



Antioxidative properties of 14-day supplementation with Yacon leaf extract in a hypercholesterolemic rat model

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ABSTRACT: (Antioxidative properties of 14-day supplementation with Yacon leaf extract in a hypercholesterolemic rat model). Yacon leaves are rich in phenolic compounds, which have antioxidant activity. The objective of this study was to evaluate the antioxidant effects of a Yacon leaf hydroalcoholic extract in hypercholesterolemic rats. Male Wistar rats were divided into seven groups: G1: normal diet (healthy) (0.9% NaCl); G2: hypercaloric diet (control) (0.9% NaCl); G3: oral suspension of 10 mg/kg simvastatin (SIM); G4: 20 mg/kg Yacon extract (YE20); G5: 40 mg/kg Yacon extract (YE40); G6: YE20 + SIM; and G7: YE40 + SIM. Lyophilized extracts were administered once daily by gavage for 14 consecutive days. Hematological, biochemical, and oxidative parameters were determined by classical methods. The groups that received the Yacon extract showed decreases in glucose and lipid levels. The extract significantly decreased serum levels of cardiac markers and reduced inflammation. Moreover, the extract reduced oxidative damage parameters and significantly increased antioxidant defenses. These results suggest that the lyophilized Yacon extract has significant antioxidant activity, possibly due to its high content of phenolic compounds.

Keywords: Yacon, leaves, antioxidant defenses, phenolic compounds.

RESUMO: (Propriedades antioxidantes da suplementação de 14 dias com extrato da folha do Yacon em um modelo de rato hipercolesterolêmico). As folhas do Yacon são ricas em compostos fenólicos, que têm atividade antioxidante. O objetivo deste estudo foi avaliar os efeitos antioxidantes do extrato hidroalcoólico da folha do Yacon em ratos hipercolesterolêmicos. Ratos Wistar machos foram divididos em sete grupos: G1: dieta normal (saudável) (NaCl 0,9%); G2: dieta hipercalórica (controle) (NaCl 0,9%); G3: suspensão oral de 10 mg/kg de sinvastatina (SIM); G4: 20 mg/kg de extrato Yacon (YE20); G5: 40 mg/kg de extrato Yacon (YE40); G6: YE20 + SIM; e G7: YE40 + SIM. Os extratos liofilizados foram administrados uma vez por dia por gavagem durante 14 dias consecutivos. Os parâmetros hematológicos, bioquímicos e oxidativo foram determinados por metodologias clássicas. Os grupos que receberam o extrato de Yacon apresentaram diminuição nos níveis de glicose e lipídios. A administração do extrato diminuiu significativamente os níveis séricos de marcadores cardíacos e reduziu a inflamação. Além disso, o extrato reduziu os parâmetros de dano oxidativo e aumentou significativamente as defesas antioxidantes. Estes resultados sugerem que o extrato do Yacon liofilizado tem uma atividade antioxidante significativa, possivelmente devido ao seu elevado teor de compostos fenólicos.

Palavras-chave: Yacon, folhas, defesas antioxidantes, compostos fenólicos.

INTRODUCTION

Oxidative stress is involved in the progression of several diseases, including cardiovascular disease (CVD) (Singh & Jialal 2006) and is the consequence of an imbalance in the redox status of an organism, with excessive production of free radicals (FR). FR are defined as any species containing one or more unpaired electrons in the outer molecular layer (Halliwell 2006). This unpaired electron makes the FR highly unstable and very reactive so that it may directly oxidize biomolecules (Valko *et al.* 2007).

The body, in turn, has an antioxidant defense system against reactive species. Antioxidants are molecules that protect against oxidative damage and include enzymes,

such as catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD), as well as nonenzymatic defenses, such as reduced glutathione (GSH), vitamin E, vitamin A, vitamin C, and polyphenols (Halliwell 2011).

The intake of natural antioxidants is associated with a decreased incidence of various diseases (Gomathi *et al.* 2012). In this context, Yacon (*Smallanthus sonchifolius*) has attracted interest for its medicinal properties. Yacon is a tuberous root that contains fructooligosaccharides as reserve carbohydrates, with β - (2 \rightarrow 1) linkages that resist hydrolysis by digestive enzymes in the colon and have bifidogenic activity (Campos, *et al.* 2012). Moreover, the beneficial properties of Yacon are due to the presence of

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phenolic compounds (Ojansivu *et al.* 2011) in the roots and leaves (Arnao *et al.* 2012).

Yacon leaves have antimicrobial (Lin *et al.* 2003), antifungal (Inoue *et al.* 1995), anti-hyperglycemic (Aybar *et al.* 2001), and antioxidant activities (Lachman *et al.* 2009) however, few studies have evaluate these effects *in vivo*. Thus, the objective of this study was to evaluate the hypolipidemic and antioxidant effects of a Yacon leaf hydroalcoholic extract in hypercholesterolemic Wistar rats.

MATERIAL AND METHODS

Chemicals

All the chemicals were from Sigma Chemical Co. (St. Louis, MO, USA) and of analytical grade. Solvents for ultra-performance liquid chromatography with diode array detection (UPLC-DAD) analysis were purchased from Tedia Company (OH, EUA).

