ACCEPTED MANUSCRIPT

Accepted manuscripts are the articles in press that have been peer reviewed and accepted for publication by the Editorial Board of the Vojnosanitetski Pregled. They have not yet been copy edited and/or formatted in the publication house style, and the text could still be changed before final publication.

Although accepted manuscripts do not yet have all bibliographic details available, they can already be cited using the year of online publication and the DOI, as follows: article title, the author(s), publication (year), the DOI.

Please cite this article THE EFFECTS OF ACUTE AND CHRONIC RED BULL® CONSUMPTION ON CARDIODYNAMICS AND OXIDATIVE STRESS IN CORONARY EFFLUENT OF TRAINED RATS

EFEKTI AKUTNE I HRONIČNE KONZUMACIJE RED BULL®-A NA KARDIODINAMIKU I OKSIDATIVNI STRES U KORONARNOM EFLUENTU TRENIRANIH PACOVA


UDC:

DOI: https://doi.org/10.2298/VSP190119040P

When the final article is assigned to volumes/issues of the Journal, the Article in Press version will be removed and the final version appear in the associated published volumes/issues of the Journal. The date the article was made available online first will be carried over.
THE EFFECTS OF ACUTE AND CHRONIC RED BULL® CONSUMPTION ON CARDIODYNAMICS AND OXIDATIVE STRESS IN CORONARY EFFLUENT OF TRAINED RATS

EFEKTI AKUTNE I HRONIČNE KONZUMACIJE RED BULL®-A NA KARDIODINAMIKU I OKSIDATIVNI STRES U KORONARNOM EFLUENTU TRENIRANIH PACOVA


*Department of Physiology, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia, †Primary Health Centre New Belgrade, Belgrade, Serbia

Correspondence: Irena Pušica, Primary Health Centre New Belgrade, Bulevar maršala Tolbuhina, 11 070 New Belgrade, Serbia. Tel.: +381 11 2222 100; +381 63 620 976; Fax: +381 11 2222 190. E-mail: irenapetrovic83@yahoo.com
Abstract

Background/Aim. Energy drinks (EDs) are widely used by athletes as ergogenic agents. The aims of this study were to determine the acute and chronic effects of Red Bull®, one of the most consumed EDs, on cardiodynamics and parameters of oxidative stress in physically trained rats. Methods. Rats were subjected to a swimming practice (1h per day, 5 days a week, during 4 weeks). They were divided into 4 groups: rats that did not consume ED either before swimming or prior to sacrificing; rats that did not consume ED before swimming, but did consume ED 30 minutes prior to sacrificing; rats that consumed ED 30 min before every swimming training, but did not consume ED prior to sacrificing; rats that consumed ED 30 min before every swimming training and 30 min before sacrificing. After sacrificing, hearts were isolated and perfused according to Langendorff technique. The parameters of cardiac function were recorded, and also the levels of prooxidants were measured in the coronary effluent during coronary autoregulation. Results. Compared to the control group, acute administration of the ED had a positive inotropic effect (manifested as significantly higher level of maximum and minimum rate of pressure development in left ventricle), while chronic administration affected the isolated increase in systolic left ventricular pressure, which could be considered the potentially negative impact of the ED. Prooxidative effect of the ED was observed, which was more pronounced in chronic consumption. Conclusion. The main conclusion of our study is that chronic administration of the ED changes the cardiovascular response and redox status in acute consumption.

Key words: cardiovascular system, energy drinks, oxidative stress, rats, swimming.

Apstrakt


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

Introduction

Energy drinks (EDs) are beverages with stimulating effects due to a combination of specific ingredients. The main active ingredient of these drinks is caffeine, but it has been shown that other components also contribute to the changes in the work of the cardiovascular system. EDs are consumed for the purpose of providing additional energy, increasing cognitive and physical performance, prolonging alertness and improving mood. Due to the positive inotropic effect, they should induce some benefit to the exercising individuals by improving skeletal muscle oxygenation and increasing aerobic metabolism and muscular performance. Thus, EDs are widely used by athletes as ergogenic agents.

