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Screening of Anti-Diabetic Potential of the Siddha Formulation Uloga Chenduram by *In-Vitro* Alpha-Amylase and Alpha-Glucosidase Enzyme Inhibition Assay

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R.Gomathi[*1], E.Preetheekha[2], T. Lakshmi Kaantham[3], S. Mohan[4]

[1] P.G. Scholar, Department of maruthuvam, National Institute of Siddha, Tambaram Sanatorium, Chennai-47, Tamil Nadu, India.

[2] P.G. Scholar, Department of kuzhanthai maruthuvam, National Institute of Siddha, Tambaram Sanatorium, Chennai-47, Tamil Nadu, India.

[3] Associate Professor, Department of maruthuvam, National Institute of Siddha, Tambaram Sanatorium, Chennai-47, Tamil Nadu, India.

[4]. Head of Department, Department of maruthuvam, National Institute of Siddha, Tambaram sanatorium, Chennai – 47, Tamil Nadu, India.

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder, characterized by hyperglycemia and carbohydrate, protein, and fat metabolic disturbances. Chronic hyperglycemia has been considered as one of the principal causes of several diabetic complications.α-amylase and α-glucosidase is carbohydrate hydrolyzing enzymes responsible for postprandial hyperglycemia. Hence, α-amylase and α-glucosidase are helping in the control of hyperglycemia by delaying carbohydrate digestion. It was observed that chronic usage of certain anti-diabetic agents may lead to hypoglycemia, headache, dizziness, nausea, hypersensitivity reactions, and weight gain. However, Siddha system of medicine has potential formulations that could better manage the condition of diabetic without major side effects. The main objective of the present study is to evaluate the anti-diabetic potential of the formulation of Uloga Chenduram (UC) by alpha-amylase and alpha-glucosidase enzyme inhibition assay. It was observed from the results of the present investigation that the test drug UC shown significant inhibition in alpha-amylase and alpha-glucosidase enzyme with the maximum inhibition of about 40.31± 17.59% and 33.06 ± 1.087% respectively, corresponding IC50 are 723.3 and 30.6 µg/ml. It was concluded from the study that the Siddha drug UC has minimal control over halting the activity of both the metabolic enzymes such as alpha-amylase and alpha-glucosidase. Further studies need to be elaborated for identifying the mechanism of action of this novel formulation with proper pre-clinical validation.

1. INTRODUCTION

Diabetes is a comparable condition with *'Madhumegam'or Neerizhivu* in Siddha literature, *Madhumegam (Neerizhivu)* is one among the *Neerinaiperukkalnoi*[1].

In the Siddha system of medicine, *Meganoi*is classified into twenty types in which four comes under Vatham, six under Pitham and ten under Kabam. *Madhumegam/Neerizhivu*((Diabetes mellitus) comes under pitham [2].

The Major cause of *Meganoikal* is the dearrangement of pithahumour. Sage Theraiyar says "*Pakarpithavinthaiyalaathumegamvaarathu"* in Pinimutharkaaranam[3].

Diabetes affects many systems in the body and, over time, can lead to serious complications. Complications from diabetes can be classified as microvascular and macrovascular. A microvascular complication involves the nervous system (diabetic neuropathy), renal system (Diabetic nephropathy) and ophthalmic system (Diabetic retinopathy) [4]. A Macrovascular complication involves the cardiovascular system (CVD), central nervous system, peripheral vascular disease. Peripheral vascular disease may lead to bruises or injuries that do not heal, gangrene, and, ultimately, amputation.

In Asia, the prevalence of diabetes is increasing at an alarming rate and expected to rise two or three folds by 2030 [5]. All forms of diabetes are characterized by chronic hyperglycemia and the development of diabetes-specific microvascular and macro-vascular complications that affect major organs such as eye, kidney, heart, brain, and lower extremities. Accumulated evidence suggested the four main hypotheses linked to hyperglycemia-induced complications in diabetic patients: increased polyol pathway flux, increased advanced glycation end products, activation of protein kinase C, and increased hexosamine pathway flux [6].

Consumed foods contain polysaccharides which are converted into monosaccharides in the small intestine by carbohydrate metabolism. The enzymes involved are mainly pancreatic alpha-amylase and alpha-glucosidase[7]. Control of postprandial blood glucose level in type 2 diabetes relies upon inhibition of these enzymes, such inhibitory action is possessed by category of drugs such as acarbose and miglitol, But these drugs have common side effects such as flatulence and abdominal bloating [8].

