УДК 615.322:615.451.1:616.379
DOI: 10.15587/2519-4852.2020.210734

SCREENING STUDY OF HYPOGLYCEMIC ACTIVITY OF THE HERBAL MIXTURES (MESSAGE 1)

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The aim. *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.*

Materials and methods. *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 officinal herbal mixtures "Arfazetin" at a dose 9 mL/kg/day and metformin tablets 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.*

Results. *The results of the study showed that the 20-day preventive treatment by the herbal mixtures reduced alimentary hyperglycemia at the 30th minutes of OGTT and helped regulate carbohydrate tolerance disorders by reducing hyperglycemia at the 15th minutes of IPGTT. The highest hypoglycemic activity showed the herbal mixtures No. 3 (12 mL/kg/day) and No. 4 (12 mL/kg/day), which was almost on a par with the comparison drug – metformin tablets, but exceeded the officinal herbal mixture "Arfazetin". In addition, the dose-dependence of the effectiveness of all five studied herbal mixtures was established.*

Conclusions*. For the first time, it was conducted the screening study of hypoglycemic activity of the herbal mixtures, which are used in folk medicine for the prevention and treatment of diabetes mellitus type 2. It was determined that the greatest effectiveness in terms of the ability to reduce alimentary hyperglycemia during OGTT and reduce impaired carbohydrate tolerance during IPGTT show the herbal mixtures No. 3 (which includes Urticea folia, Cichorii radices, Rosae fructus, Elymi repens rhizomata, Taraxaci radices) and No. 4 (which includes Arctii lappae radices, Elymi repens rhizomata, Maydis style cum sigmatis, Helichrysi arenarii flores, Rosae fructus). It was established their conditional therapeutic dose of 12 mL/kg/day*

Keywords: *herbal mixtures, hypoglycemic activity, diabetes mellitus*

1. Introduction

Diabetes mellitus is a global social and medical problem caused by the rapid spread of the disease and the development of serious complications such as angiopathies, which significantly reduce the quality and life expectancy of patients [1]. According to the official data from 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 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, as a 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 hypoglycemic 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 [5, 6]. Particular attention deserve the combinations of different medicinal plants, because such herbal mixtures will have more biologically active substances that will influence on the all links of the pathogenetic mechanism of development of diabetes mellitus and its complications [7, 8]. 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 in

Ukraine there are 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*.

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* have a tonic effect and are 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 the herbal mixtures, which are used in folk medicine for the prevention and treatment of diabetes mellitus type 2 [9], but do not have a scientific basis and to establish their conditional therapeutic dose.

2. Planning (methodology) of research

The pharmaceutical market of Ukraine is represented mainly by synthetic antidiabetic drugs, although the use of the medical herbs, especially their mixtures, has centuries of experience in folk medicine. This is evidenced by the analysis of literature sources. Therefore, the experimental study of the hypoglycemic properties of the herbal mixtures, which are used for the prevention and treatment of diabetes mellitus type 2 in folk medicine is a promising area in pharmacy and medicine. Based on this, we set ourselves the goal of conducting a screening study of the herbal mixtures, to determine their hypoglycemic activity and to establish their conditionally therapeutic dose. To achieve this goal, the following tasks were set: at the first stage to conduct an informational search of the herbal mixtures, which are used for the prevention and treatment of diabetes mellitus type 2, at the second stage – analysis of literature sources with selection of the herbal mixtures for research, at the third stage – study of safety of the herbal mixtures, by determination acute toxicity, in the fourth and fifth stages – screening study of hypoglycemic activity of the herbal mixtures in normoglycemic rats and establish their conditionally therapeutic dose.

The goal can be easily achieved, since during the experiment there are no difficulties and the study is almost safe. The experimental design is presented below Fig. 1.

