INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES

(p-ISSN: 2348-5213: e-ISSN: 2348-5221)

www.ijcrcps.com

DOI: 10.22192/ijcrcps Coden: IJCROO(USA) Volume 5, Issue 6 - 2018

Research Article

DOI: http://dx.doi.org/10.22192/ijcrcps.2018.05.06.001

In-vitro Anti Diabetic Potential of the Siddha Formulation *Vasantha Kusmakaram* Tablet Against *α*-Amylase and *α*-Glucosidase enzymes

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Abstract

Diabetes mellitus (DM) is a major endocrinal metabolic disorder affecting nearly 10% of population all over the world. Enzymes play a versatile role in maintaining normal glucose homeostasis in a healthy individual, whereas hyperactivity of certain enzymes could contribute to potential disorders. The key enzymes involved in carbohydrate metabolism are pancreatic *α*-amylase and *α*-glucosidase which convert consumed polysaccharides to monosaccharides. This enzyme action causes postprandial blood glucose level elevation due to absorption of formed glucose from polysaccharides in the small intestine. Drugs have tendency to block these core enzymes may act as a potential therapeutic lead in type 2 diabetic patients. Growing health care expenditure becomes a major economic burden on patients with diabetes further there are numerous side effects caused by conventional drugs upon long term usage. The main aim of the present research work is to evaluate the in-vitro anti diabetic potential of the siddha formulation *Vasantha Kusmakaram* tablet (VKT) by *α*-amylase and *α*-glucosidase enzyme inhibition assay. Test were carried out at the concentration varying from 100 – 500 µg/ml. Results of the present study has clearly indicates that the test drug VKT possess significant *α*-amylase enzyme inhibition property ranges from varying from 21.61 to 59.62 % and their corresponding IC50 value was 147 µg /ml. Further the test VKT revealed promising *α*-glucosidase enzyme inhibition potential ranges from 8.26 to 40.62% with IC50 value of 608.2 µg /ml. It is concluded from the data's of the present investigation that the inhibitory activity of VKT against *α*-glycosidase and *α*-amylase would prevent the uptake of glucose which subsequently restrains the increase in blood sugar. By considering the wider action of the drug the VKT this could be considered as a drug of choice for clinical management of diabetes.

Keywords: Diabetes mellitus, *VasanthaKusmakaram* tablet, *α*-amylase, *α*-glucosidase, Enzyme inhibition assay.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by clinical symptoms such as polyuria, polydipsia, polyphagia and unsolved weight loss [1-3]. In DM high blood glucose and other biochemical abnormalities results from a deficiency of β-cells of the endocrine pancreas and from resistant to insulin in target cells [4-6]. A worldwide survey reported that the estimated incidence of diabetes and projection for year 2030 is 350 million [7-8]. Diabetes mellitus can be classified into two main types, type 1 and type 2 [9], with type 1 resulting from the body's failure to produce insulin, and requires one to be injected with insulin [10]. Type 2 diabetes mellitus describes a condition of fasting hyperglycemia that occurs despite the availability of insulin [11-12].

There is growing evidence that people around the world started relying on traditional medicine for their clinical needs. Siddha medicine is one such traditional holistic medicine of south India which provided ailment for several metabolic disorders including diabetics through the versatile multi-functional formulations. Siddha formulations majorly composed of herbs, minerals and otherwise combination of both. Herbs have always been an exemplary source of drugs and herbal drugs which have been investigated all over the world to treat diabetes [13-14]. Till today more than 1 200 species of plants have been screened for activity on the basis of traditional knowledge [15]. The ability of a drug or diet to delay the production or absorption of glucose by inhibiting carbohydrate hydrolyzing enzymes such as α-amylase and α-glucosidase is one of the therapeutic approaches for decreasing postprandial hyperglycemia [16].

α-Glycosidase is an enzyme located in the intestinal brush border, which is responsible for converting oligosaccharides and disaccharides to monosaccharides, which promotes the absorption of carbohydrate and contributes to the increase in blood sugar concentration [17]. The use of *α*- glycosidase inhibitor can delay absorption of carbohydrate through competitive inhibition, thus subsequently inhibit the hydrolysis of disaccharides and the absorption of glucose [18]. Research has shown the anti-diabetes and anti-obesity effects of *α*-glycosidase inhibitor [19]. Moreover, acarbose, a recognized *α*-glycosidase inhibitor, is clinically used as anti-diabetes drug. The use of natural source*α*-glycosidase inhibitor, including plant and microorganism, has attracted attention of scientists [20]. The medical values of these natural sources should be well identified.

