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The screening study of the hypoglycemic activity of herbal mixtures (presentation 3)

Diabetes mellitus is an important social and medical problem as it causes the development of dangerous complications leading to disability and mortality. This disease is characterized by a multi-vector pathogenesis that requires a comprehensive approach to the treatment. Due to the use of mixtures of medicinal plants in the treatment of diabetes it is possible to cover all aspects of the development of this disease and its complications.

Aim. To study the hypoglycemic activity of herbal mixtures used in folk medicine for the prevention and treatment of diabetes mellitus type 2, and determine their conditional therapeutic dose.

Materials and methods. The study was performed on male albino rats weighing 180-200 g. For the preventive treatment within 20 days they received orally the aqueous extracts (1:10) of the herbal mixtures studied in the doses of 6 mL/kg/day, 9 mL/kg/day and 12 mL/kg/day and the reference drugs – the official herbal mixture "Arfazetin" in the dose of 9 mL/kg/day and metformin tablets in the dose of 60 mg/kg/day. The study of hypoglycemic properties and determination of the conditional therapeutic dose of the mixtures studied were performed using glucose loading tests (oral glucose tolerance test – OGTT and intraperitoneal glucose tolerance test– IPGTT). 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 used for scientific purposes.

Results and discussion. The results of the study showed that the 20-day preventive treatment with the herbal mixtures reduced alimentary hyperglycemia 30 minutes after OGTT and regulated carbohydrate tolerance disorders by reducing hyperglycemia 15 minutes after IPGTT. The herbal mixture No. 13 in the dose of 12 mL/kg/day showed the highest hypoglycemic activity, which was almost similar with the reference drug – metformin tablets, but exceeded the official herbal mixture "Arfazetin" by its efficiency. In addition, the dose-dependence of the effectiveness of all five herbal mixtures studied was determined.

Conclusions. For the first time, the screening study of the hypoglycemic activity of herbal mixtures used in folk medicine for the prevention and treatment of diabetes mellitus type 2 has been conducted. It has been determined that the herbal mixture No. 13 containing *Cichorii radices, Elymi repens rhizomata, Helichrysi arenarii flores, Rosae fructus, Maydis style cum stigmatis* shows the highest effectiveness by the ability to reduce alimentary hyperglycemia during OGTT and reduce impaired carbohydrate tolerance during IPGTT. Its conditional therapeutic dose, which is 12 mL/kg/day, has been determined.

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

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Скринінгове дослідження гіпоглікемічної активності рослинних зборів (повідомлення 3)

Цукровий діабет є важливою соціальною та медичною проблемою, адже спричиняє розвиток небезпечних ускладнень, що призводять до інвалідизації та смертності населення. Це захворювання характеризується багатовекторним патогенезом, що потребує комплексного підходу до лікування. Завдяки застосуванню зборів лікарських рослин у терапії цукрового діабету можна охопити усі ланки розвитку даного захворювання та його ускладнень.

Метою даного дослідження стало вивчення гіпоглікемічних властивостей рослинних зборів, які використовуються у народній медицині для профілактики та лікування цукрового діабету 2 типу та встановлення їх умовно терапевтичної дози.

Матеріали та методи. Дослідження проводилися на інтактних нормоглікемічних білих щурах самцях масою 180-200 г, які з метою профілактичного лікування впродовж 20-ти днів перорально отримували водні екстракти (1:10) досліджуваних зборів у дозі 6 мл/кг/день, 9 мл/кг/день та 12 мл/кг/день та препарати порівняння офіцинальний збір «Арфазетин» у дозі 9 мл/кг/день і таблетки метформіну у дозі 60 мг/кг/день. Вивчення гіпоглікемічних властивостей та встановлення умовно терапевтичної дози досліджуваних засобів здійснювали за допомогою тестів глюкозного навантаження. Усі досліди виконували з дотриманням загальних етичних принципів згідно з рекомендаціями Директиви ЄС 2010/63/EU про захист використовуваних тварин для наукових цілей.

