



The Effect of Garlic Pills on Serum Nitric Oxide and Preeclampsia Prevention in Healthy Nulliparous Pregnant Women: A Randomized, Controlled Clinical Trial

Fahimeh Sehhati Shafa'i¹ · Farzaneh Darvishi¹ · Fatemeh Abbasalizadeh² · Mojgan Mirghfourvand¹

Revised: 3 July 2018 / Accepted: 6 July 2018 / Published online: 1 August 2018
© Society of Fetal Medicine 2018

Abstract The present study aimed to determine the effect of garlic pills on serum nitric oxide and preeclampsia prevention in healthy nulliparous pregnant women. This randomized clinical trial was conducted among 215 nulliparous pregnant women. The participants were assigned into control and intervention groups. The intervention and control groups received garlic pills and placebo, respectively for 16 weeks from 20 week of gestation. Serum nitric oxide was measured 12 weeks after the intervention. Women were followed up for preeclampsia until childbirth in several visits. Data were analyzed using statistical package for social sciences software. The mean (standard deviation) of serum nitric oxide level was 151.4 (73.1) μmol in the intervention group and 124.1 (44.1) μmol in the control group. There was a significant difference between the two groups ($P = 0.029$). The prevalence of preeclampsia was 1 (1%) in the intervention group and 5 (5.2%) in the control group. There was no significant difference between the two groups ($P = 0.094$). The use of garlic pills has a significant effect on the elevation of serum

nitric oxide levels. Despite the absence of significant statistical differences between the two groups, garlic pills in comparison with placebo reduced the incidence of preeclampsia.

Keywords Garlic · Nitric oxide · Preeclampsia · Prevention · Nulliparous

Introduction

Preeclampsia is defined as an increase in resistant diastolic blood pressure during pregnancy up to 90 mm Hg or more in women whose blood pressure has previously been normal, plus 24-h proteinuria ≥ 0.3 g [1]. According to WHO reports, it is an important reason for pregnancy adverse consequences [2]. According to the American College of Obstetricians and Gynecologists, preeclampsia causes a large number (50,000–60,000 cases) of perinatal deaths around the world [3]. The prevalence of preeclampsia in various regions of the world is reported to be 1–2%, which increases up to 34% in the presence of other risk factors [4]. The prevalence of preeclampsia in Iran was 3.3% in a cross-sectional study in 2007 in Rasht [5].

Preeclampsia is most probably preceded by a low-oxygen environment, which creates inflammatory mediators and oxidative stress in the placenta. Then, the ratio of reactive oxygen species to vasodilators such as nitric oxide (the strongest vasodilator) is disrupted, and leads to clinical manifestations of preeclampsia [6, 7]. Once preeclampsia emerges, the only way to prevent mother's eclampsia and fetal injury is pregnancy termination [3]. Therefore, it is reasonable and valuable to adopt safe, natural, inexpensive and available approaches to prevent this syndrome. Antioxidants are thought to be effective in preventing

✉ Farzaneh Darvishi
Mwfarzane.darvishi@gmail.com

Fahimeh Sehhati Shafa'i
Fahimehsehhatie@yahoo.com

Fatemeh Abbasalizadeh
fabbasalizadeh@yahoo.com

Mojgan Mirghfourvand
mirghfourvandm@tbzmed.ac.ir

¹ Faculty of Nursing and Midwifery, Tabriz University of Medical Sciences, Tabriz, Iran

² Department of Obstetrics and Gynecology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

preeclampsia and its complications by increasing levels of nitric oxide [7]. Researchers have found no significant relationship in several review articles examining anti-oxidants such as vitamins E and C in preventing preeclampsia [8, 9]. Garlic has been shown to have potent antioxidant properties [10, 11].

Das et al. [12] showed that the garlic extract incubation in the vicinity of the prepared platelet samples and also the placental tissue increased the activity of NOS (nitric oxide production catalyst) in the platelets.

