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# Antinociceptive and antidepressant-like effects of the crude extract of *Vitex megapotamica* in rats

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ABSTRACT

*Ethnopharmacological relevance:* *Vitex megapotamica (Spreng) Moldenke* has been used in South American folk medicine to treat inflammatory diseases. However, the effects of *V. megapotamica* on animal models of nociception and depression have not been evaluated.
*Aim of the study:* This study investigated whether the crude leaf extract of *V. megapotamica* exhibits antinociceptive and antidepressant-like effects in a Freund's adjuvant-induced chronic inflammation and depression model.
*Materials and methods:* Chronic inflammation was induced in rats by the intraplantar administration of complete Freund's adjuvant (CFA; 100 μl). The effect of oral crude extract of *V. megapotamica* (VmE; 3–30 mg/kg, p.o.) on nociception (thermal hyperalgesia, mechanical allodynia and arthritis score), inflammation (edema, myeloperoxidase activity), immobility (forced swimming test), locomotor activity (open field), gastrointestinal transit, hyperalgesia and naloxone-precipitated morphine withdrawal syndrome was evaluated. Naloxone (0.4 mg/kg, i.p.) was used to investigate the involvement of opioid system in the currently described effects of VmE.
*Results:* Crude extract caused antinociceptive/antidepressant-like effects in the CFA-induced chronic inflammation model, which was prevented by naloxone. The VmE extract (10 mg/kg, p.o.) did not alter the locomotor activity, gastrointestinal function and inflammatory parameters and did not cause hyperalgesia.
*Conclusion:* *V. megapotamica* induces opioid-dependent antinociception and antidepressant-like effect, without anti-inflammatory activity. The results support the use of VmE as analgesic and antidepressant.


## 1. Introduction

The treatment of chronic inflammatory pain is one of the most challenging activities in medical practice. This occurs because chronic pain is associated with a profound decrease in the quality of life and affects daily activities of the affected patients (Orru et al., 2014). Chronic pain has a negative affective component and is closely related to anxiety and depression. It has been estimated that over 50% of patients who suffer from chronic pain also express clinically diagnosable symptoms of depression (Dworkin and Gitlin, 1991). However, the currently available analgesic drugs are devoid of anxiolytic or antidepressant action. Antidepressants and opioid analgesics are often prescribed in combination for the treatment of these disorders. However, opioid-treatment is often associated with adverse effects such as constipation, tolerance and abuse that limit their long-term utilization (Mao et al., 2011). Thus, the search for new compounds that could treat inflammatory pain and depression is welcome. In this context, plants used in folk

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medicine have been considered an important source of new molecules and therapies (Gautam and Jachak, 2009).

*Vitex megapotamica* (Spreng.) Moldenke, popularly known in Brazil as "tarumã", is a native tree from Brazil, Uruguay, Paraguay and Argentina of the *Lamiaceae* (formerly *Verbenaceae*) family (Vianna and Koehler, 2007). Folk medicine reports that *V. megapotamica* leaf infusion is indicated to treat rheumatism, skin disorders, inflammation, hypercholesterolemia and hyperglycemia (Brandt et al., 2009; Zanatta et al., 2007). In fact, initial preclinical studies have confirmed the presumed hypocholesterolemic and hypoglycemic effects of *V. megapotamica* leaf extracts and also of its ethanolic and butanolic fractions (Brandt et al., 2009; Zanatta et al., 2007). In this context, the following chemical components have been reported in leaves of *V. megapotamica*: anthocyanin glycosides, tannins, catechins, flavonoids (chlorogenic acid), triterpenoids, cardioactive glycosides, coumarins, organic acids and phenols (Brum et al., 2011). However, there is a lack of preclinical studies investigating whether *V. megapotamica* or its components have analgesic and/or anti-inflammatory activity. Therefore, the aim of this study was to investigate the antinociceptive, anti-inflammatory and antidepressant-like effects of the crude extract of *V. megapotamica* (VmE) in animals subjected to CFA-induced inflammation.

## 2. Materials and methods

### 2.1. Plant material

To prepare the plant material, the leaves of *Vitex megapotamica* were collected in Sobradinho, (Rio Grande do Sul, Brazil, coordinates: latitude 29°25′17″ south and longitude 53°01′43″ west) in March 2010. A voucher specimen number SMDB 12.526 was deposited at the Herbarium of the Botany Department, Federal University of Santa Maria (UFSM), Brazil.

### 2.2. Preparation of the extract of V. megapotamica

The crude extract of leaves of *V. megapotamica* (VmE) was obtained by maceration in ethanol/water (70:30 v/v) at room temperature during seven days, filtered and concentrated using a rotary evaporator under reduced pressure and low temperature (Boligon et al., 2013). After evaporation the VmE was obtained.

