Alpha Amylase Inhibition Activity of Some Plants Extract of *Teucrium* Species

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Abstract: Diabetes Mellitus is a metabolic disorder characterized by high blood glucose level caused due to deficiency of insulin secretion or insulin action. The inhibition of carbohydrate hydrolyzing enzymes such as α-amylase can be an important strategy in the postprandial blood glucose level in patients with type II diabetes. Plants contain different chemical constituents with potential for inhibition of α-amylase and hence maybe used as therapeutic. Three plants of *Teucrium* species were tested for α-amylase inhibition. Different concentrations of extracts were incubated with enzyme substrate solution and the activity of enzyme was measured. Also Acarbose was used as the standard inhibitor. Three plant extracts showed inhibition of α-amylase. The α-amylase inhibitory activities of extracts are *T. polium* > *T. oliverianum* > *T. Orientale*. The IC₅₀ value of Hydroalcoholic extract of *T. polium* was 3.63 mg/ml. The IC₅₀ value of *T. oliverianum* and *T. Orientale* were 3.86 and 13.93 mg/ml, respectively, when compared with acarbose (IC₅₀ value 0.037 mg/mL). This study supports that plants of *Teucrium* species exhibit considerable α-amylase inhibitory activities. All the fractions (hydroalcoholic, dichloromethane and ethyl acetate) had potent inhibitory effects on the α-amylase activity. However, the lowest inhibitory potency was observed for the dichloromethane fraction. Determination of the type of α-amylase inhibition by these plant extracts could provide by successful use of plant chemicals as drug targets.

Key words: *T. polium* • *T. oliverianum* • *T. orientale* α-Amylase Inhibitory Effects • Diabetes Mellitus

INTRODUCTION

Diabetes Mellitus (DM) is an extended metabolic disease of several etiologies characterized by chronic hyperglycemia with disorder of carbohydrate, fat and also protein metabolism resulting from defects in insulin secretion, insulin action or both of them [1]. The control of hyperglycemia is very important in the treatment of all forms of diabetes by reason that in the long term, acute and chronic complications can happen when the blood glucose concentration is not kept in the normal range [2, 3]. The drugs widely used in clinic to manage or handle diabetes are insulin, sulfonylureas, biguanide, glycosidase inhibitors, aldosederase inhibitor, thiazolidinediones, carbamoylmethyl benzoic acid [4]. Available therapies for treating type II diabetes consist of stimulation of endogenous insulin secretion, increase of activity of insulin at target tissues as well as inhibition of alpha-amylase enzyme to reduce the degradation of starch to decreasing glucose [5, 6].

One unique approach for decreasing postprandial hyperglycemia is to reduce or slow down dietary carbohydrate digestion. Inhibiting the enzymes involved, such as the α-amylase and α-glucosidase enzymes, is a strong the therapeutic goal of controlling the postprandial glycemic reaction [7]. Also, α-amylase inhibitors are one of the anti-diabetic drug families, of which Acarbose is the most well-known. These drugs have a very strong advantage and are suitable for healing noninsulin-dependent diabetes mellitus (type-2 diabetes)
[8, 9], but also induce gastrointestinal side effects that reduce their use in a Preventive approach [10]. Accordingly, several researchers are investigating and developing nutritional strategies to perfectly control postprandial glycemia, without inducing negative occasions in the digestive system, medicinal herbs due to easy accessibility and also lower negative effects have a special place in medicine to treat various diseases [11-13]. Active compounds derived from medicinal herbs may also be a source of new α-amylase inhibitors. Furthermore, some plants include a great amount of phenolic substances, therefore accordingly, they have antioxidant activity [14].

Teucrium species plants, which are rich sources of flavonoids, anthraquinone, steroid and phlobatannin, have long been used to control hypertension, inflammations, gastrointestinal disorders [15-18] and also leaves of Teucrium species have long been utilized to management diabetes mellitus worldwide [19]. The hypoglycemic results of Teucrium species have been supported by numerous researches, However, at the least to our information, there are no previous studies on the α-amylase inhibitory effect on Teucrium species. In this study, Teucrium species medicinal plants (including T. oliverianum, T. Orientale and T. polium) that are used to manage different diseases were screened to discover possible new α-amylase inhibition activity using in vitro α-amylase inhibition assay.

**MATERIALS AND METHODS**

**Chemical Materials:** All chemical substances were obtained from Sigma Aldrich (USA) and also Merck (Germany) companies. The chemicals were of analytical grade.

**Plant Materials:** Teucrium species young whole plants were collected from Ahvaz Province, Iran in May 2013. The plant was botanically identified and authenticated by local Plant Biotechnologist, Department of Natural Resources, Khuzestan, Iran. The plants were dried at ambient temperature (30-40°C) for 25-30 days Then they were into fine powder.

