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Acute and sub-chronic (28 days) oral toxicity evaluation of tincture *Baccharis trimera* (Less) Backer in male and female rodent animals

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ABSTRACT

The infusion of *Baccharis trimera* (Less) DC, popularly known as "carqueja" (broom), is popularly used in the treatment of hepatic and digestive problems. In this study, we evaluated the acute and sub-chronic oral toxicities of *B. trimera* tincture on male and female Wistar rats according to Organization for Economic Cooperation and Development (OECD, guidelines 423 e 407, respectively). The *B. trimera* tincture was administered by oral gavage in a single dose (2000 mg/kg) in doses of 100, 200 and 400 mg/kg daily for 28 days. Blood was collected to analyze hematological and biochemical parameters. Kidneys and liver were homogenized to determine lipid peroxidation and δ-aminolevulinate dehydratase (δ-ALA-D) and catalase (CAT) enzyme activities. In acute treatment, tincture did not induce any signs of toxicity or mortality. Daily oral administration produced no significant changes in the hematological and biochemical parameters, except for the hepatic enzymes alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) that showed a reduction in both sexes. Moreover, the *B. trimera* tincture did not increase lipid peroxidation or affected ALA-D and CAT activities. In conclusion, the tincture of *B. trimera* may be considered relatively safe in this protocol.

1. Introduction

The use of plants for the treatment, cure and prevention of diseases is one of the oldest medicinal practices of humanity. All information on medicinal plants wasorally transmitted to subsequent generations, then, with the advent of writing, it began to be saved as a precious treasure (Silva et al., 2014; Cunha, 2005). Although the plants may be efficient, the toxicity of preparations used is usually unknown and the population does not believe that the material or the preparation may cause harm. However, toxicology tests show that many plants currently used are highly toxic when ingested (Atsamo et al., 2011). Therefore, the great biodiversity of Brazilian plants used without scientific support encourages researchers to study the toxicity of native plants in other to determine their efficacy and safety (Brazil, 2012).

Baccharis trimera (Less) DC, popularly known as "carqueja" (broom), belongs to the Asteraceae family and is an erect, branchy, glabrous subshrub, with up to 80 cm of height. Native to South America, it is cultivated mainly in Brazil, Argentina, Paraguay and Uruguay. Its infusion is popularly used in the treatment of hepatic and digestive problems (Gene et al., 1992; Verdi et al., 2005; Abad and Bermejo, 2007).

Many biological activities have been described for *B. trimera*, such as anti-inflammatory, analgesic, anti-hepatotoxic, muscle relaxant effects, antioxidant, antidiabetic, and anthelmintic activity. (Gené et al., 1996; Torres et al., 2000; Oliveira et al., 2005; Borella et al., 2006; Dickel et al., 2007; Paul et al., 2009; Nogueira et al., 2011; Oliveira et al., 2012, 2014). The chemical composition of *B. trimera* reportedly consists of flavones, flavonols, saponins and diterpenes (Herz et al., 1977; Gosmann et al., 2010; Vieira et al.,

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2011). However, there are no studies on the chemical properties and effects of toxicity in the *B. trimera* tincture. Thus, the aim of this study is to evaluate the phytochemical characterization, as well as the toxicological profile of the *B. trimera* tincture after a single oral administration (acute) or 28 consecutive daily administrations (sub-chronic).

2. Materials and methods

2.1. Plant material

The tincture of *B. trimera* used in the experiments was purchased from Flores e Ervas Com. Farm. Ldta. (Piracicaba, São Paulo, Brazil) in 2014, registered under the number NPT.0113/0 (Farm. Resp.: Paula Mariana Pezzatti). The leaves of *B. trimera* were triturate and macerated with 63% ethanol solution.

2.2. Chemical, apparatus and general procedures

All chemicals were of analytical grade. Methanol, phosphoric acid, gallic acid, chlorogenic acid, caffeic acid and ellagic acid were purchased from Merck (Darmstadt, Germany). Quercetin, quercitrin, rutin, luteolin and kaempferol were acquired from Sigma Chemical Co. (St. Louis, MO, USA). High performance liquid chromatography (HPLC-DAD) was performed with a Shimadzu Prominence Auto Sampler (SIL-20A) HPLC system (Shimadzu, Kyoto, Japan), equipped with Shimadzu LC-20AT reciprocating pumps connected to a DGU 20A5 degasser with a CBM 20A integrator, SPD-M20A diode array detector and LC solution 1.22 SP1 software.

