In-Vitro Anti-Diabetic Evaluation of *Sambirani Poo Kuligai*by Alpha Amylase Enzyme Inhibition Assay

Abstract

Background: Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia or increased blood glucose levels with disturbances in carbohydrate, fat and protein metabolism resulting from absolute or relative lack of insulin secretion. The Siddha system of medicine is a traditional medical system that uses a scientific and holistic approach to provide preventive, promotive, curative, rejuvenating and rehabilitative health care. According to Siddha literature, diabetes is known as Innippu Neer, Madhumegam and Neerizhivu. The various reasons for the cause of this disease are attributed to food, habits and lifestyle changesand also due to hereditary causes.The inhibition of alpha amylase enzyme involved in the digestion of carbohydrates can significantly reduce the postprandial increase of blood glucose and therefore can be an important strategy in the management of blood glucose level in type-2 diabetic and borderline patients.**Aim:**The aim of the present study is to evaluate the anti-diabetic potential of the siddha formulation of Sambirani Poo Kuligai(SPK) by alpha amylase enzyme inhibition assay.**Method**: The spectrophotometric assay method was used to find alpha amylase inhibitory activity.**Results:** SPK showed presence of alpha amylase inhibitory potential with the maximum inhibition of about 48.67 ± 4.097 % and the corresponding IC_{50} is 578.6 ± 44.75 µg /ml. **Conclusion:** This in-vitro study revealed the presence of the alpha amylase activity in the siddha formulation Sambirani Poo Kuligai possess anti-diabetic activity.

Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia or increased blood glucose levels with disturbances in carbohydrate, fat and protein metabolism resulting from absolute or relative lack of insulin secretion.(1)It is estimated that 537 million (10.5%) individuals (those aged 20–79 years) worldwide are currently managing the disease.In 2021, the International Diabetes Federation (IDF) approximated that there were 537 million individuals living with diabetes, making up 10.5% of the global population, resulting in global healthcare expenses amounting to $966 billion. This health cost is predicted to rise to more than $1054 billion by 2045. It is alarming that the prevalence of DM is anticipated to increase to 643 million (11.3%) by 2030 and 783 million (12.2%) by 2045(2)

The Siddha system of medicine is a traditional medical system that uses a scientific and holistic approach to provide preventive, promotive, curative, rejuvenating and rehabilitative health care. The word siddha is derived from the Tamil word siddhi, which means “to achieve” or “perfection”or “heavenly bliss”.(3)According to Siddha literature, diabetes is known asInnippuNeer, Madhumegam andNeerizhivu. The various reasons for the

cause of this disease are attributed to food, habits, and lifestyle changes and also due to hereditary causes.Vatham, Pitham andKaphamare the basic principles of Siddha medicine which play a vital role in the pathology of Madhumegam. (4)

Amylase inhibitors are also known as starch blockers because they contain substances that prevent dietary starches from being absorped by the body. Starches are complex carbohydrates that cannot be absorbed unless they are the first broken down by the digessive enzyme amylase and other secondary enzymes. Salivary and pancreatic amylases catalyze the hydrolysis of glycosidic linkages in starch and other related polysaccharides, their inhibition have been theorized to have beneficial therapeutic effects by reducing carbohydrate-induced hyprglycemia and hyperinsulinemia.

The inhibition of alpha amylase enzyme involved in the digestion of carbohydrates, can significantly reduce the postprandial increase of blood glucose and therefore can be an important strategy in the management of blood glucose level in type-2 diabetic and borderline patients. (5)In vitro study, extracts of piper betel and syzygium aromaticum(clove) have potential alpha amylase and alpha glucosidase inhibitory activity .6,7) Based on the previous studies, SPK has the possibility of having the same pharmacological activity as alpha amylase inhibitor for potential anti-diabetic agent, but such tests have not been conducted. This research purpose is to investigate in-vitro alpha amylase enzyme inhibitor of SPK.

