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SCREENING STUDY OF HYPOGLYCEMIC ACTIVITY OF THE HERBAL MIXTURES USED IN FOLK MEDICINE (MESSAGE 4)

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Abstract

Diabetes mellitus is one of World Health Organization priorities matters, which requires immediate solutions, as the epidemiological situation is alarming – the number of patients is growing rapidly each year, leading to increased disability and mortality due to the development of macro- and microangiopathies. Herbs and their combinations due to the wide range of biologically active substances can influence on various links of the pathogenetic mechanism of development of diabetes mellitus and its complications.

The aim of our research was to study the hypoglycemic activity of the herbal mixtures, which are used in folk medicine for the prevention and treatment of diabetes mellitus type 2, but do not have a scientific basis and to establish their conditional therapeutic dose.

The study was performed on male albino rats weighing 180-200 g, which for preventive treatment during 20 days orally received aqueous extracts (1:10) of the studied herbal mixtures at a dose 6 mL/kg/day, 9 mL/kg/day and 12 mL/kg/day and comparison drugs – the official herbal mixtures "Arfazetin" at a dose 9 mL/kg/day and tablets metformin at a dose 60 mg/kg/day. The study of hypoglycemic properties and the establishment of a conditional therapeutic dose of the studied mixtures was carried out using glucose loading tests. All experiments were performed in accordance with general ethical principles with the recommendations of the EEC Council directive 2010/63/EU about the protection of animals, which are used for scientific purposes.

The results of the study showed that the 20-day preventive treatment by the herbal mixtures reduced alimentary hyperglycemia at the 30th minute of OGTT and helped regulate carbohydrate tolerance disorders by reducing hyperglycemia at the 15th minute of IPGTT. The highest hypoglycemic activity showed the herbal mixture No. 19 (*Urticae folia, Taraxaci radices, Myrtilli folia, Rosae fructus* and *Menthae folia*) in a dose 12 mL/kg/day, which was almost on a par with the comparison drug – tablets metformin but exceeded the official herbal mixture "Arfazetin". In addition, the dose-dependence of the effectiveness of all five studied herbal mixtures was established.

Key words: *herbal mixtures, hypoglycemic activity, diabetes mellitus, oral glucose tolerance test, intraperitoneal glucose tolerance test*

Introduction

Diabetes mellitus is one of WHO's priority issues. It requires immediate resolution as the epidemiological situation is gaining alarming proportions – the number of diabetic patients is increasing every year along with the number of deaths and disabilities due to the development of micro- and macro-angiopathies [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. This leads to the development of the pathological processes cascade, which leads to the development and progression of diabetic angiopathies [1, 3, 4, 5, 6].

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

One of these areas is using the herbal remedies, either as monotherapy for the prevention or in the mild stages of the disease or in the combination with traditional therapy in more severe forms of the disease. Phytotherapy is a justified method for the prevention and treatment because it has some advantages, such as relatively low toxicity, mild pharmacological effects and possibility to be used for long periods without significant side-effects, and it often well combines with synthetic drugs, has a complex activity through a number of biologically active compounds [7, 8, 9]. Particular attention deserve the combinations of different medicinal plants, as biologically active substances of plant origin have a wide range of pharmacological action and a variety of mechanisms for influencing the development of diabetes (the pathogenesis of which involves the development of insulin resistance; relative insulin deficiency, which becomes the cause of decrease the secretory activity of β-cells of the pancreatic gland) and diabetic angiopathies (the pathogenesis of which are activation of lipid peroxidation, inactivation of antioxidant protection system and development of oxidative stress) [10, 11, 12]. In addition, Ukrainian pharmaceutical market is represented mainly by synthetic antidiabetic drugs, which account for over 92 % of all oral antidiabetic drugs. Today, is registered two antidiabetic herbal mixtures – the herbal mixture "Arfazetin", which includes *Vaccinii myrtilli cormus*, *Phaseoli valvae fructum*, *Eleutherococci senticosi rhizomata et radices*, *Rosae fructus*, *Equiseti arvensis herba*, *Hyperici herba*, *Matricariae flores* and – the herbal mixture "Sadifit", which includes *Helianthi tubera*, *Steviae folia*, *Vaccini myrtilli cormus*, *Phaseoli valvae fructum*, *Thea chinensis*, *Menthae piperitae folia* in Ukraine.

However, *Vaccinii myrtilli cormus*, *Eleutherococci senticosi rhizomata et radices* and *Hyperici herba* are potent plants that can be dangerous with prolonged use. In addition, *Eleutherococci senticosi rhizomata et radices* has a tonic effect and is contraindicated in coronary heart disease, heart failure and hypertension, which are frequent complications of diabetes.