Garlic (*Allium sativum*) belongs to the *Allium* and onion family [13]. Its main compound, allicin, along with other polysulfides in garlic increase the production of nitric oxide and inhibition of angiotensin II through biological mechanisms, and as a result increase vasodilatation and decrease blood pressure [11]. In most studies, garlic has been classified as a safe medication during pregnancy with little or no effect on the mother and the fetus except for limited digestive side effects and its strong odor [14, 15]. In a systematic review, researchers also reported beneficial effects of garlic on controlling weight and fetal development [16].

Few studies have examined prevention of preeclampsia following garlic consumption, and no study was found on the effect of garlic on serum nitric oxide in pregnant women. Furthermore, review articles including Cochran review studies recommend further investigation of the effect of garlic on preeclampsia prevention, especially in areas of the world where garlic consumption is prevalent [17]. Therefore, the present study was conducted to investigate the effect of garlic on serum nitric oxide levels and prevention of preeclampsia in healthy nulliparous pregnant women through a clinical trial in Rasht, Iran, where production and consumption of garlic is prevalent.

Materials and Methods

Study Type and Participants

This randomized controlled clinical triple blind trial recruited 215 nulliparous pregnant women from April 2016 to August 2017. The inclusion criteria were willingness to participate, age of 18–35, having a record in Rasht health centers, having an ultrasound report indicating singleton pregnancy with a healthy fetus, gestational week 20, no alcohol or tobacco use, literate and having a phone number for follow-ups. Gestational age was calculated based on the date of the first day of the last menstruation or early pregnancy ultrasound. The exclusion criteria were systemic diseases such as diabetes, cardiovascular diseases, and renal diseases; history of coagulation disorders; history of any pre-pregnancy and pregnancy-related hypertension;

BMI ≥ 30 kg/m²; systolic blood pressure lower than 100 mm Hg; history of allergy to garlic; garlic intake of more than 1 serving per day or more than 10 servings per week; and participation in other similar studies.

Sample Size

Sample size was determined using Hodzic study on the biosynthesis of serum nitric oxide during normal pregnancy compared to a pregnancy complicated with preeclampsia [18] and considering $m_1 = 40.9$, $m_2 = 42.9$, $sd_1 = sd_2 = 2.8$, two-sided $\alpha = 0.05$, and power = 95% in G-power software as 52 subjects per group. However, it was increased to 60 subjects per group taking into account 20% withdrawals, and ultimately 215 subjects entered the study.

Sampling

This study was carried out in accordance with the Declaration of Helsinki guidelines after approval of the proposal by the Ethics Committee. The initial sampling was done with convenience sampling method. That is, 10 of the 15 urban health centers with their subsidiary bases were selected because of their extensive coverage of pregnant mothers. The researcher then visited the health centers and selected the eligible pregnant women presenting to the health centers by convenience method. They were briefed on study objectives and signed an informed written consent if they were willing to participate. The demographics questionnaire for mothers was completed by the researcher through an interview with the pregnant women. Blood pressure was measured once before the intervention and then in seven specified visits by a mercury sphygmomanometer and recorded in the checklist.

Randomization

Participants were randomly assigned into two groups of garlic ($n = 107$) and placebo ($n = 108$) with a 1:1 ratio with a randomized block design with block size of four and six. For allocation concealment, garlic and placebo were placed in similar, sequentially numbered opaque sealed envelopes. Blocking and preparation of envelopes were done by a person not involved in data sampling and analysis. Thus, the data collector, the participant and the data analyst were blinded to sample allocation.

Study Design

The 400 mg garlic pills (each containing 1.1–1.4 mg/kg of allicin, equivalent to 2 g of fresh garlic) and an identical placebo (in odor, color, and shape) were produced by a Pharmaceutical, Hygienic and Food company. The pills

were consumed by the pregnant women twice a day (every 12 h) from gestational week 20 for 16 weeks.

