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CHARACTERIZATION OF ANTIDIABETIC ACTIVITY OF SILVER NANOPARTICLES USING AQUEOUS SOLUTION OF *Ficus glomerata* (FIG) GUM

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ABSTRACT

Current discovery demonstrates the facile and rapid synthesis of biocompatible silver nanoparticles (AgNPs) by a novel biological route using aqueous solution of fig gum (*Ficus glomerata*). These silver nanoparticles were characterized by means of UV–vis spectroscopy, scanning electron microscopy (SEM), electron diffraction spectroscopy (EDX) and X-ray diffraction (XRD). The nanoparticles exhibited maximum absorbance at 408 nm in UV–vis spectroscopy. The XRD spectrum of silver nanoparticles exhibited 2θ values corresponding to the silver nanocrystals. SEM micrographs revealed the formation of well-dispersed silver nanoparticles of 31 nm in an average size, and the presence of silver was confirmed by EDX analysis. This *in vitro* study explores the antidiabetic properties of biosynthesized silver nanoparticles and it can be considered as a potential candidate for the management of type-II diabetes mellitus. The assay results of silver nanoparticles showed a dose dependent pattern. The percentage inhibitory activity significantly increase against α-amylase (alpha amylase) enzyme, 95.28 ± 0.33 % inhibition was seen at a concentration of 50 µg/ml and 97.74 ± 0.3 % inhibition was observed at 1000 µg/ml, similarly against α-glucosidase (alpha glucosidase) enzyme, the enzyme inhibition was observed at lower concentration of 50 µg/ml, 97.14 ± 0.02 % of inhibition and 99.98 ± 0.61 % inhibition at higher concentration of 1000 µg/ml was recorded. The results of the study also revealed that the antidiabetic activity of the AgNPs is much higher than the standard antihyperglycemic drug.

KEYWORDS: *Silver nanoparticles, antidiabetic activity, α-amylase, α-glucosidase*

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INTRODUCTION

Diabetes is one of the most challenging diseases increasing its prevalence in worldwide.[1] Type-I diabetes characterized by absolute deficiency of insulin secretion and associated with auto-immune destruction of pancreatic β-cells is likely to be prevalent among relatives of the affected person[2] whereas Type-II diabetes which accounts for 90% of cases was caused by combination of resistance to insulin action and impaired insulin secretion.[3] The intestinal enzymes, α-amylase (EC 3.2.1.1) and α-glucosidase (EC 3.2.1.20) play a key role in the degradation of starch and oligosaccharides to glucose and if suppressed would in turn delay the glucose absorption in the intestine. Eventually, the postprandial blood sugar is controlled.[4] The chronic hyperglycemia of diabetes is associated with the long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels.[5] Currently available therapies for diabetes to manage postprandial hyperglycemia at digestive level, such as glucosidase and amylase inhibitors which include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides and glinides.[6] Many of them have a large number of serious adverse effects; therefore, the search for more effective and safer hypoglycemic agents is one of the important areas of investigations.[7] Nanotechnology is a broad-based science involving manipulation of atoms, electrons, protons and neutrons in a variety of ways to generate new understanding of how materials can be developed to solve many problems in medicine, engineering, agriculture, surface science, marine science, and geology.[8] Most of the natural processes also take place in the nanometre scale regime. Therefore, a confluence of nanotechnology and biology can address several biomedical problems, and can revolutionize the field of health and medicine.[9] Among nanoparticles, silver nanoparticles in particular are known for their versatile applications in medical industries,[10] food processing industries,[11] textile industries,[12] consumer goods,[13] and for being an efficient antimicrobial agent.[14] There are different methods for the synthesis of nanoparticles. Biosynthesis of nanoparticles is advantageous over the chemical and physical methods as it is a cost-effective and environment friendly method, where it is not necessary to use high pressure, energy, temperature and toxic and redundant chemicals in solid, liquid and gaseous form.[15,16] The use of plants for the fabrication of nanoparticles is a rapid, low cost, eco-friendly and a single step method for biosynthesis process[17] which can be easily scale up. Thus in the search of new opportunities for treatment of diabetes mellitus, the current study has focused on the efficacy of gum of *Ficus glomerata* (fig). This plant belongs to the Moraceae family, and different parts of this plant are used as a traditional medicine. Medicinally it possesses various pharmacological activities, such as, antidiabetic, astringent, refrigerant, antiasthmatic, antiinflammatory, hepatoprotective, antioxidant, antiulcer, antipyretic, antidiuretic, antidiarrhoel.[18] In addition, this is the first of its kind reporting the efficiency of *F. glomerata* gum-mediated AgNPS as an antidiabetic agent. Here the aqueous solution of fig gum was used to synthesize silver nanoparticles and investigated its *in vitro* antidiabetic activity.

