analysis of Gene Expression

2.8.1. Isolation of RNA. TRizol® extraction Chemical (Invitrogen) was utilized to isolate the total genomic RNA of liver and pancreas tissues of all treated animals. After completion of the isolation procedures, RNA pellet was stored in DEPC-treated water. To digest the potential DNA residues, the pellet of isolated RNA was treated with RNase-free DNAse kit (Invitrogen, Germany). RNA aliquots were stored at -20°C or utilized immediately for reverse transcription [31].

2.8.2. Reverse transcription reaction. First Strand cDNA Synthesis Kit (RevertAidTM, MBI Fermentas) was used to synthesize the cDNA copy from liver and pancreas tissues via reverse transcription reaction (RT). A reverse transcription reaction program of 25°C for 10 min, then one hour at 42°C then 5 min at 95°C was used to obtain the cDNA copy of liver and pancreas genome. Finally, tubes of reaction containing cDNA copy were collected on ice up to use for cDNA amplification [32].

2.8.3 Quantitative Real Time-PCR. SYBR® Premix Ex TaqTM kit (TaKaRa, Biotech. Co. Ltd.) was used to perform the qRT-PCR analyses using the synthesized cDNA copies from liver tissues. Thermal cycling of the reaction was allocated to three steps; the first step was at 95.0°C for 3 min. Second step consisted of 40 cycles, each cycle was divided into 3 steps: (a) at 95.0°C for 15 sec; (b) at 55.0°C for 30 sec; and (c) at 72.0°C for 30 sec. The third step consisted of 71 cycles which started at 60.0°C and then increased about 0.5°C every 10 sec up to 95.0°C. For each reaction, a melting curve profile was conducted. The quantitative values of the target genes were normalized on the expression of the housekeeping gene (table 1). The $2^{-\Delta\Delta CT}$ method was used to determine the quantitative values of the specific genes to the β-actin gene.

2.8.4 Histopathological examinations
Different samples of the pancreas and liver tissues were obtained, fixed in buffered neutral formalin (10%), dehydrated gradually using 70%–100% ethanol, cleared using xylene, and finally inserted in paraffin. Hematoxylin and eosin (H&E) stain was used for the routinely staining of different paraffin sections (5μ in thickness) that were mounted on glass slides [36], and then examined microscopically.

2.8.5 Statistical analyses
All data were presented and analysed using IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp. All results are expressed as mean ± standard deviation (SD). A comparison among
groups was made by students’ one-way analysis of variance (ANOVA). Statistical significance was set (p < 0.05).

Table 1. Primer sequences used for qPCR

| Gene            | Primer sequence (5’–3’)$^a$ | References |
|-----------------|-----------------------------|------------|
| GLUT-2          | Forward primer-TTG GCT TTC ACT GTC TTC ACT | [33]       |
|                 | Reverse primer-CTT CCT TTT CTG TTC TCC TCA TCT C |           |
| GK              | Forward primer-AAG GAC AGG GAC CTG GGT TCC A | [34]       |
|                 | Reverse primer-TCA CTG GCT GAC TTG GCT TGC A |           |
| β-actin (Housekeeping gene) | Forward primer-GGT ATG GAA TCC TGT GAA A | [35]       |
|                 | Reverse primer-GTG TAA AAC GCA GCT CAG TAA CAG TCC G |           |

3. Results

In the present study, the therapeutic effects of Curc-NPs, ZnO-NPs and Curc/ZnO-NC were evaluated on STZ-induced diabetic rats and compared to normal rats and rats treated by traditional treatment; Diamicron.

![Figure 1. Characterization by HRTEM shows (A): Curc-NPs, (B): ZnO-NPs and (C): Curc/ZnO-NC.](image)

The obtained sizes of Curc-NPs, ZnO-NPs and Curc/ZnO-NC, together with the particle shape are shown in (figure 1). Curc-NPs had an average particle size in the range of 27-30 nm. The sizes of ZnO-NPs and Curc/ZnO-NC were in the ranges of 24-30 and 20-30 nm respectively. As clearly observed, the particles have a hexagonal geometric shape with sharp boundaries in case of ZnO, and platelet shape in case of Curc-NPs. In case of the nanocomposite, hexagonal and platelet nanoparticles are stacked together. The existence of some agglomeration is obviously due to the absence of capping agent during preparation and the poor solubility of Curcumin. The hexagonal symmetry is in line with that found from the XRD analysis for the ZnO.
Figure 2. XRD patterns of (Curc-NPs), (ZnO –NPs) and (Curc/ZnO-NC).

