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In-Vitro Antidiabetic Activity of Ethanolic Extract of *Barleria cuspidata F.Heyne Ex Nees* by Using α-Amylase and α-Glucosidase Inhibition Assay

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ABSTRACT

Background: *Barleria cuspidata, Heyne ex nees* belongs to Family: Acanthaceae Common name - Pido-Kanta-shediyo Telugu name- Radhamonoharam Tamil Name- Mancat – cemmulli *Barleria cuspidata, Heyne ex nees* has been reported to have anti-diabetic activity **Aims and Objective**: To evaluate *In vitro* anti-diabetic activity of aqueous and ethanolic leaves extracts of *BarleriaCuspidata, Heyne ex nees* α-Amylase Inhibitory Assay and α- Glucosidase Inhibition Assay. **Materials and Methods:** 6.25µg,12.5µg,25µg, 50µg, 100µg concentration of acarbose aqueous and ethanolic leaves extracts of *BarleriaC uspidata, Heyne ex nees* were used for the study. The absorbance values were taken in a spectrophotometer at 540nm and 400nm for α-Amylase and α-Glucosidase enzyme respectively. **Results and Discussion**: The extracts exhibited significant inhibition of α-Amylase, and α- Glucosidase enzyme in a dose-dependent manner. The ethanolic extract exhibited more inhibitory activity when compared with aqueous extract. **Conclusion:** Results show considerable α-Amylase inhibitory activity as well as α- Glucosidase inhibitory activity The present findings indicate that aqueous and ethanolic leaves e xtract *Barleria cuspidata, Heyne ex nees* have *in vitro* antidiabetic activity.

INTRODUCTION

Diabetes Mellitus is outstandingly a disease of sugar digestion however the metabolic yet the metabolic inconveniences in obvious took care of diabetes are not, at this point typically inconvenient and are discernibly convenient to control. It is the long time-frame issues of diabetes that are the primary intentions of grimness and Mortality. Individuals with diabetes go through far more from cardiovascular and renal illness than others, and diabetes is the overwhelming thought process of gained visual deficiency in the west. Most individuals with diabetes do presently don't kick the bucket from metabolic emergencies like ketoacidosis however from stroke, MI, or ongoing renal failure. (1)

Diabetes is related to corpulence and absence of exercise and the consistent enhance in the event in the west I being duplicated in enormous parts of the making scene as they receive that ways of lifestyle. Diabetes is in the possibility of transforming into a practically pandemic. Especially requesting is the upward hit in the occurrence of diabetes of each sort is ever young patients. This takes steps to put an intolerable weight on well-being administrations, especially in making nations. (2)

EPIDEMIOLOGY

Diabetes is known to influence over 20% of the UK populace and presumably as numerous again are probably going to have debilitated glucose resistance or even blunt diabetes whenever screened. (3)

The portrayal of egg whites in the pee as an indication of genuine kidney sicknesses in 1836 by Bright, doctor to Guy's Hospital, denoted the appearance of clinical nephrology. This perception, along with prior ones by Cotunnius in 1770 and Rollo in 1798 that pee of certain diabetics contained proteins, driven Rayer in 1840 to hypothesize that diabetes may cause a type of "Brilliant's sickness". Without a doubt, different epidemiologic investigations have exhibited that around 20-40% of diabetic subjects will create proteinuria and reformist renal disappointment on a normal of 15-20 years after the beginning of diabetes. The guess of these patients is poor and without renal help treatment, the mean endurance after the beginning of clinical proteinuria is just long term. Besides the individual and homegrown misfortune, the expense of really focusing on the diabetic patient in end-stage renal sickness is tremendous. Examinations of the WHO Renal Data System showed a sensational expansion in the rate of

ESRD that is brought about by diabetes. Somewhere in the range of 1999 and 2005 diabetes was responsible for 44% of all new instances of ESRD).[4to10]

MATERIALS AND METHODS

Collection and authentication of plant material

The leaves of *Barleria cuspidate f. Heyne ex Nees* were collected from Yercaud in December -2020. The plant has been taxonomically identified and authenticated by the botanist Dr.S.Radha. MSc. Ph.D. Central Siddha Medicinal plant Garden Mettur Dam. Tamilnadu. The authenticated plants were used for the preparation of extracts. The authenticated plant was used for the preparation of the extracts.

