Evaluation of Invitro Antidiabetic Activity of *Sphaeranthus amaranthoides* Silver nanoparticles

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Abstract

Diabetes is a syndrome which is characterized clinically as hyperglycemia due to absolute or relative deficiency of insulin production and progressive resistance of insulin. In recent years there is a fast increase in the occurrence and prevalence of diabetes mellitus. In the present study *sphaeranthus amaranthoides* was screened for antidiabetic activity in invitro. In the present search synthesis of silver phyto nanoparticles and their antidiabetic activity were studied. This is the first attempt of introducing silver herbal nanoparticles isolation and antidiabetic assessment. This is because silver nanoparticles possess a very high surface to volume ratio. This can be utilized in areas where high surface areas are critical for success. This could for example be in the catalytic industry. In biology and biochemistry nanoparticles have attracted much attention. The aim of the current work is to screen for invitro inhibition of alpha-amylase enzyme activity of extract of *sphaeranthus amaranthoides* silver nanoparticles. The silver nanoparticles showed a dose response inhibitory activity on α-amylase. Acarbose was used a standard drug. The IC50 value for plant extract is 0.28µg/ml where as for acarbose is 0.75µg/ml.

Key words: *Sphaeranthus amaranthoides*, antidiabetic activity, α-amylase, hyperglycemia, silver nanoparticles.

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Introduction:

Diabetes mellitus results from the defects in the insulin secretion and action, this may be characterized by chronic hyperglycemia, which is connected with the carbohydrates, protein and lipid metabolism [1]. Globally mortality rate 9% is recorded due to the diabetes. Diabetes mellitus a well known endocrine disorder and it is most common in India now a day. The reason may be life style and genetic factors[2]. Due these factors the diabetic monocytes produce increased superoxide anion. (O$_2^-$)[3]. In premature atherosclerosis and oxidative stress patient’s diabetes is a major risk factor. Over the centuries, herbal drugs have served as a major source of medicines for the prevention and treatment of diseases including diabetes mellitus. There are more than 200 species of plants exhibit hypoglycemic properties, including many common plants, such as pumpkin, wheat, celery, wax gourd, lotus root and bitter melon but the basis of this activity is frequently not investigated[4]. There are many synthetic hypoglycemic drugs to manage post-prandial hyper-glycaemia at digestive level, glucosidase and amylase inhibitors such as acarbose, miglitol and voglibose, but these drugs may cause many side effects. During pregnancy diabetes may cause serious problems in both mother and child, however to overcome these problems synthetic agents are used vigorously these are not suitable for continuous use due to side effects[5] such as development of hypoglycemia, weight gain, gastrointestinal disturbances, liver toxicity etc[6]. Based on the recent studies antioxidants capable of neutralizing free radicals are effective in preventing experimentally induced diabetes in animal models [7,8] as well as reducing the severity of diabetic complications[9].

Silver nanoparticles are widely used for its unique properties in catalysis, chemical sensing, biosensing, photonics, electronic and pharmaceuticals[10] and in biomedicine especially for antibacterial agent[11] and antiviral agent[12]. These properties can be extended to antidiabetic activity along with the plant extracts. The most important application of silver and silver nanoparticles is in medical industry such as tropical ointments to prevent infection against burn and open wounds[13]. Biologic synthesis of nanoparticles by plant extracts is at present under exploitation as some researchers worked on it[14, 15] and testing for antimicrobial activities[16,17].

In the present study the plant *sphaeranthus amaranthoides*...
particles of *Sphaeranthus amaranthoides* to evaluate their potential hypoglycaemic effects. The bioassay method was adopted from Conforti et al. (19). A starch solution (0.5% w/v) was obtained by stirring 0.1g of potato starch (Sigma) in 25ml of 20mM sodium phosphate buffer with 6.7mM sodium chloride, pH 6.9 at 65°C for 15min. The enzyme solution was prepared by mixing 0.0253g of (α-amylase in 100ml of cold distilled water. Silver nano particles of *Sphaeranthus amaranthoides* extract is dissolved in buffer to give a final concentration from 1mg/ml to 12.5 mg/ml. The colorimetric reagent was prepared mixing a sodium potassium tartrate solution (12.0g of sodium potassium tartrate, tetrahydrate in 8.0ml of 2M NaOH) and 96mM 3,5-dinitrosalicylic acid solution. Both control and plant extracts were added with starch solution and left to react with (α-amylase solution under an alkaline condition at 25°C. The reaction was measured over 3min. The generation of maltose was quantified by the reduction of 3,5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid. This reaction (corresponding to colour change from orange-yellow to red) is detectable at 540nm. In the presence of an (α-amylase inhibitors less maltose would be produced and the absorbance value would be decreased. Preliminary experiments were carried out to establish optimal conditions and these were found necessary: starch 0.25% w/v; (α-amylase 1 unit/ml; inhibitor concentration 1mg/ml. Statistics Data were expressed as means ± S.D. Statistical analysis was performed using Student’s t test. Differences were considered significant at p<0.05. The inhibitory concentration 50% (IC50) was calculated from the Prism dose response curve (statistical programme) obtained by plotting the percentage of inhibition versus the concentrations.

