Int Cardiovasc Res J.2015;**9**(1):34-40.

International Cardiovascular Research Journal

www.ircrj.com

# The Antihypertensive Effects of Hydroalcoholic Extract of *Allium Eriophyllum* Leaves on Rats with Simultaneous Type 2 Diabetes and Renal Hypertension

Masoud Mozafari [1], Ali Akbar Nekooeian [1,*], Zeinab Janahmadi [1]

[1] Cardiovascular Pharmacology Research Lab, Department of Pharmacology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, IR Iran

ARTICLE INFO

*Article Type:*
Research Article

*Article History:*
Received: 15 Jul 2014
Accepted: 15 Sep 2014

*Keywords:*
Diabetes
Renal Hypertension
Oxidative Stress
Rats

ABSTRACT

***Background:*** Some species of Allium family are known to have antihypertensive, anti-diabetic, and lipid lowering effects.

***Objectives:*** This study aimed to examine the possible mechanisms of antihypertensive, anti-diabetic, and anti-lipid activities of *Allium eriophyllum* which grows in Fars province, Iran in a rat model of simultaneous type 2 diabetes and renal hypertension.

***Materials and Methods:*** This study was conducted on six groups of male Spargue-Dawley rats each containing 8 - 10 animals, including a sham-control, a diabetic, a renal hypertensive, and three simultaneously hypertensive–diabetic groups receiving vehicle or 30 or 100 mg/kg/day hydroalcoholic extract of *Allium eriophyllum*. Four weeks after induction of diabetes, renal hypertension was induced and the animals started receiving the vehicle or extract for the subsequent four weeks. Afterwards, blood pressure, fasting blood sugar, serum cholesterol, triglyceride, and markers of oxidative stress were measured, and isolated studies were performed on aortic rings.

***Results:*** Systolic blood pressure, heart rate, fasting blood sugar, maximal response, and effective concentrations 50 ($EC_{50}$) of phenylephrine and acetylcholine of the hypertensive-diabetic group receiving vehicle were significantly higher compared to those of the sham-control group, and treatment with the extract led to a significant reduction in these variables. Moreover, serum superoxide dismutase and glutathione reductase and maximal response of acetylcholine were significantly lower in the hypertensive-diabetic group receiving vehicle in comparison to the sham-control group, and treatment with the extract significantly reduced these variables.

***Conclusions:*** The present study findings indicated that antihypertensive, anti-diabetic, and anti-lipid effects of the extract might be partly due to its antioxidant mechanism. It was also revealed that its antihypertensive effects may be additionally mediated by improving the release of nitric oxide as well as by sympatholytic activities.

►*Implication for health policy/practice/research/medical education:*

Currently about 115 species of Allium have been identified. One of such species is Allium eriophyllum, which grows in some areas of Fars Province, Iran. The aerial part of the plant is used by local residents as rice vegetable. In a series of preliminary experiments on isolated aortic rings we found that Allium eriophyllum hydroalcoholic extract (AEHE) of aerial parts had vasorelaxing activities. The present study was designed to examine the antihypertensive as well as blood glucose lowering activities of AEHE in rats with simultaneous renal hypertension and type 2 diabetes.

## 1. Background

Hypertension and diabetes frequently occur together. Framingham's study showed that about 58% of the subjects did have hypertension at the time that they were diagnosed with diabetes mellitus (1). Coexistence of diabetes and hypertension in human is associated with higher cardiovascular risk and mortality (2).

Despite the development of numerous medications, there is

*Corresponding author: Ali Akbar Nekooeian, Department of Pharmacology, School of Medicine, Shiraz University of Medical Sciences, Zand Street, Shiraz, IR Iran, Postal code: 71348-53185, Tel/Fax: +98-7132307591, *E-mail:* nekooeiana@sums.ac.ir

an increasing interest towards using alternative traditional remedies, especially herbal medicines, for treatment of diseases, including hypertension and diabetes. Several reports have indicated that members of genus *Allium* from Alliacea, such as garlic (*Allium sativum*), have beneficial effects on cardiovascular diseases and diabetes. Garlic was shown to have anti-diabetic (3), antihypertensive (4, 5), antihyperlipidemic (4, 6), antiplatelet (7), anticoagulant (8)), antiatherosclerotic (9), and anti-oxidative stress (10) properties.

Currently, about 115 species of Allium have been identified. One of such species is *Allium eriophyllum* which grows in some areas of Fars province, Iran. The aerial part of this plant is used by local residents as rice vegetable. In a series of preliminary experiments on isolated aortic rings, we found that *Allium Eriophyllum* Hydroalcoholic Extract (AEHE) of aerial parts had vasorelaxing activities.

## 2. Objectives

Thus, the present study aims to examine the antihypertensive as well as blood glucose lowering activities of AEHE in the rats with simultaneous renal hypertension and type 2 diabetes.

## 3. Materials and Methods

### *3.1. Plant Collection and Extract Preparation*

Aerial parts (leaves) of *Allium eriophyllum* were collected around Noorabad, Fars province, Iran in April. The exact species of the plant was determined by an herbal specialist from the Pharmacognosy Department, Faculty of Pharmacy, Shiraz University of Medical Sciences, and the voucher number 748 was assigned to the plant. Then, the leaves were dried in the shade and were ground to powder. After that, hydroalcoholic (70% ethanol and 30% distilled water v/v) extract of *Allium eriophyllum* leaves was prepared using percolation method. The yield was about 30 - 35%.