Preparation of the extracts

The collected, cleaned and powdered leaves of *Barleria cuspidata F.Heyne ex Nees* was used for the extraction purpose.300gms of powdered material was evenly packed in the soxhlet apparatus. It was then extracted with various solvents from non-polar to polar such as petroleum ether, aqueous, and ethanol.

Method of extraction

- Continuous hot percolation process.

Requirements

- Shade dried coarse powder of leaves of *Barleria cuspidata F.Heyne ex Nees*
- Soxhlet apparatus.

Solvents used

- Petroleum ether(60-80°C)
- Alcohol 90% v/v.(75-78°C)
- Distilled water.

PHARMACOLOGICAL SCREENING METHOD[11]

ANTI-DIABETIC ASSAY (α- AMYLASE INHIBITORY ASSAY) BY DNSA METHOD

Procedure:

The anti-diabetic activity of the test samples was determined according to the method described in the Worthington Enzyme Manual with slight modifications (Worthington, 1993; Kwon et al., 2006). In brief, 500µL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing 0.5 mg/mL of α- amylase enzyme and different concentrations (in µg) of the test sample as enzyme inhibitor were pre-incubated at 37°C for 10 min. After the pre-incubation, 500 µL of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube and incubated at room temperature for 5 mins. The reaction was stopped using 1.0 mL of dinitrosalicylic acid (DNSA) reagent. The test tubes were incubated in a boiling water bath for 5 min and then cooled to room temperature. The volume of the reaction mixture was made up to 10mL by adding distilled water, and the absorbance was measured at 540 nm using a UV-Visible spectrophotometer. The absorbance was compared with the controls and blank that contained buffer instead of the test sample.

C - Control with starch and without alpha-amylase

B - Control with starch and alpha-amylase

Calculation:

$$\textbf{Percentage inhibition} = \frac{(\mathbf{B} - \mathbf{A}) \times \mathbf{100}}{(\mathbf{B} - \mathbf{C})}$$

C - Absorbance of the Control with starch and without alpha-amylase

B - Absorbance of the Control with starch and alpha-amylase

A - Absorbance of the Test.

α -GLUCOSIDASE INHIBITION ASSAY

Procedure

The inhibitory activity of the sample on α-glucosidase enzyme was determined according to the method described by Kim et al. The 200 µL of diluted α-glucosidase (0.067 U/mL) was pre-incubated with the varying concentrations of sample for 10 min. The substrate solution p-nitrophenyl α-D-glucopyranoside (pNPG) was prepared in 0.1 M Sodium phosphate buffer (pH 6.9). Then 200 µL of 3.0 mMpNPG used as substrate prepared in 0.1M sodium phosphate buffer (pH 6.9) was then added to start the reaction. The reaction mixture was incubated at 37°C for 20 min and stopped by adding 2 mL of 0.1 M Na_2CO_3. The α-glucosidase activity was determined by measuring the yellow-colored para- nitrophenol released from pNPG at 400 nm. The results were expressed as a percentage of inhibition. Same procedure was done with acarbose (1mg/ml) which was used as standard.

Inhibitory activity (%) = (B-T/B-C) ×100

Where,

B is the absorbance of the blank.

T is the absorbance in the presence of the test substance.

C is the absorbance of control.

