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Preliminary, randomized, double-blinded, placebo-controlled cross-over study with resveratrol in hypertensive patients

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Hypertension is a debilitating disease affecting the population worldwide. Novel therapeutic strategies that complement the current management of hypertension will reduce the disease burden. Our randomized, cross-over, double-blinded, placebo-controlled trial studied the efficacy of plant polyphenol resveratrol (trans-3, 5, 4′-trihydroxystilbene) in managing blood pressure in pre-hypertensive and stage-1-hypertensive patients. Participants with pre-hypertension (diastolic and systolic blood pressure (BP), 80–89 mmHg and 120–139 mmHg, respectively) and 30 participants with stage-1-hypertension (diastolic and systolic BP, 90–99 mmHg and 140–159 mmHg, respectively) were assigned to receive 500 mg resveratrol, twice daily for 4 weeks, orally) or placebo twice daily for 4 weeks) in a 2 × 2 cross-over design (4 weeks treatment—4 weeks washout—4 weeks treatment). The BP of each participant was recorded every week during the study. Data analysis using the multivariable model revealed that resveratrol's effects on systolic and diastolic BP were not statistically significantly superior to those of placebo. Nitric oxide (NO) was considerably higher in the resveratrol-treated group than in the placebo-treated group. In this preliminary study, Resveratrol supplementation for a short duration enhances nitric oxide production but does not significantly lower blood pressure in pre-hypertension or stage 1 hypertension patients. This finding remains exploratory and needs confirmation through larger, long-term clinical trials. *Trial registration*: IRCT201407078129N7, first trial registration date: 15/08/2014.

Keywords Hypertension, Polyphenols: Resveratrol, Nitric oxide

Abbreviations

BP	Blood Pressure
NO	Nitric Oxide
HDL	High-Density Lipoprotein
LDL	Low-Density Lipoprotein
FBG	Fasting Blood Glucose
TC	Total Cholesterol
TG	Triglyceride
HGB	Hemoglobin
HCT	Hematocrit

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PLT	Platelet
WBC	White Blood Cells
ALP	Alkaline Phosphatase
GGT	Gamma-Glutamyl Transferase
MDA	Malondialdehyde
TAC	Total Antioxidant Capacity
OT or SGOT	Serum Glutamic-Oxaloacetic Transaminase
PT or SGPT	Serum Glutamate Pyruvate Transaminase
Cr	Creatinine
Alb	Albumin
SHR	Spontaneously Hypertensive Rat
ANOVA	Analysis of Variance
IRCT	Iranian Registry of Clinical Trials
SD	Standard Deviation
CI	Confidence Interval

Hypertension is a significant health issue worldwide due to its devastating effects on the cardiovascular, renal, and cerebrovascular systems[1]. Hypertension is considered a "silent killer" as it is one of the major asymptomatic risk factors for myocardial infarction, stroke, heart failure, dementia, renal failure, and retinopathy[2]. The population of people who have hypertension has been constantly increasing across the globe and is projected to cross 1.5 billion by 2025[1]. Hypertension management focuses on lifestyle modifications and pharmacological therapy[3]. More than 50% of patients with hypertension require multi-drug therapy for blood pressure control (The SPRINT Research Group)[4]. Effective management of hypertension may reduce the risk of incident stroke, heart attack, and cardiac failure by 35 to 40%, 15 to 25%, and up to 64%, respectively (The SPRINT Research Group). However, managing high blood pressure to less than 140/90 mm Hg is challenging. For instance, in the US, this is only achieved in about 50% of patients[5]. In the recent past, the landmark SPRINT trial reported that a systolic blood pressure of less than 120 mm Hg reduced the rates of stroke, heart attack, cardiac failure, and mortality[1,4]. Unfortunately, more adverse events were reported when treatment with currently available medications targeted a blood pressure of 120 mm Hg[4].

Functional foods and nutraceuticals are known to have comprehensive anti-hypertensive effects in pre-clinical and clinical settings[6–9]. Resveratrol is a plant polyphenol that has gained considerable interest due to its ability to prevent/reverse cardiovascular disease, including hypertension. Many previous pre-clinical studies showed that resveratrol lowers blood pressure in the spontaneously hypertensive rat (SHR) model[10,11], the angiotensin II-mediated hypertension model[12], the two-kidney one-clip hypertensive model[13], and in the partial nephrectomy model of hypertension[14,15]. Our previous pre-clinical study also reported that low-dose resveratrol treatment (2.5 mg/kg body weight/day) enhanced the blood pressure-lowering effect of a vasorelaxant, hydralazine, in SHR[16]. Resveratrol also effectively reduces pulmonary hypertension in rats[17,18]. Recently reported data also demonstrate that perinatal resveratrol treatment to SHR dams prevents the development of hypertension in adult offspring[19]. Interestingly, resveratrol prevents arterial stiffness in non-human primates (induced by a high-fat, high-sugar diet), which is considered a prognostic indicator and target in patients with hypertension[20]. However, to our knowledge, apart from one clinical trial that examined the potential of resveratrol in lowering blood pressure in persons diagnosed with hypertension, without other comorbidities (e.g., type 2 diabetes mellitus), no extensive research has been done yet[21]. We therefore hypothesised that resveratrol administration would reduce blood pressure in hypertensive patients on standard hypertensive medications. For this purpose, we tested the efficacy of resveratrol in a cross-over, randomized, double-blinded, placebo-controlled clinical trial with 16 participants, including those with pre-hypertension and stage 1 hypertension.

