Effect Of The Use Of Chia (Salvia Hispanica L.) Seeds On Antioxidant Status And Anthropometric Parameters In Obese, Type 2 Diabetics And/Or Hypertensive Patients

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ABSTRACT
Background and aims: Chia seeds have been explored owing to its dense concentrations of antioxidant and anti-inflammatory compounds, as well as recognized benefit on anthropometric measures. This study evaluated the potential effect of Chia intake on plasma antioxidant status and anthropometric parameters in individuals with chronic diseases. Methods: Fifty-nine participants were selected, including patients with Type 2 Diabetes Mellitus (T2DM) and/or hypertension (SAH) and/or obesity (group A, n=34) and healthy volunteers (group B, n=25). Plasma antioxidant status was determined by the MTT (tetrazolium salt) reduction test and weight (W), abdominal circumference (AC), waist circumference (WC) and body mass index (BMI), that were determined before (T0) and after 6 months (T6) of ingestion of 20 g/day of Chia seeds. Results: After Chia seeds consumption, the plasma antioxidant potential increased in both group A and group B (p < 0.0001). It was also observed a reduction in W, AC, WC and BMI in both groups, with all p-values being less than 0.05. High content of antioxidant compounds may explain these effects and polysaccharide gel formation in the digestive tract, which gives satiety and consequently provides a reduction in anthropometric measurements. Conclusion: In the field of nutrition, the chia seed can be a potential alternative to increase antioxidant status and reduce anthropometric measurements, improving the health status of patients, especially those who suffer from chronic disease.

Keywords: Salvia hispanica L, Antioxidant status, Type 2 Diabetes mellitus, Obesity, Anthropometric measurements.
1 INTRODUCTION

Chia is an herbaceous species and belongs to the Lamiaceae family (Ayaz et al., 2017). Since its discovery by ancient Central American civilizations, it has been cited as a food and medicinal source and more recently has become the subject of researches because of its varied chemical composition that gives potential health benefits of those who consume it (Marcinek et al., 2017; Coelho & Salas-Mellado, 2014).

Chia seeds have high concentration of protein, lipids, soluble and insoluble dietary fibers, which help intestinal transit regulation, lowering cholesterol and triglyceride levels and maintaining good glycemic control, contributing to weight loss and reducing risk factors related to chronic diseases such as obesity and Type 2 Diabetes Mellitus (T2DM) (Malta et al., 2014; Ullah et al., 2016; Oliveira-Alves et al., 2017; Ixtaina et al., 2011). Chia seeds consumption has also shown an important function in the prevention of cardiovascular disease due to its antithrombotic, anti-inflammatory and antiarrhythmic properties (Ixtaina et al., 2011; Chicco et al., 2009; Valdivia-López et al., 2009; Medina et al., 2007).

Non-communicable chronic diseases (NCDs) such as T2DM, systemic arterial hypertension (SAH) and obesity are responsible for about 70% of all deaths worldwide, approximately 38 million deaths each year (WHO, 2020). These pathologies contribute to the development of a pro-inflammatory state, with increased production of free radicals and decreased antioxidant capacity. In this context, environmental factors, poor eating habits and physical inactivity contribute to the development and progression of these diseases (WHO, 2020; Kahn et al., 2014; Medina et al., 2007). Therefore, a change in diet with addition of substances that can prevent or control health problems could be a good option.

In this way, Chia seeds, with its potential antioxidant and anti-inflammatory properties, appear as an alternative to promote beneficial health effects. Thus, this research aimed to evaluate the antioxidant status of plasma and anthropometric parameters of patients with NCDs related to a pro-inflammatory profile such as T2DM, SAH and obesity, in addition to a healthy control group before and after the use of Chia seeds (Marcinek et al., 2017; Suri et al., 2016; Lorber, 2014; Liu, 2013).

2 METHODOLOGY

2.1 STUDY DESIGN

This prospective comparative study was approved by the Ethics Committee at the Federal University of Minas Gerais (UFMG), Brazil (CAAE: 65114917.6.0000.5149) and was followed in accordance with the Declaration of Helsinki. A total of 59 individuals from the Secondary Health Care Unit of Belo Horizonte Town Hall (group A) and UFMG (group B) were selected. Samples were obtained between March 2017 and August 2018. The research protocol did not interfere with any medical conduct or prescription. All individuals were informed about the stages of the study and signed the free and informed consent form.