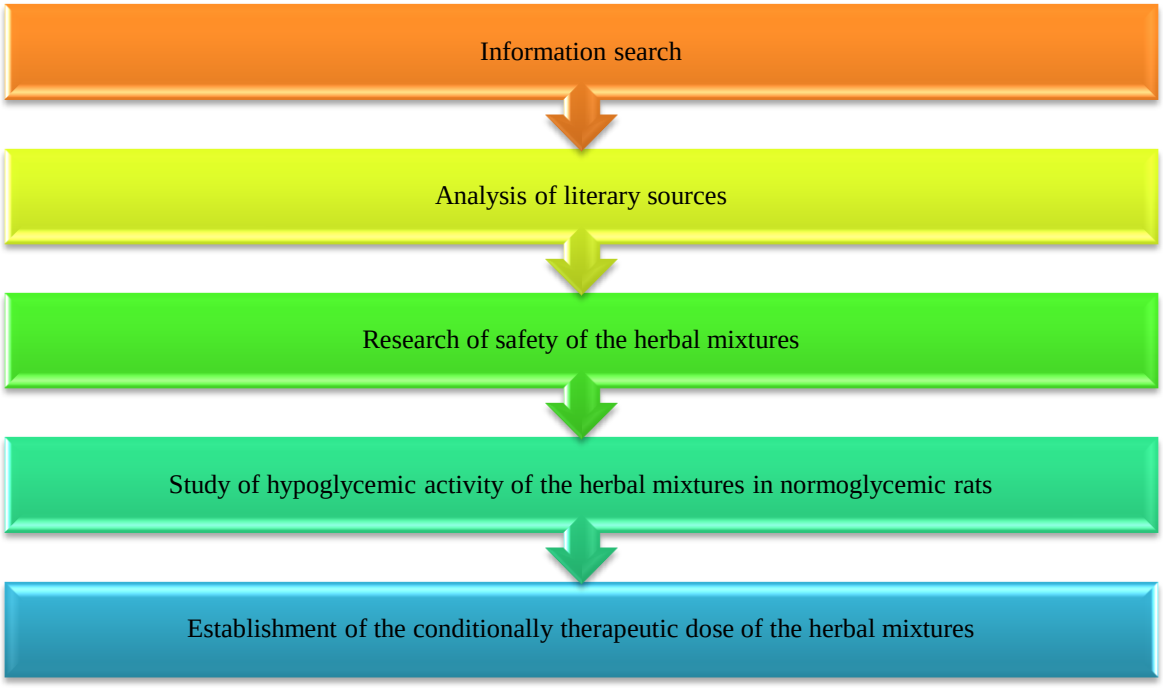

Fig. 1. Algorithm of research

3. Materials and methods

Plant materials: the herbal raw materials were harvested from June to August 2019 in Ternopil region (Ukraine). After harvesting, the raw materials were dried, crushed and brought back to standard according to the general GACP requirements [10]. 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 [9]. Composition of the mixtures are given in Table 1.

Table 1

Composition of the herbal mixtures

Herbal mixtures	Herbals	Quantity of the herbals in the mixtures, g
No. 1	*Phaseoli pericarpium*	11.76
	Cichorii radices	17.65
	Taraxaci radices.	11.76
	Helichrysi arenarii flores	17.65
	Polemonii rhizomata cum radicibus	11.76
	Ericae herba	17.65
	Melissae folia	11.76
Total: 100.0		
No. 2	*Galegae herba*	13.33
	Cichorii radices	20.0
	Helichrysi arenarii flores	13.33
	Polemonii rhizomata cum radicibus	13.33
	Ericae herba	20.0
	Melissae folia	20.0
Total: 100.0		
No. 3	*Urticea folia*	26.32
	Cichorii radices	26.32
	Rosae fructus	21.05
	Elymi repens rhizomata	15.79
	Taraxaci radices	10.52
Total: 100.0		
No. 4	*Arctii lappae radices*	26.32
	Elymi repens rhizomata	26.32
	Maydis style cum sigmatis	21.05
	Helichrysi arenarii flores	15.79
	Rosae fructus	10.52
Total: 100.0		
No. 5	*Medicago herba*	26.32
	Cichorii radices	26.32
	Elymi repens rhizomata	21.05
	Rosae fructus	15.79
	Crataegi fructus	10.52
Total: 100.0		

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 °C 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.

Comparison drugs: the officinal 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 °C, 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 [11]. 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 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.*) [12], 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. 1–5 in doses 6 mL/kg/day, 9 mL/kg/day and 12 mL/kg/day, *p.o*. 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, 30, 60 and 120 minutes after glucose loading [14].

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 [14].

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".

4. Result

At the first stage of the screening study, 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.

