EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 3
Issue: 11
299-310
Year: 2016

EVALUATION OF ANTIDIABETIC ACTIVITY OF GENISTEIN BY ENZYME INHIBITORY METHODS

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Article Received on 08/09/2016 **Article Revised on 28/09/2016** **Article Accepted on 18/10/2016**

ABSTRACT

In present study, we investigated *in vitro* antidiabetic activity of genistein (potent phytoestrogen) were evaluated for antidiabetic activity by determining their inhibitory effect on various alpha amylase and alpha-glucosidase enzymatic methods and was found to genistein exhibit potent antidiabetic activity *In vitro*. Acarbose is used as inhibitors. The future aspected that may reduce the side effect and also may increase the effect of allopathic drugs with combination.

KEYWORDS: Diabetes and Types, Genistein, Alpha Amylase, Alpha Glucosidase, Sucrase Enzymatic Methods.

INTRODUCTION

Diabetes mellitus (DM) is describes as metabolic disorder of multiple etiology which results in increased blood glucose levels and disturbances of carbohydrates, fats and protein metabolism resulting from defects in insulin secretion, insulin action or both.[1,2] Elevated levels of blood glucose (hyperglycemia) lead to spillage of glucose into the urine, hence the term sweet urine. Chronic hyperglycemia causes damage to eyes, kidneys, nerves, heart and blood vessels.[3] In diabetes, the postprandial phase is characterized by a rapid and large increase in blood glucose levels, which is called postprandial hyperglycaemia or "hyperglycaemic spikes". Diabetes mellitus is becoming the third killer of mankind after cancer and cardiovascular disease due to its high prevalence, morbidity and mortality.[4] There are two types of diabetes mellitus - **Insulin-Dependent Diabetes Mellitus (IDDM),** categorized as immune-mediated or idiopathic diabetes with β cell destruction.[5] It is occurs due to insulin insufficiency because the body does not generate any insulin and patients entirely depend on an exogenous supply of insulin. It causes severe damage to the pancreatic β-cells. People with this form of diabetes need injections of insulin every day in order to control the levels of glucose in their blood.[6,7]

Non-Insulin Dependent Diabetes Mellitus (NIDDM) or adult-onset diabetes is unable to respond to insulin and can be treated with exercise, diet management and medication. NIDDM is the most widespread type.[8] It is characterized by insulin resistance and relative insulin deficiency, either of which may be present at the time that diabetes becomes clinically manifest. Type II diabetes is the more common form of diabetes constituting 90% of the diabetic population.[9] The number of people with diabetes is expected to double to 300 million within 20 years (in 2030), of which 90% will have type 2 diabetes (non–insulin-dependent diabetes mellitus.[10] A number of plants present in nature possess marked anti-diabetic activity. Genistein is a antidiabetic phytoconstituent**. Genistein** (Biological source - Glycine max, Family -Leguminosae) is a soy isoflavone with polyphenolic compounds derived from a common class of phytoestrogens(estrogen-like chemical compound present in plants) that binds to estrogen receptors and regulate cell proliferation.[11,12] Genistein is biosynthetically the simplest isoflavonoid compound of the Leguminosae.[13] It has weak estrogenic effect.[14] Estrogen has been demonstrated to increase insulin secretion via regulation of insulin gene expression.[15] Phytoestrogens are classified into major classes according to their chemical structures. That have estrogen-like actions in the human body, They are lignans, isoflavones and coumestans.[16] Genistein has multiple molecular targets in the body, including various receptor, enzyme and pathway interactions.[17] It expresses a wide range of biological activities, such as antioxidant, anti-cancer, antimicrobial.[18] antitumor Properties[19] prevention of osteoporosis, cardiovascular diseases[20] and lowering rates of prostate, breast and colon cancers.[21] Genistein, present in soy foods at concentrations ranging from 1.9 to 229 mg/g, is reported to be the major anticancer constituent of soybean.[22]

MATERISLS AND METHODS

Table 1 - List of Chemicals and Reagent

S.No	Materials	Manufacturer/company
1.	α-glucosidase enzyme	Sigma - Aldrich, Japan
2.	Genistein	Sigma - Aldrich, Japan
3.	DNS reagent	Loba Chemie PVT LTD Mumbai India
4.	Calciun acetate	Sigma – Aldrich
5.	Potassium dihydrogen orthophosphate	Loba Chemie PVT LTD Mumbai India
6.	Sodium hydroxide pellets	Loba Chemie PVT LTD Mumbai India
7.	Sucrose	Loba Chemie PVT LTD Mumbai India
8.	Maleic acid	Loba Chemie PVT LTD Mumbai India
9.	Yeast powder	Fizmerk India chemicals
10.	Methanol	Multi Tex Science
12.	Ethanol	Excise Duty
13.	Distilled water	Columbia Institute in Pharmacy in Raipur India
14.	Sodium potassium tartarate	Fizmerk India chemicals
15.	Starch soluble	Fizmerk India chemicals
16.	Sodium dihydrogen orthophosphate	Molychem, Mumbai
17.	Sodium carbonate anhydrous	Merk life science private limited, Mumbai
18.	P-Nitrophenyl-α-D-glucopyranoside	Sigma - Aldrich, USA
19.	Acarbose	Bayer Pharmaceutical Pvt. Ltd. Baddi

Table 2 - List of equipments

S.N0.	Equipment	Model / Company
1.	Electron analytical balance	KRT 120HS / KEROY Balances PVT. LTD Varanasi India
2.	UV-visible spectrophotometer	UV-1800 24 OV / SHIMADZU Corporation Kyoto Japan
3.	Cooling micro centrifuge	Remi, C12 Ltd. Vasai India
4.	Magnetic stirrer	2-ML/ REMI equipment PVT.LTD, Mumbai
5.	Digital weighing balance	Keyroy Pvt. Ltd. Varanasi
6.	Heating mental	Suntek
7.	Deep freezer	Multitech scientific instruments
8.	Mechanical stirrer	2-ML/ REMI Equipment PVT.LTD, Mumbai

METHODS

ALPHA AMYLASE (A- AMYLASE) INHIBITORY ACTIVITY[23]

Wheat alpha amylase inhibitory activity

Extraction of wheat alpha amylase

500g of malted whole wheat was added slowly with stirring to 1 litre of 0.2% calcium acetate solution at room temperature and continuously stirred for 2 hr on a stirrer. The suspension was then centrifuged at 4˚C at 12000 rpm for 30 minutes. The resultant clear brown supernatant was stored at 2˚-3˚ prior to heat treatment.

