Original Research Paper

Volume - 13 | Issue - 09 | September - 2023 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Ayurveda

IN VITRO STUDIES ON ALPHA AMYLASE AND ALPHA GLUCOSIDASE INHIBITORY ACTIVITIES OF ASANADI KASHAYAM

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ABSTRACT **Background**: α amylase and α glucosidase are the foremost enzymes in the digestion of carbohydrate. Elevation of post prandial glucose level in the blood depends on it .Inhibition of these two enzymes can control postprandial hyperglycemia in type 2 diabetic and borderline patients. *Asanadi Kashaya* is an indigenous formulation being used since many years. However , there is finite information available on the alpha amylase and alpha glucosidase inhibitory activity of this *kashaya*. In the current study *Asanadi Kashaya* were tested for alpha amylase and alpha glucosidase inhibiting activities in vitro. **Result**: The *Asanadi kashayam* (at a concentration 100 µl/mL) showed 69.86% of alpha amylase inhibitory activity with IC50 value 42 µl/mL and alpha glucosidase inhibitory activity 64.58% with IC50 43 µl/mL.In this study *Asanadi kashaym* showed considerable alpha amylase and alpha glucosidase inhibitory effects when compared with acarbose. **Conclusion**: *Asanadi Kashaya* has the anti-diabetic property by inhibiting alpha amylase and alpha glucosidase enzymes.

KEYWORDS : Type 2 diabetes mellitus, α amylase, α Glucosidase, *Asanadi kashyam,*

INTRODUCTION:

Diabetes mellitus (DM) comprises of a group of metabolic disorders that share the phenotypic expression of hyperglycemia. Several causative factors are known to induce hyperglycemia and diabetes, yet in the vast majority of patients the etiology of the disorder is nebulous, highly complex, confusing by and large unclear. The diabetes pandemic is rapidly spreading, and mostly affects developing countries like India. Recent figures have shown that 62.4 million people currently have diabetes in India,[1] and this number is projected to rise to 101.2 million by 2030[2]. These cases fall into two major classes previously known as Juvenile onset and Maturity onset types of diabetes. In yester years, these were termed insulin-dependent diabetes mellitus(IDDM) and non-insulin dependent diabetes mellitus (NIDDM). More recently based on etiopathogenesis, the terms "type 1" and "type 2" are applied to classes of diabetes with clinical pictures similar to the above[3]. In glucose homeostasis, biochemical metabolic pathways like glycolysis, glycogenesis, gluconeogenesis and glycogenolysis plays important role in the regulation of blood glucose levels with the glucokinase (GK) enzyme. Any component that flusters the aforementioned biochemical pathways is deleterious[4]. Imbalance between blood sugar absorption and insulin secretion will results into diabetes mellitus. Postprandial hyperglycemia take part an influential role in the development of T2D[5]. One of the therapeutic approaches for decreasing postprandial hyperglycemia involves the ability of a drug or diet to inhibit carbohydrate hydrolyzing enzymes such as α-amylase and α-glucosidase, thereby delaying the production or absorption of glucose. Carbohydrate digesting enzyme inhibitors utilization plays a crucial role in managing hyperglycemia by decreasing the intestinal absorption of glucose. Acarbose stands out as a prominent inhibitor of carbohydrate metabolic enzymes in the gastrointestinal tract. However, it is linked to side effects like diarrhea and various intestinal disturbances, including bloating, flatulence, cramping, and abdominal pain[6]. In *Ayurveda* many number of therapeutic formulations have been used clinically to treat type 2 DM. They also have additional beneficial effect on various other disorders. *Asanadi gana dravya* are used for treatment of *switra, kushta, kaphaja vikaras, krimi, pandu roga, prameha* and *medodosha*[7]. They also described this group of drug in different *pramehahara yogas* in *prameha chikitsa adhyaya*. It's already proven that each ingredient of *Asanadi kashaya* is having anti-diabetic properties. So a scientific validation to prove the efficacy of this combination should be done. Hence, the main objective of present study was to investigate in-vitro, α-amylase, α-glucosidase inhibitory activity of *Asanadi kashayam.*

MATERIALS AND METHODS

Chemicals :

Phosphate – buffered saline (PBS), porcine α amylase, α Glucosidase, dinitro salicylic acid.