Table 2

Hypoglycemic effect of the herbal mixtures compared to the officinal 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 1th				
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. 1, 6 mL/kg	3.98±0.07	5.79±0.14*	5.56±0.12*	4.98±0.15*
HM No. 1, 9 mL/kg	4.11±0.09	5.69±0.16*	5.47±0.16*	5.07±0.14*
HM No. 1, 12 mL/kg	4.09±0.16	5.49±0.17*	5.38±0.11*	4.93±0.18*
HM No. 2, 6 mL/kg	4.03±0.11	5.85±0.17*	5.54±0.07*	5.06±0.08*
HM No. 2, 9 mL/kg	4.11±0.15	5.59±0.08*	5.44±0.13*	5.02±0.18*
HM No. 2, 12 mL/kg	4.06±0.11	5.36±0.17*	5.27±0.06*	5.11±0.14*
HM No. 3, 6 mL/kg	4.02±0.16	5.39±0.11*	5.27±0.17*	5.06±0.14*
HM No. 3, 9 mL/kg	4.06±0.12	5.19±0.18*	5.02±0.11*	4.87±0.12*
HM No. 3, 12 mL/kg	4.11±0.16	4.54±0.15*/**	4.43±0.12*/**	4.21±0.16*
HM No. 4, 6 mL/kg	4.14±0.15	5.49±0.11*	5.32±0.19*	5.27±0.17*
HM No. 4, 9 mL/kg	4.07±0.16	5.34±0.13*	5.11±0.16*	5.02±0.11*
HM No. 4, 12 mL/kg	3.98±0.17	4.39±0.18*/**	4.22±0.19*/**	4.15±0.18*
HM No. 5, 6 mL/kg	4.15±0.18	5.84±0.18*	5.72±0.11*	5.64±0.12
HM No. 5, 9 mL/kg	4.09±0.17	5.68±0.13*	5.47±0.16*	5.39±0.11
HM No. 5, 12 mL/kg	3.97±0.11	5.54±0.18*	5.36±0.12*	5.23±0.17*

*Note: 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"*

The results of the study showed that 20-day preventive treatment by all five herbal mixtures at doses 6 mL/kg/day, 9 mL/kg/day and 12 mL/kg/day significantly ($p<0.05$) was 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 mixtures No. 3 at dose 12 mL/kg/day and No. 4 at dose 12 mL/kg/day, because they reduced blood glucose levels by 42 % and 44 %, respectively, relative to the control group. Metformin tablets showed a similar result in efficacy, as they reduced alimentary hyperglycemia by 46 % relative to the control group of animals at 30th minute. The officinal herbal mixtures "Arfazetin" was inferior in efficiency to the herbal mixtures No. 3 at dose 12 mL/kg/day and No. 4 at dose 12 mL/kg/day and reduced glycemia by 32 % relative to the control group at the 30th minute of the test (Fig. 2).

