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Effect of the extracts and fractions of *Baccharis trimera* and *Syzygium cumini* on glycaemia of diabetic and non-diabetic mice

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Abstract

In the present study, we investigated the effects of extracts and fractions of *Baccharis trimera* and *Syzygium cumini* on glycaemia of diabetic and non-diabetic mice. Crude ethanolic extracts and aqueous and butanolic fractions of the aerial parts *of Baccharis trimera* and leaves of *Syzygium cumini* were evaluated. None of the extracts or fractions (200 or 2000 mg/kg, per os) induced any effect after acute administration. Seven-day treatment with crude ethanolic and aqueous and butanolic fractions (200–2000 mg/kg, twice daily, per os) of *Syzygium cumini* reduced glycaemia of non-diabetic mice. However, this effect was associated with a reduction of food intake and body weight, indicating that this may not be a genuine hypoglycaemic effect. In diabetic mice, only the aqueous fraction of *Baccharis trimera* (2000 mg/kg, twice daily, per os) reduced the glycaemia after a 7-day treatment. This effect was not associated with a body weight reduction. The results suggest that *Baccharis trimera* presents a potential antidiabetic activity and indicate that food intake and body weight must be determined when evaluating metabolic parameters after prolonged administration of plant extracts.

Keywords: *Baccharis trimera*; *Syzygium cumini*; Tannins; Glycaemia; Diabetes; Antidiabetic

1. Introduction

Plants have been used as sources of drugs for the treatment of diabetes in developing countries where the cost of the conventional medicines represents a burden to the population. Many species have been reported to present antidiabetic activity (Grover et al., 2002). *Baccharis trimera* (Less.) D.C. and *Syzygium cumini* (L.) Skeels (synonym: *Eugenia jambolana* Lam), from the Myrtaceae and Asteraceae families, respectively, are among the most commonly medicinal plants used to treat diabetes in Brazil. Although decoction, infusion and tincture of *Baccharis trimera* have been widely used to treat diabetes in Brazil, there is no report of their antidiabetic activity either in experimental animals or in humans. Furthermore, other species of the genus *Baccharis* may present marked toxicity (Jarvis et al., 1996; Varaschin and Alessi, 2003), raising concerns about the use of plants from this genus. On the other hand, the potential antidiabetic effect of *Syzygium cumini* has been previously evaluated. Both the aqueous and ethanolic extracts from the seeds reduced the glycaemia of diabetic animals (Prince et al., 1998; Grover et al., 2000; Sharma et al., 2003), an effect that may be associated with some inorganic constituents (Ravi et al., 2004). However, the decoction of leaves has neither presented antidiabetic activity in rats (Teixeira et al., 1997; Teixeira et al., 2000; Pepato et al., 2001) nor altered the glucose tolerance test in non-diabetic humans (Teixeira et al., 2000). Although the decoction is widely used by the population, its preparation may inactivate substances with potential pharmacological activity and may have contributed to the reported lack of effect in the last studies.

In the present study, we evaluated the potential antidiabetic effect of the crude ethanolic extract of *Baccharis trimera* and *Syzygium cumini* as well as the aqueous and butanolic fractions of these extracts. In addition, we evaluated if any

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change in the glycaemia induced by a prolonged treatment with these extracts or fractions could be associated with a change of food intake and body weight.

2. Materials and methods

2.1. Plant material

Aerial parts of *Baccharis trimera* and leaves of *Syzygium cumini* were collected in Lagoa Formosa (MG, Brazil) and identified by J.R. Stehmann, Department of Botany, Federal University of Minas Gerais (BHCB 64921 and BHCB 46216, respectively).

