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ARTICLE

Hypotensive, cardiodepressant, and vasorelaxant activities of black currant (*Ribes nigrum* 'Ben Sarek') juice[1]

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Abstract: The aim of this study was to evaluate the effects of black currant (*Ribes nigrum* L. 'Ben Sarek') juice on the blood pressure and frequency of cardiac contractions, as well as vasomotor responses of rat aortic rings. Arterial blood pressure was measured directly from the carotid artery in the anaesthetized rabbits. The aortic rings were pre-contracted with KCl (80 mmol·L^{-1}), after which black currant juice was added. An intravenous injection of black currant juice (0.33–166.5 mg·kg^{-1}) induced a significant and dose-dependent decrease of rabbit arterial blood pressure and heart rate. The black currant juice decreased arterial blood pressure of rabbit by 22.33% ± 3.76% ($p < 0.05$) and heart rate by 17.18% ± 2.93% ($p < 0.05$). Cumulative addition of the black currant juice (0.01–3 mg·mL^{-1}) inhibited concentration-dependent KCl induced contractions of the isolated rat aorta. The black currant juice, at the concentration of 3 mg·mL^{-1}, caused a maximum relaxation of 21.75% ± 3.15% ($p < 0.05$). These results demonstrate that black currant juice can induce hypotension. The hypotensive effect of the black currant may occur as the consequence of its inhibitory activity on the rate of heart contraction and vasorelaxant effects.

Key words: black currant, juices, hypotensive activity, cardiodepressant activity, vasorelaxation.

Résumé : Cette étude avait pour but d'évaluer les effets du jus de cassis (*Ribes nigrum* L. 'Ben Sarek') sur la tension artérielle et la fréquence des contractions du cœur ainsi que sur la réponse vasomotrice dans un modèle d'anneaux aortiques chez le rat. Nous avons mesuré la tension artérielle directement à partir d'artères carotides de lapins anesthésiés. Nous avons fait précontracter les anneaux aortiques avec du KCl (80 mmol·L^{-1}), puis ajouté du jus de cassis. L'administration intraveineuse de jus de cassis (de 0,33 à 166,5 mg·kg^{-1}) a entraîné une importante diminution liée à la dose de la tension artérielle et de la fréquence cardiaque des lapins. Le jus de cassis a entraîné une diminution de la tension artérielle des lapins de 22,33 ± 3,76 % ($p < 0,05$) et de leur fréquence cardiaque de 17,18 ± 2,93 % ($p < 0,05$). L'ajout de quantités cumulatives de jus de cassis (de 0,01 à 3 mg·mL^{-1}) a entraîné une inhibition liée à la concentration de la contraction provoquée par le KCl dans les aortes isolées de rats. À la concentration de 3 mg·mL^{-1}, le jus de cassis a entraîné une décontraction maximale de 21,75 ± 3,15 % ($p < 0,05$). Ces résultats montrent que le jus de cassis peut provoquer une hypotension. L'effet hypotenseur du jus de cassis pourrait être la conséquence de son activité inhibitrice sur la fréquence de la contraction du cœur et d'effets de vasorelaxation. [Traduit par la Rédaction]

Mots-clés : cassis, jus, activité hypotensive, activité de dépression de la fonction cardiaque, vasorelaxation.

