





Original Article

Effects of liquid extract from *Plinia cauliflora* fruits residues on Chinese hamsters biochemical parameters

Efeitos do extrato líquido de resíduos de frutos de *Plinia cauliflora* sobre os parâmetros bioquímicos de hamsters chineses

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Abstract

Plinia cauliflora (Mart.) Kausel, popularly known as jabuticaba, is rich in polyphenols. Phenolic compounds exhibit several biological properties, which reflect on biomarkers such as biochemical parameters. In the present study, we evaluated the plasmatic levels of glucose, total cholesterol, HDL-cholesterol, triglycerides, and uric acid of Chinese hamsters fed for 45 days with a regular diet or cholesterol-enriched diet supplemented with a liquid extract obtained from *P. cauliflora* fruits residues standardized in ellagic acid and total phenolic compounds. The results showed that the concentrated extract obtained from jabuticaba residues increased the glycemia of animals fed with a regular diet and reduced the plasmatic uric acid levels of animals fed with a cholesterol-enriched diet. Since hyperuricemia is considered to be a significant risk factor of metabolic disorders and the principal pathological basis of gout, the liquid extract from *P. cauliflora* fruits residues would be a promising candidate as a novel hypouricaemic agent for further investigation.

Keywords: biochemical parameters, Cerrado, glycemia, jabuticaba, *Myrciaria cauliflora*, uric acid.

Resumo

Plinia cauliflora (Mart.) Kausel, popularmente conhecida como jabuticaba, é rica em polifenóis. Os compostos fenólicos apresentam diversas propriedades biológicas, que refletem em biomarcadores, como os parâmetros bioquímicos. No presente estudo, avaliamos os níveis plasmáticos de glicose, colesterol total, HDL-colesterol, triglicerídeos e ácido úrico em hamsters chineses alimentados por 45 dias com dieta regular ou dieta enriquecida com colesterol suplementada com extrato líquido obtido de resíduos de frutos de *P. cauliflora* padronizado em ácido elágico e compostos fenólicos totais. Os resultados mostraram que o extrato concentrado obtido dos resíduos de jabuticaba aumentou a glicemia dos animais alimentados com dieta regular e reduziu os níveis plasmáticos de ácido úrico dos animais alimentados com dieta rica em colesterol. Uma vez que a hiperuricemia é considerada um fator de risco significativo de distúrbios metabólicos e a principal base patológica da gota, o extrato líquido dos resíduos de frutas de *P. cauliflora* seria um candidato promissor como um novo agente hipouricêmico para investigação posterior.

Palavras-chave: parâmetros bioquímicos, Cerrado, glicemia, jabuticaba, *Myrciaria cauliflora*, ácido úrico.

1. Introduction

Jabuticaba - *Plinia cauliflora* (Mart.) Kausel - is a fruit that belongs to the Myrtaceae family, occurring mainly in Brazil's southeastern in some biomes and is typical of the Brazilian Cerrado. The fruits have a great nutritional and economic potential. They are consumed *in natura*, and various products can be obtained from them, such

as liquor, jams, ice cream, and alcoholic beverages, highlighting the importance of this resource (Abe et al., 2011; Albuquerque et al., 2020; Clerici and Carvalho-Silva, 2011).

Besides, jabuticaba is popularly used to treat many diseases such as asthma, throat inflammation, and

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gastrointestinal and cardiovascular disturbances (Giraldi and Hanazaki, 2010; Borges et al., 2014). Jabuticaba peels present antioxidant, anti-inflammatory, and analgesic effects, reduced blood cholesterol and obesity-associated insulin resistance, improved glucose levels and lipid markers, and reduced adipose tissue inflammation, weight gain, dyslipidemia, and hepatic steatosis in animal models (Dragano et al., 2013; Araújo et al., 2014; Lenquiste et al., 2015; Batista et al., 2018; Brito et al., 2021).

When the jabuticaba fruits are processed, around 50% of fruit mass is discarded, representing an environmental problem. Thus, the development of a method to reuse the residues obtained from this source is necessary. The application of the jabuticaba depulping residue in drying processes was previously investigated. Silva and colleagues determined the spray-drying conditions of the aqueous jabuticaba extract to obtain the powder with functional properties standardized in total anthocyanin content and phenolic compounds (Silva et al., 2014). Moreover, jabuticaba hydroalcoholic extract presented a good performance in a spray-drying process to obtain a powder standardized in ellagic acid and total phenolic compounds (Borges et al., 2015). These works showed that there is the possibility to reuse the residues of the jabuticaba in different conditions by spray-drying process standardized powders in different chemical markers.

