Dual Effect of Curcumin–Zinc Complex in Controlling Diabetes Mellitus in Experimentally Induced Diabetic Rats

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Ultrasound-assisted extraction of curcumin from *Curcuma longa* was performed in an ultrasonic bath at 30°C using ethanol for 40 min. A successful attempt has been made to prepare curcumin–zinc (Zn) complex using a simple chemical procedure. The complex formation and its stoichiometry were characterized using elemental analysis, Fourier transform (FT)-IR and UV spectroscopy which revealed the interaction of Zn(II) ion (M) with curcumin (ligand, L) to proceed via (ML) complex type formation. Oral administration of curcumin–Zn complex at a concentration of 150 mg/kg body weight/rat/d for 45 d in streptozotocin-induced diabetic rats in comparison to curcumin and/or Zn administration exerted a hypoglycemic effect. A significant reduction in blood glucose, glycosylated hemoglobin (Hb)A1c, and lipid profile parameters with an excellent improvement in plasma insulin levels have been attained. Also, the reduced activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, and creatinine in the diabetic rats treated with the complex exhibited the non-toxic nature of the curcumin–Zn complex. Finally, the larger extent of the complex in hyperglycemic improvement in comparison to curcumin and/or Zn supplementation was interpreted by its dual action on glucose and insulin maintenance.

Key words diabetes; curcumin–zinc complex; dual action; curcumin; zinc metal; diabetes complication

Diabetes mellitus, the most common endocrine disorder characterized by a state of chronic hyperglycemia and insulin resistance. Type 2 Diabetes Mellitus (T2DM) is projected to rise over 215 million people worldwide by the year 2010.3) Sustained hyperglycemia results in glucose autoxidation and protein glycosylation which in turn leads to excessive production of reactive oxygen species (ROS).2) In other words, hyperglycemia is associated with absolute or relative deficiency in insulin secretion from the beta cells of pancreas and/or desensitization of insulin receptors.3)

Natural products have played a considerable role in the management of diabetes and its complications.5,6) Curcumin (diferuloylmethane), the most active component of turmeric isolated from the rhizomes of the plant *Curcuma longa*, has caught attention as a promising therapeutic agent in experimental diabetes and for the treatment of complications of diabetes.6,7) Moreover, it is widely used in the folk treatment of different diseases attributed to its several biological applications; mainly as a significant antioxidant agent both *in vivo* and *in vitro*.8,9) Curcumin has been suggested by many studies as an efficient therapeutic agent for treatment of diabetes, more than 215 publications with the search term “curcumin and diabetes” from the MEDLINE database in 2015. Some of these studies related to absorption, distribution, and metabolism of curcumin that possesses poor absorption and rapid metabolism that reflects its low bioavailability and reduced bioaccessibility.10,11)

Many approaches that can be used for the enhancement of curcumin bioavailability such as encapsulation, use excipient foods, emulsions, and/or micelles.12–14) An alternative approach that suggested enhancing the bioavailability of curcumin is to develop a curcumin-metal complex. The use of metals in therapeutic drugs offers a large extent of exciting and valuable metallo-pharmaceutical drugs like Cisplatin and Auranfin as anticancer and antiarthritic drugs, respectively.12) It was reported that diabetic animals and humans are zinc (Zn) deficient and that Zn supplementation will benefit or correct the diabetes-induced Zn status.13) Zn was known by its mimetic actions which enforced exploration of the antidiabetic properties of Zn complexes.14,15)

In the present study, we have designed, synthesized, and characterized a curcumin–Zn complex using safe, reproductive and efficient procedures. Comparable evaluation of the antidiabetic potentials of curcumin, Zn, and curcumin–Zn complex was explored.

MATERIALS AND METHODS

Materials All chemical reagents and solvents used were purchased from Sigma-Aldrich Company (U.S.A.); they were used without further purification. Turmeric, dried rhizomes of *C. longa* L., was purchased from local market, Cairo, Egypt. Streptozotocin (STZ) was purchased from Cornell Lab. Company, Cairo, Egypt.

