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DETERMINATION OF ANTI-HYPERTENSIVE POTENTIAL OF BEETROOT POWDER ON HYPERTENSIVE PATIENTS



Nizwa Itrat^{1,2}, Anum Nazir^{1,2*}, Aymen Shahzad¹, Roheen Shakeel³, Rashad Mahmood⁴

¹Department of Nutrition and Dietetics, University of Faisalabad, Pakistan

²Department of Nutritional Sciences, Government College University, Faisalabad, Pakistan

³Department of Physiology, Bolan Medical College, Quetta, Pakistan

⁴Department of Physiology, Mohtrma Benazir Bhutto Shaheed Medical College, Mirpur, Azad Kashmir

*Corresponding Author: Anum Nazir. E-mail: anum.nazir@tuf.edu.pk

Abstract

Hypertension is a disease in itself and also a risk factor for many other chronic cardiac conditions which are heart attacks, stroke and other CVDs. Beetroot *Beta vulgaris* is a natural root used as a vegetable, having many health potent benefits and nutraceutical cures, most prominent of them is lowering high blood pressure with the help of dietary nitrate in it. The present study was devised to analyze the beetroot powder hypotensive potential on hypertension. For this purpose, characterization (proximate analysis, TPC and TFC) of beetroot powder was conducted as part of the study. And blood tests for sodium and potassium electrolytes were done before and after the study duration. Beetroot was obtained by the washing, peeling, and size-reduction, sun drying and grinding of beetroot. Results of proximate analysis of beetroot powder showed that it contains 2.18% of protein, 70.42% of moisture, 8.25% of fiber, 5.06 % of ash, 4.00% of fat and 10.09% of nitrogen free extract. Flavonoid content of beetroot was measured at 478 nm and phenolic content showed absorbance at 67.65 nm. Human efficacy was carried out on 30 hypertensive females comprising of 3 groups, each group contained 10 subjects, provided them with capsules of beet root powder for 60 days with different dosages G₀ acted as a control group, G₁ (250 mg) and G₂ (500 mg). Their arterial systolic and diastolic blood pressures were monitored on weekly basis every 7th day for 6 weeks. The data obtained was subjected to statistical analysis for the evaluation of results which showed that 500 mg dose of beetroot powder was highly significant for hypertensive patients.

Keywords: *Beta vulgaris*, Dietary nitrate, Hypertension, Hypotensive potential, Nutraceutical cure

INTRODUCTION

Hypertension is at the top of the causes of death at 2nd and 3rd world countries and at number two in first world countries (1). Dietary habits that include ingestion of natural fruits, vegetables and nuts is not a step towards being hypertensive but habits like being a smoker and alcoholic helps a lot in getting your blood pressure pass through its threshold level (2).

Beet root (*Beta vulgaris*) is a dark bright colored vegetable which has free radical fighting properties. It's a fighter fruit for people suffering from depression, hypertension, diabetes, free radical activity and after effects of sport injuries (3). The blood pressure lowering effect of inorganic nitrate is come from the maximized production of nitric oxide (NO). Nitric oxide is a pleiotropic molecule which has its role involved in the vasodilation of blood arteries and resistance vessels (4). Beetroot consumption increases the amount of an enzyme name endothelial nitric oxide synthase It is possible on daily basis consumption of beetroot powder may cause a decrease in the risk of vascular complications (5). In recent years, there has been a surging interest in exploring the biological activity of red beetroot (*Beta vulgaris rubra*) and its potential role as a functional food for promoting health and preventing diseases. Being a rich source of nitrate, consuming beetroot offers a natural way to boost in vivo nitric oxide (NO) availability, making it a



promising approach for preventing and managing conditions linked to reduce NO levels, particularly hypertension and impaired endothelial function (6).

METHODOLOGY

SAMPLE COLLECTION

The collection process involved obtaining fresh beetroot (*Beta vulgaris*) samples from local Fresh vegetable market, Faisalabad for use in the study.

PROXIMATE ANALYSIS

Beetroot powder was evaluated for moisture, crude fat, crude protein and crude fiber according to the standard. The sample was desiccated under specified conditions which vary according to the nature of the feed. The loss in weight was determined by weighing. It was necessary to carry out preliminary drying when dealing with solid feed which has high moisture content. A clean Petri dish was taken and weighed using a weighing balance. Subsequently, the sample, along with the Petri dish, was weighed on the same weighing balance. The sample was then placed in a drying oven for 24 hours at 105°C. After the 24-hour drying period, it was removed from the oven and placed in a desiccator for 10 minutes to cool. The final weight was recorded. Subsequently, the percentage of moisture in the feed sample was calculated using the recorded weights.

