BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, March 2016. Vol. **13**(1), 279-286

Effect of Vitamin C Supplementation on Blood Pressure Level in Type 2 Diabetes Mellitus: A Randomized, Double-Blind, Placebo-Controlled Trial

Zainab Shateri[1], Seyed Ali Keshavarz[1]*, Saeed Hosseini[1], Maryam Chamari[2], Mostafa Hosseini[3] and Ensiye Nasli[4]

[1]Department of Clinical Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, IR Iran.
[2]Department of Community Nutrition, School of Nutritional Science and Dietetics, Tehran University of Medical Sciences, Tehran, IR Iran.
[3]Department of Epidemiology and Biostatistics, School of Health, Tehran University of Medical Sciences, Tehran, IR Iran
[6]Diabetes Research Center, Tehran University of Medical Sciences, Tehran, IR Iran.

http://dx.doi.org/10.13005/bbra/2031

(Received: 10 February 2016; accepted: 14 March 2016)

Hypertension is one of the common problems in diabetic people. There are evidences suggesting that oxidative stress plays an important role in the development of hypertension. It is possible that decreased antioxidant activity levels of reactive oxygen species scavengers such as vitamin C contribute to decreasing oxidative stress. This study was aimed to investigate the effect of vitamin C on blood pressure in people with type 2 diabetes. The present randomized controlled trial study was conducted on 84 patients with type 2 diabetes undergoing hypertension in the Diabetes Research Center of Tehran University of Medical Sciences, Iran, in 2014. Samples were randomly divided into two groups of vitamin C (n = 42) and placebo (n = 42) on the basis of the random permuted block method. The intervention group was given chewable vitamin C supplements at a dose of 250 mg 4 times a day and the control group was given its placebo for 45 days. The blood pressure of two groups was measured at baseline and in the end of the study. Data were analyzed using SPSS 16 software. No significant differences were observed in the average weight and body mass index, waist and hip circumference and daily intake of energy and its component between the two groups at the beginning and the end of the study. The mean of systolic (129.6±24.4 in vitamin C group and 129±14.1 in placebo, P=0.89) and diastolic (77.2±12.6 in vitamin C group and 76.5±9.65 in placebo, P=0.77) blood pressure at baseline was not significant between the two groups, but at the end of the study, findings showed that there is a significant difference between systolic (121.6±24.4 in vitamin C group and 129.2±13.5 in placebo, P<0.0001) and diastolic (72.5±11 in vitamin C group and 77.4±9.2 in placebo, P<0.0001) blood pressure of two groups. Conclusion: **According to the result of this study, the consumption of vitamin C supplement may cause a reduction in blood pressure in patients with type 2 diabetes mellitus. Therefore, in order to improve the health of these people, increasing intake of vitamin C is recommended.**

Key words: Ascorbic Acid, Blood Pressure, Diabetes Mellitus, Type 2.

Diabetes mellitus is a group of diseases characterized by high blood glucose concentrations resulting from defects in insulin secretion, insulin action, or both[1]. Diabetes is the sixth leading cause of death in the United State[2].This disease has huge health care costs. Incidence of disease has been estimated 7.7% in Iran in population 25-64 years in 2008[3]. It is

* To whom all correspondence should be addressed.
Tel.: +98-2188955897;
E-mail: s_akeshavarz@yahoo.com

anticipated that the total number of people with diabetes will increase from 171 million in 2000 to 366 million in 2030 [4].

In most cases, diabetes can be divided into two important categories of which type 2 diabetes' incidence and prevalence is increasing[4]. Diabetes has direct and indirect effects on vessels which is the main cause of morbidity and mortality[5]. One of the common problems in diabetes people is hypertension[6]. Hypertension affects about 70 percent of people with diabetes and it is on average two times more common in people with diabetes than healthy people[4].

There are numerous evidences suggesting that oxidative stress plays an important role in the development of hypertension[7-9]. It is possible that decreased antioxidant activity such as, superoxide dismutase and catalase and reduced levels of reactive oxygen species scavengers such as, vitamins E, C and glutathione contribute to oxidative stress[9] of which there are many cross-sectional studies showing that vitamin C status is inversely associated with blood pressure. Although some, but not all clinical trials have shown lowering effect of vitamin C on the level of blood pressure, scientific evidence especially in the Asian population is small[10].

