



ALPHA AMYLASE INHIBITORY ACTIVITY OF SIDDHA FORMULATION SEENTHIL CHLOORANAM-IN VITRO STUDY

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Abstract:

Background:Type 2 diabetes mellitus is a significant worldwide health issue characterized by high blood sugar levels, known as hyperglycemia. It is the most prevalent form of diabetes among affected individuals. In managing Type 2 diabetes mellitus, one effective approach is to inhibit α -amylase, an enzyme involved in breaking down starch, which helps regulate blood sugar levels. **Aim and objective:**This study aimed to assess the α -amylase inhibitory property of seenthil chooranam (SC), a siddha formulation used by siddha physicians in the management of type 2 diabetes mellitus, as it is considered a potential strategy for controlling blood sugar levels. **Method:** The spectrophotometric assay method was used to find alpha amylase inhibitory activity. **Result:** SC showed presence of α -amylase inhibitory activity (IC 50=332.3 μ g /ml) .**Conclusion:** This in-vitro study revealed the presence of the α -amylase activity in the siddha formulation seenthil chooranam.

Keywords: Diabetes mellitus, Seenthil chooranam, Madhumegam, In-vitro Alpha-amylase inhibition activity, Traditional Siddha medicine

INTRODUCTION:

There are so many indigenous systems of medicines in India. Siddha system is one among them. It has 32 forms [1] of internal medicines that have been used to treat disease. Chooranam is a form of internal medicine in which the medicines are made by powdering the ingredients manually and the final powdered product is called chooranam which is used along with anubanam (vehicle) depending upon the disease.

Seenthil Chooranam (SC), a traditional Siddha formulation is indicated for various diseases such as Vatham (imbalance in air element), Pitham (imbalance in fire element), Gunmam (Gastritis, Peptic ulcer), Madhumegam (Diabetes Mellitus), Peenisam (Sinusitis), Puzhuvettu (Alopecia), improving visual acuity and various anubanams (Vehicle) are mentioned in the Siddha text [2].

In addition Seenthil chooranam exhibits immunomodulator activity [3], bronchodilator activity [4], mast cell stabilising activity [4] and anti-microbial activity [5]. Scientific articles have also been published in supporting the clinical usage that is in the treatment of Vali Azhal Keel Vaayu (Rheumatoid arthritis) [6], Pulu vettu (Alopecia areata) [7].

Among so many diseases being treated with Siddha medicines, MadhuMegam (MM) is very common and debilitating. MM correlates with Diabetes mellitus (DM) which is not only a health burden for the nation but for the world.

DM is a medical condition marked by elevated levels of glucose in the blood, known as hyperglycemia. It encompasses two main types: Type I and Type II. Type I results from the immune system attacking the pancreas's beta cells, causing a shortage of insulin. Meanwhile, Type II arises from either insufficient insulin production or the body's reduced responsiveness to insulin. Prolonged hyperglycemia can lead to specific complications affecting the eyes (retinopathy), kidneys (nephropathy), and feet (neuropathy). This global health issue is closely linked to factors such as obesity, poor dietary habits, a sedentary lifestyle, and urbanization [8].

India has the second highest number of people with diabetes[9]. Diabetes currently affects more than 74 million Indians, which is more than 8.3% of the adult population[9]. It is estimated to be around 57% of the current cases of diabetes to be undiagnosed[10].

Among young and middle aged adults the prevalence of diabetes is 6.7% and prediabetes is 5.6% according to the National Family Health Survey-4[11]. The average age on onset is 42.5 years[12]. Nearly 1 million Indians die due to diabetes every year[12].

According to the Indian Heart Association, India is projected to be home to 109 million individuals with diabetes by 2035[13]. A study by the American Diabetes Association reports that India will see the greatest increase in people diagnosed with diabetes by 2030[14]. The high incidence is attributed to a combination of genetic susceptibility plus adoption of a high-calorie, low-activity lifestyle by India's growing middle class[15][16].

India is recognized as having the highest prevalence of diabetes worldwide, with projections estimating nearly 69.9 million affected individuals by 2030[17].

Safety and efficacy of SC is scientifically established[18] and is being used in the management of DM. Ingredients present in SC are Seenthil stem (*Tinospora cardifolia*), Karisalai (*Eclipta alba*) and Poonagam (*Eudrilus eugeniae*). Individual ingredients have anti-diabetic activity [19] [20][21]. This siddha formulation has anti diabetic effect[22].

Pancreatic α -amylase is a key enzyme in the digestive system and catalyses the initial step in hydrolysis of starch to a mixture of smaller oligosaccharides consisting of maltose, maltotriose, and a number of α -(1-6) and α -(1 - 4) oligoglucans. These are then acted on by α -glucosidases and further degraded to glucose which on absorption enters the blood-stream. Degradation of this dietary starch proceeds rapidly and leads to elevated PPHG (post-prandial hyperglycemia). It has been shown that activity of HPA (human pancreatic α -amylase) in the small intestine correlates to an increase in post-prandial glucose levels, the control of which is therefore an important aspect in treatment of type 2 diabetes[23].