XRD patterns for the nanoparticles are illustrated in (figure 2), the data revealed the crystallization of the ZnO in single phase with no additional peaks. The data of ZnO was compared and indexed with ICDD card no (01-080-6503). The reflections of ZnO were indexed as (hkl) (100), (002), (101), (102), (110), (103), (112), (201), (004). The crystal structure of the pure ZnO seen to the well-known hexagonal symmetry with space group (P63mc). The Curc-nanoparticles possessed excellent crystallinity with monoclinic symmetry as compared with ICDD card (00-063-0943) and space group. The main reflections of Curc- nanoparticles were indexed as (hkl) (-101),(101),(110),(112),(004),(-113),(-301),(114),(-303). In the nanocomposite, the reflections of ZnO nanoparticles seems to be higher than those of curcumin despite the equivalent weight ratios in the nanocomposite (50%ZnO-50%Curc). It is also obvious owing to the difference in both densities (ZnO 5.1 and Curc 1.39 g/cm³). Moreover, the number of molecules per unit cell Z (Z=4 for Curc and =2 for ZnO). The average crystallite size was calculated using the well-known Debye Scherrer’s formula [37,38,39]. It was found that the crystallite size of ZnO nanoparticles is 35.8 nm while that of Curc- is 63 nm.

Table 2. Zeta Potential for (Curc-NPs), (ZnO –NPs) and (Curc/ZnO-NC)

| Name            | Average hydrodynamic diameter DLS (nm) | Zeta potential (mV) |
|-----------------|---------------------------------------|---------------------|
| (ZnO -NPs)      | 3420                                  | -2.32               |
| (Curc-NPs)      | 2620                                  | -9.12               |
| (Curc/ZnO-NC)   | 1750                                  | -24.1               |

The data in (table 2) reported the values of zeta potential and average hydrodynamic diameter. It is clear that the nanocomposite has the lowest value for size due to the ultrasonic waves which affect directly on size as well as distribution. The zeta potential value here is small for ZnO while it revealed large one for the nanocomposite. This result means that the latter is the most stable in its use as dispersed despite the poor solubility of curcumin.

Levels of urea, creatinine, AST and ALT (table 3) showed no significant differences among all animal groups (p values were 0.944, 0.746, 0.301 and 0.516 respectively). On the other hand, there was a significant reduction in blood glucose levels in groups treated by Diamicron, ZnO-NPs, Curc-NPs and Curc/ZnO-NC compared with the positive control group (table 4) , the percentages of reduction were 57.2, 57.8, 54.5 and 66.3% respectively (P value was 0.000). There was also a significant increase in the serum insulin levels in the diabetic groups treated with Diamicron, ZnO-NPs, Curc-NPs and Curc/ZnO-NC (P value was 0.000); increment percentages were 47.2, 45.3, 42.9 and 77.1% respectively, showing that Curc/ZnO-NC induced the highest insulin secretion (table 4). As for HBA1C, there was a significant reduction in groups treated by Diamicron, ZnO-NPs, Curc-NPs
and Curc/ZnO-NC compared with the positive control group (P value was 0.000) and, noticeably, the group treated with Curc/ZnO-NC recorded the lowest value of HBA1C (table 4).

