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IN-VITRO ANTI-DIABETIC EVALUATION OF KANTHAGA PARPAM BY ALPHA AMYLASE AND ALPHA GLUCOSIDASE ENZYME INHIBITION ASSAY

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ABSTRACT

India has one of the highest numbers of people with diabetes globally. According to the National NCD Monitoring Survey, Prevalence of diabetes mellitus (DM) among adults (18-69 years) is ~9.3% and Prevalence of impaired fasting glucose (IFG) (a pre-diabetic state) is ~ 24.5%. Traditional Siddha formulations such as Kanthaga Parpam (KP), prepared by using of purified Elemental Sulphur, juices of Allium cepa, Zingiber officinalis and bark ashes of Terminalia arjuna and Tamarindus indicus is being explored as potential inhibitors of carbohydrate-hydrolyzing enzymes to mitigate postprandial hyperglycemia. This study investigates the in-vitro inhibitory effects of KP on α-amylase and α-glucosidase enzymes, key targets in the management of Type 2 diabetes. Methods: In-vitro enzyme inhibition assays against α-amylase and α-glucosidase were conducted using KP at varying concentrations (100–500 µg/ml), with Acarbose as the standard reference. Percentage inhibition and IC50 values were determined. Results: KP exhibited dose-dependent inhibition of α-amylase (maximum 26.94 ± 2.826 % , IC50 is 1177 ± 215.1 µg /ml). And α-glucosidase (maximum 43.93 ± 0.6648% IC50 is 540.8 ± 15.2 µg /ml µg/ml). Compared to Acarbose, KP showed moderate potency but measurable activity. Conclusion: The findings highlight the potential role of KP in glycemic control, supporting its further evaluation in preclinical and clinical models.

KEYWORDS: Kanthaga Parpam, Siddha medicine, Anti-diabetic, α-Amylase enzyme inhibition, α-Glucosidase enzyme inhibition.

INTRODUCTION

Diabetes Mellitus is a chronic metabolic disorder characterized by hyperglycemia due to impaired insulin secretion, insulin action, or both. According to the IDF (The International Diabetes Federation) Atlas (2025), 11.1%, or 1 in 9, of persons aged 20 to 79 have diabetes, and more than 40% are unaware that they have the disease. According to a WHO report, 830 million people globally are predicted to have diabetes by 2025. It affects millions worldwide and is associated with long-term complications such as neuropathy, nephropathy, and retinopathy. The incidence and its mortality have gone up over the past few decades. Urban populations have much higher prevalence than rural. For instance ~14.3% in urban vs ~6.9% in rural settings for diagnosed diabetes. By 2050, projections show the number of adults with diabetes in India could climb to ~156.7 million.The search for effective and safer anti diabetic drugs has drawn attention to traditional medicines. Despite the availability of modern antidiabetic drugs, issues such as side effects, cost, and limited efficacy necessitate alternative approaches.

Kanthaga Parpam, a classical herbo-mineral formulation in Siddha medicine, has been traditionally used for various chronic ailments. This article reviews the possible pharmacological basis, in-vitro evidences, and therapeutic relevance of Kanthaga Parpam in diabetes management.

Traditional Siddha formulations, including Kanthaga Parpam (KP), this formulation is prepared by purified Elemental Sulphur, triturated with extracts of Allium cepa (Vellai vengayam saaru) and Zingiber officinalis (Inji saaru). Then parpam is prepared by traditional calcinations(pudam) method by using cow dung, bark ashes of Terminalia arjuna (Marutham pattai) and Tamarindus indicus(Puliyam pattai) with excessive amount of heat. Kandhaga parpam indicated in SIDDHA VAITHIYA THIRATTU book have been widely used for many diseases.

However, there is limited systematic scientific validation of their anti-diabetic properties. The present study was designed to evaluate the in-vitro enzyme inhibitory

activities of KP to establish pharmacological evidence supporting its therapeutic role in diabetes management.

MATERIALS AND METHODS

Preparation of test sample

Kanthaga Parpam (KP) was prepared as per Siddha classical guidelines. The test solution was prepared by serial dilution to concentrations ranging from 100–500 µg/ml in double-distilled water.

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 (KP) 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 mix consist of, 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 of test drug KP on Alpha Amylase enzyme Inhibition Study

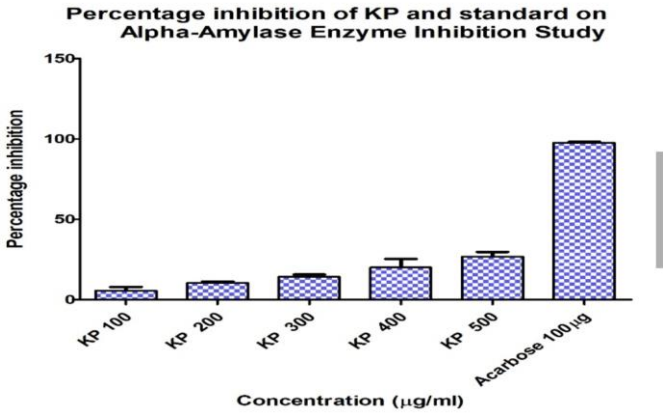
Concentration(µg/ml)	% Inhibition of KP
100 µg/ml	5.77 ± 2.239
200 µg/ml	10.5 ± 0.8236
300 µg/ml	14.34 ± 1.457
400 µg/ml	20.15 ± 5.207
500 µg/ml	26.94 ± 2.826
Standard Acarbose	97.73 ± 0.6011

Data are given as Mean ± S D (n=3)

IC50 Values for Alpha Amylase Enzyme inhibition by KP and STD.