### *3.2. Animals*

In this study, 40 male Sprague-Dawley rats, weighting 200 - 250 g, were obtained from Laboratory Animal Breeding Center, Shiraz University of Medical Sciences, Shiraz, Iran. The animals were kept under standard conditions (12 h light/dark cycle, humidity: 25 - 35%, and temperature: 22 – 24 ºC) with standard rat chow and drinking water ad libitum. All animal procedures were approved by the Institutional Animal Care and Use Committee.

### *3.3. Experimental Protocol and Design*

The rats intraperitoneally received 110 mg/kg nicotinamide (NA) and 65 mg/kg streptozotocin (STZ) (both from Sigma-Aldrich Chemical Co. Steinheim, Germany) or vehicle (distilled water). STZ and NA were dissolved in normal saline (0.9% sodium chloride in distilled water). Seven days later, the animals' Fasting Blood Sugar (FBS) levels were determined using a glucometer (Accu-check® active, Germany), and those with FBS higher than 126 mg/dL were considered with type 2 diabetes (4).

Four weeks after the induction of diabetes, control and type 2 diabetic animals were subjected to sham-operation or induction of two-kidney, one clip renal hypertension by placing self-made solid Plexiglass clips on left renal arteries as previously described (11). Briefly, the animals were anesthetized using 60 mg/kg Ketamine (Pan Pharam, Trittau, Schleswig Holstein, Germany) and 8 mg/kg Xylazine (Alfasan, Woerden, The Netherland). Then, through a left flank incision, the left renal arteries were exposed and dissected away from renal veins and the surrounding tissues. Afterwards, Plexiglass clips (internal diameter of 0.20 - 0.22 mm) were placed on the arteries. Antibiotic (Penicillin) powder (Jaber-Ebne-Hayyan Co., Tehran, Iran) was applied to the incision sites, and abdominal wall and skin were sutured using absorbable (catgut) and non-absorbable (silk) suture materials, respectively. The sham-operated animals were subjected to a similar procedure, but no clip was placed around their renal arteries. The animals were then recovered from anesthesia and kept in cages of two rats each for 4 weeks under standard conditions.

Starting from the day after the operations, the sham-operated control group (Sham-C-Veh) received the vehicle. In addition, the animals in the renal artery-clipped group were randomly assigned to 5 groups, including renal hypertensive group (HTN-Veh) receiving vehicle, type 2 diabetic group receiving vehicle (DM-Veh), simultaneous type 2 diabetes and renal hypertensive group receiving vehicle (DM + HTN-Veh), and two simultaneous type 2 diabetes and renal hypertensive groups receiving 30 mg/kg/day (DM + HTN-AEHE30) or 100 mg/kg/day AEHE (DM + HTN-AEHE 100). The vehicle (1 mL distilled water) or the extract (dissolved in the same volume of the vehicle) was administered by oral gavage for the next 4 weeks. On days 7, 14, 21, and 28, Systolic Blood Pressure (SBP) and Heart Rate (HR) were measured using non-invasive tail-cuff method (Chart 5.0 software, PowerLab 4/30, AD Instruments Inc., MA, Australia). Three consecutive blood pressure measurements, which had a difference of less than 5 mmHg, were considered as valid. The mean of such three measurements was recorded as a valid value of blood pressure in every occasion.

After measurement of SBP and HR on day 28, the animals were anesthetized using 60 mg/kg thiopental sodium (Biochem GmbH, Vienna, Australia), and a drop of tail artery blood was obtained for measurement of FBS using a glucometer. Then, the animals' chest cavities were opened, thoracic aortas were cut, and blood samples were obtained from the chest cavity blood pool for measurement of serum levels of total cholesterol, Triglyceride (TG), Superoxide Dismutase (SOD), and Glutathione Reductase (GR). The thoracic aortas were then removed and used for isolated tissue studies. The blood samples were allowed to clot for 30 minutes, centrifuged at 3000 rpm for 20 minutes, and their sera were separated and stored at -80 ºC until analysis.

### *3.4. Isolated Aortic Studies*

Thoracic aortas were cleaned of the surrounding connective tissues and were cut into 3 - 4 mm length rings which were mounted on hooks connected to force transducers (K30, Hugo Sachs Electronik, Germany). The system's organ baths were filled with 20 ml physiological solution containing the following composition (mmol/L): NaCl 118, KCl 4.7, $KH_2PO_4$ 1.2, $CaCl_2$ 2.5, $MgSO_4$ 1.2,

$NaHCO_3$ 25, and D-glucose 11.1, bubbled constantly with 95% $O_2$ and 5% $CO_2$ at pH of 7.4 and temperature of 37 ºC. Tensions were recorded by a four-channel polygraph (model 705/1, Hugo Sachs Electronik, Germany). The tissues were allowed to stabilize for 60 minutes. Then, a full dose-contraction response to phenylephrine (Phe) (Sigma-Aldrich Chemical Co., Steinheim, Germany) was performed. After two washes and 30 minutes equilibration, each ring was contracted with Phe using concentrations that caused 50% of the maximal contraction in the Sham-C-Veh group. Dose-relaxation response to Acetylcholine (Ach) or Sodium Nitroprusside (SNP) (both from Sigma-Aldrich Chemical Co., Steinheim, Germany) was performed at the plateau of contractile response to Phe. After all, dose-responses to Phe were compared using Effective Concentrations 50 ($EC_{50}$) and maximal response ($E_{max}$). Also, dose-responses to Ach or SNP were compared using Inhibitory Concentration 50 ($IC_{50}$) and $E_{max}$.

