

# The effects of unripe grape extract on systemic blood pressure, nitric oxide production, and response to angiotensin II administration

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## ABSTRACT

**Background:** Hypertension is the most common disease in the world. In Iranian folk medicine, unripe grape juice has been used as antihypertention remedy, but no data is documented for this popular belief. This study was designed to determine the effect of unripe grape extract (UGE) on blood pressure and the response to angiotensin II in rat. **Materials and Methods:** Unripe grape was collected, air dried, and extracted and concentrated. Four groups of Wistar rats received single doses of 125, 250, and 500 mg/kg of UGE or saline, respectively. The direct blood pressure and the serum nitrite level were measured one hour post UGE administration. The animals also were subjected to the infusion of various angiotensin II concentrations (100, 300, and 1000 µg/kg/min), and blood pressure was determined. **Results:** Mean arterial, systolic, and diastolic pressures (MAP, SP, and DP) in all UGE treated groups were less than the control group, but only at the dose of 125 mg/kg (Group 1) they were significantly different ( $P < 0.05$ ). The level of nitrite in groups 1-3 were significantly greater than the control group ( $P < 0.05$ ). No significant differences were detected for the MAP, SP, and DP to different concentrations of angiotensin II among these groups. **Conclusion:** UGE potentially attenuate MAP, SP, and DP via vasodilatation induced by nitric oxide production.

**Key words:** Angiotensin II, blood pressure, nitric oxide, unripe grape extract, *Vitis Vinifera*

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## INTRODUCTION

Hypertension is the most common disease in the world. Due to obesity and the change of lifestyle, the incidence of hypertension has increased mostly in the developed countries.<sup>[1-5]</sup> The prevalence of hypertension varies worldwide depending on the age, race, gender, and risk factors.<sup>[1,6-8]</sup> Accordingly, new strategies and treatment procedures have been developed to control the blood pressure in general population. In contrast, natural products also have been considered as a potential medication to control blood pressure. The land of Persia has scientific and historical background to use plants as drugs in medical treatment. In Iranian folk medicine it is believed that

unripe grape (scientifically named *Vitis Vinifera*) juice could control hypertension. Unripe grape juice also is called “verjuice”, which is much like vinegar. In other words, those grapes do not ripen up, they are getting juiced. In Persian language unripe grape is named “Ghureh” and as fresh, juice or dried has been widely used as a flavoring agent in many Iranian dishes and salads. Additionally it has been used as antigout and antiobesity in Iranian traditional medicine.<sup>[9]</sup> Unripe grape is rich in antioxidant of polyphenolic compounds.<sup>[10-15]</sup> The protective role of grape seeds in cardiovascular system is reported,<sup>[16-25]</sup> and these effects are related to the existence of polyphenolic compounds in the grape.<sup>[21,24,26]</sup> The role of unripe grape on blood pressure is not well documented yet. If we accept the population hypothesis, unripe grape may affect vascular system to promote vascular dilatation, or it may have inhibitory effect on rennin angiotensin system. Accordingly, we hypothesized that unripe grape may potentially promote nitric oxide (NO) production or limit

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the response to angiotensin II administration. In order to test this hypothesis, we measured blood pressure one hour post unripe grape extract (UGE) administration, and the response of blood pressure to graded angiotensin II infusion also was determined in a rat model.

## MATERIALS AND METHODS

### Plant material

The unripe grape (Shiraz, Iran variety) was obtained from a local market in Shiraz, Iran in July 2010. The unripe grape was grounded by a mixture to obtain juice. The unripe grape was air-dried under a controlled temperature (22°C). The plant material (450 g dry weight) was powdered and then exhaustively extracted according to percolation procedure with EthOH/H<sub>2</sub>O (70:30), then filtered and dried in freeze dryer, to obtain dried powder (90 g).

### Total phenolic contents estimation

Total phenolic contents of UGE were estimated according to the method described by Singleton *et al.*<sup>[27]</sup> Twenty microliters of the 0.85% extract was added to 1.58 mL water, and then 100 µL of the Folin–Ciocalteu reagent was added, and mixed. After 30 seconds, 300 µL of 20% Na<sub>2</sub>CO<sub>3</sub> was added and thoroughly mixed and reminded in 20°C for 2 hours. Absorbance was measured at 765 nm. Standard curve of absorbance was created versus concentration of gallic acid (50–500 µg) and total phenolic contents were reported as percentage equivalents to gallic acid.

### Animals

Adult male (212 ± 4 g) Wistar rats (Animal Centre, Ahvaz University of Medical Sciences, Ahvaz, Iran) were used for this research. The rats were individually housed at a temperature of 23–25°C. Rats had free access to water and chow. The rats were acclimatized to this diet for at least one week prior to experiment. The experimental procedures were approved in advance by the Isfahan University Medical Sciences Ethics Committee.