Introduction

Cardiovascular diseases represent a major cause of death in developed countries (Joshipura et al. 2001). Uncontrolled and prolonged elevation of blood pressure is the most common cardiovascular risk factor, contributing to the widespread morbidity and mortality (Cacanyiova et al. 2012; Louhelainen et al. 2009). Lifestyle changes, physical exercise, and intake of healthy diets can decrease blood pressure and reduce the risk for cardiovascular diseases. Scientific reports have shown that medicinal plants, fruits, and vegetables have the ability to cure, prevent, or delay the progression of cardiovascular diseases (Gopalan et al. 2012; Kruger et al. 2014; Nöthlings et al. 2008; Yang and Kortesniemi 2015). Black currant (*Ribes nigrum* L., family Grossulariaceae) has been used in both Asian and European traditional medicine for treating various ailments (Declume 1989; Suzutani et al. 2003). The contents of ascorbic acid and polyphenol, especially anthocyanins, in the berries are high and, consequently, the juice and extracts of the berries have a high antioxidant activity. Delphinidin-3-*O*-ruthinoside (2.44 mg·100 g^{-1}) was the dominant anthocyanin in *R. nigrum* 'Ben Sarek'. A detailed chemical profile and antioxidative activity of tested 'Ben Sarek' juice was reported by Miladinović et al. (2014).

Most of the chronic non-communicable diseases (CND), including the heart disease, have an inflammatory component. Among other medicinal properties, anthocyanins expressed anti-inflammatory activity (Tabart et al. 2012) and the ability to lower the risk of CND (Hakimuddin and Paliyath 2011; Ostertag et al. 2010). Generally, anthocyanins have been associated with improved cardiovascular risk profiles because they can help maintain a healthy vascular system (Taylor 2011). Thus, several clinical studies have shown that black currants can improve blood flow, decrease LDL choles-

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terol, and reduce the serum inflammatory markers in patients with peripheral artery disease (Dalgård et al. 2009; Matsumoto et al. 2005; Tahvonen et al. 2005). Gamma-linolenic acid from the oils of black currants induced a decline in blood pressure levels in rats with spontaneous hypertension (Edirisinghe et al. 2011; Engler 1993).

The aim of the present study was to evaluate the possible hypotensive and cardiac actions of black currant juice. An assessment of the existing black currant literature showed no data on its effect on the cardiovascular system. Based on our previous experimental work and the fact that the black currant has a large amount of polyphenols (Miladinović et al. 2014), which may affect the function of the cardiovascular system, our objective was to examine the in vivo effects of the juice of 'Ben Sarek' black currant on the blood pressure and frequency of cardiac contractions, and in vitro effects on the vasomotor responses of rat aortic rings.

Materials and methods

Drugs

Verapamil was obtained from Sigma–Aldrich (St. Louis, Missouri, USA). Heparin sodium salt was purchased from Hemofarm (Vrsac, Serbia) and urethane was obtained from Pliva (Zagreb, Croatia). All drugs were dissolved in distilled water and used immediately.

Plant material

The juice of black currant (*R. nigrum* 'Ben Sarek') was prepared using the procedure described by Miladinović et al. (2014). Fully ripe berries were harvested from the experimental field in Obrenovac (coordinates: 44°37′34″N, 20°13′36″E), Serbia, by hand. The juice was made from the fresh undamaged samples of berries by crushing them manually. The samples (juice) were kept in sealed bottles at –18 °C until further analyses. Supernatants obtained after centrifugation of the juice (15 000*g*, 10 min) were used for the experiments. Up until the centrifugation process, the juice was kept in the freezer for 3 months.

Animals

All animal experiments were performed according to the procedures approved by the EU Directive 2010/63/EU for animal experiments and were also approved by the Animal Care Committee of the Medical faculty in Nis (No. 01-206-7).

The rabbits (1.5–2.0 kg) and Wistar albino rats (200–250 g) used in this study were obtained from the Animal Research Center of the Medical Faculty, University of Nis, Serbia. The animals were housed in stainless steel cages under standard laboratory conditions. These animals were maintained at 20–24 °C with a 12 h light – 12 h dark cycle at least 1 week before the experiment. All animals had free access to food and water.

Blood pressure measurement in anaesthetized rabbits

In these experiments, adult male rabbits were used and arterial blood pressure was recorded as described previously (Kitic et al. 2012). The animals were anesthetized intravenously with urethane (750 mg·kg^{-1}) and the blood pressure was measured via carotid-cannulation. The arterial catheter was connected to a blood pressure transducer (P-1000-A) coupled with a Narco physiograph (NARCO Bio system, Houston, Texas, USA) for arterial pressure measurement.

