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ORIGINAL ARTICLE

Effect of Antioxidant Capsule Supplementation on Oxidative Stress Markers in Hypertensive Patients

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Abstract

Background/Aim: Hypertension is a consequence of a neuromodulatory imbalance and is directly associated with cardiovascular diseases. Oxidative stress constitutes an intermediate pathophysiological mechanism for hypertension and cardiovascular disease. Evidence suggests that there is a proportional relationship between diets high in antioxidants and a reduced risk of cardiovascular events. A high antioxidant diet could scavenge the free radicals and other toxic radicals offering better protection to the cardiovascular system. The primary study objective was to evaluate the impact of dietary supplementation with fruit extract capsules on markers of oxidative stress and antioxidant capacity in hypertensive and normotensive individuals; a secondary objective was to evaluate the impact of dietary supplementation on insulin resistance, markers of inflammation and haemodynamic variables in treated hypertensive and normotensive patients.

Methods: This clinical trial comprised 30 hypertensive patients and 29 normotensive volunteers. Study participants received placebo capsules for 4 weeks, then fruit extract capsules (blueberry, cranberry and pomegranate), one capsule of each per day for 4 weeks. Blood pressure was measured at baseline, after the use of placebo and antioxidant capsules. Catalase, thiobarbiturate acid reactive substances (TBARS), superoxide dismutase (SOD), carbonyl and ferric-reducing antioxidant powder (FRAP) were used to evaluate oxidative stress.

Results: The mean age among hypertensive participants was 49.3 ± 9.3 years. Catalase increased in both groups; reduction of TBARs, FRAP and carbonyls occurred in the hypertensive group. A significant intragroup difference in homeostatic model assessment of insulin resistance (HOMAir) was noted between normotensive and hypertensive individuals at different times. No significant difference occurred regarding inflammatory cytokines, adipocytokines and haemodynamic data after antioxidant consumption.

Conclusion: The study results suggest that supplementation with fruit capsules rich in antioxidants for 4 weeks significantly reduces oxidative stress in hypertensive patients. Studies with a larger number of patients are needed to confirm the findings.

Keys words: *Vaccinium macrocarpon*; Blueberry plants; Pomegranate; Hypertension; Oxidative stress; Antioxidants; Cardiovascular diseases.

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Introduction

Cardiovascular diseases (CVD) are the globally leading cause of death. The main risk factors for the development of CVD are arterial hypertension (AH), dyslipidaemia, obesity, sedentary lifestyle, use of alcohol and tobacco, in addition to low consumption of fruits and vegetables. All these risk factors are preventable.[1] AH is the main risk factor for CVD. A neuromodulatory imbalance is associated with oxidative stress and on the other hand, oxidative stress constitutes an intermediate pathophysiological mechanism for AH and CVD.

Low consumption of fruits and vegetables is related to myocardial infarctions and strokes. As recommended by the World Health Report (WHR) 2002, the daily consumption of fruits and vegetables, per person, should be around 400 g.[2] This is a way to supply the body with foods rich in antioxidants and achieve a balance between oxidative stress and antioxidant capacity.

There are different ways to attain the needed antioxidants. The consumption of pomegranate and cranberry juice, fruits rich in antioxidants, in humans resulted in an improvement in vascular function in patients with AH and coronary vascular disease.[3-5] The extract from the pomegranate peel has already been tested in type 2 diabetic patients, showing favourable results in blood pressure, anthropometry, body composition, lipid and glucose metabolism and oxidative stress.[6-8] Several experimental studies have tested diets enriched with antioxidants.[9, 10]

The antioxidant capacity can be assessed by different methods.[11-13] In addition to the evaluation of the antioxidant capacity, a way to assess the impact of oxidative stress on the body is through analysis of oxidative damage. Oxidative damage can be assessed by measuring thiobarbituric acid reactive substances (TBARs) and measuring carbonyl proteins.[14]

From our point of view, it is necessary to know whether the protective effects of fruit extract are present even when the extract is ingested in combination with other foods in one's personal routine, which may have large amounts of salt and/or fat. The primary objective of this study was to evaluate the impact of dietary supplementation with fruit extract capsules on markers of oxidative stress in hypertensive and normotensive patients; the secondary objective was to evaluate the impact of dietary supplementation on markers of a neuromodulatory imbalance such as inflammatory activity, insulin resistance and also haemodynamic variables in hypertensive and normotensive patients.

Methods

This was a prospective, longitudinal, cohort clinical trial. The study was submitted to and approved by the Ethics Committee for Analysis of Research Projects at the Hospital das Clínicas, Faculty of Medicine, University of São Paulo (CAPPesq) on 2 September 2015 under number 1.157.544. All participants were previously informed about the study procedures and asked to sign the Informed Consent (IC) form in accordance with Resolution 466/12 of the National Health Council.

Study population

Hypertensive patients, both sexes, aged between 18 and 59 years, body mass index (BMI) less than 35 kg/m^2, were recruited from the AH outpatient clinic of a tertiary hospital. All received medication for AH, one or more drug classes, including diuretics, angiotensin-converting enzyme inhibitors, angiotensin-receptor blockers, calcium channel blockers and central and peripheral sympatholytic agonists. Exclusion criteria were pregnancy, smoking, patients with diabetes using more than one drug in addition to metformin, glycated haemoglobin > 7 %, diagnosis of CVD (such as complex arrhythmia and valvular heart disease), LDL-cholesterol > 160 mg/dL (nondiabetics), LDL-cholesterol > 130 mg/dL (in nondiabetics using a statin), LDL-cholesterol ≥ 100 mg/dL (in diabetics using a statin), patients with pulmonary hypertension, collagen diseases, cancer, on dialysis and with any chronic disabling pathology. Normotensive volunteers were recruited from friends of hypertensive participants.

