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Green-Synthesized Silver Nanoparticles from Green Tea and Papaya Leaf Extracts: Potential for Anti-Diabetic Activity

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ABSTRACT

Introduction

Green tea and papaya leaves have been reported to be rich sources of antioxidants which contain active compounds like polyphenols, caffeine, minerals, and vitamins. Green tea, in particular, is well-known for its catechins content, which have been used to regulate blood sugar levels. Conversely, papaya leaf extract offers antimicrobial, antioxidant, and antiviral properties, and its ability to enhance insulin production contributes to its effectiveness in lowering blood sugar levels. Therefore, the aim of the present study is to explore the therapeutic potential of silver nanoparticles synthesized using extracts from green tea and papaya leaves for the treatment of type 2 diabetes mellitus and its associated complications.

Methods

In this study, silver nanoparticles were synthesized using aqueous extracts of green tea and papaya leaves. These nanoparticles were evaluated for their ability to inhibit the two key carbohydrate metabolizing enzymes, namely α-amylase and α-glucosidase. Additionally, the impact of silver nanoparticles on diabetes associated complications such as retinopathy and nephropathy were also examined. Specifically, the inhibitory effects of the synthesized silver nanoparticles on sorbitol accumulation and the formation of advanced glycation end products (AGEs) were assessed.

Results

The anti-diabetic effect of the synthesized silver nanoparticles (PL-GT-Ag NPs) and standard acarbose was evaluated at different concentrations of 5 µg/ml-160 µg/ml. The results showed that the silver nanoparticles showed marked inhibition against α-amylase and α-glucosidase with a maximum inhibition of 80% and 78% respectively at 160 µg/ml. A maximum inhibition of 88% and 84% was observed for standard acarbose at a similar concentration of 160 µg/ml. The aldolase reductase activity was dose-dependently inhibited by different concentrations of silver nanoparticles with minimum inhibitory percentage of 1.13% at 5 µg/ml to maximum inhibition of 78.93% at 160 µg/ml. Similarly advanced glycation end products (AGEs) were also inhibited in a concentration-dependent manner.

Conclusion

Overall, this study underscores the promising, enduring anti-diabetic effects of silver nanoparticles derived from green tea and papaya extracts, hinting at their potential as a therapeutic approach for diabetes treatment.

Moreover, the silver nanoparticles were found to be effective in reducing sorbitol accumulation and inhibiting AGE formation, indicating their potential in addressing diabetes-related complications.

Keywords:*Antioxidants, anti-diabetic, amylase, glucosidase, diabetic complications, catechins, polyphenols.*

INTRODUCTION

Diabetes mellitus encompasses a collection of metabolic disorders associated with abnormal glucose and lipid metabolism [1]. Among the three types of diabetes mellitus, namely Type 1 diabetes mellitus (T1DM), Type 2 diabetes mellitus (T2DM), and Gestational diabetes, T2DM is the most prevalent, accounting for over 90% of all cases, while the other two types are less common [2, 3]. T1DM typically has a sudden onset, whereas T2DM develops gradually. T2DM, also known as non-insulin dependent diabetes mellitus or adult-onset diabetes, is a complex chronic disorder, and approximately 30% of individuals with T2DM remain undiagnosed. It is characterized by disturbances in carbohydrate, lipid, and protein metabolism, leading to elevated blood sugar levels [2]. The condition arises from impaired insulin secretion, insulin resistance, or a combination of both. Various factors contribute to the rising prevalence of T2DM, including lifestyle, environmental toxins, diet, obesity, and genetic factors. The primary cause is a gradual decline in insulin secretion by the pancreatic β cells, often due to pre-existing insulin resistance in skeletal muscle, liver, and adipose tissue [3].

Postprandial hyperglycemia, which refers to high blood sugar levels after eating, plays a significant role in the development of T2DM. Effectively managing plasma glucose levels is essential in delaying or preventing the onset of T2DM [4]. One therapeutic approach to reducing postprandial hyperglycemia involves the use of medications or dietary strategies that can block carbohydrate-digesting enzymes like α-amylase and α-glucosidase, thereby slowing down the generation or absorption of glucose [5]. While the primary focus of treatment currently revolves around insulin secretagogues and sensitizers, the use of inhibitors targeting enzymes involved in carbohydrate digestion is gaining prominence in managing hyperglycemia by reducing the uptake of glucose in the intestines [6]. Although various classes of antidiabetic drugs are available in the market, their use is often associated with undesirable side effects, including diarrhea and other gastrointestinal issues like bloating, flatulence, cramps, and abdominal discomfort [7]. As a result, efforts have been made to discover substances that can impede the activity of enzymes responsible for carbohydrate breakdown.