MATERIAL AND METHODS

a) Ingredients of the Test Drug

1) Sambirani (benzoin)

2) Kirambu (clove)

3) Korosanai (korochana)

4) Vetrilai (piper betle)

The reference for this preparation was taken from the classic siddha text, AgathiyarParipooranam 400".(8) The trial drug was prepared as per a Standard Operative Procedure (SOP).

b) Drug Authentication

The requisite raw drugs were procured from a well reputed indigenous raw drug shop. The herbal raw drugs were authenticated by the Botanist of Government SiddhaMedical College, Chennai and the mineral drugs were authenticated by the HOD of *Gunapadam*Department of Govt. Siddha Medical College, Chennai.

c) PurificationofRawDrugs

Herbal and mineral drugs underwent purification as per"*SikitchaRatnaDeepamEnnumVaidhiyaNool*"(9) and "*GunapadamThathuJeevaVaguppu*" respectively.

Table 1. PurificationofRawDrugs

S.No	Name Of The Drug	Purification
1	Sambirani	The gums were purified by removing the sand, dust and odd particles
2	Kirambu	The flower buds were removed and fried slightly
3	Korosanai	The unwanted substances were removed Test for korosanai: On piercing a red hot needle into korosanai, it shows the deposition of yellow material and emission of yellow fumes
4	Vetrilai	The stalk and the middle vein were removed

Table 2. Raw Drug's Botanical Name, Family and Part Used

S.No	Name Of The Raw Drug	Botanical/ Zoological Name(10)	Family	Part Used
1	Sambirani	*Styraxbenzoin,Dryand*	Styracaceae	Resin
2	Kirambu	*Syzygiumaromaticum,Linn*	Myrtaceae	Dried Flower buds
3	Korosanai	*Felbovinumpurifactum(purified ox bile or ox gall)*	NA	NA
4	Vetrilai	*Piper betle,Linn*	Piperaceae	Leaves

Table 3. Actions and Chemical constituents of Raw Drugs

S. No	Name Of The Drug	Actions	Chemical Constituents
1	Sambirani	Stimulant(10)	Cinnamic acid, benzoic acid, bensylbenzoate, lignans, vanillin, benzaldehyde(16)

2	Kirambu	Anti-diabetic, Hepatoprotective,(12) Anti spasmodic	B caryophyllene, eugenol, acetophenone, eugenyl acetate, α humulene, γ cadinene, α phellandrene, rhammetin, kaempferol, gallic acid, vanillin(17)
3	Korosanai	Anti-spasmodic	Cholic acid, chenodeoxycholic acid, biliverdin, phospholipids, cholesterol(18)
4	Vetrilai	Stimulant, Carminative, Anti-hypercholesterolemic,13 Anti-oxidant, Anti- diabetic(14, 15)	Arecoline, choline, eugenol, chavicol, caryophyllene, limonene, allylpyrocatechol(19)

d) Preparationof the Drug

The purified Benzoin was powdered well and placed in a small pot. A paper was pasted on the inner surface of another big pot. The big pot was placed over the small pot with their mouths opposing each other. The gaps between their mouths were sealed by seven layered muddy wet cloth and was allowed to dry. Then it was subjected to sublimation process for 12 hours. After finishing sublimation process, the pot was left undisturbed to reduce heat. Then the seal was opened and the sublimed product was scrapped and collected. Clovewas powdered well and sieved through a white cloth. Korochanaalso was powdered well. Clovepowder and Korochana powder were added along with the sublimate. Then all these substances weregrounded well with Piper betel leaf juice for 48 minutes. The paste was rolled into pills to the size of the seeds of Abrusprecatorius which was equivalent to 130mg and dried in the shade and bottled up.

RESULTS

1. In-vitro Alpha Amylase Inhibition Study

Method Adopted: The spectrophotometric assay method.

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 (SPK) 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 were added to 30 µl of α-amylase enzyme solution and incubated at 37°C for 15 min. To this reaction mixture, 370 µl of 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 spectrophotometer. A control reaction was carried out without the test sample. Percentage inhibition was calculated by the following formula.

