PharmacologyOnLine

Archives • 2021 • vol.2 • 1128-1137

DETERMINATION OF HYPOGLYCEMIC ACTIVITY OF THE HERBAL MIXTURES BY MEANS OF GLUCOSE LOADING TESTS (MESSAGE 3)

Savych, Alona*[1], Gerush, Oleg[2], Basaraba, Roksolana[2]

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

[2]Department of Pharmacy, Bukovina State Medical University, Ukraine

*alonasavych@gmail.com

Abstract

Medicinal plants and their combinations due to the wide range of biologically active substances can influence on various links of the pathogenetic mechanism of diabetes mellitus development and its complications.

The aim of the reseach was to study the 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 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 during the 20 days. 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 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 mixture No. 13 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.

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 mixture No. 13, which includes *Cichorii radices, Elymi repens rhizomata, Helichrysi arenarii flores, Rosae fructus, Maydis style cum stigmatis*. It was established its conditional therapeutic dose 12 mL/kg/day.

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

Introduction

Diabetes mellitus is one of WHO's priorities matters, which requires immediate solutions, as the epidemiological situation is alarming – the number of patients is growing each year, leading to increased disability and mortality due to the development of diabetic angiopathies [1]. According to the official report of International Diabetes Federation (2019) the number of diabetics will increase to 700 million by 2045 [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, 5, 6].

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

Modern pharmacotherapy increasingly takes into account the centuries-old experience of folk medicine with the use of herbal drugs as monotherapy and in combination with synthetic drugs. This is quite justified, because phytotherapy has a number of advantages over traditional therapy with synthetic drugs, namely, it is low-toxic, has a mild pharmacological effect and can be used for a long period of time without significant side effects and combines well with synthetic drugs s [7, 8, 9]. 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 [10, 11, 12]. In addition, 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 [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) 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 °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.

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

Experimental Protocol: 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 [15]. All protocols for animals experiment were approved by the animal ethical committee of I.Horbachevsky Ternopil National Medical University.

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.*), 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.

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). 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 at 0, 30, 60 and 120 minutes after glucose loading [13].

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 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

It was studied 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. 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. 11-15 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 mixture No. 13 at dose 12 mL/kg/day, it reduced blood glucose levels by 45 %, 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. 13 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. 13 (12 mL/kg/day) was 262.2 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.

In the next stage of the screening study, the ability of the herbal mixtures No. 11-15 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. 13 (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. 13 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. 11-15, 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. 11, No. 12, No. 14 and No. 15 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. 13 (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 plant raw materials containing biologically active substances with proven hypoglycemic action. Polysaccharides, especially inulin, are the main groups of biologically active substances that can lower blood glucose are that have 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, 18]. These herbal mixtures contain herbal raw materials 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 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 [19, 20, 21]. Very 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 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 [21, 22, 23]. Herbal plant raw materials 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, screening study of the herbal mixtures No. 11-15 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.

Conclusions

It was conducted the screening study of hypoglycemic activity of the herbal mixtures No. 11-15, 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 mixture No. 13, which includes *Cichorii radices, Elymi repens rhizomata, Helichrysi arenarii flores, Rosae fructus, Maydis style cum stigmatis*. It was established its conditional therapeutic dose 12 mL/kg/day.

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. 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.
8. Savych, A., Marchyshyn, M., & Naconechna, S. (2021). Influence of some herbal mixtures on insulin resistance and glucose tolerance in rats. *PharmacologyOnLine*, *1*, 356-364.
9. Ndjaboue, R., Farhat, I., Ferlatte, C. A., Ngueta, G., Guay, D., Delorme, S., Ivers, N., Shah, B. R., Straus, S., Yu, C., & Witteman, H. O. (2020). Predictive models of diabetes complications: protocol for a scoping review. *Systematic reviews*, *9*(1), 137.
10. Savych, A., & Milian, I. (2021). Total flavonoid content in the herbal mixture with antidiabetic activity. *PharmacologyOnLine*, 2, 68-75.
11. Savych, A., & Basaraba, R. (2021). Ascorbic acid content in the herbal mixture with antidiabetic activity. *PharmacologyOnLine*. 2, 76-83.
12. 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.
13. 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.
14. WHO Guidelines on good agricultural and mixture practices (GACP) for medicinal plants (2003). *World Health Organization*, Geneva, Switzerland, 72.
15. EEC. "Council directive 2010/63/EU, of the 22nd September 2010 on the approximation of laws, regulations and administrative provisions of the member states regarding the protection of animals used for experimental and other scientific purposes". Offical Journal of the European Communities, 2010:1–29.
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.

