

EFFECTS OF PROVINOLS ON CARDIODYNAMICS AND CORONARY FLOW IN ISOLATED RAT HEARTS

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EFEKTI PROVINOLA NA KARDIODINAMIKU I KORONARNI PROTOK IZOLOVANIH SRCA PACOVA

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ABSTRACT

Provinols are an alcohol-free extract of red wine that contains a wide range of polyphenols. Polyphenols are a group of chemical compounds found in diverse plants. Polyphenols are considered to protect against cardiovascular disease. Although some older epidemiological studies have indicated that the positive effects of red wine on heart disease can be attributed to the alcohol content alone, there is now powerful evidence that polyphenols present in red wine are responsible for these positive effects. The hearts of male Wistar albino rats ($n = 36$, 12 in each experimental group, 10 weeks old, body mass 250 ± 30 g) were excised and retrogradely perfused according to the Langendorff technique at a gradually increasing perfusion pressure (40–120 cmH_2O). Parameters of cardiac function (dp/dt max, dp/dt min, SLVP, DLVP, HR, CF) were measured after perfusion with three different concentrations of provinols (5 $\mu\text{g/ml}$, 10 $\mu\text{g/ml}$ and 50 $\mu\text{g/ml}$). Administration of the highest dose (50 $\mu\text{g/ml}$) induced a significant increase in dp/dt max, dp/dt min, HR and CF compared with control conditions at CPP = 40 cmH_2O , while an intermediate dose increased dp/dt max at the same CPP. Generally viewed, the results of the present study suggest that provinols may have a beneficial effect on the intact myocardium and coronary circulation. These findings could constitute an important step in further investigation of these polyphenols under different representative experimental conditions in the heart, as well as providing a good basis for potential clinical studies in this field.

Keywords: Provinols, Isolated rat heart, Langendorff technique, Cardiodynamics, Coronary flow

SAŽETAK

Provinoli predstavljaju bezalkoholni ekstrakt crvenog vina koji sadrži širok spektar polifenola. Polifenoli su grupa hemijskih jedinjenja koje se nalaze u različitim vrstama biljaka. Smatra se da mogu da umanje rizik od nastanka kardiovaskularnih bolesti. Iako su ranije epidemioške studije ukazale da pozitivni efekti crvenog vina na razvoj oboljenja srca potiču samo od alkoholne komponente, sada postoje čvrsta saznanja da su zapravo polifenoli zaslužni za ove efekte. Srca muških Wistar albino pacova ($n = 36$, 12 u svakoj grupi, 10 nedelja starosti, telesne mase 250 ± 30 g) su izolovana i retrogradno perfundovana prema modifikovanoj Langendorff-ovoj tehnici, uz postupno povećanje koronarnog perfuzionog pritiska (40–120 cmH_2O). Parametri srčane funkcije (dp/dt max, dp/dt min, SLVP, DLVP, HR, CF) su registrovani tokom perfuzije sa tri različite doze Provinola (5 $\mu\text{g/ml}$, 10 $\mu\text{g/ml}$ i 50 $\mu\text{g/ml}$). Administracija najviše doze (50 $\mu\text{g/ml}$) je uzrokovala značajno povećanje vrednosti dp/dt max, dp/dt min, HR i CF u poređenju sa kontrolnim uslovima (pri CPP = 40 cmH_2O), dok je srednja doza povećala samo vrednosti dp/dt max na istom CPP. Generalno posmatrano, rezultati sadašnje studije sugerišu da Provinoli mogu da imaju pozitivne uticaje i na zdrav miokard i koronarnu cirkulaciju. Ova saznanja mogu da budu važan korak u daljem ispitivanju ovih polifenola u različitim eksperimentalnim uslovima na srcu, kao i dobra osnova za potencijalne kliničke studije iz ove oblasti.

Ključne reči: Provinoli, Izolovano srce pacova, Langendorff-ova tehnika, Kardiodinamika, Koronarni protok

ABBREVIATIONS

CF - Coronary flow LDL - Low density lipoprotein
CPP - Coronary perfusion pressure NO - Nitric oxide
FDRW - Freeze-dried red wine VGSCs - Voltage-gated sodium channels



INTRODUCTION

Provinols are an alcohol-free extract of red wine that contains wide range of polyphenols (1). These extracts have been studied in association with the discovery of the “French Paradox”, describing a low incidence of cardiovascular disease in French people despite a diet rich in saturated fats. Researchers believe that this may be due to high consumption of wine in this country (2). Although some older epidemiological studies have indicated that the positive effects of red wine on heart disease can be attributed to the alcohol content alone (3, 4), there is powerful evidence that polyphenols present in red wine are responsible for these positive effects (5-7).

Polyphenols are a group of chemical compounds found in a vast variety of plants (8). Polyphenols are considered to protect against cardiovascular disease (9, 10) and some cancers (11). Additionally, a large number of studies have demonstrated strong antioxidant properties and inhibition of the peroxidation of polyunsaturated fatty acids (12). These properties of polyphenols can be explained by low density lipoprotein and platelet aggregation (13). Previous studies on red wine polyphenols have shown positive effects on the oxidation of LDL-cholesterol (14), arterial hypertension (13) and vasorelaxation (15). Based on these properties, diets supplemented with foods containing polyphenols might also protect various tissues against heart injury.