Ultrasonic-Assisted Extraction of Curcumin16) *Curcuma longa* L., rhizomes were previously treated, dried, and pulverized. Thereafter, the rhizomes were extracted with absolute ethanol (approximately 10 g in 100 mL ethanol) using a 100-W ultrasonic bath at 30°C for 40 min. The reaction mixture was concentrated under vacuum, and then ethyl acetate (50% (v/v)) was added. An orange precipitate which was collected and submitted for purification using column chromatography (sili-
ca gel 60 “Merck” as a stationary phase). The two tautomeric forms of curcumin were given in Fig. 1.

Preparation of Curcumin–Zn Complex Curcumin–Zn complex was prepared according to reported method with slight modifications. An equimolar mixture of Zn sulfate (10 mmol) in 50 mL absolute ethanol and curcumin extract (10 mmol) in 50 mL absolute ethanol separately were mixed and few drops of triethylamine were added. The mixture was heated under reflux at 60°C for 2 h with simultaneous stirring. The precipitate formed after cooling was filtered off, washed with water/ethanol, and dried in an oven at 60°C.

Characterization of Curcumin and Curcumin–Zn Complex TLC was performed on Merk TLC aluminium sheets silica gel 60 F 254 with detection by UV quenching at 254 nm. Curcumin and curcumin–Zn complex were characterized using elemental and spectral analysis like Fourier transform (FT)-IR and UV spectroscopy.

Experimental Animals The study was carried on fifty male albino rats of Sprague-Dawley strain, weighing approximately (180±20 g), obtained from the Egyptian organization for biological products and vaccines, Helwan, Cairo, Egypt. Rats were randomized and maintained in the air-conditioned animal house under specific pathogen-free conditions and were subjected to a 12:12 h daylight/darkness and fed on the basal diet without any treatment for one week for acclimatization. All the ethical protocols for animal facilities were followed and supervised by the animal facilities, a research institute of Ophthalmology, Cairo, Egypt. All animal experiments received approval from the institutional animal care Committee.

Induction of Diabetes Mellitus Diabetes mellitus was induced in forty rats by a single intraperitoneal injection of STZ at 45 mg/kg dissolved in freshly prepared citrate buffer (pH 4.5, 0.1 mol/L). Soon after STZ injection, glucose water (5%) was given to rats for 2 d. After 72 h, the diabetic status was assessed by measuring fasting blood glucose.

The rats were divided into the following groups:

Group 1. Ten control rats were injected with citrate buffer to serve as a normal control group (NC).

Group 2. Ten rats representing the STZ-induced diabetic rats (DC).

Group 3. Ten diabetic rats that receive curcumin (150 mg/kg body weight was dissolved in dimethyl sulfoxide (DMSO)) daily by oral gavage for 45 d (D-Cu).

Group 4. Ten diabetic rats that receive Zn sulfate (100 mg/kg body weight was dissolved in distilled water) daily by oral gavage for 45 d (D-Zn).

Group 5. Ten diabetic rats that receive curcumin–Zn complex (150 mg/kg body weight was dissolved in DMSO) daily by oral gavage for 45 d (D-CuZ).

Collection of Blood Samples Fasting blood samples were withdrawn periodically from the tail to estimate plasma glucose, insulin, and hemoglobin (Hb)A1c. At the planned time of scarification (after 45 d), fasting blood samples were withdrawn from the retro-orbital vein and processed immediately into three tubes, one containing fluoride for immediate estimation of fasting plasma glucose, the second tube containing ethylenediaminetetraacetic acid (EDTA) for separation of plasma, and the third tube was without anticoagulant for biochemical parameters estimation. The tubes were centrifuged and the plasma and serum were separated and stored at −20°C.

