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Vazorelaxant Effect Of The PC-3 And PC-2 Polifenol Compounds Depending On Their Chemical Structure

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ABSTRACT: In the present study, on the isometric conditions of the rat aortic smooth muscle the vasorelaxant effect of the two polyphenol compounds 1-O-galloyl-6-O-bisgalloyl-2,4-valoneil-β-D-glucose (PC-3) and 1-O-galloyl-2,3-gexahydroxydiphenoil-4,6-valoneil-β-D-glucose (PC-2), isolated from Euphorbia plant speacies (encrypted as PC-3 and PC-2) depending on their chemical structure was investigated. It was obtained that the vasorelaxant effects of PC-3 (1-O-galliol-6-O-bisgalliol-2,4-valoneil-β-D-glucose) and PC-2 (1-O-galloil-2,3-hexagydroxidiphenoil-4,6-valoneil-β-D-glucose) in experiments depend on their concentration and chemical structure, as well as blockade the $Ca^{2+}{}_L$ – channel in the plasmolema of smooth muscle cells.

KEYWORDS: polyphenol compounds, blood vessels, smooth muscle, phenilephrine, blockator, receptor, $Ca^{2+}{}_L$–channel, α_1–adrenoreceptor, relaxant.

1. INTRODUCTION.

In our previous works we investigated the influence of new polyphenol compounds isolated from *Euphorbia* plant species on some functional parameters of rat liver mitochondria [1, 2]. It was observed that PC-3 and PC-2 have a positive effect to some functional parameters of mitochondria. For example, these compounds inhibit the mitochondrial permeability transition pore (mPTP) opening, activates ATP-dependant potassium channel and have high antioxidant/antiradical activities. It is particularly noteworthy that these compounds activate ATP-dependant potassium channel (mitoK_{ATP}) in mitochondria membranes. It is well known that mitoK_{ATP} channels play an important role in the ischemic reperfusion (I/R) injury and cell apoptosis [3]. Also mitoK_{ATP} have shown wide-

range of therapeutic interest as potential targets in a range of cardiovascular conditions like arrhythmias, angina and heart failure. Recent years have seen an explosive growth of interest in the role of mitoK_{ATP} channel activation in the pathogenesis of cardiac dysfunction. In this regard our futher work was to investigate the vasorelaxant activity of PC-2 and PC-3 compounds isolated from *Euphorbia* plant species growing in Uzbekistan.

Cardiovascular pathologies complications (myocardial infarction, stroke...) constitute one of the most important causes of mortality and morbidity in the world [4]. These complications, often facilitated by arterial high blood pressure, appear among the main causes of death. Indeed, according to World Health Organisation (W.H.O) experts, high blood pressure and hypercholesterolemia are more frequent in the developing countries than believed. Among the risk factors, except hypercholesterolemia, obesity, smoking addict and diabetes constitute the major contributing factors of these diseases [5]. A future scenario by the W.H.O. reveals a negative trend due to an increase in the rate of morbidity and mortality especially in Emerging Countries [4]. Considering the gravity and the frequency of these conditions, a search for compounds having vascular benefits is intensively pursued [6]. The interest of researchers in the whole world for these compounds encouraged us to study the healing plants.

Nowadays, polyphenol compounds have been isolated from more than 400 plants, and the classification, synthesis/biotransformation of these polyphenols and a wide range of pharmacological activity have been studied in detail by many researchers. One of the potential sources of polyphenols are *Euphorbia* plant species. The *Euphorbia* plant species are the most common species in the mountainous region, with two or perennial grasses. In folk medicine *Euphorbia* plants are used as an antipyretic, analgesic, as well as in the treatment of gastritis, tonsillitis, anti-inflammatory, blood pressure, liver disease, malaria treatment [7,8].

Scientific staff of the Institute of bioorganic chemistry of the Academy of Sciences of the Republic of Uzbekistan have identified a number of polyphenols that have vasorelaxant effects and it was established the structure-activity relationship. Some of these, in particular, have the valoneil (S21O15N14) group into the glucose's molecule. For example, replacing the hexaghydroxidiphenoil (S14O10N10) group in carbon (2,3) into the carbon (2,3) instead of bisgalloyl (S14O9N10) in glucose carbon (6) of the molecule PC-3 increases the vasorelaxant activity [9,10].

