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Original Research Article

Evaluation of invitro antidiabetic activity using silver nanoparticles of *Galinsoga parviflora* leaf extract

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ABSTRACT

Background: Diabetes mellitus, characterized by chronic hyperglycemia, can lead to severe complications if untreated. *G. parviflora*, a plant from the Astereaceae family, is traditionally recognized for its medicinal properties, including hypoglycemic effects. This study investigates the antidiabetic potential of silver nanoparticles synthesized from *G. parviflora* leaf extract. To evaluate the in-vitro antidiabetic activity of silver nanoparticles from *G. parviflora* by assessing their α-amylase and α-glucosidase inhibitory activities, and glucose uptake using the everted gut sac method.
Methods: Silver nanoparticles were synthesized from *G. parviflora* and tested for α-amylase and α-glucosidase inhibitory effects using colorimetric methods. Glucose uptake activity was assessed with an everted gut sac model from chicken ileum. Statistical analysis employed one-way ANOVA and Tukey's post-hoc test, with significance set at $p<0.05$.
Results: The nanoparticles exhibited significant α-amylase inhibitory activity, with 90.2% inhibition at 200 µl and 91.9% at 400 µl, surpassing the standard drug Acarbose. They also showed dose-dependent α-glucosidase inhibitory activity, achieving 34.5% inhibition at 200 µl and 32.6% at 400 µl. Glucose uptake analysis revealed significant reductions at 150 minutes for both the standard drug and nanoparticles compared to the control ($p<0.05$). However, at 300 minutes, only the standard treatment maintained significant differences.
Conclusions: Silver nanoparticles of *G. parviflora* exhibit significant in vitro antidiabetic activity by inhibiting key digestive enzymes and reducing glucose uptake. These findings suggest their potential for developing novel antidiabetic therapies.

Keywords: Antidiabetic activity, *G. parviflora*, Glucose uptake, α-Amylase inhibition, α-Glucosidase inhibition, Silver nanoparticles, Traditional medicine

INTRODUCTION

Diabetes is a metabolic disorder marked by hyperglycemia, defined by fasting plasma glucose levels ≥126 mg/dl (≥7.0 mmol/l) on two or more occasions, or plasma glucose levels ≥200 mg/dl (≥11.1 mmol/l) two hours after a 75 g glucose load (oral glucose tolerance test) or randomly.[1,2] Symptoms include thirst, polyuria, weight loss, and blurred vision. If untreated, diabetes can lead to cardiovascular, ocular, renal, and neurological complications.[3] poorly managed diabetes is responsible for nearly 1.5 million deaths each year.[4] *G. parviflora*, an herbaceous plant in the Astereaceae family, is traditionally used for its anti-inflammatory, dermatological, and anti-

scurvy properties.[5-7] It is also utilized to treat cold sores, bleeding, common colds, flu, and toothaches.[8-10] Aqueous extracts of the plant's aerial parts have shown protective effects against UV-induced damage and are used for wound and eye treatments.[11,12] The plant's juice treats injuries, and its roots are effective against beetle bites. *G. parviflora* has demonstrated significant hypoglycemic effects, making it suitable for diabetic supplementary therapy.[13,14]This plant is also valued in nutraceuticals due to its optimal levels of calcium, magnesium, and proteins. In Zimbabwe, South Africa, and Tanzania, young leaves and shoots are consumed as vegetables, while fresh leaves are used in salads, and the dried plant in soups.[15,16] *Galinsoga parviflora's* nutraceuticals and hypoglycemic properties, coupled with the enhanced absorption and efficacy of silver nanoparticles synthesized from it, make it a promising supplementary therapy for diabetes. The objectives were to evaluate in-vitro antidiabetic activity of silver nanoparticles of *G. parviflora*. Determination of α-Amylase and α-Glycosidase inhibitory activity of silver nanoparticles of *G. parviflora.* Determination of glucose uptake activity of silver nanoparticles of *G. parviflora* by everted gut sac method using chicken ileum.

