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In vitro assay of alpha amylase inhibitory activity of gymnemic acid isolated from *Gymnema Sylvestre* leaves

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ABSTRACT

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia and its type II is the major form of diabetes mellitus, accounting for 90% of cases worldwide. The management of the blood glucose level is a critical strategy in the control of diabetes mellitus complications. There are many and diverse therapeutic strategies in the management of Type II diabetes. The inhibition of carbohydrate hydrolyzing enzymes such as α-amylase can be an important strategy to lower postprandial blood glucose levels. Such inhibitors which find application in the clinical practice for management of diabetes are known to be associated with various gastrointestinal side effects. Therefore, it is the need of time to identify and explore the amylase inhibitors from natural sources having fewer side effects. In the present study, the gymnemic acid fraction of Gymnema sylvestre leaves in ancient medicine to treat diabetes were tested for their inhibitory effect on α-amylase. The HPLC analysis of gymnemic acid fraction of Gymnema sylvestre leaves with 94.68 % and 0.905 retention time may have detected The results revealed that the gymnemic acid fraction exhibited significant reduction in amylase activity. The highest inhibition i.e. 14.25% was observed at a concentration of 10 mg/mL with gymnemic acid fraction of Gymnema sylvestre leaves.

Key words: Anti-diabetic, HPLC, α-Amylase, Inhibitory effects.

INTRODUCTION

Diabetes mellitus is a multifactorial disease which is characterized by hyperglycemia, lipoprotein abnormalities and altered intermediary metabolism of major food substrates. **(1).** The management of the blood glucose level is a critical strategy in the control of diabetes complications. There are many and diverse therapeutic strategies in the management of Type II diabetes. The inhibition of carbohydrate hydrolyzing enzymes such as α-amylase can be an important strategy to lower postprandial blood glucose levels. Such inhibitors which find application in the clinical practice for management of diabetes are known to be associated with various gastrointestinal side effects. One therapeutic approach which may prove to be beneficial for treatment of diabetes is to decrease the post-prandial hyperglycemia. This can be achieved by retarding the absorption of glucose through the inhibition of the carbohydrate hydrolyzing enzymes in the digestive tract. The α glucosidase enzymes such as α-amylase are responsible for the breakdown of oligo and/or disaccharide to monosaccharides. Inhibitors of these enzymes delay carbohydrate digestion and prolong overall carbohydrate digestion time causing a marked decrease in the rate of glucose absorption thereby blunting the post prandial plasma glucose rise4. Examples of such inhibitors which find application in the clinical practice for management of diabetes are acarbose, miglitol and voglibose. Therefore, it is the need of time to identify and explore the amylase inhibitors from natural sources having fewer side effects. The Indian traditional system of medicine practiced for over thousands of years have reports of numerous anti- diabetic plants with no known side effects. Many plants and their products have been widely prescribed and used for diabetic treatment all around the world with less known mechanistic basis of their functioning. Thus, these natural products need to be evaluated scientifically in order to verify for their anti-diabetic properties. Higher plants are the source of

a large number of chemicals with wide range of medicinal, pharmacological and insecticidal properties. In recent years there is a great demand for plant-based products because of the broad biological activities, safety without any toxic side effects and low impact on environment. *Gymnema sylvestre* is a common anti diabetic plant used folklore medicine, Ayurvedic and homeopathic medicines. The plant is stomachic, stimulant, laxative and diuretic. It is good for cough, biliousness and sore eyes. If the leaves of the plant are chewed, the sense of taste for sweet and bitter substances is suppressed. The leaves are said to be used as a remedy for diabetes **(2,3).** The users of these health foods often expect weight reduction or improvement of diabetes because of their ability to suppress the taste of sweetness and inhibit glucose absorption **(4,5).** The constituents which effectively work on diabetes in ***G. sylvestre*** leaves are gymnemic acids **(6).** The present investigation was undertaken to make a study for the ability of the selected medicinal plant *Gymnema sylvestre* leaves to inhibit α-amylase activity.

MATERIALS AND METHODS

2.1 Collection and authentication

Gymnema sylvestre leaves were collected from Anna Herbal Garden, Chennai, Tamil Nadu. It was identified by Botanist, Plant Anatomy Research Centre (PARC), Tambaram, Chennai, Tamil Nadu and specimen voucher (**PARC/2012/1279**) are kept at Department of Pharmacognoscy, Vels College of Pharmacy, Chennai. The leaves of *Gymnema sylvestre* were shade dried grounded and stored dry until extraction.

