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Antihypertensive effect of allicin in dexamethasone-induced hypertensive rats

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ABSTRACT

Background: Glucocorticoid is among the most commonly prescribed medicine. Unfortunately, Excess glucocorticoid level leads hypertension in 80–90% patients. Garlic (Allium sativum) has been used since ancient times and even nowadays as a part of popular medicine for various ailments and physiological disorders. Hence this study was undertaken to investigate the antihypertensive activity of allicin in dexamethasone induced hypertension in wistar rats.

Methods: The animals were randomly divided into four groups comprising of six rats per group. Hypertension was induced by subcutaneous injection of dexamethasone (10 μg/rat/day) in hypertensive rats. Two hypertensive group animals were treated with nicorandil (6 mg/kg/day, po) and allicin (8 mg/kg/day, po) respectively for 8 weeks. While systolic blood pressure (SBP) was measured by the tail-cuff method weekly up to 8 weeks.

Results: Dexamethasone treatment resulted in significant increase in SBP while allicin treatment significantly decreases the SBP. Thus, this study confirmed that allicin treatment for 8 weeks partially reverse dexamethasone induced hypertension in rats. Allicin treatment also attenuated dexamethasone-induced anorexia and loss of total body weight.

Conclusion: This result suggests antihypertensive effects of allicin in dexamethasone induced hypertension. However, further studies are needed to explore the detailed mechanism of antihypertensive effect of allicin.

1. Introduction

Glucocorticoid is among the most commonly prescribed medicines for asthma, rheumatological syndrome, eye disorder, skin disorder, organ transplant, glomerulopathies, malignancies, pain syndrome, and other conditions.[1,2] Glucocorticoids have potent anti-inflammatory and immunosuppressant activities. Unfortunately, long-term glucocorticoid

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therapy leads to hypertension and diabetic condition. Glucocorticoid treatment causes hypertension and abnormal glucose metabolism in 80-90% of patients having Cushing syndrome.[2,3] In the current scenario, we are looking for herbal drugs to avoid such adverse effects. The usage of herbal and nutritional supplements is widespread all over the world. Now a large number of patients are using herbal drugs for cardiovascular diseases. Certain herbal drugs such as St John's wort, yohimbine, licorice, ephedra, garlic, etc. have been in use for many decades for the treatment of hypertension.[4] However, the underlying mechanism of action of herbal drugs is not clearly understood. Most prominent among these herbs is the commonly used Indian traditional spice garlic, *Allium sativum L.*, a member of the Alliaceae family, which has been used since ancient times and, even today, is a part of popular medicine for various ailments and physiological disorders.[5–7] Fresh garlic extract contains the organosulfur compound allicin, which is considered to have various pharmacological activities, including, antithrombotic, antidiabetic, antitumorigenetic, antioxidant, anticarcinogenic, antiatherosclerotic, and antihypertensive activities.[5,8–12] Therefore, the aim of the present study was to explore the effects of allicin in the treatment of dexamethasone-induced hypertension in rats. Dexamethasone is the most potent synthetic glucocorticoid that has virtually pure glucocorticoid activity.[1]

2. Methods

2.1. Animals

Wistar rats (150–200 g) of either sex were used in this study, and each experimental group included six animals. Animals bred in the animal house of Institute of Pharmaceutical Education and Research (Reg. No. 535/02/a/CPCSEA/Jan2002), Wardha (Maharashtra State), India. The rats were housed under standard laboratory conditions ($22 \pm 2\,^{\circ}C$, 12-hour light/dark cycle) with free access to food (normal pellet diet) and water. The animals were treated in accordance with the Committee for the Purpose of Control And Supervision of Experiments on Animals CPCSEA guidelines. The experimental protocol was approved by the Institutional Animal Ethics Committee (approval number 10/200910).

2.2. Chemicals

Dexamethasone and nicorandil were procured from Zydus Cadila Healthcare Ltd (Bangalore, India) and Medreich Saimirra Ltd (Chennai, India) respectively. All the other chemicals used for experimental purpose were of analytical grade.