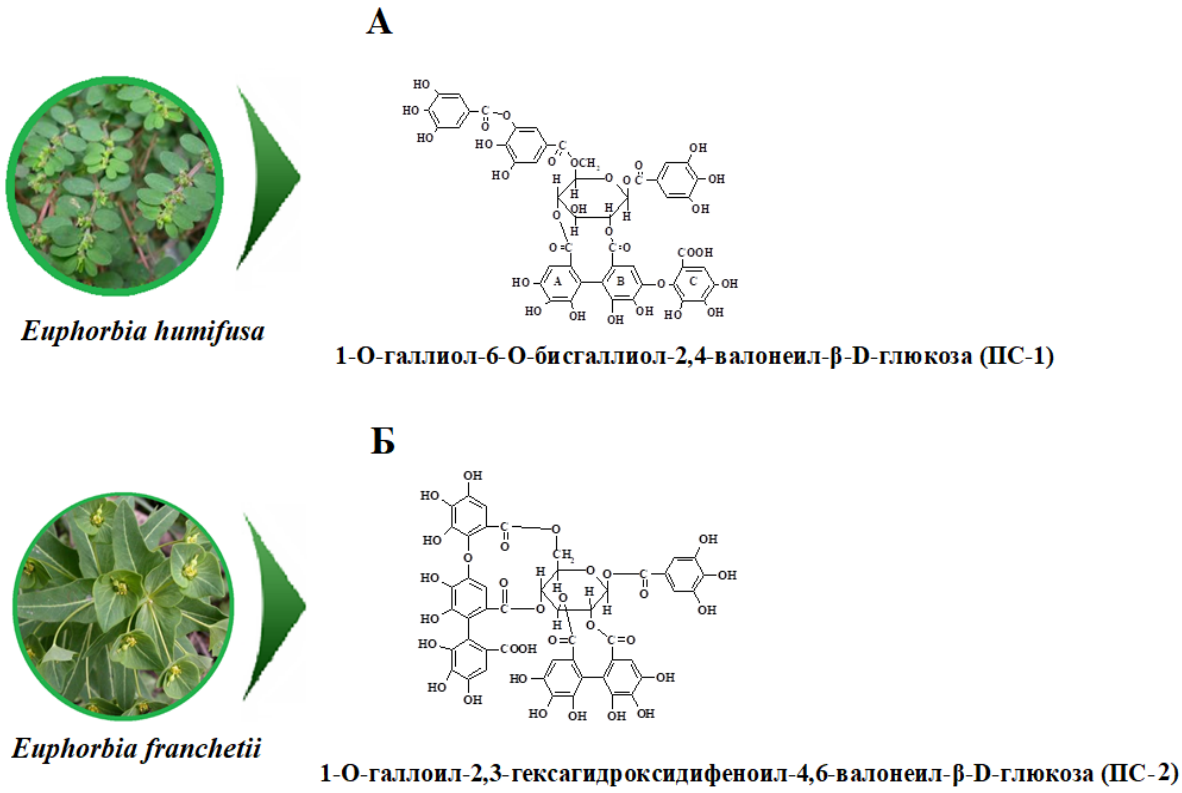


Figure-1. The chemical structure of the polyphenol compounds PC-1 (A) and PC-2 (B) isolated from *Euphorbia humifusa* and *Euphorbia franchetii* plant species.

The purpose of this study is to analyze the chemical structure-dependent vasorelaxant effect of polyphenols PC-3 and PC-2 on the isometric condition of rat aortic smooth muscle.

2. MATERIALS AND METHODS:

Aortic vascular preparation was performed using standard methods.

