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DEVELOPMENT OF MICROCAPSULES BASED ON COMBINED ANTIDIABETIC SUBSTANCE: PHARMACOLOGICAL CHARACTERISTICS

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The comparative assessment results of the hypoglycemic activity of a combined preparation containing microcapsules with a phytocomposition consisting of *Glycyrrhiza glabra* L. extracts, a dry extract of *Galega officinalis* L., *Mentha piperita* L., and gliclazide, are discussed in the article. Methods for obtaining microcapsules with an original PEG-6000 shell are described.

The aim of the study was to develop an optimal technology for obtaining microcapsules with a PEG-6000 shell containing a combined antidiabetic substance, and conduct its detailed pharmacological study on the model of type 2 diabetes mellitus, to conduct a detailed comparative pharmacological study of a microencapsulated antidiabetic composition with a shell based on PEG-6000, including gliclazide and a sum of phytoextracts on the model of type 2 diabetes mellitus.

Materials and methods. As the main objects of the study, microcapsules with a PEG-6000 shell were obtained using methyl miristate as the base liquid. The capsules contained the amount of plant extracts in their composition: a dry extract of *Glycyrrhiza glabra* L., a dry extract of *Galega officinalis* L., a dry extract of *Mentha piperita* L., and gliclazide. The study of a hypoglycemic activity was carried out after a single administration of drugs to the animals with alloxan-induced type 2 diabetes mellitus. The cumulative effect assessment of the drugs was carried out within 14 days with a test for the resistance to oral glucose on days 7 and 14.

Results. Microcapsules with the original shell were obtained by dispersion in a liquid-liquid system with the adjustment of some technological stages. The effect of the drugs under study on the glycemic profile in the rats with an experimental model of type 2 diabetes mellitus was investigated. A comparative evaluation of the pharmacological effect was carried out with a separate and combined use of microencapsulated preparations.

Conclusion. The rationality of combining phytocomponents and a synthetic antidiabetic agent in microcapsules has been proven. The obtained results testify to the rationality of plant extracts combination and a synthetic hypoglycemic agent – gliclazide in microcapsules.

Keywords: microcapsules; PEG-6000, methylmiristate; diabetes mellitus; *Glycyrrhiza glabra* extracts; *Galega officinalis* L.; *Mentha piperita* L.; gliclazide

Abbreviations: DM – diabetes mellitus; PPAR-γ – peroxisome proliferator-activated receptor; GLUT-4 – glucose transporter type 4; AMPK – adenosine monophosphate protein kinase; PEG – polyethylene glycol; PEMC(s) – plant extract microcapsules; GMC(s) – gliclazide microcapsules; GT(s) – gliclazide tablets; PBO – placebo; HGA – hypoglycemic activity.

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РАЗРАБОТКА МИКРОКАПСУЛ НА БАЗЕ КОМБИНИРОВАННОЙ АНТИДИАБЕТИЧЕСКОЙ СУБСТАНЦИИ И ЕЕ ФАРМАКОЛОГИЧЕСКАЯ ХАРАКТЕРИСТИКА

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В статье рассматриваются результаты сравнительной оценки гипогликемической активности комбинированного препарата, содержащего микрокапсулы с фитокомпозицией, состоящей из экстрактов солодки голой, козлятника лекарственного, мяты перечной и гликлазида. Описаны способы получения микрокапсул с оригинальной оболочкой из ПЭГ-6000.

Цель. Разработать оптимальную технологию получения микрокапсул с оболочкой из ПЭГ-6000, содержащих комбинированную субстанцию антидиабетического действия и провести её подробное фармакологическое исследование на модели сахарного диабета 2-го типа. Провести подробное сравнительное фармакологическое исследование микрокапсулированной антидиабетической композиции с оболочкой на основе ПЭГ-6000, включающей гликлазид и сумму фитоэкстрактов на модели сахарного диабета 2-го типа.

Материалы и методы. В качестве основных объектов исследования были получены микрокапсулы с оболочкой из ПЭГ-6000 с использованием в качестве базовой жидкости метилмиристата. Капсулы содержали в своем составе сумму растительных экстрактов: сухой экстракт солодки голой, сухой экстракт козлятника лекарственного, сухой экстракт мяты перечной, а также гликлазид. Изучение гипогликемической активности проведено после однократного введения препаратов животным с аллоксан-индуцированным сахарным диабетом 2-го типа. Оценка накопительного эффекта препаратов проведена в течение 14 суток с проведением теста на резистентность к пероральной глюкозе на 7 и 14 сутки.

Результаты. Получены микрокапсулы с оригинальной оболочкой методом диспергирования в системе жидкость-жидкость с корректировкой некоторых технологических этапов. Изучено влияние исследуемых препаратов на гликемический профиль у крыс с экспериментальной моделью сахарного диабета 2-го типа. Проведена сравнительная оценка фармакологического эффекта при раздельном и совместном применении микрокапсулированных препаратов.

