Published online in http://ijam.co.in ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 13 (2), 515-519

Revealing Anti-diabetic potential of Siddha formulation Gandhaka Sarkkarai using alpha amylase and alpha glucosidase enzyme inhibition assay

Research Article

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Abstract

Background: Diabetes mellitus (DM) is a complex metabolic disorder which involves multiple pathology manifested with increased blood glucose, neural degeneration, chronic inflammation, organ dysfunction etc. Hyperactive metabolic enzymes like alpha amylase and alpha glucosidase that are involved in digestion of starch and sucrose further upswings the postprandial hyperglycaemia by rushing excess glucose moieties into the bloodstream. Drugs that effectively inhibit the action of these digestive enzymes may be expected to better regulate the post prandial blood glucose in the diabetic patients. Conventional anti-diabetic agents offer potential side effects upon long-term usage which includes vomiting, diarrhoea, pigmentation, GI disturbance, dark urination etc. The Siddha system of medicine has excelled in the art of treating human ailments for several centuries. Aim: Present investigation designed to investigate the anti-diabetic potential of siddha formulation *Gandhaka sarkkarai* (GS) using in-vitro alpha amylase and alpha glucosidase enzyme inhibition assay model. Results: It was evidenced from the outcome of the in-vitro data's that the siddha formulation GS shown significant inhibition against alpha glucosidase enzyme with the maximum inhibition of about 50.44 % and the corresponding IC_{50} is 471.1 µg/ml, similar pattern of activity were observed against alpha amylase enzyme with the inhibition of 60.84 % (IC_{50} 400.9 µg/ml). Conclusion: Our data concludes that siddha formulation GS possess significant anti-diabetic activity via inhibiting two major carbohydrate-digesting enzymes, further studies needs to extrapolated at pre-clinical level in order to ascertain the efficacy of the formulation.

Key Words: Diabetes mellitus, Alpha amylase, Alpha glucosidase, *Siddha, Gandhaka sarkkarai*, Enzyme inhibition assay, In-vitro, Anti-diabetic activity.

Introduction

Diabetes mellitus (DM) stands unique among other metabolic disorders as it negatively impacts the metabolism of carbohydrate, fat and protein. DM predominantly classified into two categories of which type 2 prevails over 90 % of the cases than type 1. (1) Pathophysiology of type 1 signifies insulin deprivation whereas type 2 evidenced with either insulin production or insulin resistance. Despite disease pathology research also witnessed certain crucial risk factors which includes obesity, smoking, hyperlipidemia, chronic medication, sedentary lifestyle etc. (2)

The International diabetes federation predicts that nearly 366 million people are suffering from diabetes (type 1/2) and this may be expected to double by the year 2030, while considering the cases in India it was to be 40.9 million, which is expected to grow to 60.9 million by 2025. (3) Therapeutic strategies involving management of DM follows a multidimensional approach of which the enzyme inhibition plays a pivotal role in effectively combating the pathogenesis associated with DM.

Increased blood glucose level is one of the significant causes of diabetic complications. Metabolism of carbohydrate into glucose is mediated by bioactive digestive enzymes like α-amylase and α-glucosidase which contributes glucose as an digestive end product of starch ad sucrose. (4,5) Hence inhibition of α-amylase and α-glucosidase delays the glucose absorption from the small intestine and thereby adequately lowers the blood glucose level.

Conventional anti-diabetic agents that belong to the category of enzyme inhibitors (acarbose, voglibose, and miglitol) currently into clinical practice offer some potential side effects upon long-term usage which includes flatulence, diarrhoea, abdominal pain etc. (6) In order to overcome the existing clinical issues there is an urgent need for the remedy preferably from herbal origin that deserves a higher level of safety and therapeutic index. Siddha system of medicine excels at

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treating human ailments since several centuries, it is one of the holistic forms of traditional therapy whose guidelines were systematically framed by ancient siddha physicians called *siddhars*. (7,8)

Herbs become the integral part of siddha medicines, a combination of herbs and minerals considered as viable forms of medication as per siddha system of medicine. Siddha drugs grab the attention of the public for its safety and proven efficacy in managing metabolic disorders like diabetes. One such novel siddha drug is *gandhaka sarkkarai* (GS) indicated for treating diabetes as per the siddha literature. GS is a herbomineral formulation made of purified sulphur (*gandhagam*) blend with juice of *Eclipta prostrata* and *Allium cepa*.