DETERMINATION OF PROTEIN IN SAMPLE

The determination of protein in the sample was carried out using the Kjeldahl method, a well-established nitrogen determination technique dating back to the late 1800s. This method involves three fundamental steps: digestion, distillation, and titration. For the digestion phase, a clean crucible was weighed on a precision balance, and then, 1g of finely ground sample was mixed with 5g of a digestion mixture (comprising 100g potassium sulfate, 10g copper sulfate, and 5g ferric sulfate) along with 25-30 ml of sulfuric acid. This mixture was placed in a digester for approximately one and a half hours or until a light green color appeared. Distilled water was then added to bring the total volume to 250 ml in a volumetric flask. Subsequently, for distillation, 10 ml of the sample solution was introduced into a Kjeldahl distillation apparatus, and 10 ml of 40% NaOH solution was added. Simultaneously, another container held 10 ml of 4% boric acid. Boiling ensued to allow steam to enter the Kjeldahl flask, generating ammonia gas fumes that passed through a condenser into the 4% boric acid solution until the boric acid solution became colorless. Finally, for titration, the colorless boric acid solution was titrated with N/10 H₂SO₄ until the color of boric acid reappeared. The volume of acid used in the titration was recorded. The percentage of nitrogen in the sample was calculated using the formula:

$$\% \text{ of Nitrogen} = \text{Reading of N/10 vol. used} \times \text{factor} \times \text{vol. used for dilution} \times 100$$

DETERMINATION OF CRUDE ASH

This procedure was designed for the determination of the crude ash content in various types of dried, ground forages, and feeds, excluding liquid feeds or those with high sugar content. The method involved dry ashing the sample at 600°C for 3 hours and 30 minutes, followed by weighing the inorganic residue. The equipment required included porcelain crucibles, an analytical balance sensitive to 0.1 mg, a hotplate or burner, a muffle furnace, and a desiccator. To perform the procedure, a clean and dried porcelain crucible was weighed to the nearest milligram, and approximately 4 grams of the sample were added, and the weight was noted. The sample was then gently heated to eliminate fumes for at least 15 minutes, followed by the placement of the crucible in a muffle furnace set at 600°C for 3 hours and 30 minutes. The temperature was maintained until a white or light gray residue, free from carbonaceous particles, was obtained. After cooling for 1 hour, the crucible was placed in a desiccator, allowed to cool, and weighed immediately. This method allowed for the precise determination of the crude ash content in the feed.

CRUDE FAT

In the determination of crude fat, fat was extracted from the sample using petroleum ether, with subsequent distillation of the solvent and weighing of the residue. The scope of this method encompassed feeds and feed ingredients with a fat content lower than 20%. The equipment employed included an analytical balance accurate to 0.1 mg, heating apparatus with temperature control, extraction thimbles devoid of fat and ether residues, a reflux unit, Soxhlet-type extractor, electrically heated vacuum oven, desiccator, and a Buchner funnel connected to suction. Light petroleum ether with a boiling point of 40–60°C, purified for fat extraction, served as the reagent. The procedure involved weighing at least 1 g of the sample (W1) into the extraction thimble, covering it with fat-free cotton wool or filter paper, and placing it in the extractor connected to the dry flask and reflux unit. Extraction with petroleum ether was conducted for 3 to 4 hours, maintaining at least 10 siphoning cycles per hour. The solvent was distilled until the flask nearly contained 5 ml of solvent with fat, which was then transferred to a pre-weighed empty petri dish (W2) and left in an oven for half an hour to ensure complete solvent evaporation. Subsequently, the sample was cooled in a desiccator and weighed (W3).