After laboratory tests, participants were included and matched by age, sex, race, BMI and were divided into two groups according to the presence or absence of AH: normotensive (29 subjects) and hypertensive (30 subjects). All subjects were instructed not to change their eating

patterns and physical activity during the 8-week follow-up period study. At the end of the study, all participants received guidance, individually, from a nutritionist, regarding a healthy diet. Figure 1 shows a schematic representation of the recruitment process which was carried out.

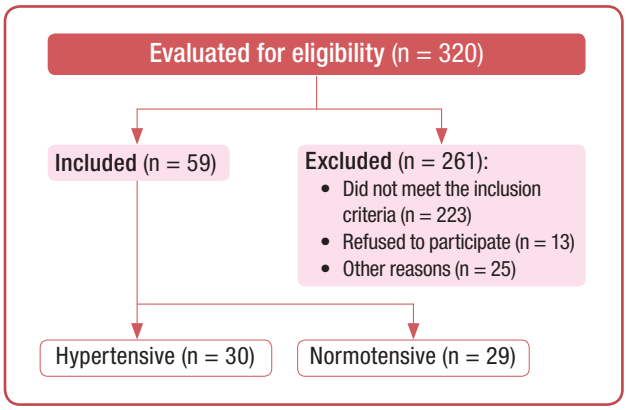

Figure 1: Consolidated flow diagram of participants from recruitment data analysis

Anthropometric data. All anthropometric assessments were performed in a standardised manner. All measurements were performed, in triplicate, using the arithmetic mean as the final measurement. A scale with a Filizola® stadiometer (Model PL 150, *Filizola Ltda*, Brazil) was used to obtain the value of body weight and height. BMI was calculated as the ratio between weight (kg) and height squared (m^2). To measure waist circumference (WC) and neck circumference (NC), an inelastic fiberglass tape with precision of 1 mm was used. The WC evaluation was performed following the technique of the midpoint between the last rib and anterosuperior iliac crest and NC was taken in the position corresponding to the midpoint of the participants' neck height.[15]

Intervention. The total duration of the study was 8 weeks. Subjects started by taking placebo capsules three times a day and at the end of 4 weeks the placebo capsules were changed to fruit extract capsules, also administered three times a day. Participants were instructed to take a cranberry capsule at breakfast, a blueberry capsule at lunch and a pomegranate capsule at dinner. The antioxidant capacity and phytochemicals such as phenolic compounds in each capsule were evaluated using the methods oxygen radical absorbance capacity (ORAC) and Folin-Ciocalteu, respectively (Table 1).

In the placebo and intervention phases, telephone contact was made with the participants to monitor adherence. Every two weeks, participants returned to deliver the remaining capsules and receive capsules for another two weeks. Adhesion was checked by pill count.

Table 1: Capsules total antioxidant capacity (ORAC method) and phenolic compounds (Folin-Ciocalteu method)

Component	Antioxidant capacity (µmol Trolox/g extract)	Phenolic compounds (mg GAE/g extract)
Cranberry	965.4 ± 27.7	96.0 ± 5.7
Blueberry	731.8 ± 28.0	87.2 ± 9.1
Pomegranate	676.3 ± 17.9	111.9 ± 2.3

GAE – gallic acid equivalent;

Anthropometric, haemodynamic and laboratory assessments were performed at baseline, after four weeks of the placebo phase and after four weeks of intervention. Capsules of placebo and extract were similar.

Laboratory tests. Blood samples were collected after 12 h of fasting to measure urea, creatinine, sodium, potassium, triglycerides, total cholesterol, LDL-cholesterol, HDL-cholesterol, C-reactive protein (CRP), glucose, insulin, tumour necrosis factor - alpha (TNF-alpha), interleukin-6 (IL-6), interleukin 2 (IL-2), interleukin-4 (IL-4), monocyte chemotactic protein-1 (MCP-1), adiponectin and leptin. Urea, creatinine, sodium, potassium, triglycerides and total cholesterol and fractions were measured only at baseline. The other exams were repeated after the intervention with placebo and antioxidant capsules.

Insulin sensitivity. Insulin sensitivity was evaluated from glucose and insulin values to calculate the homeostatic model assessment of insulin resistance (HOMAir).[16]

Antioxidant capacity marker measurements. Antioxidant capacity was measured by determining the activity of catalase, superoxide dismutase (SOD) enzymes and by the ferric-reducing antioxidant power (FRAP) method.[11-13]

Oxidative damage marker measurements. The measurement of oxidative damage markers was performed by measuring thiobarbituric acid reactive substances and measuring carbonyl proteins.[14, 17, 18]

Statistical analysis

The sample size calculation was performed con-

sidering a study on the effect of a diet rich in antioxidants on blood pressure and oxidative stress in hypertensive and normotensive individuals.[19] The statistical power considered for the calculation was 80 %, the confidence interval 95 % and the number of cases needed in each group was estimated at 27 individuals. Sample calculation was performed using the *OpenEpi* version 2013 program.

Descriptive analyses for quantitative data were performed, showing the means accompanied by the respective standard deviation or error (SD, SE). The assumptions of normal distribution in each group and the homogeneity of variances between groups were evaluated, respectively, with the Kolmogorov-Smirnov test. For quantitative variables, repeated measures ANOVA was used. For those comparisons that showed a significant difference by ANOVA, the two-by-two post-hoc test was performed, with Bonferroni correction.

When a significant difference was detected between the data obtained after ingestion of the capsules with fruit extract and the data obtained at the beginning of the study (or at the end of the placebo phase) in the hypertensive group, the unpaired Student *t* test was used for comparison with data from the normotensive group. This was performed to verify whether the significant values obtained for hypertensive individuals were bringing them closer to the normotensive group. All analyses were performed using SPSS 21 software for Windows with a significance level of $p < 0.05$.