MATERIALS AND METHODS

Collection of plant material and chemicals

Fig gum was collected from the incised trunk of *Ficus glomerata* in the forest area near Tambara, Chennai, India. The fig gum was dried to completely remove the moisture and then hydrated in distilled water for one day with intermittent stirring; extraneous materials were removed by straining through a muslin cloth. The gum was precipitated from solution using absolute acetone. The precipitate was separated and dried on water bath at 50 °C. A fine powder of dried gum was obtained using a blender and stored in air-tight container. Silver nitrate ($AgNO_3$) from Thomas Baker (Mumbai, India), and other required chemicals were purchased from Hi-Media (Mumbai, India) which are of analytical grade in analytical grade.

Biosynthesis of silver nanoparticles

A 0.5 % (w/v) homogeneous gum stock solution was prepared by dissolving 0.5 g of fig gum in 100 ml of deionized water and stirring overnight at room temperature. The solution was then centrifuged at 6000 rpm for 10 min to separate out any undissolved matter, and the supernatant is used for the experiments to be carried out in the study. 20 ml of this aqueous gum solution was mixed with 1 mM $AgNO_3$ solution. The mixture was subjected to heat at 85 °C temperature with constant stirring. The conversion of the colorless reaction mixture to the characteristic clear yellow color indicates the formation of AgNPs.

Characterization of synthesized silver nanoparticles

To verify reduction of silver ions, the solution was scanned in the range of 300–700 nm using a double-beam UV-visible spectrophotometer with water as the reference. Further characterization was done using X-ray diffraction technique. It was performed on an X-ray diffractometer operated at 45 kV and 40 mA using dried silver nanoparticles. The pattern was recorded by Cu Kα radiation in a θ–2θ configuration. The morphology of the AgNPs was examined using scanning electron microscopy. Thin films of the sample were prepared on a carbon coated copper grid by dropping a very small amount of the sample on the grid. The extra solution was removed using a blotting paper and then the film on the SEM grid was allowed to dry under a mercury lamp for 5 min. The presence of elemental silver was determined by energy dispersive X-ray (EDX) spectrometric analysis using SEM instrument equipped with EDX attachments.

Evaluation of in vitro antidiabetic activity

Inhibition of α-amylase enzyme assay

A total of 500 µl of AgNPs suspension and standard drug (50-1000 µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5 mg/ml) solution and were incubated at 25 °C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25 °C for 10 min. The reaction was stopped with 1.0 ml of

3, 5 dinitrosalicylic (DNS) acid color reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Acarbose was used as a standard drug for assay.[19] % Inhibition was calculated according to the formula:

$$\% \text{ Inhibition} = \frac{\text{A540 Control} - \text{A540 Sample}}{\text{A540 Control}} \times 100$$