Sample plant

The Yacon roots were provided by the Emater/RS, and cultivated in a property in Alegrete/RS. The leaves were duly transported fresh to Uruguaiana/RS, Brazil, where they were processed immediately.

Preparation of the extract

The hydroalcoholic Yacon leaves extract was obtained from 70% ethanol (v/v). The leaves were washed and dried at 37 °C for 5 hours and placed in contact with the solvent for 7 days in the dark, according to a slightly modified method previously described by Baroniet *al.* (2008). After the extract was filtered and concentrated under reduced pressure using a rotary evaporator at 40 °C, the remaining water portion was lyophilized. The lyophilized product was stored at -70 °C until further use.

Phytochemical analysis

UPLC-DAD was performed to evaluate the main components of the Yacon. The chemical reference substances used in this study were rutin hexahydrate, quercetin, kaempferol, gallic acid, gallocatechin, epicatechin, luteolin, gallic acid and chlorogenic acid (Sigma-Aldrich, St. Louis/EUA).

Animal Experimentation

For this study male Wistar rats (60-65 g), 30 days old, it were used. The rats were obtained from the Central Animal Laboratory of the Federal University of Santa Maria, Rio Grande do Sul, Brazil. During treatment, rats were housed at a constant room temperature, humidity, and light cycle (12:12h light-dark), with free access to tap water and fed with standard chow ad libitum.

Ethical issues

All experiments were conducted in compliance with the guidelines for biomedical research stated by the Brazilian Societies of Experimental Biology and approved by the Ethics Committee on Animal Use Experimentation of the Federal University of Pampa, CEUA, Uruguaiana, Rio Grande do Sul, Brazil (Protocol 034/2013).

Preparation of feed and induction of hypercholesterolemia

The rats had free access to water and a hypercholesterolemic diet for 30 days, as described by Fietz & Salgado (1999). After 30 days of induction, blood samples were collected (via puncture in the rat's tails), before the start of the experiments to confirm hypercholesterolemia, and then treatment with the extracts was initiated. After inducing hypercholesterolemia, the groups continued to receive the same diet until the end of the experiment.

Experimental Design

Forty-two rats were divided into 7 groups of 6 animals each: group 1 (normal diet - healthy) and group 2 (hypercaloric diet - control) received saline at a dose of 1 mL; group 3: oral suspension of 10 mg/kg simvastatin (SIM); group 4: 20 mg/kg Yacon extract (YE20); group 5: 40 mg/kg Yacon extract (YE40); group 6: YE20 + SIM; and group 7: YE40 + SIM. All treatments were administered daily by gavage. Only G1 received a normal diet, while the other groups received the hypercholesterolemic diet until the end of the experiment. All weights were recorded for each subject.

Yacon extract preparation and administration

The Yacon-based solution was obtained by the dissolution of the lyophilized extract in water. The solutions were prepared daily immediately before administration. The extracts were administered by gavage for 14 consecutive days. Animals were euthanized 24 h after the last treatment, while fasting, to obtain the whole blood.

Evaluation of antioxidant potential of Yacon extract *in vitro*

The antioxidant activity *in vitro* was determined by the classical method of DPPH (2,2-diphenyl-1-picrylhydrazyl) and the content of total polyphenols was measured in six different concentrations (0.05mg/mL, 0.10 mg/mL, 0.25 mg/mL, 0.50 mg/mL, 1.0 mg/mL and 2.0 mg/mL) of the extract.

The concentration of total polyphenols in the Yacon extract was measured spectrophotometrically using the Folin-Ciocalteu (Singleton *et al.* 1999) with modifications. Briefly, 125 µL of 1 N Folin-Ciocalteu reagent was added to a 125 µL of sample, and this mixture was allowed to stand for 6 min before the addition of 1.25 mL of 7% Na₂CO₃. The solution was then allowed to stand for 90 minutes before reading at 760 nm in Spectrophotometer (UV-1800 Shimadzu, Japan).

The standard curve of gallic acid was prepared in the same manner and total polyphenolic content was expressed in milligram of gallic acid equivalent per milliliter (mg GAE mL⁻¹). The equation obtained for standard curve of gallic acid in the range of 0.001 – 0.020 mg/mL was $y = 40.112x + 0.0581$ (R² = 0.9994).

The DPPH radical-scavenging activity of Yacon extract was determined as described by Sharma & Bhat

(2009). The DPPH scavenging capacity of the compound was calculated as:

$$\text{DPPH radical scavenging activity} = 100 - [(ABS_{\text{SAMPLE}} - ABS_{\text{BLANK}}) / ABS_{\text{CONTROL}}] \times 100$$

Where, ABS_{SAMPLE} is the absorbance of the test compound, ABS_{BLANK} is the absorbance of the blank and ABS_{CONTROL} is the absorbance of the control reaction. IC50 value (concentration of sample where absorbance of DPPH decreases 50% with respect to absorbance of blank) of the sample was determined. Ascorbic acid was used as the positive control.

