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ALPHA-AMYLASE INHIBITORY ACTIVITY OF KATANKATERYADI KWATHA (K. KWATHA) IN MANAGEMENT OF TYPE 2 DIABETES

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Keywords:

Katankateryadi Kwatha, Hyperglycaemia, Alpha amylase, Acarbose, Type 2 Diabetes

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ABSTRACT: Objectives: The objective of the study is to find out the alpha-amylase inhibitory activity of Katankateryadi Kwatha. The alpha-amylase enzyme is the key source for postprandial hyperglycaemia, which is responsible for Type 2 Diabetes. **Methodology:** The crude drugs of Katankateryadi Kwatha (K. Kwatha) were collected from a local market. The Kwatha was prepared and dried with rotatory evaporator, under reduced pressure. Different concentrations (10, 20, 40, 60, 80 and 100 µg/ml.) of Kwatha were prepared and used for α-amylase inhibitory assay using starch as a substrate. Similarly, the protocol performed with Acarbose as standard. The absorbance was read at 595 nm using spectrophotometer. The percentage of α-amylase inhibitory activity and IC_{50} values of each concentration was calculated. **Results & Discussion:** The study shows that the Katankateryadi Kwatha has alpha-amylase enzyme inhibitory property. Standard Acarbose shows IC_{50} value of 38.46±0.956 with % inhibition ranged from 23% to 80% at concentration 10, 20, 40, 60, 80 and 100 µg/ml. while the inhibitory concentration (IC_{50} value) of K. Kwatha was found to be 68.69±1.0890 with % inhibition on alpha-amylase ranged from 9% to 64% at concentration 10, 20, 40, 60, 80, and 100 µg/ml. **Conclusion:** The evidence show that K. Kwatha (a natural polyherbal product) has alpha-amylase inhibitory activity. The results of K. Kwatha are satisfactory and highly significant in comparison to standard Acarbose. Therefore K. Kwatha a polyherbal decoction, might be a future potent Ayurvedic formulation for diabetic patients.

INTRODUCTION: Diabetes is a metabolic disorder characterized by polyurea, polyphagia, and polydipsia, which affects the liver, kidney, eyes, and other organs severely. The major cause of type 2 diabetes is the improper secretion of insulin from beta cells of pancreas, insulin resistance, and beta-cell dysfunctions [1]. Diabetes mellitus is still one of the most important causes of death and disability in both developed and developing countries.

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According to the report by WHO (2015) 9% of adults in the world suffer from diabetes and this disease will be the 7th leading cause of death in 2030 [2]. The postprandial hyperglycaemia (PPHG) is the major independent risk factor for diabetes mellitus, which results in macrovascular complications [3].

PPHG is a preventive target to control type 2 diabetes. Several synthetic medicines are now available for controlling type 2 diabetes in which alpha-amylase inhibitors such as Acarbose, Voglibose, and Maglitol, *etc.*, are available in the market, but due to their several adverse effects, the people shift towards the Ayurvedic medicines to control type 2 diabetes [4].

The alpha-amylase is an enzyme secreted from the pancreas which is responsible for the metabolism of dietary starch into simple sugars like maltose, maltotriose, and oligo-glucons, which finally converted into glucose and raised the blood glucose level that is hyperglycaemia after meal [5].

Alpha-amylase enzyme catalyzes the breakdown of alpha -1, 4 linkages in dietary starch. In humans, alpha-amylase is a single polypeptide chain of 496 amino acids, which encoded as part of multigene family on chromosome [6]. Therefore alpha-amylase inhibitors are the choice of drug to control hyperglycaemia. The alpha-amylase inhibitors from Ayurvedic plants show good potency and minimum side effects in comparison to synthetic drugs [7].

Ayurveda suggests different lifestyle and food; this is considered the easiest and most cost-effective way of promoting health and prevention of diseases. The synthetic drug treatments in most of the major diseases are lifelong prescription, and in many cases, it is unsustainable. This is why a traditional system of medicine such as Ayurveda is fast emerging in the mainstream of health care today.

