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Biocompatible zinc oxide nanocrystals stabilized via hydroxyethyl cellulose for mitigation of diabetic complications

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ABSTRACT

The vascular complications of diabetes are the most serious manifestations of the disease. The hyperglycemia can directly promote an inflammatory state where the increase C-reactive (CRP) and cytokines, such as interleukins (IL-1 and IL-6), which contribute to the development of cardiovascular diseases. The current study was aimed to evaluate the role of environmentally-synthesized zinc oxide nanocrystals (ZnO-NPs) in augmentation of hyperglycemia and its complications, as well as the preservation of asymmetrical dimethylarginine (ADMA) level as a specific marker for endothelial dysfunction in streptozotocin (STZ)-induced diabetic rats. ZnO-NPs was chemically-synthesized using environmental benign biodegradable hydroxyl ethyl cellulose (HES) as both a stabilizing and directing agent in the presence of potassium hydroxide. HES is a biomaterial compound used in many biomedical applications due to its biodegradability and biocompatibility in nature. Particle size, morphological structure, purity, and crystallinity of the as-prepared ZnO-NPs were evaluated through different techniques, such as transmission electron microscopy (TEM), X-ray diffraction (XRD), and scanning electron microscopy connected to energy-dispersive X-ray spectra (SEM-EDS). Sixty male albino rats were used in this study and divided into four groups: control, ZnO-NPs, diabetic and treated groups; after the experimental period, CRP and interleukin-1 (IL-1α) were determined by ELISA. ADMA was estimated by RP-HPLC using a fluorescence detector. The results obtained indicate that CRP, IL-1α, and ADMA levels increased significantly concomitant with a reduction in NO level in the diabetic group, whereas ZnO-NPs supplementation significantly attenuated these parameters. Based on these encouraging results, the reported approach of environmental synthesis and application has the potential of leading to a new generation of nanometerials for treatment of diabetic complications with considerably enhanced selectively towards atherosclerosis.

1. Introduction

Hyperglycemia is the metabolic hallmark of both type 1 and type 2 diabetes. Type 1 progresses when the immune system destroys the pancreatic β-cells, categorically decreasing insulin production. Type 2 is defined by both insulin resistance and progressive pancreatic β-cell dysfunction. These shortages at last outcome in too little insulin to keep up blood glucose levels in the normal range. Both type 1 and type 2 diabetes particularly increment the risk of microvascular and macrovascular complications[1]. The chronic hyperglycemia can directly promote an inflammatory state, where the increase in cytokines such as interleukins (IL- α and IL-6) and tumor necrosis factoralpha (TNF-α) is involved in the advance and progress of cardiovascular diseases [2]. IL-6 is an imperative cytokine involved in numerous different immunological processes and takes part in metabolic regulation of C-reactive protein (CRP). Throughout inflammatory reaction, CRP can have adverse effects on a number of organs. Furthermore, it reduces the concentration of tissue plasminogen activator, responsible for lysing clots at the vessel wall, and increases plasminogen activator inhibitor concentrations, that inhibits the fibrinolysis process [3]. Asymmetrical dimethylarginine (ADMA) is considered as a key that companions with endothelial dysfunction; it is created by endothelial cells and presents in plasma in amounts that are suffiecient to disallow the production of nitric oxide (NO) production. Elevation of ADMA levels is almost concomitant with hypertension, hypercholesterolemia and cardiovascular diseases [4].

Unfortunately, all medical treatments, whether ancient or modern, are associated with a certain degree of risk for adverse effects, often called side effects. In general, the more drastic the intervention, the more likely and more severe the adverse outcomes. Food

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derived antioxidants have a strong potential for long term use as chemo-preventive agents in diabetes mellitus. several hundreds of plants have been examined for use in wide variety of diabetes including Caulerpa lentillifera [5] and flaxseed oil [6].

Apllication of nanotechnology in diabeties treatment has attract much attention over the last ten years. one of these nanomedicine is the zinc oxide nanocrystals (ZnO-NPs) which is considered as one of the multifunctional inorganic nanoparticles and has attracted much attention due to its distinctive combination of superior physical, chemical, biological, electrical, optical, long-term environmental stability, biocompatibility, low cost and non-toxic properties [7]. All these superior properties encourage the use of this compound in medicine and other applicable domains [7]. It is well stated that, zinc has been described to play an important role in glucose homeostasis by enhancing hepatic glycogenesis through its action on the insulin signaling pathway and thus inhibits intestinal glucose absorption and increases glucose uptake in skeletal muscle and adipose tissue [8]. Moreover, zinc has been stated to inhibit glucagon secretion [9], consequently reducing gluconeogenesis and glycogenolysis, augmenting the structural integrity of insulin [10]. The focal function of zinc in diabetes mellitus was revealed by supplementation studies in diabetic rats [11].

