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Inotropic and lusitropic effects of Perilla frutescens (L.) Britton extract on the rabbit myocardium

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Key words: Perilla frutescens, myocardial contractility, inotropic effect, lusitropic effect.

Summary. Background. Common perilla (Perilla frutescens (L.) Britton) is a plant cultivated in many countries around the world. Although its immunomodulating and antioxidative properties are well known, there is a lack of data about the cardiotropic activity of the plant.

The objective of this study was to determine the influence of Perilla frutescens extract on the myocardial contractility in vitro and as a food supplement in vivo.

Material and methods. Rabbits of the experimental group were fed with a supplement of 100 mg/kg of Perilla frutescens extract for 14 days. Rabbits of control group were fed with ordinary food. The maximal mechanical activity of isolated myocardial preparations, obtained from the rabbits of both groups, was tested during the perfusion with Ringer’s solution containing 5 μM of adrenaline and 4.5 mM of CaCl₂. For the assessment of the direct influence of Perilla frutescens extract on the myocardial contractility in vitro isolated heart preparations were perfused with 0.01, 0.1, and 1.0 mg/ml of Perilla frutescens extract.

Results. The maximum force of isometric contraction, maximum velocity of force development, and maximum velocity of relaxation were higher among the atrial and ventricular preparations from the experimental group, as compared with the control group. Perfusion of the myocardial preparations with different concentrations of Perilla frutescens extract revealed slight dose-dependent increase in the parameters of contraction and relaxation.

Conclusions. The consumption of Perilla frutescens extract as a food supplement leads to an increase in the contractility of the rabbit myocardium. Perilla frutescens extract in vitro had a dose-dependent positive inotropic and lusitropic effect on the rabbit myocardium.

Introduction

Common perilla, Perilla frutescens (L.) Britton, plant of the Laminaceae family native to South-East Asia, which is successfully cultivated all over the world, is becoming very promising herb in Lithuania. Its well-known antimicrobial, antiallergic, antitumor, and antioxidative properties are used extensively and constantly investigated (1–4). In spite of widespread occurrence there are only few references regarding the influence of P. frutescens preparations on cardiovascular system. Some authors describe cytoprotective effects, using cardiotoxic drugs, which are related to the antioxidative properties of the substances isolated from P. frutescens (5, 6). O. Ezaki et al. report long-term influence of perilla oil-containing diet on the cardiovascular system, which is related to the increase of serum omega-3 polyunsaturated fatty acids (7).

Although the recent investigations refer to the vasoactive properties of certain flavonoids (8, 9), influence of P. frutescens preparations on contractile function of myocardium still remains undiscovered.

The aim of the present study was to determine the influence of P. frutescens extract on the mechanical properties of the contractile myocardium in vitro and effect of P. frutescens extract used as a food supplement on contractility of the myocardium.

Material and methods

Frozen-dried P. frutescens extract was produced by Department of Food Technology of Kaunas University of Technology.

Chinchilla rabbits of both sexes (3–3.5 kg, 6–10

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months old) were divided into two groups: control and experimental. Rabbits of the control group were fed with ordinary food. Rabbits of the experimental group were fed with an addition of 100 mg/kg of P. frutescens extract during 14 days before the experiments.

The experiments were carried out in vitro on the isolated preparations of rabbits left atria and right ventricle. Animals were killed in accordance with ethical guidelines approved by Lithuanian State Food and Veterinary Service (License No. 0112). The chest was opened; the heart promptly excised and immersed in a modified Ringer’s solution consisting of 139.3 mM NaCl, 3.5 mM KCl, 0.58 mM NaH₂PO₄, 2.1 mM Na₂HPO₄, 2.5 mM CaCl₂, 1.3 mM MgCl₂, 11.1 mM glucose; pH was adjusted to 7.2–7.4 with NaHCO₃.

The preparation ready for experiment, stripe of the auricle from the left atria or papillary muscle from the right ventricle, was transferred into the organ bath perfused with the oxygenated Ringer’s solution. The bath temperature was maintained at 25±0.5°C by means of thermostatically controlled water jacket.

Mechanical activity of the preparations was registered by micromechanographic device “Mioton-CAO12” under isometric conditions. Preparation was stimulated by suprathreshold rectangular electrical pulses of 0.3 Hz, 20–40 V, 3–10 ms, delivered by a pair of Ag/AgCl electrodes placed inside the organ bath. Electrodes were arranged along the papillary muscle or stripe of the atria in order to provide simultaneous excitation of the entire preparation.

The preparation was stretched to an optimal length, until the amplitude of isometric contraction reached its maximum. Before starting the experimental procedures, the preparation was allowed to stabilize for about 1.5 hours. The preparation was considered to be well adapted when its force amplitude remained unchanged.

To determine the possible differences in the mechanical properties of myocardium obtained from experimental and control groups of rabbits, preparations were superfused with Ringer’s solution containing 5 µM of adrenaline and 4.5 mM of CaCl₂. Previous studies have shown that in general the combination of adrenaline and calcium chloride is optimal in order to reach the state of “maximum contraction” comparing the contractility of the myocardium preparations obtained from the different groups of rabbits (10).