Inactivation of beta-amylase

Since beta amylase interferes with the enzymatic determination of alpha amylase it was inactivated by this treatment at pH between 6.5 and 8.0. The pH of the extract was first adjusted to 6.6 with cold 4% ammonium hydroxide. Heat treatment was carried out at 85˚C to 90˚C and other at 72˚C to 74˚C using a water bath with continuous stirring. The extract was then cooled to 2˚C to 3˚C until use.

Determination of wheat alpha amylase inhibitory activity

The assay mixture containing 200µl of 0.02 M sodium phosphate buffer, 20µl of enzyme and the plant extracts in concentration ranges 20, 40, 60, 80 and 100 µg/ml were incubated for 10 minutes at room temperature followed by addition of 200µl of starch solution in all test tubes. The reaction was terminated with the addition of 400µl DNS reagent and placed in boiling water bath for 5 minutes, cooled and diluted with 15ml of distilled water and absorbance was measured at 540nm.

The control samples were prepared without addition of any extracts and the blank solution used phosphate buffer.

The percent inhibition was calculated according to the formula

$$\text{Inhibition (\%)} = \frac{\text{Abs(control)} - \text{Abs(extract)}}{\text{Abs (control)}} \text{ X } 100$$

The IC_{50} values were determined from plots of percent inhibition verses log inhibitor concentration and were calculated by non linear regression values. Acarbose was used as the reference alpha amylase inhibitor.

Salivary alpha amylase

Collection and preparation of salivary alpha amylase sample

Rabbit saliva samples were collected from the rat mouth with the help of cotton bird and dissolve in 0.1 M tris-

buffer (pH 7.0) and centrifuged at 13,000 rpm for 20 min. An aliquot of the supernatant was diluted with water. These extract were then used in the assay.

Salivary alpha amylase inhibitory activity

Salivary alpha amylase was assayed by the method reported previously with a single modification. the assay mixture containing 200µl of 0.02 M sodium phosphate buffer, 20µl of enzyme and the plant extract in concentration range 20-100µl were incubated for 10min. at room temperature followed by addition of 200µl of starch in all test tubes. The reaction was terminated with the addition of 400µl DNS reagent and placed in boiling water bath for 5 min. cooled and diluted with 15ml of distilled water and absorbance was measured at 540nm. The control samples were prepared without any plant extract. The inhition was calculated according to the formula.

$$\text{Inhibition (\%)} = \frac{\text{Abs(control)} - \text{Abs(extract)}}{\text{Abs (control)}} \text{ X } 100$$

The IC50 values were determine from plots of percent inhibition vs log inhibitor concentration and calculated by non-linear regression analysis from the mean inhibitory values.

Fungal alpha amylase inhibitory activity[24]

Isolation of fungal alpha amylase

Diastase powder was weighted and kept for 24 hours in temperature 4˚C. then dissolved in 100mM phosphate buffer, pH 6.8 and stirred for 2 hours and centrifuged at 12000 rpm in 4˚C for 10 min. The resultant solution was used as enzyme.

Inhibition activity of fungal alpha amylase

The assay mixture containing 200 µl of 0.02M sodium phosphate buffer, 20 µl of enzyme and the plant extracts in concentration range 20-100 µg/ml were incubated for 10 minutes at room temperature followed by addition of 200 µl of starch in all test tubes. The reaction was terminated with the addition of 400 µl DNS reagent and placed in boiling water bath for 5 minutes, cooled and diluted with 15 ml of distilled water and absorbance was measured at 540 nm. The control samples were prepared without any plant extracts. The % inhibition was calculated according to the formula.

$$\text{Inhibition (\%)} = \frac{\text{Abs(control)} - \text{Abs(extract)}}{\text{Abs (control)}} \text{ X } 100$$

The IC50 values were determine from plots of percent inhibition vs log inhibitor concentration and calculated by non-linear regression analysis from the mean inhibitory values.

ALPHA GLUCOSIDASE INHIBITORY ASSAY

Alpha Glucosidase (B. Stearothermophil) inhibitory activity[25]

α-glucosidase activity was performed according to the method of Lam et al. α-Glucosidase type IV enzyme (Sigma Co., St. Louis, USA) from B. stearothermophilus was dissolved in 0.5 M phosphate buffer (pH 6.5) (3 U/ml).

The α-glucosidase inhibitory activity was determined by measuring the release of 4-nitrophenol from p-nitrophenyl α-D glucopyranoside. The assay mixtures for these experiments contained 0.3 ml of 10mM p-nitrophenyl α-D-glucopyranoside, 1.0 ml of potassium phosphate (0.1M, pH: 6.8), 0.2 ml of enzyme solution and 0.2 ml of inhibitor test sample, all in a final volume of 1.7 ml. Following an incubation time of 30 min at 37ºC, the reaction was terminated by the addition of 2.0 ml of 100 mM sodium carbonate. The liberated p-nitrophenol was determined at 400 nm using spectrophotometer.

The % inhibition rates were calculated using the formula,

$$\text{Inhibition (\%)} = \frac{\text{Abs(control)} - \text{Abs(extract)}}{\text{Abs (control)}} \text{ X } 100$$

Suitable reagent blank and inhibitor controls were also carried out and subtracted. Dose dependent variation in the α-glucosidase inhibition was measured using 25 µl to 200 µl of the aqueous extracts. The IC50 values were determine from plots of percent inhibition vs log inhibitor concentration and calculated by non-linear regression analysis from the mean inhibitory values.

