In-vitro evaluation of the anti-diabetic activity of alcoholic extracts of certain plants belonging to families meliaceae and fabaceae

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ABSTRACT

The leaves of 4 plants belong to families Meliaceae and Fabaceae were exhaustively extracted with hot 80% Ethanol, under reflux. The dry residues of the alcoholic extracts were tested using Sucrase enzyme inhibitory activity test to evaluate their anti-diabetic activity. The alcoholic extracts exhibited significant in vitro anti-diabetic activity.

INTRODUCTION

Herbal medicines have been used since centuries by different cultures worldwide for treatment of diabetes. Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin action, insulin secretion, or both[1]. Control of post-prandial hyperglycemia is critical in the early treatment of diabetes mellitus[31] as it could induce non enzymatic glycosylation of various proteins, resulting in the development of chronic complications such as, micro- and macro-vascular diseases[39], and it has also been proposed as an independent risk factor for cardiovascular diseases[44,5]. Postprandial hyperglycemia can be controlled by decreasing the absorption of glucose through the inhibition of enzymes responsible for hydrolysis carbohydrate such as sucrase enzyme, in the digestive tract[6]. Phytochemicals exhibit their hypoglycemic effect by several mechanisms, such as, inhibition of carbohydrate metabolizing enzymes, manipulation of glucose transporters, α-cell regeneration, and enhancing the insulin releasing activity[37]. The present study deals with the investigation of the in-vitro anti-diabetic activity of several 4 plants belong to families Meliaceae (such as Azadirachta indica) and Fabaceae (such as Acacia glauca, Acacia Senegal and Acacia nilotica) through the performance of Sucrase inhibitory activity test.

MATERIALS AND METHODS

Preparation of the extracts

Powdered, air-dried leaves of Azadirachta indica, Acacia glauca, Acacia Senegal and Acacia nilotica (250 g) were exhaustively extracted with hot 80% Ethanol (4x1 L), under reflux. After filtration the extracts were concentrated under vacuum at 50°C. The dry residues obtained were kept in the refrigerator.

Assay of sucrase inhibitory activity

A crude enzyme solution of rat intestinal sucrase
enzyme was prepared according to the method of Dahlqvist[8]. The effect of samples on sucrase enzyme activity was assayed according to the method of Honda and Hara[9]. Enzyme solutions (10 µL) were incubated together for 10 min at 37°C, and the volume was made up to 200 µL with maleate buffer (pH 6.0) in case of control or up to 200 µL with buffer solubilized sample (100 µg/mL in maleate buffer with pH 6.0). The enzyme reaction was initiated by adding 100 µL of sucrose solution (60 mM). After 30 min, the reaction was terminated by adding 200 µL of 3,5-dinitrosalysilic acid reagent and placing the mixture in a boiling water bath for five min. The absorbance of solution was read at 540 nm. The percent inhibitory activities were calculated using the following formula:

\[
\text{% Inhibition} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100
\]

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample), and the Abs sample is the absorbance of the test sample; an untreated enzyme solution was used as the control. All experiments were carried out in triplicate.

**RESULTS**

**Results of the anti-diabetic activity (Sucrase inhibitory activity)**

*Acacia nilotica* decreases the activity of sucrase enzyme by (61.38 ± 2.46) followed by *Acacia glauca* (59.43 ± 3.42), *Acacia Senegal* (47.54 ± 1.33) and *Azadirachta indica* (41.34 ± 2.37), as shown in TABLE 1.

**TABLE 1: Sucrase enzyme activity**

<table>
<thead>
<tr>
<th>Tested extract</th>
<th>Sucrase %±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Azadirachta indica</em></td>
<td>41.34 ± 2.37</td>
</tr>
<tr>
<td><em>Acacia glauca</em></td>
<td>59.43 ± 3.42</td>
</tr>
<tr>
<td><em>Acacia Senegal</em></td>
<td>47.54 ± 1.33</td>
</tr>
<tr>
<td><em>Acacia nilotica</em></td>
<td>61.38 ± 2.46</td>
</tr>
</tbody>
</table>

SD is the standard deviation

**DISCUSSION**

It is a general opinion that medicinal plants inhibit sucrase activity due to the presence of several possible factors and mechanisms, such as polyphenolic concentration[14]. In our present investigation, the effect of various extracts of tested plants on carbohydrate hydrolyzing enzyme, namely, rat intestinal sucrase, have been studied using *in vitro* model systems. The extracts of tested plants significantly inhibited sucrase enzyme activity (Figure 1). The Sucrase inhibitory activity of *tested plants* supposed to be due to the presence of flavonoid glycosides and/or hydrolysable tannins[10]. With a constant rise in the incidence of type II diabetes around the world it appears that more anti-diabetic drugs with complementary mechanisms of action should be developed, in order to achieve durable glycemic control by inhibiting, in a reversible way, the hydrolysis of disaccharides and the ultimate steps of the digestion of dietary polysaccharides, to reduce the rise of postpran-
dial blood glucose in diabetics\textsuperscript{[11]}. Rat intestinal sucrase occurs as a complex of sucrase and isomaltase, which converts sucrose into glucose\textsuperscript{[12]}. So the tested plants (i.e. \textit{Azadirachta indica}, \textit{Acacia nilotica}, \textit{Acacia glauca} and \textit{Acacia Senegal}) may offer a support to control carbohydrate hydrolysis in diabetic disease.

**CONCLUSION**

The ethanol extract of the leaves of \textit{Azadirachta indica} (family \textit{Meliaceae}), \textit{Acacia nilotica}, \textit{Acacia glauca} and \textit{Acacia Senegal} (family \textit{Fabaceae}) leaves exhibited significant in-vitro anti-diabetic activity using Sucrase inhibitory activity test. But previous studies observed that those plants which belonging to the family \textit{Fabaceae} (Mimosaceae) contain the toxic alkaloid mimosine\textsuperscript{[13,14]}, so we recommend in vitro and in-vivo toxic test to be done to evaluate their safety to be used as complementary drugs to help main medicines in treatments of diabetic patients.

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