METHODS

Study design

This study is an experimental study conducted in a laboratory setting, assessed the antidiabetic potential of *G. parviflora* silver nanoparticles by evaluating their effects on enzyme inhibition and glucose uptake in chicken ileum.

Study place

This experimental study was conducted in the Department of Pharmacology at Sri Adichunchangiri College of Pharmacy, Rajiv Gandhi University of Health Sciences, Bengaluru, Karnataka, India.

Study duration

The duration of the study was from 08/10/2021 to 26/04/2022

Ethical Considerations

Ethical considerations were not required for this study because it was an in vitro and laboratory-based study.

The study utilized a comprehensive array of chemicals and equipment, meticulously cataloged in Table 1, ensuring precise and accurate experimental procedures.

Procurement of Galinsoga parviflora's silver nanoparticles

Galinsoga parviflora's silver nanoparticle was obtained from the Department of Pharmacology, Sri Adichunchangiri College of Pharmacy, B. G. Nagara.

Alpha amylase inhibitory activity

Principle

Principle is based on colorimetric method. The enzyme α-amylase converts starch in to maltose. This maltose released from the starch is measured by the reduction of 3, 5 DNSA. Maltose reduces the pale-yellow colour of alkaline 3, 5 DNSA to orange red colour. The intensity of the colour is directly proportional to the maltose present in the sample. Intensity of colour change is measured using spectrophotometer at 540 nm.[17]

Preparation

Preparation of Phosphate buffer: 2.8 g of Disodium hydrogen Phosphate and 3.1 g of Sodium dihydrogen Phosphate were accurately weighed and dissolved in 100 ml distilled water to produce 0.2 M phosphate buffer. The PH was adjusted to 6.9 using 3N NaOH and 3N HCL. Preparation of DNS reagent: Solution A- 0.8 g of NaOH was dissolved in 3 ml of water and 0.1 g of DNS was dissolved in 2 ml of water and mixes both the solutions to get 5 ml of solution A. Solution B-3 g of Sodium Potassium tartrate was dissolved in 5 ml of water. Mix both solution A and Solution B to get DNS reagent.

Procedure

The enzyme, test, standard was prepared as 1 mg/ml solution using distilled water. Required amount of Sodium Phosphate buffer (0.2M PH 4.5) (for reaction volume 100 microliter), Alpha amylase enzyme 10 µl and Test samples 200 ml and 400 µl were added to the 1.5 ml micro centrifuge tubes and incubated at room temperature for 20 minute. 100 µl of starch solution (1% Starch in PBS) was added to all the tubes except reagent blank and incubated for 3 minutes at room temperature. Then 100 µl of freshly prepared DNS reagent was added to all the test tubes and heated on water bath at 95 °C for 5 min to stop the reaction. Similarly Control (with 100% enzyme activity) and standard was prepared and the absorbance is recorded at 540 nm.[18] The experiment is repeated thrice, the procedure is depicted in Figure 1. The alpha amylase inhibitory activity was calculated by using the formula:

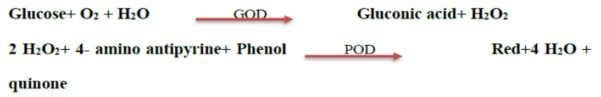
$$\%\ Inhibition = \frac{Absorbance\ of\ test - Absorbance\ of\ control}{Absorbance\ of\ test} \times 100$$

Alpha glucosidase inhibitory activity

Principle

Glucose is converted to gluconic acid and hydrogen peroxide by the enzyme glucose oxidase. Hydrogen peroxide then split to form water and nascent oxygen. The nascent oxygen then combines with a chromogen (e.g 4-aminophenazone+phenol) forms a pink color. Glucose oxidase enzyme specifically acts on glucose. So, this

method gives true value of glucose levels. Intensity of the pink color is proportional to the glucose concentration and is measured at 505 nm.[19, 20]



