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Functionalization of gold nanoparticles as antidiabetic nanomaterial

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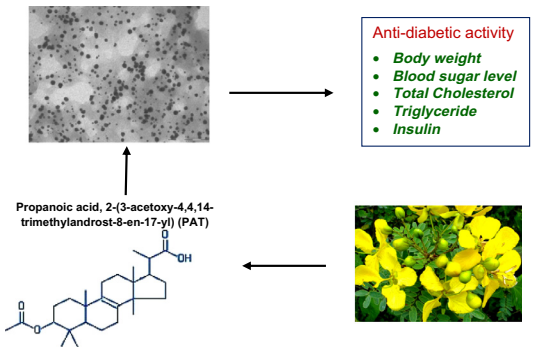
HIGHLIGHTS

- Pharmacologically active molecule for gold nanoparticles synthesis.
- Functionalization of gold nanoparticles achieved without doping molecules.
- Anti-diabetic potent gold nanoparticles.

GRAPHICAL ABSTRACT



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ABSTRACT

In the present investigation, functionalization of gold nanoparticles synthesized using propanoic acid 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl) (PAT) an active biocomponent isolated from *Cassia auriculata* is studied in detail. On reaction of PAT with aqueous $HAuCl_4$, rapid formation of stable gold nanoparticles was achieved. Formation of gold nanoparticles was confirmed by UV–vis spectroscopy, XRD, GC–MS, FTIR, TEM and SEM with EDAX. Gold nanoparticles mostly were monodisperse, spherical in shape and ranged in size 12–41 nm. Gold nanoparticles synthesised using PAT was administered to alloxan (150 mg/kg body weight) induced diabetic male albino rats at different doses (0.25, 0.5, 0.75 and 1.0 mg/kg body weight) for 28 days. Plasma glucose level, cholesterol and triglyceride were significantly ($p < 0.001$) reduced in experimental animals treated with gold nanoparticles at dosage of 0.5 mg/kg body weight and plasma insulin increased significantly. The newly genre green gold nanoparticles exhibit remarkable protein tyrosine phosphatase 1B inhibitory activity.

Introduction

Ethnopharmacological approach on the synthesis of nanoparticles is an exciting technology in creating symbiosis between nanoscience and medical science. In this context, the idea of functionalizing gold nanoparticles as antidiabetic nanomaterial by synthesizing with pharmacologically important plant material has been conceived. Large numbers of plants having antidiabetic nature have been subjected for the synthesis of gold nanoparticles. As a result, the flower of *Cassia auriculata* has been found to possess biosynthesizing property of gold nanoparticles.*Cassia auriculata* is a shrub with bright yellow flower and found throughout India [1]. Various parts of the plant are reported to have a number of therapeutic activities to manage diseases like leprosy, asthma, gout, rheumatism and diabetes [2]. It is also used as antipyretic, anticancer and skin infection treatments [3], hepatoprotective [4] and antihyperglycemic [5], hypolipidemic [6] and anti-microbial

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activity [7]. Indeed, it is expected that gold nanoparticles synthesized using the flower of the shrub*Cassia auriculata* might have medical properties. Accordingly, efforts are made to identify the newly generated gold nanoparticles medicinal value. As a result, their antidiabetic activity has been explored. As a number of people with diabetes multiplies worldwide, the disease takes an ever increasing proportion of national and international health care budgets. It is projected to become one of the world's main disablers and killers within the next 25 years. It is most common serious metabolic disease in the world. The number of people suffering from the disease worldwide is increasing at an alarming degree with a probable 552 million people likely to be diabetic by the year 2030 as against 366 million estimated in 2011 [8]. Ninety percent of these patients suffer from type 2 diabetes, which is characterized by a resistance to insulin. Nanoparticles can be engineered as nanoplatforms for effective and targeted delivery of drugs and imaging labels by overcoming the many biological, biophysical and biomedical barriers [9,10]. The fascinating potential applications of metal nanoparticles are determined by its chemical compositions, size, shapes and controlled dispersities [11]. Gold nanoparticles are considered to be more important for their unique and tunable surface Plasmon resonance (SPR). Indeed, the surface chemistry of nanoparticles can modify their interactions with external systems [12]. Herein we address an active principle of the flower of *Cassia auriculata* possessing biosynthesizing property of gold nanoparticles further the capping molecule found to functionalize newly genre gold nanoparticles as antidiabetic nanomaterial.