AIM AND OBJECTIVE:

This study aimed to assess the α -amylase inhibitory activity of Seenthil chooranam (SC), a siddha formulation used by Siddha physicians to treat type 2 diabetes mellitus, as it is considered a potential strategy for controlling blood sugar levels.

MATERIALS AND METHODS:

STANDARD OPERATIVE PROCEDURE

Source of raw drugs

The required raw drugs were procured from a well reputed indigenous drug shop. The raw drugs taken for study was authenticated by botanist, Govt Siddha medical college, poonagam was authenticated by Gunapadam department, Govt Siddha medical college, Chennai.

Purification of raw drugs

- Seenthil stem was washed 21 times in water and was soaked in milk and dried [2].
- Debris was removed from karisalai and karisalai was washed to get rid of soil, wiped with clean cloth and dried[24].
- Poonagam was soaked in milk to empty its intestine and dried [2].

Preparation of sample medicine

Each dried ingredient was powdered separately. Equal parts of 3 powdered ingredients was mixed together. Then this powder (chooranam) was stored in an air tight container.

Sample of 20gm of study medicine was sent to the Noble Research Solutions, Chennai, to evaluate the alpha amylase inhibitory activity.

The chemicals and method adapted are as follows.

Table:1 List of chemicals used for α -amylase inhibitory activity

Name of the Chemical	Source
α -amylase enzyme	Sigma-Aldrich
2-Chloro-4-Nitrophenyl- α -Maltotrioxide	Hi-Media
Phosphate buffer	Merck

Method: The spectrophotometric assay method [25].

Procedure:

The enzyme α -amylase (0.5 U/ml) was prepared by mixing 3.24 mg of α -amylase in 100 ml of phosphate buffer (pH 6.9). Test Sample (SC) was prepared in the serial dilution of the concentration ranges from 100,200,300,400 and 500 μ g/ml using DD water. Acarbose 100 μ g/ml used as a reference standard. About 600 μ l of test sample were added to 30 μ l of α -amylase enzyme solution and incubated at 37°C for 15 min. To this reaction mixture, 370 μ l of substrate, 2-Chloro-4-Nitrophenyl- α -Maltotrioxide (CNP3- 0.5 mg/ml) was added, mixed and for incubated 37°C for 10 min. Finally, absorbance was measured at 405 nm against blank in spectrophotometer.

A control reaction was carried out without the test sample. Percentage inhibition was calculated by the following formula.

$$\text{Percentage inhibition} = \frac{\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Test}}}{\text{Absorbance}_{\text{Control}}} \times 100$$

Absorbance_{Control}

RESULT:

In the study, the Alpha Amylase inhibition assay by CNPG3 revealed that the SC showed inhibition of α -amylase enzyme activity. SC at 100,200,300,400 and 500 μ g/ml concentration showed 28.53 ± 4.539 , 36.11 ± 4.882 , 46.66 ± 8.368 , 55.78 ± 7.13 and 68.29 ± 7.362 27.94 ± 5.76 , 59.91 ± 1.36 , 70.83 ± 0.83 , 84.58 ± 0.48 And 94.02 ± 4.7 percentage inhibition of α -amylase activity respectively and IC_{50} value was found to be 332.3 ± 52.41 μ g/ml [Table 1].

However, Acarbose was used as reference standard, and showed 31.52 ± 0.1 , 72.49 ± 0.14 and 82.92 ± 0.09 percentage inhibition of α -amylase activity at concentrations of 0.1, 0.5 and 1.0 μ g/ml [Table 3]; the calculated IC_{50} value was found to be 0.312 μ g/ml [Table 2].

Table:1 Inhibitory activity of SEENTHIL CHOORANAM(SC) against α -amylase(n=3)

Concentration (μ g/ml)	% Inhibition of SC
100 μ g/ml	28.53 ± 4.539
200 μ g/ml	36.11 ± 4.882
300 μ g/ml	46.66 ± 8.368
400 μ g/ml	55.78 ± 7.13
500 μ g/ml	68.29 ± 7.362
Standard Acarbose	95.96 ± 1.465

*Data are given as Mean $\pm$ SD (n=3)

Table: 2 IC_{50} Values for Alpha Amylase Enzyme inhibition by SC and STD

Test Standard	Drug	/	IC_{50} Value of Alpha Amylase enzyme inhibition $\pm$ SD (μ g /ml)
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SC	332.3 ± 52.41
Standard-Acarbose	20.61 ± 5.146

*Data are given as Mean ± SD (n=3)

Table: 3 Percentage inhibition of Standard Acarbose on Alpha Amylase enzyme

Inhibition Study

Concentration (µg/ml)	% Inhibition of Acarbose
10 µg/ml	27.94 ± 5.76
20 µg/ml	59.91 ± 1.36
40 µg/ml	70.83 ± 0.83
80 µg/ml	84.58 ± 0.48
100 µg/ml	94.02 ± 4.7

