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In Vitro Anti-Diabetic Activity of Silver Nanoparticles Extract of Scales of *Catla catla* (Hamilton, 1822)

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Abstract: Diabetes mellitus is a chronic illness that causes hyperglycemia and improper glucose, protein, and fat metabolism, and it causes a failure of insulin production. International Diabetes Federation estimates that 366 million people worldwide have diabetes, which is expected to double by 2030. Anti-diabetic medications with multimodal mechanisms of action are necessary to address the starting point of the problem's progression and consequences. Anti-diabetic medications typically have a single mechanism of action and may cause unwanted side effects. Herbal remedies may offer a comparative advantage due to the existence of bioactive secondary metabolites that work through various pathways. In the present study, the scale extract of *Catla catla* was studied for anti-diabetic activity. Silver nanoparticles were synthesized from ethanol extract of *Catla catla* scales and used for anti-diabetic activity. This study was carried out to investigate the potential of silver nanoparticle extract of *Catla catla* scales to inhibit the activity of alpha amylase and alpha-glucosidase enzymes. The alpha-amylase and alpha-glucosidase activity of the silver nanoparticle extract of *Catla catla* scales was assayed and observed as 88.50% (100 µg) and 92.14% (100 µg), respectively. From the present study, it was found that the silver nanoparticle extract of *Catla catla* scales possessed excellent anti-diabetic activity.

Keywords: Alpha-amylase, Alpha-glucosidase, *Catla catla*, Silver nanoparticles, Anti-diabetic

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Introduction

Diabetes mellitus is a severe, chronic, and diverse group of metabolic disorders characterized by persistent high blood glucose levels caused by changes in carbohydrate, fat, and protein metabolism prompted by inherited or acquired insulin deficiency or ineffective insulin production. Chronic hyperglycemia during diabetes is linked to dysfunction of the eyes, kidneys, nerves, heart, and blood vessels. The problem is primarily connected with dyslipidemia, which increases oxidative stress along, as a result, alters the body's antioxidant

defence system. Due to modern lifestyles and increased intake of carbohydrate-rich foods, the condition continues to be a global health concern as well as an economic burden in economically downtrodden countries (Mattei *et al*., 2015; Zakir *et al*., 2022).

According to the International Diabetes Federation, over 366 million people worldwide have diabetes, with the number expected to double by 2030. Diabetes affects 40.9 million individuals in India, with a total population anticipated to increase to 60.9 million by 2025 (Roth *et al*., 2020). Type 2 diabetes (T2D) is more frequent than type 1 and accounts for more than 90% of all diabetic patients. An imbalance between blood sugar absorption and insulin production causes type 2 diabetes. Hyperglycemia is a hallmark of T2DM, contributing to the majority of the disease's harmful depicts (Himanshu *et al*., 2020).

Among the different treatments used to manage diabetes complications, one of the most important therapeutic targets is the suppression of alpha-amylase and alpha-glucosidase enzymes. Alpha amylase is an essential pancreatic and salivary gland secretory product that catalyzes the initial step in the breakdown of complex polysaccharides into oligosaccharides and disaccharides in the intestinal mucosa (Kashtoh and Baek, 2023).

Acarbose, a well-known anti-amylase and glucosidase medication, is an essential part of the antidiabetic pharmacological family and can be used to treat insulin-dependent diabetes mellitus problems. However, this medicine is associated with gastrointestinal adverse effects such as flatulence, bowel bloating, and hypoglycemia, limiting its usage in the treatment of diabetes mellitus (Blahova *et al*., 2021). As a result, research and development of alternative ways for optimal management of postprandial hyperglycemia with no adverse effects are required. Therefore, there has been a lot of interest in screening for alternative medications or bioactive chemicals that inhibit alpha-amylase and alpha-glucosidase enzymes.

Nanotechnology has created new prospects in a variety of industries, including food packaging, animal husbandry, electronics, agriculture, medicine, and healthcare. It is also among the most recent industrial developments. Nanoparticles are a significant advancement in medicine and healthcare, and they have been utilized to address many of the most common actions, including antibacterial, antifungal, and antioxidant properties (Nagesh *et al*., 2022). Hence, the present study was undertaken to elucidate the ethanol extract of silver nanoparticles of scales of *Catla catla* for anti-diabetic activity

Materials and Methods

In the present study, silver nanoparticle was synthesized from ethanol extract of *Catla catla* scales and used for anti-diabetic activity.

