PharmacologyOnLine
Archives • 2021 • vol. 2 • 1450-1456

INHIBITION OF PANCREATIC α- GLUCOSIDASE BY WATER EXTRACTS OF SOME HERBAL MIXTURES

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Abstract

Diabetes mellitus is an important social and medical problem, as it causes the development of dangerous complications that lead to disability and mortality. This disease is characterized by a multi-vector pathogenesis that requires a comprehensive approach to treatment. Inhibition of pancreatic α-glucosidase activity is an important mechanism in the prevention and treatment of type 2 diabetes.

The aim of our research was to study an inhibitory α-glucosidase activity of the herbal mixtures, which are used in folk medicine for the prevention and treatment of diabetes mellitus type 2 in Ukraine and with established hypoglycemic, hypolipidemic, antioxidant, hepatoprotective, pancreatoprotective activity in pharmacological study *in vivo* and the defined phytochemical composition that determines such pharmacodynamics.

During the study of antidiabetic activity *in vitro* it was established the α-glucosidase IC50 was 328.16 μg/mL of the sample 1, 359.57 μg/mL of the sample 2, 342.73 μg/mL o of the sample 3, 369.50 μg/mL of the sample 4 and 292.55 μg/mL of the sample 5.

The present study showed a high inhibitory activity of herbal mixtures to pancreatic α-glucosidase, which suggests the effectiveness of the studied herbal mixtures for the prevention and treatment of type 2 diabetes

Keywords: diabetes mellitus, herbal mixtures, α-glucosidase activity, acarbose

Introduction

Diabetes mellitus is a global social problem in the field of health care, due to rapid spread of this disease and the development of serious complications such as micro- and macroangiopathies, which significantly reduce the quality and life expectancy of patients [1]. According to the official information of International Diabetes Federation (2019), the number of patients is projected to increase to 642 million by 2040 [2].

An important problem of pharmacovigilance is that existing pharmacotherapy can effectively reduce hyperglycemia, but it is not always able to stabilize fluctuations in glycemic values during the day and maintain it at an optimal level.

A promising approach for management of diabetes, particularly type 2 diabetes, is to decrease postprandial hyperglycemia by inhibiting carbohydrate hydrolyzing enzymes in gastrointestinal tract [3, 4, 5, 6]. α-Amylase is involved in degrading long chain of starch and α-glucosidase breaks down oligosaccharides and disaccharides [7, 8, 9, 10]. Inhibitors of these enzymes slow down carbohydrate digestion thus prolong overall digestion time, causing a reduction in glucose absorption and consequently blunting postprandial plasma glucose [11, 12, 13, 14]. Postprandial hyperglycemia is a major risk factor for diabetic vascular complications leading to disabilities and mortality in diabetics [15, 16].

Currently there are several antidiabetic drugs such as acarbose that act by inhibiting α-amylase and α-glucosidase. Acarbose is an oligosaccharide of microbial origin (*Actinoplanes*) that potently inhibits *in vitro* and *in vivo* such brush-border enzymes as glucoamylase, dextrinase, maltase and sucrase as well as the pancreatic α-amylase [17, 18, 19, 20]. Due to the presence of an intramolecular nitrogen, acarbose attaches to the carbohydrate binding site of α-glucosidase enzyme with an affinity exceeding that of the normal substrate by a factor of 104–105. The enzymatic reaction stops because the C–N linkage in the acarviosine unit of acarbose cannot be cleaved [21, 22, 23]. While efficient in attenuating the rise in blood glucose, continuous uses of acarbose and other similar drugs are often associated with undesirable effects [24, 25]. It is for this reason that there is a need for natural α-glucosidase and α-amylase inhibitors that would possess no adverse or unwanted side effects.

Particular attention deserves the combinations of different medicinal plants because such herbal mixtures will have more biologically active substances that will influence on all links of the pathogenetic mechanism of development of diabetes mellitus and its complications [26, 27].

In addition, acarbose is a medication clinically used to inhibit α-glucosidase and α-amylase. Unfortunately, its long-term administration resulted in side effects including abdominal distention and diarrhea. Alternative plant-derived products with better safety potential may also be used for the management of diabetes mellitus [28, 29, 30].