Data are given as Mean ± SD (n=3)

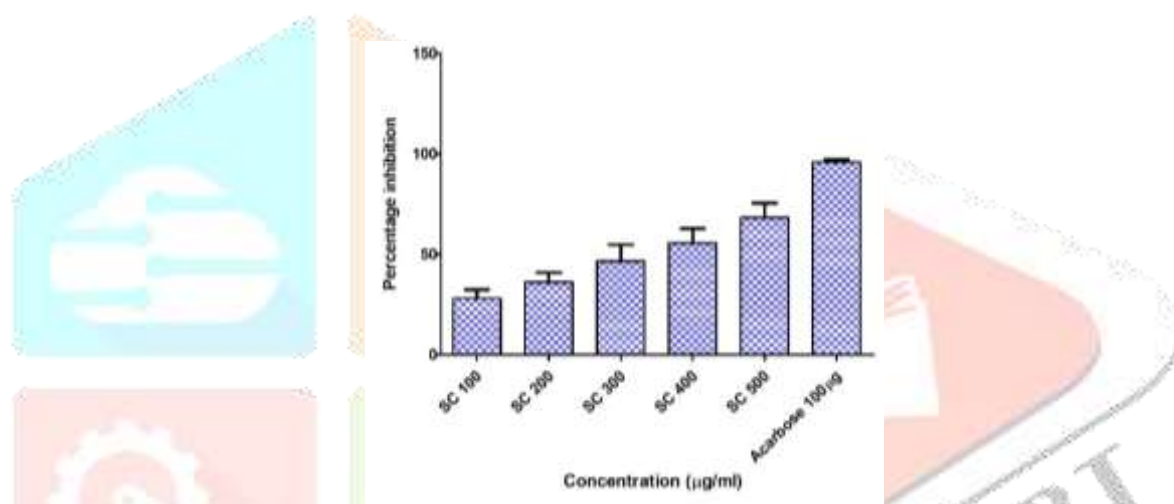


Fig:1 Percentage inhibition of test drug SC and Standard on Alpha Amylase Enzyme Inhibition Assay

DISCUSSION

The antidiabetic properties of plants can be evaluated by several methods; one such model is the *in vitro* α -amylase inhibitory activity by 2-chloro-4-nitro phenyl α -maltotrioxide (CNPG3) assay (Spectrophotometric assay method). Alpha-amylase, an enzyme found in salivary, intestinal mucosal, and pancreatic secretions, functions in the breakdown of the α -1-4-glycosidic bonds in starch, thereby increasing the bioavailability of glucose in the blood. For a substance to be antidiabetic, it should either reduce the amount of glucose in the blood or increase the efficacy of insulin. Inhibiting carbohydrate hydrolyzing enzymes has been proven to decrease postprandial hyperglycemia and improve impaired glucose metabolism without promoting insulin secretion in non-insulin dependent diabetes mellitus (NIDDM) patients.

The phytochemical analysis of the sample SC reveals the presence of bioactive phytocomponents such as flavonoids, steroids, triterpenoids, phenols, tannins, saponins, proteins, and carbohydrates. Each of these components contributes to the antidiabetic properties of the plant in unique ways. For instance, flavonoids are known for their antioxidant activity, which can help combat oxidative stress often seen in diabetes.

These findings align with other studies that have investigated the antidiabetic properties of similar plants or formulations. This consistency across different studies strengthens the validity of this results and underscores the potential of plant-based treatments for diabetes.

The potential clinical implications of findings are significant. If these plants can effectively inhibit α -amylase and thus reduce postprandial hyperglycaemia, they could potentially be used as a natural treatment for NIDDM. This could provide a cost-effective and accessible treatment option for many patients, particularly in regions where access to conventional diabetes medications is limited.

However, this study has some limitations. Further research is needed to determine the optimal dosage of the plant extract for maximum antidiabetic effect, and to explore its effects in combination with other antidiabetic treatments. Additionally, while this study focused on in-vitro models, future studies should aim to validate these findings in in vivo models and clinical trials.

In conclusion, this study adds to the growing body of evidence supporting the antidiabetic properties of plants, and highlights the need for further research in this area. By continuing to explore the potential of plant-based treatments, we can hope to expand our arsenal of effective, accessible treatments for diabetes.

CONCLUSION:

It was observed from the results of the present investigation that the formulation SC shown promising alpha amylase enzyme inhibition potential with the maximum inhibition of about $68.29 \pm 7.362\%$ and the corresponding IC_{50} is $332.3 \pm 52.41 \mu\text{g/ml}$. Standard acarbose exhibited significant inhibition in alpha glucosidase enzyme with the maximum inhibition of about $95.96 \pm 1.465\%$ and the corresponding IC_{50} is $20.61 \pm 5.146 \mu\text{g/ml}$.

This *in-vitro* study demonstrates α -amylase inhibitory activity of Seenthil Chooranam.

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Competing of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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