### Experimental protocol

Four groups of rats were randomly assigned. Group 1–3 received a single doses of UGE; 125 (Group 1), 250 (Group 2), or 500 (Group 3) mg/kg, ip, respectively. The control group (Group 4) received saline in equal volume. Thirty minute later, the animals were anesthetized (Inactin; thiobutabarbital, 150 mg/kg, i.p.; Sigma, St Louis, MO, U.S.A.) and trachea was isolated to insert air ventilation tube. Catheters were implanted into the jugular vein and carotid artery. Catheterization time was about 15 minutes for each animal. The animals with catheterization times more than 15 minutes were omitted from the study. The direct blood pressure was measured continuously, and after 15 minutes of equilibrium time (exactly one hour post

UGE administration) the blood pressures were recorded, and blood samples were obtained. The data at this time was considered as one hour post UGE administration. Then a series of intravenous (via jugular vein) infusions of Ang II (100, 300, and 1000 ng/kg/min) were commenced. Each dose was administered until stability for arterial blood pressure was achieved (about 10 minutes period), and then the data was collected for about 5 minutes.

The level of serum nitrite was determined one hour after UGE administration. The serum level of nitrite (stable NO metabolite) was measured using a colorimetric assay kit (Promega Corporation, USA) that involves the Griess reaction. Briefly, after adding sulphanilamide solution and incubation, N-1-naphtylethylenediamine dihydrochloride solution was added. Then, absorbance was measured by a microreader in 540 nm wavelength. The nitrite concentration of samples was determined by comparison to nitrite standard reference curve.

### Statistical analysis

Data are expressed as mean ± SEM. To compare the trend of blood pressure response to graded angiotensin II infusion between the groups, repeated measured analysis was applied. Unpaired *t*-tests also was applied to compare the blood pressure between the experimental groups. Values of *P* < 0.05 were considered statistically significant.

## RESULTS

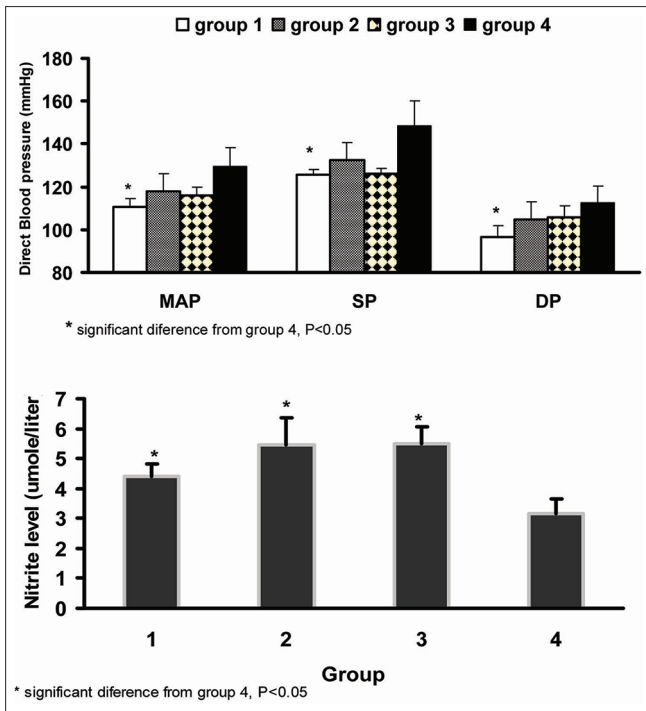
The total phenolic content of UGE was estimated as 8.5% gallic acid equivalents.

### The effect of UGE on blood pressure

The data obtained for mean arterial, systolic, and diastolic pressures (MAP, SP, and DP) are shown in [Figure 1]. In all UGE treated groups, MAP, SP, and DP were less than the control group, but only they were significant at the dose of 125 mg/kg (Group 1) (*P* < 0.05). Mean heart rates in groups 1–4 were recorded as 286 ± 12, 300 ± 20, 358 ± 12, and 341 ± 24/minute, respectively. The statistical analysis indicated that one hour post UGE administration, the heart rate in group 1 was significantly different from the control group (*P* < 0.05). UGE increased the serum nitrite level in groups 1–3 significantly when compared with the control group (*P* < 0.05) [Figure 1].

### Blood pressure response to graded angiotensin II infusion

The data obtained for blood pressure one hour post UGE was considered as base for angiotensin II infusion, and since these data were not statistically the same in all groups, therefore instead of using absolute data for blood pressure in response to angitensin II infusion, we used the percentage