Herein, the aim of this research was planned to get simple and facile route for the preparation of zinc oxide nanocrystals (ZnO-NPs) via wet chemical process using natural biodegradable and biocompatible polymer; nominated hydroxyethyl cellulose (HES) to act as both stabilizing agent to avoid the agglomeration of ZnO-NPs and in the same time as structure directing agents. The final product of ZnO-NPs was fully characterized using several techniques such as Transmission electron microscopy (TEM), X-Ray diffraction (XRD) and scannine electron microscopy connected to Energy dispersive x-ray spectra (SEM-EDS) to call attention to the nature of the formed ZnO-NPs. In addition, The research work was extended to evaluate the positive role of ZnO-NPs on ameliorating hyperglycemia and its complications as well as preservation of ADMA level as a specific marker for endothelial dysfunction in STZ-induced diabetic rats.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

Zinc nitrate hexahydrate [Zn(NO3)2· 6H2O] was purchased from Sigma Co. (USA). Hydroxyethyl cellulose was obtained commercially from Aldrich Co (USA). Asymmetric dimethylarginine (HPLC standard), streptozotocin (STZ), 5-sulfosalicylic acid (5-SSA), β mercaptoethanol and o-phthalaldehyde (OPA)were purchased from Sigma Chemicals Co. (St Louis, Missouri, USA). Tetrahydrofuran (THF),methanol, ethanol and all used chemicals were HPLC grade and purchased from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leics, UK. Ultrapure water was used to prepare all solutions.

2.1.2. Animals

Sixty male albino rats (Sprague–Dawley strain) were supplied by the animal house of the National Research Centre, Giza, Egypt. The age of each rat was 6 months and the average mean weight was 180 ± 20 g. The animals were housed in individual suspended stainless steel cages at a temperature range of 25 ± 2oC, under a 12- h light/12-h dark cycle, and allowed to acclimatize for a period of 7 days to the experiment. The animals had free access to water and a standard rodent chow diet. The guidelines of the ethical care and treatment of the animals followed the regulations of the ethical committee of National Research Centre, Giza, Egypt.

2.2. Methods

2.2.1. Preparation of zinc oxide nanocrystals (ZnO-NPs)

A typical procedure for preparing ZnO-NPs is by wet chemical method. In a typical procedure, 0.4 g of HES was dissolved in 75 mL of deionized water at room temperature under mechanical stirring to get completely soluble HES. Then 0.9 g of zinc nitrate [$(Zn(NO_3)_2{\cdot}6H_2O$] dissolved in 15 mL of deionized water was added slowly to HES solution with contentious stirring at room temperature. After 30 min of addition, an aqueous solution of KOH (2 M) was added drop wise along the side walls of the solution vessel with the solution of zinc cations coordinated with HES solution. The reaction mixture was held under continuous stirring for 4 h. The as formed white colloidal solution of zinc hydroxide ($Zn(OH)_2$) was centrifuged at 6000 rpm for 1 h, washed several times with deionized water to remove the unreacted byproducts then dried at 80 °C in hot air oven.The obtained dried composite was calcinated for 1 h at 800 ◦C to get pure ZnO-NPs.

2.3. Characterization

2.3.1. Characterization of zinc oxide nanocrystals (ZnO-NPs)

The morphological feature of the synthesized ZnO-NPs were investigated by making use of field emission scanning electron microscopy (FESEM; Quanta FEG 250 with field emission technique. Energy dispersive X-ray spectr (EDS) was connected to FESEM to determine the existence element of the tested nanocomposite The particle size and size distribution of the resulted ZnO-NPs was examined using high-resolution transmission electron microscope connected energy dispersive X-ray (HRTEM-EDS; JEOL-JEM-1200). The samples for FESEM were prepared by surface coating with gold. On the other hand, the samples of TEM were prepared by dropping dilute suspension of ZnO-NPs onto copper coated grids. Structural characteristics and crystallinity of the obtained ZnO-NPs powder were further investigated by making use of X-ray diffraction (Philips PW3040) with CuKα with 2 θ ranging between 5° and 80°.

2.3.2. Induction of diabetes

Streptozotocin (STZ) was dissolved in 50 mM sodium citrate solution (pH 4.5) containing 150 mM sodium chloride. The solution (6 mg/100 g body weight was subcutaneously administered in rats). Fasting blood sugar was estimated after 3 days to confirm the development of diabetes mellitus [12,13].

2.4. Experimental design

Sixty male albino rats (Sprague–Dawley strain) were divided into 4 groups; each group contains 15 rats. All groups were categorized as follows: Group I (control group) stated as normal control rats and received a vehicle; while Group II (ZnO-NPs) is the healthy rats that received ZnO-NPs (10 mg/kg body weight/day orally). On the other hand, Group III (diabetic group) defined as diabetic control rats and received a vehicle. Group IV (treated group) is the diabetic rats received ZnO nanocrystals (10 mg/kg body weight/day orally for 30 days.

2.4.1. Samples collection

After the experimental period, animals were kept fasting for 12 h, then anesthetized under light ether anesthesia, and the blood was withdrawn from the retro-orbital venous plexus of the eye using heparinized capillary tubes. Blood samples were collected in two tubes; the first one contains sodium fluoride for determination of fasting blood sugar and the second tube was dry clean tube without anti-coagulant for serum separation. All tubes were centrifuged at 3000 rpm using cooling centrifuge (Laborzentrifugen,

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