Reports describing the oral administration of red wine polyphenolic compounds, including provinols, indicate their ability to decrease blood pressure in normotensive rats (16). In addition, an accelerated decrease in blood pressure and improvement of structural and functional cardiovascular characteristics occur as a consequence of chronic inhibition of nitric oxide (NO) synthesis (17). All of these effects of provinols are associated with a greater increase in NO synthase (NOS) activity in the left ventricle and aorta (17, 18). Because of the above properties, polyphenols may interfere with the atherogenesis process and/or the thrombotic phenomena associated with atherosclerosis, which could at least partially explain the beneficial effects of these substances.

On the other hand, there is a lack of data on the direct effects of red wine polyphenols on myocardial function, especially on coronary circulation in the intact heart. Thus, this study aimed to assess the direct and acute influence of provinols on cardiac function and coronary flow, using an isolated rat heart model.

MATERIALS AND METHODS

Isolated heart preparation

Hearts of male Wistar albino rats ($n = 36$, 12 in each experimental group, 10 weeks old, body mass 250 ± 30 g) were isolated and perfused via retrograde perfusion using the Langendorff technique (Langendorff apparatus, Experimetria Ltd, 1062 Budapest, Hungary). After brief ketamine/xylazine narcosis, the animals were euthanized via cervical dislocation

(Schedule 1 of the Animals/Scientific procedures, Act 1986, United Kingdom) and premedicated with heparin. This was followed by immediate thoracotomy and sudden cardiac arrest induced by superfusion with ice-cold isotonic saline. The hearts were rapidly excised, and the aortas were cannulated and retrogradely perfused at a pressure in the range of 40 to 120 cmH₂O. They were subsequently perfused in a reverse fashion via the aorta with Krebs–Henseleit solution (nutrient-rich, oxygenated solution). The composition of the noncirculating Krebs-Henseleit perfusate was as follows (mM/L): NaCl 118, KCl 4.7, CaCl₂·2H₂O 2.5, MgSO₄·7H₂O 1.7, NaHCO₃ 25, KH₂PO₄ 1.2, and glucose 11, equilibrated with 95% O₂ plus 5% CO₂ and warmed to 37 °C (pH 7.4).

All experimental procedures were performed in accordance with prescribed legislation (EU Directive for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes 86/609/EES) and the principles of ethics.

Physiological Assay and Experimental Protocol

After successful heart perfusion at a CPP of 60 cmH₂O, a 30 min period was allowed for stabilisation of the preparation. To test coronary vascular reactivity, all hearts were challenged via short-term occlusion (5–30 s), followed by a bolus injection of 5 mM/L adenosine (60 µL at a flow rate of 10 mL/min to elicit maximum coronary flow (CF)) during the stabilisation period. The hearts were discarded if the flow did not increase by 100% over the control value for both tests (approximately 25% of hearts). When the flow was considered stable (three measurements of the same values), coronary effluent samples were collected.

After control sets of experiments (control conditions), hearts were perfused with

- (1) provinols at a dose 5 µg/ml
- (2) provinols at a dose 10 µg/ml
- (3) provinols at a dose 50 µg/ml.

After establishing a stable heart rate, removal of the left atrium and rupture of the mitral valve allowed a sensor to be inserted (transducer BS4 73-0184, Experimetria Ltd., Budapest, Hungary) in the left ventricle for direct and continuous monitoring of the following parameters of left ventricular function:

1. dp/dt max - maximum rate of pressure development in the left ventricle,
2. dp/dt min - minimum rate of pressure development in the left ventricle,
3. SLVP - systolic pressure of the left ventricle,
4. DLVP - diastolic pressure of the left ventricle,
5. HR - heart rate.

Coronary flow was measured using flowmetry. The substances tested within a series of acute experiments were administered via continuous perfusion under changing perfusion pressures, starting from a pressure of 60 cmH₂O, followed by 80 cmH₂O, 100 cmH₂O, and 120 cmH₂O, and finally, 40 cmH₂O at end of the experiment. For each perfusion pressure, functional parameters of the left ventricle were registered.