Biochemical Assays Fasting plasma glucose was determined using the glucose oxidase method and plasma insulin was estimated using a previously described enzyme-linked immunosorbent assay (ELISA) method. HbA1c was determined according to Trivelli et al. The serum lipid profile parameters were measured using commercially available biochemical kits (Span Diagnostics, Bangalore) according to standard procedures. The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and albumin in serum were assayed according to the method described by King. Blood urea and serum creatinine were estimated according to reported procedures.

Statistical Analysis The statistical analysis was carried out by using SPSS, PC statistical software (version 16.0; SPSS Inc., Chicago, U.S.A.). The results were expressed as the mean±standard deviation (S.D.). Data were analyzed by one-way ANOVA. The differences between means were tested for significance using Bonferroni–Dunn test at (p<0.05).

RESULTS

Characterization of Curcumin and Curcumin–Zn Complex The experimental elemental analysis for the curcumin–Zn complex revealed C 54.33% and H 4.19%. The calculated mass of Zn (II) ion (M) with curcumin (ligand, L) type complexes are C_{29}H_{27}O_{2}Zn (53.87%) and C_{29}H_{27}O_{2}Zn (54.14%). Therefore, the structure of the complexes is suggested as shown in Fig. 2.

The UV spectroscopy revealed bands at 380, 413, 432, and 456 nm for curcumin in DMSO. While the curcumin–Zn complex showed the same with the difference in the third band to appear at 438 nm. FT-IR spectra of curcumin showed the following characteristic absorption bands at 1320, 1332, 1406 (C–O) vibration, 1603 (C=O), 1627, 1725 (CO), 1857, 1882 (CH-aliphatic), 3563, 3600 cm⁻¹ (free OH groups). The curcumin–Zn complex exhibited a red shift in its IR spectrum to give absorption band at 1596 (C=O), and 1624 cm⁻¹ for CO while the broadband for the hydroxyl groups was disappeared.

Body Weight and Body Weight Gain (BWG) The diabetic groups showed remarkably body weight loss after STZ-induction. A significant decrease in BWG (p<0.05) for diabetic control was observed compared with normal control group; while the treated groups showed gradual improvements in BWG (Table 1).

Plasma Glucose Level Table 2 and Fig. 3 counts the changes in fasting plasma glucose levels for normal and
The plasma insulin levels for control groups (NC and DC) were not significantly different on day 15, 30, and/or 45. On the other hand, treated groups showed a significant gradual increase \( (p<0.05) \) in the mean plasma insulin levels among diabetic rats; DC (9.15 µIU/mL), D-Cu (8.71 µIU/mL), D-Zn (8.84 µIU/mL), and D-CuZ (8.96 µIU/mL) on comparison with the normal control (21.50 µIU/mL).

The plasma insulin levels for control groups (NC and DC groups) did not show any significant difference on day 15, 30, and/or 45. The plasma insulin levels for control groups showed a significant gradual increase \( (p<0.05) \) in the mean plasma insulin levels which increased after 45 d to reach 11.60 and 12.69 µIU/mL for D-Cu and D-Zn, respectively. While in case the treated group D-CuZ approaches nearly the normal range (10.88, 0.78, and 16.68% for the rats administrated curcumin, Zn, and curcumin–Zn complex, respectively.

During six weeks trial, an overall significant decrease in plasma glucose level in diabetic groups was observed to be 10.88, 0.78, and 16.68% for the rats administrated curcumin, Zn, and curcumin–Zn complex, respectively.

**Plasma Insulin Level** In order to gain insights into the correlation between plasma glucose and the insulin secretory capacity of the pancreatic islets, we monitored fasting plasma insulin levels for the diabetic and control groups through the whole experiment via a two-week determination. Table 3 and Fig. 4 revealed that after STZ injection, a significant decrease \( (p<0.05) \) was observed in plasma insulin levels in diabetic rats, DC (9.15 µIU/mL), D-Cu (8.71 µIU/mL), D-Zn (8.84 µIU/mL), and D-CuZ (8.96 µIU/mL) on comparison with the normal control (21.50 µIU/mL).