Результати та їх обговорення. Результати дослідження показали, що 20-ти денне профілактичне введення рослинних зборів знижувало аліментарну гіперглікемію на 30-й хв ОТТГ та сприяло зменшенню порушень толерантності до вуглеводів шляхом зниження гіперглікемії на 15-й хв ВОТТГ. Найбільшу гіпоглікемічну активність проявив рослинний збір № 13 у дозі 12 мл/кг/день, яка була практично на рівні з препаратом порівняння таблетками метформіну, але перевищувала за ефективністю офіцинальний збір «Арфазетин». Окрім цього було встановлено дозозалежність ефективності усіх п'яти досліджуваних рослинних зборів.

Висновки. Вперше проведено скринінгове дослідження гіпоглікемічної активності рослинних зборів, що застосовуються в народній медицині для профілактики та лікування цукрового діабету 2 типу. Визначено, що

найбільшу ефективність за здатністю знижувати аліментарну гіперглікемію під час ОТТГ та знижувати порушення толерантності до вуглеводів під час ВОТТГ проявив рослинний збір № 13, до складу якого входять цикорію корені, пирію кореневища, цмину квітки, шипшини плоди, кукурудзи стовпчики з приймочками. Встановлено його умовно терапевтичну дозу, яка складає 12 мл/кг/день.

Ключові слова: рослинні збори; гіпоглікемічна активність; цукровий діабет; оральний тест толерантності до глюкози; внутрішньоочеревинний тест толерантності до глюкози

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Скрининговое исследование гипогликемической активности растительных сборов (сообщение 3)

Сахарный диабет является важной социальной и медицинской проблемой, поскольку приводит к развитию опасных осложнений, приводящих к инвалидизации и смертности населения. Это заболевание характеризуется многовекторным патогенезом и требует комплексного подхода к лечению. Благодаря применению сборов лекарственных растений в терапии сахарного диабета можно охватить все звенья развития данного заболевания и его осложнений.

Цель. Изучение гипогликемических свойств растительных сборов, используемых в народной медицине для профилактики и лечения сахарного диабета 2 типа, и установление их условно терапевтической дозы.

Материалы и методы. Исследования проводились на интактных нормогликемических белых крысах самцах массой 180-200 г, которые с целью профилактического лечения в течение 20-ти дней перорально получали водные экстракты (1:10) исследуемых сборов в дозе 6 мл/кг/день, 9 мл/кг/день и 12 мл/кг/день и препараты сравнения официнальный сбор «Арфазетин» в дозе 9 мл/кг/день, а также таблетки метформина в дозе 60 мг/кг/день. Изучение гипогликемических свойств и установление условно терапевтической дозы исследуемых средств осуществляли с помощью тестов глюкозной нагрузки. Все опыты проводили с соблюдением общих этических принципов согласно рекомендациям Директивы ЕС 2010/63/EU о защите используемых животных для научных целей.

Результаты и их обсуждение. Результаты исследования показали, что 20-дневное профилактическое использование растительных сборов снижало алиментарную гипергликемию на 30-й минуте ОТТГ, что способствовало уменьшению нарушений толерантности к углеводам путем снижения гипергликемии на 15-й минуте ВЧТТГ. Наибольшую гипогликемическую активность проявил растительный сбор № 13 в дозе 12 мл/кг/день, которая была практически на уровне с препаратом сравнения таблетками метформина, но превышала по эффективности официнальный сбор «Арфазетин». Кроме этого была установлена дозозависимость эффективности всех пяти исследуемых растительных сборов.

Выводы. Впервые было проведено скрининговое исследование гипогликемической активности растительных сборов, применяемых в народной медицине для профилактики и лечения сахарного диабета 2 типа. Определено, что наибольшую эффективность по способности снижать алиментарную гипергликемию при ОТТГ и снижать нарушение толерантности к углеводам при ВЧТТГ проявляет растительный сбор № 13, в состав которого входят цикория корни, пырея корневища, бессмертника цветки, шиповника плоды, кукурузы рыльца. Установлено его условно терапевтическую дозу, которая составляет 12 мл/кг/день.

Ключевые слова: растительные сборы; гипогликемическая активность; сахарный диабет; оральный тест толерантности к глюкозе; внутрибрюшинный тест толерантности к глюкозе

Diabetes mellitus is a global social problem in the field of healthcare due to a 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 the 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 the 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 formation of a cascade of pathological processes – excessive glycation and inactivation of the body's antioxidant defense system, triggering the processes of free radical oxidation of lipids and, consequently, the development of oxidative stress, which leads to the development and progression of diabetic angiopathies [1, 3, 4].