Rat intestinal alpha Glucosidase inhibitory activity[25]

Preparation of crude alpha Glucosidase from rat small intestine

Rat small intestine homogenate was prepared according to the method described by Alagesan Kathirvel et.al 2012 with slight modifications. Rats were anaesthetized by using halothane (usually 8-10ml) in a jar. Small intestine was removed and washed with 30ml of 0.9% of NaCl and placed in ice cold 0.9% NaCl. The small intestine was minced with a surgical knife and homogenized using Potter-Elvehjem type of homonizer in 50ml of 0.1 M potassium phosphate buffer of pH 6.8. After 30 min, the homogenates were centrifuged for 30min at 10,000 rpm at 4˚C. The supernatant was used as crude enzyme source.

Alpha Glucosidase inhibitory activity

The alpha - glucosidase inhibitory activity was determined by measuring the release of 4-nitrophenol from p-nitrophenyl alpha-D glucopyranoside. The assay mixtures for these experiments contained 0.3 ml of 10 mM p-nitrophenyl alpha – glucopyranoside, 1.0 ml of potassium phosphate solution (0.1M, pH 6.8), 0.2 ml of crude enzyme solution and 0.2 ml of inhibitor extract, all in a final volume of 1.7 ml. Following an incubation time of 30 min at 37˚C, the reaction was terminated by the addition of 2.0 ml of 100 mM sodium carbonate solution. The librated p-nitrophenol was determined at 400 nM using spectrophotometer.

The % inhibition rates were calculated using formula,

$$\text{Inhibition (\%)} = \frac{\text{Abs(control)} - \text{Abs(extract)}}{\text{Abs (control)}} \text{ X } 100$$

The control samples were prepared without addition of any extracts and the blank solution used phosphate buffer.

The IC50 values were determine from plots of percent inhibition vs log inhibitor concentration and calculated by non-linear regression analysis from the mean inhibitory values.

Yeast alpha Glucosidase inhibitory activity[26]

Preparation of Enzyme

The baker's yeast was weighted and kept for 24 hours in temperature 4˚C. then yeast was dissolved in 100mM phosphate buffer, pH 6.8 and stirred for 2 hours and centrifuged at 12000 rpm in 4˚C for 10 min. The resultant solution was used as enzyme.

Inhibition activity

The yeast glucosidase was dissolve in 100 mM phosphate buffer, pH 6.8 was used as enzyme source. 100mM P-nitrophenyl-α-d-glucopyranoside was used as substrate. The test sample was weighted and dissolves with water to get a concentration of 20-100 µg/ml. the test sample was mixed with 320 µl of 100mM phosphate buffer (pH 6.8) and 50 µl of 10mM PNPG in the buffer and then it was incubated at 30ºC for 5 minutes. After the incubation, 20 µl of the buffer containing 0.5 mg/ml of the enzyme was added and further incubated at 30ºC for 5 minutes. Finally, 3.0 ml of 50 mM sodium hydroxide was measured at 410 nm on a spectrophotometer.

The % inhibition rates were calculated using formula,

$$\text{Inhibition (\%)} = \frac{\text{Abs(control)} - \text{Abs(extract)}}{\text{Abs (control)}} \text{ X } 100$$

The enzyme without test sample was used as a control.

The IC50 values were determine from plots of percent inhibition vs log inhibitor concentration and calculated by non-linearregression analysis from the mean inhibitory values.

SUCRASE INHIBITORY ASSAY[27]

Isolation of Sucrase enzyme

Rats were sacrificed, intestine removed and chilled with ice cold 80 mM phosphate buffer. The intestine was then cut open, the mucosa scraped off with a piece of glass rod and homogenized with four parts (v/v) of cold buffer. Nuclei and large cell debris were removed by centrifugation at 2000 to 4000 rpm for 10 mins and supernatant aliquoted into 1.5 ml vials and stored at -20ºC.

Determination of sucrase inhibitory assay

The effect of test sample on sucrase activity was assayed according to the method of Honda and Hara 10µL of the enzyme along with varying concentrations of the sample is incubated for 10 minutes at 37°C. Malate buffer (pH 6.0) is used to make up the volume up to 200 µL. The enzyme reaction is initiated by adding 100 µL sucrose solution (60 mM) and incubated for 30 minutes. The reaction is terminated by the addition of 200 µL of 3, 5-dinitrosalysilic acid reagent and treating the mixture in a boiling water bath for 5 minutes. The absorbance of the solution is read at 540 nm.

Sucrase inhibition rate is calculated as follows

The % inhibition rates were calculated using formula,

$$\text{Inhibition (\%)} = \frac{\text{Abs(control)} - \text{Abs(extract)}}{\text{Abs (control)}} \text{ X } 100$$

Where, AC control is the absorbance of the control reaction (all reagents to be added except for the test sample) and the AS sample is the absorbance of the test sample. Models to target Specific/Particular enzyme.

The enzyme without test sample was used as a control.

The IC50 values were determine from plots of percent inhibition vs log inhibitor concentration and calculated by non-linear regression analysis from the mean inhibitory values.

RESULT

The inhibitory activity of phytoconstituent Genistein on alpha amylase enzyme (wheat alpha amylase, salivary alpha amylase and fungalalpha amylase), alpha Glucosidase enzyme (Bacillus stearothermophylus alpha glucosidase, Baker˝s yeast alpha Glucosidase, Rat intestinal alpha glucosidase) and Sucrase enzymes was evaluated. Acarbose is a reported antidiabetic drug and potent alpha amylase, alpha Glucosidase and sucrose enzyme inhibitor so that is used as a standard and a ayurvedic drug Madhunashinivati that is a marketed product of patanjali used as a reference drug it also have antidiabetic activity.

The % inhibition and IC_{50} of Acarbose, Madhunashinivati (patanjali) and Genistein are mentioned in the tables and graphs are plotted according to their percent inhibition with various concentrations and In the alpha amylase inhibition assay.