Percentage inhibition

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

Table 4. Percentage inhibition of test drug SPK on Alpha Amylase enzyme Inhibition Study

Concentration (µg/ml)	% Inhibition of SPK
100 µg/ml	16.77 ± 3.228
200 µg/ml	25.37 ± 8.351
300 µg/ml	35.39 ± 19.16
400 µg/ml	42.38 ± 11.04
500 µg/ml	48.67 ± 4.097
Standard Acarbose	94.02 ± 4.742

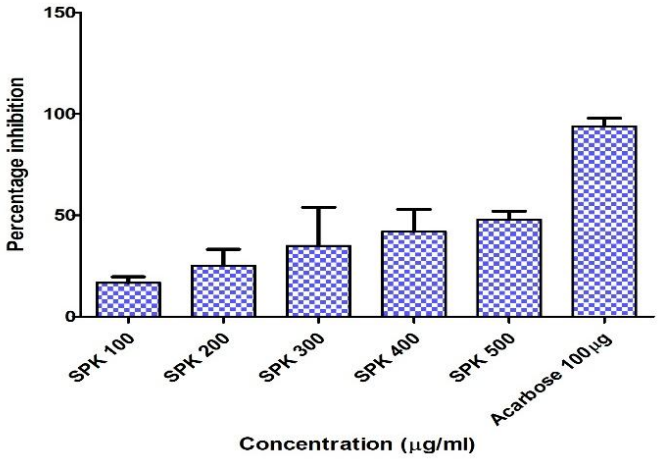
Data are given as Mean ± SD (n=3)

Table 5. IC50 Values for Alpha Amylase Enzyme inhibition by SPK and STD

Test Drug / Standard	IC50 Value of Alpha Amylase enzyme inhibition ± SD (µg /ml)
SPK	578.6 ± 44.75
Standard- Acarbose	26.41 ± 2.526

Data are given as Mean ± SD (n=3)

Fig 1. Percentage inhibition of test drug SPK and Standard on Alpha Amylase Enzyme Inhibition Assay



DISCUSSION

This study investigated the alpha- amylase inhibition activity of SPK as a potential therapeutic strategy for type 2 diabetes. Our findings demonstrated that SPK exhibited significant alpha-amylase inhibitory potential with the maximum inhibition of about 48.67 ± 4.097 % and the corresponding IC_{50} is 578.6 ± 44.75 µg /ml. Standard acarbose exhibited significant inhibition in alpha amylase enzyme with the maximum inhibition of about 94.02 ± 4.742 % and the corresponding IC_{50} is 26.41 ± 2.526 µg /ml.

The alpha amylases are the calcium metalloenzymes which can't function in the absence of calcium. There are many digestive enzymes in humans and among them the most important one is pancreatic alpha – amylase , that act as a catalysis in the reaction which involves the hydrolysis of the alpha-1,4 glycosidic linkages of the starch, amylopectin, amylose, glycogen and numerous maltodextrins and is responsible for starch digestion. The large molecules like starch cannot cross the blood brain barrier as glucose has to reach the brain thus; to overcome this problem alpha-amylase cleaves the large starch molecules into smaller fragments of sugars in order to cross the blood brain barrier. If there will be excess conversion of starch to sugars, it will increase the sugar level in blood, then the role of insulin will come into action by ordering cells to metabolize the excess sugar moieties and store as energy sources i.e., glycogen. This cycle is endlessly happening in a healthy person. But in some cases, due to excess activity of amylase enzyme and insulin deficiency or resistance to insulin, level of blood glucose arises which might results in hyperglycaemia.(20).

In the management of post prandial 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.(21)It was observed from the results of the present investigation that the formulation SPK shown promising alpha amylase enzyme inhibition potential with the maximum inhibition of about 48.67 ± 4.097 % and the corresponding IC_{50} is 578.6 ± 44.75 µg /ml. Standard acarbose exhibited significant inhibition in alpha amylase enzyme with the

maximum inhibition of about 94.02 ± 4.742 % and the corresponding IC_{50} is 26.41 ± 2.526 µg /ml.