Preparation of kashayam:

The drug *Asanadi kashaya* consists of 23 drugs. The drug was procured from a reliable source. *Kashaya* was made as per the standard method mentioned in the Sargdhara samhitha.

In vitro assay

α amylase inhibition assay:

Different concentrations of the sample, such as 6.25µl/mL,12.5 µl/mL, 25 µl/mL ,50 µl/mL,100µl/mL, were prepared from a stock concentration of 10mg/mL and were made up to 100µl using 25mM phosphate buffer pH 6.9. The buffer contained 25µl of porcine α amylase at a concentration of 0.5 mg/ml. The mixtures were incubated at 25°C for 10 minutes. After pre-incubation, 25µl of a 0.5% starch solution in 25mM phosphate buffer pH 6.9 was added. The reaction mixtures were then incubated at 25°C for 10 minutes. The reaction was halted with 50µl of 96mM 3,5-dinitrosalicylic acid color reagent. The microplate was incubated in a boiling water bath for 5 minutes and subsequently cooled to room temperature. Absorbance was measured at 540nm using a microplate reader (Erba, Lisascan).[8]

Calculation

$$\% \text{ inhibition} = \frac{\text{control} - \text{test}}{\text{control}} \times 100$$

α Glucosidase inhibition assay :

Different concentrations of the sample, such as 6.25µl/mL,12.5 µl/mL, 25 µl/mL ,50 µl/mL,100µl/mL,, were prepared from a stock concentration of 10mg/mL and made up to 100µl using 0.1M phosphate buffer pH 7.2. The buffer contained 25µl of αGlucosidase, and the mixture was incubated at 25°C for 10 minutes. After pre-incubation, 1ml of 0.1 M phosphate buffer (pH 7.2) containing 37mM sucrose was added. Then, the reaction mixture was incubated for 30 minutes at 37°C, and the reaction was stopped by incubating in a boiling water bath for 2 minutes. A tube with phosphate buffer and enzyme was maintained as a control. The tubes were added with 250µL of glucose reagent and incubated for 10 minutes, followed by measuring absorbance at 510nm using a microplate reader (Erba, Lisascan). Acarbose was included as a standard. Each experiment was performed in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula,[9]

Calculation

$$\% \text{ inhibition} = \frac{\text{control} - \text{test}}{\text{control}} \times 100$$

Statistical Analysis

All experiments were done in triplicates and results represented as Mean+/- SE. One-way ANOVA and Dunnett's test were performed to analyse data. IC 50 was calculated using ED50 PLUS V 1.0 Software .

RESULT

In the present study, *Asanadi kashayam* was evaluated for its inhibitory effect on α-amylase and α-glucosidase enzymes by in-vitro method.

Alpha amylase inhibition

Sample set 1 of concentration of 6.25,12.5,25,50,100 exhibited 16.44,33,42.01,52.43,70.05 percentage of inhibition respectively with IC50 value of 42.711 µl/mL. Sample set 2 of concentration of 6.25,12.5,25,50,100 exhibited 16.77,33.66,42.48,52.57,69.24 percentage of inhibition respectively with IC50 value of 42.41 µl/mL and sample set 3 of concentration of 6.25,12.5,25,50,100 exhibited 16.23,32.49.42.12,52.79,70.26 percentage of inhibition respectively with IC50 value of 42.41 µl/mL. Average percentage of inhibition is 16.48,33.05,42.20,52.60,69.86 as mentioned in the table (2).Acarbose was used as a standard reference drug, which showed α-amylase inhibitory activity with an IC50 value of 54.99 µg/mL(table no.1).Among all, 100 µl/mL concentration of *Asanadi kashayam* has shown best enzyme inhibitory activity with an average of 69.86 percentage of inhibition.