Results

From all participants who were selected; no one dropped out. The percentage of adhesion, calculated by counting the capsules, was approximately 98 % among normotensive individuals and 95 % among hypertensive individuals. Among hypertensive patients, only 2 (6.7 %) had diabetes and 8 (26.7 %) had prediabetes.

Table 2: Demographic profile and some clinical parameters at baseline (mean ± SD)

Parameter	Normotensive (n = 29)	Hypertensive (n = 30)	p
Women	16 (55.2 %)	16 (55.2 %)	0.961
Men	13 (44.8 %)	13 (44.8 %)	
Age (years)	45.5 ± 8.9	45.5 ± 8.9	0.185
Ethnicity (white/non-white)	21/8	21/8	0.456
Urea (mg/dL)	28 ± 5	28 ± 5	0.024
Creatinine (mg/dL)	0.8 ± 0.2	0.8 ± 0.2	0.380
Sodium (mEq/L)	141 ± 3	141 ± 3	0.540
Potassium (mEq/L)	4.5 ± 0.3	4.5 ± 0.3	0.900
Total cholesterol (mg/dL)	191 ± 35	191 ± 35	0.585
LDL-cholesterol (mg/dL)	112 ± 28	112 ± 28	0.907
HDL-cholesterol (mg/dL)	51 ± 13	51 ± 13	0.721
Triglycerides (mg/dL)	110 ± 69	110 ± 69	0.721

LDL - low density lipoprotein; HDL - high density lipoprotein;

Demographic data and results of initial biochemical tests are shown in Table 2. Subjects were compared according to weight, BMI, waist circumference and neck circumference before and after ingestion of the antioxidant capsules. Anthropometric data did not change after the consumption of antioxidants (Table 3).

A significant intragroup difference for HOMAir between normotensive individuals at different times ($p = 0.001$) was found. The same was true for the hypertensive subjects ($p = 0.001$), as shown in Figure 2. Bonferroni's post-hoc test showed that both for normotensive and hypertensive individuals, initial measurements were similar to post-placebo measurements and that post intervention measurements were statistically smaller than the previous ones. The post intervention HOMAir declined to values below half of the initial values.

There was no significant difference regarding inflammatory cytokines and adipocytokines after consumption of antioxidants, as shown in Table 4.

Haemodynamic data were not different after consumption of antioxidants (Table 5). A reduction occurred in TBARs and carbonyls in the hypertensive subjects after consumption of antioxidant fruit capsules (Figure 3 and 4).

Regarding antioxidant capacity, there was an increase in catalase in normotensive and hypertensive subjects after 4 weeks of consumption of capsules with fruit extract (Figure 5).

Table 3: Anthropometric profile of normotensive and hypertensive individuals at baseline, post-placebo (PP) and post-fruit extract (PFE) use (mean ± SD)

Parameter	Normotensive (n = 29)				Hypertensive (n = 30)			
	Basal	PP	PFE	p	Basal	PP	PFE	p
Weight (kg)	77 ± 12	77 ± 2	77 ± 12	0.074	77 ± 12	78 ± 15	78 ± 15	0.425
BMI (kg/m²)	28 ± 4	28 ± 4	28 ± 6	0.122	29 ± 4	29 ± 4	29 ± 4	0.452
NC (cm)	37 ± 3	37 ± 3	37 ± 3	0.972	38 ± 3	37 ± 3	38 ± 4	0.205
WC (cm)								
Women	94 ± 8	94 ± 7	93 ± 7	0.243	91 ± 10	91 ± 10	91 ± 10	0.286
Men	89 ± 19	94 ± 9	94 ± 8	0.750	101 ± 11	101 ±12	101 ± 12	0.540

BMI – Body mass index; WC – Waist circumference; NC – Neck circumference;

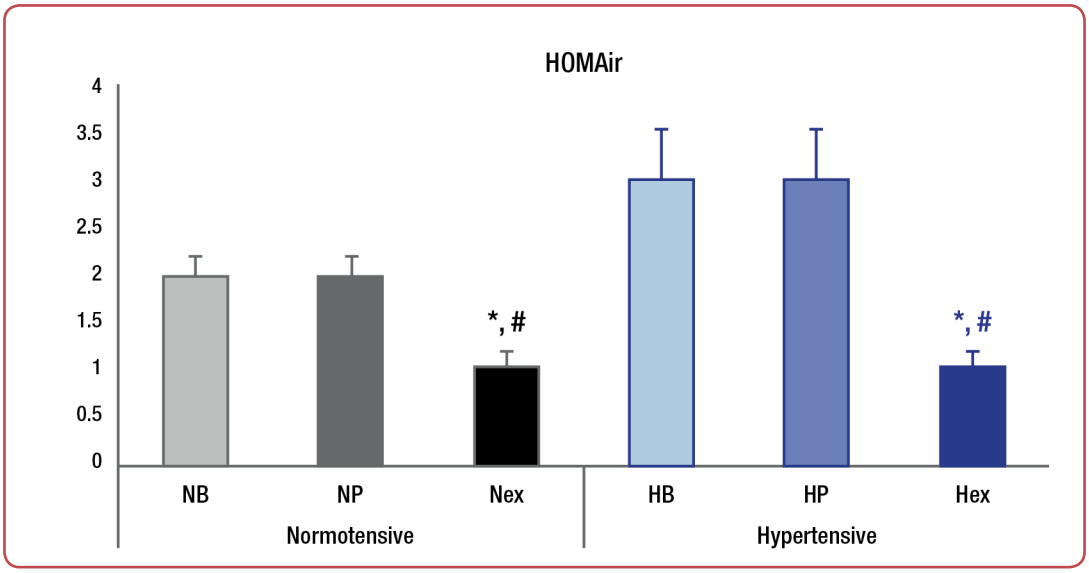

Figure 2: Homeostatic model assessment of insulin resistance (HOMAir) in normotensive and hypertensive subjects at baseline, after placebo and extract (mean ± SE)