**Table 3.** Biochemical effect of Diamicron, Curc-NPs, ZnO-NPs and Curc/ZnO-NC on STZ-induced diabetic rats.

|                          | Control −ve | Control +ve | Diam | Curc-NPs | ZnO-NPs | Curc/ZnO-NC | F    | P value |
|--------------------------|-------------|-------------|------|----------|---------|-------------|------|---------|
| Urea (mg/dl)             | Mean 21.917 | 23.333      | 22.583 | 22.083   | 22.667  | 21.917      | 0.239| 0.944   |
|                          | ±SD 4.575   | 4.048       | 3.040 | 3.302    | 3.197   | 2.325       |      |         |
| Creatinine (mg/dl)       | Mean 0.753  | 0.707       | 0.725 | 0.7147   | 0.741   | 0.67        | 0.539| 0.746   |
|                          | ±SD 0.124   | 0.148       | 0.146 | 0.146    | 0.118   | 0.114       |      |         |
| AST U/L                  | Mean 22.833 | 22.917      | 20   | 19.6     | 21      | 23.4        | 1.245| 0.301   |
|                          | ±SD 6.4     | 4.5         | 4.8  | 5.9      | 3.1     | 5.3         |      |         |
| ALT U/L                  | Mean 22.08  | 21.5        | 20.5 | 19.9     | 20.6    | 23          | 0.857| 0.516   |
|                          | ±SD 5.7     | 3.9         | 5.7  | 5.6      | 3.09    | 4.6         |      |         |

**Table 4.** Effect of Diamicron, Curc-NPs, ZnO-NPs and Curc/ZnO-NC on serum Glucose, HBA1C and Insulin levels of STZ-induced diabetic rats.

|                          | Control −ve | Control +ve | Diam | Curc-NPs | ZnO-NPs | Curc/ZnO-NC | F    | P value |
|--------------------------|-------------|-------------|------|----------|---------|-------------|------|---------|
| Initial glucose (mg/dl)  | Mean 103.7  | 242.2       | 240.8 | 243.8    | 240.9   | 241.2       | 480.661| .000*   |
|                          | ±SD 8.05    | 11.48       | 5.84 | 7.48     | 5.85    | 8.68        |      |         |
| Final glucose (mg/dl)    | Mean 101.3  | 347.1       | 148.7 | 158      | 146.4   | 116.8       | 2141.111| .000*   |
|                          | ±SD 4.64    | 10.32       | 4.57 | 4.59     | 5.18    | 5.24        |      |         |
| Initial insulin (μIU/ml) | Mean 12.25  | 8.04        | 8.39 | 8.07     | 8.23    | 12.02       | 583.311| .000*   |
|                          | ±SD 0.20    | 0.22        | 0.26 | 0.32     | 0.27    | 0.30        |      |         |
| Final insulin (μIU/ml) U/L| Mean 12.62  | 6.95        | 10.23 | 9.93     | 10.1    | 12.31       | 375.036| .000*   |
|                          | ±SD 0.28    | 0.50        | 0.26 | 0.34     | 0.22    | 0.29        |      |         |
| HBA1C                    | Mean 5.481  | 8.467       | 6.5  | 6.75     | 6.447   | 6.2083      | 106.351| .000*   |
|                          | ±SD 0.371   | 0.286       | 0.404| 0.272    | 0.351   | 0.232       |      |         |

* The mean difference is significant at the 0.001 level (P<0.001).

Results of the GLUT-2 and GK genes (tables 5, 6) and (figures 3, 4) showed that the expression levels in liver tissues were significantly lower than those in pancreatic tissues (P < 0.0001). Compared to the positive control group, all the other groups showed significant reduction in the expression levels of GLUT-2 and GK genes in liver and pancreas tissues (P < 0.0001). Furthermore, treatment with Curc/ZnO-NC reduced the expression levels of GLUT-2 and GK genes significantly compared to Curc-NPs alone, ZnO-NPs alone or with the Diamicron treatment (P < 0.0001) in both liver and pancreatic tissues.
Table 5. Comparison between the mean values of GLUT-2-mRNA in liver and pancreas with respect to the different rats groups

|                  | Liver Mean± SD | Pancreas Mean± SD | t-statistic | P value |
|------------------|----------------|-------------------|-------------|---------|
| Control –ve      | 0.62 ± 0.05    | 0.96 ± 0.05       | 15.205      | 0.000*  |
| Control +ve      | 2.35 ± 0.09    | 2.91 ± 0.04       | 17.981      | 0.000*  |
| Diamicron        | 1.18 ± 0.02    | 1.39 ± 0.06       | 17.981      | 0.000*  |
| Curc-NPs         | 1.42 ± 0.06    | 1.74 ± 0.07       | 10.500      | 0.000*  |
| ZnO-NPs          | 1.23 ± 0.08    | 1.43 ± 0.06       | 10.976      | 0.000*  |
| Curc/ZnO-NC      | 0.84 ± 0.04    | 1.19 ± 0.05       | 6.325       | 0.000*  |

*P < 0.0001

Figure 3. The alterations of GLUT-2-mRNA in liver and pancreas tissues (negative control) and positive control and DM-rats treated with Curc-NPs, ZnO-NPs, Curc/ZnO-NC and Diamicron.