Заключение. Доказана рациональность комбинирования фитокомпонентов и синтетического средства сахароснижающего действия в микрокапсулах. Полученные результаты свидетельствуют о рациональности комбинации растительных экстрактов и синтетического сахароснижающего средства – гликлазида в микрокапсулах.

Ключевые слова: микрокапсулы; ПЭГ-6000, метилмиристат; сахарный диабет; экстракт солодки голой; козлятник лекарственный (галега); мята перечная; гликлазид

Список сокращений: СД – сахарный диабет; PPAR-γ – рецептор-активатор пролиферации пероксисом; GLUT-4 – глюкозный транспортёр тип 4; AMPK – аденозинмонофосфатная протеинкиназа; ПЭГ – полиэтиленгликоль; МКЭ – микрокапсулы с растительными экстрактами; МКГ – микрокапсулы с гликлазидом; ТГ – таблетки гликлазида; Пл – плацебо; ГА – гипогликемическая активность.

INTRODUCTION

One of the most problematic aspects of modern medicine and pharmacy, in particular epidemiology, is diabetes mellitus (DM) which can be considered as a non-infectious epidemic of the present. The prevalence of this disease reaches more than 6% in some countries [1, 2]. At the same time, the disease is unstable; it is usually accompanied by a frequent disability [3] and lethality [4]. This has been especially acute in recent years associated with the pandemic [5, 6]. It was during this period that the problem of diabetes reached its maximum.

Interest in preventive measures (diets, dietary regimens) has grown. However, this did not affect

the need for the development and improvement of pharmacotherapy, in which the basic emphasis had also to be revised. The interest of diabetologists in medicinal plants, which occupy an important position in the treatment and prevention of DM today, has sharply increased [7–8]. The search for new antidiabetic drugs made out of medicinal plants, is an important and strategically significant task, since they contain bioactive phytochemicals that are more active and safe than conventional therapy. At the same time, it is impossible to underestimate the role of synthetic drugs that have been solving these problems for many years quite successfully – these are, first of all, metformin, gliclazide, glibenclamide, and other well-known drugs which are constantly in demand [9–11].

The main component of the proposed phytocomposition is an extract of *Glycyrrhiza glabra* L., which has a wide range of a pharmacological activity. Biologically active substances in the *Glycyrrhiza glabra* L. extract can significantly bind to gamma *peroxisome* proliferator-activated receptors (PPAR-γ), which leads to a decrease in blood glucose levels. Therefore, chalcone and amorphrutin, obtained from *Glycyrrhiza glabra* L., promote the differentiation of adipocytes and improve glucose and lipid metabolism. Amorphrutin increases the sensitivity of cells to insulin and, as a result, glucose tolerance. Glabridin prevents glucose intolerance and ensures its maximum utilization by translocation of GLUT-4 using adenosine monophosphate protein kinase (AMPK) [12]. In addition, glycyrrhizic acid, which is one of the main bioactive components of *Glycyrrhiza glabra* L., inhibits the activity of peroxidase, hemoglobin esterase and hemoglobin-mediated oxidative damage, while not affecting the ability of the protein to bind oxygen. This effect is especially important in the treatment of DM, given the complications associated with an oxidative stress [13–15]. In addition, there are studies [16] on the positive effect of the *Glycyrrhiza glabra* L extract on the intestinal microbiota, which is extremely important, since it is known that the pathogenesis of the onset and severity of DM is indirectly associated with a violation of the enzymatic and biochemical balance of the vital activity of the normal intestinal microflora.

One of the medicinal plants with a pronounced hypoglycemic effect is *Galega officinalis* L. In the 1920s, it was found out that guanidine, an active component in the herb of *Galega officinalis* L., reduces blood glucose levels, which became the basis for the synthesis of several antidiabetic compounds and, in particular, metformin [17]. The hypoglycemic effect is also associated with the alkaloid of galegin in the plant [18]. However, the study of anti-diabetic properties of *Galega officinalis* L. is ongoing. To date, a decrease in the concentration of glucose and glycosylated hemoglobin in the blood of animals against the background of the galega extract administration, as well as an increase in the cell tolerance to glucose, an increase in the content of C-peptide and insulin in blood plasma, have been proven. The *in vivo* studies confirmed the cytoprotective effect of the *Galega officinalis* L. extract on pancreatic cells, expressed in an increase in the number of Langerhans islets, their average area, diameter, volume, and the number of β-cells [19, 20]. It has been established that the *Galega officinalis* L. extract regulates disorders of the proliferation, function and apoptosis of leukocytes associated with DM, thereby having a pronounced immunocorrective effect [21].

Aromatic plants rich in essential oils, such as *Mentha piperita* L., *Melissa officinalis* L., *Cuminum cyminum* L., are among the potential new sources of drugs. These plants are very promising due to their diverse chemical composition and multiple mechanisms of action. Thus, the antidiabetic effect of *Mentha piperita* L. is associated with the inhibition of ATP-sensitive K+channels on the β-cell membrane, increased insulin exocytosis under the action of menthol. In addition, menthol increases the survival of β-cells by stimulating the expression of Bcl-2, an anti-apoptotic factor, and protects pancreatic β-cells from apoptosis in a rat model of diabetes [22, 23]. In addition, the hypoglycemic effect of the *Mentha piperita* L. extract is associated with an inhibitory effect on α-amylase and α-glucosidase, which, in turn, has a significant inhibitory effect on postprandial hyperglycemia [24].

