THE EFFECTS OF DIRECT RED BULL ADMINISTRATION TO ISOLATED HEARTS OF TRAINED AND UNTRAINED RATS WHO REGULARLY CONSUMED OR DID NOT CONSUME ENERGY DRINK: FOCUS ON CARDIODYNAMICS AND OXIDATIVE STRESS

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EFEKTI DIREKTNE ADMINISTRACIJE RED BULL-A U IZOLOVANOM SRCU TRENIRANIH I NETRENIRANIH PACOVA KOJI SU REDOVNO KONZUMIRALI ILI NISU KONZUMIRALI ENERGETSKO PIĆE: FOKUS NA KARDIODINAMIKU I OKSIDATIVNI STRES

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ABSTRACT

Energy drinks (EDs) contain caffeine and other active ingredients which affect cardiovascular system. The aims of this study were to examine direct effects of Red Bull (RB) on cardiodynamics and oxidative stress in isolated hearts of rats. The rats were divided into four groups: untrained rats who never consumed ED (dED-UT); untrained rats who consumed ED 5 days a week during 4 weeks (ch+dED-UT); rats trained 5 times a week for 4 weeks, but did not consume ED (dED-T); rats trained and consumed ED 5 times a week for 4 weeks (ch+dED-T). After sacrificing, hearts were isolated and perfused according to Langendorff technique. Through the isolated heart of all rats in each group, RB was administered. The parameters of cardiac function were recorded, and the levels of prooxidants were measured in the coronary effluent during coronary autoregulation. Rats in ch+dED-UT group had significantly lower rates of myocardial contraction and relaxation compared to rats in dED-UT group. The same effect was recorded in the dED-T group compared to dED-UT group. The levels of hydrogen peroxide were significantly higher in trained rats. Rats in ch+dED-T group also had significantly higher levels of superoxide anion radical and index of lipid peroxidation, as well as lower levels of nitrites when compared to ch+dED-UT group, while opposite effect was recorded in rats in dED-T group compared to dED-UT group. The RB could have a potentially negative inotropic effect in chronic consumers. Prooxidative effect of RB was most pronounced in trained chronic consumers.

Keywords: *cardiovascular system, energy drinks, oxidative stress, rats, swimming*

SAŽETAK

Energetska pića (EP) sadrže kofein i druge aktivne sastojke koji utiču na kardiovaskularni sistem. Ciljevi ovog istraživanja bili su da se utvrde direktni efekti Red Bull-a (RB) na kardiodinamiku i oksidativni stres u izolovanim srcima pacova. Pacovi su bili podeljeni u četiri grupe: netrenirani pacovi koji nikada nisu konzumirali EP (dED-UT); netrenirani pacovi koji su konzumirali EP, 5 dana nedeljno tokom 4 nedelje (ch+dED-UT); trenirani pacovi 5 puta nedeljno, tokom 4 nedelje, koji nisu konzumirali EP (dED-T); trenirani pacovi koji su konzumirali EP, 5 puta nedeljno tokom 4 nedelje (ch+dED-T). Nakon žrtvovanja, srca pacova su izolovana i perfundovana prema tehnici po Langendorff-u. Kroz izolovana srca svih pacova u svakoj grupi, administriran je RB. Određivani su parametri funkcije srca, kao i nivo prooksidativnih vrsta u koronarnom efluentu tokom koronarne autoregulacije. Pacovi u grupi ch+dED-UT imali su značajno niže stope kontrakcije i relaksacije miokarda u poređenju sa pacovima u grupi dED-UT. Isti efekat zabeležen je u grupi dED-T u odnosu na grupu dED-UT. Nivoi vodonik peroksida bili su značajno viši u grupi treniranih pacova. Pacovi u grupi ch+dED-T, imali su takođe značajno više nivoe superoksid anjon radikala i indeksa lipidne peroksidacije, kao i niže nivoe nitrita u poređenju sa grupom ch+dED-UT, dok je suprotan efekat zabeležen kod pacova u dED-T grupi u poređenju sa dED-UT grupom. RB bi mogao imati potencijalno negativan inotropan efekat kod hroničnih konzumera. Prooksidativni efekat RB bio je najizraženiji kod treniranih hroničnih konzumera.