Materials and methods

Chemicals and plant material

The flowers of *Cassia auriculata* were collected from Thiruvalam area of Vellore district of Tamilnadu, India. The plant was identified by Dr. M. Jayakumar, a plant taxonomist, Thiruvalluvar University, Vellore. The voucher specimen of the plant was prepared and kept in the Department of Zoology, Thiruvalluvar University, Vellore, India. The flowers were shade dried at room temperature for 4–5 days and the dried flowers were crushed into coarse powder and stored in a sterile plastic container. Choloroauric acid ($HAuCl_4.6H_2O$) was obtained from Loba Chem. All other chemicals, solvents and reagents used for this study were of analytical grade.

Preparation of extract and isolation of the active compound

The dried coarse powder of *C. auriculata* was extracted with hydromethanol (water: methanol 1:1 ratio) and the extract was evaporated to reach the dry condition. Further hydromethonlic extract was fractionated with chloroform, acetone, hexane, ethyl acetate and *n*-butanol. Among the fractionated extracts *n*-butanol extract was found to be more potent in synthesizing gold nanoparticles rapidly. A portion of *n*-butonal extract was subjected to TLC separation using chloroform: methanol (100:0;80:20;60:40;50:50;20:80;0:100) as mobile phase. Fractions obtained from the usage of chloroform and methanol (60:40) was collected respectively for further studies. The fraction was subjected to silica gel column (35×2.5 cm) and fractions were collected using chloroform and methanol in the ratio of 60:40. The fractions collected were lyophilized and subsequently subjected to spectroscopic characterization *viz.,* Fourier Transform Infrared (FTIR) spectroscopy using FTIR spectrophotometer (Perkin Elmer Spectrum1) and Gas Chromatography-mass spectrometry (GC–MS) using JEOL GCMATE II GC–MS.

Synthesis of gold nanoparticles

Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl) (PAT) (0.6 mg) was dissolved in 45 mL of deionized water and mixed with 5 mL of 1 mM aqueous $HAuCl_4$ solution. The color of the reaction solution turned from light yellowish brown to ruby red color within 3 min of reaction.

Characterization

The bioreduction of $HAuCl_4$ in aqueous solution was monitored by periodic sampling of aliquots (0.2 mL) of the suspension, diluting the sample with 2 mL of deionized water and subsequently UV–vis spectra were recorded. UV–vis spectrum was recorded as a function of time of reaction on a spectrophotometer TECHCOMP 2300 with a resolution of 1 nm.

X-ray diffraction measurements of the bioreduced choloroauric acid solution drop coated onto glass substrates were taken on a X-ray difractometer (Bruker Model D8, Germany) operated at a voltage of 40 kV and a current of 30 mA with Cu Kα radiation.

For High Resolution Scanning Electron Microscopic (HRSEM) image and Energy Dispersive X-ray (EDAX) analysis samples were prepared on carbon coated copper grids, which was dried and measured on a FEI Quanta FEG 200 HRSEM equipped with EDAX.

The morphology of the nanoparticles was measured using High Resolution Transmission Electron Microscopy (HRTEM) on carbon coated copper grids. The films on the grids were allowed to dry prior to measurements on a transmission electron microscope (JEOL 3010) operated at an accelerating voltage of 120 keV.

For FTIR measurement, the solution of gold nanoparticles was centrifuged at 10,000 rpm for 15 min. The pellet was dried and mixed with KBr pellet and analyzed on a FT-IR instrument (Perkin Elmer Spectrum1).

Antidiabetic studies

Healthy male albino rats weighing 180–200 gms were used for the present study. Rats were housed in an air-conditioned room at 22 ± 1 °C with a photocycle of 12 h light and 12 h dark. Animals were fed with commercial rat diet (Hindustan lever, Mumbai, India) and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC).

Induction of diabetes

Diabetes was induced in rats by the administration of single intraperitonial dose of alloxan monohydrate (150 mg/kg) [13]. After 48 h of alloxan injection, the animals were screened for hyperglycemia condition. Induction of diabetes was confirmed with a glucose level of above 250 mg/dl and by such the methodology these animals alone were selected for this study [14].

Experimental design

Animals were divided into 6 groups, each group containing six rats. Group I served as normal untreated control rats; Group II served as diabetic control rats; Group III served as positive control and fed with a standard drug glibenclamide 0.5 mg/kg of body weight [15]; Group IV Diabetic rats fed with gold nanoparticles synthesized using PAT of *C. auriculata* (0.5 mg/kg body weight in aqueous solution); Group V Normal rats fed with gold nanoparticles synthesized using PAT of *C. auriculata* (0.5 mg/kg body weight in aqueous solution); Group VI diabetic rats fed with PAT of *C. auriculata* (0.5 mg/kg of body weight in aqueous solution).