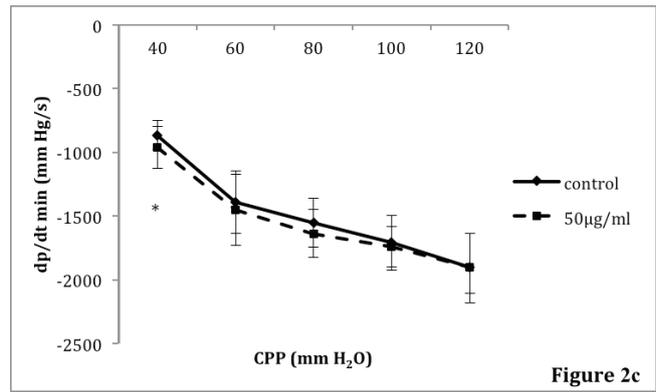
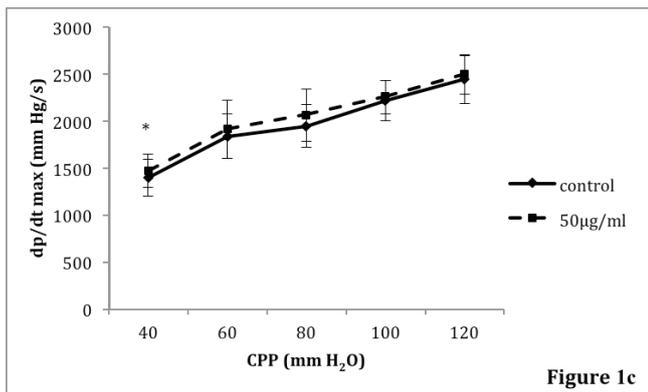
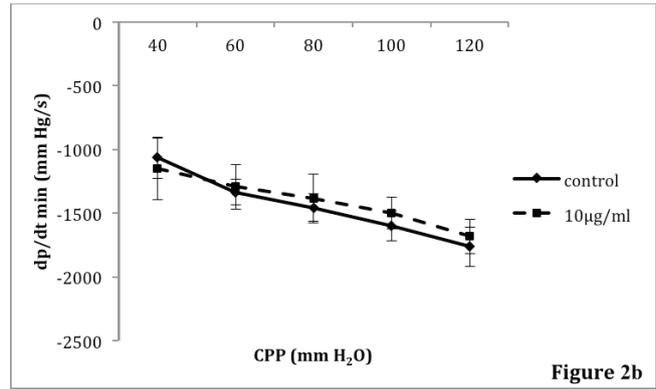
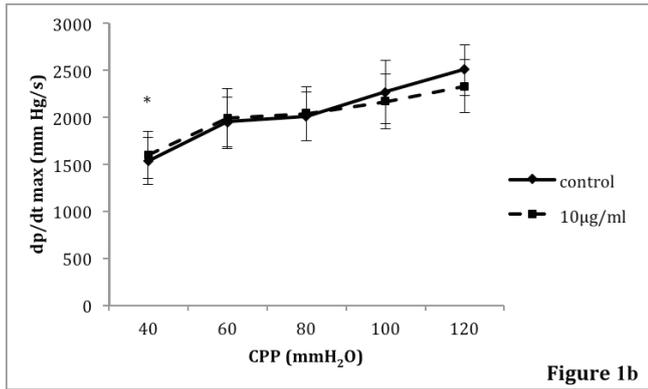
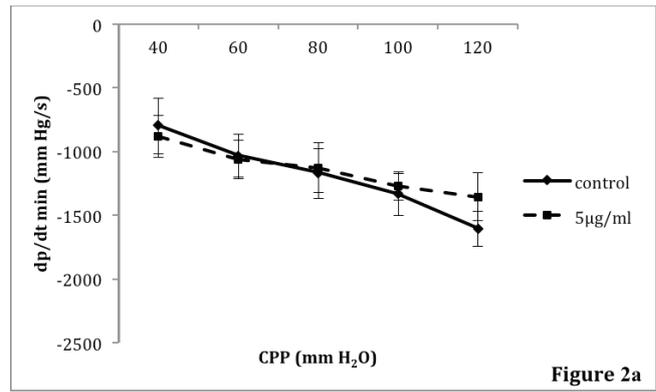
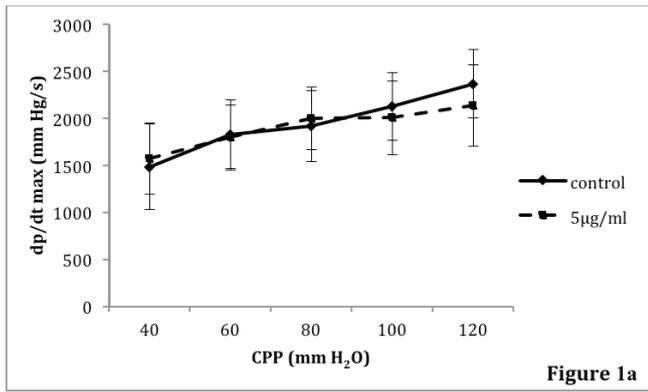


Figure 1a-c. The effects of 5 µg/ml provinol (1a), 10 µg/ml provinol (1b) and 50 µg/ml provinol (1c) on dp/dt max. The values represent $X \pm SE$; * $p < 0.05$, ** $p < 0.01$

Figure 2a-c. The effects of 5 µg/ml provinol (2a), 10 µg/ml provinol (2b) and 50 µg/ml provinol (2c) on dp/dt min. The values represent $X \pm SE$; * $p < 0.05$, ** $p < 0.01$

In the experimental work, the rules regarding the welfare of laboratory animals and the rules for the use of experimental animals of the Faculty of Medical Sciences, University of Kragujevac, were respected, which are compliant with the European Directive in this area.