Standard

Table (1) percentage of inhibition of standard

Standard concentration(µg/mL)	Percentage inhibition
62.5	53.17
125	79.56
250	83.54
500	90.65
1000	95.29

Sample

Table (2) Average percentage of inhibition of sample

Sample concentration (µl/mL)	Percentage inhibition (Average)
6.25	16.4851
12.5	33.055
25	42.2053
50	52.6023
100	69.862

Standard

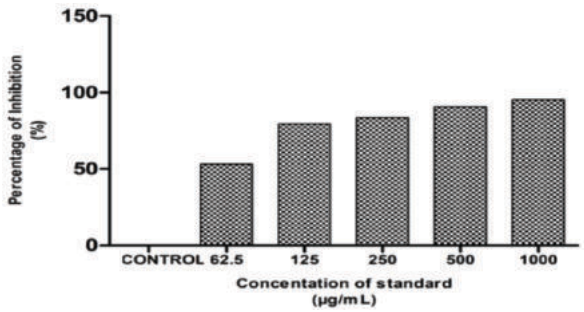

Figure:3 Graphical representation depicting the alpha amylase activity of Standard- Along Y axis Percentage inhibition, Along X axis varied concentration of Standard.

Sample

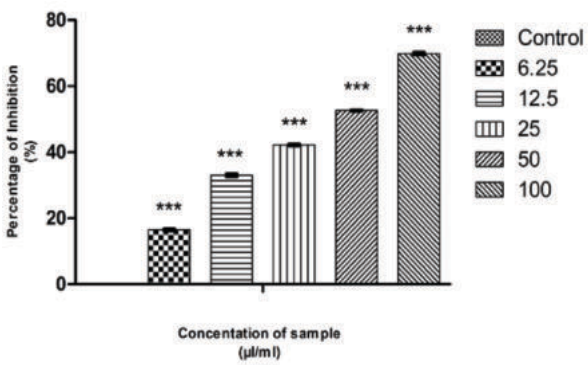

Figure 4: Graphical representation depicting the alpha amylase activity of Sample- Along Y axis Percentage inhibition, Along X axis varied concentration of Sample. ***p< 0.001 compared to control group.

Alpha glucosidase inhibition

Sample set 1 of concentration of 6.25,12.5,25,50,100 exhibited 16.40 , 27.86,40.65,53.32,65.70 percentage of inhibition respectively with IC50 value of 42.853 µl/mL. Sample set 2 of concentration of 6.25,12.5,25,50,100 exhibited 14.83,29.02,39.21,52.72,64.12 percentage of inhibition respectively with IC50 value of 43.711µl/mL and sample set 3 of concentration of 6.25,12.5,25,50,100 exhibited 14.36,29.79,40.45,52.30,63.93 percentage of inhibition respectively with IC50 value of 43.574 µl/mL. Average percentage of inhibition15.19,28.89,40.10,52.78,64.58 as mentioned in the table (2). Acarbose was used as a standard reference drug, which showed α-amylase inhibitory activity with an IC50 value of 88.53 µg/mL.(table no 1). Among all, 100 µl/mL concentration of *Asanadi kashayam* has shown best enzyme inhibitory activity with an average of 64.58 percentage of inhibition.