Inhibition of α-glucosidase enzyme assay

The α-glucosidase inhibitory effect was determined according to the standard method using different concentration (50-1000 μg/ml) of AgNPs.[20] 10 μl of α-glucosidase enzyme solution and varying concentrations of the nanoparticles suspension was incubated together for 10 min, at 37 °C, and the volume was made up to 210 μl with maleate buffer, pH 6.0. The enzyme reaction was started by adding 200 μl of 2 mM p-nitrophenyl-α-D-glucopyranoside (pNPG) solution and further incubated at 37 °C for 30 min. Enzymatic reaction was stopped by adding 2 ml 0.2 M sodium carbonate solution. After the addition of 1.0 ml of 0.1 M disodium hydrogenphosphate solution, the absorption of liberated p-nitrophenol was read at 400 nm. Each test was performed three times and the mean absorption was used to calculate the percentage α-glucosidase inhibition. The similar reaction was performed with the drug, Acarbose at varying concentration (50-1000 μg/ml) and compared the results with the test samples containing the AgNPs. % Inhibition was calculated according to the formula:

$$\% \text{ Inhibition} = \frac{\text{A400 Control} - \text{A400 Sample}}{\text{A400 Control}} \times 100$$

STATISTICAL ANALYSIS

All determinations were carried out in triplicates and data were analyzed by ANOVA followed by Tukey's multiple comparisons test for significant differences. Values were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Visual observations and UV–vis spectroscopy

UV–visible spectroscopy could be used to examine the formation of the metal nanoparticles by reduction of metal ions in aqueous solutions when exposed to the aqueous gum solution of *F. glomerata*. The progress of the reaction between metal ions and the gum extracts were monitored by recording the absorption spectra as a function of time. The absorption peak is assigned to the surface plasmon resonance (SPR) band of Ag nanoparticles formed by the reduction of Ag^{+1} ions. In the current synthesis, it is observed that the surface plasmon resonance (SPR) peak occurs at 408 nm in the visible range of spectrum and is shown in Figure 1. The initial color of the reaction mixture changes to yellowish brown color soon after the addition of plant gum extract. The intensity of this brown color rapidly enhances with time. Increase in the intensity of brown color clearly indicates the formation of AgNP in the reaction mixture (Fig 1). The absorption spectrum of the yellowish-brown silver nanoparticles solution showed a surface plasmon vibration band with a maximum of 408 nm, indicating the presence of Ag nanoparticles. The metal nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. Due to the tiny dimensions, silver nanoparticles have distinctive color in colloidal solution.[21] The process of biosynthesis is carried out at ambient environmental conditions and the total reaction is completed within few minutes

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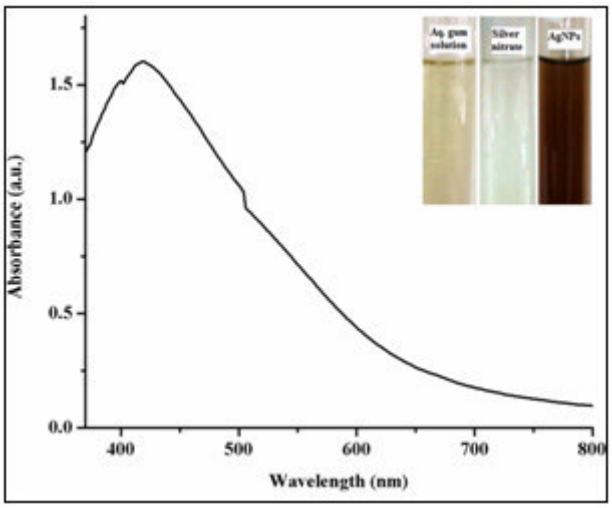

Figure 1

UV-vis absorption spectrum of biosynthesized AgNPs aqueous gum solution of F. glomerata. Inset shows the corresponding AgNPs solution after reduction.

Scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) analysis

The localization of silver nanoparticles was done in SEM using a secondary electron detector. These particles were found to be in cubic in nanoscale dimensions (Fig 2a) with diameter of 31±3 nm. EDX analysis of the nanoparticles shows strong signals for silver atoms which confirm the presence of metallic silver in the sample in higher percentages (Fig 2b). There are some weak signals could have arisen from macromolecules like proteins of gum extract.