Furthermore, chronic hyperglycemia over the long term can lead to microvascular complications such as diabetic neuropathy, nephropathy, and retinopathy. Research has illuminated the crucial role played by the interaction between Advanced Glycation End-products (AGEs) and their corresponding receptor, known as RAGE, in the emergence of vascular complications associated with diabetes [8]. AGEs also significantly influence the advancement of diabetic retinopathy, ultimately causing a decline in retinal cell function and their demise [9]. Besides, in the insulin-resistant tissues of individuals with diabetes, such as the lens and retina, elevated sorbitol levels are generated due to increased glucose availability through the polyol pathway [10]. Aldose reductase (AR), serving as the initial enzyme in the polyol pathway, catalyzes the conversion of excess D-glucose into D-sorbitol, concurrently converting NADPH in to NADP +. The initial accumulation of sugar alcohols primarily occurs in the lens epithelium, ultimately contributing to the development of cataracts [11]. Consequently, the development of pharmacological compounds capable of inhibiting AGEs formation and sorbitol accumulation could represent a promising therapeutic breakthrough in preventing complications associated with diabetes [12].

In recent years, nanotechnology has emerged as a highly versatile field with broad applications in various disciplines such as environmental remediation, agriculture, chemicals, and pharmaceuticals. Green synthesis refers to the environmentally friendly production of nanoparticles using plant extracts or other natural sources. This approach offers numerous advantages over conventional synthesis methods, such as being cost-effective, sustainable, and free from toxic chemicals [13, 14]. Among the diverse range of nanoparticles, silver nanoparticles (AgNPs) are particularly well-suited for clinical applications due to their distinctive properties [15, 16]. Several medicinal plants in India have been utilized to synthesize silver nanoparticles [17-19]. Green tea is known for its potential to have anti-diabetic effects. It has been linked to various benefits in managing diabetes, along with its other biological activities [20]. Green tea has been found to positively influence the regulation of blood sugar and insulin sensitivity [21, 22]. Research suggests that regular consumption of green tea may reduce the risk of developing type 2 diabetes, improve glycemic control, and lower the incidence of diabetic complications [23].

Carica papaya leaves have also been studied for their potential in managing diabetes. Extracts from these leaves have shown properties that could assist in diabetes management [24, 25]. Based on this background, this study aims to examine the synergistic effect of green tea and papaya leaf extract-assisted silver nanoparticles on enzymes involved in the onset of diabetes and its associated complications.

MATERIALS AND METHODS

α- amylase inhibitory activity

In vitro amylase inhibition was studied by the method of Wickramaratne et al. 2016 [26]. Briefly, 100µL of different concentrations (5, 10, 20, 40, 80 & 160µg/ml) of papaya leaf and green tea extracts mediated silver nanoparticles (PL-GT-Ag NPs) was incubated with 200µL of α-amylase enzyme (Hi-media RM-638) and 100µL of 2mM of phosphate buffer (pH-6.9). After 20-min incubation, 100µl of 1% starch solution was added. The same was performed for the controls where 200µl of the enzyme was replaced by a buffer. After incubation for 5 min, 500µl of Dinitrosalicylic acid reagent was added to both control and test. They were kept in a boiling water bath for 5 min. The absorbance was recorded at 540 nm using spectrophotometer and the percentage inhibition of α-amylase enzyme was calculated using the formula:

$$\% \text{ inhibition} = [(\text{Control-Test})/\text{Control}] * 100$$

Suitable reagent blank and inhibitor controls were simultaneously carried out.

α-glucosidase inhibitory activity

The enzyme inhibition activity for α-glucosidase was evaluated according to the method previously reported by Sanchet*iet al*. (2011) [27] with minor modifications. The reaction mixture consisted of 50µL of 0.1M phosphate buffer (with pH of 7.0), 25µL of 0.5mM 4-nitrophenyl α-D-glucopyranoside (dissolved in 0.1M phosphate buffer, with pH of 7.0), 50µL of different concentrations (5, 10, 20, 40, 80 & 160µg/ml) of PL-GT-Ag NPs and 25µL of α-glucosidase solution (a stock solution of 1mg/mL in 0.01M phosphate buffer, with pH of 7.0 was diluted to 0.1Unit/mL with the same buffer, with pH of 7.0 just before assay). This reaction mixture was then incubated at 37°C for 30 min. Then, the reaction was terminated by the addition of 100µL of 0.2M sodium carbonate solution. The enzymatic hydrolysis of the substrate was monitored by the amount of p-nitrophenol released in the reaction mixture at 410 nm using a microplate reader. Individual blanks were prepared for correcting the background absorbance, where the enzymes were replaced with buffers. Controls were conducted in an identical manner replacing the plant extracts with methanol. Acarbose was used as positive control. All experiments were carried out in triplicates.

