Journal of Ethnopharmacology 191 (2016) 115–124

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Tabernaemontana catharinensis ethyl acetate fraction presents antinociceptive activity without causing toxicological effects in mice

Evelyne da Silva Brum [a], Laís da Rosa Moreira [a], Andreia Regina Haas da Silva [b], Aline Augusti Boligon [c], Fabiano Barbosa Carvalho [d], Margareth Linde Athayde [c], Ricardo Brandão [b], Sara Marchesan Oliveira [a,d,*]

[a] *Laboratory of Neurotoxicity and Psychopharmacology, Center of Natural and Exact Sciences, Federal University of Santa Maria, Santa Maria, RS, Brazil*
[b] *Laboratory of Toxicological Analysis, Pharmaceutical Sciences Graduate Program, Center of Health Sciences, Federal University of Santa Maria, Santa Maria, RS, Brazil*
[c] *Laboratory of Phytochemistry, Pharmaceutical Sciences Graduate Program, Center of Health Sciences, Federal University of Santa Maria, Santa Maria, RS, Brazil*
[d] *Department of Biochemistry and Molecular Biology, Graduate Program in Biological Sciences: Biochemical Toxicology, Center of Natural and Exact Sciences, Federal University of Santa Maria, Santa Maria, RS, Brazil*

ARTICLE INFO

Article history:
Received 15 January 2016
Received in revised form
5 May 2016
Accepted 13 June 2016
Available online 15 June 2016

Studied compounds:
Capsaicin (CID: 1548943)
ethyl acetate (CID: 8857)
formalin (CID: 712)
glutamate (CID: 33032)
morphine (CID: 5288826)
MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (CID: 64965)
naloxone (CID: 5284596)
sodium diclofenac (CID: 5018304)

Keywords:
Apocynaceae
Antinociception
Inflammatory pain
Cobrina

ABSTRACT

Ethnopharmacological relevance: *Tabernaemontana catharinensis* (Apocynaceae) is a medicinal plant used for the treatment of painful and inflammatory disorders. Here, we investigated the antinociceptive potential of the ethyl acetate fraction (Eta) from *T. catharinensis* leaves and assessed its toxic effects in mice to validate its popular use.

Materials and methods: Adult male Swiss mice (30–35 g) were used. The Eta antinociceptive effect (200–800 mg/kg, oral route (p.o.)) was evaluated in the acetic acid, formalin, capsaicin and tail-immersion tests. Adverse effects were analyzed using rotarod and open-field tests, body temperature, biochemical analysis and gastric lesions assessment. To evaluate the acute (OECD 423) or sub-acute (OECD 407) toxicity of the Eta, it was administered orally at a single (2000 mg/kg) or repeated doses (100–400 mg/kg/day for 28 days), respectively. Mortality, behavioral changes, biochemical and hematological parameters were evaluated. The Eta effect on cellular viability also was evaluated.

Results: Eta (200–800 mg/kg) inhibited the nociception caused by acetic acid ($93.9 \pm 1.5\%$), formalin ($86.2 \pm 10.8\%$) or capsaicin ($75.4 \pm 3.3\%$) without inducing gastric lesions. Moreover, Eta neither altered the body temperature, biochemical parameters, nor forced or spontaneous locomotor activity of mice. The acute administration of the Eta (2000 mg/kg) promoted a decrease in blood glucose levels and alanine aminotransferase activity. In the sub-acute toxicity study, Eta increased the aspartate aminotransferase activity (400 mg/kg) and platelet distribution width (200 mg/kg). Furthermore, Eta did not alter the cellular viability in cortical slices.

Conclusions: Eta presents antinociceptive effects and mild toxicity in mice. These results support its traditional use as a potential analgesic.