*3.5. Biochemical Measurements*

Serum levels of cholesterol and TG were determined using Pars Azmun commercial kits (Pars Azmun Co, INC, Karaj, Iran). Besides, serum levels of SOD and GR were determined using Biorexfars chemical kits (Shiraz, Iran).

*3.6. Statistical Analysis*

The data, presented as mean ± SEM, were analyzed using One-way Analysis of Variance (ANOVA) followed by Duncan's multiple range test in case of statistical significance. Besides, $P \leq 0.05$ was considered as statistically significant. The analyses were performed using Sigmastat statistical software, version 3.0.

## 4. Results

*4.1. Effects on Hemodynamics*

The results showed no significant difference between Sham-C-Veh and DM-Veh groups regarding SBP at weeks 1, 2, 3, and 4 of the treatment (Figure 1A). However, the SBP of HTN-Veh and DM + HTN-Veh groups was significantly higher than that of the Sham-C-Veh group at weeks 1, 2, 3, and 4 (Figure 1A). Moreover, the SBP of DM + HTN-AEHE30 and DM + HTN-AEHE100 groups was significantly lower compared to the DM + HTN-Veh group at weeks 1, 2, 3, and 4 (Figure 1A).

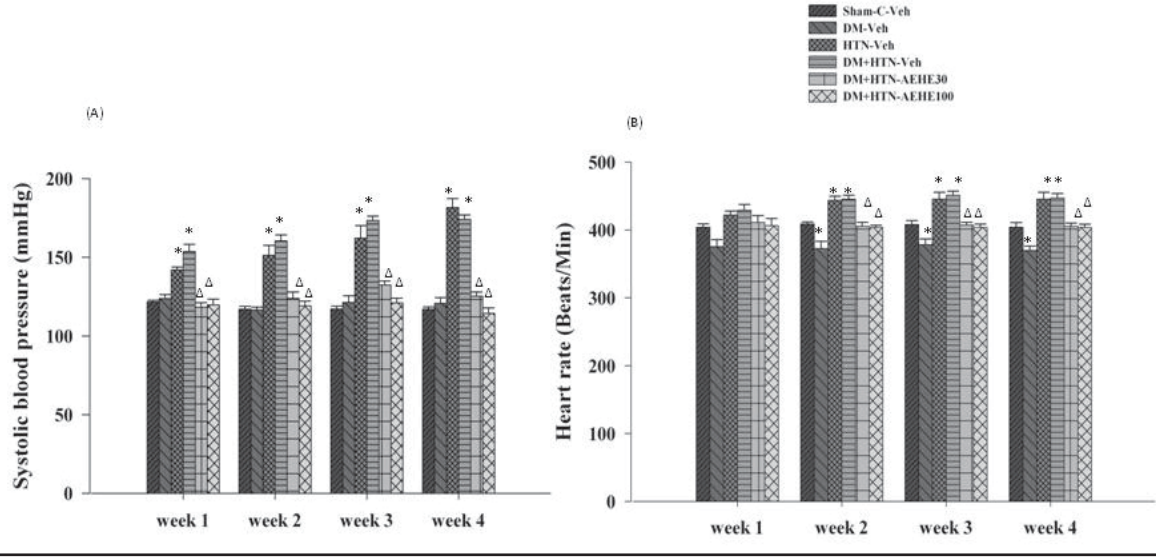
The HR of the DM-Veh group was significantly lower than that of the Sham-C-Veh group at weeks 2, 3, and 4, but not at week 1 (Figure 1B). However, the HR of HTN-Veh and DM + HTN-Veh groups was significantly higher compared to the Sham-C-Veh group at weeks 2, 3, and 4, but not at week 1 (Figure 1B). Moreover, the HR of DM + HTN-AEHE30 and DM + HTN-AEHE100 groups was significantly lower than that of the DM + HTN-Veh group at weeks 2, 3, and 4, but not at week 1 (Figure 1B).

*4.2. Effects on Biochemical Parameters*

After 4 weeks of treatment, FBS level of the DM-Veh group was significantly higher than that of the Sham-C-Veh group, but was not significantly different from those of the HTN-Veh and Sham-C-Veh groups. Besides, FBS level of the DM + HTN-Veh group was significantly higher than that of the Sham-C-Veh group, but not significantly different from that of the DM-Veh group. Moreover, FBS levels of DM + HTN-AEHE30 and DM + HTN-AEHE100 groups were significantly lower than that of the DM + HTN-Veh group (Table 1).

The results demonstrated no significant difference between Sham-C-Veh and HTN-Veh groups with respect to serum TG and cholesterol levels (Table 1). The serum TG and cholesterol levels of DM-Veh and DM + HTN-Veh groups were significantly higher than those of the Sham-C-Veh group. However, the serum TG and cholesterol levels of DM + HTN-AEHE30 and DM + HTN-AEHE100 groups

**Figure 1.** Systolic Blood Pressure (A) and Heart Rate (B) of the Sham-Operated Control Group Receiving Vehicle (Sham-C-Veh), Type 2 Diabetes Group Receiving Vehicle (DM-Veh), Renal Hypertensive Group Receiving Vehicle (HTN-Veh), Simultaneous Type 2 Diabetes and Renal Hypertension Group Receiving Vehicle (DM + HTN-Veh), and Simultaneous Type 2 Diabetes and Renal Hypertension Groups Receiving *Allium eriophyllum* Leave Extract at 30 mg/kg/day (DM + HTN-AEHE30) and 100 mg/kg/day (DM + HTN-AEHE100) after Four Weeks of Treatment.