Sulfonylureas limits gluconeogenesis in the liver, decrease the breakdown of lipids into fatty acids and also by blocking KATP channels increases insulin secretion in the pancreas [9]. Sulfonylureas are contraindicated in patients with hepatic and renal diseases as it creates side effects like hypoglycemia, headache, dizziness, hypersensitivity reaction and weight gain. However, many of these conventional drugs have been reported for their inefficiency with prominent adverse side effects [10]. These limitations have largely prompted the exploration of management strategies involving the use of natural therapy reported to be cost-effective antidiabetic agents with fewer or no reported side effects [11]. Increasing evidence also suggested that the intake of anti-oxidant may also tend to halt the progression of diabetes in several cases [12,13].The present study aimed at evaluating the anti-diabetic potential of the Siddha formulation Uloga Chenduram (UC) by alpha-amylase and alpha-glucosidase enzyme inhibition assay.

MATERIALS AND METHODS:

2. RAW DRUGS, PURIFICATION & DRUG PREPARATION

2.1 INGREDIENTS OF ULOGA CHENDURAM [14]

1. Purified ulogamanduram (ferrouso ferric oxide) - 336gm

2. Naavalpattaichaaru (stem Bark juiceofSyzygium*cumini*) - 672 ml

3. Kaiyaanchaaru (juice of Eclipta*prostrata*) - 672 ml

Decoction of following herbs,

4. Kadukkai (Terminalia *chebula*) - 100 ml

5. Thandrikai (Terminalia *bellirica*) -100ml

6. Nellikai (Phyllanthus *Emblica*) - 100 ml

2.2 SOURCE OF RAW DRUGS:

The required raw drugs for the preparation of ULOGA CHENDURAM were procured from a reputed country medical shop, Paris corner, Chennai. The Ulogamanduramauthenticated by the competent authority of the Gunapadam Department, NIS. Naavalpattai and kaiyaan leaves were collected from pallavaram and authenticated by the Assistant professor, medicinal botany, NIS Chennai. The raw drugs were purified and medicine was prepared as per Siddha literature in Gunapadam laboratory at National Institute of Siddha.

2.3 METHOD OF PURIFICATION: [15]

Mandooram was placed in theural and pounded and then it was placed in a pan, added 4 parts of tamarind leaves (Tamarindusindica) and 8 parts of water, boiled for 3hrs (1saamam). Then the leaves and powder were washed and dried, after that mandooram was powdered in a kalvam and placed it in a pan with 4 parts of cow's urine, boiled until it dried then washed with water.

2.4 METHOD OF PREPARATION: [14]

At first instance, the purified ulogamandooram was well soaked in both naavalpattaichaaru, kaiyaanchaaru for seven days and then dried well. Now it was ground well with Triphala decoction and made into small round cakes (villai) and subjected to pudam (7 times). Allowed to cool itself then chenduram was collected.

3. MATERIALS AND METHODS

3.1. *In-vitro* Alpha-Amylase Inhibition Study [16]

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 (UC) was prepared in the serial dilution of the concentration ranges from 100,200,300,400 and 500 µg/ml using DD water. Acarbose 100 µg/ml used as a reference standard. About 600 µl of test sample was added to 30 µl of α-amylase enzyme solution and incubated at 37°C for 15 min. To this reaction mixture, 370 µl of the substrate, 2-Chloro-4-Nitrophenyl-α-Maltotrioside ($CNPG_3$- 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 a spectrophotometer. A control reaction was carried out without the test sample. Percentage inhibition was calculated by the following formula.

Percentage inhibition

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

3.2. *In-vitro* α-Glucosidase Enzyme Inhibition Study [17]

The α-glucosidase enzyme solution was 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 each of the test sample at varying concentration along with Acarbose 100 µg/ml used as a reference standard was added to 250 µl of 20 mM p-nitrophenyl-α-D -glucopyranoside and 495 µl of 100 mM phosphate buffer (pH 7.0). It was 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 it was incubated at 37°C for exactly 15 min. 250 µl of phosphate buffer was added instead of enzyme for blank. The reaction was then stopped by the addition of 1000 µl of 200 mM Na_2CO_3solution and the amount of p-nitrophenol released was measured by reading the absorbance of the sample against a sample blank (containing PBS with no sample) at 405 nm using UV visible spectrophotometer.

