



α -AMYLASE AND α -GLUCOSIDASE INHIBITORY ACTIVITY OF DIFFERENT EXTRACTS OF *STEVIA REBAUDIANA* AND *TINOSPORA CORDIFOLIA*

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ABSTRACT

One of the therapeutic approach to treat Type II Diabetes is to lower the postprandial blood glucose level by inhibition of carbohydrate hydrolyzing enzyme such as α -amylase. The current study evaluated the anti-diabetic potential of different extracts of *Stevia rebaudiana* and *Tinospora Cordifolia* via *in-vitro* inhibition of α -amylase and α -glucosidase using the petroleum ether, chloroform and methanol extracts. The α -amylase inhibitory potentials of the extracts were investigated by reacting different concentrations of the extracts with α -amylase and starch solution while α -glucosidase inhibition was determined by pre-incubating α -glucosidase with different concentrations of the extracts followed by the addition of p-nitrophenylglucopyranoside (pNPG). IC_{50} values clearly indicate that methanol extract of *T. Cordifolia* (METC) showed higher α -amylase inhibition (IC_{50} value-14.03 μ g /ml) in comparison with acarbose (IC_{50} value-36.22 μ g /ml) followed by methanol extract of *S. rebaudiana*, MESR (IC_{50} value-84.63 μ g /ml), petroleum ether extract of *T. Cordifolia*, PEETC (IC_{50} value-151.51 μ g /ml),), petroleum ether extract of *S. rebaudiana*, PEESR (IC_{50} value-163.83 μ g /ml), chloroform extract of *T. Cordifolia*, CETC (IC_{50} value-258.34 μ g /ml), and chloroform extract of *S. rebaudiana*, CESR (IC_{50} value-599.87 μ g /ml). For α - glucosidase METC showed higher inhibition (IC_{50} value-35.64 μ g /ml) in comparison with acarbose (IC_{50} value-50.43 μ g /ml) followed by MESR (IC_{50} value-87.62 μ g /ml), PEETC (IC_{50} value-136.47 μ g /ml), CETC (IC_{50} value-161.94 μ g /ml), PEESR (IC_{50} value-192.01 μ g /ml) and CESR (IC_{50} value-298.35 μ g /ml). The results of the work indicate that the methanol extracts of *Stevia rebaudiana* and *Tinospora Cordifolia* possessed considerable in vitro anti diabetic activity.

KEYWORDS: Amylase Inhibition, α -glucosidase, Antihyperglycaemic Activity.

INTRODUCTION

Diabetes is a chronic metabolic disorder characterized by impaired carbohydrate metabolism resulting in an increased blood glucose level i.e. hyperglycemia. The prevalence of diabetes is increasing annually, according to WHO, the global prevalence of diabetes is estimated to increase from 4% in 1995 to 5.4% by the year 2025 majorly in the developing countries.^[1] India has earned the dubious distinction of being termed the “Diabetes Capital of the World” by leading the world with largest number of diabetic subjects and the prevalence of diabetes is consistently increasing.^[2] Herbal drugs are effective, safer and acceptable to the general society.^[3] One of the therapeutic approach for treatment of diabetes is to decrease hyperglycemia. The medicinal plants or natural products involve retarding the absorption of glucose by inhibiting the carbohydrate hydrolyzing enzymes α -amylase and α -glucosidase. α -amylase which is a prominent enzyme in pancreatic juice breaks starch into absorbable molecules, maltose.^[4] α -glucosidase, present in small intestine catalyzes the digestion of starch and disaccharides.^[5] Inhibitors of α -amylase and α -

glucosidase delay the breakdown of carbohydrate in the small intestine and decrease the postprandial blood glucose levels. The inhibition of activity of these enzymes is very useful and effective to lower the levels of postprandial hyperglycemia.^[6,7] *Stevia rebaudiana* is plant of Asteraceae family. stevia leaves contain a complex mixture of natural diterpene glycosides namely, steviol, steviolbioside, stevioside, rebaudiosides and ducloside A.^[8] It is used as Antidiabetic Antimicrobial and Antifertility agent. It is also used for its immunological potential. *Tinospora Cordifolia* belonging to menispermaceae family. A large number of compounds belonging to different classes such as alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds and polysaccharides have been isolated from *T.cordifolia*.^[9] Traditionally it is used as antipyretic, antidiabetic, cardiostonic, antiseptic and analgesic. The aim of the current study was to evaluate the α -Amylase and α -Glucosidase inhibitory effects of various extracts of *S. rebaudiana* and *T. Cordifolia*.