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**Glycosylated Hemoglobin (HbA1c)** There was a significant elevation in HbA1c % \( (p<0.05) \) for the diabetic control (13.23%) regarding the NC group (5.35%) on day 45. All over the experimental period, there was no significant change in the control groups NC and DC on days 15, 30, and 45.

The initial % of HbA1c for the treated groups D-Cu, D-Zn, and D-CuZ recorded 12.97, 13.24, and 13.48%, respectively. The three treated groups showed significant dropping
Values differ significantly from 15th d (p < 0.05). ** Values differ significantly from 30th d (p < 0.05).

Experimental Groups

** Values differ significantly from 45th d (p < 0.05). *** Values differ significantly from G3 (D-Cu) (p < 0.05).

Values differ significantly from G4 (D-Zn) (p < 0.05).

Liver and Kidney Functions Table 6 revealed the effects of curcumin, Zn, and curcumin–Zn complex administration on the liver functions (liver enzymes: aspartyl aminotransferase “AST,” alanine aminotransferase “ALT” and albumin) of control and diabetic groups. We found that, the oral administration of curcumin, Zn, and curcumin–Zn complex induced a significant decrease in high-density lipoprotein cholesterol (HDL-C) for the non-treated diabetic control rats compared to normal control.

Lipid Profile Our results accounted for a significant increase (p < 0.05) in serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C); and a significant decrease in high-density lipoprotein cholesterol (HDL-C) for the non-treated diabetic control rats compared to normal control.

Protection with curcumin, Zn and curcumin–Zn complex administration for 45d resulted in significant reduction in serum TC, TG, and LDL-C levels and conversely, serum HDL-C was significantly increased in comparison with the diabetic control (Table 5).

** Values differ significantly from normal control (p < 0.05).
a markedly significant reduction ($p<0.05$) of the serum activities of the enzymes S.ALT (38.20, 32.10, and 31.10 U/L), S.AST (85.70, 80.20, and 75.20 U/L); when they are compared to the diabetic control (DC) enzymes S.GPT (42.10 U/L), S.GOT (102.80 U/L) respectively. On the other hand, serum albumin results showed significant changes ($p<0.05$) in diabetic groups D-Zn and D-CuZ (4.57, 4.91 mg/dL), respectively, regarding the diabetic control group “DC” (3.75 mg/dL).

Moreover, table 6 demonstrated the change in blood urea and serum creatinine for the normal and diabetic rats. The diabetic control was found to show a markedly significant reduction ($p<0.05$) in comparison with normal control 32.80 and 1.05 mg/dL compared to the negative control 32.80 and 0.62 mg/dL respectively. The increase in the levels of urea and creatinine in diabetic rats were restored to near normal in curcumin, Zn, as well as curcumin–Zn complex treated diabetic rats.

**DISCUSSION**

Diabetes is considered as a pivotal global that not yet have effective management. Several drugs including hypoglycemic agents and insulin were established to control the blood glucose level only when they are regularly administrated. Jin et al. approved that, such founded treatments are tedious and tend to pose several clinical challenges. Medicinal plants played an excellent role in replacing traditional drugs; they are offering a similar degree of efficacy without so many troublesome side effects.

Curcumin was explored by many researchers as a hypoglycemic agent. As curcumin quenches singlet oxygen effectively, leading to adduct formation and can be used as a powerful antioxidant agent. Thereafter, it can be used as a cytoprotective agent for pancreatic islet cells via inhibition of islet apoptosis as it inhibited oxidative stress, more the less, several studies has been investigated the role of curcumin in experimental T2DM. Zn supplementation was known by its essential and beneficial role in diabetes. Especially, the Zn levels in pancreatic islets are amongst the highest concentration in the body (about 20 µg/g).

Insulin biosynthesis and maturation depend on one side on its complexation with Zn forming a hexamer. Such feature means that Zn is essential for both prolongs the duration of action and facilitate the storage of insulin.