2.3. Induction of hypertension

In the experimental rats, hypertension was induced by subcutaneous injection of dexamethasone (10 μg/kg/d) in the evening.[13,14]

2.4. Preparation of aqueous extract of garlic

Garlic (*A. sativum* L.) grown in Wardha was acquired from the market. Garlic bulbs were identified and authenticated by Dr Alka Chaturvedi, Post graduate Teaching Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, with a voucher specimen (No. 9803). Garlic bulbs were stored at $4\,^{\circ}C$ and used for analysis within 30 days. Allicin-containing garlic extract was prepared from 10 g of garlic cloves. The cloves were crushed with an electric vegetable crusher, and the juice was poured into a sterile centrifuge tube and centrifuged at 5000 rpm (3000 *g*) for 10 minutes in order to separate the majority of the pulp from the supernatant liquid. The supernatant garlic extract (allicin) was either used immediately for activity or stored at $4\,^{\circ}C$; it was relatively stable during the experimental weeks. Accordingly, animals were administered with 8 mg allicin/kg body weight.[15,16]

2.5. Analysis of allicin in fresh garlic extract

Allicin in garlic extract (10 μL, 20 μL, 30 μL, and 40 μL of the extract were used) was reacted with cysteine via the thiol-disulfide exchange reaction, and the remaining cysteine was subsequently determined by reaction with Ellman's reagent 5,5′-dithiobis-(2-nitrobenzoic acid) to produce the 2-nitro-5-thiobenzoate anion. Absorbance was measured at a wavelength of 412 nm. One mole of thiosulfinite reacts with 2 mole cysteine, and since allicin makes up 60–80% of the thiosulfinates produced in garlic, multiplication of the total thiosulfinate content by a factor of 0.7 gives the approximate allicin content.[15]

2.6. Experimental design

Animals were randomly divided into four groups, each consisting of six animals. Animals in group I (normal control rats) received 1 mL (po/d) 1% acacia gum suspension, group II (hypertensive control rats) received 1 mL (po/d) 1% acacia gum suspension, animals in group III (hypertensive rats) received nicorandil 1 mL (6 mg/kg/po/d) in 1% acacia gum suspension,[17,18] and group IV (hypertensive rats) received allicin 1 mL (8 mg/kg/po/d) in 1% acacia gum suspension[16] during the course of the entire study (8 weeks).

2.7. Estimation of systolic blood pressure

Systolic blood pressure (SBP) measurements were recorded weekly by the same investigator, between 10 am and 12 noon, using the integrated BIOPAC -is an instrument use to measure blood pressure and NIBP-Non Invasive Blood Pressure 200A system. The animal is placed in the restrainer (animal holder) leaving the tail outside and adjusted to the position where the animal has limited movement. The restrainer is placed in the heating chamber and heated up to $32\,^{\circ}C$. BSL PRO software is used for recording SBP. The basic software setup is done, and IR sensors are calibrated prior to starting the measurement. An IR sensor is then connected to the tail of the animal inside the restrainer. After the required setup and calibration of IR sensors, SBP was recorded.

2.8. Estimation of body weight, food, and water intake

Body weight, food, and water intake of each animal of every group were measured weekly, for 8 weeks.

2.9. Statistical analysis

Statistical analysis was carried out by two-way analysis of variance followed by Bonferroni post-tests. All values were expressed as mean ± standard error of the mean. All groups were compared with hypertensive control animals. A p value of <0.05 was considered to be statistically significant.

3. Results

3.1. Quantification of allicin from fresh garlic aqueous extract

Garlic extracts contained approximately 12.8 mg allicin/mL.

3.2. Effect of allicin on SBP in hypertensive rats

Table 1 shows that SBP ($^{*}p < 0.05$) increased significantly in hypertensive control animals when compared with the normal control group. Allicin-treated animals were found to have significantly ($\dagger p < 0.05$) decreased SBP when compared with hypertensive control group .

3.3. Effect of allicin on body weight

A significant decrease in body weight was recorded in all groups of dexamethasone-treated animals. Hence, all treated animals did not show any significant changes when compared with group II (hypertensive control) animals. However, body weight of group I (normal) animals significantly increased in the 7th ($^{*}p < 0.05$) and 8th ($\dagger p < 0.001$) weeks (Table 2) as compared with that of group II (hypertensive control) animals.

