9.

α - Glucosidase Inhibitory Assay

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia (high blood sugar) with disturbance of carbohydrate, fat and protein metabolism, resulting from defects in insulin secretion and/or insulin action. Insulin is the hormone responsible for maintaining rising blood glucose level at a normal range (80 to 126 mg/dl).

Only monosaccharides, such as glucose and fructose, can be transported out of the intestinal lumen into the blood stream. Complex starches, oligosaccharides, and disaccharides must be broken down into individual monosaccharides before being absorbed through the intestine. This digestion is facilitated by enteric enzymes, including pancreatic α-amylase, and α-glucosidases that are attached to the brush border of the intestinal cells.

α -Glucosidase is an enzyme that hydrolyses 1,4- α glucosidic bonds. Oral anti-diabetic drugs are α-glucosidase inhibitors that prevent the digestion of carbohydrates (such as starch and table sugar). Hence, α-glucosidase inhibitors reduce the impact of carbohydrates on blood sugar levels. α - Glucosidase inhibitors act as competitive inhibitors of enzymes needed to digest carbohydrates. Inhibition of these enzyme systems reduces the rate of digestion of carbohydrates. Less glucose is absorbed because the carbohydrates are not broken down into glucose molecules.

There are naturally occurring substances, found mainly in plant materials such as fruits, leaves, seeds etc that have an inhibitory effect on α-glucosidase. These substances would be safer and cheaper alternatives to synthetic pharmaceutical drugs. In the following procedure α-Glucosidase inhibitory activity is determined by measuring the release of *p*-nitrophenol from *p*-nitrophenyl-α-D-glucopyranoside (PNPG) and monitored by the change in absorbance at 400 nm

In vitro Antidiabetic Assay

In vitro analysis for anti-diabetic activity of different natural samples such as herbs and food is performed by measuring the inhibition of α-glucosidase enzyme against the extracts of respective sources.

α-Glucosidase Inhibitory Assay

Reagents

1. Enzyme - α-glucosidase (type V - Sigma G-9259)
   The enzyme stock is prepared by dissolving 10 units of enzyme with 10ml of acetate buffer (pH 6) containing 0.2 % (w/v) bovine serum albumin. The stock enzyme solution is diluted five times with acetate buffer (pH 6.0) just before use.

2. Substrate - p-nitrophenyl-α-D-glucopyranoside (PNPG) (Sigma N-1377)
   20 mM PNPG solution is prepared with de-ionized water and store in an amber bottle at -20 °C.

3. 100 mM Sodium acetate buffer (pH 6.0) [0.81g sodium acetate in 100ml dist.H_2O]

4. 200 mM Sodium carbonate solution [10.6g Sodium carbonate in 500ml dist.H_2O]

Sample preparation

Water or solvent extracts of the sample can be used in the assay. All extracts should be filtered and centrifuged before concentrating to dryness (freeze drying, vacuum drying or rotary evaporation). A known quantity of the dried sample should be dissolved in water or acetate buffer for the assay.

Assay Procedure

1. Add buffer (see table 1) in to 2 ml vial
2. Add 250 µl of PNPG and mix well.
3. Pre-incubate at 37°C for 5 min.

4. Add 250 µl of enzyme solution and mix by inverting the vials
5. Incubate at 37°C for 30 min.
6. Cool down to room temperature and add 500 µl of Sodium carbonate and mix well.
7. Read the absorbance at 400 nm (use buffer as reference)

Experiment Design

Each experiment (for one sample) should include blank, control, treatment and treatment blank (Table 1.). In order to determined IC_{50} (inhibition concentration 50 %) of a sample five different concentration of the sample (within the linear region) should be used (for screening purposes 3 point IC_{50} can be used). At each concentration at least 3 independent replicates should be used. Generally, one experiment contains 34 vials (Blank 1; control 3; treatment 5x3; treatment blank 5x3).

Table 1. Experiment Design.

	Sample (µl)	Substrate (µl)	Enzyme (µl)	Buffer (µl)
Blank	0	250	0	750
Control	0	250	250	500
Treatment	50	250	250	450
Treatment blank	50	250	0	700

Calculation of %inhibition of α-glucosidase activity.

The following formula is used to calculate the %inhibition of α-glucosidase enzyme by a given concentration of the sample.

$$\% \text{ inhibition} = \frac{C_o - (S_o - Sb_o)}{C_o} \times 100$$

Where;

C_o is mean absorbance of controls

S_o is absorbance of sample

Sb_o is absorbance of respective sample blank

Calculation of IC_{50} [concentration that inhibits 50% of enzyme activity]

Plot the % inhibition activity vs. sample concentration and get the sample concentration at 50 % inhibition. (When the inhibition of highest tested concentration is below 40%, the inhibition activity may not be dose dependent and when the intercept of the curve is >15 % the calculation of IC_{50} may lead to a false value).