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IN-VITRO EVALUATION OF α-AMYLASE AND α-GLUCOSIDASE INHIBITORY ACTIVITY OF WATER EXTRACT OF INDIVIDUAL AND COMBINED KANTAKI PANCHAMOOLA

Dr. Nivedita Bagewadi [1] Dr. Manjunath Ajanal [2] Dr. Shashikala Bani [3]

1. MD Scholar, Department of Dravyaguna, Rajiv Gandhi Education Society Ayurvedic Medical College and Hospital Ron, Gadag District Karnataka- 582209.
2. Professor, Department of Dravyaguna, Rajiv Gandhi Education Society Ayurvedic Medical College and Hospital Ron, Gadag District Karnataka- 582209.
3. Professor and HOD, Department of Dravyaguna, Rajiv Gandhi Education Society Ayurvedic Medical College and Hospital Ron, Gadag District Karnataka- 582209.

Abstract

Every living organism is subjected to exposure to electromagnetic radiation. This EMF shows adverse effects on the living organisms. They have been found to alter the growth and development of animals, bees and plants. Studies have shown that plants grown under the influence of electromagnetic radiation have physiological, morphological and adverse cell characteristics. Transformers are high electromagnetic field generator and plants that grow in vicinity to them has bad impact. Although many studies have reported to cause negative impact on plants. In Ayurveda special attention has been given for collection, cultivation and propagation of medicinal plants to avoid environmental and ecological influence which includes collection of plants which grows near graveyards, anthills, temples, walking lanes etc.

Introduction

Diabetes Mellitus is a chronic, multifactorial, non-communicable, life-threatening metabolic disease characterized by hyperglycaemia, insulin resistance, and relative insulin deficiency which results from interaction between genetic, environmental and behavioural risk factors. 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. Insulin is key

player to regulate carbohydrate, fat and protein metabolism. Insulin deficiency may affect the metabolism. Mainly two α-amylase and α-glucosidase are the carbohydrate hydrolysing enzymes are responsible for postprandial hyperglycaemia. Thus, inhibition of these enzymes, involved in the digestion of carbohydrates, fats and proteins can significantly reduce PPHG. In conventional system of medicine, DM is managed by controlling blood sugar through diet, exercise, insulin therapy, oral medications such as Metformin, Sulfonylureas like Glimepiride, Glipizide etc. and Acarbose, Miglitol and Voglibose are used to inhibits both α-amylase and α- glucosidase used for controlling PPHG. Many drugs are used under stimulation of insulin secretion, increasing peripheral absorption of glucose etc. which are known for their side effect and tolerance. Hence, α-amylase and α-glucosidase enzyme inhibitive drugs would be best drug of choice for the management.

Acharya Sushruta suggested *Kantaki Panchamoola* (5 herbal drugs) comprises of roots of *Karamarda (Carissa carandas* Linn.), *Gokshura (Tribulus terrestris* Linn.), *Saireyaka (Barleria prionitis* Linn.), *Shatavari (Asparagus racemosus* Willd) and *Himsra (Capparis sepiaria* Linn.) under the management of *Sarvameha*. Hence drugs with α-Amylase and α-glucosidase inhibitory activity are considered as antidiabetic. So present study was intended to evaluate α-Amylase and α-glucosidase inhibitory activity as indicated in Sarvameha.

The existing management methods for DM is developing the Drug resistance, chronicity of the disease is life threatening with undue complications, affected by macrovascular and microvascular diseases. It is indeed the responsibility of the researcher to screen the Safe, Longing and Efficacious Drug as the Remedial choice. The various pathophysiological changes related to DM increased glucose level in post-ponderal state, worsen the DM. In this perspective, the present study is to observe and evaluate the pharmacological activities of Kantaki Panchamoola in Diabetes Mellitus described by Acharya Sushruta has been undertaken.

Materials and Methods

Chemicals

α-glucosidase (*Saccharomyces cerevisiae*), α-amylase (procaine pancreas) and 3, 5, di-nitro salicylic acid (DNS) were purchased from Sigma-Aldrich, Bangalore. P-nitro-phenyl-α-D-glucopyranoside (p-NPG), sodium carbonate (Na_2 CO_3), sodium dihydrogen phosphate, di-sodium hydrogen phosphate were purchased from Hi-Media, Mumbai.

Plant Material

The Matured individual trial part of the drugs was collected from Natural habitat of Kappatagudda, Gadag, identified by Botanist and authenticated at Central research Laboratory of RGES AMC Ron. The collected plant materials were washed under running tap water and dried under shade, coarsely powdered, and stored in the neatly labelled airtight container.