3.4. Effect of allicin on food intake

No significant changes in food intake were found in any group of animals until the 7th week, while in the 8th week a significant increase ($^{*}p < 0.05$) in food intake was recorded in group I (normal) and group IV (allicin-treated) animals when compared with hypertensive control animals (Table 3).

4. Discussion

In the present study, we found that dexamethasone increased SBP in rats, and this hypertension was attenuated by allicin; this result showed the antihypertensive effect of allicin. Various studies have suggested that dexamethasone causes overproduction of reactive oxygen species such as superoxide, interacts with nitric oxide (NO) produced by the vascular endothelium—a vasodilator, causes NO–redox imbalance, and reduces its NO bioavailability, which may lead to hypertension.[13,14,19–21] Further, dexamethasone can also block NO synthase gene expression at the transcriptional level.[22] Thus, dexamethasone can act by several

Table 1 – Effect of allicin on systolic blood pressure in hypertensive rats.

Group(s)	Systolic blood pressure (mmHg)								
	0th week	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
I	109.5 ± 2.52	108.66 ± 1.56	105.00 ± 2.09	103.00 ± 1.21	99.83 ± 1.66	102.83 ± 2.57	100.16 ± 2.68	100.83 ± 3.17	101.16 ± 2.90
II	107.8 ± 2.34	119.0 ± 0.89	125.5 ± 1.11	130.5 ± 1.31	133.5 ± 0.84*	134.6 ± 0.49*	132.6 ± 1.33*	132.0 ± 0.85*	133.6 ± 0.80*
III	118.5 ± 1.25	113.0 ± 1.41	107.5 ± 1.23	110.5 ± 1.47	113.0 ± 1.36	109.3 ± 0.92	105.6 ± 0.66	110.8 ± 0.91	108.6 ± 0.98
IV	118.3 ± 2.18	112.3 ± 2.23	108.8 ± 2.41	108.1 ± 3.70	105.6 ± 4.06	104.5 ± 3.28†	102.0 ± 2.04†	100.1 ± 2.31†	103.8 ± 1.90†

Data are expressed as mean ± SEM ($n = 6$). Significance was determined by two-way ANOVA followed by Bonferroni post-tests. A p value of <0.05 was considered significant. All treated groups were compared with group II.
ANOVA, analysis of variance; group I, normal control animals; group II, hypertensive animals; group III, nicorandil-treated animals; group IV, allicin-treated animals; SEM, standard error of the mean.
* $p < 0.05$ (group I vs. group II).
† $p < 0.05$ (group II vs. group IV).

Table 2 – Effect of allicin on body weight in hypertensive rats.

Group(s)	Body weight (g)								
	0th week	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
I	160.33 ± 10.50	163.33 ± 12.80	165.83 ± 11.30	168.00 ± 9.70	170.17 ± 9.58	179.00 ± 8.70	193.50 ± 10.65	205.17 ± 13.80*	220.67 ± 12.60***
II	165.83 ± 12.40	162.50 ± 10.50	159.00 ± 13.43	155.83 ± 11.75	152.16 ± 9.80	153.33 ± 10.60	151.66 ± 10.37	149.83 ± 11.45	147.33 ± 8.32
III	164.67 ± 18.90	160.16 ± 16.55	156.66 ± 13.34	153.66 ± 15.87	151.83 ± 10.84	149.16 ± 9.60	150.83 ± 12.53	148.66 ± 11.55	148.16 ± 9.89
IV	165.83 ± 17.70	163.16 ± 15.66	160.83 ± 12.74	158.66 ± 11.95	155.50 ± 14.75	153.00 ± 10.55	150.50 ± 9.50	147.33 ± 11.43	144.83 ± 9.30

Data are expressed as mean ± SEM ($n = 6$). Significance was determined by two-way ANOVA followed by Bonferroni post-tests. A p value of <0.05 was considered significant. All groups were compared with group II.