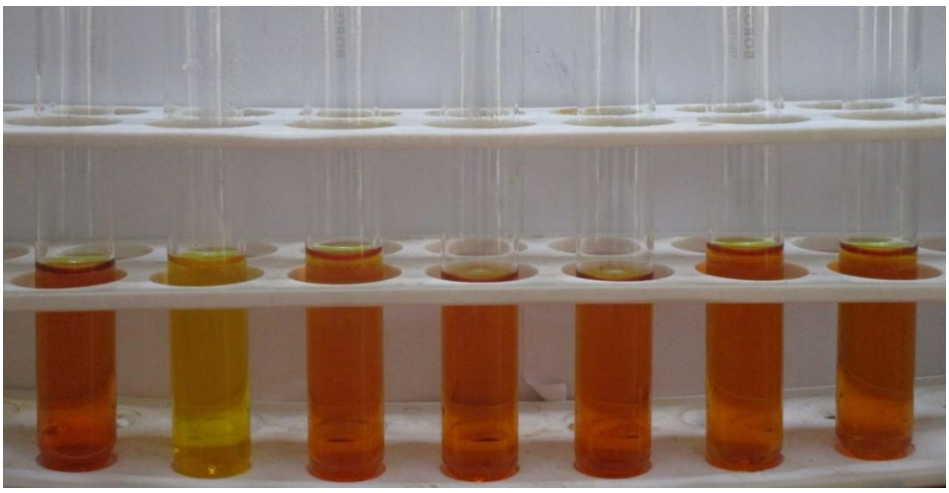

Fig no:1 α-amylase inhibition activity of Anti-Diabetic Activity of Ethanolic Extract of *Barleria cuspidate F.Heyne ex Nees*