The direct influence of P. frutescens extract on the mechanical properties of contractile myocardium was also examined. Preparations from the part of rabbits of control group were perfused with the Ringer’s solution containing three different concentrations of extract: 0.01 mg/ml, 0.1 mg/ml, and 1.0 mg/ml.

In all cases mechanical activity of preparations was registered during the sixth minute after the change of the solution in the organ bath.

Data were recorded by the polygraph and stored in a computer for off-line processing using the original software. To evaluate mechanical activity of preparations, the following parameters were measured:
- maximum force of isometric contraction, Pₒ;
- maximum velocity of force development, +dP/dt;
- maximum velocity of relaxation, −dP/dt.

Data are reported as mean±SEM. Each parameter is expressed in percent, comparing to its baseline value. Data obtained during investigation of the direct influence of P. frutescens extract on the mechanical properties of contractile myocardium are also expressed in absolute values (mN) and standardized per square unit of section of the preparation (mm²).

The Student’s t-test was used to determine differences between the means. Results were considered to be statistically different when p<0.05.

Results
All parameters of mechanical activity registered under the influence of 5 µM of adrenaline with higher concentration of CaCl₂ on the myocardial preparations obtained from the rabbits of experimental group were increased, as compared with the preparations obtained from the control group. Maximum force of isometric contraction and maximum velocity of force development were significantly higher testing the preparations of left atria (p<0.01) and right ventricle (p<0.05) from the rabbits of experimental group, than the same parameters of preparations obtained from the control group (Fig. 1). Maximum velocity of relaxation was also increased among the preparations of left atria from the experimental group (p<0.001), while the increase in this parameter during the test of the right ventricle from the experimental group was not statistically significant (Fig. 1).

Superfusion of myocardial preparations with different concentrations of P. frutescens extract enhanced their mechanical activity even when lowest concentration of extract (0.01 mg/ml) was used. At this concentration the increase of maximum force of isometric contraction and maximum velocity of force development was statistically significant (p<0.05) on the preparations of right ventricle (Fig. 2B, Table), in contrast to the preparations of left atria (Fig. 2A, Table). Higher concentrations of extract (0.1 and 1.0 mg/ml) caused further increase of these parameters of both left atria and right ventricle.
Fig. 1. Parameters of isometric contraction registered on the preparations of left atria (A) and right ventricle (B) from the rabbits of experimental and control group during the perfusion with Ringer’s solution containing 5 μM of adrenaline and 4.5 mM of CaCl₂.

A

$P_0$ – maximum force of isometric contraction; $+dP/dt$ – maximum velocity of force development; $-dP/dt$ – maximum velocity of relaxation. * – $p<0.05$; ** – $p<0.01$; *** – $p<0.001$ as compared to control group.

B

A statistically significant increase ($p<0.05$) in maximum velocity of relaxation of the preparation from right ventricle was observed only at concentrations of extract, 0.1 and 1.0 mg/ml (Fig. 2B, Table), whereas none of the concentrations could cause a statistically significant increase in the maximum velocity of relaxation among the preparations of left atria (Fig. 2A, Table).
**Fig. 2.** Effect of different concentrations of *Perilla frutescens* extract on the parameters of isometric contraction of the preparations from left atria (A) and right ventricle (B) of the rabbits

- $P_0$: maximum force of isometric contraction; $+dP/dt$: maximum velocity of force development; $-dP/dt$: maximum velocity of relaxation; PFE – *Perilla frutescens* extract. Baseline parameters were registered during the perfusion with Ringer’s solution. * – $p<0.05$; ** – $p<0.01$ as compared to baseline.

**Discussion**

In the present study we investigated the influence of *P. frutescens* extract on the mechanical properties of mammalian myocardium. The preparations from the left atria and right ventricle of the rabbits, which were fed with an addition of *P. frutescens* extract during two weeks before the experiment, demonstrated an increase in the maximum mechanical response...
Table. Effect of different concentrations of *Perilla frutescens* extract on the parameters of isometric contraction of the preparations from left atria and right ventricle of the rabbits (absolute values)

<table>
<thead>
<tr>
<th>Concentration of PFE</th>
<th>Left atria (n=5)</th>
<th>Right ventricle (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P₀ mN/mm²</td>
<td>+dP/dt mN/s/mm²</td>
</tr>
<tr>
<td>Baseline</td>
<td>8.15±1.46</td>
<td>92.61±14.59</td>
</tr>
<tr>
<td>0.01 mg/ml</td>
<td>9.01±1.15</td>
<td>102.31±16.24</td>
</tr>
<tr>
<td>0.1 mg/ml</td>
<td>9.14±1.30</td>
<td>108.64±16.19*</td>
</tr>
<tr>
<td>1.0 mg/ml</td>
<td>10.30±1.48**</td>
<td>122.42±22.06*</td>
</tr>
</tbody>
</table>

P₀ – maximum force of isometric contraction; +dP/dt – maximum velocity of force development; −dP/dt – maximum velocity of relaxation; PFE – *Perilla frutescens* extract. Baseline parameters were registered during the perfusion with Ringer’s solution.

