Original Article

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Comparison of Cardiovascular Effects of Ribes Khorassanicum Fractions with Its Total Extract in Normotensive Rats

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ABSTRACT

BACKGROUND AND OBJECTIVE: Ribes khorassanicum has beneficial cardiovascular effects in normotensive and hypertensive animals. For further investigation, the effect of three fractions of aqueous (polar), ethyl acetate (polar and non-polar) and n-hexane (non-polar) of the plant's hydroalcoholic extract on cardiovascular parameters in normotensive rats was compared with its total extract.

METHODS: In this experimental study, 42 rats were divided into 7 groups (6 in each group): control, three doses of total extract 4, 12 and 24 mg/kg and three fractions of aqueous, ethyl acetate and n-hexane (20 mg/kg). Femoral artery cannulation and heart rate (HR), mean arterial pressure (MAP) and systolic blood pressure (SBP) were recorded throughout the experiment and then the changes (Δ: difference before and after injection) were calculated and statistically analyzed.

FINDINGS: 24 mg/kg of the extract significantly increased ΔHR (117.5±11.6 beats per minute) compared to control (5.3±2.7, $p<0.001$) and 12 mg/kg of the extract significantly decreased ΔMAP (-15.1±2.8 mm Hg) and ΔSBP (-18.4±3 mm Hg) compared to the control ($p<0.05$). The fractions increased ΔHR, which was not significant but was significant compared to 24 mg/kg of the extract ($p<0.05$). Furthermore, the increase of ΔMAP and ΔSBP in n-hexane fraction (19.45±3.1 mm Hg and 34.6±11.5 mm Hg, respectively) was significant compared to the control and 12 mg/kg of the extract ($p<0.01$ to $p<0.05$).

CONCLUSION: The active ingredients in the cardiovascular effects of Ribes khorassanicum extract are spread in all fractions and the total extract has a better effect than each of the fractions alone due to its various non-polar and polar compounds as well as the synergistic effect.

KEY WORDS: *Ribes Khorassanicum, N-Hexane Fraction, Blood Pressure, Heart Rate.*

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Introduction

Cardiovascular disease is one of the most common causes of death in Iran and the world. Every year, more than 51 million people die due to this disease (1). Many factors are known as risk factors for cardiovascular disease, one of the most important of which is hypertension (2). Although several chemical drugs are available to control blood pressure, due to the side effects of drugs and their high cost, the general popularity of herbal medicines to control and treat diseases such as hypertension has increased recently. Among the medicinal plants that have traditionally been effective in lowering blood pressure are Ribes plants (3).

About 150 species of this genus have been reported worldwide, among which Ribes khorassanicum, R. orientale, R. Biebersteinii, R. uva-crispa and R. melananthum can be found in Iran (3, 4). One of the rare species of this plant with the scientific name of Ribes khorassanicum (R. Khorasanicum) from the genus Ribes and the genus Grossulariaceae is native to Khorasan and was introduced in 1996 from northeastern Khorasan by Adibi et al. and Assadi. (3, 5). Local name R. khorasanicum is a cranberry as well as a grossularia that is used to treat hypertension and digestive problems (3, 5). There is little information about the active ingredients and pharmacological effects of this plant. In previous studies, the antimicrobial and antioxidant effect of the plant has been observed (3). It also contains active ingredients such as flavonoids, anthocyanins, tannins and saponins (6).

