Exploration of antihyperglycaemic potential of the siddha formulation *Vallarai choorana tablet* by *In vitro* α-amylase and α-glucosidase enzyme inhibition assay

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Category: Original Research Communication

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Abstract

Diabetes mellitus is a chronic metabolic disease that can be suspected or recognized clinically by the onset of one or more of the characteristic symptoms such as polyuria, polydipsia, polyphagia, and unsolved weight loss. As per the statistics established in the year 2013, the prevalence of diabetes in India was 9.1%, which was just slightly higher than the global average of 8.3%. On the other hand, because of the country's enormous population, India has the second-largest diabetic population in the world, behind China. Pancreatic enzymes alpha-amylase and alpha-glucosidase catalyse the breakdown of polysaccharides into simpler sugars. These enzymes are responsible for the absorption of glucose produced from polysaccharides, which causes an increase in blood glucose levels. Currently available enzyme inhibitors like acarbose offers some potential side effects like flatulence, diarrhoea, and abdominal pain. It is a well-known fact that traditional methods of treatment have

always played an important part in contributing to the fulfilment of the health care requirements of people all over the world. Siddha system of medicine holds greater importance as it has been fully integrated into Indian culture, and it has received enhanced visibility among the global audience due to versatile ideology of dealing dreadful diseases. The main objective of the present investigation is to explore the possible mechanism underlying the antihyperglycaemic potential of the siddha formulation *Vallarai choorana* tablet by *in-vitro* alpha amylase and glucosidase enzyme inhibition assays. Findings of the current study, evident that the siddha formulation VCT shown considerable inhibition of both the alpha amylase and glucosidase enzymes dose dependently. The formulation reveals the maximum inhibition of about 78.21% (IC_{50} is 290.6 µg/ml) against alpha amylase enzyme and 56.16 % (IC_{50} is 438.1µg/ml) against alpha glucosidase enzymatic activity. Outcome of the present investigation clearly signifies that the siddha formulation *Vallarai choorana* tablet exhibit significant enzyme inhibition property against alpha amylase and glucosidase enzymes, thereby it may be considered as viable drug of choice for managing postprandial hyperglycemia.

Keywords: Diabetes mellitus, Siddha, Alpha-amylase, Alpha-glucosidase, *Vallarai choorana* tablet, Antihyperglycaemic.

DOI Number: 10.48047/nq.2022.20.19.NQ99028 **NeuroQuantology2022; 20(19):329-338**

1. Introduction

Diabetes mellitus is a collection of metabolic abnormalities that often implicates the metabolism of carbohydrates, but also the metabolisms of protein and fat. A recent study on the prevalence of diabetes mellitus (DM) across the globe suggests that there are currently 463 million people living with the condition. According to statistical forecast it was observed that there is a potential increase in global prevalence of diabetes as much as 10.2% (578 million by 2030) and 10.9% (700 million by 2045) correspondingly [1]. Diabetes mellitus is typically brought on by an insufficiency or absence of insulin secretion; however, it may also be brought on by a decrease in the cells' capacity to make use of insulin [2]. Diabetes mellitus is widely regarded as the single most important risk factor that contributes to the development of a wide variety of clinical conditions, including ischemic heart disease, peripheral neuropathies, ulcerations, and delayed wound healings, all of which have a negative impact on a patient's life expectancy [3].

Obesity, genetics, environmental and lifestyle factors play a crucial role in the development of type 2 diabetes [4]. It has been demonstrated beyond a reasonable doubt that a poor diet is a major contributor to the genesis and progression of diabetes [5]. In addition, dietary changes have been shown to be effective in preventing type 2 diabetes, as well as in delaying the onset of diabetes and improving glucose control in patients [6].

The American Diabetes Association estimates that the total expenditures connected with diagnosed diabetes have

increased by 41% in the past five years, reaching a total of $245 billion in 2012 from $174 billion in 2007. This represents an increase of 41% over the course of just five years [7]. The World Health Organization (WHO) is asking decision makers in the health care industry to establish efficient management methods to reverse the rising trend of diabetes through treatment modalities that are both trouble-free and cost-effective.

Patients with diabetes managed with some degree of relief as a result of the availability of antidiabetic medications such as insulin, biguanides, sulphonylureas, glucosidase inhibitors, and other categories of antidiabetic pharmaceuticals [8]. However, several of these medications offers unwanted side effects, such as hypoglycemia, dizziness, and lactic acidosis, as well as a high price tag and limited availability, particularly in developing countries [9,10]. Additionally, some of these medications cause severe hypoglycemia. Because of these issues, there has been a significant push toward the creation of effective ethnomedicines [11].