Drug

Provinols were purchased from the French company VITIMED Groupe UDM Distillerie du Vivarais (Route de Ruoms BP 47, 07150 Vallon Pont d'Arc France).

Statistics

The statistical analysis of the experimental data included the following basic descriptive statistics: the mean value (X) \pm the standard deviation (SD). The following statistical

test was used to test the statistical significance of the results and to confirm the hypothesis: paired-samples T test. A database analysis of the results was performed using the software package SPSS 20th (SPSS Inc., Chicago, IL, USA). P values lower than 0.05 ($p < 0.05$) were considered to be significant, while P values lower than 0.01 ($p < 0.01$) were considered to be highly significant.

RESULTS

Maximum rate of pressure development in the left ventricle (dp/dt max)

Parameters related to contractile force and systolic performance showed no significant changes between the control and

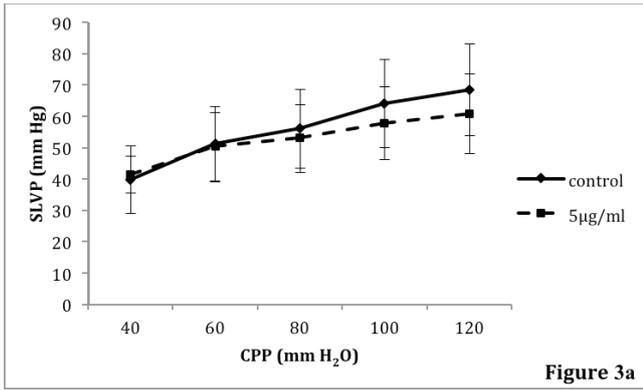


Figure 3a

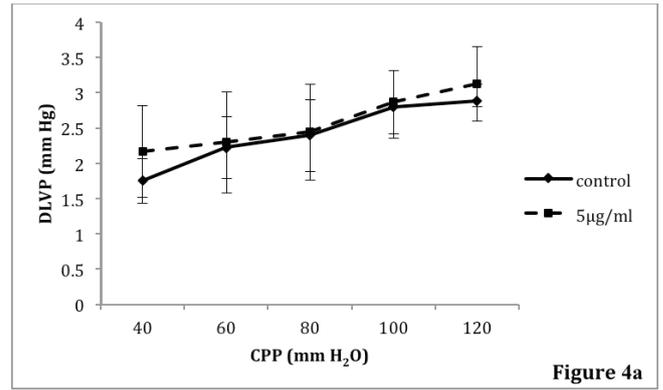


Figure 4a

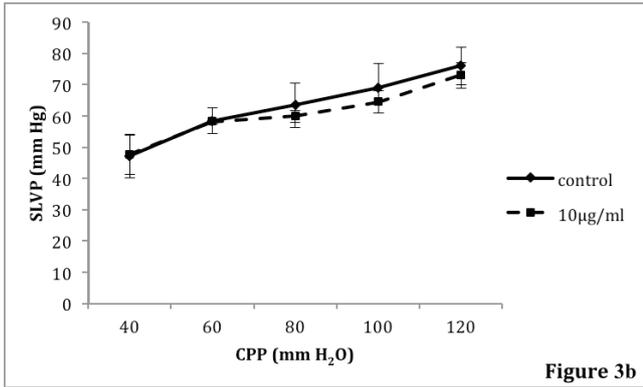


Figure 3b

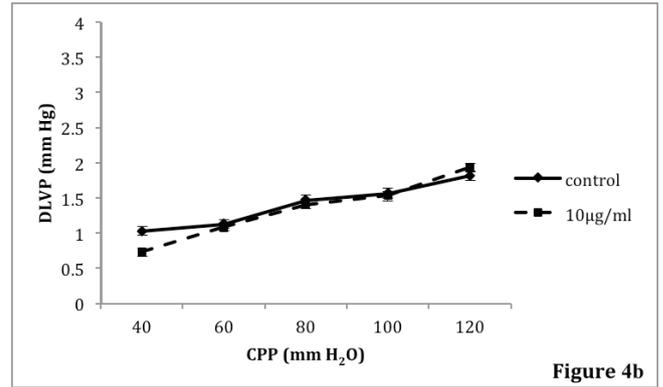


Figure 4b

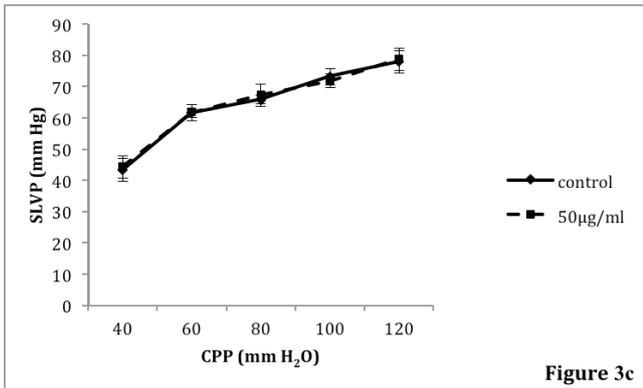


Figure 3c

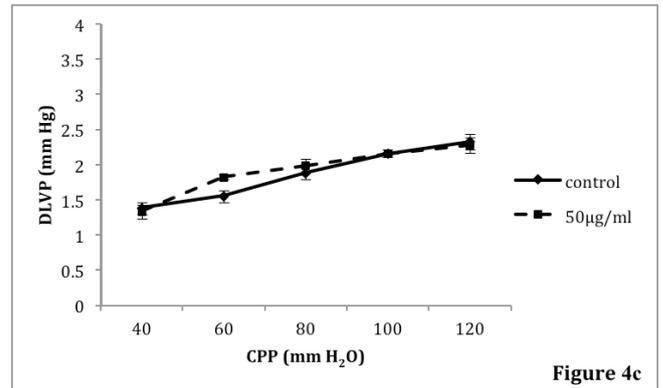


Figure 4c

Figure 3a-c. The effects of 5 µg/ml provinol (3a), 10 µg/ml provinol (3b) and 50 µg/ml provinol (3c) on SLVP. The values represent $X \pm SE$; * $p < 0.05$, ** $p < 0.01$

Figure 4a-c. The effects of 5 µg/ml provinol (4a), 10 µg/ml provinol (4b) and 50 µg/ml provinol (4c) on DLVP. The values represent $X \pm SE$; * $p < 0.05$, ** $p < 0.01$