In previous studies, R. khorasanicum extract was quantitatively studied in terms of phenolic compounds, flavonoids and anthocyanins and the amount of these compounds were 44.6, 37.9 and 9.7 mg/g of crude extract, respectively. The effect of different doses of total extract (4, 12 and 24 mg/kg) of this plant on cardiovascular responses in rats with normal blood pressure was investigated and it was shown that different doses of this plant in normotensive animals have different effects on cardiovascular parameters and low doses reduced blood pressure and high doses caused tachycardia (7, 8). In pilot studies, doses higher than 35 mg/kg were also toxic, causing severe tachycardia and animal death. This plant reduces the hypertensive effects of angiotensin II and has antioxidant effects on heart tissue (7). Furthermore, the effect of R. khorasanicum on hypertension induced by L-NAME - which is a nitric oxide synthesizing enzyme inhibitor - was investigated and its beneficial effects were shown (8). Due to the fact that increasing oxidative stress by increasing the activity of angiotensin II as one of the important factors affecting the arteries, causes increase in blood pressure (9), reducing oxidative stress through antioxidants in this plant such as flavonoids, anthocyanins and phenolic compounds can reduce blood pressure by affecting the activity of angiotensin II (7). Considering the different effects of total extract doses on cardiovascular responses, for further investigation, the total extracts in non-polar (n-hexane fraction), polar-non-polar (ethyl acetate fraction), and polar (aqueous fraction) phases were isolated and the effects of these fractions on cardiovascular responses in normotensive rats were compared with total extracts to determine which of the polar, polar or non-polar substances had an effect on the cardiovascular system.

Methods

Animals and study groups: In this experimental study, 42 male Wistar rats with an average weight of 240±10 g were used. Rats were kept in a standard environment and had no restrictions on water or food. Their storage conditions were 12 hours of light and 12 hours of darkness. This study was approved with the code of ethics IR.MUMS.fm.REC.1396.30 in Mashhad University of Medical Sciences. The animals were divided into 7 groups (6 rats in each group) as follows:

1) Control group: Rats in this group were intraperitoneally injected with saline instead of the extract.

2, 3, 4) Groups of hydroalcoholic extract of R. khorassanicum: rats of these groups intraperitoneally received the hydroalcoholic extract of the plant at doses of 4, 12 and 24 mg/kg (7).

5, 6, 7) Groups of aqueous, ethyl acetate and n-hexane fractions of R. khorassanicum extract: In rats of the mentioned groups, 20 mg/kg aqueous, ethyl acetate and n-hexane fractions of the extract were injected intraperitoneally.

Preparation of hydroalcoholic extract and related fractions: hydroalcoholic extract of R. Khorassanicum was obtained from 100 g powder of the dried plant mixed with 540 ml distilled water and 1260 ml ethanol 70% at 42 °C and the mixture was cleared after 72 hours and the solvent was removed by rotary apparatus at 50 °C. The dried extract was weighed and the desired concentrations were prepared by adding the appropriate amount of distilled water (10). To prepare the fractions, 10 g of the prepared extract was mixed with 100 ml of ethanol and transferred to a decanter funnel. N-hexane solvent was added to the decanter funnel and the

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n-hexane fraction was extracted. Then, the remaining solution from the previous step was combined with ethyl acetate solvent and the ethyl acetate fraction was extracted and finally, the remaining solution in the decanter funnel was considered as the aqueous fraction (11, 12) (Figure 1).

Method: First, the animals were anesthetized intraperitoneally with urethane at a dose of 1.5 g/kg (13). After anesthesia, to record blood pressure and heart rate, the femoral artery of rats was cannulated by angiocatheter filled with heparin saline solution. The angiocatheter was then connected to the power label via a pressure transducer. Then blood pressure and heart rate were transferred from PowerLab to the computer and continuously recorded by Lab chart 8 software. After recording, the animal was rested for 10 minutes so that the cardiovascular parameters would be fixed (14). Then, rat in the experimental, saline, extract and fractions groups were injected intraperitoneally with a volume of 0.5 ml. Responses were recorded for 30 minutes after injection (15).

Data collection and analysis: Cardiovascular responses including systolic blood pressure, mean arterial pressure and heart rate were collected and then their changes (Δ) were calculated after injection of extracts and fractions. The results were expressed as mean and standard error (Mean±SEM) of expression changes and were statistically analyzed in different groups using ANOVA and Tukey post - hoc test and $p<0.05$ was considered significant.