*NB – normotensive at baseline; NP – normotensive on placebo; Nex – normotensives on extract; HB – hypertensive at baseline; HP – hypertensive on placebo; Hex – hypertensive on extract. * = extract vs baseline; # = extract vs placebo; & = hypertensive at baseline vs normotensive at baseline;*

Table 4: Plasma dosage of inflammatory cytokines and adipocytokines at baseline, post-placebo (PP) and post-fruit extract (PEF) use (mean ± SD)

Parameter	Normotensive (n = 29)				Hypertensive (n = 30)			
	Basal	PP	PFE	p	Basal	PP	PFE	p
CRP (mg/dL)	2.3 ± 1.8	4.0 ± 0.0	2.0 ± 0.1	0.441	3.4 ± 3.2	3.2 ± 3.1	3.3 ± 3.1	0.724
TNF-alpha (pg/mL)	19.0 ± 15.3	21.5 ± 18.1	18.6 ± 14.2	0.680	12.0 ± 11.2	14.2 ± 10.8	12.9 ± 11.2	0.663
IL-6 (pg/mL)	2.9 ± 2.0	2.8 ± 1.9	2.6 ± 2.0	0.849	2.9 ± 1.7	2.6 ± 1.2	2.7 ± 2.1	0.606
IL-2 (pg/mL)	1.0 ± 1.7	0.6 ± 1.2	0.7 ± 1.0	0.325	1.7 ± 3.6	1.2 ± 2.8	0.8 ± 1.6	0.071
IL-4 (pg/mL)	1.0 ± 0.8	1.0 ± 0.7	0.9 ± 0.7	0.834	0.6 ± 0.5	0.7 ± 0.5	0.7 ± 0.5	0.724
MCP-1 (pg/mL)	1.9 ± 1.3	1.8 ± 1.4	1.9 ± 1.6	0.951	1.4 ± 0.9	1.5 ± 0.9	1.4 ± 1.2	0.953
Adiponectin (mg/L)	11.2 ± 6.1	10.6 ± 6.7	11.2 ± 7.8	0.494	14.6 ±10.6	13.6 ± 9.6	13.9 ± 9.9	0.310
Leptin (ng/mL)	13.1 ± 10.2	11.9 ± 9.2	14.4 ± 11.9	0.111	13.5 ± 9.5	12.2 ± 8.8	13.5 ± 9.3	0.246

CPR – C reactive protein; TNF-alpha – tumour necrosis factor alpha; IL-6 – Interleukin 6; IL-2 – Interleukin 2; IL-4 – Interleukin 4; MCP-1 – monocyte chemoattractant protein 1;

Table 5: Blood pressure and heart rate at baseline, post-placebo (PP), post-fruit extract (PFE) use (mean ± SD)

Parameter	Normotensive (n = 29)				Hypertensive (n = 30)			
	Basal	PP	PFE	p	Basal	PP	PFE	p
SBP (mmHg)	121.8 ± 15.6	122.0 ± 13.4	120.3 ± 11.7	0.557	141.7 ± 22.6	143.4 ± 28.6	137.0 ± 21.9	0.113
DBP (mmHg)	74.2 ± 9.6	74.3 ± 9.4	71.9 ± 8.7	0.124	83.6 ± 13.9	86.3 ± 17.9	79.7 ± 15.5	0.137
HR (bpm)	64.7 ± 11.2	66.0 ± 12.0	67.3 ± 10.7	0.179	63.7 ± 10.0	60.6 ± 10.5	61.7 ± 9.0	0.081

SBP – Systolic blood pressure; DBP – Diastolic blood pressure; HR – heart rate (beats per minute);

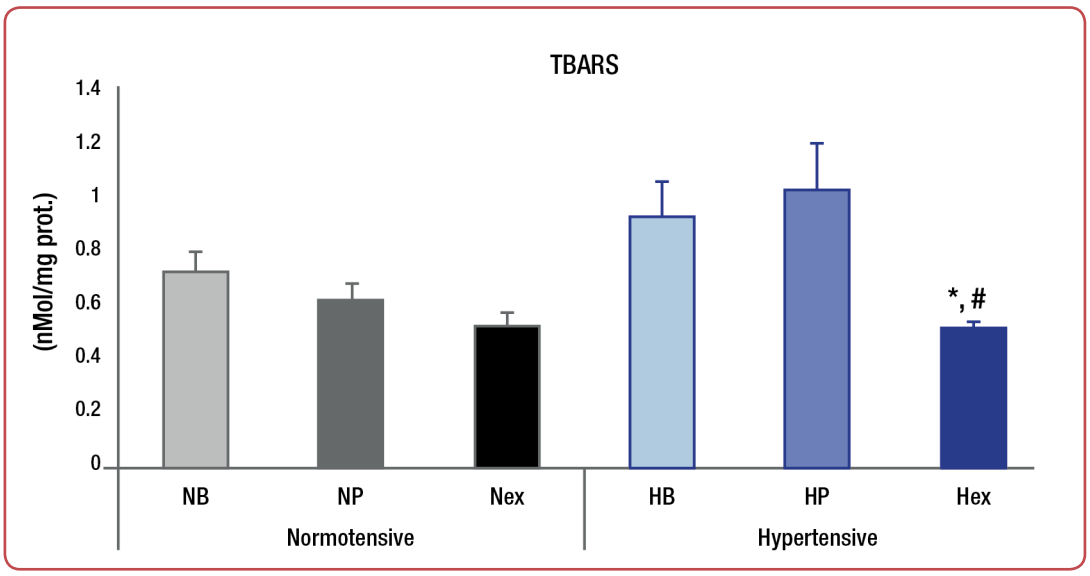

Figure 3: Thiobarbiturate acid reactive substances (TBARS) levels in normotensive and hypertensive subjects at baseline, after placebo and extract (mean ± SE)