Unfortunately, the insolubility of curcumin in water and its very poor solubility in organic solvents render its usage as a promising anti-hyperglycemic agent. In the present study, we would like to improve the bioavailability of orally administrated curcumin via its complexation with Zn and evaluate the antidiabetic effect of the novel curcumin–Zn complex in comparison with curcumin and Zn.

Our work showed that induction of diabetes using STZ led to a significant increase in plasma glucose and glycosylated hemoglobin and a significant decrease in plasma insulin levels regarding the basal levels. Our results of curcumin treated group are in agreements with previous which emphasized the effect of curcumin administration on lowering blood glucose levels in STZ-induced diabetic rats. Also, Zn was proven to facilitate normoglycemia by mediating the translocation of glucose transporter type 4 (GLUT4) in between cytosol and plasma membrane resulted in the increased glucose uptake by tissues.

Moreover, serum insulin level exhibited a marked elevation in diabetic rats administrated curcumin–Zn complex (D-CuZ) regarding weak elevation on other treated groups (G3 & G4). Vijayaraghava et al. reported that Zn metallo-complex exhibited better results in controlling blood glucose in experimental diabetic rats when administrated for 30d. As our results shown in Tables 2, and 3 at 45th d, the curcumin–Zn complex almost improved plasma insulin levels while it showed a relatively small improvement on plasma glucose levels. The reason for this discrepancy is not clear at present.

As well as Vijayaraghava et al. accounted for the interesting effect of Zn metallo-complexes in insulin level maintenance. On the other hand, Best et al. showed that curcumin induced electrical activity in rat pancreatic cells via activating the volume-regulated anion channel, with depolarization of the cell membrane potential, generating electrical activity, and improved insulin release.

It is well known that in diabetes mellitus the protein glycation is a common feature. It causes hemoglobin levels drastically reduced due to the irreversible glycation, which in turn leading to glycosylated hemoglobin formation. The glycosylated HbA1c levels are the markers to assess the glycemic status for a period 2–3 months and prognosis of the disease. HbA1c is obviously increased in diabetic patients and the degree of increase is directly proportional to the fasting blood glucose levels, STZ induced diabetic rats showed an increase in HbA1c levels. The curcumin–Zn treatment to diabetic rats reduced the HbA1c levels to a larger extent than administration of curcumin or Zn. Such findings can be accounted for the ameliorative potential of the complex during hyperglycemia as well as the antioxidant properties of such complex, which in agreement with findings of Rochette et al. for the effect of antioxidant on controlling HbA1c levels.

Hyperlipidemia is a recognized complication of DM char-

### Table 6. Effect of Daily Oral Administration of Curcumin, Zn, and Curcumin–Zn Complex in Serum Liver and Kidney Functions for Normal and Diabetic Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>S.ALT (U/L)</th>
<th>S.AST (U/L)</th>
<th>Albumin (g/dL)</th>
<th>Urea (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (NC)</td>
<td>25.30±2.21$^{(b)}$</td>
<td>61.50±4.03$^{(b)}$</td>
<td>3.77±0.21</td>
<td>32.80±3.94$^{(b)}$</td>
<td>0.62±0.07$^{(b)}$</td>
</tr>
<tr>
<td>G2 (DC)</td>
<td>42.10±2.77$^{(a)}$</td>
<td>102.80±6.75$^{(a)}$</td>
<td>3.75±0.18</td>
<td>59.70±2.75$^{(a)}$</td>
<td>1.05±0.08$^{(a)}$</td>
</tr>
<tr>
<td>G3 (D-Cu)</td>
<td>38.20±2.09$^{(a)}$</td>
<td>85.70±5.03$^{(a)}$</td>
<td>3.49±0.45</td>
<td>44.80±3.91$^{(a)}$</td>
<td>0.77±0.05$^{(a)}$</td>
</tr>
<tr>
<td>G4 (D-Zn)</td>
<td>32.10±2.28$^{(a)}$</td>
<td>80.20±5.69$^{(a)}$</td>
<td>4.57±0.33$^{(a)}$</td>
<td>41.60±3.89$^{(a)}$</td>
<td>0.76±0.06$^{(a)}$</td>
</tr>
<tr>
<td>G5 (D-CuZ)</td>
<td>31.10±2.60$^{(a)}$</td>
<td>75.20±5.43$^{(a)}$</td>
<td>4.91±0.26$^{(a)}$</td>
<td>40.10±4.75$^{(a)}$</td>
<td>0.65±0.11$^{(a)}$</td>
</tr>
</tbody>
</table>