Thus, the literature data on the spectrum of hypoglycemic activity of the *Glycyrrhiza glabra* L. extract; *Galega officinalis* L. and *Mentha piperita* L. confirm the rationality of including extracts of these medicinal plants in the proposed phytocomposition and the development of combined antidiabetic dosage forms based on them.

THE AIM of the study was to develop an optimal technology for obtaining microcapsules with a PEG-6000 shell containing a combined antidiabetic substance, and conduct its detailed pharmacological study on the model of type 2 diabetes mellitus, to conduct a detailed comparative pharmacological study of a microencapsulated antidiabetic composition with a shell based on PEG-6000, including gliclazide and a sum of phytoextracts on the model of type 2 diabetes mellitus.

MATERIALS AND METHODS

Objects of study

The main objects of the study were microcapsules containing a composition of dry extracts of *Glycyrrhiza glabra*, *Galega officinalis* L. and *Mentha piperita* L. in the ratio of 6:1:3, respectively, as well as gliclazide in a traditional dosage.

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PEG-6000 with a density of 1200 kg/m^3, soluble in water and alcohol, non-toxic, was chosen as a film former for microcapsules. Methylmiristat is a process medium, it is a clear, colorless liquid with a low melting point. To obtain microcapsules, dry extracts of *Glycyrrhiza glabra* L., *Galega officinalis* L. and *Mentha piperita* L. obtained from the manufacturer of LLC Kazan Extract Plant (Russia), were used. The moisture content of the extracts was not more than 4.8%.

Reference drug

For a comparative assessment of the hypoglycemic activity, the tablet form of gliclazide (60 mg, manufacturer LLC SERVIER RUS, Russia) had been chosen as the reference drug. For this purpose, model tablets with an active ingredient content of 2.5 mg were used. The drug was administered using a gastric tube, taking into account the interspecies conversion factor, in several doses to achieve the required dose of gliclazide.

Equipment

Measurements of blood glucose concentration in the animals with an experimental model of type 2 diabetes mellitus was carried out using an Accu-Chek Performa Nano glucometer (Roche Diagnostics GmbH, Germany).

The preparation of microcapsules was carried out using an overhead stirrer US-1170D with a four-bladed propeller nozzle (ULAB, Russia). A vessel with a hemispherical bottom with a volume of 500 ml was used as a reactor for obtaining microcapsules.

A six-seater water bath LOIP LB-161 (LC LOIP, Russia) was also used.

The administration of the study drugs was carried out using an intragastric metal probe for rodents.

Microcapsules obtaining

Taking into account the originality of the PEG-6000 microcapsule shell used for the first time, the most reliable technological method of dispersion in the liquid-liquid system was chosen. A saturated complex was sequentially delivered in a thin stream: 25.0 g of the total extracts solutions and 25.0 g of a gliclazide solution together with 100 g of the solution forming the shell of PEG-6000 microcapsules. The complex was delivered into the reactor with the base medium in the form of methyl miristat at the temperature of 40°C. Herewith, defining the technological novelty was the choice of the base solution and an active mixing device – a propeller mixer operating at the speed of 500–600 rpm. The temperature factor also changed, quickly passing from a state of heating to artificial cooling.

The drops shaped in the oil, quickly solidified, spherical particles were formed from them, and then they were separated from the methyl miristate solution and washed with 90% ethyl alcohol, making sure that the shell did not dissolve. The standardization of the finished product of microcapsules was carried out according to their flowability and the angle of repose, which was 30° and which indicated a normal technological cycle [25].

Experimental animals

The hypoglycemic activity (GA) of the studied preparations was investigated on male rats of the Wistar line weighing 200–220 g, with alloxan-induced DM. The animals were obtained from the Novosibirsk Nursery of Laboratory Animals (State Unitary Enterprise of Laboratory Animals, the RAS Siberian branch) and kept in the vivarium of Krasnoyarsk State Medical University of the Ministry of Health of Russia. The work with laboratory animals was carried out in accordance with Directive N 2010/63/EU of the European Parliament and the Council of the European Union "On the protection of animals used for scientific purposes" and GOST 33044-2014 "Principles of good laboratory practice". The animals were kept in conventional cages with an area of 820 cm^2, with litter of non-coniferous sawdust, the indoor temperature of 22±2°C, a the relative air humidity of 65±5%, with a free access to water and food. The study protocol was approved by the ethics committee of Krasnoyarsk State Medical University of the Ministry of Health of Russia (protocol No. 104/2021 dated April 17, 2021).