Among four ingredients of Sambirani Poo Kuligai, styrax benzoin contains free balsamic acid chiefly cinnamic 10% and benzoic acid 6% and vanillin. In a study conducted by Veronica F Salau et al, the fructose-streptozotocin induced diabetic rats were given vanillin at a low (150mg/kg body weight) or high (300mg/kg body weight) dose of vanillin for 5 weeks intervention period and there levels of blood glucose observed significant reduction in blood given. (22) Rahman M Hafizur et al. conducted a study on mechanism of antidiabetic activity of cinnamic acid in in-vitro and in-vivo non obese type 2 diabetic rats and finally concluded that cinnamic acid exerts antidiabetic activity by improving glucose tolerance in vivo and stimulating insulin secretion in-vitro.(23)

Sabbir Ahmed et al conducted a study on molecular docking and dynamics stimulation of natural compounds from betel leaves(piper betleL.),In this study, a new molecule, apigenin-7-*O*-glucoside (screened out from 123 compounds of *Piper betle* L.), which inhibited both enzymes (alpha-amylase and alpha-glucosidase) activity by binding with its active sites, ASP-197, GLU-233, and ASP-300 and ASN-258, ASP-327, ILE-143, ASP-382, respectively. (24) Sindhu S Nair et al, conducted in-vitro studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts, among them methanolic extracts of piper betleshowed that (84.63µg/mL) exhibited 50% alpha amylase inhibition activity at the mentioned concentrations. The alpha glucosidase IC50 for the plant extracts Piper betle was found to be 96.56± 12.93µg/mL respectively.(6)Stephen Adeniyi Adefegha et al. conducted a study on In vitro inhibition activity of polyphenol-rich extracts from Syzygium aromaticum (L.) Merr. & Perry (Clove) buds against carbohydrate hydrolyzing enzymes linked to type 2 diabetes and Fe^{2+}-induced lipid peroxidation in rat pancreas. The result revealed that both extracts inhibited alpha-amylase and alpha-glucosidase in a dose-dependent manner. However, the alpha-glucosidase inhibitory activity of the extracts were significantly (P<0.05) higher than their alpha-amylase inhibitory activity. The free phenolics (31.67 mg/g) and flavonoid (17.28 mg/g) contents were significantly (P<0.05) higher than bound phenolic (23.52 mg/g) and flavonoid (13.70 mg/g) contents.(7)

Studies mentioned in this review, reveal that among the four ingredients of Sambirani Poo Kuligai, Piper betle, syzygium aromaticum and styrax benzoin has potential anti-diabetic properties.In the present study, the results of alpha amylase inhibitory assay displayed a considerable inhibitory activity with the extract of Sambirani Poo Kuligai.Hence it can be concluded that Sambirani Poo Kuligai is a siddha formulation with multi-nodal antidiabetic actions and may serve as a potent anti-diabetic agent.

CONCLUSION

From this study, we can state that siddha formulation Sambirani Poo Kuligai showed significant inhibition of alpha amylase enzyme activity. Thus, the siddha formulation SPK may consider as a remedy for diabetes and other insulin resistance- related diseases like poly

cystic ovarian syndrome; however, animal and human studies are needed to confirm this activity.

Reference

1 . Narkhede MB. Investigation of in vitro α-amylase and α-glucosidase inhibitory activity of polyherbal extract. Int. J. Pharm. Res. Dev. 2011;3:97-103.

2 . Hossain MJ, Al-Mamun M, Islam MR. Diabetes mellitus, the fastest growing global public health concern: Early detection should be focused. Health Science Reports. 2024 Mar;7(3):e2004.

3 . Dubey NP, Dubey N. Basic principles of integrated medicine. World Association of Integrated Medicine. 2013.

4 . Logisha MS, Nivetha G, Karpagavalli K, Muthukumar NJ, Mahalakshmi MV, Meenakumari M. A Review on Anti-Diabetic Herbs of Siddha System Based on Their Organoleptic Characteristic. International Journal of Ayurveda and Pharma Research. 2023 Feb 5:87-91.

5 . Pillegowda-Smitha K, Srinivas-Kulashekar K, Krishnappa-Pavithra T, Srinivas-Ashok-Kumar B, Setty-Saran G. Alpha-amylase Inhibitory Activity of Sitopaladi Churna: An Ayurvedic Formula. Traditional and Integrative Medicine. 2016:40-3.

6 . Nair SS, Kavrekar V, Mishra A. In vitro studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts. European journal of experimental biology. 2013;3(1):128-32.

7 . Adefegha SA, Oboh G. In vitro inhibition activity of polyphenol-rich extracts from Syzygium aromaticum (L.) Merr. & Perry (Clove) buds against carbohydrate hydrolyzing enzymes linked to type 2 diabetes and Fe2+-induced lipid peroxidation in rat pancreas. Asian Pacific Journal of Tropical Biomedicine. 2012 Oct 1;2(10):774-81.