the lowest dose of provinols (5 µg/ml) when compared at the same CPP over the entire CPP range ($p > 0.05$) (Fig 1a). Administration of the intermediate (10 µg/ml) and highest (50 µg/ml) doses induced a significant increase in dp/dt_{max} compared with the control conditions at CPP = 40 cmH₂O ($p < 0.05$) (Figs 1b, 1c).

Minimum rate of pressure development in the left ventricle (dp/dt_{min})

There were no statistically significant changes in the values of parameters describing the lusitropic effect (diastolic function) during the application of 5 µg/ml provinols or 10 µg/ml provinols over the entire CPP range ($p > 0.05$) (Figs 2a, 2b). Perfusion with the highest dose of provinols (50 µg/ml) induced a significant increase in dt/dp_{min} at CPP = 40 cmH₂O ($p < 0.05$) (Figure 2c).

Systolic pressure of the left ventricle (SLVP)

After the administration of provinols at doses of 5 µg/ml, 10 µg/ml and 50 µg/ml, we did not observe any statistically significant changes in systolic left ventricle pressure or parameters of myocardial function over the entire CPP range ($p > 0.05$) (Figs 3a, 3b, 3c).

Diastolic pressure of the left ventricle (DLVP)

Diastolic left ventricular pressure did not change significantly with an increase in CPP in the control or in all other groups ($p > 0.05$). There was no significant difference between the control and any of the groups at any of the set CPPs ($p > 0.05$) after the administration of all three doses of provinols (5 µg/ml, 10 µg/ml and 50 µg/ml) (Figs 4a, 4b, 4c).

