

Effects of grape powder supplementation on inflammatory and antioxidant markers in hemodialysis patients: A randomized double-blind study

Efeitos da suplementação de farinha de uva sobre marcadores inflamatórios e antioxidantes em pacientes em hemodiálise: Estudo duplo-cego randomizado

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ABSTRACT

Introduction: Polyphenols contained in natural sources such as grapes, have been considered pharmacological agents to combat oxidative stress and inflammation, common features in Chronic Kidney Disease patients. **Objective:** To evaluate the effects of grape powder supplementation on inflammatory and antioxidant biomarkers in hemodialysis (HD) patients. **Methods:** The double-blind placebo-controlled randomized clinical trial evaluated non-diabetic HD patients that received grape powder (500 mg of polyphenols/day) (n = 16, 9 men, 53.0 ± 9.8 years of age, 111.6 ± 58.2 HD months) or placebo (n = 16, 9 men, 52.7 ± 13.7 years of age, 110.4 ± 93.1 HD months) for five weeks. The glutathione peroxidase (GSH-Px) activity and C-reactive protein (CRP) levels were evaluated by ELISA method. **Results:** After the intervention period, the patients receiving grape powder showed an increase in the GSH-Px activity (16.5 (41.0) to 42.0 (43.3) nmol/min/ml) (*p* < 0.05) and they did not have the CRP levels increased as seen in placebo group (2.6 (0.28) to 2.8 (0.23) mg/L) (*p* < 0.05). **Conclusion:** The use of grape powder as phenolic source could play an important role as an antioxidant and anti-inflammatory agent in non-diabetic HD patients.

Keywords: inflammation; oxidative stress; polyphenols; renal dialysis.

RESUMO

Introdução: Polifenóis contidos em fontes naturais, como as uvas, têm sido considerados agentes farmacológicos no combate ao estresse oxidativo e inflamação, condições comuns na Doença Renal Crônica. **Objetivo:** Avaliar os efeitos da suplementação de farinha de uva sobre marcadores inflamatórios e antioxidantes em pacientes submetidos à hemodiálise (HD). **Métodos:** Estudo randomizado, duplo-cego, placebo-controlado, no qual foram avaliados pacientes não diabéticos em HD que receberam farinha de uva (500 mg de polifenóis/dia) (n = 16, 9 homens, 53,0 ± 9,8 anos, 111,6 ± 58,2 meses em HD) ou placebo (n = 16, 9 homens, 52,7 ± 13,7 anos, 110,4 ± 93,1 meses em HD) por cinco semanas. A atividade da glutathione peroxidase (GSH-Px) e os níveis plasmáticos de proteína C-reativa (PCR) foram mensurados por meio do método ELISA. **Resultados:** Após o período de intervenção, os pacientes que receberam farinha de uva apresentaram elevação na atividade da GSH-Px (16,5 (41,0) para 42,0 (43,3) nmol/min/ml) (*p* < 0,05) e não foi observada elevação nos níveis de PCR, como visto no grupo placebo (2,6 (0,28) para 2,8 (0,23) mg/L) (*p* < 0,05). **Conclusão:** O uso da farinha de uva como fonte de polifenóis pode desempenhar um importante papel anti-inflamatório e antioxidante em pacientes não diabéticos submetidos à HD.

Palavras-chave: diálise renal; estresse oxidativo; inflamação; polifenóis.

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INTRODUCTION

Cardiovascular diseases (CVD) are the most common cause of mortality and morbidity in hemodialysis (HD) patients. This increased risk of CVD is not only related to traditional risk factors but also to nontraditional and uremia-specific risk factors, such as hypervolemia, anemia, calcium-phosphorus metabolism disorders, inflammation and oxidative stress.¹

The oxidative stress in HD patients has been reported as a result of increased pro-oxidant activity (advanced age, diabetes, chronic inflammatory state, uremic syndrome, bioincompatibility of dialysis membranes) and reduced antioxidant systems (vitamin C, intracellular levels of vitamin E, glutathione system and phenolic compounds).^{2,3}