Extraction and Fractionation

Individual as well as combined *Kantaki panchamoola* powdered (4 g) material will be extracted with 40 mL of distilled water at a temperature from 80 to 100 °C in reflux for 3 h to give an initial extract (fraction I). The residues will be extracted with 60 mL of distilled water at a temperature from 80 to 100 °C for 0.5 h to give fraction II. After cooling to room temperature and then filtering (Whatman No 2), the two fractions will be combined and dried under vacuum below 40 °C and weighed to determine the yield. The extracts will be completely dried in a freeze-drier and stored at −20 °C until further use.

In-vitro Assay

α-amylase inhibitory activity

This activity was carried out according to the standard method. In a 96-well plate, reaction mixture containing 50µl phosphate buffer (100 mM, pH = 6.8), 10µl α–amylase (2 U/ml), and 20µl of varying concentrations of extracts were pre incubated at 37°C for 20 min. Then, the 20µl of 1% soluble starch (100 mM phosphate buffer pH 6.8) were added as a substrate and incubated further at 37°C for 30 min; 100µl of the DNS color was then added and boiled for 10 min. The absorbance of the resulting mixture was measured at 540 nm using Intech UV-Spectrophotometer. Acarbose at various concentrations (0.1–0.5 mg/ml) were used as a standard. Without test (extracts), substance was set up in parallel as control and each experiment was performed in triplicates. The results were expressed as percentage inhibition, which were calculated using the formula,

Inhibitory activity (%) = (1 − As/Ac) ×100

Where, As is the absorbance in the presence of test substance

Ac is the absorbance of control.

α-glucosidase inhibitory activity

This activity was carried out according to the standard method. In a 96-well plate, reaction mixture containing 50 µl phosphate buffer (100 mM, pH=6. 8), 10µl alpha-glucosidase (1U/ml), and 20 µl of varying concentrations of extracts were preincubated at 37°C for 15 min. Then, 20µl P-NPG (5 mM) was added as a substrate and incubated further at 37°C for 20 min. The reaction was stopped by adding 50µl Na2CO3 (0.1 M). The absorbance of the released *p*-nitrophenol was measured at 405 nm using Intech UV-Spectrophotometer. Acarbose at various concentrations (0.1–0.5 mg/ml) was included as a standard. Without test, substance was be set up in parallel as a control and each experiment was performed in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula,

Inhibitory activity (%) = (1 − As/Ac) ×100

Where, As is the absorbance in the presence of test substance

Ac is the absorbance of control.

Statistical Analysis

Statistical analysis was done by 'ANOVA' test.

Results

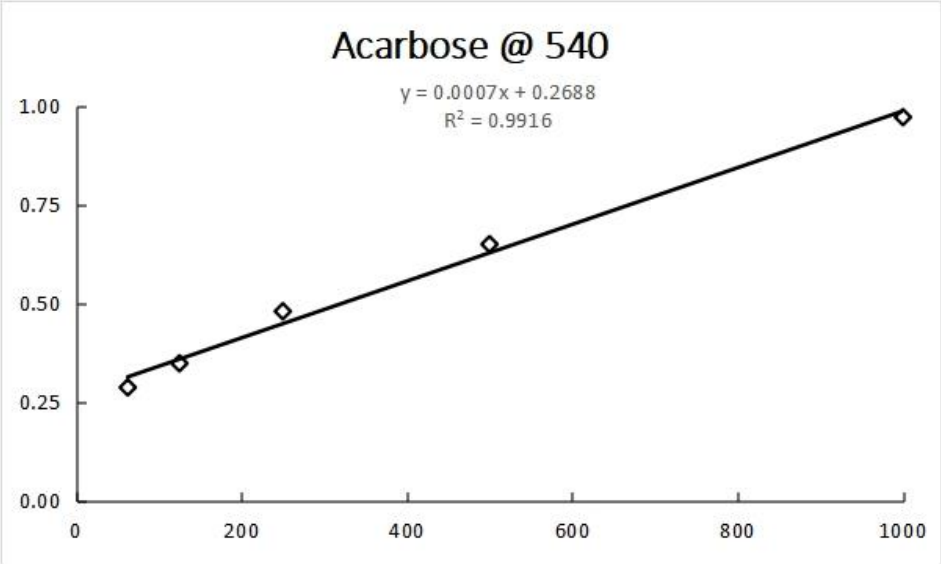
Aqueous extract of individual and combined Kantaki panchamoola were evaluated for their inhibitory effect on α-amylase and α-glucosidase enzymes by *in-vitro* method. In α- amylase inhibitory activity Gokshura, Himsra, Karamarda, Saireyaka, Shatavari and K.P have shown inhibitory activity 5.255, 5.154, 5.113, 5.062, 4.955, 5.113 mg/ml respectively. On comparison, maximum inhibitory activity shown by Gokshura 5.255 mg/ml and least inhibitory activity shown by Shatavari 4.955 mg/ml. In α-glucosidase inhibitory activity, Gokshura, Himsra, Karamarda, Saireyaka, Shatavari and K.P have shown inhibitory activity 4.997, 4.640, 4.548, 4.457, 4.396 and 4.569 mg/ml respectively, which on comparison, maximum inhibitory activity shown by Gokshura 4.997 mg/ml and least inhibitory activity shown by Shatavari 4.396 mg/ml.