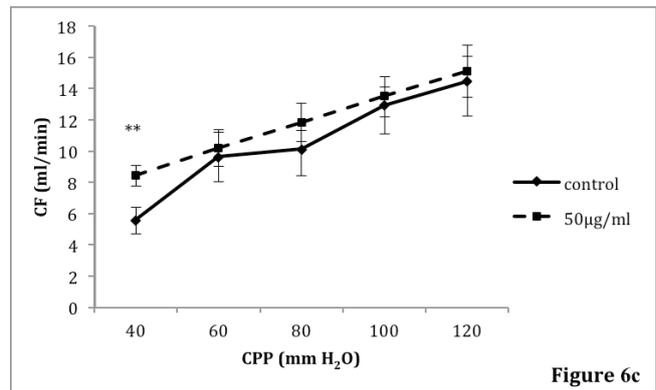
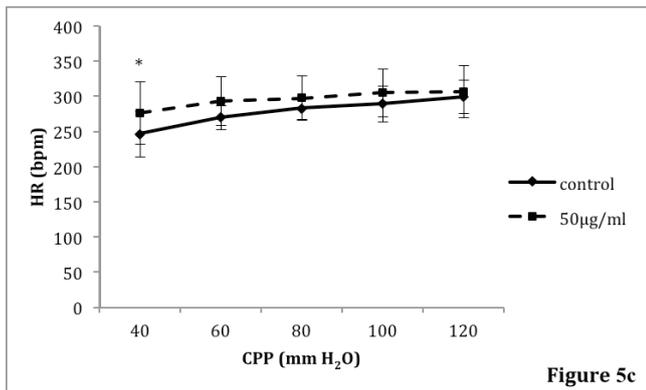
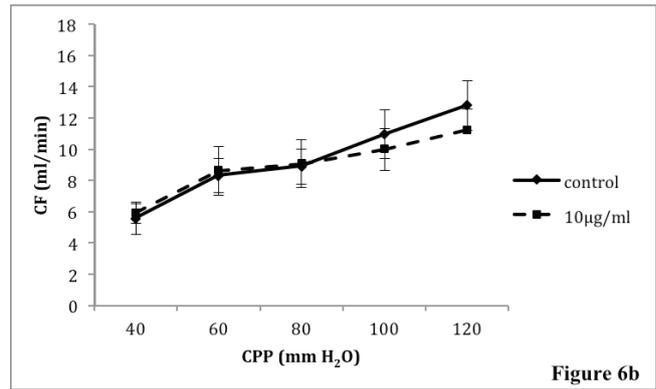
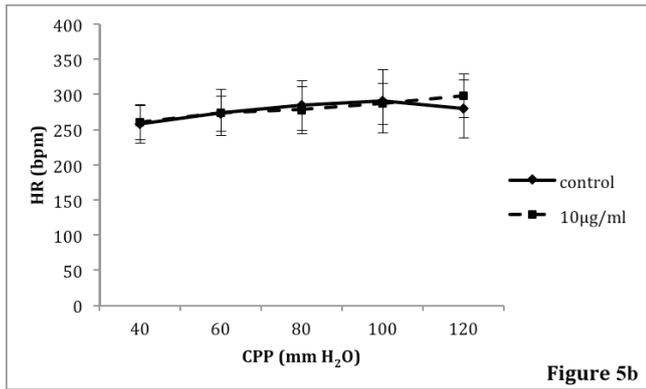
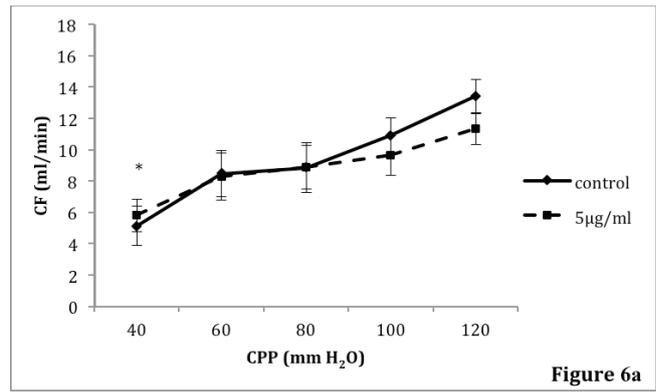
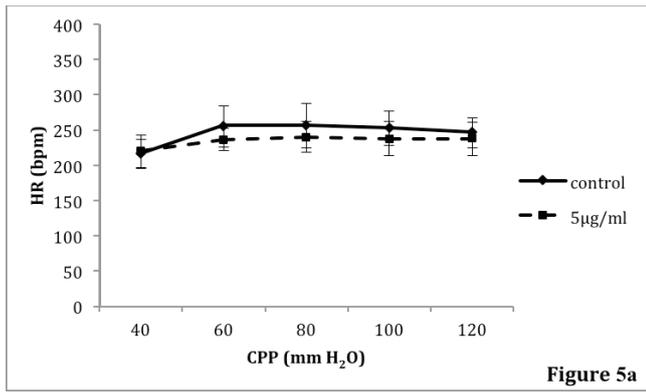


Figure 5a-c. The effects of 5 µg/ml provinol (5a), 10 µg/ml provinol (5b) and 50 µg/ml provinol (5c) on HR. The values represent X ± SE; *p<0.05, **p<0.01

Figure 6a-c. The effects of 5 µg/ml provinol (6a), 10 µg/ml provinol (6b) and 50 µg/ml provinol (6c) on CF. The values represent X ± SE; *p<0.05, **p<0.01.

Heart rate (HR)

The heart rate did not change significantly under the lowest (5 µg/ml) and intermediate (10 µg/ml) doses of provinols with an increasing CPP (p>0.05) (Figs 5a, 5b). The administration of the highest dose of provinols (50 µg/ml) induced a significant increase in HR (p>0.05) compared with control conditions (Figure 5c).

Coronary flow (CF)

This parameter was significantly increased after the application of provinols at dose of 5 µg/ml (p<0,05) at CPP = 40 cmH₂O (Figure 6a). After the administration of an intermediate dose of provinols (10 µg/ml), there was no significant difference detected over the entire CPP range (p>0.05) (Figure 6b). Compared with the controls, the cor-

onary flow was increased in the 50 µg/ml group at CPP = 40 cmH₂O (p<0,01) (Figure 6c).

DISCUSSION

As previously noted, red wine polyphenols have been reported to possess beneficial properties for the prevention of cardiovascular diseases (13, 19), but the molecular mechanisms underlying their haemodynamic effects on the cardiovascular and renal systems are much more poorly understood (19). Polyphenolic compounds have been documented to relax precontracted smooth muscle of arteries with an intact endothelium. Moreover, some of these compounds were shown to relax endothelium-denuded ar-



teries (20, 21). Another therapeutically relevant effect of flavonoids may be their ability to interact with the generation of NO from the vascular endothelium, which leads not only to vasodilatation but also to the expression of genes that protect the cardiovascular system (22-24). Provinols have been shown to improve human endothelial vascular function (25) and reduce blood pressure in animal studies (16, 17, 19), but the results of human intervention studies investigating the effect of red wine polyphenols on blood pressure are inconsistent.