### 2.3. Analysis of phenolic compounds in VmE

Separations were carried out on a UHPLC 1260 Infinity Binary system (Agilent, Santa Clara, CA, USA), which was able to operate at pressures up to 600 bar. A Zorbax SB-C18 Rapid Resolution HD column (2.1 × 50 mm, 1.8 μm, Agilent) was used at a temperature of 40 °C. The injection volume was 5 μl, and the injected aliquots were acidified to a final concentration of 0.1% acetic acid (v/v). The phenolic compounds were separated using a gradient elution composed of 0.1% acetic acid in water (A) and acetonitrile (B) as the mobile phase at a constant flow rate (0.8 ml $min^{-1}$) according to the following elution program: 8.0% B (0.00–0.10 min); 8.0–25.8% B (0.10–3.45 min); 25.8–54.0% B (3.45–6.90 min); 54.0–100.0% B (6.90–7.00 min); and 100.0% B (7.00–9.00 min). The detection of the phenolic compounds by tandem mass spectrometry (MS/MS) was carried out by using an electrospray ionization source (ESI) as described elsewhere (Faccin et al., 2016).

### 2.4. Drugs and reagents

The following reagents were purchased from Sigma: Complete Freund's Adjuvant (CFA −1 mg/ml of heat killed *Mycobacterium tuberculosis* oil suspension), hexadecyltrimethylammonium bromide (HTAB), 5-(N,N-diethylamino)-pentyl-3,4,5-trimethoxybenzoate (TMB). Naloxone and morphine sulfate were purchased from Cristália, São Paulo, Brazil. All other reagents were of analytical grade and were purchased from local supplier.

### 2.5. Animals

The present study was conducted in accordance with the internationally accepted principles for laboratory animal use and care, and all procedures were approved by the local Ethics Committee (process number 116/2013). The number of animals and the intensity of nociceptive stimuli used were the minimum necessary to demonstrate the consistent effects of drug treatments. The behavior evaluation was performed blindly with respect to drug administration. All experiments were performed using adult male Wistar rats (3 months old; 250–300 g). The animals were housed in a room with controlled temperature (22 ± 1 °C) and a 12 h light/12 h dark cycle with standard lab chow and water *ad libitum*.

### 2.6. Animal treatment

The VmE was dissolved in 5% Tween 80, 20% polyethylene glycol and 75% saline (0.9% NaCl) prior oral administration by gavage (p.o.). VmE was administered at the dose of 3 – 30 mg/kg. Morphine was used as positive control at the dose of 10 mg/kg and administered intraperitoneally (i.p.) (Tonello et al., 2014).

### 2.7. CFA-induced inflammation

The antinociceptive, anti-inflammatory and antidepressant-like activities of VmE were evaluated in rats subjected to the CFA-induced paw inflammation, an animal model of chronic pain. Animals were anesthetized with isofluorane and 100 μl of CFA (1 mg/ml) or saline were injected intraplantarly (i.pl.) in the right hind paw (Rossato et al., 2014). Forty-eight hours after CFA injection, inflammatory and nociceptive parameters were evaluated (Rossato et al., 2014). On the other hand, the antidepressant-like parameter was assessed 7 days after CFA injection (Maciel et al., 2013).

### 2.8. Nociceptive parameters

To investigate the possible effect of VmE (3–30 mg/kg) on the nociceptive changes induced by CFA, we measured mechanical allodynia (defined as pain in response to a non-nociceptive stimulus; Khan et al., 2014), thermal hyperalgesia (defined as an increased pain sensitivity; Khan et al., 2014), and arthritis score. Morphine (10 mg/kg, i.p.) was used as a positive control.

#### 2.8.1. Mechanical allodynia measurement

Rats were placed in cages with a wire mesh bottom that allowed full access to the paws. Mechanical allodynia was evaluated through *up-and-down* method using von Frey filaments (Dixon, 1980). A sequence of von Frey filaments, with different forces and logarithmic increments (6, 8, 10, 15, 26, 60 and 100 g) was used. In case of a positive response (paw withdrawal), the next filament with smaller force was applied; in case of negative response (no paw withdrawal response), the next filament with greater force was applied. This was repeated until a total of 6 applications (Rossato et al., 2014).

#### 2.8.2. Thermal hyperalgesia

Thermal hyperalgesia was assessed using Plantar test with minor modifications (Hargreaves et al., 1988). Briefly, animals were habituated in a Plexiglas chamber for 20 min; a radiant light beam generated by a 60 W light bulb was then directed onto the right