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

RESULTS AND DISCUSSION

4. RESULTS

4.1. Effect of UC on alpha-amylase enzyme inhibition assay

It was observed from the results of the present investigation that the Siddha formulation UC shown significant inhibition in alpha-amylase enzyme with the maximum inhibition of about 40.31± 17.59%and the corresponding IC50 is 723.3 ± 219.9 µg /ml. Standard acarbose exhibited significant inhibition in alpha-glucosidase enzyme with the maximum inhibition of about 95.76 ± 2.761% and the corresponding IC50 is 34.35 ± 2.26 µg /ml. As shown in Table 1,3 and Figure 1.

Table No. 1: Percentage inhibition of test drug UC on Alpha-Amylase Enzyme Inhibition Study

Sr. No.	Concentration (µg/ml)	% Inhibition of UC
1.	100 µg/ml	9.129 ± 3.192
2.	200 µg/ml	19.04 ± 4.86
3.	300 µg/ml	25.28 ± 3.133
4.	400 µg/ml	33.02 ± 3.823
5.	500 µg/ml	40.31 ± 7.596
6.	Standard Acarbose	95.76 ± 2.761

Data are given as Mean ± SD (n=3).

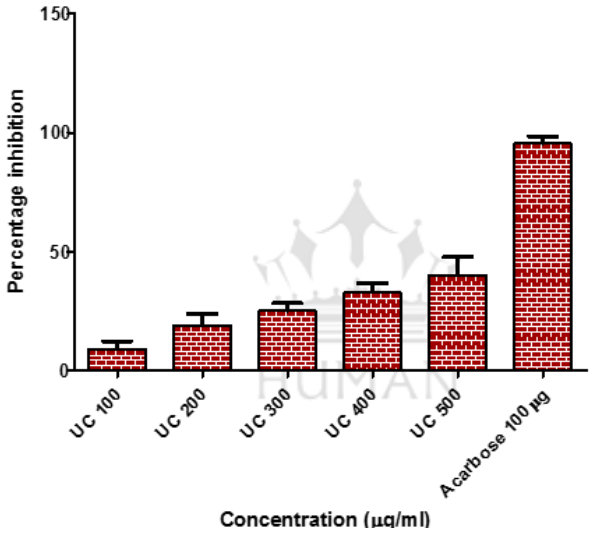

Figure No. 1: Percentage inhibition of test drug UC and standard acarbose on Alpha-Amylase Enzyme Inhibition Study

4.2. Effect of UC on α-glucosidase enzyme inhibition assay

It was observed from the results of the present investigation that the Siddha formulation UC shown significant inhibition in alpha-glucosidase enzyme with the maximum inhibition of about 33.06 ± 1.087% and the corresponding IC50 is 130.6 ± 35.25 µg/ml. Standard acarbose exhibited significant inhibition in alpha-amylase enzyme activity with the maximum inhibition of about 93.63 ± 0.95 % and the corresponding IC50 is 6.61 ± 5.06 µg /ml. As shown in Table 2,3 and Figure 2.

Table No. 2: Percentage inhibition of test drug UC on Alpha Glucosidase Enzyme Inhibition Study

S.No	Concentration (µg/ml)	% Inhibition of UC
1.	100 µg/ml	2.916 ± 0.6685
2.	200 µg/ml	10.73 ± 2.424
3.	300 µg/ml	20.54 ± 0.2971
4.	400 µg/ml	26.14 ± 0.6744
5.	500 µg/ml	33.06 ± 1.087
6.	Standard Acarbose	93.63 ± 0.9502

Data are given as Mean ± SD (n=3).

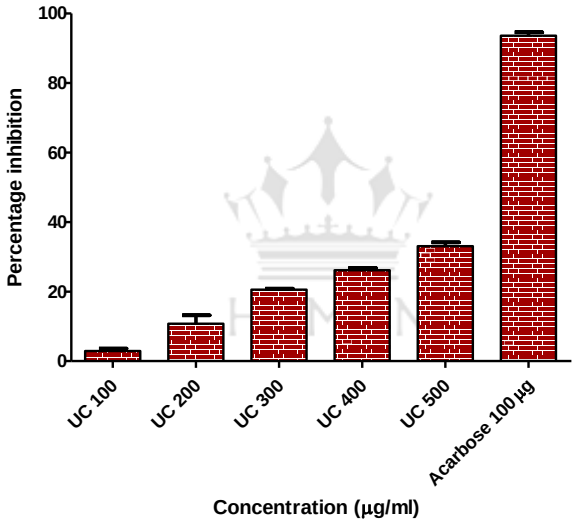

Figure No. 2: Percentage inhibition of test drug UC and standard acarbose on Alpha GlucosidaseEnzyme Inhibition Study

Table No. 3: IC50 Values for Alpha-Amylase Enzyme inhibition by UC and STD

Test Drug / Standard	IC50 Value of Alpha-Amylase enzyme inhibition ± SD (µg /ml)	IC50 Value of α-Glucosidase enzyme inhibition ± SD (µg /ml)
UC	723.3 ± 219.9	130.6 ± 35.25
Standard- Acarbose	34.35 ± 2.262	6.61 ± 5.066

Data are given as Mean ± SD (n=3).