Assessment of Variables

The examined outcomes of this study were serum nitric oxide levels and the prevalence of preeclampsia. Venous blood samples were collected from mothers (5 cc) after 8–10 h of fasting (to control the nutrients affecting the amount of nitric oxide) to measure serum nitric oxide level, 12 weeks after the start of the intervention (gestational week 32). Samples were stored at -18°C . Once 215 samples were collected, they were tested with a 200-assays Nitric Oxide Assay Kit using Griess test at a wavelength of 450–630 nm by a laboratory expert. In order to follow-up the incidence of preeclampsia, blood pressure was measured once before the start of the intervention in the first visit (gestational week 20) and then after the start of the intervention in the second visit (gestational weeks 26–30), the third visit (gestational weeks 31–34), the fourth visit (gestational weeks 35–37), the fifth visit (gestational week 38), the sixth visit (gestational week 39) and the seventh visit (gestational week 40) by mercury sphygmomanometer with a precision of 99.9% and a maximum error of ± 2 mm with a proper cuff from the right arm in sitting position after 15 min of rest. The systolic and diastolic blood pressure criteria were considered as the first and fifth Korotkoff sounds, respectively. If the systolic blood pressure was equal to or greater than 140 mm Hg or diastolic blood pressure was equal to or greater than 90 mm Hg, a urine sample was collected by the researcher in a special container provided by the laboratory and sent to a single laboratory to measure the random urine protein, which was recorded in the checklist. The participating pregnant women filled out the questionnaire at 8 and 16 weeks after the intervention in order to assess side effects and participants' level of satisfaction with the medication.

Statistical Analysis

Data were analyzed in SPSS-21 software using independent *t* test, Chi square, Fisher, and round Chi square with $\alpha = 0.05$ at all stages. Normality of quantitative data was investigated, all of which had a normal distribution. All analyses were performed on the basis of intention to treat. The significance level was considered at $P < 0.05$.

Results

Four hundred pregnant women presenting to health centers were evaluated for inclusion criteria. Among them, 215 were eligible, of whom 14 were excluded due to lack of

cooperation in consuming pills (Fig. 1). Demographic characteristics of mothers including age, BMI, educational level, and socioeconomic status were compared before treatment and no significant differences were observed between these parameters ($P > 0.05$).

Approximately more than three-fourths of women (81.4%) were 18–34 years old. More than two-thirds of the participants (67%) had a normal BMI. Most of the subjects (93%) were housewives. More than one-third of the subjects (39.5%) had a high school diploma. About two-thirds of the participants (63.3%) reported that their income was somehow sufficient to cover the cost of living (Table 1).

On gestational week 32, the mean (standard deviation) of serum nitric oxide in the garlic and placebo groups was 151.4 (73.1) μmol and 124.1 (44.1) μmol , respectively. According to independent *t*-test, it was significantly higher in the garlic group (mean difference: 27.3; confidence interval: 95%; 2.8–51.7) ($P = 0.029$) (Table 2).

The prevalence of preeclampsia was 1 (1%) in the garlic group and 5 (5.2%) in the placebo group. There was no significant difference between the two groups based on Chi square test (OR: 5.4; CI: 95%; 0.6–47.9 ($P = 0.094$)) (Table 3).

Comparison of mothers' satisfaction with the medications in both garlic and placebo groups did not show a significant difference ($P = 0.464$), and about half of women (58%) reported that they were pleased. The side effects of the pills in the two groups were negligible. The only complication reported by the majority of mothers was mild nausea: 29 cases (28.2%) in the garlic group and five cases (5.1%) in the placebo group. The next most common complaint was the pill odor; three cases (2.8%) in garlic group of and one case (0.9%) in the placebo group. There was one case (0.9%) of severe constipation in the placebo group. No serious complications were reported following the consumption of pills by the pregnant women.

Discussion

The present study is the first study to investigate the effect of garlic pills on serum nitric oxide and preeclampsia prevention in healthy pregnant women. Similar studies are rare at the national and international levels, and most of the studies evaluated the effect of garlic pills on prevention of preeclampsia in pregnant women with high risk for preeclampsia.