Some studies have shown that lowering effect of vitamin C is through the dilation of vessels and improvement the activity of nitric oxide (10). A study was conducted by Zibaee Nezhad et. al. in Shiraz in 2009 with the goal of blood pressure adjustment in people with hypertension by vitamin C. This study was done on 50 people with hypertension. These people were given 250 mg vitamin C twice a day for a month. The results showed significant reduction in systolic and diastolic blood pressure levels in people taking vitamin C [11].

The study was done on 26 healthy people and surveyed the effect of oral and acute administration of vitamin C on oxidative stress, arterial stiffness or hypertension. This study showed that this vitamin has no effect on systolic and diastolic blood pressure in healthy people[12]. The important point is that high blood pressure in patients with diabetes leads to significant elevation of vascular complications and both make people susceptible to chronic kidney diseases. Overlapping diabetes and hypertension significantly increase ischemic cerebrovascular disease risk, retinopathy and sexual dysfunction[4].

Therefore, regarding the importance of hypertension in people with type 2 diabetes mellitus and its complications and with regard to possible role of vitamin C in blood pressure levels in these people, we decided to evaluate its effects on people with type 2 diabetes mellitus.

Objectives

The following directional hypothesis was set for the present study: the blood pressure reduces intervention group more than a placebo group.

Patients and methods

The present study was recorded with registration number IRCT2014093019342N1. This study was a randomized double blind placebo controlled clinical trial study. The sample size was calculated using the following formula in which power 80%, á=0.05, level of confidence=95%, ó= 14 and (n=42).

$$n = \frac{2\delta^2\left(z_{1-\frac{\alpha}{2}}+z_{1-\beta}\right)^2}{(\mu_1-\mu_2)^2}$$

Considering 15 percent more, each group consisted of 49 people. In this study, 98 patients with diabetes and hypertension who had records in the Diabetes Research Center of Tehran University of Medical Sciences (Tehran, Iran, in 2014) were included at the beginning of which 14 patients were excluded at the end of study (Figure 1).

As a result, 84 patients were selected as a sample for the chrrent study. Subjects were randomly divided on the basis of the random permuted block method into intervention and control group. It was given to intervention group vitamin C in dose of 250 mg four times a day during 6 weeks and its placebo was given to the control group. Vitamin C and its placebo were provided by pharmaceutical Daroupaksh Company. Participants were blocked based on age, sex, drugs and duration of diabetes.

Inclusion criteria for this study were as follows: 30-70 years-old diabetic and hypertensive, fasting blood sugar of 126 to 250 mg/dl, literate (for recording three-day record), consumption lowering blood sugar and blood pressure pills, tend to keep the weight during the study, lack of kidney

stones, body mass index ranging from 25 to 40, non-use of contraceptives pills among women, non-smoking and alcohol, lack of specific physical activity and the desire to maintain normal activities and current lifestyle during the study. During the study, patients who did not use drugs or willingness to cooperate or had changed their medication were excluded from the study.

After obtaining informed consent from the patients, one code was assigned to each patient for confidentiality. Their profile and anthropometric indices (weight, height, waist circumference, hip circumference and body mass index) were included in the questionnaire. Medications weren't discontinued due to medical and ethical problems and duration of diabetes and medication names were recorded. The patients were asked to note three days of their foods at the beginning and at the end of the study.

From each patient 15 ml blood was drawn after 12 hours of fasting at the beginning and in the last step of the study. Fasting blood glucose was measured by glucose oxidase method. The blood pressure of people intervention and control was measured by Germany mercury sphygmomanometer at baseline and the end of the study. Blood pressure was measured from right hand. The participants who were sensitive to the supplement were excluded from the study.

To check normally distributed data, the Kolmogorov-Simonov test was used and the normal distribution of the data was verified. Data analysis was performed using SPSS version 16. Also, Paired t test, Independent t test and analysis of covariance (ANCOVA) were utilized. P value of <0.05 were considered as significant.

RESULTS

The participants were composed of 42 males and 42 females and the ratio of women to men in this study was 1 to 1. The mean age of the participating patients in this study was 58 years. It was 56.8 years in vitamin C and 59.2 years in the placebo group. It should be noted that there wasn't a significant difference between the two groups in terms of age (P =0.17). Duration of diabetes in vitamin C group was 8.9 years and in the placebo group was 8.98 years which shows no significant difference between two groups(P =0.94). Schooling level of patients is shown in table 1.The data of these patients are shown in Table 2.