Table 1. Composition of the herbal mixtures

Herbal mixtures	Herbals	Quantity of the herbals 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

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 third				
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*

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 three				
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

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"

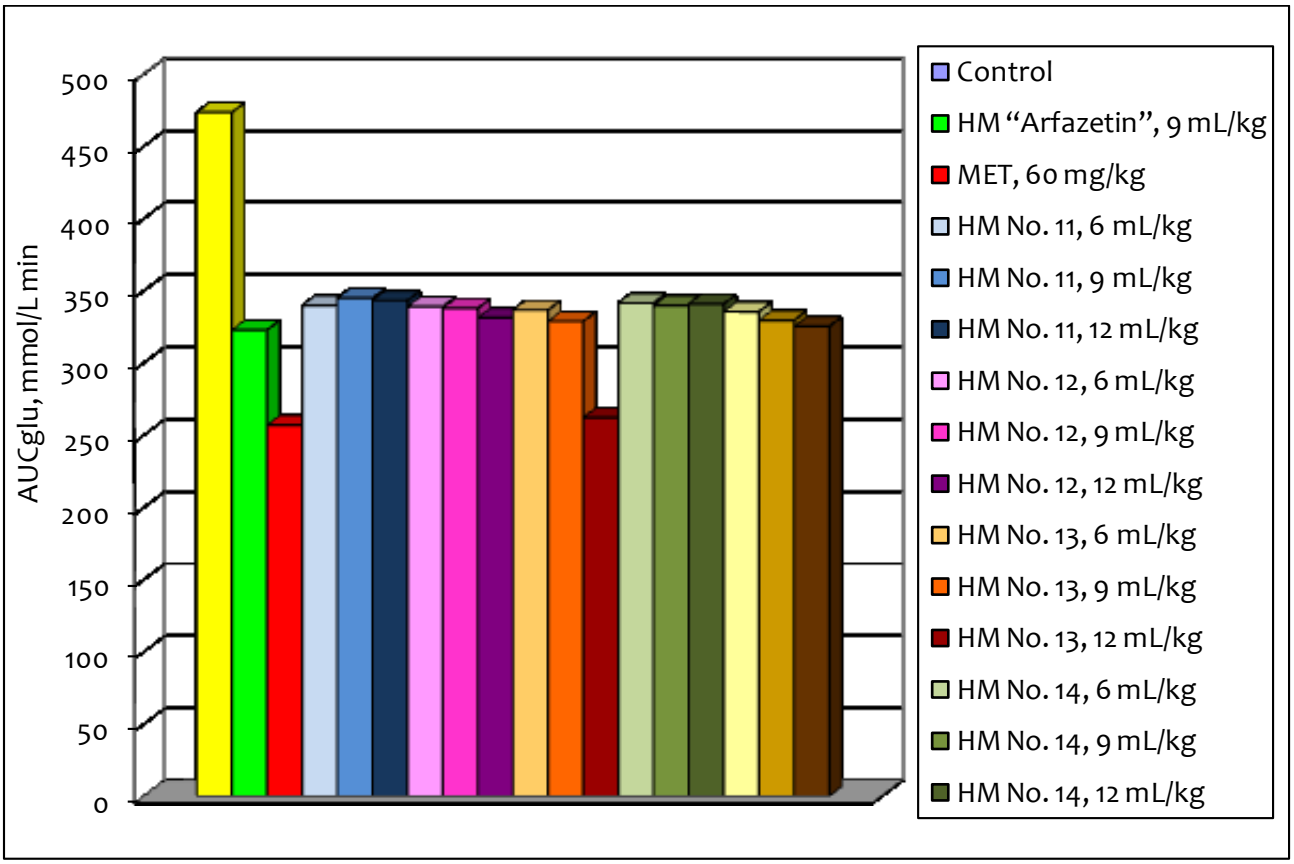

Figure 1. Hypoglycemic effect of the herbal mixtures compared to the official herbal mixture "Arfazetin" and tablets metformin by OGTT after 20 days of preventive treatment of normoglycemic rats, mean ± SEM, n=8.