8 . *R.C.Mohan, Agasthiyar paripooranam 400 page no: 62.*

9 . C. kannuswamy pillai, SikitchaRatnaDeepamEnnumVaidhiyaNool(2018)page no: 28,29,31.

10 . *Vaithya Ratnam K.S.Murukase Muthaliyar, Gunapadam (mooligai vaguppu), 2013*

11. Dr.R.Thiyaga rajan L.I.M, Gunapadam part 2&3 (Thathu-Jeeva vaguppu), 2013.

12 . Sammy J, Tamuno-Emine D, Nwachuku E. Evaluation of Anti-diabetic, Hepatoprotective and Antilipidemic Potentials of Syzygium aromaticum (Clove) on Albino Rat. JOCAMR. 2020;1:38-50.

13. Venkadeswaran K, Muralidharan AR, Annadurai T, Ruban VV, Sundararajan M, Anandhi R, Thomas PA, Geraldine P. Antihypercholesterolemic and antioxidative potential of an extract of the plant, Piper betle, and its active constituent, eugenol, in triton WR-1339-induced hypercholesterolemia in experimental rats. Evidence-Based Complementary and Alternative Medicine. 2014 Jan 1;2014.

14. Perumal PA, Saravanabhavan K. Antidiabetic and antioxidant activities of ethanolic extract of Piper betle L. leaves in catfish, Clarias gariepinus. Asian J Pharm Clin Res. 2018;11(3):194-8.

15. Jing X, Sarker MM, Alam S, Gifari AJ, Akhter S, Khan F, Soma MA. Piper betel juice improved lipid profile and hepatic oxidative stress in high-fat-diet induced hyperlipidemic rats.

16 . Sohail Akhtar M, Alam T. Chemistry, Biological Activities, and Uses of Benzoin Resin. InGums, Resins and Latexes of Plant Origin: Chemistry, Biological Activities and Uses 2022 Mar 31 (pp. 1-22). Cham: Springer International Publishing.

17 . Nassar MI, Gaara AH, El-Ghorab AH, Farrag A, Shen H, Huq E, Mabry TJ. Chemical constituents of clove (Syzygium aromaticum, Fam. Myrtaceae) and their antioxidant activity. Revista Latinoamericana de Química. 2007;35(3):47.

18.

19 . Rekha VP, Kollipara M, Gupta BR, Bharath Y, Pulicherla KK. A review on Piper betle L.: nature's promising medicinal reservoir. American Journal of Ethnomedicine. 2014;1(5):276-89

20 . Agarwal P, Gupta R. Alpha-amylase inhibition can treat diabetes mellitus. Res. Rev. J. Med. Health Sci. 2016 Sep;5(4):1-8.

21 . Kaantham, L., & Mohan, S. (2020). Screening of Anti-Diabetic Potential of the Siddha Formulation Uloga Chenduram by In-Vitro Alpha-Amylase and Alpha- Glucosidase Enzyme Inhibition Assay.

22 . Salau VF, Erukainure OL, Olofinsan KO, Msomi NZ, Ijomone OM, Islam MS. Vanillin improves glucose homeostasis and modulates metabolic activities linked to type 2 diabetes in fructose–streptozotocin induced diabetic rats. Archives of Physiology and Biochemistry. 2024 Mar 3;130(2):169-82.

23 . Hafizur RM, Hameed A, Shukrana M, Raza SA, Chishti S, Kabir N, Siddiqui RA. Cinnamic acid exerts anti-diabetic activity by improving glucose tolerance in vivo and by stimulating insulin secretion in vitro. Phytomedicine. 2015 Feb 15;22(2):297-300.

24 . Ahmed S, Ali MC, Ruma RA, Mahmud S, Paul GK, Saleh MA, Alshahrani MM, Obaidullah AJ, Biswas SK, Rahman MM, Rahman MM. Molecular docking and dynamics simulation of natural compounds from betel leaves (Piper betle L.) for investigating the potential inhibition of alpha-amylase and alpha-glucosidase of type 2 diabetes. Molecules. 2022 Jul 15;27(14):4526.