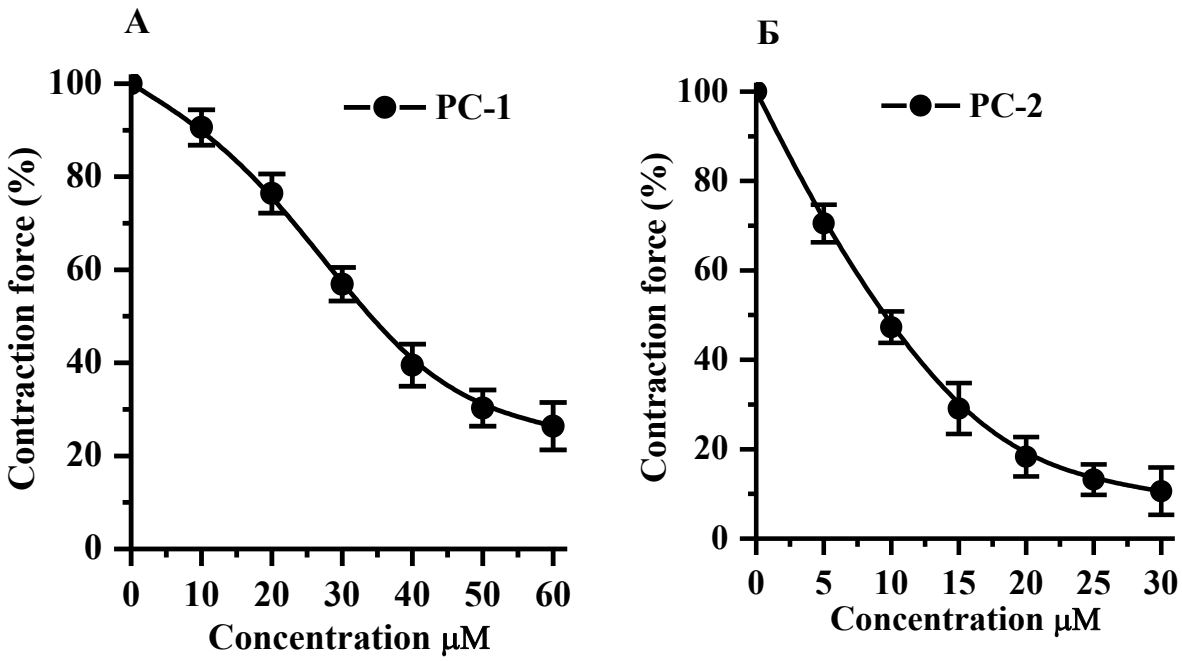
The experiments were performed on healthy white, non-breeding rats (150–200 g), fed under standard nutrient and water conditions. The experimental animals were surgically removed after cervical dislocation, and the ring segments were cut (l = 2–4 mm; ø = 1–2 mm). In the experiments the Krebs-Henseleit physiological solution with the following chemical composition (in mM) was used: (mM): NaCl - 120,4; KCl - 5; $NaHCO_3$ - 15.5; NaH_2PO_4 - 1,2; $MgCl_2$ - 1,2; $CaCl_2$ - 2.5; $C_6H_{12}O_6$ - 11.5 (pH = 7.4) [11]. The physiological solution was aerated with carbogen (O_2–95% and CO_2–5%), and the temperature constant (t=+37±0.5°C) was provided by ultrasound (U – 8; Bulgaria). The aortic vascular contraction activity was recorded under isometric conditions using the FT–03 (Grass Instrument Co., USA) power sensor, signal amplifier (Reapamil hydrochloride [12, 13] (Sigma-Aldrich, Germany), NaHCO3, CaCl2, MgSO4, glucose, NaCl, KSl, NaH2PO4 (Russia) were used in the experiments. The effect of alkaloids on the Ca2 + L-channel studied in the experiments

was evaluated by the amplitude of the contraction force induced by KCl (50 mM) [14]. The effect of alkaloids studied on experiments on reserve Ca2 +-channel (SOCC - store-operated Ca2 + -channels) / receptor-dependent Ca2 + -dependent (ROCC - receptor-operative Ca2 + -channels) amplification force induced by phenylephrine (1 μM). was evaluated by [15]. Obtained by OriginPro c. 8.5 Statistical processing using special software package SR1 (EULA, Northampton, MA 01060–4401, USA).Grass Instrument, USA) using Endem 621.02 (Czech) standard method (mechanography). Results However, G.F. Mathematical and statistical processing was performed using the methods provided by (1990) [16]. The results are presented in the form of Mm of the results of experiments performed in n times, where M represents the arithmetic mean and m represents the standard error value. Also, the results of the experiments were calculated based on the Student's t -test on statistical significance of values between groups and were estimated as statistically significant at $p < 0.05$, $p < 0.01$.
Initially, the rat aortic drug was incubated at –45–60 minutes until a normal electromechanical activity was recorded based on a voltage of 1 g (9.8 ~ 10 mN).

