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Original Article

Effect of processed Berberis vulgaris in apple vinegar on blood pressure and inflammatory markers in type 2 diabetic patients

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Abstract

Background: Hypertension and increased inflammatory markers in type 2 diabetic patients are two major risk factors of cardiovascular diseases. Therefore, management of these two cardiovascular diseases risk factors is of the first magnitude in type 2 diabetic patients. This study was aimed to determine effect of processed Berberis vulgaris on blood pressure and inflammatory markers in type 2 diabetic patients.

Methods: Type 2 diabetic patients were recruited into a clinical trial (n =57) and randomly assigned into 3 groups: 1) processed B. vulgaris (n =19), daily consumption of 1 Tsp processed B.vulgaris in apple vinegar, 2) apple vinegar group (n =19), daily consumption of 2 Tsp apple vinegar and 3) control group with no intervention. Nutritional intake, anthropometric indices, inflammatory markers and systolic- and diastolic blood pressure were measured at the baseline and the end of 8th week.

Results: At the end of 8 weeks, mean of nutritional intake, anthropometric indices, hs CRP concentration, systolic- and diastolic blood pressure did not change in processed B.vulgaris, apple vinegar and control groups significantly. Interleukin-6 concentration did not shift in processed B.vulgaris and control groups but in apple vinegar group decreased significantly.

Conclusion: These findings had shown processed B.vulgaris had no effect on systolic- and diastolic blood pressure but apple vinegar had positive effect on interleukin-6. Nevertheless, further investigations about B.vulgaris effect on blood pressure and inflammatory markers are necessary.

Key words: Berberis, Systolic Pressure, Diastolic Pressure, Interleukine 6.

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Introduction

Hypertension is a very common co morbid condition in diabetes mellitus [1,2], affecting 60% to 68% of individuals with diabetes mellitus. Hypertension is well-reported as independent risk factor for many disorders like coronary artery disease, cerebrovascular disease, end-stage renal disease, and eye disease. When diabetes mellitus and hypertension co-exist, the risk of these morbid consequences further increases. However, there is ample evidence to demonstrate a significant reduction of these disease incidences by lowering the blood pressure in individuals with diabetes mellitus. In the UK Prospective Diabetes Study (UKPDS), for example, reductions of 10 mmHg in systolic blood pressure decreased 24% diabetes-related end-points, 37% micro-vascular end-points, 44% strokes and 32% deaths related to diabetes mellitus. A recently-published work indicated that only 36% of diabetics had a blood pressure control of <130/80 mmHg [1]. Although suitable blood pressure control achieves more significant effects than good glucose control on the morbidity and mortality due to diabetes [2].

Markers of acute-phase response, including C-reactive protein (CRP), and IL-6, the main mediators of the response, also have been shown to be elevated in patients with type 2 diabetes [3]. These acute-phase markers are associated with increased cardiovascular diseases and atherosclerosis risk [4,5]. Therefore, interventional planning to reduce blood pressure and inflammatory markers in type 2 diabetic patients has significant importance.

Berberis vulgaris (B.vulgaris) species from Berberidaceae family have been used in traditional medicine and is used in the treatment of infectious fever and dysentery. Evidence shows hypotensive [6] and anti-inflammatory effects of B.vulgaris fruit [7] in animal models. However, B.vulgaris effects on blood pressure and inflammatory markers have not been assessed yet. This study was aimed to investigate the effect of processed Berberis vulgaris on blood pressure and inflammatory markers in type 2 diabetic patients.

Methods

In the present study, according to inclusion and exclusion criteria and medical records, 291 patients that attended the Endocrine and Metabolism Clinic of Sina hospital of Tabriz city in Iran, were chosen. The inclusion criteria were defined as: type 2 diabetes, and exclusion criteria were defined as: renal, hepatic, gastrointestinal and parathyroid disease and insulin therapy. After face to face interview and explanation of the objectives of the trial, 65 patients consented to participate in the study. They were randomly assigned into three groups: 1) those who consumed processed B.vulgaris (n =23), 2) those who consumed apple vinegar (n =23) and 3) the control group (n =19) and were followed up for 8 weeks. Eight patients were excluded after two weeks because of intervention intolerance and 57 patients (n =19 in each groups) completed the study (Figure1). Based on reduced systolic pressure observed in Fatehi et al [6] study conducted in rats, the amount of B.vulgaris for processed B.vulgaris group has been set at 5gr/day (300gr during experiment). To improve lifetime and taste and easy consumption of B.vulgaris fruit, it was incubated with apple vinegar for 40 days at room temperature out of sunlight. After washing, 300g fruit of B.vulgaris for each person was incubated in apple vinegar. 770cc apple vinegar was used for each person. After 20 days, the complex of B.vulgaris and apple vinegar was blended and mixed and remained for another 20 days. The processed B.vulgaris group was fed 1Tsp of processed B.vulgaris with meal each day for 8 weeks. The apple vinegar group was fed 2Tsp of apple vinegar daily to assess and omit the probable effects of apple vinegar on blood pressure and inflammatory markers. The control group was not given any intervention. We had demanded

