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Science

Evaluation of *In vitro* Antidiabetic activity of silver nanoparticles synthesized from *Azima tetracantha* leaf

Manimegalai B Research Scholar, P.G and Research Department, Marudupandiyar College, Thanjavur,

Velavan S Associate Professor, P.G and Research Department, Marudupandiyar College, Thanjavur,

ABSTRACT The aim of the current work is to screen *in vitro* inhibition of alpha-amylase and α-glucosidase enzyme activities in silver nanoparticles synthesized from *Azima tetracantha* leaf extract. 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 present findings exhibited a concentration dependent inhibition of α-amylase and α-glucosidase activity by the *Azima tetracantha* leaf extract and AgNPs. The half inhibition concentration (IC_{50}) of *Azima tetracantha* leaf extract, AgNPs and Acarbose tested against α-amylase were 288.79, 262.18µg/ml-1 and 246.14µg/ml-1 respectively. The half inhibition concentration (IC_{50}) of *Azima tetracantha* leaf extract, AgNPs and Acarbose tested against α-glucosidase were 315.23, 271.78µg/ml^{-1} and 266.72µg/ml^{-1} respectively. The results of the study revealed that the antidiabetic activity of the AgNPs is much higher than the *Azima tetracantha* leaf extract and near to the standard antihyperglycemic drug.

KEYWORDS : Diabetes mellitus, *Azima tetracantha* leaf, Silver nanoparticles, Acarbose, α-amylase and α-glucosidase

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 (WHO, 1999). 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 (Riserus et al., 2009). Due these factors the diabetic monocytes produce increased superoxide anion. (O_2) (Venugopal et al., 2002). 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.

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 (Lamer, 1985) such as development of hypoglycemia, weight gain, gastrointestinal disturbances, liver toxicity etc (Dey et al., 2002). Based on the recent studies antioxidants capable of neutralizing free radicals are effective in preventing experimentally induced diabetes in animal models as well as reducing the severity of diabetic complications9 Silver nanoparticles are widely used for its unique properties in catalysis, chemical sensing, biosensing, photonics, electronic and pharmaceuticals and in biomedicine especially for antibacterial agent and antiviral agent (Rai et al., 2009). 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. Biologic synthesis of nanoparticles by plant extracts is at present under exploitation as some researchers worked on it (Bhyan et al., 2007). In the present study is to screen for *in vitro* inhibition of alpha-amylase ad α-glucosidase enzyme activities of silver nanoparticles synthesized from *Azima tetracantha* leaf extract.

MATERIALS AND METHODS:

Preparation of leaf extract

The dried leafs were pulverized well with mortar and pestle to make a powder. Twenty grams of powder sample was mixed into 100 ml of deionized water and the mixture was boiled for 10 min. After cooling the leaf extract was filtered with Whatman No. 1 filter paper. The filtrate was stored at 4◦C for further use.

Synthesis of Ag nanoparticles using leaf extracts

For the Ag nanoparticles synthesis, 5 ml of *Azima tetracantha* leaf extract was added to 45 ml of 1 mM aqueous AgNO3 solution in a 250 ml Erlenmeyer flask. The flask was then incubated in the dark at 5hrs (to minimize the photo activation of silver nitrate), at room temperature. A control setup was also maintained without leaf extract. The Ag nanoparticle solution thus obtained was purified by repeated centrifugation at 10,000 rpm for 15 min followed by re-dispersion of the pellet in de-ionized water. Then the Ag nanoparticles were freeze dried using SEM analysis (Arunachalam et al., 2012).

In vitro antidiabetic activity

In vitro α-amylase inhibition assay was carried out by the method of Apostolidis (2007). The α-glucosidase inhibitory activity was determined according to the method described by Apostolidis et al., (2007).

RESULTS AND DISCUSSION:

The synthesize and characterization of AgNPs from *Azima tetracantha* leaf extract showed the particle size between 10-80nm as well the cubic structure of the nanoparticles reported in our earlier report (Manimegalai and Velavan, 2015). In the preset study to investigate the antidiabeticc activity of AgNPs tested against alpha-amylase ad α-glucosidase enzymes.

There are several possible mechanisms through which these herbs can act to control the blood glucose level (Tanira, 1994). In that one of the mechanism is that an alteration of the activity of some enzymes that are involved in glucose metabolism. 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 oligosaccharides, which would in turn cause a decrease in the absorption of glucose and consequently in the reduction of postprandial blood glucose level elevation (Davis et al., 2001). Alpha amylase and glucosidase inhibitors are the potential targets in the development of lead compounds for the treatment of diabetes (Subramanian et al., 2008) Thus in this study, AgNPs were used as inhibitors of these intestinal enzymes.

