Exercício –sala de aula:

Comentar sobre e criticar o material and methods deste trabalho.

Lack of antidiabetic effect of a *Eugenia jambolana* leaf decoction on rat streptozotocin diabetes

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Abstract

Streptozotocin-diabetic rats were treated for 17 days with a decoction of *Eugenia jambolana* (Myrtaceae) leaves (15%, w/v) as a substitute for water. Body weight, food and fluid intake, urine volume, glycemia, urinary glucose and urea were evaluated every 5 days. The animals were sacrificed by decapitation and blood samples collected for the determination of glycemia, serum cholesterol, HDL-cholesterol, triglycerides and angiotensin-converting enzyme. The weight of adipose and muscle tissues was also determined. There were no statistically significant differences between treated and untreated rats for any of the biochemical or physiological parameters. We conclude that, at least in this experimental model, *Eugenia jambolana* leaf decoction has no antidiabetic activity.

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Introduction (parte)

*Eugenia jambolana* is frequently used for the treatment of diabetes, and it has been shown that the bark, fruits, seeds or leaves of this plant collected from diverse regions of the world and administered in different pharmaceutical preparations (e.g., tinctures and aqueous extracts) decrease blood glucose levels in diabetic animals (17-22). Also, infusions (simple aqueous extracts prepared with hot water but without boiling) and decoctions (boiled infusions) of E. jambolana have been used in popular medicine for the treatment of diabetes mellitus.

In a southern Brazilian study, most Jambolão users interviewed stated that they either infused or decocted leaves of both E*. jambolana* and *S. jambos* in water at an average concentration of 2.5 g/l and drank it in place of water at a mean daily intake of about 1 liter. It was found, however, that *S. jambos* collected in southern Brazil and prepared by these methods did not affect the glycemia of normal individuals (5). Subsequent studies on normal and streptozotocin-diabetic rats have also shown that aqueous extracts and decoctions of southern Brazilian *E. jambolana* have no hypoglycemic effects (2,23).

The majority of the studies cited above in which the presence or absence of hypoglycemic effects was studied were restricted to the evaluation of these effects in animals or nondiabetic humans. Moreover, studies of the effects of chronic treatment with plant extracts are infrequently found in the literature. Because of this, and the contradictory reports appearing in the rare studies which have been carried out with Brazilian Eugenia, the present study with streptozotocin-diabetic rats was carried out to investigate the effects of subchronic administration of *E. jambolana* leaf decoction on several metabolic parameters usually altered in diabetes mellitus.

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Material and Methods

Plant material and decoction preparation

A tree located in the Medicinal Plants Garden of the School of Pharmacy, Araraquara, SP, Brazil, was identified as *Eugenia jambolana* by Dr. Gilberto Dolejal Zanetti and authenticated material was deposited in the Herbarium of the Department of Industrial Pharmacy, Federal University of Santa Maria, Santa Maria, RS, Brazil, under accession No. 118. Leaves from this tree were collected in September and October (the end of winter/beginning of spring in southeast Brazil) and used to prepare a decoction according to the recipe used by the local population of Araraquara, i.e., boiling 150 g of fresh leaves in 1 liter of water for 5 min, allowing the decoction to stand for 30 min and filtering through simple filter paper. Such procedure resulted in a decoction with a 15% higher concentration than that produced by the method described by Teixeira et al. (5). A fresh decoction was prepared every 2 or 3 days and kept in dark bottles at 4oC.

Animals and treatment

A group of male Wistar rats were adapted to metabolic cages for 2 or 3 days. The animals were then anesthetized with ethyl ether and 40 mg/kg body weight streptozotocin (STZ) dissolved in 0.01 M citrate buffer, pH 4.5, was injected into the jugular vein. The rats were fasted for 14-16 h and their mean weight was 158 ± 2 g. Another group of rats weighing 112 ± 4 g were treated in the same way except that they received STZ at the dose of 60 mg/kg body weight because young rats proved to be more resistant than older ones to the diabetogenic action of STZ (24). All rats were returned to their metabolic cages where they had free access to water and food, being housed under a 12:12-h light/dark cycle at 22-25oC. The animals were fed a normal laboratory chow diet containing (w/w) 16% protein, 66% carbohydrate and 8% fat. All experimental protocols were approved by the Ethics Committee.

Decoction administration

Three days after STZ (40 mg/kg body weight) administration, the STZ group had their body weight, plasma glucose (339-387 mg/dl), urinary glucose and food intake measured and these parameters were used to obtain matched pairs of rats with a similar degree of diabetes. One rat in each pair was randomly assigned to a group to be treated with *E. jambolana* decoction (treated group) and the other to an untreated (control) group. Five days after STZ injection, animals in the treated group received *E. jambolana* decoction in place of water, while the control group received water. Body weight, food and liquid intake, urine volume, plasma and urinary glucose were measured every 5 days at about 9 a.m. During the experiment blood samples for plasma glucose were collected from the tip of the tail. The rats were sacrificed by decapitation 22 days after STZ injection (17-day treatment with *E. jambolana* decoction) when free running blood was collected for the determination of plasma glucose and serum cholesterol, HDL-cholesterol, triacylglycerol and angiotensin-converting enzyme (ACE). The epididymal fat pad and retroperitoneal adipose tissue overlying the psoas, all pancreatic tissue and the soleus and extensor digitorum longus (EDL) muscles were removed and weighed.

Insulin administration

Five days after STZ (60 mg/kg body weight) injection, body weight, food and liquid intake, urine volume, plasma and urinary glucose and urinary urea were measured. The rats were treated twice a day (9 a.m. and 6 p.m.) with subcutaneous injections of 3 units of NPH insulin (Biohulin N U-100, BioBRÁS, Montes Claros, MG, Brazil) for 17 days, after which the same metabolic parameters were again evaluated. These insulin-treated rats served as a further control for the experimental model.

Chemical and statistical analysis

Urinary glucose was determined by the o-toluidine method (25) and urinary urea by a modified diacetyl method (26). ACE was determined by a spectrophotometric assay (27). Plasma glucose and serum cholesterol, HDL-cholesterol and triglycerides were determined with an autoanalyzer (Bayer Technicon RA-100, Tarrytown, NY, USA). Data were analyzed by randomized ANOVA and the paired and unpaired Student t-test (GB-STAT program, version 5.0), with the level of significance set at P<0.05.