Evaluation of Yacon extract in vivo

Biochemical and hematological analysis

The hemograms (complete blood count) were determined using an automated hematology analyzer Cell-Dyn 3200 (Abbott Diagnostic, Abbot Park, IL, USA). Total cholesterol, high-density lipoprotein cholesterol (HDL- cholesterol), triacylglycerol total, and glucose levels were measured using the automatic analyzer A-25 Biosystems (Biosystems SA, Barcelona, Spain) for *in vitro* diagnostics. Low-density lipoprotein cholesterol (LDL- cholesterol) values were computed according to the Friedewald formula.

Enzymatic markers for liver function (aspartate transaminase - AST, alanine transaminase - ALT), renal (creatinine, uric acid) and heart (creatinine kinase - CK, its isoform CK-MB and lactate dehydrogenase - LDH) were determined by automated equipment (A25 Biosystem) for *in vitro* diagnostics. Homocysteine levels were measured by high-performance liquid chromatography coupled to mass spectrometry (LC-MS/MS), according to Nelson *et al.* (2003). All biochemical assays were carried out in triplicate.

Oxidative damage

Oxidative parameters, lipid peroxidation (Ohkawa *et al.* 1979) and protein carbonyls (Levine 2002) in the plasma were measured using spectrophotometric methods. The assessment of DNA damage was made by the frequency of micronucleus (Schmid 1975) in leukocytes.

Antioxidants defenses

The levels of polyphenols (Singleton *et al.* 1999) and vitamin C (Jacques-Silva *et al.* 2001) in the plasma were quantified by spectrophotometry.

The levels of GSH (Akerboom & Sies 1981) the activity of SOD (kit RANSOD - RANDOX Brasil LTDA), CAT (Aebi 1984), and GPx (kit RANSEL - RANDOX Brasil LTDA) were determined in erythrocytes. All assays were carried out in triplicate.

Statistical Analysis

Data were expressed as mean \pm standard deviation (SD). Comparisons between groups were performed using two-way analysis of variance (ANOVA), followed by Bonferroni's multiple comparison test for *post hoc*

analysis. Results were considered statistically significant when $p < 0.05$. The statistical analysis was performed using GraphPad Prism (version 5.0, GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS AND DISCUSSION

Total polyphenol content and DPPH assay

Total phenolic contents in the Yacon leaf hydroalcoholic extract were 2.28–49.26 mg GAE/mL in a dose-dependent manner. The Yacon leaf extract inhibited DPPH radicals 18.15–86.84%.

A study by Arnao *et al.* (2011) showed that the IC50 values of a Yacon leaf hydroalcoholic extract for capturing DPPH radicals were 44.2–110.3 μg dry leaf/mL, and total phenolic content was 7.7–22.7 mg GAE/g.

These results suggest that Yacon leaf extracts have high antioxidant activity *in vitro*, which may be related to the large content of phenolic compounds.

Phytochemical analysis

The major peaks on the chromatogram were characteristic of flavonoids and phenolic acids; however, none of the compounds showed retention times or a spectral profile similar to the standards used. These results differ from those presented by Simonovska *et al.* (2003) and Valentová *et al.* (2003) who revealed the presence of caffeic acid, ferulic acid, chlorogenic acid, and protocatechuic acid, among others. Different extracts and/or origins of the plant material could explain these differences.

The flavonoid rutin was identified in the chromatogram obtained from the leaf extract. Mean rutin content in the leaf extract was approximately 0.81 μg /mL. Rutin is one of the most abundant polyphenolic compounds in the flavonoid class.

Effect of the Yacon leaf extract on the rat hematological profile

The hypercholesterolemic diet fed to the rats significantly increased ($p < 0.05$) leukocyte, platelet, and monocyte counts in the G2 control group. After 14 days of supplementation with the Yacon leaf extract, significant decreases in leukocyte and platelet counts were observed in all groups compared to those in the G2 and G1 control groups (Table 1).

A significant difference in monocyte count was detected in the G2 control group compared to that in the Yacon-supplemented groups. Administering the extract without simvastatin (SIM) (G4) resulted in a lower monocyte count than that in the healthy control group (G1).

Inflammation is highly associated with the development of atherosclerosis (Berbée *et al.* 2015) and platelets, which are stimulated in response to inflammation, are responsible for atherothrombotic events, which are both determinants of CVD (Rafieian-Kopaei *et al.* 2014) Monocytes are key cells in the formation of atherosclerotic plaques (McLaren *et al.* 2011). Thus, our results demonstrate that administering the Yacon

Table 1. Hematological parameters of the hypercholesterolemic rats exposed to different treatments. Data are expressed as means \pm SD.