Table No 1: Percentage inhibition of α-amylase enzyme activity at different concentrations of drugs

Conc mg/mL	Gokshura	Himsra	Karamarda	Saireyaka	Shatavari	KP
0.008	1.425	1.154	1.667	1.795	1.368	1.538
0.016	2.828	2.545	2.727	2.727	2.000	2.54
0.031	3.367	2.778	3.131	3.182	2.828	3.030
0.063	3.794	3.415	3.484	3.415	3.066	3.206
0.125	4.215	3.879	3.851	3.822	3.621	3.592
0.250	4.676	4.208	4.042	4.000	4.063	4.021
0.500	4.923	4.538	4.400	4.523	4.538	4.538
1.000	5.255	5.154	5.113	5.062	4.955	5.113

α - AMYLASE INHIBITION ACTIVITY

Figure 1: Calibration curve for Acarbose @ 540 for α-amylase enzyme inhibition activity


Acarbose @ 540
y = 0.0007x + 0.2688
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0.75
0.50
0.25
0.00
0
200
400
600
800
1000

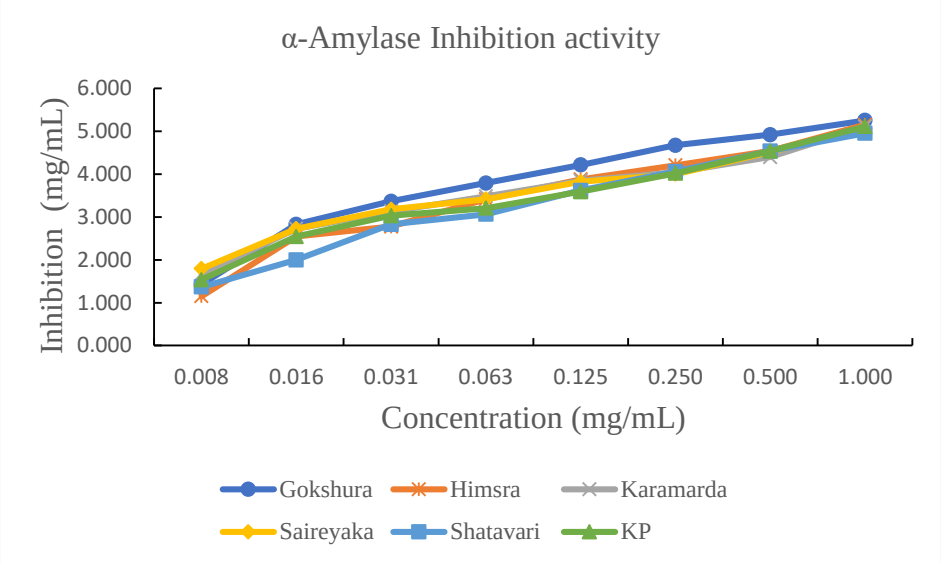
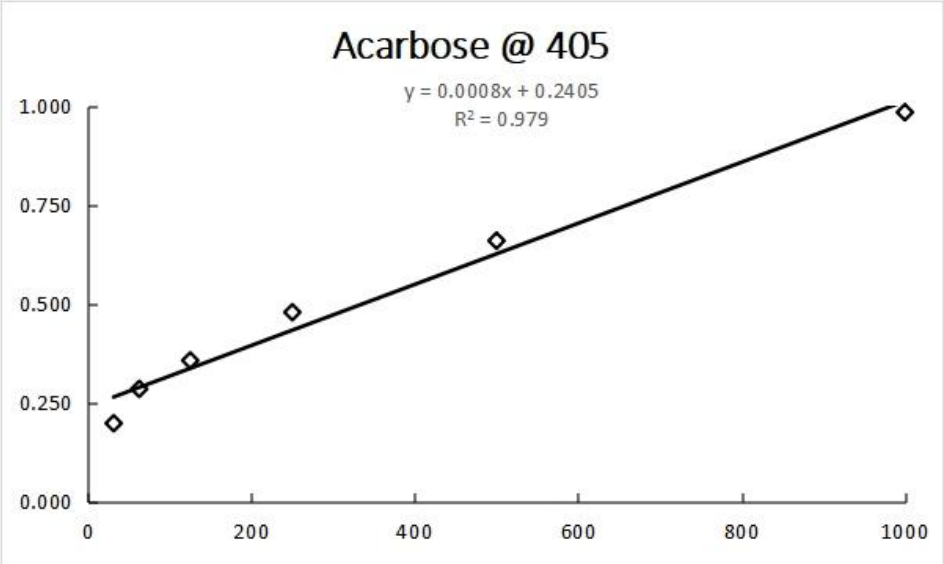
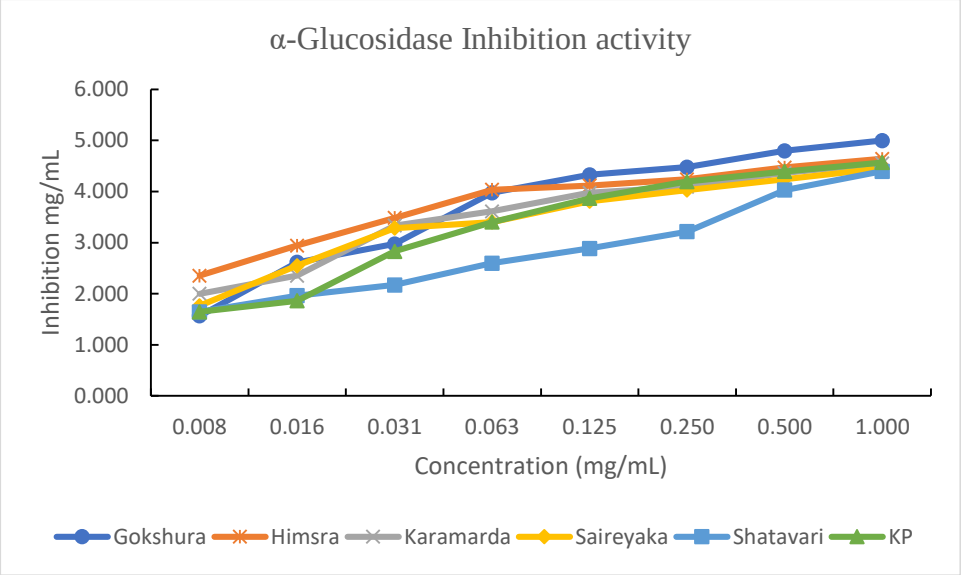