Alpha amylase inhibitory activity

Wheat alpha amylase inhibitory activity

Table 3 – The percent inhibition of wheat alpha amylase by *Madhunashini Vati (Patanjali), Acarbose, Genistein* at varying concentrations

Concentration (µg/ml)	Madhunashinivati (Patanjali)		Acarbose		Genistein	
	% inhibition	IC_{50}(µg/ml)	% inhibition	IC_{50}(µg/ml)	% inhibition	IC_{50}(µg/ml)
20	23.89±0.85	160.91	20.53±4.44	139.01	31.28±3.43	161.43
40	25.89±1.35		26.41±0.58		38.22±2.58	
60	29.99±2.91		28.52±0.67		39.23±1.33	
80	30.23±2.178		33.07±0.88		40.2±0.18	
100	41.28±0.45		42.3±1.33		42.25±0.58	

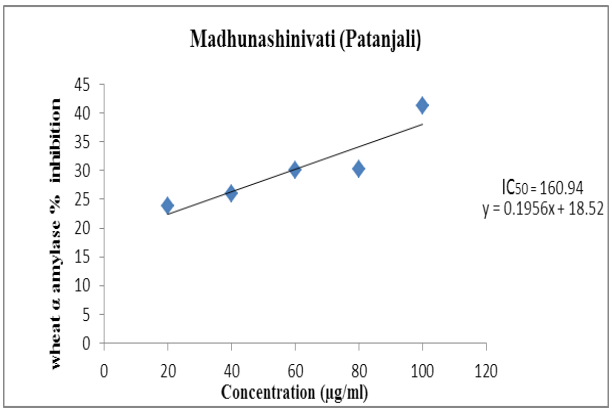

Fig 1: % inhibition of wheat alpha amylase by Madhunashinivati (Patanjali)

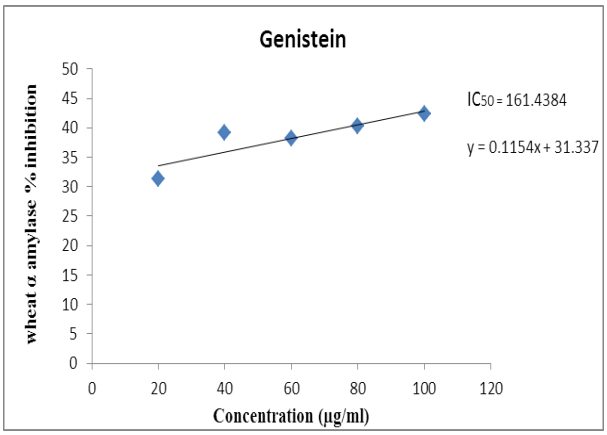

Fig 3: % inhibition of wheat alpha amylase by Genistein

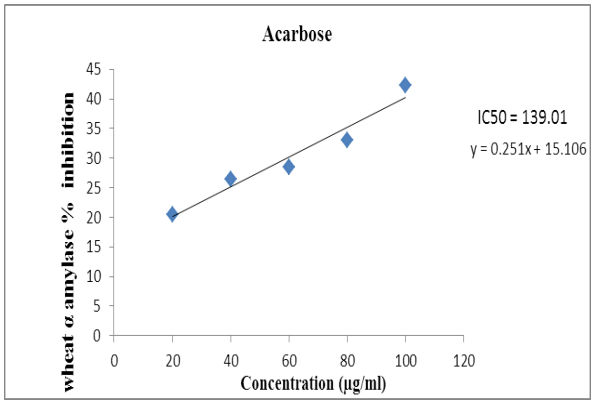

Fig 2: % inhibition of wheat alpha amylase by Acarbose

Salivary alpha amylase inhibitory activity

Table 4 – The percent inhibition of salivary alpha amylase by *Madhunashini Vati (Patanjali), Acarbose, Genistein* at varying concentrations

Concentration µg/ml	Madhunashinivati (Patanjali)		Acarbose		Genistein	
	% inhibition	IC_{50}(µg/ml)	% inhibition	IC_{50}(µg/ml)	% inhibition	IC_{50}(µg/ml)
20	48.35±0.94	29.02	45.32±0.94	33.80	35.15±1.18	166.2
40	61.18±0.99		52.46±2.96		35.18±1.68	
60	63.01±1.42		58.23±1.86		40.18±1.02	
80	64.61±0.99		65.11±2.94		41.55±0.58	
100	65.42±2.94		72.13±0.60		42.53±1.33	

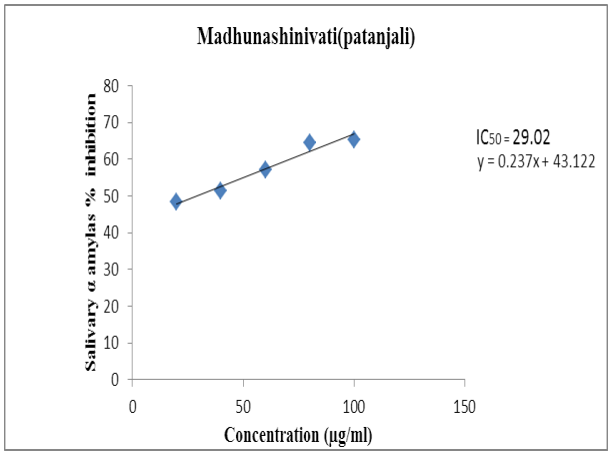

Fig 4: % inhibition of salivary alpha amylase by Madhunashinivati (Patanjali)