Test Drug	Standard IC50 Value of Alpha Amylase enzyme inhibition ± SD(µg/ml)
KP	1177 ± 215.1
Standard- Acarbose	35.56 ± 3.1

Data are given as Mean ± SD (n=3)



2. In-vitro α-Glucosidase Enzyme Inhibition Study

Method Adopted: **The spectrophotometric assay** method.

Test Solution: Test Sample (KP) was 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 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 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 2 CO 3 solution 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 KP and STD on α-Glucosidase Enzyme Inhibition Study.

Concentration (µg/ml)	% Inhibition of KP
100 µg/ml	7.983 ± 0.7397
200 µg/ml	22.84 ± 1.133
300 µg/ml	29.21 ± 1.428
400 µg/ml	39.56 ± 1.197
500 µg/ml	43.93 ± 0.6648
Standard- Acarbose	99.45 ± 0.6365

Data are given as Mean ± SD (n=3)

IC50 Values for α-Glucosidase Enzyme Inhibition Assay by KP and STD

Test Drug	Standard IC50 Value of α-Glucosidase enzyme inhibition ± SD (µg /ml)
KP	540.8 ± 15.2
Standard- Acarbose	35.75 ± 7.129

Data are given as Mean ± SD (n=3)

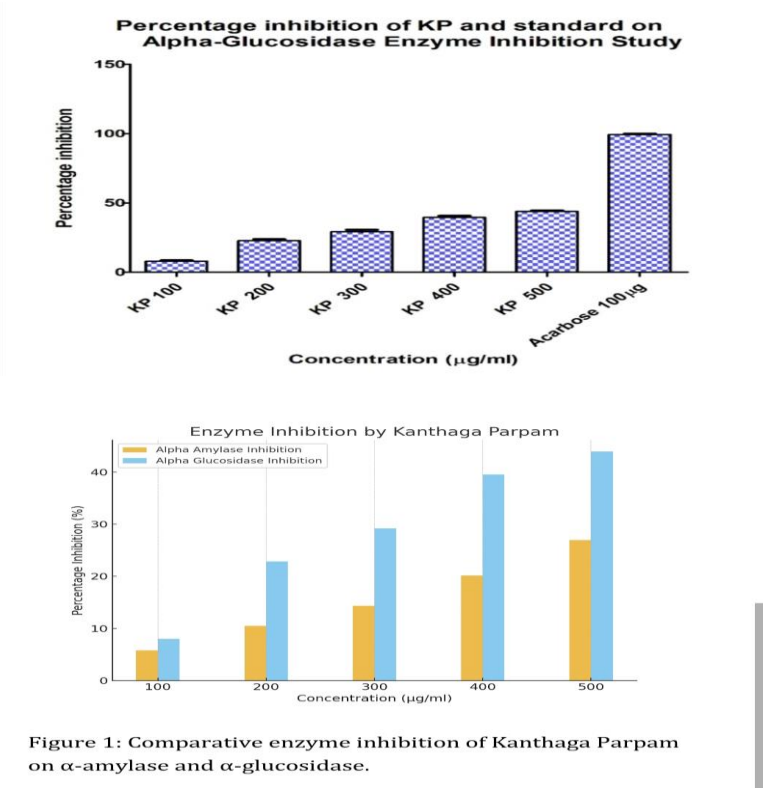


Figure 1: Comparative enzyme inhibition of Kanthaga Parpam on α-amylase and α-glucosidase.

RESULTS

- It was observed from the results of the present investigation that the formulation KP shown promising alpha amylase enzyme inhibition potential with the maximum inhibition of about 26.94 ± 2.826 % and the corresponding IC 50 is 1177 ± 215.1 µg /ml. Standard acarbose exhibited significant inhibition in alpha amylase enzyme with the maximum inhibition of about 97.73 ± 0.601% and the corresponding IC 50 is 35.56 ± 3.1µg /ml.
- It was observed from the results of the present investigation that the formulation KP shown promising glucosidase enzyme inhibition potential with the maximum inhibition of about 43.93 ± 0.6648 % and the corresponding IC 50 is 540.8 ± 15.2 µg /ml. Standard acarbose exhibited significant inhibition in alpha glucosidase enzyme with the maximum inhibition of about 99.45 ± 0.6365 % and the corresponding IC 50 is 35.75 ± 7.129 µg/ml.

DISCUSSION

The current investigation confirmed that KP exhibits concentration-dependent inhibitory activity against both α-amylase and α-glucosidase enzymes, although the potency was considerably lower compared to Acarbose. The observed effects may be attributed to the presence of phytoconstituents such as flavonoids, alkaloids, and phenolic compounds in the formulation. These classes of compounds are known to modulate carbohydrate metabolism and delay glucose absorption.

Previous reports on Siddha and herbal formulations demonstrated similar inhibitory activities, validating the role of traditional medicines in glycemic regulation. However, the relatively high IC50 values of KP indicate that higher concentrations may be required for significant clinical effects. Nonetheless, its traditional use, safety profile, and potential for synergistic effects in polyherbal combinations justify further in-vivo and clinical studies.

CONCLUSION

Kanthaga Parpam demonstrated measurable α-amylase and α-glucosidase inhibitory activity in vitro. Though less potent than Acarbose, its activity supports its traditional use in Siddha medicine for diabetes management. Future directions should include isolation of bioactive compounds, in-vivo efficacy testing, and clinical validation.

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