Statistical analyses were performed using the statistical package Origin 6 (OriginLab Corporation, USA). The data was evaluated using parametric Student's t-test, we expressed as M ± m. Deemed authentic results are expressed at * - $P<0.05$; ** - $P<0.01$; ***- $P<0.001$.

3. RESULTS.

Due the beneficial effects of polyphenols to numerous disease states, including the cardiovascular diseases, studies has been performed to determine the vasorelaxant effects of polyphenol compounds PC-3 and PC-2 isolated from *Euphorbia* plants. Obtained data revealed that polyphenol compounds PC-3 and PC-2 have a significant vasorelaxant effect on isometric contraction activity (*in vitro*) of the rat aorta by pretreatment of the 50 mM KCl. Specifically, it was found that PC-3 at the minimum concentration of 10 μM reduces the compressive strength by 9.4 ± 3.8% and at a maximum concentration of 60 μM (n = 4–6) to 70.6 ± 5.1% compared to the control. It was also observed that the PC-2 at a minimum concentration of 5 μM reduces the compressive strength by 29.5 ± 4.9% and at the maximum concentration30 μM to 89.4 ± 5.3% compared to the control. The (EC_{50}) values for PC-3 and PC-2 were 33.9 μM and 9.6 μM, respectively (Figure 2 A and B).



2-расм. Concentration dependent vasorelaxant effect of PC-3 (A) and PC-2 (B) polyphenols on aorta preparation contraction induced by KCl (50 мМ). Y-axis – contraction force induced by 50 мМ KCl (100%). X-axis – concentration of polyphenol compound мкМ. (* – $p < 0,05$; ** $p < 0,01$; n=4–6).

It is well known that L-type calcium channels are responsible for the excitation-contraction coupling of cardiac muscles. That's why our future investigation was to study the role of voltage-dependent L-type Ca^{2+} channels in the relaxant effect of PC-3 and PC-2 polyphenols. Their effects on the contraction of aortic preparation induced by cumulative addition of $CaCl_2$ to a calcium-free environment with 50 mM KCl was investigated. In these experiments, increasing the concentration of $CaCl_2$ (0–2.5 mM) in the incubation medium resulted in a gradual increase of the aortic contraction strength due to the entry of Ca^{2+} ions through the L-type Ca^{2+} channels (Figure 3 A). The presence of the studied polyphenols in the incubation medium significantly reduced the development of contraction forces in response to increased $CaCl_2$ (Figure 3 A). These results indicate that the relaxant effect of studied polyphenols depend on the reducing of Ca^{2+} ions permeability through L-type Ca^{2+}-channels and may be associated with decreasing of $[Ca^{2+}]_{in}$ in the cell.

To clarify the participation of the L-type Ca^{2+} channels in the relaxant effects of PC-3 and PC-2 polyphenols in the experiments the specific blocker of this channel verapamil was

used. It was observed that in the presence of verapamil EC_{50} = 0.1 μM, the relaxant effect of polyphenols was decreased. In this conditions, the concentrations of PC-3 (EC_{50} = 33.9 μM) and PC-2 (EC_{50} = 9.6 μM) were additionally reduced to 5.1 ± 3.3% (55.1 ± 4.3 compared to the control) and 9.7 ± 3.8% (59.7 ± 5.4% compared to the control), respectivly (Figure 3 B).