ANOVA, analysis of variance; group I, normal control animals; group II, hypertensive animals; group III, nicorandil-treated animals; group IV, allicin-treated animals; SEM, standard error of the mean.

* $p < 0.05$.

*** $p < 0.001$.

Table 3 – Effect of allicin on food intake in hypertensive rats.

Group(s)	Food intake (g/d)								
	0th week	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
I	14.00 ± 3.91	13.00 ± 1.79	11.50 ± 1.64	9.00 ± 2.60	12.00 ± 2.39	10.60 ± 2.06	13.80 ± 1.62	15.60 ± 1.60	17.30 ± 1.17*
II	12.30 ± 2.67	8.50 ± 1.48	9.00 ± 2.12	10.00 ± 2.77	6.50 ± 1.71	8.30 ± 1.83	11.40 ± 1.92	9.20 ± 1.51	7.80 ± 1.84
III	15.30 ± 1.98	9.33 ± 1.57	9.00 ± 1.74	8.33 ± 2.09	10.50 ± 2.48	8.50 ± 3.43	11.20 ± 3.22	15.00 ± 4.37	13.66 ± 3.11
IV	18.30 ± 17.70	12.60 ± 1.96	7.90 ± 1.55	8.00 ± 2.34	7.66 ± 1.68	11.33 ± 2.05	13.40 ± 1.47	15.70 ± 1.31	17.66 ± 2.80*

Data are expressed as mean ± SEM ($n = 6$). Significance was determined by two-way ANOVA followed by Bonferroni post-tests. A p value of <0.05 was considered significant. All groups were compared with group II.

ANOVA, analysis of variance; group I, normal control animals; group II, hypertensive animals; group III, nicorandil-treated animals; group IV, allicin-treated animals; SEM, standard error of the mean.

* Shows the level of significance ($^{*}p < 0.05$).

pathways to deplete the supply of NO, consequently leading to vasoconstriction.[23] Another possible explanation may be that dexamethasone increases the levels of enzymes such as angiotensin-converting enzyme, which might be responsible for a significant elevation in blood pressure.[24,25] The hypertensive effect of dexamethasone can be reduced by elimination of the above risk factors via increases in NO level, angiotensin-converting enzyme inhibitor activity, and free radical-scavenging activity. It has already been established that antioxidants are effective in the treatment of dexamethasone-induced hypertension. In the present study, experimental animals of hypertensive groups, such as group I (normal) and group IV (allicin treated) animals, showed lower SBP (†$p < 0.05$) when compared with group II (hypertensive control) rats (Table 1).

Various mechanisms for the antihypertensive effect of garlic have been reported, including reduction in vascular resistance and a subsequent fall in total peripheral resistance contributing substantially to the antihypertensive action by activating NO synthase, the enzyme that produces NO.[26,27] Further, Park et al[28] reported that fermented garlic contains nitrite, which is converted to NO in the body, and increases protein kinase (PKG) and eNOS protein expressions in aortic tissues. This further leads to antihypertensive effects via the Soluble guanylyl cyclase (sGC)–Cyclic guanosine monophosphate cGMP–protein kinase pathway. In addition, garlic (allicin) has antioxidant property, which might be responsible for the antihypertensive effect of allicin by lowering the level of oxidative stress in hypertensive rats, consequently restoring NO–redox imbalance and increasing NO bioavailability.[26,27] Recently, Hiramatsu et al[29] reported that aged garlic extract increases the expression of nuclear factor erythroid 2-related factor 2, which is responsible for the antioxidant activity. They confirmed that aged garlic extract decreases oxidative stress and maintains cellular redox balance via the nuclear factor erythroid 2-related factor 2–antioxidant response element signaling pathway.