Eclipta prostrata is a potential medicinal herb belonging to the family Asteraceae known for its folklore therapeutic ailment in treating liver and kidney disease. Studies emphasize the versatile mechanism of this herb in acting as anti-inflammatory (9), antioxidant (10) and anti-hyperlipidaemic agent. (11) *Allium cepa* called by onion in vernacular terminology belongs to the family Amaryllidaceae has been substantially investigated for its anti-hyperlipidaemia (12), anti-hypertensive (13) and anti-diabetic properties (14). Sulphur is a core ingredient of biologically significant amino acids like cysteine and methionine which mediates enzymatic actions in executing fundamental physiological activity essential for human survival, further sulphur based drugs showcase broad spectral antimicrobial activity. (15,16) Sulphur injections are proven to limit the blood glucose level in tested diabetic patients. (17) Still now there is no documentary evidence claiming the anti-diabetic potential of the formulation GS, hence the present research work aimed at evaluating the anti-diabetic activity of GS using in-vitro alpha amylase and α-glucosidase enzyme inhibition assay.

Materials and Methods

Chemicals and Reagent

The origin of the enzyme alpha glucosidase is revealed to be from saccharomyces cerevisiae, further the origin of alpha amylase identified as procaine pancreas. Sources of procurement of other fine chemicals such as 3, 5, di-nitro salicylic acid (DNS), P-nitro-phenyl-α-D-glucopyranoside (p-NPG), Sodium carbonate (Na2 CO3), Bovine serum albumin (BSA), Sodium dihydrogen phosphate, di-sodium hydrogen phosphate were from Sigma-Aldrich and Hi-Media suppliers.

In-vitro α-amylase enzyme inhibitory assay

In-vitro α-amylase enzyme inhibitory potential of the test formulation GS accomplished by the established laboratory procedure using spectrophotometric assay method. (18, 19) The enzyme α-amylase with the concentration of 8.30 ng/ml was processed by mixing 3240 µg of α-amylase in 0.lL of phosphate buffer saline (pH 6.9). Test Sample (GS) were constructed in varying dilutions with appropriate concentration from 100 to 500 µg/ml. Standard drug acarbose (0.1mg/ml) were utilised as a reference to predict the efficacy of the test formulation. About 0.6 ml of serially diluted test sample (GS) were mixed with 0.03 ml of the prepared α-amylase enzyme solution and subjected to incubation at 37°C for 15 min. To this reactant mixture, 0.37 ml of substrate (CNPG3- 500 µg /ml) was added and allowed for brief incubation at 37°C for 10 min. Final absorbance of resultant coloured product was spectrophotometrically measured at 405 nm by using respective blank solution (control reaction mix without test sample) to extrapolate the calibration curve. Percentage inhibition of the test sample and standard drug was calculated by the following formula.

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

In-vitro α-a glucosidase enzyme inhibitory assay

The α-glucosidase enzyme solution were constructed by dissolving 500µg of α-glucosidase in 10000 µl phosphate buffer (pH 7.0) containing 20000 µg BSA. 10 µl of serially diluted (100 -500 µg/ml) test sample (GS) along with standard Acarbose (0.1mg/ml) were added to 0.25 ml of 20 mM p-NPG, 0.495 ml of 100 mM phosphate buffer (pH 7.0) and subjected to incubation at 37°C for 5 min. Final reaction in the medium was triggered upon addition of 0.25 ml α-glucosidase enzyme solution containing BSA and was subjected to incubation at 37°C for 15 min. The reaction was then terminated by addition of 1ml of 200 mM Na_2CO_3 solution, further the quantum of release of the p-nitrophenol was spectrophotometrically measured at 405 nm by using respective blank solution (control reaction mix without test sample) to extrapolate the calibration curve. (20,21) Percentage inhibition of the test sample and standard drug was calculated by the following formula.

