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Garlic Powder Effect on Plasma Renin Activity, and Cardiovascular Effects of Intravenous Angiotensin I and Angiotensin II in Normotensive and Hypertensive Male Rats

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Background: Hypertension is amongst major health issues in developed countries, which results in cardiovascular morbidity and mortality. Garlic plays an important role in the reduction of hypertension.
Objectives: The aim of the this study was to investigate the effect of consumption of garlic powder on blood pressure and renin angiotensin system in a nitric oxide deficiency hypertension model in male rats.
Materials and Methods: In this study, thirty-two adult male Wistar rats (200 - 250 g) were randomly divided to four groups as follows: normotensive, normotensive-garlic, hypertensive and hypertensive-garlic. Oral N-nitro-L-arginine methyl ester (L-NAME) was used to induce hypertension. Normotensive-garlic and hypertensive-garlic groups were fed 900 mg of garlic powder daily by oral gavage for forty-five days. At the beginning of the experiments, systolic blood pressure was measured. Immediately after the end of the experimental procedure animals were anaesthetized by pentobarbital, blood samples were collected and, after injection of angiotensin I, angiotensin II and captopril, blood pressure was re-measured. Plasma renin activity was also measured.
Results: At the beginning and at the end of experiments, systolic blood pressure was higher in the hypertensive groups than the normotensive group. However, at the end of the study, diastolic blood pressure and mean arterial pressure were higher in the hypertensive groups than the normotensive group. Angiotensin I level was low in the normotensive group whereas plasma renin activity was high in the hypertensive group. After different doses of injection of angiotensin I and angiotensin II, mean arterial pressure was significantly increased in the normotensive-garlic and the hypertensive-garlic groups, respectively. By using captopril, mean arterial pressure and its changes were not significant after injection of different doses of angiotensin I in different groups.
Conclusions: Garlic powder consumption can reduce blood pressure, yet in this model, it did not have an effect on the interaction between renin angiotensin system and nitric oxide inhibition.

Keywords: Blood Pressure; Garlic; Renin-Angiotensin System; Rats

1. Background

It is believed that food factors have a vital role on development of various human diseases such as cardiovascular and metabolic diseases, atherosclerosis, hyperlipidemia, thrombosis, diabetes and hypertension (1). Epidemiological studies have shown that diets rich in fruits, vegetables and spices decrease cardiovascular diseases (2-4). Garlic (*Allium sativum*) is known in many countries as a strong inhibitor of several diseases (5). Garlic has selenium, oligosaccharides and flavonoids, which can promote garlic efficacy. Garlic is full of flavonoids such as quercetin and sulfur components, including allyl propyl disulfide, which is good for human health. In addition, garlic and onion have sulfur contents such as S-alkyl cysteine sulfoxide. These substances are turned to volatile compounds, including thiosulfinates and polysulfides, by alliinase enzyme during garlic crush (1, 6). Garlic, onion, green onion and scallion are the major sources *Allium* in the human diet. In Indian traditional medicine garlic is used for treatment of heart disease and arthritis (7).

Hypertension is an important disease, which is commonly diagnosed in developed countries (4). Hypertension can induce degenerative diseases especially cardiovascular disease. Many documents have indicated the effect of some functional foods and nutrients (8, 9) on blood pressure, for example, garlic decreases blood pressure and aortic rigidity due to aging (6, 10, 11). In a research done on a group of patients with high diastolic blood pressure, garlic powder consumption for 12 weeks could decrease diastolic pressure compared to the control group (10). Another research on men with hypercholesterolemia, indicated that garlic extract decreased systolic blood pressure compared to the control group (12). In a double blind study on healthy adults, consumption of garlic pill three times per day for 12 weeks could not change blood pressure. Changes in blood pressure were not observed in another study on patients with hypercholesterolemia who consumed garlic (13). Mechanisms of the garlic effect on cardiovascular function are to increase nitric oxide synthase (NOS) activity (5), decrease prostaglandin production (14) and decrease Angiotensin (ANG) II (15). It has been reported that garlic decreases vessels wall thickness while ANG II increases vessels wall thickness (16, 17).