Thus, **the aim of our research** was to study an inhibitory α- glucosidase activity of the herbal mixtures, which are used in folk medicine for the prevention and treatment of diabetes mellitus type 2 and with established hypoglycemic, hypolipidemic, antioxidant, hepatoprotective, pancreatoprotective activity in pharmacological study *in vivo* [23, 24, 25, 26] and the defined phytochemical composition that determines such pharmacodynamics [16, 17, 18, 19, 20, 21].

Methods

Plant materials: The herbal raw materials harvested in June to August 2019 in Ternopil region (Ukraine) were used. After harvesting, the raw materials were dried, crushed and brought back to standard according to the general GACP requirements [31]. The plants were identified by Department of Pharmacognosy with Medical Botany, I.Horbachevsky Ternopil National Medical University, Ternopil, Ukraine. The voucher specimens of the herbal raw materials have been deposited in Departmental Herbarium for future record.

For the study were used the five different herbal mixtures, composition of which is given in Table 1.

Chemicals and standards: chemical reference substance (CRS) of acarbose were of primary reference standard grade (≥ 95 % purity HPLC) and were purchased from Sigma-Aldrich Chemical Company (Germany), as well as α-glucosidase. Water used in the studies was produced by MilliQ Gradient water deionizaton system (USA).

Extraction procedure: the samples of herbal raw materials (10 g) were placed into a 100 mL conical flask with120 mL of distilled water. The extractions were carried out in a water bath for 30 min. The resulting extracts were filtered using Whatmann filter paper No1. Then the filtrates were evaporated by rotary evaporator and were lyophilized to dryness. The lyophilized powders of each herbal mixture were stored at 4 °C for further use.

Inhibition of α-glucosidase enzyme: the method is based on the inhibition of α-glucosidase that catalyses the hydrolysis of *p*-nitrophenyl-*D*-glucopyranoside to *p*-nitrophenol. A total of 20 µL of samples of the studied extracts with a range of concentrations100-1000 µg/mL were added to 50 µL potassium phosphate buffer 0.1 M (pH 6.8) and 10 µL α-glucosidase 0.25 U/mL and were incubated at 37 °C for 10 min. Then 10 µL 5 of mM *p*-nitrophenyl-α-*D*-glucopyranoside was added and further incubated for 30 min. For the termination of reaction, it was added 50 µL of Na_2CO_3 0.1 M. The absorbance was measured at 405 nm using the spectrophotometer Shimadzu 1800-UV (Japan). Experiment was performed in triplicate. Acarbose was used as a positive control [5].

Calculation of 50% Inhibitory Concentration (IC50): the inhibitory concentration of the water extracts of the herbal mixtures required to inhibit the activity of the enzyme by 50%, IC50 was calculated by regression analysis using the percentage scavenging activities at five different concentrations of the extracts. Inhibition (I %) was calculated by:

$$\%\ Inhibition = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Results and Discussion

During the development of type 2 diabetes, insulin's ability to stimulate cellular uptake of glucose from blood is compromised [32, 33, 34]. The most effective and beneficial therapy is to regain the optimal level of blood glucose as soon as possible after meal [35, 36]. Thus, inhibitors of α-glucosidase that catalyzes cleavage of glucose from disaccharide are effective in delaying glucose absorption and managing diabetes [37]. Acarbose is widely used in treatment of patients with type 2 diabetes via inhibiting the upper gastrointestinal glucosidases that convert complex polysaccharides into monosaccharides in a dose-dependent manner and result in a delayed glucose absorption and a depressed postprandial hyperglycemia. However, gastrointestinal side effects, mainly flatulence and sometimes soft stools or abdominal discomfort, have often been reported [38]. Inhibitory effects against α-glucosidase of water extracts of herbal mixtures by taking acarbose as a positive control.