Ključne reči: *kardiovaskularni sistem, energetska pića, oksidativni stres, pacovi, plivanje*

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ABBREVATIONS:

ED - energy drink, **RB** - Red Bull, **CPP** - coronary perfusion pressure,
dp/dt max - maximum rate of left ventricular pressure development,
dp/dt min - minimum rate of left ventricular pressure development,
SLVP - systolic left ventricular pressure, **DLVP** - diastolic left ventricular pressure,
HR - heart rate, **CF -** coronary flow, O_2^- - superoxide anion radical,
H_2O_2 - hydrogen peroxide, **NO** - nitrogen monoxide,
TBARS - thiobarbituric acid reactive substances

INTRODUCTION

The term "energy drink" (ED) is used for caffeinated beverages, which are consumed in order to improve physical and mental performance (1). Extensive advertising, the colourful packaging and noted short-term improvement in performance after consumption have resulted in the high popularity of EDs. They are most popular among athletes, drivers and young people (2).

The effects of EDs are associated with the frequency and amount of consumption, as well as with the concentration and interaction of ingredients (3). The health hazard data on EDs are found to be limited; therefore the assessment of their safety is based on the impact of individual ingredients. Red Bull (RB) is one of the most commonly consumed EDs (4). The ingredients of the original RB are as follows: caffeine, taurine, glucuronolactone, carbohydrates, and B-group vitamins (5). Caffeine, a methylxanthine, increases sympathetic nerve activity (6). Taurine, a derivative of the amino acid cysteine (6), increases muscle strength, improves endurance, reduces physical exercise-induced DNA damage and accelerates recovery after training (7). The consumption of glucose or other carbohydrates before and during physical activity delays the onset of fatigue, conserves muscle glycogen and improves performance (8). Glucuronolactone is a naturally occurring substance (5), which is formed from glucose in the liver (5, 9) and it is added to EDs to fight fatigue and provide a sense of well-being (10). The B-group vitamins belong to a group of water-soluble vitamins that have the role of coenzyme and are important for the proper function of cells, especially mitochondrial function and energy production (11).

EDs exhibit a maximum effect 30-60 minutes after ingestion (12). It is assumed that most of the biological effects of EDs are mediated by a positive inotropic effect, which implies increase in heart rate, cardiac output, myocardial contractility, stroke volume and arterial blood pressure (13). Particularly important is the effect of EDs on the changes in ventricular repolarization (14). The results of meta-analysis have shown that acute consumption of EDs significantly raises systolic and diastolic arterial pressure, while there is no significant effect on the heart rate (15). In athletes, acute consumption of RB had a positive inotropic effect (enhancement of left ventricular and left atrial contractility) in the recovery period after physical exercise (16). But also, the case series of ED-associated acute adverse cardiovascular events were described (17). ED consumption may lead to the increased cardiomyocytes apoptosis, which could be the cause of cardiovascular disorders in the ED consumers (2). Chronic ED consumption may be the cause of hypertensive heart disease, coronary artery disease, cerebrovascular disease and peripheral arterial disease (18). EDs affect the increase in blood glucose level, total cholesterol, triglycerides and low-density lipoprotein cholesterol, which all contribute to an increase in cardiovascular risk (19, 13). Endothelial dysfunction and increased risk of myocardial ischaemia, which may occur due to the loss of nitrogen monoxide (NO) activity in the blood vessel wall, have also been linked to ED consumption (20). However, it has also been demonstrated that acute RB consumption may exhibit a positive effect on the endothelial function (21, 22). Furthermore, results of previous studies have shown that acute ED administration induced lipid peroxidation and oxidative stress in the liver and brain of rats (23), and that the chronic use of energy drinks leads to toxicity, an inflammatory response and oxidative stress (24-30). However, it was shown that RB administration in rats for 14 days caused the acceleration of soft tissue healing and that was explained by the antioxidant effects of the RB ingredients (31).

Previous studies have shown various results regarding the effects of EDs on heart and cardiovascular system, depending on the dose, population, existence of risk factors and other protocol variables. To our knowledge, there is currently no published research examining the direct effect of ED on the heart. Thus, the main aim of this study was to examine direct effects of RB on cardiodynamics, coronary flow and oxidative stress in isolated rat hearts. Furthermore, the aim of the study was to examine if regular training or daily ED consumption change the effects that direct ED administration has on previously mentioned parameters.