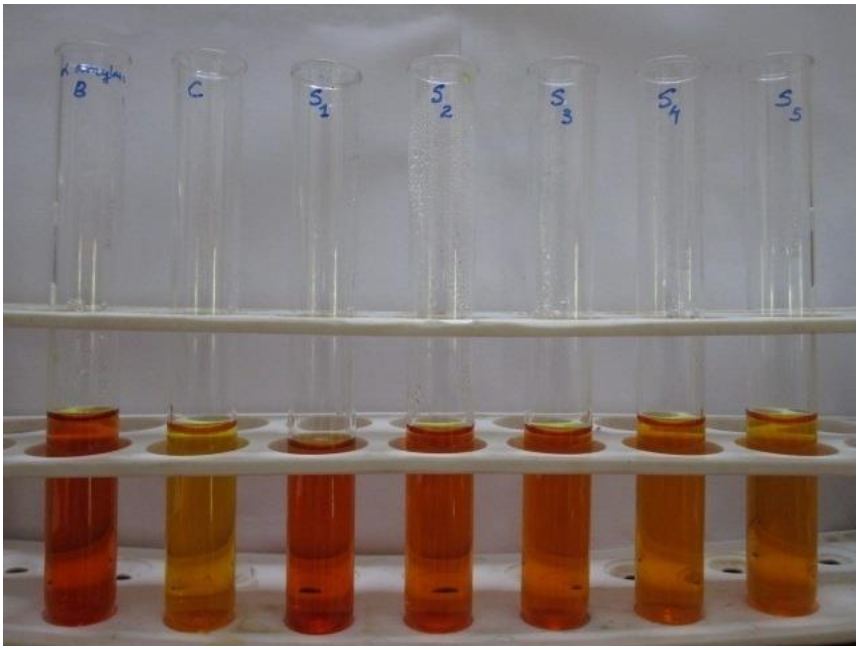

Fig no:2 α-Amylase Inhibition of Anti-Diabetic Activity of Standard Acarbose

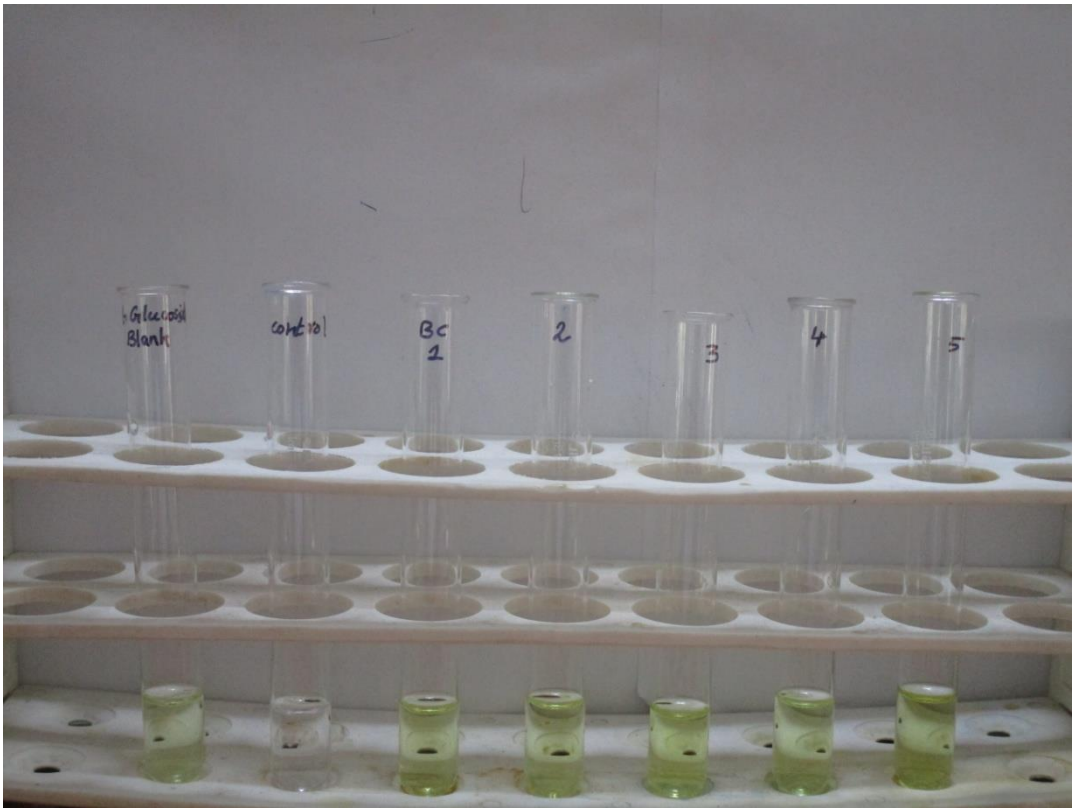

Fig no :3 α-Glucosidase Inhibition Assay of anti-diabetic Activity of Ethanolic Extract of *Barleria cuspidate F.Heyne ex Nees*

