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In vitro Anti-diabetic evaluation of Pereechangai Nei by alpha amylase and alpha glucosidase enzyme inhibition assay

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Abstract
Ancient Indian system of medicine believed that a healthy soul can only be developed through a healthy body. Any derangements in body's three humors namely, vatham, pitham, kabam results in disease. Siddha system is one of the oldest among Indian systems of medicine originated in southern parts of India. In its texts, Siddha system classified diseases into 4448 types. *Madhumegam* is one among the pitha types of Meganoi which is also called as *Thithippu Neer* or *Inippuneer*. The causes, signs and symptoms of *Madhumegam* could be correlated with Diabetes Mellitus in modern system. Diabetes mellitus is one of the major health problem in developing countries whose management is still a challenge for modern system of medicine. As a solution Siddha system serves as a hope with its pure herbal medicines which serve the purpose for long term use in the management of chronic diseases like Diabetes. This article discusses and presents the study of Anti-Diabetic Evaluation of Pereechangai Nei (PCN) by Invitro methods in Alpha Amylase and Alpha Glucosidase Enzyme Inhibition Assays. The results of the study indicate that the drug Pereechangainei possess anti diabetic activity.

Keywords: Siddha, madhumegam, diabetes, pereechangainei, *in vitro* methods

1. Introduction
Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia and glucosuria either due to insufficient insulin secretion of pancreas or due to insulin resistance of target cells [1]. It is one among the lifestyle disorders which is associated with metabolic complications that subsequently lead to premature death. As of 2017, an estimated 425 million people had diabetes worldwide, with type 2 diabetes making up about 90% of the cases. In 2016, an estimated 1.6 million deaths were directly caused by diabetes. Mathumegam is a clinical condition characterized by frequent and excessive passage of urine with 'sweetness' eventually leading to deterioration of seven body constituents [2, 3]. The complications due to long term poorly controlled hyperglycemia causes detoriation of these body constituents which are described as Madhumega Avathaigal [4]. For controlling high blood sugar levels diet modifications, physical exercises, insulin therapy, and oral medications are advised [5]. However, several allopathic hypoglycemic agents produce adverse effects. Thus the management without any side effects by herbal medicines are now gaining more importance and popularity across the world. From ancient time's mankind have been successfully treated with traditional medicines for all diseases. In siddha classical texts, many herbal, mineral, herbal mineral formulations have been mentioned for the treatment of madhumegam. One among such classical text called Therayar *Maha Karisal* mentions about *''Pereechangainei''* a polyherbal medicated ghee formulation recommended exclusively for the treatment of *Madhumegam* [6]. Each ingredient of *Pereechangainei* have been identified for their individually proven Antihyperglycemic activity [8, 9]. Hence this current study was carried out to prove the antidiabetic activity of the drug *Pereechangainei* by Invitro methods in Alpha Amylase and Alpha Glucosidase Enzyme Inhibition Assays.

2. Materials and Methods
2.1 Identification of raw drugs: The herbal ingrediants were authenticated from Assistant Professor of Medicinal botany, National Institute of Siddha, Tambaram sanatorium, Chennai.

2.2 Ingredients of *Pereechangainei:* (Medicated ghee of Date fruit)
1. *Pereechangai* (*Phoenix dactylifera*, Linn)
2. *Peraamutti* (*Pavonia Odorata*, Willd)
3. *Kodiveli* (*Plumbago Zeylanica*, Linn)

4. *Peipudal* (*Trichosanthes Cucumerina*, Linn)
5. *Nannari* (*Hemidesmus indicus*, R. Br)
6. *Sirupeelai* (*Aerva lanata*, Linn)
7. *Kondrai* (*Cassia fistula*, Linn)
8. Senbagam (*Michelia champaca*, Linn)
9. *Balli poondu* (*Strigustutea*, Linn)
10. *Inji* (*Zingiber officinale*, Rosc)
11. *Milagu* (*Piper nigrum*, Linn)
12. *Thippili* (*Piper longum*, Linn)
13. *Yelam* (*Elettaria cardamomum*, Maton)
14. *Lavangam (Syzygium aromaticum*, Linn)
15. *Pasu Nei* (Cow's ghee) [6].

2.3 Method of purification: Purification of raw drugs were done as per the methods given in Siddha text *Sigichaarathnadeepam* [7].

