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Original Article

Composition and cardioprotective effects of *Primula veris* L. solid herbal extract in experimental chronic heart failure

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ABSTRACT

Background: High interest in chronic heart failure (CHF) is accounted for by its high incidence, poor prognosis, growing number of hospital admissions due to the heart failure relapse, and inadequate treatment. These facts necessitate a search for new pharmacological agents for the CHF correction. Herbal medicinal products appear to be very promising as they have a noticeable therapeutic effect and tend to be more harmless in comparison to the most of synthesized medications.

Purpose: Our aim was to study the composition of the *Primula veris* L. solid herbal extract (PVSHE) and its effects on the myocardial contractile function in animals with experimental CHF.

Study design: The study design involved the identification of the raw material composition of the *P. veris* L. extract. For the experimental part of our research, we used the model of CHF to elucidate the cardioprotective properties of PVSHE.

Methods: The active extract constituents were isolated by thin-layer chromatography and column chromatography; the extract components were identified by high-performance liquid chromatography, ultraviolet spectroscopy (UVS), and nuclear magnetic resonance spectroscopy (NMRS). To model CHF, L-isoproterenol at a dose of 2.5 mg/kg was intraperitoneally injected to the experimental rats twice a day for 21 days. Cardiac output was assessed with the loading test, adrenoreactivity test, and maximum isometric loading test; CHF markers adrenomedullin and copeptin were detected in blood plasma with ELISA kit for adrenomedullin and copeptin (CoudClone Corp., USA).

Results: P. veris L. solid herbal extract contains flavonoid aglycons (apigenin, quercetine, kaemferol), flavonoid glycosides (cinarozid, rutin, hyperozid), as well as polymethoxylated flavonoids acting as chemotaxonomic markers for the genus Primula (8-methoxy-flavone; 3′,4′methylenedioxy-5′-methoxyflavone). The substance 3′,4′methylenedioxy-5′-methoxyflavone has been isolated from the primrose herb for the first time. We showed that the PVSHE has a cardioprotective effect when it was administered at a dose of 30 mg/kg in the experimental CHF, as evidenced by a lower number of animal death, lower level of CHF markers in the blood plasma of the experimental animals, the higher increase in rate of myocardial contraction and relaxation, the higher level of left ventricular pressure (LVP) and of maximum intensity of structural performance (MISP), as compared to the control group.

Conclusion: P. veris L. solid herbal extract contains flavonoid aglycons, flavonoid glycosides, and polymethoxylated flavonoids. The herbal agent increases the myocardial contractility in experimental CHF.

Abbreviations: AM, adrenomedullin; BAC, biologically active compounds; BAS, biologically active substances; CHF, chronic heart failure; ELISA, Enzyme-Linked Immunosorbent Assay; HPLC, high-performance liquid chromatography; HR, heart rate; LPO, lipid peroxidation; LVP, left ventricular pressure; MISP, maximum intensity of structural performance; NMR, nuclear magnetic resonance; PVSHE, *Primula veris* L. solid herbal extract; ROS, reactive oxygen species; SSS, standard sample solutions; TLC, thin-layer chromatography; UV, ultaviolet; UVS, ultraviolet spectroscopy

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Introduction

The global unremitting interest in CHF is accounted for by a high incidence of chronic heart failure and its poor prognosis, a growing number of hospital admissions due to the heart failure relapse, inadequate treatment, and a rise of economic costs to combat this pathology (Seferovic et al., 2013; Akwo et al., 2017; Farré et al., 2017; Korda et al., 2017; Piccinni et al., 2017).

The pharmaceutical agents for CHF therapy even in cases of adequate and regular administration are not always effective (Mikhin, Khlebodarov, 2010). These factors stimulate a search for new medications for CHF pharmacological correction. Herbal medicinal products appear to be very promising as they have a noticeable pharmacological effect and tend to be more harmless than synthesized agents.

The World Health Organization reports that herbal medicinal products are one of the major sources of adapted biologically active substances used to treat and boost immunity in various diseases (Tilburt and Kaptchuk, 2008). In the literature, there are findings of adverse effects in uncontrolled intake of some herbal medicinal products (e.g., flavonoid-induced allergy or inhibition of iron, folate, and vitamin C absorption or antithyroid and estrogenic activity) (Egert and Rimbach, 2011; Boušová and Skálová, 2012; Posadzki et al., 2013), however, as pointed out by most authors, the herbal medicine can successfully compete with synthesized drugs as they have a wider therapeutic index, lower number of adverse reactions and can be administered continuously (Ahmad et al., 2010; Rivera et al., 2013; Al-Zuaidy et al., 2016).

Numerous experimental and clinical data indicate an active search for medicinal plants with cardioprotective effect.

It was shown that a member of bioflavonoid family *baicalein* isolated from Scutellaria baicalensis limits the myocardial remodeling in CHF *in vivo* and *in vitro* (Zhao et al., 2016).

There is an evidence that Qi-shen-yi-qi (QSYQ), one of the most well-known traditional Chinese medicine formulas, has a cardioprotective effect in rats with experimental CHF. It was showed that QSYQ exerts its cardiac protective efficacy mainly by inhibiting the inflammatory response and down-regulation of apoptosis. The anti-inflammatory and antiapoptotic activity of QSYQ is probably achieved by inhibition of COXs-induced P53/FasL pathway (Wang et al., 2015b).

Multiple studies of Ginkgo biloba extract showed its protective effect on cardiovascular diseases including cardiac injury (Boghdady, 2013), ischemia (Wang et al., 2016), and myocardial infarction (Liu et al., 2014) due to its antioxidant, antiapoptotic, anti-inflammatory effects, and stimulation of cellular respiration and phosphorylation in mitochondria. The authors pointed at the fact that Ginkgo biloba extract minimizes ischemic and reperfusion injury of myocardium (Lu et al., 2011).

Randomized controlled trials of the patients with CHF showed that the dry extract of Crataegus (Hawthorn), WS 1442, increases cardiac performance functioning, alleviates disabling symptoms, and improves quality of life of the patients with CHF. The Hawthorn extract has an increased safety profile in both single agent therapy and add-on therapy, no drug-to-drug interactions, and no adverse reactions. It is possible that WS 1442 exerts the cardioprotective effect in heart failure II-III (Holubarsch et al., 2018).

Genus *Primula* L., and cowslip primrose in particular (*Primula veris* L. or *P. officinalis* (L.) Hill.), is of special importance in relation to the study of CHF pharmacological compensation. *P. veris* L. represents the flora of the European part of the Russian Federation, and it was used by us to produce a solid extract as a dosage form with the optimal concentration of biologically active substances (BAS) (Latypova et al., 2009, 2011).

Earlier revealed endothelioprotective, angioprotective, antioxidant, antihypoxant, and anticoagulant effects of the solid extract from cowslip primrose (*P. veris* L.) (Latypova et al., 2009; Iksanova et al., 2014) make it possible to suggest its cardioprotective properties.

In view of the aforesaid, the purpose of our study was to study the composition of the *P. veris* L. solid herbal extract (PVSHE) and its effects on the myocardial contractile function in experimental CHF.