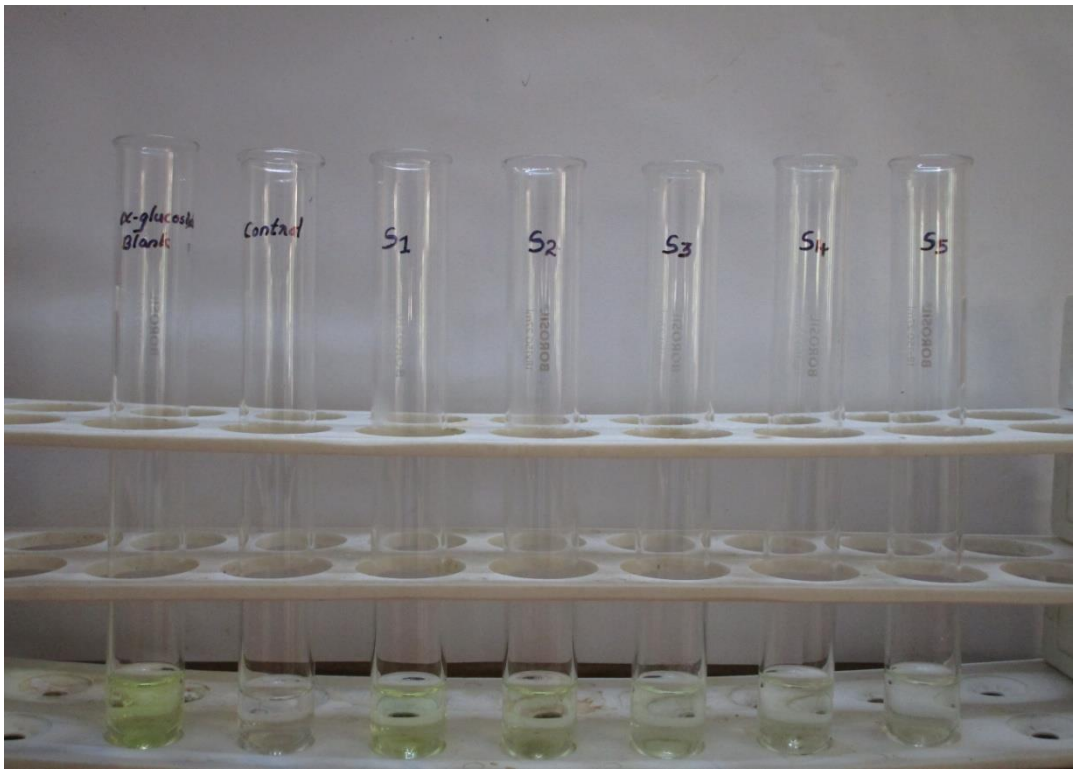

Fig no: 4 α-Glucosidase Inhibition Assay of Anti-Diabetic Activity Standard Acarbose

RESULTS AND DISCUSSION

α-Amylase inhibitor activity of *Barleria cuspidata F.Heyne ex Nees*

One therapeutic approach is the prevention of carbohydrate absorption after food intake, which is facilitated by the inhibition of enteric enzymes including α-glucosidase and α-amylase present in the brush borders of the intestine.

The alpha-amylase inhibition action of *Barleria cuspidata F.Heyne ex nees* may be responsible for the diabetic treatment. The α -amylase inhibition is otherwise called a starch blocker. It prevents or slows the absorption of starch in the body mainly blocking the glucosidic linkage of starch and other oligosaccharides, maltose, maltotriose, and other simple sugars.

The alpha-amylase from the three different sources that are mainly used pancreatic alpha-amylase, serum alpha-amylase, α-amylase from the fermented body. The leaves extract showed the significant in pancreatic amylase inhibitor activity concentration range from(6.25µg, 12.5µg, 25µg,50µg,100µg) The percentage of inhibition was found to be (24.46%,33.33%,38.36%,55.63%,61.63%) respectively. The standard acarbose the

concentration range (6.25µg,12.5µg,25µg,50µg, 100µg) of percentage of inhibition was found to be (11.40%,13.26%,15.91%,17.24%,18.56%) respectively.

α-Glucosidase inhibitor activity of *Barleria cuspidate F.Heyne ex Nees*

The number of glucosidases located in the brush border surface membrane of intestinal cells and key enzymes of carbohydrates metabolism. α -glucosidase inhibition activity blocks the action of alpha-glucosidase enzymes in the small intestine. The rate limits the conversion of oligosaccharides and disaccharides to mono-saccharides and it is necessary for gastrointestinal absorption.

The *In-vitro* alpha glucosidase inhibition activity of Ethanolic leaf extract of *Barleria cuspidata F.Heyne ex Nees*. In the various concentration range (6.25µg,12.5µg, 25µg, 50µg, 100µg) and the percentage of inhibition was found to be (17.32%, 34.81%, 55.94%, 72.77%, 85.47%). When compared to standard acarbose concentration range (6.25µg,12.5µg, 25µg, 50µg 100µg) the percentage of inhibition was found to be (28.4%, 37.9%, 56.90%, 68.24%, 79.58%) respectively.

