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In Vitro Study Of Anti-Diabetic Effect Of Broccoli Mediated Selenium Nanoparticles

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ABSTRACT

The SeNPs have paved their way into the field of biomedicine by serving as an anticarcinogenic agent, antidiabetic agents, antioxidant agent, or as a nutritional supplement, also as a drug supplement against AIDS. Broccoli accumulates Se many-fold beyond the concentration of Se in the soil. The present investigation aims to evaluate the possible antidiabetic effect of selenium nanoparticles mediated by broccoli. Preparation of nanoparticles powder was done by centrifuging the NP's solution at 8000 rpm for 10 minutes, then collected, washed twice with distilled, water dried at 600 C and stored. SeNP concentrations of 10,20,30,40 and 50 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. The current study demonstrated results in which Se-

NPs had an antidiabetic effect close to that of the standard used. 57.2% of alpha amylase enzyme inhibition was seen at 20µl concentration which was significantly comparable to the standard acarbose used. From the current study we can confer that Selenium nanoparticles has an inhibitory effect on alpha amylase and this inadvertently has a therapeutic effect on Diabetes Mellitus by controlling the blood glucose level. SeNPs synthesized using broccoli have an evident antidiabetic potential. They show a considerably good amount of alpha-amylase inhibition with respect to acarbose. Thus, SeNPs could provide a possible alternative for conventional antidiabetic drugs in the future.

KEYWORDS: anti-diabetic; broccoli;green synthesis; in vitro; innovative technique; selenium nanoparticles

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder in which the metabolism of carbohydrate, fat and protein is affected. It is mainly characterized by hyperglycemia, where elevated blood sugar levels are seen either due to the lack of production of insulin by the pancreas or due to the improper response of the cells of the body to the insulin produced. Some long-term complications of Diabetes mellitus include heart disease, stroke, dysfunction and failure of various organs [1,2]. Diabetes can occur in both genders and at any age. There are three main types of diabetes, type 1, type 2, and gestational diabetes. Type 1 diabetes can only be treated by the substitution of insulin and type 2 can be treated by a number of therapeutic methods. Some conventional methods of treatment for diabetes include the reduction of the insulin demand, stimulating secretion of endogenous insulin, enhancing insulin potential at the target tissues and preventing degradation of oligosaccharides and disaccharides [3]. The major types of drugs involved in the management of type 2 diabetes the inhibitors of enzymes α-glucosidase and α-amylase. The inhibitory action of these enzymes prevents breakdown of starch into simpler sugars and delays the absorption rate of glucose and thus, leads to a decrease in blood glucose level [3,4]. Inhibitors of glycosidases currently in clinical use are acarbose and miglitol that inhibit α-glucosidase and α-amylase and voglibose, an inhibitor of α-glucosidase. However, synthetic hypoglycemic agents have limitations such as they are non-specific, side effects like bloating, abdominal discomfort, diarrhea and flatulence and failure to elevate diabetic complications [5,6].

Nanomedicine refers to the application of nanotechnology based techniques and methods for the purpose of medical research and clinical practice that aids in treatment, diagnosis, monitoring and control of biological systems [7]. Nanoparticles (NPs) provide a solution for many of the existing biopharmaceutical and pharmacokinetic (PK) problems that are associated with many drugs used currently in treatment of various diseases. They possess the ability to boost the therapeutic potential of ionised drugs and improve the penetration of water soluble compounds, proteins, peptides, vaccines, siRNA, miRNA, DNA etc. The drug delivery system can also be made versatile by surface modification of nanoparticles with targeting ligands , thus enabling them to selectively deliver the drug at target site [8]. The field of nanomedicine and nanoparticles in particular, have gained popularity owing to their targeted delivery, excellent biological activity and bioavailability, low toxicity and dual functionality in terms of therapy and diagnosis [9]. The nanoparticles can be engineered according to the desired size, gene and drug loading capacity and controlled release [10]. Research indicates the use of nanoparticles in treatment of diabetes [2], such as silver nanoparticles which are reported to have an anti-diabetic effect [11]. Studies have also reported the anti-hyperglycemic activity of phyto-derived extracts and nanoparticles [12–18].

