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Antioxidant potential of dietary chia seed and oil (*Salvia hispanica* L.) in diet-induced obese rats

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ABSTRACT

This study aimed to investigate the effects of dietary chia seed and oil on plasma and liver oxidative status in diet-induced obese rats. Thirty-six *Wistar* rats were divided in six groups (6 animals each): control group was fed the American Institute of Nutrition (AIN)-93 M diet; HFF group was fed a high-fat and high-fructose (HFF) diet; chia seed short (6-weeks) and long (12-weeks) treatments received an HFF diet with chia seed; chia oil short (6-weeks) and long (12-weeks) treatments received an HFF diet with chia oil. Plasma and hepatic biomarkers of lipid peroxidation, endogenous enzymatic and non-enzymatic antioxidant systems and antioxidant capacity were determined. HFF diet induced weight gain, oxidative stress and lipid peroxidation in plasma and liver of animals. Compared to HFF group chia seed and chia oil (12 and 6 weeks) intake increased plasma reduced thiol (GSH) levels, plasma catalase (CAT) and glutathione peroxidase (GPx) activities. In the liver glutathione reductase (GRd) activity was enhanced, while CAT and GPx activities did not change. There were no differences in plasma and liver superoxide dismutase activity among chia diets and HFF group. Chia (seed and oil) intake did not modify liver lipid peroxidation, but was able to reduce plasma thiobarbituric acid reactive substances (TBARS) and 8-isoprostane levels increased by HFF group. Plasma and hepatic antioxidant capacity values were increased in chia seed and oil groups about 35% and 47%, respectively, compared to HFF group. Chia groups presented similar antioxidant potential, regardless of treatment time. Dietary chia seed and oil reduced oxidative stress *in vivo*, since it improved antioxidant status and reduced lipid peroxidation in diet-induced obese rats.

1. Introduction

Obesity has become a worldwide epidemic and may be a state of chronic oxidative stress, which consists in an imbalance between protector endogenous antioxidant systems and free radicals generation (Fernández-Sánchez et al., 2011). The reactive oxygen species (ROS) excess can damage cell proteins, lipids and DNA, which might result in loss of function and even cellular death. This mechanism has been linked to many diseases, such as cardiovascular disease, neurodegeneration, cancer and diabetes (Brambilla et al., 2008; Pandey & Rizv, 2010). Furthermore, obesity impairs the enzymatic antioxidant system, with reduction of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GRd) activities, and also affects the non-enzymatic antioxidant systems (reduced thiol, minerals, vitamins and polyphenols) which play essential roles in many antioxidant mechanisms (Brambilla et al., 2008; Fernández-Sánchez et al., 2011). Many investigations have shown that consumption of natural dietary sources (fruits, nuts, vegetables) with antioxidant bioactive compounds (polyphenols, tocopherols, carotenoids, vitamins) may support the prevention of oxidative stress and could be a natural alternative for preventing and controlling chronic diseases (Avignon, Hokayem, Bisbal, & Lambert, 2012; Bullo, Lamuela-Raventos, & Salas-Salvado, 2011; Landete, 2012). Several mechanisms have been proposed for this health protection, such as the improvement of antioxidant defense system which protects and reduces ROS damage in the organism (Avignon et al., 2012; Brambilla et al., 2008). Therefore, the evaluation of potential functional foods that may improve antioxidant systems efficacy or alleviate reactive oxygen and nitrogen species formation could be a strategy to prevent obesity complications.

In this context, chia (*Salvia hispanica* L.) is an herbaceous plant that belongs to *Lamiaceae* family native from southern Mexico and northern Guatemala (Ayerza, 1995). Today it is commercially available for human consumption as whole seeds, flour, and oil in the Americas, Australia, Europe and Southeast Asia (Mohd Ali et al., 2012). Chia seed is the richest known botanical source of omega-3 α-linolenic acid (C18:3,

Abbreviations: AIN, American Institute of Nutrition; ALA, alpha-linolenic acid; CAT, catalase; FAME, fatty acids methyl esters; FRAP, ferric reducing antioxidant power; GC, gas chromatography; GPx, glutathione peroxidase; GRd, glutathione reductase; GSH, reduced thiol; HFF, high-fat and high-fructose diet; LDL, low-density lipoprotein; MDA, malondialdehyde; PB, phosphate buffer; ROS, reactive oxygen species; SOD, superoxide dismutase; TBA, 2-thiobarbituric acid; TBARS, thiobarbituric acid reactive substances; TCA, trichloroacetic acid.