Red Bull® (RB®) is considered to be one of the most consumed EDs. It has been shown that 355 ml of RB® leads to a significant increase in the systolic and diastolic blood pressure, heart rate, stroke volume, and a double product, which is an indirect indicator of oxygen consumption in the myocardium. Because of the significant increase in myocardial function of the right and left ventricles, a positive inotropic effect of EDs has been suggested. EDs affect the increase in glycemia, cholesterol, and triglycerides, so
the overall effect of EDs is an increase in cardiovascular risk \(^\text{15}\). Acute cardiovascular adverse effects of EDs include: the effect on hemodynamics and electrophysiological changes, the effect on endothelial function, as well as the association with vascular pathology \(^\text{16}\). The association between EDs consumption and cardiovascular changes include supraventricular and ventricular arrhythmias, ischemia and myocardial infarction, QT interval prolongation, aortic dissection, and death \(^\text{7}\). Although moderate consumption of EDs is considered relatively safe in healthy population \(^\text{10}\), it is not recommended that they are consumed in sport and during exercise, and special caution is recommended for people with cardiovascular diseases \(^\text{17,18}\).

The occurrence of oxidative stress in a blood vessel and damage to the endothelium-dependent vasodilation is associated with a reduction in the production of nitric oxide (NO) or the increased production of the reactive oxygen species (ROS), in particular of superoxide anion (\(O_2^-\)) \(^\text{19}\). The effect of caffeine on blood vessel regulation is manifested through the balance of vasoconstrictor and vasodilatory effect \(^\text{20}\). At rest, caffeine either does not alter \(^\text{21}\) or improves the endothelial function \(^\text{20}\), but reduces it during physical activity \(^\text{21}\). Current data suggest that EDs decrease endothelial function at rest \(^\text{22,23}\). In mice, it was shown that the application of ED affects the reduction of peri-intestinal fat tissue, but also increases the pericardial fat tissue, which represents a significantly greater source of chemokines and cytokines with proinflammatory properties, compared to the subcutaneous fat tissue \(^\text{24}\), which further implies a larger production of ROS \(^\text{25}\). Particular importance is attributed to oxidative stress because it plays an important role in pathogenesis and the development of cardiovascular diseases.

There are very few preclinical studies in the literature examining the influence of EDs on the cardiovascular system. The aims of this study were to determine the acute and chronic effects of RB\(^\circ\) on cardiodynamics and oxidative stress in the coronary effluent in physically trained rats, as well as the effect of chronic RB\(^\circ\) consumption on acute consumption of RB\(^\circ\) for the indicated parameters (cardiodynamics and oxidative stress).

**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. Good Laboratory Practice and the European Council Directive (86/609EEC) were followed during the conception, design and performance of the study.

Subjects

The study was performed using the Wistar albino rats. The sample size calculation, based on a study published by Barcelos et al. 26, revealed that 24 rats were requisite to perform the study. At the beginning of the study rats were eight weeks old and their weight was 200-250g. They were kept in cages (8 rats in one cage) and fed with commercial rat food (20% protein food, Veterinary Institute Subotica) and water ad libitum. Temperature in the room was set to 25 °C, and 12 hours of light were provided.

Training protocol

The study lasted 4 weeks. All rats 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 constantly monitored during swimming.

ED consumption

Initially, rats were divided into two groups based on ED consumption during the study period (rats that did and did not consume ED 30 minutes before swimming). Later those two groups were further divided into groups based on ED consumption before the sacrificing (rats that did and did not consume ED before they were sacrificed). Thus, groups were as follows:

1) Control group (C-T): rats that did not consume ED either before swimming or prior to sacrificing (n = 6);

2) Acute ED group (acED-T): rats that did not consume ED before swimming, but did consume ED 30 minutes prior to sacrificing (n = 6);

3) Chronic ED group (chED-T): rats that consumed ED 30 min before every swimming training, but did not consume ED prior to sacrificing (n = 6);
4) Chronic + Acute group (ch + acED-T): rats that consumed ED 30 min before every swimming training and 30 min before sacrificing (n = 6).

The ED administration was performed 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. 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).

Cardiodynamic parameters

After short ketamine/xylazine narcosis, rats were sacrificed and their hearts were excised and attached to the Langendorff apparatus via aortic cannula. Krebs–Henseleit buffer was used during performance of retrograde perfusion according to the Langendorff technique. First, an equilibration period, during which coronary perfusion pressure (CPP) was kept at 70 cmH₂O, was performed. After that, CPP was changed in the following order: 1) 60 cmH₂O, 2) 80 cmH₂O, 3) 100 cmH₂O, 4) 120 cmH₂O, and 5) 40 cmH₂O.

