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Inhibition Of Aldolase Reductase And Protein Glycation End Product By Avocado Seed Extract Bio-Assisted Zinc Oxide Nanoparticles

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Abstract

Background

Zinc oxide nanoparticles (ZnO NPs) possess a broad range of pharmacological properties, including anticancer, anti-diabetic, and antioxidant effects. Green synthesis of ZnO NPs offers enhanced bioactivity and reduced toxicity, positioning them as promising candidates for therapeutic applications. Specifically, ZnO NPs have shown potential in inhibiting key enzymes such as aldose reductase (AR) and advanced glycation end products (AGEs), which are implicated in diabetes and other health conditions.

Methods

In this study, ZnO nanoparticles were synthesized via a green chemistry approach using avocado seed extract. The inhibitory effects of the green-synthesized ZnO NPs on aldose reductase (AR) and advanced glycation end products (AGEs) were assessed using enzyme inhibition assays. The bioactivity of the nanoparticles at varying concentrations was further compared to standard drugs, quercetin and aminoguanidine to assess their potential advantages in enzyme inhibition and overall therapeutic efficacy.

Results

The green-synthesized ZnO NPs exhibited superior enzyme inhibition activity compared to conventionally synthesized ZnO NPs. At low concentrations, the green-synthesized nanoparticles showed significant inhibition of both AR and AGEs, suggesting their potential in preventing diabetic complications. Furthermore, the eco-friendly synthesis process minimized toxicity while enhancing the bioactivity of ZnO NPs, supporting their application in therapeutic interventions.

Conclusion

The green-synthesized ZnO nanoparticles demonstrated potent inhibition of AR and AGEs, suggesting their potential as a safe and effective therapeutic agent. Their eco-friendly production method further enhances their viability for use in multi-disease pathways, particularly in diabetes management.

Keywords: *Glycation, Aldolase reductase, nanoparticles, avocado seed, zinc oxide*

INTRODUCTION

Despite significant progress in managing diabetes-related hyperglycemia through methods such as diet, hypoglycemic medications, insulin therapy, insulin pumps, and islet transplantation, the long-term complications of diabetes continue to be major contributors to mortality [1].These complications arise from changes in proteins that lead to permanent tissue damage. A key effect of hyperglycemia is the overproduction of nonenzymatic protein glycation [2]. Glycation is the non-enzymatic reaction between amino and carbonyl groups of reducing sugars, leading to protein modifications. Accumulated advanced glycation end-products (AGEs) contribute to diabetic complications through receptor-dependent and direct pathways. Microvascular complications include neuropathy, nephropathy, retinopathy, and cataracts, while macrovascular ones involve atherosclerosis, coronary heart disease, and cerebrovascular disease. Although hyperglycemia drives diabetic complications, AGE formation also increases with normal aging [3-5].

Hyperglycemia increases glucose influx into cells, where aldose reductase (AR) catalyzes the conversion of excess glucose into sorbitol using nicotinamide adenine dinucleotide phosphate (NADPH) as a coenzyme. This reaction is a critical part of the polyol pathway [6,7]. The buildup of sorbitol leads to osmotic stress, the formation of advanced glycation end (AGE) products, and depletion of NADPH, which worsens oxidative stress and impairs cellular function. Furthermore, the metabolism of fructose by aldose reductase results in the production of diacylglycerol and the activation of protein kinase C (PKC), which drives low-grade inflammation, increases vascular permeability, and stimulates angiogenesis [8,9]. Inhibiting AGEsand AR offers an alternative approach to treating diabetes that doesn't rely on controlling blood glucose levels. This method could be beneficial in preventing or reducing some diabetic complications [10, 11]. Traditionally, plant extracts have played a role in diabetes management. Various studies have demonstrated the ability of crude plant extracts or their bioactive components to effectively reduce blood glucose levels [12]. However, synthesis of nanoparticles from active phytoconstituents has gained huge interest among researchers as novel emerging applications in the field of pharmaceuticals [13]. Eco-friendly nanoparticle synthesis has become popular among researchers for its cost-effectiveness, ambient condition processing, non-toxicity, and environmental compatibility. The resulting nanoparticles are water-soluble, biocompatible, and free of toxic stabilizers, enabling easy application [14]. The seed extract, pulp and leaves of *Persea americana* (avacado) are abundant in phytochemicals, antioxidants vitamins and micronutrients [15].

Studies have recognized *Persea americana* as an effective agent for lowering blood sugar levels [15, 16].In the present study, the seed extract of avacado has been used as a reducing/capping agent for the synthesis of zinc oxide nanoparticles. Further, the green synthesized avocado seed extract bio-assisted zinc oxide nanoparticles (As-ZnO NP) was evaluated for its effect against AGE products and AR enzyme for the management of diabetes complications.