The blood pressure was measured before and after black currant juice administration. Arterial pressure was allowed to return to the resting level between injections. Changes in blood pressure were recorded as the difference between the steady state values before and the peak readings after the injection. Animals were treated with black currant juice, which was administered in rising concentrations (0.33–166.5 mg·kg^{-1}) at intervals of 20–25 min. The juice of black currant was diluted from a stock solution in sterile isotonic saline immediately before its administration.

Mean blood pressure was calculated according to the following formula: diastolic pressure + 1/3 pulse pressure (mm Hg). The heart rate was estimated by counting the arterial pressure recordings. The blood pressure and heart rate were recorded at a chart speed of 10 mm·s^{-1}.

Isolation of the rat aorta and recording of contractions

Isolated rat aorta was used for the study. Aortic rings were mounted in 10 mL tissue bath containing Krebs solution at 37 °C and aerated with carbogen. The composition of Krebs solution was (mmol·L^{-1}): NaCl 118.2, KCl 4.7, $CaCl_2{\cdot}2H_2O$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 11.7, $NaHCO_3$ 25.0, and EDTA 0.026.

An equilibrium period of 60 min was given before any drug was added. High K^+ (80 mmol·L^{-1}) doses were used to induce sustained contractions. The black currant juice (0.01–3 mg·mL^{-1}) was then added to the organ bath, and the relaxation was evaluated as percentage of the induced vasoconstriction. In the second experimental series, the aortic rings were pre-contracted with KCl (80 mmol·L^{-1}), and then verapamil (0.015–1.5 μg·mL^{-1}), a calcium channel blocker, was added.

Tension changes in the tissue were recorded using a transducer (TSZ-04-E; Experimetria Ltd, Budapest, Hungary) and analyzed with a SPEL Advanced ISOSYS Data Acquisition System (Experimetria Ltd, Budapest, Hungary).

Statistical analysis

All data were presented as mean ± standard error of the mean of 6 determinations. Statistical evaluation was performed using the Student's *t* test. Significant differences were accepted when the *p* value was <0.05. The mean effective doses, EC_{50}, that is the concentration which elicited 50% of maximal response, were established by regression analysis. Correlations among the amount of total polyphenols, dominant anthocyanins, and the values of EC_{50} for heart rate and blood pressure were calculated following Pearson's correlation coefficient method ($p < 0.05$). Statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, Illinois, USA).

Results

Effects of the black currant juice on blood pressure

In anaesthetized rabbits, baseline mean blood pressure did not vary and mean value of mean blood pressure was 95.72 ± 8.57 mm Hg. Intravenous administration of the black currant juice produced fall in systolic, diastolic, and mean arterial blood pressure. Their hypotensive effects were dose-dependent. The black currant doses of 166.5 mg·kg^{-1} induced significantly fall in the mean blood pressure by 22.33% ± 3.76% ($p < 0.05$), with EC_{50} value of 14.33 ± 2.77 mg·kg^{-1} (Fig. 1). After the hypotensive peak, the blood pressure increased progressively and reached the initial basal value in about 3–5 min depending on the dose. Intravenous administration of 0.9% NaCl (1 mL·kg^{-1}) did not cause significant changes in the blood pressure parameters.

Effects of the black currant juice on heart rate

The intravenous injection of the black currant juice induced a dose-dependent decrease of heart rate in anaesthetized rabbits. The maximum fall in heart rate by 17.18% ± 2.93% beats·min^{-1} was recorded at the dose of 166.5 mg·kg^{-1}, with EC_{50} value of 17.51 ± 2.34 mg·mL^{-1} ($p < 0.05$) (Fig. 2). Intravenous administration of 0.9% NaCl (1 mL·kg^{-1}) did not cause significant changes in the heart rate.