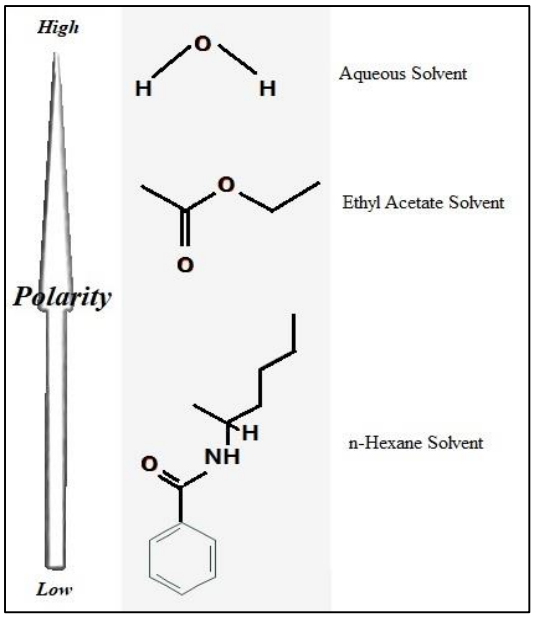

Figure 1. The polarity of the solvents used to prepare fractions

Results

Effects of different doses of total R. khorasanicum extract on cardiovascular responses: Heart rate changes increased at doses 4 and 12 (6.4±3.1 and 6.8±4.8 beats per minute), but these changes were not significant compared to control group (5.3±2.7). 24 mg/kg of R. khorasanicum extract significantly increased heart rate (117.5±11.6 beats per minute, $p<0.001$) (Figure 2). 4 and 12 mg/kg doses reduced mean arterial pressure (-8.5±3.2 and -15.1±2.8 mm Hg, respectively) and systolic blood pressure (-10.8±4.2 and -18.4±3.3 mm Hg, respectively). This decrease was significant only at 12 mg/kg compared to the control group (mean arterial: 2.5±1.24 and systolic: 4.6±3.2 mm Hg) ($p<0.05$). 24 mg/kg dose had no significant effect on mean arterial pressure (2.8±1.99 mm Hg) and systolic blood pressure (-2.7±1.09 mm Hg) compared to the control group (Figures 2 and 3).

The effect of different fractions of hydroalcoholic extract of R. khorasanicum on cardiovascular responses: In this part of the study, the effect of 20 mg/kg of each of the three fractions of the extract on changes in cardiovascular parameters was evaluated and it was found that all three fractions significantly increased heart rate (aqueous fraction: 9.4±4.5, ethyl acetate fraction: 32.5±7.5 and n-hexane fraction: 34.6±11.5 beats per minute) and these values were significantly different from the dose of 24 mg/kg (117.5±11.6) ($p<0.01$). In addition, there was no significant difference between the three fractions (Figure 2). Changes in mean arterial pressure (aqueous fraction: -2.3±1.5, ethyl acetate fraction: 4.4±2.7 and n-hexane fraction: 9.45±3.1 mm Hg) and systolic blood pressure (aqueous fraction: -3.82±1.5, ethyl acetate fraction: 5.38±2.6 and n-hexane fraction: 34.6±11.5 mm Hg) were different in the three fractions; the aqueous fraction had no effect on cardiovascular responses but ethyl acetate and n-hexane fractions increased them but these effects were not significant compared to control group (Figures 3 and 4). There was no significant difference between the three fractions.

Comparison of the effects of different fractions with total extract: The three fractions increased heart rate. However, changes caused by these fractions were not significant compared to doses of 4 and 12 mg/kg of total extract. Neverthelss, all fractions were significant in increasing heart rate at the dose of 24 mg/kg (117.5±11.6 beats per minute) ($p<0.05$). Changes in mean arterial pressure and systolic blood pressure increased in ethyl acetate and n-hexane fractions and

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these effects showed significant differences only compared to 12 mg/kg of total extracts ($p<0.01$ to $p<0.05$) (Figures 2-4).

