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Research article

Monoaminergic neurotransmission is mediating the antidepressant-like effects of *Passiflora edulis* Sims fo. *edulis*

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ABSTRACT

The genus *Passiflora* is popularly used to treat anxiety. Recent studies showed antidepressant-like effects of two varieties of *P. edulis* (*edulis* and *flavicarpa*) in mice. However, the mechanisms of antidepressant actions are still unknown. Here, the effects of *P. edulis* fo. *edulis* aqueous extract (AE, 100–300 mg/kg, po), and ethyl acetate (AcOEt, 25–50 mg/kg, po), butanol (BuOH, 25–50 mg/kg, po) and residual aqueous (25–100 mg/kg, po) fractions were investigated in the mouse forced swimming test. In addition, the involvement of monoamines in the *P. edulis* fractions-induced antidepressant actions was approached. HPLC analyses showed that AcOEt and BuOH, but not residual, fractions shared with AE the main peaks between 25 and 70 min (UV 340 nm), which are suggestive of flavonoids. Nortriptyline and fluoxetine reduced the immobility time and similar results were observed for AE, AcOEt and BuOH but not residual fractions. PCPA (inhibitor of 5-HT synthesis), AMPT (inhibitor of catecholamine synthesis) and sulpiride (selective D2 receptor antagonist), but not DSP-4 (noradrenergic neurotoxin), blocked the antidepressant actions of AcOEt and BuOH. In conclusion, AcOEt and BuOH fractions shared with AE similar phytochemical composition and antidepressant actions. Preserved 5-HT and dopamine transmissions were required for the antidepressant effects of *P. edulis* fractions.

1. Introduction

Passiflora is a genus belonging to Passifloreaceae family. Up to now there are described around to 600 species of *Passiflora* worldwide, which more than the half occurring in the American territory, and 120 being native from Brazil [1]. *Passiflora* species are currently used worldwide in traditional folk medicine to the treatment of anxiety, insomnia, epilepsy, spasm, and aches [2,3]. Due to the quality of its fruits, yielding, juiciness, and the consumer's choice, *P. edulis* fruit accounts for 95% of the passion fruit-cultivated area in Brazil [4]. However, due to genetic and morphological dissimilarities, it is accepted nowadays that there are varieties of *P. edulis*: *P. edulis* Sims fo. *edulis* (purple passion fruit) and *P. edulis* fo. *flavicarpa* Degener (yellow passion fruit). However, the infraspecific taxonomy of *P. edulis* is still contradictory and it deserves further discussion (for review see [4]).

Pharmacological *in vivo* studies have described relevant biological effects to *P. edulis Sims*, such as antioxidant [5], anti-inflammatory [6–8], anti-hypertensive [9], and anxiolytic-like actions [10–12]. Recently, experimental studies have contributed to highlight the antidepressant activity of *P. edulis* alcohol and aqueous extracts of stems and leaves in mice [12,13]. In our previous study [12], the central effects of the two varieties of *P. edulis* (*e.g.*, *edulis* and *flavicarpa*) were assessed in mice, and the variety *edulis* showed the most promising antidepressant-like actions.

No information is still available about the mechanisms by which *P. edulis* promotes antidepressant-like effects. It is well known that the biological activities of plant specimen are explained by their effects on neuronal communication, interaction of plant metabolites with receptors and with neurotransmitters synthesis [14]. Based on the monoaminergic hypothesis of depression [15], it is possible that the antidepressant effects of *P. edulis* may be related to the modulation of monoamine neurotransmission. Therefore, the present study aimed to

Abbreviations: AE, aqueous extract; AcOEt, ethyl acetate fraction; AMPT, α-methyl-DL-tyrosine chloride; BuOH, butanol fraction; DSP-4, *N*-(2-chloroethyl)-*N*-ethyl-bromo-benzylamine; FST, forced swimming test; PCPA, *P*-chlorophenylalanine

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investigate the effects of butanolic (BuOH), ethyl acetate (AcOEt), and residual fractions of *P. edulis* Sims fo. *edulis* aqueous extract in the forced swimming test (FST), a predictive test of antidepressant activity in rodents. In a second set of experiments, this study investigated the putative involvement of the monoaminergic transmission in the *P. edulis* fractions-induced antidepressant-like effects.

2. Materials and methods

2.1. Animals

Experiments were performed using male Swiss mice (10–12 weeks old; 35 ± 5 g) breaded at the Federal University of Rio Grande do Norte (Natal, Brazil). Mice were housed in cages (41 × 34 × 16 cm, 13 mice/cage) covered with sawdust bedding under standard conditions (22 ± 2 °C, 12 h light-dark cycle, lights on 6.00a.m.) with food and water *ad libitum*. A total number of 532 mice were used to develop this study; being 154 mice used in the experiment 1, and 378 animals for developing the experiment 2. Animals were randomically assigned into experimental or control groups. All experiments were performed in 3 distinct days, and a number of 3–4 animals/group/day were employed. An exception was the experiment with AMPT which was replicated during 5 days (3–4 animals/group/day) due to the huge animal behavior variability. Behavioral procedures were conducted between 1.00 and 5.00 p.m. All animals were used just once. in vivo studies have been reported according to ARRIVE guidelines [16] and in accordance with Brazilian law no. 11.794/2008 for Care and Use of Experimental Animals and were approved by Local Ethics Committee for Animal Use of the Federal University of Rio Grande do Norte (License #005/2015; #032/2010).