Angiotensin II is the main product of renin angiotensin system (RAS), which affects adrenal cortex, brain, cardiovascular system and kidneys (18). The RAS system is an important modulator of blood pressure and vascular function/disease (19, 20). Furthermore, plasma renin activity and angiotensin converting enzyme are important for determination of renin angiotensin system activity. Plasma renin activity also affects the activity of constitutive NOS (cNOS), which reduces secondary renin angiotensin system activity after cNOS reinforcement (21). Since nitric oxide (NO) has a central role in blood pressure regulation, increasing NOS activity by garlic can interfere with the anti-hypertensive effect of garlic. Chronic inhibition of NOS by analogues of L-arginine, namely N^{ω}-nitro-L-arginine-methyl-ester (L-NAME), can increase arterial blood pressure in rats.

2. Objectives

According to studies on the effects of garlic on reducing blood pressure and stimulating NOS, our research aimed to determine possible mechanisms of garlic on blood pressure via renin angiotensin system after inhibition of NO production by L-NAME. For this purpose, this experimental study included two groups of rats with normal blood pressure and high blood pressure (with L-NAME) to determine the effects of garlic powder on plasma renin activity, angiotensinogen and cardiovascular system responses to angiotensin I and II.

3. Materials and Methods

3.1. Animals

Thirty-two adult male Sprague-Dawley rats weighing between 200 - 250 g were purchased from the Pasteur institute of Iran. The animals were kept in an animal room with 12 hours light/dark cycle with temperatures ranging between 22 and 25°C. Rats were fed with standard rat chow and given free access to tap water throughout the study. The study was approved by the ethical committee of Hamadan University of Medical Sciences. The animals were acclimatized for one week and were randomly assigned to four groups: normotensive (C), normotensive-garlic (CG), hypertensive (H) and hypertensive-garlic (HG).

3.2. Induction of Hypertension

For induction of hypertension, NO inhibitor or L-NAME (made by the Fluca Company) was used. Drinking water containing L-NAME (370 μmol/L) within a black container (ad libitum) was given to the animals. Water containing L-NAME was provided daily.

3.3. Feeding Method of Garlic

Nine hundred milligrams of garlic powder (Hamadan-Iran Grandis Company) was dissolved in 2 mL of water and transferred to the animals' stomach via a catheter, on a daily basis.

3.4. Blood Pressure Measurement

At the beginning and after induction of hypertension, the animals were kept in a temperature-controlled restrainer. Indirect systolic blood pressure was measured by using tail pressure cuff which was connected to NARCO BIO system. At the end of study, the animals were anaesthetized by pentobarbital (50 mg/kg; i.p.) and direct blood pressure and its changes in response to injection of Ang I and Ang II were measured via a catheter (PE-50) inserted into femoral artery. Blood was taken for subsequent determination of plasma renin activity.

3.5. Different Injection Doses of Angiotensin I and II

Angiotensin I was dissolved in normal saline and concentrations of 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9} M were prepared. These doses in a total volume of 0.1 mL were injected via a catheter with one-minute intervals and the changing rate of systolic and diastolic blood pressure was recorded. After blood pressure stabilization in animals, different doses of angiotensin II with concentrations of 10^{-4} to 10^{-9} M in the same volume were injected to the femoral venues via a catheter with one-minute intervals and the changing rate of systolic and diastolic blood pressure were recorded in the animals again. At the end of this section, captopril (10 mg/kg) in volume of 0.1 mL was injected and then different doses of angiotensin I were re-injected using the above-mentioned method and the changes of blood pressure were recorded.

3.6. Plasma Renin Activity Measurement

Plasma renin activity (PRA) was measured using the gamma coat PRA radioimmunoassay kit (DiaSorin Inc). Plasma Renin activity was measured at the end of the study.

3.7. Statistical Methods

The Statistical Package for Social Sciences (SPSS), version 16 was used for standard statistical analysis. Data were presented as mean ± standard Deviation (SD). Student's t-test and one-way Analysis of variance (ANOVA) were used to determine the difference between groups, and $P < 0.05$ denoted statistical significance.

4. Results

4.1. Blood Pressure

The results showed that systolic, diastolic and mean arterial blood pressure weren't significantly different in normotensive groups; while in the normotensive-garlic group these variables had decreased (Figure 1). In the hypertensive group, systolic, diastolic and mean arterial blood pressure had increased compared to the beginning of the study although, in the hypertensive-garlic group these variants had decreased.

4.2. Angiotensin I and Plasma Renin Activity

The lowest amount of serum angiotensin I was seen in the normotensive group. In the normotensive-garlic group, serum angiotensin I had significantly increased compared to the normotensive group. While there was an increase in serum angiotensin I in the hypertensive group compared to the normotensive group, it didn't differ significantly in the normotensive-garlic group (Table 1). At the end of the study, the highest plasma renin activity was seen in the hypertensive group while the lowest was in the normotensive group. No significant difference was seen in plasma renin activity in the normotensive-garlic group compared to the normotensive group. There was a significant decrease in plasma renin activity in the hypertensive-garlic group compared to the hypertensive group at the end of the study (Table 1).