The relationship between the increase in the inhibitory activity of α-glucosidase and the concentration of aqueous extracts of herbal mixtures was revealed. It was experimentally established that the concentration required for 50% inhibition (IC50) of α-glucosidase enzyme was 328.16 µg/mL of the sample 1; 359.57 µg/mL of sample 2; 342.73 µg/mL of the sample 3; 369.50 µg/mL of the sample 4; 292.55 µg/mL of the sample 5 (Table 2). The IC50 value of standard drug acarbose against α-glucosidase was 202.62µg/mL.

Inhibition of intestinal pancreatic α-glucosidase are used for tretment of diabetes mellitus type 2 that work by preventing the digestion of carbohydrates, such as starch [38]. The search for a new α-glucosidase inhibitor from herbal mixtures is a striking method for the management of postprandial hyperglycemia. Secondary metabolites such as tannins, phenolic acids, and flavonoids are the main phytoconstituents that possess α-glucosidase inhibitory activity [39].

Conclusions

For the first time, it was conducted the study of α-glucosidase inhibition in the water extracts of the herbal mixtures, which are used in folk medicine for the prevention and treatment of diabetes mellitus type 2 and with established hypoglycemic, hypolipidemic, antioxidant, hepatoprotective, pancreatoprotective activity in pharmacological study *in vivo* and the defined phytochemical composition that determines such pharmacodynamics. The present study showed a high inhibitory activity of herbal mixtures to pancreatic α-glucosidase, which is one of the mechanisms of prevention and treatment of type 2 diabetes.

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Table 1. Composition of the herbal mixtures

Herbal mixtures	Herbal drug component	Portion in the mixture, %	Relative ratio
Sample 1	*Urtica dioica* leaf	26.32	5
	Cichorium intybus roots	26.32	5
	Rosa majalis fruits	21.05	4
	Elymus repens rhizome	15.79	3
	Taraxacum officinale roots	10.52	2
Sample 2	*Arctium lappa* roots	26.32	5
	Elymus repens rhizome	26.32	5
	Zea mays columns with stigmas	21.05	4
	Helichrysum arenarium flowers	15.79	3
	Rosa majalis fruits	10.52	2
Sample 3	*Inula helenium* rhizome with roots	10.0	1
	Helichrysi arenarium flowers	20.0	2
	Zea mays columns with stigmas	20.0	2
	Origanum vulgari herb	20.0	2
	Rosa majalis fruits	20.0	2
	Taraxacum officinale roots	10.0	1
Sample 4	*Cichorium intybus* roots	26.32	5
	Elymus repens rhizome	26.32	5
	Helichrysum arenarium flowers	21.05	4
	Rosa majalis fruits	15.79	3
	Zea mays columns with stigmas	10.52	2
Sample 5	*Urtica dioica* leaf	20.0	1
	Taraxacum officinale roots	20.0	1
	Vaccinium myrtillus leaf	20.0	1
	Rosa majalis fruits	20.0	1
	Mentha piperita herb	20.0	1

Table 2. α- glucosidase inhibition of water extracts of the samples of the herbal mixtures

Herbal mixtures	Concetration, µg/mL	Inhibition, %	IC50, µg/mL
Sample 1	100	30.69± 3.12	328.16
	200	41.16± 3.17	
	400	54.95± 2.98	
	800	62.10± 3.08	
	1000	73.86± 3.05	
Sample 2	100	29.49± 2.92	359.57
	200	39.58± 3.18	
	400	52.64± 3.14	
	800	60.86± 3.03	
	1000	71.46± 3.28	
Sample 3	100	28.94± 3.09	342.73
	200	40.48± 2.67	
	400	53.82± 2.75	
	800	60.69± 3.04	
	1000	70.93± 3.15	
Sample 4	100	29.83± 3.04	369.50
	200	38.94± 3.15	
	400	51.99± 3.03	
	800	64.04± 2.61	
	1000	70.96± 2.19	
Sample 5	100	32.85± 3.08	292.55
	200	43.91± 3.24	
	400	57.07± 3.31	
	800	65.19± 2.98	
	1000	75.38± 2.82	
Acarbose (standart)	100	42.19± 2.12	202.62
	200	49.84± 1.98	
	400	62.11± 1.59	
	800	74.09± 2.37	
	1000	89.73± 2.65	

Note: Values are expressed as mean ± SD (n=3).