Setting up DM model type 2

Setting up the model of alloxan-induced DM was carried out in the classical way in accordance with the guidance by A.M. Mironov[1]. The animals were intraperitoneally injected with a 5% solution of nicotinamide at the dose of 230 mg/kg, followed by the administration of a 5% solution of alloxan at the rate of 150 mg/kg after 15 minutes. On the 4th day after the injection of alloxan, 5 groups of animals (n=8) with an average blood glucose level of more than 20 mmol/l, were formed according to the principle of paired analogs. Taking into account the previous studies, the doses of the administered drugs were selected [26, 27] and calculated based on the interspecies dose conversion factor. The studied substances and the reference drug were administrated into the stomach using a metal probe.

The administration of drugs was carried out for 14 days according to the following scheme:

Group 1 – microcapsules with plant extracts (PEMCs) – 750 mg/kg;

[1] [Guidelines for conducting preclinical studies of drugs (Part 1)]. Mironova AN, editor. M: Grif and K; 2012. 944 p. Russian

Group 2 – microcapsules with gliclazide (GMCs) – 150 mg/kg;

Group 3 – gliclazide tablets (GTs) – 60 mg/kg – reference group;

Group 4 – combination of microcapsules (PEMCs + GMCs);

Group 5 – 2.5 ml of purified water (PBO) – control group.

Determination of hypoglycemic effect

The assessment of hypoglycemic activity (HGA) was carried out by monitoring the concentration of glucose in the blood of animals for 24 hours after a single administration of the study drugs. Taking into account the established cumulative hypoglycemic effect, characteristic of this phytocomposition and confirmed for microcapsules with a gelatin shell, the effectiveness of the developed microcapsules with a shell based on PEG-6000, was studied for a long-term use. For this purpose, tests for the resistance to oral glucose were carried out on days 7 and 14 according to the methods [26, 27]. The general study design of the hypoglycemic activity of the microencapsulated preparations is shown in Fig. 1. The scheme of the test for the glucose resistance is shown in Fig. 2.

Statistical analysis

Statistical processing of the obtained results was carried out using a Microsoft Excel software package (2016), by a descriptive statistics method, by calculating the mean value of the studied indicator and the standard deviation (σ). The normality of the sample distribution was determined using the Shapiro-Wilk test. The Wilcoxon's t-test was used to assess differences between quantitative non-normally distributed data of related samples, the Student's t-test was used for normally distributed data of unrelated samples ($p < 0.05$).

RESULTS AND DISCUSSION

The results of the HGA study after a single administration of microcapsules containing the composition of plant extracts, gliclazide, as well as their combination, are presented in Table1.

The comparison of the presented data in Table 1 shows that the decrease in the animals' blood plasma glucose concentration was established in all the groups after a single administration of the studied drugs, except the control group. There was no significant decrease in blood glucose levels in the animals of the control group within 24 hours after starting the experiment. Against the background of the use of microcapsules with plant extracts, a hypoglycemic activity (HGA) develops after 4 hours and is maintained within 8 hours. After the administration of GMCs, HGA manifests itself after 2 hours and continues to grow within 10 hours. However, the achievement of the physiological blood glucose level in the animals after a single administration of monopreparations is not observed within 24 hours. With a combination of PEMCs+GMCs, the pharmacological effect occurs after 2 hours, and after 6 hours, the animals' blood glucose concentration reaches its physiological level. After a single administration of the developed microcapsules and the reference drug, a stable hypoglycemic effect is observed from 6 to 12 hours. At the same time, the average animals' blood glucose level in the in this time interval was as follows: for group 1 – 18.0 ± 1.4 mmol/l; group 2 – 11.8 ± 0.9 mmol/l; group 3 – 6.6 ± 0.2 mmol/l; group 4 – 7.7 ± 0.6 mmol/l; the blood glucose level in the control animals' group was 24.7 ± 1.9 mmol/L.

According to the statistical analysis of the obtained results, the HGA combination of PEMCs+GMCs is comparable to the gliclazide tablet form. In addition, in the pharmacological activity, the combined composition of microcapsules is superior to the microcapsules containing only one component. 6 hours after the administration of the drugs, the animals' blood glucose level in group 4 decreased by 68.8% ($p < 0.05$), while the blood glucose concentration in the animals of groups 1 and 2 decreased by 22.9% ($p < 0.05$) and 51.4% ($p<0.05$), respectively. It should be also notified that 6 hours after the administration of the PEMCs+GMCs combination and the reference drug, there are significant differences in the average values of the animals' blood glucose levels of the respective groups. The glucose concentration in the blood of the animals receiving a combination of PEMCs+GMCs (group 4) is 23.3% ($p < 0.05$) higher than in the blood of the animals treated with GTs. The absence of statistically significant differences in the initial values of the animals' blood plasma glucose concentration in these groups indicates different pharmacokinetic characteristics of the dosage forms of the studied drugs, i.e., a different degree of elongation.