Effects of the black currant juice on isolated aorta

The addition of KCl (80 mmol·L^{-1}) generated sustained contractions in the isolated aorta. Cumulative addition of the black currant juice inhibited the concentration-dependent KCl induced contractions of isolated rat aorta. The black currant juice at the concentration of 3 mg·mL^{-1} caused a maximum relaxation of

Fig. 1. Hypotensive effect of the black currant (*Ribes nigrum* L.) juice in anaesthetized rabbits. The results show that juice of black currant decreased the mean blood pressure significantly (*p < 0.05). Values are mean ± standard error of the mean of 6 experiments.

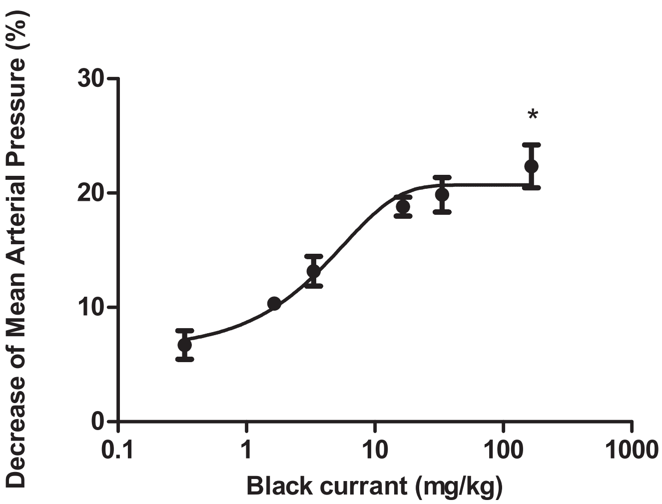


Fig. 2. Effect of the black currant (*Ribes nigrum* L.) juice on heart rate in anaesthetized rabbits. The results show that juice of black currant decreased the heart rate significantly (*p < 0.05). Values are mean ± standard error of the mean of 6 experiments.

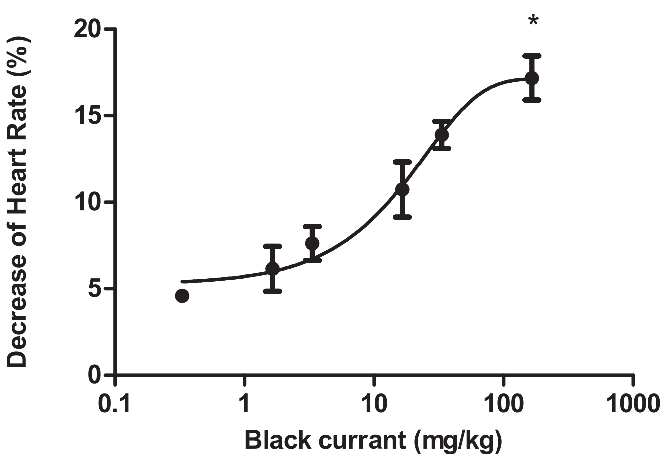
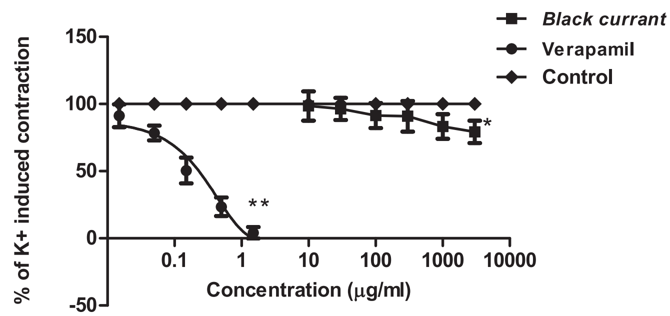


Fig. 3. Effects of the verapamil and the black currant (*Ribes nigrum* L.) juice on the KCl (80 mmol·L^{-1})-induced contractions in isolated rat aorta preparations. The results show that juice of black currant decreased the contractions of aorta significantly (*p < 0.05 and **p < 0.01). Values are mean ± standard error of the mean of 6 experiments.



