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ALPHA GLUCOSIDASE INHIBITORY ACTIVITY OF SIDDHA FORMULATION SEENTHIL CHOORANAM-IN VITRO STUDY

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ABSTRACT

According to the World Health Organization (WHO), Diabetes Mellitus (DM) is one of the top ten morbid conditions, characterized by high blood sugar level, or hyperglycemia. Type 2 diabetes is the most common type of diabetes. Inhibition of α-glucosidase enzyme is an effective method of managing diabetes.This study assess the α-glucosidase inhibitory activity of seenthil chooranam (SC), a siddha formulation practised by siddha physicians in the management of type 2 diabetes mellitus. SC is regarded as an effective medicine for reducing blood sugar level.The alpha glucosidase inhibitory activity of SC is determined using spectrophotometric assay technique.SC exhibits α-glucosidase inhibitory activity (IC 50 = 380.8 ± 104.5µg/ml).

KEYWORDS: Diabetes mellitus, Seenthil chooranam, Madhumegam, In-vitro Alpha-glucosidase inhibition activity, ancient medicine, Siddha.

1. INTRODUCTION

Siddha Medicine is an ancient medicine practicised by siddhars, who were masters in creating unique formulations of medicines to treat and manage various illnesses. Seenthil chooranam

is of those formulations. In Siddha literature, Yugi, a renowned siddhar, mentions MadhuMegam (MM) as one of the 20 types of Meganeer which is classified under Neerinai perukkal noi.[1] The symptoms listed in MM corresponds to the metabolic disorder, Diabetes Mellitus (DM), which is a highly prevalent health burden not just in India but also around the world. According to the World He alth Organization, Diabetes is one of the top ten fatal illnesses.

DM is a metabolic disorder characterized by hyperglycemia. Type I and Type II are its two primary subtypes. Type I is caused by an error in the immune response targeting the beta cells in the pancreas resulting in absolute insulu. Type II diabetes, on the other hand, is caused by either inadequate insulin synthesis or a decreased body's ability to respond to insulin. Long-term hyperglycemia can affect the kidneys (nephropathy), foot (neuropathy), and eyes (retinopathy). Urbanization, poor eating habits, obesity, and sedentary lifestyles are all strongly associated with this global health concern.[2]

India has the second highest number of people with diabetes.[3] Diabetes currently affects more than 74 million Indians, which is more than 8.3% of the adult population.[3] It is estimated to be around 57% of the current cases of diabetes to be undiagnosed.[4]

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.[5] The average age on onset is 42.5 years.[6] Nearly 1 million Indians die due to diabetes every year.[6]

According to the Indian Heart Association, India is projected to be home to 109 million individuals with diabetes by 2035.[7] A study by the American Diabetes Association reports that India will see the greatest increase in people diagnosed with diabetes by 2030.[8] 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.[9][10]

India is recognized as having the highest prevalence of diabetes worldwide, with projections estimating nearly 69.9 million affected individuals by 2030.[11]

SC contains Seenthil stem (Tinospora cardifolia), Karisalai (Eclipta alba), and Poonagam (Earthworm).[12] Individual components of SC demonstrates anti-diabetic action.[13][14][15] This formulation as a whole has anti-diabetic effect.[16] Its safety has previously been proved[17] and is being practised in the management of DM.

Alpha-glucosidase is an enzyme found in the brush border of small intestine. It catalyses the final stage in the breakdown of ingested carbohydrates, converting disaccharides and oligosaccharides into glucose and other monosaccharides. This process permits these simple carbohydrates to enter the bloodstream, resulting in an increase in postprandial blood glucose level. Therefore, managing this is a crucial part in the management of type 2 diabetes.

Assessing the α-glucosidase inhibitory activity would help to scientifically validate the usage of siddha formulation, SC in the management of type 2 diabetes mellitus.

MATERIALS AND METHODS

STANDARD OPERATIVE PROCEDURE

Ingredients of the Seenthil Chooranam

Seenthil chooranam is a siddha formulation mentioned in "The Siddha Formulary Of India"under the heading chooranam.The ingredients of the same is as below.