Collection of scales of Catla catla

Scales of *Catla catla* (Cc) were collected from Pulicat fish market, Tamil Nadu, India and it was transported to the laboratory. The scales of *C. catla* were washed in freshwater, followed by double distilled water to remove all the debris, and dried for three weeks. The dried scales of *C. catla* were taken for further research.

Preparation of scales of Catla catla extract

10 g of fish scale was solvent-extracted with 200 ml of ethanol (Merck Chemicals) for 4 h and kept at rotary evaporator at 80^0C. The extracts were filtered using Whatman filter paper before being utilized to make Cc-AgNPs and kept at 4°C for further studies (Nagesh *et al*., 2022).

Synthesis of Silver Nanoparticles of scales of Catla catla extract

Preparation of 1mM silver nitrate aqueous solution ($AgNO_3$)

1 mM of silver nitrate was prepared in double distilled water and stored in the amber colour bottle until further use.

Biosynthesis of Ag NPs using fish scale extract

Prepared scales of *Catla catla* extract was utilized for the synthesis of Ag NPs. To prepare, 1 mM $AgNO_3$ was treated with scales of *Catla catla* extract. Then, the extract, along with optimized Ag NPs, was kept immediately under the bright sunlight for 10-15 min; as a result the colourless solution turned into brownish colour, which revealed the reduction of Ag^+ into Ag^0 nanoparticles. Visible colour change was observed at 1:9 ratios, and this reaction was subjected to further process (Amin and Sutoyo, 2022).

Separation of silver nanoparticles

The derived silver nanoparticles were separated with minor alterations by centrifugation at 5000 rpm for 20 min. After the necessary reacting duration, the supernatant liquid was removed, and the pellets were extracted and rinsed with double distilled water before being dried to eliminate moisture in a hot air oven at 35°C - 40°C. The dry powder was collected and stored at 4°C for further characterization (Amin and Sutoyo, 2022; Nagesh *et al*., 2022).

Alpha amylase activity of Silver Nanoparticles of scales of Catla catla extract

α- amylase activity assay was carried out by following the method of Sudha *et al.* (2011). The assay mixture included 40 µl of 0.02 Sodium phosphate buffer (pH 6.9, 6 mM), 0.02 units of PPA (α-amylase) solution, silver nanoparticle extract of *Catla catla* scales (100-500 µg/ml), and acarbose (as positive control) at concentrations ranging from 100-500 µg/ml (w/v) and was incubated at 37°C for 10 min. Then, soluble starch (1% w/v) was added to each reaction well and incubated at 37°C for 15 min. To inhibit the enzymatic reaction, 1 M HCl (20 µl) was added, followed by 100 µl of iodine reagent (5 mM iodine and 5 mM KI). The wavelength of the absorbance was measured at 620 nm using a microplate reader. A dark blue color showed the existence of starch; yellow indicated the absence of starch; and brown represented partially degraded starch in the reaction mixture. For the positive control in anti-diabetic assays, acarbose was used. The α-amylase inhibitory activity was expressed as per cent inhibition and was calculated using the equation given below:

$$\% \text{ inhibition} = \text{O.D. of control} - \text{O.D. Of extract} / \text{O.D. of control} \times 100$$

The alpha-glucosidase activity of Silver Nanoparticles of scales of Catla catla extract

The inhibitory activity of α-glucosidases (Vennila and Pavithra, 2015) was measured by incubating 1 ml of starch solution (2% w/v maltose) with 0.2 M Tris buffer (pH 8) and silver nanoparticle extract of *Catla catla* scales (100-500 µg/ml). The reaction mixture was incubated at 37 °C for 10 min. To start the reaction, added 1 ml of α-glucosidase enzyme (1 U/ml) and incubated it at 35°C for 40 min. The reaction was then halted by adding 2 ml of 6 N HCl. The intensity of the color was measured at 540 nm in a spectrophotometer. The results are expressed as % inhibition using the formula:

$$\% \text{ inhibitory activity} = (Ac-As)/Ac \times 100$$

Where, 'Ac' is the absorbance of the control, and 'As' is the absorbance of the sample.

Statistical analysis

All the data obtained in the present study were statistically analyzed using the statistical software SPSS version 16.0. To determine the significant difference between extract concentrations, a one-way ANOVA with the Bonferroni test was used.