Phenolic compounds act as antioxidant for their ability to donate hydrogen or electrons and prevent the oxidation of various compounds, particularly fatty acids and oils.⁴ Fruits, especially those with red or blue color (such as grapes, plums, cherry), are the most important sources of polyphenols.⁵

In fact, increased uptake of food-based antioxidants is a promising alternative measure to reduce oxidative cell damage and stress response.⁶ In HD patients, only four studies^{2,6-8} evaluated the effects of polyphenolic-rich fruit juices on antioxidant capacity, oxidative stress and lipid profile. However, studies of dietary supplementation with grape powder as source of polyphenols never were performed in HD patients and it seems interesting because the fluid restriction is an important part of diet management in these patients. Thus, the aim of the present work was to assess the effects of grape powder supplementation on C-reactive protein and glutathione peroxidase levels in HD patients.

METHODS

SUBJECTS

The double-blind placebo-controlled randomized clinical trial evaluated clinically stable HD patients from *Centro Integrado de Nefrologia* in Rio de Janeiro, Brazil. Inclusion criteria were age > 18 years and patients on maintenance dialysis for at least 6 months. Patients with *diabetes mellitus*, inflammatory diseases, cancer, AIDS, autoimmune diseases, potassium levels above 5.5 mg/dL,

use of catheter access for HD and antioxidant vitamin supplements were excluded. Of the 35 HD patients that met eligibility criteria, one declined to participate. Thus, 34 patients were randomized. After randomization, one patient in each group (experimental and placebo) was lost to follow-up (by transfer from another clinic and change of treatment modality) and thirty-two patients concluded the study (16 in each group).

The dialysis duration was 3.5-4.0 h/session, three times per week, the blood flow greater than 350 ml/min, and the dialysate flow was of 500 ml/min. The etiology of Chronic Kidney Disease (CKD) was hypertensive nephrosclerosis (13), chronic glomerulonephritis (6), polycystic kidney disease (3), and other diseases or unknown causes (10). As antihypertensive medications, 2 patients were receiving ACE inhibitors, four taken β -blockers and 2 patients, calcium channel blocker. Four patients received a combination of β -blockers and ACE inhibitors. Furthermore, patients received antianemia treatment encompassing erythropoietin and iron preparations. None patient had taken cholesterol-lowering drugs during study period.

Weight and height measurements were obtained immediately after the HD session by a trained researcher using standard techniques and body mass index (BMI) was calculated as body weight divided by square height. The study protocol was reviewed and approved by the Ethics Committee of the Faculty of Medicine of the University Hospital Antônio Pedro (n. 264/10). The patients were aware of the study and signed an informed consent after reading such document.

ANALYSIS OF POLYPHENOLS CONTENT IN GRAPE POWDER

The total phenolic content was determined by the method described by Singleton & Rossi⁹ in six different grape powders available in the market, which was carried out interpolation of the absorbance of the samples against a calibration curve constructed from standards of gallic acid (mg/ml) and expressed as mg the gallic acid equivalent/g of extract. The grape powder with higher amount of polyphenols was purchased from Linho Lev Company Inc[®] (Santo Ângelo, RS, Brazil). The antioxidant capacity analysis was done by using DPPH radical degradation activity method.¹⁰

The specified supplement of grape powder used (12 g) for the study contained 18.8 kcal (1.2 g of protein, 2.6 g of carbohydrates, and 0.6 g of lipids), 1.8 mg sodium, 234 mg potassium and 500 mg of total polyphenols (using gallic acid as calibrator). Sodium and potassium correspond to 0.12% and 7.8% from recommendation to these patients, respectively.

SUPPLEMENTATION

Grape powder was pre-weighed and mixed to grape jelly in a way that both jelly (with and without grape powder addition) had minor difference regarding sensorial characteristics. Jellies were packaged in dull plastic containers in an amount sufficient for one week and each week a new pack of jelly was offered. Thus, it was possible a more accurate assessment of compliance and adverse events.

The patients were instructed to consume one tablespoon of grape jelly daily during five weeks in the afternoon snack (in dialysis day, therefore, after HD procedure). The placebo group ingested only grape jelly while experimental group consumed grape powder (in the amount of 12 g - 500 mg of total polyphenols) added to jelly.