The present study showed that garlic consumption can increase serum nitric oxide levels in pregnant women. The result is consistent with a study by Das et al. (1995) in the UK investigating the activation of nitric oxide synthase (NOS) by garlic in vitro as well as on a human sample in vivo. Researchers showed that the garlic extract

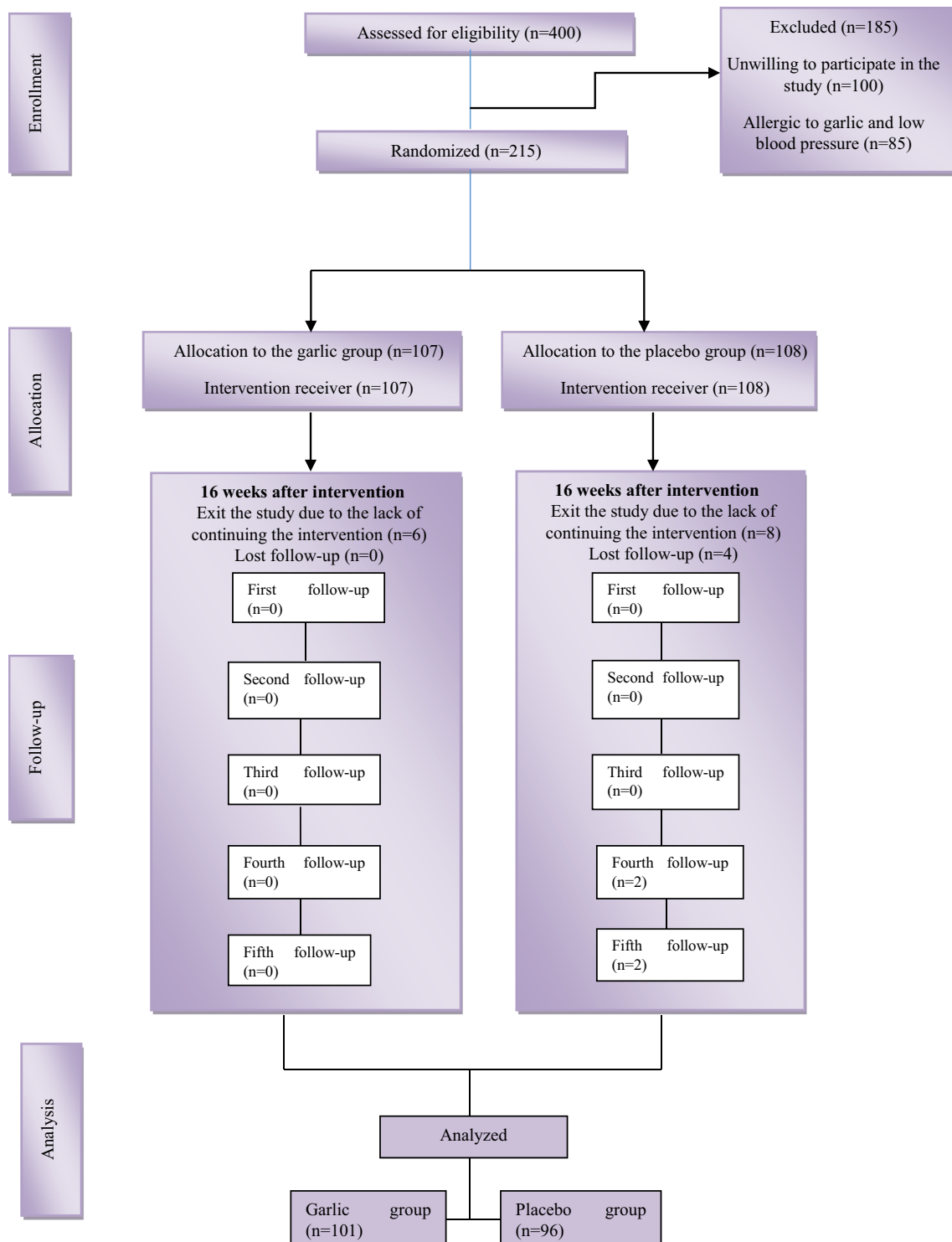


Fig. 1 Summary of patient flow

incubation in the vicinity of the prepared platelet samples and also the placental tissue increased the activity of NOS in the platelets. The consumption of 4 g of garlic also increased the activity of NOS from 0.21 to 0.45 nm in

platelets. Plasma nitrite levels increased from 3.9 to 7.8 μmol , too [12].