Results and Discussion

The present study illustrated the anti-diabetic activity of silver nanoparticles of ethanol extract of scales of *Catla catla*. Inhibitors of alpha-amylase and alpha-glucosidase, which are particularly useful in postprandial alpha-glycemic control, are good candidates for preventing diabetic complications. Because hyperglycemia has been proposed as an independent risk factor for diabetes, controlling postprandial hyper-glycemia is suggested to be important in

Table 1: Alpha-amylase activity of silver nanoparticles of ethanol extracts of scales of *Catla catla*

S. No.	Concentrations (µg)	Silver nanoparticles of ethanol extracts of scales of *Catla catla*	Acarbose
1	100	12.33 ± 0.04	38.23 ± 0.15
2	200	35.44 ± 0.16	41.20 ± 0.18
3	300	56.20 ± 0.23	59.17 ± 0.23
4	400	72.21 ± 0.29	78.44 ± 0.32
5	500	88.50 ± 0.31	89.20 ± 0.32

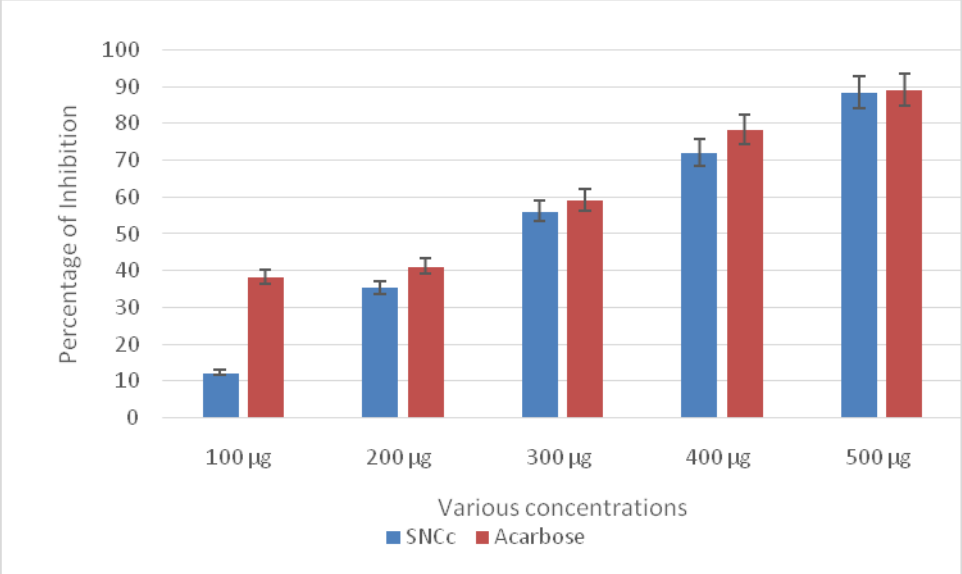

Fig. 1: Alpha-amylase activity of silver nanoparticles of ethanol extracts of scales of *Catla catla.*

treatment of diabetes. The present study assessed the inhibitory impact of the anti-diabetic activity of silver nanoparticles of ethanol extracts of scales of *Catla catla* on α-amylase (Table 1; Fig. 1) and α-glucosidase (Table 2; Fig. 2) using an *in-vitro* approach.

Silver nanoparticles of ethanol extracts of scales of *Catla catla* of 100, 200, 300, 400, and 500µg doses inhibited the α-amylase enzyme by 12.33%, 35.44%, 56.20%, 72.21% and 88.50%, respectively (Table 1; Fig. 1). Likewise, Acarbose at 100, 200, 300, 400, and 500 µg doses inhibited the α-amylase enzyme by 38.23%, 41.20%, 59.17%, 78.44% and 89.20%, respectively (Table 1; Fig.1). Dose-dependent inhibition of α-amylase enzyme was observed in silver nanoparticles of ethanol extracts of scales of *Catla catla*. Acarbose was used as a standard reference drug, which showed maximum inhibition when compared to silver nanoparticles of ethanol extracts of scales of *Catla catla*. Alpha-glucosidase inhibition activity of silver nanoparticles of ethanol extracts of scales of *Catla catla* was also investigated. Our results demonstrated that the highest and lowest potential α-glucosidase inhibitory activity was shown in silver nanoparticles of ethanol extracts of scales of *Catla catla* at 500 µg and 100 µg and it was found to be 92.14 % and 14.24%, respectively (Table 2; Fig. 2). The percentage of inhibition was significantly different ($P \leq 0.05$). Values are expressed as mean ± SD of triplicates. Experimental results showed that silver nanoparticles of ethanol extracts of scales of *Catla catla* significantly inhibited the α-glucosidase and α-amylase enzymes. This result is in agreement with previous reports, which indicated that