Materials and methods

A solid extract of cowslip primrose (*P. veris* L.) as the most optimal dosage form containing the maximum amount of hydrophilic and lipophilic compounds was manufactured in laboratory settings by the method of partial maceration.

Methods of investigating phenol compounds

Methods of thin-layer chromatography and column chromatography were used to assess the composition of the raw materials and of the herbal extract and to isolate biologically active compounds (BAC). To perform thin-layer chromatography, Sorbfil plates (PTSH-P-A and PTCH-A-UF lines, ZAO Sorbpolymer, Russia) were used. The optimal solvent systems were as follows: ethyl acetate – glacial acetic acid – water (5:1:1); glacial acetic acid – concentrated formic acid – chloroform (10:2:2); n-butanol – glacial acetic acid – water (4:1:2). High grade and analytic grade reagents were employed.

To develop chromatograms, specific chromogenic reagents were used: 10% alcoholic sodium hydroxide, 5% alcoholic aluminum chloride followed by heating the plate in a drying chamber at a temperature of 100–105 °C (2–3 min); 0.1% vanillin diluted in sulfuric acid subsequently heated at 80 °C (3 min). The chromatograms were visually examined in visible and UV light before and after the development (Latypova et al., 2009; 2011).

Isolation and identification of the basic groups of BAC

Preparative-scale separation of BAC was performed by the methods of fractional extraction and column chromatography involving the use of silica gel L, 40/100 и L 100/110 μm. The fractions were purified on microcolumns with silica gel L 40/100, polyamide (Woelm DC, Germany). The methods of thin-layer chromatography (TLC), and ultraviolet spectroscopy (UVS) were employed to control BAC fractionation. Individual BACs from the studied plants were isolated by adsorption column chromatography with the use of a polyamide sorbent with ethanol–chloroform mixtures. A method of rechromatography on microcolumns with a polyamide sorbent (eluant – ethanol–water mixtures) was also applied.

Identification of phenol compounds using high-performance liquid chromatography (HPLC)

A Gilston chromatograph (model 305, France) and a manual injector (Rheodyne 7125 model, USA) were employed to conduct the study. The data were processed with Multichrome software for Windows (Microsoft, USA). The chromatographic procedure comprised the following phases: stationary phase – metal column Kromasil C (AkzoNobel, Sweden) 18, 4.6 × 250 mm in size (size of particles is 5 μm); mobile phase: methyl alcohol–purified water–concentrated phosphoric acid in the ratio of 400:600:5 and acetonitrile – water – concentrated phosphoric acid in the ratio of 400:600:5.

The rate of the eluent supply was 0.5–0.8 ml/min; the procedure lasted from 70 to 120 min; at room temperature; UV detector (Gilston UV/VIS, model 151, country); the wavelength was 254 nm; the volume of the studied solutions and standard sample solutions (SSS) introduced into the chromatograph was 20 μl. To prepare the mobile phase methyl alcohol, concentrated phosphoric acid, and distilled water were used. The separated substances were identified by comparing the retention times of the chromatographic peaks obtained in the samples with those of SSS. To assess the relative concentration of phenol compounds in the

investigated samples, the method of internal normalization was employed.

Identification of the isolated substances using UVS

Electronic absorption spectra were registered in the 200–600 nm area using a Specord 40 spectrometer (Analytik Jena, Germany) for the liquid cells with an absorption layer thickness of 10 mm. Differential UV spectrophotometry in the spectrum region was applied to quantify and express total flavonoids in terms of rutin equivalent.

To obtain specific UV spectra, a reaction of complexation of flavonoids with aluminum chloride in 70% acetified ethanol medium was carried out (Latypova et al., 2009, 2011).

Identification of the isolated substances using NMR spectroscopy

NMR spectra were registered using a Bruker Avance III 500 MHz spectrometer (BrukerBiospin, Rheinstetten, Germany) at room temperature in $CDCl_3$ solution. The proton frequency was 500 MHz. The calibration of the instrument involved a standard test sample: line form (3% $CHCl_3$, 0.2% TMS, acetone-d6), sensitivity of nuclei ^1H (0.1% ethylbenzene, $CDCl_3$). Signal-to-noise ratio (S/N) was calculated with the use of the standard test sample (0.1% ethylbenzene, $CDCl_3$), and it was 750:1.

For the original Lorentzian line of the chloroform signal, the full width at half maximum was 0.15 Hz when the «Lineshape test sample» was employed (3% $CHCl_3$, 0.2% TMS, acetone-d6). The following parameters of NMR ^1H spectra were registered: spectral width – 10.0 kHz, number of accumulation points – 64 K, number of scan – 8, accumulation time – 3.3 s, relaxation delay – 5.0 s, length of 90° impulse was 115 ms. Before Fourier transformation, free induction decay was multiplied by Lorentzian window function (lb = 1 Hz).

NMR spectra ^{13}C with power-gated decoupling which involved the use of composite pulses WALTZ-16 were registered in the following conditions: spectral window – 29.8 kHz, number of points – 64 K, length of excitation impulses (30°) – 3.2 μs, relaxation delay - 0.9 s, number of scans – 256. The ^{13}C NMR spectra were edited on the basis of the DEPT-135 experiment.

^1H and ^{13}C NMR spectra were recorded on a Bruker Avance III 500 MHz spectrometer (BrukerBiospin, Rheinstetten, Germany) at room temperature in $CDCl_3$ solution with TMS as the internal standard. All chemical shifts were reported in ppm. NMR spectra were referenced to the residual solvent peak; chemical shifts δ were in ppm, and apparent scalar coupling constants J were in Hertz. Mass spectroscopic data were obtained on a Finnigan DSQ IIGC-MC instrument (Thermo Scientific, USA). Analytic TLC was carried out on Sorbfil plates of the grade PTSKh-AF-A (”Sorbpolymer” Co., Russia).

All target compounds were characterized and determined to be at least >95% pure by ^1H NMR and analytical HPLC.

Experimental animals

The study of cardioprotective effects of the *P. veris* L. solid herbal extract on the myocardial contractile function in experimental CHF was performed on the adult male rats (Wistar strain) weighing 270–320 g. They were purchased from Rappolovo Animal Farm (Leningrad region, Russia). The animals were kept in standard vivarium conditions and cared for according to the Good Laboratory Practice guidelines for preclinical studies (GOST 3 51000.396 and 1000.4. −96, Russia). The experimental study was approved by the Regional Independent Ethics Committee of the Volgograd Medical Research Centre (protocol N. 176-2013 of 08.05.2013; Volgograd, Russia).

In our study, four groups were formed: 1) intact group – rats (n = 10) which received purified water and were intraperitoneally injected by normal saline solution at a dose of 0.1 ml/100 g body weight twice a day for 21 days; 2) control group (CHF + purified water) – rats (n = 20) with isoproterenol-induced CHF; they received purified water and were intraperitoneally injected by L-isoproterenol; 3) experimental group (CHF + PVSHE) – rats (n = 15) with isoproterenol-induced CHF; they were administrated with the solid herbal extract of *P. veris* L. at a dose of 30 mg/kg; 4) experimental group treated by a comparator medication (CHF + mildronate) – rats (n = 15) with isoproterenol-induced CHF; they were administrated by a comparator drug of mildonium (Mildronate, Grindex pharmaceuticals, Latvia) at a dose of 50 mg/kg.