ANALYTIC PROCEDURES AND SAMPLE PROCESSING

Blood samples were drawn from each subject in the morning, after fasting overnight. Blood was drawn from the arteriovenous fistula, before the dialysis session, into a syringe containing EDTA (1.0 mg/ml). Plasma was separated (15 min, 3000 x g, 4 °C) and stored at -80 °C until analysis. No difference was found in interdialytic weight gain on blood collection day corresponding to the beginning and end of supplementation. Thus, differences in plasma measurements before and after supplementation should not be attributed to fluid retention fluctuations.

DETERMINATION OF THE ACTIVITY OF GLUTATHIONE PEROXIDASE

The GSH-Px activity was determined by ELISA (Cayman Chemical, Ann Arbor, MI, USA). Cayman's GSH-Px assay measures GSH-Px activity indirectly by a coupled reaction with glutathione reductase (GR). Oxidized glutathione, produced upon reduction of hidroperoxide by GSH-Px, is recycled to its reduced state by GR and NADPH. The oxidation of NADPH to NADP⁺ is accompanied by a decrease in

absorbance at 340 nm. Under conditions in which the GSH-Px is rate limiting, the rate of decrease in the absorbance at 340 nm is directly proportional to the GSH-Px activity in the sample. The intra- and inter-assays CVs were 5.7 and 7.2%, respectively.

DETERMINATION OF THE HIGH-SENSITIVITY CRP, ALBUMIN, POTASSIUM, Kt/V_{sp}

Plasma high-sensitivity CRP was determined by ELISA method using R&D systems® kits (Minneapolis, MN, USA). The intra- and inter-assays CVs were < 8.5 and 7%, respectively. Measurements of serum albumin and potassium levels were done according to routine requirements for HD patients (by bromocresol green and selective electrode method, respectively). The single-pool Kt/V (Kt/V_{sp}) was used to represent the dialysis dose.

STATISTICAL ANALYSIS

Data are presented as mean ± standard deviation or median (interquartile range), when appropriate. Statistical analysis for change from baseline was carried out using Wilcoxon or paired t-test. Differences between placebo and experimental group were analyzed by Mann-Whitney or *t*-student test. Statistical significance was accepted as *p* < 0.05. All analyses were performed using S-PLUS 8.0 statistical software (Chicago, IL, USA).

RESULTS

Baseline characteristics of placebo and experimental groups are listed in Table 1. Gender, age, dialysis vintage, dialysis dose, BMI, and albumin levels were similar in both groups. The jelly consumption was not different between experimental and placebo group (3.5 (1.75) versus 4.5 (2) containers, respectively; *p* = 0.11).

TABLE 1 BASELINE CHARACTERISTICS OF HEMODIALYSIS PATIENTS

	Groups	
	Placebo	Experimental
Male/female	9/7	9/7
Age (years)	52.7 ± 13.7	53.0 ± 9.8
Dialysis vintage (months)	110.4 ± 93.1	111.6 ± 58.2
Kt/V _{sp}	1.22 ± 0.3	1.25 ± 0.2
BMI (kg/m ²)	22.6 ± 3.6	22.0 ± 2.1
Albumin (mg/L)	3.8 ± 0.3	3.9 ± 0.2

BMI: Body mass index; Kt/V_{sp}, represent the dialysis dose.

In experimental group, the consumption of grape powder was effective to increase the activity of GSH-Px. The placebo group showed low activity of GSH-Px before supplementation when compared to experimental group and, although the activity of GSH-Px has been also increased after intervention, the values before and after grape jelly consumption were not statistically different (Table 2). The individual values for GSH-Px activity in experimental and placebo group before and after intervention are presented in the Figures 1 and 2, respectively. In relation to inflammation, the CRP levels were maintained after grape powder consumption, however, CRP levels increased significantly in the end of study period in placebo group (Table 2).

Potassium levels were maintained before and after intervention in both groups (4.9 ± 0.5 versus 4.8 ± 0.6 mg/dL, respectively, in experimental group; and 4.7 ± 0.6 versus 4.8 ± 0.7 mg/dL, respectively, in placebo group). Moreover, any adverse events were associated to the grape powder supplementation (constipation or diarrhea, for example). There was no correlation among variables.