Materials and methods

Design overview and ethics approval

This study was designed as a single-center, cross-over, randomized, double-blinded, and placebo-controlled, 2×2 trial with an allocation ratio of 1:1 (Fig. 1).

The rationale for the study and the protocol details, including pre-specified sample size and primary/secondary outcome measures, are published by us as a trial protocol[22]. The Materials and Methods section of this report is thus similar to the previously published protocol[22]. The participants, physicians, principal investigator, the laboratory technician, and statistical consultant were blinded to the allocation status during the study. The methodology consultant kept the allocation records confidential until blinded statistical analyses were over. This trial was an investigator-initiated study sponsored by the Persian Gulf Tropical Medicine Research Center affiliated with Bushehr University of Medical Sciences (BPUMS), Bushehr, Iran (Grant number: 3172, 93/4/17). The study was approved by the regional research ethics committee of BPUMS (approval NO: B-93-16-4). The trial has been registered with the Iranian Registry of Clinical Trials NO: IRCT201407078129N7. All methods were carried out in accordance with relevant guidelines and regulations, including the Declaration of Helsinki. Written informed consent was obtained from all participants prior to their inclusion in the study.

Study settings, population, and recruitment

The study was conducted at the School of Medicine of BPUMS, Bushehr, Iran. Inclusion criteria included pre-hypertensive (the mean of two measurements in a 15-minute interval; diastolic and systolic blood pressure, 80–89 mmHg and 120–139 mmHg, respectively) and stage-1 hypertensive (the mean of two blood pressure measurements in a 15-minute interval; diastolic and systolic blood pressure, 90–99 mmHg and 140–159 mmHg, respectively) males or females, aged between 20 and 60 years, having ability to provide informed consent, the

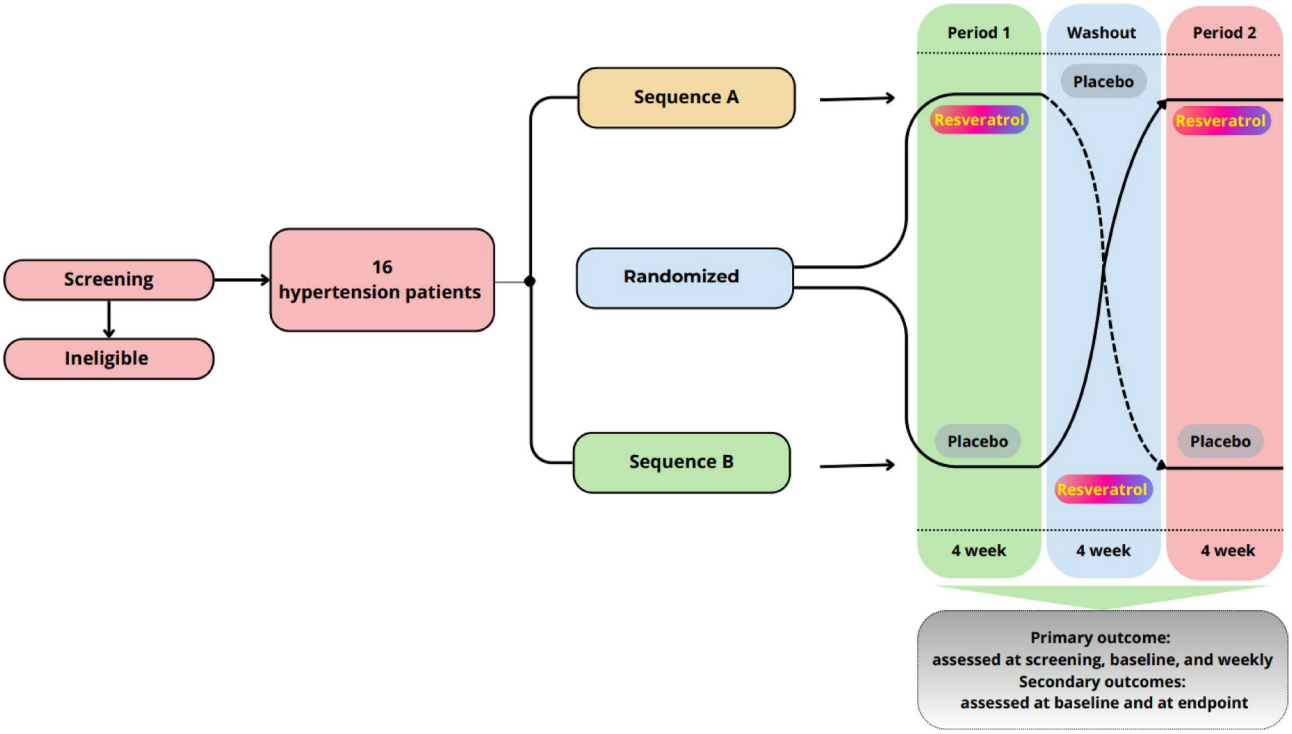

Fig. 1. Randomized Cross-over Study Design.

study started on 15 August 2014 and ended on 20 August 2018. Exclusion criteria can be seen in the protocol already published[22]. These classifications were based on the 2013 ESH/ESC Guidelines for the Management of Arterial Hypertension[23].