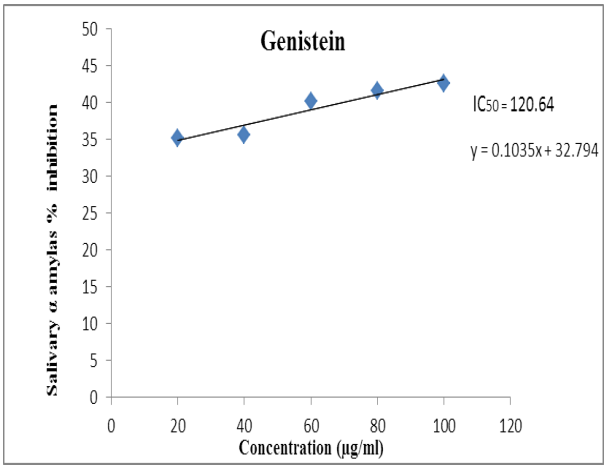

Fig 6: % inhibition of salivary alpha amylase by Genistein

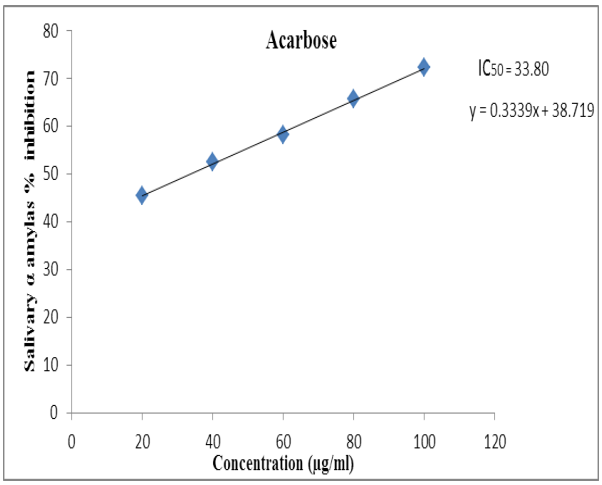

Fig 5: % inhibition of salivary alpha amylase by Acarbose

Fungal Alpha Amylase inhibitory activity

Table 5– The percent inhibition of Fungal Alpha Amylase by *Madhunashini Vati (Patanjali), Acarbose, and Genistein* at varying concentrations

Concentration µg/ml	Madhunashinivati (Patanjali)		Acarbose		Genistein	
	% inhibition	IC_{50}(µg/ml)	% inhibition	IC_{50}(µg/ml)	% inhibition	IC_{50}(µg/ml)
20	23.62±0.01	105.33	36.24±1.78	79.19	20.84±1.71	120.64
40	31.39±0.94		39.81±1.33		24.92±0.23	
60	34.62±1.71		44.66±1.39		34.48±1.71	
80	45.62±1.12		52.42±0.56		36.53±1.40	
100	46.57±0.58		53.89±1.78		44.34±1.12	

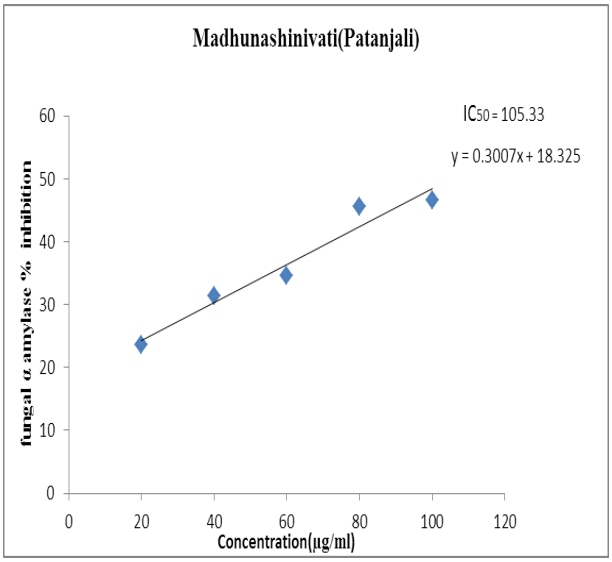

Fig 7: % inhibition of fungal alpha amylase by Madhunashinivati (Patanjali)

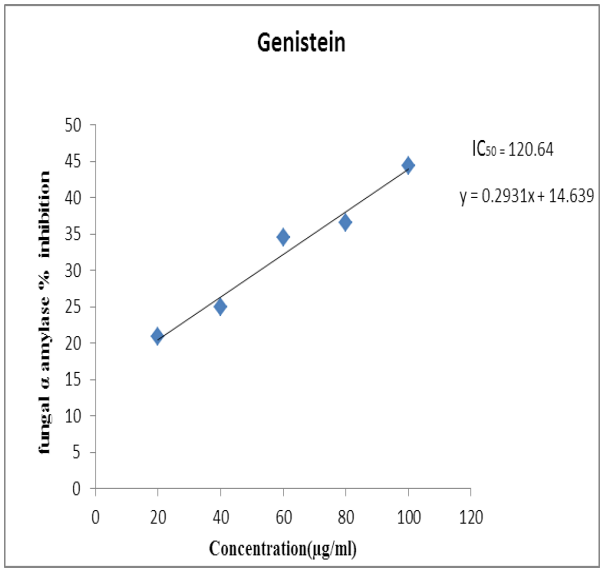

Fig 9: % inhibition of fungal alpha amylase by Genistein

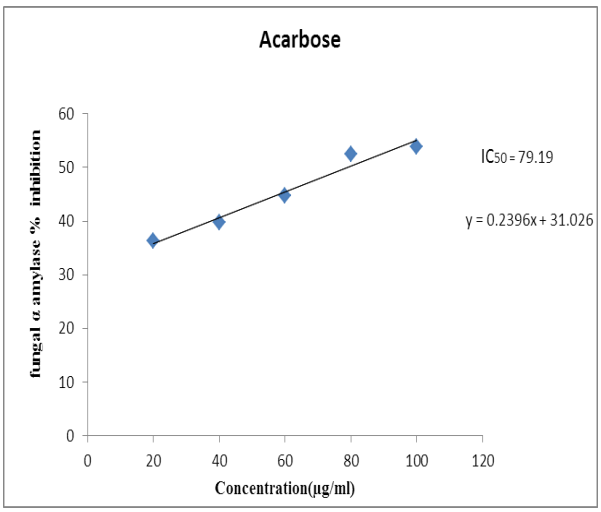

Fig 8: % inhibition of fungal alpha amylase by Acarbose

Bacillus strearothermophillus alpha Glucosidase inhibitory activity

Table 6– The percent inhibition of Bacillus strearothermophillus alpha Glucosidase by *Madhunashini Vati (Patanjali), Acarbose, Genistein* at varying concentrations

Concentration µg/ml	Madhunashinivati (Patanjali)		Acarbose		Genistein	
	% inhibition	IC_{50}(µg/ml)	% inhibition	IC_{50}(µg/ml)	% inhibition	IC_{50}(µg/ml)
20	25.25±0.67	85.01	41.41±0.45	46.74	23.23±1.35	102.66
40	36.36±0.60		43.43±3.77		25.25±0.67	
60	38.38±2.58		53.53±3.96		35.35±1.20	
80	47.47±0.71		66.66±2.94		45.45±	
100	56.56±2.15		74.74±1.74		47.47±1.77	