Procedure

The enzyme, test, and standard solutions were prepared as 1 mg/mL in distilled water. Sodium phosphate buffer (0.2 M, pH 4.5), 1 µl of α-glucosidase enzyme, and 20 µl or 40 µl of test samples or standard drug were added to 1.5 ml micro centrifuge tubes and incubated at room temperature for 5 minutes. Then, 50 µl of 37 mm sucrose solution was added (except for the reagent blank) and incubated for 30 minutes at room temperature. The tubes were heated at 100°C for 5 minutes to stop the reaction, and then diluted with phosphate buffer to 200 µl. From this, 100 µl was transferred to another tube and mixed with 1 mL of GOD-POD reagent, incubated for 10 minutes. Control (with 100% enzyme activity) and standard were similarly prepared. Absorbance was measured at 505 nm in a 96-well plate. The experiment was repeated three times, depicted in Figure 2. The α-glucosidase inhibitory activity was calculated using the formula.[21]

$$\%\ Inhibition = \frac{Absorbance\ of\ control - Absorbance\ of\ test}{Absorbance\ of\ control} \times 100$$

Glucose uptake in chicken ileum by everted gut sac

Procedure

In this study, the method of Viviyan Therasa et al was followed with modifications: The ileum was isolated from a chicken and placed in Krebs solution. A moistened glass rod with a rounded end and constriction was used to slip the intestine over, tying it securely with moistened thread at the constriction. The intestine was gently everted over the rod without touching the mucosal surface to avoid damage, trimmed to slightly longer than the rod, and cut into 3-4 cm segments. These segments were mounted onto the everted gut sac apparatus, dipped in Krebs solution, and aerated for tissue survival. A drop of phenol red was added inside the tissue to check for leakage or damage. Three beakers were prepared: a control with Krebs solution and glucose, a standard with Krebs solution, glucose, and Acarbose, and a test with Krebs solution, glucose, and *G. parviflora* nanoparticles. After 2 and 5 hours, the glucose concentration within the sac was measured using a glucomewter, 22 depicted in Figure 3.

Statistical analysis

Data were entered into Microsoft excel spreadsheets and cross-checked for accuracy. The statistical analysis was performed using IBM SPSS Statistics software for Windows, version 22 (Armonk, NY, USA). All experiments were performed in triplicate, and the data were presented as mean±standard deviation. The data were analyzed using one-way ANOVA followed by post hoc Tukey's test, with p values<0.05 considered statistically significant.

RESULTS

Alpha amylase inhibition activity of silver nanoparticles from G. parviflora

In this study, silver nanoparticles synthesized from *G. parviflora* were evaluated for their in-vitro Alpha amylase inhibition activity. Statistical analysis, depicted in Table 1, was conducted using a one-way ANOVA, revealing a highly significant difference among group means ($F=1181.42$, $p<0.05$), indicating substantial variation between treatments. The between-group sum of squares (SS) was 499.695 with a mean square (MS) of 166.565, while the within-group SS was 1.1281 with an MS of 0.141. Subsequent Tukey's HSD post hoc test, detailed in Table 2, confirmed these findings by demonstrating statistically significant differences in all pairwise comparisons, where mean differences exceeded the HSD value of 0.587.

These results underscore the distinct and significant impacts of each treatment, highlighting the potential clinical and experimental implications of both Acarbose and *G. parviflora* in Alpha amylase inhibition. The significant outcomes of the one-way ANOVA, supported by Tukey's HSD post hoc test, validate the marked differences observed among treatment groups, contributing valuable insights into the differential effects of these treatments and emphasizing their potential therapeutic relevance in managing conditions influenced by alpha amylase activity (Table 1 and 2).

Alpha glucosidase invitro antidiabetic activity

In this study, the alpha-glucosidase inhibitory activity of *G. parviflora* silver nanoparticles were assessed and compared with the standard drug Acarbose. The results indicated that the silver nanoparticles exhibited inhibitory activity in a dose-dependent manner, as detailed in Table 3. Statistical analysis, also depicted in Table 3, showed a significant difference among group means ($F(3, 8) = 559.08$, $p<0.05$).

The Tukey's HSD post hoc test, depicted in Table 4, further demonstrated that all pairwise comparisons between groups were statistically significant, with mean differences exceeding the HSD value of 0.587. This suggests that each treatment, including different concentrations of Acarbose and *G. parviflora*, produced distinct effects on the outcome variable. These findings highlight the unique impact of each treatment, providing crucial insights for decision-making in clinical and experimental contexts (Table 3 and 4).