Further, garlic has potent vasorelaxant and K^+ channel opener activities, which lead to vasodilation, thus exerting an antihypertensive effect.[5,6,11,30,31] This report was further supported by the finding that allicin selectively opens SUR2, a type of K^+ channel receptor, present in blood vessels, leading to dilation of blood vessels, which may be a possible mechanism for the antihypertensive activity of allicin.[32] Other studies have also revealed that fresh garlic extract (allicin) has inhibitory activity against angiotensin 1 converting enzyme and diuretic activity, which may be involved in the antihypertensive action of garlic.[10–12,28,33,34]

Previous evidence supports the theory that corticosteroids induce an imbalance between vasoconstriction and vasodilation, favoring vasoconstriction, resulting in hypertension.[34] Corticosteroids seem to negatively affect the production of other vasodilatory substances, such as prostaglandin I2 and prostaglandin, and to have a positive effect on vasoconstrictor prostaglandins such as thromboxane-B2 in the vascular endothelium.[35–37] Garlic causes more reduction in the synthesis of thromboxane B2, thereby reducing hypertension. Garlic was also found to inhibit endothelin-1-induced contraction.[37,38] Recently, a randomized database search clinical trial reports suggested that garlic is an effective and safe therapeutic approach for the treatment of hypertension.[39]

In addition, dexamethasone causes a decrease in body weight and food intake. The possible mechanism could be that dexamethasone increases leptin mRNA expression in the adipose tissue and induces long-lasting hyperleptinemia in rats; plasma leptin may play a role in dexamethasone-induced anorexia. Additionally, increased expression of monoamine oxidase A and 5-hydroxytryptamine (5-HT) reuptake transporter genes by repeated dexamethasone administration appears to be implicated in decreases of brain 5-HT levels in the hypothalamus.[40,41] Synergistically low level of 5-HT and high plasma leptin level suppress food intake and increase energy expenditure, resulting in a loss of both fat and lean mass; this mechanism may be involved in the reduction of total body weight and food intake in dexamethasone-treated animals.

This study indicates that long-term treatment with allicin-containing garlic extract shows significant effect in reversing dexamethasone-induced SBP as well as improves body weight and food intake in dexamethasone-induced hypertension in rats. However, this study has some limitations; the molecular mechanism involved in antihypertensive effects needs to be explored. Moreover, it has already been established that allicin is the major compound of garlic, and all sulfoxides except cyloalliin are converted into allicin by enzymatic reactions.[42] Hence, we need to evaluate cyloalliin-related activities. Further, it is well documented that allicin has a very short half-life, and it is difficult to maintain its long-term therapeutic activities. There are some other compounds such as stable active metabolite of allicin may be is responsible for antihypertensive activity. Hence, further studies exploring the molecular mechanism and active molecule responsible for antihypertensive action are required.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. Ong SLH, Zhang Y, Whitworth JA. Mechanisms of dexamethasone-induced hypertension. *Curr Hypertens Rev* 2009;5:61–7.
2. Bernal-Mizrachi C, Weng S, Feng C, Finck BN, Knutsen RH, Leone TC, et al. Dexamethasone induction of hypertension and diabetes is PPAR-α dependent in LDL receptor-null mice. *Nature Med* 2003;9:1069–75.
3. Szemeredi K, Bagdy G, Kopin IJ, Goldstein DS. Neurocirculatory regulation in cortisol-induced hypertension. *Clin Exp Hypertens* 1989;11:1425–39.
4. Mansoor GA. Herbs and alternative therapies in the hypertension clinic. *Am J Hypertens* 2001;14:971–97.
5. Ashraf MZ, Hussain ME, Fahim M. Antiatherosclerotic effects of dietary supplementations of garlic and turmeric: restoration of endothelial function in rats. *Life Sci* 2005;77:837–57.
6. Baluchnejadmojarad T, Roghani M, Homayounfar H, Hosseini M. Beneficial effect of aqueous garlic extract on the vascular reactivity of streptozotocin-diabetic rats. *J Ethnopharmacol* 2003;85:139–44.