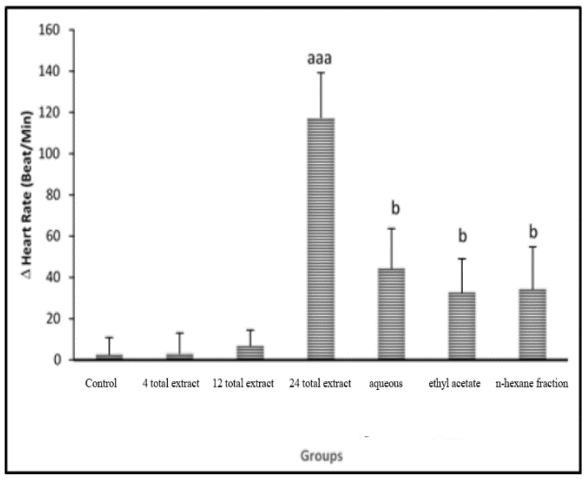

Figure 2. Effect of different doses of R. khorasanicum extract and aqueous, ethyl acetate and n-hexane fractions on heart rate changes. The dose of 24 mg/kg of extracts caused a significant increase. All three fractions were significant at the dose of 24 mg/kg ($p<0.001$); aaa: significant difference compared to the control group ($p<0.05$); b: significant difference compared to the extract group with a dose of 24 mg/kg, Δ: Changes before and after injection

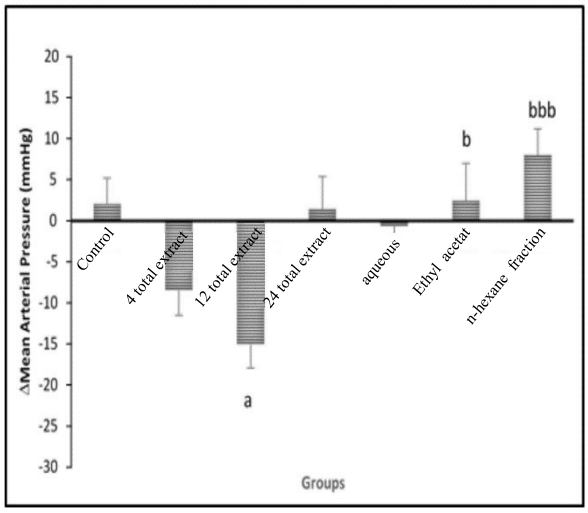

Figure 3. Effect of different doses of R. khorasanicum extract and 20 mg/kg aqueous, ethyl acetate and n-hexane fractions on mean arterial pressure. 12 mg/kg of total extracts caused a significant increase ($p<0.05$; a), ethyl acetate and n-hexane fractions were significantly higher than 12 mg/kg total extract ($p<0.05$; b and $p<0.001$; bbb). Number of rats was 6 in each group. Significant difference compared to the control group, $p<0.05$; significant difference compared to the control group, $p<0.05$; b and $p<0.001$; bbb significant difference compared to 12 mg/kg total extract group, Δ: Changes before and after injection

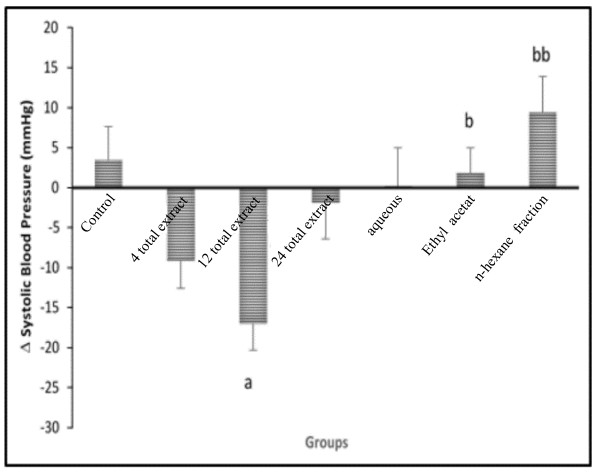

Figure 4. Effect of different doses of R. khorasanicum extract and 20 mg/kg aqueous, ethyl acetate and n-hexane fractions on systolic blood pressure. Only the dose of 12 mg/kg total extract caused a significant reduction ($p<0.05$; a). Ethyl acetate and n-hexane fractions showed significant increase compared to the dose of 12 mg/kg total extract ($p<0.05$; b and $p<0.001$; bbb). The number of rats in each group was 6. Δ: Changes before and after injection

Discussion

The present study showed that intraperitoneal injection of total plant extract had different effects depending on the injection dose; dose of 12 mg/kg significantly reduced mean arterial pressure and systolic blood pressure but had no significant effect on heart rate changes. On the other hand, dose of 24 mg/kg extract only increased heart rate significantly without significant changes in blood pressure, and doses above 30 mg/kg were lethal (pilot studies).