1. Seenthil Thandu (Stem of Tinospora cardifolia)
2. Karisalai (Eclipta alba)
3. Poonagam (Earthworm)

Authentication of raw drugs

The required raw medicines were collected. Botanist of Govt Siddha Medical College certified the plants, while the Gunapadam department of Govt Siddha Medical College in Chennai authenticated the poonagam (earthworm).

Purification of raw drugs

- Seenthil stem is washed 21 times in water and soaked in milk and dried.[12]
- Debris were removed from karisalai and washed to get rid of soil, wiped with clean cloth and dried.[18]
- Poonagam is soaked in milk to empty its intestine and dried.[12]

Preparation of medicine

Each dried ingredient is powdered separately. Equal parts of all three powdered ingredients are mixed and stored in an air tight container.

Study center

Sample of 20gm of SC was sent to "The Noble Research Solutions, Chennai" for evaluation

Method adapted

The spectrophotometric assay.[19]

The chemicals and method adapted are as follows.

Chemical List

Name of the Chemical	Source
p-nitrophenyl-α-D –glucopyranoside	Hi-Media
$Na_2 CO_3$	Hi-Media
Glucosidase enzyme	Sigma-Aldrich
Phosphate buffer	Merck

Machine

Spectrophotometer	Name of the instrument SPEKTRON 721S

Test Solution: Test Sample (SC) is prepared in the serial dilution of the concentration ranges from 100,200,300,400 and 500 µg/ml using DD water.

PNPG (p-nitrophenyl-α-D -glucopyranoside**)**: 20 ml PNPG is prepared by dissolving 603 mg PNPG in 100 ml of phosphate buffer solution (PBS)

Enzyme: The α-glucosidase enzyme solution is prepared by dissolving 0.5 mg α-glucosidase in 10 ml phosphate buffer (pH 7.0) containing 20 mg bovine serum albumin. About 10 µl of the test sample at varying concentration along with Acarbose 100 µg/ml used as a reference standard were added to 250 µl of 20 mM p-nitrophenyl-α-D -glucopyranoside and 495 µl of 100 mM phosphate buffer (pH 7.0). It is pre-incubated at 37°C for 5 min and the reaction started by addition of 250 µl of the α-glucosidase enzyme solution prepared by 0.5 mg α-glucosidase in 10 ml phosphate buffer (pH 7.0) containing 20 mg bovine serum albumin, after which is then incubated at 37°C for exactly 15 min. 250 µl of phosphate buffer is added instead of enzyme for blank. The reaction is then stopped by addition of 1000 µl of 200 mM $Na_2 CO_3$ solution and the amount of p-nitrophenol released is measured by reading the absorbance of sample against a sample blank (containing PBS with no sample) at 405 nm using UV visible spectrophotometer.

RESULT

In this study, the Alpha glucosidase inhibition assay reveals that SC shows inhibition of α-glucosidase enzyme activity.SC at 100,200,300,400 and 500µg/ml concentration showed 33.88 ± 2.193, 36.64 ± 0.3181,44.37 ± 0.1383,57.53 ± 1.318 and 65.53 ± 3.025 percentage

inhibition of α-glucosidase activity respectively and IC_{50} value was found to be 380.8 ± 104.5µg/ml [Table 2].

However, Acarbose is used as reference standard, and shows 33.25 ± 4.14,56.57 ± 2.03,74.87 ± 0.67,81.39 ± 1.85 and 96.07 ± 2.21 percentage inhibition of α- glucosidase activity at concentrations of 10, 20,40,80 and 100µg/ml [Table 3]; the calculated IC_{50} value is found to 28.76 ± 21.27µg/ml[Table 2].