| PARAMETERS | GROUPS | | | | | | |
|-------------------------------------|----------------|-----------------|----------------|---------------|---------------|---------------|---------------|
| | G1= HEALTHY | G2= CONTROL | G3= SIM | G4= YE20 | G5=YE40 | G6=YE20+SIM | G7=YE40+SIM |
| Erythrocytes ($10^6/\text{mm}^3$) | 8 \pm 0.4 | 6 \pm 0.5 | 7 \pm 0.8 | 6 \pm 0.4 | 6 \pm 0.4 | 6 \pm 0.4 | 6 \pm 0.3 |
| Hemoglobin (g/dL) | 15 \pm 0.4 | 14 \pm 0.6 | 13 \pm 1.5 | 14 \pm 0.5 | 14 \pm 0.5 | 14 \pm 0.4 | 14 \pm 0.6 |
| Hematocrit (%) | 50 \pm 1.3 | 39 \pm 1.0 | 40 \pm 4.2 | 39 \pm 1.2 | 39 \pm 0.9 | 39 \pm 0.9 | 39 \pm 1.4 |
| Leukocytes ($10^3/\text{mm}^3$) | 6 \pm 0.2 | 8 \pm 0.5* | 6 \pm 0.5 | 5 \pm 0.4 | 4 \pm 0.6 | 4 \pm 0.3 | 3 \pm 0.2 |
| Lymphocytes (%) | 69 \pm 2.5 | 67 \pm 10.5 | 69 \pm 7.2 | 6 \pm 9.9 | 63 \pm 10.5 | 63 \pm 5.5 | 59 \pm 9.8 |
| Neutrophils (%) | 26 \pm 1.5 | 26 \pm 6.2 | 25 \pm 7.1 | 34 \pm 10.1 | 25 \pm 9.3 | 31 \pm 6.1 | 31 \pm 10.8 |
| Monocytes (%) | 7 \pm 1.7 | 12 \pm 5.8* | 4 \pm 0.7 | 4 \pm 0.9 | 8 \pm 4.7 | 3 \pm 0.5 | 6 \pm 5.6 |
| Eosinophils (%) | 1 \pm 0.3 | 1 \pm 0.5 | 2 \pm 0.4 | 0.4 \pm 0.8 | 0.3 \pm 0.5 | 0.5 \pm 0.5 | 0.2 \pm 0.4 |
| Basophil (%) | 0 \pm 0 | 0 \pm 0 | 0 \pm 0 | 0 \pm 0 | 0 \pm 0 | 0 \pm 0 | 0 \pm 0 |
| Platelets ($10^3/\text{mm}^3$) | 392 \pm 23.1 | 531 \pm 12.5* | 400 \pm 18.2 | 318 \pm 5.1 | 306 \pm 7.0 | 307 \pm 6.1 | 295 \pm 2.7 |

Data are expressed as means \pm SD.* indicate statistically significant differences ($p < 0.05$) in relation to all other groups by two-way ANOVA followed by Bonferroni's comparison *pos hoc* test.

extract decreased the inflammatory process and the risk of developing atherosclerosis in hypercholesterolemic rats. These effects may be due to the high concentrations of flavonoids present in this plant. Studies have shown that flavonoids have important anti-inflammatory activities and prevent platelet aggregation (Nikfarjam *et al.* 2017). Choi *et al.* (2015) observed that rutin inhibited thrombus formation in mice, suggesting that rutin is a potent anti-thrombotic agent against CVD. Oliveira *et al.* (2013) reported a topical anti-inflammatory effect of Yacon leaves.

None of the other parameters evaluated were significant.

Lipid and glycemic profiles

Plasma glucose decreased significantly in all groups receiving the Yacon extract with or without SIM compared to that in the control group (G2). The glucose-lowering effect of the G5 (YE40) treatment was better than all other groups, showing an independent effect of SIM. Although this study used a lower extract dose and for a shorter period, these findings corroborate a study by Honoré *et al.* (2012) where administration of a Yacon leaf extract (dry extract, 70 mg/kg body weight) for 4 weeks significantly reduced hyperglycemia in rats with streptozotocin (STZ)-induced diabetes. Aybar *et al.* (2001) evaluated the hypoglycemic effect of an aqueous extract of Yacon leaves in normoglycemic rats transiently

induced to be hyperglycemic and STZ-induced diabetic rats. As results, administration of a 2% Yacon extract for 30 days produced a significant hypoglycemic effect in rats with STZ-induced diabetes.

An abnormal lipid profile is a major cardiovascular risk factor and is characterized by elevated levels of total cholesterol, triacylglycerol, and low-density lipoprotein (LDL)-cholesterol, and a decrease in high-density lipoprotein (HDL)-cholesterol (Plana *et al.* 2014).

Serum levels of total cholesterol decreased significantly ($p < 0.05$) in the G4, G5, G6, and G7 groups compared to that in the G2 group. Triacylglycerol and LDL-cholesterol levels also decreased significantly in the same groups compared to those in G2 and G1. A significant increase ($p < 0.05$) in HDL-cholesterol levels was observed in the G4, G5, G6, and G7 groups compared to that in G2 control group, and these levels were significantly higher in groups that received SIM (G6 and G7) compared to that in the healthy control group (G1). These results demonstrate that administration of a Yacon leaf extract to hypercholesterolemic rats improved their lipid profile, suggesting a possible protective effect on the progression of CVD.

Miura *et al.* (2004) reported that administering an aqueous extract of Yacon leaves (500 mg/kg) for 6 weeks reduces hyperglycemia and hyperlipidemia in type 2 diabetic mice.

