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Antidepressant-like action of the ethanolic extract from *Tabebuia avellanedae* in mice: Evidence for the involvement of the monoaminergic system

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ABSTRACT

The antidepressant-like effect of the ethanolic extract obtained from barks of *Tabebuia avellanedae*, a plant widely employed in folk medicine, was investigated in two predictive models of depression: forced swimming test (FST) and tail suspension test (TST) in mice. Additionally, the mechanisms involved in this antidepressant-like action and the effects of the association of the extract with the antidepressants fluoxetine, desipramine and bupropion in the TST were investigated. The extract from *T. avellanedae* produced an antidepressant-like effect, in the FST (100 mg/kg, p.o.) and in the TST (10–300 mg/kg, p.o.), without accompanying changes in ambulation when assessed in the open-field test. The anti-immobility effect of the extract (30 mg/kg, p.o.) in the TST was prevented by pre-treatment of mice with ketanserin (5 mg/kg, i.p., a preferential 5-HT_{2A} receptor antagonist), prazosin (1 mg/kg, i.p., an α_1-adrenoceptor antagonist), yohimbine (1 mg/kg, i.p., an α_2-adrenoceptor antagonist), propranolol (2 mg/kg, i.p., a β-adrenoceptor antagonist), sulpiride (50 mg/kg, i.p., a dopamine D_2 receptor antagonist) and SCH23390 (0.05 mg/kg, s.c., a dopamine D_1 receptor antagonist). The combined administration of a subeffective dose of WAY100635 (0.1 mg/kg, s.c., a selective 5-HT_{1A} receptor antagonist) and a subeffective dose of the extract (1 mg/kg, p.o.) produced a significant reduction in the immobility time in the TST. In addition, the combination of fluoxetine (1 mg/kg, p.o.), desipramine (0.1 mg/kg, p.o.), or bupropion (1 mg/kg, p.o.) with a subeffective dose of the extract (1 mg/kg, p.o.) produced a synergistic antidepressant-like effect in the TST, without causing hyperlocomotion in the open-field test. It may be concluded that the extract from *T. avellanedae* produces an antidepressant-like effect in the FST and in the TST that is dependent on the monoaminergic system. Taken together, our results suggest that *T. avellanedae* deserves further investigation as a putative alternative therapeutic tool that could help the conventional pharmacotherapy of depression.

1. Introduction

Depression is an important global public-health issue, both because of the relatively high lifetime prevalence ranging from 2% to 15% and because it is associated with substantial disability (Moussavi et al., 2007). Although commonly used antidepressants such as the selective serotonin reuptake inhibitor (SSRI) fluoxetine are often effective, full efficacy is only apparent after several weeks and many patients only partially respond, and some remain refractory. Therefore, considerable efforts are invested in the search for better drugs and even combined treatments approaches for treatment of depression (Morilak and Frazer, 2007).

According to the most accepted hypothesis of depression, the monoamine theory, the major neurochemical process in depression is the impairment of monoaminergic neurotransmission and the decrease in extracellular concentrations of noradrenaline and serotonin (Hindmarch, 2002; Kiss, 2008; Schildkraut, 1965). Therefore, the classical antidepressants agents are designed to increase monoamine transmission, either by inhibiting neuronal reuptake (for example, SSRIs such as fluoxetine) or by inhibiting degradation (for example, monoamine oxidase inhibitors (MAOi) such as iproniazide) (Krishnan and Nestler, 1998). In addition to serotonergic and noradrenergic systems, preclinical and clinical data support the role of the dopaminergic system in depression (Bonhomme and Esposito, 1998; Kulkarni et al., 2008).

Tabebuia avellanedae (Bignoniaceae), is a tree native to tropical rain forests in the northeast of Brazil commonly known as “pau d'arco” or “ipê-roxo”. Some studies have reported that this plant exerts a number of pharmacological activities, such as antinociceptive,

Abbreviations: ANOVA, analysis of variance; DMSO, dimethylsulfoxide; DRL, differential-reinforcement-of-low-rate; FST, forced swimming test; MAOi, monoamine oxidase inhibitor; SCH23390, (*R*)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride; 5-HT, serotonin; SSRI, selective serotonin reuptake inhibitor; TST, tail suspension test; WAY100635, N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2-pyridynyl) cyclohexanecarboxamide.

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antiedematogenic (De Miranda et al., 2001), antiulcerogenic (Twardowschy et al., 2008), anti-angiogenic (Kung et al., 2007), anti-inflammatory (Byeon et al., 2008), anti-cancer (Kim et al., 2007; Pardee et al., 2002), antimicrobial (Pereira et al., 2006), anti-fungal (Portillo et al., 2001) and diuretic (Byeon et al., 2008).

Considering that: a) *T. avellanedae* is extensively used as analgesic in Brazil (Byeon et al., 2008); b) depression is frequent in chronic pain patients and several studies have indicated that pain and depression share common neurochemical mechanisms (Micó et al., 2006); c) some antidepressant drugs afford an integral alleviation of pain (Krell et al., 2005; Micó et al., 2006), the ethanolic extract from this plant was investigated in two behavioral models commonly used to detect antidepressant activity: forced swimming test (FST) and tail suspension test (TST) in mice. Moreover, the involvement of the monoaminergic system and the effect of the combined administration of subeffective doses of the extract of *T. avellanedae* and antidepressants were investigated in the TST.