7. Londhe VP, Gavasane AT, Nipate SS, Bandawane DD, Chaudhari PD. Role of garlic (*Allium sativum)* in various diseases: an overview. *J Pharm Res Opin* 2011;1:129–34.
8. Liu C, Wong P, Li C, Hse H, Sheen L. Antidiabetic effect of garlic oil but not diallyl disulfide in rats with streptozotocin induced diabetes. *Food Chem Toxicol* 2006;44:1377–84.
9. Eidi A, Eidi M, Esmaeili E. Antidiabetic effect of garlic (*Allium sativum* L.) in normal and streptozotocin-induced diabetic rats. *Phytomedicine* 2006;13:624–9.
10. Hosseini M, Shafiee SM, Baluchnejadmojarad T. Garlic extract reduces serum angiotensin converting enzyme (ACE) activity in nondiabetic and streptozotocin-diabetic rats. *Pathophysiology* 2007;14:109–12.
11. Ali M, Al-Qattan KK, Al-Enezi F, Khanafer RM, Mustafa T. Effect of allicin from garlic powder on serum lipids and blood pressure in rats fed with a high cholesterol diet. *Prostaglandins Leukot Essent Fatty Acids* 2000;62:253–9.
12. Oron-Herman M, Rosenthal T, Mirelman D, Miron T, Rabinkov A, Wilchek M, et al. The effects of S-allylmercaptocaptopril, the synthetic product of allicin and captopril, on cardiovascular risk factors associated with the metabolic syndrome. *Atherosclerosis* 2005;183:238–43.
13. Ong SLH, Vickers JJ, Zhang Y, McKenzie K, Walsh CE, Whitworth JA. Role of xanthine oxidase in dexamethasone-induced hypertension in rats. *Clin Exp Pharmacol Physiol* 2007;34:517–9.
14. Zhang Y, Croft KD, Mori TA, Schyvens CG, McKenzie KUS, Whitworth JA. The antioxidant tempol prevents and partially reverses dexamethasone-induced hypertension in the rat. *Am J Hypertens* 2004;17:260–5.
15. Curtis H, Noll U, Stormann J, Slusarenko AJ. Broad-spectrum activity of the volatile phytoanticipin allicin in extracts of garlic (*Allium sativum* L.) against plant pathogenic bacteria, fungi and oomycetes. *Physiol Mol Plant Pathol* 2004;65:79–89.
16. Elkayam A, Mirelman D, Peleg E, Wilchek M, Miron T, Rabinkov A, et al. The effects of allicin and enalapril in fructose-induced hyperinsulinemic hyperlipidemic hypertensive rats. *Am J Hypertens* 2001;14:377–81.
17. Xu J, Nagata K, Obata K, Ichihara S, Izawa H, Noda A, et al. Nicorandil promotes myocardial capillary and arteriolar growth in the failing heart of Dahl salt-sensitive hypertensive rats. *Hypertension* 2005;46:719–24.
18. Trongvanichnam K, Mitsui-Saito M, Ozaki H, Karaki H. Effects of chronic oral administration of a high dose of nicorandil on *in vitro* contractility of rat arterial smooth muscle. *Eur J Pharmacol* 1996;314:83–90.
19. De Gennaro Colonna V, Rossoni G, Rigamonti A, Bonomo S, Manfredi B, Berti F, et al. Enalapril and quinapril improve endothelial vasodilator function and aortic eNOS gene expression in L-NAME-treated rats. *Eur J Pharmacol* 2002;450:61–6.
20. Rojas A, Figueroa H, Re L, Morales MA. Oxidative stress at the vascular wall: Mechanistic and pharmacological aspects. *Arch Med Res* 2006;37:436–48.
21. Cannon RO. Role of nitric oxide in cardiovascular disease: focus on the endothelium. *Clin Chem* 1998;44:1809–19.
22. Kanno K, Hirata Y, Imai T, Iwashina M, Marumo F. Regulation of inducible nitric oxide synthase gene by interleukin-1 beta in rat vascular endothelial cells. *Am J Physiol* 1994;267:H2318–24.
23. Wallwork CJ, Parks DA, Schmid-Schonbein GW. Xanthine oxidase activity in the dexamethasone-induced hypertensive rat. *Microvasc Res* 2003;66:30–7.