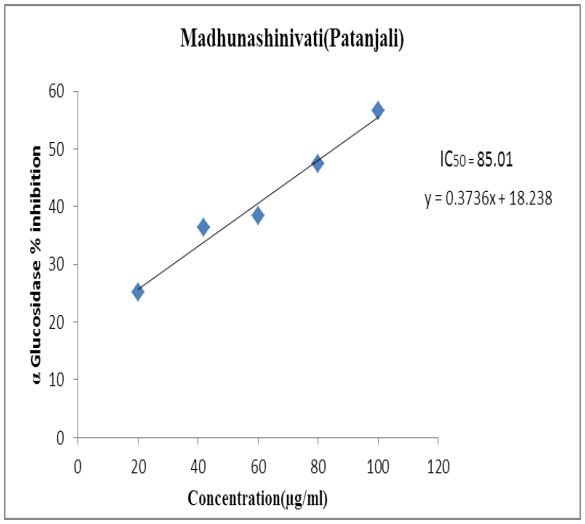

Fig 10: % inhibition of bacillus stearothermophillus alpha glucosidase by Madhunashini (Patanjali)

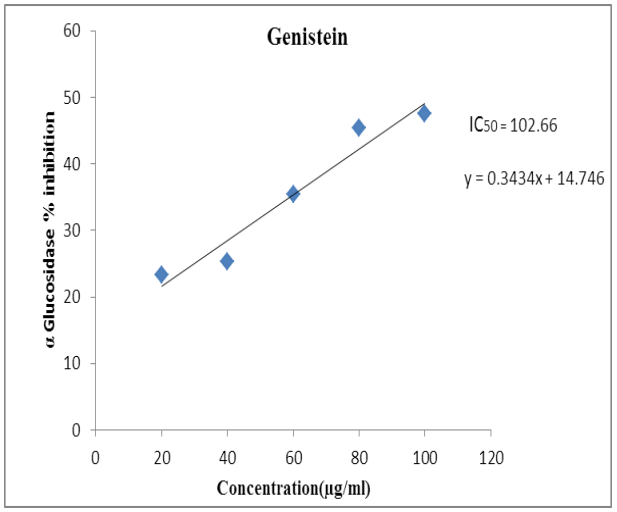

Fig 12: % inhibition of bacillus strearothermophillus alpha glucosidase by Genistein

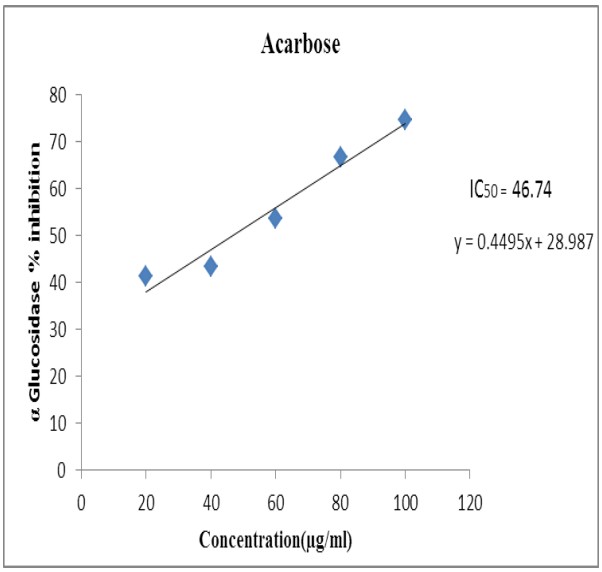

Fig 11: % inhibition of bacillus strearothermophillus alpha glucosidase by Acarbose

Yeast alpha Glucosidase inhibitory activity

Table 7 – The percent inhibition of yeast alpha glucosidase by *Madhunashini Vati (Patanjali), Acarbose, Genistein* at varying concentrations

Concentration µg/ml	Madhunashinivati (Patanjali)		Acarbose		Genistein	
	% inhibition	IC_{50}(µg/ml)	% inhibition	IC_{50}(µg/ml)	% inhibition	IC_{50}(µg/ml)
20	33.95±0.88	85.13	42.02±1.33	53.23	24.48±1.71	101.80
40	36.41±1.20		49.01±0.62		30.41±4.13	
60	43.82±0.77		53.33±1.78		41.77±1.11	
80	47.53±0.72		54.15±0.35		43.24±0.58	
100	54.93±0.35		57.65±0.71		47.89±1.33	

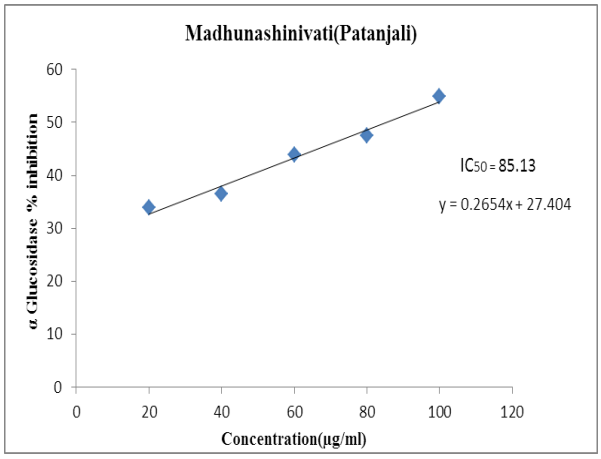

Fig 13: % inhibition of Yeast alpha Glucosidase by Madhunashinivati (Patanjali)