Hodzic et al. [18] conducted a cross sectional study in Bosnia and Herzegovina (2017) to compare nitric oxide biosynthesis between healthy pregnant women and

Table 1 Demographic and obstetric information in two groups of intervention and control

Demographic information	Garlic group N = 107 Number (percent)	Placebo group N = 108 Number (percent)	P
Age (year)	25.8 (4.4)**	25.6 (4.3)**	0.760*
BMI	23.8 (2.5)**	24.01 (2.5)**	0.671*
Level of education			0.453‡
Primary school	11 (10.3)	5 (4.6)	
Secondary school	12 (11.2)	21 (19.4)	
High school	16 (15.0)	17 (15.7)	
High school diploma	49 (45.8)	32 (29.6)	
Academic	19 (17.8)	33 (30.6)	
Job			0.175†
Housewife	97 (90.7)	103 (95.4)	
Employed	10 (9.3)	5 (4.6)	
Husband's job			0.285†
Unemployed	4 (3.7)	4 (3.7)	
Shopkeeper	18 (16.8)	13 (12.0)	
Laborer	26 (24.3)	26 (24.1)	
Employee	19 (17.8)	11 (10.2)	
Self-employed	40 (37.4)	54 (50.0)	
Husband's educational level			0.444‡
Primary school	8 (7.5)	5 (4.6)	
Secondary school	14 (13.1)	24 (22.2)	
High school	11 (10.3)	14 (13.0)	
High school diploma	47 (43.9)	38 (35.2)	
Academic	27 (25.2)	27 (25.0)	
Sufficient monthly family income for living expenses			0.732‡
Sufficient	27 (25.2)	17 (15.7)	
Fairly sufficient	59 (55.1)	77 (71.3)	
Insufficient	21 (19.6)	14 (13.0)	
Residence			0.622†
Owned house	16 (15.0)	20 (18.5)	
Husband's parents' home	15 (14.0)	13 (12.0)	
Wife's parent's home	6 (5.6)	10 (9.3)	
Rented house	70 (65.4)	65 (60.2)	
Abortion history			0.395†
Yes	27 (25.2)	22 (20.4)	
No	80 (74.8)	86 (79.6)	

*Independent T-test; **Mean (Standard deviation); ‡Trend Chi square test; †Chi square test

Table 2 Comparison of serum nitric oxide levels between intervention and control groups

Variable	Garlic group (n = 101) Mean (SD)	Placebo group (n = 100) Mean (SD)	MD (95% CI); P
Serum nitric oxide (µmol)	151.4 (73.1)	124.1 (44.1)	27.3 (2.8–51.7) 0.029*

*Independent T-test

pregnant women with preeclampsia, and reported that the serum nitric oxide levels in women with preeclampsia were lower than those in healthy pregnant women (30.7 vs. 35.1 µmol).

Ziaee et al. (2001) in Tehran conducted a study to determine the effect of garlic pills on prevention of preeclampsia on 100 pregnant women with positive roll-over test. Their results are consistent with the present study

Table 3 Comparison of preeclampsia prevalence in intervention and control groups

Variable	Garlic group (n = 101) Number (percent)	Placebo group (n = 96) Number (percent)	OR (95% CI); <i>P</i>
Preeclampsia			5.4 (0.6–47.9); 0.094 [†]
Positive	1 (1)	5 (5.2)	
Negative	100 (99)	91 (94.8)	

[†] χ^2 test

as the administration of garlic pills for 8 weeks (gestational weeks 28–32) was not successful in preventing preeclampsia and the observed difference in preeclampsia incidence was not significant between the two groups [seven cases (14%) in the intervention group versus nine cases (18%) in the control group] [19].