MATERIALS AND METHODS

Preparation of Avocado seed extract

Avocado seed extract was rinsed with deionized water to remove impurities and were dried at 50ºC to eliminate the moisture content. The dried seeds were ground into a fine powder using a blender. Subsequently, 5 grams of this avocado powder were combined with 500 milliliters of deionized water to create a brown extract solution. This mixture was then heated at 60 °C for 30 minutes while being stirred.

Preparation of avocado seed extract mediated zinc oxide nanoparticles

ZnO nanoparticles (ZnO NPs) were synthesized using avocado seed extract. Initially, 1M zinc acetate was dissolved in 50 ml of Milli-Q water and stirred continuously on a magnetic stirrer for 1 hour. Subsequently, 20 ml of sodium hydroxide solution was added dropwise to the zinc acetate solution. Following this, 25 ml of the prepared avocado seed extract solution was introduced into the mixture. The resulting solution was stirred for 2 hours at 35–40 °C and maintained at a pH of 12. The appearance of a yellow color after incubation indicated the formation of ZnO NPs. The nanoparticles were then separated from the reaction mixture by centrifugation at 8000 rpm and 60 °C for 15 minutes. The resulting pellet was collected and dried.

Advanced Glycation end product (AGE) assay

Advanced glycation end products (AGEs) are formed by non-enzymatic glycosylation of proteins that enhance vascular permeability in both micro and macro vascular structures by binding to specific macrophage receptors. AGE reaction mixture was constituted as follows; 1 mg/mL bovine serum albumin in 50mM sodium phosphate buffer (pH 7.4) and 0.02% sodium benzoate into 0.2M fructose and 0.2M glucose. The reaction mixture (2.75mL) was treated with different volumes As-ZnO nanoparticles (5, 10, 20, 40, 80 & 160µg/ml).

Determination of Aldose Reductase Inhibition

A total of 531µL of 0.1 M potassium buffer (pH 7.0), 90µL of NADPH solution (1.6 mM in potassium buffer), 90µL of recombinant human aldolase reductase (AR) (6.5U/mg) (Sigma, USA - SRP6371-100UG), 90µL of ammonium sulphate solution (4 M in potassium buffer), and 90 µL of DL-glyceraldehyde (25 mM in potassium buffer) were mixed with 9µL of different concentrations of As-ZnO nanoparticles (5, 10, 20, 40, 80 & 160µg/ml) in a cuvette, and the activity of AR was assessed spectrophotometrically by measuring the decrease in NADPH absorbance at 340 nm for 3 min using a spectrophotometer (Biotic Synergy H4 multimode reader, USA). Quercetin was used as positive controls. The inhibition of AR (%) was calculated using the following equation: (1 − (△A sample/min) – (△A blank/min)/(△A control/min) − (△A blank/min)) × 100%, where △A sample/min is the decrease in absorbance over 3 min with reaction solution, test sample, and substrate, and △A control/min without the test sample

RESULTS

Effect of the synthesized As-ZnO nanoparticle on aldolase reductase enzyme

Elevated activity of aldose reductase (AR) has been associated with the buildup of intracellular sorbitol, which has been connected to various secondary complications related to diabetes. The results of the present study showed dose-dependent increase in the inhibition of aldolase reductase enzyme activity by As-ZnO nanoparticles compared with that of standard quercetin (Figure 1). The maximum inhibition was 78.14% for As-ZnO nanoparticle and 80.95% for quercetin respectively at the highest concentration of 160µg/ml.

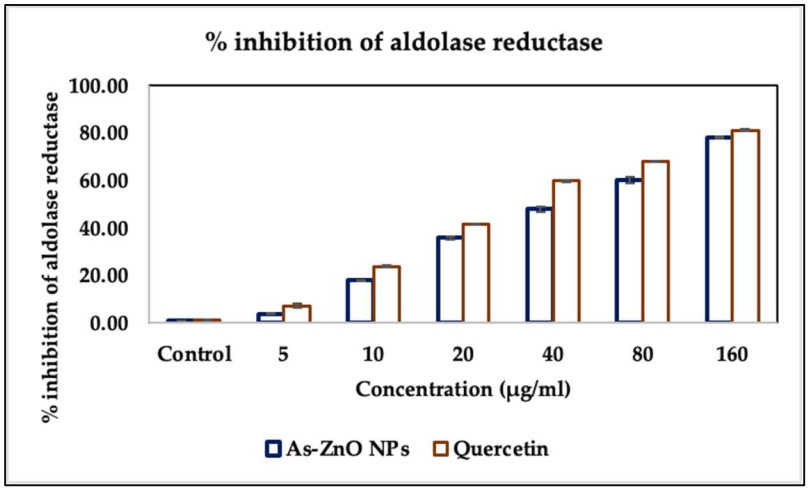

Figure 1: In vitro aldolase reductase inhibitory activity of avacado seed extract bio-assisted zinc oxide nanoparticles. Data are expressed as Mean±SEM (n=3).