Table 2. Biochemical parameters of hypercholesterolemic rats treated with Yacon leaf extract. Data were expressed as mean \pm SD.

| PARAMETERS | GROUPS | | | | | | |
|---------------------------|-----------------|-------------------------------|---------------------------------|-----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| | G1= HEALTHY | G2= CONTROL | G3= SIM | G4= YE20 | G5=YE40 | G6=YE20+SIM | G7=YE40+SIM |
| Glucose (mg/dL) | 93.0 \pm 3.3 | 152.0 \pm 17.6 ^a | 130.0 \pm 8.4 ^{a,b} | 118.2 \pm 17.7 ^{a,b} | 101.0 \pm 16.4 ^{b,c} | 115.0 \pm 14.9 ^{a,b} | 113.9 \pm 25.9 ^{a,b} |
| Total cholesterol (mg/dL) | 174.0 \pm 6.8 | 207.0 \pm 5.0 ^a | 137.8 \pm 4.2 ^{a,b} | 188.2 \pm 10.6 ^{a,b,c} | 184.5 \pm 4.4 ^{a,b,c} | 166.8 \pm 7.5 ^{a,b,c} | 149.9 \pm 5.2 ^{a,b,c} |
| Triglycerides (mg/dL) | 308.0 \pm 2.9 | 327.0 \pm 4.1 ^a | 213 \pm 19.6 ^{a,b} | 312.0 \pm 3.8 ^{b,c} | 307.8 \pm 4.1 ^{b,c} | 305.5 \pm 5.1 ^{b,c} | 305.0 \pm 3.5 ^{b,c} |
| LDL-cholesterol (mg/dL) | 158.0 \pm 3.0 | 172.9 \pm 5.9 ^a | 125.9 \pm 11.5 ^{a,b} | 163.8 \pm 6.4 ^{b,c} | 159.2 \pm 4.0 ^{b,c} | 157.8 \pm 6.1 ^{b,c} | 153.4 \pm 8.3 ^{b,c} |
| HDL-cholesterol (mg/dL) | 38.6 \pm 2.4 | 27.8 \pm 2.7 ^a | 30.8 \pm 2.8 ^{a,b} | 34.1 \pm 2.3 ^{a,b} | 38.7 \pm 2.0 ^{b,c} | 44.3 \pm 3.0 ^{b,c} | 50.6 \pm 1.4 ^{b,c} |
| AST (U/L) | 352.0 \pm 1.7 | 351.3 \pm 6.0 | 350.0 \pm 1.7 | 353.5 \pm 7.4 | 350.5 \pm 5.7 | 353.5 \pm 6.0 | 354.2 \pm 5.6 |
| ALT (U/L) | 55.0 \pm 1.7 | 53.0 \pm 3.4 | 51.4 \pm 1.8 | 52.7 \pm 4.0 | 52.3 \pm 2.9 | 51.7 \pm 2.5 | 53.4 \pm 3.3 |
| Urea (mg/dL) | 53.7 \pm 0.6 | 50.8 \pm 2.5 | 52.0 \pm 1.7 | 51.6 \pm 2.1 | 50.8 \pm 1.8 | 50.8 \pm 2.1 | 50.9 \pm 1.8 |
| Creatinine (mg/dL) | 0.70 \pm 0.1 | 0.66 \pm 0.1 | 0.64 \pm 0.1 | 0.67 \pm 0.1 | 0.67 \pm 0.1 | 0.65 \pm 0.1 | 0.67 \pm 0.1 |

(Two-way ANOVA followed by Bonferroni Test). Data were expressed as mean \pm SD. a: Significantly different from Group 1; b: Significantly different from Group 2; c: Significantly different from Group 3. $P < 0.05$ was considered significant.

The serum hepatic and renal biochemical markers (Table 2) were within normal limits in all groups, demonstrating that the extract is not nephrotoxic or hepatotoxic at the administered doses.

Markers of cardiac damage

Creatine kinase (CK) and its isoform CK-MB are used as markers of heart disease (Ghomarde *et al.* 2014). Homocysteine is a sulfhydryl amino acid formed from methionine in the liver. Homocysteinemia has been associated with an increased risk of atherothrombotic events (Kalra 2004). Lactate dehydrogenase (LDH) is a key enzyme in carbohydrate metabolism that is found in many tissues. Serum levels of LDH increase in a variety of disorders, particularly cardiac and hepatic

diseases (Huijgen *et al.* 1997) The hypercholesterolemic diet induced an increase in the levels of CK, CK-MB, homocysteine, and LDH in the G2 group (Fig. 1A–D), whereas the groups receiving the Yacon extract showed decreased ($p < 0.05$) levels of these markers when compared to G2 and lower or similar levels compared to G1. These results indicate that the extract reversed the damage caused by the hypercholesterolemic diet, suggesting a cardioprotective effect by the extract. These effects may be attributable to rutin and other phenolic compounds, as several studies have demonstrated a cardioprotective effect of these compounds (Annapurna *et al.* 2009, Li *et al.* 2013, Saklaniet *et al.* 2016, Wang *et al.* 2017).