Inhibition of *in-vitro* α-amylase enzyme assay

Alpha amylase is an enzyme that hydrolyses alpha bonds of large alpha linked polysaccharide such as lycogen 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 (Gupta *et al*., 2012). The α-amylase inhibitors act as an anti-nutrient that obstructs the digestion and absorption of carbohydrates (Narkhede *et al*., 2011). The present findings exhibited a concentration dependent inhibition of α-amylase activity by the *Azima tetracantha* leaf extract and AgNPs. The lowest inhibition of α-amylase activity of *Azima tetracantha* leaf extract, AgNPs and Acarbose were 15.45%, 19.57% and 22.45% in the concentration of 100µg/ml respectively while the highest inhibition of α-amylase

activity of *Azima tetracantha* leaf extract, AgNPs and Acarbose were 82.65%, 88.39% and 92.84% in the concentration of 500µg/ml respectively. The greatest effect of AgNPs (500 µg/ml) was found to be near to standard Acarbose. The half inhibition concentration (IC50) of *Azima tetracantha* leaf extract, AgNPs and Acarbose were 288.79, 262.18µg/ml-1 and 246.14µg/ml-1 respectively. From the present study it can be concluded that AgNPs showed marked *in vitro* antidiabetic effect against the α-amylaseactivity(Table 1 and Figure 1). Present finding is in agreement with Merina Paul Das and Jeyanthi Rebecca (2017) study.

Table 1: *In vitro* α-amylase inhibition of *Azima tetracantha*, AgNPs and Acarbose

Groups	Concentrations	% of inhibition		
		Azimate tracantha	AgNPs	Standard
Group I	100µg/ml	15.45 ± 1.08	19.57 ± 1.36	22.45 ± 1.57
Group II	200µg/ml	33.25 ± 2.32	40.58± 2.84	39.61 ± 2.77
Group III	300µg/ml	48.65 ± 3.40	61.74 ± 4.32	65.74 ±4.60
Group IV	400µg/ml	68.48 ± 4.79	72.43 ± 5.07	78.31 ± 5.48
Group V	500µg/ml	82.65 ± 5.78	88.39 ± 6.18	92.84 ± 6.49
IC_{50} (µg/ml)		288.79	262.18	246.14

Values are expressed as Mean ± SD for triplicates

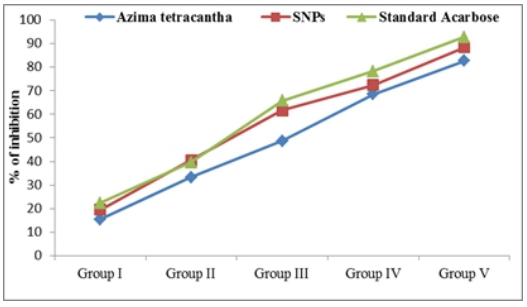

Figure 1: *In vitro* α-amylase inhibition of *Azima tetracantha*, AgNPs and Acarbose

Inhibition of *in-vitro* α-glucosidase enzyme assay

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 (Bhat *et al*., 2011). The present findings exhibited a concentration dependent inhibition of α- glucosidases activity by the *Azima tetracantha* leaf extract and AgNPs.

The lowest inhibition of α- glucosidase activity of *Azima tetracantha* leaf extract, AgNPs and Acarbose were 16.54%, 18.65% and 20.45% in the concentration of 100µg/ml respectively while the highest inhibition of α-amylase activity of *Azima tetracantha* leaf extract, AgNPs andAcarbose were 73.45%, 83.67% and 84.65% in the concentration of 500µg/ml respectively. The greatest effect of AgNPs (500 µg/ml) was found to be near to standard Acarbose. The half inhibition concentration (IC_{50}) of *Azima tetracantha* leaf extract, AgNPs and Acarbose were 315.23, 271.78µg/ml^{-1} and 266.72µg/ml^{-1} respectively. From the present study it can be concluded that AgNPs showed marked *in vitro* antidiabetic effect against the α- glucosidase activity (Table 2 and Figure 2). Present finding is in agreement with Merina Paul Das and Jeyanthi Rebecca (2017) study.

Table 2: *In vitro* α-glucosidase inhibition of *Azima tetracantha*, AgNPs and Acarbose

Groups	Concentrations	% of inhibition		
		Azimatetracantha	AgNPs	Standard Acarbose
Group I	100µg/ml	16.54 ± 1.15	18.65 ± 1.30	20.45 ± 1.43
Group II	200µg/ml	32.45 ± 2.27	41.23 ± 2.88	34.45 ± 2.41
Group III	300µg/ml	50.74 ± 3.55	56.35 ± 3.94	62.35 ± 4.36
Group IV	400µg/ml	65.65 ± 4.59	72.84 ± 5.09	76.45 ± 5.35
Group V	500µg/ml	73.45 ± 5.14	83.67± 5.85	84.65 ± 5.92
Group VI (Standard)	IC_{50} (µg/ml)	315.23	271.78	266.72

Values are expressed as Mean ± SD for triplicates

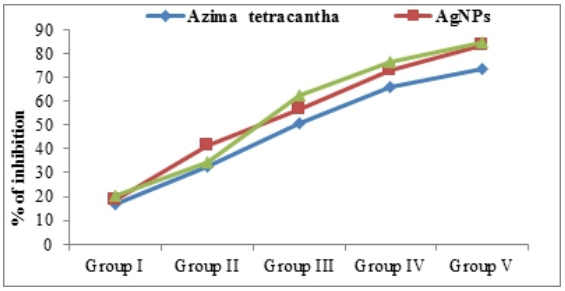

Figure 2: *In vitro*α-glucosidaseinhibition of *Azima tetracantha*, AgNPs and Acarbose

Conclusion:

The synthesized AgNPs possess potential antidiabetic activity compared to *Azima tetracantha* leaf extract and near to commercial drug Acarbose and hence clearly proved their pharmaceutical and medicinal importance.

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