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

One of the areas is phytotherapy since 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 in a number of biologically active compounds [5, 6]. Particular attention deserve the combinations of different medicinal plants as these herbal mixtures have more biologically active substances that will influence on all links of the pathogenetic mechanism of diabetes mellitus development and its complications [7-9]. In addition, the pharmaceutical market of Ukraine is represented mainly by synthetic antidiabetic drugs, which account for over 92 % of all oral antidiabetic drugs. Today there are

two antidiabetic herbal mixtures in Ukraine – the herbal mixture "Arfazetin" containing *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" containing *Helianthi tubera, Steviae folia, Vaccini myrtilli cormus*, *Phaseoli valvae fructum, Thea chinensis, Menthae piperitae folia*.

However, *Vaccinii myrtilli cormus*, *Eleutherococci senticosi rhizomata et radices* and *Hyperici herba* are potent plants that can be dangerous with the 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 often complications of diabetes.

Thus, **the aim** of our research was to study the hypoglycemic activity of herbal mixtures used in folk medicine for the prevention and treatment of diabetes mellitus type 2 [10], but without a scientific basis and determine their conditional therapeutic dose.

Materials and methods

The herbal raw material harvested in June to August 2019 in the Ternopil region (Ukraine) was used. After collecting the raw material was dried, crushed and standardized according to the general GACP requirements [11]. The plants were identified by the Department of Pharmacognosy with Medical Botany, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine. The voucher specimens of the herbal raw materials were deposited in the Departmental Herbarium for future record.

For the study, five different herbal mixtures used in folk medicine for the prevention and treatment of diabetes mellitus type 2 in Ukraine were applied [10]. The composition of the mixtures is given in Tab. 1.

The samples of the herbal raw material were powdered by a 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 on the boiling water bath for 30 min. The extracts were filtered using Whatmann filter paper No. 1. Then the filtrates were evaporated by a rotary evaporator and lyophilized to dryness. The lyophilized powders of each herbal mixture were stored at 4 °C for further use.

The aqueous extract of the reference drug – the official herbal mixture "Arfazetin" was prepared using 5 g of a 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, metformin tablets were crushed and mixed with 2 mL of distilled water.

Table 1

The composition of the herbal mixtures

Herbal mixtures	Medicinal plants	The amount of the plants in the mixtures, g
No. 11	*Helianthi tuberosi tuber*	23.53
	Maydis style cum stigmatis	11.76
	Elymi repens rhizomata	23.53
	Leonuri cardiacae herba	11.76
	Betulae verrucosae folia	5.89
	Plantaginas majoris folia	11.76
	Crataegi fructus	11.76
		Total: 100.00
No. 12	*Helianthi tuberosi tuber*	11.11
	Callunae herba	22.22
	Polygoni avicularis herba	22.22
	Origani herba	11.11
	Althaeae radices	22.22
	Crataegi fructus	11.11
		Total: 100.00
No. 13	*Cichorii radices*	26.32
	Elymi repens rhizomata	26.32
	Helichrysi arenarii flores	21.05
	Rosae fructus	15.79
	Maydis style cum stigmatis	10.52
		Total: 100.00
No. 14	*Myrtilli fructus*	16.67
	Cichorii radices	25.00
	Elymi repens rhizomata	25.00
	Crataegi fructus	16.67
	Rosae fructus	16.67
		Total: 100.00
No. 15	*Myrtilli folia*	33.33
	Galegae herba	33.33
	Taraxaci radices	33.33
		Total: 100.00

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

The study was performed on male albino rats weighing between 180-200 g bred in the vivarium of the Central Research Laboratory at I. Horbachevsky Ternopil National Medical University where they were kept under appropriate conditions (at a constant room temperature of 22 ± 1 °C, 40-70 % humidity conditions and a 12-hour light/dark cycle). Throughout the experimental period the animals received a 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 [12]. All protocols for animals experiment were approved by the animal ethical committee of I.Horbachevsky Ternopil National Medical University.