DISCUSSION

The present study was the first to supplement grape powder as phenolic source in HD patient and, considering a total polyphenols dosage of 500 mg, our observations provide evidence that food-based antioxidants could be an appropriate therapeutic to combat the oxidative stress and avoid inflammation progression.

The oxidative stress is increased in HD patients and it has been reported to mediate inflammatory process.¹¹ To counter the destructive effects of the oxidants, there are endogenous antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and GSH-Px, which help to detoxify oxidative stress.¹²⁻¹⁴ Levels of GSH-Px and its redox forms in blood have been considered an index of the

whole-organism oxidative status and are a useful indicator of cardiovascular disease. Restoring the GSH levels by substituting the glutathione precursor N-acetylcysteine or overexpressing GSH-Px prevents cardiac dysfunction. Finally, a negative relationship between vascular endothelial function and GSH redox ratio was reported in CKD patients, and highlights the important role of this marker in the regulation of endothelial dysfunction in these patients.¹²

Polyphenols have been shown to increase serum antioxidant capacity due the induction of the nuclear factor E2-related factor 2 (Nrf2),¹⁵ a transcription factor responsible for both constitutive and inducible expression of antioxidant response element-regulated genes.¹⁶ In fact, in our study it was observed a significantly increase in activity of GSH-Px in patients that received grape powder as polyphenols source. Interestingly, the GSH-Px activity also increased in placebo group (although not statistically significantly) probably due the content of polyphenols present in grape jelly.

Regarding inflammatory markers, CRP levels were increased in placebo group but it was maintained in experimental group. Although an increase of only 0.2 mg/L in placebo group may not be clinically relevant, this result shows that grape powder supplementation can avoid the inflammation progression. In fact, Overman *et al.*¹⁷ observed that the extract of grape powder attenuates lipopolysaccharide (LPS) - mediated inflammation in macrophages, possibly decreasing the activation of inflammatory cytokines, chemokines and prostaglandins.

In HD patients, previous studies showed beneficial effects of polyphenols. After dietary supplementation with 100 ml concentrated red grape juice in 26 HD patients during 14 days, Castilla *et al.*⁷ observed an improvement in the lipoprotein profile (reduced Apo B-100 and increased Apo AI), reduced plasma concentrations of monocyte chemoattractant protein 1 (MCP-1) and oxidized LDL levels and increased of total antioxidant capacity. Additionally, comparing

TABLE 2 CHANGES IN OXIDATIVE STRESS AND INFLAMMATORY BIOMARKERS ACCORDING INTERVENTION

	Experimental Group		Placebo Group	
	Before	After	Before	After
GSH - Px activity (nmol/min/ml)	16.5 (41.0) ^a	42.0 (43.3) ^b	1.0 (3.46)	17.8 (50.3)
C-reactive protein (mg/dL)	2.6 (0.3)	2.7 (0.3)	2.6 (0.3)	2.8 (0.2) ^c

^a $p < 0.05$ between experimental and placebo groups before supplementation; ^b $p < 0.05$ experimental group before and after supplementation;

^c $p < 0.05$ placebo group before and after supplementation.

Figure 1. Glutathione peroxidase (GSH-Px) activity for each patient in experimental group before and after intervention.