* Significantly different from the Sham-C-Veh group ($P < 0.05$); Δ Significantly different from the DM + HTN-Veh group ($P < 0.05$)

**Table 1.** The Serum Levels of Biochemical Markers of All Experimental Groups

| | FBS (mg/dL) | Triglyceride (mg/dL) | Cholesterol (mg/dL) | Superoxide Dismutase (Unit/mL) | Glutathione Reductase (Unit/mL) |
|---|---|---|---|---|---|
| **Sham-C-Veh** | 106.6 ± 2.9 | 65.6 ± 5.3 | 47.0 ± 4.6 | 265.3 ± 44.5 | 72.86 ± 3.8 |
| **DM-Veh** | 207.6 ± 4.9 [a] | 88.5 ± 6.6 [a] | 60.5 ± 3.5 [a] | 89.0 ± 12.0 [a] | 27.0 ± 3.1 [a] |
| **HTN-Veh** | 120.1 ± 2.4 [a] | 65.5 ± 5.5 | 50.5 ± 1.9 | 150.4 ± 22.1 [a] | 39.8 ± 3.5 [a] |
| **DM+HTN-Veh** | 198.3 ± 5.7 [a] | 87.6 ± 9.2 [a] | 60.7 ± 3.7 [a] | 115.2 ± 15.6 [a] | 28.7 ± 2.2 [a] |
| **DM + HTN-AEHE30** | 105.7 ± 4.5 [b] | 40.3 ± 3.5 [b] | 49.1 ± 2.5 [b] | 244.4 ± 72.2 [b] | 65.8 ± 3.8 [b] |
| **DM + HTN-AEHE100** | 103.4 ± 5.8 [b] | 38.5 ± 4.4 [b] | 49.5 ± 2.6 [b] | 266.6 ± 51.3 [b] | 64.0 ± 3.8 [b] |

Abbreviations: Sham-C-Veh, sham-control group treated with vehicle; DM-Veh, type 2 diabetes mellitus, renal hypertensive treated with vehicle; DM + HTN-Veh, simultaneous type 2 diabetes mellitus and renal hypertensive group; DM + HTN-AEHE30, simultaneous type 2 diabetes and renal hypertension groups receiving *Allium eriophyllum* leave extract at 30 mg/kg/day; DM + HTN-AEHE100, simultaneous type 2 diabetes and renal hypertension groups receiving the extract at 100 mg/kg/day; FBS, fasting blood glucose
[a] Significant difference ($P < 0.05$) from Sham-C-Veh; [b] Significant difference ($P < 0.05$) from DM + HTN-Veh; The values are mean ± SEM, n = 8 - 10 each.

were significantly lower compared to the DM + HTN-Veh group (Table 1).

Serum SOD and GR levels of HTN-Veh, DM-Veh, and DM + HTN-Veh groups were significantly lower than those of the Sham-C-Veh group (Table 1). In addition, the serum SOD and GR levels of DM + HTN-AEHE30 and DM + HTN-AEHE100 groups were significantly higher compared to the DM + HTN-Veh group (Table 1).

*4.3. Isolated Aortic Ring Studies*

The $E_{max}$ of Phe from DM-Veh, HTN-Veh, and DM + HTN-Veh groups were significantly higher than those of the Sham-C-Veh group (Table 2, Figure 2A). However, the $E_{max}$ of DM + HTN-AEHE30 and DM + HTN-AEHE100 groups were significantly lower compared to the DM + HTN-Veh group (Table 2, Figure 2A). Moreover, the $EC_{50}$ of Phe concentration response in DM-Veh, HTN-Veh, and DM + HTN-Veh groups were significantly lower than those of the Sham-C-Veh group (Table 2). Also, the $EC_{50}$ Phe concentration responses in DM + HTN-AEHE30 and DM + HTN-AEHE100 groups were significantly lower in comparison to the DM + HTN-Veh group (Table 2).

The $_{Emax}$ of relaxation response to Ach in DM-Veh, HTN-Veh, and DM + HTN-Veh groups were significantly lower than that of the Sham-C-Veh group (Table 2, Figure 2B). Moreover, the $E_{max}$ of relaxation response to Ach in DM + HTN-AEHE30 and DM + HTN-AEHE100 groups were significantly higher compared to the DM + HTN-Veh group (Figure 2B). The $IC_{50}$ of Ach concentration response in DM-Veh, HTN-Veh, and DM + HTN-Veh groups were significantly higher than that of the Sham-C-Veh group (Table 2). Besides, the $IC_{50}$ of Ach concentration response in DM + HTN-AEHE30 and DM + HTN-AEHE100 groups were significantly lower in comparison to the DM + HTN-Veh group (Table 2). However, no significant difference was observed among Sham-Veh, DM-Veh, HTN-Veh, DM + HTN-Veh, DM + HTN-AEHE, and DM + HTN-AEHE100 groups regarding the $E_{max}$ and $IC_{50}$ of SNP concentration response (Figure 2C).