The screening study of the hypoglycemic activity of herbal mixtures and determination of their conditionally therapeutic dose was performed in intact normoglycemic rats. Animals were randomly divided into eight groups of eight animals (n=8) each and received different preventive treatment once daily for 20 days. Group I (control) received distilled water (12 mL/kg/day) *per os* (*p.o.*), group II (HM "Arfazetin") – an aqueous extract of the official herbal mixture "Arfazetin" (9 mL/kg/day, *p.o.*) [13], group III (MET) – a suspension of metformin (60 mg/kg/day, *p.o.*) [14], group IV-VIII (HM) – aqueous extracts of the herbal mixtures No. 11-15 in the doses 6 mL/kg/day, 9 mL/kg/day and 12 mL/kg/day, *p.o*. The last oral administration of the products studied was 2 hours before the glucose load tests.

Fasting blood glucose (basal glycemia) was measured in tail blood samples after a 6-hour fast on the 20th day of the experiment using a glucose analyzer (Accuk-Check glucometer, Germany). The oral glucose tolerance test (OGTT) was performed after measuring basal glycemia by administering glucose solution (3 g/kg, *p. o).* Blood glucose levels were determined in 0, 30, 60 and 120 minutes after glucose loading [15].

Measurements of the intraperitoneal glucose tolerance test (IPGTT) were performed on the 21st day of the experiment after overnight fasting (16-18 hours) by intraperitoneal administration of glucose solution (2 g/kg, *i. p*.) to rats 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 in 15, 45 and 60 minutes after using a glucose analyzer [15].

The values were expressed as mean ± SEM. The data were analyzed using GraphPad Prism software, version 5.03. The results were compared using the ANOVA-One-Way test followed by *Mann-Whitney U test*. The difference was considered to be 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 stage of the screening study, the effect of the herbal mixtures and the reference drugs on basal glycemia and on glycemia after carbohydrate loading by OGTT after 20 days of the preventive treatment was studied. This test allows simulating alimentary hyperglycemia that occurs after eating. The hypoglycemic activity of the herbal mixtures and reference drugs was manifested by their ability to reduce blood glucose levels in 30 minutes of the test during its maximum increase in response to oral carbohydrate load.

The results of the study showed that the 20-day preventive treatment by all five herbal mixtures (No. 11-15) in the doses of 6 mL/kg/day, 9 mL/kg/day and 12 mL/kg/day significantly ($p<0.05$) reduced glycemia in 30 minutes after OGTT compared to the control group. However, the herbal mixture No. 13 in the dose of 12 mL/kg/day showed the best results of the hypoglycemic activity in 30 minutes after the test, and it reduced blood glucose levels by 45 %, relative to the control group. Metformin tablets demonstrated a similar result in efficacy as they reduced alimentary hyperglycemia by 46 % relative to the control group of animals in 30 minutes. The official herbal mixtures "Arfazetin" was inferior in efficiency to the herbal mixture No. 13 in the dose of 12 mL/kg/day and reduced glycemia by 32 % relative to the control group in 30 minutes after the test (Tab. 2).

During the determination of the 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. 13 (12 mL/kg/day) was 262.2 mmol/L min. Regarding the results of the reference drugs the AUC_{glu} of metformin (60 mg/kg/day) was lower and amounted to 256.8 mmol/L min, and that of the herbal mixture "Arfezetin" (9 mL/kg/day) was higher and amounted to 322.8 mmol/L min.

At the second stage of the screening study the ability of the herbal mixtures No. 11-15 and the reference drugs to improve carbohydrate tolerance was determined using IPGTT. The hypoglycemic effect of the herbal mixtures and the reference drugs was assessed by their ability to reduce hyperglycemia in 15 minutes after IPGTT during the maximum rise of blood glucose in 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 in 15 minutes of the test (hyperglycemic peak), exceeding the initial data by 2.0 times. The best ability to reduce the hyperglycemic peak of IPGTT showed the herbal mixture No. 13 (12 mL/kg/day); the blood glucose level was lower by 26 % relative to the control group. Metformin tablets showed a similar effect and reduced hyperglycemia in 15 minutes 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. 13 in the dose of 12 mL/kg/day and reduced hyperglycemia by 21 %. By the end of the experiment in 60 minutes after IPGTT the blood glucose level returned to baseline in all groups of animals (Tab. 3).