Thus, **the aim** of our research was to study the hypoglycemic activity of the herbal mixtures, which are used in folk medicine for the prevention and treatment of diabetes mellitus type 2 [13], but do not have a scientific basis and to establish their conditional therapeutic dose.

Methods

Plant materials: The herbal raw materials harvested in June to August 2019 in Ternopil region (Ukraine) and in Charpathians (Ukraine) (*Myrtilli folia*) were used. After harvesting, the raw materials were dried, crushed and brought back to standard according to the general GACP requirements [14]. 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, which are used in folk medicine for the prevention and treatment of diabetes mellitus type 2 in Ukraine [13]. Composition of the mixtures is given in Table 1.

Extraction procedure: The samples of the herbal raw material were grinded into a powder by laboratory mill. Then 10 g of each powdered herbal mixture was put into a 100 mL conical flask and 120 mL of distilled water was added to each. The aqueous extracts were obtained by heating in the boiling water bath for 30 min. The extracts were filtered using Whatmann filter paper No. 1. Then the filtrates were evaporated by rotary evaporator and were lyophilized to dryness. The lyophilized powders of each herbal mixture were stored at 4 ℃ for further use.

The aqueous extract of the comparison preparation – the official herbal mixture "Arfazetin" was prepared using 5 g of dry raw material and 110 mL of distilled water (as indicated in the instructions for use) under the same conditions.

To prepare the metformin suspension, the metformin tablets were crushed and mixed with 2 mL of distilled water.

Drugs: The official herbal mixture "Arfazetin" was purchased from PJSC Pharmaceutical Factory "Viola" (Ukraine), the standard drug – metformin SANDOZ® from Lek S.A. (Poland).

Experimental Animals: The study was performed on male albino rats weighing between 180 g and 200 g, which were bred at the animal house of the Central Research Laboratory of I.Horbachevsky Ternopil National Medical University, where they were kept under appropriate conditions (at a constant room temperature of 22 ± 1 ℃, 40-70 % humidity conditions and a 12-hour light/dark cycle). Throughout the experimental period, the animals received standard rat diet and water *ad libitum.* The animals were treated in accordance with the internationally accepted standard ethical guidelines for laboratory animal use and care as described in the European Community Guidelines [15]. All protocols for animals experiment were approved by the animal ethical committee of I.Horbachevsky Ternopil National Medical University.

Experimental Protocol: Screening study of hypoglycemic activity of the herbal mixtures and determination of their conditionally therapeutic dose was performed on intact normoglycemic rats. Animals were randomly divided into eight groups of eight animals (n=8) each and received different preventive treatment once daily during the 20 days. Group I (Control) received per os (*p.o.*) distilled water (12 mL/kg/day), group II (HM "Arfazetin") – aqueous extract of the official herbal mixture "Arfazetin" (9 mL/kg/day, *p.o.*) [13], group III (MET) – suspension of metformin (60 mg/kg/day, *p.o.*) [13], group IV-VIII (HM) – aqueous extracts of the studied herbal mixtures No. 16-20 in doses 6 mL/kg/day, 9 mL/kg/day and 12 mL/kg/day, *p.o.* Metformin was chosen as a comparison drug because, according to the recommendations of the American Diabetes Association and the International Diabetes Federation, it is recognized as the "gold standard" in the treatment of diabetes mellitus type 2 [13]. The official herbal mixture "Arfazetin" was chosen as a reference drug because it is similar in origin and mechanism of action in relation to the studied mixtures. The last oral administration of the researched means was carried out 2 hours before the glucose load tests.

Measurement of Oral Glucose Tolerance Test (OGTT): Fasting blood glucose (basal glycemia) was measured in tail blood samples after a 6-hour fast on 20th day of the experiment using a glucose analyzer (glucometer Accuk-Check, Germany). OGTT was performed after measuring basal glycemia by administering glucose solution (3 g/kg, *p. o).* Blood glucose levels were determined at 0, 30th, 60th and 120th minute after glucose loading [13].

Measurement of Intraperitoneal Glucose Tolerance Test (IPGTT): After overnight fasting (16-18 hours) on 21th day of the experiment, rats were injected intraperitoneally with glucose solution (2 g/kg, *i. p.*) in the morning. The level of glucose in the blood obtained from the tail vein of animals was determined before the introduction of glucose and after 15, 45 and 60 minutes using a glucose analyzer [13].