Standard

Table(4) percentage of inhibition of standard

Standard concentration(µg/mL)	Percentage inhibition
62.5	39.24
125	65.07
250	79.39
500	87.18
1000	94.04

Sample

Table(5) Average percentage of inhibition of sample

Sample concentration (µl/mL)	Percentage inhibition (Average)
6.25	15.1984
12.5	28.8963
25	40.1086
50	52.7847
100	64.5862

Standard

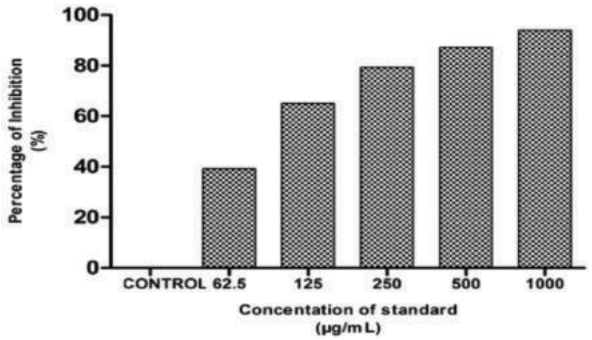

Figure 3: Graphical representation depicting the alpha glucosidase activity of standard- Along Y axis Percentage inhibition, Along X axis varied concentration of standard.

Sample

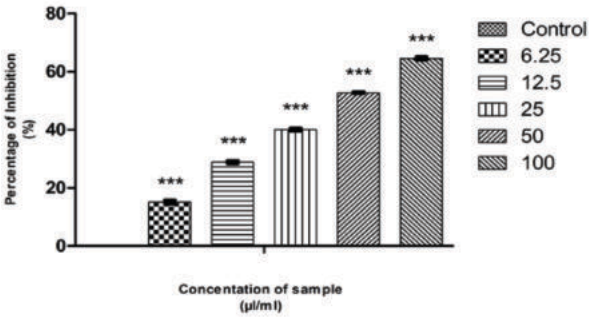

Figure 4: Graphical representation depicting the alpha glucosidase activity of Sample- Along Y axis Percentage inhibition, Along X axis varied concentration of Sample.***p< 0.001 compared to control group.

DISCUSSION

Alpha amylase and alpha glucosidase are the main two types of carbohydrate hydrolysing enzyme which responsible for post prandial hyperglycemia. Alpha amylase in the pancreatic juice hydrolyse the alpha- 1,4 glycosidic linkages(polysaccharides) randomly , so as to

produce smaller subunits like maltose, isomaltose ,dextrins and branched or unbranched oligosaccharides (disaccharides). And conversion of disaccharides to monosaccharides is done by alpha glucosidase which expedite postprandial hyperglycemia. Hence, inhibitions of alpha amylase and alpha glucosidase are effective in the control of hypoglycemia because they delay carbohydrate digestion which consequently lessen the post prandial plasma glucose level. There was no details available in the literature about the invitro anti diabetic studies of *Asanadi kashyam*. Hence the present study focused to evaluate alpha amylase and alpha glucosidase inhibitory action of *Asanadi Kashaya.* Based on the results, *Asanadi kashayam* showed significant activity with alpha amylase and alpha glucosidase inhibition. The *Asanadi kashayam* (at a concentration 100 µl/mL) showed 69.86% of alpha amylase inhibitory activity with IC50 value 42 µl/mL and alpha glucosidase inhibitory activity 64.58% with IC50 43 µl/mL.In this study *Asanadi kashaym* showed considerable alpha amylase and alpha glucosidase inhibitory effects when compared with acarbose.

Limitations of the study:
In the realm of studying biological processes, cell lines are frequently employed as substitutes for primary cells. Nevertheless, caution is essential while interpreting the outcomes, as cell lines might not consistently replicate primary cells with precision. The study of these entities occurs in the absence of their natural surroundings, which commonly involve interactions with different cell types. So the results may vary. There is high chances of contamination of cell lines.

CONCLUSION
The result of study designate that Asanadi Kashaya showed significant alpha amylase and alpha inhibitory effects. This study supports the ayurvedic concept that Asanadi kashyam presumptively useful in management of diabetes.

Acknowledgments
This study was supported by Biogenix Research center , center for molecular biology and applied science,Thirumala, Thiruvananthapuram.

Financial Support And Sponsorship
Nil

Conflicts Of Interest
There are no conflicts of interest.

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