CRUDE FIBER

The principle of this method involved sequentially treating the defatted sample with boiling dilute sulfuric acid and boiling sodium hydroxide solution, with the resulting loss in mass during incineration corresponding to the mass of crude fiber. Equipment used included an analytical balance, filter crucibles, heating apparatus, filtration equipment, desiccator, a ventilated drying oven capable of maintaining a temperature of 103 ± 2 °C, and a muffle furnace capable of maintaining a temperature of 550 ± 20 °C. Reagents employed were petroleum ether (boiling point 40 to 60 °C), 0.15 M sulfuric acid, and 0.23 M sodium hydroxide. In the pretreatment phase, the defatted sample was quantitatively transferred into a beaker. For digestion, 200 ml of sulfuric acid was added to the beaker containing the sample and boiled for 30 ± 1 minutes. The resulting mixture was filtered through a crucible, and the residue was washed with hot distilled water until neutral. Subsequently, the residue was transferred quantitatively to a beaker, and 200 ml of sodium hydroxide was added, followed by boiling for 30 ± 1 minute. The mixture was again filtered through a crucible, and the residue was washed until rinsing was neutral. Afterward, the residue was placed in a crucible. For drying and incineration, the crucibles were positioned in an oven set at 103 ± 2 °C and dried for 4-24 hours, with the drying time commencing when the oven reached 103°C. After cooling in a desiccator, the crucibles were weighed directly (W2). The crucibles were then placed in a muffle furnace, and the samples were incinerated for 2-3 hours at 550 ± 20 °C, with the incineration time commencing when the furnace reached 550 °C. After cooling in a desiccator, the crucibles were again weighed directly to obtain W3 using an analytical balance.

DATA COLLECTION

Thirty subjects were enrolled in the study and divided into 3 groups evenly i.e. G0 as controlled group (receiving no beetroot powder capsule), G1 as group 2 (consuming 250 mg of beetroot powder capsule each day) and G2 as group 3 (consuming 500 mg of beetroot powder capsule each day). The Intervention period was 63 days. Serum sodium and potassium tests were performed by electrolyte panel (A routine test in hospital) before the initiation of plan on individuals of each group to get the baseline values and after the trail. Random systolic and diastolic blood pressure were checked every 7th day for 9 weeks. Serum sodium test was carried out by a statistically designed, multi laboratory study to evaluate a flame atomic emission spectroscopic (FAES) method for serum sodium as a Reference Method. The cooperating laboratories performed the method with either manual or semi-automated pipetting. Although both modes of pipetting satisfied our acceptability criteria, only the method with semi-automated pipetting is described here as the Reference Method.

STATISTICAL ANALYSIS



Results were analyzed by the use of statistical package of social sciences (SPSS) version 16 using Duncan's multiple range test (DMRT) following analysis of variance (ANOVA) to establish the level of significance. Values of $p \leq 0.05$ were considered significant.

RESULTS

Hypertension had become a widespread chronic problem that had also heightened the risk of heart diseases. This escalation in such health issues had ultimately translated into a significant economic burden across various dimensions. The dash diet had been employed to address high blood pressure, with a primary focus on the intake of fresh fruits and vegetables. Beetroot had been recognized for its substantial fiber content (Table I a), which played a crucial role in maintaining blood pressure within the normal range. Furthermore, beetroot was rich in total phenolic compounds (TPC) and total flavonoids compound (TFC) (Table I b), which had the potential to mitigate inflammation within the body (Fig. 1).

Table I. a) Proximate Composition (%) of Beetroot powder. b) TFC and TPC of Beetroot powder

a. Proximate parameters	Composition (%)
Moisture	70.42±0.20
Ash	5.06±0.25
Crude protein	2.18±0.01
Crude fat	4.00±0.02
Crude fiber	8.25±0.02
NFE	10.06±2.15
b. Compounds	Content
Flavonoids	478.0±0.014
Phenolic	67.65±0.353

