Original Article

The effects of unripe grape extract on systemic blood pressure and serum levels of superoxide dismutase, malondialdehyde and nitric oxide in rat

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Abstract

Backgrounds: The new lifestyle increases the incidence of hypertension. In Iranian folk medicine, it is believed that Verjuice obtained by unripe grape (*Vitis vinifera)* could control blood pressure. We tested the effects of unripe grape extract (UGE) in blood pressure alteration, serum antioxidant level and aorta endothelial permeability in rats.

Materials and Methods: Four groups of rats were treated daily by placebo and three different doses of UGE (50, 150 and 300 mg/kg/day). Four weeks later, the animals were anesthetized and catheterized. The direct mean arterial, systolic and diastolic pressures (MAP, SP and DP) were recorded. The endothelial permeability was determined and the serum levels of superoxide dismutase (SOD), malonaldialdehyde (MDA) and nitrite were measured.

Results: High dose of UGE increased MAP and SP significantly ($P < 0.05$) when compared with the control group. Decrease of MDA and increase of SOD and nitrite also were detected statistically in animals treated with high dose of UGE ($P < 0.05$). No difference in aorta endothelial permeability was observed between the groups.

Conclusion: The effect of UGE on blood pressure was dose dependent. High dose of UGE increased MAP and SP although its antioxidant activity was significantly high. Such observation mechanisms need to be defined.

Key Words: Blood pressure, malonaldialdehyde, nitrite, nitric oxide, superoxide dismutase, unripe grape extract, unripe grape (*Vitis vinifera*)

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INTRODUCTION

Hypertension is one the most common diseases around the world and its prevalence are accompanied with age, race, and other risk factors.[1-3] However, obesity and the change of lifestyle in developed countries are the main important risk factors to increase the incidence of hypertension.[4-6] Many synthetic drugs the world, with complex mechanism have been synthesized to treat hypertension, but special attentions also have been made by pharmacognosists to develop natural drugs to prevent or to treat circulation hemodynamic disturbances. Grape (*Vitis vinifera)* has been found as a source of natural antioxidants[7,8] to protect cardiovascular system.[9-12] In the other hand, unripe grape also is a source of polyphenolic and carateneoids antioxidants.[13,14] In Persian language unripe grape

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named "Ghureh", and it has been widely used as a flavoring agent in many Iranian dishes and salads as fresh, juice (verjuice) or dried forms. Additionally, in Iranian folk medicine it is believed that verjuice can control hypertension. Anti-atherosclerotic effects of verjuice in animal and its effect in plasma lipid profile and blood pressure in human were studied by others.[15,16] In addition, unripe grape is known as a powerful antioxidant to enhance vascular endothelial function by nitric oxide formation. We reported before that mean arterial, systolic and diastolic pressures and heart rate as well as serum nitrite level reduced after one hour post administration of 125 mg/kg of unripe grape extract (UGE) in rat.[17] However, in related to long time effect of unripe grape, the general population believed that daily uses of verjuice prevent hypertension and coronary artery diseases. In this study, we attempted to test the long time use of UGE in hemodynamic alteration. Therefore, we measured systemic blood pressure and serum level of superoxide dismutase (SOD), malonaldialdehyde (MDA) and nitric oxide metabolite in rats treated daily with UGE for a period of 4 weeks.

MATERIALS AND METHODS

Fresh unripe grapes (*Vitis vinifera* L. cv Shiraz) (40 kg) were bought from local green grocer in Shiraz, Fars province, Iran, in June 2012 and authenticated by Pharmacognosy Department of Pharmacy School, Isfahan University of Medical Sciences. The fruits were dried under shadow at 22°C. The dried material was powdered and then extracted at room temperature according to percolation method using 70% ethanol. The extract was concentrated by rotary evaporator and freeze dryer respectively to obtain dry powder (400 g).

Total phenolic content evaluation

According to Folin-Ciocalteu method, total polyphenolic compounds of the hydroalcoholic UGE were evaluated at 763 nm. Total phenolic contents were reported as galic acid equivalent.[18]

Animals

Adult male (200 ± 20 g) Wistar rats (Animal Centre, School of Pharmacy, Isfahan University of Medical Sciences, Isfahan, Iran) were used for this research. The animals were housed at a standard room temperature, and had free access to water and chow. The experimental procedures were approved in advance by the Isfahan University Medical Sciences Ethics Committee.