Results are presented as mean±S.D. a) Values differ significantly from normal control ($p<0.05$). b) Values differ significantly from diabetic control ($p<0.05$).
acterized by elevated levels of cholesterol, triglycerides and phospholipids and changes in lipoprotein composition. One of the major pathogenesis of lipid metabolism disturbances in diabetes is the increased mobilization of free fatty acids from adipose tissue and secondary elevation of free fatty acid levels in the blood due to insulin deficiency or insulin resistance. The excessive lipolysis in diabetic adipose tissue may lead to increased free fatty acids in circulation which enter the liver and are esterified to form triglycerides. The fatty acid compositions of various tissues are altered in both experimental and human diabetes, \(^{31}\) as well as the finding of the present study is in correlation with the findings of Pepato et al. and Sharma et al. \(^{42, 43}\) Moreover, the targeted group (curcumin–Zn complex group) showed unique findings for reducing the LDL levels, which were in agreements with the explored role of curcumin in decreasing the LDL level. \(^{40}\) The LDL level for G5 administered curcumin–Zn complex showed a reduction to a larger extent (15.66 mg/dL) regarding the control group (26.60 mg/dL). On the other hand, the administration of curcumin alone decreases the LDL level (30.70 mg/dL) to a shorter extent than complex owing to the evidence for the excellent improvements of curcumin bioavailability by Zn complexation.

The observed increase in activities of the liver enzymes AST and ALT investigated in the serum of diabetic rats may be due to the deficiency of these enzymes from the liver cytosol into the bloodstream as a consequence of the hepatic tissue damage. \(^{45}\) The reversal of AST and ALT activities in curcumin–Zn complex treated diabetic rats towards near normal indicate the hepatoprotective nature. Interestingly, some of the beneficial effects of curcumin in diabetes owing to its ability to reduce blood glucose and triglyceride concentrations in diabetic rats, decreasing oxidative stress and consequence improvement of liver enzymes.

Diabetes is generally accompanied with many complications such as renal dysfunctions known as diabetic proteinosis. It was reported that the accelerated proteolysis of uncontrolled diabetes is due to the deranged glucagon-mediated regulation of cAMP formation. \(^{46}\) These are reflected by a considerable increase in the serum creatinine levels. The amount of serum creatinine is used to assess the renal function and to detect treatment-related toxic effects of compounds on the renal function of experimental rats. The significant decrease in the levels of serum creatinine in the curcumin–Zn group to a larger extent than other treated diabetic groups emphasized the proper regulation of carbohydrate and protein metabolism for the group administrated curcumin–Zn complex. Furthermore, the normalized serum creatinine levels indicate the normal kidney function as well as the renoprotective effect of such complex.

Blood urea levels are elevated in the diabetic rats due to increased protein catabolism and renal dysfunction. The damage to renal cells is mainly due to glucose-mediated osmotic diuresis, reactive oxygen species, and glucose overload. In the present work, the observed elevated urea levels in diabetic rats were restored to the near normal levels by the treatment of curcumin–Zn complex as a consequence of improved glycemic control.

**CONCLUSION**

Successful attempts at the design, synthesis, and character-
ization of a new curcumin–Zn complex as a novel hyperglycemic agent have been achieved. Curcumin–Zn complex has been exerted a dual action in diabetes, where the beneficial effect of curcumin in glycemic status was explored besides the action of Zn metal in insulin maintenance. Moreover, in such complex an effective soluble curcumin has been attained.

**Conflict of Interest**
The authors declare no conflict of interest.

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