Studies on Antidiabetic Activity of Indian Medicinal Plants Using $\alpha$-Amylase and $\alpha$-Glucosidase Inhibitory Activity - A Pathway to Antidiabetic Drugs

R.M. Balaji, Chitra Jeyaram, K. Meenakshi Sundaram and M.S. Ramasamy

Abstract: Diabetes mellitus is a multifactorial disease. The nature has a plentiful sources as a remedy for this slow-killing condition. The aim of the present study was to assess the medicinal plants for anti-diabetic activity by using $\alpha$-amylase and $\alpha$-glucosidase inhibition assay. Anti-diabetic activities of medicinal plants were determined by $\alpha$-amylase and $\alpha$-glucosidase inhibition assay. Results revealed that methonolic extract of plants possesses inhibitory properties of alpha amylase and alpha glucosidase in vitro. The $\text{IC}_{50}$ of W. somnifera (0.54 mg/ml), O. sanctum (0.55 mg/ml) and A. paniculata (0.57 mg/ml) showed equal inhibitory activity with the positive control, acarbose (0.54 mg/ml). A. calamus ($\text{IC}_{50}$-0.3 mg/ml) showed nearly 50% higher activity than the acarbose in the present study. W. somnifera (0.58 mg/ml), O. sanctum (0.58 mg/ml) were lower than the positive control (acarbose, 0.8 mg/ml) against $\alpha$-glucosidase enzyme ($p<0.001$). In conclusion, the results indicate that crude extracts of multifarious plant possesses anti-diabetic effect through alpha amylase and alpha glucosidase inhibitory concentration ($\text{IC}_{50}$) and this research conformed the need of further study.

Key words: $\alpha$-amylase, $\alpha$-glucosidase, W. somnifera, O. sanctum, Anti-diabetic activity

INTRODUCTION

Diabetes mellitus (DM) is a multifactorial disease [1]. 171 million people had been affected by diabetes mellitus in 2000 and the ratio would be expected to be raised to 366 million in 2030. The antioxidants were used to reduce the risk of DM related diseases and the synthetic antioxidants such as Butylated Hydroxytoluene (BHT) and Butyl Hydroxyanisole (BHA) possessed side effects. It is an endocrine and metabolic disorder characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action or both. This is done by retarding the absorption of glucose through the inhibition of the carbohydrate hydrolyzing enzymes, alpha-amylose and alpha-glucosidase. Examples of such inhibitors which are in clinical use were acarbose, miglitol and voglibose. Plants continue to play an important role in the treatment of diabetes, particularly in developing countries where most people have limited resources and do not have an access to modern treatment. The increase in demand in industrially-developed countries to use alternative approaches to treat diabetes, such as plant-based medicines, is also due to the side effects associated with the use of insulin and oral hypoglycaemic agents [2]. Higher plants, animals and microorganisms are able to produce different type of alpha amylases and alpha glucosidases to regulate their digesting power [3, 4]. The enzymes may vary with the action potential based on the site of the activity. In animals alpha amylase inhibitors decrease the high glucose levels that can occur after a meal by slowing the speed with which alpha amylase can convert starch to simple sugars. This is a characteristic symptom that the diabetic people face low insulin, which prevents the effective glucose metabolism in the blood. So, they need to be given alpha amylase inhibitors in order to keep their glucose levels under control. The plants use alpha amylase inhibitors as a major defensive mechanism to prevent infestation [5]. Apart from anti-diabetic activity these plants also has anti-inflammatory, expectorant, anti-bacterial, anti-oxidant, anti-fertility, anti-viral, anti-cancer, anti-rheumatic, aromatic, anti-venom, anti-helminthic anti-allergic activity [6] etc. Alpha
glucosidase inhibitors are most useful in oral treatment of type 2 diabetic mellitus. These enzymes act as a preventing agent in the digestion of carbohydrates such as starch. Alpha glucosidase inhibitors act as competitive inhibitors of alpha glucosidase enzyme needed to digest carbohydrates. The intestinal alpha glucosidases hydrolyze complex carbohydrates to glucose and other monosaccharides in the small intestine. Inhibition of these enzyme systems reduces the digestion of carbohydrates. The existing synthetic drugs cause gastrointestinal side effects such as iarrhoea, flatulence, abdominal bloating etc. Therefore naturally existing alpha amylase and glucosidase inhibitors could be a valuable source for treating post prandial hyperglycemia with minimal side effects [7]. The present study was carried out to investigate the inhibitory potentials of the methanolic extracts on alpha amylase and alpha glucosidase, the key enzymes responsible for carbohydrate hydrolysis.