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. 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 (O₂⁻), 2) hydrogen peroxide (H₂O₂), 3) nitrogen monoxide (NO), and 4) index of lipid peroxidation.
(TBARS). The exact protocols for measurement of those prooxidative species may be found in our previously published papers \(^{31}\) or in the original sources \(^{32-35}\).

**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**

**Cardiodynamics**

Cardiodynamic parameters of isolated rat hearts in four groups (C-T, acED-T, chED-T, ch + acED-T) are shown in Figures 1-6.

1. Acute application of the ED

In relation to the C-T group, the following were recorded in the acED-T group: 1) at all CPPs, statistically significantly higher level of dp/dt max (p < 0,05; t test for independent samples), significantly higher level of HR (p < 0,05; Mann-Whitney) and a significantly lower level of DLVP (p < 0,05; t test for independent samples); 2) at CPP 60-100 cmH\(_2\)O, statistically significantly higher level of SLVP (p < 0,05; t test for independent samples); 3) at CPP 60-120 cmH\(_2\)O, statistically significantly higher levels of dp/dt min and CF (p < 0,05; t test for independent samples).

2. Chronic application of the ED

In relation to the C-T group, the following were recorded in the chED-T group: 1) at all CPPs, statistically significantly higher level of HR (p < 0,05; t test for independent samples) and significantly lower level of DLVP (p < 0,05 Mann-Whitney); 2) at CPP 60-120 cmH\(_2\)O, statistically significantly higher level of CF (p < 0,05; Mann-Whitney); 3) at all CPP, higher level of SLVP, but only statistically significantly higher at CPP 60, 100 and 120 cmH\(_2\)O (p < 0,05; t test for independent samples) and higher level of dp/dt min,
but statistically significantly only at CPP 100-120 cmH₂O (p < 0.05; t test for independent samples); 4) at all CPP, higher level of dp/dt max, but without statistical significance (p > 0.05; t test for independent samples).

3. Chronic + acute application of the ED

In relation to the group acED-T, the following were recorded in the group ch + acED-T: 1) at all CPPs, lower level of dp/dt max and higher level of DLVP, but statistically significant for both parameters only CPP 40 cmH₂O (p < 0.05; t test for independent samples); 2) at all CPP, lower level of dp/dt min and CF, but without statistical significance (p > 0.05; t test for independent samples). There were no statistically significant differences in the levels of SLVP and HR between these two groups (p > 0.05; Mann-Whitney).

In relation to the chED-T group, in the group ch+acED-T were recorded lower level of dp/dt max and CF, as well as the higher level of DLVP, but without statistical significance (at all CPPs) (p > 0.05; t test for independent samples; Mann-Whitney test). There were no statistically significant differences in the levels of SLVP, dp/dt min and HR between these two groups (p > 0.05; Mann-Whitney; t test for independent samples).

In relation to the C-T group, the following were recorded in the group ch+acED-T: 1) at all CPPs, significantly higher level of HR (p < 0.05; t test for independent samples), higher level of SLVP, but only CPP 60 and 80 cmH₂O statistically significantly higher (p < 0.05; Mann-Whitney) and higher level of dp/dt min, but at CPP 60 -120 cmH₂O statistically significantly higher (p < 0.05; t test for independent samples); 2) at all CPPs, higher level of dp/dt max and CF, and lower level of DLVP, but without statistical significance (p > 0.05; t test for independent samples).

Oxidative stress

Prooxidative parameters in the effluent during coronary autoregulation of isolated rat hearts in four groups (C-T, acED-T, chED-T, ch + acED-T) are shown in Figures 7-10.

1. Acute application of the ED

In relation to the C-T group, the following were recorded in the acED-T group: 1) at all CPP, the level of TBARS (lipid peroxidation index) was statistically significantly higher (p < 0.05; Mann-Whitney); 2) at all CPP, levels of O₂⁻ and nitrites (NO) were higher, but
without statistical significance (p > 0,05; Mann-Whitney; p > 0,05; t test for independent samples, respectively). There was no statistically significant difference in the level of H$_2$O$_2$ between these two groups (p > 0,05; Mann-Whitney).