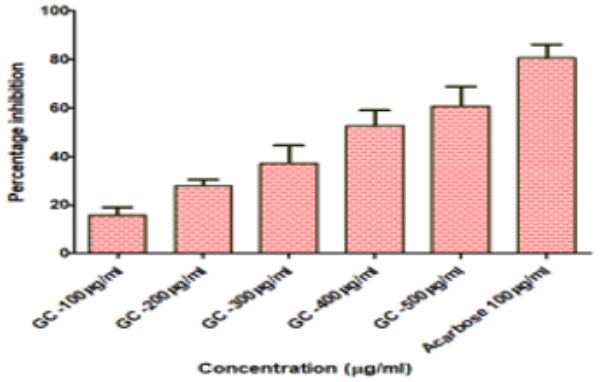
$$\%\text{inhibition} = \frac{\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Test}}}{\text{Absorbance}_{\text{Control}}} \times 100$$

Results

Effect of GS on Alpha amylase enzyme inhibition assay

Alpha-amylase enzyme inhibitory potential of the siddha drug GS were illustrated in the figure 1. Measurable inhibition activity was observed from lowest (100 µg/ml) to highest concretion (500 µg/ml) of the test drug GS in the tested in-vitro model. Inhibition of α-amylase activity by GC was found to be dose dependent from 100 to 500 µg/ml concentrations. A maximum of 37.3, 52.7 and 60.8 % inhibition of α-amylase enzyme activity was observed at concentration ranges from 300 to 500 µg/ml in comparison with reference standard Acarbose with the maximum inhibition of 80.63 % at 100 µg/ml. Enzyme inhibition potential of the formulation GS measured in terms of IC_{50} value as it was hypothesised that lower the IC_{50} value greater the potency in terms of inhibition. Calibration plot sequence of the drug GS reveals significant α-amylase inhibition potential with an IC_{50} value of 400.9 µg/mL when compared with Acarbose with an IC_{50} value of 42.15 µg/mL. Results were tabulated in table 1 and 2.

Figure 1: Percentage inhibition of GS and Acarbose on Alpha amylase enzyme inhibition assay



Effect of GS on Alpha glucosidase enzyme inhibition assay

Alpha- glucosidase enzyme inhibitory potential of the siddha drug GS were illustrated in the figure 2. There is notable increase in inhibition potential revealed from lowest (100 µg/ml) to highest concentration (500 µg/ml) of the test drug GS. Inhibition of α- glucosidase activity by GC was found to be absolute dose dependent with concentration ranges from 100 to 500 µg/ml. A maximum of 36.7, 45.5 and 50.5 % inhibition of α-glucosidase enzyme activity was observed at concentration ranges from 300 to 500 µg/ml in comparison with reference standard Acarbose with the maximum inhibition of 88.3 % at 100 µg/ml. Data of calibration plot provoked by the drug GS reveals significant α- glucosidase enzyme inhibition potential with an IC_{50} value of 471.1 µg/mL when compared with Acarbose with an IC_{50} value of 30.54 µg/mL. Results were tabulated in Table 1 and 2.

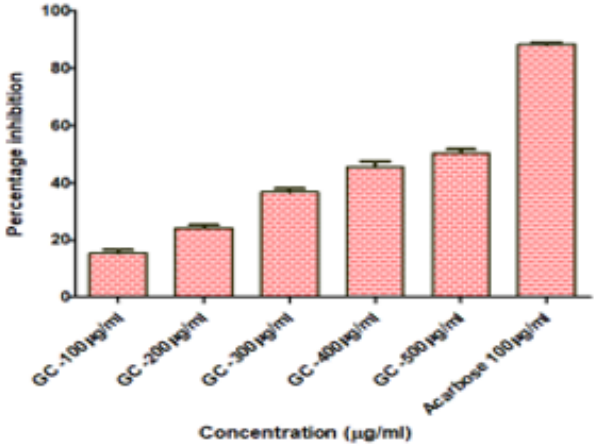
Table 1: Percentage inhibition of test drug GS and STD on Alpha Amylase and Alpha Glucosidase enzyme Inhibition assay.

Concentration (µg/ml)	% Inhibition of Alpha amylase enzyme	% Inhibition of Alpha Glucosidase enzyme
GS-100 µg/ml	15.91±3.15	15.36±1.22
GS-200 µg/ml	27.93±2.78	24.17±0.97
GS-300 µg/ml	37.39±7.23	36.76±1.33
GS-400 µg/ml	52.71±6.47	45.5±1.91
GS-500 µg/ml	60.84±8.11	50.44±1.31
Standard Acarbose	80.63 ± 5.70	88.32 ± 0.73

Table 2: IC50 Values for α-Amylase and α-Glucosidase Enzyme inhibition by GS and Acarbose

Drug	IC_{50} (µg /ml)	
	Alpha Amylase	Alpha Glucosidase
GS	400.9 ± 58.84	471.1 ± 16.27
Standard-Acarbose	42.15 ± 2.26	30.54 ± 1.87