Available evidenced strongly suggested that natural products derived from a wide variety of herbal therapeutics can serve as an important viable measure of therapy for diabetes, and that they are capable of effectively controlling the condition of diabetes as well as preventing and delaying the complications of diabetes.

Ayurveda, Siddha, Unani and Homeopathy are the officially recognized alternative health systems in India, and each of these practices makes use of a variety of herbal medicines in a way that is both safe and consistent over the course of centuries. On a regular basis, millions of Indians use herbal drugs in the form of spices, home remedies, health foods, and over-the-counter (OTC) medications for self-medication or as drugs prescribed in non-allopathic systems [12]. Herbal drugs can also be used as a substitute for conventional pharmaceuticals in some non-allopathic medical systems.

In siddha system of medicine single herb formulation gaining momentum because of its extensive pharmacological activity. Siddha literatures evident numerous herbal formulation for the management of metabolic disorders like diabetes, one such vital formulation is *Vallarai choorana* tablet which comprises of *centella asciatica* (*Vallarai*) as the major ingredient. *Centella asiatica* belongs to the family Apiaceae, is a potential folklore herb widely used for the management of psoriasis, diarrhoea, fever, amenorrhea, inflammation, relieving anxiety and improving cognition [13]. Some clinical evidenced suggested that this herb under investigation for managing diabetic neuropathy. The main objective of the present investigation is to explore the possible mechanism underlying the anti-diabetic efficacy of the siddha formulation *Vallarai choorana* tablet by suitable in-vitro assays.

2.Materials and Methods

2.1.Method of preparation

The roots of the herb *centella asciatica* were acquired from the traditional vendor and was subjected to

the process of purification [14]. The purified shade dried roots were then grounded in to fine powder and sieved as per standard protocol. In order to facilitate tablet compression, the resulting powder is granulized by mixing it with a specified proportion of additives such as gum arabic and talcum powder to get *Vallarai choorana* tablet.

2.2.Alpha Amylase enzyme Inhibition assay [15]

α-amylase enzyme inhibition potential of the test formulation evaluated by mixing 3.24 mg of -amylase with 100 ml of phosphate buffer yields a solution of 0.5 U/ml of the α enzyme (pH 6.9). The test formulation (VCT) at the concentrations of 100, 200, 300, 400, and 500 µg/ml were prepared by serial dilution with DMSO. The reference standard (acarbose) were prepared at 100 micrograms per millilitre concentration. About 600 µl of the test sample was mixed with 30 µl of the amylase enzyme solution and then incubated at 37 °C for 15 minutes. The substrate 2-Chloro-4-Nitrophenyl—Maltotrioside (CNPG3- 0.5 mg/ml) was added to the reaction mixture (370 µl total), which was then stirred and incubated at 37°C for 10 minutes. Finally, spectrophotometer absorbance at 405 nm was measured against blank. The reaction was repeated without the test sample to serve as a control. The percentage of inhibition nature of the test dug was ascertained by using the formula.

Percentage inhibition

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

2.3.Alpha glucosidase enzyme Inhibition assay [16]

α-glucosidase enzyme was prepared by dissolving 0.5 milligrams of α-glucosidase into 10 millilitres of phosphate buffer with a pH of 7.0 comprises of 20 milligrams of bovine serum albumin. About 10 µl of each of the test samples at varying concentrations (100- 500 µg/ml), in addition to acarbose at a concentration of 100 µl g/ml, were added to 250 µl of 20 mM p-nitrophenyl—D -glucopyranoside and 495 µl of 100 mM phosphate buffer. The mixture was then mixed thoroughly (pH 7.0). It was pre-incubated at 37°C for 5 minutes before the reaction was started by adding 250 µl of the α-glucosidase enzyme solution. The reaction was then carried out at 37°C for exactly 15 minutes after the enzyme solution was added. In place of the enzyme, a blank containing 250 µl of phosphate buffer was performed. After that, the reaction was halted by adding 1000 µl of a solution containing 200 mM Na2 CO3, and the amount of p-nitrophenol that was released was measured by comparing the absorbance of the sample to the absorbance of a sample blank, which contained PBS but no sample, at 405 nm using a UV visible spectrophotometer.

