Revista Brasileira de Farmacognosia 25 (2015) 158–163

Revista Brasileira de Farmacognosia
BRAZILIAN JOURNAL OF PHARMACOGNOSY
www.sbfgnosia.org.br/revista

Original Article

Involvement of GABAergic pathway in the sedative activity of apigenin, the main flavonoid from *Passiflora quadrangularis* pericarp

Andressa C. Gazola[a,*], Geison M. Costa[a,b], Leonardo Castellanos[b], Freddy A. Ramos[b], Flávio H. Reginatto[a], Thereza C.M. de Lima[c], Eloir P. Schenkel[a]

[a] *Programa de Pós-graduação em Farmácia, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil*
[b] *Departamento de Química, Universidad Nacional de Colombia, Bogotá, Colombia*
[c] *Programa de Pós-graduação em Farmacologia, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil*

ARTICLE INFO

Article history:
Received 6 September 2014
Accepted 13 March 2015
Available online 1 April 2015

Keywords:
Apigenin
Passiflora quadrangularis
Passion flower
Passion fruit
Sedative

ABSTRACT

In the current study we showed that oral administration of an aqueous extract of *Passiflora quadrangularis* L., Passifloraceae, pericarp results in a significant prolongation of the sleep duration in mice evaluated in the ethyl ether-induced hypnosis test which indicates sedative effects. Apigenin, the main flavonoid of the extract, induced a similar sedative response when applied alone, at a dose equivalent to that found in the extract, suggesting that apigenin is mediating the sedative effects of *P. quadrangularis* extract. In addition, the sedative effect of apigenin was blocked by pretreatment with the benzodiazepine antagonist flumazenil (1 mg/kg), suggesting an interaction of apigenin with gamma-aminobutyric acid type A ($GABA_A$) receptors. However, apigenin at concentrations 0.1–50 μM failed to enhance GABA-induced currents through $GABA_A$ receptors ($\alpha_1\beta_2\gamma_{2S}$) expressed in *Xenopus* oocytes. Nevertheless, based on our results, we suggest that the *in vivo* sedative effect of the *P. quadrangularis* extract and its main flavonoid apigenin maybe be due to an enhancement of the GABAergic system.

Introduction

The genus *Passiflora* has the most economical significance among all other genera in the family Passifloraceae. Its species are mainly distributed throughout Latin America. *Passiflora quadrangularis* L. is usually cultivated at altitudes below 2500 m (Killip, 1938). In Brazil, the occurrence of this species is described in the states of Amazonas and Maranhão (Cervi, 1997) and also in Colombia, in the regions of Chocó, Meta, Huila and Santander. Popularly known as "maracujá-açu" in Brazil (Cervi, 1997) and "badea" in Colombia (Hernández and Bernal, 2000), this species is widely consumed in Colombia as juice. The fruits are much bigger than the other fruits of *Passiflora* species showing pale yellowish green color when ripe (Vanderplank, 2000).

To date, only few pharmacological and chemical studies with pericarps of *Passiflora* species is available. In traditional medicine, preparations using leaves of some species are used as sedatives and mild tranquilizers (Lewis and Elvin-Lewis, 1977; Schindler, 1884). In particular, sedative, anxiolytic-like and anticonvulsant effects of leaves extracts from different species of *Passiflora* have been reported (for a review, see Dhawan et al., 2004).

Regarding the chemical composition, most of the studies is also related to leaves extracts and reported the presence of flavonoids (Petry et al., 1998; Ulubelen et al., 1982; Zucolotto et al., 2012) and saponins (Orsini et al., 1985; Reginatto et al., 2001). In this respect, due the lack in the literature about neuropharmacological and chemical data of pericarp from *Passiflora* species and considering that the sedative and anxiolytic-like activities of *P. edulis* var. *flavicarpa* pericarp were previously described (Sena et al., 2009), the aim of the present study was to evaluate the sedative activity of *P. quadrangularis* pericarp aqueous extracts, the correlation of the sedative activity to its flavonoids constituents besides to study the involvement of the gamma-aminobutyric acid system in the action of these compounds.