Randomization/blinding procedures

A stratified complete block randomization method was used in this trial. Blocks of four were used for this purpose. The randomization scheme was generated using a random number formula in Microsoft Excel. Patients with pre-hypertension or stage 1 hypertension were separately randomized to receive the active drug. The study was double-blinded (the patients, those participating in the research, and those who analyzed the results were unaware of the patient's state regarding receiving the active drugs or placebo). For this purpose, participants were blinded by using a placebo identical to the active drug in appearance, but the content was neutral cellulose. To blind those who conducted the study, the person who delivered or checked the study drug differed from those who examined the patients, and unique numbers identified all the drug packages.

Treatment regimen

Patients with pre-hypertension or stage-1-hypertension were separately randomized to receive 500 mg resveratrol (99% pure, Biotivia, Bioceuticals International SrI, Italy) twice daily for 4 weeks (sequence A), or placebo (500 mg neutral microcellulose capsules, Biotivia, Bioceuticals International SrI, Italy), twice daily for 4 weeks (sequence B), in a 2×2 cross-over design. Patients took Resveratrol or placebo at 7 to 8 a.m. in the mornings on an empty stomach and at 8 to 9 p.m. with 200 cc of water. Patients were asked to record their conditions after taking the capsules, if any unusual effects were experienced. At the end of the 4 weeks, another 4-week washout period was followed, during which the patients in both sequences did not receive either resveratrol or placebo. 4-week washout period was based on the pharmacokinetic profile of resveratrol and prior studies[24,25], ensuring complete elimination of any residual biological effect before crossover. All the participants were followed up for an additional 1-month period to assess any possible effects after the completion of the study.

Among the participants who completed the study, 4, 3, 2, 1, 1, 1,1,1,1,1,1,1 patients were on Captopril, Aspirin, Losartan, Atorvastatin, Caprin, Valsartan, Metformin, Atenolol, Spironolactone, Amlopres, Metformin, Citalopram, respectively. Three participants were not on any medication. The patients reported no comorbidities except for high blood pressure.

Compliance

Compliance was quantified by counting the capsules consumed between the two visits and presented as percentages (number consumed/number expected to be consumed) × 100. If compliance was less than 60%, we considered the case noncompliant. All sixteen patients who completed the study had 100% compliance. And, there were 13 noncompliant participants. Every participant was asked to complete a questionnaire during each visit to monitor the type of foods consumed during the study, particularly to confirm that they did not have food products that may contain resveratrol.

Physical examination

Blood pressure was assessed twice on the right arm after a 15-minute rest in the sitting position, using a standard mercury sphygmomanometer (BP AG1-20 BASIC – Aneroid Blood Pressure Kit – Microlife). To reduce variability, participants were instructed in advance to avoid caffeine intake, intense physical activity, smoking, or emotional stress at least two hours before the measurement. The participants'anthropometric parameters and clinical characteristics, including age, sex, height, and weight, were measured. The participants were asked to fill out a standard questionnaire form (developed by the United States Department of Agriculture) regarding their typical food intake, including the amount of salt, alcohol, green tea, coffee, grapes, peanuts, wine, berries, and lifestyle (exercise, smoking, sleeping habits, and rest)[15,18]. In addition, the amount of vitamins and other micronutrients supplemented to the diet will be included. At baseline, the patients were asked to fast (10-hour to 12-hour overnight fast) for blood collection. The blood samples were collected before the first stage of the study, after the 1-month intervention, after the 1-month washout, and at the end of the study. Then, the serum were separated, given a code number, and stored at − 80 °C until analysis.

Biochemical analysis

Fasting blood glucose (FBG), serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL), triglyceride (TG), hemoglobin (HGB), hematocrit (HCT), platelet (PLT), and white blood cells (WBC) were measured using enzymatic methods with the Selectra 2 autoanalyzer (Vital Scientific, Spankeren, Netherlands)[16], employing commercial kits supplied by Pars Azmoon Co. (Tehran, Iran). The Friedewald formula was used to calculate serum low-density lipoprotein (LDL) cholesterol[17]. To evaluate liver function, serum levels of alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), bilirubin, and albumin were also measured using Pars Azmoon enzymatic kits on the same autoanalyzer platform. Levels of malondialdehyde (MDA) and nitric oxide (NO) were determined using validated colorimetric assay kits provided by Karmania Pars Gene (Kerman, Iran). Total antioxidant capacity (TAC) was measured with a commercial colorimetric kit from BioVision Inc. (Milpitas, CA, USA).

Informed consent

Participants were enrolled with their voluntary informed consent. The consented individuals were referred to the biochemistry laboratory, and the study coordinator invited them to attend a first study visit by the concerned clinicians on a specified day, when a prescreening was conducted to exclude participants based on the inclusion/exclusion criteria. The tests were repeated if participants showed abnormal values for laboratory tests during prescreening. If the tests showed similar results (abnormal values), the participants were excluded from the study. If the criteria were met, the study coordinator reviewed the consent form designed in the local language (Persian).