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

3.Results

3.1.Effect of the formulation VCT on α-amylase enzyme inhibition assay

Based on the findings of the current study, it was identified that the siddha formulation VCT shown considerable inhibition of the alpha amylase enzyme. The sample VCT exhibited 16.22, 38.28, 57.31, 67.57 and

78.21% of inhibitory action against alpha amylase enzyme activity at the concentration ranging from 100 -500 µg/ml. Further it was also documented that the sample VCT revealed IC_{50} value of 290.6 ± 7.87 µg/ml. Standard acarbose was shown to inhibit the alpha amylase enzyme, with a maximum inhibition of around 95.71% and an IC_{50} value of 42.87 ± 7.96 µg/ml. As shown in table 1 and figure 1.

3.2.Effect of the formulation VCT on α-glucosidase enzyme inhibition assay

It was found in the present investigation that the siddha formulation VCT significantly inhibited alpha glucosidase enzyme activity. At concentrations between 100 and 500 g/ml, the sample VCT showed inhibitory actions of 22.02, 28.75, 35.73, 45.33, and 56.16 percent against alpha glucosidase enzyme activity. Further it was also documented that the sample VCT revealed IC50 value of 438.1 ± 6.31µg/ml. Standard acarbose was shown to inhibit the alpha glucosidase enzyme, with a maximum inhibition of around 88.44% and an IC_{50} value of 29.45 ± 2.10 µg/ml. As shown in table 1 and figure 1.

Table 1: Percentage inhibition of test drug VCT and Acarbose on α-glucosidase and α-amylase inhibition assay

Concentration (µg/ml)	% Inhibition of α-amylase activity	% Inhibition of α-glucosidase activity
VCT 100 µg/ml	16.22±5.065	22.02±1.395
VCT 200 µg/ml	38.28±3.761	28.75±1.892
VCT 300 µg/ml	57.31±4.926	35.73±2.369
VCT 400 µg/ml	67.57±2.907	45.33±0.7402
VCT 500 µg/ml	78.21±2.248	56.16±0.9483
STD -Acarbose	95.71±0.5456	88.44 ± 0.62

Data are given as Mean ± SD (n=3)

Table 2: Estimated inhibitory concentration 50 (IC_{50}) values for α-glucosidase and amylase inhibition for VCT and Acarbose.

	Inhibitory Concentration, IC_{50} (µg/ml)	
Enzyme inhibition assay	Test formulation (VCT)	Standard (Acarbose)
α-amylase	290.6 ± 7.87	42.87 ± 7.96
α-glucosidase	438.1 ± 6.31	29.45 ± 2.10

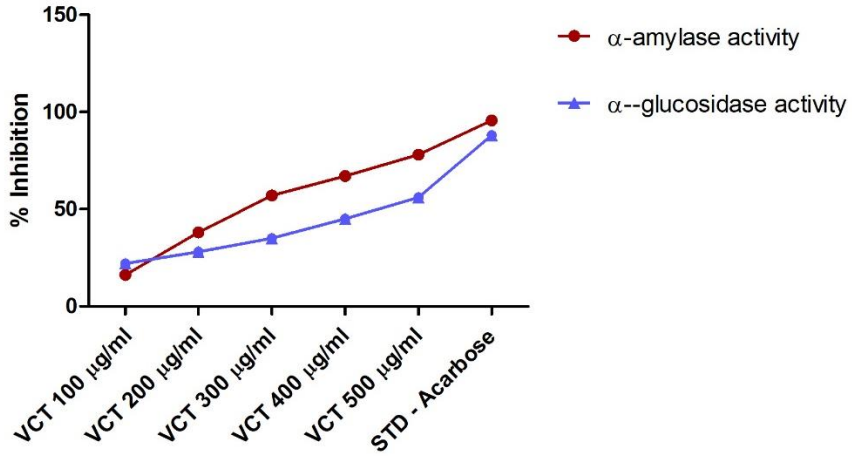

Figure 1: Effect of VCT and Acarbose on for α-amylase and α-glucosidase enzyme inhibition assay

4. Discussion

Recent research from the International Diabetes Federation indicates that diabetes is more frequent in developing countries. Countries in which people have adopted high-calorie westernized diets, but have less access to opportunities for physical activity. Noninsulin-dependent diabetes mellitus (NIDDM), more generally known as type 2 diabetes (T2D), is responsible for roughly 90%–95% of all instances of diabetes [17].