## 5. Discussion

The present study aimed to examine the possible antihypertensive and anti-diabetic effects of *AEHE* in the rats with simultaneous type 2 diabetes and renal hypertension. The main findings of the study were that *AEHE* prevented development of hypertension and endothelial dysfunction and decreased blood glucose and lipid profile. This study showed that the SBP of the rats with simultaneous renal hypertension and type 2 diabetes was higher compared to the Sham-control group, which was in agreement with a previous report (12). Such a significantly higher SBP might be partly mediated by impairment of endothelium-dependent relaxation, characterized by lower $E_{max}$ and higher $IC_{50}$ of Ach concentration response. This suggestion is similar to that of the others using the same model (13) or spontaneously hypertensive rats (14).

The current study demonstrated that simultaneous type 2 diabetes and renal hypertension was associated with increased HR, whereas type 2 diabetes and hypertension alone were associated with decreased and increased HR,

**Table 2.** The Values of Maximal Response ($E_{max}$) and Effective Concentration 50 ($EC_{50}$) and Inhibitory Concentration 50 ($IC_{50}$) of Phenylephrine and Acetylcholine

| | Phenylephrine | | Acetylcholine | |
|---|---|---|---|---|
| | $E_{max}$ | $EC_{50}$ | $E_{max}$ | $IC_{50}$ |
| **Sham-C-Veh** | 100.0 ± 00.0 | -6.72 ± 0.06 | 84.3 ± 3.6 | -7.18 ± 0.30 |
| **DM-Veh** | 154.7 ± 18.5 [a] | -7.05 ± 0.09 [a] | 64.9 ± 5.8 [a] | -5.41 ± 0.28 [a] |
| **HTN-Veh** | 168.0 ± 15.5 [a] | -7.33 ± 0.09 [a] | 67.9 ± 5.8 [a] | -5.97±0.27 [a] |
| **DM + HTN-Veh** | -141.3 ± 5.8 [a] | -7.31 ± 0.07 [a] | 52.7 ± 6.2 [a] | -4.61 ± 0.51 [a] |
| **DM + HTN-AEHE30** | 122.0 ± 4.6 [b] | -7.13 ± 0.05 [b] | 85.4 ± 1.1 [b] | -7.09 ± 0.20 [b] |
| **DM + HTN-AEHE100** | 116.5 ± 3.4 [b] | -7.15 ± 0.06 [b] | 87.9 ± 3.2 [b] | -7.69 ± 0.30 [b] |

Abbreviations: Sham-C-Veh, sham-control group treated with vehicle; DM-Veh, type 2 diabetes mellitus, renal hypertensive treated with vehicle; DM + HTN-Veh, simultaneous type 2 diabetes mellitus and renal hypertensive group; DM + HTN-AEHE30, simultaneous type 2 diabetes and renal hypertension groups receiving *Allium eriophyllum* leave extract at 30 mg/kg/day; DM + HTN-AEHE100, simultaneous type 2 diabetes and renal hypertension groups receiving the extract at 100 mg/kg/day; FBS, fasting blood glucose
[a] Significant difference ($P < 0.05$) from Sham-C-Veh; [b] Significant difference ($P < 0.05$) from DM + HTN-Veh; The values are mean ± SEM, n = 8 - 10 each.