The animals of intact and control groups orally received purified water at a dose of 0.1 ml per 100 g of body weight once a day from the 1st day of L-isoproterenol injection and over the next three weeks.

CHF modeling

CHF was modeled by intraperitoneal injection of L-isoproterenol (Sigma-Aldrich, USA) at a dose of 2.5 mg/kg twice a day for 21 days (Ennis et al., 2003).

Functional tests

To assess the functional condition of the heart the following loading tests were used: volume loading test (intravenous injection of saline solution 0.3 ml per 100 g of body weight), test for adrenoreactivity (intravenous introduction of adrenalin 0.1 ml per 100 g of body weight at a 10^7 g/l dilution), and maximum isometric loading test (occlusion of the ascending aortic arch for 30 s) (Mironov, 2012).

Preparation for surgery

The assessment of cardiodynamic changes in all the animal groups was performed after the preparation for surgery: the narcotized animals (hydrochloride, 400 mg/kg) were put on artificial lung ventilation (UgoBasile, Italy, a lung mechanical ventilation pump for rodents). Subsequently, thoracotomy and pericardiotomy were performed in the fourth intercostal space. A catheter connected to the pressure sensor (Biopac systems, USA) was inserted through the heart apex into the left ventricle. The interface universal module (UIM100C) of the UIM100C polygraph (Biopac systems, USA) and AcqKnowledge 4.0 software (Biopac systems, USA) were used to register the myocardial contraction rate (+dP/dt max, mm Hg/s) and relaxation rate (−dP/dt max, mm Hg/s), left ventricular pressure (LVP) (mm Hg), and heart rate (HR) (beats per min). The maximum intensity of structural performance (MISP) was calculated as follows: ((LVP avg. × HR avg. / (left ventricle mass + 1/3 mass of interventricular septum (mm Hg/mg*min) (Mironov, 2012).

Determining of CHF markers

The markers of CHF, adrenomedullin and copeptin, were measured in the plasma of rats by a competitive enzyme-linked immunosorbent assay (ELISA) technique. To obtain plasma, blood from the abdominal aorta was mixed with ethylenediaminetetraacetic acid (EDTA) (9:1) and centrifuged at 1000 g/min for 15 min immediately after sampling. The resulting plasma was stored at −20 °C and defrosted on the day of marker detection. To measure the concentration of adrenomedullin, the ELISA Kit for Adrenomedullin (Cloud-Clone Corp., USA) was employed. After determination of wells for diluted standard, blank and samples, and preparation of reagents, the assay procedure went through the following steps: incubation of the plasma samples and calibrator dilutions (on 50 μL for each well) with 50 μL of prepared Detection Reagent A for 1 h at 37 °C; aspiration/wash process for 3 times with a reaction buffer; incubation with 100 μl of prepared Detection Reagent B for 30 min at 37 °C; aspiration/wash process for total 5 times as conducted previously; incubation with 90 μl of the substrate solution in the dark for 20 min at 37 °C. The reaction was stopped by the addition of 50 μl of

Stop Solution to each well, and the optical density of the solutions was read at 450 nm by the vertical microplate reader (Tecan, Austria) immediately. The concentration of adrenomedullin was read from the standard calibration curve and expressed in pg/ml.

In a similar scheme, the concentration of copeptin was determined with ELISA Kit for Copeptin (Cloud-Clone Corp., USA).

Statistical analysis

Statistical analysis was performed with Statistica 10 Software program (StatSoft Inc., USA). The Shapiro–Wilk was used to test the distribution of the data. When the studied parameters were normally distributed, the Newman–Keuls test was employed. In case the distribution differed from the normal one, the Kruskal–Wallis test and Dunn's post hoc test were applied. The numerical values were presented as M ± m и M ± σ, in which M is an arithmetic mean value, m is a standard error of the mean, σ is a standard deviation. P value < 0.05 was considered statistically significant.

Results

Our previous studies and other published data have demonstrated that *P. veris* L. herb contains a combination of hydrophilic and lipophilic BAC (Latypova et al., 2009, 2011, 2015).

Isolation and identification of individual compounds

To isolate individual compounds, herb of *P. veris* L. gathered in the European part of the Russian Federation was ground up to the granules of 1–3 mm size. It has been experimentally found, that the maximum output of phenol compounds (both qualitatively and quantitatively) from *P. veris* L. can be obtained by extracting with 70% ethanol (Latypova et al., 2011). To identify the composition of the phenol compounds, the method of HPLC was employed. Our previous studies have demonstrated that polyphenolic compounds and flavonoids, in particular, appear to have great potential.

To separate BAC, vacuum evaporated aqueous extracts were applied on the columns filled with silica gel L 40/100 и L 100/110 μm. The extractives were eluted with chloroform, alcohol-chloroform mixtures, and ethanol. The obtained eluates were divided into fractions of identical composition; this was followed by their vacuum evaporation. The individual substances were isolated with rechromatography on the columns with a polyamide sorbent (Woelm DC, Germany); they were eluated with water and hydroalcoholic solutions at concentrations of 20%, 40%, 70%, and 96%.

The fractions containing identical substances were combined, evaporated if necessary. The precipitation was separated and recrystallized from ethanol and hydroalcoholic solutions.

The process of BAC fractioning was supervised with TLC and UVS techniques. To perform TLC, Sorbfil plates (PTSH-P-A and PTCH-A-UF), and a number of solvent systems were applied. UV spectra of the solutions of the isolated substances and hydroalcoholic extracts were registered with a Specord 40 (Analytik Jena, Germany). The solvent system n-butanol-acetic acid-water (BAW) at the ratio 4:1:2 has experimentally proved to be optimal.

The structure of isolated compounds was identified by chromatography mass-spectrometry, UVS, and NMR-spectroscopy, as well as by the investigation of their physicochemical properties and by the comparison of those with standard samples.

As part of our study, we have identified a number of polymethoxylated flavonoids acting as *Primula* L. genus chemotaxonomic markers (8-methoxy-flavone, flavones, 3′,4′methylenedioxy-5′-methoxyflavone) which are promising for further study (Table 1).

We have isolated a substance from which the signals of eight quaternary carbon atoms, one CH_2 group, seven CH groups, and one OCH_3 group were detected in NMR ^{13}C spectra. The signals of carbonyl C-4 (δ_C 178.34) carbon atom as well as carbon atoms with double bonds C-2 (δ_C 162.96) and C-3 (δ_C 106.93) have characteristic chemical shifts in 1.3 substituted (i.e. position 3 in relation to C═O) enone system. The position of 5′-methoxy-1,3-benzodioxol substitute in 4H-chromen-4-one was detected using the upfield signal from proton H-3 (δ_H 6.72) and its carbon C-3 (δ_C 106.93). It is typical for the enone double bond proton located in the shielding zone of magnetically anisotropic group C═O to shift the signal to a more intense field in the spectrum (if the proton were in C-2 position and the benzodioxol substitute were in C-3, the H-2 signals would be registered in a less intense field in the region of 8.1. − 8.5 Ppm).