2. Methods

2.1. Plant material and preparation of the ethanolic extract from T. avellanedae

T. avellanedae barks were provided by Chamel Indústria e Comércio de Produtos Naturais Ltda (Campo Largo, Brazil), lot 4753. The identification was performed by botanist Elide Pereira dos Santos and a voucher specimen has been deposited at the Herbarium of the Department of Botany at the Universidade Federal do Paraná, Brazil. Dried and powdered barks (5 kg) were extracted three times by maceration with 95% ethanol for 7 days at room temperature. The combined ethanolic extract was filtered, the solvent evaporated under reduced pressure (40–50 °C) and lyophilized to give a red-brown solid (919.2 g).

2.2. Animals

Adult female Swiss mice (30–40 g) were maintained at constant room temperature (22–25 °C) with free access to water and food, under a 12:12 h light:dark cycle (lights on at 07:00 h). The cages were placed in the experimental room 24 h before the test for acclimatization. All manipulations were carried out between 9:00 and 17:00 h, with each animal used only once ($N = 6$–9 animals per group). The procedures in this study were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the local Ethics Committee. All efforts were made to minimize animal suffering and the number of animals used in the experiments.

2.3. Drugs and treatment

The following drugs were used: ketanserin tartarate, N-{2-[4-(2-methoxyphenyl)-1 piperazinyl]ethyl}-N-(2-pyridynyl) cyclohexanecarboxamide (WAY100635), prazosin hydrochloride, yohimbine hydrochloride, propranolol, (*R*)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (SCH23390), sulpiride, fluoxetine, desipramine and bupropion (all from Sigma Chemical Co., St. Louis, U.S.A.). Drugs were dissolved in saline, except sulpiride and prazosin that was diluted in saline with 5% dimethylsulfoxide (DMSO) and fluoxetine, desipramine and bupropion that were dissolved in distilled water. Ketanserin, prazosin, yohimbine, propranolol and sulpiride were administered by intraperitoneal (i.p.) route. SCH 23390 and WAY100635 were administered by subcutaneous (s.c.) route. Fluoxetine, desipramine and bupropion were administered by oral (p.o.) route). The drugs were administered in a constant volume of 10 ml/kg body weight. Appropriate vehicle-treated groups were also assessed simultaneously.

The extract from *T. avellanedae* (1–300 mg/kg) was dissolved in distilled water with 5% Tween 80 and was administered acutely by oral route (p.o.) by gavage 60 min before the FST, TST or the open-field test. The dissolution of the extract was freshly done from the liophylized power immediately before its administration. A control group received distilled water with 5% Tween 80 as vehicle. Fluoxetine (10 mg/kg, p.o., a conventional antidepressant) was used as a positive control.

In the experiments designed to study the time-course effect of the extract from *T. avellanedae* (30 mg/kg, p.o.), the immobility time in the TST was assessed in an independent group of mice, 30, 60 or 120 min after the administration of the extract (Posser et al., 2009).

To address some of the mechanisms by which the extract causes antidepressant-like action in the TST, animals were treated with different drugs. In the experiments performed to assess the interaction of the extract with the monoaminergic system, mice were pre-treated with vehicle, ketanserin (5 mg/kg, a preferential 5-HT_{2A} receptor antagonist), prazosin (1 mg/kg, an α_1-adrenoceptor antagonist), yohimbine (1 mg/kg, an α_2-adrenoceptor antagonist), propranolol (2 mg/kg, a β-adrenoceptor antagonist), SCH23390 (0.05 mg/kg, a dopamine D_1 receptor antagonist) or sulpiride (50 mg/kg, a dopamine D_2 receptor antagonist), and 30 min later they received vehicle or extract (30 mg/kg, p.o.) before being tested in the TST after 60 min, as described previously (Binfaré et al., 2009; Machado et al., 2009).

In a separate series of experiments, the involvement of the 5-HT_{1A} receptor subtype in the effect of the extract in the TST was investigated. In order to verify a possible synergistic effect of the extract with WAY100635 (0.1 mg/kg, a selective 5-HT_{1A} receptor antagonist), mice were pre-treated with a subeffective dose of the extract (1 mg/kg, p.o.) or vehicle and after 30 min they received a subeffective dose of WAY100635 (0.1 mg/kg, s.c.) or saline by s.c. route before being tested in the TST 30 min later (Brocardo et al., 2008).

We also assessed the ability of the extract to potentiate the antidepressant-like effect of conventional antidepressants. To this end, mice received by p.o. route vehicle, fluoxetine (1 mg/kg, a serotonin reuptake inhibitor), desipramine (0.1 mg/kg, a noradrenaline reuptake inhibitor) or bupropion (1 mg/kg, a dopamine reuptake inhibitor) and immediately after, a subeffective dose of the extract (1 mg/kg, p.o.) or vehicle were administered. Sixty minutes later, the TST or the open-field test were carried out (Binfaré et al., 2009).

The doses of the drugs used were chosen on the basis of literature and are previously reported not to increase locomotor activity (Binfaré et al., 2009; Brocardo et al., 2008; Kaster et al., 2005; Machado et al., 2007, 2009; O'Neill and Conway, 2001; Rodrigues et al., 2002).

2.4. Forced swimming test (FST)

Mice were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), containing 19 cm of water (depth) at 25 ± 1 °C; the total amount of time each animal remained immobile during a 6-min session was recorded (in seconds) as immobility time, as described previously (Brocardo et al., 2008; Kaster et al., 2005; Machado et al., 2007). Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. A decrease in the duration of immobility is indicative of an antidepressant-like effect (Porsolt et al., 1977).

2.5. Tail suspension test (TST)

The total duration of immobility induced by tail suspension was measured according to the method described by Steru et al. (1985). Mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the