Oxidative damage

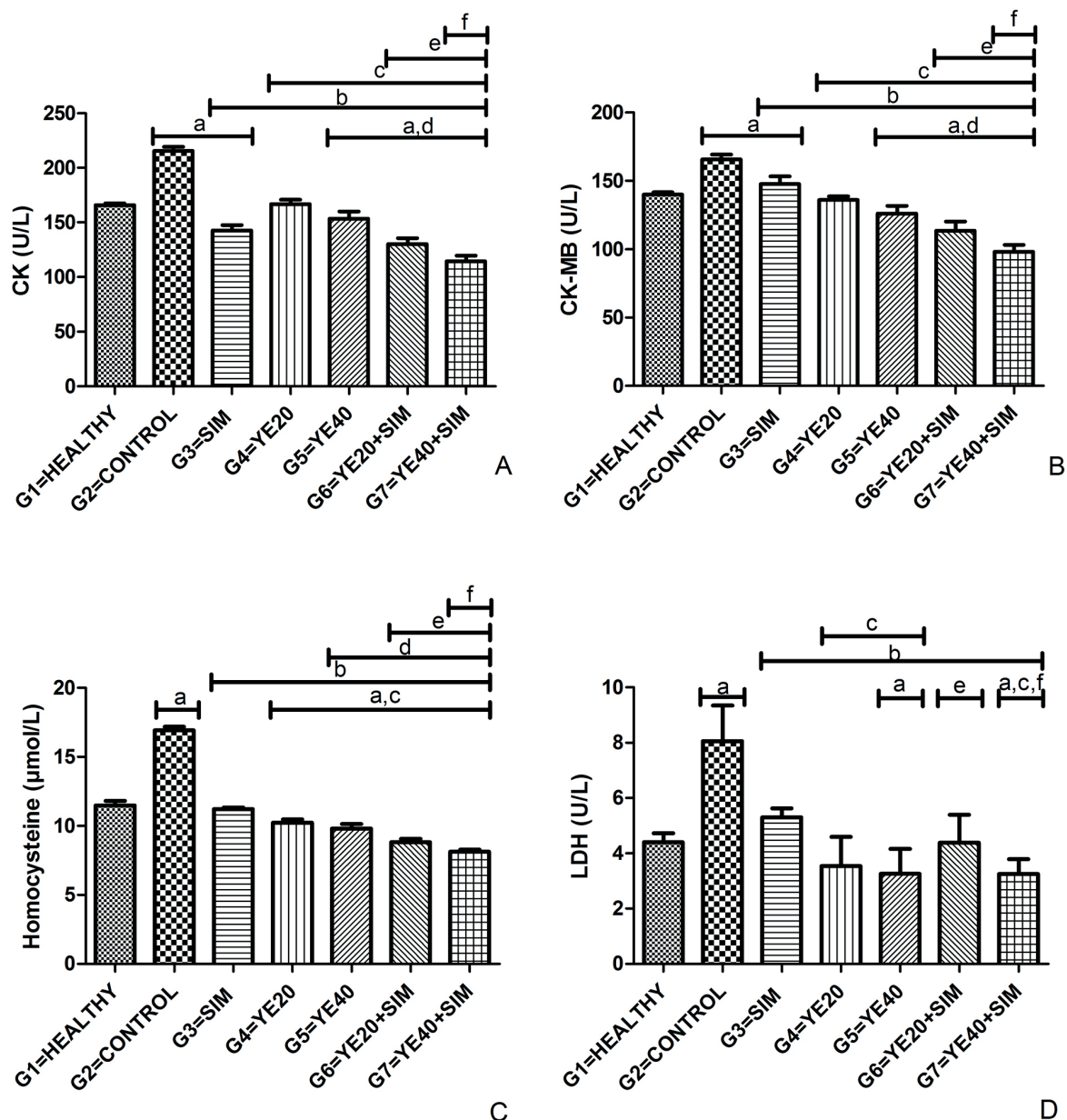


Figure 1. Markers of cardiac damage in the hypercholesterolemic rats exposed to different treatments. A. CK-Total. B. CK-MB. C. Homocysteine. D. LDH. Comparisons were made as a: Significantly different from Group 1; b: Significantly different from Group 2; c: Significantly different from Group 3; d: Significantly different from Group 4; e: Significantly different from Group 5; f: Significantly different from Group 6. $P < 0.05$ was considered significant.

FRs directly oxidize lipids (lipid peroxidation), proteins (carbonylation and/or nitration), and genetic material (DNA) (oxidation of nitrogenous bases) (Halliwell 2006). Lipid peroxidation is a complex process that involves the interaction of reactive oxygen species with polyunsaturated fatty acids, resulting in a wide variety of highly reactive electrophiles aldehydes and malondialdehyde (MDA), the main product (Halliwell & Chirico 1993). The control group (G2) had high levels of MDA (Fig. 2A). However, the groups treated with the Yacon extract showed significant decreases in plasma MDA levels ($p < 0.05$) compared to that in the control group (G2). The decrease in plasma MDA level indicates reduced lipid peroxidation. The groups supplemented with the Yacon extract also showed a significant reduction ($p < 0.05$) in protein carbonyls and frequency of micronuclei compared to those in G2 (Fig. 2B, C). Administering the Yacon extract (G4) resulted better protein carbonylation than that in the groups given SIM. These results show that supplementation with Yacon reduced oxidative damage of the biomolecules induced by a hypercholesterolemic diet, showing a promising future in the prevention or treatment of diseases associated with oxidative stress.

