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Rajesh S Mony
Head (QA/QC), Vaidyaratnam Oushadhasala Pvt. Ltd., Vaidyaratnam Road, Thaikkattussery P.O, Ollur, Thrissur, Kerala, India

Corresponding Author:
Rajesh S Mony
Head (QA/QC), Vaidyaratnam Oushadhasala Pvt. Ltd., Vaidyaratnam Road, Thaikkattussery P.O, Ollur, Thrissur, Kerala, India

Alpha-glucosidase and Alpha-amylase inhibitory activity by poly herbal extract with reference to positive control acarbose

Rajesh S Mony

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Abstract
It is well known that type 2 diabetes is an endocrine disease. Among glucose-lowering medications, α glucosidase which is a membrane bound enzyme at the epithelium of the small intestine that catalyses the cleavage of glucose from disaccharide are effective for delaying for glucose absorption. i.e., inhibitors delay the absorption of ingested carbohydrates, reducing the postprandial glucose.
Pancreatic α-amylase is a key enzyme in the digestive system and catalyses the initial step in hydrolysis of starch to a mixture of smaller oligosaccharides consisting of maltose, maltotriose and a number of a-(l-6) and a-(1-4) oligoglucans. These are then acted on by alpha-glucosidases and further degraded to glucose which on absorbtion enters the blood-stream. Degradation of this dietary starch proceeds rapidly and leads to elevated PPHG (post-prandial hyperglycemia). It has been shown that activity of HPA (human pancreatic alpha-amylase) in the small intestine correlates to an increase in post-prandial glucose levels, the control of which is therefore an important aspect in treatment of type 2 diabetes. Pancreatic a-amylase inhibitors offer an effective strategy to lower the levels of post-prandial hyperglycemia via control of starch breakdown. By this study the Herbal extract demonstrated potent inhibitory activity for both the enzymes.

Keywords: α-glucosidase, α-amylase, *Salacia chinensis, Turmeric, Terminalia chebula, Momordica charantia, Trigonella foenum-graecum*

Introduction

A. Study of anti-diabetic activity by inhibitory activity of α-glucosidase

A.1 Objective

The aim of the present study is to test the alpha glucosidase inhibitory effect of the Herbal extract.

A.2 Principle

The αlpha glucosidase inhibitory activity is determined by measuring the release of 4-nitrophenol from 4-nitrophenyl-dglucopyranoside (4NPGP) using Spectrophotometer at 405 nm absorbance.

A.3 Materials

10 grams each of following crushed (coarse powder) drugs are extracted using Hydro alcoholic mixture (50:50) in a Soxhlet extraction apparatus. After siphoning for six cycle, extracted solvent is taken and evaporated to Dryness over a boiling water bath.

a) *Salacia chinensis* (Bark).
b) *Curcuma longa* (Tuber).
c) *Terminalia chebula* (Dried fruit).
d) *Momordica charantia* (Fruit).
e) *Trigonella foenum-graecum* (seed).

This is repeated for three times and yield is standardized to 6-8 gm.

Equipment and Reagents

1. 0.1M sodium phosphate buffer (pH 6.8).
2. 2mM 4-NPGP.
3. 0.1U alpha glucosidase (from yeast).
4. Positive control Acarbose (Prandase/Precose-Brand name).
5. Negative control without extract.
6. Solvent control (DMSO).

7. α Glucosidase (from yeast), Sigma (AGH 9U/mg).
8. 4 nitro phenyl 2D glucopyranoside (4-NPGP), Merck, Germany.
9. Bio-Rad microplate reader.

A.4 Method

The α Glucosidase (AGH) inhibitory activity was determined by Matsui *et al*., (1996) [1] and Andrade-Cetto (2008) [2]. For that the a known (for e.g. 20mg) quantity of the extract was dissolved in DMSO to make stock solution and serially diluted to make different concentrations. The solvent DMSO control, positive Acarbose drug control and (n=5) were also be maintained. The concentration of AGH inhibitor required to inhibit 50% (IC 50) of the AGH activity under the assay conditions was calculated (based on the absorbance). The Bio-Rad microplate absorbance reader was used for the assay.