Experimental design

Four groups of rats were randomly assigned and treated daily by a feeding tube for period of 4 weeks as followings: Group 1 as the control group received saline as vehicle. Group 2 received a single dose of UGE (50 mg/kg/day, treat1) dissolved and homogenized in 1 ml of saline. Similarly, two other single doses of UGE (150 mg/kg/day, treat2) and (300 mg/kg/day, treat3) were given to the animals in Groups 3 and 4, respectively. Four weeks later, the animals were anesthetized (urethane, 1800 mg/kg i.p.; Merck, Germany) and trachea was subjected to insert air ventilation tube. The polyethylene catheter was implanted into the carotid artery. The animals were subjected to direct blood pressure measurement continuously via catheter connected to pressure transducers and a bridge amplifier (Scientific Concepts, Vic., Melbourne, Australia), and after 15 to 30 min of equilibrium time, the mean arterial, systolic and diastolic pressures (MAP, SP, DP) were recorded for 5 min and average values were considered as final measured values. Blood sample also was obtained, and Evan Blue (EB, 10 mg/kg) was injected via carotid line.

After 15 min, the animal was killed by over dose of anesthetic drug. In order to determine vascular permeability, the aorta was removed, opened and washed in saline. Excess water was removed and the aorta wet weight was determined. To extract the EB from the tissue, the aorta was kept in 4 ml of formamide at 80°C overnight. The concentration of EB in formamide solution was determined photometrically at a wavelength of 623 nm using standard curves. The concentration of extracted EB in formamide solution was consider as vessel permeability.[19]

The serum level of nitrite (stable NO metabolite) was measured using a colorimetric assay kit (Promega Corporation, USA) that involves the Griess reaction. The serum levels of MDA were measured by manual method. Briefly, 0.5 mL of the sample was mixed with 1 mL of 10% trichloroacetic acid (TCA). The mixture was centrifuged at 2000 g for 10 minutes. Then, 500 μL of the supernatant was added to 500 μL of 0.67% thiobarbituric acid (TBA) and was incubated in boiling water for 10 minutes. After cooling down, the absorbance was read at the wavelength of 532 nm, and finally the serum level of SOD was measured by ELISA kit (Glory Inc., USA).

Statistical analysis

Data are expressed as mean ± SEM. ANOVA with LSD *post-hoc* was applied to compare the measured parameter between the experimental groups. Values of $P < 0.05$ were considered statistically significant.

RESULTS

The total phenolic content of UGE was estimated as 6.5% gallic acid equivalent.

The effect of UGE on systemic blood pressure

MAP, SP and DP are demonstrated in Figure 1. MAP and SP in group 3 treated with 300 mg/kg of UGE was significantly higher than control group ($P < 0.05$), and such observation was not seen in DP.

The effect of UGE on serum levels of MDA, SOD and nitrite

The data for serum level of MDA, SOD and nitrite are tabulated in Table 1. The serum level of MDA in Groups 3 and 4 treated with 150 or 300 mg/kg of UGE was statistically less than the control group. On the contrary, the serum levels of SOD and nitrite in these groups were higher than the control group. In addition, the serum level of SOD and nitrite in Group 4 were significantly higher than vehicle-treated group ($P < 0.05$).