2.4 Method of drug preparation: *Pereechangai Nei* was prepared according to the procedure mentioned in Siddha classical text *Therayar Mahakarisal* [6].
Pereechangainei

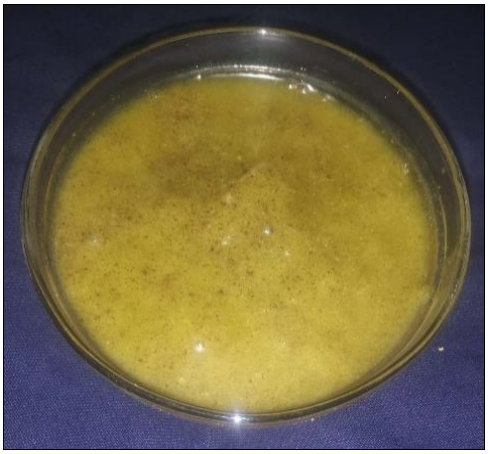

Fig 1: *Pereechangai Nei*

2.5 Solubility profile of *Pereechangainei*: The solubility of PCN was tested in various solvents and its results are mentioned below.

Table 1: Solubility Profile in solvents

S.No.	Solvent used	Solubility/Dispersibility
1	Chloroform	Soluble
2	Ethanol	Partly soluble
3	Water	Insoluble
4	Ethyl acetate	Soluble
5	Hexane	Soluble
6	DMSO	Insoluble

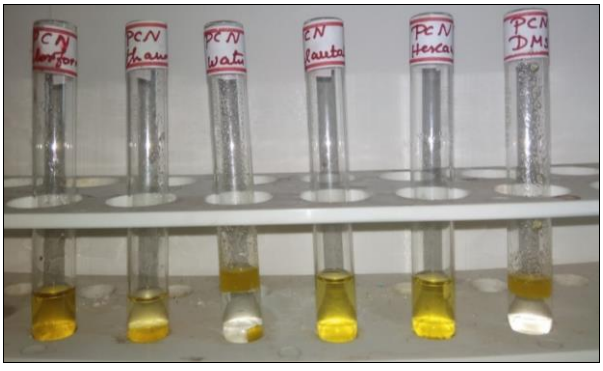

Fig 2: Solubility of PCN

2.6 *In vitro* alpha amylase inhibition study [11]
Method adopted: The spectrophotometric assay method.
Test sample extraction: Chloroform
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 (PCN) was prepared in the serial dilution of the concentration ranges from 100,200,300,400 and 500 µg/ml using Chloroform. 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

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

2.7 *In vitro* α-Glucosidase Enzyme Inhibition Study [12]
Method Adopted: The spectrophotometric assay method.
Test Solution: Test Sample (PCN) was prepared in the serial dilution of the concentration ranges from 100,200,300,400 and 500 µg/ml using Chloroform.
PNPG (p-nitrophenyl-α-D -glucopyranoside): 20 mM PNPG prepared by dissolving 603 mg PNPG in 100 ml of PBS

Enzyme: 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 vaying 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 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 addition of 1000 µl of 200 mM Na_2CO_3solution and the amount of p-nitrophenol released was measured by reading the absorbance of sample against a sample blank (containing PBS with no sample) at 405 nm using UV visible spectrophotometer.

3. Results

Table 2: Percentage inhibition of test drug PCN on α-Glucosidase Enzyme Inhibition Study

Concentration (µg/ml)	% Inhibition of PCN
100 µg/ml	10.6±8.11
200 µg/ml	18.67±6.81
300 µg/ml	39.72±5.35
400 µg/ml	49.3±7.71
500 µg/ml	73.53±4.82
Standard Acarbose	93.6±0.96

Data are given as Mean ± SD (n=3)

Table 3: IC50 Values for α-Glucosidase Enzyme inhibition by PCN and STD

Test Drug / Standard	IC50 Value of Alpha Amylase enzyme inhibition ± SD (µg /ml)
PCN	420.1±104.5
Standard- Acarbose	10.81±2.64

Data are given as Mean ± SD (n=3)