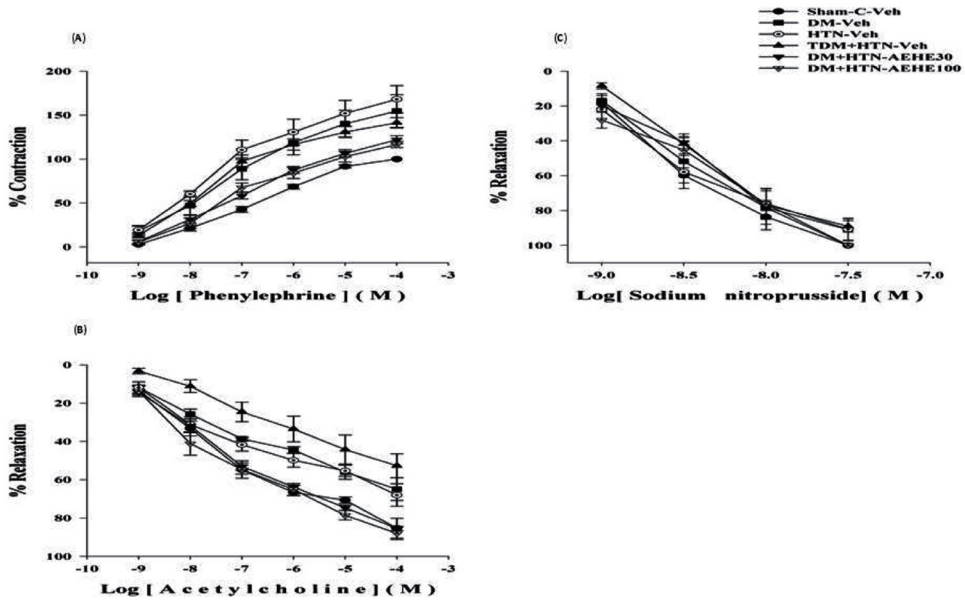



**Figure 2.** Concentration-Response Curves of A) Phenylephrine, B) Acetylcholine, and C) Sodium Nitroprusside in Aortic Rings from the Sham-Operated Control Group Receiving Vehicle (Sham-C-Veh), Type 2 Diabetes Group Receiving Vehicle (DM-Veh), Renal Hypertensive Group Receiving Vehicle (HTN-Veh), Simultaneous Type 2 Diabetes and Renal Hypertension Group Receiving Vehicle (DM + HTN-Veh), and Simultaneous Type 2 Diabetes and Renal Hypertension Groups Receiving *Allium eriophyllum* Leaves Extract at 30 mg/kg/day (DM + HTN-AEHE30) and 100 mg/kg/day (DM + HTN-AEHE100) after Four Weeks of Teatment. The Responses Were Calculated as Mean ± SEM and as the Percentage of Maximal Response to Each Compound in the Control Group. The data have been presented as Mean ± SEM, N = 8 - 10 in each group.

respectively. These results suggest that hypertension has offset the diabetes-induced decrease of HR. The diabetes-induced decrease in HR is in agreement with the earlier reports (12) and has been attributed to autonomic imbalance (5) and changes in pacemaker activity of the heart (6). The hypertension-induced increase in HR is also in agreement with an earlier report (12, 15) and was attributed to increased sympathetic activity (16).

To the best of our knowledge, the present study was the first to report the favorable effects of *AEHE* in a model of simultaneous type 2 diabetes and hypertension. The study indicated that *AEHE* reduced blood pressure. This is consistent with the previous studies showing that other Allium species had antihypertensive effects in the same model (17) or had vasodilatory effects on thoracic aortic preparations (13). The antihypertensive effects of the extract may be related to extract-induced reduction of number of vascular alpha receptors (down-regulation) or an alpha antagonism. This conclusion is evidenced from the finding that *AEHE* reduced the $E_{max}$ and increased the $EC_{50}$ of Phe concentration response in aortic rings of the rats treated with the extract. This indicates that the extract may have reduced the number of vascular alpha receptors (down-regulation) or have played an alpha antagonist activity role. Yet, both of these speculations need to be investigated. A similar effect on alpha receptor-induced contraction in isolated aortic rings has been reported for antihypertensive herbs, such as sesamin (18), and naturals products, such as quercetin (19), resveratrol (20), and oleuropein (21). Moreover, the antihypertensive effects of the extract might be related to a beta receptor antagonism. This suggestion is based on the finding that HR was lower in the rats receiving the extract. Beta receptor antagonists, a class of antihypertensive drugs, are known to reduce blood pressure by reducing HR (22). Given the effects of the extract on alpha and beta receptor-mediated effects, the extract may have acted on these receptors separately, or alternatively, on a site upstream these receptors in the sympathetic nervous system.

We also investigated whether changes in the status of endothelial release of Nitric Oxide (NO) was involved in the antihypertensive effects of *AEHE*. Our findings showed that the extract increased $E_{max}$ and decreased $IC_{50}$ of Ach concentration response in isolated aortic ring studies. Such changes have been taken as increased endothelium-dependent relaxation and enhanced release of endothelium-derived NO (3, 21). Therefore, this finding indicated that a part of the extract's antihypertensive effects might be related to enhanced release of NO. Enhanced release of NO has been reported as an antihypertensive mechanism for other herbs, such as sesamin (23), and natural products, such as resveratrol (9) and oleuropein (21).

Earlier studies have reported that anti-oxidative stress activities underlay the antihypertensive mechanism of herbs, such as resveratrol (20) and oleuropein (21). Therefore, we measured the serum levels of SOD and GR and found that the extract did decrease them in the rats with simultaneous renal hypertension and diabetes. Consequently, it might be possible to suggest that, in agreement with previous reports (21), a part of the antihypertensive activity of *AEHE* is attributed to decrease of oxidative stress. Increased oxidative stress has been reported to be involved in development of hypertension by causing up-regulation of angiotensin II signaling (24), decreasing NO bioavailability (25), dysfunction of endothelial NO synthase (26), and reduction of the levels

of reactive oxygen species scavenger (27). Therefore, it can be speculated that, by virtue of anti-oxidative stress activity, the extract may have opposed one or more of such mechanisms and thereby has prevented the increase of blood pressure in the present study.

The current study revealed that *AEHE* reduced FBS and improved lipid profile in the rats with simultaneous type 2 diabetes and renal hypertension. Our findings were in line with those of the previous studies which showed that the extracts of other species of Allium decreased blood glucose (28), total cholesterol (29), and TG (30) in STZ-induced diabetic rats. They are also in agreement with a research demonstrating that S-methyl cysteine sulfoxide from bulb of *Allium cepa* reduced TG and cholesterol levels in high cholesterol diet fed rats (31). Nevertheless, the present study cannot speculate the mechanism of hypoglycemic and hypolipidemic effects of *AEHE*. Earlier studies, however, attributed the hypoglycemic effects of other Allium extracts to increased secretion of insulin from pancreatic B cells (32), enhanced insulin sensitivity (33), and antioxidant activities (34). The lipid lowering effects of such extracts have also been attributed to inhibition of cholesterol synthesis (35) and depressed hepatic activity of lipogenic and cholesterogenic enzymes (36, 37). Our study demonstrated that *AEHE* did reduce serum SOD and GR levels. Therefore, the anti-oxidative stress activity of the extract may partly explain the reduction of blood glucose and lipid profile. Whether one or more of the above-mentioned mechanisms underlie the hypoglycemic or lipid lowering effects of *AEHE* remains to be investigated.