MATERIALS AND METHODS

The study was conducted in the Laboratory for Cardiovascular Physiology at the Faculty of Medical Sciences, University of Kragujevac. It was approved by the Ethics Committee of the Faculty. The conception, design and performance of the study were followed by Good Laboratory Practice criteria and the European Council Directive (86/609EEC).

Subjects

The Wistar albino rats were used in this study. The sample size calculation, based on a study published by Barcelos et al. (32), revealed that 24 rats were required to perform the study. At the beginning of the study, rats were eight weeks old and their weight was 200-250g. They were housed in conventional cages in groups of 8 animals per cage. They were fed with commercial rat food (20% protein food, Veterinary Institute Subotica) and water ad libitum. Room temperature was set to 25 °C and 12 hours of light were provided.

ED consumption and training protocol

The study lasted 4 weeks. The rats were divided into four groups (six rats in each group) depending on chronic ED consumption during the study period (rats who did and did not consume ED every day) and depending on whether they were subjected to the training protocol or not. After sacrificing the animals, ED was administered to the isolated heart of all rats in each group (all hearts were perfused with ED, as explained bellow).

Thus, groups were as follows:

dED-UT group: untrained (sedentary) rats who never consumed ED,

ch+dED-UT group: untrained rats who consumed ED 5 days a week during 4 weeks,

dED-T group: trained rats 5 times a week for 4 weeks, which did not consume ED,

ch+dED-T group: trained rats which consumed ED 5 times a week for 4 weeks.

The rats of two groups were subjected to a swimming practice (1h per day, 5 days a week) in a 80x60x100cm pool for experimental animals. An electric heater was used to keep the water temperature at 34 C°. During swimming, the pump installed in the pool made constant waves, in order to prevent the rats from floating. Rats were monitored the whole time during swimming. The rats in other two groups were untrained (they were not subjected to a swimming practice).

The ED was administered to rats in two groups by an intragastric gavage (p.o.). RB was used in the amount of 3.75 ml/kg, as determined on the basis of the previously published studies (32, 33). The indicated dose corresponds to a dose of caffeine close to the maximum recommended (about 6 mg /kg). A standard can of 250 ml RB contains: 80 mg of caffeine, 1000 mg of taurine, 21.5 g of sucrose, 5.25 g of glucose, 600 mg of glucuronolactone, 20 mg of vitamin B3 (niacinamide), 5 mg of vitamin B5 (calcium pantothenate), 5 mg of vitamin B6 (pyridoxine hydrochloride), 50 mg of inositol, 5 µg of vitamin B12 (cyanocobalamin), 100 mg of sodium citrate, as well as natural and artificial flavors and colors (caramel, riboflavin) (34,11,35).

After one month, rats were sacrificed by short ketamine/xylazine narcosis. After that, their hearts were excised and attached to the Langendorff apparatus via aortic cannula. Krebs–Henseleit buffer was used during the performance of retrograde perfusion according to the Langendorff technique. First, an equilibration period, during which coronary perfusion pressure (CPP) was kept at 70 cmH_2O, was performed. After that, CPP was changed in the following order: 1) 60 cmH_2O, 2) 80 cmH_2O, 3) 100 cmH_2O, 4) 120 cmH_2O, and 5) 40 cmH_2O. Through the isolated heart of all rats in each group, Krebs–Henseleit buffer, in which 150 µmol of ED was dissolved, was perfused by the Langendorff retrograde perfusion method.

Cardiodynamic parameters

Parameters of myocardial function were measured using the pressure sensor (transducer BS4 73-0184, Experimentria Ltd, Hungary) which was attached to the latex ballon, filled with bubble-free saline, which was inserted into the left chamber (36). Cardiodynamic parameters were continuously measured. The following parameters of myocardial function were recorded: 1) maximum and minimum rate of pressure development in LV (dp/dt max and dp/dt min), 2) systolic and diastolic left ventricle pressure (SLVP and DLVP) and 3) heart rate (HR). Furthermore, coronary flow (CF) was measured flowmetrically.