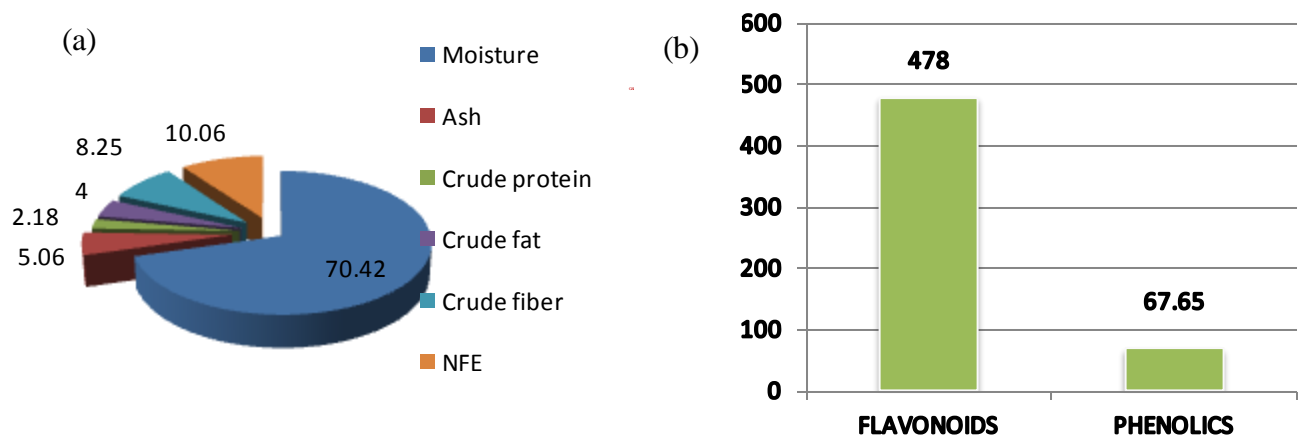


Fig. 1 (a). Characterization of beetroot powder **(b).** TPC and TFC

Table II. Effect of time duration in lowering Blood pressure

Tests of Between-Subjects Effects					
Dependent Variable	Potassium				
Source	SS	df	MS	F	P value
Groups	7.317	2	3.658	28.063	0.000
Duration Day	.229	1	3.229	11.760	0.019
Groups * Duration Day	5.895	2	2.948	22.610	0.000
Error	7.040	54	.130		
Total	873.029	60			

Table III. Statistical analysis for serum sodium level of patients

Source	SS	df	MS	F	P value
Groups	341.742	2	170.871	8.853	0.000
Duration Day	14.701	1	14.701	7.762	0.038
Groups * Duration Day	287.629	2	143.814	7.452	0.001
Error	1042.197	54	19.300		
Total	1232948.670	60			

Table IV. Statistical analysis for systolic and diastolic blood pressures of patients

Dependent Variable Systolic					
Source	SS	df	MS	F	P value
Groups	21179.607	2	10589.803	145.732	0.000
Duration weeks	6489.563	9	721.063	9.923	0.000
Groups * Duration weeks	2921.127	18	162.285	2.233	0.003
Error	19619.900	270	72.666		
Total	5743831.000	300			

Dependent Variable Diastolic					
Source	SS	df	MS	F	P value
Groups	14090.667	2	7045.333	162.446	0.000
Duration weeks	4370.000	9	485.556	11.196	0.000
Groups * Duration weeks	2821.000	18	156.722	3.614	0.000
Error	11710.000	270	43.370		
Total	2674400.000	300			

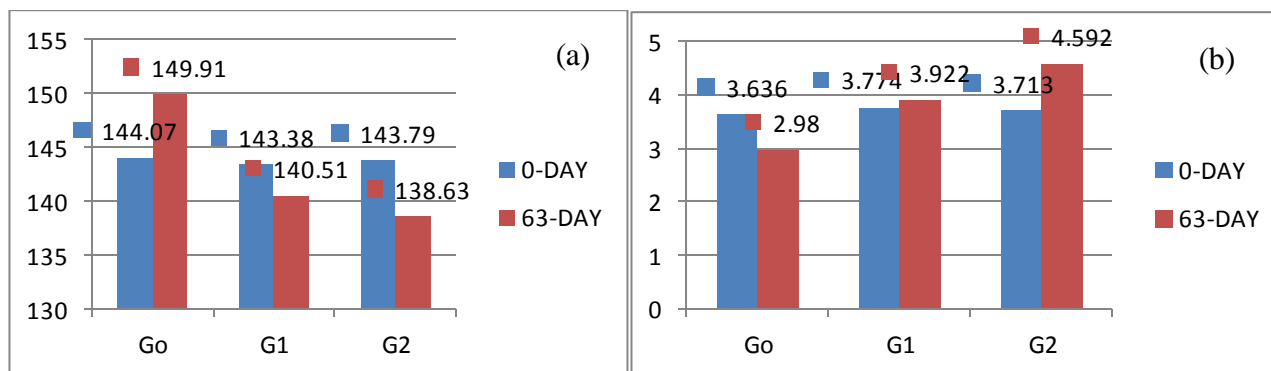


Fig. 2 (a). Means sodium among patients before and after the trial **(b).** Means potassium among patients before and after the trial (Go= control group. G1 250 mg, G2 500 mg)

