Research Article

Antidiabetic activity of silver nanoparticles synthesized using neem and *Aloe vera* plant formulation

K. Sathvika, S. Rajeshkumar*, T. Lakshmi, Anitha Roy

ABSTRACT

Introduction: Diabetes mellitus (DM) is a serious and chronic disease that can occur either when the pancreas fails to produce enough insulin (Type 1 DM/insulin-dependent DM) or when the body fails to use the insulin, it produces effectively (Type 2 DM/non-insulin-dependent DM). Since the prevalence of DM has only been exponentially increasing, the need for finding alternative forms of medication and treatment is of immense importance. Here, we have aimed to analyze the possible antidiabetic potential of silver nanoparticles (Ag-NPs) synthesized using neem and *Aloe vera* through an alpha-amylase assay. **Materials and Methods:** Leaves of *Azadirachta indica* and *A. vera* were made into a filtered herbal formulation. A metallic solution of silver nitrate was added to it and the conjunction was kept in a magnetic stirrer/orbital shaker for the synthesis of its nanoparticles. After centrifugation, the resultant pellet was powdered. The antidiabetic potential was studied through the inhibition of alpha-amylase. Ag-NP concentrations of 50, 100, and 150 µL were preincubated with 100 µL of alpha-amylase solution (1 U/mL) at room temperature for 30 min. About 100 µL of starch solution (1% w/v) was then added to it and the mixture was incubated at room temperature for 10 min. About 100 µL of 96 mM 3,5-dinitrosalicylic acid reagent was added to it to stop the reaction and the solution was heated in a water bath. Control was maintained where equal quantities of the enzyme and extract were replaced by a sodium phosphate buffer maintained at a pH of 6.9. The reading was measured at 540 nm. The experiment was performed thrice. Acarbose was used as a positive control and the percentage of inhibition was calculated. **Results:** A color change was observed after the synthesis of Ag-NPs. The prepared particles were then characterized by a peak seen at 425 nm in ultraviolet-visible spectroscopy. The results of the assay showed that 150 µg of our Ag-NPs exhibited an alpha-amylase inhibition which was close to that of acarbose with a variation of only 2.67%. **Conclusion:** Ag-NPs synthesized using neem and *A. vera* have an evident antidiabetic potential. They show a considerably good amount of alpha-amylase inhibition with respect to acarbose. They are also easy to synthesize, eco-friendly, and inexpensive. Thus, Ag-NPs could be used as a possible alternative for conventional antidiabetic drugs.

KEY WORDS: *Aloe vera*, Antidiabetic, Diabetes, Neem, Silver nanoparticles

INTRODUCTION

Believed to be the 7th most common cause of death in the world in 2016, diabetes mellitus (DM) or diabetes poses to be a threat that can lead to severe bodily damage if left unchecked.[1] It is a serious and chronic disease that can occur either when the pancreas fails to produce enough insulin (Type 1 DM/insulin-dependent DM) or when the body fails to use the insulin, it produces effectively (Type 2 DM/non-insulin-dependent DM).[2] Since the prevalence of DM has only been on the rise, the need for finding alternative forms of medication and treatment is of immense importance – thus introducing the need for studying the possible antidiabetic efficacy of nanoparticles (NPs) which, according to many, is a Panacea of various disciplines.

Access this article online	
Website: jprsolutions.info	**ISSN:** 0975-7619

Nanotechnology is currently an enthusiastically developing field in the era of modern science. The size-dependent properties and versatility of NPs give its multifunctional nature. It has been extensively used in the fields of agriculture, medicine, physics,[3] optics, and electronics.[4,5] As of late, its specificity in drug delivery has also been under active research.[6,7]

Silver has long been used for dressing wounds, in creams and as an antibiotic coating on medical

Department of Pharmacology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India

***Corresponding author:** Dr. S. Rajeshkumar, Department of Pharmacology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai - 600 077, Tamil Nadu, India. Tel.: +91-9629739263. E-mail: ssrajeshkumar@hotmail.com

Received on: 13-03-2019; Revised on: 22-05-2019; Accepted on: 27-05-2019

appliances.[8,9] Since silver has a low toxicity, it can be used in medicine with a relatively minimal risk.[10] Silver also possesses a very apparent antibacterial potential. Thus, silver appears as an attractive proposition in the field of nanotechnology. Silver NPs (Ag-NPs) can be synthesized using various methods. Although popular, chemical forms of synthesis can include the use of hazardous chemicals – causing its biosynthesis to be an eco-friendlier alternative. Biosynthesis can be done from microorganisms, enzymes, and plant extracts. Plant-based extracts are preferably used as they eliminate the elaborate need for maintaining cell cultures.