2.2. Preparation of extracts and fractions

The plant material was dried, powdered and defated with hexane and then with ethanol 70%. The extracts were evaporated to dryness at 40 °C. A part of this crude ethanolic extract was used in the experiments. The other part was resuspended in water and successively extracted with dichloromethane and *n*-butanol. The fractions were evaporated to dryness at 40 °C and the aqueous fractions were lyophilised. The crude ethanolic extracts (EE), aqueous (AF) and butanolic fractions (BF) of *Baccharis trimera* and *Syzygium cumini* were used in the experiments.

2.3. Animals

Female Swiss mice with 22–27 g were used. The animals were maintained on a 12:12 h light–dark cycle and food and water were available ad libitum. Experiments were performed in accordance with the guidelines specified by the Ethics Committee on Animal Experimentation of the Federal University of Minas Gerais. We used five to seven animals in each experimental group throughout the study.

2.4. Administration of extracts, fractions and drugs

Solutions or suspensions of extracts and fractions were prepared in saline immediately before the experiments. Tannic acid (TA), a mixture of gallotannin and tannin (Sigma, USA), was also suspended in saline. Solutions or suspensions were administered per os in a volume of 100 μl/25 g. Streptozotocin (200 mg/kg, Sigma, USA) and human NPH insulin (2 U/kg, Biobrás, Brazil) were injected intravenously and subcutaneously, respectively.

2.5. Determination of glycaemia

Mice were decapitated and blood was collected in tubes containing 50 μl of Na_2EDTA:NaF (60/30 mg in 1 ml of distilled water). Glycaemia was determined by an enzymatic method (glucose oxidase and aminoantipyrine – Labtest, Brazil).

2.6. Determination of body weight and food consumption

Daily food consumption of the whole experimental group and body weight of each animal treated with the extracts, fractions or TA were determined daily for 7 days.

2.7. Experimental protocols

2.7.1. Acute treatment of non-diabetic mice

Two doses of the extracts or fractions (200 and 1000 or 200 and 2000 mg/kg, depending on the protocol) or insulin were administered, food was removed and glycaemia was determined 3 h after.

2.7.2. Seven-day treatment of non-diabetic mice

Two daily doses (08:00 and 18:00 h) of the extracts or fractions (200, 1000, 1500 or 2000 mg/kg, depending on the protocol) or TA (50 or 500 mg/kg) were administered and glycaemia determined 3 h after the last dose. The first dose of the extracts, fractions or TA was administered at 18:00 h on day 1 and the last dose was administered at 08:00 h on day 8. Food was removed immediately after the last dose. One experimental group was treated with insulin in the last day and glycaemia was determined 3 h later.

2.7.3. Seven-day treatment of diabetic mice

Streptozotocin (200 mg/kg) was dissolved in buffer citrate (pH 4.5) and injected i.v. In this protocol, the animals were treated with the extracts or fractions (200, 1500 or 2000 mg/kg, depending on the protocol) for 7 days, two daily doses (08:00 and 18:00 h), with the first dose administered 24 h after the treatment with streptozotocin. The treatment with the extracts, fractions and insulin and also glycaemia analysis were carried out as described for the non-diabetic mice.

2.7.4. Acute treatment of non-diabetic mice submitted to glucose overload

To evaluate the effect of the extracts, fractions or TA on the elevation of glycaemia induced by the per os administration of glucose, non-diabetic mice were fasted for 15 h, before treatment. The extracts, fractions or TA were administered 30 min before glucose (1000 mg/kg) and glycaemia determined 1 h after.

2.8. Chemical characterization of extracts and fractions

The assays were performed using a HPLC Hewlett Packard model 1100, with a DAD detector. Column: RP-18 Chromolith 100 × 4.6 mm (Cat. 1.02129.0001, Merck). The eluent was acetonitrile:water (EM Science, AX0142-1), with gradient of 10–90% of acetonitrile in 30 min. Detection: UV spectra at 254 nm. Injection volume: 20 μl. Each sample (1 ml) was evaporated to dryness at less than 50 °C, dissolved in acetonitrile 20%, filtered (Minisart RC-15, 0.45 μm,