2.3. Quantification of compounds by HPLC-DAD

Reverse phase chromatographic analyses were carried out under gradient conditions using C_{18} column (4.6 mm × 250 mm) packed with 5 μm diameter particles; the mobile phase was water containing 2% phosphoric acid (A) and methanol (B), and the composition gradient was: 17% of B until 10 min and then changed to obtain 20%, 30%, 50%, 60%, 70%, 20% and 10% of B at 20, 30, 40, 50, 60, 70 and 80 min, respectively, following the method described by Klimaczewski et al. (2014) with slight modifications. The *B. trimera* tincture was analyzed at a concentration of 25 mg/ml. The presence of nine antioxidants compounds was investigated, namely, gallic acid, chlorogenic acid, caffeic acid, ellagic acid, quercetin, quercitrin, rutin, luteolin and kaempferol. The identification of these compounds was performed by comparing their retention time and UV absorption spectrum with those of the commercial standards. Flow rate was 0.6 ml/min, injection volume was 40 μl and wavelength was 270 nm for gallic acid and ellagic acid; 325 nm for caffeic acid and chlorogenic acid; and 365 nm for quercetin, quercitrin, rutin, kaempferol and luteolin.

The samples of the mobile phase were filtered through 0.45 μm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use. Stock solutions of standards references were prepared in the HPLC mobile phase at a concentration range of 0.030–0.250 mg/ml for quercetin, quercitrin, rutin, kaempferol and luteolin; and 0.020–0.300 mg/ml for gallic, caffeic, ellagic and chlorogenic acids. The chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200–600 nm). Calibration curve for gallic acid: $Y = 13479x + 1175.8$ ($r = 0.9997$); caffeic acid: $Y = 11965x + 1285.3$ ($r = 0.9999$); chlorogenic acid: $Y = 12649x + 1304.7$ ($r = 0.9995$); ellagic acid: $Y = 13582x + 1196.5$ ($r = 0.9998$); quercetin: $Y = 13087x + 1265.3$ ($r = 0.9999$); quercitrin: $Y = 11678x + 1264.6$ ($r = 0.9998$); rutin: $Y = 11912x + 1345.9$ ($r = 0.9997$); kaempferol: $Y = 13638x + 1195.4$ ($r = 0.9999$) and luteolin $Y = 12537x + 1271.2$ ($r = 0.9996$). All chromatography operations were carried out at ambient temperature and in triplicate. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated based on the standard deviation of the responses and the slope using three independent analytical curves, as defined by Abbas et al. (2014). LOD and LOQ were calculated as 3.3 and 10 σ/S, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve.

2.4. Animals

Adult male and female Wistar rats, 6- to 8-weeks old, weighing 160–200 g, were supplied by the production facility of the Biotério Central da Universidade Federal de Santa Maria (UFSM). The animals were segregated according to gender and were allowed to adjust to the new environment for 5 days before the study started. All rats were housed in polypropylene cages, at an ambient temperature of 24 °C ± 2 °C and 45–55% relative humidity, with a 12:12 h light/dark cycle. They were provided with commercial food pellets and water *ad libitum* unless otherwise stated. The animals were handled and the experiments carried out in conformity with the Ethics and Animal Welfare Committee of UFSM (CEUA UFSM; Protocol 050/2014).

2.5. Acute toxicity study

The acute oral toxicity study was performed as per the Organisation for Economic Cooperation and Development (OECD) 423 guidelines (OECD, 2001). Rats of either sexual category ($n = 6$) were divided into two groups, i.e., control and test groups. Control group received ethanol 63% as vehicle while the test group received an oral dose of 2000 mg/kg tincture in the *B. trimera*. The dose used in the experiment of acute toxicity was selected from one of four fixed levels, 5, 50, 300, or 2000 mg/kg, as described in the OECD guide.

All the experimental animals were observed for their mortality and clinical signs of toxicity (general behavior, respiratory patterns, cardiovascular signs, motor activities, reflexes and changes in skin and fur texture) at 30 min, 2, 6 and 12 h and thereafter once a day for 14 days following the treatment. Body weights and food consumption were recorded daily throughout the study period. On the 15th day, the rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.), received tramadol analgesia (8 mg/kg i.p.) and euthanized by cardiac puncture and subjected to gross pathological examination of all the major internal organs such as brain, heart, lung, liver, kidney, spleen, adrenals and gonads.

2.6. Sub-chronic 28 days study

The sub-chronic 28 days study was performed according to the OECD guideline 407 (OECD, 2008) with some modifications. In the present study, tincture in the *B. trimera* was administered with a gavage at three different doses, i.e., at 100, 200 and 400 mg/kg/day for a period of 28 consecutive days. Group I served as control and received 63% ethanol orally at a dose of 10 ml/kg bwt for the same period. Both sexes of rats were divided in to 4 groups with 10 animals (5 males + 5 females) in each.

The animals were maintained on an *ad libitum* diet and tap water throughout the test, with body weight and food consumption monitored weekly, and behavioral and clinical signs daily. On day 29 of the study, all the animals were anesthetized with pentobarbital sodium (50 mg/kg, i.p.), received tramadol analgesia (8 mg/kg i.p.) and euthanized by cardiac puncture. The blood samples were collected in two tubes: one with anticoagulant, EDTA, and the other without anticoagulant. Afterwards, the rats were euthanized and the kidney and liver were removed and rapidly homogenized in