The isolated substance is a deep green heavy oily liquid composed of $C_{17}H_{12}O_5$, 3′,4′-methylenedioxy-5′-methoxyflavone (2-(7-methoxy-1,3-benzodioxol-5-il)-4H-chromen-4-on); its UVS in ethanol was λ_{max} 219, 337, 410 nm.

The chemical compound 3′,4′- methylenedioxy-5′-methoxyflavone has been isolated from *P. veris* L. herb for the first time ever (Fig. 1) (Latypova et al., 2015).

In addition to polymethoxylated flavonoids, we have also isolated the flavonoid aglycons (apigenin, quercetine, kaempferol), flavonoid glycosides (cinarozid, rutin, hyperozid) (Table 1) (Latypova et al., 2009, 2011).

On the basis of the chemical composition, we conducted a study to develop a way to produce a pharmaceutical substance from *P. veris* L. herb with the maximum output of active ingredients. The solid herbal extract (PVSHE) meets all these requirements, and it was further investigated to reveal its biological properties.

A solid extract was produced by the method of partial maceration. 100.0 g of ground raw materials were extracted by 1000 ml of 70% ethanol. The extract portions were purified by clarification at a temperature of 8 °C for 72 h, filtered, and evaporated with a rotary evaporator (IR-1LT model, Labtex) to be transformed into a solid extract.

The produced extract is a deep green heavy liquid with a pleasant odor and slightly bitter taste and no more than 25% of moisture.

Our findings have demonstrated that rutin is a dominant BAC in the obtained extract, which is important for standardization of production. The content of total flavonoids in terms of rutin equivalent was no less than 5.0% in each solid extract assay sample (Latypova et al., 2009).

The further study was focused on the effects of PVSHE on the myocardial contractile function in the animals with experimental CHF.

Animal death in CHF modeling

Totally, 11 animals from 60 rats died. Among them, 5 animals died in the control group with modeled CHF; 1 and 5 rats died in the experimental groups receiving PVSHE at a dose of 30 mg/kg and mildronate at a dose of 50 mg/kg, respectively.

Level of markers in blood plasma of the animals with CHF

The CHF markers used in our study, AM and copeptin, were higher by 50.4% and 66.1% ($P < 0.05$), respectively, in the animals with CHF as compared to the intact animals; this suggests a pathological process. The concentrations of AM were 23.4% and 26.3% lower ($P < 0.05$) in the CHF rats receiving PVSHE and in the CHF rats receiving mildronate as compared to the control group, respectively; the concentrations of copeptin in the same groups were 35.7% and 37.2% lower ($P < 0.05$) as compared to the controls, respectively (Table 2).

Changes in cardiac functional reserve in the animals with CHF

The maximum increase in myocardial contraction and relaxation rates and in LVP in response to loading stress in the control CHF animals was registered on 10 s after the introduction of saline solution, and it was lower than that in the intact group. The rats of the experimental groups receiving PVSHE and mildronate showed a higher rise in the

Table 1
Phenol compounds identified in the herb and extract of *P. veris*.

