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In Vitro Study Of Antidiabetic Effect Of Green Synthesised Titanium Dioxide Nanoparticles

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ABSTRACT:

Introduction: Nanoparticles have been defined by others as objects with at least one of their three dimensions in the 1–100 nm range. Titanium (Ti), the earth's tenth most common element, is widely dispersed. The use of nanoparticles in drug delivery systems, antimicrobial materials, cosmetics, sunscreens, and electronics has increased dramatically as a result of the development of nanotechnology. Diabetes mellitus is defined as a group of metabolic disorders characterized by high blood glucose levels (hyperglycemia). Neem is widely utilised in Ayurveda, Unani, and Homoeopathic treatment, and has become a modern medical cynosure. The therapeutic properties of neem leaf have been documented in detail. Immunomodulatory, anti-inflammatory, antihyperglycemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic, and anticarcinogenic effects have been demonstrated in neem leaf and its compounds. The aim of this study is to determine the antidiabetic effect exhibited by neem mediated Titanium dioxide nanoparticles.

Materials and Method: 40 ml of 1mM Titanium dioxide solution was mixed with 40 ml of Diluted Neem extract (2 ml of extract in 80 ml of Distilled Water). This solution was kept on a magnetic stirrer for 24 hours for nanoparticle formation. The synthesized nanoparticles were preliminarily confirmed with UV-vis Spectroscopy. The nanoparticles powder was collected by dry heating the solution at 70 degrees Celsius. The Antidiabetic effect was determined using Alpha Amylase inhibitory assay. The α-amylase (0.5 mg/mL) was premixed with extract at various concentrations (20-100 µg/ml) and starch as a substrate was added as a 0.5% starch solution to start the reaction. The reaction was carried out at 37°C for 5 min and terminated by addition of 2 mL of DNS (3,5-dinitrosalicylic acid) reagent. The reaction mixture was heated for 15 min at 100°C and diluted with 10 mL of distilled water in an ice bath. α- amylase activity was determined by measuring spectrum at 540 nm. The % α-amylase inhibitory activity is calculated by the following formula % Inhibition = (Control OD-Sample OD / Control OD) × 100. The IC50 value was defined as the concentration of the sample extract to inhibit 50% of α-amylase activity under assay conditions. The results were tabulated and graphically represented.

Results and Discussion: A color change was visually observed after the synthesis of NPs. The extract had turned a white from its initial green color. This color change from green to dark brown preliminarily confirms the presence of TiO2-NPs. The bioreduction of TiO2 to NPs was characterized using UV-vis spectroscopy. This was carried out to oversee the formation and stability of the NPs. It was done using a UV-vis spectrometer under 300–700 nm of wavelength. The peak was seen at a wavelength of 425 nm. From the results of our study, it is clear that TiO2 -NPs inhibit the function of the enzyme alpha-amylase. There is an increase in the level of alpha-amylase inhibition when 50 µg/mL of TiO2-NPs are administered. This shows that TiO2-NPs synthesized using neem 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 97.2% is seen with 50 µg/mL of TiO2-NPs which are in positive correlation with the effect brought about by the standard acarbose.

Conclusion: The results of the assay showed that 50 µg of our TiO2-NPs exhibited 97.2% alpha-amylase inhibition which is close to that of acarbose (84%). TiO2-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, TiO2-NPs could be used as a possible alternative for conventional antidiabetic drugs.

INTRODUCTION:

