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Screening of Anti-Diabetic Potential of the Siddha Formulation Aavari Kirutham by In-Vitro Alpha –Amylase and Alpha – Glucosidase Enzyme Inhibition Assay

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Abstract: *Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues. α-amylase and α-glucosidase is carbohydrate hydrolyzing enzymes responsible for postprandial hyperglycemia. These enzymes helps in the control of hyperglycemia by delaying carbohydrate digestion. It was observed that chronic usage of certain anti-diabetic drugs may lead to hypoglycemia, headache, dizziness, nausea,Intoralance and weight gain. However, Siddha system of medicine has potential herbal and herbomineral formulations for the Management of the condition of diabetic without major side effects. The main objective of the present study is to evaluate the anti-diabetic potential of the herbal formulation of Aavari Kirutham (AAK) by alpha-amylase and alpha-glucosidase enzyme inhibition assay. It was observed from the results of the present investigation that the test drug AAK shown significant inhibition in alpha-amylase and alpha-glucosidase enzyme with the maximum inhibition of about 56.26± 6.76% and 41.13± 0.71% respectively, corresponding IC50 are 419± 117.5 and 596.5± 18.8µg/ml. It was concluded from the study that the Siddha Herbaominaral drug Aavari Kirutham has significant control over the activity of both the metabolic enzymes such as alpha-amylase and alpha-glucosidase. Further clinical and preclinical studies is needed to identify the mechanism of action*

Keywords: *Diabetes Mellitus, Hyperglycemia, Alpha-amylase, Alpha-glucosidase, Siddha system, Aavari kirutham.*

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I. Introduction

Siddha system of medicine is an ancient Dravidian medical system treated as an holistic approach, has been practiced in southern part of India and north-east Sri Lanka for more than 2000 years of period.

According to siddha system of medicine diseases are classified into 4448 in number [1], one among them is madhumegam that can be correlated with diabetes mellitus

DM is leading cause of morbidity and mortality world over. The prevalence of Diabetes Mellitus among various countries ranges from 1% - 30% and it is higher in the developed countries compared with the developing countries. By 2036,844 million people will be affected with DM .As of 2016,422 million people have diabetes worldwide, up from an estimated 382 million people in 2013 and from 108 million in 1980.

Accounting for the shifting age structure of global population, the prevalence of DM is 8.5% among adults in 2016, nearly double the rate of 4.7% in 1980. Type II makes up 90%of the cases. The WHO estimates that DM resulted in 1.5 million deaths in 2012, making it 8th leading cause of death [2]

There is no satisfactory effect on anti diabetic modern drug on diabetic complication. But in Siddha system we have many anti diabetic herbal and herbomineral formulation, Present study aimed at evaluating the

anti-diabetic potential of the siddha formulation Aavari kirutham by alpha amylase and alpha glucosidase enzyme inhibition assay.

STANDARD OPERATING PROCEDURE FOR AAVARI KIRUTHAM

INGREDIENTS OF AAVARAI KIRUTHAM: [3]

1**.** PurifiedAavaraisamoolam(Cassia *auriculata Linn*)
2. Purified Thenampoo(Cocos *nusifera)*
3. Purified Vaazhaikizhangu(Musa *paradisiacal Linn)*
4. Purified Vaazhaipoocharu*(*Musa *paradisiaca Linn*)

} Each 335 ml

5. Purified Jathikkai*(*Myristica*fragrans. Houtt)*
6. Purified Seeragam*(*Cuminum*cyminum .Linn)*
7. Purified Milagu(Piper *longum. Linn)*
8. Purified Kirambu(Syzygium*aromaticum .Linn)*
9. Purified Amukkura kizhangu(Withania*somnifera .Linn)*
10. Purified Lavangapattai(Cinnamomum*verum. Linn)*
11. Purified Sathisaaranaikizhangu(Trianthema*decandra .Linn*)
12. Purified Panai karkandu (Borassus*flabellifer. Linn*)
13. Purified Cow's ghee

} Each 8.75 gms

- 1340ml

SOURCES OF RAW DRUGS

The required raw drugs for preparation of **"AAVARAI KIRUTHAM"** were purchased from a well reputed country shop at Chennai .The raw drugs were authenticated by the Botanist, National Institute of Siddha Tambaram Sanatorium Chennai 600 047.The raw drugs were purified as per the standard Siddha guideline and medicine were prepared at Gunapadam laboratory of National Institute of Siddha.