Statistical Analysis: The values were expressed as mean ± SEM. The data were analysed by using GraphPad Prism software version 5.03. The results were compared by using the ANOVA-One-Way test followed by *Mann-Whitney U test*. The difference was considered statistically significant at $p<0.05$. The value of the integrated glycemic index of the area under glycemic curve (AUC_{glu}, mmol/L min) was calculated using the statistical software package "MedCalk, v.9.3.7.0".

Results and Discussion

At the first time, the effect of the herbal mixtures and the comparison drugs on basal glycemia and on glycemia after carbohydrate loading by OGTT after 20 days of preventive treatment was studied. This test allows simulate alimentary hyperglycemia that occurs after eating. Hypoglycemic activity of the herbal mixtures and reference drugs was manifested by their ability to reduce blood glucose levels at the 30th minute of the test, during its maximum increase in response to oral carbohydrate load.

The results of the study showed that 20-day preventive treatment by all five herbal mixtures No. 16-20 at doses 6 mL/kg/day, 9 mL/kg/day and 12 mL/kg/day significantly (p<0.05) reduced glycemia at the 30th minute of OGTT compared with the Control group. However, the best results of hypoglycemic activity at the 30th minute of the test showed the herbal mixture No. 19 at dose 12 mL/kg/day, it reduced blood glucose level by 44 %, relative to the Control group. Tablets metformin showed a similar result in efficacy, as they reduced alimentary hyperglycemia by 46 % relative to the Control group of animals at 30th minute. The official herbal mixtures "Arfazetin" was inferior in efficiency to the herbal mixture No. 19 at dose 12 mL/kg/day and reduced glycemia by 32% relative to the Control group at the 30th minute of the test (Table 2).

During the determination of integrated glycemic index based on the results of OGTT, it was found that the area under glycemic curve (AUC_{glu}) of the herbal mixture No. 19 (12 mL/kg/day) was 263.4 mmol/L min. Regarding the results of the comparison drugs, the AUC_{glu} of metformin (60 mg/kg/day) was lower and amounted to 256.8 mmol/L min, and the herbal mixture "Arfezetin" (9 mL/kg/day) was higher and amounted to 322.8 mmol/L min.

At the second time of the screening study, the ability of the herbal mixtures No. 16-20 and comparison drugs to improve carbohydrate tolerance was determined using IPGTT. The hypoglycemic effect of the herbal mixtures and comparison drugs was assessed by their ability to reduce hyperglycemia at 15th minute of IPGTT during the maximum rise of blood glucose in the animals in response to intraperitoneal carbohydrate load.

During the study, a significant (p<0.05) increase in blood glucose levels was observed in animals from the Control group at the 15th minute of the test (peak hyperglycemic), exceeding the initial data by 2.0 times. The best ability to reduce the hyperglycemic peak of IPGTT showed the herbal mixture No. 19 (12 mL/kg/day) because blood glucose level was lower by 26 % relative to the Control group. Tablets metformin showed a similar effect and reduced hyperglycemia at the 15th minute of the test by 27 % relative to the Control group, and the official herbal mixture "Arfazetin" was slightly inferior to the effectiveness of the herbal mixture No. 19 at dose 12 mL/kg/day and reduced hyperglycemia by 21 %. By the end of the experiment at the 60th minute of IPGTT, the blood glucose level returned to baseline in all groups of animals (Table 3).

The results of a screening study using OGTT and IPGTT of the herbal mixtures No. 16-20, which are used in folk medicine for the prevention and treatment of diabetes mellitus type 2, indicated dose-dependent hypoglycemic activity. The best hypoglycemic effect of the studied objects was shown at a dose 12 mL/kg/day.

The study using glucose load tests showed that the herbal mixtures No. 16, No. 17, No. 18 and No. 20 at doses 6 mL/kg/day, 9 mL/kg/day and 12 mL/kg/day showed hypoglycemic activity, but it is slightly lower compared to the herbal mixture No. 19 (12 mL/kg/day) and comparison drugs – the official herbal mixture "Arfazetin" (9 mL/kg/day) and tablets metformin (60 mg/kg/day) (Tables 2, 3).

Hypoglycemic activity of the studied herbal mixtures is quite predictable because they include medicinal herbal raw materials containing biologically active substances with proven hypoglycemic action. The main groups of biologically active substances that can lower blood glucose are carbohydrates, that have ability to increase glucagon-like peptide-1 (GLP-1) and insulin secretion, to inhibit the glucagon secretion, to stimulate the β-cells proliferation and neogenesis [16, 17, 18]. Presented herbal mixtures contain plant raw materials that are rich in carbohydrates, such as *Avenae sativae semina* (the herbal mixtures No. 16

and No. 17), *Taraxaci radices* (the herbal mixtures No. 17 and No. 19), *Elymi repens rhizomata* (the herbal mixture No. 16), *Lini semina* (the herbal mixtures No. 16, No. 17 and No. 18), *Arctii lappae radices* (the herbal mixture No. 18).