The attention of nanotechnologists on selenium and its beneficial effects are increasing at a fast rate. Plant-based synthesis provides an advantage as they do so without adding additional stabilizing and capping agents to prepare nanoparticles and they thus possess excellent biocompatible nature [19–21]. Plant-based SeNPs can be one promising alternative strategy to tackle diabetes because of its non-toxic nature. SeNPs derived from plant sources are considered superior in comparison to other metal nanoparticles, such as platinum, silver, and gold due to their biocompatibility and degradability potential in vivo. Their unique optical and photoelectric properties have made the phyto-fabricated SeNPs as a novel therapeutic form of drug delivery vehicles [22,23]. Our team has extensive knowledge and research experience that has translate into high quality publications[24–33]. Studies have reported the anticancer, antiviral, antioxidant roles of SeNPs in drug therapy, however there is a lack of evidence regarding the anti-diabetic effect of SeNP. The aim of the current study is to investigate the antidiabetic potential of selenium nanoparticles derived from broccoli in vitro.

METHODOLOGY

Broccoli extracts preparation

Broccoli florets were collected fresh and washed with running tap water and double washed with Milli-Q water and left to dry at room temperature. They were then cut into small pieces using a sterile scalpel. 10 g of broccoli was crushed using mortar and pestle and mixed with 100 mL of Milli-Q water and this solution was heated at 80°C. This was later filtered using Whatman filter paper no.1. Re-filtration of the solution was performed using Whatman filter paper of smaller pore size. This filtrate was used as an extract.

Selenium nanoparticle formation

Preparation of nanoparticles powder began by centrifuging the NP's solution at 8000 rpm for 10 minutes. The pellet was collected, washed twice with distilled, water dried at 600 C and stored.

Antidiabetic effect determination

Different concentrations of selenium nanoparticles such as 20, 40, 60, 80, 100 µL were pre-incubated with 100 µL of αamylase solution (1 U/mL) for 30 minutes at room temperature. This was followed by addition of 100 µL of starch solution (1% w/v) to the mixture and then incubated at room temperature for 10 minutes. DNSA reagent (3, 5- dinitrosalicylic acid solution) was added at a concentration of 100 µL of 96 mM to stop the reaction. The solution was then heated in a water bath for 5 minutes. Acarbose was used as a positive control and was maintained such that equal quantity of enzyme extract was replaced by sodium phosphate buffer which was maintained at a pH of 6.9. Reading was measured at 540 nm. The experiment was performed in triplicate. % inhibition was calculated using the formula: % inhibition = $C - TC * 100$ (Where, C= control, T= test sample)

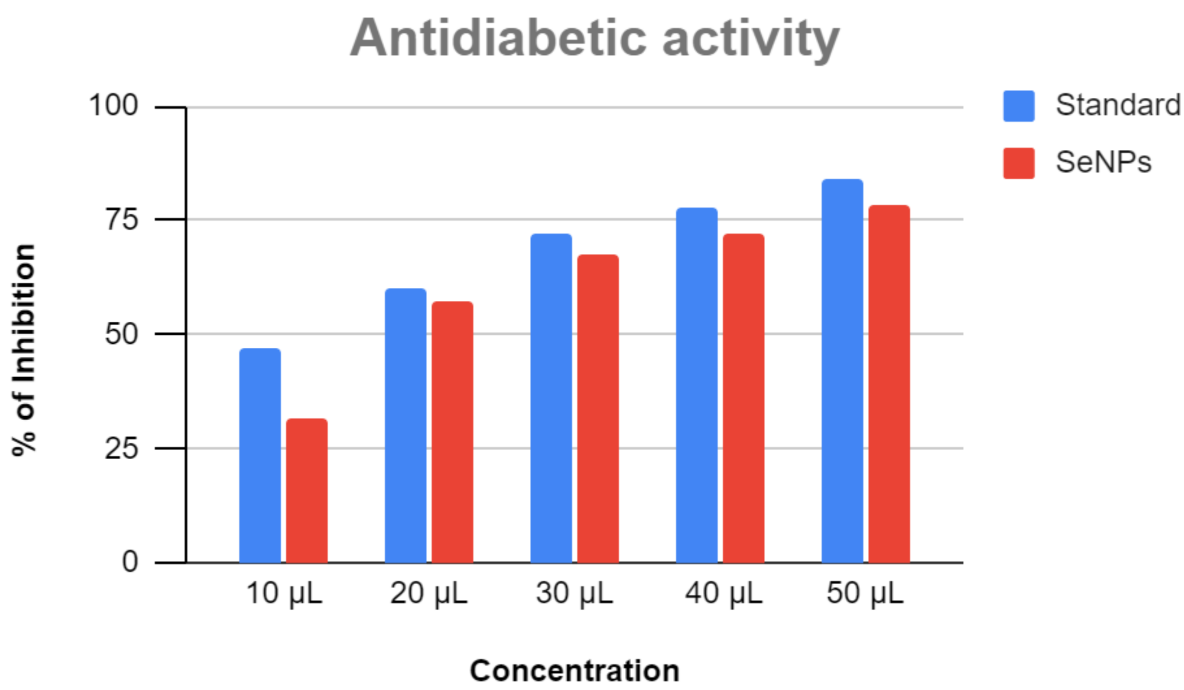
Data was collected and tabulated for both control and SeNPs at various concentrations.