The advantages of Ayurvedic drugs are effectiveness, safety, and wide acceptability [8]. The Katankateryadi Kwatha (Decoction) is a polyherbal preparation that is recommended in ancient literature for the management of Diabetes Mellitus Type 2. The formulation of Katankateryadi Kwatha contains *Barberis aristata* DC (Stem), *Glycyrrhiza glabra* Linn (Stem), *Terminalia chebula* (Fruit), *Terminalia bellirica* (Fruit), *Emblica officinalis* (Fruit), and *Plumbago zeylanica* Linn. (Root) [9].

MATERIALS AND METHODS:

Plant Material and Chemicals: All crude drugs of *Barberis aristata* DC (Stem), *Glycyrrhiza glabra* Linn. (Stem), *Terminalia chebula* (Fruit), *Terminalia bellirica* (Fruit), *Emblica officinalis* (Fruit), and *Plumbago zeylanica* Linn (Root) was purchased from Gola Dinanath a local market in Varanasi during the summer season in dry form and authenticated from Department of Dravyaguna, Institute of Medical Sciences, Banaras Hindu University (Authentication no: DG/18-19/206, 207, 208, 209, 210). Alpha-amylase and Acarbose were purchased from Hi-Media.

All chemicals and reagents are from analytical grade used in experimentation, and the experiment was performed in 2020 in Pharmacology Lab of Department of Dravyaguna IMS BHU Varanasi India.

Preparation of K. Kwatha (Decoction): Katankateryadi Kwatha was prepared using six drugs *i.e. Barberis aristata* DC (Stem), *Glycyrrhiza glabra* Linn (Stem), *Terminalia chebula* (Fruit), *Terminalia bellirica* (Fruit), *Emblica officinalis* (Fruit), and *Plumbago zeylanica* Linn (Root).

All the ingredients of K. Kwatha were subjected for size reduction using the pulvarizer. Equal amount of all crude drugs was soaked in 4 times the water in vessel and kept overnight for 12 h. After 12 h contents were boiled at 90 ºC - 95 ºC with stirring.

Water was evaporated till 1/4th amount was remains and galenicals was filtered through cotton cloth. Filtrate was dried with a rotatory evaporator, and dried powder was used for their Physico-chemical analysis and alpha amylase inhibitory assay [10].

Physico-chemical Evaluation of K. Kwatha:

Organoleptic Properties: Color, odor, taste, and texture of K. Kwatha were analyzed.

Phytochemical Screening: The Phytochemical screening of K. Kwatha was carried out to find out the active chemical constituents that are responsible for alpha-amylase inhibitory activity. The procedure was as follows [11].

A. Test for Alkaloids: K. Kwatha were dissolved in dil. hydrochloric acid and filtered. The filtrate was used for the Alkaloids test.

i. Wagner's Test: Filtrate was treated with few drops of Wagner's reagent. A yellow or brown precipitate indicates the presence of alkaloids.

ii. Mayer's Test: Filtrate was treated with few drops of Mayer's reagent. A white or pale yellow precipitate was formed indicates the presence of alkaloids.

B. Test for Carbohydrates: K. Kwatha were dissolved in 5 ml distilled water and filtered. The filtrate was used to test the presence of carbohydrates.

i. Benedict's Test: Filtrate was treated with 5 ml of Benedict's solution and boiling for 5 min. The formation of a brick-red colored precipitate is due to the presence of carbohydrates.

ii. Fehling's Test: Filtrate was hydrolyzed with dil. Hcl, neutralized with alkali and heated with 1 ml of a mixture of equal parts of Fehling's solution 'A' and Fehling's solution 'B' and boiling the contents of the test tube for few minutes. A red or brick red precipitate is formed indicates the presence of carbohydrates.

iii. Molisch's Test: Filtrate was treated with 2 drops of alcoholic alpha naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.

C. Test for Flavonoids: K. Kwatha was treated with few drops of lead acetate solution. The formation of a yellow color precipitate indicates the presence of flavonoids.

D. Test for Proteins:

i. Biuret's Test: To 1 ml of K. Kwatha add 5-8 drops of 10% w/v sodium hydroxide solution followed by 1 or 2 drops of 3% w/v copper sulphate solution. A red or violet color indicates the presence of protein.

ii. Millon's Test: Dissolved a small quantity of K. Kwatha in 1 ml of distilled water and adding 5-6 drops of Millon's reagent. A white precipitate is formed, which turns red on heating indicates the presence of protein.