participants not to change their lifestyle and physicians not to shift type and dose of patients' medications until the end of study. Nutritional intake was assessed for three days (two workdays and one holiday) at baseline and the end of 8th week by use of 24-hour Food Recall Inventory, and analyzed by Nutrition III software. Weight was measured using scale (Germany), height was measured using a tape, both at baseline and the end of week 8 and body mass index (BMI) was estimated. Systolic and diastolic blood pressures were measured at baseline and the end of week 8 by digital manometer (EZ VIEW model KM-7000, Japan) after 10 minutes resting. Fasting venous blood samples were collected at baseline and the end of 8th week. After serum isolation, hs CRP concentration was determined by immunoturbidometric methods using auto-analyzer (model Alcyon 300 Abbott, USA and Germany) and IL-6 concentration was determined by ELISA methods (Stat Fax, USA).

Data was presented as mean and standard deviation (SD). Means before and at the end of week 8 were analyzed by paired t-test. One-way ANOVA was used to compare mean differences among groups and followed by Bonferroni's post hoc test. The level of significance was defined at $P<0.05$.

Results

In the processed B.vulgaris group, mean age was 59.1±12.2 years, 73.9% had systolic hypertension, 52.1% had diastolic hypertension and 22.7% were in inflammatory phase; in the apple vinegar group, the mean age was 54.6±13.1 years, 65.2% had systolic hypertension, 43.3% had diastolic hypertension and 27.3% were in inflammatory phase; in the control group, the mean age was 53.8±9.0 years, 52.6% had systolic hypertension, 52.6% had diastolic hypertension and 23.5% were in inflammatory phase.

There were no significant differences in the nutritional intake, weight and BMI in the processed B.vulgaris, apple vinegar and control groups over intervention (Table1).

hs CRP and IL-6 concentration were not significantly different among the groups. hs CRP concentration did not show significant differences in three groups. In the processed B.vulgaris and control groups IL-6 concentration did not change significantly during experiment, but reduced significantly in apple vinegar group (Table2).

The effect of processed B.vulgaris and apple vinegar, on inflammatory markers, did not differ between patients with and without inflammation status (Table3).

No differences between processed B.vulgaris, apple vinegar and control groups were observed for systolic- and diastolic blood pressure. These parameters did not differ significantly over study (Table2).

The effect of processed B.vulgaris and apple vinegar on systolic- and diastolic blood pressure in hypertensive patients were the same as normotensive patients (table4).

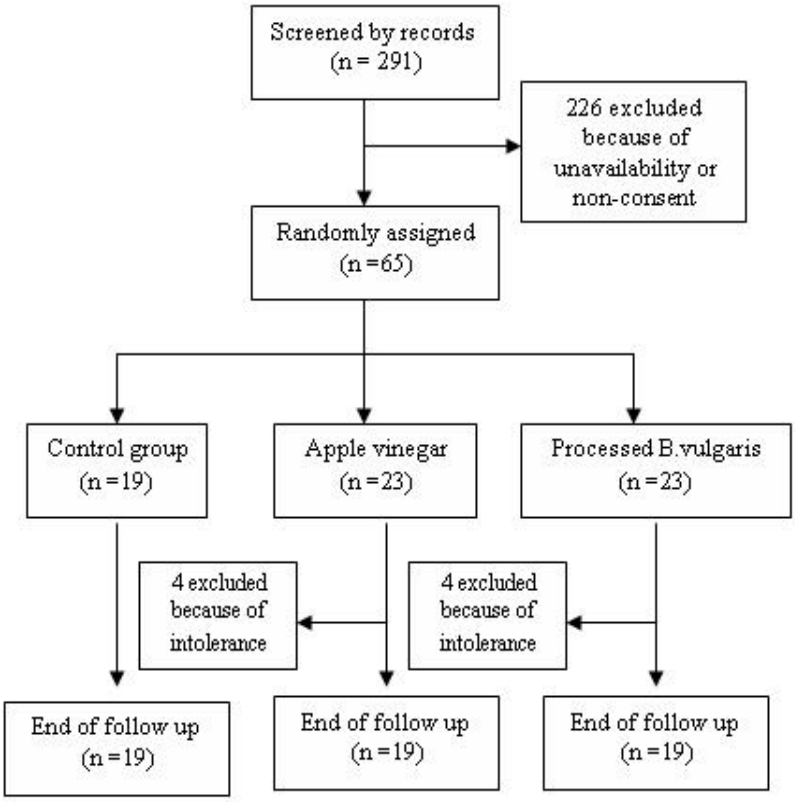

Fig1. Study design and disposition of subjects

Table1. Comparison of anthropometry indices and nutritional intake at baseline and end of 8 weeks in processed B.vulgaris, apple vinegar and control groups