α-Amylase Inhibition activity
Inhibition (mg/mL)
6.000
5.000
4.000
3.000
2.000
1.000
0.000
0.008
0.016
0.031
0.063
0.125
0.250
0.500
1.000
Concentration (mg/mL)
Gokshura
Himsra
Karamarda
Saireyaka
Shatavari
KP

Table No 2: Percentage inhibition of α-Glucosidase enzyme activity at different concentrations of drugs

Conc mg/mL	Gokshura	Himsra	Karamarda	Saireyaka	Shatavari	KP
0.008	1.569	2.353	2.000	1.765	1.647	1.647
0.016	2.614	2.941	2.353	2.549	1.961	1.863
0.031	2.974	3.485	3.333	3.283	2.172	2.828
0.063	3.977	4.035	3.614	3.404	2.596	3.404
0.125	4.326	4.118	3.978	3.810	2.885	3.866
0.250	4.477	4.238	4.092	4.029	3.215	4.196
0.500	4.798	4.470	4.364	4.242	4.030	4.394
1.000	4.997	4.640	4.548	4.457	4.396	4.569

Figure 2: Calibration curve for individual and combined Kantaki panchamoola @ 405 for α-glucosidase enzyme inhibition activity





Discussion

α-amylase and α- glucosidase are carbohydrate hydrolysing enzymes helps for hydrolysis and catalysation of poly-carbohydrate to mono-carbohydrate thus helps in absorption of glucose from GIT which interns increase in hyperglycaemia, inhibition of these enzyme by using drug would help in reducing risk of DM. The drugs of *Kantaki Panchamoola* have shown Antidiabetic, Anti-inflammatory activity, Antioxidant activity, Antidiarrheal activity, Anticonvulsant activity etc. Individually these herbs are known to contain of saponins, tannins, flavonoids, alkaloids, glycosides, sitosterol's, which are known for inhibition of α-amylase and α-glucosidase enzymes. Previously, the antidiabetic activity of Kantaki panchamoola has been reported in the literature. But there was no information available in the literature about the in-vitro antidiabetic (α- amylase and α-glucosidase inhibitory activity) studies. Hence, the present study aimed to evaluate α-amylase and α-glucosidase inhibitory activity of aqueous extract of individual and Kantaki panchamoola.

Conclusion

The results of the present study are proven that Gokshura is effective for α-amylase and α-glucosidase inhibition, which may helpful to reduce the postprandial glucose levels. However, the principle compounds responsible for the inhibitory action of α-amylase and α-glucosidase need to be further identified and characterized. This may be useful for the development of new antidiabetic agents from native plant resources.