Table 6. Comparison between the mean values of GK-mRNA in liver and pancreas with respect to the different rat groups.

|                  | Liver Mean± SD | Pancreas Mean± SD | t-statistic | P value |
|------------------|----------------|-------------------|-------------|---------|
| Control –ve      | 0.72 ± 0.02    | 1.13 ± 0.04       | 28.991      | 0.000*  |
| Control +ve      | 1.86 ± 0.03    | 2.74 ± 0.05       | 47.725      | 0.000*  |
| Diamicron        | 1.21 ± 0.05    | 1.55 ± 0.04       | 16.791      | 0.000*  |
| Curc-NPs         | 1.28 ± 0.06    | 1.69 ± 0.06       | 15.280      | 0.000*  |
| ZnO-NPs          | 1.19 ± 0.05    | 1.41 ± 0.05       | 9.839       | 0.000*  |
| Curc/ZnO-NC      | 0.97 ± 0.04    | 1.28 ± 0.03       | 19.606      | 0.000*  |

*P < 0.0001
Figure 4. The alterations of GK-mRNA in liver and pancreas tissues (negative control) and positive control and DM-rats treated with Curc-NPs, ZnO-NPs, Curc/ZnO-NC and Diamicron.

Histopathological illustration (figure 5) showed that the liver of normal rats had normal histoarchitecture. The hepatic parenchyma and hepatic sinusoids appeared normal with normal distribution of Kupffer cells in contrast to the liver of diabetic rats where necrotic changes, dilatation of liver sinusoids, Kupffer cells activation and cytoplasmic vacuolization of hepatocytes were prominent. Variable degrees of improvements were noticed after treatment; liver of Diamicron-treated group, as a traditional treatment, showed moderate hepatic sinusoids (BS) dilatation with a slightly high number Kupffer cells (KC) and mild degenerative changes. In the Curc-NPs-only treated group, histoarchitecture of the liver appeared more or less normal with very mild necrobiotic changes (NT) and degeneration (FCH). In addition, mild dilatation of blood sinusoids (BS) and mild increase in number and distribution of (KC) was noticed. The Liver of ZnO-NPs-only treated group had normal tissue histoarchitecture with mild blood sinusoids (BS), widening and normal number and distribution of (KC) and congested central vein (CV). While the Curc/ZnO-Nc, treated group showed marked dilatation of liver sinusoids with the presence of (KC) (LS), mild necrotic changes and congested (CV).

Histopathological illustration (figure 6) of the pancreas of the normal control rats showed normal histological architecture in the form of normal acinar structure with normal islets of Langerhans. In contrast, pancreas of positive control group showed significant decrease in islets of Langerhans (IL) with marked atrophy of acini, vacuolar degeneration and necrosis. When diabetic groups received treatments, the following was noticed; pancreas of Diamicron-treated group showed restoration of normal size of (IL) with normal acinar cells and mild necrotic changes. Pancreas of Curc-NPs-treated group showed moderate restoration of cells of (IL) with mild necrotic changes. Pancreas of ZnO-NPs-treated group had normal-sized (IL) with mild central vacuolation and necrotic changes. Pancreas of Curc/ZnO-NC-treated group appeared more or less normal with restoration of (IL) and mild necrotic changes.

4. Discussion

Significant hyperglycemia in rats was induced by the STZ (60 mg/kg b.wt.) injection. This dose is reported to cause damage to β-cells of the (IL). Moreover, clinical diabetes emerged within 2–4 days due to autoimmune process [40]. Similar results were reported by previous studies [41], where STZ induced significant hyperglycemia which may be caused by gluconeogenesis enhancement due to the absence of insulin [42]. In this study, we evaluated the possible therapeutic effect of zinc oxide,
curcumin and their nanocomposite on diabetic rats (streptozotocin-induced), compared to the diamicron treatment. There are numerous types of nanomaterials tested for their potential use in drug delivery systems [43]. Metallic nanoparticles (e.g. zinc, silver, iron and gold) and metal oxides nanoparticles have a great role in biomedical and biotechnological applications [44]. ZnO –NPs act as potent antidiabetic agents [45].