	Processed B.vulgaris		Apple vinegar		Control	
	baseline	**Week 8**	**baseline**	**Week 8**	**baseline**	**Week 8**
Weight (kg)	74±10	73±14	78±8	77±8	77±17	78±18
BMI (kg/m2)	29.3±3	28.7±4	31.1±4	31.4±4	31.0±6	31.1±6
Energy (kcal)	1742.0±624.7	1740.5±673.0	1916.8±838.9	1719.9±694.6	1486.5±343.1	1580.7±340.2
Carbohydrate(g)	207.9±77.0	225.2±104.5	232.3±159.5	213.4±62.6	176.9±59.5	199.5±53.9
Protein (g)	65.9±34.9	65.1±26.6	61.7±25.3	62.2±44.6	60.6±17.3	61.4±23.0
Fat (g)	78.1±24.7	78.5±18.3	86.9±28.5	86.2±32.1	75.3±15.8	74.9±25.7

In this experimental study, means before and at the end of week 8 were analyzed by paired t-test, and One way ANOVA was used to compare mean differences among groups. Values without sign indicate insignificant difference.
The sign of ± shows Mean ± SD. n=19 in each group

Table2. Comparison of inflammatory markers and systolic- and diastolic blood pressure at baseline and at the end of week 8 in processed B.vulgaris, apple vinegar and control groups

	Processed B.vulgaris		**Apple vinegar**		**Control**	
	baseline	Weeks 8	baseline	Weeks 8	baseline	Weeks 8
hs CRP(mg/L)	2.35±2.11	2.47±2.63	1.97±2.08	2.10±1.99	2.06±2.10	1.91±1.64
IL-6(pg/ml)	3.65±4.66	2.81±1.79	3.91±3.60	2.20±1.56*	2.91±2.71	2.82±1.39
Systolic pressure (mmHg)	158±22	150±18	149±22	144±20	150±15	147±13
Diastolic pressure(mmHg)	91±13	90±13	88±17	87±14	89±8	87±11

In this experimental study, means before and at the end of week 8 were analyzed by paired t-test and One way ANOVA was used to compare mean differences among groups. Values without sign indicate insignificantly difference.
The sign of ± shows mean ± SD. n=19 in each group

Table3. Comparison of inflammatory markers at baseline and at the end of week 8 in processed B.vulgaris, apple vinegar and control groups based on inflammatory status

inflammatory markers		Processed B.vulgaris		Apple vinegar		Control	
		baseline	Weeks 8	baseline	Weeks 8	baseline	Weeks 8
Non-inflammatory phase	hs CRP(mg/L)	1.47±0.95	2.05±2.22	0.86±0.62	1.39±1.65	0.96±0.85	1.24±1.18
	IL-6(pg/ml)	3.35±5.94	2.95±1.80	3.38±2.97	2.81±1.36	3.45±4.17	2.26±0.64
Inflammatory phase	hs CRP(mg/L)	5.40±2.33	5.03±3.98	5.16±1.55	3.94±1.70	6.75±1.34	5.05±0.63
	IL-6(pg/ml)	1.55±0.77	2.00±289	3.37±4.47	2.37±1.37	3.98±2.71	2.56±1.83

In this experimental study, means before and at the end of week 8 were analyzed by paired t-test and One way ANOVA was used to compare mean differences among groups. Values without sign indicate insignificantly difference.
The sign of ± shows Mean ± SD. n=19 in each group

Table4. Comparison of systolic- and diastolic pressure at baseline and end of week 8 in processed B.vulgaris, apple vinegar and control groups based on inflammatory status

blood pressure		**Processed B.vulgaris**		**Apple vinegar**		**Control**	
		baseline	**Weeks 8**	**baseline**	**Weeks 8**	baseline	Weeks 8
Normotensive							
	systolic pressure(mmHg)	128±5	133±15	125±5	133±11	126±5	132±14
	diastolic pressure(mmHg)	78±8	80±6	79±7	80±10	81±5	78±9
Hypertensive							
	systolic pressure(mmHg)	168±15	159±12	158±15	152±23	151±7	148±14
	diastolic pressure(mmHg)	102±8	101±11	102±16	94±17	99±4	95±4

In this experimental study, means before and at the end of week 8 were analyzed by paired t-test and One way ANOVA was used to compare mean differences among groups. Values without sign indicate insignificantly difference.
The sign of ± shows Mean ± SD. n=19 in each group

Discussion

In the present study, the effect of processed B.vulgaris in apple vinegar on the blood pressure and inflammatory markers in type 2 diabetic patients was assessed. In this study, nutritional intake and anthropometric indices had been presumed as confounding factors. Therefore, neither nutritional intake change nor anthropometric indices, show the lack of these variables effect on blood pressure and inflammatory markers.