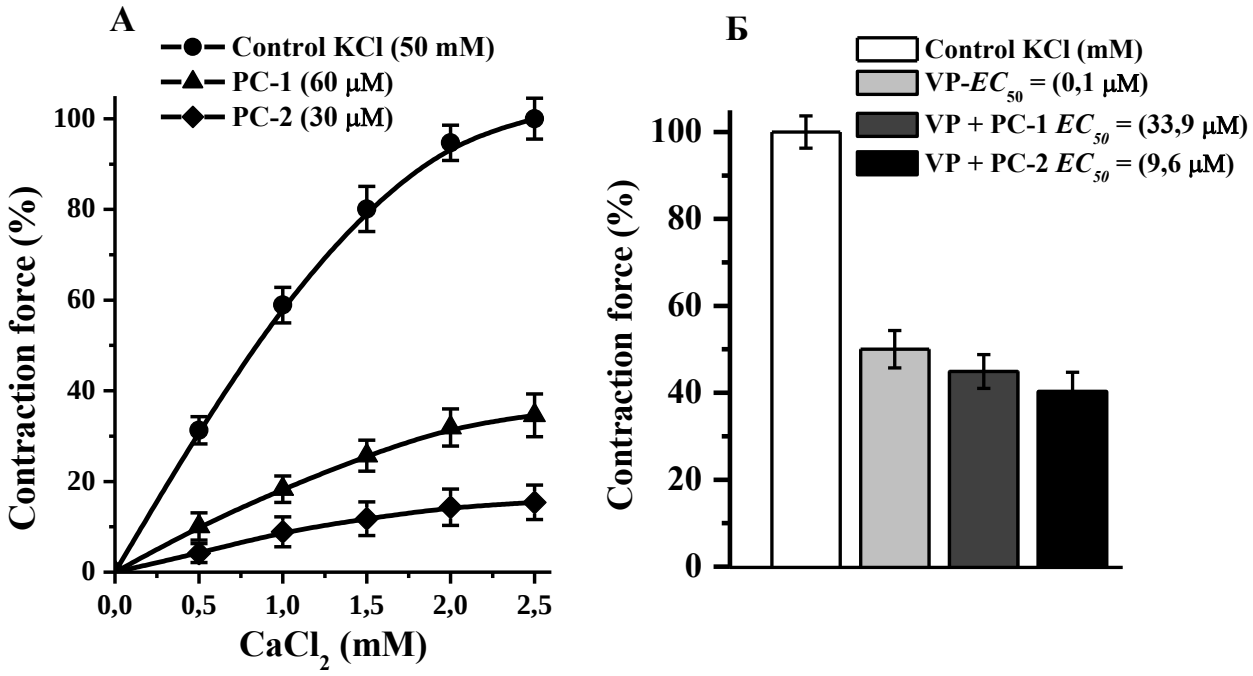


Figure-3. Dependence of the relaxant effect of PC-3 and PC-2 polyphenols on the $[Ca^{2+}]_o$ concentration and the state of voltage-dependent L-type Ca^{2+}-channels. A – the effect of PC-3 and PC-2 polyphenols on rat aortic contraction pretreatment by cumulative addition of $CaCl_2$ to A-Krebs solutions.Y axis – 100 % of contraction pretreatrment by 50 мМ KCl. X axis – the concentration of $CaCl_2$. Б – the relaxant effect of PC-3 and PC-2 on muscule contraction pretreatment by 50 мМ KCl under existing of verapamile амил (0,1 мкМ) (* – p<0,05; **p<0,01; n=4–6).

Based on the results, we can conclude that the relaxant effect of PC-3 and PC-2 polyphenols is due to blockade of L-type Ca^{2+} channels. However, manifestation of the minor vasorelaxant effect of PC-3 and PC-2 in the presence of verapamil in incubation medium, along with the blockade of L-type Ca^{2+} channels, other mechanisms may be involved in the reduction of Ca^{2+} ions transport in smooth muscle cells.

In addition besides voltage-dependent Ca^{2+}-channels, receptor-controlled Ca^{2+} channels play an important role in regulating the Ca^{2+} homeostasis in smooth muscle cells [17]. In order to evaluate the effect of polyphenols on receptor-controlled Ca^{2+} channels in

subsequent experiments, aortic muscle contraction induced by phenylphrine (FE), which is α1-adrenorceptor agonist, was investigated.