№	Substance name	Spectral parameters
1	Flavone; (2-phenyl-4H-chromen-4-on)	NMR^{1}H ($CDCl_3$): 6.82 (1H, c, H^3), 7.40 (1H, dd, *J* = 8.2, 1.0, H^6), 7.51 (2H, м, $H^{3'}$, $H^{5'}$), 7.55 (2H, м, $H^{4'}$, H^8), 7.68 (1H, dd, *J* = 8.2, 1.0, H^7), 7.91 (2H, м, $H^{2'}$, $H^{6'}$), 8.23 (1H, dd, *J* = 8.2, 1.7, H^5). NMR^{13}C ($CDCl_3$):107.3 (C^3), 117.9 (C^8), 123.7 (C^{10}), 124.9 (C^6), 125.4 (C^5), 126.0 ($C^{6'}$, $C^{2'}$), 128.8 ($C^{3'}$, $C^{5'}$), 131.3 ($C^{4'}$), 131.5 ($C^{1'}$), 133.5 (C^7), 156.0 (C^9), 163.0 (C^2), 178.0 (C = O).
2	8-methoxy-flavone; (8-methoxy-2-phenyl-4H-chromen-4-on)	NMR^{13}C ($CDCl_3$): 56.2 (OCH_3), 107.1 (C^3), 114.2 (C^5), 116.1 (C^7), 124.1 (C^{10}), 124.6 (C^6), 126.1 ($C^{6'}$, $C^{2'}$), 128.7 ($C^{3'}$, C^5), 131.2 ($C^{4'}$), 131.6 ($C^{1'}$), 146.1 (C^9), 148.8 (C^8), 162.6 (C^2), 178.0 (C═O).
3	3′,4′-methylenedioxy-5′-methoxyflavone	NMR^{1}H ($CDCl_3$): 4.0 (3H, s, OCH_3), 6.10 (2H, s, H-2′), 6.71 (1H, s, H-3), 7.11 (1H,d, *J* = 1.5hz, H-6′), 7.14 (1H, d, *J* = 1.5 hz, H-4′), 7.43 (1H, dd,*J* = 7.9, 7.3 hz,H-6), 7.55 (1H, d,*J* = 8.2 hzH-8), 7.69 (1H, ddd, *J* = 8.2, 7.3, 1.5hz, H-7), 8.22 (1H, dd, *J* = 7.9, 1.5 hz, H-5). NMR^{13}C ($CDCl_3$): 56.36 (OCH_3), 100.74 ($C^{6'}$), 102.34 ($C^{2'}$), 106.93 (C^3, $C^{4'}$), 117.98 (C^8), 123.89 (C^{10}), 125.23 (C^6), 125.69 (C^5), 126.14 ($C^{5'}$), 133.71 (C^7), 138.45 ($C^{1'}$), 143.87 ($C^{7'}$), 149.55 ($C^{3'}$), 159.13 (C^9), 162.96 (C^2), 178.34 (C = O).
4	Apigenin; (5,7,4-trihydroxyflavone, 5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-chroman-4-on)	NMR^{1}H (DMF-d7): 8.01 (2H,d,*J* = 8.7, $H^{2'}$, $H^{6'}$), 7.05 (2H,d, *J* = 8.7, $H^{3'}$, $H^{5'}$), 6.79 (1H,c, H^3), 6.62 (1H,d, *J* = 2.0, H^8), 6.31 (1H,d, *J* = 2.0, H^6). NMR^{13}C (DMF-d7):182.24 (C^4), 164.85 (C^2), 164.34 (C^7), 162.25 (C^5), 161.80 ($C^{4'}$), 157.89 (C^9), 128.59 ($C^{2'}$, $C^{6'}$), 121.79 ($C^{1'}$), 116.11 ($C^{3'}$, $C^{6'}$), 104.11 (C^{10}), 103.05 (C^3), 98.96 (C^6), 94.12 (C^8).
5	Quercetin; (3,5,7,3′,4′-pentahydrooxyflavone)	NMR^{1}H (CD_3OD): 6.18 (1H, d, *J* = 2.1, H^6), 6.38 (1H, d,*J* = 2.1, H^8), 6.87 (1H, d,*J* = 8.5, H^5), 7.6 (1H, dd, *J* = 8.5, 2.1, H^6), 7.74 (1H, d,*J* = 2.1, H^2). NMR^{13}C (CD_3OD):94.65 (C^8), 99.48 (C^6), 104.76 (C^3), 116.22 (C^{10}), 116.47 ($C^{5'}$), 121.92 ($C^{6'}$), 124.38 ($C^{2'}$), 137.48 ($C^{1'}$), 146.46 (C^9), 148.23 ($C^{3'}$), 149.01 ($C^{4'}$), 158.45 (C^5), 162.73 (C^7), 165.83 (C^2), 177.57 (C^4).
6	Kaempferol; (3,5,7,4′- tetrahydrooxyflavone)	ЯМР ^{1}H (DMSO):8.10 (2H, d, *J* = 8.0, $H^{2'}$, $H^{6'}$), 7.0 (2H, d, *J* = 8.0, $H^{3'}$, $H^{5'}$), 6.54 (1H, d, *J* = 2.0, H^8), 6.28 (1H, d, *J* = 2.0, H^6). ЯМР ^{13}C (DMSO):93.50 (C^8), 98.21 (C^6), 103.16 (C^{10}), 115.42 ($C^{3'}$, $C^{5'}$), 121.74 ($C^{1'}$), 129.57 ($C^{2'}$, $C^{6'}$), 135.67 (C^3), 146.81 (C^2), 156.23 (C^9), 159.25 ($C^{4'}$), 160.72 (C^5), 163.96 (C^7), 175.90 (C^4).
7	Cinarozid; (luteolin-7-O-β-D-glucopyranoside)	NMR^{1}H (Aceton-d6 + D_2O):3.42 (1H, t, *J* = 9.0, $H^{4''}$), 3.49 (1H, t, *J* = 9.0, $H^{2''}$), 3.56 (1H, t, *J* = 9.0, $H^{3''}$), 3.60 (1H, m, $H^{5''}$), 3.68 (1H, dd, *J* = 12.2, 5.6, $H^{6a''}$), 3.85 (1H, dd, *J* = 12.2, 1.8, $H^{6b''}$), 5.10 (1H, d, *J* = 7.8, $H^{1''}$), 6.44 (1H, d, *J* = 1.8, H^6), 6.63 (1H, s, H^3), 6.83 (1H, d, *J* = 1.8, H^8), 6.95 (1H, d, *J* = 8.0, $H^{5'}$), 7.41 (1H, д, *J* = 8.0, $H^{6'}$), 7.43 (1H, br.s., $H^{2'}$). NMR^{13}C (Aceton-d6 + D_2O):61.7 ($C^{6''}$), 70.3 ($C^{4''}$), 73.8 ($C^{2''}$), 76.8 ($C^{3''}$), 77.4 ($C^{5''}$), 95.8 (C^8), 100.5 (C^6), 100.7 ($C^{1''}$), 103.7 (C^3), 106.3 (C^{10}), 113.8 ($C^{2''}$), 116.5 ($C^{5'}$), 120.3 ($C^{6'}$), 122.6 ($C^{1'}$), 146.3($C^{3'}$), 150.4 ($C^{4'}$), 158.0 (C^9), 161.8 (C^5), 163.9 (C^7), 165.8 (C^2), 183.1 (C^4).
8	Rutin; (3-O-rutinozidquercetin)	NMR^{1}H (CD_3OD): 1.12 (3H, d*J* = 6.3, $H^{6'''}$), 3.30- 3.85 (10H, м, $H^{2''}$, $H^{3''}$, $H^{4''}$, $H^{5''}$, $H^{6''a}$, $H^{6''b}$, $H^{2'''}$, $H^{3'''}$, $H^{4'''}$, $H^{5'''}$), 4.52 (1H, d, *J* = 1.5, $H^{1'''}$), 6.10 (1H, d, *J* = 7.5, $H^{1''}$), 6.20 (1H, d, *J* = 2.1, H^6), 6.38 (1H, d, *J* = 2.1, H^8), 6.88 (1H, d, *J* = 8.4, $H^{5'}$), 7.63 (1H, dd, *J* = 8.5, 2.2, $H^{6'}$), 7.70 (1H, d, *J* = 2.05, H2′). NMR^{13}C (CD_3OD):18.34 ($C^{6'''}$), 68.97 ($C^{6''}$), 70.11 ($C^{5'''}$), 71.76 ($C^{4''}$), 72.48 ($C^{2'''}$), 72.64 ($C^{3'''}$), 74.35 ($C^{4'''}$), 76.15 ($C^{2''}$), 77.52 ($C^{5''}$), 78.55 ($C^{3''}$), 95.35 (C^8), 100.40 (C^6), 102.82 ($C^{1'''}$), 105.26 ($C^{1''}$), 105.95 (C^{10}), 116.47 ($C^{5'}$), 118.17 ($C^{2'}$), 123.48 ($C^{1'}$), 124.04 ($C^{6'}$), 136.08 (C^3), 146.16 ($C^{3'}$), 150.18 ($C^{4'}$), 158.80 (C^2), 159.69 (C^9), 166.41 (C^5), 166.42 (C^7), 179.70 (C^4).
9	Hyperozid; (quercetin-3-O-β-D-galactopyranoside)	NMR^{1}H (CD_3OD):3.49 (1H, т, *J* = 6.0, $H^{5''}$), 3.56 (1H,м, $H^{3''}$), 3.65 (2H,dd, *J* = 11.2, 6.0, $H_{6''}$), 3.81(1H, dd, *J* = 9.5, 8.0, $H^{2''}$), 3.86 (1H,м, $H^{4''}$), 5.26 (1H,d, *J* = 7.6, $H^{1''}$), 6.23 (1H,d, *J* = 2.0, H^6), 6.43 (1H,d, *J* = 2.0, H^8), 6.89 (1H,dd, *J* = 8.6, 2.0, $H^{5'}$), 7.62 (1H,dd, *J* = 8.6, 1.1, $H^{6'}$), 7.82 (1H,d, *J* = 2.0, $H^{2'}$). NMR^{13}C (CD_3OD):62.10 ($C^{6''}$), 70.12($C^{4''}$), 73.25 ($C^{2''}$), 75.18 ($C^{3''}$), 77.37 ($C^{5''}$), 94.91 (C^8), 100.07 (C^6), 105.16 ($C^{1''}$), 105.66 (C^{10}), 116.39 ($C^{5'}$), 117.92 ($C^{2'}$), 123.16 ($C^{6'}$), 123.34 ($C^{1'}$), 135.66 (C^3), 146.12 ($C^{3'}$), 150.06 ($C^{4'}$), 158.56 (C^9), 158.92 (C^2), 163.19 (C^5), 166.13 (C^7), 179.61 (C^4).

investigated indices as compared to the control animals with CHF (Table 3).