The aim of the present study was to assess the influence of acute administration of provinols on cardiac function and coronary flow in the isolated rat heart.

Cardiac contractility was estimated according to the maximum and minimum rate of left ventricle pressure development (dp/dt max and dp/dt min). The first parameter (dp/dt max) represents an indirect indicator of the contractile force of the myocardium (inotropic properties), while dp/dt min reflects the ability of the cardiac muscle to relax during diastole (lusitropic properties). The lowest dose of provinols did not induce any significant changes in the contractile force of the myocardium (dp/dt max), while the intermediate and the highest doses increased the values of this marker (at CPP=40 cmHg) (Figs 1b, 1c). Another parameter related to contractility (dp/dt min) showed the same trend of reactivity (Figs 2a-c). Furthermore, our results revealed a decrease in SLVP (Figs 3a-c) and DLVP (Figs 4a, 4b, 4c) following the acute administration of provinols at all tested doses. Using the similar study model, Ferrara and coauthors examined the effects of freeze-dried red wine (FDRW) on cardiac function and ECG in Langendorff-perfused rat hearts. These authors noted reduced left ventricular pressures, but at a 10 percent higher concentration in comparison with our highest dose (26). However, FDRW has a different content of polyphenols than red wine extracts produced without using a freeze-drying technique.

For HR, the results of present study showed that the highest dose of provinols induced an increase of HR, while the other doses did not significantly change this parameter (Figs 5a, 5b). Dillenburg and colleagues recently investigated the effects of the red wine polyphenol resveratrol on HR and other haemodynamic parameters in hypertensive rats. Their results indicated that resveratrol did not alter HR values in these rats (16). Differences between this previous study and the present study regarding the different experimental models used may be a likely explanation for the obtained results. On the contrary, human studies have documented that daily consumption of red wine (40 grams) for 4 weeks results in an increased 24-hour systolic HR in normotensive humans (27). Based on all of the above results, it seems that red wine may alter HR predominantly in the absence of hypertensive conditions.

The data describing the potential mechanism underlying the effects of polyphenols on the heart are still insufficient. Studies on the effects of the red grape polyphenols

quercetin, catechin and resveratrol on cardiac voltage-gated sodium channels (VGSCs) suggest that some of their cardioprotective effects may involve inhibition of the Na⁺ current (28). This protective mechanism involves improved myocyte calcium handling and contractility, downstream of the inhibition of the late Na⁺ current.

On the other hand, the influence of red wine extracts on the coronary endothelium are less well understood. CF was increased after the administration of the lowest and highest doses of provinols tested in this work (Figs 6a, 6c), as shown by Shimada et al., who investigated the effects of red wine in healthy volunteers (29). The positive effects of polyphenols on the coronary endothelium could be related to a reduced oxidant status and increased production of NO, as indicated by the increase in NO synthase activity in both cardiac and aortic tissues (13, 17, 19). Such enhanced NO production could contribute to the relaxation of vascular smooth muscles and the increase in CF induced by provinols.

CONCLUSIONS

Generally viewed, the results of the present study suggest that provinols may have a beneficial effect on the intact myocardium and coronary circulation. These findings could be an important step in further investigation of these polyphenols under different representative experimental conditions in the heart (ischaemic/reperfusion injury), as well as providing a good basis for potential clinical studies in this field.

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Disclosures

The authors declare that they have no conflicts of interests relevant to the manuscript.

REFERENCES

1. Seruga M, Novak I, Jakobek L. Determination of polyphenols content and antioxidant activity of some red wines by differential pulse voltammetry, HPLC and spectrophotometric methods. *Food Chem.* 2011; 124: 1208–16.
2. Renaud S, De Lorgeril M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. *The Lancet.* 1992; 339(8808): 1523-6.
3. Hennekens CH, Willett W, Rosner B, et al. Effects of beer, wine and liquor in coronary death. *JAMA.* 1979; 242: 1973-4.
4. Rimm EB, Giovannucci E, Willet WC, et al. Alcohol and mortality. *Lancet.* 1991; 338: 1073 – 4.



