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Original Article

Evaluation of the effects of passion fruit peel flour (*Passiflora edulis* fo. *flavicarpa*) on metabolic changes in HIV patients with lipodystrophy syndrome secondary to antiretroviral therapy

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ABSTRACT

This study evaluated the effects of using passion fruit peel flour together with diet therapy and counseling in 36 patients with HIV lipodystrophy who were in an ambulatory clinic in a university hospital. The patients were divided into two groups. One received 30 g of passion fruit peel flour daily for 90 days and diet therapy counseling. The other group received only diet therapy counseling. The metabolic changes were analyzed before and after the intervention, with a significance level predetermined at $p \leq 0.05$. The use of passion fruit peel flour was effective in reducing total cholesterol and triacylglycerides after 30 days. The concentrations of LDL-C decreased, while HDL-C increased in the blood of lipodystrophy patients after 90 days passion fruit peel flour treatment. No significant differences in food consumption were seen between groups. The use of 30 g of passion fruit peel flour for 90 days together with diet therapy counseling was effective in improving plasma concentrations of total cholesterol, LDL-C, HDL-C and triacylglycerides.

Introduction

The treatment of acquired immunodeficiency syndrome (AIDS) with reverse transcriptase and protease inhibitors represented a positive impact on the survival rate of individuals carrying the human immunodeficiency virus, or HIV (Smith, 2014). However, when HAART or highly active antiretroviral therapy (Smith, 2015) became a routine treatment, changes in body fat distribution and dyslipidemia were observed (Guimarães et al., 2007). These changes are called HIV Lipodystrophy syndrome (Tien et al., 2006; Guimarães et al., 2007; Araújo et al., 2007), which can increase the risk of cardiovascular diseases (Friis-Møller et al., 2003).

Soluble fiber may increase short-chain fatty acid synthesis (Wong et al., 2006), thereby reducing endogenous cholesterol production and associated with a diet low in cholesterol and saturated fat can reduce blood levels of LDL-C and triacylglycerides (Liu et al., 2000; Schneeman, 2002; Solà et al., 2007). The American Heart Association (2006) recommended the use of 5–10 g/day of soluble fiber for the reduction of dyslipidemia. A fiber-rich diet may be beneficial in preventing the development of fat deposition in people with HIV (Hendricks et al., 2003).

Passion fruit is the common name for various species of plants in the genus *Passiflora* (Zeriak et al., 2010). The two species with the most commercial value are *P. edulis* fo. *edulis* (red passion fruit) and *P. edulis* fo. *flavicarpa* O. Deg. (yellow), with the yellow species being the most widely cultivated (Zeriak et al., 2010). It has several health effects, including lowering the concentrations of LDL-C and cholesterol in the blood (Ramos et al., 2007). The peels can be dried

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and made into a food product called passion fruit peel flour (PFPF) that has several health effects and is sold in Brazil as an adjuvant treatment for diabetes due to its hypoglycemic effect (Smith et al., 2012). Flour prepared from yellow passion fruit peels has also been shown to reduce blood glucose in diabetic people. In a phase I clinical study, passion fruit peel flour was well tolerated in 36 people between ages 20 and 60, of both sexes. They received 10 g of flour three times a day and were told to put it in their choice of juice, soup, or any other food or beverage. There was an average reduction of blood glucose, triacylglycerides, total cholesterol and LDL of 5.2, 15.0, 18.2 and 19.0%, respectively.

In phase II studies, flour prepared from yellow passion fruit peels reduced blood glucose, cholesterol, LDL, blood pressure and body weight in diabetic patients.

The peels, or rinds, of Hawaiian yellow passion fruit have been fed to cattle and found an increase in milk production (Otagaki and Matsumoto, 1958). There are many anecdotal accounts of it increasing milk production and preventing bacterial infection, but these effects have not been quantified.

PFPF contains about 10% moisture, 7.5% ash, 4% protein, 19% soluble fiber, 38% insoluble fiber and 21% soluble carbohydrates (Córdova et al., 2005; Pinheiro et al., 2008). The major compound in PFPF is pectin, a dietary fiber that is rich in polygalacturonic acid and its methyl ester (Smith et al., 2012). However, to the best of our knowledge, the soluble carbohydrates and other soluble compounds have never been identified or analyzed by either HPLC or NMR.

Thus, considering that PFPF is a good source of soluble fiber, this study evaluated the effectiveness of PFPF in improving the blood lipid profile of individuals carrying HIV and receiving HAART who developed lipodystrophy. In addition, the hot, pressurized methanolic extract (100 °C and 10 MPa pressure) was analyzed by HPLC and NMR.

Materials and methods

Chemicals

Chloroform ($CHCl_3$), deuterated chloroform ($CDCl_3$), methanol (CH_3OH) and deuterated methanol (CD_3OD) were from Sigma–Aldrich, St. Louis, MO.