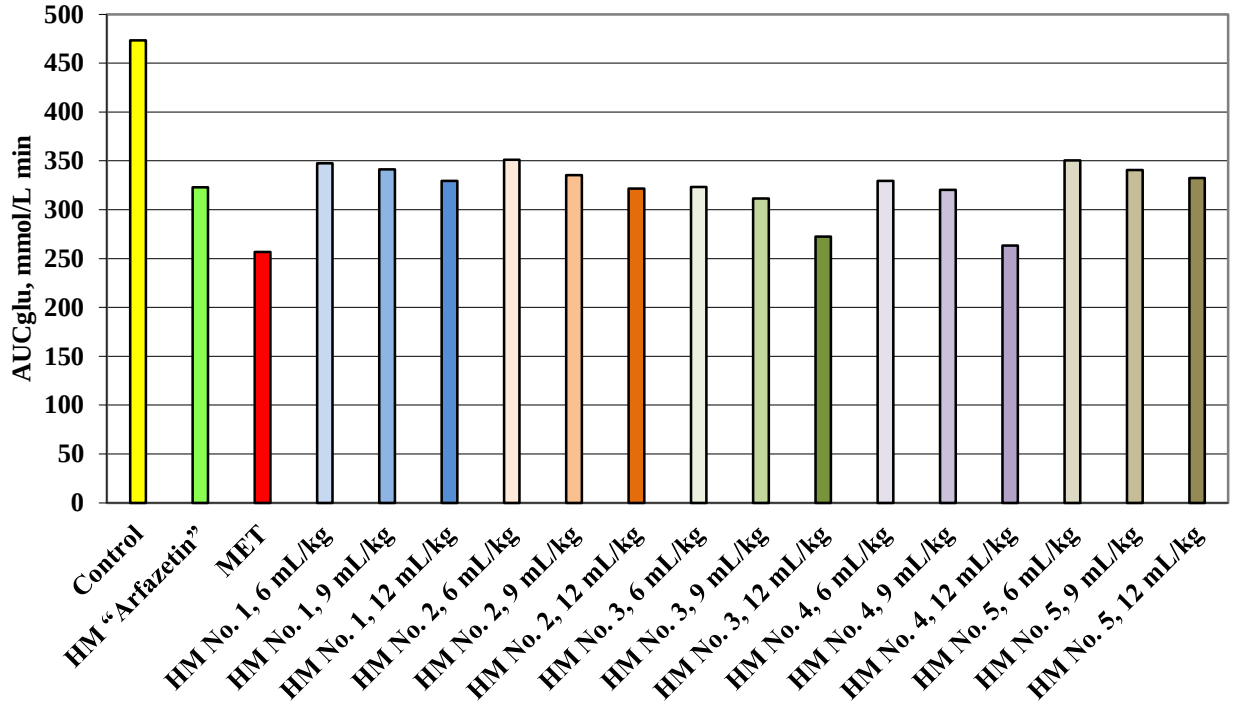

Fig. 2. Hypoglycemic effect of the herbal mixtures compared to the officinal herbal mixture "Arfazetin" and tablets metformin by OGTT after 20 days of preventive treatment of normoglycemic rats, mean ± SEM, n=8

During the determination of integrated glycemic index based on the results of OGTT, it was found that the area under glycemic curve (AUC_{glu}) in the herbal mixtures No. 3 (12 mL/kg/day) and No. 4 (12 mL/kg/day) was 272.4 mmol/L min and 263.4 mmol/L min, respectively. 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.

In the second stage of the screening study, the ability of the herbal mixtures No. 1–5 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 mixtures No. 3 (12 mL/kg/day) and No. 4 (12 mL/kg/day), because blood glucose levels were 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 officinal herbal mixture "Arfazetin" was slightly inferior to the effectiveness of the herbal mixtures No. 3 at dose 12 mL/kg/day and No. 4 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 (Table 3).

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 1th				
1	2	3	4	5
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. 1, 6 mL/kg	4.21±0.17	7.21±0.18*	5.13±0.17	4.38±0.18
HM No. 1, 9 mL/kg	4.17±0.18	7.16±0.19*	5.09±0.16	4.23±0.17
HM No. 1, 12 mL/kg	4.19±0.11	7.11±0.18*	5.03±0.15	4.26±0.16
HM No. 2, 6 mL/kg	4.17±0.15	7.39±0.16*	5.19±0.14	4.26±0.17
HM No. 2, 9 mL/kg	4.19±0.19	7.28±0.13*	5.17±0.16	4.22±0.19

Continuation of Table 3

1	2	3	4	5
HM No. 2, 12 mL/kg	4.21±0.18	7.17±0.18*	5.12±0.17	4.31±0.18
HM No. 3, 6 mL/kg	4.11±0.17	7.09±0.17*	5.09±0.18	4.20±0.19
HM No. 3, 9 mL/kg	4.09±0.18	7.01±0.17*	5.08±0.19	4.17±0.19
HM No. 3, 12 mL/kg	4.16±0.18	6.39±0.18*/**	4.93±0.19*	4.22±0.18
HM No. 4, 6 mL/kg	4.23±0.18	7.17±0.18*	5.22±0.16	4.28±0.19
HM No. 4, 9 mL/kg	4.21±0.15	7.15±0.16*	5.18±0.16	4.27±0.09
HM No. 4, 12 mL/kg	4.17±0.16	6.40±0.15*/**	4.95±0.14*	4.22±0.18
HM No. 5, 6 mL/kg	4.27±0.18	7.38±0.18*	5.24±0.16	4.41±0.19
HM No. 5, 9 mL/kg	4.18±0.16	7.29±0.16*	5.15±0.17	4.30±0.11
HM No. 5, 12 mL/kg	4.11±0.17	7.15±0.18*	5.09±0.18	4.24±0.18