Data quality control and management

All staff members who collected and handled the data were well trained for managing clinical data. Adequate attention was given to collecting accurate and valid data and setting up a regular monitoring scheme by qualified staff. Original hard copies of patient records are kept at the recruitment center. The data would be available only to designated researchers involved in the trial. All patient documents are stored after considering the safety and security issues. Necessary schemes were set up to control the quality of drug delivery, storage, and handling, clinical examinations, and laboratory tests.

Adverse effects

In the present study, one patient (female) reported feeling of heaviness, hyperthermia, and discomfort in the body, after consuming resveratrol on the first day; she was also on oral medications (50 mg Hydrochlorothiazide and 50 mg Amlodipine). Another patient (male) reported pain and swelling in his feet and face; he was also on oral medications (Losartan, Metoral, Atorvastatin, and Aspirin). Both patients were immediately given medical attention, asked to discontinue resveratrol capsules, and excluded from the study. The other patients reported no other adverse reports.

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Statistical analysis

This is the final analysis on data obtained from the participants completing both study periods; the scientific steering committee made this decision.

Demographic, socioeconomic, and anthropometric variables and baseline values of outcome variables were described using mean and standard deviation (SD) for quantitative variables and frequency and percentage for categorical variables in the sequence.

The study's sample size was calculated using PASS 11 power and sample size software. Treatment, sequence, period, and carryover effects were estimated using an analysis of variance model for all outcome variables (systolic blood pressure, diastolic blood pressure, and biochemical measures). The treatment effect was defined as the value of the outcome variable before each period minus the value of the outcome variable after that period.

Carryover effect was defined as the baseline values of the outcome variable in period 1 minus the baseline values of systolic or diastolic blood pressure in period 2. Treatment period was defined as period 1 for the sequence AB and period 2 for the sequence BA. Sequence and period variables were described as dichotomous categorical variables.

A cross-over model was used for different parameterization types. The variables that remained in the final model included treatment effect, period, and treatment-by-period interaction, assuming no carryover or sequence effect. Data were analyzed using the pk cross-over menu of Stata/SE 13.0 statistical software. P values ≤ 0.05 were considered significant. The data are mean with a standard deviation of 95% confidence interval.

Results

A total of 30 patients, including 21 men and 9 women, were allocated to sequence AB or BA to receive resveratrol or placebo in a 2 × 2 cross-over trial design. Sixteen participants (4 females and 12 males) with a mean age of 49.0 ± 9.2 years completed the trial and were included in the data analyses. Figures 1 and 2 illustrate the study design and the CONSORT flow diagram of recruitment. and follow-up process, respectively.

The participants allocated to sequence AB or BA were comparable in terms of baseline values of systolic and diastolic blood pressure. Table 1 shows the participants' baseline characteristics. Table 2 shows mean systolic and diastolic blood pressure at the baseline and different times during the trial by the sequence and period of receiving resveratrol or placebo, respectively.

Data analyzed using the multivariate ANOVA model revealed that resveratrol's effects on systolic and diastolic blood pressure were not statistically significant compared with placebo (Table 3). Except for nitric oxide (NO), which showed a statistically significant increase following resveratrol treatment ($P = 0.042$), none of the other biochemical parameters—including FBS, TG, TC, HDL, creatinine (Cr), liver enzymes (ALP, GGT, ALT), oxidative stress markers (MDA, TAC), and hematologic indices (Hgb, HCT, PLT, WBC)—exhibited significant treatment effects (all $P > 0.05$). These findings, detailed in Table 4. No multiple comparisons were performed in this analysis. Table 5 presents the descriptive mean ± SD values of biochemical parameters at baseline and after each treatment period, separately for each treatment sequence. This table is intended for illustrative purposes and to support interpretation of patterns over time. No statistical comparisons were performed within Table 5. The significance of treatment effects was formally assessed using the crossover ANOVA model, the results of which are shown in Table 4.

Discussion

This randomized clinical study showed that short-term treatment with resveratrol for one month did not lower blood pressure in pre-hypertensive/stage I hypertensive patients. Nevertheless, resveratrol treatment improved NO levels in hypertensive patients compared to placebo. Resveratrol has been shown to induce endothelial NO synthase[26,27] and reduce the uncoupling of the endothelial NO synthase[27].

Our study suggests that resveratrol may be beneficial in improving vascular health in hypertensive patients, but longer-term resveratrol treatment may be needed to bring about a clinically significant lowering in blood pressure.

A key finding of our study is the statistically significant increase in nitric oxide (NO) levels following resveratrol supplementation, which did not translate into a significant reduction in either systolic or diastolic blood pressure. This apparent dissociation between a key vasodilatory molecule and its expected physiological outcome warrants a careful examination of the underlying pathophysiological mechanisms and study limitations. Several potential explanations for this phenomenon exist.

First, the regulation of blood pressure is a complex multifactorial process that extends beyond the NO pathway. In hypertensive patients, potent vasoconstrictor systems, such as the Renin-Angiotensin-Aldosterone System (RAAS) and the sympathetic nervous system, are often chronically overactive[28]. It is plausible that the modest increase in NO bioavailability induced by resveratrol was insufficient to overcome the powerful pressor effects exerted by these countervailing systems. The net hemodynamic effect is a balance of vasodilator and vasoconstrictor inputs, and in this context, the effect of NO may have been functionally negated.