5. DISCUSSION

According to an estimation of the International Diabetes Federation, approximately 366 million people are suffering from diabetes and this may double by 2030, in India to be 40.9 million, which is expected to grow to 60.9 million by 2025 [18]. Between two types of diabetes, type 2 is more prevalent than type 1, with more than 90% of the total diabetic patients suffering from it. Type 2 diabetes (T2D) is a disease caused by an imbalance between blood sugar absorption and insulin secretion. Postprandial hyperglycemia plays an important role in the development of T2D [19]. Because diabetes is regarded as a chronic metabolic disease, numerous antidiabetic therapies with conventional drugs are often not a single-dose program as most drugs require frequent injections, sometimes for the entire life of the diabetic patient.

Multiple strategies have been devised or explored in the management of DM. Stimulation of Adenosine monophosphate-dependent protein kinase (AMPK) (Biguanides-Metformin); blockage of ATP-gated K^+ channels in β cells (Sulfonylureas-Glipizide); stimulation of peroxisome proliferator-activated receptors activities (PPAR Υ) (Thiazolidinediones-Rosiglitazone); and glucagon-like peptide-1 (GLP-1) (Exenatide-Byetta) modulation [20,21].

In the management of postprandial hyperglycemia (PPH) enhancement of insulin secretion, insulin sensitivity or reducing glucose production in the liver are achieved by inhibiting the activity of alpha-amylase and alpha-glucosidase, the major risk factor for cardiovascular complication in DM patient is glycation end product (a metabolite), hence by reducing PPH reduces this metabolite[22]. It was observed from the results of the present investigation that the Siddha formulation UC shown significant inhibition in alpha-amylase enzyme with the maximum inhibition of about 40.31± 17.59%and the corresponding IC50 is 723.3 ± 219.9 µg /ml. Standard acarbose exhibited significant inhibition in alpha-glucosidase enzyme with the maximum inhibition of about 95.76 ± 2.761% and the corresponding IC50 is 34.35 ± 2.26 µg /ml.

Acarbose inhibits both α-amylase and α-glucosidase, but Miglitol and Voglibose inhibit only α-glucosidase. Though effective in controlling PPHG, these inhibitors are not desirable for long-term treatment due to their gastrointestinal side effects [23,24]. Given the fact that about 80 % of diabetic people are living in low and middle-income countries [25], these drugs are expensive also. Therefore, several groups have made their efforts to find α-amylase and α-

glucosidase inhibitors from alternate sources like herbs and microbes [26]. It was observed from the results of the present investigation that the Siddha formulation UC shown significant inhibition in alpha-glucosidase enzyme with the maximum inhibition of about 33.06 ± 1.087% and the corresponding IC50 is 130.6 ± 35.25 µg/ml. Standard acarbose exhibited significant inhibition in alpha-amylase enzyme activity with the maximum inhibition of about 93.63 ± 0.95 % and the corresponding IC50 is 6.61 ± 5.06 µg /ml.

6. CONCLUSION

Diabetes is a multifactorial disease that has a significant adverse impact on health and mortality, particularly from cardiovascular diseases. Studies have shown large regional and socioeconomic differences in the prevalence of type 2 diabetes in India. Now, a day's traditional medicines are gaining popularity in the treatment of diabetes and its related complications. From the data of the present study, it was concluded that the formulation UlogaChenduram possessed a significant level of α-amylase and α-glucosidase enzyme inhibition property in the tested models.

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	**R. Gomathi** PG scholar, Department of Maruthuvam, National institute of Siddha, Tambaram sanatorium, Chennai 47.
	**E.Preethekha** PG scholar, Department of Kuzhanthai Maruthuvam, National institute of Siddha, Tambaram sanatorium, Chennai 47.
	**T.Lakshmikantham** Associate professor, Department of Maruthuvam, National institute of Siddha, Tambaram sanatorium, Chennai 47.
	**S.Mohan** Head of Department, Department of Maruthuvam, National institute of Siddha, Tambaram sanatorium, Chennai 47.