*Note: 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"*

The results of a screening study using OGTT and IPGTT of the herbal mixtures No. 1–5, which are used in folk medicine for the prevention and treatment of diabetes mellitus type 2, indicate 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. 1, No. 2 and No. at 5 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 mixtures No. 3 (12 mL/kg/day), No. 4 (12 mL/kg/day) and comparison drugs – the officinal herbal mixture "Arfazetin" (9 mL/kg/day) and metformin tablets (60 mg/kg/day) (Tables 2, 3).

5. Discussion

Hypoglycemic activity of the studied herbal mixtures is quite predictable, because they include medicinal plant raw materials containing biologically active substances with 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 [15, 16]. Presented herbal mixtures contain herbal raw materials that are rich in inulin, such as *Cichorii radices* (the herbal mixtures No. 1, No. 2, No. 3 and No. 5), *Taraxaci radices* (the herbal mixtures No. 1 and No. 3), *Elymi repens rhizomata* (the herbal mixtures No. 3, No. 4 and No. 5), *Arctii lappea radices* (the herbal mixture No. 4).

In addition, medicinal plants that are part of the studied herbal mixtures contain polyphenolic compounds that 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 [17–19]. 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 species (ROS) formation either by inhibition of enzymes or by chelating trace elements involved in free radical generation; scavenging ROS; inhibition the enzymes involved in ROS generation – microsomal monooxygenase, glutathione S-transferase, mitochondrial succinoxidase, nicotinamide adenine dinucleotide phosphate (NADH) oxidase, and so forth [19–21]. Medicinal plant raw materials containing phenolic compounds are *Phaseoli pericarpium* (the herbal mixture No. 1), *Helichrysi arenarii flores* (the herbal mixtures No. 1, No. 2 and No. 4), *Ericae herba* (the herbal mixtures No. 1 and No. 2), *Melissae folia* (the herbal mixtures No. 1 and No. 2), *Galegae herba* (the herbal mixture No. 2), *Urticea folia* (the herbal mixture No. 3), *Rosae fructus* (the herbal mixtures No. 3, No. 4 and No. 5), *Medicago herba* (the herbal mixture No. 5), *Crataegi fructus* (the herbal mixture No. 5), *Maydis style cum sigmatis* (the herbal mixture No. 4).

Thus, screening study of the herbal mixtures No. 1–5 shows their hypoglycemic activity by OGTT, IPGTT and confirms the effectiveness of their use in folk medicine for the prevention and treatment of diabetes mellitus type 2.

Study limitations. Studies have shown the potential hypoglycemic properties of the herbal mixtures in normoglycemic rats by glucose loading tests, but no studies on animals with experimental pathology have been shown.

Prospects for further research. The herbal mixtures No. 3 and No. 4 showed the highest hypoglycemic activity during the screening study. The next stage will be the phytochemical determination of biologically active substances of these promising herbal mixtures, as well as pharmacological research *in vitro* and *in vivo* and establishing the relationship between the chemical composition of the mixtures and their pharmacodynamics.

6. Conclusions

For the first time, it was conducted the screening study of hypoglycemic activity of the herbal mixtures No. 1–5, which are used in folk medicine for the prevention and treatment of diabetes mellitus type 2. It was determined that the greatest effectiveness in terms of the ability to reduce alimentary hyperglycemia during OGTT and reduce impaired carbohydrate tolerance during IPGTT showed the herbal mixtures No. 3 (which includes *Urticea folia, Cichorii radices, Rosae fructus, Elymi repens rhizomata, Taraxaci radices*) and No. 4 (which includes *Arctii lappae radices, Elymi repens*

rhizomata, Maydis style cum sigmatis, Helichrysi arenarii flores, Rosae fructus). It was established their conditional therapeutic dose as 12 mL/kg/day.

Conflict of interests

The authors declare that they have no conflicts of interest.

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Received date 24.06.2020
Accepted date 21.07.2020
Published date 31.08.2020

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