Analyzing the results of monitoring the level of the animals' blood plasma glucose concentration, it can be concluded that a stable hypoglycemic effect after a single administration of the developed microcapsules was not observed immediately, just as after a single administration of the reference drug, in the time range from 4 to 12 hours. However, in this time interval, a significant decrease in blood glucose was observed with the use of gliclazide tablets (group 3 of the animals), with the use of gliclazide microcapsules (group 2) and with the administration of a combination of microcapsules (group 4). At the same time, the levels of glucose in the animals' blood plasma of groups 3 and 4 are almost equivalent and slightly lower than in group 2 which may indicate the presence of potentiation of the hypoglycemic effect when the herbal components are combined with a synthetic drug.

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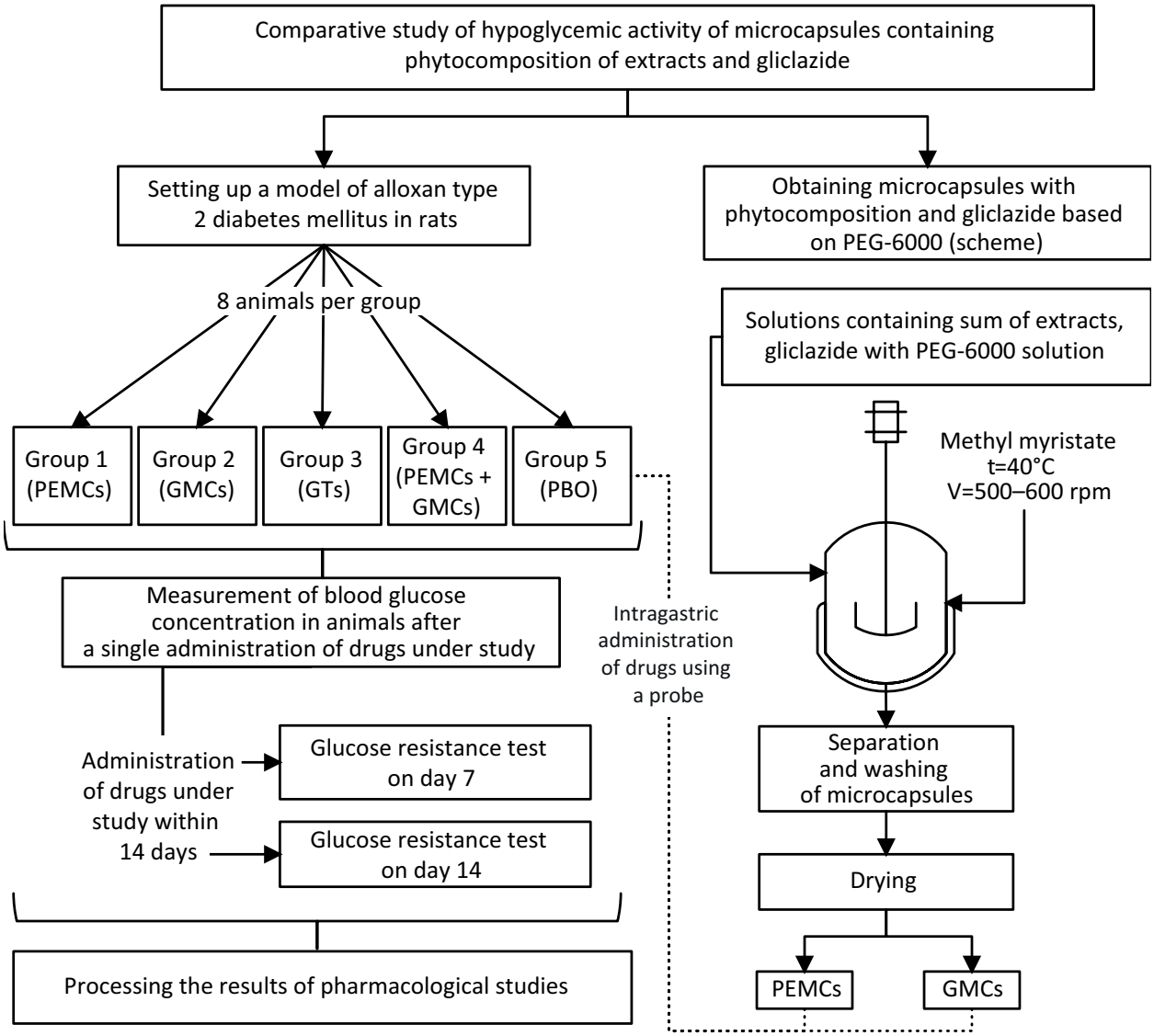