Medicinal plants that are part of the studied herbal mixtures contain polyphenolic compounds, which exhibit antidiabetic activity by different mechanism of actions, including stimulation of insulin secretion, improvement of pancreatic β-cell functionality, inhibition of gluconeogenesis, intensification of glucose uptake, delay of carbohydrate digestion and glucose absorption, inhibition of protein glycation and insulin fibrillation [19, 20, 21]. No less important is their antioxidant activity in the treatment and prevention of diabetes and its complications because they can include suppression of reactive oxygen spesies (ROS) formation; inhibition the enzymes involved in ROS generation, and so forth [21, 22, 23]. Medicinal plant raw materials containing phenolic compounds are *Phaseoli pericarpium* (the herbal mixtures No. 16 and No. 20), *Myrtilli folia* (the herbal mixtures No. 16, and No. 19 and No. 20), *Rosae fructus* (the herbal mixtures No. 17, and No. 19 and No. 20), *Melissae folia* (the herbal mixture No. 17), *Veronicae herba* (the herbal mixture No. 18), *Betulae verrucosae folia* (the herbal mixtures No. 18 and No. 20), *Urticae folia* (the herbal mixtures No. 18 and No. 19), *Menthae folia* (the herbal mixtures No. 19 and No. 20).

Thus, screening study of the herbal mixtures No. 16-20 showed their hypoglycemic activity by OGTT, IPGTT and confirmed the effectiveness of their using in folk medicine for the prevention and treatment of diabetes mellitus type 2.

Conclusions

During the study, it was conducted the screening study of hypoglycemic activity of the herbal mixtures No. 16-20, which are used in folk medicine for the prevention and treatment of diabetes mellitus type 2.

Acoding to the result of the study, the greatest effectiveness in terms of the ability to reduce alimentary hyperglycemia during OGTT and reduce impaired carbohydrate tolerance during IPGTT showed the herbal mixture No. 19, which includes *Urticae folia, Taraxaci radice, Myrtilli folia, Rosae fructus* and *Menthae folia.* It was established its conditional therapeutic dose 12 mL/kg/day.

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Table 1. Composition of the herbal mixtures

Herbal mixtures	Herbals	Quantity of the herbals in the mixtures, g
No. 16	*Phaseoli pericarpium*	20.00
	Avenae sativae semina	20.00
	Myrtilli folia	20.00
	Lini semina	20.00
	Elymi repens rhizomata	20.00
		Total: 100.00
No. 17	*Taraxaci radices*	20.00
	Rosae fructus	20.00
	Lini semina	20.00
	Melissae folia	20.00
	Avenae sativae semina	20.00
		Total: 100.00
No. 18	*Veronicae herba*	20.00
	Betulae verrucosae folia	20.00
	Urticae folia	20.00
	Arctii lappae radices	20.00
	Lini semina	20.00
		Total: 100.00
No. 19	*Urticae folia*	20.00
	Taraxaci radices	20.00
	Myrtilli folia	20.00
	Rosae fructus	20.00
	Menthae folia	20.00
		Total: 100.00
No. 20	*Betulae verrucosae folia*	20.00
	Myrtilli folia	20.00
	Rosae fructus	20.00
	Menthae folia	20.00
	Phaseoli pericarpium	20.00
		Total: 100.00

Table 2. Hypoglycemic effect of the herbal mixtures compared to the official herbal mixture "Arfazetin" and tablets metformin by OGTT after 20 days of preventive treatment of normoglycemic rats