Azadirachta indica (commonly referred to as neem) is a tree that belongs to the family Meliaceae and is typically found in tropical and subtropical regions such as India, Nepal, Pakistan, and Bangladesh. Although its properties are not well documented, it can potentially be used to reduce dental plaque formation, stomach and intestinal ulcers, psoriasis, gingivitis, and diabetes.[11-13] *Aloe vera* is a succulent evergreen perennial that grows in tropical climates around the world. It is commonly known to have beneficial effects in the fields of medicine and cosmetology, but its effectiveness and safety has no solid evidence as of yet. It is currently being used to treat burns and wounds, psoriasis, herpes simplex, and constipation.[14] Both *A. indica* and *A. vera* are commonly available and easily affordable, making it a potentially better solution for DM when compared to the rising costs of analog insulin for the cost constrained.

In this current investigation, we have synthesized Ag-NPs using a formulation of *A. indica* and *A. vera,* and it was characterized by ultraviolet-visible (UV-vis) spectroscopy. Finally, the antidiabetic potential of the synthesized NPs was evaluated through an alpha-amylase assay.

MATERIALS AND METHODS

Preparation of the Plant Extract

Leaves of *A. indica* and *A. vera* were freshly collected from Chennai in November 2018 and were thoroughly washed 3–4 times in distilled water. They were then shade dried for 7–14 days. The well-dried leaves were powdered using a mortar and a pestle and were stored in airtight containers. Later, 1 g of the powdered *A. indica* was dissolved in distilled water and was boiled for 5–10 min at 60–70°C and the same was done using the powdered *A. vera*. The two solutions were then filtered using Whatman No. 1 filter papers. Finally, the filtered plant extracts were collected and stored at 4°C for further use.

Synthesis of NPs

About 1 mM of silver nitrate was dissolved in 80 ml/90 ml of double-distilled water. The plant extracts of *A. indica* and *A. vera* were added to this metal solution and were then made into a formulation of 100 ml. Its color change was visually observed and photographs were taken for systematic recording. The formulation was later kept in a magnetic stirrer/orbital shaker for the synthesis of its NPs.

Characterization of the Synthesized Ag-NPs

Primarily, the synthesis of Ag-NPs is characterized using UV-vis spectroscopy: 3 ml of the formulation was taken in a cuvette and was scanned in a UV-vis spectrometer under 300 nm–700 nm of wavelength. The results were recorded for its graphical analysis.

Preparation of the Ag-NP Powder

The Ag-NP solution was centrifuged in a refrigerated centrifuge (Lark). The centrifugation was done at around 8000 rpm for the duration of 10 min and the resultant pellet was collected and washed with distilled water twice. The final pellet was purified and dried at 60°C. In the end, the Ag-NP powder was collected and stored in an airtight tube (Eppendorf).

Study of Antidiabetic Activity of Ag-NPs

The inhibition of alpha-amylase was determined by measuring the amount of maltose liberated during the experiment which was adapted from that of Bhutkar and Bhise's.[15]

Different concentrations of Ag-NPs (50, 100, and 150 µL) were preincubated with 100 µL of alpha-amylase solution (1 U/mL) at room temperature for 30 min. About 100 µL of starch solution (1% w/v) was then added to it and the mixture was incubated at room temperature for 10 min. About 100 µL of 96 mM 3,5-dinitrosalicylic acid solution reagent was added to it to stop the reaction and the solution was heated in a water bath for 5 min. Control was maintained where equal quantities of the enzyme and extract were replaced by a sodium phosphate buffer maintained at a pH of 6.9. The reading was measured at 540 nm. The experiment was performed 3 times. Acarbose was used as a positive control.

