PharmacologyOnLine

Archives • 2021 • vol.2 • 1443-1449

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

Savych, Alona*[1], Marchyshyn, Svitlana[1], Milian, Ivanna[2]

[1]Department of Pharmacognosy with Medical Botany, I. Horbachevsky Ternopil National Medical University, Ukraine

[2] Department of General Chemistry, I. Horbachevsky Ternopil National Medical University, Ukraine

*alonasavych@gmail.com

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-amylase 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-amylase 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-amylase IC50 was 699.49 μg/mL of the sample 1, 758.15 μg/mL of the sample 2, 781.76 μg/mL of the sample 3, 700.17 μg/mL of the sample 4 and 646.52 μg/mL of the sample 5.

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

Keywords: diabetes mellitus, herbal mixtures, α-amylase 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 [3, 4, 5, 6]. A sudden rise in blood glucose levels, causing hyperglycemia in type 2 diabetes patients happens due to hydrolysis of starch by pancreatic α-amylase and uptake of glucose by intestinal α-glucosidases [7, 8, 9, 10]. The inhibition of enzymes involved in the breakdown of starch (α-amylase) and uptake of glucose (α–glucosidase) has been suggested to be a useful approach to the management and prevention of type 2 diabetes and dietary phytochemicals, have promising potential [11, 12, 13, 14]. Amylase inhibitors are also known as starch blockers because they contain substances that prevent dietary starch from being absorbed by the body. Starches are complex carbohydrates that cannot be absorbed unless they are first broken down by the digestive enzyme amylase and other secondary enzymes [15, 16, 17, 18].

Therefore, the optimization of pharmacotherapy, search and study of new drugs with antidiabetic activity for the prevention and treatment of this disease and its dangerous complications is a topical issue of pharmacy and medicine.

One such area is phytotherapy, as it has a number of advantages over traditional therapy with using oral synthetic agents, namely, it is low-toxic, has a mild pharmacological effect and can be used for long periods without significant side effects, is well combined with synthetic drugs, has a complex activity through a number of biologically active compounds [19, 20, 21, 22]. 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 [23, 24, 25, 26].

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 [27, 28, 29, 30].

Thus, **the aim of our research** was to study an inhibitory α-amylase 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-amylase. 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-amylase enzyme: the method is based on enzyme inhibition, so the transformation of starch to reducing oligosaccharides that react with 3,5-dinitrosalicylic acid is blocked. A total of 500 µL of samples of the studied extracts with a range of concentrations100-1000 µg/mL were added to 500 µL of 0.20 mM phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing α-amylase solution (0.5mg/mL) and were incubated at 25°C for 10 min. Thereafter, it was added 500 µL of (1% w/v) starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) to each tube and was incubated at 25°C for 10 min. The reaction was stopped with 1.0 mL of 3,5-dinitrosalicylic acid colour reagent (12.0 g of sodium potassium tartrate tetrahydrate in 8 mL of 2 M NaOH and 96 mM 3,5- dinitrosalicylic acid solution). Then the tubes were incubated in the boiling water bath for 5 min and cooled to room temperature. The reaction mixture was diluted by adding 10 mL of distilled water and absorbance was measured at 540 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

Management of the blood glucose level is a critical strategy in the control of diabetes complications. Inhibitors of saccharide hydrolysing enzymes (α-amylase) have been useful as oral hypoglycemic drugs for the control of hyperglycemia especially in patients with type-2 diabetes mellitus [32, 33, 34]. Inhibition of this enzyme delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently reducing the postprandial plasma glucose rise [35, 36, 37].

The experimental studies of antidiabetic activity *in vitro* of investigated herbal mixtures with a range of concentrations100-1000 µg/mL were performed by inhibition of α-amylase activity compared with acarbose.