Group of animals	Glucose level, mmol/L			
	0 min	30 min	60 min	120 min
Series fourth				
Control	4.17±0.07	7.89±0.09	7.62±0.12	5.85±0.13
HM "Arfazetin", 9 mL/kg	4.08±0.08	5.38±0.11*	5.33±0.15*	4.92±0.14*
MET, 60 mg/kg	3.91±0.16	4.28±0.17 */**	4.17±0.18 */**	4.02±0.14 */**
HM No. 16, 6 mL/kg	3.93±0.17	5.48±0.16*	5.33±0.18*	5.29±0.18*
HM No. 16, 9 mL/kg	4.01±0.16	5.49±0.17*	5.39±0.13*	5.26±0.17*
HM No. 16, 12 mL/kg	4.04±0.15	5.44±0.17*	5.31±0.18*	5.17±0.19*
HM No. 17, 6 mL/kg	4.03±0.18	5.59±0.16*	5.42±0.14*	5.33±0.11*
HM No. 17, 9 mL/kg	4.06±0.17	5.51±0.18*	5.39±0.18*	5.21±0.19*
HM No. 17, 12 mL/kg	4.04±0.11	5.42±0.19*	5.32±0.13*	5.18±0.11*
HM No. 18, 6 mL/kg	4.02±0.18	5.62±0.15*	5.48±0.13*	5.31±0.18*
HM No. 18, 9 mL/kg	4.07±0.15	5.54±0.15*	5.39±0.10*	5.18±0.17*
HM No. 18, 12 mL/kg	4.08±0.11	5.49±0.16*	5.36±0.14*	5.17±0.15*
HM No. 19, 6 mL/kg	3.92±0.14	5.32±0.15*	5.21±0.18*	5.11±0.18*
HM No. 19, 9 mL/kg	4.02±0.17	5.25±0.13*	5.19±0.18*	5.09±0.14*
HM No. 19, 12 mL/kg	4.05±0.16	4.39±0.15 */**	4.24±0.16 */**	4.12±0.18*
HM No. 20, 6 mL/kg	4.09±0.17	5.63±0.20*	5.59±0.18*	5.32±0.18*
HM No. 20, 9 mL/kg	4.03±0.14	5.51±0.17*	5.39±0.19*	5.19±0.19*
HM No. 20, 12 mL/kg	4.06±0.11	5.48±0.28*	5.32±0.18*	5.18±0.11*

Values are expressed as mean ± SEM from 8 rats; * $p<0.05$ with respect to Control group; ** $p<0.05$ with respect to the herbal mixture "Arfazetin".

Table 3. Hypoglycemic effect of the herbal mixtures compared to the official herbal mixture "Arfazetin" and tablets metformin by IPGTT after 20 days of preventive treatment of normoglycemic rats

Group of animals	Glucose level, mmol/L			
	0 min	15 min	45 min	60 min
Series fourth				
Control	4.21±0.11	8.62±0.17*	5.23±0.18	4.42±0.11
HM "Arfazetin", 9 mL/kg	4.19±0.18	6.82±0.19*	5.01±0.17	4.43±0.15
MET, 60 mg/kg	4.14±0.19	6.32±0.17*/**	4.92±0.18	4.21±0.13
HM No. 16, 6 mL/kg	4.18±0.15	7.37±0.12*	5.36±0.15	4.31±0.18
HM No. 16, 9 mL/kg	4.13±0.14	7.26±0.18*	5.21±0.17	4.23±0.14
HM No. 16, 12 mL/kg	4.16±0.19	7.19±0.14*	5.19±0.13	4.27±0.21
HM No. 17, 6 mL/kg	4.20±0.22	7.39±0.17*	5.38±0.11	4.31±0.11
HM No. 17, 9 mL/kg	4.12±0.17	7.25±0.13*	5.22±0.18	4.23±0.17
HM No. 17, 12 mL/kg	4.17±0.21	7.09±0.15*	5.17±0.16	4.25±0.13
HM No. 18, 6 mL/kg	4.18±0.17	7.36±0.15*	5.39±0.16	4.34±0.17
HM No. 18, 9 mL/kg	4.23±0.16	7.21±0.12*	5.28±0.13	4.36±0.22
HM No. 18, 12 mL/kg	4.17±0.15	7.17±0.15*	5.15±0.16	4.28±0.15
HM No. 19, 6 mL/kg	4.16±0.15	7.08±0.16*	5.14±0.13	4.26±0.19
HM No. 19, 9 mL/kg	4.09±0.16	7.07±0.22*	5.07±0.15	4.18±0.15
HM No. 19, 12 mL/kg	4.18±0.13	6.35±0.15*/**	4.93±0.14*	4.24±0.19
HM No. 20, 6 mL/kg	4.17±0.16	7.34±0.16*	5.37±0.15	4.28±0.12
HM No. 20, 9 mL/kg	4.18±0.13	7.23±0.19*	5.26±0.16	4.31±0.11
HM No. 20, 12 mL/kg	4.09±0.16	7.13±0.17*	5.16±0.18	4.23±0.21

Values are expressed as mean ± SEM from 8 rats; * $p<0.05$ with respect to Control group; ** $p<0.05$ with respect to the herbal mixture "Arfazetin"