A.5 Statistical analysis

The AGH inhibitory activity was statistically analyzed by one-way ANOVA followed by Tukey's test. Plasma glucose levels are expressed as the mean±standard error mean.

A.6 Results

The results of AGH inhibitory activity of extract are shown in table 1 and Fig.1. From this data extract exhibits potential AGH inhibitory activity. The percentage of inhibition was seen to increase with an increase in concentration of the drug (extract). The IC50value of the sample extract was observed as 25 µg/ml (P = <0.001). The IC50 value of Acarbose for alpha glucosidase is 128 µg/ml (ref. Andrade-Cetto *et al*., 2008) [2].

B. Study of anti-diabetic activity by inhibitory activity of α-amylase

B.1 Objective

The aim of the present study is to test the alpha amylase inhibitory effect of the Herbal extract.

B.2 Principle

The α amylase inhibitory activity is determined by measuring the presence of starch in the assay medium. A dark-blue colour indicates the presence of starch; a yellow colour indicates the absence of starch; brownish colour indicates partially degraded starch in the reaction mixture.

In the presence of inhibitors from the extracts the starch added to the enzyme assay mixture is not degraded and gives a dark blue colour complex No colour complex is developed in the absence of the inhibitor, indicating that starch is completely hydrolysed by a-amylase.

B.3 Materials

10 grams each of following crushed (coarse powder) drugs are extracted using Hydro alcoholic mixture (50:50) in a Soxhlet extraction apparatus. After siphoning for six cycle, extracted solvent is taken and evaporated to Dryness over a boiling water bath.

a) *Salacia chinensis* (Bark)
b) *Curcuma longa* (Tuber)
c) *Terminalia chebula* (Dried fruit)
d) *Momordica charantia* (Fruit)
e) *Trigonella foenum-graecum* (Seed)

This is repeated for three times and yield is standardized to 6-8 gm.

Equipment and Reagents

1. Sodium phosphate.
2. α-amylase.
3. Soluable starch, HCL.
4. Iodine reagent (5mM iodine & 5mM KI).
5. Sodium chloride.
6. Positive control Acarbose (Prandase/Precose –Brand name).
7. DMSO.
8. Bio-Rad Microplate reader.

B.4 Method

The αlpha-amylase inhibitory activity was determined by following the method of Sudha *et al*., 2011and Xiao *et al*., 2006. The total assay mixture composed of 40 µl 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride), 0.02 units of PPA solution and extract at concentration from 0.1-1.5 mgml-1 (w/v) were incubated at 37 °C for 10 min. Then soluble starch (1%, w/v) was added to each reaction well and incubated at 37 °C for 15 min. 1 M HCl (20 µl) was added to stop the enzymatic reaction, followed by the addition of 100 µl of iodine reagent (5 mM I2 and 5 mM KI). The absorbance was read at 620 nm on a microplate reader. The IC50 values were determined from plots of percent inhibition versus concentration. Solvent control DMSO, Positive control (Acarbose) and (n=8) were also be maintained.

B.5 Statistical analysis

The α-amylase inhibitory activity was statistically analyzed by one-way ANOVA followed by Tukey's test. Plasma glucose levels are expressed as the mean±standard error mean.

B.6 Result

The results of α-amylase inhibitory activity of extract are shown in table 2 and Fig. 2. From this data extract exerted potential α-amylase inhibitory activity. The percentage of inhibition increased with an increase in concentration of the drug (extract). extract exhibited appreciable alpha-amylase inhibitory effects with IC 50 50 µg/ml µg/ml (P = <0.001) when compared to Acarbose (IC50 = 90.8 µg/ml).