In our experiments, the effect of polyphenols on the aortic contraction strength induced by FE under the L-type Ca^{2+}-channel blocker-verapamil was studied. In these conditions, the muscle contraction induced by FE was 13.7 ± 2.8% lower than non-verapamil conditions. Under these conditions, polyphenols PC-3 (40 μM) and PC-2 (25 μM) reduced the aortic contraction induced by FE to a maximum of 89.4 ± 5.4% and 83.3 ± 4.9%, respectively (Fig. 4. A and B).

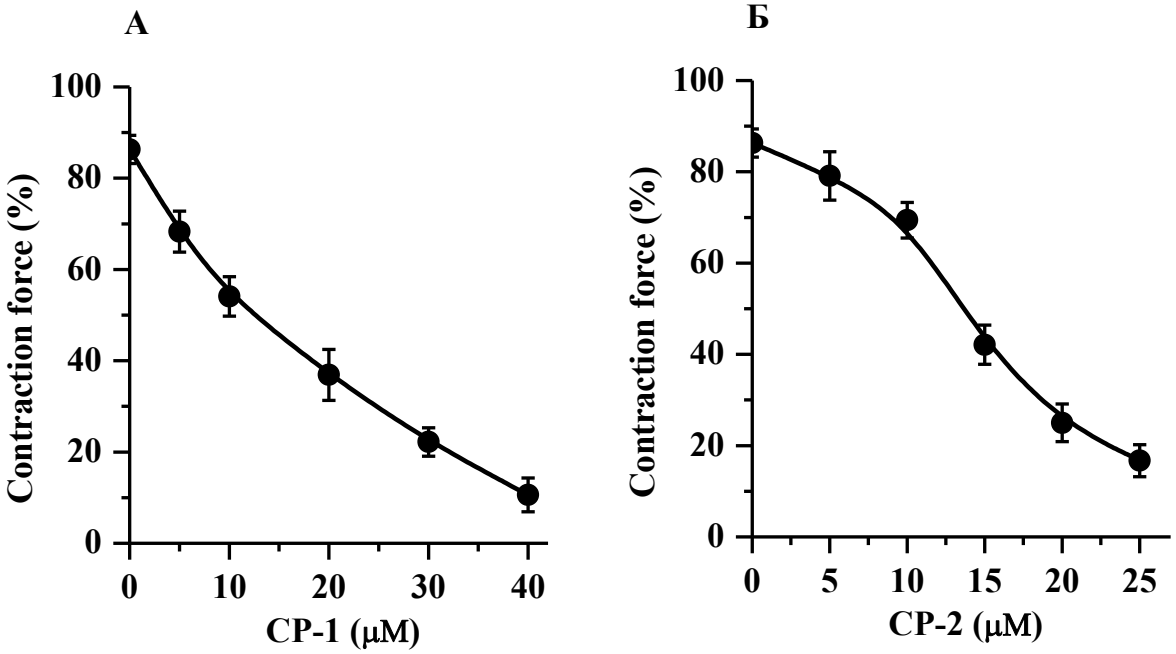


Figure-4. Influence of PC-3 and PC-2 to the rat aorta contraction induced by phenilephrine. Y axis – 100 % aorta contraction force pretreatment by 1 мкМ ФЭ. X axis – concentration of polyphenols. (* – $p<0,05$; ** $p<0,01$; $n=4–6$).

In these experiments, the EC_{50} values of PC-3 and PC-2 polyphenols were 12.6 μM and 13.5 μM, respectively. The results show that the observed effects of polyphenols can be attributed to the blockade of receptor-regulated Ca^{2+}-channels. Additional evidence of the effects of studied polyphenols on receptor-regulated Ca^{2+}-channels can be explained by experiments with α-adrenoreceptor blocker-phentolamine (FA). In our experiments, the relaxant effect of PC-3 (40 μM) and PC-2 (25 μM) polyphenols in the presence of 10 μM of phentolamine showed a reduction of FE by 48.9 ± 5.7 and 43.2 ± 4.1 compared to phentolamine-free conditions (Fig. 5 B).