Also, the investigations over this raw material's biological activities are important to know the potential functional properties of the jabuticaba residues (Guo et al., 2003; Soong and Barlow, 2004). According to Andrade et al. (2015), the concentrated liquid extract from jabuticaba residues presented *in vitro* activity to reduce the vascular tension and the blood pressure of isolated aortas from rats. This result revealed the great potential for the reuse of this raw material due to its biological properties. Therefore, the present study's objective was to evaluate the effects of concentrated liquid extract obtained from jabuticaba residues on biochemical parameters of diet-induced hypercholesterolemic Chinese hamsters.

2. Materials and Methods

2.1. Plant material

Winery Jabuticabal gently donated the residues of the *P. cauliflora* fruit peels at Hidrolândia, Goiás State, Brazil, in November 2011.

2.2. Preparation of the hydroethanolic extract

The dried and powdered blades of jabuticaba residues were exhaustively extracted using ethanol:water (45:55 v/v) as a solvent mixture. The resulted extract was filtered and evaporated under reduced pressure on a rotary evaporator at 40 °C, obtaining the concentrated liquid extract.

2.3. Characterization of the concentrated liquid extract

The density and pH of the extract were determined according to the Brazilian Pharmacopoeia V (Brasil, 2010). The total solids content was determined by a gravimetric

method in a halogen lamp analyzer (MB 35; Ohaus Inc., Pine Brook, NJ), and viscosity was measured with a viscometer (Brookfield DV-III+; Brookfield Engineering Laboratories, Inc., Middleboro, MA).

2.4. HPLC-PDA Ellagic Acid quantification (EAc)

HPLC analysis was performed using Waters LC system (Milford, Massachusetts, USA) comprising a quaternary pump, a degasser, an autosampler, and a photodiode array detector, model 2998. Enpower 2.0 software was employed to control the equipment and the acquisition and treatment of data. Chromatographic separation was performed with a Zorbax Eclipse RP-C₁₈ reverse-phase column (250 × 4.6 mm, 5 µm particle size). The mobile phase was methanol and water (60:40, v/v) at a flow rate of 0.5 mL·min⁻¹ at ambient temperature. The injection volume of all samples was 10 µL, the detection wavelength was 252 nm, and the run time was 12 min. The HPLC method was validated (data not shown) according to Agência Nacional de Vigilância Sanitária (ANVISA–Brazilian National Health Surveillance Agency) guidelines (Brasil, 2013).

2.5. Total phenolic quantification

Total phenolic content (TPC) was determined according to Hagerman and Butler (1978), adapted by Mole and Waterman (1987). Ferric chloride was added to aqueous extract solution under alkaline conditions to yield a colored complex with phenolic compounds, read at 510 nm. Standard curves were prepared with tannic acid.

2.6. Total flavonoid quantification

Total flavonoid content (TFC) was determined following the method described by Rolim et al. (2005) with modifications. Sample absorbance was measured at 361 nm. Standard curves were prepared with rutin.

2.7. Animals and diets

Experiments were performed on male Chinese hamsters (80–120 g) from the Centro de Experimentação Animal da Pontifícia Universidade Católica de Goiás (PUC Goiás). The animals were individually housed at 25 °C, under 12:12 h light:dark cycle with free access to chow and tap water until the day of the experiment. Animal care and handling procedures followed the International Association for the Study of Pain guidelines for the use of animals in pain research (Zimmermann, 1983) and Institutional Animal Care and Use Committee-PUC GO 0013-1.

Animals were randomly in four groups (n = 8/cage). Group 1 (control): animals received a regular rodent diet (RD); Group 2: animals received a regular diet supplemented with the concentrated liquid extract (1% liquid extract; w/w) (E-RD); Group 3: animals received a cholesterol-enriched diet (1% cholesterol, w/w) (CD); Group 4: animals received a cholesterol-enriched diet (1% cholesterol, w/w) supplemented with the concentrated liquid extract (1% liquid extract, w/w) (E-CD). The liquid extract from jabuticaba residues was added daily directly at the animal's feed. After 45 days of feeding, the animals were anesthetized (isoflurane inhalation), and blood was

collected by cardiac puncture into EDTA flasks. The plasma was separated by centrifugation and stored at $-70\text{ }^{\circ}\text{C}$ for further analysis.

2.8. Biochemical analysis

Fasting blood glucose, total cholesterol, HDL-cholesterol, triglycerides, and uric acid were measured by enzymatic colorimetric assays using commercial kits (Labtest, Vista Alegre, MG, Brazil) according to the manufacturer's instructions.

2.9. Statistical analysis

Data are presented as means \pm SEM of measurements made on eight animals in each group. Comparisons between groups that receive or not the liquid extract obtained from *P. cauliflora* fruits residues were determined using t-test (RD x E-RD or CD x E-CD). The data were analyzed using the Prism 4 computer software (GraphPad, San Diego, USA). The value of $p < 0.05$ was considered significant.