Another randomized controlled trial in 2001 by Akbari et al. in Tehran aimed to determine “the effect of garlic pills on prevention of preeclampsia in high-risk nulliparous mothers”. One hundred pregnant women with a positive roll-over test in gestational weeks 28–32 were recruited. Fifty subjects in the intervention group received 800 mg garlic pills daily and 50 subjects in the control group received placebo pills for 6–8 weeks. Consistent with this study, there was no significant difference in the prevalence of preeclampsia and pregnancy outcomes between the two groups ($P > 0.05$) [20].

A'jami Harandi et al. in Kashan (2015) determined the effect of garlic pills on pregnancy outcomes in pregnant women at risk of preeclampsia and 44 pregnant primigravid women with a positive roll-over test from gestational weeks 27. Nine weeks of consuming garlic pills in the intervention group reduced the incidence of preeclampsia in this group compared to the control group (four cases in the placebo group vs. one case in the control group), but the difference was not statistically significant [21]. This finding is in line with the results of the present study.

The findings of the above studies, together with the results of the present study suggest the effect of garlic in increasing serum nitric oxide, which can be associated with prevention of pregnancy-related hypertension disorders.

In our study, consumption of garlic pills was associated with a reduction in preeclampsia prevalence in the intervention group, but this was not significantly different in comparison to the control group. This finding is statistically consistent with the results of recent clinical trials. However, the observed difference in the incidence of preeclampsia between the two groups is much higher than that in other studies, and if a larger population is studied, the statistical difference may be significant.

The present study had the following limitations; it has a small sample size, and it is not possible to generalize the

results to rural communities and pregnant women high-risk for preeclampsia. It is suggested that further studies measure the level of potential biomarkers associated with the preeclampsia pathogenesis (sFlt-1, sEng, PIGF, VEGF) in order to achieve better results. Considering that the pathophysiological changes associated with pre-eclampsia are at the very early stages of pregnancy [2], it is recommended that in future studies, the onset of intervention begin from the lower weeks of pregnancy.

One of the strengths of this study is its large sample size compared to previous studies, the onset of intervention with the onset of the second half of pregnancy, the long duration of intervention, and the blinding of the study.

Conclusion

The findings of this study showed that the serum nitric oxide level was significantly higher in the intervention group than in the control group, which indicates the effectiveness of the garlic pills. Considering the role of nitric oxide in vasodilatation and prevention of blood pressure disorders such as preeclampsia, the presence of antioxidant compounds in the garlic, and the absence of significant side effects, garlic pills can be used to prevent pregnancy related hypertension, especially preeclampsia, and, consequently, to promote maternal and neonatal health and reduce health costs.

Acknowledgements This study was extracted from a postgraduate thesis registered at Iranian Registry of Clinical Trials (IRCT201602163027N33) and approved by the Ethics Committee of Tabriz University of Medical Sciences (TBZMED.REC.1394.1028) on February 1, 2016. The authors hereby thank all the participants; the staff of health centers in Rasht; Dr. Adel Montazeri, the laboratory technical director of the Razi and Hashmat Hospitals in Rasht and his staff, especially Ms. Rona Namazi and Ms. Atefeh Alavi, and Dr. Abbas Momeni; the honorable staff of Dr. Momeni laboratory of Rudсар; and Mr. Janbaz, in charge of the blood bank of Rudсар, who have cooperated in the implementation of this research project.

Compliance with Ethical Standards

Conflict of interest The Authors declares that they have no conflict of interests.