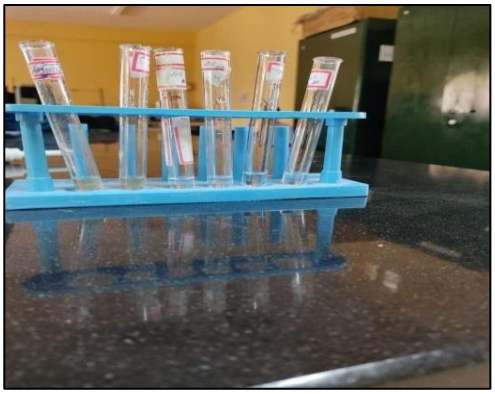


Figure 1: Alpha-amylase in vitro antidiabetic activity.

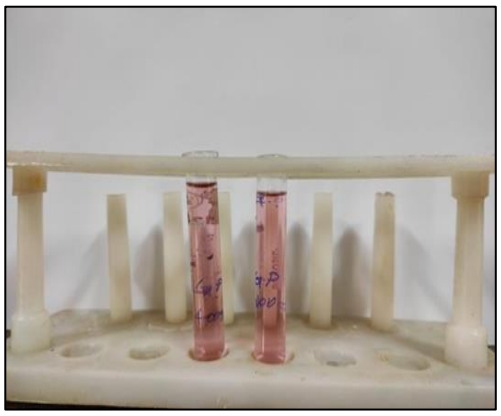


Figure 2: In-vitro antidiabetic activity of alpha-glucosidase.

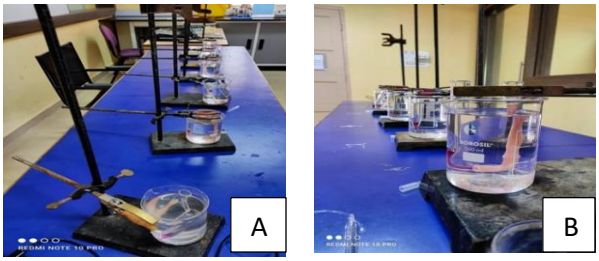


Figure 3 (A and B): Glucose uptake in chicken ileum by everted gut sac.

Glucose uptake in chicken ileum by everted gut sac

The glucose uptake of *G. parviflora* silver nanoparticles from chicken ileum were assessed and compared with Acarbose, showing a reduction in glucose uptake as observed by measuring the glucose level in the serosal compartment. Statistical analysis of these results revealed significant differences between treatments. Both the standard drug and *G. parviflora* silver nanoparticles significantly reduced glucose uptake at 150 minutes compared to the control ($p<0.05$). However, at 300 minutes, only the standard treatment maintained a significant reduction, while the *G. parviflora* nanoparticles did not. These findings suggest that *G. parviflora* silver nanoparticles could be a promising alternative to standard treatments for reducing glucose absorption, particularly at earlier time points, highlighting their potential in developing new therapeutic strategies for managing glucose levels. Results are depicted in Table 4.

Table 1: List of the chemicals and equipment used in the study.

S. No.	Name of materials and equipment
1	*Galinsoga parviflora* extract
2	*Galinsoga parviflora* nanoparticles
3	Acarbose (yarrow chem products, molecular weight 645.60)
4	α- amylase (AGD biomedicals)
5	α- glucosidase (SISCO research laboratory pvt ltd)
6	GOD-POD Reagent (AGD biomedicals)
7	NaCl (SD fine-chem ltd, molecular weight 58.44)
8	KCl (SD fine-chem ltd, molecular weight 74.55)
9	CaCl2 (SD fine-chem ltd, molecular weight 110.98)
10	NaOH (SD fine-chem ltd, molecular weight 40)
11	Di- Sodium Orthophosphate (sd fine-chem ltd, Molecular Weight 141.96)
12	Starch (SD fine-chem ltd)
13	Potassium sodium tartrate (sd fine-chem ltd, molecular weight 282.22)
14	MgS04 (Thomas baker ltd, Molecular weight 246.48)
15	Sodium dihydrogen orthophosphate (Molychem pvt ltd, Molecular weight 156.01)
16	Glucose (Thermo fisher scientific India pvt ltd, Molecular weight 180.16)
17	NaHCO3 (Thermo fisher scientific India pvt ltd, Molecular weight 84.01)
18	Maltose monohydrate (Thermo fisher scientific India pvt ltd, Molecular weight 360)
19	Phenol red reagent (Qualigens fine chemicals)
20	DNS solution (Amanda health care pvt ltd)
21	Weighing balance (ACCULAB)