The percentage of inhibition was calculated using the formula:

$$\% \text{ Inhibition}=\frac{C-T}{C}*100$$

Where,
C – Control
T – Test sample.

RESULTS AND DISCUSSION

Visual Observation

A color change was visually observed after the synthesis of NPs. The extract had turned a dark brown from its initial green color. This color changes from green to dark brown preliminarily confirms the presence of Ag-NPs Figure 1.

UV-vis Spectroscopy Analysis

The bioreduction of pure silver nitrate to Ag-NPs was characterized using UV-vis spectroscopy shown in Figure 2. This was carried out to oversee the formation and stability of the AgNPs. It was done using a UV-vis spectrometer under 300–700 nm of wavelength. The peak was seen at a wavelength of 425 nm.

Antidiabetic Activity

From the results of our study, it is clear that Ag-NPs inhibit the function of the enzyme alpha-amylase Figure 3. The latter is an enzyme that hydrolyzes alpha bonds of alpha-linked polysaccharides such as glycogen and starch to yield monosaccharides such as glucose and maltose.[16] Therefore, by preventing the action of this enzyme, we are indirectly inducing a therapeutic effect on DM by controlling the level of glucose in the blood.

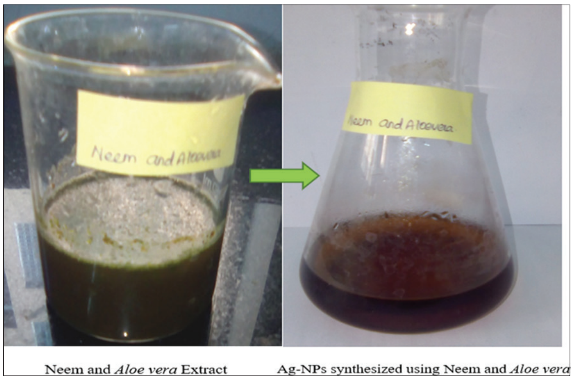

Figure 1: Visual observation

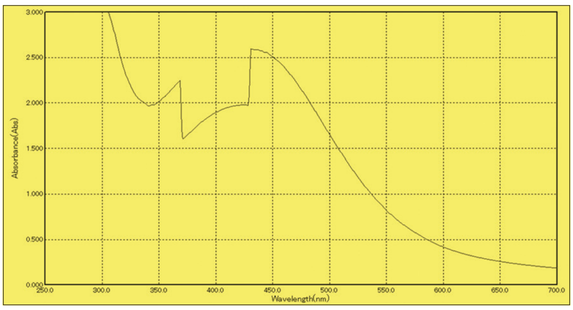

Figure 2: Ultraviolet-visible spectroscopy analysis

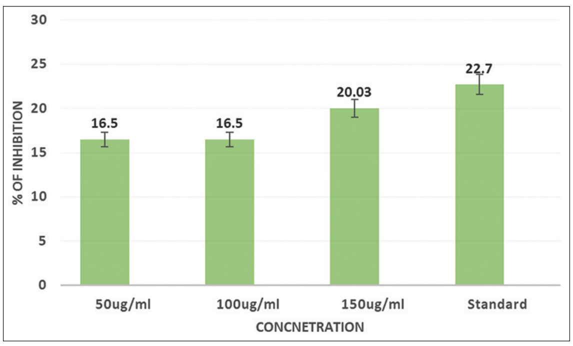

Figure 3: Antidiabetic activity

There is no increase in the amount of inhibition initially as seen with 50 and 100 µg/mL of Ag-NPs. Moreover, then, there is an increase in the level of alpha-amylase inhibition when 150 µg/mL of Ag-NPs are administered. This shows that Ag-NPs synthesized using neem and *A. vera* produce an antidiabetic efficacy that is constant at first until it crosses a certain concentration threshold and then it increases. An inhibitory percentage of up to 20.03% is seen with 150 µg/mL of Ag-NPs which are in positive correlation with the effect brought about by the standard acarbose. Acarbose is an alpha-glucosidase inhibitor that is commonly used in China. It showed 22.7% inhibition which is close to that of our Ag-NPs. Similar studies have been done using Ag-NPs with other mediators such as lemongrass and *Punica granatum*.[17,18]

CONCLUSION

The results of the assay showed that 150 µg of our Ag-NPs exhibited 20.03% alpha-amylase inhibition which is close to that of acarbose (22.7%) with a variation of only 2.67%. Ag-NPs are eco-friendly, easy to synthesize, and cheap. They are also established sources of other additional benefits such as antibacterial, antifungal, antiviral, and antioxidant properties. Thus, Ag-NPs could be used as a possible alternative for conventional antidiabetic drugs.