2.2 Extraction of Gymnemic acid by Hoopers method(7)

Step 1: Extraction with petroleum ether (Defatting process)

I kg of dried *Gymnema sylvestre* dry leaf powder was packed into a clean soxhlet extraction unit. Seven litres of petroleum ether (60-80^{o}C) was added and extracted for 24-36 hrs till all components are soluble in petroleum. petroleum ether extract is collected and distilled . Then a net of 240gm of petroleum extracts was obtained. petroleum ether extracts was obtained.

Step 2: Extraction with 90% methanol

The plant material is then extracted with 90% methanol. 90% methanol is added and the extraction was carried out for 24-36hrs till total methanol soluble extracts was obtained. Then methanol soluble extract was distilled and finally 185gm of thick paste were obtained.

Step 3: Isolation of pure gymnemic acid from methanol extract

175gm thick paste of methanol soluble extract was dissolved in 1% aq.KOH solution on continuously stirring for 45 min to 1hr.The solution is then filtered through filter paper to separate the undissolved particles. Diluted HCL was added slowly under constant stirring, during which the gymnemic acids were precipitated. Precipitated solution was filtered under suction and precipitate was dried. The pure gymnemic acid was obtained. The yield of crude gymnemic acid fraction was found to be 29.6%. The isolated gymnemic acid fraction was subjected to qualitative chemical test and thin layer studies and positive tests for steroids, terpenoids and glycosides. The gymnemic acid fraction was dissolved in ethanol used for further studies.

2.3 Chromatographic system(8)

Chromatographic measurements were made on Shimadzu integrated liquid chromatographic system which consisted of a solvent delivery pump (model LC-10AT), injector (Model SC), UV visible absorbance detector (model SPD-10A) and the instrument was connected to the computer with Class-VP software. HPLC analysis was performed on a lichrosorb (Phenomenex) Luna 5 µ C 18, 250 X 4.6 mm column. The mobile phase was consisted of acetonitrile: 0.1% ortho Phosphoric acid (23:77 v/v) and pumped at a flow rate of 2.0 ml/min. The mobile phase was filtered through 0.45 µm Millipore filter and degassed by sonication for 30 min. The detection was carried out at 205 nm. An injection volume of the sample was 20µl. The temperature in laboratory was maintained at ambient conditions.

Preparation of sample solutions

The isolated gymnemic acid was dissolved in 1:1 mixture of methanol and HPLC grade water, followed by acidifying with concentrated hydrochloric acid. The acidified sample was transferred to a 10 ml volumetric flask and the volume was made up to 10 ml with methanol (50%) and filtered through Whatman filter paper and used for further HPLC analysis. The HPLC estimation was carried out by injecting 20 µl of the sample solution. Percentage of gymnemic acid was estimated using the area under the curve obtained from the sample by comparing the same with standard.

Calibration curve

Eight different concentrations of gymnemic acid fraction solution after dilution upto one mL (50 to 800 µg mL-1) with mobile phase were injected in triplicates. Regression equation with slope, intercept and co-efficient of correlation (r2) was derived. (Table 1).

2.4 Assay for α-amylase inhibition:(9)

The determination of α-amylase inhibition was carried out by quantifying the reducing sugar (maltose equivalent) liberated under the assay conditions. The enzyme inhibitory activity was expressed as a decrease in units of maltose liberated. A modified dinitrosalicylic acid (DNS) method was adopted to estimate the maltose equivalent.8 1mL of the aqueous solution gymnemic acid fraction of the *Gymnema sylvestre* plant were pre-incubated with α-amylase 1 U/mL for 30 min and thereafter 1 mL (1% w/v) starch solution was added. The mixture was further incubated at 37°C for 10 min. Then the reaction was stopped by adding 1 mL DNS reagent (12.0 g of sodium potassium tartrate tetrahydrate in 8 mL of 2 M NaOH and 96 mM 3, 5- dinitrosalicylic acid solution) and the contents were heated in a boiling water bath for 5 min. A blank was prepared without test drug and another without the amylase enzyme, replaced by equal quantities of buffer (20 mM Sodium phosphate buffer with 6.7 mM Sodium chloride, pH 6.9 at 20o C). The absorbance was measured at 540 nm. The reducing sugar released from starch was estimated as maltose equivalent from a standard graph. Acarbose was used as positive control. The aqueous plant extracts from different plant parts were diluted in buffer to give a final concentration of 5mg/mL, 10mg/mL. The anti-diabetic activity was determined through the inhibition of α-amylase which was expressed as a percentage of inhibition and calculated by the following equations:

$$\% \text{ reaction} = (\text{maltose}) \text{ test} / (\text{maltose}) \text{ control} \times 100 \quad \% \text{ inhibition} = 100\% \text{ reaction}$$

2.5 Statistical analysis

All the analyses were carried out in triplicate and the results were expressed in mean ± SD.