α-amylases are enzymes that catalyses the hydrolysis of internal α-1,4-glycosidic linkages in starch in low molecular weight products, such glucose, maltose and maltotriose units [21]. Such enzymes hydrolyze the starch molecules into polymers composed of glucose units. Starch is an important constituent of the human diet and is a major source of glucose .Increased intake of starch promotes high level of amylase action which results in abnormal hike in blood glucose level. Drug has tendency to halt the activity of α-Amylases could be better therapeutic candidate for clinical management of DM. The main aim of the present investigation is to evaluate the anti-diabetic potential of the traditional siddha formulation *VasanthaKusmakaram* tablet against *α*-amylase and *α*-glucosidase enzymes by in-vitro enzyme inhibition assay.

2. Materials and Methods

2.1. Ingredients

The formulation *VasanthaKusmakaram* tablet comprises of the following ingredients

1.	Lingam	- *Mercuric Sulphide*
2.	Vengaram	- *Borax*
3.	Lavangam	- *Myrtus caryophyllus*
4.	Thippili	- *Piper longum*
5.	Kostam	- *Saussurea lappa*
6.	Akkirakaram	- *Anacyclus pyrethram*
7.	Adhimathuram	- *Glychyrrhiza glabra*
8.	Korosanai	- *Purified Ox bile*
9.	Kunguma Poo	- *Crocus sativus*
10.	PachaiKarpooram	- *Camphora officinarum*
11.	Ginger	- *Zingiber officinale*
12.	Cow's milk	- Quantity sufficient

2.2. Preparation of *Vasantha Kusmakaram* Tablet [22]

Each ingredient was purified well as per literature. Followed by this each ingreadients were powdered inkalvam with Ginger juice for 2 days, and then with cow's milk for 2 days. Finally each tablet of 100mg were made and allowed for shade dry.

2.3. *In-vitro* Alpha Amylase Inhibition Study [23]

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 (VKT) was prepared in the serial dilution of the concentration ranges from 100,200,300,400 and 500 µg/ml.About 600 µl of test sample (VKT) were added to 30 µl of α-amylase enzyme solution and incubated at 37°C for 15 min. To this reaction mixture, 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 was calculated by the following formula.

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

2.4. *In-vitro* Alpha Glucosidase enzyme Inhibition Study [24]

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 (VKT) 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_2CO_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.

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

3. Results

3.1. Effect of VKT on Alpha Amylase enzyme Inhibition activity

It was observed from the results of the present investigation that the formulation VKT has shown significant inhibition of alpha amylase enzyme with the percentage inhibition ranges from 21.61 ± 5.917 to 59.62 ± 11.56 % at the concentration of 100 µg/ml to 500µg/ml. The corresponding IC50 was found to be 147 ± 16.34µg /ml, which reveals the anti-diabetic potential of the formulation. The results are summarized in Table 1 and 2.

Table 1: Percentage inhibition of test drug VKT on Alpha Amylase Inhibition Study

Concentration (µg/ml)	% Inhibition of VKT
100 µg/ml	21.61 ± 5.917
200 µg/ml	32.84 ± 7.632
300 µg/ml	42.81 ± 8.242
400 µg/ml	52.13 ± 6.685
500 µg/ml	59.62 ± 11.56

Data are given as Mean ± SD (n=3)

Table 2: IC50 Values for Alpha Amylase Inhibition Assay by VKT

Test Drug / Standard	IC50 Value of Alpha Amylase enzyme inhibition ± SD (µg /ml)
VKT	147 ± 16.34

Data are given as Mean ± SD (n=3)

3.2. Effect of VKT on alpha Glucosidase enzyme Inhibition activity

From the results of alpha glucosidase enzyme inhibition assay it was observed that the formulation VKT reveals highest percentage inhibition ranges from 8.26 ± 1.72 to 40.62 ± 3.032 % at the concentration of 100 µg/ml to 500µg/ml. The corresponding IC50 was found to be 608.2 ± 35.67µg /ml. The results are summarized in Table 3 and 4.

