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Solvent-free and one pot synthesis of silver and zinc nanoparticles: Activity toward cell membrane component and insulin signaling pathway in experimental diabetes

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ABSTRACT

Objective: To investigate and compare between the effect of both silver nanoparticles (AgNPs) and zinc oxide nanoparticles (ZnONPs) on insulin signaling pathway and insulin sensitivity in experimental diabetes. Preparation of AgNPs and ZnONPs in their solid state were carried out using pullulan (Natural polymer) as both reducing and stabilizing agent. The synthesis of thesenanoparticles in a large scale were carried out without using any solvents. The experimental male albino rats received diluted solutions of AgNPs and ZNONPs. After the experimental period, blood was withdrawn; erythrocyte membrane lipids were extracted and fatty acids were determined by HPLC. Oxidant, antioxidant profile and phosphatidylinositol 3-kinase (PI_3K) were estimated.
Results: It was observed that the as synthesized AgNPs and ZnONPs have nearly spherical shape with small size due to the stabilization effect of pullulan as proved by UV-vis spectroscopy (UV-vis), Transmission electron microscy (TEM) and Field emission scanning electron microscopy (FESEM), Zeta potential, Dynamic light scattering (DLS) and X-ray diffraction (XRD) techniques. The average hydrodynamic size of the formed AgNPs was 15 nm which is considered as very small size when compared with that of ZnONPs (above 50 nm). Fasting blood sugar was significantly increased in diabetic group along with elevation of MDA and DNA damage indicating the oxidative properties of streptozotocin. Whereas, the treatment with nanoparticles significantly attenuated these elevations.
Conclusion: AgNPs and ZnONPs represent promising materials in attenuating diabetic complications and insulin resistance in experimental diabetes; no Impressive differences were observed between the effect of ZnONPs and AgNPs in this current research.

1. Introduction

Diabetes mellitus, is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced [1], it is characterized by chronic hyperglycemia with disturbances in carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, action or both [2,3].

Insulin resistance is likely to be associated with defects in transmembrane glucose transport [4,5] which may be linked to decreased translocation and internalization of glucose transporter- 4 (GLUT-4) to the sarcolemma [6] and to defects in insulin receptor binding and signaling [7].

The fatty acid (FA) profile of cell membrane phospholipids is associated with insulin sensitivity in rodents and in humans [8]. Several studies demonstrated a positive correlation between a high content of polyunsaturated fatty acids (PUFA) and insulin sensitivity. It has been demonstrated *in vitro* and *in vivo* studies that changes in the phospholipid composition of membranes are associated with changes in the number of insulin receptors per cell, the affinity of insulin to the insulin receptor, and membrane glucose transport [9].

Protein kinase C (PKC), a key enzyme in insulin action, is a family of protein kinase enzymes that are involved in controlling the function of other proteins through the phosphorylation of hydroxyl groups of serine and threonine amino acid residues on these proteins. PKC enzymes play important roles in several signal transduction cascades [10].

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Upon activation, protein kinase C (PKC) isozymes are translocated to the plasma membrane, where they begin to activate and phosphorylate a wide variety of protein targets. PKC binding to membranes is not only regulated by lipid structures but also by specific membrane lipids [11].

The use of nanoparticles [12], nanofibers [13–17]and polymer nanoparticles [18] in smart textile [19–21] and medicine [13–17] are an attractive proposition due to their targeted action; increase the efficacy of the drugs. Indeed, their small size gives them an edge as they can elope immune responses and also gives them the ability to cross relatively impenetrable membranes [22]. The metals nanoparticles, as zinc, silver, iron and gold, oxides of nanoparticles, have important roles in medical and biological applications [23,24]. Silver is safe and effective bactericidal metal because it is non-toxic to animal cells whereas it is highly toxic to bacteria [24–27]. AgNPs are considered one from the most commonly used nanomaterials and they are known to have antioxidant and antimicrobial properties [24–27].

On the other hand, Zinc, the essential metal, is an activator for more than three hundred enzymes in the body [28]. It plays a key role in different metabolic pathways including glucose metabolism, also, it promotes hepatic glycogenesis through insulin pathways and thus improves glucose utilization. In addition, it could improve insulin signaling pathway by different mechanisms, including increased insulin receptor phosphorylation, enhancing phosphatidylinositol 3-kinase (PI_3K) activity and inhibition of glycogen synthase kinase-3 [29]. The beneficial role of zinc in diabetes has been implicated by studies of the zinc supplies in diabetic rats [12,30,31].

There are several methods used for the preparation of these nanoparticles mentioned in literature such as chemical reduction, electrochemical reduction, sonication, radiation, biological and sol gel process [17,25,32–34]. However, the solid state synthesis is considered as one of the most efficient methods due to many reasons such as purity due to washing after preparation, scaled up, easy transportation, cost- effective, used less chemicals and solvent free [35–41].