METHOD OF PURIFICATION OF RAW DRUGS [4]

1. Aavaraisamoolam(Cassia *auriculata Linn*)- Clean with water then dry it under shadow.
2. Thenampoo(Cocos *nusifera)*Clean with white cloth.
3. Vaazhaikizhangu(Musa *paradisiacal Linn)*Clean with water then dry it under shadow
4. Vaazhaipoocharu*(*Musa *paradisiaca Linn*) Clean with water then dry it under shadow.
5. Jathikkai*(*Myristica*fragrans. Houtt)*Outer peel is to be removed & cut into small pieces Then dry it under shadow.
6. Seeragam*(*Cuminum*cyminum .Linn)*Fruit-dried it in sunlight and fried it like as golden yellow colour.
7. Milagu(Piper *nigrum. Linn)*Soak in cow's butter milk for 3 hours and dry it under shadow.
8. Kirambu(Syzygium*aromaticum .Linn)* Clean with white cloth and dry under shadow
9. Amukkura kizhangu(Withania*somnifera .Linn)*Tuber boiled with milk and dried and then powder it
10. Lavangapattai(Cinnamomum*verum. Linn)* Clean dry under shadow.
11. Sathisaaranaikizhangu(Trianthema*decandra .Linn*) Tuber boiled with milk and dried and then powder it
12. Panai karkandu (Borassus*flabellifer. Linn*) -175 grams
13. Cow's ghee - 1340ml

METHOD OF PREPARATION:

Step: 1.

Decoction:

Whole plant of Aavarai was pounded and made into coarse powder & it was added with 1300 ml of water then it wasboiled and reduced into 325 ml

2. Juice extracted from thennam poo
3. Juice extracted from vaazhai kizhangu
4. Juice ectracted from vaazhai poo
5. Cow s ghee – 1340 ml.

} Each 335 ml

Step: 2.
6. Sathikkai
7. Seeragam
8. Milagu
9. Kirambu
10. Elavanga pattai
11. Amukkura kizhangu
12. Sathisaaranai kizhangu
13. Panai karkandu – 175 grams

Each 8.75grams

PROCEDURE:

Ingredients of step1 is mixed together and allowed it for one night, Next day boil this mixture untill it get "Vandal mezhugupatham" and filtered. Then ingredients in step 2 with panai karkandu 175 grams added to this mixture that was at the time of melted stage of ghee [3]

The trial drug **AAVARAI KIRUTHAM** were be stored in a clean and dry wide mouthed airtight glass bottles

II. Materials And Methods

1. In-vitro Alpha Amylase Inhibition Study [5]

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 (AAK) 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

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

1. In-vitro α-Glucosidase Enzyme Inhibition Study [6]

Method Adopted: The spectrophotometric assay method.

Test Solution: Test Sample (AAK) 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.

Percentage inhibition of test drug AAK on Alpha Amylase enzyme Inhibition Study

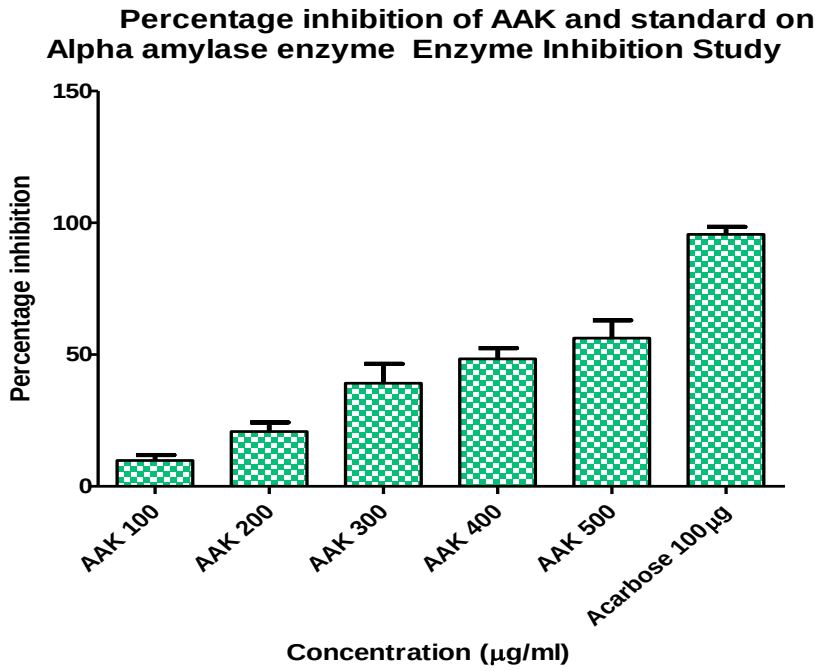
Concentration (µg/ml)	% Inhibition of AAK
100 µg/ml	9.78 ± 2.14
200 µg/ml	20.84 ± 3.45
300 µg/ml	39.15 ± 7.36
400 µg/ml	48.38 ± 4.07
500 µg/ml	56.26 ± 6.76
Standard Acarbose	95.65 ± 2.87

Data are given as Mean ± SD (n=3)

IC50 Values for Alpha Amylase Enzyme inhibition by AAK and STD

Test Drug / Standard	IC50 Value of Alpha Amylase enzyme inhibition ± SD (µg /ml)
AAK	419.8 ± 117.5
Standard- Acarbose	36.31 ± 1.83