C. Comparative analysis of α-glucosidase & α- amylase inhibitory activity of extract

The 50% inhibitory activity (IC50) of α-glucosidase & α-amylase occurred at 25 µg/ml and 50 µg/ml respectively. The result showed that significant inhibition of both the enzymes by extract. It was observed that the α-amylase inhibitory activity was lower than α-glucosidase inhibitory activity.

Table 1: Inhibition of α Glucosidase activity of extract

S. No.	Concentration µg/ml	OD value	% inhibition
1.	2.5	0.059±0.001	2.98
2.	5	0.082±0.002	5.3
3.	10	0.186±0.025	15.68
4.	15	0.253±0.036	22.42
5.	20	0.380±0.049	35.1
6.	25	0.535±0.047	50.6
7.	30	0.600±0.083	57.12
8.	35	0.726±0.030	69.68
9.	40	0.854±0.035	82.48
10.	45	0.935±0.033	90.60
11.	50	1.048±0.052	101.92
12.	250 (Acarbose)	1.382±0.035	135.3
13.	Solvent (DMSO) blank	0.028±0.002	-

Table 2: Inhibition of α amylase activity of extract

S. No.	Concentration µg/ml	OD value (±SD)	% inhibition
1	2.5	0.072±0.001	1.8
2	5	0.101±0.002	4.733333
3	10	0.107±0.003	5.266667
4	15	0.223±0.002	16.86667
5	20	0.239±0.031	18.57
6	25	0.284±0.048	23.03333
7	37.5	0.336±0.002	28.2
8	50	0.563±0.019	50.93333
9	62.5	0.652±	100.466
10	250 (Acarbose)	1.193±0.125	113.9
11	Solvent (DMSO) Blank	0.056±0	-