© 2016 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Medicinal plants have been used almost in all cultures as sources of medicine to treat, cure or prevent diseases (Melo et al., 2013; Silva et al., 2012). Most of these plants have been evaluated using experimental animal models (Al-Sayed and El-Naga, 2015; Silva et al., 2013) as well as in humans (Dapas et al., 2014) in order to confirm their activities. Such extracts have shown great importance to research and consequently to the development of new drugs. However, products derived from plants are used with a misconception that they did not cause toxic or adverse effects, but when used in exaggerated form may be harmful (da Silva et al., 2014).

Literature data shows that several plant extracts are commonly used for the treatment of painful and inflammatory conditions

Abbreviations: Eta, ethyl acetate fraction, limit of detection; LOQ, limit of quantification; GLU, glucose; AST, aspartate aminotransferase; ALT, alanine aminotransferase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; LDH, lactate dehydrogenase; NSAIDs, non-steroidal anti-inflammatory drugs

* Correspondence to: Graduate Program in Biological Sciences: Biochemical Toxicology, Center of Natural and Exact Sciences, Federal University of Santa Maria, Camobi, 97105-900 Santa Maria, RS, Brazil.
E-mail address: saramarchesan@hotmail.com (S.M. Oliveira).

http://dx.doi.org/10.1016/j.jep.2016.06.036
0378-8741/© 2016 Elsevier Ireland Ltd. All rights reserved.

(Melo et al., 2013; Silva et al., 2012, 2013). Pain is one of the most prevalent conditions that limits productivity and reduces the quality of patient's life. Although there is an arsenal of effective and widely used analgesics, some concern regarding their safety, side effects and abuse potential, make their clinical use problematic (Cazacu et al., 2015). As many people are now choosing alternative medicine for treatment (Silva et al., 2012), it is interesting to investigate the biological effects of these plants and verify their possible adverse effects in order to prove their popular use and to provide an improved safety profile for the patients.

According to folk medicine, *Tabernaemontana catharinensis* A. DC., previously classified as *Peschiera fuchsiaefolia,* belongs to the Apocynaceae family and it is used for the treatment of various disorders, including antidote for snake bites, to relieve toothache, and as a vermifuge (Spitzer et al., 1995). It is popularly known as "jasmim" (jasmine), "leiteira de dois irmãos" (milkweed), "cobrina" or "casca de cobra" (snake skin). Other biological activities of *T. catharinensis* include antioxidant, antiviral, antimicrobial, trypanocidal and antileishmanial activities (Boligon et al., 2014, 2013a).

Previous studies showed that the crude extracts from *T. catharinensis* seeds and leaves produced in vivo antineoplastic activity and the aqueous or alcoholic extracts from its leaves or stem barks produced significant dose-related anti-inflammatory and analgesic effects in animal models (Gomes et al., 2009; Rates et al., 1993). The biological activities of *T. catharinensis* were attributed to its polyphenol and flavonoid compounds (Boligon et al., 2014). Until now, there are not scientific preclinical studies supporting the traditional use of ethyl acetate fraction (Eta) of *T. catharinensis* leaves, neither its analgesic activity nor its security and its possible toxic effect. The aim of this study was to investigate the Eta antinociceptive effects in screening tests for new analgesics in mice, the possible acute and sub-acute toxicity of the Eta through hematological and biochemical parameters, and determine its effects on cellular viability.

2. Materials and methods

2.1. Plant collection, extraction and fraction preparation

The leaves of the *T. catharinensis* were collected in Bossoroca (Rio Grande do Sul, Brazil) in September of 2009 (coordinates 28°65′93″ S and 55°01′27″ W). A dried voucher specimen is preserved in the herbarium of the Department of Biology at Federal University of Santa Maria (register number SMBD 12,355). The parts of the plant were dried at room temperature and powdered in a knife mill. The leave powder (1580.02 g) was macerated at room temperature with ethanol 70% for a week with daily shake-up. After filtration, the crude extract was evaporated under reduced pressure to remove the ethanol. The extract was suspended in water and partitioned with ethyl acetate to obtain the ethyl acetate fraction (Eta).