Second, the efficacy of NO is contingent upon the integrity of its downstream signaling pathway. Even with increased NO production, its vasodilatory effect can be blunted if there is an impairment in the sensitivity of its receptor, soluble Guanylate Cyclase (sGC), within the vascular smooth muscle cells[29]. This state, sometimes referred to as "NO resistance," is known to occur in pathological conditions like hypertension and diabetes. Consequently, higher levels of NO may not lead to a proportional increase in cGMP production and the subsequent smooth muscle relaxation required to lower blood pressure.

Third, the prevailing oxidative stress in hypertensive individuals poses a significant challenge. Reactive oxygen species (ROS), particularly the superoxide anion (O2·−), rapidly scavenge NO to form peroxynitrite (ONOO−), thereby reducing its bioavailability and contributing to endothelial damage[30]. Furthermore, high oxidative stress can cause the "uncoupling" of the eNOS enzyme itself, leading it to produce superoxide instead of NO. Thus, while resveratrol may have stimulated eNOS activity, a portion of the generated NO could have been immediately quenched in the pro-oxidant milieu of the hypertensive vasculature.

A recent report by Theodosius et al. showed that treatment for six months with a 50 mg/day micronized formulation of resveratrol was not able to reduce blood pressure in stage I and II hypertensive patients[21]. However, there was a statistically significant reduction in the measurement of systolic and diastolic blood pressure at the end of the study in participants who had and had not received micronized formulation of resveratrol[21]. This suggested that blood pressure was not significantly different between patients treated with placebo and micronized formulation of resveratrol[21]. Interestingly, these results are in conjunction with our study, even though both studies have significant differences, such as a smaller dose of resveratrol. This is an important aspect,

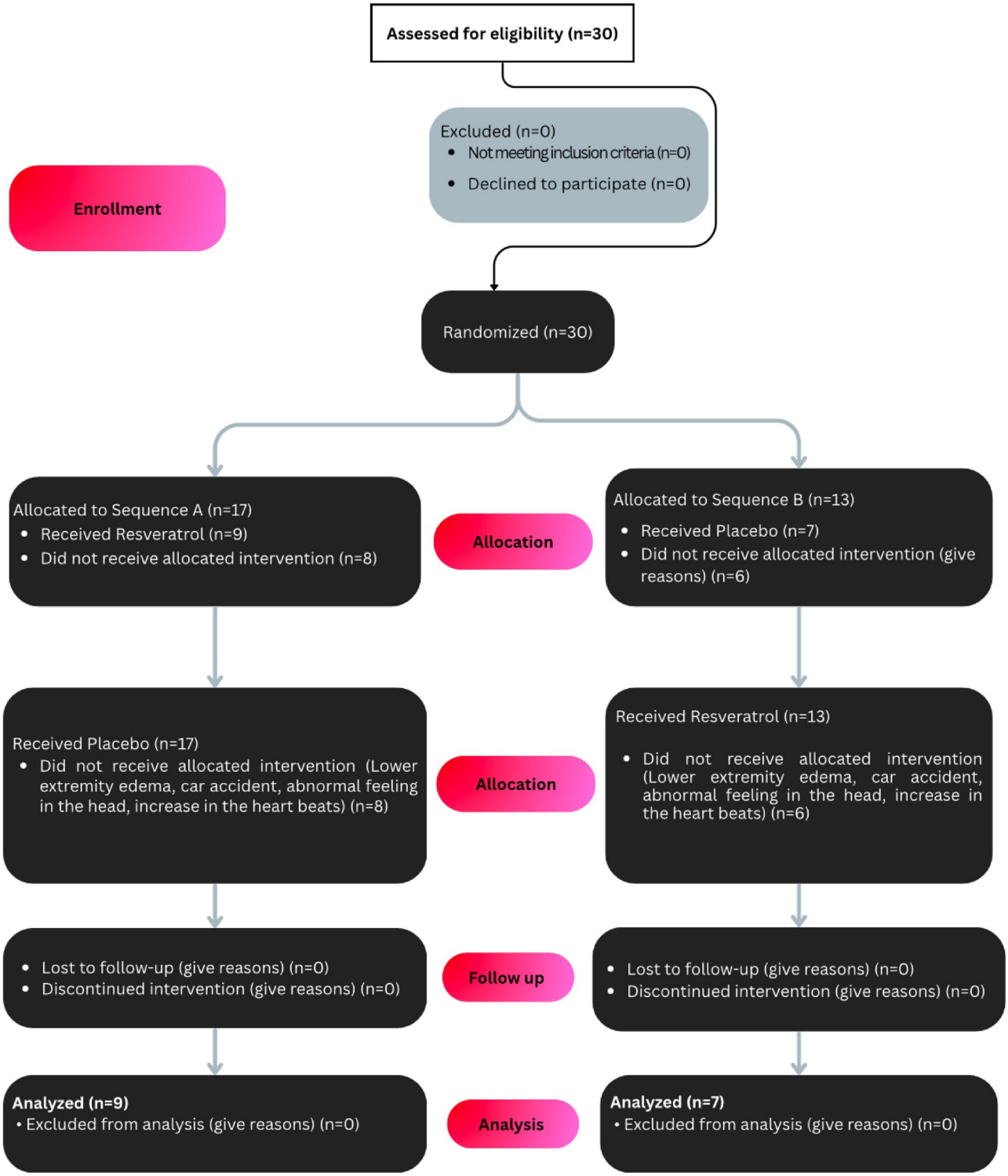

Fig. 2. Consort flow diagram of trial.