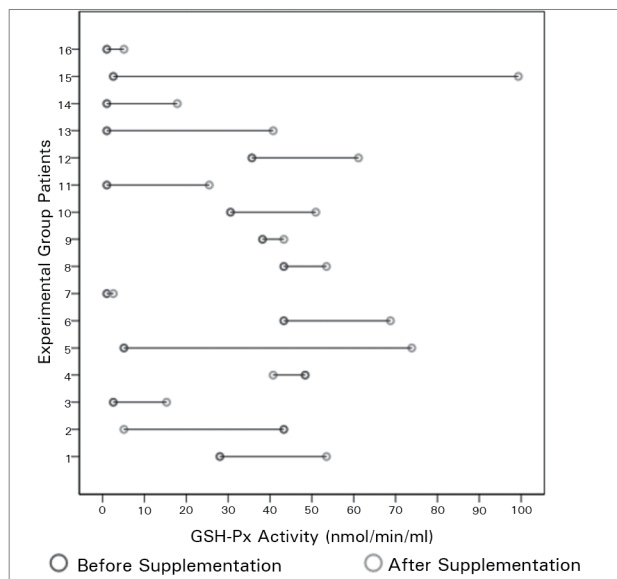
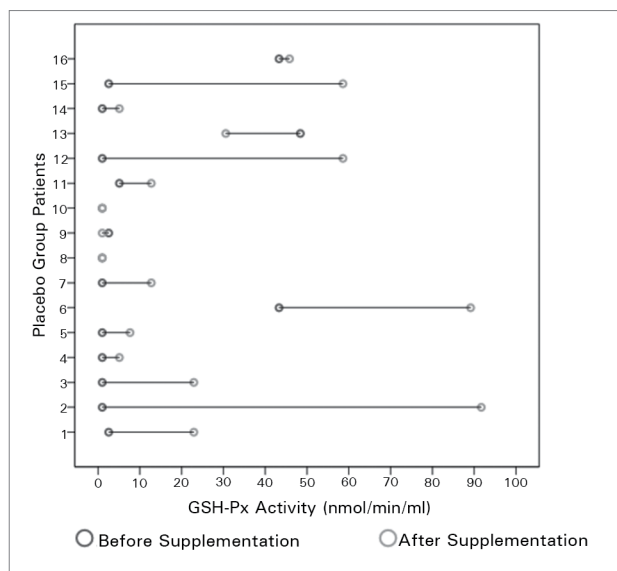


Figure 2. Glutathione peroxidase (GSH-Px) activity for each patient in placebo group before and after intervention.



dietary supplementation of grape juice and vitamin E in HD patients for 14 days, Castilla *et al.*² observed that only the juice was effective in reducing plasma concentrations of total cholesterol and Apo-B levels and increase HDL-cholesterol and both (juice and vitamin E) reduced plasma concentrations of oxidized LDL and NADPH oxidase activity in neutrophils. These effects are enhanced when the supplements were used in combination.

In addition, Spormann *et al.*⁶ observed a significant decreased of DNA oxidation damage, protein and lipid peroxidation and nuclear factor-kB binding activity, a transcription factor responsible for expression

of inflammatory genes, during the consumption of 200 ml per day of anthocyanin/polyphenolic-rich fruit juice. Moreover, the study reported an increase of glutathione level and status, similarly to that in our study. Thus, regular intake of polyphenols from juices and also grape powder could reduce the oxidative stress and, consequently, the cardiovascular disease risk in HD population.

The safety of grape powder supplementation has not been evaluated in subjects with impaired renal function. Although adverse health effects cannot be excluded after consumption of a high dosage of individual compounds taken as dietary supplements,¹⁸ they are unlikely to arise from the consumption of polyphenol-rich food.⁶ In fact, any adverse event was reported in a previous study conducted by Spormann *et al.*⁶ in which 200 ml of fruit juice contained 700 mg of total polyphenols was ingested for 21 HD patients during 4 weeks. In the present study, adverse events related to the grape powder supplementation were also not observed.

Our study has some limitations. With regards to inflammatory measures, the inclusion of the only PCR measurement at two time points is problematic. Adding more time points and additional markers of inflammation would provide a clearer picture of the inflammatory profile of HD patients. However, due logistical problems other multiple inflammatory as well as oxidative stress biomarkers could not be assessed. Furthermore, the analysis of phenolic content in grape jelly could explain the augment in GSH-Px activity also in placebo group.

CONCLUSION

In summary, this study showed for the first time, that the consumption of 500 mg/day of polyphenols from grape powder is effective to increase the activity of GSH-Px and avoid the inflammation progression in HD patients. Compared with fruit juices, use of grape powder as a source of polyphenols could be a good strategy for HD patients due fluid restrictions that these patients are subjected. Thus, grape powder supplementation could play an important role as an antioxidant and anti-inflammatory agent in HD patients and further studies should be designed to recommendations for the use of phenolic compounds in CKD patients.

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