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Evidence of hypoglycemic, lipid-lowering and hepatoprotective effects of the Bixin and Bixin: β-CD inclusion compound in high-fat-fed obese mice

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ABSTRACT

Associations between obesity, diabetes type II, and steatosis have long been recognized. However, a pharmacotherapy that acts in a multifactorial manner controlling the interactions between these conditions is not available. A variety of natural plants, functional fatty acids, and other natural dietary compounds have been used in various anti-obesity products. We investigated the effects of oral administration of an antioxidant carotenoid pigment Bixin and Bixin: β-Cyclodextrin in an obese murine model. C57BL/6 male mice (4–5 weeks) received standard diet (2.18 kcal per 1 g) (CT) and high-fat diet (4.38 kcal per 1 g) (CT/OB, BIX and BIX/βCD) (n = 10 per group). After 16 weeks, the BIX and BIX/βCD were treated by gavage (100 μL day-1) for six weeks, with water (CT and CT/OB groups) and (50 mg kg-1 day-1), Bixin (BIX group) or Bix: β-CD (BIX/βCD). Body weight, Lee's Index, adiposity, CHT, TG, CHT/HDL-c, glucose levels (metabolic markers) and, liver markers (AST and ALT) were determined. All metabolic and liver parameters exhibited down-regulation after oral administration of BIX and BIX/βCD. Particularly relevant was Lee's Index and adiposity in BIX- and BIX/βCD-treated groups (339.18 g/cm -BIX and 327.58 g/cm -BIX/βCD vs. 360.68 g/cm -CT/OB animals), this finds associated with the insulin sensitivity test, showed a clear association between reduction of adipose tissue and decrease of peripherical insulin resistant. In conclusion, our study suggested that the oral administration of the Bixin and Bix: β-CD inclusion compound improved the metabolic parameters evaluate in obese mice, being more palatable and hepatoprotective.

1. Introduction

Obesity and overweight are major contributors to the global burden of chronic diseases and their complications, including diabetes, cardiovascular diseases, hypertension, osteoarthritis, some cancers and inflammation-based pathologies, which suggests that the obese are likely to have a disproportionate use of the health care system [1,2]. The World Health Organization (WHO) reported that in 2014, more than 1.9 billion adults were overweight worldwide, and of these adults, over 600 million were obese [3]. The high prevalence of overweight and obesity, combined with their concomitant risks, poses a particularly relevant worldwide public health challenge. At the same time, it is well established that obesity and metabolic syndrome are commonly associated with nonalcoholic fatty liver disease (NAFLD), one of the most common causes of chronic liver disease worldwide. In fact, it is so closely associated that hepatic steatosis has been proposed as a diagnostic criterion of metabolic syndrome. Hepatic steatosis is present in greater than 60% of obese and 90% of morbidly obese adults, and the prevalence of elevated alanine aminotransferase (ALT) in obese youth is a biological marker of this disease [4].

Abbreviations: BIX, Bixin (*BixaOrellana*); BIX:β-CD, Bixin and β cyclodextrins (inclusion complex); CT, control; CT/OB, obese control; HFD, high fat diet; NAFLD, nonalcoholic fatty liver disease; BCRJ, Banco de Células de Rio de Janeiro; DEX, dexamethasone; IST, insulin sensitivity test; CHT, cholesterol; TG, triglycerides; GLU, glucose; ALT/GTP, alanine aminotransferase; AST/GOT, aspartate amino transferase; CEUA, Ethics Committee of Animal Experimentation

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A variety of natural plants (e.g., herbs, fruits, and vegetables), functional fatty acids (e.g., polyunsaturated fatty acids and conjugated fatty acids), and other natural dietary compounds have been used in various anti-obesity products [5]. There are also alternative strategies to treat obesity and diabetes using natural products, such as the polysaccharide portion of wolfberry, garlic-derived monomers, red grape-derived resveratrol, and milk thistle–derived substances [6,7], as well as peptides or proteins isolated from many animal sources [8,9] that have different action mechanisms in specific signaling pathways related to glucose and lipid metabolism. Among these natural products, antioxidant carotenoid pigments extracted from *Bixa Orellana L.* seeds, a shrub native to tropical America, and especially Bixin, its main constituent, are shown to have several bioactive properties including antioxidant effects [10] [11], activity in cancer cells [12,13], and anti-inflammatory activity [14,15]. The antioxidant effect of Bixin is important in the prevention of hyperlipidemia and arteriosclerosis [16] and in vitro and in vivo reports have shown that the partially purified extract of *Bixa Orellana L.* and purified Bixin showed hypoglycemic effects [17–20]. These results suggest that Bixin should potentially replace the actions of hypoglycemic drugs as thiazolidinediones and statins in the treatment of diabetes type 2 or hyperlipidemia. Unfortunately, the clinical relevance of Bixin is limited by its low solubility and stability against light and oxygen during processing and storage, owing to its largely hydrophobic structure [21,22].

In the pharmaceutical arsenal to increase water solubility and physicochemical stability, cyclodextrins are used in drug: cyclodextrin inclusion compound. Cyclodextrins have also been considered to be used in delivery systems based on their ability to form inclusion compounds and improve the solubility and bioavailability of drugs [23–29]. Some reports described the interaction between Bixin and cyclodextrins (α and β-CD) as a strategy to stabilize food formulations against air, ozone, light, and high temperatures [30,31]. Thus, we hypothesize that an inclusion compound of Bixin and cyclodextrin can be useful for treating obesity, hypoglycemia, and lipid-lowering or nonalcoholic fatty liver steatosis; thus, the present report aims to investigate in vitro or in vivo tests using Bix: β-CD inclusion complex oral formulations.