To further investigate the effects of this plant, fractions of three phases of polar (aqueous fraction), semi-polar (ethyl acetate fraction) and non-polar (n-hexane fraction) were prepared and the effect of each one on cardiovascular activity was investigated. Considering that the total extract contains solvent in all three fractions, we conclude that there are different compounds with a wide polarity spectrum in R. khorasanicum extract. Due to the presence of phenolic compounds, flavonoids and anthocyanins (7) in the plant, which have beneficial cardiovascular and antioxidant effects, it is likely that the cardiovascular effect of this plant is through these compounds (3). Given that different species of the genus Ribes have beneficial cardiovascular effects with mechanisms including blockade of angiotensin II type 1 (16), angiotensin - converting - enzyme inhibition, and nitric oxide release from the vascular endothelium (18, 19), it is highly probable that these mechanisms affect

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cardiovascular activity. According to the results obtained in this study, the total extract of R. khorasanicum had better effects in lowering blood pressure in normotensive rats than any of the aqueous, ethyl acetate and n-hexane fractions of the extract. According to the results, R. khorasanicum extract in low doses (4 and 12 mg/kg) had more effect on blood vessels and with increasing dose up to 12 mg/kg, it showed good vasodilatory effects and reduced blood pressure compared to the control group. However, with increasing the dose of the extract (24 mg/kg), the vasodilatory effects of the extract were overshadowed by its chronotropic effects.

It should be noted that cardiac output has an important effect on blood pressure and increase in heart rate can increase blood pressure. Therefore, at a dose of 24 mg/kg of the extract, due to the increase in heart rate, blood pressure also increases and the vasodilatory effects of the extract fade. Regarding the fractions of the extract, it can be said that there is an increase in heart rate in all groups treated with the fractions. One reason may be the relatively high dose of the fractions (20 mg/kg), but the important point is that the major vasodilatory effect of the extract is probably due to the presence of -OH group compounds (20). Therefore, in aqueous fraction and to a lesser extent ethyl acetate fraction of the extract, despite the increase in heart rate, these compounds were able to exert their vasodilatory effect on the arteries and prevent an increase in blood pressure following the increase in heart rate. In the group treated with n-hexane fraction of the extract, due to the lack of polar compounds, the vasodilatory effects are minimized and due to the increase in heart rate, blood pressure also increases. According to the quantitative analysis of the active ingredients of this plant, phenolic compounds, flavonoids and anthocyanins are of special importance in the beneficial effects of the plant (21). The aqueous fraction of the extract containing polar compounds is mainly total phenolic compound and the ethyl acetate and n-hexane fractions of the extract containing semi-polar and non-polar compounds mainly contain flavonoids and anthocyanins. According to the results, polar and semi-polar compounds have better effects in lowering blood pressure than non-polar compounds. Therefore, it can be concluded that compounds with full or partial polarity may have played a very important role in the antihypertensive effects of R. khorasanicum extract. It can also be said that non-polar compounds of the plant in the n-hexane phase, although did not show any effect on lowering blood pressure by itself, together with other compounds with polarity, they were able to further reduce blood pressure in the group receiving the total extract of R. khorasanicum. This can confirm the synergistic effect of compounds with a wide polarity spectrum in the total extract.

The results showed that the hydroalcoholic extract of R. khorasanicum had a better effect on lowering blood pressure in normotensive rats than any of its fractions. Therefore, it can be concluded that the total extract of R. khorasanicum has a better effect than any of the fractions of the extract separately due to having a variety of non-polar and polar compounds such as flavonoids and phenols and having a synergistic effect. Therefore, it is suggested that one uses its total extract in future studies.

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