Advanced Glycation end product (AGE) assay [28]

Advanced glycation end products (AGEs) are formed by non-enzymatic glycosylation of proteins that enhance vascular permeability in both micro and macro vascular structures by binding to specific macrophage receptors. The silver nanoparticles were evaluated for its activity on AGEs formation. AGE reaction mixture was constituted as follows; 1mg/mL bovine serum albumin in 50mM sodium phosphate buffer (pH 7.4) and 0.02% sodium benzoate into 0.2M fructose and 0.2M glucose. The reaction mixture (2.75mL) was treated with 50µL of different concentrations of PL-GT-Ag nanoparticles (5, 10, 20, 40, 80 & 160µg/ml). Amino guanidine was used as positive control. After incubating at 37°C for 3 days, the fluorescence intensity of the reaction was determined at excitation and emission wavelengths of 350 nm and 450 nm, respectively, using Biotek synergy multi-mode reader, USA. The percentage activity was calculated with respect to solvent control.

Determination of Aldose Reductase Inhibition [29]

A total of 531µL of 0.1 M potassium buffer (pH 7.0), 90µL of NADPH solution (1.6 mM in potassium buffer), 90µL of recombinant human aldolase reductase (AR) (6.5U/mg) (Sigma, USA - SRP6371-100UG), 90µL of ammonium sulphate solution (4 M in potassium buffer), and 90 µL of DL-glyceraldehyde (25mM in potassium buffer) were mixed with 9µL of different concentrations of PL-GT-Ag nanoparticles (5, 10, 20, 40, 80 & 160µg/ml) in a cuvette, and the activity of AR was assessed spectrophotometrically by measuring the decrease in NADPH absorbance at 340 nm for 3 min using a spectrophotometer (Biotek Synergy H4 multimodereader, USA). Quercetin was used as positive controls. The inhibition of AR (%) was calculated using the following equation: $(1 - (\triangle A \text{ sample/min}) - (\triangle A \text{ blank/min})/(\triangle A \text{ control/min}) - (\triangle A \text{ blank/min})) \times 100\%$, where $\triangle A$ sample/min

is the decrease in absorbance over 3 min with reaction solution, test sample, and substrate, and △A control/min without the test sample.

STATISTICAL ANALYSIS

All data obtained were analyzed by One way ANOVA followed by Student's t-test using SPSS software. Data were represented as Mean±SEM for triplicates. The level of statistical significance was set at $p<0.05$.

RESULTS

UV-Visible spectra of the synthesized silver nanoparticles

The UV-Vis spectra of the green tea and papaya leaf extract mediated silver nanoparticles is shown in Fig 1. The results of the UV spectra confirmed the synthesis of silver nanoparticles by exhibiting the absorption maximum between 400-450nm. Metal nanoparticles possess free electrons, leading to the formation of a surface plasmon resonance (SPR) absorption band. This occurs due to the collective oscillation of the electrons in resonance with the light wave. The observed peaks reflect the surface plasmon resonance characteristics specific to silver nanoparticles [30].