Figure1 – Study design

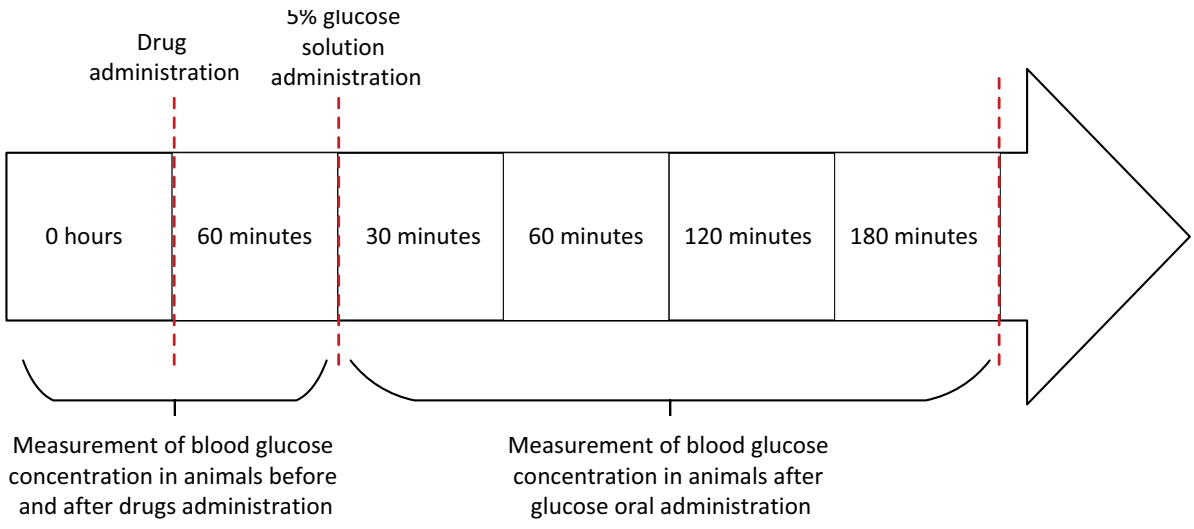

Figure 2 – Scheme of glucose resistance test on days 7 and 14

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Table 1 – Blood glucose level in animals with experimental model of type 2 diabetes mellitus after a single administration of drugs under study

Group	Time, hours							
	0	2	4	6	8	10	12	24
1	25.3±3.8	23.5±4.1#	20.9±3.4#	19.5±2.3*#	19.1±4.3*#	16.8±5.3*#	16.8±4.5*#	23.6±4.4#
2	25.1±3.3	18.5±2.6	15.3±1.9*#	12.2±1.7*#	11.2±1.0*#	11.0±0.7*#	13.0±0.9*#	24.3±4.2#
3	27.7±2.4	15.1±3.8*	8.4±2.4*	6.6±1.1*	6.4±0.9*	6.5±0.7*	6.8±0.5*	12.9±1.8*
4	27.6±4.0	17.6±3.0*	10.2±2.2*	8.6±2.0*#	7.3±1.7*	7.3±1.5*	7.5±1.3*	26.5±3.0#
5	21.7±1.2	22.1±0.6	21.7±0.8	25.7±0.5	26.4±0.9	24.5±0.6	22.2±1.2	26.1±1.4

Note: results are presented as Mean±σ; * – mean blood glucose concentration values significantly different from the control group (p <0.05) and # – mean blood glucose concentration values significantly different from the comparison group (p <0.05), according to the results of the analysis by the Student's t-criterion.

Table 2 – Study results of cumulative effect on day 7

Group	Mean glucose concentration, mmol/l					
	Drug administration		Glucose administration			
	0	60 min	30 min	60 min	120 min	180 min
1	25.3 ± 4.4#	24.4 ± 4.4#	24.0 ± 4.2#	25.8 ± 3.3#	25.5 ± 4.6#	24.0 ± 4.2#
2	23.5 ± 4.8#	20.7 ± 5.5*#	18.0 ± 4.6*#	16.6 ± 3.8*#	14.4 ± 3.4*#	13.6 ± 3.5*#
3	6.4 ± 0.7*	6.2 ± 0.9*	6.9 ± 1.1*	6.0 ± 1.1*	5.8 ± 0.8*	5.8 ± 0.8*
4	19.8 ± 1.8*#	12.4 ± 1.5*#	9.9 ± 1.0*#	6.8 ± 0.6*	6.8 ± 0.5*#	6.8 ± 0.4*#
5	23.9 ± 3.5	24.9 ± 4.0	28.5 ± 2.9	28.7 ± 4.2	26.7 ± 2.8	25.4 ± 2.0

Note: results are presented as Mean±σ; * – mean values of blood glucose concentration significantly different from the control group (p <0.05) and # – mean values of blood glucose concentration significantly different from the comparison group (p <0.05), according to the results of the analysis by the Student's t-criterion.

Table 3 – Study results of cumulative effect on day 14

Group	Mean glucose concentration, mmol/l					
	Drug administration		Glucose administration			
	0	60 min	30 min	60 min	120 min	180 min
1	18.2 ± 3.2*#	17.9 ± 3.3*#	16.5 ± 4.2*#	17.6 ± 4.4*#	16.0 ± 3.4*#	12.2 ± 2.5*#
2	14.0 ± 2.3*#	12.7 ± 1.4*#	12.7 ± 1.6*#	12.4 ± 1.5*#	11.6 ± 0.9*#	11.1 ± 0.7*#
3	6.7 ± 1.0*	5.6 ± 1.3*	5.6 ± 1.2*	5.9 ± 1.1*	6.1 ± 1.3*	6.3 ± 1.2*
4	12.6 ± 1.4*#	9.0 ± 0.5*#	8.5 ± 0.5*#	6.6 ± 0.4*	6.5 ± 0.4*	6.5 ± 0.4*
5	21.7 ± 3.3	21.3 ± 3.0	27.0 ± 2.8	28.7 ± 1.0	28.3 ± 2.4	28.1 ± 1.4

Note: results are presented as mean ± standard deviation; * – mean values of blood glucose concentration significantly different from the control group (p <0.05) and # – mean values of blood glucose concentration significantly different from the comparison group (p <0.05), according to the results of the analysis by the Student's t-criterion.