Table No: 01 *In-Vitro* Anti-Diabetic Activity of α-Amylase (Standard Acarbose) by α-Amylase Method

Standard	Concentration (µg)	OD at 540nm	% of Inhibition
Blank	-	0.446	-
Control	-	0.029	-
Acarbose	6.25	0.344	24.46
	12.5	0.307	33.33
	25	0.296	38.36
	50	0.214	55.63
	100	0.189	61.63

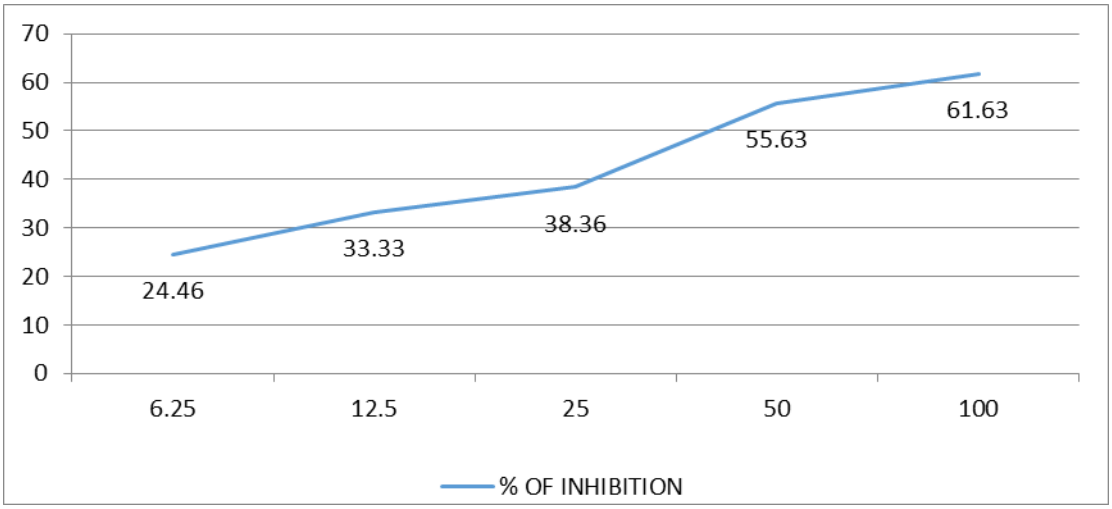

Figure No:05 *In-Vitro* Anti-Diabetic Activity of α-Amylase (Standard Acarbose) by α-Amylase Method

Table No: 02 *In-Vitro* Anti-Diabetic Activity of α-Amylase of Ethanolic Extract of *Barleria cuspidate F.Heyne ex Nees*

Sample	Concentration (µg)	OD at 540nm	% of Inhibition
Blank	-	0.444	-
Control	-	0.067	-
BC	6.25	0.401	11.40
	12.5	0.394	13.26
	25	0.384	15.91
	50	0.379	17.24
	100	0.374	18.56

