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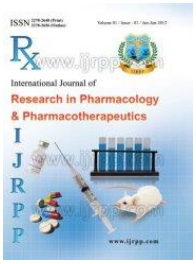

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A randomized double blinded placebo controlled trail on efficacy of resveratrol in controlling hypertension

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ABSTRACT

High blood pressure is classified as either primary (essential) high blood pressure or secondary high blood pressure. The target of this study is to assess the efficiency of resveratrol in managing blood pressure in members diagnosed with prehypertension and stage 1 hypertension. The main objectives of this trial are to regulate the BP-lowering actions of resveratrol on systolic, diastolic, and mean arterial BP in members diagnosed with prehypertension and stage 1 hypertension. The outcomes from this study will help to decide the effectiveness of short-term resveratrol treatment in hypertensive patients and connect the gap with regards to the recent preclinical and clinical evidence. The primary results in this study will be BP. Systolic and diastolic BPs will be measured by using a mercury sphygmomanometer twice on patient in a sitting position after a 10-minute rest and with a 15-minute interval. The measurement will be done every week during the intervention period. The mean systolic and diastolic BP will be computed. Mean arterial pressure will be calculated as systolic blood BP plus two times the diastolic BP divided by three $[(SBP + 2 \times DBP) \div 3]$. This study will also help in gathering further data with regard to recognizing a therapeutically effectual dose of resveratrol in reducing cardiovascular disease risk factors. Importantly, if a positive outcome is identified, it will provide us with an effectual therapeutic strategy to fight against hypertension and would pave the way for reaching enormous population well-being sake. Pharmacokinetics may have assisted to draw a relation between the plasma bioavailability and the actual physiological effect. In this trial, stage 1-hypertensive patients will be on quality therapy for hypertension as well; therefore, it may not be feasible to understand the standalone effictiveness of resveratrol in lowering BP in these patients.

Keywords: Sphygmomanometer, Enormous, Therapeutically, Resveratrol

INTRODUCTION

Elevated blood pressure is categorized as either primary (essential) elevated blood pressure or secondary elevated blood pressure.[1] About 90–95% of cases are primary, defined as elevated blood pressure due to unspecific lifestyle and genetic agents.[2][3] Lifestyle agents that increase the risk include excess salt, high body

weight, smoking, and alcohol.[3][4] The remaining 5–10% of cases are classified as secondary elevated blood pressure, defined as elevated blood pressure due to an identifiable cause, such as chronic kidney disease, narrowing of the kidney arteries, an endocrine disorder, or the use of birth control pills.[5]

Blood pressure is indicated by two lengths, the systolic and diastolic pressures, which are the maximal and minimal pressures, respectively.[6] Normal blood pressure at relax position is within the span of 100–140 millimetres mercury (mmHg) systolic and 60–90 mmHg diastolic.[7] Elevated blood pressure is present if the relaxing blood pressure is persistently at or above 140/90 mmHg for most adults .[7] Different numbers implemented to children. [8] Ambulatory blood pressure monitoring over a 24-hour period appears more exact than office best blood pressure measurement.[9][10]

Lifestyle alters and medications can lower blood pressure and decrease the threat of health complications.[9] Lifestyle alter include weight loss, low salt intake, physical exercise, and a healthy diet.[5] If lifestyle alters are not adequate then blood pressure medications are used.[9] Up to 3 medications can reduce blood pressure in 90% of people.[5]The therapy reasonably elevated arterial blood pressure (defined as >160/100 mmHg) with medications is combined with an improved life expectancy.[11] The effect of treatment of blood pressure between 140/90 mmHg and 160/100 mmHg is less clear, with some reviews finding satisfaction [11][12] and others finding a lack of proof for satisfaction.[13] High blood pressure affects between 16 and 37% of the population globally.[13] In 2010 hypertension was believed to have been a factor in 18% (9.4 million) deaths.