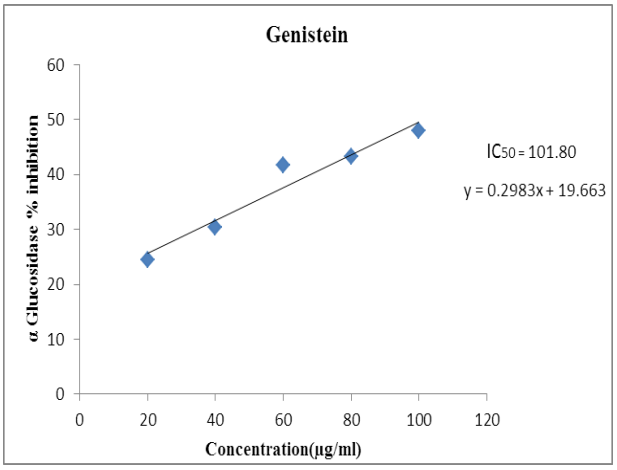

Fig 15: % inhibition of Yeast alpha Glucosidase by Genistein

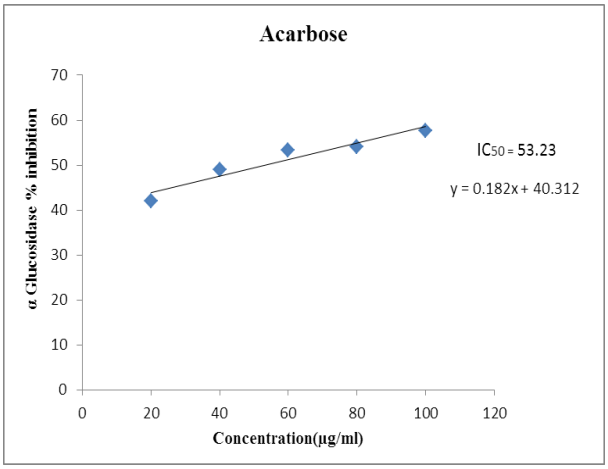

Fig 14: % inhibition of Yeast alpha Glucosidase by Acarbose

Rat intestinal alpha Glucosidase inhibitory activity

Table 8: – The percent inhibition of rat intestinal alpha glucosidase by *Madhunashini Vati (Patanjali), Acarbose, Genistein* at varying concentrations

Concentration µg/ml	Madhunashinivati (Patanjali)		Acarbose		Genistein	
	% inhibition	IC_{50}(µg/ml)	% inhibition	IC_{50}(µg/ml)	% inhibition	IC_{50}(µg/ml)
20	37.2±0.67	58.91	40.31±1.02	42.60	29.30±3.06	86.56
40	40.31±0.38		46.12±2.54		33.33±1.39	
60	47.67±1.73		62.89±1.25		37.25±0.66	
80	53.88±1.76		63.63±1.02		44.96±1.39	
100	73.25±0.67		73.64±0.66		58.52±2.15	

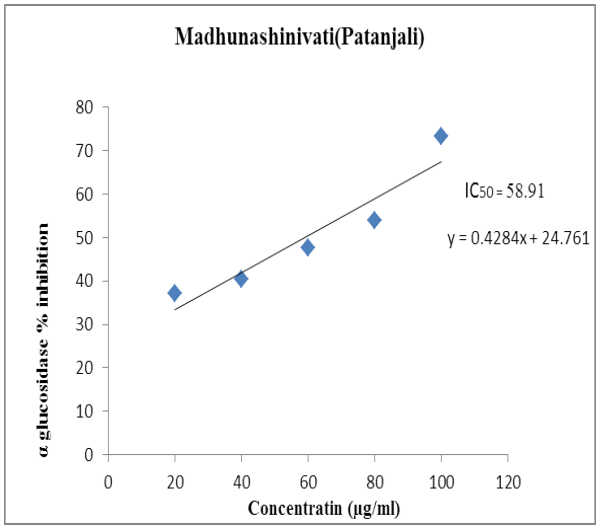

Fig 16: % inhibition of Rat intestinal alpha Glucosidase by Madhunashinivati(Patanjali)

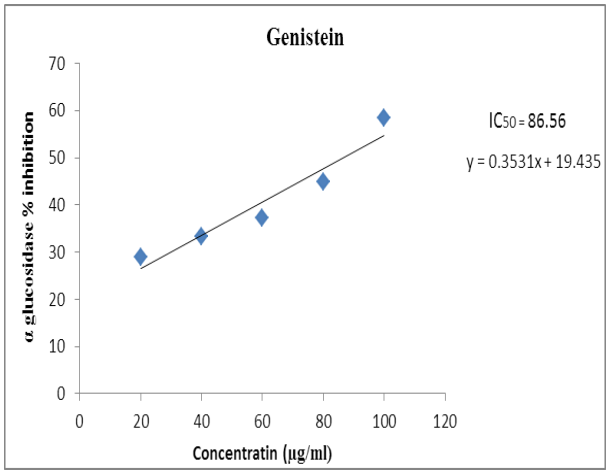

Fig 18: % inhibition of Rat intestinal alpha Glucosidase by Genistein

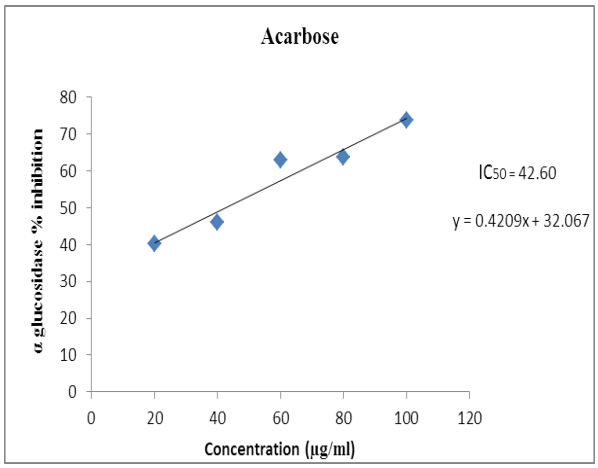

Fig 17: % inhibition of Rat intestinal alpha Glucosidase by Acarbose

Sucrose inhibitory activity

Table 9 – The percent inhibition of sucrase by *Madhunashini Vati (Patanjali), Acarbose, Genistein* at varying concentrations