2. Chronic application of the ED

In relation to the C-T group, the following were recorded in the chED-T group: 1) at all CPP, the level of TBARS was statistically significantly higher (p < 0,05; Mann-Whitney); 2) at CPP 60, 80 and 120 cmH$_2$O, the level of O$_2^-$ was statistically significantly higher (p < 0,05; Mann-Whitney); 3) at all CPP, level of H$_2$O$_2$ was higher but statistically significant only at CPP 60 cmH$_2$O (p < 0,05; Mann-Whitney); 4) at all CPP, levels of nitrites (NO) were lower, but without statistical significance (p > 0,05; Mann-Whitney).

3. Chronic + acute application of the ED

In relation to the acED-T group, the following were recorded in the group ch+acED-T: 1) at all CPPs level of TBARS was statistically significantly higher (p < 0,05; t test for unbound samples); 2) at CPP 40, 80, 100 and 120 cmH$_2$O, the levels of nitrites (NO) were statistically significantly lower (p < 0,05; Mann-Whitney); 3) at all CPP, level of H$_2$O$_2$ was higher, but without statistical significance (p > 0,05; Mann-Whitney). There was no statistically significant difference in the level of O$_2^-$ between these two groups (p> 0,05; Mann-Whitney).

In relation to the chED-T group, the following were recorded in the group ch+acED-T: 1) at all CPPs, the levels of nitrites (NO) were lower, but statistically significant only at CPP 80-120 cmH$_2$O (p < 0,05; Mann-Whitney); 2) at all CPPs, the level of H$_2$O$_2$ was lower, but without statistical significance (p > 0,05; Mann-Whitney). There were no statistically significant differences in the levels of O$_2^-$ and TBARS between these two groups (p > 0,05; Mann-Whitney; p > 0,05; t test for independent samples).

In relation to the C-T group, the following were recorded in the group ch + acED-T: 1) at all CPPs, the level of TBARS was statistically significantly higher (p < 0,05; Mann-Whitney), while the level of O$_2^-$ was higher, but statistically significant only at CPP 60 and 80 cmH$_2$O (p < 0,05; Mann-Whitney); 2) at all CPPs, the levels of nitrites (NO) were lower, but statistically significant at CPP 40, 80-120 cmH$_2$O (p < 0,05; Mann-Whitney).
There was no statistically significant difference in the level of \( \text{H}_2\text{O}_2 \) between these two groups (\( p > 0.05; \) t test for independent samples).

**Discussion**

In this research, we studied the effect of RB\(^\circledR\) on cardiovascular parameters and coronary auto-regulation in the isolated heart of trained rats, as well as the level of pro-oxidative parameters in the coronary venous effluent. The training included swimming during the period of four weeks (1 hour/day, 5 days/week), which is considered to be a moderate intensity exercise. The specificity of swimming as an exercise is in engaging the muscles of the entire body, which improves the capacity of the cardiovascular system. The influence of RB\(^\circledR\) was evaluated at three levels: acute consumption, chronic consumption, as well as a combination of chronic and acute consumption.

Coronary autoregulation implies intrinsic cardiac ability to maintain blood flow relatively constant in response to a change in perfusion pressure when myocardial oxygen demand is constant \(^{36}\). The maximum rate of pressure change in the left ventricle (dp/dt max) occurs at the end of the isovolumetric contraction and is used to estimate the inotropic properties of the myocardium \(^{37}\), while the minimum rate of pressure change in the left ventricle (dP/dtmin) represents the relaxation rate (luzitropic properties of the myocardium) and reflects the maximum rate of pressure drop in the left ventricle \(^{38}\). In our study, the increase in the level of dp/dt max and dp/dtmin within the acED-T group was registered, when compared to the C-T group. In line with that, acute consumption of ED contributed to an increase in SLVP, HR, and CF, while DLVP was lower in the acED-T group.

