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Hypoglycaemic activity and molecular mechanisms of *Caesalpinia ferrea* Martius bark extract on streptozotocin-induced diabetes in Wistar rats

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ABSTRACT

Ethnopharmacological relevance: The tea from the stem bark of *Caesalpinia ferrea* Martius (Leguminosae) has been popularly used in the treatment of diabetes in Brazil.
Aim of the study: To investigate the hypoglycaemic properties and to elucidate the mechanisms by which the aqueous extract of the stem bark of *Caesalpinia ferrea* reduces blood glucose levels in streptozotocin-induced diabetic rats via the enzymatic pathways of protein kinase B (PKB/Akt), AMP-activated protein kinase (AMPK) and acetyl-CoA carboxylase (ACC).
Materials and methods: The aqueous extract of the stem bark of *Caesalpinia ferrea* (300 and 450 mg/kg/day), vehicle and metformin (500 mg/kg/day) were administered orally to STZ-diabetic rats ($n = 7$/group) for 4 weeks. Changes in body weight, food and water intake, fasting glucose levels and oral glucose tolerance were evaluated. Phosphorylation (P) and the expression of Akt, AMPK and ACC in the liver and skeletal muscle were determined using Western blot.
Results: The aqueous extract of the stem bark of *Caesalpinia ferrea* reduced blood glucose levels and improved the metabolic state of the animals. P-Akt was increased in the liver and skeletal muscle of the treated animals, P-AMPK was reduced only in the skeletal muscle of these animals and P-ACC was reduced in both when compared with untreated rats.
Conclusion: The results indicate that the aqueous extract of the stem bark of *Caesalpinia ferrea* has hypoglycaemic properties and possibly acts to regulate glucose uptake in liver and muscles by way of Akt activation, restoring the intracellular energy balance confirmed by inhibition of AMPK activation.

1. Introduction

Diabetes is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels (ADA, 2010).

The pharmacological treatment of diabetes includes oral hypoglycaemic and insulin. Although these drugs are effective in reducing glycaemia, they may cause undesirable side effects (such as weight gain, hypoglycemia, edema, gastrointestinal disturbances and insulin resistance) that can discourage patient compliance.

On the other hand, ethnopharmacological evidence has shown that the use of plants is a viable alternative for the treatment of diabetes. The advantages of herbal medicine include significant efficacy, low incidence of side effects, low cost and relative safety (Ali et al., 2006). In fact, the medicinal plants are considered an important source of molecules with potential hypoglycaemic effects. Grover et al. (2002) have reported about 800 plants with these molecules, which may act through different mechanisms, including the inhibition or stimulation of enzymatic activity and/or protein expression. The wide diversity of species has led scientists to make great efforts to bioprospect plants that may contribute to the management of diabetes.

Caesalpinia ferrea Martius (Leguminosae), popularly known as "pau-ferro" or "jucá", is a large tree that is found mainly in the North and Northeast of Brazil. In folk medicine, the tea of the

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stem bark of *Caesalpinia ferrea* has been used for the treatment of diabetes (Araújo et al., 2008). Other therapeutic properties of this plant include anti-inflammatory, antiulcer (Bacchi and Sertié, 1994; Bacchi et al., 1995), analgesic (Carvalho et al., 1996), anticancer (Nozaki et al., 2007), antibacterial (Sampaio et al., 2009) and antihypertensive (Menezes et al., 2007). In view of its ethnomedicinal importance, the Brazilian Ministry of Health has included this species on the National List of Medicinal Plants important to the Health System.

Phytochemical investigation of the hydroalcoholic extract of the stem bark and leaves of *Caesalpinia ferrea* has revealed flavonoids, saponins, tannins, coumarins, steroids and other phenolic compounds (Gonzalez et al., 2004). Tannins were the main compounds found (Souza et al., 2006). One component isolated from the fruit is ellagic acid, inhibitor of aldose reductase, which is an enzyme involved in the complications of diabetes (Ueda et al., 2001).

Although *Caesalpinia ferrea* is widely used in folk medicine, there is no experimental evidence proving its hypoglycaemic properties. The aim of this study was, therefore, to investigate the hypoglycaemic properties of the stem bark of *Caesalpinia ferrea* in streptozotocin-induced diabetic rats and to examine the effects of its aqueous extract on the phosphorylation and expression of protein kinase B (PKB/Akt), AMP-activated protein kinase (AMPK) and acetyl-CoA carboxylase (ACC) in skeletal muscle and the liver. The activation of these enzymes contributes to reduction of glycaemia by increasing glucose uptake in the tissues (Farese et al., 2005).