as it is known that resveratrol's blood pressure-lowering effect is dose-dependent. Another study also showed that a single 300 mg resveratrol improved endothelial function in women with high LDL-cholesterol but did not reduce blood pressure[31]. However, it should be noted that this study was an acute treatment and employed a very low dose, which may not have been enough to bring about anti-hypertensive effects. Interestingly, it should be noted that resveratrol has been reported to lower blood pressure in diabetic patients[8,32,33]. This includes a report from our group showing a significant reduction in blood pressure with resveratrol treatment in Iranian patients with type 2 diabetes. A meta-analysis showed that resveratrol reduced systolic blood pressure in patients with type 2 diabetes[34]. In contrast, another meta-analysis of available randomized, controlled clinical trials across

Variable	Sequence AB ($n=9$)	Sequence BA ($n=7$)
Sex	9 males	3 males, 4 females
Smoking (Never)	9 (100%)	7 (100%)
Age (years)	51.3 ± 5.1	46.0 ± 12.6
Duration of hypertension (months)	14.8 ± 7.3	40.0 ± 70.0
Mean systolic blood pressure (mmHg)	140.0 ± 8.6	141.1 ± 9.7
Mean diastolic blood pressure (mmHg)	96.7 ± 21.2	92.8 ± 16.7
Body mass (kg)	75.5 ± 33.4	80.5 ± 12.0

Table 1. Baseline characteristics of participants. Continuous variables are presented as mean ± standard deviation; categorical variables are shown as frequency (percentage).

	Mean Systolic Blood Pressure (mmHg)				Mean Diastolic Blood Pressure (mmHg)			
	Sequence AB ($n=9$)		Sequence BA ($n=7$)		Sequence AB ($n=9$)		Sequence BA ($n=7$)	
	Period 1 (Resveratrol)	Period 2 (Placebo)	Period 1 (Placebo)	Period 2 (Resveratrol)	Period 1 (Resveratrol)	Period 2 (Placebo)	Period 1 (Placebo)	Period 2 (Resveratrol)
Baseline	142.2 (10.9)	141.7(11.5)	141.4 (9)	130.6 (10.1)	90.6 (9.8)	89.4 (7.7)	87.9 (6.8)	85.1 (6.9)
Week 1	142.7 (10.4)	132.2 (10.7)	134.1 (8.1)	133.6 (12.8)	88 (5.6)	85.6 (6.9)	86.4 (8.9)	86.4 (5.4)
Week 2	144.4 (9.7)	136.9 (8.5)	129.3 (7.2)	137.9 (16.3)	9.1 (3.5)	85.3 (7.0)	83.6 (5.7)	87.5 (7.8)
Week 3	139.6 (8.3)	136.9 (12.0)	132.4 (6.5)	131.4 (7.3)	87.5 (6.3)	86.7 (4.8)	82.9 (3.7)	82.8 (3.0)
Week 4	136.3 (5.2)	135.0 (7.4)	129.3 (11.7)	132.5 (6.9)	87.5 (5.3)	86.9 (3.0)	85.0 (5.0)	84.7 (5.7)

Table 2. Systolic and diastolic blood pressure (mmHg) during the intervention course. *Data are mean (standard deviation).

Source of Variation	Partial SS	df	MS	F	*P* value
Treatment effect	449.56	1	449.56	5.43	0.027
Period effect	327.06	1	327.06	3.95	0.057
Treatment * Period	7.38	1	7.38	0.09	0.768
Residuals	2319.94	28	82.85		
Total	3018.88	31			

Table 3. Analysis of variance (ANOVA) for a 2 × 2 cross-over study. SS (Sum of Squares): The total variation attributable to each source. df (Degrees of Freedom): The number of values that are free to vary for each source. MS (Mean Square): The average variation, calculated by dividing the sum of squares (SS) by its respective degrees of freedom (df). F (F-statistic): The test statistic obtained by dividing the MS of a factor by the MS of the residuals; used to determine statistical significance.

heterogenous samples (metabolic syndrome, type 2 diabetes mellitus, obesity, etc.) that assessed the effect of resveratrol on blood pressure reported that administration of resveratrol did not significantly affect systolic blood pressure, diastolic blood pressure or pulse pressure[35]. The efficacy of resveratrol in lowering blood pressure may vary when other comorbidities are present.

The 1 g/day dose was chosen based on pre-clinical studies conducted in animal models of hypertension. These studies indicated that resveratrol may have a dose-dependent effect on blood pressure, because many studies, including those from our group, observed no significant effect with low-dose resveratrol, and[2] the success of our aforementioned study in which 1 g/day resveratrol lowered blood pressure in type 2 diabetic patients. Additionally, several clinical trials, including ours, reported 1 g/day resveratrol as a safe and tolerable dose[36].