MATERIALS AND METHODS

Plant Material: Fresh parts of eight plants Coriandrum sativum (leaves and stems), Withania somnifera (leaves and roots), Ocimum sanctum (leaves), Glycyrrhiza glabra (leaves and roots), Acorus calamus (rhizome), Aegle marmelos (leaves), Cyamopsis tetragonoloba (pod), Nigella sativa (seed) and Rographis paniculata (leaves) were collected from the harma Herbal Research Foundation, Chennai.

Preparation of Plant Extracts: The plant materials were washed with distilled water without squeezing to remove debris and dust particles. Then it was shade dried under the room temperature until all the water molecules evaporated. After drying, the plant material was powdered. The powdered plant material (250g) was extracted in the methanol for a period of 48hours. After filtration, the process was repeated 2 times using additional methanol each time. Concentrated methanol extracts is obtained by distillation process.

Alpha Amylase Inhibition Assay: Screening of plant extracts and acarbose (as positive control) of anti-diabetic activities and being used in ayurveda for the treatment of diabetes. Therefore, screening of alpha glucosidase (EC 3.2.1.20) and alpha amylase (EC 3.2.1.1) enzymes in plant has received more attention.

RESULTS

Many herbal extracts has been reported for their anti-diabetic activities and being used in ayurveda for the treatment of diabetes. Therefore, screening of alpha glucosidase and alpha amylase enzymes in plant has received more attention.
Table 1: Inhibitory potential of plant methanolic extracts on \( \alpha \)-amylase and \( \alpha \)-glucosidase enzymes. The medicinal plants were extracted with methanol and the extracts were tested for their \( \alpha \)-amylase and \( \alpha \)-glucosidase inhibitory activity (in triplicates). IC\(_{50}\) value was calculated using non-linear regression.

<table>
<thead>
<tr>
<th>S.no</th>
<th>Name of the plant</th>
<th>( \alpha )-amylase IC(_{50}) (mg/ml)</th>
<th>( \alpha )-glucosidase IC(_{50}) (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Acarbose</td>
<td>0.54</td>
<td>0.8</td>
</tr>
<tr>
<td>2.</td>
<td>G. glabra</td>
<td>2.8</td>
<td>1.22</td>
</tr>
<tr>
<td>3.</td>
<td>W. somnifera</td>
<td>0.54</td>
<td>0.58</td>
</tr>
<tr>
<td>4.</td>
<td>O. sanctum</td>
<td>0.55</td>
<td>0.58</td>
</tr>
<tr>
<td>5.</td>
<td>C. sativum</td>
<td>2.24</td>
<td>1.25</td>
</tr>
<tr>
<td>6.</td>
<td>A. paniculata</td>
<td>0.57</td>
<td>1.13</td>
</tr>
<tr>
<td>7.</td>
<td>A. marmelos</td>
<td>2.49</td>
<td>0.8</td>
</tr>
<tr>
<td>8.</td>
<td>A. calamus</td>
<td>0.3</td>
<td>1.26</td>
</tr>
<tr>
<td>9.</td>
<td>N. sativa</td>
<td>1.26</td>
<td>1.53</td>
</tr>
</tbody>
</table>

Inhibition of \( \alpha \) Amylase Activity by Selected Medicinal Plants: The inhibitory activity of methanolic extracts of C. sativum, W. somnifera, O. sanctum, G. glabra, A. calamus, A. marmelos, C. tetragonaloba, N. sativa was investigated for \( \alpha \) amylase inhibiting activity and the results were shown in Figure 1. Compared to positive control, the plant extracts showed effective activity in the assay. The IC\(_{50}\) of W. somnifera (0.54 mg/ml), O. sanctum (0.55 mg/ml) and A. paniculata (0.57 mg/ml) showed equal inhibitory activity with the positive control, acarbose (0.54 mg/ml). A. calamus (IC\(_{50}\)=0.3 mg/ml) showed nearly 50% higher activity than the acarbose in the present study (Table 1). The present study indicated that A. calamus could be useful in management of \( \alpha \)-amylase inhibition among the studied plants.