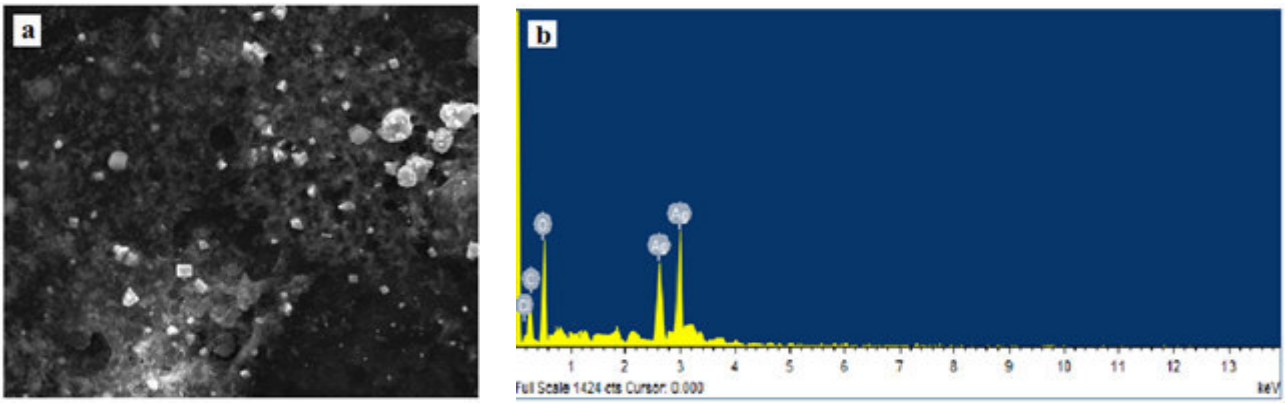

Figure 2
SEM micrograph (a) and energy dispersive X-ray spectrum (b) of biosynthesized metallic AgNPs.

X-ray diffraction (XRD) analysis

A representative XRD profile of the cubical silver nanoparticles displaying the structural information and crystallinity are shown in Fig 3. After reaction, the diffraction peaks at 2θ values at 37.21°, 45.36°, 66.45°, 78.65° and 85.88° corresponding to (111), (200), (220), (311) and (222) of Bragg reflections planes of a faced centre cubic (fcc) lattice of silver metals were obtained. The average crystallite size of AgNPs were found to be 31 nm in diameter from the full width at half maximum (FWHM) of the slowly scanned peak using Debye-Scherrer's equation (1):[22] $t = 0.9\lambda / \beta \cos\theta$ where t is the particle diameter, λ is the wavelength of the incoming X-rays (Cu Kα radiation), B is the breadth of the peak (or full width at half maximum, FWHM) in radian units, and θ is the half angle of diffraction.

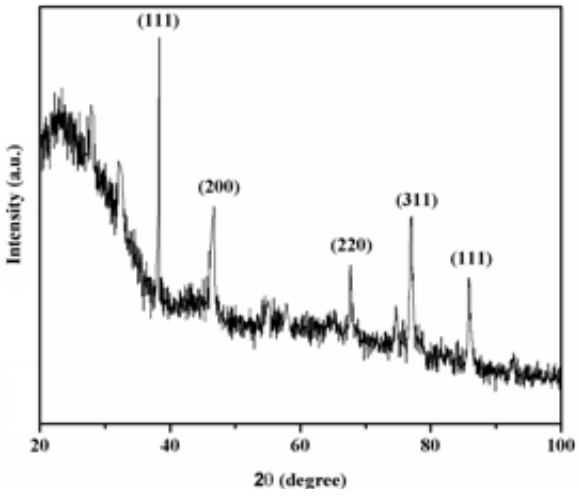

Figure 3
XRD pattern of synthesized silver nanoparticles.

Evaluation of in vitro antidiabetic activity

The intestinal enzymes like α-amylase and α-glucosidase are found to be very important in carbohydrate digestion and glucose absorption. The suppression of the activity of such digestive enzymes would delay the degradation of starch and oligo saccharides, which would in turn cause a decrease in the absorption of glucose and consequently in the reduction of postprandial blood glucose level elevation.[23] Alpha amylase and glucosidase inhibitors are the potential targets in the development of lead compounds for the treatment of diabetes.[24] Thus in this study, AgNPs were used as inhibitors of these intestinal enzymes.