24. Rajashree S, Puvanakrishnan R. Dexamethasone induced alterations in the levels of proteases involved in blood pressure homeostasis and blood coagulation in rats. *Mol Cell Biochem* 1999;197:203–8.
25. Barreto-Chaves MLM, Heimann A, Krieger JE. Stimulatory effect of dexamethasone on angiotensin-converting enzyme in neonatal rat cardiac myocytes. *Braz J Med Biol Res* 2000;33:661–4.
26. Schwartz IF, Hershkovitz R, Iaina A, Gnessin E, Wollman Y, Chernichowski T, et al. Garlic attenuates nitric oxide production in rat cardiac myocytes through inhibition of inducible nitric oxide synthase and the arginine transporter CAT-2 (cationic amino acid transporter-2). *Clin Sci* 2002;102:487–93.
27. Bozin B, Mimica-Dukic N, Samojlik I, Goran A, Igic R. Phenolics as antioxidants in garlic (*Allium sativum* L., Alliaceae). *Food Chem* 2008;111:925–9.
28. Park BM, Cha SA, Kim HY, Kang DK, Yuan K, Chun H, et al. Fermented garlic extract decreases blood pressure through nitrite and sGC–cGMP–PKG pathway in spontaneously hypertensive rats. *J Funct Foods* 2016;22:156–65.
29. Hiramatsu K, Tsuneyoshi K, Ogawa T, Morihar N. Aged garlic extract enhances heme oxygenase-1 and glutamate-cysteine ligase modifier subunit expression via the nuclear factor erythroid 2-related factor 2-antioxidant response element signaling pathway in human endothelial cells. *Nutr Res* 2016;36:143–9.
30. Sharifi AM, Darabi R, Akbarloo N. Investigation of antihypertensive mechanism of garlic in 2K1C hypertensive rat. *J Ethnopharmacol* 2003;86:219–24.
31. Ojewole JAO, Adewunmi CO. P-1: possible mechanisms of antihypertensive effect of garlic: evidence from mammalian experimental models [abstract]. *Am J Hypertens* 2001;14(S1):29A.
32. Dubey H, Singh A, Patole AM, Tenpe CR. Allicin, a SUR2 opener: possible mechanism for the treatment of diabetic hypertension in rats. *Braz J Pharmacog* 2012;22:1053–9.
33. Suetsuna K. Isolation and characterization of angiotensin I-converting enzyme inhibitor dipeptides derived from *Allium sativum* L (garlic). *J Nutr Biochem* 1998;9:415–9.
34. Pedraza-Chaverri J, Tapia E, Medina-Campos ON, Granados MDLA, Franca M. Garlic prevents hypertension induced by chronic inhibition of nitric oxide synthesis. *Life Sci* 1998;2:71–7.
35. Peppa M, Krania M, Raptis SA. Hypertension and other morbidities with Cushing's syndrome associated with corticosteroids: a review. *Integr Blood Press Control* 2011;4:7–16.
36. Falardeau P, Martineau A. Prostaglandin I2 and glucocorticoid-induced rise in arterial pressure in the rat. *J Hypertens* 1989;7:625–32.
37. Al-Qattan KK, Khan I, Alnaqeeb MA, Ali M. Thromboxane-B2, prostaglandin-E2 and hypertension in the rat 2-kidney 1-clip model: a possible mechanism of the garlic induced hypotension. *Prostaglandins Leukot Essent Fatty Acids* 2001;64:5–10.
38. Banerjee SK, Maulik SK. Effect of garlic on cardiovascular disorders: a review. *Nutr J* 2002;1:1–14.
39. Xiong XJ, Wang PQ, Li SJ, Li XK, Zhang YQ, Wang J. Garlic for hypertension: a systematic review and meta-analysis of randomized controlled trials. *Phytomedicine* 2015;22:352–61.
40. Jahng JW, Kim NY, Ryu V, Yoo SB, Kim BT, Kang DW, et al. Dexamethasone reduces food intake, weight gain and the hypothalamic 5-HT concentration and increases plasma leptin in rats. *Eur J Pharmacol* 2008;581:64–70.
41. Carlos R, Salaman P. Dexamethasone inhibits food intake suppression induced by low doses of interleukin-1β administered intracerebroventricularly. *Brain Res Bull* 1991;27:737–8.
42. Amagase H. Clarifying the real bioactive constituents of garlic. *J Nutr* 2006;136:716S–25S.