MATERIALS AND METHODS

Chemicals: The α -amylase α -glucosidase and acarbose were purchased Sigma-Aldrich and. Chemicals like 3, 5-Dinitro salicylic acid, sodium potassium tartarate, sodium chloride and sodium hydroxide were obtained from Himedia, Mumbai. Chemicals used in present investigation were of analytical grades.

Preparation of extracts: The plant materials leaves of *S. rebaudiana* and *T. cordifolia* leaves and stems were collected from the areas in and around Bhopal and authenticated by the Botany Department Saifia Science college, Bhopal (M.P) and voucher specimens were deposited (420/ Bot./saf./14 and 420/ Bot./saf./14). Plant material were shade dried and ground into powder, subjected for successive solvent extraction using petroleum ether (60-80°C), chloroform and methanol as solvent respectively. The final extracts were filtered and the filtrate were concentrated under vacuum.

In-vitro Alpha-Amylase inhibition assay

α -amylase inhibitory activity was studied using enzyme-starch system. Sample (1%) was mixed by stirring with 25 mL of potato starch (4%) in a beaker; 100 mg of α -amylase was added to the starch solution, stirred vigorously and incubated at 37 °C for 60 min. After the incubation period 0.1 M NaOH (2 mL) was added to terminate enzyme activity. The mixture was centrifuged for 15 min (3,000 × g) and glucose content was determined in the supernatant. A control test was also run without the addition of test sample.^[10]

% Inhibition = Absorbance of control – Absorbance of test / Absorbance of control X 100

IC₅₀ (concentration of extract required to inhibit 50% of PPA) values of Acarbose and extracts were determined from graphs of percentage inhibition versus concentration (μ g/ml).

In-vitro α -Glucosidase inhibition assay

100 μ l of a sample of different extracts was incubated with 50 μ l α -glucosidase (1.0 U/ml) in phosphate buffer (0.1 M, pH 6.8) for 10 min at 37°C. The reaction was initiated by addition of 50 μ l of substrate: 5 mM, p-

nitrophenyl- α -D glucopyranoside in a 0.1 M phosphate buffer at pH 6.8. P-nitrophenol's release kinetics were measured spectrophotometrically for 5 min with intervals of 30 seconds at 405 nm. Acarbose was used as reference.^[11] The IC₅₀ (concentration required to decrease the reaction rate to 50%) was then determined from the concentration-dependence curve.

% Inhibition = Absorbance of control – Absorbance of test / Absorbance of control X 100

where Ac and Ae are the absorbance of the control and extract respectively. The concentration of extract resulting in 50 % inhibition of enzyme activity (IC₅₀) was determined graphically.

RESULTS AND DISCUSSION

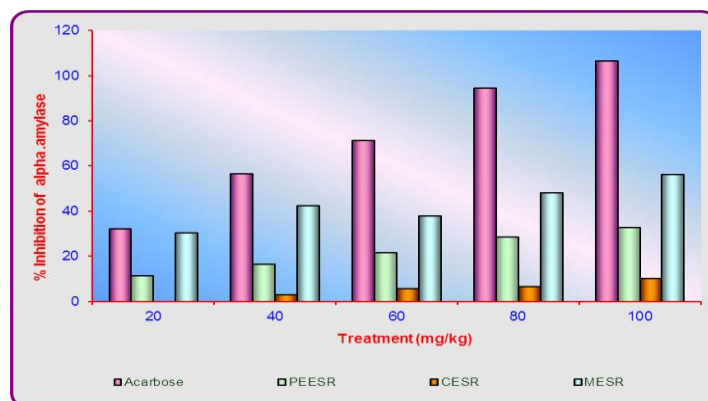
Diet rich in carbohydrate causes sharp rise in the blood glucose level as the complex carbohydrates in the food is rapidly absorbed in the intestine aided by the α -glucosidase enzyme which breaks disaccharides into absorbable monosaccharides, α -glucosidase inhibitor inhibits the disaccharide digestion and impedes the postprandial glucose excursion to enable overall smooth glucose profile.^[12] Management of blood glucose is essential in treatment of diabetes and for decreasing the risk of microvascular and macrovascular complications. Many antidiabetic plant extracts have been in use to inhibit starch digesting enzymes and maintaining the blood glucose level and thus provide protection to human against hyperglycemia. Acarbose represents a pharmacological approach to achieving the metabolic benefits of a slower carbohydrate absorption in diabetes, by acting as a potent competitive inhibitor of intestinal α -glucosidases. Acarbose molecules attach to the carbohydrate binding sites of α -glucosidases, with a much higher affinity constant than the normal substrate.^[13] *In-vitro* α -amylase and α -glucosidases inhibitory activity data of Acarbose are given in Table-1. *In-vitro* α -amylase inhibitory activity of different extracts of *S. rebaudiana* data are given in Table-2. *In-vitro* α -amylase inhibitory activity of different extracts of *T. cordifolia* data are given in Table-3. *In-vitro* α -glucosidases inhibitory activity data of *S. rebaudiana* are given in Table-4.