RESULTS

A color change was observed after the synthesis of SeNPs. The prepared particles were then characterized by a peak seen at 540 nm in ultraviolet-visible spectroscopy. The results of the assay showed inhibitory effects close to that of the standard. The highest % inhibition was seen at 50 µL with 78.4% followed by 72% at 40 µL and 67.4% at 30 µL (table 1). The closest % inhibition was seen at 20 µL with 57.2% and acarbose was 60% (graph 1). Results are tabulated in Table 1.

S.No	Concentration	Wavelength	ABS	Standard	SeNP
1.	10 µL	540 nm	0.28	47	31.8
2.	20 µL	540 nm	0.326	60	57.2
3.	30 µL	540 nm	0.682	72	67.4
4.	40 µL	540 nm	0.216	78	72
5.	50 µL	540 nm	0.428	84	78.4

Table 1: Table representing % inhibition of standard (acarbose) and SeNP at various concentrations



Graph 1: Bar graph represents antidiabetic activity. X-axis represents concentration and Y-axis represents the % inhibition. Color blue denotes standard (acarbose) and color red denotes SeNP.

DISCUSSION

Currently, Diabetes mellitus (DM) has reached epidemic dimensions across the globe and still remains a great challenge in treatment owing to its pathological complexity. The increased incidence of DM places a heavy burden on the individual and the society. Studies proven that phytomedicines and natural medicines have prominent antidiabetic effects [34]. Phyto-derived nanoparticles are a promising candidate as an alternative to conventional therapy for a number of diseases. The methods generally used in the production of nanoparticles on a large scale employ toxic chemicals, are time consuming and uneconomical. Thereby, a biogenic protocol is followed to manufacture nanoparticles from plant extracts [35,36]. In this study Selenium nanoparticles were obtained from broccoli.

SeNPs had a comparable % inhibitory effect to acarbose which was used as a control in this study (graph 1). However, SeNPs did not prove to be better than the standard. At no concentration did SeNPs demonstrate an % inhibition greater than or equal to acarbose (table 1). The inhibitory potential of SeNPs increased with increased concentration with the highest seen at 50 µL with inhibition of 78.4%.

The closest SeNPs performed compared to acarbose was at 20 µL with SeNPs inhibition at 57.2% and acarbose at 60%.

A number of studies exist which prove the anticancer and antioxidant potential of SeNP[19,37]. However, studies reporting the antidiabetic effects of selenium are rare. Some studies have reported the synergistic effect of selenium when used alongside traditional drugs and have shown increased efficacy of the drugs [38]. Other studies indicate the use of SeNPs as an oral hypoglycemic drug delivery system [34]. One study reported favourableantidiabeticpotency of selenium nanoparticles that were delivered in liposomes as it preserves the integrity of pancreatic β cells and results in increase in insulin secretion and consequent depletion of glucose [39]. The present study suggests that SeNPs possess promising potential to be employed as a hypoglycemic drug in treatment of Diabetes, as the results are comparable with acarbose and synthesis of SeNPs are easy and economical. However, to accurately determine their efficacy in the treatment of diabetes, large scale studies and in vivo studies can be conducted in the future.

CONCLUSION

SeNPs synthesized using broccoli have an evident antidiabetic potential. Apart from being easy to synthesize, eco-friendly, and inexpensive, they displayed a considerably good amount of alpha-amylase inhibition with respect to acarbose. Thus, we can conclude within the limits of the present study that SeNPs could be employed as an alternative to conventional antidiabetic drugs. Large scale studies could further provide substantial evidence for the antidiabetic potential of selenium nanoparticles.

SOURCE OF FUNDING

The present project is funded by the following agencies

- Saveetha Institute of Medical and Technical Sciences
- Saveetha Dental College and Hospitals
- Saveetha University
- Prompt paper products private LTD.

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