Continued.

S. No.	Name of materials and equipment
22	Water bath (Anamatrix instrumental technologies pvt ltd)
23	Micro pippete (RV instruments)
24	Glucometer (ACCU-CHEK)
25	Shimadzu UV-Visible spectrophotometer, Model 1700

Table 2: In-vitro alpha amylase inhibition activity of silver nanoparticles from *G. parviflora*.

S. No	Drug	Volume(µl)	Absorbance	Percentage of inhibition	sum of squares	Degrees of freedom	Mean square	F ratio	P value
1	Acarbose	200	0.037±0.001	75.6±0.65	499.695	3	166.565	1181.42	<0.05
		400	0.053±0.001	83.01±0.32	1.1281	8	0.141		
2	*G. parviflora* silver nanoparticles	200	0.092±0.0015	90.2±0.16	500.8231	11			
		400	0.113±0.0105	91.9±0.76					

*values are the means of three replicates±Standard Deviation.

Table 3: Tukey's HSD post-hoc test for in-vitro alpha amylase inhibition activity of silver nanoparticles from *G. parviflora*.

Comparison	Mean difference	HSD (0.05)	Significant (Yes/No)
Acarbose 200 µl vs Acarbose 400 µl	7.4	0.587	Yes
Acarbose 200 µl vs *G. parviflora* 200 µl	14.6	0.587	Yes
Acarbose 200 µl vs *G. parviflora* 400 µl	16.3	0.587	Yes
Acarbose 400 µl vs *G. parviflora* 200 µl	7.2	0.587	Yes
Acarbose 400 µl vs *G. parviflora* 400 µl	8.9	0.587	Yes
***G. parviflora* 200 µl vs *G. parviflora* 400 µl**	1.7	0.587	Yes

Table 4: Alpha-glucosidase-induced antidiabetic activity.

Drug	Vol (µl)	Absorbance	% of inhibition	Source of variation	Sum of squares	Degrees of freedom	Mean square	F ratio	P value
Acarbose	200	0.33±0.01	27.152±2.207	Between groups	1094.523	3	364.841	559.08	<0.05
Acarbose	400	0.406±0.001	10.264±0.156	Within groups	5.221	8	0.652	-	-
***G. parviflora* silver nanoparticles**	200	0.296±0.002	34.510±0.459	Total	1099.744	11	-	-	-
***G. parviflora* silver nanoparticles**	400	0.305±0.0015	32.597±0.337	-	-	-	-	-	-

*Values are the means of three replicates±Standard Deviation.

Table 5: Tukey's HSD Post Hoc Test Results.

Comparison	Mean difference	HSD (0.05)	Significant (yes/no)
Acarbose 200 µl vs Acarbose 400 µl	7.4	0.587	Yes
Acarbose 200 µ vs *G. parviflora* 200 µl	14.6	0.587	Yes
Acarbose 200 µl vs *G. parviflora* 400 µl	16.3	0.587	Yes
Acarbose 400 µl vs *G. parviflora* 200 µl	7.2	0.587	Yes
Acarbose 400 µl vs *G. parviflora* 400 µl	8.9	0.587	Yes
***G. parviflora* 200 µl vs *G. parviflora* 400 µl**	1.7	0.587	Yes

Table 6: Glucose uptake in chicken ileum by everted gut sac.