MATERIALS AND METHODS

Study design and ethics approval

This study is a crossover, randomized, double-blinded, and placebo-controlled, single-center trial with an allotment ratio of 1:1. The participants, physician, principal investigator and physician and statistical consultant will be blinded to the allocation status. The data of allotments will be kept private by technical consultant and will be revealed only after blinded statistical analyses or by requirement from Data and Safety Advisory Board. The study is accepted by the regional research ethics committee.

Study settings, population, and recruitment

The study was conducted at the Prime Hospital Hyderabad, Prehypertensive (the mean of two measurements in a 15-minute interval; diastolic and systolic BP, 80–89 mmHg and 120–139 mmHg, respectively) and stage-1 hypertensive (the mean of two measurements in a 15-minute interval; diastolic and systolic BP, 90–99 mmHg and 140–159 mmHg, respectively) males or females, aged between 20 and 60 years will be registered for the trial. The patients for this study will be enrolled through doctor referral at the clinic and randomized to the treatment arm after the first screening and with their voluntary consent.

General objective

The objective of this trial is to regulate whether resveratrol (99 % pure) therapy for 4 weeks will lower BP in prehypertensive and stage 1-hypertensive patients.

Specific objectives

Primary objectives: The principal objectives of this trial are to determine the resveratrol effect in lowering the BP on systolic, diastolic, and mean arterial BP in sufferers diagnosed with prehypertension and stage 1 hypertension.

Secondary objectives: The subordinate objectives are as follows:

- To determine if therapy with resveratrol decreases the levels of renin, angiotensin II, endothelin, norepinephrine, tumor necrosis factor-α (TNF-α), and oxidative stress markers and elevate the level of nitric oxide in prehypertensive and stage 1-hypertensive patients.
- To determine the effects of resveratrol on hematologic indices in participants with prehypertension and stage 1 hypertension.
- To determine the effects of resveratrol on lipid profile in these patients.
- To determine the effects of resveratrol on liver function markers in patients with prehypertension and stage 1 hypertension.
- To determine the effects of resveratrol on renal function markers in patients with prehypertension and stage 1 hypertension.

Specific procedures

Serum renin, angiotensin II, endothelin, norepinephrine, and TNF-α will be studied by ELISA kits. Nitric oxide (NO), malondialdehyde and urinary isoprostanes will be studied by ELISA Kits, spectrophotometry, gas chromatography/mass spectrometry, respectively. Hematocrit (HCT) and platelet (PLT) will be evaluated using a hematology cell counter. Prothrombin time (PT) and partial thromboplastin time (PTT) will be studied using a blood coagulameter. Analyses for biochemical parameters, involving fasting blood glucose (FBG), Selectra 2 autoanalyzer. Serum total cholesterol and high-density lipoprotein cholesterol (HDL) will be deterimined using cholesterol oxidase phenol amino antipyrine enzymatic method and triglyceride (TG) using the glycerol-3-phosphate oxidase phenol amino antipyrine enzymatic method. Serum low-density lipoprotein (LDL) cholesterol will be measured using the Friedewald formula. Creatinine and blood urea nitrogen (BUN) levels will be determined using enzymatic method. In order to calculate liver function in the patients, alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), bilirubin and albumin will be calculated by enzyme kinetic methods on a Selectra 2 autoanalyzer.

Eligibility criteria

Inclusion criteria are as follows

- Prehypertensive (mean of two measurements in a 15-minute interval; diastolic and systolic BP, 80–89 mmHg and 120–139 mmHg, respectively)
- Stage 1 hypertensive (mean of two measurements in a 15 minute interval; diastolic and systolic BP, 90–99 mmHg and 140–159 mmHg, respectively)
- Male or female
- Age between 20 and 60 years
- Ability to provide informed consent