References

- World Health Organization. WHO recommendations for prevention and treatment of pre-eclampsia and eclampsia. Geneva; 2013. p. 14–17.
- World Health Organization. Prevention and treatment of pre-eclampsia and eclampsia. Geneva; 2011. p. 1–38.
- ACOG. Hypertension in pregnancy. Washington: Library of Congress Cataloging-in-Publication Data; 2013. p. 17–33.
- Fauvel JP. Hypertension during pregnancy: epidemiology, definition. *Presse Med.* 2016;45(7–8 Pt 1):618–21.
- Soroori ZZ, Sharami SH, Faraji R. Seasonal variation of the onset of preeclampsia and eclampsia. *J Res Med Sci.* 2007;12(4):198–202.
- Foo L, Tay J, Lees CC, McEniery CM, Wilkinson IB. Hypertension in pregnancy: natural history and treatment options. *Curr Hypertens Rep.* 2015;17(5):545.
- Matsubara K, Higaki T, Matsubara Y, Nawa A. Nitric oxide and reactive oxygen species in the pathogenesis of preeclampsia. *Int J Mol Sci.* 2015;16(3):4600–14.
- Polyzos N, Mauri D, Tsappi M, Tzioras S, Kamposioras K, Cortinovic I, et al. Combined vitamin C and E supplementation during pregnancy for preeclampsia prevention: a systematic review. *Obstet Gynecol Surv.* 2007;62(3):202–6.
- Conde-Agudelo A, Romero R, Kusanovic JP, Hassan SS. Supplementation with vitamins C and E during pregnancy for the prevention of preeclampsia and other adverse maternal and perinatal outcomes: a systematic review and metaanalysis. *Am J Obstet Gynecol.* 2011;204(503):e1–12.
- Moosavi T, Zakavi A, Hosseini-valiki F, Yousef pour M, Fakhari M, Rafiei A, et al. Nutritional properties of garlic according to traditional and modern medicine: a review study. *J Mazandaran Univ Med Sci.* 2016;26(139):227–45.
- Ried K, Fakler P. Potential of garlic (*Allium sativum*) in lowering high blood pressure: mechanisms of action and clinical relevance. *Integr Blood Press Control.* 2014;7:71–82.
- Das I, Khan NS, Sooranna SR. Potent activation of nitric oxide synthase by garlic: a basis for its therapeutic applications. *Curr Med Res Opin.* 1995;13(5):257–63.
- Amagase H, Petesch BL, Matsuura H, Kasuga S, Itakura Y. Intake of garlic and its bioactive components. *J Nutr.* 2001;131:955–62.
- Dugoua J-J. Herbal medicines and pregnancy. *J Popul Ther Clin Pharmacol.* 2010;17(3):370–8.
- ESCOPE. Garlic 2017 [cited 2017 2017.5.5]. <http://www.koop-phyto.org/en/medicinal-plants/garlic.php>.
- Sharma R, Jaitawat A, Kantwa SM, Jain N, Rani D. Role of garlic and fenugreek during gestation and lactation: a review. *UJERT.* 2014;4(5):265–79.
- Meher S, Duley L. Garlic for preventing pre-eclampsia and its complications (review). *Cochrane Database Syst Rev.* 2006;19(3):CD006065.
- Hodžić J, Izetbegović S, Muračević B, Iriškić R, Štimjanin Jović H. Nitric oxide biosynthesis during normal pregnancy and pregnancy complicated by preeclampsia. *Med Glas.* 2017;14(2):211–7.
- Ziaei S, Hantoshzadeh S, Rezasoltani P, Lamyian M. The effect of garlic tablet on plasma lipids and platelet aggregation in nulliparous pregnant at high risk preeclampsia. *Eur J Obstet Gynecol Reprod Biol.* 2001;99:201–6.
- Akbari N. The effect of garlic pills on the prevention of preeclampsia in primipara who are at risk. *J Sabzevar Sch Med Sci.* 2001;8(2):8–71.
- Aalami-Harandi R, Karamali M, Asemi Z. The favorable effects of garlic intake on metabolic profiles, hs-CRP, biomarkers of oxidative stress and pregnancy outcomes in pregnant women at risk for pre-eclampsia: randomized, double-blind, placebo-controlled trial. *J Matern Fetal Neonatal Med.* 2015;28(17):2020–7.