**Extraction and Fractionation Procedure:** The air-dried plants were carefully cleaned under running tap water in order to remove any type of pollution and also air-dried in shade, powdered in grinder and passed via sieve of mesh size no-40. The extraction was handled aqueous ethanol (30: 70) mixture by hot Soxhlet extraction technique and the extract was condensed in a rotary evaporator under decreased pressure, crude hydroalcoholic extract (HAC). The dried crude extract was conserved in airtight glass bottle at 4-8°C. With serious model of indomethacin-induced ulcer, HAC was discovered extremely active also it was after that fractionated with solvents of improving polarity. At first, HAC was partition-fractionated with 1 : 1 (volume ratio) of n-hexane and ethanol (50%) and the mixture was shaken intensely and kept for about 30 min to make the two layers separate. The upper layer comprising of n-hexane was removed and concentrated in a rotary evaporator to obtain n-hexane extract. The same procedure was repeated with the bottom part layer by utilizing equivalent volume of additional solvents such as, dichloromethane and ethyl acetate, DCMC and EAC, respectively.

**Assessment of α-amylase Inhibition:** The starch solution (0.5% w/v) was obtained by stirring and boiling 0.25 g of soluble potatostarch in 50 ml of deionized water for 15 min. The enzyme solution (0.5 unit/ml) was prepared by mixing 0.001 g of α-amylase (EC 3.2.1.1) in 100 ml of 20 mM sodium phosphate buffer (pH 6.9) containing 6.7 mM sodium chloride. The extracts and/or fractions were dissolved in DMSO to give suitable concentrations for the assay. The color reagent was a solution containing 96 mM 3,5-dinitrosalicylic acid (20ml ), 5.31 M sodium potassium tartrate in 2 M sodium hydroxide (8 ml) and deionized water (12 ml ). One ml of each the extracts and/or fractions and 1 ml of the enzyme solution were mixed in a test tube and incubated at 25°C for 30 min. To 1ml of this mixture was added 1 ml of the starch solution and the tube was further incubated at 25°C for 3 min. Then, 1 ml of the color reagent was added and the stopped tube was placed into an 85°C water bath. After 15 min, the reaction mixture was removed from the water bath and cooled thereafter, diluted with 9 ml distilled water and the absorbance value determined at 540 nm using a Shimadzu Multispect-1501 spectrophotometer (Japan).

Individual blanks were prepared for correcting the background absorbance. In this case, the color reagent solution was added before the addition of starch solution and then, the tube was placed into the water bath. Then, the method was followed as described above. Controls were conducted in an identical manner, replacing extracts and/or fractions with 1 ml DMSO. Acarbose solution (at the concentrations of 0.0094, 0.0184, 0.036, 0.07, 0.11, 0.21 μg/ml) was used as positive control. The inhibition percentage of α-amylase was assessed by the following formula:
I\(\alpha\)-amylase % = \(100 \times (\Delta A_{\text{Control}} - \Delta A_{\text{Sample}}) / \Delta A_{\text{Control}}\)

\(\Delta A_{\text{Control}} = A_{\text{Test}} - A_{\text{Blank}}\)

\(\Delta A_{\text{Sample}} = A_{\text{Test}} - A_{\text{blank}}\)

The I\(\alpha\)-amylase % was plotted against sample concentration and a logarithmic regression curve was obtained in order to calculate the IC50 value which is concentration of sample (mg/ml) necessary to decrease the absorbance of \(\alpha\)-amylase solution by 50%.

**Statistical Analysis:** All of the statistical analyses were performed using GraphPad Prism 3.02 statistical. The data were expressed as mean ± SEM for here experiments in each group. The IC50 values were estimated by nonlinear curve-fitting and presented as their respective 95% confidence limits.

**RESULTS AND DISCUSSION**

Diabetes mellitus is a chronic metabolic disorder identified by hyperglycemia due to insulin insufficiency and/or insulin resistance contributing to excess blood glucose. It affected approximately 171 million people all around the world in the year 2000 and the number is projected to increase to around 366 million by 2030 [19]. Management of the blood glucose level is an essential approach in the control of diabetes complications. Inhibitors of carbohydrates hydrolysing enzymes (\(\alpha\)-amylase and \(\alpha\)-glucosidase) have been helpful as oral hypoglycemic medicines for the control of hyperglycemia exclusively in patients with type-2 diabetes mellitus [5, 6, 20, 21]. Inhibition of these enzymes holds of carbohydrate digestion and extend the total carbohydrate digestion time, leading to a decrease in the rate of glucose absorption and therefore reducing the postprandial plasma glucose rise [22]. Commonly used synthetic inhibitory drugs such as acarbose and miglitol 18 possessed negative effects.