As for the impact of RB\(^\circledR\) and other EDs on cardiodynamics, majority of previously published papers have focused on acute consumption and have been conducted on young, healthy people in a state of rest. As far as athletes are concerned, after consuming RB\(^\circledR\) and during their recovery phase following physical exercise, a significant increase in contractility of the left atrium and ventricle was registered \(^3\). It is believed that most of the biological effects of EDs are mediated by a positive inotropic effect \(^{15}\), which is in line with our results. As for humans, it has been demonstrated that the acute consumption of 250 ml
of RB® affects the increase in mean arterial pressure \(^2\), but there are also studies that have shown no effect on systolic and diastolic arterial pressure in rest \(^2\). Also, in terms of the effect of ED on the HR, non-homogeneous results were obtained (mostly with no effect or increase in the HR) \(^2\), but there was also a study published, showing how ED influenced the reduction of the HR \(^3\). It has been shown that a higher dose of RB® (355 ml) affects the increase in systolic and diastolic arterial pressure and the increase in the HR \(^4\), as well as that RB® at a dose of 500 ml affects the increase in the activity of the sympathetic nervous system \(^1\). The previously said can be explained by the effect of EDs on the increase in norepinephrine levels \(^2\), which increases the HR and blood pressure, triggers the release of glucose from energy stores and increases blood flow to skeletal muscles \(^6\).

The dP/dt min level within the chED-T group was higher when compared to the C-T group, indicating a positive lysithropic effect of the ED, while a decrease in DLVP was also registered. As it is the case with acute ED consumption, chronic consumption has also affected the increase in the HR and coronary flow. The fact that there was no significant difference in the dp/dt max level between the chED-T and the C-T group, as well as that SLVP was significantly higher in the chED-T group, can be interpreted as a negative influence of chronic ED consumption. Hypertension can cause left ventricular hypertrophy, which is a risk factor for future cardiovascular events \(^4\). In pre-clinical studies, chronic use of ED has mainly been evaluated through their effect on heart metabolism and, in accordance with our results, a negative effect on the heart of the rats has been registered \(^1\). Regular moderate exercise has beneficial effects on heart \(^8\), but our results show the chronic consumption ED can disrupt that effect.

Compared to acED-T, significantly lower levels of dp/dt max were observed in the ch+acED-T group, which may indicate slight depression in cardiac contractile force and systolic function. Also, when compared to acED-T group, the lower values of dp/dt min (less negative) and CF were registered in the ch+acED-T group and, although they were not statistically significant, in combination with a significantly higher level of DLVP, they may be interpreted as mild changes in diastolic function. The obtained results suggest that EDs affect in a different way on the cardiovascular system in chronic consumers when they acutely consume EDs than when it comes to occasional acute consumption. Given that the consumption of EDs by athletes is still a controversial topic, in terms of whether the benefits for improving the performance are greater than potential health hazards, these
results which describe how chronic consumption of EDs affects cardiovascular response in acute consumption can be useful for further research on this topic.

In groups acED-T and chED-T, when compared to the C-T, there was a significant increase in lipid peroxidation index level (estimated through the TBARS level), which indicates the deterioration of redox status. Also, a significant increase in TBARS level was observed in the ch+acED-T group, when compared to the acED-T group, which suggests that in chronic consumers acute ED consumption continues to deteriorate redox status. The intense lipid peroxidation in biological membranes leads to the loss of fluidity, a decrease in the membrane potential, the increased permeability for H⁺ and other ions and, in the end, a membrane rupture may also occur with the release of cellular content into extracellular space. Our results are consistent with an increase in lipid peroxidation observed in the liver and brain of rats, after 14 days of using another commercially available ED. In the chED-T group, an increased level of prooxidative species, O₂⁻ and H₂O₂, was also registered, when compared to the C-T. It is known that O₂⁻ and H₂O₂ affect the activation of the mitochondrial permeability transition pores, which leads to the loss of cytochrome C from mitochondria and the activation of caspases with the development of apoptosis. Generally, larger amount of O₂⁻ reacts with NO, reducing its bioavailability and damaging endothelium-dependent vasodilatation. A moderate intensity training leads to a reduction in TBARS, O₂⁻, and H₂O₂, while, in our study, chronic ED consumption, in combination with moderate intensity training, had the opposite effect and caused an increase in TBARS, O₂⁻, and H₂O₂.