Oxidative stress

Coronary flow, which was collected during each CPP, was used to measure the levels of oxidative stress in coronary venous effluent. Spectrophotometer (Analytic Jena Specord S 600, UK) was used to determine the levels of 1) superoxide anion radical (O_2^-), 2) hydrogen peroxide (H_2O_2), 3) nitrogen monoxide (NO) and 4) index of lipid peroxidation (thiobarbituric acid reactive substances, TBARS). The exact protocols for measurement of those prooxidative species may be found in our previously published paper (37) or in the original sources (38-41).

Statistics

SPSS 23.0 was used to perform the statistical analysis. Comparison of groups was performed using the parametric (t-test for independent samples) or nonparametric test (Mann-Whitney U test), depending on the results of the Shapiro-Wilk test for data distribution. The results on the figures are shown as the mean ± standard error of the mean (X ± SE).

RESULTS

Cardiodynamic parameters of isolated rat hearts in four groups (dED-T, ch+dED-T, dED-UT, ch+dED-UT) are shown in Figures 1-6. Prooxidative parameters in the effluent during coronary autoregulation of isolated rat hearts in four groups (dED-T, ch+dED-T, dED-UT, ch+dED-UT) are shown in Figures 7-10.

1) The direct effect of the ED on the heart of untrained rats who chronically consumed the ED and those who did not consume ED

Cardiodynamics

In relation to the dED-UT group, the following were recorded in the ch+dED-UT group: 1) at all CPPs, level of dp/dt max was lower, but statistically significant only at CPP 60-100 cmH_2O ($p < 0.05$); 2) at all CPPs, level of dp/dt min was lower (more positive), but statistically significant only at CPP 60-120 cmH_2O ($p < 0.05$); 3) at all CPPs, level of CF was higher, but statistically significant only at CPP 80-120 cmH_2O ($p < 0.05$); at all CPPs, statistically significantly lower level of SLVP ($p < 0.05$) and statistically significantly higher level of DLVP ($p < 0.05$); 4) at all CPPs, higher level of HR, but only statistically significantly higher at CPP 60-80 cmH_2O ($p < 0.05$).

Oxidative stress

In relation to the dED-UT group, the following were recorded in the ch+dED-UT group:

1) at all CPPs, level of O_2^- was lower, but statistically significantly only at CPP 40, 80-120 cmH_2O ($p < 0.05$); 2) at all CPPs, level of TBARS was statistically significantly lower ($p < 0.05$); 3) at all CPPs, levels of nitrites (NO) were higher, but statistically significant only at CPP 80-120 cmH_2O ($p < 0.05$); 4) at all CPPs, level of H_2O_2 was higher, but without statistical significance ($p > 0.05$).

2) The direct effect of the ED on the heart of trained rats who chronically consumed the ED and those who did not consume ED

Cardiodynamics

Although at all CPP levels dp/dt max, dp/dt min, CF and SLVP were lower in ch+dED-T than in dED-T group, and levels of DLVP higher, no statistical significance was observed in any cardiodynamic parameter between the groups ($p > 0.05$). Also, there was no statistically significant difference in the level of HR between these two groups ($p > 0.05$).

Oxidative stress

Levels of H_2O_2 at CPP 60, 100-120 cmH_2O were significantly higher in ch+dED-T when compared to dED-T group ($p < 0.05$). There was no statistically significant difference between those two groups in the levels of other prooxidative species.

3) The direct effect of the ED on the heart of trained and untrained rats who did not consume ED

Cardiodynamics

In relation to the dED-UT group, the following were recorded in the dED-T group:1) at all CPPs, statistically significantly lower level of dp/dt max, dp/dt min and SLVP ($p < 0.05$); 2) at all CPPs, level of HR was lower, but statistically significant only at CPP 40, 60 and 100 cmH_2O ($p < 0.05$); 3) at all CPPs, level of CF was higher, but statistically significant only at CPP 60-120 cmH_2O ($p < 0.05$). There was no statistically significant difference in the level of DLVP between these two groups ($p > 0.05$).

Oxidative stress

In relation to the dED-UT group, the following were recorded in the dED-T group: 1) at all CPPs, higher level of O_2^-, but without statistical significance ($p > 0.05$); 2) at all CPPs, level of H_2O_2 was higher, but statistically significant only at CPP 60-120 cmH_2O ($p < 0.05$); 3) at all CPPs, level of TBARS was lower, but statistically significant only at CPP 40-80 cmH_2O ($p < 0.05$); 4) at all CPPs, levels of nitrites (NO) were higher, but statistically significant only at CPP 80-120 cmH_2O ($p < 0.05$).