AgNPs and ZnONPs are essential materials in a huge number of metabolic processes. However, there is no data about the effective power of AgNPs on the glucose status. Such suggestion encourages us to study the effect of AgNPs in comparison with ZnONPs. So, from this point of view, we aimed to (1) prepare these nanoparticles (AgNPs and ZnONPs) in their solid form using pullulan as both reductant and stabilizing agent in presence of KOH as activating agent. (2) investigate and compare between the effect of both AgNPs and ZnONPs on insulin signaling pathway and glucose transporting in order to improve insulin sensitivity in experimental model of diabetes.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

Pullulan was purchased from Sigma-Aldrich Co (USA). Silver nitrate ($AgNO_3$) and Zinc nitrate ($Zn(NO_3)_2.6H_2O$) ≥99% were purchased from Sigma Aldrich Co (Germany). Potassium hydroxide (KOH) was purchased by the company of HmbG Chemicals (India). Fatty acids standards (HPLC grade) and streptozotosin (STZ) were purchased from Sigma Chemicals Co. (Munih, Germany). Acetonitrile, methanol, ethanol, N-hexane, 2-propanol and all other laboratory chemicals in this work were HPLC grade.

2.1.2. Experimental animals

Forty male albino rats weighting 180 ± 10 g were obtained from the animal house of National Research Centre (NRC), Giza, Egypt. The animals were housed in stainless steel cages at the temperature range of 22 + 2 °C, under 12-h light/12-h dark cycle, and allowed to acclimatize for a period of 10 days to the experiment; the guidelines of the ethical care and treatment of the animals were followed the regulations of the ethical committee of NRC (ethical number16303).

2.2. Methods

2.2.1. Solid state synthesis of silver nanoparticles (AgNPs)

A definite quantity of pullulan (0.75 g) and potassium hydroxide (KOH) (0.15 g) were mechanically mixed together and milled for 5 min at ambient temperature. After the milling, 0.22 g of silver nitrate ($AgNO_3$) was added and the mechanical grinding was continued in air at ambient temperature for another 20 min. Afterward the fine homogeneous was obtained, the solid mixtures was obtained as a deep yellow was collected. To remove the unreacted reactants, the as synthesized AgNPs powder was suspended in 5 ml of deionized water in a centrifugation tube, followed by the centrifugation at 6000 rpm for 30 min. After decanting the centrifuge solution, the residue was freeze dried using freeze dryer instrument to obtain the dry product of AgNPs. The purified freeze dried AgNPs was kept for further characterization and application.

2.2.2. Preparation of pullulan stabilized zinc oxide nanoparticles (ZnONPs) in its solid state

In a typical synthetic experiment, 0.75 g of pullulan was grinded with 0.15 g of potassium hydroxide (KOH) for 5 min. After complete grinding, the solid mixture was mixed and grinded with zinc nitrate hexahydrate $Zn(NO_3)_2{\cdot}6H_2O$ (0.22 g) for 15 min. The obtained white product was washed several times with ethanol and deionized water, respectively, to remove any unreacted compound or any of the formed by-products. Finally, the dispersed product in deionized water was centrifuged at 6000 rpm for 30 min to discard the supernatant, the resultant solid powder was dried in air at 80 °C for 60 min and calcined at 800 °c for 120 min to convert the formed zinc hydroxide to ZnONPs. To improve the purification of the resultant calcined powder, it was washed with ethanol and deionized water several times and dried at 50 °C overnight. Then, the purified powder of ZnONPs was kept also for further characterization and application.

2.2.3. Induction of diabetes mellitus

Streptozotocin (STZ) was dissolved in 50 mM sodium citrate (pH 4.5) solution containing 150 mM of NaCl. The solution (6.0 mg/0.5 ml/100 g body weight) was subcutaneously administrated in rats; fasting blood sugar was estimated after 3 days to confirm the development of diabetes mellitus [42].

2.2.4. Experimental design

After the acclimatization period, animals were divided into four groups, 10 rats in each group as follow: Group I (control group): healthy rats received a vehicle. Group II (diabetic group): diabetic rats received a vehicle. Group III (treated group I): diabetic rats received ZnONPs (10 mg/kg bw/day orally). Group IV (treated group II): diabetic rats received AgNPs (10 mg/kg bw/day orally). After the experimental period (30 days), animals were kept fasting for 12 h before blood sampling; blood was withdrawn from the retro-orbital venous plexus of the eye using capillary tubes and collected in (1) Tubes contain sodium fluoride for blood glucose estimation and (2) heparinized tubes for other biochemical parameters.

One part of the heparinized blood was centrifuged at 2000 rpm for 10 min using cooling centrifuge (Laborzentrifugen, 2K15, Sigma, Germany). Plasma was separated and immediately frozen. Packed RBCs were used for several biochemical parameters.

2.2.5. Characterization of AgNPs, ZnONPs and the treated rats with these nanoparticles

2.2.5.1. Physical characterization of AgNPs and ZnONPs. For UV–vis spectroscopy, the resultant nanopowder from each of the reactions was re-suspended in equal amount of deionized water and spectrum scans were performed using UV–vis Spectrophotometer (T80 UV/vis