The intervention was carried out in both groups and 20 grams of chia seeds were provided daily for 6 months to each participant. The amount of chia seeds varied between 7 to 30 grams per day (Ferreira et
The variables of the antioxidant status of plasma and anthropometric parameters presented in the study were measured before (T0 - before the introduction of Chia seeds) and after the intervention (T6 - 6 months after the introduction of Chia seeds). Fifty-nine participants were allocated between two intervention groups: group A (n=34) which included patients who were previously diagnosed with at least one of these NCDs (T2DM, SAH and obesity), and group B (n=25), composed by healthy volunteers. Both groups were matched for sex and age.

2.2 INCLUSION AND EXCLUSION CRITERIA

In both groups the participants were adults (aged 18-65 years) of both genders. Group A included patients with a BMI $\geq 30$ kg/m$^2$, previous diagnosis of at least one of these NCDs (T2DM, SAH and obesity) and preserved digestive tract (Abeso, 2018). The exclusion criteria of group A were surgeries for partial or total removal of the digestive tract, diagnosis or history of coagulopathies, use of anticoagulants, presence of other concomitant diseases, such as neoplasms, human immunodeficiency virus (HIV) and neurodegenerative disease.

We considered inclusion criteria of group B, BMI between 18.5 and 24.9 kg/m$^2$, non-regular use of medication, no clinical history of chronic diseases, nonsmokers and no alcoholics, no use of food supplements and digestive tract preserved. The exclusion criteria were previous medical diagnosis of any diseases and chronic use of anti-inflammatory drugs.

2.3 BODY COMPOSITION

At the beginning (T0) and at the end (T6) of the follow-up, some parameters were determined in all participants: weight (kg), height (m), abdominal (cm) and waist circumferences (cm). Weight and height were measured on a calibrated scale with a Marte® with branded stadiometer, a maximum load of 200 kilograms and a sensitivity of 50 grams, as standardized by WHO/FAO (Kahn et al., 2014). The circumferences were determined with inelastic tape measure. All measures were determined with patients barefoot and wearing light clothing. From weight and height measurements, BMI was determined by the ratio of weight to height squared and classified according to existing cutoff points (Wiltgen et al., 2009).

2.4 BLOOD SAMPLES

Venous blood samples were obtained after fasting of at least 8 hours and up to 12 hours, in vacuum tubes containing branded serum separation and EDTA anticoagulant (VACUETTE®). Samples were transported in refrigerated coolers until processing, not exceeding the maximum time of 2 hours between collection and centrifugation. The samples were centrifuged at 2195 g for 15 minutes to obtain plasma and serum samples, aliquoted and kept in a freezer at -80°C (Jouan®, model VX380) until use.
2.5 DETERMINATION OF PLASMA ANTIOXIDANT STATUS BY REDUCTION OF MTT

To determine the antioxidant capacity of plasma after 6 months of intervention with Chia seeds (T6), an assay was performed with the [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] (MTT) reagent. It is a tetrazolium salt that can be reduced by antioxidants such as ascorbate, urate, α-tocopherol, albumin and the group of sulfidril proteins, forming formazan crystals, which are purple and soluble in isopropanol.

A mixture containing plasma, phosphate buffered saline (PBS) and MTT was incubated at 37°C for 60 minutes, protected from light. Subsequently, an acid solution of isopropanol HCl (0.04 M) was added and vortexed for 30 seconds. The samples were centrifuged at 1372 g for 10 minutes and the supernatant absorbance measured by spectrophotometer VersaMax® microplate reader (Molecular Devices® - USA) at 570 nm (Duarte et al., 2015).

2.6 STATISTICAL ANALYSIS

Statistical analyzes were performed using GraphPad PRISM (version 6.0). Data normality was tested by the Shapiro-Wilk test. The comparison between groups was performed using Student's t-test for continuous and parametric variables. For nonparametric variables, the Mann-Whitney and Wilcoxon tests were used. The presence of outliers was evaluated by the ROUT test. Parametric variables were presented as mean and standard deviation (SD) and nonparametric variables as median, minimum and maximum. Significant differences were considered as p-value < 0.05.