Exclusion criteria are as follows

- Approved or doubtful secondary hypertension
- History of chronic or acute kidney disease
- History of heart failure
- History of chronic or acute liver diseases
- History of diabetes mellitus
- History of prior cardiovascular events (acute myocardial infarction, cardiovascular diseases, percutaneous coronary angioplasty or coronary artery bypass graft)
- Pregnancy or breast feeding
- Blood arterial pressure > 180/110
- History of bowel disease of any etiology that may affect absorption/or distribution of any drug administered orally
- History of electrolyte imbalance during 3 months prior to the enrolment
- History of alcohol abuse 4 weeks prior to the enrolment
- Requiring a major surgical procedure (abdominal, thoracic, neurovascular, urological, or gynecological) during the course of the study
- Consumption of steroid hormones or nonsteroidal anti-inflammatory drugs 1 month prior to the enrolment
- History of hormonal changes (thyroid and adrenal)
- Receiving lipid-lowering drugs
- History of bleeding disorders
- Receiving blood thinners
- Regular intake of omega-3 fatty acid, vitamins, and mineral supplements
- Intention of having high intake of table salt or salty foods

Informed consent

The patients will be educated about the study by their doctor during clinic visit. Interested patients will contact the study coordinator by telephone/email. Interested patients will be asked to attend a first study visit by the specific clinicians on a particular day, when a pre-screening will be organized to eliminate participants based on the inclusion/exclusion criteria. If individuals show up with unusual values for laboratory tests during pre-screening, the tests will be repeated. If tests show similar results (abnormal values) the participants will be eliminated from the trail. If the criteria are met, study coordinator will go through the consent form designed in local language. Finally, clinical trial particulars will be outlined and the participants will be given an opportunity to ask any questions/concerns. If they consent to the study, allotment will be completed. Individuals will be registered only with their voluntary informed consent.

Randomization and intervention

After the allotment of the patient is completed, randomization will be done to allot them to placebo or resveratrol arms. A stratified complete block randomization method will be used in this study. Blocks of four will be used for this purpose. The randomization scheme will be created using random number formulae in Microsoft Excel. Patients with prehypertension or stage 1 hypertension will be individualy randomized to accept active drug (resveratrol) in 500 mg capsules, twice daily for 4 weeks (sequence A), or placebo (500 mg neutral microcellulose capsules), twice daily for 4 weeks (sequence B), in a 2 × 2 crossover design. The drug or placebo will be taken by patients at 7 to 8 a.m. in the morning before food and at 8 to 9 p.m. with 200 cc water. The patients will be asked to document their conditions after taking the capsules in case any unusual effects are experienced. At the end of the 4 weeks, another 4-week washout period will follow, during which the patients in both sequences will receive placebo. All the participants will be followed up for an additional 1-month period for assessing any possible after effects.

The taril will be double-blinded (the patients, those who will be participating in the study and those who analyze the results will be unaware of the state of the patient with regard to receiving the active drugs or placebo). For this purpose, individuals will be blinded by using a placebo that is identical to active drug in impression, but the content is neutral cellulose. To blind those who perform the study, the person who delivers or checks the study drug will be different from those who check the patients, and all the drugs packages will be identified by unique numbers. Finally, the randomization table will be concealed from research staff by using closed envelops.

Compliance will be quantified by counting the number of capsules taken during the interval between the two visits and shown as percentages (number consumed/number expected to be consumed) × 100). If compliance is less than 60 %, we will consider this case as noncompliant. Noncompliant patients will be included under the intention-to-treat analysis. Besides, at the analysis stage, we will compare intention-to-treat (if randomized, then we will analyze) and per-protocol analyses (those who have received the treatment compared to those who have not) and interpret the results.

Outcome measures

The main outcome in this taril will be BP. Systolic and diastolic BPs will be calculated by using a mercury sphygmomanometer twice on patient in a sitting position after a 10-minute rest and with a 15-minute interval. The data collection will be done every week during the intervention period. The average systolic and diastolic BP will be computed. Average arterial pressure will be calculated as systolic blood BP plus two times the diastolic BP divided by three [(SBP + 2 × DBP) ÷ 3]. The secondary outcome will include the biochemical analysis of plasma or serum for various biochemical and hematological markers. Table 1 provides a list of secondary results measures. Table 2 shows the flow chart of the study.