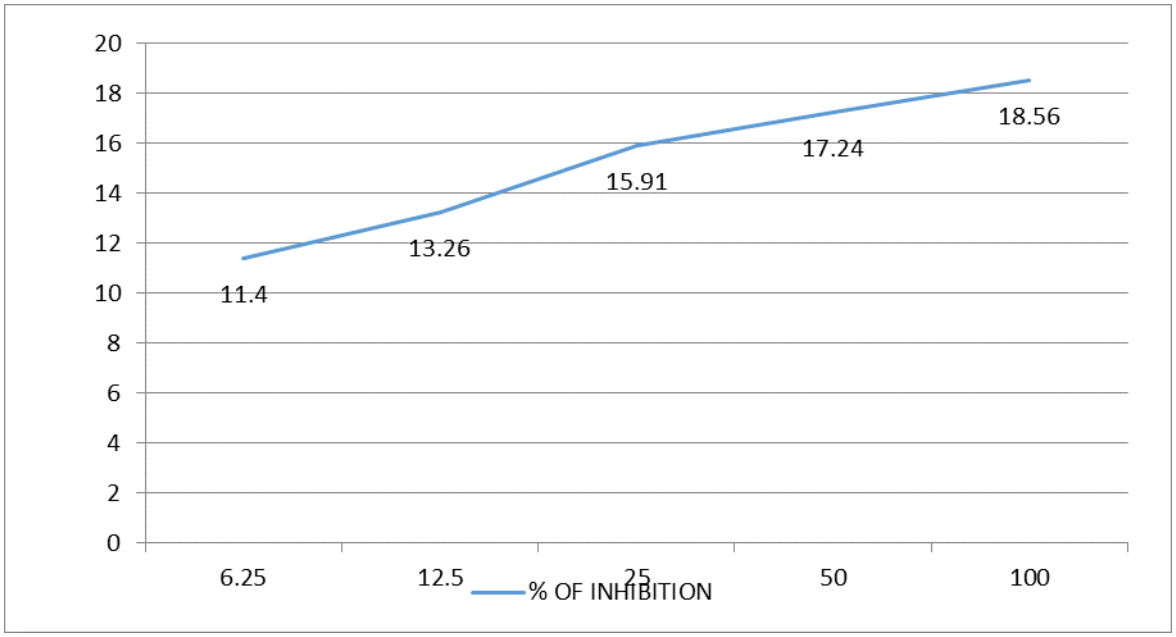

Figure No 06: ***In-Vitro*** **Anti-Diabetic Activity of α-Amylase of Ethanolic Extract of** ***Barleria cuspidata F. Heyne ex Nees***

Table No: 03 ***In-Vitro*** **Anti-Diabetic Activity of α-Glucosidase Inhibitor Assay (Standard Acarbose)** ***By*** **α- Glucosidase Method**

Standard	Concentration (µg)	OD at 400nm	% of Inhibition
Blank	-	0.631	-
Control	-	0.025	-
Acarbose	6.25	0.526	17.32
	12.5	0.420	34.81
	25	0.292	55.94
	50	0.190	72.77
	100	0.113	85.47

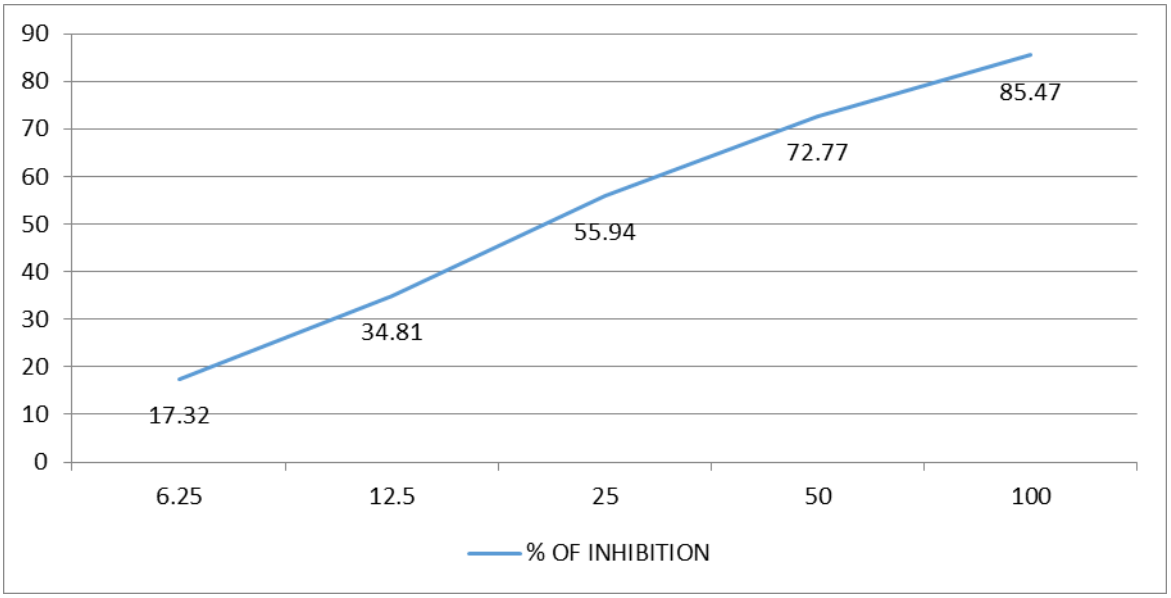

Figure No:07 In-Vitro Anti-Diabetic Activity of α-Glucosidase Inhibitor Assay (Standard Acarbose) By α-Glucosidase Method