As a result, 84 patients were selected as a sample for the chrrent study. Subjects were randomly divided on the basis of the random permuted block method into intervention and control group. It was given to intervention group vitamin C in dose of 250 mg four times a day during 6 weeks and its placebo was given to the control group. Vitamin C and its placebo were provided by pharmaceutical Daroupaksh Company. Participants were blocked based on age, sex, drugs and duration of diabetes.

Inclusion criteria for this study were as follows: 30-70 years-old diabetic and hypertensive, fasting blood sugar of 126 to 250 mg/dl, literate (for recording three-day record), consumption lowering blood sugar and blood pressure pills, tend to keep the weight during the study, lack of kidney stones, body mass index ranging from 25 to 40, non-use of contraceptives pills among women, non-smoking and alcohol, lack of specific physical activity and the desire to maintain normal activities and current lifestyle during the study. During the study, patients who did not use drugs or willingness to cooperate or had changed their medication were excluded from the study.

After obtaining informed consent from the patients, one code was assigned to each patient for confidentiality. Their profile and anthropometric indices (weight, height, waist circumference, hip circumference and body mass index) were included in the questionnaire. Medications weren't discontinued due to medical and ethical problems and duration of diabetes and medication names were recorded. The patients were asked to note three days of their foods at the beginning and at the end of the study.

Table 1. Schooling level of participants taking 1000 milligrams of vitamin C and placebo per day

Variables		Groups	
		Vitamin C group	Placebo group
Education	Primary	6	7
	Middle and high school	12	8
	Diploma	13	13
	College	11	14

Table 2. Characteristics of the participants before and after taking 1000 milligrams of vitamin C and placebo per day

Variables	Time	Groups		P value
		Vitamin C(n=42) Mean±SD	Placebo(n=42) Mean±SD	
Height(cm)	Baseline	164.2±11	165.1±10.8	0.706‡
Weight(kg)	Baseline	82.6±15	80.9±14.2	0.598‡
	End	82.1±15.2	80.6±14.3	0.639‡
Body mass index	Baseline	30.6±4.6	29.5±3.2	0.23‡
	End	30.6±4.6	29.4±3.2	0.27‡
Waist	Baseline	98.2±9.9	97.6±8.1	0.764‡
circumference(cm)	End	97.9±9.9	97.3±8.3	0.786‡
Hip	Baseline	108.1±10.1	104.7±6.5	0.068‡
circumference(cm)	End	108.1±10	104.6±6.7	0.071‡
Waist to hip ratio	Baseline	0.9±0.06	0.9±0.07	0.112‡
	End	0.9±0.06	0.9±0.07	0.123‡
Systolic blood	Baseline	129.6±24.4	129±14.1	0.892‡
pressure(mmHg)	End	121.6±24.4	129.2±13.5	<0.0001§
Diastolic blood	Baseline	77.2±12.6	76.5±9.65	0.772‡
pressure(mmHg)	End	72.5±11	77.4±9.2	<0.0001§

‡ Independent t test § ANCOVA

Table 3. Mean and standard deviation of findings related to food intake in patients receiving vitamin C (1000mg/day) & placebo

Variables	Time	Groups		P value
		Vitamin C(n=42) Mean±SD	Placebo(n=42) Mean±SD	
Energy (Kcal/day)	Baseline	2024±251	1986±206	0.44
	End	2070±249	2036±225	0.50
Carbohydrate(g/day)	Baseline	257±48	252±44	0.64
	End	264±43	255±44	0.37
Protein (g/day)	Baseline	65±15	62±12	0.37
	End	65±15	63±12	0.47
Total fat (g/day)	Baseline	84±17	82±19	0.56
	End	87±19	87±14	0.90
Fiber (g/day)	Baseline	16±4	14±5	0.1
	End	15±3	16±4	0.3
Saturated fatty acid(g/day)	Baseline	22±8	19±7	0.07
	End	21±6	19±6	0.35
PUFA(g/day)	Baseline	22±5	24±7	031
	End	24±6	25±5	0.40
Cholesterol(mg/day)	Baseline	148±91	209±123	0.64
	End	198±95	166±84	0.10
Calcium (mg/day)	Baseline	658±314	659±248	0.15
	End	640±264	541±216	0.06
Potassium (mg/day)	Baseline	2245±644	2148±509	0.44
	End	2256±592	2133±497	0.30
Sodium (mg/day)	Baseline	956±437	813±421	0.13
	End	862±325	780±406	0.30
Magnesium (mg/day)	Baseline	261±67	240±44	0.09
	End	264±77	242±46	0.11
Vitamin C(mg/day)	Baseline	90±60	75±53	0.21
	End	82±45	81±60	0.94