Table 1. The percentage inhibition of α -amylase and α -glucosidase by Acarbose.

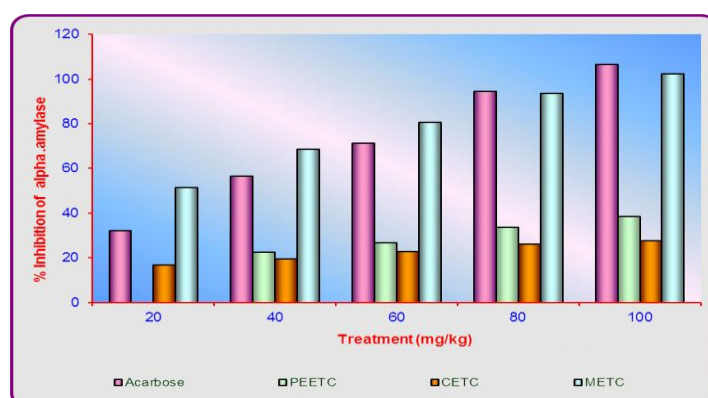
S. No	Concentration (μ g/ml)	% Inhibition of α -amylase	% Inhibition of α -glucosidase
1.	20	32.15	29.12
2.	40	56.64	42.16
3.	60	71.20	61.28
4.	80	94.36	69.3
5.	100	106.40	78.54
6.	IC ₅₀	36.22	50.43

Table 2. The percentage inhibition of α -amylase by different extracts of *S. rebaudiana*

S. No	Concentration ($\mu\text{g/ml}$)	% Inhibition of alpha amylase		
		PEESR	CESR	MESR
1.	20	11.46	00	30.36
2.	40	16.32	2.80	2.80
3.	60	21.54	5.72	37.82
4.	80	28.42	6.54	48.20
5.	100	32.78	10.24	56.16
6.	IC50	163.83	559.87	84.63

Graph: 1 α -amylase inhibition by extracts of *S. rebaudiana* in comparison with acarboseTable 3. The percentage inhibition of α -amylase by different extracts of *T.cordifolia*

S. No	Concentration ($\mu\text{g/ml}$)	% Inhibition of alpha amylase		
		PEETC	CETC	METC
1.	20	20.10	16.85	51.24
2.	40	22.54	19.42	68.62
3.	60	26.72	22.64	80.46
4.	80	33.48	25.98	93.52
5.	100	38.50	27.56	102.38
6.	IC50	151.51	258.34	14.03

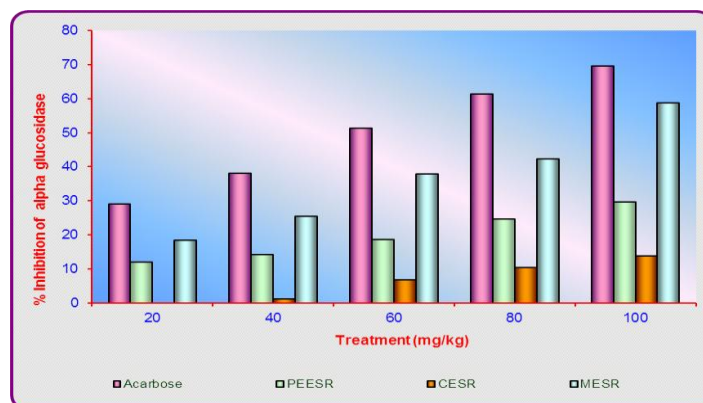
Graph: 2 α -amylase inhibition by extracts of *S. rebaudiana* in comparison with acarbose.