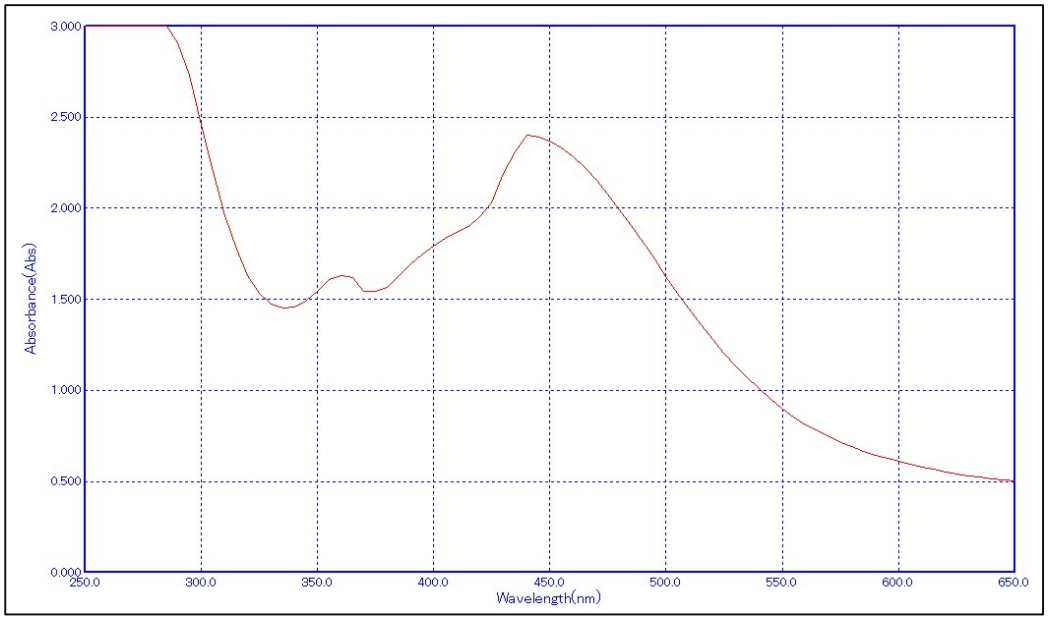

Fig 1: UV-Visible Spectra of the synthesized silver nanoparticles exhibiting absorption maximum between 400-450nm.

α-amylase inhibitory activity of green synthesized silver nanoparticles

The α-amylase inhibitory activity of silver nanoparticles synthesized using green tea and papaya leaf extracts is shown in Fig 2. The inhibitory effect of the silver nanoparticles was found to be dose – dependent. At 5 µg/ml, the nanoparticles exhibited 0.85% inhibition and at highest concentration of 160 µg/ml, a 79.28% inhibition was observed. The inhibitory activity was compared to that of standard drug Acarbose. Acarbose exhibited 88.02% inhibition of α-amylase activity at 160 µg/ml.

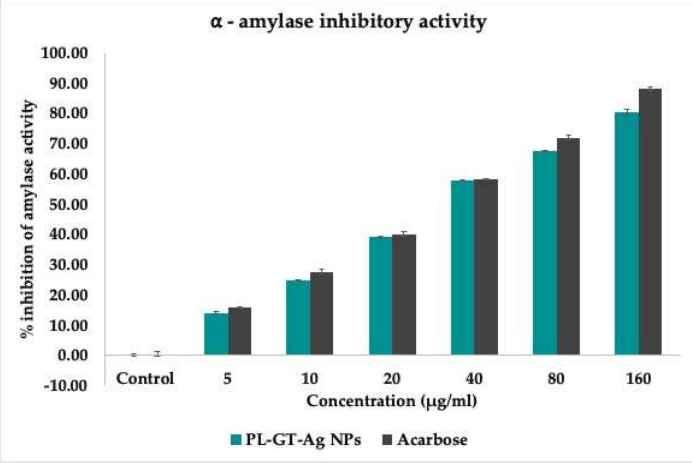

Fig 2: Represents the α-amylase inhibitory activity of silver nanoparticles synthesized using green tea and papaya leaf extracts as compared to standard drug Acarbose. 'X' axis represents different concentrations of silver nanoparticles and the 'Y' axis represents the % of inhibition. Green color bar graph denotes silver nanoparticles synthesized using green tea and papaya leaf extracts and black color bar graph represents standard drug (Acarbose). Results are expressed as Mean±SEM (n=3).

α-glucosidase inhibitory activity of green synthesized silver nanoparticles

The silver nanoparticles synthesized from green tea and papaya leaf extract have exhibited potential inhibition onα-glucosidase enzyme (Fig 3). The inhibition was found to be in the range of 0.15% to 78.42% from a low concentration of 5µg/ml to a higher concentration of 160µg/ml respectively. The inhibitory effect was found to be concentration - dependent and was compared with the effect of standard acarbose.