Table No: 04 *In-Vitro* Anti-Diabetic Activity of α-Glucosidase of Ethanolic Extract of *Barleria cuspidate F.Heyne ex Nees*

Sample	Concentration (µg)	OD at 400nm	% of Inhibition
Blank	-	0.589	-
Control	-	0.020	-
BC	6.25	0.905	28.4
	12.5	0.893	37.9
	25	0.830	56.90
	50	0.758	68.24
	100	0.712	79.58

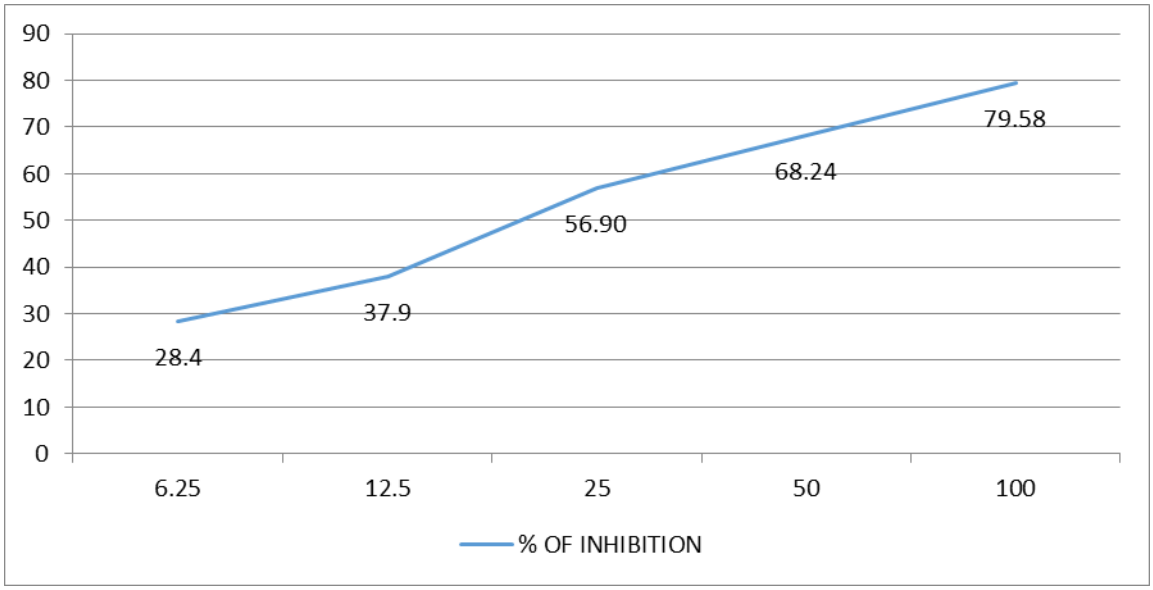

Figure No:08 - *In-Vitro* Anti-Diabetic Activity of α-Glucosidase Inhibitor Assay (sample)

CONCLUSION

From the results, we concluded the ethanolic extract of leaves of *Barleriacuspidata F. Heyne ex Nees* exhibited good anti-diabetic activity by the ability to inhibit the αalpha-amylase Inhibitory Assay and α- Glucosidase Inhibition Assay. The extent of inhibition by the extracts at different concentrations was compared with standard acarbose. This study confirms that leaves of *BarleriaCuspidataF.Heyne ex Nees* can mitigate postprandial hyperglycemia and assist in combating diabetic complications.

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