The results of the screening study of the herbal mixtures No. 11-15 used in folk medicine for the prevention and treatment of diabetes mellitus type 2 using OGTT and IPGTT indicate a dose-dependent hypoglycemic activity. The best hypoglycemic effect of the objects studied was shown in the dose of 12 mL/kg/day.

Table 2

The hypoglycemic effect of the herbal mixtures compared to the official herbal mixture "Arfazetin" and metformin tablets by oral glucose tolerance test in 20 days of the preventive treatment in normoglycemic rats

Group of animals	Glucose level, mmol/L			
	0 min	30 min	60 min	120 min
Series 3 of the experiments				
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. 11, 6 mL/kg	4.07±0.18	5.66±0.17*	5.52±0.15*	5.34±0.14*
HM No. 11, 9 mL/kg	3.92±0.16	5.58±0.11*	5.39±0.13*	5.22±0.18*
HM No. 11, 12 mL/kg	4.18±0.11	5.72±0.17*	5.55±0.16*	5.31±0.19*
HM No. 12, 6 mL/kg	4.02±0.17	5.65±0.18*	5.39±0.17*	5.24±0.18*
HM No. 12, 9 mL/kg	4.07±0.17	5.63±0.18*	5.47±0.17*	5.29±0.19*
HM No. 12, 12 mL/kg	4.05±0.15	5.52±0.12*	5.33±0.15*	5.25±0.14*
HM No. 13, 6 mL/kg	3.96±0.11	5.61±0.18*	5.43±0.18*	5.20±0.17*
HM No. 13, 9 mL/kg	4.01±0.16	5.48±0.18*	5.31±0.18*	5.11±0.19*
HM No. 13, 12 mL/kg	3.92±0.17	4.37±0.19*/**	4.23±0.15*/**	4.04±0.14*
HM No. 14, 6 mL/kg	4.07±0.11	5.69±0.12*	5.53±0.18*	5.31±0.10*
HM No. 14, 9 mL/kg	4.02±0.15	5.66±0.17*	5.43±0.15*	5.19±0.19*
HM No. 14, 12 mL/kg	4.04±0.16	5.68±0.17*	5.38±0.11*	5.20±0.18*
HM No. 15, 6 mL/kg	4.11±0.08	5.59±0.11*	5.42±0.16*	5.31±0.18*
HM No. 15, 9 mL/kg	4.03±0.17	5.49±0.17*	5.32±0.18*	5.21±0.12*
HM No. 15, 12 mL/kg	4.11±0.17	5.42±0.18*	5.38±0.17*	5.19±0.17*

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

Table 3

The hypoglycemic effect of the herbal mixtures compared to the official herbal mixture "Arfazetin" and tablets metformin by intraperitoneal glucose tolerance test in 20 days of the preventive treatment in normoglycemic rats

Group of animals	Glucose level, mmol/L			
	0 min	15 min	45 min	60 min
Series 3 of the experiments				
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. 11, 6 mL/kg	4.16±0.16	7.44±0.17*	5.31±0.13	4.28±0.12
HM No. 11, 9 mL/kg	4.18±0.15	7.31±0.18*	5.27±0.16	4.27±0.17
HM No. 11, 12 mL/kg	4.18±0.16	7.21±0.16*	5.09±0.17	4.29±0.13
HM No. 12, 6 mL/kg	4.19±0.17	7.41±0.18*	5.38±0.16	4.31±0.17
HM No. 12, 9 mL/kg	4.22±0.18	7.32±0.16*	5.28±0.19	4.29±0.22
HM No. 12, 12 mL/kg	4.20±0.18	7.21±0.18*	5.11±0.18	4.32±0.21
HM No. 13, 6 mL/kg	4.09±0.17	7.23±0.19*	5.15±0.13	4.21±0.18
HM No. 13, 9 mL/kg	4.17±0.16	7.09±0.17*	5.09±0.12	4.22±0.21
HM No. 13, 12 mL/kg	4.16±0.18	6.38±0.18*/**	4.94±0.17*	4.21±0.12
HM No. 14, 6 mL/kg	4.19±0.16	7.37±0.16*	5.32±0.19	4.28±0.16
HM No. 14, 9 mL/kg	4.18±0.16	7.29±0.13*	5.29±0.13	4.27±0.12
HM No. 14, 12 mL/kg	4.16±0.12	7.09±0.16*	5.17±0.16	4.28±0.16
HM No. 15, 6 mL/kg	4.18±0.17	7.29±0.13*	5.22±0.13	4.25±0.11
HM No. 15, 9 mL/kg	4.22±0.17	7.21±0.15*	5.06±0.17	4.29±0.27
HM No. 15, 12 mL/kg	4.20±0.12	7.16±0.19*	5.04±0.13	4.29±0.17