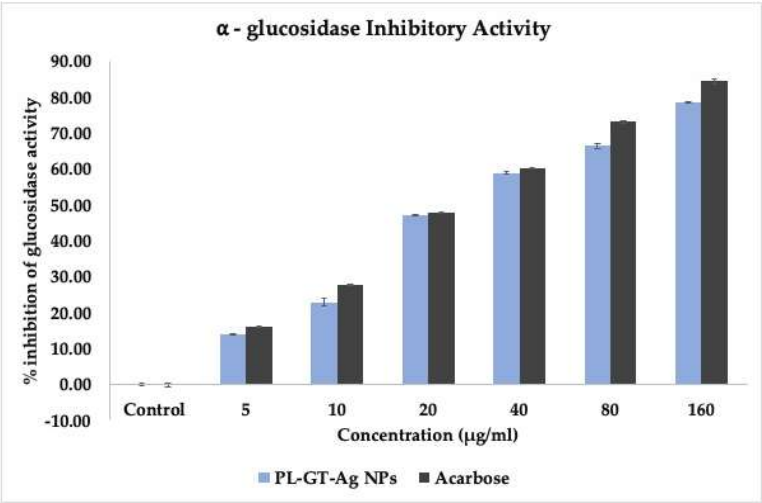

Fig 3: Represents the α-glucosidase inhibitory activity of green tea and papaya leaf extract assisted silver nanoparticles compared to standard drug Acarbose. 'X' axis represents the different concentrations of silver nanoparticles and the 'Y' axis represents the % of inhibition. Purple color bar graph denotes silver nanoparticles synthesized using green tea and papaya leaf extracts and black color bar graph represents standard drug (Acarbose). Results are expressed as Mean±SEM (n=3).

Effect of Silver Nanoparticles on Sorbitol accumulation

The inhibitory activity of green synthesized nanoparticles against sorbitol accumulation, a primary cause of diabetic cataract, was evaluated. The results showed significant inhibition against sorbitol accumulation in all the tested concentrations between 5-160µg/ml. The inhibitory activity was compared to that of Standard quercetin. A maximum inhibition of 78.93% was observed at160µg/ml of silver nanoparticles while the standard quercetin showed 80.95% inhibition at the similar concentration (Fig 4).

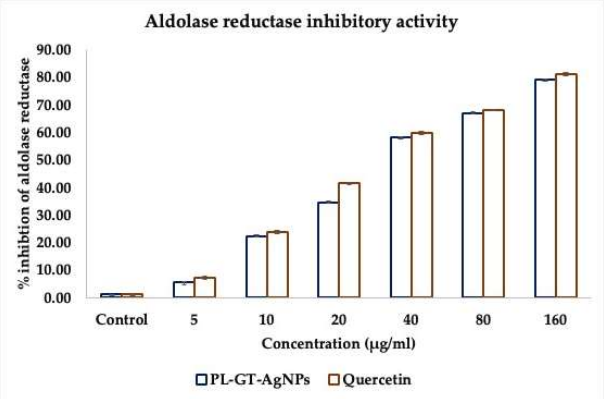

Fig 4: Represents the sorbitol accumulation inhibitory activity of green tea and papaya leaf extract assisted silver nanoparticles compared to standard drug Quercetin. 'X' axis represents the different concentrations of silver nanoparticles and the 'Y' axis represents the % of inhibition. Blue outlined bar graph denotes silver nanoparticles synthesized using green tea and papaya leaf extracts and maroon outlined bar graph represents Quercetin. Results are expressed as Mean±SEM (n=3).

Effect of Silver Nanoparticles on Advanced Glycation End product

The effect of the silver nanoparticles synthesized from green tea and papaya leaf extracts on formation of advanced glycation end products was evaluated. The results demonstrated that the synthesized silver nanoparticle was effective in inhibiting the AGEs formation. A dose-dependent inhibition of AGEs by silver nanoparticles at concentrations between 5µg/ml to 160µg/ml was observed. The maximum percentage of inhibition at 160µg/ml was found to be 73.85% for silver nanoparticles and 83.61% for standard Aminoguanidine respectively (Fig 5).

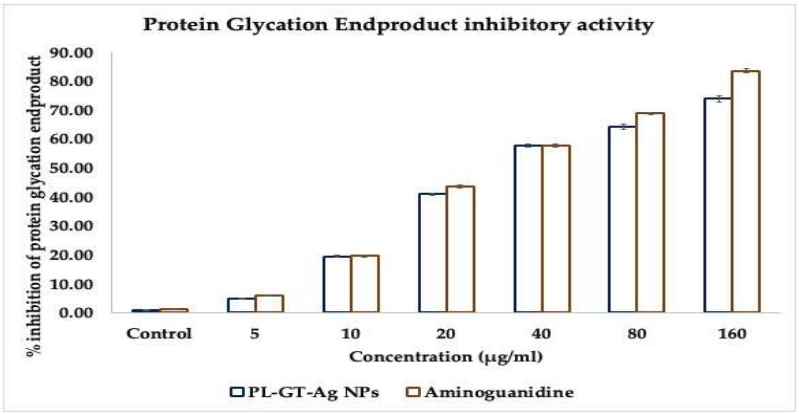

Fig 5: Represents the advanced glycation endproducts inhibitory activity of green tea and papaya leaf extract assisted silver nanoparticles compared to standard drug Quercetin. 'X' axis represents the different concentrations