Thus, after a single administration, the HGA profile of the preparations under study was investigated and confirmed. It has been reliably established that the above described hypoglycemic effect of microcapsules based on PEG-6000 and containing a combination of PEMCs+GMCs, can be characterized as more delayed and prolonged.

As it has been notified in the previous studies[2] [25], the composition of medicinal plants extracts is characterized by a cumulative hypoglycemic effect. In support of this trend, PEG-6000 based microcapsules were tested for resistance to the alimentary glucose administration on the 7th and 14th days of the study. For this purpose, one hour after the administration of the studied preparations, the animals of all experimental groups were administrated with 2.5 ml of a 5% glucose solution using an intragastric metal probe, and the blood glucose level was measured within the next 3 hours. The study results of the resistance to oral glucose on the 7th day of therapy are presented in Table 2.

According to the data presented in Table 2, it can be notified that before the administration of drugs on day 7, the blood glucose level in the animals' blood of the studied groups was higher than a physiological one in all experimental groups, except group 3. In the control group, the blood glucose level in the animals was high throughout the entire time of the experiment. It is also worth notifying that in the animals of groups 1 and 2 before the administration of drugs, the blood glucose concentration does not have significant changes compared to the control group.

The blood glucose concentration level in the animals receiving microcapsules with plant extracts (group 1) was high throughout the experiment, no statistically

[2] Ibid.

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significant changes were detected (p >0.05) either against the background of the drug administration and or against the background of a glucose oral administration.

The glucose content in the animals' blood of group 2 decreased by 13.5% (p <0.05) from the initial value. This trend persisted within the group throughout the experiment, and at the end of the test, there was a decrease in the glucose content in the animals' blood by 42.1% (p <0.05), which indicates a smooth increase in the pharmacological effect. Despite the fact that the decrease in the blood glucose concentration to the physiological level was not observed, against the background of the use of microcapsules with gliclazide, the animals showed resistance to oral glucose.

In the animals receiving the comparison drug (group 3), before the administration of the drug, the blood glucose level was at the level of the physiological norm, which is associated with the manifestation of a stable hypoglycemic effect characteristic of gliclazide tablets. This trend continued throughout the experiment. Against the background of the alimentary load, an increase in the blood glucose concentration in the animals of group 2 was not observed, which also indicates the stability of the reference drug.

Before the administration of the drug, the blood glucose level in the animals receiving combined therapy with microcapsules (group 4) on day 7 was above the physiological norm. At the same time, the glucose concentration was 67.7% (p <0.05) higher than in the animals of the comparison group, but 17.2% (p <0.05) lower than in the control group. However, after the administration of the drug, the hypoglycemic effect increased, and already after 60 minutes, a statistically significant (the Wilcoxon T-test) decrease by 37.4% (p <0.05) was established in the blood glucose concentration in the animals of group 4 with the dynamics unchanged. 60 min after the glucose oral administration, the level of blood glycemia in the of animals of group 4 decreased by 65.7% (p <0.05) with the maintenance of a physiologically significant level till the end of the test, which indicates the animals' resistance to receiving a combination of microcapsules.

According to the statistical analysis results, it can be said that the administration of the microcapsules combination (group 4) contributed to the manifestation of a tolerance effect comparable to that of the reference drug (group 3) 60 minutes after the glucose oral administration.

According to the test results on the resistance to alimentary glucose on the 7th day of the study, therapy with a combination of PEMCs+GMCs gives a more pronounced pharmacological effect compared to the separate use of the drugs.

Table 3 presents the study results of the resistance to oral glucose on the 14th day of the administration of the investigated drugs.

According to the results presented in Table 3, it can be said that before the administration of drugs on the 14th day, the blood plasma glucose concentration in the animals of the studied groups was also higher than the physiological norm in all the experimental groups, except the comparison one. The blood glucose level in the animals treated with the reference drug (group 3) was at the physiological level both before and after the administration of the drug. Based on the statistical analysis results of blood glycemia in the animals of the 3rd group (the Wilcoxon T-test), it can be said that the observed changes after the alimentary load are statistically insignificant (p >0.05).

It should be notified that before the administration of drugs, the glucose level on the 14th day in groups 1, 2 and 4 decreased by 28.1% (p <0.05), 40.4% (p <0.05) and 36.4% (p <0.05) compared with the values of this parameter in the respective groups on the 7th day.