Preparation and characterization of the PFPF

The PFPF was produced from sanitized ripe yellow passion fruits (*Passiflora edulis* fo. *flavicarpa* O. Deg), that were identified by AUOS-S and stored at the Federal University of Rio de Janeiro for further reference. The peel was separated from the pulp, dehydrated, and transformed into flour using a knife mill. In order to characterize the PFPF, 10 g of it was mixed with enough HydroMatrix™ (Sigma–Aldrich, St. Louis, MO) to fill the 100 ml stainless steel sample cell used in an Accelerated Solvent Extractor (ASE, ThermoFisher Scientific, Sunnyvale, CA). Then, CH_3OH was added while the temperature and pressure were increased to 100 °C and 10.3 MPa (1500 psi, 100 atm) over a 3 min time (static time). Next, the solvent was purged into a collection vessel. A total of four cycles were run to statically extract the sample, resulting in a total volume of about 160 ml. The solvent was evaporated off and the oily residues remaining were weighed. A portion of the residue remaining after evaporating the CH_3OH from the methanolic extract of each sample was redissolved in CD_3OD for NMR analysis. Another portion was dissolved in methanol for HPLC analysis.

NMR analyses were done using an Agilent DD2 600 MHz NMR (Santa Clara, CA). A 30° pulse width and 1 s pulse delay were used for the ^1H NMR, while a 30° pulse width and 2 s pulse delay were used for the ^1H-coupled ^{13}C-NMR spectra, also known as $^{13}C\{^1H\}$-NMR. Chemical shifts were referenced to the CD_3OD signals at 3.35 and 4.78 ppm (for ^1H) and 49.30 ppm (for ^{13}C) for the spectra of the methanolic extracts and to the $CDCl_3$ signals at 7.27 and 77.23 ppm, for ^1H and $^{13}C\{^1H\}$-NMR, respectively.

The FTIR spectrum of the residue remaining from the methanolic extract, after evaporating off the methanol was acquired using ATR attachment on Shimadzu IRAffinity-1S. The UV–Vis spectrum of a 5 μg/ml solution of the extract in methanol was obtained using Shimadzu UV-2600. Plain methanol was used as blank. The source of light was shifted to deuterium bulb at 300 nm and the spectrum window was opened between 900 nm and 185 nm.

Experimental design

A clinical therapeutic randomized trial was performed with individuals carrying HIV, presenting Lipodystrophy Syndrome, receiving HAART, and showing dyslipidemia (hypercholesterolemia, hypertriglyceridemia, or both) as defined by the National Cholesterol Education Program (2002). These individuals were monitored at the Lipodystrophy Ambulatory Center in the João de Barros Barreto University Hospital (HUJBB), during the period of January to December 2009, and fulfilled the criteria for participation in the study. The sample was calculated through the calculation of Proportion: two samples. For the first sample (from the group using PFPF), the estimated proportion of plasma cholesterol improvement was 50%; for the second sample, this estimate was of 10%. The ratio between these samples was stipulated as 1:1. The power of the test was fixed as 0.8, and the unilateral alpha level in 0.05. The sample was calculated from sixteen individuals in each group.

The 36 individuals participating in the study were systematically divided into two groups. Group 1 ($n = 18$) received diet therapy counseling and 30 g/day of PFPF, consumed as a dilute solution in water, juices, and/or fruit smoothies. Group 2 ($n = 18$) received diet therapy counseling only. There were 12 men and 6 women in each group.

Information about pathology, weight, height, and food intake was assessed in the initial evaluation by the 24 h recording method (R24) at the time of the initial (beginning of the intervention) and last assessments (end of the study).

Individuals from the two groups were instructed to have biochemical tests performed monthly (total cholesterol – TC, LDL-C, HDL-C, and triacylglycerides) at the HUJBB's laboratory. These tests were performed in the morning, after 12 h fasting and at 4 time points in the study: before the intervention (T0), at 30 (T30), 60 (T60), and 90 days (T90) after the intervention.

The following parameters were followed as inclusion criteria in the study: be an adult patient (any gender) receiving HAART, attending treatment at the Lipodystrophy ambulatory center at the HUJBB, presenting changes in the TC and/or LDL-C and/or triacylglycerides, presenting undetectable HIV viral load and LTCD4+ above 300 cells/mm^3 and agreeing to participate in the study by voluntarily signing an informed consent form.

The following criteria were followed for study exclusion: individuals presenting triacylglycerides above 700 mg/dl, or using lipid-lowering drugs, who could not complete the 90 days of monitoring, did not tolerate the use of PFPF, missed appointments, displayed mental illness, were under age 18 (children, and adolescents), and did not accept to participate in the study.

The data obtained were tabulated and analyzed using the Biostat 5.0 program (2007) with a significance level of $p \leq 0.05$. The dietpro program (Esteves and Monteiro, 2002) was used for the evaluation of R24 h. The bilateral t-test was used for the verification of homogeneity in the observed values between the groups.