In conclusion, the present study revealed that *AEHE* had antihypertensive effects in the rats with simultaneous type 2 diabetes and renal hypertension, which might be related to enhancement of release of endothelium-derived NO, antagonism of beta receptors, antagonism and/or down-regulation of alpha receptors, and anti-oxidative stress activities. The results also showed that the extract had blood glucose and serum lipid lowering activities, which may be partly attributed to anti-oxidative stress activities.

**Acknowledgements**

The present study was financially supported by the Research Vice-Chancellor of Shiraz University of Medical Sciences, Shiraz, Iran (grant No. 6357).

**Authors' Contribution**

Study concept and design: Masoud Mozafari, Ali Akbar Nekooeian; Analysis and interpretation of data: Masoud Mozafari, Ali Akbar Nekooeian; Drafting of the manuscript: Masoud Mozafari, Ali Akbar Nekooeian, Zeinab Janahmadi; Critical revision of the manuscript for important intellectual content: Ali Akbar Nekooeian; Statistical analysis: Masoud Mozafari, Ali Akbar Nekooeian, Zeinab Janahmadi

**Financial disclosure**

There is no financial disclosure.

**Funding/Support**

The present study was supported by a research grant from Vice-Presidency in Research, Shiraz University of Medical Sciences.

**References**

1. Chen G, McAlister FA, Walker RL, Hemmelgarn BR, Campbell NR. Cardiovascular outcomes in framingham participants with diabetes: the importance of blood pressure. Hypertension. 2011;57(5):891–7.
2. Colivicchi F, Mettimano M, Genovesi-Ebert A, Schinzari F, Iantorno M, Melina G, et al. Differences between diabetic and non-diabetic hypertensive patients with first acute non-ST elevation myocardial infarction and predictors of in-hospital complications. J Cardiovasc Med (Hagerstown). 2008;9(3):267–72.
3. Al-Qattan KK, Khan I, Alnaqeeb MA, Ali M. Mechanism of garlic (Allium sativum) induced reduction of hypertension in 2K-1C rats: a possible mediation of Na/H exchanger isoform-1. Prostaglandins Leukot Essent Fatty Acids. 2003;69(4):217–22.
4. Nekooeian AA, Khalili A, Khosravi MB. Effects of Short-term Renovascular Hypertension and Type 2 Diabetes on Cardiac Functions in Rats. Iran J Med Sci. 2014;39(1):51–9.
5. Thayer JF, Yamamoto SS, Brosschot JF. The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. Int J Cardiol. 2010;141(2):122–31.
6. Howarth FC, Jacobson M, Shafiullah M, Adeghate E. Long-term effects of streptozotocin-induced diabetes on the electrocardiogram, physical activity and body temperature in rats. Exp Physiol. 2005;90(6):827–35.
7. Allison GL, Lowe GM, Rahman K. Aged garlic extract and its constituents inhibit platelet aggregation through multiple mechanisms. J Nutr. 2006;136(3 Suppl):782S–8S.
8. Chan KC, Yin MC, Chao WJ. Effect of diallyl trisulfide-rich garlic oil on blood coagulation and plasma activity of anticoagulation factors in rats. Food Chem Toxicol. 2007;45(3):502–7.
9. Toklu HZ, Sehirli O, Ersahin M, Suleymanoglu S, Yiginer O, Emekli-Alturfan E, et al. Resveratrol improves cardiovascular function and reduces oxidative organ damage in the renal, cardiovascular and cerebral tissues of two-kidney, one-clip hypertensive rats. J Pharm Pharmacol. 2010;62(12):1784–93.
10. Dillon SA, Burmi RS, Lowe GM, Billington D, Rahman K. Antioxidant properties of aged garlic extract: an in vitro study incorporating human low density lipoprotein. Life Sci. 2003;72(14):1583–94.
11. Nekooeian AA, Mashhoodi T. Solid plexiglass clips to induce reproducible renal hypertension in the rat. Indian Journal of Pharmacology. 2007;39(1):25.
12. Khalili A, Nekooeian AA, Khosravi MB, Fakher S. Simultaneous renal hypertension and type 2 diabetes exacerbate vascular endothelial dysfunction in rats. Int J Exp Pathol. 2012;93(3):210–7.
13. Al-Qattan KK, Thomson M, Al-Mutawa'a S, Al-Hajeri D, Drobiova H, Ali M. Nitric oxide mediates the blood-pressure lowering effect of garlic in the rat two-kidney, one-clip model of hypertension. J Nutr. 2006;136(3 Suppl):774S–6S.
14. Sekiguchi F, Miyake Y, Hirakawa A, Nakahira T, Yamaoka M, Shimamura K, et al. Hypertension and impairment of endothelium-dependent relaxation of arteries from spontaneously hypertensive and L-NAME-treated Wistar rats. J Smooth Muscle Res. 2001;37(2):67–79.
15. Zhong G, Chen F, Cheng Y, Tang C, Du J. The role of hydrogen sulfide generation in the pathogenesis of hypertension in rats induced by inhibition of nitric oxide synthase. J Hypertens. 2003;21(10):1879–85.
16. Souza HC, Martins-Pinge MC, Dias da Silva VJ, Borghi-Silva A, Gastaldi AC, Blanco JHD, et al. Heart rate and arterial pressure variability in the experimental renovascular hypertension model in rats. Autonomic Neuroscience. 2008;139(1):38–45.
17. Al-Qattan KK, Alnaqeeb MA, Ali M. The antihypertensive effect of garlic (Allium sativum) in the rat two-kidney--one-clip Goldblatt model. J Ethnopharmacol. 1999;66(2):217–22.
18. Kong X, Yang JR, Guo LQ, Xiong Y, Wu XQ, Huang K, et al. Sesamin improves endothelial dysfunction in renovascular hypertensive rats fed with a high-fat, high-sucrose diet. Eur J Pharmacol. 2009;620(1-3):84–9.
19. Ajay M, Achike FI, Mustafa AM, Mustafa MR. Effect of quercetin on altered vascular reactivity in aortas isolated from streptozotocin-induced diabetic rats. Diabetes Res Clin Pract. 2006;73(1):1–7.
20. Bhatt SR, Lokhandwala MF, Banday AA. Resveratrol prevents endothelial nitric oxide synthase uncoupling and attenuates development of hypertension in spontaneously hypertensive rats. Eur J Pharmacol. 2011;667(1-3):258–64.