‡ Independent t test § ANCOVA

From each patient 15 ml blood was drawn after 12 hours of fasting at the beginning and in the last step of the study. Fasting blood glucose was measured by glucose oxidase method. The blood pressure of people intervention and control was measured by Germany mercury sphygmomanometer at baseline and the end of the study. Blood pressure was measured from right hand. The participants who were sensitive to the supplement were excluded from the study.

To check normally distributed data, the Kolmogorov-Simonov test was used and the normal distribution of the data was verified. Data analysis was performed using SPSS version 16. Also, Paired t test, Independent t test and analysis of covariance (ANCOVA) were utilized. P value of <0.05 were considered as significant.

The mean differences of weight, waist circumference, hip circumference, waist-to-hip ratio and body mass index were not significant between the two groups before and after intervention. These variable's mean differences within the group were also compared before and after the intervention which was not significant. At the baseline, there was no statistically significant difference between the two groups in terms of mean baseline systolic (P =0.89) and diastolic blood pressure (P =0.77) but at the end of the study significant difference between mean systolic (P<0.0001) and diastolic (P<0.0001) blood pressure in the two groups were observed.

There weren't significant differences in terms of mean energy, carbohydrate, fat, protein, fiber, saturated fatty acids, polyunsaturated fatty acid (PUFA), cholesterol, calcium, sodium, potassium, magnesium and vitamin C in two groups

Table 4. Findings related to physical activity in patients receiving vitamin C (1000mg/day) and placebo

		Groups	
		Vitamin C(n=42)	Placebo(n=42)
Physical activity at the baseline	HeavyModerate	03	13
	Light	39	42
Physical activity in the end	Heavy	0	1
	Moderate	2	0
	Light	38	42

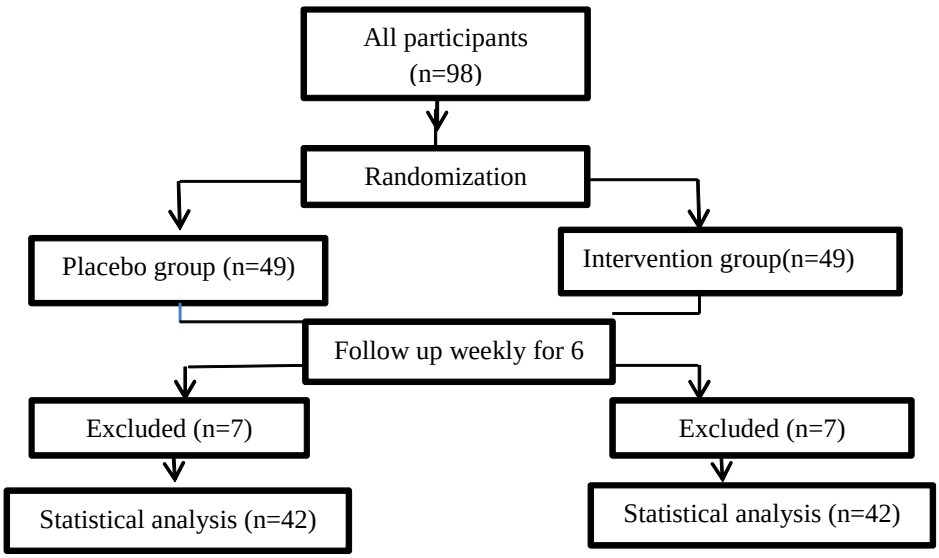

Fig. 1. Flow Diagram of Participants

before and after the intervention (Table 3).

Patients were divided into three groups according to the International Physical Activity Questionnaire (IPAQ): The first group included people who had intense physical activity such as heavy lifting, gardening, aerobics, fast cycling, football, basketball and running 7 days prior to admission. The second group were people who had moderate physical activity (activity will moderate force and makes breathing a little faster) like carrying light loads and cycling at moderate speed 7 days prior to admission moderate physical activity (activity will moderate force and makes breathing a little faster) like carrying light loads and cycling at moderate speed. The third group was people who had light physical activity such as walking at least ten minutes per day 7 days prior to admission (Table 4).