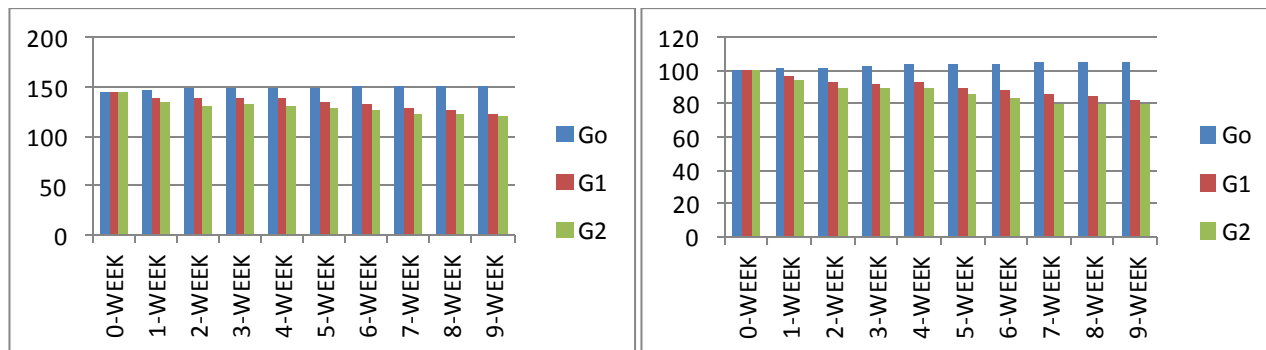


Fig. 3 (a). Mean values of systolic blood pressure among patients before and after the trial **(b).** Means diastolic blood pressure among patients before and after the trial (Go= control group. G1 250 mg, G2 500 mg)

According to the Fig. 2 (a), mean scores results showed significant effect of beetroot powder intake on blood sodium levels. G2 with the highest dosage of beetroot powder capsules (500 mg) on 0 day showed a mean score (143.790±3.5288 mmol/L) and on 63rd day (138.630±3.7313 mmol/L) showed a highly significant result as shown in Fig. 2 (a).

According to the Fig. 2 (b) mean scores results showed significant effect of beetroot powder intake on Serum potassium levels. G2 with the highest dosage of beetroot powder capsules (500 mg) on 0 day showed a mean score (3.7130±0.53091 mmol/L) and on 63rd day (4.5920±0.42240 mmol/L) showed a highly significant result as shown in Fig. 2 (b). The mean values of systolic blood pressure level G2 with the highest dosage of beetroot powder capsules (500 mg) on 1st day showed a mean score (145.500±14.9907 mmHg) and on 63rd day (120.000±2.9887 mmHg) showed a highly significant result as shown in Fig. 3 (a). Recently used beetroot powder supplementation showed significant results (p<0.05); however, the time period for the research was increased and was conducted on post-menopausal women (7). The mean values of Go, G1 and G2 are mentioned in Fig. 3 (b). The mean values of systolic blood pressure level G2 with the highest dosage

of beetroot powder capsules (500 mg) on 0 day showed a mean score (100.00 ± 10.54 mmHg) and on 63rd day (80.00 ± 1.012 mmHg) showed a highly significant result.

DISCUSSION

Beetroot can be consumed for its marvelous potential in therapeutic meals of hypertension (7). It seems like the nature designed beetroots for lowering bad cholesterol by its fiber and reducing inflammation by phenolic content. Recent study also proves the positive correlation with beetroot consumption is associated with reducing systolic blood pressure by lowering the cholesterol due to valuable amount of fiber (8). Beetroot bread acutely increased endothelium-independent vasodilation and also led to a reduction in diastolic blood pressure (DBP). Consequently, fortifying bread with beetroot could serve as an effective means to boost dietary intake of heart-protective beetroot and might open up new therapeutic avenues for managing hypertension (9). Use of beetroot juice while following a diet low in nitrates could potentially decrease blood pressure (BP), consequently lowers the risk of cardiovascular incidents. Nonetheless, it remains unclear whether incorporating beetroot juice into a regular diet has a comparable impact on BP (10). Randomized clinical trials have yet to undergo a systematic assessment concerning the blood pressure-reducing effects of inorganic nitrate and beetroot (11). Few studies highlighted notable hypotensive effects resulting from a modest dose (100 g) of beetroot, and these effects remained consistent regardless of processing methods or the presence of betacyanins. These data further solidifies the existing evidence supporting the cardiovascular protective benefits of vegetables rich in dietary nitrates (12). Dietary nitrate has been shown to exert a variety of advantageous vascular effects. These include blood pressure reduction, inhibition of platelet aggregation, preservation or enhancement of endothelial function, and improved exercise performance, both in healthy individuals and those with peripheral arterial disease. Additionally, preclinical investigations involving nitrate or nitrite have indicated the potential to provide protection against ischaemia-reperfusion injury and to decrease arterial stiffness, inflammation, and intimal thickness (13). Another study suggests a role for dietary nitrate as an affordable, readily-available, adjunctive treatment in the management of patients with hypertension (14).