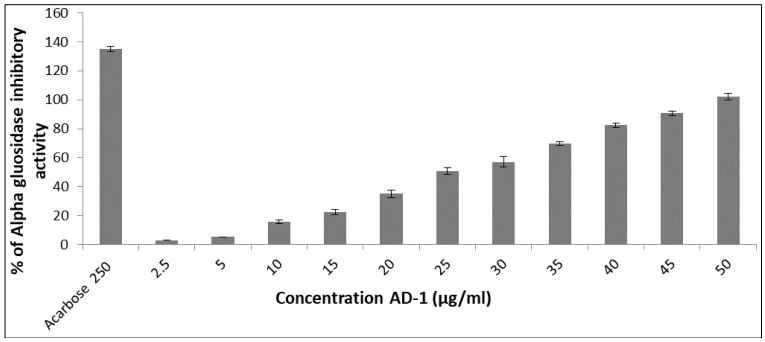


Fig 1: α Glucosidase inhibitory activity of extract

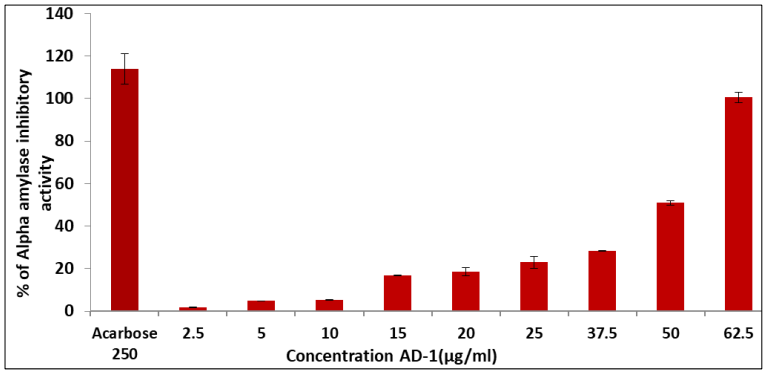


Fig 2: α-amylase inhibitory activity of extract

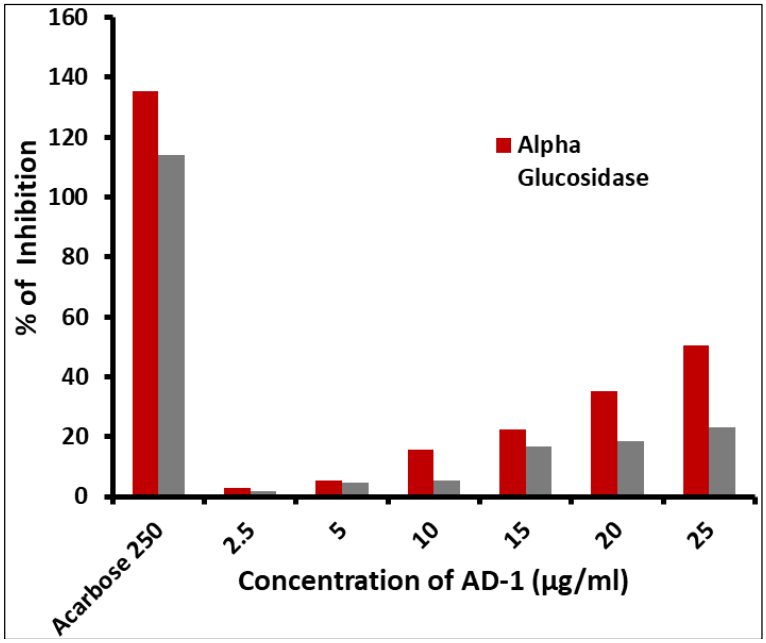
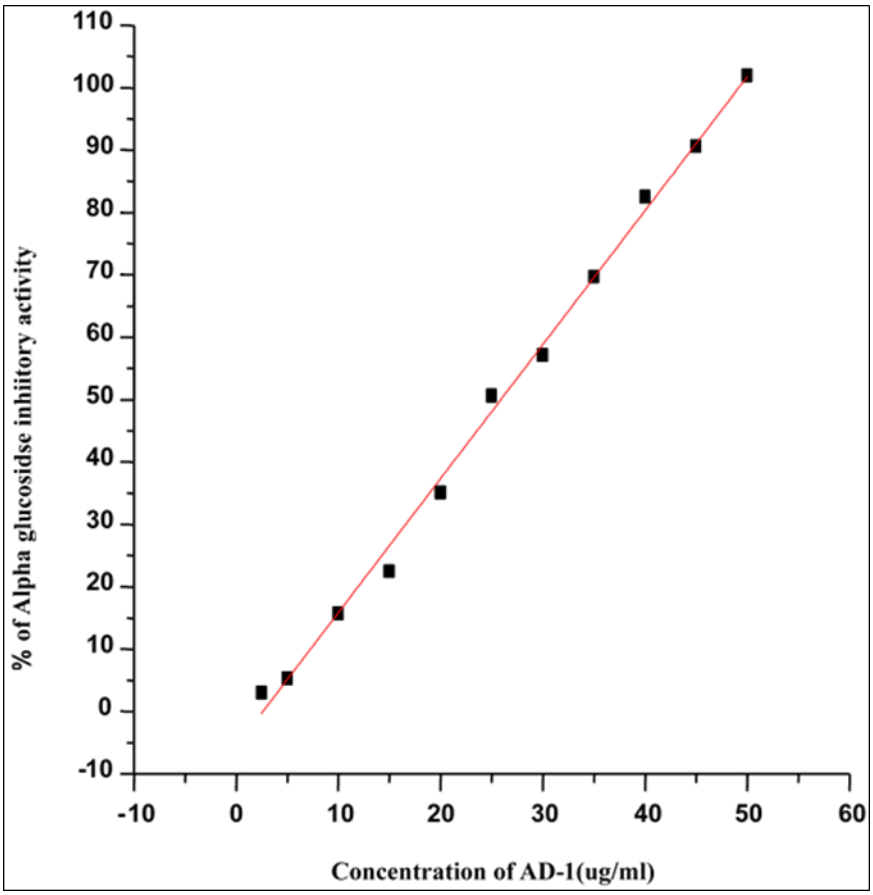
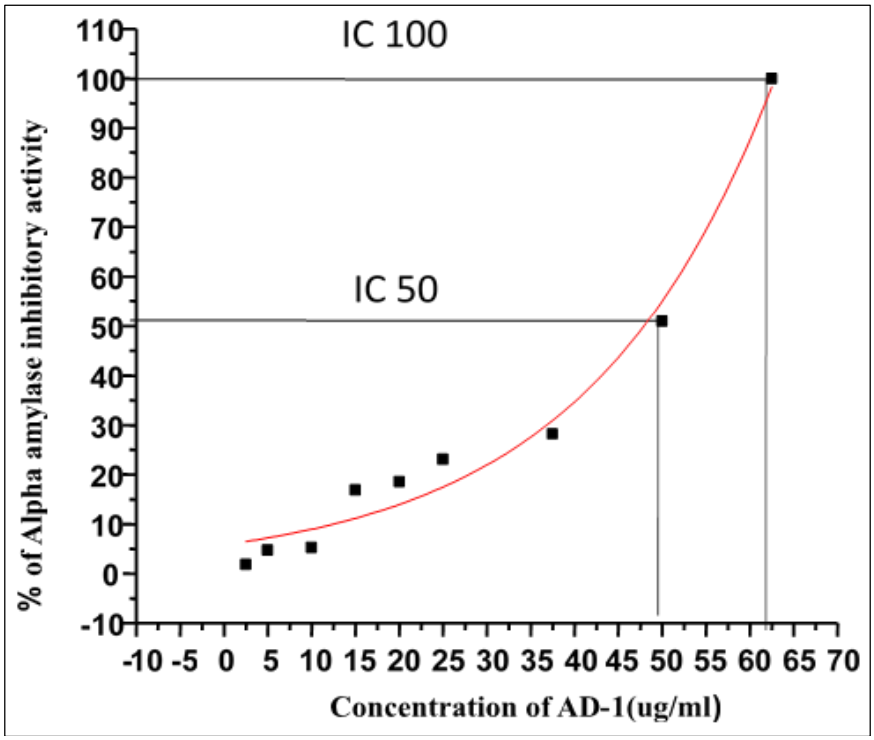