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

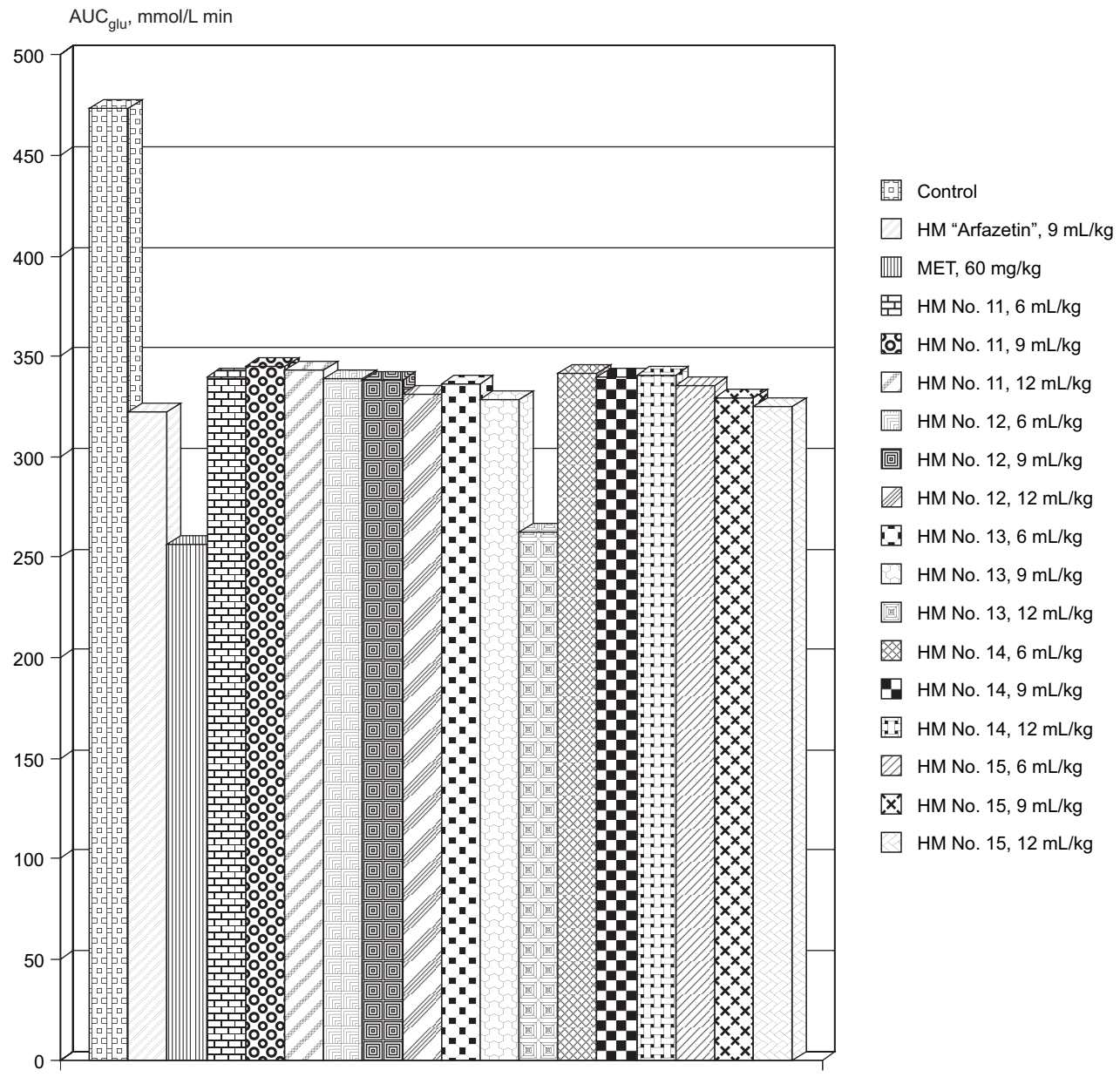

Fig. The hypoglycemic effect of the herbal mixtures compared to the official herbal mixture "Arfazetin" and metformin tablets by OGTT in 20 days of the preventive treatment of normoglycemic rats, mean ± SEM, n=8