The use of nanoparticles in drug delivery systems, antimicrobial materials, cosmetics, sunscreens, and electronics has increased dramatically as a result of the development of nanotechnology(1). Nanomaterials were defined by the European Union in October 2011 as a natural, accidental, or produced material containing particles in an unbound state or as an aggregate or agglomeration, with 50% or more of the particles having one or more exterior dimensions in the size range 1–100 nm. Nanoparticles have been defined by others as objects with at least one of their three dimensions in the 1–100 nm range(2). Titanium (Ti), the earth's tenth most common element, is widely dispersed. Ti has an average density of 4400 mg/kg in the earth's crust. Ti does not exist in the metallic state in nature due to its high affinity for oxygen and other elements. Titanium dioxide has been increasingly popular in industrial and consumer applications in recent years(3). Their reduced sizes, which allowed for more surface area per unit mass, have been credited with the increase in catalytic activity. Concerns have been expressed that Titanium dioxide nanoparticles may have a unique bioactivity and pose a threat to human health. TiO2 is a white pigment that is commonly used due to its brightness and high refractive index. This pigment is consumed in the amount of four million tonnes per year all over the world(4). Furthermore, TiO2 is one of the top five Nanoparticles used in consumer products, accounting for 70% of total pigment production volume worldwide. Paints, coatings, plastics, papers, inks, medicines, pharmaceuticals, food, cosmetics, and toothpaste all contain TiO2(5). It can even be used to whiten skim milk as a colour. Sunscreens also contain TiO2 nanoparticles. Furthermore, TiO2 has long been employed as an articulating component in prosthetic implants, particularly for the hip and knee(6).TiO2 Nanoparticles are being studied in the field of nanomedicine as potential instruments for enhanced imaging and nanotherapeutics. TiO2 Nanoparticles, for example, are being studied as potential photosensitizers for photodynamic treatment (PDT). TiO2 Nanoparticles are also suited for usage in a variety of skin care products due to their unique physical features(7). Acne vulgaris, recurrent condylomaaccuminata, atopic dermatitis, hyperpigmented skin lesions, and other non-dermatologic illnesses are all being studied with nano-preparations containing TiO2 nanoparticles. Under UV light irradiation, TiO2 Nanoparticles also display antimicrobial capabilities(8).

Diabetes mellitus is defined as a group of metabolic disorders characterized by high blood glucose levels (hyperglycemia). Advances in Nanotechnology have aided in Diagnosis, Disease monitoring, Glucose sensors and Insulin delivery. While it is still a developing field, numerous innovations have aided in faster diagnosis and prompt treatment of diabetes mellitus(9).

Our team has extensive knowledge and research experience that has translate into high quality publications(10–18),(19–24),(25–29)

Because of its extensive spectrum of medical benefits, Azadirachtaindica, also known as neem, has gained worldwide reputation in recent years. Neem is widely utilised in Ayurveda, Unani, and Homoeopathic treatment, and has become a modern medical cynosure. Neem produces a wide range of physiologically active chemicals that are both chemically and structurally varied. From various portions of the neem plant, more than 140 chemicals have been identified. The leaves, blossoms, seeds, fruits, roots, and bark of the neem tree have long been used to cure inflammation, infections, fever, skin ailments, and dental problems. The therapeutic properties of neem leaf have been documented in detail. Immunomodulatory, anti-inflammatory, antihyperglycemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic, and anticarcinogenic effects have been demonstrated in neem leaf and its compounds(30).

The aim of this study is to determine the antidiabetic effect exhibited by neem mediated Titanium dioxide nanoparticles.

MATERIALS AND METHOD:

Preparation of the Plant Extract:

In January 2021, A. indica leaves were newly gathered from Chennai and carefully cleaned 3–4 times in distilled water. They were then dried in the shade for 7–14 days. The well-dried leaves were crushed and kept in airtight containers using a mortar and pestle. After that, 1 g of powdered A. indica was dissolved in distilled water and heated at 60–70°C for 5–10 minutes. After that, the solution was filtered through Whatman No. 1 filter sheets. Finally, the filtered plant extracts were collected and kept at 4°C until needed.

Synthesis of NPs:

Titanium dioxide was dissolved in 80 ml/90 ml of double-distilled water at a concentration of around 1 mM. The A. indica plant extracts were mixed with the metal solution and formed into a 100 mL formulation. Its colour change was seen visually, and images were taken for meticulous documentation. For the synthesis of its NPs, the formulation was maintained in a magnetic stirrer/orbital shaker.

Preparation of the Ti-NP Powder:

In a chilled centrifuge, the Ti-NP solution was centrifuged (Lark). The centrifugation was carried out at 8000 rpm for 10 minutes, and the resulting pellet was collected and washed twice with distilled water. At 60°C, the final particle was filtered and dried. The Ti-NP powder was collected and stored in an airtight tube at the end (Eppendorf).

Study of Antidiabetic Activity of Ti-NPs:

The amount of maltose released during the experiment, which was developed from Bhutkar and Bhise's, was used to determine alpha-amylase inhibition. For 30 minutes, different concentrations of Ti-NPs (10, 20, 30, 40, and 50 L) were pre- incubated at room temperature with 100 L of alpha-amylase solution (1 U/mL). The mixture was then incubated at room temperature for 10 minutes with 100 L of starch solution (1 percent w/v). To stop the reaction, 100 L of 96 mM 3,5-dinitrosalicylic acid solution reagent was added, and the solution was heated in a water bath for 5 minutes. Equal amounts of enzyme and extract were replaced with a sodium phosphate buffer maintained at a pH of 6.9 as a control. At 540 nm, the reading was taken. The experiment was carried out three times. As a positive control, acarbose was used. The percentage of inhibition was calculated using the formula: % Inhibition= C-T/C *100 − Where, C – Control T – Test sample.