4) The direct effect of the ED on the heart of trained and untrained rats who chronically consumed the ED

Cardiodynamics

In relation to the group ch+dED-UT, the following were recorded in the group ch + dED-T: 1) at all CPPs, lower level of dp/dt max, CF and SLVP but without statistical significance ($p > 0.05$); 2) at all CPPs, level of dp/dt min was lower, but statistically significant only at CPP 40-100 cmH_2O ($p < 0.05$); 3) at all CPPs, level of DLVP was lower, but statistically significant only at CPP 40, 80-120 cmH_2O ($p < 0.05$); at all CPPs, statistically significantly lower level of HR ($p < 0.05$).

Oxidative stress

In relation to the group ch+dED-UT, the following were recorded in the group ch + dED-T: at all CPPs, level of O_2^- was higher, but statistically significant only at CPP 40, 100-120 cmH_2O ($p < 0.05$); at all CPPs, statistically significantly higher level of H_2O_2 and TBARS ($p < 0.05$); at all CPPs, levels of nitrites (NO) were lower, but statistically significant only at CPP 60-100 cmH_2O ($p < 0.05$).

FIGURES

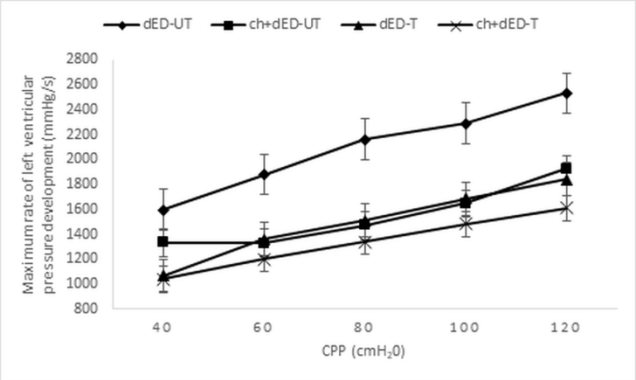

Figure 1.

Values of maximum rate of left ventricular pressure development during coronary autoregulation of the isolated trained rat hearts in the following groups: dED-UT, ch+dED-UT, dED-T, ch+dED-T. CPP, coronary perfusion pressure. Data are means ± SE.

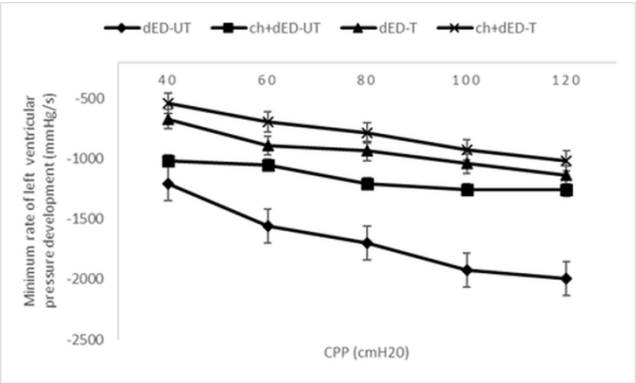

Figure 2.

Values of minimum rate of left ventricular pressure development during coronary autoregulation of the isolated trained rat hearts in the following groups: dED-UT, ch+dED-UT, dED-T, ch+dED-T. CPP, coronary perfusion pressure. Data are means ± SE.

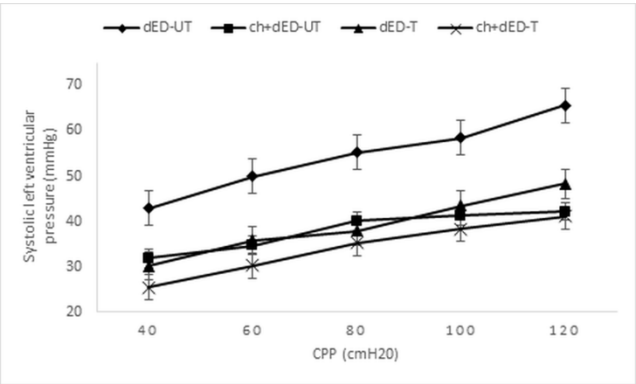

Figure 3.