BLOOD SODIUM AND POTASSIUM LEVELS

Fig. 2 (a) and Fig. 2 (b) indicate substantial effects of beetroot powder on blood sodium and potassium levels, respectively. Notably, the group receiving the highest dosage of beetroot powder (G2 with 500 mg) experienced significant reductions in sodium levels and substantial increases in potassium levels over the course of the study. These findings are particularly promising as maintaining a healthy balance of sodium and potassium is crucial for blood pressure regulation. Elevated sodium levels are associated with increased blood pressure, while potassium has the opposite effect. The substantial increase in potassium levels, in particular, suggests that beetroot powder may play a pivotal role in mitigating the harmful effects of high sodium intake, which is prevalent in modern diets.

SYSTOLIC AND DIASTOLIC BLOOD PRESSURE

Fig. 3 (a) and Fig. 3 (b) display the mean values of systolic and diastolic blood pressure, respectively, before and after the trial. It's noteworthy that G2, the group receiving the highest beetroot powder dosage (500 mg), exhibited significant reductions in both systolic and diastolic blood pressure levels. This reduction in blood pressure is a key indicator of beetroot's efficacy in hypertension management. High blood pressure, if left uncontrolled, can lead to serious health complications, including heart disease and stroke. The observed decrease in blood pressure aligns with previous research (7) and highlights beetroot's potential as a natural intervention for individuals with pre-hypertension and hypertension.

PROXIMATE COMPOSITION OF BEETROOT POWDER

Table I (a) provides a breakdown of the proximate composition of beetroot powder, revealing its nutritional profile. Beetroot powder is particularly rich in moisture and fiber, constituting 70.42% and 8.25%, respectively. This high fiber content is of significance as dietary fiber has been associated with blood



pressure regulation. Fiber contributes to overall cardiovascular health by promoting satiety, aiding in weight management, and modulating blood pressure.

PHENOLIC COMPOUNDS AND FLAVONOIDS

Table I (b) presents the content of phenolic compounds and flavonoids in beetroot powder. Phenolic compounds and flavonoids are bioactive compounds with antioxidant and anti-inflammatory properties. These compounds are abundant in beetroot and are believed to contribute to its potential health benefits. Inflammation is increasingly recognized as a contributing factor to hypertension and related cardiovascular diseases. The presence of significant phenolic compounds and flavonoids in beetroot suggests its role in reducing inflammation, furthermore, flavonoids found in fruits and vegetables could offer protection against cardiovascular disease (15). Recent years have witnessed an increasing body of evidence suggesting that polyphenols found in plant-based foods, owing to their inherent biological properties, could serve as distinctive nutraceuticals and complementary therapeutic approaches for addressing various facets of type 2 diabetes mellitus (16).

The results of this study suggest that beetroot powder can be a valuable component of dietary interventions for individuals with pre-hypertension, stage 1, and stage 2 hypertension. Its ability to modulate sodium and potassium levels, reduce blood pressure, and provide a rich source of dietary fiber and bioactive compounds makes it a promising natural remedy. Future research should explore the long-term effects of beetroot consumption on blood pressure and cardiovascular health in larger and more diverse populations. Additionally, investigations into the mechanisms through which beetroot exerts its blood pressure-lowering effects, such as its impact on endothelial function and nitric oxide production, would provide valuable insights.

CONCLUSION

This study showed that beetroot powder intake had a highly significant effects on all the 4 parameters which have a role in either keeping blood-pressure normal or abnormal, reducing systolic blood pressure levels, diastolic blood pressure levels, blood sodium level and increasing blood potassium levels. Thus, beetroot powder could be a beneficial adjuvant therapy in the high blood pressure control of patients with pre-hypertension, stage 1 and 2 hypertension.

Authors' Contributions:

Conceptualized and designed the experiments: Aymen Shahzad. Performed the experiments: Aymen Shahzad. Analyzed the data: Nizwa Itrat Contributed materials/ analysis/ tools: Aymen Shahzad, Roheen Shakeel and Rashad Mahmood. Wrote the paper: Aymen Shahzad. Revised by Nizwa Itrat and Anum Nazir.

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