E. Test for Saponins: In a test tube containing 5 ml of K. Kwatha, add a drop of sodium bicarbonate solution, shake the mixture vigorously and leave for 3 min. Honeycomb-like froth is formed, which indicates the presence of saponins.

F. Test for Steroids:

i. Liebermann-Burchard's Test: K. Kwatha was treated with chloroform and filtered. The filtrate was treated with few drops of acetic anhydride, boiled and cooled. The formation of brown ring at the junction indicates the presence of phytosterol.

ii. Salkowski Reaction: K. Kwatha was treated with chloroform and filtered. The filtrate treated with few drops of Conc. Sulphuric acid carefully, from the side of the test tube, shaken and allowed to stand. Red color is produced in the chloroform layer, which indicates the presence of phytosterol.

G. Test for Tannins: Took 1-2 ml of K. Kwatha in a test tube, few drops of 5% $FeCl_3$ solution was added. A green color indicates the presence of tannins.

H. Test for Glycosides: K. Kwatha was hydrolyzed with dil. Hcl and then treated with sodium nitropruside in pyridine and sodium hydroxide. The formation of pink to blood-red color indicates the presence of cardiac glycosides.

Alpha-Amylase Inhibitory Assay: The α-amylase inhibitory assay was performed by starch iodine method [12]. 1 ml of Katankateryadi Kwatha and standard (Acarbose) of different concentrations (10, 20, 40, 60, 80, and 100 µg/ml) was used in pre-labeled test tubes. A volume of 20 µl of α-amylase was added in each test tube and incubated for 10 min at 37 ºC. After the incubation 200 µl of 1%, starch solution was added to each test tube, and the mixture was re-incubated for 1 h at 37 ºC. Then 200 µl of 1% iodine solution was added to each test tube, and after that, 10 ml distilled water was added. The absorbance of the mixture was taken at 565 nm. Sample blank, substrate blank, and α-amylase blank were taken under the same conditions. Each experiment was done in triplicate. IC_{50} was calculated by using regression analysis.

$$\% \text{ α-amylase inhibition} = [1- ((SA-SBB)-SMB) / AAB] \times 100$$

SA= Sample absorbance, SMB = Sample blank, SBB = Substrate blank, AAB= α-amylase blank

Statistical Analysis: All data are represented in Mean ± SEM and analysed by regression analysis on Graph pad Prism 7 software.

RESULTS:

Physico-chemical Analysis of K. Kwatha:

Organoleptic properties of K. Kwatha: The physical properties of liquid K. Kwatha having yellowish- brown in colour, astringent taste, pungent in odour and pH 7.2. After dry, the powder of Kwatha shows the same physical properties.

Phytochemical Analysis of K. Kwatha: Phytochemical analysis was performed under

standard protocol, and the result shows the presence of Phytochemicals **Table 1**.

TABLE 1: PHYTOCHEMICAL ANALYSIS OF K. KWATHA

S. no.	Chemical Test	Observation
1	Test for alkaloids	
	Wagner's test	+
	Mayer's test	+
2	Test for Carbohydrates	
	Benedict's test	+
	Fehling's test	+
	Molisch's test	+
3	Test for Flavonoids	
	Shinoda's test	+
4	Test for Proteins	
	Biuret's test:	+
	Millon's test:	+
5	Test for Saponins Glycosides	+
6	Test for steroids	+
7	Test for Tannins & phenols	+
8	Test for Glycosides	+

Alpha-Amylase inhibitory assay of K. Kwatha: The study shows the inhibitory concentration (IC_{50} value) of K. Kwatha was 68.69 ± 1.0890 with % inhibition ranged from 9% to 64 % at concentration 10 to 100 µg/ml. Similarly, Acarbose was used as standard drug shows IC_{50} value of 38.46 ± 0.956 with % inhibition ranged from 23% to 80% at 10 to 100 µg **Table 2**. The graphical comparison of % inhibition and concentration between standard and K. Kwatha was plotted after regression analysis, and IC_{50} value of K. Kwatha and Acarbose was plotted, which shows significant inhibitory action of K. Kwatha **Fig. 1** and **2**.