2. Materials and methods

2.1. Plant material and extraction

Bark from the stem of *Caesalpinia ferrea* Mart. Ex Tul. was collected from the Amazon Research National Institute (INPA) experimental culture, in the Brazilian State of Amazonas (03°05′48.0″S and 59°59′55.0″W.Gr). The voucher specimen of the plant was deposited at the INPA herbarium under number 228022. The bark was collected in September 2009, March 2010 and September 2010, with approximately 3 kg being harvested on each occasion. The material collected was dried, first at room temperature for 48 h, and then taken to an oven with circulating air at a temperature of 45 ± 2 °C until its weight stabilized. Subsequently, the material was ground in a 1 mm mesh knife mill, thereby providing the raw material (MPV). The aqueous extract of *Caesalpinia ferrea* was prepared by raw material infusion (7.5:100, w/v) using boiling distilled water as the extractive solvent for a period of 15 min. The aqueous extract presented a solid soluble content of 0.6 ± 0.02 g%. The aqueous extract was dried using a MSD 1.0 Labmaq Mini Spray Dryer. The drying process was performed using the following parameters: inlet temperature of 120 °C, compressed air pressure of 2 bar, diameter rotor of 0.7 mm and power flow of 10 mL/min. The yield of dry extract after drying of aqueous extract was 98%, representing 5.88 g of dry extract per liter of aqueous extract of *Caesalpinia ferrea.*

2.2. Phytochemical analysis of Caesalpinia ferrea

2.2.1. Thin layer chromatographic (TLC) analysis of Caesalpinia ferrea

The methods described by Wagner and Bladt (1996) were used to screen the dried bark extract for the hydrolysable tannins (gallic and ellagic acids), condensed tannins (catechins), flavonoids, saponins, coumarins, phenylpropanoids, cinnamic acid derivatives, alkaloids, triterpenoids/steroids, monoterpenes, sesquiterpenes, iridoids, sugars and luteolin. The phytochemical profile was drawn up using thin layer chromatography (TLC) on silica gel plates (Merck® art. 105553, UV 250–366 nm) using the appropriate mobile phase, reagents and standards.

2.2.2. Estimation of total tannin content in Caesalpinia ferrea

The total tannin content was determined using the spray dried extract (SDE) of *Ceasalpinia ferrea* aqueous extract at 7.5% (w/v), by way of the difference between redissolved SDE before and after precipitation with 150 mg of casein (Merck® Germany). The measurements were performed at 270 nm and the total tannin content was calculated as gallic acid (mg/g of SDE). The results represent the mean of three measurements.

2.2.3. High performance liquid chromatography (HPLC) analysis of Caesalpinia ferrea

The main phytochemical markers (gallic acid, ellagic acid, catechin and epicatechin) were quantified by way of LC-DAD analysis using a Shimadzu system (LC-20AT) equipped with a photo diode array detector (SPD-M20A). The chromatographic separation was performed using a Gemini RP-18 column 240 × 4 mm i.d. (Phenomenex), protected by a pre-column packet of the same material. A gradient elution was performed by varying the proportion of solvent B (methanol) to solvent A (acetic acid 0.5%, w/w) at a flow rate of 0.8 mL/min, according to the following gradient program: 20–40% B (10 min), 40–60% B (10 min), 60% B (10 min), 60–40% B (10 min), and 40–20% B (10 min). The SDE of *Caesalpinia ferrea* and standard were dissolved in methanol:water (20/80, v/v) and filtered through a 0.45 μm membrane (Millipore®, USA) prior to injection of 20 μL.

The peaks of each marker substance in the dried extract were initially identified by comparing the retention time and UV-spectrums. After that, the peaks were confirmed by spiking the sample with a small amount of the standards.

2.3. Animals

Male Wistar rats (*Rattus norvegicus* var. *albinus*) (aged 2 months, weighing 280–300 g) were obtained from the Department of Physiology and Pharmacology at the Federal University of Pernambuco (UFPE), Pernambuco, Brazil. The animals were kept under standard environmental conditions (22 ± 2 °C); 12:12 h dark/light cycle. Water and industrialized dry food (Labina®, Purina, Brazil) were made available *ad libitum*. The experimental protocol was approved by the Animal Experimentation Ethics Committee of UFPE (Process no. 01411), in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals.

2.4. Induction of experimental diabetes

Diabetes was induced using streptozotocin (STZ) from Sigma–Aldrich®, St. Louis, MO, USA. The animals fasted overnight and diabetes was induced by way of a single intra peritoneal injection of a freshly prepared solution of STZ (50 mg/kg b.w.) in a 0.1 M citrate buffer (pH 4.5). On the third day of STZ-injection, the animals with fasting glycaemia higher than 200 mg/dL and with signs of polyuria and polydipisia were considered to be diabetic and included in the study.

2.5. Diabetic animals

2.5.1. Treatment

In the experiment, the animals were randomly divided into five groups ($n = 7$/group). Group 1 (NDC-non-diabetic control) and group 2 (DC-diabetic control) consisted of rats treated with vehicle (water); group 3 (MTD-diabetic rats treated with metformin 500 mg/kg/day b.w.), groups 4 and 5 (diabetic rats treated with