Antioxidants defenses

The enzymatic (SOD, CAT, and GPx) and non-enzymatic (vitamin C, GSH, and polyphenols) antioxidant defense biomarker results are shown in Figure 3A–F.

G2 demonstrated low antioxidant enzyme activities and reduced levels of vitamin C, GSH, and polyphenols. Enzyme activities increased significantly ($p < 0.05$) (SOD, CAT, and GPx) after 14 days of Yacon supplementation compared to those in G2, and GPx levels were similar to those in G1. The combination of the extract with SIM (G5 and G7) significantly increased CAT activity, suggesting its concomitant use in the clinic; however, more studies are necessary to confirm this result. The levels of GSH, vitamin C, and polyphenols were significantly higher in the extract-supplemented groups compared to those in G2. The polyphenol levels in the groups supplemented with the highest dose of Yacon (G5 and G7) with or without SIM were similar to those of G1. These results show that Yacon increased antioxidant activities in hypercholesterolemic rats, possibly due to the presence of phenolic compounds, such as rutin. Sun *et al.* (2017) reported significant increases in the levels of these antioxidants in rats with oxidation-induced damage treated with rutin.

The continuous production of reactive species by metabolic processes induces various antioxidant defense mechanisms to protect biomolecules from oxidative damage (Sies 1993). Flavonoids are powerful antioxidants that inhibit a wide range of reactive species (Halliwell 2008). Studies strongly suggest a contribution of polyphenols to the prevention of various diseases, among them is the ability to inhibit LDL oxidation (Amarowicz & Pegg 2017).

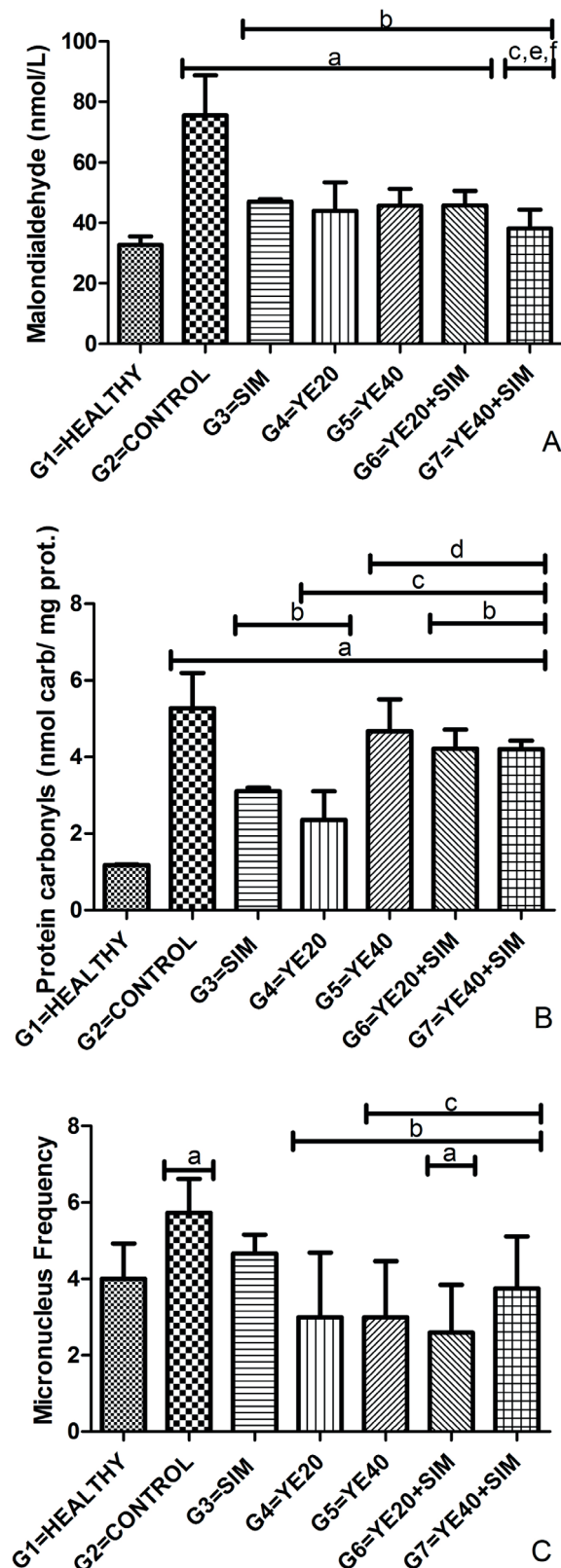


Figure 2. Biomarkers of oxidative damage in hypercholesterolemic rats after treatment of Yacon extract. A. Lipid peroxidation levels. B. Carbonyl protein contents. C. Frequency of micronucleus. Comparisons were made as a: Significantly different from Group 1; b: Significantly different from Group 2; c: Significantly different from Group 3; d: Significantly different from Group 4; e: Significantly different from Group 5; f: Significantly different from Group 6. $P < 0.05$ was considered significant.