The average differences and standard deviation of fasting blood sugar in intervention groups was 152.9 ±23.8 and in placebo groups were 143.2 ±15.3, showed no significant difference in terms of fasting blood sugar between two groups(P=0.07).

DISCUSSION

The findings of this study showed that vitamin C could reduce blood pressure in patients with type 2 diabetes with 1000 mg per day. This effect was associated with both significant systolic and diastolic blood pressure reduction. The meta-analysis study was conducted by Juraschek et al., This research was conducted on studies from 1966 to 2011, in 29 clinical trials, and the mean dose of 500 mg per day of vitamin C was evaluated. The study took 8 weeks and the subjects ranged from 10 to 120 people. The results showed that short-term supplementation of this vitamin could reduce systolic and diastolic blood pressure(13). A study in 2011 was conducted by Fernades et al. to maintain blood pressure and vitamin C vasodilator response in obese children with psychological stress. This research was done on children 8 to 12 years obesity in the 3 groups to whom vitamin C supplement was given to one group (n=11) in dose 500 mg and the other group was given placebo (n=10) during 45 days. 8 thin children in the same age were chosen as control group. Findings showed that vitamin C supplementation could reduce mean blood pressure[14]. In conclusion, findings from the present study support the findings from other studies.

At the baseline, mean intake vitamin C was 80 mg in women and it was 85 mg in men and according to dietary reference intake vitamin C is 60 mg and 75 mg in women and men older than 31 years, respectively(15). So, dietary intake amounts of this vitamin were sufficient in women and men in the baseline. At the end of the study mean intake of vitamin C was 82 mg (women) and 80 mg(men) that in the end of the research, intake was sufficient but in summary difference mean intake of vitamin C wasn't significant in the two groups(intervention and control) in baseline and the end(P=0.21 and P=0.94, respectively).

Research has shown that serum vitamin C was low in the patient with hypertension in comparison to total population[11].This research didn't investigate levels vitamin C in patients in the baseline and in the total, in conclusion we can't discuss this association.

Oxidative stress was considered as the main cause of multi chronic diseases such as diabetes and its complications; so, antioxidants like vitamin C can be useful in patients with diabetes[16, 17].

Several mechanisms are responsible for reductive effects of vitamin C on blood pressure. According to research, increasing blood pressure can cause increasing oxidative stress[18]. One study showed that vitamin C reduced oxidative stress and improved vascular structure and function which may be mediated by adjusting enzymatic systems that produce free radicals[19].Vitamin C is known as an antioxidant that eliminates superoxide anions and other reactive oxygen species[14]. Effect of blood pressure lowering drugs such as ACE inhibitors, calcium channel blockers II are partly due to antioxidant activity. ACE II inhibitors increase access nitric oxide by reducing the production of angiotensin II and catalysis Bradykinin[7].

In one study, it was shown that vitamin C improves arterial vasodilatation by improving and restoring the activity of nitric oxide in high blood pressure and helps to lower blood pressure[10].There is evidence that vitamin C increase concentration of Tetrahydrobiopterin that is a cofactor for nitric oxide synthase[13].

Ascorbic acid effects on prostaglandin

production as an antioxidant. Prostaglandins are made from unsaturated fatty acids, especially linoleic acid and they are prone to spontaneous oxidation. Some of these prostaglandins are vasodilators such as prostacyclin (PG1-2)and thus they lower blood pressure, and others are vasoconstrictors and increase blood pressure such as thromboxane 2 (TXA-2)[20].

Since vitamin C is a water soluble vitamin and not stored in the body surplus, it is recommended that the effectiveness of this supplement could be done in populations with more volume and a longer time to verify the results of this study.

According to researchers' searches, this study is the first study conducted in Iran which investigated the effect of vitamin C on this dose and this sample size. The present study did not measure serum concentrations of vitamin C at the beginning and the end of the study, which can be considered as the weakness of current study. Matching the drugs of blood pressure with blood sugar reduction is considered as the strengths of this study that was a very difficult work.