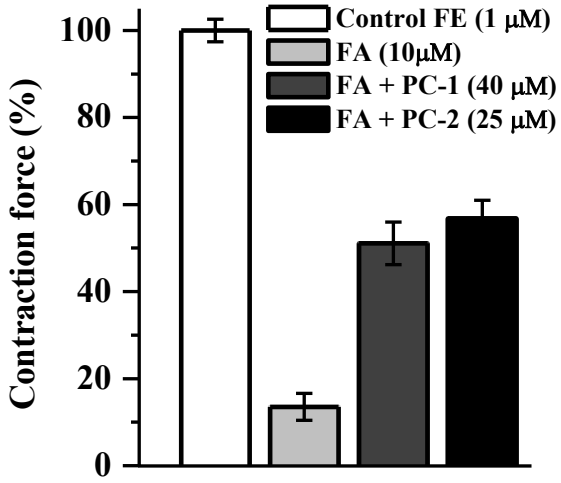


Figure-5. Influence of phentoamine (10 μM) to the relaxant effect of PC-3 and PC-2. Y axis – 100 % aorta contraction force pretreatment by 1 мкМ ФЭ. (* – p<0,05; **p<0,01; n=4–6).

These results indicate that the relaxant effect of the studied polyphenols may be related to the blockade of receptor-controlled Ca^{2+}-channels.

Data from the present study confirm that polyphenols PC-3 and PC-2 isolated from *Euphorbia* plant species can induce a relaxation in rat aortic rings. The vasorelaxation induced by polyphenols is now well documented. The magnitude of the relaxation obtained and the role played by endothelium vary among the substances which have been tested so far. Polyphenolic compounds isolated from *Euphorbia* plant species display a relatively large relaxation which mainly relies on the presence of endothelium and NO release.

It is known that the biological activity of polyphenolic compounds largely depends on their chemical structure, on the number of hydroxyl groups in the benzene ring, their position, degree of screening, and also on the nature of other substituents that affect the electron density of the benzene ring [18]. The literature also cites data that the location of hydroxyl groups in the benzene ring has a greater role in the manifestation of biological activity than their number [19]. The presence of several hydroxyl groups in the benzene ring, especially in the ortho- and para- positions to each other, is especially sensitive to the action of oxidizing agents. Such phenols are extremely easy to oxidize and are good reducing agents. For example, hydroxyl groups in the ortho- position undergo intramolecular repulsion; therefore, their AOA increases compared to isomers in which the hydroxyl groups are in the meta-position.

However, despite the great interest in polyphenols, there are few publications on the

effect of the structure of their molecules on the cardioprotective activity. Nevertheless, given that these compounds have 16 hydroxyl groups in their molecule, PC-2 exhibits a more pronounced vasorelaxant activity than PC-3. Perhaps this is due to the presence of a hexahydroxydiphenyl group in the PC-2 molecule. We previously described that a more pronounced antioxidant activity was observed in the case of PC-2 than PC-3. Perhaps this is due to the large number and location of hydroxyl groups in their molecule, which can serve as electron-donor functional groups of the molecules as well as the presence of hexahydroxydiphenyl functional group.

We described above that the study of the vasorelaxant effect of these compounds was based on the fact that they activate the ATP-dependent potassium channel, which plays an important role in cardioprotection. The correlation coefficient between the activation of the ATP-dependent potassium channel and the vasorelaxant effect of PC-2 is $r^2 = 0.78$.

4. CONCLUSIONS

Polyphenols - PC-3 and PC-2 have a relaxant effect and effectively relieve hypercalcium solution and phenylephrine-induced rat aortic contraction. It is obtained that PC-2 has more pronounced vasorelaxant effect.

Based on the analysis of the literature and experimental results, vasorelaxant effect of the polyphenol compounds of PC-3 and PC-2 on the isometric contraction of the rat aortic vasculature *in vitro* conditions may be mainly related to the receptor-regulated Ca^{2+} channel blockade.

The scientific/experimental results could be used as a theoretical basis for the development of antiperentative pharmacological drugs based on polyphenols.

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