* – p<0.05; ** – p<0.01 as compared to baseline.

during the perfusion with Ringer’s solution containing 5 μM of adrenaline and 4.5 mM of CaCl₂, as compared with the preparations obtained from the control group. These data conform to the results obtained in another part of the study, where perfusion of the preparations with the Ringer’s solution, containing different concentrations of *P. frutescens* extract, revealed dose-dependent positive inotropic and positive lusitropic effects on the myocardium of left atria and right ventricle of the rabbits. Such effects can probably be explained by the presence of certain substances in the extract which affect several aspects of calcium metabolism in the cardiomyocyte: 1) increase of inward calcium current through the L-type calcium membrane channels; and 2) accelerate an uptake of calcium into the sarcoplasmatic reticulum.

*P. frutescens* leaves and fruits are known to be rich of flavonoids and polyphenolics. Among them, catechin, ferulic acid, apigenin, luteolin, rosmarinic acid, and caffeic acid are mentioned as the most important active ingredients of the plant (11). Although polyphenolics, rosmarinic, caffeic acids and their derivatives are intensively tested due to their cytoprotective antioxidative properties using cardiotoxic drugs (6, 12) or during ischemia-reperfusion injury (13–15), there are no references regarding their direct influence on the myocardial contractility. Some flavonoids, such as ferulic acid, apigenin, luteolin, besides their antioxidative characteristics also possess vasorelaxing properties (8, 9, 16, 17). In addition, as it is reported by M. Itoigawa et al., apigenin and luteolin produced a moderate positive inotropic effect on guinea pig papillary muscle, while catechins did not produce any positive inotropic effect. Authors suppose the presence of cyclic AMP-dependent mechanism for the positive inotropic effect of flavonoids (18). During another study, luteolin derivative, luteolin-7-glucoside, was tested on isolated Langendorff-perfused guinea pig heart. Observed slight positive inotropic and positive lusitropic effects were explained by the possible inhibition of phosphodiesterase (19).

Thus, on the supposition that *P. frutescens* contains substances which increase the amount of cyclic AMP and/or inhibit cyclic AMP splitting enzyme in cardiomyocytes, it is possible to explain contractile effects of *P. frutescens* extract. Cyclic AMP via protein kinase A increases the entry of calcium ions through the L-type calcium channels and enhances calcium-induced calcium release from sarcoplasmatic reticulum. As a consequence, peak force and velocity of contraction increase (positive inotropic effect). Due to phosphorylation of phospholamban the rate of uptake of calcium into the sarcoplasmatic reticulum rises, causing an increase in velocity of relaxation (positive lusitropic effect).

Although some flavonoids seem to be able to affect contractility of the myocardium, it is important to emphasize that since *P. frutescens* extract is a mixture of several substances, it cannot be excluded that we are observing the result of several simultaneous influences. That is why additional studies of composition of *P. frutescens* extract and its influence on the myocardium are required.

Conclusions
The consumption of *Perilla frutescens* extract as a food supplement leads to an increase in the contractility of the rabbit myocardium. *Perilla frutescens* extract in vitro has a dose-dependent positive inotropic and positive lusitropic effects on the rabbit myocardium.

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Κρυμίνης περίλε (Perilla frutescens (L.) Britton) εκστρακτό inotropinis ir luzitropinis poveikis triušių miokardu

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Raktažodžiai: krūminė perilė, miokardo kontraktiliškumas, inotropinis poveikis, luzitropinis poveikis.


Tyrimo tikslas. Nustatyti Perilla frutescens ekstrakto poveikį miokardo kontraktiliškumui in vitro ir jo kaip maisto papildo vartojimą in vivo.

Medžiaga ir metodai. Eksperimentinės grupės triušių prieš tyrimą kasdien (14 dienų) kartu su maistu gaudavo 100 mg/kg Perilla frutescens ekstrako. Kontrolės grupės triušių buvo maitinami įprastu maistu. Po 14 dienų atlikti abiejų grupių triušių izoliuotų miokardo preparatų maksimalaus mechanizmo aktyvumo tyrimai perfuzuojant juos Ringerio tirpalu, turinčiu 5 µM adrenalinio ir 4,5 mM CaCl₂. Tiriant tiesioginį Perilla frutescens ekstrakto poveikį in vitro miokardo preparatai buvo perfuzuojami Ringerio tirpalu su trimis skirtingomis krūminės perilės ekstrako koncentracijomis: 0,01 mg/ml; 0,1 mg/ml ir 1,0 mg/ml.

Rezultatai. Eksperimentinės grupės triušių kairiojo priesūriūdio ir dešiniojo skilvelio preparatų maksimali izOMETRINIO susitraukimo įėja, maksimalus izOMETRINIO susitraukimo greitis ir maksimalus izOMETRINIO atsipalaidavimo greitis buvo didesnis nei kontrolės grupės triušių. Perfuozuojant skirtingomis Perilla frutescens ekstrako koncentracijomis, nustatytas priklausomos miokardo preparatų susitraukimo ir atsipalaidavimo rodmenų padidėjimas, priklausomas nuo dozės.


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