3 RESULTS

The clinical and demographic characteristics of groups A and B are presented in Table 1. A total of 59 subjects were selected for this study, including 34 patients with NCDs (T2DM, SAH and obesity) and 25 healthy volunteers, who were evaluated in two stages: T0 (before the introduction of Chia seeds) and T6 (6 months after the introduction of Chia seeds). In group A, most patients were female (85.3%) and the average age was 49, ranging from 19 to 65 years old. Regarding the presence of NCDs, 64.7% of participants had SAH, 61.8% had T2DM and 70.6% were obese. The control group (group B) also showed a predominance of female gender, with an average age of 38 (Cochain & Zernecke, 2017; Nieman et al., 2015).
**Table 1. Clinical and demographic characterization of participants**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group A (n = 34)</th>
<th>Group B (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender [n (%)]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>05 (14.70%)</td>
<td>02 (8.00%)</td>
</tr>
<tr>
<td>Female</td>
<td>29 (85.29%)</td>
<td>23 (92.00%)</td>
</tr>
<tr>
<td>Age (Years)</td>
<td>49 (19-65)</td>
<td>38 (24-59)</td>
</tr>
<tr>
<td>Body composition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>88.00 (50-125)</td>
<td>64.80 (41-84)</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>33.98 (19-43)</td>
<td>23.51 (18-28)</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>96.00 (70-134)</td>
<td>71.00 (53-94)</td>
</tr>
<tr>
<td>AC (cm)</td>
<td>101.00 (73-130)</td>
<td>81.00 (63-97)</td>
</tr>
<tr>
<td>SAH [n (%)]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>22 (64.7%)</td>
<td>NA</td>
</tr>
<tr>
<td>No</td>
<td>12 (35.3%)</td>
<td>NA</td>
</tr>
<tr>
<td>T2DM [n (%)]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>21 (61.8%)</td>
<td>NA</td>
</tr>
<tr>
<td>No</td>
<td>21 (61.8%)</td>
<td>NA</td>
</tr>
</tbody>
</table>
| SAH = systemic arterial hypertension; T2DM = type 2 Diabetes mellitus. Group A: patients with hypertension and/or T2DM and/or obesity; Group B: healthy volunteers; NA: Not applicable. The data were presented as absolute value (n) and frequency (%) for gender, SAH and T2DM and as median with interquartile range for the others parameters.

Regarding anthropometric parameters, we found a significant reduction of these variables in T6, after Chia consumption in relation to T1, before the introduction of Chia. We observed a reduction in W [T0 = 88.00 x T6 = 85.01 Kg (p < 0.0001)]; AC [T0 = 101.00 x T6 = 99.50 cm (p < 0.0001)]; WC [T0 = 96 x T6 = 91 cm (p < 0.0001)] and BMI [T0 = 33.98 x T6 = 32.78 Kg/m2 (p < 0.0001)] considering group A. There was also a reduction in W, AC, WC and BMI when we compared T0 and T6 for group B: W [T0 = 64.80 x T6 = 63.01 Kg (p < 0.0001)]; AC [T0 = 81.00 x T6 = 78.00 cm (p < 0.0003)]; WC [T0 = 71 x T6 = 70 cm (p < 0.0001)] and BMI [T0 = 23.51 x T6 = 22.77 Kg/m2 (p < 0.0001)]. These results are presented in Table 2.

**Table 2. Anthropometric profile before and after 6 months of Chia seeds consumption for Groups A and B**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T0 (n = 41)</td>
<td>T6 (n = 34)</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>88.00 (50-25)</td>
<td>85.01 (50-116)</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>33.98 (19-43)</td>
<td>32.78 (19-40)</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>96.00 (70-134)</td>
<td>91.00 (66-119)</td>
</tr>
<tr>
<td>AC (cm)</td>
<td>101.00 (73-130)</td>
<td>99.50 (84-115)</td>
</tr>
</tbody>
</table>

T0 = initial time, before introduction of Chia seeds; T6 = final time, 6 months after introduction of Chia seeds; BMI = body mass index; Statistical difference was obtained from median. WC = waist circumference; AC = abdominal circumference. Group A: patients with hypertension (SAH) and/or type 2 Diabetes mellitus (T2DM) and/or obesity; Group B: healthy volunteers. All data were presented as median (minimum and maximum). P-values less than 0.05 were considered significant.

In this work, the antioxidant capacity was assessed by the formation of formazan crystals with the MTT reagent. The results exhibited a significant rise in plasma antioxidant capacity in T6 compared to T0, with p < 0.0001, both in group A, patients with T2DM and/or SAH and/or obesity (Figure 1A) and group B, healthy volunteers (Figure 1B).
Effect Of The Use Of Chia (Salvia Hispanica L.) Seeds On Antioxidant Status And Anthropometric Parameters In Obese, Type 2 Diabetics And/Or Hypertensive Patients

A: Mean absorbance levels obtained from the MTT test for group A before and after 6 months of Chia use; B: Mean absorbance levels obtained from the MTT test for group B before and after 6 months of Chia use; MTT = \[3-(4,5\text{-Dimethylthiazol-2-yl}) - 2,5\text{-Diphenyltetrazolium Bromide}\]; T0 = absorbance measured by reduction of MTT at time 0 (before Chia use); T6 = absorbance measured by reduction of MTT at the end time (6 months after Chia use). Group A: patients with type 2 Diabetes mellitus (T2DM) and/or hypertension (SAH) and/or obesity. Group B: healthy volunteers. Results are presented as mean and standard deviation. P-values less than 0.05 were considered significant.