Figure 5. Histology illustration of liver for six groups (A) Negative control (B) positive control (C) Diamicron treated group (D) Curc-NPs treated group (E) ZnO-NPs treated group (F) Curc/ZnO-NC treated group.

Figure 6. Histology illustration of pancreas for six groups (A) Negative control (B) positive control (C) Diamicron treated group (D) Curc-NPs treated group (E) ZnO-NPs treated group (F) Curc/ZnO-NC treated group.

Medicinal plants are recognized for their great role in replacing traditional synthetized drugs; with a similar degree of efficacy and less side effects. Curcumin was explored by many studies as a hypoglycemic agent [46], because curcumin quenches singlet oxygen effectively, leading to adduct formation. Moreover, it can be used as a cytoprotective agent for pancreatic islet cells, due to its inhibition of islet apoptosis as it inhibits the oxidative stress. However, curcumin has low bioavailability due to its sparingly solubility in water [47]; so nanocurcumin was used in this study to enhance its bioavailability, having a larger surface area in contact with the solvent leading to enhanced solubility.

Our results show the potent antidiabetic effect of the investigated nanoparticles. However, Curc/ZnO-NC induces the highest reduction in blood glucose levels and the highest elevation in insulin levels compared to Curc-NPs, ZnO–NPs and Diamicron, revealing the potent synergistic effect of Curc/ZnO-NC that improves glucose utilization and metabolism. This can be attributed to its enhancement of hepatic glycogenesis via insulin signaling pathway [9] (Table 4).

All tissues which respond to insulin can express insulin receptors (IRs) with different levels. Moreover, insulin potential requires that it binds to the receptors α–subunits leading to the phosphorylation of β–subunits to fulfill its role [45]. This supports our experimental results which demonstrated that diabetic rats liver and pancreas tissues revealed elevated GLUT-2 and GK mRNAs expression levels compared to the treated groups. Furthermore, tissues of pancreas showed elevated GLUT-2 and GK genes expression than the liver tissues of DM-rats. Also, the Curc/ZnO-NC showed the highest reduction GLUT-2 and GK gene expression compared to other treatments. These findings were asserted biochemically and histopathologically [48].

5. Conclusions
This research evaluates different nanoparticles; Curc-NPs, ZnO-NPs and Curc/ZnO-NC for their antidiabetic activities. All treated groups showed significant reduction in blood glucose, elevated
insulin levels, regulated GLUT-2 and GK genes, however, Curc/ZnO-NC revealed the most potent anti-diabetic activity, as it showed a significant reduction in blood glucose by 66.3% and also elevated insulin levels by 77.1%. Compared to the positive control group, the histopathological findings correlate with the achieved data. Future work will be focused on the variation of both size and concentration of nanoparticles and its antidiabetic effect.