TABLE 2: TABLE SHOWS % ALPHA-AMYLASE INHIBITION & IC_{50} VALUE OF K. KWATHA AND STANDARD ACARBOSE

Drug	Concentrations µg/ml	% alpha-amylase inhibition	IC_{50} value
Katankatareyadi Kwatha	10	9	68.69 ± 1.0890
	20	17	
	40	29	
	60	44	
	80	58	
	100	64	
Acarbose	10	23	38.46 ± 7.1625
	20	46	
	40	52	
	60	66	
	80	69	
	100	80	

*Values are expressed as mean ± SD, n=3

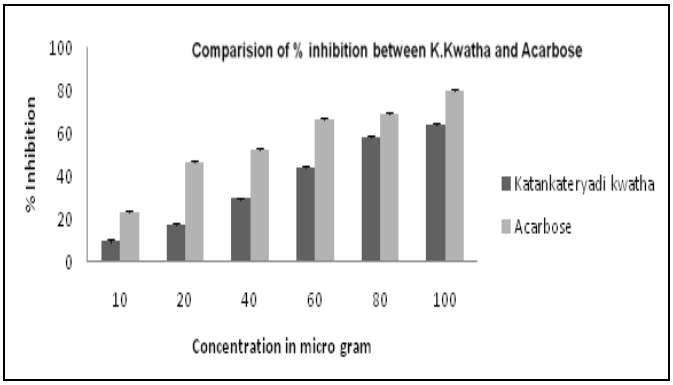

FIG. 1: SHOWS COMPARISON OF % INHIBITION BETWEEN K. KWATHA AND ACARBOSE

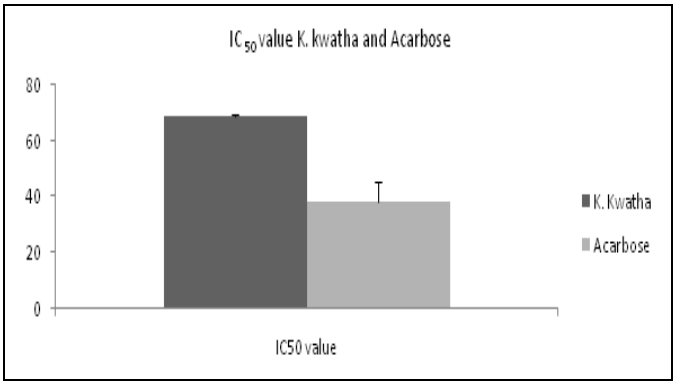

FIG. 2: COMPARISON OF IC_{50} VALUES BETWEEN K. KWATHA AND ACARBOSE. Y-AXIS SHOWS CONCENTRATION IN µg/ml

DISCUSSION: Diabetes mellitus type 2 now a day is one of the major causes of death. Postprandial hyperglycaemia is one of the major factors of diabetes mellitus Type 2. Metabolism of dietary starch by pancreatic alpha-amylase is the major source of high blood glucose levels, and the inhibition of this enzyme is a very effective target in the treatment of diabetes type 2. The drugs that inhibit carbohydrate metabolism decrease postprandial hyperglycaemia without affecting insulin secretion. Now a day world focuses on Ayurvedic drugs for the prevention and control of diabetes mellitus type 2 [13]. Although the effect of Ayurvedic drugs on α amylase inhibition also has benefits on obesity and insulin resistance patients [14]. The K. Kwatha contents *Barberis aristata* DC [15], *Glycyrrhiza glabra* Linn [16], *Terminalia chebula* [17], *Terminalia bellirica* [18], *Emblica officinalis* [19], and *Plumbago zeylanica* Linn [20], have antidiabetic properties individually in different solvents. In combination with the listed six drugs shows the additive and synergistic effect of Kwatha in animal experimentation. In the present study, we found that K. Kwatha shows good alpha-amylase

inhibitory activity and needs future research to find molecular activity.

CONCLUSION: Above study shows that K. Kwatha has Alpha-amylase inhibitory activity. The inhibitory concentration (IC_{50} value) was found to be 68.69 ± 1.0890 with % inhibition ranged from 9% to 64% at concentrations 10 to 100 µg/ml. The value was significant with respect to standard Acarbose. The Kwatha contains a high amount of flavonoids and tannins suggested for the alpha-amylase enzyme inhibitory activity. Therefore the K. Kwatha is a beneficial approach to control postprandial hyperglycaemia in type 2 diabetes mellitus.

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CONFLICTS OF INTEREST: The authors declare no conflicts of interest.

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