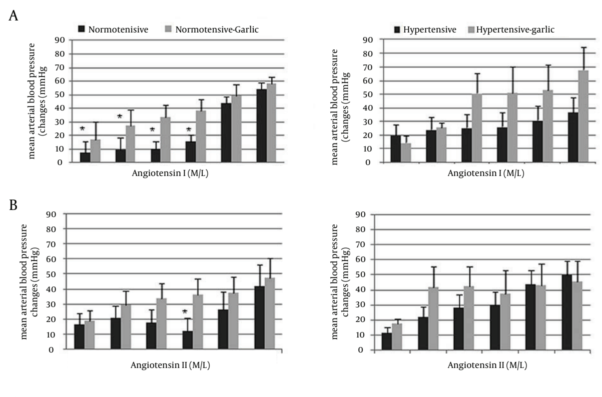
4.3. The Effect of Angiotensin and Captopril Injection on Vessels Responsiveness

The mean arterial blood pressure changes for different injection doses of angiotensin I was significantly different in both normotensive and normotensive-garlic groups. In the normotensive-garlic group, mean arterial blood pressure changes in response to 10^{-6} - 10^{-9} M of angiotensin I injection was higher against 10^{-4} and 10^{-5} M doses in the normotensive group. Vessels responsiveness to different doses of angiotensin I was not significantly different in the hypertensive and hypertensive-garlic groups (Figure 2, A). The mean arterial blood pressure showed significant changes for different injection doses of angiotensin II in the normotensive group, by decreasing at low doses and increasing at high doses. In the normotensive-garlic group, mean arterial blood pressure significantly increased at different doses. Thus, vessel responsiveness demonstrated significant differences in the normotensive and normotensive-garlic groups (Figure 2, B).

The mean arterial blood pressure at different injection doses of angiotensin II showed an increase in the hypertensive group, while this was not seen in the hypertensive-garlic group. As a result, the responsiveness of vessels to injection of angiotensin II was significantly different in the hypertensive-garlic compared to the hypertensive group (Figure 2, B).

Mean arterial blood pressure in different animal groups didn't show statistically significant differences after captopril injection. In the normotensive-garlic group after treatment with captopril, the mean arterial blood pressure increased by higher doses of angiotensin I injection although, angiotensin I concentrations was equaled in other higher doses. These changes weren't observed in the normotensive group. The mean arterial blood pressure at different injection doses of angiotensin I after treatment with captopril, increased in the same manner for both hypertensive and hypertensive-garlic groups (Figure 2, C).

Figure 1. Systolic, Diastolic and Mean Arterial Blood Pressure in Normotensive, Normotensive-Garlic, Hypertensive and Hypertensive-Garlic Groups