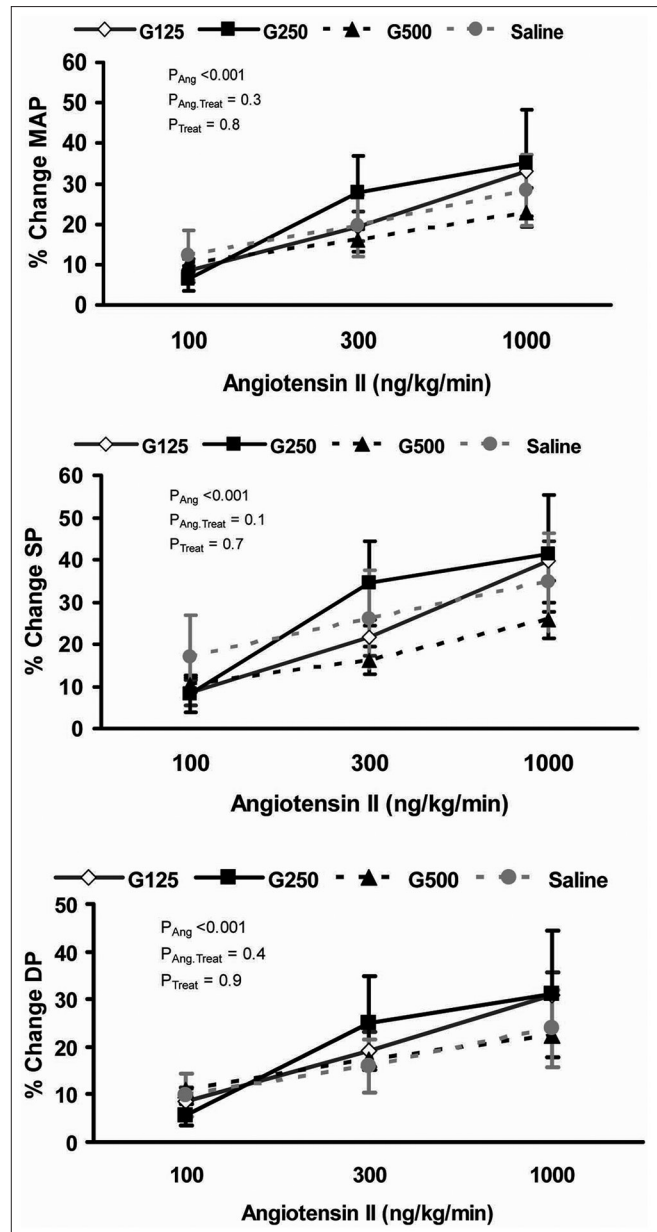


**Figure 1:** Mean arterial, systolic, and diastolic pressures (MAP, SP, and DP) and nitric oxide (nitrite) levels in four experimental groups. The groups 1-4 stand for animals treated with 125, 250, and 500 mg/kg of unripegrape extract (UGE) and placebo (control), respectively. Data are presented as mean  $\pm$  SEM and were analyzed using unpaired *t*-test

change in blood pressures. Angiotensin II infusion increased MAP, SP, and DP in all experimental groups ( $P < 0.05$ ). However, statistical analysis for percentage change of blood pressures indicated no significant difference between the groups [Figure 2]. This analysis indicated that UGE did not limit angiotensin II effects.

## DISCUSSION

The effects of UGE on MAP, SP, DP, and nitrite level were the main objectives of this study. The blood pressures response to graded angiotensin II infusion also was determined. One hour post UGE administration, the blood pressure in the first group (UGE: 125 mg/kg) was reduced significantly. This reduction was assumed to be related to the level of nitrite or NO. UGE contains polyphenolic compounds,<sup>[21,24,26]</sup> and activation of endothelial NO synthase by polyphenols has been reported.<sup>[28]</sup> NO act as a vasodilator via different mechanisms, and it plays a pivotal role in endothelial function.<sup>[29-35]</sup> Our findings for the first time support the idea that UGE administration stimulates NO production and promotes systemic vasodilatation. Another interpretation for this result is related to antioxidant role of unripe grape,<sup>[10,11]</sup> which may preserve endothelium-dependent vasodilatation.<sup>[36]</sup>



**Figure 2:** The percentage change of mean arterial, systolic, and diastolic pressures (MAP, SP, and DP) response to graded angiotensin II infusion in four experimental groups. The groups 1-4 stand for animals treated with 125, 250, and 500 mg/kg of unripe grape extract (UGE) and placebo (control), respectively. Data are presented as mean  $\pm$  SEM and were analyzed using a repeated-measures ANOVA (for percentage change of MAP, SP, and DP from angiotensin II 0–1000 ng/kg/min), using the factors treatment; UGE or placebo and angiotensin administration ( $P_{\text{Treat}}$ ), Ang II ( $P_{\text{Ang}}$ ), and the interaction between treatment and angiotensin II ( $P_{\text{Ang Treat}}$ )

UGE did not affect blood pressure response to graded angiotensin II. We hypothesized that reduction of blood pressure by UGE may occur through inhibition of RAS activities either by an effect on angiotensin converting enzyme (ACE) or angiotensin II receptors (AT1 or AT2). However, our findings reject this hypothesis. The antioxidant effect of ACE has been reported,<sup>[37]</sup> and administration of

angiotensin II decreases renal antioxidant enzyme activities.<sup>[38]</sup> The ATI antagonist; losartan, also has antioxidant effect.<sup>[39-42]</sup> It seems the antioxidant effect of unripe grape potentially had no effects on RAS or its receptors.

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