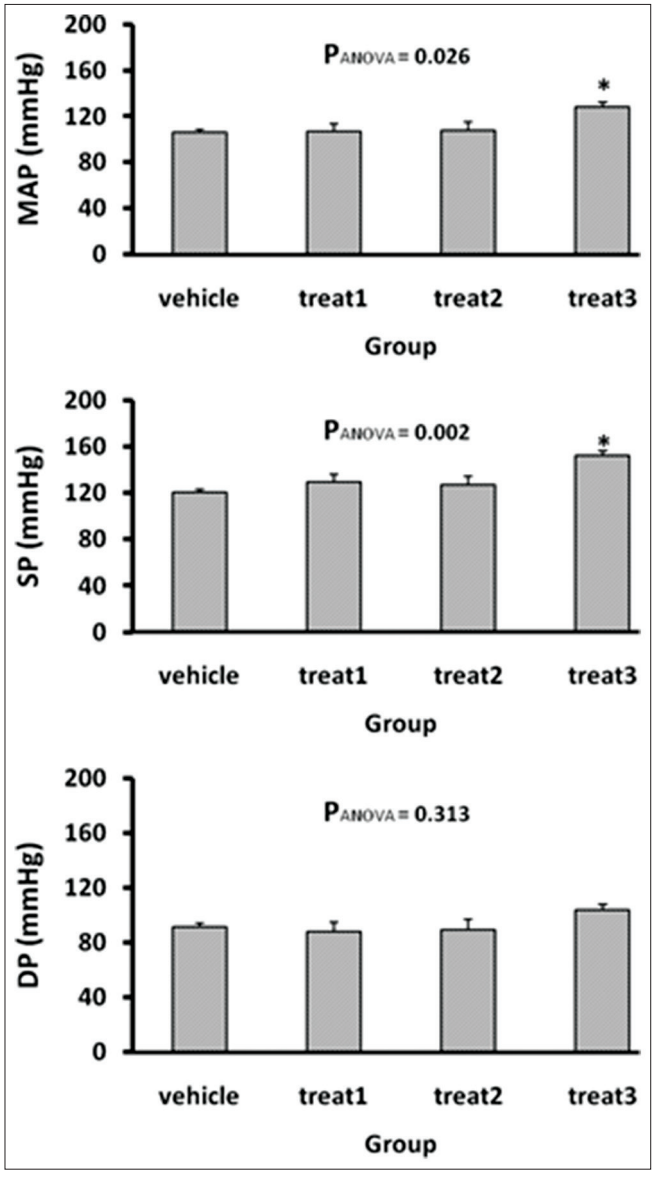

Figure 1: Mean arterial, systolic and diastolic pressures (MAP, SP, DP) in four groups of experiments. The animals treated with vehicle and unripe grape extract (50, 150 and 300 mg/kg; treat1–3) for period of 4 weeks. *Significant difference from others groups ($P < 0.05$)

The effect of UGE on aorta permeability

The data for aorta permeability as μg of EB/g tissue also is demonstrated in Table 1. The data analysis indicated that UGE did not alter the aorta endothelial permeability when compared with the control group.

DISCUSSION

In the new days, many research works have been focused on the herbal medicine implementation in hypertension therapy. In this study, we evaluated chronic effect of UGE on blood pressure, antioxidant level and endothelial permeability in normal rats. We found that high dose of UGE may increase MAP and SP. On the contrary to this finding, previously we demonstrated that single dose of UGE may attenuate blood pressure.[17] This controversy may relate to accumulated dose of UGE. UGE has antioxidant effect[13] as we found by serum level of MDA and SOD. However, antioxidants may act differently and promote the disturbances about their role in hypertension therapy.[20] Although high dose of UGE (300 mg/kg) increased SP, however, it did not alter the DP. In addition, the endothelial permeability of aorta did not change by UGE. Accordingly, it seems that UGE may not disturb the vascular resistance system, and its effect is directly related to heart function. This result of blood pressure has convergence with some previous researches that grape seed extract and vitamin C as antioxidant could increase blood pressure permeability of aorta.[21] In the current study, we measured the blood pressure under general anesthesia. It was preferred to measure the blood pressure in conscious rats to obtain more reliable data. Accordingly, it was one of our limitations, but

Table 1: Serum levels malonaldialdehyde, nitrite, superoxide dismutase and aorta endothelial permeability (evan blue uptake) in the four groups of experiments. The animals treated with vehicle and unripe grape extract (50, 150 and 300 mg/kg) for a period of 4 weeks

Group	MDA (nmole/dL)	Nitrite (μmole/L)	SOD (ng/dL)	EB ptake (μg/g tissue)
Control (vehicle)	5.28±0.82	7.15±1.81	137.1±1.06	25.6±9.6
50 mg/kg	4.96±0.78	10.49±1.07	149.84±2.99	26.6±4.9
150 mg/kg	2.55±0.50*†	11.72±2.96	147.04±4.11	24.3±9.2
300 mg/kg	2.78±0.46*†	16.35±2.83*	160.29±5.47*	34.1±7.6
P value	0.012	0.06	0.047	0.66

Statistical difference ($P<0.05$) from (*) control group (*) or from Group 2 (†).
MDA: Malonaldialdehyde, SOD: Superoxide dismutase, EB: Evan blue

the measurement condition was similar in all of the experimental groups.

The second point was the antioxidant effect of UGE. UGE is the source of antioxidant, because the result of Folin–Ciocalteu analysis of UGA verified polyphenolic constituents in the extract and also was confirmed by other.[13,14] According to antioxidant activities of this kind of compounds, antioxidant activity of the extract could be expected. Our results confirmed that MDA decreased and SOD increased as the dose of UGE was increased. The increase of serum nitrite level also may support the antioxidant activity of UGE, because other antioxidant such as melatonin, *N*-acetylcysteine increase the level of nitric oxide.[22] Finally, nitric oxide and antioxidants have vasodilatory effects, and based on MDA, nitrite and SOD levels in serum from animals in Group 4 that received high dose of UGE, the antioxidant and nitrite levels were high, and accordingly a vasodilation process to reduce blood pressure was expected.[23] Such expectation was not achieved and high dose of UGE increased the blood pressure; possibly, UGE could act as some dietary supplements that could increase the blood pressure.[24]

CONCLUSION

The chronic effect of UGE on blood pressure was dose depended. High dose of UGE increased MAP and SP although its antioxidant activity was significantly high. Such observation mechanisms need to be defined, but it seems UGE has cardiotonic effect.

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