In control rats with CHF, the increase in +dp/dt max and -dp/dt max and LVP in response to adrenaline administration on 10 sec was lower than in the intact animals. The animals with CHF receiving PVSHE at a dose of 30 mg/kg showed a significantly higher increase in myocardial contraction and relaxation rates, LVP and HR, on 10 sec as compared to the control animals with CHF. Moreover, some parameters in the CHF rats receiving PVSHE exceeded those in the group receiving a comparator drug of mildronate (Table 4).

After the occlusion of the ascending aortic arch, the increase in myocardial contraction and relaxation rates, LVP, and MISP on 5 s and 30 s was lower in the rats with isoproterenol-induced CHF as compared to the intact group. Moreover, HR was elevated in the animals with CHF (Table 5). The maximum isometric load caused a noticeable rise in the study parameters on 5 s and 30 s in the animal group receiving PVSHE in relation to the control group. It should be pointed out that the increase in LVP and MISP on 30 s in this experimental group exceeded

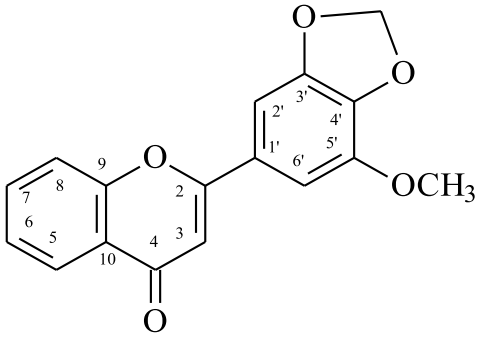

Fig. 1. Structural formula of 3′,4′- methylenedioxy-5′-methoxyflavone.

Table 2
Changes in the levels of adrenomedullin and copeptin in the animals with experimental CHF (M ± σ).

Animal groups	Adrenomedullin, pg/ml	Copeptin, pg/ml
Intact + purified water (*n* = 8)	34.3 ± 7.9	23.6 ± 1.6
CHF + purified water (*n* = 8)	51.6 ± 9.9[a]	39.2 ± 2.6[a]
CHF + PVSHE 30 mg/kg (*n* = 10)	39.5 ± 8.2[b]	25.2 ± 1.3[b]
CHF + mildronate (*n* = 7)	38.0 ± 6.1[b]	24.6 ± 1.6[b]

Note.
[a] In relation to the indices of the intact animal group, $p < 0.05$.
[b] In relation to the indices of the control group of animals with isoproterenol-induced CHF, $p < 0.05$.

Table 3.
Effects of the studied substances on the indices of myocardial contractility, LVP and HB in the animals with experimental CHF during volume load test (M ± m).

Animal groups	Volume load test, % increase of the index is provided in brackets							
	+dP/dt max mm Hg/s		−dP/dt max mm Hg/s		LVP, mm Hg/s		HB, bpm	
	Baseline	10 s	Baseline	10 s	Baseline	10 s	Baseline	10 s
Intact group + purified water (n = 10)	5878.5 ± 637.2	8313.8 ± 872.4 (45.8 ± 15.1)	−4506.0 ± 616.7	−5868.3 ± 625.0 (36.3 ± 10.0)	113.6 ± 9	128.5 ± 6.3 (15.5 ± 5.0)	301.4 ± 26.9	416.6 ± 13.3 (46.8 ± 14.6)
CHF + purified water (n = 15)	5242.9 ± 785.0	5673.1 ± 869.5 (9.4 ± 4.4)[a]	−3525.8 ± 665.1	−3945.7 ± 601.3 (15.9 ± 5.9)	97.3 ± 8.9	100.5 ± 9.0 (3.8 ± 2.7)[a]	260.2 ± 21.6	298.3 ± 25.7 (17.4 ± 11.3)
CHF + PVSHE 30 mg/kg (n = 14)	3937.2 ± 718.7	5458.7 ± 949.5 (37.6 ± 10.9)[b]	−2878.2 ± 671.3	−3692.2 ± 711.3 (32.8 ± 10.3)	94.9 ± 10.9	103.6 ± 10.5 (11.3 ± 3.2)	221.1 ± 30.3	282.6 ± 32.4 (35.4 ± 5.4)[b]
CHF + mildronate (n = 9)	3258.6 ± 234.2	5079.5 ± 508.2 (58.8 ± 16.9)[b]	−2467.2 ± 271.0	−3379.2 ± 410.3 (41.5 ± 17.4)[b]	93.2 ± 5.2	103.2 ± 6.8 (10.6 ± 3.9)	186.4 ± 35.3	275.7 ± 33.1 (69.6 ± 23.2)[b]

Note.
[a] In relation to the indices of the intact animal group, $p < 0.05$.
[b] In relation to the indices of the control group of animals with isoproterenol-induced CHF, $p < 0.05$% increase of the indices is provided in brackets.

Table 4.
Effects of the studied substances on the indices of myocardial contractility, LVP and HB in the animals with experimental CHF during adrenoreactivity test (M ± m).

Animal groups	Adrenoreactivity test, % increase of the index is provided in brackets							
	+dP/dt max mm Hg/s		−dP/dt max mm Hg/s		LVPmm Hg/s		HB, bpm	
	Baseline	10 s	Baseline	10 s	Baseline	10 s	Baseline	10 s
Intact group + purified water (n = 10)	5086.7 ± 628.8	12,654.0 ± 974.5 (144.1 ± 27.3)	−3380.4 ± 375.6	−8973.5 ± 650.2 (180.4 ± 24.8)	114.9 ± 8.8	197.4 ± 10.2 (79.2 ± 17.1)	358.9 ± 24.2	548.0 ± 21.9 (58.3 ± 13.1)
CHF + purified water (n = 15)	4912.9 ± 709.7	6229.7 ± 801.6 (31.4 ± 8.4)[a]	−3484.7 ± 647.1	−5040.3 ± 716.3 (64.0 ± 23.3)[a]	94.4 ± 8.7	142 ± 11.6 (55.0 ± 13)	261.0 ± 19.0	489.5 ± 39.2 (92.5 ± 18.5)
CHF + PVSHE 30 mg/kg (n = 14)	3963.4 ± 781.3	9 324.9 ± 905.3 (160.5 ± 38.4)[b]	−3517.0 ± 762.3	−6690.3 ± 1221.4 (129.1 ± 63.3)[b]	91.3 ± 12.4	136.7 ± 15.9 (57.2 ± 16.6)	263.6 ± 29.4	501.8 ± 26.1 (104.7 ± 21.1)
CHF + mildronate (n = 9)	3320.6 ± 569.7	6453.1 ± 848.5 (76.4 ± 15.0)[b]	−2296.8 ± 475.1	−4637.9 ± 800.0 (127.5 ± 28.1)[b]	89.1 ± 7.5	145.6 ± 10.1 (66.2 ± 11.2)	222.5 ± 31.5	517.4 ± 17.9 (165.3 ± 34.2)[b]

Note.
[a] In relation to the indices of the intact animal group, $p < 0.05$.
[b] In relation to the indices of the control group of animals with isoproterenol-induced CHF,$p < 0.05$% increase of the indices is provided in brackets.