Our current study also revealed that 1 g resveratrol treatment was well-tolerated by participants; however, it did result in non-serious adverse effects in two hypertensive subjects. Mild effects such as nausea and gastrointestinal disturbances have been reported earlier; nevertheless, several clinical trials carried out with 1 g resveratrol have shown no adverse effects. Thus, it is essential to acknowledge that treatment with 1 g resveratrol may have resulted in negative interaction with standard medications, and this may have contributed to the observed adverse effects in hypertensive patients.

Despite a sound mechanistic rationale and several encouraging secondary outcomes, this study has some important limitations that must be acknowledged. The primary limitation was the small final sample size, which fell well short of the original target. Persistent recruitment challenges—exacerbated by the COVID-19 pandemic and nationwide lockdowns in Iran—led to the premature termination of the trial. Although fewer participants completed the study than initially planned, the crossover design conferred a methodological

Source Parameter	Effect of Treatment (F, *p*-value)	Effect of Treatment Sequence (F, *p*-value)	Effect of Sequence (F, *p*-value)
FBS	0.11 (0.749)	0.20 (0.659)	3.11 (0.100)
HCT	1.08 (0.318)	0.14 (0.712)	**6.97 (0.020*)**
Hgb	0.39 (0.541)	0.45 (0.513)	2.50 (0.138)
PLT	0.58 (0.459)	0.36 (0.560)	0.05 (0.818)
WBC	0.69 (0.690)	0.005 (0.944)	**9.59 (0.008*)**
TG	0.17 (0.688)	0.08 (0.784)	0.07 (0.791)
Chol	0.02 (0.877)	0.47 (0.504)	0.47 (0.305)
HDL	0.59 (0.454)	0.09 (0.769)	0.008 (0.930)
ALP	0.001 (0.971)	0.16 (0.692)	0.11 (0.739)
GGT	2.65 (0.126)	0.75 (0.400)	0.09 (0.760)
OT	0.05 (0.823)	1.86 (0.194)	0.01 (0.905)
Pt	0.24 (0.630)	0.02 (0.890)	0.001 (0.977)
Cr	0.76 (0.396)	0.77 (0.393)	0.77 (0.394)
Alb	1.69 (0.215)	4.56 (0.051)	0.72 (0.409)
NO	**4.98 (0.042*)**	0.43 (0.524)	1.92 (0.187)
MDA	2.65 (0.126)	0.27 (0.610)	0.04 (0.847)
TAC	0.01 (0.934)	0.19 (0.689)	0.18 (0.677)

Table 4. The biochemical results of the analysis of variance for the two-period cross-over design. * $P < 0.05$ (statistically significant). FBS: Fasting Blood Sugar; HCT: Hematocrit; Hgb: Hemoglobin; PLT: Platelet; WBC: White Blood Cell; TG: Triglyceride; CHOL: Cholesterol; HDL: High-Density Lipoprotein; ALP: Alkaline Phosphatase; GGT: Gamma-Glutamyl Transferase; OT: Serum Glutamic Oxaloacetic Transaminase; PT: Serum Glutamate Pyruvate Transaminase; Cr: Creatinine; Alb: Albumin; NO: Nitric Oxide; MDA: Malondialdehyde; TAC: Total Antioxidant Capacity.