*NB – normotensives at baseline; NP – normotensives on placebo; Nex – normotensives on extract; HB – hypertensive at baseline; HP – hypertensive on placebo; Hex – hypertensive on extract; * = extract vs baseline; # = extract vs placebo;*

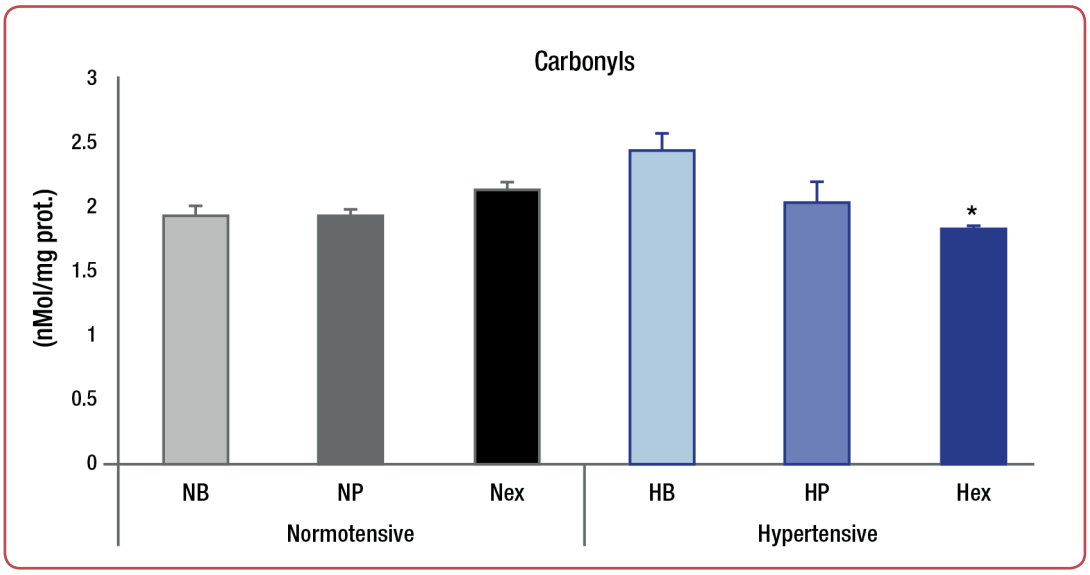

Figure 4: Carbonyls levels in normotensive and hypertensive subjects at baseline, after placebo and extract (mean ± SE)

*NB – normotensive at baseline; NP – normotensive on placebo; Nex – normotensive on extract; HB – hypertensive at baseline; HP – hypertensive on placebo; Hex – hypertensive on extract. * = extract vs baseline; # = extract vs placebo;*

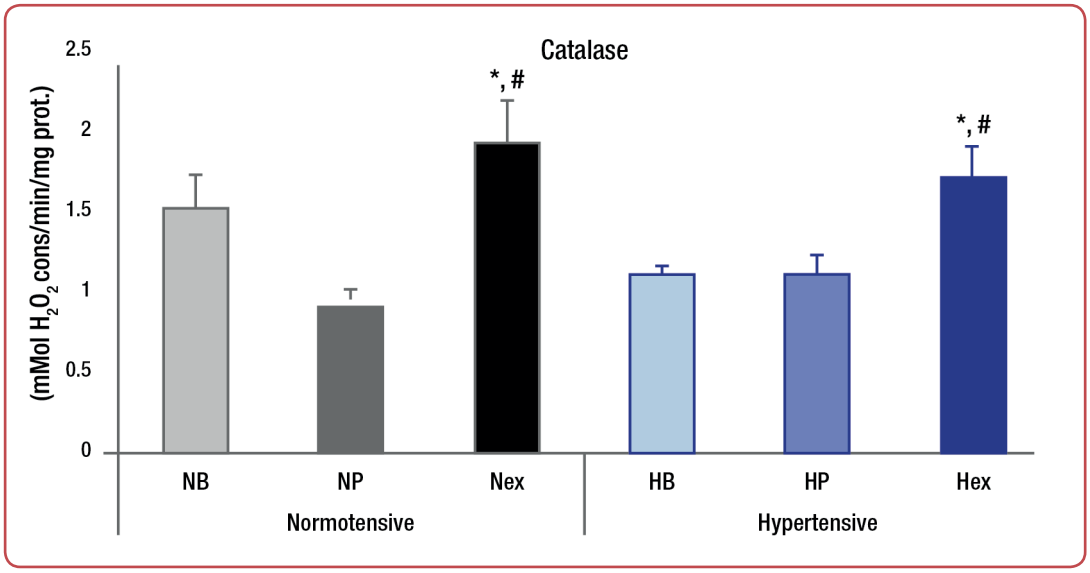

Figure 5: Catalase levels in normotensive and hypertensive subjects at baseline, after placebo and extract (mean ± SE)

*NB – normotensive at baseline; NP – normotensive on placebo; Nex – normotensive on extract; HB – hypertensive at baseline; HP – hypertensive on placebo; Hex – hypertensive on extract. * = extract vs baseline;*

Intragroup analyses demonstrate that catalase values after supplementation were significantly different from baseline and after the placebo period.

Discussion

Presented study shows that capsules containing fruit extracts conserve antioxidant power and have a beneficial effect on preventing the oxidation of lipids and proteins in hypertensive individuals. The antioxidant effect observed points to the fact that dietary supplementation with fruit extract capsules somehow interrupted the vicious cycle that causes oxidative damage to lipids and proteins in hypertensive patients. Certainly, the increased activity of the catalase enzyme, also observed in this study, is involved in decreasing oxidative stress, due to its ability to neutralise hydrogen peroxide. In addition to this electrochemical effect, the data presented here show a reduction in insulin resistance after consumption of fruit extracts. This metabolic effect can be considered important because it improves one of the main mechanisms of the metabolic syndrome, the insulin resistance.