REFERENCES

1. World Health Organisation. The Top 10 Causes of Death. Available from http://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death. [Last retrieved on 2018 May 24].
2. World Health Organisation. Diabetes. Available from https://www.who.int/news-room/fact-sheets/detail/diabetes. [Last retrieved on 2018 Oct 30].
3. Agarwal H, Kumar SV, Rajeshkumar S. A review on green synthesis of zinc oxide nanoparticles an eco-friendly approach. Resour Efficient Technol 2017;3:406-13.
4. Santhoshkumar J, Kumar SV, Rajeshkumar S. Phyto-assisted synthesis, characterization and applications of gold nanoparticles a review. Biochem Biophys Rep 2017;11:46-57.
5. Menon S, Rajeshkumar S, Kumar SV. A review on biogenic synthesis of gold nanoparticles; characterization and its applications. Resour Efficient Technol 2017;3:516-27.
6. Rajeshkumar S, Naik P. Synthesis and biomedical applications of cerium oxide nanoparticles a review. Biotechnol Rep 2017;17:1-5.
7. Agarwal H, Menon S, Kumar SV, Rajeshkumar S. Mechanistic study on antibacterial action of zinc oxide nanoparticles synthesized using green route. Chem Biol Interact 2018;286:60-70.
8. Rajeshkumar S, Bharath LV. Mechanism of plant-mediated synthesis of silver nanoparticles a review on biomolecules involved, characterisation and antibacterial activity. Chem Biol Interact 2017;273:219-27.
9. Rajeshkumar S. Green synthesis of different sized antimicrobial

silver nanoparticles using different parts of plants a review. Int J Chem Tech Res 2016;9:197-208.
10. Rajeshkumar S. Phytochemical constituents of fucoidan (*Padina tetrastromatica*) and its assisted AgNPs for enhanced antibacterial activity. IET Nanobiotechnol 2017;11:292-9.
11. Halim EM. Lowering of blood sugar by water extract of *Azadirachta indica* and *Abroma augusta* in diabetes rats. Indian J Exp Biol 2003;41:636-40.
12. Bandyopadhyay U, Biswas K, Sengupta A, Moitra P, Dutta P, Sarkar D, *et al.* Clinical studies on the effect of neem (*Azadirachta indica*) bark extract on gastric secretion and gastroduodenal ulcer. Life Sci 2004;75:2867-78.
13. Pandey S, Jha A, Kaur V. Aqueous extract of neem leaves in treatment of psoriasis vulgaris. Indian J Dermatol Venereol Leprol 1994;60:63-7.
14. Aloe. Available from: https://www.drugs.com/mca/aloe. [Last retrieved on 2017 Oct 17].
15. Bhutkar MA, Bhise SB. *In vitro* assay of alpha amylase inhibitory activity of some indigenous plants. Int J Chem Sci 2012;10:457-62.
16. Pugh MB. Stedman's Medical Dictionary. 27th ed. Baltimore, Maryland, USA: Lippincott Williams and Wilkins; 2000. p. 65.
17. Agarwal H, Kumar V, Shanmugam R. Antidiabetic effect of silver nanoparticles synthesized using lemongrass (*Cymbopogon citratus*) through conventional heating and microwave irradiation approach. J Microbiol Biotechnol Food Sci 2018;7:371-6.
18. Saratale RG, Shin HS, Kumar G, Benelli G, Kim DS, Saratale GD, *et al.* Exploiting antidiabetic activity of silver nanoparticles synthesized using *Punica granatum* leaves and anticancer potential against human liver cancer cells (HepG2). Artif Cells Nanomed Biotechnol 2018;46:211-22.

Source of support: Nil; Conflict of interest: None Declared