The study using glucose load tests showed that the herbal mixtures No. 11, No. 12, No. 14 and No. 15 in the doses of 6 mL/kg/day, 9 mL/kg/day and 12 mL/kg/day exhibited the hypoglycemic activity, but it was slightly lower compared to the herbal mixture No. 13 (12 mL/kg/day) and the reference drugs – the official herbal mixture "Arfazetin" (9 mL/kg/day) and metformin tablets (60 mg/kg/day) (Tab. 2, 3).

The hypoglycemic activity of the herbal mixtures studied is quite predictable since they include the medicinal plant raw material containing biologically active substances with the proven hypoglycemic action. The main groups of biologically active substances that can lower blood glucose are polysaccharides, especially inulin that has the ability to increase glucagon-like peptide-1 (GLP-1), which increases insulin secretion, inhibits glucagon secretion, causes proliferation and neogenesis of β-cells and increases the response of β-cells to glucose [16, 17]. The herbal mixtures under research contain the herbal raw material that are rich in carbohydrates, such as *Cichorii radices* (the herbal mixtures No. 13 and No. 14), *Taraxaci radices* (the herbal mixture No. 15), *Elymi repens rhizomata* (the herbal mixtures No. 11, No. 13 and No. 14), *Helianthi tuberosi tuber* (the herbal mixtures No. 11 and No. 12), *Plantaginas majoris folia* (the herbal mixture No. 11), *Althaeae radices* (the herbal mixture No. 12).

In addition, medicinal plants that are part of the herbal mixtures studied contain polyphenolic compounds that exhibit the 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 [18-20]. No less important is their antioxidant activity in the treatment and prevention of diabetes and its complications since they can include suppression of forming reactive oxygen species (ROS) either by inhibition of enzymes or by chelating trace elements involved in free radical generation; scavenging ROS; inhibition of the enzymes involved in ROS generation – microsomal monooxygenase, glutathione S-transferase, mitochondrial succinoxidase, nicotinamide adenine dinucleotide phosphate (NADH) oxidase, and so forth [20-22]. The medicinal plant raw material containing phenolic compounds are *Leonuri cardiacae herba* (the herbal mixture No. 11), *Betulae verrucosae folia* (the herbal mixture No. 11), *Origani herba* (the herbal mixture No. 12), *Callunae herba* (the herbal mixture No. 12), *Maydis style cum stigmatis* (the herbal mixtures No. 11 and No. 13), *Polygoni avicularis herba* (the herbal mixture No. 12), *Helichrysi arenarii flores* (the herbal mixture No. 13), *Rosae fructus* (the herbal mixtures No. 13 and No. 14), *Galegae herba* (the herbal mixture No. 15), *Crataegi fructus* (the herbal mixtures No. 11, No. 12 and No. 14), *Myrtilli fructus* (the herbal mixture No. 14) and *Myrtilli folia* (the herbal mixture No. 15).

Thus, the screening study of herbal mixtures No. 11-15 shows their hypoglycemic activity in OGTT, IPGTT and confirms the effectiveness of their use in folk medicine for the prevention and treatment of diabetes mellitus type 2.

CONCLUSIONS

1. For the first time, the screening study of the hypoglycemic activity of herbal mixtures No. 11-15 used in folk medicine for the prevention and treatment of diabetes mellitus type 2 has been conducted.

2. It has been determined that the herbal mixture No. 13 containing *Cichorii radices, Elymi repens rhizomata, Helichrysi arenarii flores, Rosae fructus, Maydis style cum stigmatis* shows the highest effectiveness by the ability to reduce alimentary hyperglycemia during OGTT and reduce impaired carbohydrate tolerance during IPGTT. Its conditional therapeutic dose, which is 12 mL/kg/day, has been determined.

Conflict of interests: authors have no conflict of interests to declare.

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