\[
%\text{Inhibition} = \left( \frac{\text{Maltose}_{\text{test}}}{\text{Maltose}_{\text{control}}} \right) \times 100
\]

**Results and discussion:**

**Characterization of synthesized silver nanoparticles:**

Silver nanoparticles are formed by reduction of the aqueous Ag+ during exposure to the ethanol extract of *sphaeranthus amaranthoides* were followed by UV–vis spectroscopy. It is well known that silver nanoparticles exhibit reddish-brown in water[20]. After 24 h of the incubation process silver nanoparticles showed reddish-brown color, suggested the formation of silver nanoparticles in solution. These colors arise due to excitation of surface plasmon vibrations in the silver metal nanoparticles.[21] **Fig.1a** and Fig1b shows the UV–vis spectra recorded from the ethanol extract of *sphaeranthus amaranthoides* silver nanoparticles. It is observed that the silver surface plasmon resonance band occurs at 340 nm and steadily increases in intensity as a function of time of reaction without any shift in the peak wavelength. The frequency and width of the surface plasmon absorption depends on the size and shape of the metal nanoparticles as well as on the dielectric constant of the metal itself and the surrounding metal [22, 23]. Generally the shape of the silver nanoparticles will be changed when in contact with water or any biological sample. It is generally recognized that UV–vis
The herrer formula is 50
e XRD pattern of pure silver ions is known to
displays the number of x-ray counts whilst the horizontal
axis displays energy in KeV. Identification lines for the
major emission energies for silver (Ag) are displayed and
these correspond with peaks in the spectrum, thus giving
confidence that silver has been correctly identified. The
FTIR spectrum analysis of nanoparticles were shown in
Fig 5 which manifest absorption peaks located at the region
about 3500cm⁻¹ and 650cm⁻¹. The absorption peak at around
638cm⁻¹ can be assigned as Secondary amide N-H wagging,
likewise the peak at 1036cm⁻¹ as phosphorus ester P-OH
stretching,1297cm⁻¹ as Nitrate NO₂ symmetric
stretching,1384cm⁻¹ as Aliphatic nitro compound NO₂
symmetric,1630cm⁻¹ as Primary amide NH₂ bending-amide
II band,1713cm⁻¹ as Aliphatic hydrocarbons C-H
stretching,2922cm⁻¹ as Carboxylic acids O-H stretching,3429cm⁻¹ as O-H stretching.
The invitro inhibition activity of silver nanoparticles
There are several possible mechanisms through which these
herbs can act to control the blood glucose level[28]. In that
one of the mechanism is that an alteration of the activity of
some enzymes that are involved in glucose metabolism.
The α-amylase inhibitors act as an anti-nutrient that
obstructs the digestion and absorption of carbohydrates[29]. One of the Synthetic α-amylase
inhibitors is acarbose is a complex oligosaccharides that
delay the digestion of carbohydrates. It inhibits the action
of pancreatic amylase in breakdown of starch. Synthetic
inhibitor causes side effect such as abdominal pain,
diarrhoea and soft faeces in the colon. The reference drug
arcabose was not a potent inhibitor of α
amylase under the
similar principle to that of acarbose with IC
50 value of about 1 mg/ml) or no inhibition of α
amylase with IC
50 value of 0.28
µg/ml similar principle to that of acarbose with IC
50 value 0.75µg/ml. It should be mentioned here that the calculated
IC
50 values in the current studies is correlated with earlier
studies[32]. The calculated IC
50 values of Ag nanoparticles
of Sphaeranthus amaranthoides showing lower value
Fig. 6. Lineweaver-Burk plot of the activity of α-amylase in the absence or presence of S. amaranthoides Ag Nano particles extract compared the standard suggest that these nanoparticles are potent than acarbose in inhibiting α-amylase. Furthermore, fig 6 line weaver burk plot which is constructed against the substrate concentration vs velocity of the enzyme in the presence or absence of plant silver nanoparticles suggests that the mode of inhibition of acarbose towards α-amylase can be mixed non-competitive, this is correlated with earlier studies [31]. In the current study, the mode of inhibition of sphaeranthus amaranthoides silver nanoparticles was found to be competitive. This finding suggest that some of the α-amylase inhibitory components in sphaeranthus amaranthoides ethanol extract may be structural analogs of the substrate of α-amylase.

Conclusion:
According to the present study we can conclude that plant medicines are showing a better inhibitory activity when compared to the synthetic inhibitors. Natural medicines have lesser or no side effects when compared with chemical inhibitors. From the present study we can conclude that the sphaeranthus amaranthoides is showing better antidiabetic activity when compared with the acarbose which is synthetic inhibitor for α-amylase.

References:

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