21. Nekooeian AA, Khalili A, Khosravi MB. Effects of oleuropein in rats with simultaneous type 2 diabetes and renal hypertension: a study of antihypertensive mechanisms. J Asian Nat Prod Res. 2014;16(9):953–62.
22. Feringa HH, Bax JJ, Boersma E, Kertai MD, Meij SH, Galal W, et al. High-dose beta-blockers and tight heart rate control reduce myocardial ischemia and troponin T release in vascular surgery patients. Circulation. 2006;114(1 Suppl):I344–9.
23. Wu XQ, Kong X, Zhou Y, Huang K, Yang JR, Li XL. Sesamin exerts renoprotective effects by enhancing NO bioactivity in renovascular hypertensive rats fed with high-fat-sucrose diet. Eur J Pharmacol. 2012;683(1-3):231–7.
24. Touyz RM, Tabet F, Schiffrin EL. Redox-dependent signalling by angiotensin II and vascular remodelling in hypertension. Clin Exp Pharmacol Physiol. 2003;30(11):860–6.
25. Touyz RM. Reactive oxygen species, vascular oxidative stress, and redox signaling in hypertension: what is the clinical significance? Hypertension. 2004;44(3):248–52.
26. Fujii S, Zhang L, Igarashi J, Kosaka H. L-arginine reverses p47phox and gp91phox expression induced by high salt in Dahl rats. Hypertension. 2003;42(5):1014–20.
27. Taddei S, Virdis A, Ghiadoni L, Magagna A, Salvetti A. Vitamin C improves endothelium-dependent vasodilation by restoring nitric oxide activity in essential hypertension. Circulation. 1998;97(22):2222–9.
28. Thomson M, Al-Amin ZM, Al-Qattan KK, Shaban LH, Ali M. Anti-diabetic and hypolipidaemic properties of garlic (Allium sativum) in streptozotocin-induced diabetic rats. Int J Diabetes & Metabolism. 2007;15:108–15.
29. Eidi A, Eidi M, Esmaeili E. Antidiabetic effect of garlic (Allium sativum L.) in normal and streptozotocin-induced diabetic rats. Phytomedicine. 2006;13(9-10):624–9.
30. Madkor HR, Mansour SW, Ramadan G. Modulatory effects of garlic, ginger, turmeric and their mixture on hyperglycaemia, dyslipidaemia and oxidative stress in streptozotocin-nicotinamide diabetic rats. Br J Nutr. 2011;105(8):1210–7.
31. Kumari K, Augusti KT. Lipid lowering effect of S-methyl cysteine sulfoxide from Allium cepa Linn in high cholesterol diet fed rats. J Ethnopharmacol. 2007;109(3):367–71.
32. Liu CT, Hse H, Lii CK, Chen PS, Sheen LY. Effects of garlic oil and diallyl trisulfide on glycemic control in diabetic rats. Eur J Pharmacol. 2005;516(2):165–73.
33. Mathew PT, Augusti KT. Studies on the effect of allicin (diallyl disulphide-oxide) on alloxan diabetes. I. Hypoglycaemic action and enhancement of serum insulin effect and glycogen synthesis. Indian J Biochem Biophys. 1973;10(3):209–12.
34. Rahimi R, Nikfar S, Larijani B, Abdollahi M. A review on the role of antioxidants in the management of diabetes and its complications. Biomed Pharmacother. 2005;59(7):365–73.
35. Singh DK, Porter TD. Inhibition of sterol 4alpha-methyl oxidase is the principal mechanism by which garlic decreases cholesterol synthesis. J Nutr. 2006;136(3 Suppl):759S–64S.
36. Yeh YY, Liu L. Cholesterol-lowering effect of garlic extracts and organosulfur compounds: human and animal studies. J Nutr. 2001;131(3s):989S–93S.
37. Gebhardt R, Beck H. Differential inhibitory effects of garlic-derived organosulfur compounds on cholesterol biosynthesis in primary rat hepatocyte cultures. Lipids. 1996;31(12):1269–76.