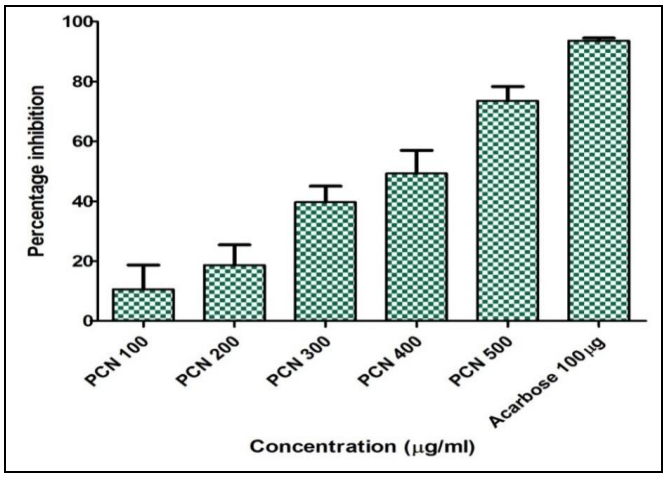

Fig 3: Percentage inhibition of PCN and standard on alpha glucosidase enzyme inhibition study

Table 4: Percentage inhibition of test drug PCN and STD on Alpha Amylase Inhibition Study

Concentration (µg/ml)	% Inhibition of PCN
100 µg/ml	12.8±4.89
200 µg/ml	30.24±4.92
300 µg/ml	45.04±7.87
400 µg/ml	57.33±8.00
500 µg/ml	75.42±5.83
Standard- Acarbose	95.72±1.93

Data are given as Mean ± SD (n=3)

Table 5: IC50 values for alpha amylase inhibition assay by PCN and STD

Test Drug / Standard	IC50 Value of Alpha Amylase enzyme inhibition ± SD (µg /ml)
PCN	311.9±29.14
Standard- Acarbose	43.46±4.51

Data are given as Mean ± SD (n=3)
Data are given as Mean ± SD (n=3)

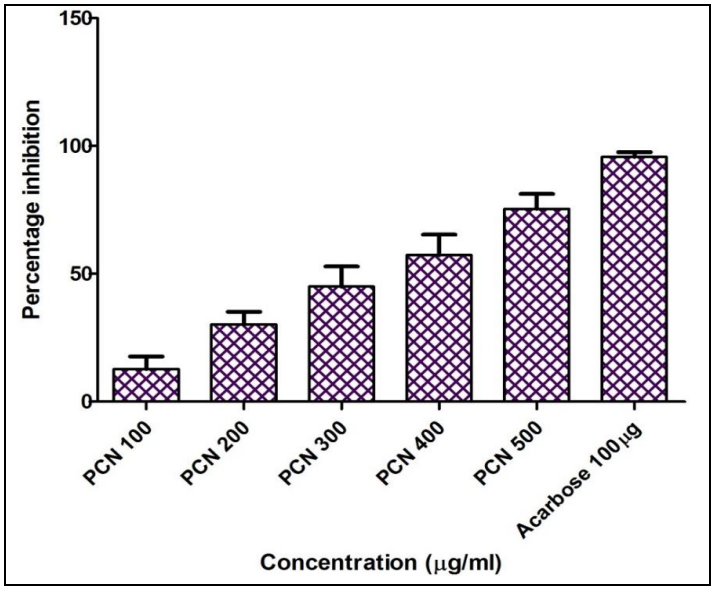

Fig 4: Percentage Inhibition of PCN and standard on alpha amylase Enzyme Inhibition Study

Discussion

- It was observed from the results of the present investigation that the siddha formulation PCN shown significant inhibition in alpha glucosidase enzyme with the maximum inhibition of about 73.53±4.82% and the corresponding IC50 is 420.1±104.5 µg /ml. Standard acarbose exhibited significant inhibition in alpha glucosidase enzyme with the maximum inhibition of about 93.6±0.96% and the corresponding IC50 is 10.81±2.64µg /ml.
- It was observed from the results of the present investigation that the siddha formulation PCN shown significant inhibition in Alpha Amylase enzyme with the maximum inhibition of about 75.42±5.83% and the corresponding IC50 is 95.72±1.93µg/ml. Standard acarbose exhibited significant inhibition in alpha amylase enzyme activity with the maximum inhibition of about 95.72±1.93% and the corresponding IC50 is 43.46±4.51µg /ml.

Conclusion

From this study, we can state that drug *Pereechangainei*s howed significant inhibition of alpha amylase enzyme and alpha glucosidase enzyme activity. Thus, the drug possess antidiabetic property and has beneficial effects on controlling high blood sugar levels. This can be further studied in experimental diabetic rats for potential future research.

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