Concentration µg/ml	Madhunashinivati (Patanjali)		Acarbose		Genistein	
	% inhibition	IC_{50}(µg/ml)	% inhibition	IC_{50}(µg/ml)	% inhibition	IC_{50}(µg/ml)
20	38.35±2.58	66.24	43.76±2.77	26.51	29.29±3.06	110.16
40	45.87±0.94		50.47±0.74		34.46±1.61	
60	49.46±0.62		58.06±1.06		38.54±2.57	
80	51.67±1.24		59.78±3.99		40.79±0.60	
100	57.7±1.56		60.85±0.99		49.19±1.39	

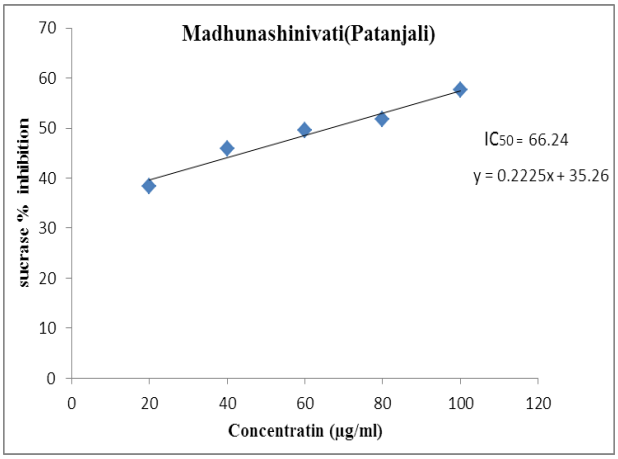

Fig 19: % inhibition of Sucrase by Madhunashinivati(Patanjali)

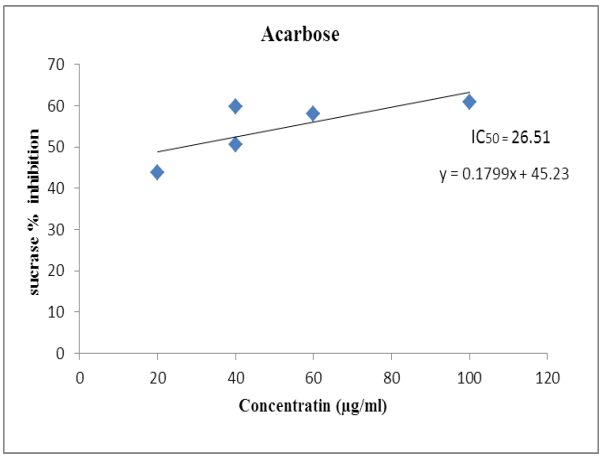

Fig 20: % inhibition of Sucrase by Acarbose

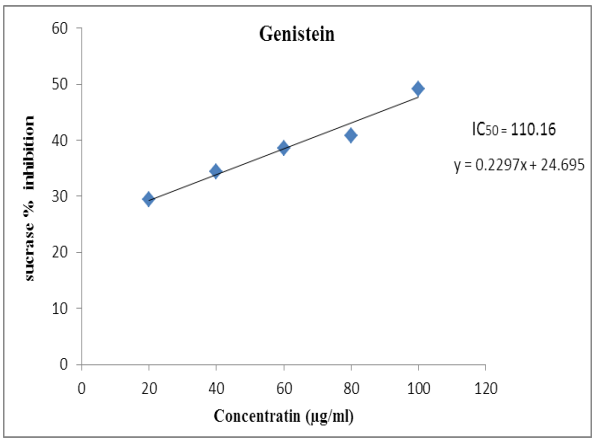

Fig 21: % inhibition of Sucrase by Genistein

DISCUSSION

The results are indicates that the phytoconstituent genistein was shown the **dose** dependent increase in percentage inhibitory activity against different enzymes. The phytoconstituent genistein is good alpha amylase inhibitory activity in fungal alpha amylase than wheat and salivary alpha amylase, greater alpha Glucosidase inhibitory activity in rat intestinal alpha glucosidae the phytoconstituent genistein is showed IC_{50} values.

Fungal alpha amylase **(120.64 µg/ml)** <Wheat alpha amylase **(161.43 µg/ml)**< salivary alpha amylase**(166.2 µg/ml).**

Rat intestinal alpha Glucosidase**(86.60 µg/ml)**<Yeast alpha Glucosidase **(101.80 µg/ml)**<B. alpha glucosidase and IC_{50} of sucrase **(110.16 µg/ml),** respectively in the different alpha amylase, different alpha Glucosidase and sucrase enzymes that indicates The phytoconstituent genistein showed the greater IC50 value in the fungal alpha amylase as compared to salivary alpha amylase and wheat alpha amylase and better inhibitory activity in rat intestinal alpha Glucosidase as campared to yeast and bacillus alpha Glucosidase and in sucrase enzyme it shown appreciable inhibitory activity also found that the genistein is greater % inhibition activity in alpha Glucosidase enzyme. Acarbose is α-Glucosidase inhibitor (AGIs) is a class of oral glucose-lowering drugs used exclusively for treatment or prevention of type 2 diabetes mellitus. It acts by altering the intestinal absorption of carbohydrates through inhibition of their conversion into simple sugars (monosaccharides) and thus decreases the bioavailability of carbohydrates in the body, significantly lowering blood glucose levels. Because of Acarbose is allopathic drug so it can be produces various types of side effects, like gastrointestinal side effects can limit its use, The predominant gastrointestinal symptom associated with acarbose is flatulence; though loose stools and abdominal discomfort have also been reported. These result from undigested carbohydrates entering from the small intestine directly into the colon, mimicking malabsorption.

Genistein is a isoflavone and a potent phytoestrogen and anticancer drug used in various cancers like breast cancer, overian cancer and also shows antioxidant activity. Because it's a natural pure drug and also shows the antidiabetic activity, it can be safe than Acarbose and can be reduces the side effects when using with combination so that's why it have been choosen for the evaluation of its mechanism in different in vitro enzymatic activity.

CONCLUSION

The inhibitory activity of genistein is evaluated on different in vitro enzymatic inhibitory methods and we found that the genistein is showed greater % inhibition activity in the alpha Glucosidase enzymatic activity and it shown the activity near about Madhunashinivati (patanjali). The present study indicates the phytoconstituent genistein can be use in the treatment of diabetes and it also can be combine with any allopathic drug for reducing their side effects.

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