of silver nanoparticles and the 'Y' axis represents the % of inhibition. Blue outlined bar graph denotes silver nanoparticles synthesized using green tea and papaya leaf extracts and maroon outlined bar graph represents Aminoguanidine. Results are expressed as Mean±SEM (n=3).

DISCUSSION

Glucosidase and amylase are essential enzymes involved in the metabolism of carbohydrates. It plays a crucial role in the final step of carbohydrate digestion by catalyzing the breakdown of oligosaccharides and disaccharides into absorbable monosaccharides, such as glucose [31]. Inhibition of α-amylase and α-glucosidase enzyme activity has been recognized as an effective approach to reducing blood sugar levels and managing conditions like diabetes [32]. Several studies have explored the use of amylase and glucosidase inhibitors or starch blockers to impede the absorption of dietary starches and subsequently reduce the postprandial rise in blood sugar levels [33-35].

The use of silver nanoparticles synthesized using natural products as amylase and glucosidase inhibitors has been investigated in several studies [36, 37]. For example, a study by Yarrappagaari et al. (2020) [16] demonstrated the inhibitory effect of green-synthesized silver nanoparticles on α-amylase activity. The researchers used the whole plant of Cleome*viscosa* to synthesize the nanoparticles and observed significant inhibition of amylase activity. Similarly, another study by Balan et al. (2019) [36] reported the synthesis of silver nanoparticles using the aqueous leaf extract of *Lonicera japonica* and found that these nanoparticles effectively inhibited α-amylase activity. Moreover, studies have also explored the inhibitory activity of silver nanoparticles on α-glucosidase. The inhibitory effect of green-synthesized silver nanoparticles using *Ocimumbasilicum* and *Ocimum sanctum (L.)* extract exhibited significant inhibition of α-glucosidase activity [37]. Similarly, *Allium cepa* mediated silver nanoparticles were evaluated for their inhibitory activity against α-glucosidase. The study demonstrated significant inhibition of the carbohydrate metabolism enzyme [38]. Few other studies have demonstrated that silver nanoparticles synthesized using *Allium sativum*, *Cassia auriculata* leaves extracts have also shown significant inhibition of both amylase and glucosidase enzymes highlighting the potential of these nanoparticles as inhibitors for controlling blood sugar levels [39, 40]. In concordance with these results the present study also exhibited marked inhibition of both α-amylase and α-glucosidase enzymes by green tea and papaya leaf extract mediated silver nanoparticles suggesting their potential in preventing post-meal spikes in blood sugar levels.

The formation of advanced glycation end products (AGEs) is intricately connected to the generation of Reactive Oxygen Species (ROS), and the interaction between AGEs and their receptors, can also trigger the production of ROS. AGEs have a tendency to accumulate in various locations associated with diabetic complications, such as the kidney, retina, and atherosclerotic plaques [41]. Further, sorbitol accumulation and advanced glycation end-products formation was also found to be markedly reduced indicating the potential of the green synthesized silver nanoparticles for the management of diabetes associated complications. The anti-diabetic effect of the synthesized silver nanoparticles in our study might be attributed to the polyphenols, especially the tannins and flavonoids present in green tea and papaya leaf [21, 24]. With the aforementioned explanation in consideration, the present study was endeavored to ascertain the involvement of AgNPs synthesized using green tea and papaya leaf extract in inhibiting the formation of AGEs and also in preventing the accumulation of sorbitol.

CONCLUSION

The synthesized PL-GT-Ag NPs showed significant inhibitory effect against the key enzymes involved in carbohydrate metabolism and hence may be considered for the management of diabetes owing to its efficacy. However, further *in vivo* and molecular studies are much needed in order to further substantiate the anti-diabetic efficacy of the PL-GT-Ag NPs and to elucidate its anti-diabetic mechanism.

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CONFLICT OF INTEREST

The authors declared no conflict of interest pertaining to the study.

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