Traditionally, various parts of herbs were used directly as a medication. Clinically effective substances are now being obtained from plants, even those that have not been categorized before as medicinal herbs. Recently, traditional medicine (Phytotherapy) is often used to treat several diseases, besides modern medicine. A lot of natural extracts have been reported to have antidiabetic activities and are utilized for the treatment of diabetes. Herbal extracts have been used perfectly or ultimately for the processing of numerous modern medicines [10-12].

*Teucrium* species are the most widespread plant in the world that is used for medical purposes. In traditional medicine, this plant is used as a diuretic, treatment of gout, rheumatism, hyperlipidemia, hyperglycemia, hypertension, spleen and liver disease [14-17].

In this work, the inhibition activities of the extracts obtained from *T. polium*, *T. Orientale* and *T. oliverianum* were investigated on the \(\alpha\)-amylase enzyme and IC50 values were calculated. Among the plants studied, three species, demonstrated inhibitory concentration dependent effects on the \(\alpha\)-amylase activity. The strongest activity (at Concentration: 25mg/ml) was shown by the HAC extract of *T. polium* (97.77%) *T. Orientale* DCMC extract revealed a weaker activity. It is possibly because of the fact that at high extract concentrations, there is certainly a confirmation alter derived from authentic of substances to the enzyme [23, 24]. The percentage inhibition and IC50 values displayed by each extract is shown in Tables 1, 2 and 3. The IC50 value of the positive control, acarbose, was measured as 0.037 mg/mL.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Extracts</th>
<th>Concentration (mg/mL)</th>
<th>Inhibition (%)</th>
<th>IC50 (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. Polium</em></td>
<td>DCMC</td>
<td>1.56</td>
<td>41.59 ± 0.64</td>
<td>3.01</td>
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<td></td>
<td></td>
<td>3.12</td>
<td>50.45 ± 0.73</td>
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<td></td>
<td></td>
<td>6.25</td>
<td>59.34 ± 1.01</td>
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<td></td>
<td></td>
<td>12.5</td>
<td>68.20 ± 0.92</td>
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<td></td>
<td></td>
<td>25</td>
<td>77.07 ± 0.49</td>
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<td><em>EAC</em></td>
<td></td>
<td>1.56</td>
<td>49.32 ± 0.56</td>
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<td></td>
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<td>3.12</td>
<td>57.83 ± 1.11</td>
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<td>6.25</td>
<td>66.37 ± 0.97</td>
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<td></td>
<td>12.5</td>
<td>74.89 ± 1.21</td>
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<td>25</td>
<td>83.41 ± 0.83</td>
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<tr>
<td><em>HAC</em></td>
<td></td>
<td>1.56</td>
<td>25.36 ± 0.12</td>
<td>3.63</td>
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<tr>
<td></td>
<td></td>
<td>3.12</td>
<td>43.45 ± 0.63</td>
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<td>6.25</td>
<td>61.58 ± 0.76</td>
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<td>12.5</td>
<td>79.67 ± 1.02</td>
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<td>25</td>
<td>97.77 ± 1.21</td>
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The outcomes of this study show that the administration of some of Teucrium species may possibly control the postprandial blood glucose ranges and confirm the use of these herbs suggested as a treatment of diabetes in Traditional Medicine in Iran and other areas. *T. polium* and *T. oliverianum* extract displayed a good inhibitory activity on $\alpha$-amylase. Generally, our *in vitro* studies indicated that Teucrium species, especially *T. polium* and *T. oliverianum*, can function as organic $\alpha$-amylase inhibitors and might possess beneficial anti-diabetic property in the type II diabetes mellitus.

This inhibitory activity of the Teucrium species extracts may be as a result of the existence of various phytochemicals like flavonoids, tannins, saponins, anthraquinone, steroid, phlobatannin, terpenoid, in them [25]. Previous research studies on $\alpha$-amylase inhibitors identified from medicinal herbs recommend that a number of capability inhibitors belong to flavonoid class that has features of inhibiting $\alpha$-amylase activities [26]. In general, the enzyme inhibitory activity of plant extracts not just rely on the amount of especial phytochemicals but additionally may depend on the quality of especial phytochemicals. Additional researchers also have reported that biological activities of phytochemicals depend on the extent of hydroxylation and also conjugation [27, 29]. Further, *in vitro* and *in vivo* research are required to confirm the present observations, findings on the isolation of active substances contained in the extract and *in vivo* studies are necessary to recognize a potential chemical substance entity for clinical utilize in the therapy of diabetes and other related disorders.

**RESULTS**

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REFERENCES