2. Materials and methods

2.1. Materials

Bixin (Bix) powder was extracted from seeds according to the methodology established by Barbosa et al. [32]. Beta-cyclodextrin (β-CD) was purchased from Cerestar Co, IN, USA. All other reagents (Acetone, $NaHCO_3$) were of analytical grade. For in vitro assays, 3T3-L1 mouse pre-adipocyte fibroblasts (BCRJ Code: 0019) were purchased from Banco de Células de Rio de Janeiro (BCRJ), and Dulbecco's Modified Eagle's Medium High Glucose (DMEM), fetal bovine serum (FBS), penicillin-streptomycin solution, 1-methyl-3-isobutyl xanthine (IBMX), dexamethasone (DEX) and insulin (INS) were all purchased from Gibco-Invitrogen, Brazil.

2.2. Inclusion compound preparation

The Bix: β-CD inclusion compound was prepared in a 1:1 molar ratio. Briefly, the first solution of 39.5 mg of Bix in 5 mL acetone was mixed in 50 mL $NaHCO_3$ solution (0.05%) to complete solubilization, and a mixture of a solution of 113.5 mg β-CD in 30 mL of distilled water was prepared using magnetic stirring for 12 h. The final solution was freeze-dried. For comparison, a 1:1 molar ratio physical mixture (PM) of Bix (3.9 mg) and β-CD (11.3 mg) was also prepared.

2.3. Physicochemical characterization of an inclusion compound

Physicochemical characterization was performed using Fourier transformed-infrared (FTIR) spectra collected with a Perkin Elmer Spectrum GX spectrophotometer, KBr pellets, and scanning between 4000 and 400 cm^{-1}. The X-ray diffraction patterns (XRD) were obtained on a Shimadzu XRD-7000 X-Diffractometer with Cu Kα = 1.54051 radiation. The scanning speed was 2θ/min. The thermal stability of the Bixin, Bixin-β-CD inclusion compound and physical mixture were assessed using an SDT Q600 TA analyzer (TG/DTG-DTA) over a temperature range of 25 °C–700 °C at a heating rate of 10 °C/min under a nitrogen purge. The one-dimensional NMR spectra (^1H) and two-dimensional spectra (2D ROESY) were obtained using a Bruker Avance DPX-400 (400 MHz). Samples were prepared in NMR tubes 8.00 inches in length with 5-mm external diameter.

2.4. In vitro assays

2.4.1. Culture and induce insulin resistance in 3T3-L1 cells

The 3T3-L1 pre-adipocytes was cultivated at a density of 10^5 cells/cm^2 in complete DMEM with 10% FBS and 37 °C and 5% CO_2 conditions until confluence. On the second day after confluence, the complete medium was replaced with differentiation medium (DMEM supplemented with IBMX 0.5 mM, DEX 0.1 μM and INS 10 mg mL^{-1} (1.72 mM), and cells were maintained for h. After this treatment, the cells were washed with PBS (pH 7.4), and the culture medium was replaced and changed every two days until completion after eight days [33]. Differentiated adipocytes were exposed for eight days by DMEM supplemented with DEX at a concentration of 1000 nM for 48 h. After exposure to DEX, the medium was replaced with DMEM with low glucose content. After one hour, the medium was supplemented with insulin (2500 nM) for glucose uptake stimulation for 24 h, and the residual glucose in the medium was quantified [34]. Differentiated adipocytes were exposed for 48 h to different concentrations of the Bix and Bix: β-CD inclusion compound. After this treatment, the cells were washed twice with PBS, and the medium was replaced with complete culture medium supplemented with DEX (1000 nM) and maintained for 48 h. The medium was replaced by DMEM with low glucose content and, after one hour, was supplemented with insulin (2500 nM). After 24 h of exposure to insulin, the residual glucose in the medium was quantified using the glucose liquiform enzyme-colorimetric endpoint assay (Labtest, Brazil) according to the instructions and standards of the manufacturer, and reading was performed with a spectrophotometric microplate format (96 well plates) in triplicate. The evaluation included the quantification of residual glucose after treatment in normal cells and the insulin resistance cell phenotype was induced by DEX [35,36].

2.5. In vivo studies

Male C57BL/6 mice aged 4–5 weeks were obtained from the animal care center at the Universidade Federal de Minas Gerais (CEBIO/UFMG) and kept under control conditions at room temperature (24 ± 2 °C), with alternating 12 h light and dark cycles with free access to food and water. They were maintained according to the ethical guidelines of our institution (experimental protocol approved by the Animal Ethics Committee at the university, CETEA/CEUA – UFMG, number 318/2015).

First, the mice were divided into two groups and treated for 16 weeks: the control group (n = 10) was fed with the Nuvilab CR-1® Quimtia mouse diet (Colombo, Brazil) with regular maintenance composed of 56.1% carbohydrate, 29.4% protein and 14.8% fat (3.03 kcal per 1 g of diet); the second group (n = 30, 10 animals per group) received the high-fat diet (HFD) to induce metabolic changes. The HFD diet was composed of 45% fat (42.1% lipid, 21.9% protein and 35.9% carbohydrates). The high-fat diet was prepared following the protocols described previously [37–39]. Lee index (calculated as one-third of body weight (grams)/Nasal-anal length (centimeters) x 1000) were used to determine obesity. Lee index > 344.32 after administration of the HF diet for 16 weeks were considered obese [37,40–42]. After 16 weeks, the mice from the HF diet group were redistributed equally into