Results showed that processed B.vulgaris had no effect on hs CRP and IL-6 concentrations. Shamsa et al [7] has reported anti-inflammatory effect of B.vulgaris fruit on guinea pigs. Barberry fruit (100g) were extracted by boiling in water for 20-30 min; after filtering, the filtrate (aqueous extract) was concentrated in rotary vacuum evaporator. Thick syrup was obtained. The thick syrup was dried by freeze drying to yield adhesive powder. The desired concentrations were prepared from this powder. These researchers have noted adding B.vulgaris fruit extract to terminal segments of ileum as dexchlorpheniramine and atropine declined histamine and acetylcholine concentrations, respectively. Histamine is an amine that releases stored IL-6 from T-cell [8]. In addition, histamine causes local vasodilatation and inflammation. Therefore, B.vulgaris consumption may reduce IL-6 concentration through decreased histamine. However, the lack of B.vulgaris effect on IL-6 and hs CRP concentrations may be due to insufficient B.vulgaris consumption. Despite this, IL-6 concentration decreased significantly in apple vinegar group.

Apple was identified as a major dietary source of flavonoids especially flavan-3-ols (catechins) in the epidemiologic studies that inhibits cyclooxygenase enzyme [9,10]. Cyclooxygenase enzyme is a first involving enzyme in prostaglandins metabolism and exists in two forms: cyclooxygenase-1 and cyclooxygenase-2. Cyclooxygenase-2 is responsible for prostaglandins synthesis which produces inflammation, pain and fever and stimulates inflammatory responses in body [11]. Inhibition of cyclooxygenase-2 suppresses proinflammatory cytokines production by lymphocytes and macrophages and leads to decreased cytokines concentrations [12]. The mechanism mentioned explains decreased IL-6 concentration in patients who were fed apple vinegar. Xagorari et al [13] showed that flavonoids consumption inhibited the production of proinflammatory molecules from macrophages and inhibition of both IL-6 and TNF-α release through protein tyrosine phosphorylation, and also nuclear factor-kappa B-mediated gene expression and proinflammatory cytokine production in macrophages. Krakauer [14] has reported that flavonoids inhibited T-cell proliferation and production of IL-1, TNF, IL-6 and interferon gamma by human peripheral blood mononuclear cells. Hung et al [15] and Kaur et al [16] have suggested that polyphenolic compounds inhibit proinflammatory cytokines expression including IL-6, IL-1 and CRP expression.

In addition, higher lowering effect of apple vinegar on IL-6 concentration than B.vulgaris may be due to processing [9] or more storage duration than apple vinegar. Studies have shown that storage of food can increase, decrease or not affect flavonoids levels in foods. However, the effect of food shortage on flavonol content in foods is unclear [17]. In the present study, processed B.vulgaris had no effect on systolic- and diastolic blood pressure. Fatehi et al [6, 18] assessed B.vulgaris fruit extract on arterial blood pressure in rats. Some 10g dried B.vulgaris, boiled in 100ml water for 5 min, and filtrated aqueous extract were concentrated and yielded 522mg solid material. The stock solution of the extract (10mg/ml) was prepared from this solid material on the day of experiment. After 20 min, injection of the prepared extract in the right and left jugular veins of hypertensive rats, arterial blood pressure and heart rate

were measured. Findings showed that administration of the extract (0.005-1mg/100g body weight of rat) significantly reduced the mean arterial blood pressure and heart rate in hypertensive rats. This also showed an enhancement in magnitude of the outward potassium currents from cells. Augmentation of potassium currents caused repolarization of membrane, inhibition of excitability of cell, vasodilatation and hypotensive effects. They have suggested that polyphenolic compounds of B.vulgaris are responsible for hypotensive effects in rats. The evidence indicates that polyphenolic compounds increase potassium channels activity and may contribute to hypotension [6]. Most of the investigations conducted on polyphenolic compounds [19-24] support the hypotensive effects of polyphenolic compounds. However, lack of B.vulgaris effect on blood pressure may be due to anticholinergic activity of B.vulgaris fruit [7]. Acetylcholine, an amine, causes vasorelaxation and hypotension [23]. Therefore, decreased acetylcholine can increase systolic- and diastolic blood pressure and explain lack of B.vulgaris effect in the present study.

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