Data are given as Mean ± SD (n=3)



Percentage inhibition of test drug AAK and STD on α-Glucosidase Inhibition Study

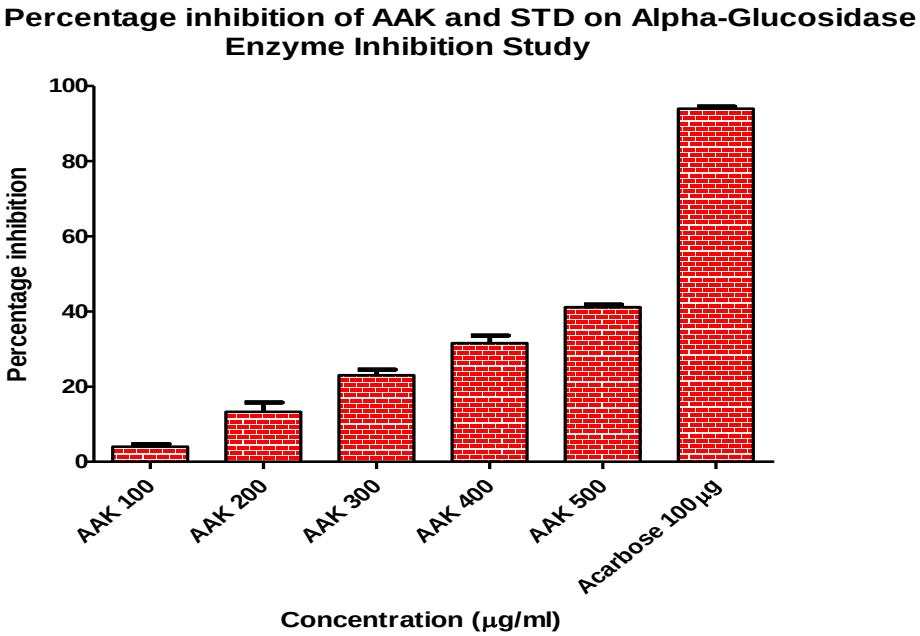
Concentration (µg/ml)	% Inhibition of AAK
100 µg/ml	4.033 ± 0.65
200 µg/ml	13.32 ± 2.43
300 µg/ml	23.04 ± 1.48
400 µg/ml	31.58 ± 2.02
500 µg/ml	41.13 ± 0.71
Standard- Acarbose	93.96 ± 0.57

Data are given as Mean ± SD (n=3)

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

Test Drug / Standard	IC50 Value of α-Glucosidase enzyme inhibition ± SD (µg /ml)
AAK	596.5 ± 18.8
Standard- Acarbose	8.594 ± 3.128

Data are given as Mean ± SD (n=3)



III. Discussion

Type 2 diabetes (T2D) is a disease caused by an imbalance between blood sugar absorption and insulin secretion. Considering the fact that diabetes is regard as a chronic metabolic disease, various 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 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 [7,8].

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[9].It was observed from the results of the present investigation that the siddha formulation aavarai kirutham shown significant inhibition in alpha amylase enzyme with the maximum inhibition of about 56.26± 6.76%and the corresponding IC50 is 419.8 ± 117.5 µg /ml. Standard acarbose exhibited significant inhibition in alpha amylase enzyme with the maximum inhibition of about 419.8 ± 117.5% and the corresponding IC50 is 36.31 ± 1.83 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 the diabetic people are living in low and middle income countries [10], 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 [11].It was observed from the results of the present investigation that the siddha formulation AAK shown significant inhibition in alpha glucosidase enzyme with the maximum inhibition of about 41.13 ± 0.71% and the corresponding IC50 is 596.5 ± 18.8 µg/ml. Standard acarbose exhibited significant inhibition in alpha glucosidase enzyme activity with the maximum inhibition of about 93.96 ± 0.71 % and the corresponding IC50 is 8.594 ± 3.128µg /ml.

IV. Observation and Conclusion

- It was observed from the results of the present investigation that the siddha formulation AAK shown significant inhibition in alpha amylase enzyme with the maximum inhibition of about 41.13 ± 0.71 % and the corresponding IC50 is 596.5 ± 18.8 µg /ml. Standard acarbose exhibited significant inhibition in alpha glucosidase enzyme with the maximum inhibition of about 93.96 ± 0.57 % and the corresponding IC50 is 8.594 ± 3.128 µg /ml.

- It was observed from the results of the present investigation that the siddha formulation AAK shown significant inhibition in alpha glucosidase enzyme with the maximum inhibition of about 56.26 ± 6.76 % and the corresponding IC50 is 419.8 ± 117.5µg/ml. Standard acarbose exhibited significant inhibition in alpha amylase enzyme activity with the maximum inhibition of about 95.65 ± 2.87 % and the corresponding IC50 is 36.31 ± 1.83 µg /ml.

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