5. De Whalley CV, Rankin SM, Hoult JR, Jessup W, Leake DS. Flavonoids inhibit the oxidative modification of low density lipoproteins by macrophages. *Biochem Pharmacol.* 1990; 39: 1743 -50.
6. Teissedre PL, Frankel EN, Waterhouse AL, et al. Inhibition of in vitro human LDL oxidation by phenolic antioxidants from grapes and wines. *Sci Food Agric.* 1996; 70: 55 - 61.
7. Lavy A, Puhman B, Markel A, et al. Effect of dietary supplementation of red or white wine on human blood chemistry, hematology and coagulation: favourable effect of red wine on plasma high-density lipoprotein. *Ann NutrMetab.* 1994; 38 (5) 287 - 94.
8. Martin S, Andriambelison E, Takeda K, Andriantsitohaina R. Red wine polyphenols increase calcium in bovine aortic endothelial cells: a basis to elucidate signaling pathways leading to nitric oxide production. *British journal of pharmacology.* 2002; 135(6): 1579-87.
9. Hertog MGL, Feskens EJM, Hollman PCH, Katan MB, Kroumhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet.* 1993; 342: 1007–11.
10. Keli SO, Hertog MGL, Feskens EJM, Kroumhout D. Flavonoids, antioxidant vitamins and risk of stroke. The Zutphen Study. *Arch Intern Med.* 1996; 156: 637–42.
11. He S, Sun C, Pan Y. Red wine polyphenols for cancer prevention. *Int J Mol Sci.* 2008; 9(5): 842-53.
12. Pietta P, Simonetti P, Gordana C, Brusamolino A, Morazzoni P, Bombardelli E. Relationship between rate and extent of catechin absorption and plasma antioxidant status. *BiochemMolBiol Int.* 1998; 46: 895- 903.
13. Pechanova O, Bernátová I, Babál P, et al. Red wine polyphenols prevent cardiovascular alterations in L-NAME-induced hypertension. *J Hypertens.* 2004; 22(8): 1551-9.
14. Pal S, Ho N, Santos C, et al. Wine Polyphenolics Increase LDL Receptor Expression and Activity and Suppress the Secretion of ApoB100 from Human Hep G2 Cells. *Nutr.* 2003; 133 (3): 700-6.
15. Zenebe W, Pechánová O, Andriantsitohaina R. Red wine polyphenols induce vasorelaxation by increased nitric oxide bioactivity. *Physiol Res.* 2003; 52(4): 425-32.
16. Dillenburg DR, Mostarda C, Moraes-Silva IC, et al. Resveratrol and grape juice differentially ameliorate cardiovascular autonomic modulation in L-NAME-treated rats. *Auton Neurosci.* 2013; 179(1-2): 9-13.
17. Bernátová I, Pechánová O, Babál P, Kyselá S, Stvrtina S, Andriantsitohaina R. Wine polyphenols improve cardiovascular remodeling and vascular function in NO-deficient hypertension. *Am J Physiol Heart Circ Physiol.* 2002; 282(3): H942-8.
18. Fitzpatrick DF, Hirschfield SL, Coffey RG. Endothelium-dependent vasorelaxing activity of wine and other grape products. *Am J Physiol.* 1993; 265 (2 Pt 2): H774-8.
19. Pechánová O, Rezzani R, Babál P, Bernátová I, Andriantsitohaina R. Beneficial Effects of Provinols™: Cardiovascular System and Kidney. *Physiol. Res.* 2006; 55 (1): S17-S30.
20. Fuster V, Badimon L, Badimon JJ, Chesebro JH. The pathogenesis of coronary artery disease and the acute coronary syndromes. *New Engl J Med.* 1992; 326: 242-50.
21. Andriambelison E, Kleschyov A, Muller B, Beretz A, Stoclet JC, Andriantsitohaina R. Nitric oxide production and endothelium-dependent vasorelaxation induced by wine polyphenols in rat thoracic aorta. *Br J Pharmacol.* 1997; 120: 1053-8.
22. Middleton EJR, Kandaswami C, Theoharides TC. The effect of plant flavonoids on mammalian cells: Implications for inflammation, heart disease and cancer. *Pharmacol Rev.* 2000; 52: 673-751.
23. Zenebe W, Pechánová O. Effects of red wine polyphenolic compounds on the cardiovascular system. *BratislLekListy.* 2002; 103: 159-65.
24. Curin Y, Andriantsitohaina R. Polyphenols as potential therapeutical agents against cardiovascular diseases. *Pharmacol Rep.* 2005; 57: 97-107.
25. Perez-Vizcaino F, Duarte J, Andriantsitohaina R. Endothelial function and cardiovascular disease: effects of quercetin and wine polyphenols. *Free radical research.* 2006; 40(10): 1054-65.
26. Ferrara A, Fusi F, Gorelli B, Sgaragli G, Saponara S. Effects of freeze-dried red wine on cardiac function and ECG of the Langendorff-perfused rat heart. *Can J Physiol Pharmacol.* 2014; 92(2): 171-4.
27. Zilkens RR, Burke V, Hodgson JM, Barden A, Beilin LJ, Puddey IB. Red wine and beer elevate blood pressure in normotensive men. *Hypertension.* 2005; 45(5): 874-9.
28. Wallace CH, Baczkó I, Jones L, Fercho M, Light PE. Inhibition of cardiac voltage-gated sodium channels by grape polyphenols. *Br J Pharmacol.* 2006; 149(6): 657-65.
29. Shimada K, Watanabe H, Hosoda K, Takeuchi K, Yoshikawa J. Effect of red wine on coronary flow-velocity reserve. *Lancet* 1999; 354:1002.