Inhibition of in-vitro α-amylase and α-glucosidase enzyme assay

Alpha amylase is an enzyme that hydrolyses alpha-bonds of large alpha linked polysaccharide such as glycogen and starch to yield glucose and maltose. Alpha amylase inhibitors bind to alpha-bond of polysaccharide and prevent break down of polysaccharide in mono and

disaccharide.[25] The *in vitro* α-amylase inhibitory studies showed that biosynthesized AgNPs have significant α-amylase inhibitory activity. There was a dose-dependent increase in percentage inhibitory activity against α-amylase enzyme. At a concentration of 50 µg/ml extract showed a percentage inhibition 95.28 ± 0.33 and for 1000 µg/ml, it was 97.74± 0.3 (Figure 4). There is slight variation in α-amylase inhibitory effect among the different concentration of test solutions, the AgNPs are more effective even in minimum concentration (50 µg/ml). The nanoparticles also exhibited much higher α-amylase inhibition as compared with the standard drug, Acarbose (77.1 % at 1000 µg/ml). The intestinal α-glucosidases hydrolyze complex carbohydrates to glucose and other monosaccharides in the small intestine. Inhibition of these enzyme systems helps to reduce the rate of digestion of carbohydrates.[26] Percent of α-glucosidase inhibition of the AgNPs was plotted as a function of concentration in comparison with Acarbose as shown in Fig 4.

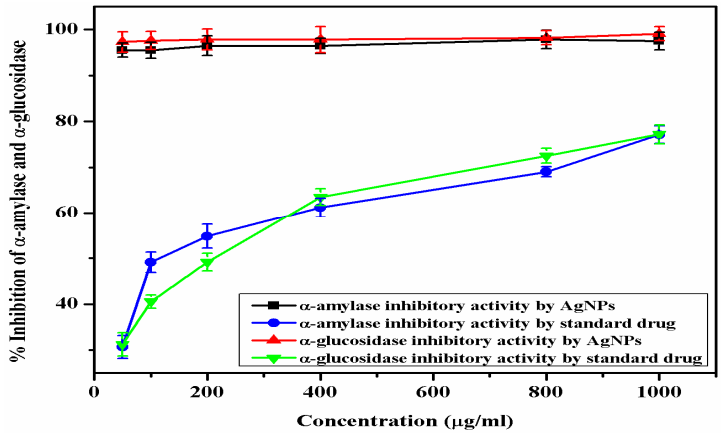

Figure 4
In vitro α-amylase and α-glucosidase inhibitory activity of silver nanoparticles.

The results indicate that AgNPs exhibited well anti α-glucosidase activity for all concentration variation. Like α-amylase, here also similar dose-dependent pattern was observed. The inhibition activity of AgNPs is much higher than the commercial drug, Acarbose. The drug showed 77.58 ± 0.51 at maximum concentration (1000 µg/ml), where biosynthesized AgNPs exhibited 99.98 ± 0.61 at same concentration (Fig 4). Most interestingly, the nanoparticles showed almost same α-glucosidase inhibition activity even in lower concentrations (97.14 ± 0.02 % at 50 µg/ml) which direct that the dosage of AgNPs can be easily reduced to control the blood sugar level. In addition, the effect of biosynthesized AgNPs using gum solution of *F. glomerata* on α-amylase and α-glucosidase inhibition is much higher compared to the using gum extract alone.[27]

CONCLUSION

In this study, we have synthesized cubic shaped AgNPs using aqueous gum solution of *F. glomerata* as a reducing agent for the first time. The synthesized AgNPs are very well dispersed, well defined in shape and polycrystalline in nature. The synthesized AgNPs possess significant antidiabetic activity compared to commercial drug and hence clearly proved their pharmaceutical and medicinal importance. Furthermore, the use of plant material for nanoparticles synthesis offers the benefits of ecofriendliness and amenability for large scale production.

CONFLICT OF INTEREST

Conflict of interest declared none.

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