Effect of the synthesized As-ZnO nanoparticle on advanced glycation end products formation

As shown in the Figure 2 the As-ZnO nanoparticles has markedly inhibited the formation of AGEs. The inhibitory effect observed was almost on par with the standard aminoguanidine. The pattern of AGEs inhibition by As-ZnO nanoparticles and standard aminoguanidine were similar indicating concentration-dependent effect. The minimum inhibition was 1.03% at 5µg/ml and maximum inhibition was found to be 76.37% at 160µg/ml for As-ZnO nanoparticles whereas aminoguanidine exhibited a minimum inhibition of 1.17% and a maximum of 83.61% at similar concentrations.

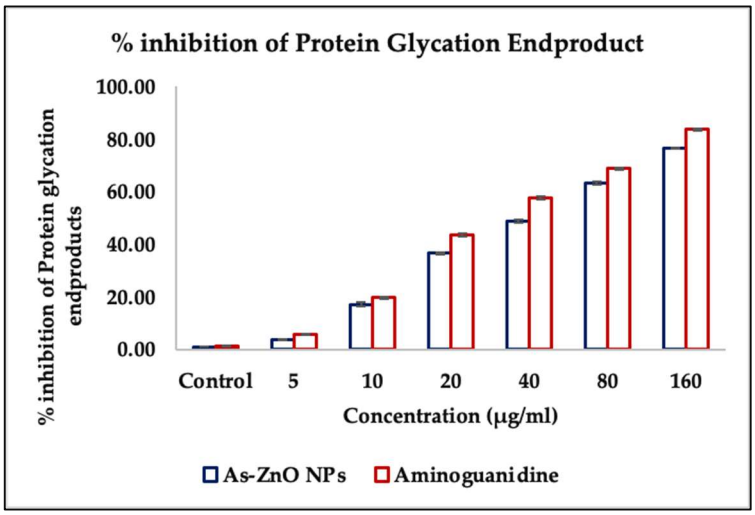

Figure 2: In vitro advanced glycation end product formation inhibitory effect of avacado seed extract bio-assisted zinc oxide nanoparticles. Data are expressed as Mean±SEM (n=3).

DISCUSSION

The current study demonstrated the potential of green-synthesized zinc oxide nanoparticles (ZnO NPs) mediated by avocado seed extract in inhibiting aldose reductase activity and the formation of advanced glycation end products (AGEs). While the exact inhibitory mechanism is not fully understood,

previous research has suggested that substances with strong antioxidant properties may bind to sugars or proteins, thus preventing the production of Amadori products, such as aldose reductase or sorbitol, and subsequently inhibiting AGE formation [1, 2, 17]. Avocado seed extract is known to contain bioactive compounds like alkaloids, terpenes, and flavonoids, which possess antioxidant properties [18]. Therefore, it is proposed that the inhibitory effects of the As-ZnO nanoparticles may be linked to these bioactive components in the avocado seed extract.

Free radicals promote the formation AGEs by triggering the autoxidation of sugars. The phenolic compounds, flavonoids and tannins exhibit considerable free radical scavenging activities and metal ion chelating properties [19]. Most phenolic compounds and flavonoids from the edible plants have been reported to possess antiglycation effect. It has also been reported that the inhibitory activity of flavonoids against protein glycation was strongly related to their scavenging effect on free radicals derived from the glycoxidation process [20]. In line with the given hypothesis, it can be assumed that phenolic compounds and flavonoids in the avocado seed may contribute to the antioxidant activity and antiglycation effect. Further, the As-ZnO nanoparticles has also exhibited potent inhibitory effect against AR activity.

Zinc oxide nanoparticles (ZnO NPs) possess a broad spectrum of pharmacological properties, including anticancer, anti-diabetic, and antioxidant effects [21, 22]. A significant advantage of using green-synthesized ZnO nanoparticles is their potent ability to inhibit key enzymes implicated in the development of various health conditions, even at minimal concentrations [23]. This eco-friendly approach not only enhances their bioactivity but also minimizes potential toxicity, making them a promising candidate for therapeutic interventions across multiple disease pathways. In line with previous findings, the present study demonstrated strong inhibition of aldose reductase (AR) and advanced glycation end products (AGEs) by the green-synthesized ZnO nanoparticles, further supporting their therapeutic potential.

CONCLUSION

In conclusion, avocado seed extract has potent inhibitory effects on protein glycation and inhibition of aldolase reductase. Furthermore, the results suggest that avocado seed extract acts as an antioxidant with suppressing effect on the formation of AGEs and AR.

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CONFLICT OF INTEREST

All the authors declare that there was no conflict of interest in the present study.

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