The relationship between the increase in the inhibitory activity of α-amylase and the concentration of aqueous extracts of herbal mixtures was revealed. During the study of inhibition of α-amylase enzyme it was established that the IC50 of the water extracts of the sample 1 was 699.49 µg/mL; the sample 2 – 758.15 µg/mL; the sample 3 – 781.76µg/mL; the sample 4 – 700.17 µg/mL; the sample 5 – 646.52 µg/mL (Table 2). The IC50 value of standard drug acarbose against α-amylase was 246.22 µg/mL.

Inhibition of intestinal pancreatic α-amylase activities results in delayed carbohydrate digestion of absorbable monosaccharides leading to a drop in postprandial hyperglycemia [38]. The search for a new α-amylase 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-amylase inhibitory activity [39].

Conclusions

For the first time, it was conducted the study an inhibitiry α-amylase activity of 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-amylase, which is one of the mechanisms of prevention and treatment of type 2 diabetes.

References

1. American Diabetes Association (2020). Standards of Medical Care in Diabetes. *Diabetes care*, *43*, 1212.
2. International Diabetes Federation (2019). *IDF Diabetes Atlas, 9th ed.* Brussels, Available at: https://www.diabetesatlas.org
3. Marchyshyn, S., Polonets, O., Savych, A., & Nakonechna, S. (2020). Determination of carbohydrates of *Chrysanthemum morifolium L.* leaves and flowers by GC-MS. *Pharmakeftiki*, 32(4), 202-212.
4. Savych, A., Marchyshyn, S., Harnyk, M., Kudria, V., & Ocheretniuk, A. (2021). Determination of amino acids content in two samples of the plant mixtures by GC-MS. *Pharmacia*, *68*(1), 283-289.
5. Savych, A., Marchyshyn, S., Kyryliv, M., & Bekus, I. (2021). Cinnamic acid and its derivatives in the herbal mixtures and their antidiabetic activity. *Farmacia*, 69(3), 595-601.
6. Skyler, J. S., Bakris, G. L., Bonifacio, E., Darsow, T., Eckel, R. H., Groop, L., Groop, P. H., Handelsman, Y., Insel, R. A., Mathieu, C., McElvaine, A. T., Palmer, J. P., Pugliese, A., Schatz, D. A., Sosenko, J. M., Wilding, J. P., & Ratner, R. E. (2017). Differentiation of Diabetes by Pathophysiology, Natural History, and Prognosis. *Diabetes*, *66*(2), 241–255.
7. Shanaida, M., Hudz, N., Korzeniowska, K., Wieczorek, P. (2018). Antioxidant activity of essential oils obtained from aerial part of some *Lamiaceae* species. *International Journal of Green Pharmacy*, 12 (3), 200–204.