Percentage inhibition of test drug SC on α-Glucosidase enzyme Inhibition Study

Concentration (µg/ml)	% Inhibition of SC
100 µg/ml	33.88 ± 2.193
200 µg/ml	36.64 ± 0.3181
300 µg/ml	44.37 ± 0.1383
400 µg/ml	57.53 ± 1.318
500 µg/ml	65.53 ± 3.025
Standard- Acarbose	95.57 ± 1.905

Data are given as Mean ± SD (n=3)

IC50 Values for α-Glucosidase enzyme Inhibition Assay by SC and STD

Test Drug / Standard	IC50 Value of α-Glucosidase enzyme inhibition ± SD (µg /ml)
SC	380.8 ± 104.5
Standard- Acarbose	28.76 ± 21.27

Data are given as Mean ± SD (n=3)

Table 3: Percentage inhibition of Standard Acarbose on α- Glucosidase enzyme Inhibition Study.

Concentration (µg/ml)	% Inhibition of Acarbose
10 µg/ml	33.25 ± 4.14
20 µg/ml	56.57 ± 2.03
40 µg/ml	74.87 ± 0.67
80 µg/ml	81.39 ± 1.85
100 µg/ml	96.07 ± 2.21

*Data are given as Mean ± SD (n=3)

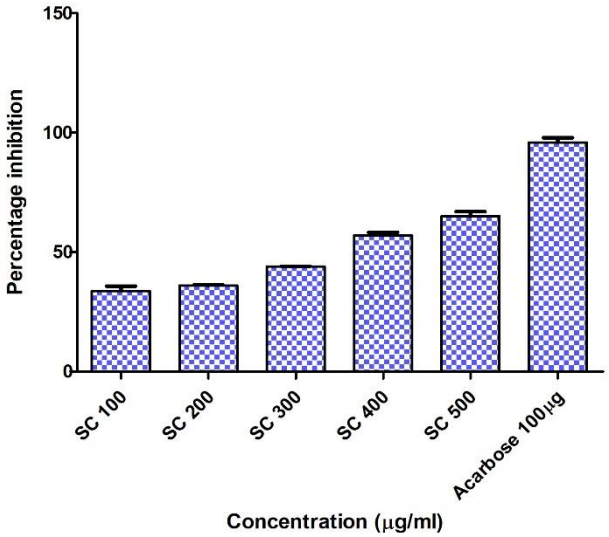

Fig. 1: Percentage inhibition of test drug SC and Standard on α- Glucosidase enzyme Inhibition Assay.

DISCUSSION

The antidiabetic properties of plants can be evaluated by several methods; one such method is the in vitro α-glucosidase inhibitory activity by Spectrophotometric method. Alpha-glucosidase, 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.[20]

In a study, the dichloromethane (DCM) extract of Tinospora cordifolia shows 100% inhibition of alpha glucosidase, while salivary amylase was inhibited to the extent of 75% and pancreatic amylase to 83%.[21] In another study, eclipta alba shows alpha glucosidase activity in streptozotocin-induced diabetic rats.[22] More over the earthworm extract enhanced

organ functions in diabetic rats by ameliorating physiological and structural changes.[23]

These findings are consistent with prior studies on the antidiabetic benefits of similar formulations. This uniformity among research improves the validity of the findings and highlights the possibility of plant-based management of diabetes. The findings could have important clinical implications too. Plants that inhibit α-glucosidase may lower postprandial hyperglycemia, making them a viable natural treatment for NIDDM. This could be a cost-effective and accessible treatment option for many people to over come gastro-intestinal and other side effects in conventional treatment.

However, this study has limitations. This study focused only on in-vitro models, future studies should aim to validate these findings in in vivo models and clincal trials and to explore its effects in combination with other antidiabetic treatments.

In conclusion, this study evidence supporting the antidiabetic properties of siddha formulations, and highlights the need for further research in this area. By continuing to explore the potential of siddha medicines, we can hope to expand our arsenal of effective, accessible treatments for diabetes.

CONCLUSION

It is observed from the results of the present investigation that the formulation SC has shown alpha glucosidase enzyme inhibition potential with the maximum inhibition of about 65.53 ± 3.025% and the corresponding IC_{50} is 380.8 ± 104.5 µg /ml. Standard acarbose exhibited significant inhibition in alpha glucosidase enzyme with the maximum inhibition of about 96.07 ± 2.21% and the corresponding IC_{50} is 28.76 ± 21.27µg /ml.

This *in-vitro* study demonstrate α-glucosidase 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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