HPLC CHROMATOGRAM OF GYMNEMIC ACID FRACTION OF *GYMNEMA SYLVESTRE* LEAVES

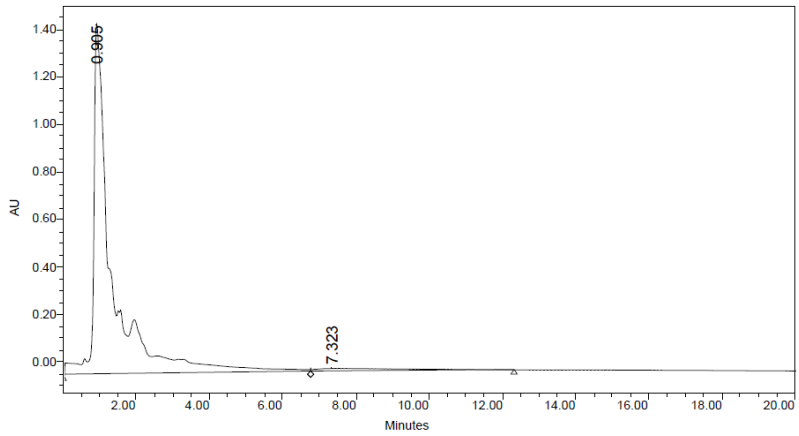

Table: I **HPLC CHROMATOGRAM OF GYMNEMIC ACID FRACTION OF *GYMNEMA SYLVESTRE* LEAVES**

	RT (min)	Peak Type	Area (µV*sec)	% Area	Height (µV)	% Height	Integration Type	Points Across Peak	Start Time (min)	End Time (min)
1	0.905	Unknown	52247349	94.68	1477449	98.93	BV	405	0.017	6.767
2	7.323	Unknown	2937016	5.32	15960	1.07	VB	334	6.767	12.333

	Baseline Start (min)	Baseline End (min)	Slope (µV/sec)	Offset (µV)
1	0.017	12.333	1.945200e-003	-5.299618e-002

Table: II **α-AMYLASE INHIBITORY ACTIVITY OF GYMNEMIC ACID FRACTION OF *GYMNEMA SYLVESTRE* LEAVES**

Sample	Concentration	% inhibition
Gymnemic acid fraction	5mg/ml	17.49±0.25
	10mg/ml	14.23±0.15
Acarbose	1mg/ml	48.21±0.21

RESULTS

In the present study gymnemic acid fraction isolated from *Gymnema sylvestre* leaves by Hoopers method. The isolated gymnemic acid fraction shows the presence of steroids, terpenoids and glycosides. HPLC was applied for testing the presence of number of organic compounds available of gymnemic fraction of *Gymnema Sylvestre* and this gymnemic acid fraction were Isolated . One of the major organic components with 94.68 % and 0.905 retention time may have detected. Fig 1 & Table 1.The α-amylase inhibition activity of gymnemic acid fraction of *Gymnema sylvestre* leaves was investigated and reported. The concentration of 5 mg/ml and 10mg/mi of gymnemic acid fraction showed the highest inhibition of 17.49% and 14.23, which is compared with the standard drug Acarbose Table2. From the results, it can be concluded that use of these plant extracts will be greatly beneficial to reduce the rate of digestion and absorption of carbohydrates and thereby contribute for effective management of diabetes by decreasing the post-prandial hyperglycemia.

CONCLUSION

Analytical study suggests that gymnemic acid fraction contain various constituents which are given in the table 1. Preparative HPLC study revealed presence constituents were Isolated of acetonitrile: 0.1% ortho Phosphoric acid (23:77 v/v) from gymnemic acid fraction of *Gymnema Sylvestre*. The results concluded showed that the gymnemic acid fraction of *Gymnema Sylvestre* posses good α amylase inhibition and only one constituents were Isolated of acetonitrile: 0.1% ortho Phosphoric acid (23:77 v/v) from gymnemic acid fraction of *Gymnema Sylvestre*. and further investigations are in progress to identify the active structure and synthesis for application of this compound and its pharmacological action.

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