Table 5.
Effects of the studied substances on the indices of myocardial contractility, LVP and HB in the animals with experimental CHF in response to maximum isometric load test (M ± m).

Animal groups	Maximum isometric load test, % increase of the index is provided in brackets					
	+dP/dt max mm Hg/s			−dP/dt max mm Hg/s		
	Baseline	5 s	30 s	Baseline	5 s	30 s
Intact group + purified water (n = 10)	6089.6 ± 925.6	11,720.7 ± 123-4.4 (115.0 ± 25.4)	6212.1 ± 964.7 (22.9 ± 8.7)	−4367.2 ± 686-.5	−6871 ± 924.4 (126.2 ± 20.8)	−3846.8 ± 385-.6 (36.6 ± 6.2)
CHF + Purified water (n = 15)	3928.6 ± 618.9	5441.5 ± 730.5 (43.8 ± 12.9)[a]	3034.3 ± 431.8 (−16.6 ± 10.8)[a]	−2920.3 ± 437-.8	−3418.7 ± 412-.1 (17.3 ± 7.5)[a]	−2381.7 ± 398-.9 (−13.1 ± 9.5)[a]
CHF + PVSHE 30 mg/kg (n = 14)	3348.7 ± 784.4	5789.7 ± 1155.-4 (102.8 ± 24.3)[b]	3596.8 ± 802.2 (17.4 ± 5.4)[b]	−2583.8 ± 668-.1	−3535.5 ± 725-.7 (48.7 ± 11.4)	−2666.1 ± 550-.8 (19.8 ± 7.3)
CHF + mildronate (n = 9)	3000.2 ± 337.3	5117.5 ± 561.1 (69.1 ± 10.5)	3796.4 ± 466.6 (27.2 ± 9.4)[b]	−2216.2 ± 182-.4	−3946.9 ± 409 (83.6 ± 23.3)[b]	−3123 ± 376.5 (41.4 ± 17.4)[b]

Animal groups	Maximum isometric load test, % increase of the index is provided in brackets					
	LVP, mm Hg/s			HB, bpm		
	Baseline	5 s	30 s	Baseline	5 s	30 s
Intact group + purified water (n = 10)	99.8 ± 4.3	209.5 ± 7.0 (112.4 ± 11.3)	162.3 ± 5.8 (63.4 ± 4.2)	315.7 ± 27.1	449.8 ± 27.4 (43.9 ± 7.0)	352.9 ± 31.7 (18.0 ± 15.6)
CHF + Purified water (n = 15)	86.0 ± 5.9	140.6 ± 9.4 (66.4 ± 10.7)[a]	98.0 ± 6.6 (17.0 ± 9.7)[a]	310.7 ± 27.1	549.8 ± 27.4 (50.5 ± 16.1)	619.6 ± 379.9 (78.2 ± 83.2)
CHF + PVSHE 30 mg/kg (n = 14)	102.7 ± 7.0	178.8 ± 1.8 (76.1 ± 8.5)	156.5 ± 9.0 (53.9 ± 5.3)[b]	334.7 ± 30.9	483.1 ± 24.2 (50.7 ± 12.4)	375.9 ± 33.5 (15.5 ± 10.6)
CHF + mildronate (n = 9)	86.3 ± 5.1	168.4 ± 4.6 (99.9 ± 12.4)[b]	125.5 ± 8.2 (47.0 ± 9.2)[b]	308.4 ± 35.6	482.9 ± 21.9 (78.5 ± 27.9)	397.6 ± 18.5 (43.5 ± 19.4)

Note.
[a] In relation to the indices of the intact animal group, $p < 0.05$.
[b] In relation to the indices of the control group of animals with isoproterenol-induced CHF, $p < 0.05$% increase of the indices is provided in brackets.

that in the animals receiving mildronate after the occlusion of the ascending aortic arch (Table 6).

Discussion

Cardiovascular diseases is an urgent medico-social priority as mortality and morbidity rates for cardiovascular diseases remain the highest worldwide (Rivera et al., 2013; Akwo et al., 2017; Farré et al., 2017; Korda et al., 2017; Piccinni et al., 2017).

CHF is one of the most widespread and steadily rising diseases with unfavorable prognosis. CHF is characterized by the number of pathological processes in cardiomyocytes, such as cytoplasmic accumulation of sodium and potassium ions, impairment of high-energy compound synthesis, acidification of cardiomyocyte cytoplasm. These result in disturbances of myocardial functioning and cause a decrease in their power and rates (Zhong et al., 2013).

Our study has not demonstrated any statistically significant differences in the initial indices of myocardial contraction and relaxation rates and LVP between the intact animals and the control animals with CHF.

When loading tests were performed, the increase in the studied parameters was significantly lower in the control rats with CHF than in the intact animals; this supports the conclusion about a decrease in cardiac ino- and chronotropic reserves in the CHF rats in comparison to the intact animals.

Nowadays, some biomarkers are used for diagnosis, monitoring, and disease prognosis in patients with CHF. A number of studies have described the prognostic potential of copeptin as a predictor of cardiac decompensation or even death of patients with heart failure. (Morgenthaler, 2010; Balling et al., 2012). Adrenomedullin (AM), a vasoactive peptide belonging to the calcitonin gene-related peptide family, is another biomarker with similar prognostic potential in relation to cardiac failure. The patients with CHF demonstrated elevated AM level in their blood plasma in proportion to the severity of CHF (Nishikimi et al., 2013).

In our research, the levels of copeptin and AM in the animals with CHF were 1.5 and 1.7 higher as compared to the indices of the intact group, respectively, which suggested a pathological process progress.

Current CHF therapy (angiotensin-converting enzyme inhibitors, beta-adrenergic blocking agents, diuretics, implantable cardioverter defibrillators, cardiac resynchronization therapy devices) has yielded a significant decline in morbidity and mortality caused by heart failure. Despite significant achievements in the treatment of CHF, the longevity and quality of life in patients with heart failure remain low, and the cardiac patient management requires the improvement of therapeutic approach for CHF compensation (Brown et al., 2017). Recently, there has been growing interest in medicinal plants as many of them have cardioprotective effects (Ahmad et al., 2010).

It was shown that the proanthocyanidin extract of grape seeds (grape seed proanthocyanidin extract, GSPE) reduces H_2O_2-induced oxidative stress in cardiomyocytes, and this contributes to the survival of cardiomyocytes and improvement of their contractile function. There was an evidence of a cardioprotective effect of GSPE in reperfusion syndrome through removing, directly or indirectly, free radicals from the myocardium in an isolated rat heart (Wang et al., 2007).