3. Results and Discussion

3.1. Characterization of the concentrated extract

The concentrated extract showed a density of 0.978 g/mL , a solid content of 12% (w/w), a pH of 3.23 , and a viscosity of 4.28 mPa . The contents of EAc, TPc, and TFC relative to the total solid (w/w) mass were 0.027% , 17.89% , and 4.5% .

3.2. Biochemical parameters

The concentrated liquid extract from jabuticaba residues increased the glycemia (27.5%) of Chinese hamsters with a regular diet (Figure 1), which could be explained by the presence of molecules that arrived from the fermentation process, such as soluble sugars (Silva et al., 2008). The significant amount of low mass carbohydrates present in jabuticaba residues could be better absorbed by the animals, resulting in glycemia increase in E-RD group. Meta-analysis data pointed that humans' berry polyphenol consumption did not clearly affect biomarkers of glucose metabolism compared with placebo or no treatment. Although some studies indicate significant effects, these effects were too small to be clinically relevant (Rambaran et al., 2020).

The concentrated liquid extract from jabuticaba residues did not alter the plasmatic levels of triglycerides, total cholesterol, and HDL-cholesterol in Chinese hamsters with a regular diet or a cholesterol-enriched diet (Figures 2, 3). In opposite to what was observed in this study, previous studies showed a reduction in serum cholesterol and triglycerides and an increase in HDL-cholesterol of rats and mice fed with jabuticaba peel (Lenquist et al., 2012; Araújo et al., 2014; Brito et al., 2021; Batista et al., 2018). These advantages over serum biochemical markers could be explained by anthocyanins' presence (Kong et al., 2003), which may have been extracted during the fermentation process to obtain the jabuticaba beverage. Anthocyanins are generally extracted using alcoholic solutions (Khoo et al., 2017).

The concentrated liquid extract from jabuticaba residues reduced the plasmatic uric acid (47%) of animals fed with a cholesterol-enriched diet (Figure 4). Uric acid is the terminal product of the purine nucleotide catabolism. The high level of uric acid in blood, hyperuricemia, may result in urate crystals deposition in joint tissues, which promotes inflammation and causes gout, a form of arthritis accompanied by intense pain. Also, hyperuricemia is probably associated with a diet rich in cholesterol and triglycerides, obesity, hypertension, metabolic syndrome, and cardiovascular disease (Carneiro et al., 2000; Chen et al., 2015; Jiang et al., 2020). So, the hyperuricemia treatment could help to reduce the morbidity and mortality of many patients.

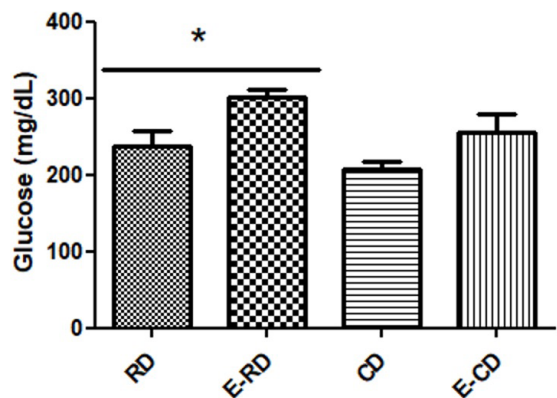


Figure 1. Blood glucose in Chinese hamsters' groups. RD= animals received a regular diet; E-RD = animals received a regular diet with the concentrated liquid extract from jabuticaba residues; CD = animals received a cholesterol-enriched diet; E-CD = animals received a cholesterol-enriched diet with the concentrated liquid extract from jabuticaba residues. Data are presented as mean \pm SE. * represents statistical differences obtained by the t-test ($p < 0.05$) between groups that received or not the extract (RD x E-RD or CD x E-CD).

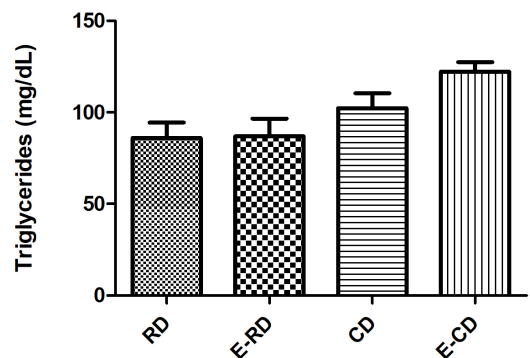


Figure 2. Plasmatic triglycerides in Chinese hamsters' groups. RD = animals received a regular diet; E-RD = animals received a regular diet with the concentrated liquid extract from jabuticaba residues; CD = animals received a cholesterol-enriched diet; E-CD = animals received a cholesterol-enriched diet with the concentrated liquid extract from jabuticaba residues. Data are presented as mean \pm SE.