2.2. Material plant

The leaves of *Passiflora edulis* fo. *edulis* was collected in Santa Sofia, Boyacá, Colombia (the Global Position Systems – GPS – location at 05°43′01″ N) in January 2009, and identified by the botanist Luis Carlos Jimenez (Instituto Nacional de Ciencias, Universidad Nacional de Colombia). A voucher specimen was deposited in the herbarium at the same university (COL 530661).

2.3. Preparation of extract and fractions

The leaves of *P. edulis* Sims fo. *edulis* (100 g) were air-dried at 40 °C during 7 days in a airflow oven, powdered and extracted using hot water (90 °C) by infusion (plant:solvent, 1:10, w/v) for 10 min. The same plant was extracted two times by the infusion. The crude extracts obtained from extraction 1 and 2 were grouped, resulting in the aqueous extract (AE). After that, this extract was submitted to a liquid–liquid fractionation with solvents of increased polarity, 3 × 300 ml ethyl acetate and 3 × 300 ml *n*-butanol, resulting in 1.2 g ethyl acetate (EtAOc), 7.1 g butanolic (BuOH), and 9.1 g aqueous residual (AR) fractions.

2.4. Thin–layer chromatography (TLC) analysis

The fractions were analyzed by TLC using aluminum pre-coated sheets with silica gel (Merck, Darmstadt, Germany) as adsorbent while as mobile phases were employed ethyl acetate:formic acid:water (8:1:1 v/v/v). The chromatograms were analyzed under 254 and 365 nm UV light and then sprayed with Natural Product Reagent 0.5%, generally used to flavonoid detection.

2.5. High performance liquid chromatography (HPLC) analysis

HPLC analysis was performed in a Chromaster® WWR Hitachi chromatograph, quaternary pump, auto injector coupled to DAD detector and EZChrom Elite (version 3.3.2) software. The column used was a Phenomenex-Luna 5 μm C18(2) 100Å (250 × 4.6 mm). The mobile phase was (A) acetonitrile and (B) acetic acid 0.3%, using the following gradient 0–10 min 10:90% (A) in (B), 10–40 min 20:80% (A) in (B), and 40–90 min 20:80% (A) in (B) at a flow rate of 1 ml/min and UV 340 nm. The aqueous extract (7.5 mg/ml) and fractions (1 mg/ml) were analyzed in triplicate.

2.6. Drugs

The aqueous extract (AE), and ethyl acetate (AcOEt), butanolic (BuOH), and residual (AR) fractions of *P. edulis* fo. *edulis* were solubilized in saline and given orally (po) in a volume of 10 ml/kg 60 min before testing. Nortriptyline (Novartis Biociências S.A., São Paulo, Brazil) and fluoxetine (Cristália Lab. Farm., São Paulo, Brazil) were solubilized in saline and injected intraperitoneally (ip) 30 or 60 min before test, respectively. *P*-chlorophenylalanine (PCPA) (Sigma-Aldrich, San Louis, USA) was solubilized in tween 80 (0.5%) and saline. The α-methyl-DL-tyrosine chloride (AMPT) (Sigma-Aldrich, San Louis, USA) and *N*-(2-chloroethyl)-*N*-ethyl-bromo-benzylamine (DSP-4) (Sigma-Aldrich, San Louis, USA) were solubilized in distillated water. DSP-4 solution was stored from light until the use. Sulpiride (Sigma-Aldrich, San Louis, USA) was solubilized in saline and glacial acetic acid (0.1 M). The pH solution was adjusted with sodium hydroxide. Control groups were treated with the same volume, via of administration and using identical vehicles as applied for the respective experimental groups.

2.7. Forced swim test (FST)

This test was performed according to Porsolt et al. [17]. Mice were individually forced to swim in a transparent glass cylinder (24 cm in height, 18 cm in diameter) containing 18 cm of water at 23 ± 1 °C. The time spent immobile (*e.g.*, time spent floating in the water without doing any further attempts to escape) was measured by an experienced observer during the last 4 min of a single 6 min test session. After behavioral assessment, animals were kept warm by using a warming pad until they were completely dried when returned to their home cages.

2.8. Open field test

The mice spontaneous locomotor activity was measured using open field. The apparatus (40 × 40 × 40 cm) had black floor and walls. Each mice was placed in the center of open field and the distance traveled (in meters) were registered every 5 min during 30 min through a video camera connected to an automated activity monitoring system (Anymaze, Stoelting Co.,Wood Dale, USA). After the behavioral evaluation of each animal, the arena was cleaned with 10% ethanol solution.

2.9. Experimental procedure

2.9.1. Experiment 1 – effects of P. edulis fractions in the FST

This series of experiment aimed to test effects of distinct doses of aqueous extract (100 and 300 mg/kg, po), BuOH (25 and 50 mg/kg, po), AcOEt (25 and 50 mg/kg, po), and residual (25–100 mg/kg, po) fractions of *P. edulis* in mice in the FST. In a distinct experimental series, the effects of BuOH and AcOEt fractions, both at the active dose–50 mg/kg (po), were also assessed on spontaneous locomotion in the open field test.

2.9.2. Experiment 2–involvement of monoaminergic transmission in the antidepressant-like effects of P. edulis fractions

The involvement of 5-HT transmission on the antidepressant-like actions of *P. edulis* fractions was evaluated by pretreating mice with