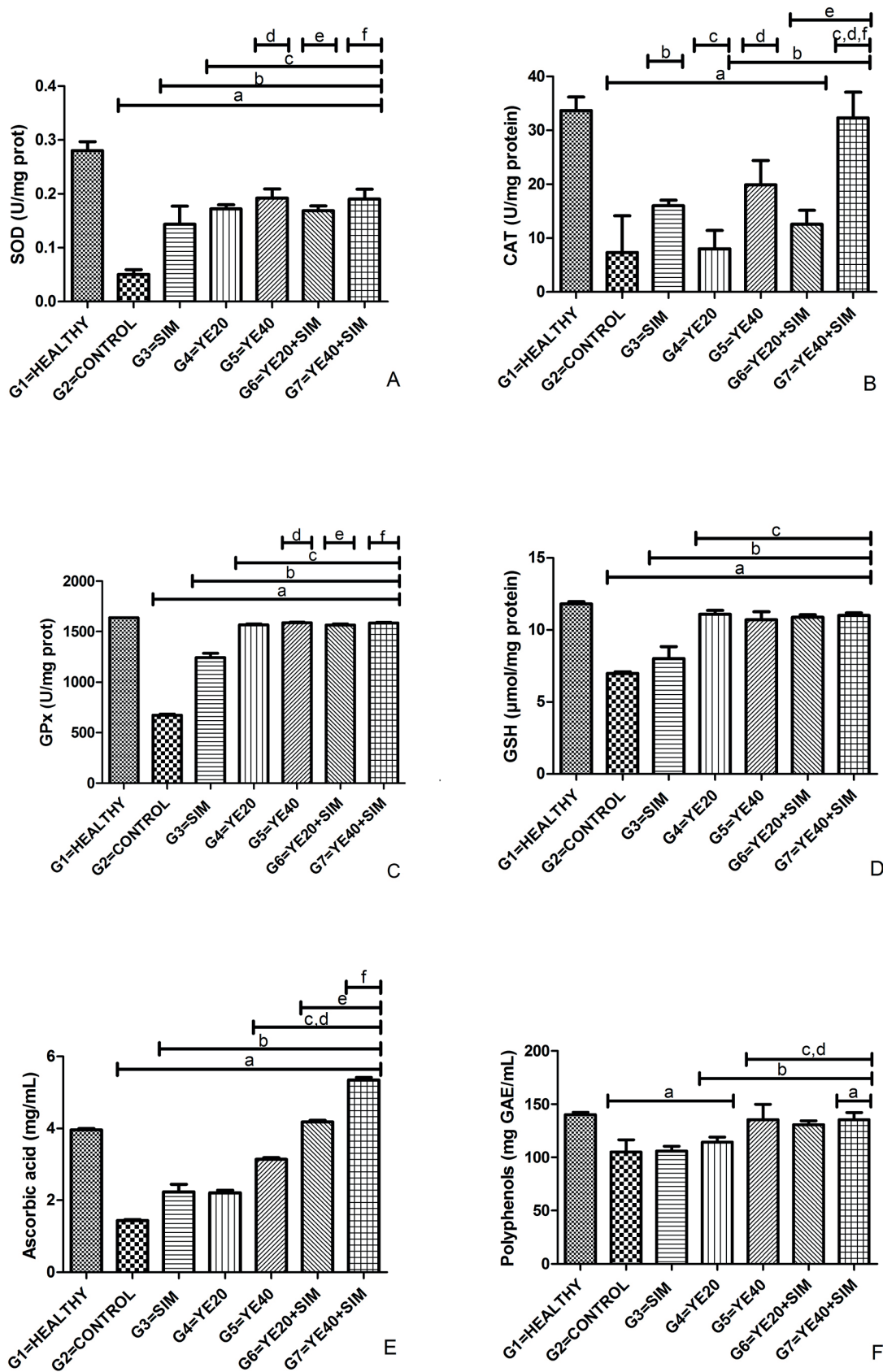


Figure 3. Antioxidant defenses markers in hypercholesterolemic rats after treatment of Yacon extract. A. SOD activity. B. CAT activity. C. GPx activity. D. GSH levels. E. Ascorbic acid contents. F. Polyphenols contents. Comparisons were made as a: Significantly different from Group 1; b: Significantly different from Group 2; c: Significantly different from Group 3; d: Significantly different from Group 4; e: Significantly different from Group 5; f: Significantly different from Group 6. P<0.05 was considered significant.

CONCLUSION

Administration of a Yacon leaf extract to hypercholesterolemic rats reduced oxidative damage to lipids, proteins, and DNA and enhanced antioxidant defenses. The extract also showed high antioxidant activity *in vitro*, possibly due to its high polyphenol contents, including rutin, which may be the main metabolite responsible for the positive effects of this plant.

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The authors declare that they have no competing interests.

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