Table 2: Alpha-glucosidase activity of silver nanoparticles of ethanol extracts of scales of *Catla catla*

S. No.	Concentrations (µg)	Silver nanoparticles of ethanol extracts of scales of *Catla catla*	Acarbose
1	100	14.24± 0.06	36.67 ± 0.18
2	200	39.03 ± 0.18	46.29 ± 0.32
3	300	63.11 ± 0.28	68.53 ± 0.38
4	400	77.36 ± 0.33	82.20 ± 0.40
5	500	92.14 ± 0.42	94.65 ± 0.42

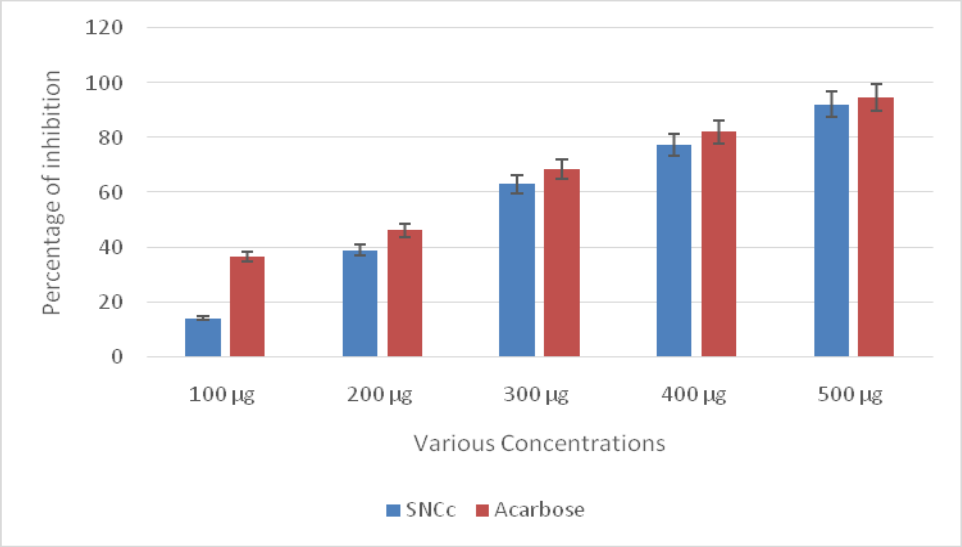

Fig. 2: Alpha-glucosidase activity of silver nanoparticles of ethanol extracts of scales of *Catla catla.*

hypoglycemic activity through either increased secretion of insulin from the pancreas or similar action to the insulin (Davis, 2006). Kainat and Ali (2021) explored the anti-diabetic by evaluating the inhibitory activity of biosynthesized *Hibiscus rosa sinensis* MgO-NPs against α-amylase and α-glucosidase.

Kumar *et al*. (2011) also revealed that secondary metabolites have significant inhibitory activity against alpha-amylase and could be used as an effective treatment for postprandial hyperglycemia. Similarly, Mohanasundaram *et al*. (2019) showed that alpha-glucosidase is a membrane-bound enzyme located in the small intestinal epithelium that converts disaccharides to glucose. Inhibitors can restrict dietary carbs from being absorbed hence, controlling postprandial hyperglycemia. Carbohydrate digestion, as well as glycoprotein and glycolipid processing need the presence of glucosidases. Inhibiting this enzyme delays carbohydrate absorption from the GI tract and reduces the rate of postprandial glucose. This delayed digestion and breakdown of starch may have beneficial effects on insulin resistance and glycemic index control in people with diabetes (Uddin *et al*., 2004). Results showed considerable α-amylase inhibitory activity as well as α-glucosidase inhibitory activity. The present findings indicated that silver nanoparticles of ethanol extracts of scales of *Catla catla* possessed *in vitro* anti-diabetic activity.

Conclusion

This study suggested that one of the mechanisms by which scales of *Catla catla* displayed its anti-diabetic potential is by the inhibition of alpha-amylase and alpha-glucosidase. However, further study is needed to isolate the active principle(s) in this plant which are responsible for this activity. This provides scientific evidence for the use of scales of *Catla catla* and baseline data for further work to characterize and could also be utilized in the pharmaceutical industry.

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