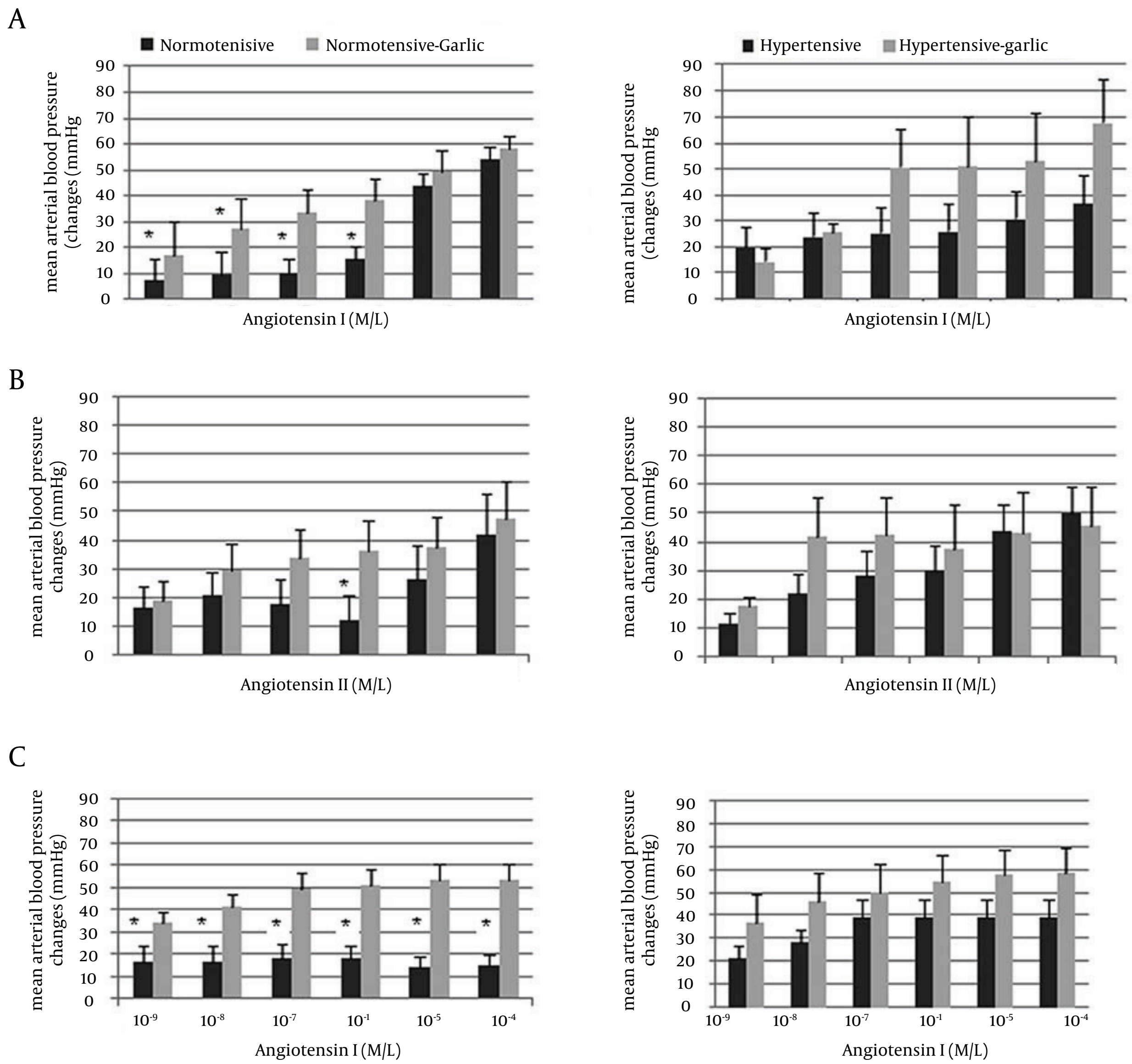
* $P < 0.05$ versus normotensive groups.

Table 1. Serum Angiotensin I and Plasma Rennin Activity in the Normotensive and Hypertensive Groups

Group	Angiotensin I, ng/mL	Plasma Rennin Activity, ng/mL/h
Normotensive	9.81 ± 5.99 [a]	254.16 ± 70.31
Normotensive-Garlic	37.61 ± 1.23	335.82 ± 49.71
Hypertensive	40.01 ± 11.78	512.99 ± 94.80 [a]
Hypertensive-Garlic	44.39 ± 13.81	297.95 ± 31.39

[a] $P < 0.05$ versus other groups.

Figure 2. Mean Arterial Blood Pressure Change by Injection of 10^{-4} - 10^{-9} M/L of Angiotensin I (A), Angiotensin II (B), and Re-Injection of Angiotensin I After Captopril (10 mg/kg) Intake (C), in Normotensive, Normotensive-Garlic, Hypertensive and Hypertensive-Garlic Groups



* $P < 0.05$ versus normotensive groups.