The results were tabulated and graphically represented.

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 change from green to dark brown preliminarily confirms the presence of Ti-NPs(31).

Antidiabetic Activity:

From the results of our study, it is clear that Ti-NPs inhibit the function of the enzyme alpha-amylase. Alpha amylase is an enzyme that hydrolyzes alpha bonds of alpha-linked polysaccharides such as glycogen and starch to yield monosaccharides such as glucose and maltose(32). Therefore, by inhibiting the action of this enzyme, there is an indirect therapeutic effect in Diabetes Mellitus by controlling the level of glucose in the blood(33). There is no increase in the amount of inhibition initially as seen with 10 and 20 µg/mL of TiO2 NPs.

Moreover, then, there is an increase in the level of alpha-amylase inhibition when 50 µg/mL of TiO2-NPs are administered. This shows that TiO2-NPs synthesized using neem 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 97.2% is seen with 50 µg/mL of TiO2-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 84% inhibition which is close to that of our TiO2-NPs. Similar studies have been done using Ag-NPs with other mediators such as lemongrass and Punicagranatum(34).

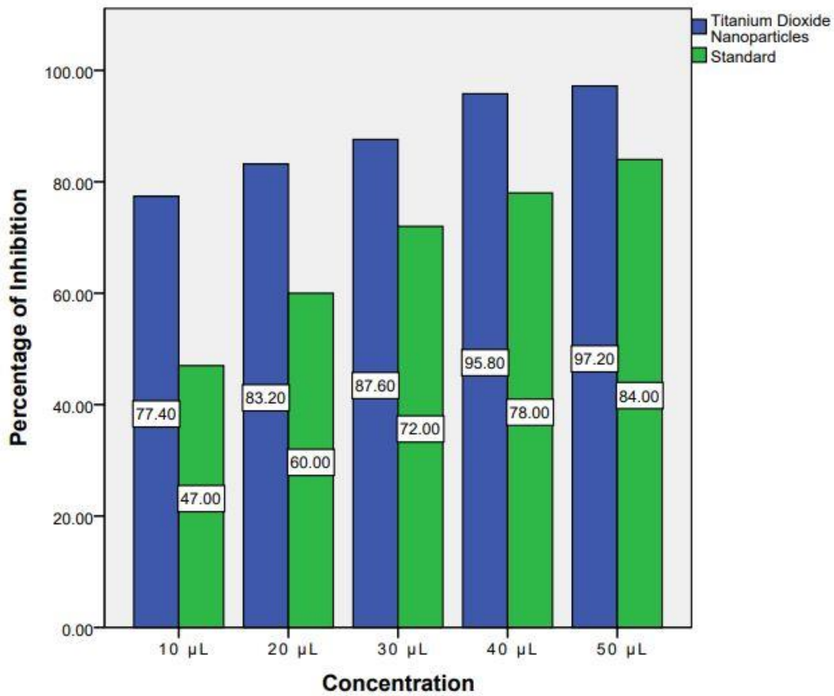

Fig. 1: Bar graph showing the Percentage of inhibition of Alpha Amylase by the standard and Titanium Dioxide nanoparticles. X axis represents the concentration of the solutions and Y axis represents the percentage of inhibition. Titanium dioxide nanoparticles (blue) are consistently producing a satisfactory amount of inhibition when compared to the standard (green).

CONCLUSION:

The results of the assay showed that 50 µg of our Ag-NPs exhibited 87.73% alpha-amylase inhibition which is close to that of acarbose (88.7%). Ti-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, Ti-NPs could be used as a possible alternative for conventional antidiabetic drugs.

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AUTHOR CONTRIBUTIONS:

Samyuktha P S contributed to nanoparticle synthesis, analysis and interpretation and drafting of the article. Rajeshkumar contributed to the nanoparticles synthesis, characterisation, anti-diabetic assay and interpretation of the results. Dhanraj contributed to the critical revision of the article.

CONFLICT OF INTEREST:

No potential conflict of interest relevant to this article was reported.

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