Fig 3: Comparative study α-Glucosidase and α-amylase inhibitor activity of AD1



% of Alpha glucosidase inhibitory activity Linear Fit of B

Fig 4: Alpha Glucosidase inhibition of extract at varying concentrations



% of Alpha amylase inhibitory activity Boltzmann of B

Fig 5: Alpha Amylase inhibition of extract at varying concentrations

The data is indicated as the mean± SEM; (n = 5). (p< 0.001)

D. Discussion and Conclusion

The present study was established to study the potential AGH inhibitory activity of extract. The inhibitory activity is dose dependent. From this experiment, the AGH inhibitory activity of extract was approximately 5 times more active than Acarbose. This implies the potential inhibitory activity of the given drug extract. The results obtained in this study based on extract exhibits a much lesser IC 50 value (25 µg/ml) and more hypoglycemic activity than that of the standard drug Acarbose (128 µg/ml (ref. Andrade-Cetto *et al.*, 2008)) [2].

Further this study supports that this drug could act as a hypoglycemic activity based on their inhibitory activity on glucose absorption at the gut level.

Drugs that reduce post-prandial hyper glycemia by suppressing hydrolysis of starch such as αlpha-amylase inhibitors have been found useful in the control of diabetes mellitus. Many traditional Indian systems of medicines have been reported for their anti-diabetic activities and are currently being used for the treatment of diabetes. This implies the potential alpha amylase inhibitory activity of the given drug extract. The results obtained in this study based on the given sample of extract exhibits a much lesser IC 50 value (50 µg/ml) and more hypoglycemic activity than that of the standard drug Acarbose (90.8 µg/ml (Tamil *et al*., 2010)) [7]. From this experiment, the α-amylase inhibitory activity of extract was approximately 2 times more active than Acarbose. Further this study supports that this drug (extract) could act as a hypoglycemic activity based on their competitive type of inhibition.

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