4 DISCUSSION

The role of a balanced diet for the benefit of health has aroused the interest of the scientific community, which aims to prove that certain foods might influence reduced risk of NCDs (Malta et al., 2014).

Some researchers have shown a potential benefit of Chia consumption in cardiovascular disease, lipid and glycemic profiles, blood pressure, body composition and inflammatory markers. However, these studies vary greatly in sample size, individual profiles, and sample groups and there are more studies in animals than in humans. In addition, the quantities and presentation forms of Chia seeds (ground, whole, oil and insertion in food production) are different in several studies (Silva et al., 2019; Vuksan et al., 2017; Nieman et al., 2015).

Animal models have revealed that Chia seeds utilization may result in an improvement of the lipid profile associated with cardioprotective and hepatoprotective actions (Marcinek et al., 2017; Coelho & Salas-Mellado, 2014). In their study, Poudyal et al. found a reduction of fat in the liver and heart in rats (Poudyal et al., 2013). Chicco et al., (2009) also had used animal models. They showed improvement in dyslipidemia and insulin resistance after the intervention with chia seeds for 5 weeks (Medina et al., 2007). Other authors found low concentrations of glucose, triglycerides, LDL-c, VLDL-c and increased levels of HDL-c, in addition to hypertrophy of the intestinal muscle layers and good protein digestibility in Wistar rats (Da Silva et al., 2017; Marineli et al., 2015; Ho et al., 2013).

In addition, Fortino et al., (2017) found fatty acid oxidase elevation, free fatty acids reduction in plasma, less hepatic steatosis and hypercholesterolemia in pregnant rats when the corn oil diet was replaced with chia seeds (Fortino et al., 2017). In another study, the possible mechanisms related to the beneficial

Figure 1 - Mean absorbance levels obtained from the MTT test for group A before and after 6 months of Chia use
effects of chia seeds on skeletal muscle, lipotoxicity and insulin resistance in rats were investigated. The results indicated that chia seeds have reduced the content of lipids in skeletal muscle and increased muscle protein levels. In addition, the activities of the lipogenic enzyme decreased and the proportion of n-3 and n-6 fatty acids increased. This study also has demonstrated an increase in the levels of muscular oxidative capacity and a reduction in the lipogenic pathway (Ferreira et al., 2020; Marcinek & Krejpcio, 2017).

In the present study, a prevalence of females was observed in demographic characterization (Table 1). This greater participation of women over men is related to eating habits and has been the subject of research. In the United States, for example, women are approximately three times more vulnerable to eating disorders than men and approximately twice as susceptible to obesity (Liu, 2013). The higher prevalence of obesity in women compared to men can also be found in Brazil. The prevalence of obesity was 18.7% for men and 20.7% for women in 2018. However, a greater number of women had healthy eating habits in the same year, 27.2%, compared to men, 18.4%, which helps to explain the fact that women seem more susceptible to new diets and/or dietary changes and justifies their greater participation in our study (Nieman et al., 2009).

When we considered anthropometric parameters, a significant reduction in weight, abdominal circumference, waist circumference and BMI were found in both groups after intervention with Chia (Table 2). Possibly, the use of Chia seeds may have contributed to weight loss. After the intervention, there was a significant reduction in the BMI degrees, providing a better BMI classification of the individuals who have participated in this study. Some studies confirmed this weight loss property, correlating satiety to regular consumption of Chia seeds (Malta et al., 2014; Duarte et al., 2015).

In another work with overweight and obese adults, Ayaz et al., 2017 have observed weight loss, decreased BMI and circumferences (abdominal / waist) after a diet with 25 g of Chia for 6 months, which have been corroborated with our own research (Table 2). Another study demonstrated the ability of Chia seeds to increase satiety and decrease appetite, due to being rich in dietary fiber and low in carbohydrates (Ayaz et al., 2017; Poudyal et al., 2013). These observations can be associated with other results which have demonstrated that the consumption of 30 g/day of Chia in overweight adults and diabetics for six months may result in weight loss and reduced waist circumference (Chim-Chi et al., 2018), which is according to our study (Table 2). In addition, Toscano et al., (2015) showed a significant reduction in weight and circumferences in overweight and obese adults who received 35 g/day for 12 weeks (Toscano et al., 2015). On the other hand, in another study, also with adults, the authors found no difference regarding weight loss, reduced BMI, waist circumference or insulin resistance after consuming 25 g/day of Chia, for 10 days (Vuksan et al., 2017). In this case, time may be one of the factors that led to different results both in our study and in the other studies previously mentioned.