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ALA, up to 68%) (Ayerza, 1995) compared to flaxseed (50.6%), rapeseed (8.1%), soybean (7.6%) and sunflower (1.8%) oils (Tuberoso, Kowalczyk, Sarritzu, & Cabras, 2007); and has been also described as an important source of protein, dietary fiber, minerals and bioactive compounds (Marineli et al., 2014; Reyes-Caudillo, Tecante, & Valdivia-López, 2008). Literature data indicates that chia seed and oil possess high antioxidant potential confirmed by different *in vitro* assays (Marineli et al., 2014; Martínez-Cruz & Paredes-López, 2014; Reyes-Caudillo et al., 2008). Chia seed and chia oil are considered new sources of natural antioxidants, due to the content of tocopherols, phytosterols, carotenoids (Álvarez-Chávez, Valdivia-López, Aburto-Juárez, & Tecante, 2008; Ixtaina et al., 2011) and phenolic compounds (Martínez-Cruz & Paredes-López, 2014; Reyes-Caudillo et al., 2008), which have the potential to protect consumers against many diseases and also promotes beneficial effects on human health (Avignon et al., 2012; Landete, 2012). Chia lipids could be an omega-3 alternative source to vegetarians and people with fish allergies, since chia seed and oil have not shown problems associated with other nutritional sources such as flaxseed and marine products, including fish flavor, animal weight loss and digestive problems (Mohd Ali et al., 2012).

Our previous study Marineli et al. (2015) reported that chia ingestion induced heat shock protein expression and restored SOD and GPx expression in skeletal muscle, with a higher potential of oil relative to seed. However, chia seed and oil has been little explored from a scientific point of view, especially in relation to its antioxidant potential *in vivo*. To the best of our knowledge, this is the first report about antioxidant effects in liver and plasma after chia seed and oil intake. We hypothesized that dietary chia seed and chia oil could protect against oxidative stress by improving antioxidant defenses and reducing lipid peroxidation in diet-induced obese rats, due to the content of bioactive compounds. Therefore, the present study aimed to evaluate the effects of dietary chia seed and chia oil on oxidative stress and antioxidant biomarkers of plasma and liver in diet-induced obese *Wistar* rats.

2. Materials and methods

2.1. Chemical, chia seed and oil

Solvents were all analytical grade from J.T. Baker (Phillipsburg, NJ, USA). Metaphosphoric acid was purchased from Vetec Química Fina (Sao Paulo, Brazil). L-Glutathione reduced, glutathione reductase, glutathione oxidized form disodium salt, β-nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt hydrate (NADPH), 5′5′-dithio-bis-2-nitrobenzoic acid (DTNB), albumin from bovine serum (BSA), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,4,6- tris(2-pyridyl)-s-triazine (TPTZ), 2-thiobarbituric acid (TBA) were all obtained from Sigma-Aldrich (St. Louis, MO, USA). Trichloroacetic acid (TCA) was purchased from Synth Laboratorios (Sao Paulo, Brazil), Bradford reagent was purchased from BioAgency (Sao Paulo, Brazil) and malondialdehyde (MDA) was purchased from Cayman Chemical Company (Ann Arbor, MI, USA). Fresh chia seed and chia oil from FTP S.A. Santiago, Chile were purchased from R&S Blumos Comercial de Produtos Alimentícios Ltda., Brazil. According to the producer, chia oil was obtained by cold pressing (<40 °C) and stored at 2–8 °C until use in amber glass bottles without head space. Seeds were ground in a laboratory impact mill (Marconi MA 630/1, Piracicaba — Sao Paulo, Brazil) and passed through a 0.850 mm sieve. Chemical analysis and antioxidant activity of Chilean chia seed and oil samples used in this study were previously determined by Marineli et al. (2014) in the same raw material.