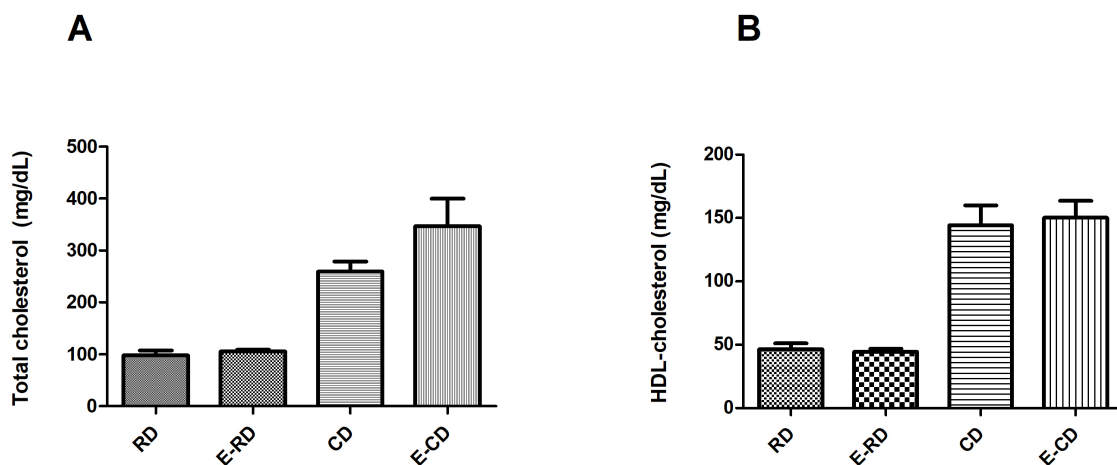


Figure 3. Plasmatic total cholesterol (A); HDL-cholesterol (B) in Chinese hamsters' groups. RD = animals received a regular diet; E-RD = animals received a regular diet with the concentrated liquid extract from jabuticaba residues; CD = animals received a cholesterol-enriched diet; E-CD = animals received a cholesterol-enriched diet with the concentrated liquid extract from jabuticaba residues. Data are presented as mean±SE.

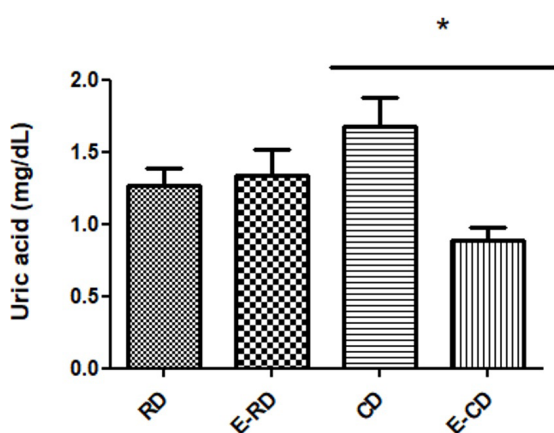


Figure 4. Plasmatic uric acid in Chinese hamsters' groups. RD = animals received a regular diet; E-RD = animals received a regular diet with the concentrated liquid extract from jabuticaba residues; CD = animals received a cholesterol-enriched diet; E-CD = animals received a cholesterol-enriched diet with the concentrated liquid extract from jabuticaba residues. Data are presented as mean±SE. An asterisk represents statistical differences obtained by the t-test ($p < 0.05$) between groups that received or not the extract (RD x E-RD or CD x E-CD).

Currently, the clinical drugs available to treat hyperuricemia exhibit many side effects. In this sense, studies on the treatment of hyperuricemia with plant-based functional foods have received increasing attention. (Jiang et al., 2020). Oligonol®, a phenolic substance produced from lychee fruit polyphenols, containing a mixture of catechin, epicatechin, proanthocyanidins, and others, lowered serum uric acid through inhibition of xanthine oxidase, a key enzyme involved in uric acid production

(Moriwaki et al., 2011). Green tea polyphenols, mainly represented by epigallocatechin-3-gallate, epigallocatechin, epicatechin-3-gallate, epicatechin, gallic acid, and catechin, lowered uric acid by decreasing the uric acid production and increasing uric acid excretion (Chen et al., 2015). Moreover, flavonoids, phenolic acids, saponins, alkaloids, polysaccharides, among others, have been identified as bioactive components from plant-based functional foods that present anti-hyperuricemia activities (Jiang et al., 2020).

In summary, the results point that 45 days treatment of Chinese hamsters with the concentrated extract obtained from jabuticaba residues can alter some biochemical parameters, increasing glycemia in animals fed with a regular diet and reducing the plasmatic uric acid levels in animals fed with a cholesterol-enriched diet. Thus, even the raw material being a residue, its extract possesses biological activities over the biochemical parameters evaluated in this paper. The data presented here suggest that liquid extract from *Plinia cauliflora* fruits residues would be a promising candidate as a novel hypouricemic agent for further investigation.

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