Values of systolic left ventricular pressure during coronary autoregulation of the isolated trained rat hearts in the following groups: dED-UT, ch+dED-UT, dED-T, ch+dED-T. CPP, coronary perfusion pressure. Data are means ± SE.

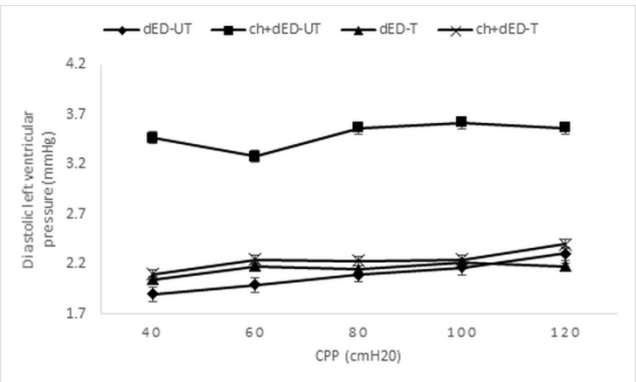

Figure 4.

Values of diastolic left ventricular pressure during coronary autoregulation of the isolated trained rat hearts in the following groups: dED-UT, ch+dED-UT, dED-T, ch+dED-T. CPP, coronary perfusion pressure. Data are means ± SE.

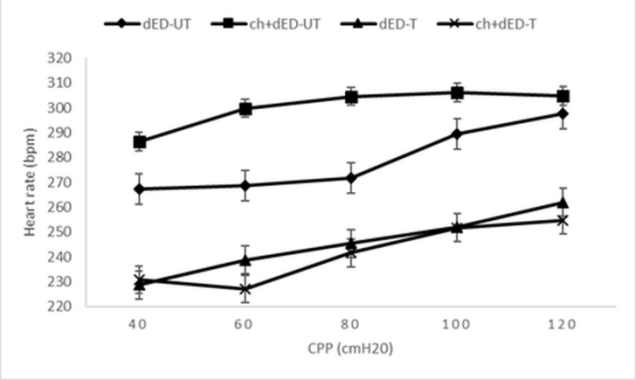

Figure 5.

Values of heart rate during coronary autoregulation of the isolated trained rat hearts in the following groups: dED-UT, ch+dED-UT, dED-T, ch+dED-T. CPP, coronary perfusion pressure. Data are means ± SE.

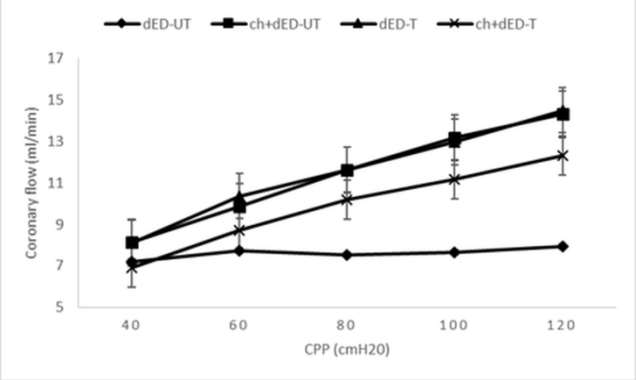

Figure 6.

Values of coronary flow during coronary autoregulation of the isolated trained rat hearts in the following groups: dED-UT, ch+dED-UT, dED-T, ch+dED-T. CPP, coronary perfusion pressure. Data are means ± SE.

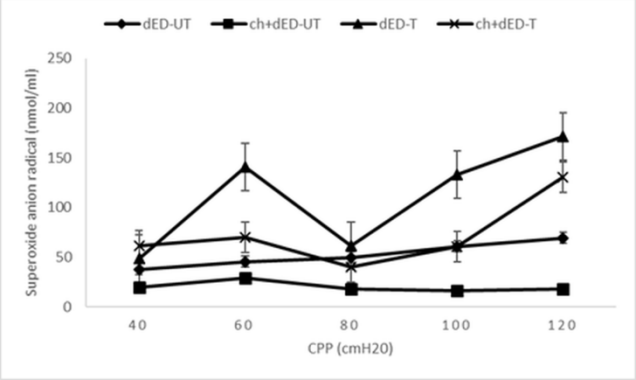

Figure 7.