Drug	Time interval (min)	Concentration in serosal compartment (mg/dl)	Replicate 1	Replicate 2	Replicate 3	Mean	P value	Significant differences
Control	150	286.33	285	287	287	286.33	<0.05	Yes
Control	300	293.33	292	294	294	293.33	<0.05	Yes
Standard	150	235.33	234	236	236	235.33	<0.05	Significant (Control-150 vs Standard-150)
Standard	300	258.66	257	260	259	258.66	<0.05	Significant (Control-300 vs Standard-300)
***G. parviflora* silver nanoparticles**	150	250.5	249	251	251	250.5	<0.05	Significant (Control-150 vs Galinsoga-150)
***G. parviflora* silver nanoparticles**	300	286.5	285	288	287	286.5	>0.05	Not Significant (Control-300 vs Galinsoga-300)
Standard	150	-	-	-	-	-	>0.05	Not Significant (Standard-150 vs Galinsoga-150)
Standard	300	-	-	-	-	-	<0.05	Significant (Standard-300 vs Galinsoga-300)

*Values are the means of three replicates±Standard Deviation.

DISCUSSION

In our research, *G. parviflora* demonstrated significant antidiabetic activity through its silver nanoparticles, which exhibited potent inhibitory effects on both α-amylase and α-glucosidase enzymes, surpassing the standard drug Acarbose in efficacy. The nanoparticles achieved 90.2% and 91.9% inhibition of α-amylase at 200 µl and 400 µl, respectively, compared to Acarbose's 75.6% and 83.01%. Similarly, for α-glucosidase inhibition, the nanoparticles showed 34.51% and 32.60% inhibition at the same volumes, outperforming Acarbose's 27.15% and 10.26%. Ferheen S, Rehman also demonstrated *G. parviflora's* strong inhibitory activity against α-glucosidase enzyme.[23] Furthermore, the nanoparticles significantly reduced glucose uptake in the chicken ileum at 150 minutes, indicating their effectiveness in managing postprandial blood glucose levels. However, their reduced efficacy at 300 minutes suggests the need for optimized formulations or combined treatments for sustained antidiabetic effects. A similar study showed the hypoglycemic potential of *G. parviflora* ethanolic extract using an in vivo model, where at 400 mg/kg; the ethanolic extract exhibited a strong hypoglycemic effect comparable to the standard glibenclamide medication (5 mg/kg) in practically all respects.[24] Overall, our findings highlight the potential of *G. parviflora* silver nanoparticles as a natural alternative for diabetes management, emphasizing their superior enzyme inhibition and early-stage glucose uptake reduction compared to both acarbose and ethanolic extracts.

Limitations of this study include its in vitro nature, conducted solely in a laboratory setting, which may not fully replicate the complexities of in vivo conditions. The study's short duration from 08/10/2021 to 26/04/2022 limits the assessment of long-term effects and efficacy of *G. parviflora* silver nanoparticles. The use of chicken ileum as a model for assessing glucose uptake simplifies the biological context and may not fully translate to human physiological responses. Conducted at a single site, Sri Adichunchangiri College of Pharmacy, the findings' generalizability to broader populations or settings may be limited. Furthermore, the absence of clinical data restricts direct application of the results to human subjects,

suggesting a need for further research to validate these findings in clinical settings and over extended periods.

Conclusion

The study advances knowledge and understanding in the field of diabetes management by demonstrating that *G. parviflora* silver nanoparticles act as a potent antidiabetic agent with strong inhibitory effects on α-amylase and α-glucosidase enzymes, surpassing the standard drug Acarbose in enzyme inhibition. The nanoparticles' ability to significantly reduce glucose uptake in the chicken ileum at early time points highlights their potential for managing postprandial blood glucose levels. However, the diminished efficacy at later time points suggests the need for combined treatments or controlled administration to sustain their hypoglycemic effect. In summary, *G. parviflora* silver nanoparticles offer significant advantages in enzyme inhibition and early-stage glucose uptake reduction, representing a promising natural alternative for diabetes management. Future research should focus on optimizing the formulation and delivery of these nanoparticles to enhance their sustained efficacy and explore their potential in clinical settings.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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