In addition, before the administration of drugs, on day 14, the glucose concentration in groups 1, 2 and 4 was lower by 16.1% (p <0.05), 35.5% (p <0.05) and 41.9% (p <0.05) than the glucose concentration in the blood plasma of the control group. As a whole, these factors indicate the manifestation of a cumulative antidiabetic effect of the studied microcapsule preparations.

60 minutes after the administration of microcapsules, a significant change by 9.3% (p <0.05) and 28.6% (p <0.05), respectively, from the initial level, was found out in the blood glucose concentration in the animals of groups 2 and 4. This trend persisted throughout the experiment, and 60 minutes after the oral administration of glucose, group 4 showed a decrease in the glucose concentration by 47.6% (p <0.05) while maintaining a physiologically significant level. Meanwhile, in group 2, at the end of the experiment, a decrease in the animals' blood glucose content was observed only by 20.7% (p <0.05). The average blood glucose level of animals treated with microcapsules with plant extracts (group 1), had significantly decreased by 32.9% (p <0.05) from the initial level only by the end of the test. At the same time, a decrease in the blood glucose concentration of the animals in groups 1 and 2 to the normal level was not established.

In the control group, the animals' blood glucose level was high throughout the entire time of the experiment, and 30 min after the administration of oral glucose there was a significant increase in its blood concentration.

The blood glucose level in the animals' control group was high throughout the entire time of the experiment, and 30 min after the administration of oral glucose there was a significant increase in its blood concentration.

Analyzing the dynamics of changes in the blood

glucose concentration of the studied groups' animals, it is worth notifying that a significant decrease in glucose was observed in groups 2, 3 and 4 both after the treatment and against the background of the glucose administration, in comparison with the control group. However, as previously stated, a decrease in the blood glucose concentration in the animals treated with monopreparations in microcapsules up to the physiological norm was not observed, while the administration of PEMCs+GMCs combination gives a hypoglycemic effect comparable to the reference drug 2 hours after the administration, both on days 7 and 14.

A more pronounced therapeutic effect caused by the combination of plant extracts and gliclazide, is most likely associated with a potentiation of the action due to a broader focus of the plant components. This is a decrease in blood glucose associated with the interaction of biologically active substances of *Glycyrrhiza glabra* L. extract with PPAR-γ receptors and due to an increase in the utilization by the GLUT-4 transport system and an increase in the cell sensitivity to insulin [12]. The active components of *Galega officinalis* L. extract increase the number of islets of Langerhans and increase the sensitivity of cells to insulin [19, 20]. The versatile orientation of the mechanisms of the hypoglycemic action of the plant components used is of particular importance, given the hypoglycemic effect of gliclazide realized by stimulating insulin secretion. Additional inhibition of α-amylase and α-glucosidase by the active components of *Mentha piperita* L. extract [24] contributes to an increase in resistance to alimentary factors in the development of hyperglycemia. As a whole, these effects contribute to the versatile orientation of therapy, which is a positive characteristic of the proposed composition of microcapsules.

Thus, according to the results of studying the cumulative effect of the investigated drugs for 14 days, the administration of PEMCs+GMCs combination gives a more pronounced pharmacological effect compared to the separate use of the drugs. The HGA combination is comparable to the reference drug and has significant statistical differences compared to the control group of animals. A pharmacological potentiation is characteristic for the developed original antidiabetic substance, consisting of the sum of phytoextracts (dry extracts of *Glycyrrhiza glabra* L., *Galega officinalis* L., *Mentha piperita* L.) with the addition of gliclazide, i.e. a combined composition. As a dosage form and a delivery vehicle, the developed microcapsules based on PEG-6000, containing the entire complex of active substances, showed a rather original HGA profile. The combination of biologically active substances of the plant origin, given their versatile action direction, with a synthetic antidiabetic agent in one dosage form, has a number of advantages over classical hypoglycemic drugs, i.e., the range and variability of the action. The use of microcapsules also makes it possible to achieve a reduction in the irritating effect of the dosage form on the gastrointestinal tract, which is characteristic of tablets.

In addition, microcapsules have certain technological and economic advantages – this is the possibility of reducing the dose of gliclazide, and given their prolongation, the obtained microcapsules can later be used as an intermediate and preparatory stage for constructing a form – a spansule. Therefore, the pharmacological role of the developed microcapsules is obvious, and its fixation and discussion presented in this article, are necessary.

CONCLUSION

A technology for obtaining microcapsules with a shell based on PEG-6000, has been developed and substantiated. It has been shown that microcapsules containing the studied complex – the sum of plant extracts and gliclazide – have a pronounced positive property of the gradual development of a hypoglycemic effect. The administration of a microcapsules combination causes a more pronounced pharmacological response, comparable to the reference drug in terms of the hypoglycemic activity, but not inferior to it in terms of the elongation. In addition, it causes a comparable resistance to the alimentary glucose administration, which confirms the feasibility of the chosen technology and the originality of the microcapsule shell structure.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

AG – development of the composition and production of microcapsules with a composition of extracts and gliclazide, article writing; EFS – study design development, microcapsule composition development, general study management, article writing; OFV, SES – conducting pharmacological studies, statistical processing of the results, article writing.

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