Values of superoxide anion radical in effluent, during coronary autoregulation of the isolated trained rat hearts in the following groups: dED-UT, ch+dED-UT, dED-T, ch+dED-T. CPP, coronary perfusion pressure. Data are means ± SE.

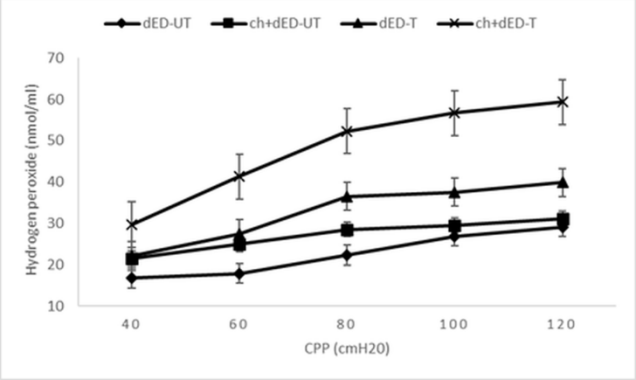

Figure 8.

Values of hydrogen peroxide in effluent, during coronary autoregulation of the isolated trained rat hearts in the following groups: dED-UT, ch+dED-UT, dED-T, ch+dED-T. CPP, coronary perfusion pressure. Data are means ± SE.

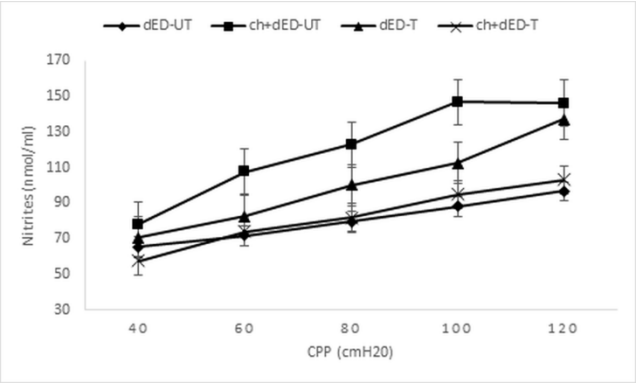

Figure 9.

Values of nitrites in effluent, during coronary autoregulation of the isolated trained rat hearts in the following groups: dED-UT, ch+dED-UT, dED-T, ch+dED-T. CPP, coronary perfusion pressure. Data are means ± SE.

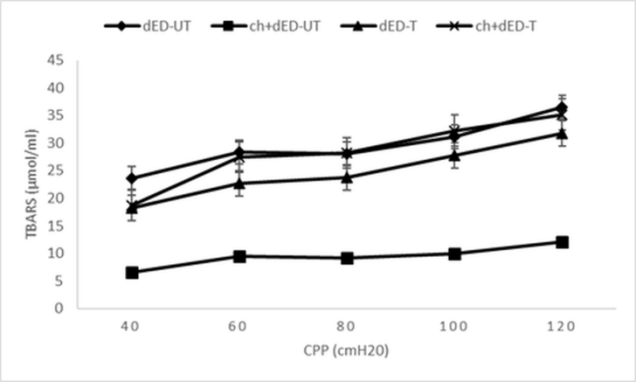

Figure 10.

Values of index of lipid peroxidation in effluent, during coronary autoregulation of the isolated trained rat hearts in the following groups: dED-UT, ch+dED-UT, dED-T, ch+dED-T. CPP, coronary perfusion pressure. Data are means ± SE.

DISCUSSION

In this research, we studied the direct effects of RB on cardiodynamics, coronary flow and oxidative stress in isolated hearts of trained and untrained rats, who chronically consumed RB in comparison with those who did not consume RB.