Table 3: Percentage inhibition of test drug VKT on α-Glucosidase Enzyme Inhibition Study

Concentration (µg/ml)	% Inhibition of VKT
100 µg/ml	8.26 ± 1.725
200 µg/ml	20.54 ± 3.052
300 µg/ml	27.29 ± 2.079
400 µg/ml	33.6 ± 1.873
500 µg/ml	40.62 ± 3.032

Data are given as Mean ± SD (n=3)

Table 4: IC50 Values for α-Glucosidase Enzyme inhibition by VKT

Test Drug / Standard	IC50 Value of α-Glucosidase enzyme inhibition ± SD (µg /ml)
VKT	608.2 ± 35.67

Data are given as Mean ± SD (n=3)

4. Discussion

It is estimated that the global prevalence of diabetes is increasing each year causing a major burden to the health sector, especially in the developing countries. It is estimated that the prevalence of diabetes is high among the urban population [25]. Only 5% of the diabetes in the world is Type 1 (insulin dependent), and the remaining 95% falls in Type 2 (noninsulin dependent). The prevalence of diabetes is increasing globally, particularly in developing countries. Conventionally, DM was treated with herbal remedies (plants), diet, and physical exercise. Thousands of plants were used to control DM, though one-third only investigated for phytochemicals and its pharmacological activities [26].The World Health Organization reported that worldwide global population is in the midst of a diabetes epidemic. The people in Southeast Asia and Western Pacific are being under greater risk, and the majority of patients have type 2 diabetes. Insulin resistance typically precedes the onset of type 2 diabetes and is commonly accompanied by other cardiovascular risk factors such as dyslipidemia, hypertension, and prothrombotic factors [27].

α-amylase and *α*-glucosidase enzymes action causes elevation of postprandial blood glucose level due to absorption of formed glucose from polysaccharides in the small intestine. Currently, available drugs in this category are acarbose and miglitol, which competitively inhibit above enzymes. But these drugs have common side effects such as flatulence and abdominal bloating. Drugs having an inhibitory action on both of these enzymes possess an ability to control of postprandial blood glucose level specifically in type 2 diabetic patients. New drugs or formulations which are devoid of the above side effects will improve the compliance in type 2 diabetic patients. From the results of alpha glucosidase enzyme inhibition assay it was observed that the formulation VKT reveals highest percentage inhibition ranges from 8.26 ± 1.72 to 40.62 ± 3.032 % at the concentration of 100 µg/ml to 500µg/ml. The corresponding IC50 was found to be 608.2 ± 35.67 µg /ml.

Human pancreatic α-amylase (HPA) inhibitors offer an effective strategy to lower postprandial hyperglycemia via control of starch breakdown [28]. It was observed from the results of the present investigation that the formulation VKT has shown significant inhibition of alpha amylase enzyme with the percentage inhibition ranges from 21.61 ± 5.917 to 59.62 ± 11.56 % at the concentration of 100 µg/ml to 500µg/ml. The corresponding IC50 was found to be 147 ± 16.34µg /ml, which reveals the anti-diabetic potential of the formulation.

5. Conclusion

Currently available treatments for clinical management of DM have several side effects such as hypoglycemia, weight gain and other complications which necessitate the need for development of new antidiabetic targets and therapies for glycemic control. Siddha system of traditional medicine offers versatile medicines for treating dreadful metabolic disorders like diabetes. Once such novel formulation is *VasanthaKusmakaram* tablet which comprises of biological active phytocomponents which acts my multiple mechanisms.

Our present study results clearly demonstrated that the siddha formulation *Vasantha Kusmakaram* tablet possesses potent *α*-amylase and *α*-glucosidase inhibition activity in in-vitro which further has to be confirmed through proper *in vivo* models.

Acknowledgments

I wish to acknowledge my thanks to The Noble research solutions, Chennai for their technical and analytical support for this research work.

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Subject: Siddha Medicine

Quick Response Code

DOI: 10.22192/ijcrcps.2018.05.06.001

How to cite this article:
M. N. Parandhaman, S.Balamani. (2018). *In-vitro* Anti Diabetic Potential of the Siddha Formulation *Vasantha Kusmakaram* Tablet Against *α*-Amylase and *α*-Glucosidase enzymes. Int. J. Curr. Res. Chem. Pharm. Sci. 5(6): 1-6.
DOI: http://dx.doi.org/10.22192/ijcrcps.2018.05.06.001