In the ch + acED-T group, when compared with the acED-T group, a significant decrease in the NO level was registered (estimated through the level of nitrite). It is known that atherosclerosis occurs due to the mechanism of vascular inflammation, which is defined by the increased production of reactive oxygen species and due to the fact that endothelial dysfunction is characterized by the reduced production of NO. NO is produced from L arginine and represents an important endogenous basal coronary tone regulator, while reactive hyperaemia and shear stress are a stimulus for the release of NO from the endothelium and the formation of vasodilatation. NO leads to the relaxation of the smooth muscles of coronary vessels, inhibits adhesion and platelet aggregation, inhibits leukocyte activation and reduces the consumption of oxygen in the myocardium. High glucose levels in EDs can be a factor that contributes to the damaging of platelet function and the
occurrence of endothelial dysfunction. Hyperglycemia contributes to an increase in oxidative stress markers, and lipid peroxidation in erythrocytes is directly proportional to in vitro glucose concentration.

As already mentioned, EDs reduce endothelial function in humans at rest, and our results in rats show that this also applies to physical activity, for chronic consumers when they consume EDs acutely. On the other hand, acute administration of RB® at a dose of 250 ml and 355 ml has been shown to improve the endothelial function, and this topic is an open field for further studies. Due to endothelial dysfunction, EDs consumption is associated with an increased risk of myocardial ischaemia. Previous evidence linking ED consumption with myocardial ischaemia are mainly based on case reports. The lack of randomized and prospective researches is a major obstacle to the impossibility to establish an unambiguous connection between excessive ED consumption and ischemia or myocardial infarction.

Conclusion

While the acute effects of EDs on the cardiovascular system are fairly clarified, chronic effects are much less studied and further researches are suggested. The conclusion of our study is that acute administration of the ED had a positive inotropic effect, while chronic administration affected the isolated increase in SLVP, which could be considered the potentially negative impact of the ED. Chronic administration of EDs changed the cardiovascular response in acute consumption. Also, the prooxidative effect of the ED was observed. Due to the potential association of ED consumption with the onset of endothelial dysfunction and potential morbidity combined with physical exercise, further researches are needed to clarify action mechanisms and significance of their effects, i.e. the correlations with clinical outcomes.

Acknowledgement

This work was supported by Grant No. 175043 from the Ministry of Science and Technical Development of the Republic of Serbia and Junior project 01/15 by Faculty of Medical Sciences, Kragujevac, Serbia.

REFERENCES


34. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Anal Biochem 1982; 126(1): 131-8.


Figure legends

Fig. 1 − Values of maximum rate of left ventricular pressure development during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure. Data are means ± SE.

Fig. 2 − Values of minimum rate of left ventricular pressure development during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure. Data are means ± SE.
Fig. 3 – Values of systolic left ventricular pressure during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure. Data are means ± SE.

Fig. 4 – Values of diastolic left ventricular pressure during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure. Data are means ± SE.

Fig. 5 – Values of heart rate during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure. Data are means ± SE.

Fig. 6 – Values of coronary flow during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure. Data are means ± SE.

Fig. 7 – Values of superoxide anion radical in effluent, during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure. Data are means ± SE.

Fig. 8 – Values of hydrogen peroxide in effluent, during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure. Data are means ± SE.

Fig. 9 – Values of nitrites in effluent, during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure. Data are means ± SE.

Fig. 10 – Values of index of lipid peroxidation in effluent, during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure. Data are means ± SE.
Figures

Fig. 1 – Values of maximum rate of left ventricular pressure development during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure. Data are means ± SE.

Fig. 2 – Values of minimum rate of left ventricular pressure development during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure. Data are means ± SE.
Fig. 3 – Values of systolic left ventricular pressure during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure. Data are means ± SE.

Fig. 4 – Values of diastolic left ventricular pressure during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure. Data are means ± SE.
Fig. 5 – Values of heart rate during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure. Data are means ± SE.

Fig. 6 – Values of coronary flow during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure. Data are means ± SE.

Fig. 7 – Values of superoxide anion radical in effluent, during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure. Data are means ± SE.
Fig. 8 – Values of hydrogen peroxide in effluent, during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure. Data are means ± SE.

Fig. 9 – Values of nitrites in effluent, during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure. Data are means ± SE.
Fig. 10 – Values of index of lipid peroxidation in effluent, during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure. Data are means ± SE.

Received on January 19, 2019.
Revised on March 7, 2019.
Accepted March 11, 2019.
Online First March, 2019.