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References
[1] American Diabetes Association 2014 Diabetes Care 37 81
[2] Tiwari A K and Rao J M 2002 Curr. Science 30
[3] Zheng Y, Ley S H and Hu F B 2018 Nat. Rev. Endocrinol. 14 88
[4] Albrecht M A, Evans C W and Raston C L 2006 Green Chem. 8 417
[5] Salata O V 2004 J. Nanobiotechnology 2 3
[6] Ahmed H H, El-Maksoud M D A, Moneim A E A and Aglan H A 2017 Biol. Trace Elem. Res. 177 267
[7] Thompson K H, Lichter J, LeBel C, Scaife M C, McNeill J H and Orvig C 2009 J. Inorg. Biochem. 103 554
[8] Chausmer A B 1998 J. Am. Coll. Nutr. 17 109
[9] Jansen J, Karges W and Rink L 2009 J. Nutr. Biochem. 20 399
[10] Haase H, Overbeck S and Rink L 2008 Exp. Gerontol. 43 394
[11] Wink M 2015 Medicines 2 251
[12] Hatcher H, Planalp R, Cho J, Torti F M and Torti S V 2008 Cell. Mol. Life Sci. 65 1631
[13] Gupta S C, Patchva S and Aggarwal B B 2013 AAPS J. 15 195
[14] Rahimi H R, Mohammadpour A H, Dastani M, Jaafari M R, Abnous K, Mobaran M G and Oskuee R K 2016 Avicenna J. Phytomed. 6 567
[15] Matschinsky F M 2009 Nat. Rev. Drug Discov. 8 399
[16] Tahran A A, Piya M K, Kennedy A and Barnett A H 2010 Pharmacol. Ther. 125 328
[17] Im S S, Kang S Y, Kim S Y, Kim H I, Kim J W, Kim K S and Ahn Y H 2005 Diabetes 54 1684
[18] Orci L, Unger R H, Ravazzola M, Ogawa A, Komiya I, Baetens D, Lodish H F and Thorens B 1990 J. Clin. Invest. 86 1615
[19] Hozyen H F, Ibrahim E S, Khairy E A and El-Dek S I 2019 Vet. World 12 1225
[20] Shirsath S R, Sable S S, Gaikwad S G, Sonawane S H, Saini D R and Gogate P R 2017 Ultrasound. Sonochem. 38 437
[21] Farghali A A, Khedr M H, El-Dek S I and Megahed A E 2018 Ultrason. Sonochem. 42 556
[22] Murali R, Srinivasan S and Ashokkumar N 2013 Biochimie 95 1848
[23] El-Gharbawy R M, Emara A M and Abu-Risha S E S 2016 Biomed. Pharmacother. 84 810
[24] Zhang D W, Fu M, Gao S H and Liu J L 2013 Evid. Based Complement. Alternat. Med. 2013 636053
[25] Pulido N, Suarez A, Casanova B, Romero R, Rodriguez E and Rovira A 1997 Metabolism 46 10
[26] Temple R, Clark P M S and Hales C N 1992 Diabet. Med. 9 503
[27] Cohen M P 1992 Eur. J. Clin. Chem. Clin. Biochem. 30 851
[28] Edrees H M, Elbehiry A and Elmosaad Y M 2017 Nanotechnol. 3 4
[29] Murray R and Kaplan A 1984 Clinical Chemistry (Toronto: The CV Mosby Co.)
[30] Chaney A L and Marbach E P 1962 Clin. Chem. 8 130
[31] Salem N A, Wahba M A, Eisa W H, El-Shamarka M and Khalil W 2018 Inflammopharmacology 26 1025
[32] Khalil W K B, Booles H F, Hafiz N A and El-Bassyouni G E 2018 Int. J. Pharmacol. 14 477
[33] Amalan V, Vijayakumar N, Indumathi D and Ramakrishnan A 2016 Biomed. Pharmacother. 84 230
[34] Baldini S F, Steenackers A, Olivier-Van Stichelen S, Mir A M, Mortuaire M, Lefebvre T and Guinez C 2016 Biochem. Biophys. Res. Commun. 478 942
[35] Serrano R, Villar M, Martinez C, Carrascosa J M, Gallardo N and Andres A 2005 J. Mol. Endocrinol. 34 153
[36] Bancroft JD, Gamble M, editors 2008 Theory and Practice of Histological Techniques. Elsevier Health Sciences, pp. 135-160
[37] Mansour S F, Abdo M A and El-Dek S I 2017 J. Magn. Magn. Mater. 422 105
[38] El-Dek S I 2010 Philos. Mag. Lett. 90 233
[39] Khedr M H and Farghali A A 2005 J. Mater. Sci. Technol. 21 675
[40] Weiss R B 1982 Cancer Treat Rep 66 427
[41] Sellamuthu P S, Muniappan B P, Perumal S M and Kandasamy M 2009 Glob. J. Health Sci. 55 206
[42] Yao X H, Chen L and Nyomba B G 2006 J. Appl. Physiol. 100 642
[43] Yih T C and Al-Fandi M 2006 J. Cell. Biochem. 97 1184
[44] Hirst S M, Karakoti A S, Tyler R D, Sriranganathan N, Seal S and Reilly C M 2009 Small 5 2848
[45] Alkaladi A, Abdelazim A and Afifi M 2014 Int. J. Mol. Sci. 15 2015
[46] Abdel Aziz M T, El-Asmar M F, El Nadi E G, Wassef M A, Ahmed H H, Rashed L A and Abdel Aziz A T 2010 Angiology 61 557
[47] Modasiya M K and Patel V M 2012 Int. J. Pharm. Life Sci. 3 1490
[48] Nasr M, Raslan M, Ashour S, Mahmoud S and El-Nesr K 2018 Biosci. Res. 15 4520