Given the studies concerning the effects of oxidative stress in the pathogenesis of hypertension and diabetes and according to results of the current study, we recommend increasing the intake of vitamin C to reduce blood pressure and diabetes complications in diabetic patients.

ACKNOWLEDGEMENTS

This study was conducted with funding provided by Tehran University of Medical Sciences and it is a part of the thesis of the first author. We appreciate Tehran University of Medical Sciences for its financial support and all the patients and Diabetes Research Center of Tehran University of Medical Sciences as well as all those who somehow helped us in this study.

REFERENCES

1. Mahan L, Escottstump S, Raymond J. Krause's Food and the nutrition care process. 13, editor. United States of America: Elsevier 2012.
2. Mokdad A, Ford E, Bowman B, Dietz W, Vinicor F, Bales Vea. Prevalence of obesity, diabetes, and obesity-related health risk factors. JAMA. 2003; **289**: 76-9.
3. Esteghamati A, Gouya M, Abbasi M, Delavari A, Alikhani S, Alaedini Fea. Prevalence of diabetes and impaired fasting glucose in the adult population of Iran. *Diabetes care* 2008; **31**: 96-8.
4. Lago R, Singh P, Nesto R. Diabetes and hypertension. *Endocrinology and metabolism.* 2007; **3**(10):667.
5. Fowler M. Microvascular and macrovascular complications of diabetes. *Clinical diabetes.* 2008; **26**(2): 77-82.
6. Mahan L, Escott S, Raymond J. Krause's food and the nutrition care process. United States of America: Elsevier 2012.
7. Hirata Y, Satonaka H. Hypertension and oxidative stress. *JAMA* 2001;**44**(12):540-5.
8. Rodrigo R, Prat H, Passalacqua W, Araya J, Guichard C, Bachler J. Relationship between oxidative stress and essential hypertension. *Hypertens Res.*2007; **30**:1159-67.
9. Ceriello A. Possible role of oxidative stress in the pathogenesis of hypertension. *Diabetes care.* 2008; **31**(2):181-4.
10. Taddei S, Virdis A, Ghiadoni L, Magagna A, Salvetti A. Vitamin c improves endothelium-dependent vasodilation bu restoring nitric oxide activity in essential hypertension. Circulation 1998; **97**: 2222-9.
11. Zibaee Nezhad M, Eftekhari M, Aghasadeghi K. Modulation of blood pressure in hypertensive patients by vitamin C. *Iranian Cardiovascular Research Journal.* 2009; **31**(1): 16-20.
12. Kelly R, Yeo K, Issac H, Lee C, Huang S, Teng Lea. Lack of effect of acute oral ingestion of vitamin C on oxidative stress, arterial stiffness or blood pressure in healthy subjects. *Free Radical Research.* 2008; **42**(5):514-22.
13. Juraschek S, Guallar E, Appel L, Miller E. Effects of vitamin C supplementation on blood pressure. Am J Clin Nutr. 2012;95:1079-88.
14. Fernades P, Lira F, Borba V, Costa M, Trombeta I, Santos M, et al. Vitamin C restores blood pressure and vasodilator response during mental stress in obese children. *Arg Bras Cardiol.* 2011; **96**(6): 490-7.
15. Ross A, Caballero B, Cousins R, Tucker K, Ziegler T. Modern nutrition in health and disease. 11, editor. Philadelphia: Lippincott Williams & Wilkins; 2014.
16. Wei W, Liu Q, Tan Y, Liu L, Li X, Cai L. Oxidative stress, diabetes, and diabetic complications. *Hemoglobin 2*009;**33**(5):370-7.
17. Giacco F, Brownlee M. Oxidative stress and diabetic complications. *Circulation Research.*

2010; **107**: 1058-70.

18. Briones A, Touyz R. Oxidative Stress and Hypertension: *Current Concepts*. *Curr Hypertens Rep*. 2010;**12**:135-42.
19. Chen X, Touyz R, Park J, Schiffrin E. Antioxidant effects of vitamins C and E are associated with altered activation of vascular NADPH oxidase and superoxide dismutase in stroke-prone SHR. *Hypertension* 2001;**38**:606-11.
20. Ravanshad S, Sadeghi S, Zibaeinejad M, Setoudehmaram E, Montaseri H. Effect of vitamin C supplementation on hypertension. *The Journal of Qazvin Univ of Med Sci*. 2005; **35**: 94-8.