The results of our study showed that, after direct RB administration to the heart, untrained rats who chronically consumed RB had statistically significantly lower levels of dp/dt max, dp/dt min and SLVP, while levels of DLVP were significantly higher, compared to untrained rats who never consumed RB. The same was observed when trained rats regular RB consumers were compared to trained rats non-consumers of RB, except that no statistically significant difference between groups was found. This could be interpreted as either a potentially harmful effect of RB on the myocardial contraction and relaxation in chronic consumers, or their decreased reactivity to RB ingredients due to adaptation induced by daily consummation. Trained rats that never consumed RB had statistically significantly lower levels of dp/dt max, dp/dt min and SLVP when compared to untrained rats that never consumed RB, while trained rats that chronically consumed RB had only statistically significantly lower levels of dp/dt min when compared to untrained chronic RB consumers. This might suggest that trained rats respond less to direct RB administration, as well as that chronic RB consumption decreases the effects that acute/direct consummation has on the heart, both in trained and untrained rats. In chronic untrained RB consumers, DLVP significantly increased after direct RB administration to the heart when compared to untrained non-consumers, while this change was not so pronounced in trained rats. Regarding the HR, untrained rats that chronically consumed RB responded to direct RB administration with higher HR than those who did not use to consume RB, while both trained groups, chronic RB consumers and non-consumers of RB, had lower levels of HR when compared to their untrained matched controls. Trained rats also had higher CF than untrained rats. This also suggests that regular training depresses the effects of direct RB on the heart cardiodynamics, while CF is preserved.

As we have previously mentioned, there are no other studies that included administration of ED directly to the isolated rat heart, except for the study that has been recently published by our team (42). In that paper, we have shown that, in trained rats, acute consummation of the RB had a positive inotropic effect (manifested as significantly higher level of dp/dt max and dp/dt min compared to the levels measured in control rats), while chronic administration affected the isolated increase in SLVP, which could be considered the potentially negative impact chronic ED consumption (42). There were no significant differences in cardiodynamic parameters after acute RB consummation (30min before sacrificing) between trained rats that regularly drank RB and those who did not (42), which is in consent with the results presented in this paper (direct instead of acute RB administration). Those results

suggest that acute/direct RB administration affects cardiodynamics to the greater extent in untrained, than in trained rats, i.e. that regular training affects the effects of both acute and chronic ED consumption.

The results regarding the levels of prooxidant species in the coronary effluent showed that, after direct RB administration to the isolated heart, levels of H2O2 were the highest in trained rats: both trained chronic RB consumers and non-consumers had higher H_2O_2 levels than their untrained matched controls, and trained chronic consumers had higher levels of H_2O_2 in comparison to trained non-consumers. Prooxidative effect of direct RB administration was most prominent in trained chronic RB consumers, since they had significantly higher levels of O_2 H_2O_2, TBARS and lower levels of NO when compared to untrained chronic RB consumers. Except in the case of H_2O_2, opposite was observed when trained non-consumers of RB were compared to untrained non-consumers of RB: levels of TBARS were lower and levels of NO higher in trained rats. This suggests that positive effects of regular training on redox state (43) may be diminished by chronic ED consumption. Pusica et al. (42) have shown that both rats who chronically consumed RB, and rats who consumed RB acutely, had significantly increased levels of lipid peroxidation in coronary effluent when compared to control rats, as well as that acute RB consummation increased the levels of TBARS to the greater extent in rats who chronically consumed RB than in those who have not consumed it before, which suggests that in chronic consumers acute ED consumption continues to deteriorate redox status. Interestingly, in this research, untrained rats who consumed RB on a daily basis had lower levels of O_2, TBARS and higher levels of NO in coronary effluent after direct RB administration to the isolated rat heart than untrained rats who did not used to consume RB. Thus, in untrained rats, chronic RB consumption did not negatively affect cardiac oxidative state, which is the opposite to the results related to the cardiodynamics. Finally, the relationship between cardiodynamics and oxidative stress in coronary effluent in our study may be discussed in terms of the relationship between NO levels and CF: in groups that had increased levels of CF levels of NO in coronary effluent were also significantly higher than in their matched controls. This supports the role of NO in endothelial function and vasodilatation (44).

CONCLUSIONS

The conclusion of this study is that the RB could have a potentially negative inotropic effect in chronic consumers. However, it may also be considered as their decreased reactivity to RB ingredients due to adaptation induced by daily consummation. It seems that trained rats respond less to direct RB administration, as well as that chronic RB consumption decreases the effects that direct administration has on the heart, both in trained and untrained rats. Our results suggest that cardiac prooxidative effect of direct RB administration was the most pronounced in trained chronic RB consumers, while chronic RB consumption did not deteriorate oxidative status in isolated hearts of untrained rats.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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