	Sequence A				Sequence B			
Parameter	Baseline	Period 1 (Resveratrol)	After washout	Period 2 (Placebo)	Baseline	Period 1 (Placebo)	After washout	Period 2 (Resveratrol)
HCT	44.58 ± 2.11	46.788 ± 5.64	44.62 ± 4.01	42.04 ± 3.63	45.03 ± 4.475	41.27 ± 2.37	43.39 ± 2.73	45.08 ± 2.62
Hgb	15.31 ± 0.98	15.36 ± 1.19	14.52 ± 1.42	14.24 ± 1.41	14.36 ± 1.16	14.26 ± 1.05	14.64 ± 1.73	14.81 ± 1.34
PLT	260.11 ± 59.95	265.75 ± 76.43	261.37 ± 78.84	273.29 ± 69.11	277.86 ± 60.07	239.23 ± 97.31	295.43 ± 40.65	265.89 ± 64.37
WBC	6.08 ± 0.71	5.70 ± 1.22	5.96 ± 1.82	7.74 ± 1.34	7.700 ± 1.15	7.857 ± 1.10	7.99 ± 1.32	6.02 ± 1.80
FBS	92.56 ± 18.61	104.00 ± 17.38	90.00 ± 24.52	90.14 ± 8.82	91.43 ± 12.49	94.29 ± 21.78	89.57 ± 13.88	103.33 ± 16.02
TG	205.56 ± 111.55	196.22 ± 100.52	162.78 ± 56.77	190.14 ± 65.37	170.714 ± 62.73	177.14 ± 73.19	198.14 ± 69.48	193.78 ± 109.34
CHOL	177.78 ± 26.55	185.00 ± 19.76	177.00 ± 27.34	192.86 ± 30.69	190.86 ± 31.23	198.00 ± 30.47	205.57 ± 32.02	181.78 ± 21.87
HDL	39.66 ± 14.84	36.70 ± 13.21	40.38 ± 7.51	37.13 ± 6.85	38.86 ± 7.29	38.39 ± 9.58	37.46 ± 10.12	39.56 ± 7.49
LDL	97.00 ± 32.34	113.73 ± 23.812	103.89 ± 31.63	117.54 ± 26.89	117.86 ± 28.81	124.14 ± 28.53	128.51 ± 31.47	103.58 ± 23.65
ALP	205.22 ± 49.89	199.00 ± 34.81	187.79 ± 41.20	185.86 ± 69.27	185.57 ± 57.43	191.86 ± 37.28	167.14 ± 19.45	191.78 ± 54.22
GGT	23.78 ± 6.92	23.68 ± 7.71	26.00 ± 10.72	21.43 ± 7.34	19.00 ± 5.54	31.29 ± 17.35	19.86 ± 5.01	26.68 ± 9.53
OT	21.11 ± 3.65	22.11 ± 5.13	22.11 ± 4.54	19.43 ± 5.13	17.43 ± 5.47	22.86 ± 7.99	18.43 ± 3.41	19.68 ± 5.48
PT	15.44 ± 4.56	17.56 ± 8.44	19.78 ± 10.46	17.00 ± 9.47	14.00 ± 7.26	19.00 ± 11.67	11.57 ± 4.31	18.68 ± 10.10
Cr	0.99 ± 0.28	4.714 ± 11.36	1.06 ± 0.31	0.90 ± 0.22	0.99 ± 0.12	0.92 ± 0.18	0.94 ± 0.15	0.95 ± 0.5
Alb	4.41 ± 0.51	4.68 ± 0.32	4.64 ± 0.15	4.44 ± 0.24	4.43 ± 0.31	4.67 ± 0.21	4.54 ± 0.41	4.62 ± 0.22
NO	36.17 ± 18.15	46.69 ± 27.56	32.34 ± 23.63	38.94 ± 22.17	23.67 ± 21.94	51.94 ± 22.21	22.50 ± 20.04	22.92 ± 15.71
MDA	1.13 ± 1.01	1.39 ± 0.74	1.62 ± 0.55	1.02 ± 0.66	0.97 ± 0.34	2.14 ± 0.85	1.50 ± 0.82	1.96 ± 2.38
TAC	0.18 ± 0.06	0.28 ± 0.18	0.33 ± 0.15	0.34 ± 0.19	0.18 ± 0.09	0.31 ± 0.29	0.33 ± 0.19	0.30 ± 0.20

Table 5. The results of biochemical parameters during the two sequences. HCT: Hematocrit; Hgb: Hemoglobin; PLT: Platelet; WBC: White Blood Cell; FBS: Fasting Blood Sugar; TG: Triglyceride; CHOL: Cholesterol; HDL: High-Density Lipoprotein; LDL: Low-Density Lipoprotein; ALP: Alkaline Phosphatase; GGT: Gamma-Glutamyl Transferase OT: Serum Glutamic Oxaloacetic Transaminase; PT: Serum Glutamate Pyruvate Transaminase; Cr: Creatinine; Alb: Albumin; NO: Nitric Oxide; MDA: Malondialdehyde; TAC: Total Antioxidant Capacity.

advantage by allowing each participant to serve as their own control. This approach reduces inter-individual variability and increases the power to detect treatment effects, even in smaller samples. The original sample size calculation, based on an expected large effect size on systolic blood pressure, indicated that 24 completers would be sufficient. However, only 16 participants completed both treatment phases. As such, we acknowledge the heightened risk of a Type II error and regard the findings as preliminary and hypothesis-generating. This report therefore presents initial data from a single-center trial conducted in a relatively homogeneous population of Iranian adults. Another limitation is the lack of sex-stratified analysis. Although both males and females were enrolled, the limited sample size precluded meaningful comparison of sex-specific responses to resveratrol. As a result, the study may have been underpowered to detect modest treatment effects on systolic or diastolic blood pressure. Given these constraints, the absence of statistically significant effects on the primary outcome should be interpreted cautiously. Further randomized, controlled trials with larger, multiethnic populations and longer treatment durations are warranted to confirm and extend these preliminary observations.

Conclusions

In conclusion, our preliminary findings indicate that daily administration of 1 g resveratrol for 30 days does not produce a statistically significant reduction in blood pressure among Iranian patients with pre-hypertension or stage I hypertension. However, due to limited statistical power, definitive conclusions cannot be drawn. Therefore, further well-powered, long-term randomized clinical trials are warranted to clarify the antihypertensive potential of resveratrol.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Author contributions

AM was primarily responsible for conducting the clinical trial, getting the human ethics approvals, and collecting and storing clinical trial samples and confidential data. TN and AM originated the concept for this human trial. AM, PR, JMS, and TN were involved in the planning and design of the study. AM and AO were involved in the preparation of the study protocol. AM, MR, and ES will identify patients and recruit. MK, AA, MA, NH, and SA collected and organized data. AM MM analyzed all the data from the study. AM, PR, JS, and TN have contributed to writing and editing the trial manuscript. All authors have read and approved the final manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Ethical approval and consent to participate

The study was approved by the regional research ethics committee of BPUMS (approval NO: B-93-16-4). The trial has been registered with the Iranian Registry of Clinical Trials NO: IRCT201407078129N7.

Additional information

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