5. Discussion

Results of this study showed that inhibition of Nitric oxide (NO) production in treated groups with L-NAME led to hypertension. However, in normotensive groups, adding garlic powder to their diet could not decrease arterial blood pressure significantly. Meanwhile, in hypertensive groups despite the tendency to increase blood pressure, adding garlic to the subjects' diet did not make significant changes in arterial blood pressure. Measurement of Plasma Renin Activity (PRA) showed that induction of hypertension in hypertensive groups caused a significant increase in PRA. Furthermore adding garlic to the subjects' diet led to slight and non-significant increase in PRA in groups, which had natural blood pressure compared to the normotensive group, while in the hypertensive group, garlic made a significant reduction in PRA compared to the the non-treated group. Comparison of basal levels of angiotensin I showed that adding garlic to the diet of the normotensive group led to an increase in angiotensin I levels in this group, with this finding being consistent with the slight rise of PRA in the normotensive group. On the other hand, adding garlic to the diet of the hypertensive group caused a slight and non-significant increase in angiotensin I compared to the controlled hypertensive group. This finding is consistent with the slight rise of PRA in the hypertensive group. One of the most striking findings was the significant increase of angiotensin I level in normotensive animals after adding garlic to their diet for a long duration of time. This finding along with the increase of PRA in this group indicated that the long-term consumption of garlic had stimulated renin angiotensin system. Also this finding is consistent with the results of other studies indicating that an increase of NO production and reduction in arterial blood pressure and glomerular filtration in kidneys may enhance active renin production through the kidneys (14, 22, 23). After blood pressure reduction, glomerular filtration reduction is the strongest and the main mechanism of physiological stimulation of active renin production through the kidneys (24-26). Besides, increasing of PRA is one the most important factors that activates renin angiotensin system and leads to hypertension in the long term (24-26). Therefore, it is alarming for people with normal blood pressure and long term and excessive consumption of garlic and again it reminds about the principle of balance in the diet. It was observed that adding garlic to the diet of hypertensive animals led to a slight increase in arterial blood pressure. Hypertension models that have been induced by inhibition of NO production, in this study and previous studies, indicated garlic's intervention in NO production process. Our findings demonstrated that in hypertensive animals according to inhibition of NO production by L-arginine analog, not only reduction did not occur, but also a slight increase occurred in the average level of arterial blood pressure. This increase indicates changes in vascular endothelium, because vascular endothelium is the main place for both NO production and Angiotensin converting enzyme (ACE) production and is also the place for vascular effects of Angiotensin II (27, 28). Previous studies have shown that the levels of NO production in vascular endothelium affect the activity of ACE and receptors of angiotensin II (29-31).

The results of intravenous infusion of angiotensin I on blood pressure in the studied groups showed that induction of hypertension significantly increased response to dilute doses of angiotensin I. Angiotensin I does not have direct biological effects and it should be converted to angiotensin II by ACE to act its roles (32). Therefore, probable causes of these changes can be increasing of ACE activity and/or increasing of angiotensin II receptors. Whereas changes that are created by response to angiotensin I are more tangible than those changes created by response to angiotensin II (affected by induction of hypertension), the possibility of changes in ACE activity is more likely, because because opposite to the angiotensin I, response to angiotensin II is independent of ACE. Moreover, inhibition of NO can cause an increase in the number of angiotensin II receptors and also produce ACE in endothelium (30, 31), therefore this part of our findings is consistent with the results of other studies. On the other hand, the investigation of the response to angiotensin I, after inhibition of ACE by treatment with captopril, indicates that captopril vehemently reduces response to angiotensin I, and also arterial blood pressure in animals of control normotensive group. Also this finding can be the reason for the increase in conversion of angiotensin I to angiotensin II as the most likely change through induction of hypertension. It should be mentioned that in addition to ACE, other enzymes such as kimase and tonin are also able to convert angiotensin I to angiotensin II (33, 34). It is interesting to know that other studies have indicated the intervention of NO in producing kimase (35). Moreover, the results of this study showed that adding garlic to the diet of both normotensive and hypertensive animals increases response to angiotensin I rather than angiotensin II. In simpler terms, adding garlic to the diet has shifted the dose-response diagram of angiotensin I and angiotensin II to the left and has increased sensitivity to these two peptides. Alignment of these changes in response to angiotensin I and angiotensin II indicates that the process of response to angiotensin II at the receptor's level and or mediation in response at the receptor or post-receptor levels may be affected by garlic powder. Also angiotensin II in addition to vessels can change blood pressure and heartbeat by affecting the central nervous system (24, 36). This part of the findings is confirmed by garlic's effects on arterial blood pressure in normotensive and hypertensive animals. We used garlic powder in this study, because this product is crude and not divided, thus there may be some materials in garlic garlic which affect endothelial or RAS function. The results of this study are inconsistent with the results of Sharifi et al., which have shown that fresh garlic can inhibit Goldblatt hypertension (37). Meanwhile, our other study showed that garlic powder does not affect hypertension induction in two-kidney-one-clip (2K1C) rats (38). The comparison of our previous study with Sharifi's study, suggests that fresh garlic and garlic powder may affect the hypertension process and function of renin angiotensin system differently.

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Authors' Contributions

Study concept and design: Aliasghar Vahidinia and Behnam Heshmatian. Analysis and interpretation of data: Mohammad Zarei and Aliasghar Vahidinia. Drafting of the manuscript: Mohammad Zarei, Aliasghar Vahidinia and Behnam Heshmatian. Statistical analysis: Aliasghar Vahidinia and Iraj Salehi.

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