8. Shanaida, M., Hudz, N., Jasicka-Misiak, I., Wieczorek, P. (2021). Polyphenols and Pharmacological Screening of a *Monarda fistulosa* L. dry Extract Based on a Hydrodistilled Residue By-Product. *Frontiears in Pharmacology*, 12, 1-10.
9. Shanaida, M., Adamiv, S., Yaremchuk, O., Ivanusa, I. (2021). Pharmacological study of the polyphenol-containing phytosubstance obtained from the Anise Hyssop herb. *PharmacologyOnLine*, 2, 105-112.
10. Savych, A., Marchyshyn, M., Basaraba, R., & Lukanyuk, M. (2020). Antihyperglycemic, hypolipidemic and antioxidant properties of the herbal mixtures in dexamethasone-induced insulin resistant rats. *PharmacologyOnLine*, 2, 73-82.
11. Savych, A., Marchyshyn, M., & Naconechna, S. (2021). Influence of some herbal mixtures on insulin resistance and glucose tolerance in rats. *PharmacologyOnLine*, *1*, 356-364.
12. Savych, A., & Milian, I. (2021). Total flavonoid content in the herbal mixture with antidiabetic activity. *PharmacologyOnLine*, 2, 68-75.
13. Savych, A., & Basaraba, R. (2021). Ascorbic acid content in the herbal mixture with antidiabetic activity. *PharmacologyOnLine*. 2, 76-83.
14. Savych, A., Basaraba, R., Muzyka, N., & Ilashchuk, P. (2021). Analysis of fatty acid composition content in the plant components of antidiabetic herbal mixture by GC-MS. *Pharmacia*, *68*(2), 433-439.
15. Savych, A., Marchyshyn, M., & Basaraba, R. (2020). Screening study of hypoglycemic activity of the herbal mixtures (Message 1). *ScienceRise: Pharmaceutical Science*, *4*(26), 40-46.
16. Savych, A., Marchyshyn, S., Kozyr, H., & Yarema, N. (2021). Determination of inulin in the herbal mixtures by GC-MS method. *Pharmacia*, *68*(1), 181-187.
17. Savych, A., Marchyshyn, S., & Basaraba, R. (2020). Determination of fatty acid composition content in the herbal antidiabetic collections. *Pharmacia*, *67*(3), 153–159.
18. Savych, A., Marchyshyn, S., & Milian, I. (2021). Determination of carbohydrates in the herbal antidiabetic mixtures by GC-MC. *Acta Pharmaceutica*, *71*(3), 429-443.
19. Savych, A., Marchyshyn, S., Basaraba, R., & Kryskiw, L. (2021). Determination of carboxylic acids content in the herbal mixtures by HPLC. *ScienceRise: Pharmaceutical Science*, *2*(30), 33-39.
20. Savych, A., & Nakonechna, S. (2021). Determination of amino acids content in two herbal mixtures with antidiabetic activity by GC-MS. *Pharmakeftiki*, 33 (2), 116-123.
21. Savych, A., Bilyk, O., Vaschuk, V., & Humeniuk, I. (2021). Analysis of inulin and fructans in *Taraxacum officinale* L. roots as the main inulin-containing component of antidiabetic herbal mixture. *Pharmacia*, 68(3), 527-532.