It was also found that green tea extract contributes to protection against cardiovascular and renal diseases studied on the models of these diseases in vitro and in vivo. The main mechanisms of green tea therapeutic benefit are provided by vascular, antioxidative, antithrombogenic, anti-inflammatory, and hypolipidemic effects of the flavonoids identified in the green tea extract (Stangl et al., 2006). Catechins of green tea inhibit the formation of free radicals or interrupt their chain reactions; this is considered as the basis of the mechanism of catechin protection against atherosclerosis and thrombosis. Green tea polyphenols also act as antioxidative agents due to their ability to absorb reactive oxygen species (ROS) and nitrogen, and to form chelate complexes with redox-active transition metal ions (Wang et al., 2014).

A retrospective analysis of clinical trials has shown that Hawthorn extract in CHF patients increased cardiac resistance to physical stress while its side effects were rare, mild, and short-term. The trial results support an opinion that Hawthorn extract should be used as an additional therapeutic agent for the treatment of chronic heart failure (Pittler et al., 2003). The cardioprotective effects of Hawthorn extract are likely to be related to its antiarrhythmic impacts (Garjani et al., 2000), and ability to increase coronary blood flow and cardiac output (Brixius et al., 1998). Inhibition of type III and IV phosphodiesterase (Schussler et al., 1995), stimulation of antioxidative and anti-inflammatory mechanisms contribute to the therapeutic benefit of Hawthorn extract in CHF (Bahorun et al., 2003).

It was identified that natural polyphenol curcumin has a number of pharmacological effects such as endothelioprotective, anti-inflammatory, antioxidative, and it can be considered as a potentially inexpensive, well-tolerated, and harmless therapeutic agent to support the function of endothelial cells (Santos-Parker et al., 2017; Bai et al., 2018).

Catechin exerts cardioprotection through treating many kinds of angiocardiopathy (Zhang et al., 2014). Loke et al. (2008) showed a significant increase in NO concentration in healthy men under the influence of quercetin and epicatechin (Loke et al., 2008). There is an evidence that catechins neutralize superoxide anions, prevent the reduction of NO synthesis, and reduce the effects of angiotensin II *in vitro* (Wu et al., 2009).

Oxidative stress and lipid peroxidation (LPO) play an important role in the pathogenesis of CHF. ROS can cause dysfunction and death of cardiomyocytes by destroying their membranes and membrane proteins. The damage of cell membrane bilipid layer by free radicals results in remodelling of the myocardium, which leads to a deterioration of myocardium contractile function (Gorshunova et al., 2011; Mattera et al., 2017). It was showed that ROS stimulate the cardiac fibroblast proliferation, and expression and posttranslational activation of matrix metalloproteinases which play a pivotal role in extracellular remodeling. Oxidative stress can additionally activate apoptosis and contribute to maladaptive myocardial remodeling (Tsutsui et al., 2011; Hassanpour et al., 2018). Furthermore, ROS cause damage to the vascular endothelium; this decreases nitric oxide (NO) synthesis, which results in vasoconstriction, hypercoagulation, and proliferation of smooth muscle cells.

Previous studies indicate that CHF progresses rapidly from a compensatory hypertrophic state to a state of decompensated failure when vascular growth cannot keep pace with pathological myocyte growth. In the hypertrophied heart, myocardial angiogenesis is maintained by vascular endothelial growth factor (VEGF), which is induced by hypoxia-inducible factor 1(Hif-1) in relative hypoxic conditions. However, in the advanced hypoxic condition of the failing heart, Hif1 is inhibited by p53 accumulation in the myocardium, resulting in the suppression of myocardial angiogenesis and cardiac dysfunction (Izumiya et al., 2006; Oka et al., 2014).

The herbal medicinal product obtained by us contains a rich combination of hydrophilic and lipophilic BACs among which polyphenol compounds, namely flavonoids, are of particular importance. The flavonoid constituents are represented by aglycons and glycosides as well as polymethoxylated flavonoids. Our previous experimental studies have found that PVSHE has antioxidant, antihypoxant (Latypova et al., 2011), endothelio- and angioprotective effects (Latypova et al., 2011; Iksanova et al., 2014).

It is possible that the PVSHE prevents the formation of ROS due to the flavonoids which are known to be able to suppress peroxide processes at the initial stage of the chain. They act as free radical scavengers preventing the further formation of more toxic congeners (Maietti et al., 2017).

Also, the indications are that flavonoids have the inhibitory effect on apoptosis of myocardial cells in chronic heart failure, and the

Table 6.
Effects of the studied substances on MISP in the animals with experimental CHF (M ± m).

Animal groups	MISP (M ± m).% increase of the index is provided in brackets		
	baseline	5 s	30 s
Intact + purified water (n = 10)	63.9 ± 4.6	191.2 ± 13.9 (204.9 ± 20.1)	122.2 ± 11.3 (84.1 ± 9.9)
CHF + purified water (n = 15)	34.5 ± 3.5	82.4 ± 8.6 (139.4 ± 12.4)[a]	44.9 ± 3.7 (34.7 ± 10.6)[a]
CHF + PVSHE 30 mg/kg (n = 14)	66.3 ± 19.0	168.3 ± 46.3 (159.9 ± 15.4)	115.5 ± 32.9 (74.8 ± 13.2)[b]
CHF + mildronate 50 mg/kg (n = 9)	29.1 ± 2.8	95.6 ± 5.7 (234.0 ± 30.5)[b]	58.0 ± 4.7 (102.4 ± 18.7)[b]

Note.
[a] In relation to the indices of the intact animal group, $p < 0.05$.
[b] In relation to the indices of the control group of animals with isoproterenol-induced CHF, $p < 0.05$% increase of the indices is provided in brackets.

mechanism may be closely related to the regulation of Cx43 expression (Wang et al., 2015a).

In addition, it was found that herbs with a high percentage of phenolic constituents stimulate the synthesis of VEGF and blood vessel density, and exert cardioprotection through promoting angiogenesis in the animal models of myocardial infarction (Junqing et al., 2015; Yu et al., 2017).

The above-mentioned effects of the studied herbal medicinal product are likely to be crucial for ensuring its cardioprotective benefit.

Conclusion

Therefore, the *P. veris* L. solid herbal extract contains flavonoid aglycons (apigenin, quercetine, kaempferol), flavonoid glycosides (cinarozid, rutin, hyperozid), and polymethoxylated flavonoids acting as chemotaxonomic markers for the genus *Primula* L. (8-methoxy-flavone, flavone, 3′,4′methylenedioxy-5′-methoxyflavone). The agent 3′,4′methylenedioxy-5′-methoxyflavone has been isolated from the primrose herb for the first time.

In the experimental CHF, PVSHE at a dose of 30 mg/kg exerts a cardioprotective effect which is evidenced by a smaller number of animal deaths, the lower level of CHF plasma markers, a higher increase in myocardial contraction and relaxation rates, LVP, and MISP in the test for adrenoreactivity, in the volume loading and maximum isometric loading tests as compared to the control group.

Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no financial support for this work that could have influenced its outcome.

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