The inhibition of α -amylase might be attributed to several possible factors such as fiber concentration, the presence of inhibitors on fibers, encapsulation of starch and enzyme by the fibers present in the sample thereby

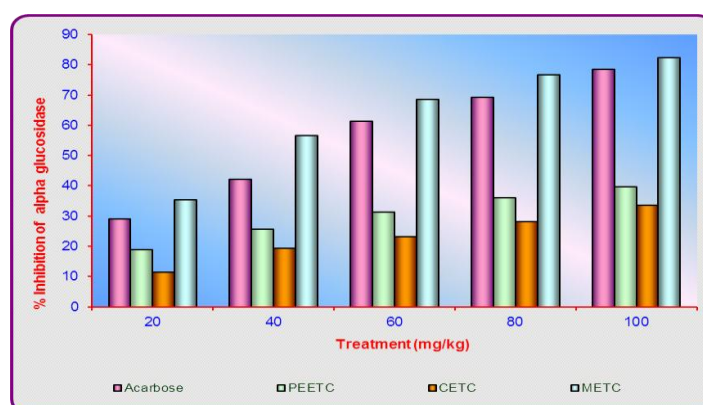
reducing accessibility of starch to the enzyme and direct adsorption of the enzyme on fibers leading to decreased amylase activity.^[14]

Table 4. The percentage inhibition α -glucosidase by different extracts of *S. rebaudiana*

S. No	Concentration ($\mu\text{g/ml}$)	% Inhibition of alpha glucosidases		
		PEESR	CESR	MESR
1	20	11.94	00.00	18.46
2	40	14.20	1.12	25.50
3	60	18.54	6.80	37.82
4	80	24.70	10.26	42.35
5	100	29.65	13.73	58.80
6	IC50	192.01	298.35	87.62

Graph: 3 α -glucosidase inhibition by extracts of *S. rebaudiana* in comparison with acarboseTable 4. The percentage inhibition of α -glucosidase by different extracts of *T. cordifolia*

S. No	Concentration ($\mu\text{g/ml}$)	% Inhibition of alpha glucosidase		
		PEETC	CETC	METC
1.	20	18.92	11.52	35.34
2.	40	25.74	19.26	56.67
3.	60	31.20	23.20	68.50
4.	80	36.00	28.14	76.72
5.	100	39.64	33.56	82.45
6.	IC50	136.47	161.94	35.64

Graph: 4 α -glucosidase inhibition by extracts of *T. cordifolia* in comparison with acarbose.

An effective strategy for the management of T2D patients involving the profound inhibition of intestinal α -glucosidases.^[15] is warranted. The glucosidase enzyme is located in the brush border of the small intestine and is required for the breakdown of carbohydrates and absorption of monosaccharids. The α -glucosidase inhibitory agent delay, the absorption of ingested carbohydrates and reducing the postprandial glucose.^[16] Several natural resources have been evaluated for their

ability to suppress the production of glucose from carbohydrates in the gut or glucose absorption from the intestine.^[17] Data indicate that methanol extracts of *T. cordifolia* and *S. rebaudiana* showed more inhibition as compared to petroleum ether and chloroform extracts. IC₅₀ values clearly indicate that METC showed higher α -amylase inhibition (IC₅₀ value-14.03 $\mu\text{g/ml}$) in comparison with acarbose (IC₅₀ value-36.22 $\mu\text{g/ml}$) followed by MESR (IC₅₀ value-84.63 $\mu\text{g/ml}$),

PEETC (IC₅₀ value-151.51 µg /ml), PEESR (IC₅₀ value-163.83 µg /ml), CETC (IC₅₀ value-258.34 µg /ml), and CESR (IC₅₀ value-599.87 µg /ml).

IC₅₀ values clearly indicate that METC showed higher α-glucosidase inhibition (IC₅₀ value-35.64 µg /ml) in comparison with acarbose (IC₅₀ value-50.43 µg /ml) followed by MESR (IC₅₀ value-87.62 µg /ml), PEETC (IC₅₀ value-136.47 µg /ml), CETC (IC₅₀ value-161.94 µg /ml), PEESR (IC₅₀ value-192.01 µg /ml) and CESR (IC₅₀ value-298.35 µg /ml).

CONCLUSION

In our study acarbose was used as the positive control; it inhibited the α-amylase and α-glucosidase activity with an IC₅₀ value 36.22 µg/ml for α-amylase and IC₅₀ value 50.43 µg/ml for α-glucosidase. IC₅₀ values of the METC showed that this is very potent α-amylase and α-glucosidase inhibitors in comparison with acarbose.

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