The weight reduction which has been found in the current study can be attributed to the rich fiber content present in Chia seeds and its high viscosity, giving this food an additive effect on satiety and consequently on weight loss (Ayaz et al., 2017). Unlike our study, in an investigation with overweight
adults who consumed 35 g/day of Chia seed for 12 weeks, the authors found no significant change in BMI or waist circumference (Toscano et al., 2015). Likewise, Vuksan et al., (2017) found no weight loss in individuals with TDM2 who consumed 37 g/day of Chia and in 62 postmenopausal women who ingested 25 g/day of ground chia for 10 weeks. However, they demonstrated increased plasma levels of alpha-linolenic acid (ALA) and docosahexaenoic acid (EPA) (Nieman et al., 2015). In contrast, the consumption of chia seeds in the present study led to significant weight loss in healthy and chronically ill patients.

In a review, Grancieri et al., (2019) showed the bioactive potential of the chia seed peptides, their chemical and nutritional composition and their beneficial effects on human health. They found hypotensive, hypoglycemic, antitoxic and anticholesterolemic effects (Grancieri et al., 2019).

In addition, more beneficial effects of chia have been reported in the literature, such as: laxative, analgesic and cardiovascular actions (Ferreira et al., 2018; Coelho & Salas-Mellado, 2014; Ixtaina et al., 2011) and increased plasma levels of alpha-linolenic acid (ALA) and docosahexaenoic acid (EPA) (Ferreira et al., 2020; Chicco et al., 2009). Besides, reduction of circumferences (CA, CC), improved lipid profile, decreased blood glucose levels and weight loss were found by other authors after the consumption of Chia (Vuksan et al., 2017; Cochain & Zernecke, 2017; Vuksan et al., 2010). Toscano et al., (2015) showed a reduction in total cholesterol and VLDL lipoprotein and an increase in HDL lipoprotein with consumption of 35 grams of chia / day by overweight and obese adults (Toscano et al., 2015).

In our study, in the MTT assay it was possible to observe an increase in the plasma antioxidant capacity after consuming Chia seeds, both in the patients and in the control group. It is known that in chronic conditions such as aging, hypertension, obesity and diabetes, reactive oxygen species and other oxidizing molecules can be continuously generated, contributing to both the inflammatory process and the worsening of these conditions (Nieman et al., 2015).

Some studies have associated the daily intake of bioactive components from Chia seeds with a protective effect against plasma oxidative stress and obesity (Oliveira-Alves et al., 2017; Marineli et al., 2015). The importance of this finding lies in the idea that reducing oxidative stress could be a natural alternative for prevention and control of some chronic diseases (Marineli et al., 2015). However, the main mechanism for improving the antioxidant status of plasma remains under discussion, as there is a hypothesis of a reduction in pro-oxidative compounds or an increase in antioxidant compounds (Chim-Chi et al., 2018). Further studies are needed to elucidate such mechanisms. The antioxidant potential of Chia is characterized by its ability to inhibit the production of reactive oxygen species (ROS) or their direct scavenging activity. They can transform into active molecules reducing cell damages, as well as helping to regulate gene expression and signal transduction. In contrast, the same antioxidant molecules can generate mild oxidative stress. Therefore, the organism generates feedback mechanisms, increasing the defense and survival of cells (Xanthis et al., 2021). Additionally, lipids and biologically active substances may be protected by the antioxidant properties from Chia during storage or use in thermal processes and in novel foods (Kulczynski et al., 2019).
Despite its longitudinal design, which allows for more decisive conclusions, this study was limited precisely by its experimental model, since the non-adherence to the proposed intervention resulted in the abandonment of participants and reduction in the sample size. However, despite this limitation, the study showed that Chia seeds could be a beneficial food source that could cause weight loss and increase antioxidant capacity. Further studies are needed to prove the effects of Chia on human health.

5 CONCLUSION

The regular consumption of Chia seeds was associated with an increase in the antioxidant capacity of plasma, in addition to contributing to the reduction of anthropometric measures, circumferences (abdominal and waist), BMI and weight loss. Six months after the intervention of using Chia, the study has already found promising results for patients with non-communicable chronic diseases and healthy individuals. Further studies are needed to confirm the effectiveness of these seeds as a complementary treatment and as preventive agents, especially in relation to chronic disease.

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REFERENCES