21.75% ± 3.15%, with EC_{50} value of 132.06 ± 18.92 mg·mL^{-1} (Fig. 3). In comparison, verapamil induced vasorelaxation in aortic rings constricted with KCl, with an EC_{50} value of 0.15 ± 0.06 μg·mL^{-1}.

Discussion

The aim of the present work was to investigate the effects of black currant (*R. nigrum* 'Ben Sarek') juice on blood pressure and evaluate the possible mechanism responsible for this effect. The results showed that the black currant juice induced a hypotensive effect in anesthetized rabbits. The intravenous injection of the black currant juice produced a dose-dependent decrease in the mean blood pressure. The hypotensive effect was short-term and the blood pressure reached the basal value in about 3–5 min. Those results were consistent with a study of Engler (1993) who showed that gamma-linolenic acid from the oils of black currants induced a decline in blood pressure levels in rats with spontaneous hypertension. Furthermore, Toyoshi and Kohda (2004) reported that administration of anthocyanin cyanidin-3-*O*-glucoside lowered the blood pressure of spontaneously hypertensive rats. It has also been suggested that anthocyanins express an endothelium-dependent inhibitory effect on arterial contraction (Mendes et al. 2003). Xu et al. (2004) reported that cyanidin-3-*O*-glucoside upregulates eNOS. Endothelium-dependent relaxation activity in porcine coronary arteries caused by anthocyanin extracts from chokeberry, bilberry, and elderberry was noted (Toufektsian et al. 2008). Statistical analysis established a high correlation between EC_{50} value of blood pressure and the content of total anthocyanins (r = −0.99), as well as delphinidine-3-*O*-glucoside (r = −0.965) and cyanidine-3-*O*-glucoside (r = −0.988). High correlation coefficients were also found between the percentage of blood pressure and heart rate decrease with the same bioactive compounds.

Edirisinghe et al. (2011) reported that black currant concentrates made from cultivars 'Ben Gairn' and 'Ben Hope' activated an eNOS via Akt/PI3 kinase pathway in vitro in human endothelial cells.

Blood pressure is a product of cardiac output and vascular resistance; hence, black currant juice was studied for its possible inhibitory effects on heart rate and isolated rat aorta. We found that the intravenous injection of the black currant juice induced a dose-dependent decrease of heart rate. The negative chronotropic effect of black currant may be responsible for its hypotensive activity.

Isolated rat aorta was used for the possible mode study of the black currant hypotensive action. The present results demonstrated that the black currant juice concentration-dependently relaxed the contractions of the isolated rat aorta induced by KCl. These findings suggested that black currant possessed vasodilatory activities. Hypotensive effect of black currant was the outcome of vascular smooth muscle relaxation and decrease of peripheral resistance. It was reported that the black currant extract increased eNOS activation in human umbilical vein endothelial cells (Edirisinghe et al. 2011).

The literature has shown that the contraction of smooth muscle preparation is dependent upon the increase in the cytoplasmic free Ca^{2+} which activates the contractile elements. The KCl-induced smooth muscle contraction is mediated by the cell membrane depolarization and an increase in Ca^{2+} influx through voltage-operated calcium channels (Bolton 1979; Karaki and Weiss 1984). The possibility that the black currant could induce vasodilatory effect via decrease of calcium influx was examined in this study. The finding of this study was that the juice of black currant was able to relax the KCl-induced contractions of rat aorta.

Using a combined in vivo and in vitro approach, the present investigation shows that the juice of black currant induced hypotension. The hypotensive effect of the black currant may result from its inhibitory activity on rate of heart contraction (negative chronotropic effect) and vasorelaxant effects.

Conflict of interest

The authors declare that there is no conflict of interest, financial or otherwise, associated with this work.

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