The fact that a decrease occurred in protein oxidation is of great relevance due to its implications, because the carbonylation of proteins widely affects cellular function, reaching enzymes, receptors, membrane transport proteins, modulating immune response proteins and causing irreversible damage when it affects repair enzymes in the genetic material. Furthermore, oxidised proteins can contribute to the generation of secondary damage to other biomolecules.[20]

According to the Haytowitz and Bhagwat[21] database, a blueberry capsule is equivalent to approximately six units of the fresh fruit, a cranberry capsule has an antioxidant capacity similar to seven units of the fruit and a pomegranate capsule is equivalent to approximately half of a small unit of fruit. Thus, it is possible to obtain the benefits shown here with a small dose supplementation.

The fact that we did not use larger doses of fruit extract is directly related to the problem of medication adherence. Low adherence to drug treatment in chronic diseases is very common; however, in AH it is even more pronounced because most individuals do not have symptoms.[22] The number of prescribed doses is one of the factors that contribute to low adherence to treatment. In presented case, to ensure better adherence, it was decided to administer 3 doses daily. For us to achieve a meaningful dose of the extracts, the capsules had to be large in size.

More than 90 % adherence to supplementation was achieved and rates of 80 % can be considered acceptable.[23] The success of presented intervention can be attributed to two factors. The first was to carry out weekly telephone contacts as a way to bring the patient closer to the researcher and monitor adherence. The other important factor was that the study started with the administration of the placebo, so the habit of ingesting the capsules was incorporated into the participants' routine during the placebo phase, in order to prevent the low consumption of fruit extract capsules.

A limitation of this study is that the dose of antioxidants is relatively low. It is known that the concentration of polyphenols in a juice, for example, depends on the type of processing the food has undergone. Many marketed fruit juices are filtered to produce a sediment-free liquid. With that, the fibre and the number of polyphenols present in the drink are removed.[21] Although the drink contains a higher concentration of polyphenols when it is not filtered, from a commercial point of view, it is not interesting. For this reason, researchers chose to indicate the consumption of capsules with the dry fruit extract, as a way to maintain the antioxidant properties of the fruit with greater integrity.

To authors' knowledge this is the first study using fruit extract capsules as a source of antioxidants in hypertensive and normotensive patients. Therefore, it is difficult to compare data with those in the literature. Even when presentations like juices, frozen fruits, or dried fruits are used, it is difficult to establish comparisons because the processing differs a lot between studies.[24]

Artificial antioxidants are potentially carcinogenic and therefore of limited use. Accordingly, there is a great effort in research to use waste products from fruits rich in polyphenols as antioxidant agents.[25] Blueberries, cranberries and pomegranates, in general, are processed to remove juice and bagasse and seeds are disposed of. Bagasse and seeds are very rich in polyphenols, which can be used to produce capsules containing fruit extracts.

In the study by Lopes et al[19] a reduction in blood pressure levels was observed in obese hypertensive individuals following a standard DASH diet, ingesting 4 to 5 servings of fruit per day. Our proposal of supplementation with fruit extract is limited compared with the use of the DASH diet, in which a large number of antioxidants from vegetables, legumes and fruits have been used. Perhaps this is an explanation for why there was a drop in blood pressure levels in hypertensive patients. The DASH diet is the gold standard for preventing and treating high blood pressure. The combination of foods present in the DASH diet is superior to supplementation with only fruit capsules when the objective is to reduce blood pressure levels.

In addition, it should be taken into account that some antioxidants have a greater specificity to control AH, as is the case with quercetin present in blueberries.[26] It is interesting to note that the structural modification that occurs in polyphenols during food processing alters their antioxidant capacity: supplementation with wine capsules reduced BP, whereas grape juice capsules had no similar effect.[27]

The anthropometric indices used in the present study were weight, height, WC, NC and BMI. All these measurements were performed for the clinical assessment of metabolic risk associated with body adiposity, because they are easy, simple, practical, non-invasive measures. WC measurement is a way to estimate visceral adiposity. In this study, women (hypertensive and normotensive) had an increased WC above 88 cm. At first, this confers a greater cardiovascular risk.[28] In relation to men, the WC was less than 102 cm.

NC is a simpler and more practical measure compared with WC and is not influenced by postprandial abdominal distension or respiratory movements.[29] The increase in NC is associated with abdominal visceral fat. The consumption of fruit extract did not modify the NC in the study groups.

Hypertensive and obese individuals have a low-intensity inflammatory process and increased oxidative stress.[30, 31] This condition leads to alterations in the levels of cytokines and free radicals.[32] It has long been known that antioxidants present in fruits affect the functions of inflammatory cells, usually in-

hibiting the secretion of pro-inflammatory cytokines and cell proliferation. Therefore, flavonoids are recognised as immunomodulators.[33] As a result, the levels of C-reactive protein (CRP), interleukins 2, 4 and 6, TNF-alpha, monocyte chemotactic factor (MCP1), leptin and adiponectin, an adipocytokine with anti-inflammatory action were evaluated. There was no difference in cytokine levels in the two groups after intervention with antioxidant capsules.