Materials and methods

Plant material and extraction

Fruits of *Passiflora quadrangularis* L., Passifloraceae, were collected in Colombia [Neiva, Huila (2°59′55″, −75°18′16″)], identified by Prof. Luis Carlos Jimenez (Instituto Nacional de Ciencias,

* Corresponding author.
E-mail: andressa@unibave.net (A.C. Gazola).

http://dx.doi.org/10.1016/j.bjp.2015.03.009
0102-695X/

Universidad Nacional de Colombia) and a voucher specimen was deposited in the Herbarium of the Universidad Nacional de Colombia (COL 572634).

Extract for the ethyl ether-induced hypnosis test as well as the HPLC-DAD analyses was obtained as follows. Fresh pericarp was directly extracted by infusion with hot water (90 °C; plant:solvent, 1:3, w/v) for 10 min. The aqueous extracts were subsequently filtered and freeze-dried.

HPLC-DAD analyses

The analyses were performed on a PerkinElmer Series 200 high-performance liquid chromatography (HPLC) system equipped with diode array detection (DAD), quaternary pump, online degasser and auto-sampler (injection volume was 20 μl). The separation was performed with a PerkinElmer Brownlee Analytical C_{18} column (250 mm × 4.6 mm i.d.; 5 μm). The flow was maintained at a constant rate of 1.2 ml/min. The mobile phase used consisted of solvent A (acetonitrile) and solvent B (0.5% formic acid in water) in a linear gradient elution (0–20 min– 15% – 35% of A; 20–25 min – isocratic 35% A). The chromatograms were monitored at 340 nm and UV spectra were recorded in the 200–400 nm range. The data were processed with Chromera software® (Version 3.2.0.4847) (PerkinElmer®, Waltham, MA, USA).

Qualitative and quantitative analyses were performed with the extract in a concentration of 5000 μg/ml. For qualitative analyses the reference standards co-eluted with the extract were apigenin (≥95% – Sigma–Aldrich®, USA), isoorientin (≥98% – Extrasynthèse®, France), vitexin-2″-*O*-rhamnoside (≥98% – Sigma–Aldrich®, USA), vitexin-2″-*O*-xyloside (Costa et al., 2013), isovitexin (≥98% – Fluka®, USA) and swertisin (Santos et al., 1996).

The quantitative analyses were developed using the external standard method, being apigenin used as a reference substance to create a calibration curve with six points in a 0.5–40 μg/ml concentration range. All of the standard solutions were analyzed in triplicate, and the average peak areas were measured. The validation of the analytical procedures was performed in accordance with ICH guidelines (2005). In particular, the following parameters were evaluated: specificity, linearity, accuracy, precision (both repeatability and intermediate precision), the limit of quantification (LOQ) and the limit of detection (LOD).

Animals

Male adult Swiss mice (3–4 months) weighing 35–50 g were used for behavioral evaluations. Animals were maintained on a 12-h light-dark cycle (lights on at 7 a.m.) at a constant room temperature (23 ± 2 °C). Mice were housed in groups of twenty per plastic cage (30 cm × 37 cm × 16 cm) with food and water provided *ad libitum* except during the experiments. All animals were allowed to adapt to the laboratory conditions for at least one week prior to the behavioral assessments of the study. On the day of the experiment, animals were housed in the experimental room for at least 1 h prior to the start of the testing procedures. Each animal was used only once. Experiments were conducted in accordance with national and international standards of animal welfare (Brazilian Law #11,794, 10/08/2008; NIH publication #85-23, revised in 1996) and approved by the local Committee for Animal Care in Research (#23080.044085/2009-37/CEUA/UFSC). All efforts were made to minimize the number of animals used and their suffering.