Table 1: List of biochemical markers

Outcome	Method of measurement
LFT (ALP, GGT, albumin, bilirubin)	Autoanalyzer (spectrophotometry)
Lipid profile (total cholesterol, HDL, LDL, TG)	Autoanalyzer (spectrophotometry)
RFT (serum creatinine, BUN)	Autoanalyzer (spectrophotometry)
Biochemical assessments(endothelin, TNF-α, NO, renin, angiotensin II, norepinephrine, malondialdehyde, urinary isoprostanes)	ELISA kits, spectrophotometry, gas chromatography/mass spectrometry
Hematological markers (PLT, HCT, PT, PTT)	Autoanalyzer assay kits
Fasting blood glucose	Autoanalyzer (spectrophotometry)

Table 2: Flow chart of the study

1	2	3	Washout	7	10	11	12	
Week	**Screening**	**Baseline**	**4**	**(4 weeks)**	**10**	**11**	**12**	**16**
In/exclusion criteria checking	×							
Enrolment	×							
Obtaining informed consent		×						
Physical examination	×							
Randomization	×	×						
Blood pressure	×	×	×					
LFT	×	×	×					
RFT	×	×	×					
Lipid profile measurement		×	×					
Blood markers measurement		×	×					
Hematological indices and FBG measurement	×	×	×					
Compliance measurement			×					
Adverse effect checking			×					

Sample size

The specimen size for the study was measured by using PASS 11 power and sample size software. Depending on the software output, a two-sided *t* test will attain approximately 80 % power when the total sample size of a 2 × 2 cross-over design is $n = 44$, the actual mean difference for systolic BP (as primary outcome) is 10, the square root of the within mean square error is 10, and the significance level is 0.05. Allotment of 44 patients would provide an 80 % power to demonstrate a significant difference between the study arms. We expect approximately 10 % loss to follow-up. To attain the measured statistical power, we decided to recruit 50 patients in each strata (prehypertension and stage 1 hypertension group), equally allocated to sequences A or B. In total, 100 patients will participate in this trial. To reduce loss to follow-up, we give the participants a visit card. The next visit time is documented on the card based on the trail protocol. In addition, we call the individulas, the day before, to evock them of their visit time, and again on the day after if they fail to attend their visit on time to re-invite them to reschedule soon.

Adverse effects

No data exist of severe adverse effects in any of the past human studies with the 1-g resveratrol treatment, involving a recent study from our group where we used a similar dosage of resveratrol in diabetic participants. Nevertheless, any adverse event at the time of the study will be documented. If the adverse effect is serious enough to require medical attention, it will be reported as soon as possible, and the Data and Safety Monitoring Board (DSMB) will make a decision on whether or not blinding should be removed and whether the patient should be elimenated from the study. In the case of a fatal or severe event requiring hospital admission, documenting should occur on the same day to the principal investigator. For any adverse effect in its early stages (before any link with the intervention is established) necessary therapy will be given. The DSMB will be accountable for studying each case individually to verify a possible link to trail products. The principal investigator will be accountable for managing the adverse effects clinically. Required services will be provided free of charge. If any unexpected serious or fatal adverse effect occurred that is believed to be due to the consumption of

resveratrol, the study will be terminated. The decision will be made by the DSMB.

Data quality control and management

All staff individuals who will gather and handle the data are well educated for managing clinical data. Adequate attention will be given to gather accurate and valid data and set up a regular monitoring scheme by qualified staff. Original hard copies of patient datas will be kept at the recruitment center, a copy will be sent to the research deputy of BPUMS, and the data will be available only to designated researchers involved in the trial. All patient documents that are sent or received will be stored after taking into consideration safety and security issues.