There are currently three agents that can be used, and they are called acarbose, miglitol, and voglibose [18]. Because of its mode of action, their features are distinct from those of other diabetes medications. Over the past two decades, acarbose has been successfully utilized in the management of hyperglycemia [19]. The majority of adverse effects are digestive in nature and include symptoms such as flatulence, diarrhoea, and abdominal pain. These symptoms are often modest; however, they have the potential to impair compliance, and they are the most prevalent cause for patients to stop receiving therapy [20].

The pancreatic enzymes alpha-amylase and alpha-glucosidase are essential for carbohydrate metabolism in the small intestine. These enzymes break down the polysaccharides that have been digested into monosaccharides. The action of this enzyme leads to an increase in the amount of glucose in the blood after a meal as a result of the absorption of glucose generated from polysaccharides in the small intestine [21]. To manage the postprandial blood glucose level in individuals with type 2 diabetes, medications that have an inhibitory activity on both of these enzymes have the capacity to limit the level of glucose in the blood. Acarbose and miglitol, both of which decrease enzyme activity by competing with it, are the only medicines in this class that are currently on the market. On the other hand, these medications are known to cause adverse effects such as belly bloating and flatulence [22]. Patients who have type 2 diabetes will have a higher rate of compliance if new medications or

formulations are developed that do not have the negative effects listed above.

The enzyme alpha-amylase kicks off the process of carbohydrate digestion by hydrolyzing the 1, 4-glycosidic bonds of polysaccharides (starch, glycogen) to disaccharides, which ultimately results in postprandial hyperglycemia [23,24]. Therefore, inhibitors of alpha-amylase are effective in the treatment of hyperglycemia because they delay the digestion of carbohydrates, which ultimately results in a reduction in the amount of postprandial plasma glucose. Based on the findings of the current study, it was identified that the siddha formulation VCT shown considerable inhibition of the alpha amylase enzyme. The sample VCT exhibited 16.22, 38.28, 57.31, 67.57 and 78.21% of inhibitory action against alpha amylase enzyme activity at the concentration ranging from 100 -500 µg/ml. Further it was also documented that the sample VCT revealed IC_{50} value of 290.6 ± 7.87 µg/ml. Standard acarbose was shown to inhibit the alpha amylase enzyme, with a maximum inhibition of around 95.71% and an IC_{50} value of 42.87 ± 7.96 µg/ml.

Alpha-glucosidase is responsible for the conversion of oligosaccharides and disaccharides to monosaccharides, which in turn improves the absorption of carbohydrates and contributes to an increase in the concentration of blood sugar [25]. Through a process known as competitive inhibition, the administration of a glycosidase inhibitor can cause a delay in the absorption of carbohydrates. This, in turn, can block the hydrolysis of disaccharides and the absorption of glucose [26]. Glycosidase inhibitors have been proven to have anti-diabetic and anti-obesity benefits, according to research [27]. The utilization of glucosidase inhibitors derived from natural sources has captured the attention of researchers in recent years [28]. It is important to accurately assess the therapeutic potential of these natural sources. It was found in the present investigation that the siddha formulation VCT significantly inhibited alpha glucosidase enzyme activity. At concentrations between 100 and 500 g/ml, the sample VCT showed inhibitory actions of 22.02, 28.75, 35.73, 45.33, and 56.16 percent against alpha glucosidase enzyme activity. Further it was also documented that the sample VCT revealed IC50 value of 438.1 ± 6.31µg/ml. Standard acarbose was shown to inhibit the alpha glucosidase enzyme, with a maximum inhibition of around 88.44% and an IC_{50} value of 29.45 ± 2.10 µg/ml.

5. Conclusion

More than 90 percent of all of the morbidity and death that is associated with diabetes mellitus can be attributed to type 2 diabetes mellitus (also known as T2DM), which is the most common form of diabetes mellitus. The majority of the time, this is characterized by over expressive intestinal enzymes like alpha amylase and glucosidase enzyme. Conventional enzyme inhibitors offers potential side effect that results in poor patient compliance. Outcome of the present investigation clearly signifies that the siddha formulation *Vallarai choorana* tablet offers remarkable enzyme inhibition property against alpha amylase

and glucosidase enzymes, thereby it may be considered as alternate drug of choice for managing postprandial hyperglycemia in near future.

Acknowledgement

We wish to acknowledge our 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.

6. References

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