Drugs and treatments

Mice were orally treated (*p.o.*) through an intragastric cannula, in a constant volume of 0.1 ml/10 g of animal weight, with *P. quadrangularis* aqueous extract or apigenin, both dissolved in distilled water. In the ethyl ether-induced hypnosis test, the extract was tested at the doses of 100, 300 and 600 mg/kg and apigenin was tested at the doses of 0.1, 0.3, 0.6 and 1.0 mg/kg. An equal volume of distilled water (vehicle, *p.o.*) treated mice of the control groups. Diazepam (DZP – Hoffman-La Roche®, Switzerland) was used as positive drug control, dissolved in water with 10% of propylene glycol, and administered *p.o.* at a dose of 1 mg/kg in the sedative test (Sena et al., 2009). Animals were individually tested 1 h after their treatments with the extract or apigenin and 30 min after their treatment with DZP.

In order to assess the involvement of the GABAergic system, a group of animals were pretreated (*i.p.*) with physiological saline solution (Quimibrás Indústrias Químicas S.A., Brazil) or flumazenil (Flumazen®, União Química Lab., Brazil – 1 mg/kg – Carvalho et al., 2011) 15 min prior the oral treatment with the test compound apigenin (0.6 mg/kg), the reference drug DZP (1 mg/kg) or distilled water (control group).

Ethyl ether-induced hypnosis test

Animals were placed in an ethyl ether-saturated glass cage (6 ml, 13 min of saturation, 30 cm × 20 cm glass cage) after treatments. A stopwatch was used to record the duration of hypnosis in seconds (s). The hypnosis time was measured by the loss of the righting reflex, and the recovery of this reflex was considered as the end-point of the hypnosis (Vieira, 2001).

Voltage clamp analysis

Preparation of stages V–VI oocytes from *Xenopus laevis* and synthesis of capped off run-off poly(A+) cRNA transcripts from linearized cDNA templates (pCMV vector) were performed as previously described (Khom et al., 2006). Briefly, female *Xenopus Laevis* frogs (Nasco, Fort Atkinson, USA) were anaesthetized by exposing them for 15 min to a 0.2% solution of MS-222 (methane sulfonate salt of 3-aminobenzoic acid ethyl ester; Sigma, Vienna, Austria) before surgically removing parts of the ovaries. Follicle membranes from isolated oocytes were digested with 2 mg/ml collagenase solution (Type 1A). Selected stages V–VI oocytes were injected with about 10–50 nl of DEPC-treated water (diethyl pyrocarbonate) containing the cRNAs at a concentration of approximately 300–3000 pg/nl. The amount of cRNA was determined by means of a NanoDrop ND-1000 (Kisker Biotech, Steinfurt, Germany). To ensure expression of the gamma-subunit, cRNAs for expression of $\alpha_1\beta_2\gamma_{2s}$ receptors were mixed in a ratio of 1:1:10, respectively. After injection, oocytes were stored at 18 ± 8 °C for 24–48 h in ND96 solution containing penicillin G (10,000 IU/100 ml) and streptomycin (10 mg/100 ml). Electrophysiological experiments on $GABA_A$ receptors were performed using the two-microelectrode-voltage-clamp method at a holding potential of −70 mV, making use of a TURBO TEC 01 C amplifier (npi electronic, Tamm, Germany) and an Axon Digidata 1322A interface (Molecular Devices, Sunnyvale, CA). Data acquisition was done using pCLAMP v.9.2. The bath solution contained 90 mM NaCl, 1 mM KCl, 1 mM $MgCl_2.6H_2O$, 1 mM $CaCl_2$ and 5 mM HEPES (pH 7.4). Microelectrodes were filled with 3 M KCl.

GABA and apigenin were applied by means of a fast perfusion system (Screening Tool, npi electronic, Tamm, Germany) (Baburin et al., 2006) to study the GABA-induced chloride current (I_{GABA}) modulation. To elicit I_{GABA}, the chamber was perfused with 120 μl of GABA containing solution at a volume rate between 300 and 1000 ml/s. The I_{GABA} rise time ranged from 100 to 250 ms (Khom et al., 2006). To exclude voltage-clamp errors, oocytes with maximal current amplitudes >3 mA were discarded.

Potentiation of the I_{GABA} in percent was defined according to the formula: $I_{GABA}\ (\%) = [I_{(GABA\ +\ apigenin)}/I_{GABA} - 1] \times 100$, where $I_{(GABA+apigenin)}$ is the current response in the presence of different