Figure 2: Percentage inhibition of GS and Acarbose on Alpha glucosidase enzyme inhibition assay



Discussion

In diabetes uncontrolled hyperglycemia may be expected to trigger certain biochemical pathways (polyol, flux, hexosamine, protein kinase C (PKC)) that accelerates the production of unstable oxygen radicals. (22) Increased production of reactive oxygen species sets up the oxidative stress condition which in turn kick-starts the inflammatory process that paves way for degenerative changes on the morphology of βcells of the pancreas. (23)

Growing prevalence of diabetes mellitus urges the need of effective medication with higher level of efficacy and biocompatible upon long term administration. Existing enzyme inhibitors for the management of diabetes like Acarbose is a fermented oligosaccharide compound found to be a gold standard anti-diabetic agent that inhibits the digestive action of both alpha glucosidase and alpha amylase enzymes. (24) Despite its clinical legacy extensive usage of this drug Acarbose results in clinical complications like gastrointestinal disturbance, diarrhoea, pigmentation, dark urination etc. (25) By considering this fact there is a huge demand and market potential for drugs from alternate traditional medicines with high safety and wider therapeutic window.

More than 1000 herbal species are currently proven to be effective in the management of DM. (26) Herbal derivatives exerts its mechanism by rejuvenating the β cells thereby restoring the insulin production and also ensures effective glycaemic control of diabetes patients. Medicinal plants have an impressive track record of serving as a trustworthy source of delivering notable entities in treating disease and disorders. (27) Presence of pharmacologically active phytotherapeutics in the form of alkaloids, flavonoids, tannins, glycosides, saponins etc synergise the mode of action of herbal drugs against specified targets. (28)

The enzyme alpha amylase pioneers the action of starch digestion and converts them into maltose similarly the enzyme alpha glucosidase expressing at brush borders of small intestines involved in conversion of maltose and sucrose into glucose and fructose. Absorption of these simple sugar moieties derived from carbohydrate metabolism triggers the blood glucose

level after a healthy meal called postprandial hyperglycemia. (29) In the present study inhibition of α-amylase activity by GC was found to be dose dependent from 100 to 500 µg/ml concentrations. A maximum of 37.3, 52.7 and 60.8 % inhibition of α-amylase enzyme activity was observed at concentration ranges from 300 to 500 µg/ml in comparison with reference standard Acarbose with the maximum inhibition of 80.63 % at 100 µg/ml. Enzyme inhibition potential of the formulation GS measured in terms of IC_{50} value as it was hypothesised that lower the IC_{50} value greater the potency in terms of inhibition. Calibration plot sequence of the drug GS reveals significant α-amylase inhibition potential with an IC_{50} value of 400.9 µg/mL when compared to that of Acarbose with an IC_{50} value of 42.15 µg/mL.

The enzyme alpha glucosidase plays a crucial role in monitoring postprandial blood glucose levels in humans. Agents that tend to inhibit alpha glucosidase activity may possess better regulation in controlling postprandial hyperglycemia and are considerably used for the treatment of type II diabetes. (30) A maximum of 36.7, 45.5 and 50.5 % inhibition of α- glucosidase enzyme activity was observed at concentration ranges from 300 to 500 µg/ml in comparison with reference standard Acarbose with the maximum inhibition of 88.3 % at 100 µg/ml. Data of calibration plot provoked by the drug GS reveals significant α- glucosidase enzyme inhibition potential with an IC_{50} value of 471.1 µg/mL when compared to that of Acarbose with an IC_{50} value of 30.54 µg/mL.

Conclusion

In-vitro techniques substantially aids the researcher in understanding the mode of drug action and also to narrow down research in the path of newer drug discovery. Due to its reliability, relevance and reproducibility these techniques attain greater importance ever. Outcome of the present study clearly implies that the siddha formulation *gandhaka sarkkarai* effectively inhibits both α-amylase and α-glucosidase enzymes. However, the principle phytotherapeutics present in the formulation that are majorly responsible for mediating the inhibitory action against the enzymes α-amylase and α-glucosidase need to be identified and characterized in future. This attempt paves a way for the development of newer anti-diabetic agents from traditional siddha medicines.

Aknowledgement

I wish to acknowledge my thanks to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, Tamil Nadu, India and The Noble research solutions, Chennai, Tamil Nadu, India for their support.

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