22. Savych, A., & Mazur, O. (2021). Antioxidant activity *in vitro* of antidiabetic herbal mixtures. *PharmacologyOnLine*, 2, 17-24.
23. Savych, A., & Polonets, O. (2021). Study of hypoglycemic activity of antidiabetic herbal mixture on streptozotocin-nicotinamide-induced rat model of type 2 diabetes. *PharmacologyOnLine*, 2, 62-67.
24. Savych, A., Basaraba, R., & Gerush, O. (2021). Comparative analysis of hypoglycemic activity of herbal mixtures by glucose tolerance tests (message 2). *PharmacologyOnLine*, 2, 1118-1127.
25. Savych, A., Gerush, O., & Basaraba, R., (2021). Determinationof hypoglycemic activity of the herbal mixtures by means of glucose loading tests (message 3). *PharmacologyOnLine*, 2021, 2, 1128-1137.
26. Savych, A., & Sinichenko, A. (2021). Screening study of hypoglycemic activity of the herbal mixtures used in folk medicine (message 4). *PharmacologyOnLine*, 2021, 2, 1254-1262.
27. Budniak, L., Slobodianiuk, L., Marchyshyn, S., Kostyshyn, L., Horoshko, O. (2021). Determination of composition of fatty acids in *Saponaria officinalis* L. *ScienceRise: Pharmaceutical Science*, 1(29), 25-30.
28. Budniak, L., Slobodianiuk, L., Marchyshyn, S., Klepach, P., Honcharuk, Y. (2021). Determination of carbohydrates content in *Gentiana cruciata* L. by GC/MS method. *International Journal of Applied Pharmaceutics*, 13(1), 124-128.
29. Slobodianiuk, L., Budniak, L., Marchyshyn, S., Kostyshyn, L., Zakharchuk, O. (2021). Analysis of carbohydrates in *Saponaria officinalis* L. using GC/MS method. *Pharmacia*, 68(2), 339-345.
30. Slobodianiuk, L., Budniak, L., Marchyshyn, S., Skrynchuk, O., Kudria, V. (2021) HPLC analysis of amino acids content in *Crambe cordifolia* and *Crambe koktebelica* leaves. *International Journal of Applied Pharmaceutics*, 13(4), 111-116.
31. WHO Guidelines on good agricultural and mixture practices (GACP) for medicinal plants (2003). *World Health Organization*, Geneva, Switzerland, 72.
32. Slobodianiuk, L., Budniak, L., Marchyshyn, S., Parashchuk, E., Levytska, L. (2021). Experimental studies on expectorant effect of extract from *Pimpinella saxifraga* L. *PharmacologyOnLine*, 1, 404-410.
33. Darzuli, N., Budniak, L., Slobodianiuk, L. (2021). Investigation of the antibacterial and antifungal activity of the *Pyrola rotundifolia* L. leaves dry extract. *Pharmacologyonline*, 1, 395-403.
34. Budniak, L., Slobodianiuk, L., Marchyshyn, S., Klepach, P. (2021). Investigation of the influence of the thick extract of common centaury (*Centaurium erythraea* Rafn.) herb on the secretory function of the stomach. *Pharmacologyonline*, 2, 352-360.
35. Slobodianiuk, L., Budniak, L., Marchyshyn, S., Demydiak, O. (2021). Investigation of the anti-inflammatory effect of the dry extract from the herb of *Stachys sieboldii* Miq. *Pharmacologyonline*, 2, 590-597.
36. Slobodianiuk, L., Budniak, L., Marchyshyn, S., Berdey, I., Slobodianiuk, O. (2021). Study of the hypoglycemic effect of the extract from the tubers of *Stachys sieboldii* Miq. *Pharmacologyonline*, 2, 167-178.
37. Budniak, L., Vasenda, M., Slobodianiuk, L. (2021). Determination of flavonoids and hydroxycinnamic acids in tablets with thick extract of *Primula denticulata* Smith. *PharmacologyOnLine*, 2, 1244-1253.
38. Budniak, L., Slobodianiuk, L., Marchyshyn, S., Basaraba, R., Banadyga, A. (2021). The antibacterial and antifungal activities of the extract of *Gentiana cruciata* L. herb. *Pharmacologyonline*, 2, 188-197.
39. Budniak, L., Slobodianiuk, L., Darzuli, N., Honcharuk, Ya. (2021). The antibacterial activity of the tablets with dry extract of round-leaved wintergreen leaves. *Pharmacologyonline* 2, 672-679.

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

the samples of the herbal mixtures

Herbal mixtures	Concetration, µg/mL	Inhibition, %	IC50, µg/mL
Sample 1	100	22.17± 3.65	699.49
	200	30.97± 2.98	
	400	41.15± 3.51	
	800	52.97± 4.28	
	1000	61.64± 3.49	
Sample 2	100	20.47± 3.31	758.15
	200	28.58± 4.42	
	400	39.13± 5.28	
	800	51.27± 3.63	
	1000	59.34± 3.75	
Sample 3	100	21.58± 3.53	781.76
	200	32.11± 3.94	
	400	38.28± 2.37	
	800	50.56± 3.63	
	1000	60.18± 3.74	
Sample 4	100	20.65± 3.62	700.17
	200	31.07± 2.86	
	400	40.95± 3.73	
	800	53.01± 3.85	
	1000	58.75± 3.92	
Sample 5	100	23.04± 3.76	646.52
	200	31.65± 4.93	
	400	42.82± 2.71	
	800	54.47± 3.83	
	1000	63.18± 3.17	
Acarbose (standart)	100	33.98± 1.92	246.22
	200	47.37± 2.13	
	400	58.75±2.46	
	800	69.58± 2.06	
	1000	75.94± 1.99	

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