Required schemes will be set up to control the quality of drug delivery, storage and handling, clinical examinations, and laboratory tests.

Physical examination

Anthropometric parameters and clinical characteristics of the participants, including age, sex, height, weight, and body mass index (BMI) will be measured. The participants will also be 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). In addition, the amount of vitamins and other micronutrients supplemented to the diet will be included. At baseline, the patients will be asked to fast (10-hour to 12-hour overnight fast) for blood collection. The blood samples will be 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 will be separated and given a code number and stored at −80 °C until analysis.

Statistical analysis

Data will be analysed on an intention-to-treat basis, defined as all randomized patients who received at least one dose of study medication. Patients with no data recorded for a parameter will be excluded from the analysis of that particular parameter. The statistician will remain blinded to the status of the patients with regard to the intervention. Data will be analysed stratified by prehypertension or stage 1-hypertension groups.

Final analyses will be purformed after the trial is completed or a decision has been made to stop the trial by scientific steering committee, which may occur after the interim analysis if the members are satisfied with the strength of the proof.

Because of a comparatively long washout period, we do not expect a significant carryover effect. However, we will check for a carryover effect by using a two-group independent *t* test to compare the average effects of outcomes for the two sequences. Therapy, sequence, and period effects will be estimated by using an analysis of variance model for all outcome variables. Baseline values of results variables as well as potential confounding factors will be controlled by using repeated calculation analysis of variance models. Appropriate post-hoc analysis using Bonferroni correction for multiple comparisons will be carried out.

Data will be analyzed by using the pk crossover menu of Stat/SE 11.0 statistical software.

DISCUSSION

The elevating need for alternative strategies for controlling hypertension can be addressed by recognizing promising nutraceutical individuals. In this regard, resveratrol has revealed great potential in interrupting and/or reversing cardiovascular diseases involving hypertension in preclinical studies. Recent meta-analyses that reviewed successful clinical trials ended that resveratrol may be considered as an adjuvant therapeutic individual for managing type 2 diabetes. In light of these promising clinical findings and previously reported preclinical evidence, resveratrol appears to be a potential antihypertensive agent. The efficiency of resveratrol has yet to be clinically investigated in patients with hypertension. This study will be the first study to investigate the potential of therapeutic use of resveratrol for the management of BP in patients diagnosed with prehypertension and type 1 hypertension. The results from this study will help to determine the efficiency of short-term resveratrol treatment in hypertensive patients and bridge the gap with regards to the present preclinical and clinical conformation. This trail will also help in gathering further data with regard to identifying a therapeutically effective dose of resveratrol in controlling cardiovascular disease risk factors. Importantly, if a positive result is identified, it will provide us with an effective therapeutic strategy to reduce hypertension and

would have the way for achieving expensive public health benefit.

LIMITATIONS

This study is designed as a pilot study to investigate the efficiency of resveratrol as a blood-pressure-lowering agent in a particular population.

In addition, the study has a small sample size. Hypertension is a disease that requires long-term medication. Because the study duration is only 4 weeks, longer-term studies need to be conducted to measure the efficiency of resveratrol as a sustained therapy option.

In this trail, Individuals will receive daily only a single, high dose of resveratrol, which will be taken twice throughout the study (a high dose that is well tolerated). Further study may be needed to recognize whether resveratrol can lower BP at a much lower daily dose.

Another limitation is that a pharmacokinetics study will not be done in this study to understand the absorption, distribution, metabolism, and excretion of resveratrol in hypertensive patients;

Pharmacokinetics may have helped to draw a correlation between the plasma bioavailability and the actual physiological effect. In this study, stage 1-hypertensive patients will be on standard treatment for hypertension as well; therefore, it may not be possible to understand the stand alone efficiency of resveratrol in decreasing BP in these patients. Extensive toxicological analysis will also not be managed as part of this trail.

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