2.2. Drugs, reagents, apparatus and general procedures

Acetonitrile and phenolic acids were purchased from Merck (Darmstadt, Germany). Flavonoids and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained 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. The laboratorial kits used for biochemical analysis were acquired from Wiener Laboratórios SAIC (São Paulo, São Paulo, Brazil) and were used in a semi-automatic biochemical analyzer (Genz, Bioplus: Bio-2000). Morphine sulfate, naloxone (Cristália, São Paulo, Brazil), sodium diclofenac and capsaicin were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of analytical grade and were purchased from local suppliers.

2.3. HPLC analysis

The polyphenol analysis of the Eta of *T. catharinensis* leaves was carried out under gradient conditions using C18 column (4.6 mm × 250 mm) packed with 5 μm diameter particles. The mobile phase was constituted by solvent A=water: acetic acid (98:2 v/v) and B=acetonitrile. The gradient program was started with 95% of A and 5% of B until 2 min and changed to obtain 25%, 40%, 50%, 60%, 70% and 80% B at 10, 20, 30, 40, 50 and 80 min, respectively, following the method described by Boligon et al. (2012) with modifications. The flow rate was 0.7 ml/min and the injection volume was 40 μl. Detection was performed with four wavelengths, 254 nm for gallic acid, 280 nm for catechin, 327 nm for caffeic, *p*-coumaric, and chlorogenic acids, and 365 for quercitrin, quercetin, rutin and kaempferol. The mobile phase was filtered through a membrane filter 0.45 μm and then degassed by an ultrasonic sound before use. The fraction and standards solutions were prepared in the acetonitrile: water (1:1, v/v). Standards calibration curves were constructed at the concentration range of 0.015–0.300 mg/ml. The chromatographic peaks were confirmed by comparing their retention time with those of reference standards and by DAD spectra; the quantification was performed by peak integration using the external standard method. All chromatography operations were carried out at ambient temperature and in triplicate. The method sensitivity was assessed by calculating the limit of detection (LOD) and limit of quantification (LOQ), using the following formulas: $LOD=(3.3 \times SD)/S$; $LOQ=(10 \times SD)/S$. Both standard deviation (SD) and slope (S) values were derived from three independent analytical curves, whereby SD corresponds to the standard deviation of the analytical response and the S value represents the slope of the calibration curve (Boligon et al., 2013b).

2.4. Animals

The experiments were carried out on adult male Swiss mice (30–35 g) obtained from the animal house of the Federal University of Santa Maria. All mice were kept under constant environmental conditions with 12:12 h light–dark cycle, ambient temperature of 25 ± 1 °C and 45–55% of relative humidity. Mice were fed with commercial food pellets and water ad libitum. The experimental protocols were performed with the approval of the Ethics Committee of the Federal University of Santa Maria (process number 23076.038594/2014-57) and were carried out in accordance with the current guidelines for the care of laboratory animals (Zimmermann, 1983). The number of animals and intensities of noxious stimuli used were the minimum necessary to demonstrate the consistent effects of the treatments. The behavior evaluation was performed blindly with respect to drug administration. A total of 347 mice were used in this study distributed as follows: Acetic acid-induced abdominal writhing test: n=6/group (total 30). Formalin test: n=6/group (total 30). Tail-immersion test: n=5/group (total 25). Evaluation of capsaicin-induced nociception: n=6/group (total 126). Opioid receptor involvement in the Eta antinociceptive activity: n=6/group (total 36). Rotarod and open-field tests and body temperature measurement: n=6/group (total 24). Gastric lesions assessment and biochemical analysis: n=6/group (total 24). Toxicological analysis: n=8/group (total 48). Cellular viability assays: n=4 (total 4).

Download English Version:

https://daneshyari.com/en/article/2544579

Download Persian Version:

https://daneshyari.com/article/2544579

Daneshyari.com