The effects of antioxidants on inflammatory markers in humans are controversial, because the amount of antioxidants ingested in the usual diet can vary greatly between individuals, in addition to being a parameter that is difficult to control and monitor. To overcome this difficulty, studies with a large number of participants are needed. For example, in a multicentre, international study with 315 participants without associated chronic diseases, it was observed that lower levels of active polyphenols are associated with higher levels of plasma CRP and *vice versa*, indicating an inversely proportional relationship between inflammatory markers and polyphenols.[34]

Data in the literature show the effect of flavonoids in regulating glucose by increasing insulin secretion.[35, 36] In the present study, an increase in plasma insulin after consumption of extracts and fruits was not observed. However, the HOMAir index declined in both groups after consumption of fruit extracts, indicating an improvement in insulin sensitivity.

The assessment of enzyme activity, SOD, gives us an idea of the body's immediate response to oxidative stress. In presented study, there was no change in SOD levels after the use of fruit extract capsules. In the study by Riso et al[37] dietary supplementation with blueberry juice in adult men did not change SOD levels after six weeks of intervention. In this study, an increase in catalase levels in both groups was observed, which suggests an increase in antioxidant levels. Regarding FRAP, the values did not change after using the antioxidant capsules. On the other hand, one month supplementation of 500 g of fresh strawberries in healthy volunteers resulted in an increase in antioxidant capacity, assessed by the FRAP and oxygen radical absorbance capacity tests.[38] Probably the number of antioxidants offered in that study was higher than in presented study.

As is well known, consuming fruit is a way of ingesting polyphenols, important antioxidants. Although this study has some limitations, results encourage us to consume fruit extract capsules, as it is easy and practical.

Conclusion

Supplementation with cranberry, blueberry and pomegranate extract capsules reduced insulin resistance in hypertensive patients and normotensive volunteers and also had an impact on decreasing oxidative stress, through increased catalase enzyme activity and reduced lipid peroxidation and oxidative damage to proteins.

Ethics

The study was submitted to and approved by the Ethics Committee for Analysis of Research Projects at the Hospital das Clínicas, Faculty of Medicine, University of São Paulo (CAPPesq), decision number 1.157.544, dated 2 September 2015.

Acknowledgement

None.

Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

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References

1. World Health Organization [Internet]. Nutrition Unit. (2003). Fruit and vegetable promotion initiative: a meeting report, 25-27/08/03. World Health Organization. [Cited: 1-Sep-2024]. Available from: https://iris.who.int/handle/10665/68395.
2. World Health Report 2002. Reducing risks, promoting healthy life. Geneva: World Health Organization; 2002.
3. Dohadwala MM, Holbrook M, Hamburg NM, Shenouda SM, Chung WB, Titas M, et al. Effects of cranberry juice consumption on vascular function in patients with coronary artery disease. Am J Clin Nutr. 2011;93:934–940. doi: 10.3945/ajcn.110.004242.
4. Asgary S, Keshvari M, Sahebkar A, Hashemi M, Rafieian-Kopaei M. Clinical investigation of the acute effects of pomegranate juice on blood pressure and endothelial function in hypertensive individuals. ARYA Atheroscler. 2013;9(6):326-31. PMID: 24575134.
5. Asgary S, Sahebkar A, Afshani MR, Keshvari M, Haghjooyjavanmard S, Rafieian-Kopaei M. Clinical evaluation of blood pressure lowering, endothelial function improving, hypolipidemic and anti-inflammatory effects of pomegranate juice in hypertensive subjects. Phytother Res. 2014;28(2):193-9. doi: 10.1002/ptr.4977.
6. Grabež M, Škrbić R, Stojiljković MP, Vučić V, Grujić VR, Jakovljević V, et al. Rev Cardiovasc Med. 2022;23(2):57. doi: 10.31083/j.rcm2302057.
7. Grabež M, Škrbić R, Stojiljković MP, Rudić-Grujić V, Paunović M, Arsić A, et al. Beneficial effects of pomegranate peel extract on plasma lipid profile, fatty acids levels and blood pressure in patients with diabetes mellitus type-2: a randomized, doubleblind, placebo-controlled study. Journal of Functional Foods. 2020; 64:103692. doi: 10.1016/j.jff.2019.103692.
8. Grabež M, Škrbić R, Stojiljković M, Rudić-Grujić V, Šavikin K, Menković N, et al. Beneficial effects of pomegranate peel extract treatment on anthropometry and body composition of overweight patients with diabetes mellitus type-2: a randomized clinical trial. Scr Med. 2020;51:21–7. doi: 10.5937/scritamed51-25763.
9. Vinson JA, Bose P, Proch J, Al Kharrat H, Samman N. Cranberries and cranberry products: powerful in vitro, ex vivo, and in vivo sources of antioxidants. J Agric Food Chem. 2008 Jul 23;56(14):5884-91. doi: 10.1021/jf073309b.
10. Elks CM, Reed SD, Mariappan N, Shukitt-Hale B, Joseph JA, Ingram DK, et al. A blueberry-enriched diet attenuates nephropathy in a rat model of hypertension via reduction in oxidative stress. PLoS ONE. 2011;6(9):e24028. doi: 10.1371/journal.pone.0024028.
11. Boveris A, Chance, B. The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. Biochem J. 1973;134(3):707–16. doi: 10.1042/bj1340707.
12. Marklund, S, Marklund G. Involvement of the superoxide anion radical in the auto oxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974;47:469-474. doi: 10.1111/j.1432-1033.1974.tb03714.x.
13. Benzie IF, Strain JJ. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous

measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol. 1999;299:15-27. doi: 10.1016/s0076-6879(99)99005-5.
14. Buege JA, Aust S. Microsomal lipid oxidation. Methods Enzymol. 1978;52:302-10. doi: 10.1016/s0076-6879(78)52032-6.
15. Assyov Y, Gateva A, Tsakova A, Kamenov Z. A comparison of the clinical usefulness of neck circumference and waist circumference in individuals with severe obesity. Endocrine Res. 2016;42(1):6-14. doi: 10.3109/07435800.2016.1155598.
16. Mari A, Ahrén B, Pacini G. Assessment of insulin secretion in relation to insulin resistance. Curr Opin Clin Nutr Metab Care 2005;8(5):529-33. doi: 10.1097/01.mco.0000171130.23441.59.
17. Lissi E, Salim-Hanna M, Pascual C, del Castillo MD. Evaluation of total antioxidant potential (TRAP) and total antioxidant reactivity from luminol-enhanced chemiluminescence measurements. Free Radic Biol Med. 1995;18(2):153-8. doi: 10.1016/0891-5849(94)00117-3.
18. Reznick AZ, Packer L. Oxidative damage to proteins: spectrophotometric method for carbonyl assay. Methods Enzymol. 1994;233:357-63. doi: 10.1016/s0076-6879(94)33041-7.
19. Lopes HF, Martin KL, Nashar K, Morrow JD, Goodfriend TL, Egan BM. DASH diet lowers blood pressure and lipid-induced oxidative stress in obesity. Hypertension. 2003;41(3):422-30. doi: 10.1161/01.HYP.0000053450.19998.11.
20. Halliwell B, Whiteman M. Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? Br J Pharmacol. 2004 May;142(2):231-55. doi: 10.1038/sj.bjp.0705776.
21. Haytowitz DB, Bhagwat S. [Internet]. USDA Database for the Oxygen Radical Absorbance Capacity (ORAC) of Selected Foods, Release 2. U.S. Department of Agriculture 2010. [Cited: 1-Sep-2024]. Available from: http://www.ars.usda.gov/nutrientdata/orac.
22. Hori PC, da Silva GV. Adherence to antihypertensive treatment: approach, measurement methods and strategies for good outcomes Rev Bras Hipertens. 2016;23(4):84-9.
23. Osterberg L, Blaschke T. Adherence to medication. N Engl J Med. 2005;353(5):487-97. doi: 10.1056/NEJMra050100.
24. Klavins L, Kviesis J, Klavins M. Comparison of methods of extraction of phenolic compounds from American cranberry (Vaccinium macrocarpon L.) press residues. Agronomy Research. 2017;15:1316-29.
25. Duda-Chodak A, Tarko T. Antioxidant properties of different fruit seeds and peels. Acta Scientiarum Polonorum: Technologia Alimentaria. 2007; 6:29-36.
26. Johnson SA, Figueroa A, Navaei N, Wong A, Kalfon R, Ormsbee LT, et al. Daily blueberry consumption improves blood pressure and arterial stiffness in postmenopausal women with pre- and stage 1-hypertension: a randomized, double-blind, placebo-controlled clinical trial. J Academy Nutrit Dietet. 2015;115(3):369-77. doi: 10.1016/j.jand.2014.11.001.
27. Draijer R, Graaf Y, Slettenaar M, Groot E, Wright CI. Consumption of a polyphenol-rich grape-wine extract lowers ambulatory blood pressure in mildly hypertensive subjects. Nutrients. 2015; 7:3138-53. doi: 10.3390/nu7053138.
28. Lean MEJ, Han TS, Morrison CE. Waist circumference as a measure for indicating need for weight management. BMJ. 1995;311:158-61. doi: 10.1136/bmj.311.6998.158.
29. Ben-Noun L, Sohar E, Laor A. Neck circumference as a simple screening measure for identifying overweight and obese patients. Obes Res. 2001; 9(8):470-7. doi: 10.1038/oby.2001.61.
30. Chae CU, Lee RT, Rifai N, Ridker PM. Blood pressure and inflammation in apparently healthy men. Hypertension 2001;38:399-403. doi: 10.1161/01.hyp.38.3.399.
31. Ellulu MS, Patimah I, Khaza'ai H, Rahmat A, Abed Y. Obesity and inflammation: the linking mechanism and the complications. Arch Med Sci. 2017;13:851–63. doi: 10.5114/aoms.2016.58928.
32. Dinh QN, Drummond GR, Sobey CG, Chrissobolis S. Roles of inflammation, oxidative stress, and vascular dysfunction in hypertension. Biomed Res Int. 2014;2014:406960. doi: 10.1155/2014/406960.
33. Middleton E Jr, Kandaswami C. Effects of flavonoids on immune and inflammatory cell functions. Biochem Pharmacol. 1992;43(6):1167-79. doi: 10.1016/0006-2952(92)90489-6.
34. Harms L, Scalbert A, Zamora-Ros R, Sabina R. Plasma polyphenols associated with lower high-sensitivity C-reactive protein concentrations: a cross-sectional study within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. Br J Nutr. 2020;123(2):198–208. doi: 10.1017/S0007114519002538.
35. Ehrenkranz JR, Lewis NG, Kahn CR, Roth J. Phlorizin: a review. Diabetes Metab Res Rev. 2005 Jan-Feb;21(1):31-8. doi: 10.1002/dmrr.532.
36. Fraga CG, Croft KD, Kennedy DO, Tomás-Barberán FA. The effects of polyphenols and other bioactives on human health. Food Funct. 2019 Feb 20;10(2):514-528. doi: 10.1039/c8fo01997e.
37. Riso P, Klimis-Zacas D, Del Bo' C, Martini D, Campolo J, Vendrame S, et al. Effect of a wild blueberry (Vaccinium angustifolium) drink intervention on markers of oxidative stress, inflammation and endothelial function in humans with cardiovascular risk factors. Eur J Nutr. 2013;52:949–61. doi: 10.1007/s00394-012-0402-9.
38. Alvarez-Suarez JM, Giampieri F, Tulipani S, Casoli T, Di Stefano G, González-Paramás AM, et al. One-month strawberry-rich anthocyanin supplementation ameliorates cardiovascular risk, oxidative stress markers and platelet activation in humans. J Nutr Biochem. 2014; 25(3):289–94. doi: 10.1016/j.jnutbio.2013.11.002.