2.2. Animals, diets and interventions

This work was approved by the Ethics Commission on Animal Use (CEUA/UNICAMP, protocol no. 2936–1) and followed the University guidelines for the use of animals in experimental studies. Thirty-six male *Wistar* rats aged 21 to 23 days were obtained from Multidisciplinary Center for Biological Investigation, University of Campinas. Animals remained in individual cages for growth with free access to water and chow diet for 4 weeks and were maintained under controlled conditions (22 ± 1 °C, 60–70% humidity, 12 h light/dark cycle). After growth, animals (234.08 g ± 19.12) were randomly divided in six experimental groups (n = 6/group) for 12 weeks.

Diets were based on the American Institute of Nutrition (AIN)-93 M diet (Reeves, Nielsen, & Fahey, 1993) with protein concentration of 12%. Control group received the standard diet (AIN-93 M); high-fat and high-fructose (HFF) group received a diet containing 4% (w/w) soybean oil, 31% (w/w) lard and 20% fructose (w/w) (Shapiro, Tumer, Gao, Cheng, & Scarpace, 2011); chia seed short and long treatments received an HFF diet with 13.3% (w/w) of chia seed; chia oil short and long treatments received an HFF diet with 4% (w/w) of chia oil. There were a long (12 weeks) and a short (6 weeks) treatment with chia seed or chia oil. Animals from the long treatment were fed only an HFF diet containing chia seed or chia oil for 12 weeks. Short groups were initially fed only an HFF diet for the first 6 weeks, followed by an additional 6 weeks with an HFF diet containing chia seed or chia oil. Soybean oil for both chia seed and oil diet was replaced by the oil content of chia seed or chia oil. Chia diets were formulated to provide equal quantities of oil and ALA from chia (Ayerza & Coates, 2007). The seeds contained 30.2% of oil (Marineli et al., 2014). Protein and dietary fiber content in HFF-chia seed groups was balanced taking into account the amount of such nutrients present in chia seed (Marineli et al., 2014). Diets composition is presented in Table 1. Diets were prepared monthly and packed in dark polyethylene bags and stored at −20 °C to minimize the oxidation of fatty acids. Weight gain was monitored weekly and food intake every 2 days.

2.2.1. Diet analyses

Calorie values of diets were determined using isoperibol automatic calorimeter (PARR 1261) instrument equipped with oxygen bomb

Table 1
Ingredients and fatty acid composition of experimental diets.

	AIN-93 M	HFF	Chia seed	Chia oil
Ingredients (g/kg diet)				
Casein[a]	139.53	139.53	106.53	139.53
Maltodextrin	155.0	45.40	45.40	45.40
Corn starch	465.69	136.44	123.44	136.44
Sucrose	100.0	29.32	29.32	29.32
Soybean oil	40	40	–	–
Chia oil	–	–	–	40
Chia seed	–	–	133	–
Lard	–	310	310	310
Fructose	–	200	200	200
Cellulose	50	50	3.6	50
Mineral mixture	35	35	35	35
Vitamin mixture	10	10	10	10
L-Cystine	1.8	1.8	1.8	1.8
Choline bitartrate	2.5	2.5	2.5	2.5
tert-Butyl hydroquinone	0.008	0.008	0.008	0.008
Energy density (kcal/g diet)	3.74	5.89	5.84	5.88
Fatty acids[b]				
SFA	7.38	127.86	125.35	125.99
MUFA	10.20	146.94	138.87	140.11
PUFA	22.42	71.10	81.11	79.54
Linoleic (C18:2n−6)	19.89	66.74	54.97	53.53
α-Linolenic (C18:3n−3)	2.20	4.06	25.88	25.73
n−6/n−3 ratio	9.04	16.44	2.12	2.08

AIN-93 M group: control standard diet; HFF group: high-fat and high-fructose diet; Chia seed groups: HFF diet plus 13.3% (w/w) chia seed; Chia oil groups: HFF diet plus 4% (w/w) chia oil. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

[a] Amount was calculated based on protein content equal to 86% to provide 12 g of protein/100 g of diet.

[b] Fatty acids expressed as g/kg diet.