Inhibition of \( \alpha \) Glucosidase Activity by Selected Medicinal Plants: The inhibitory activity of methanolic extracts of C. sativum, W. somnifera, O. sanctum, G. glabra, A. calamus, A. marmelos, C. tetragonaloba, N. sativa was investigated for \( \alpha \) glucosidase inhibiting activity and the results was shown in Figure 2. The IC\(_{50}\) of W. somnifera (0.58 mg/ml), O. sanctum (0.58 mg/ml) were lower than the positive control (acarbose, 0.8 mg/ml) as showed in Table 1. A. marmelos showed similar activity with the positive control.

The other plants showed slightly higher concentration than the acarbose in the present study. This study confirmed that the W. somniferum and O. sanctum have higher ability to reduce the risk of postprandial hyperglycemia.

Phytochemical Analysis: The preliminary phytochemical screening tests for the methanolic extracts of the selected medicinal plants in the present study revealed the presence of alkaloids, proteins, amines, amino acids, flavonoids, tannins, phenols, carbohydrates, terpenes, steroids, hydroquinone derivatives (Table 2).

Any of these secondary metabolites, singly or in combination with others could be responsible for the anti-diabetic activity of the plant extract as showed in Figures 1, 2 and Table 1.
Fig. 1: Inhibition of α-amylase activity of eight plants (% of inhibition ±SEM) (n=3)

Fig. 2: Inhibition of α-glucosidase activity of eight plants (% of inhibition ±SEM) (n=3)

**Statistical Analysis:** Experimental results were presented as the mean ± standard Error (SE) of three parallel measurements. The statistical analyses were performed by one-way ANOVA, followed by Dunnett’s t. test. The difference was considered to be statistically significant when the p value was less than 0.05.

**DISCUSSION**

α-glucosidase (EC 3.2.1.20) and α-amylase (EC 3.2.1.1) enzymes inhibition play a major role in type 2 diabetic patients. This rise of type 2 DM has become a serious concern in the medical world, which accounts for more than 9% death globally. This awakened the medicinal practitioners of allopathy and other systems of medicines to search for novel therapeutic agents to stalk the DM progress. Although the research has given prominent drugs since last decade, drug resistance is also on the upswing in the patients. This particular concern has urged us to deal with effective approaches. One of the effective strategies to monitor blood glucose for diabetes mellitus is to either inhibit or reduce the production of glucose from the small intestine in type II diabetes patients is one of the effective strategy. Many α-amylase and α-glucosidase inhibitors impede with the carbohydrate digestion and aid in controlling the glycemic content at better ways. The synthetic drugs end up [13] in various side effects, but, the naturally occurring inhibitors due to their structural diversity, give a promising hope for being as best source for the same. This scenario motivates us to explore, biologically active enzyme inhibitors especially from the higher plants.
CONCLUSION

Many herbal extracts have been reported to have the anti-diabetic activity. These have been used directly or indirectly by modern medicines. In this present study the methanolic extract of nine plants were screened for anti-diabetic activity using α-amylase and α-glucosidase enzyme inhibitory activity. The concentration dependent inhibitory activity of eight crude methanolic extracts were subjected to screening in the present study. This analysis exhibited higher Α-amylase inhibitory activity than α-glucosidase inhibitory activity among the plant extracts. The phytochemical analysis of the methanolic extract of eight plants revealed the presence of alkaloids, phenols, flavonoids, tannins, terpenes, hydroquinone, proteins and glycosides.

REFERENCES


