Phytomedicine 17 (2010) 940–949

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.de/phymed

Passiflora incarnata L. (Passionflower) extracts elicit GABA currents in hippocampal neurons *in vitro*, and show anxiogenic and anticonvulsant effects *in vivo*, varying with extraction method

S.-M. Elsas[a,*], D.J. Rossi[b], J. Raber[a,b,c], G. White[a], C.-A. Seeley[a], W.L. Gregory[d], C. Mohr[b], T. Pfankuch[b], A. Soumyanath[a]

[a] *Department of Neurology, Oregon Health and Science University, Portland, OR 97239, USA*
[b] *Department of Behavioral Neuroscience, Oregon Health and Science University, Portland, OR 97239, USA*
[c] *Division of Neuroscience, ONPRC, Oregon Health and Science University, Portland, OR 97239, USA*
[d] *Helfgott Research Institute, National College of Natural Medicine, Portland, OR 97201, USA*

ARTICLE INFO

Keywords:
Passiflora incarnata
Passian flower
Flavonoid
$GABA_A$ receptor
Epileptic seizure
Elevated plus maze
Rotarod
Pentylenetetrazol

ABSTRACT

Potential mechanisms of *Passiflora incarnata* extracts and the effect of extraction methods on ingredients and biological effects were explored. Using the same batch of plant material, total flavonoid yields as measured by high-performance liquid chromatography coupled to diode array detection (HPLC–DAD) increased substantially with hot versus cold extraction methods.

Whole *Passiflora* extract induced prominent, dose-dependent direct $GABA_A$ currents in hippocampal slices, but the expected modulation of synaptic $GABA_A$ currents was not seen. GABA was found to be a prominent ingredient of *Passiflora* extract, and GABA currents were absent when amino acids were removed from the extract.

Five different extracts, prepared from a single batch of *Passiflora incarnata*, were administered to CF-1 mice for 1 week in their drinking water prior to evaluation of their behavioral effects. Anticonvulsant effects against PTZ-induced seizures were seen in mice that received 2 of the 5 *Passiflora* extracts. Instead of the anxiolytic effects described by others, anxiogenic effects in the elevated plus maze were seen in mice receiving any of the 5 *Passiflora* extracts.

Introduction

Passiflora incarnata (Purple Passionflower) is an indigenous American vine with white and blue or purple flowers and an edible fruit (Dhawan et al. 2001a). Its medicinal use originated with native Americans (Spinella 2001), and its most popular uses are for insomnia and anxiety (Carlini 2003) as well as epilepsy (Spinella 2001). *Passiflora incarnata* is listed in the pharmacopoeias of Great Britain, United States, India, France, Germany, Switzerland and others (Dhawan et al. 2001b). The active ingredients have not been conclusively defined (Carlini 2003). Most available data suggests flavonoids as possible active ingredients (Speroni and Minghetti 1988; Dhawan et al. 2001b, 2003).

Studies in animal models show efficacy of *Passiflora* extracts and flavonoid fractions against pentylenetetrazol (PTZ) induced seizures (Speroni and Minghetti 1988; Speroni and Billi 1996; Nassiri-Asl et al. 2007; Nassiri-Asl et al. 2008). This effect of *Passiflora* can be inhibited by the benzodiazepine site antagonist Ro 15-1788, suggesting the involvement of $GABA_A$ receptors (Medina et al. 1990). Flavonoids bind with high affinity to the benzodiazepine site of the $GABA_A$ receptor (Medina et al. 1997; Marder and Paladini 2002), but appear to modulate $GABA_A$ and also $GABA_C$ receptor currents by a different mechanism than benzodiazepines (Goutman et al. 2003; Kavvadias et al. 2004).

In 3 clinical trials, *Passiflora* extracts showed anxiolytic efficacy. One of the trials compared *Passiflora* to placebo (Movafegh et al. 2008), and two others showed *Passiflora* to have anxiolytic efficacy similar to benzodiazepines (Mori et al. 1993; Akhondzadeh et al. 2001b). In addition, *Passiflora* extract showed sedative effects in 2 clinical trials (Akhondzadeh et al. 2001a; Movafegh et al. 2008).

In preparation for a clinical trial in epilepsy patients, potential mechanisms of *Passiflora* extracts and the effect of extraction method on ingredients and biological effects were explored. An initial extract was tested with full and with reduced amino acid content in a hippocampal slice preparation. Using several extraction methods, another 5 extracts were prepared from the same original

* Corresponding author at: OHSU Epilepsy Center CR-120, Department of Neurology, Oregon Health and Science University, 3181 SW Sam Jackson Park Road, Portland, OR 97219, USA. Tel.: +1 503 494 4016; fax: +1 503 494 1653.
E-mail address: elsas@ohsu.edu (S.-M. Elsas).

0944-7113/$ – see front matter © 2010 Elsevier GmbH. All rights reserved.
doi:10.1016/j.phymed.2010.03.002

plant material. All 5 extracts were analyzed for flavonoid and amino acid contents, and tested for neurological effects in mice using the elevated plus maze, the rotarod, and the subcutaneous PTZ model of epileptic seizures after application in the drinking water for 1 week.

Methods

Passiflora extracts for in vitro testing in hippocampal slices

Whole extract: An extract of passionflower (Lot# PAS 02034C) was obtained from a local dietary supplement manufacturer, Oregon's Wild Harvest (OWH), Sandy, OR. Fresh passionflower, collected from the wild, was steeped in 44% ethanol for 35 days. The extract was distilled to remove ethanol, and freeze-dried to a dry powder (1 g equivalent to 25.78 g of fresh Passionflower herb or 5.6 g of dried plant material).

Amino acid reduced extract: The freeze-dried powder from above (3 g) was dissolved in a minimum amount of water and applied to a column containing EMD C-18 silica gel 60 (Sigma; 100 g), previously conditioned with methanol and water. Amino acids were eluted with water (1 l), and flavonoids by elution with 80% aqueous methanol containing 1% ammonia (400 ml), methanol:chloroform; water (48:30:12; 150 ml), chloroform (100 ml) and finally dichloromethane; methanol; ammonia (200:75:5; 175 ml). The water elution containing amino acids was freeze-dried (extracted amino acids, 2.4 g). The combined organic elutions were dried on a centrifugal evaporator (amino acid reduced extract, 0.8 g).

Thin-layer chromatography (TLC) of amino acids in Passiflora extracts: Amino acids in the various *Passiflora* extracts (50 mg/ml; 10 μl) were compared by TLC on silica gel with n-butanol–acetone–glacial acetic acid–water 35:35:10:20 as mobile phase. Standards applied (1 mg/ml; 10 μl) were alanine, aspartic acid, cystine, GABA, glutamine, glycine, methionine, serine, tryptophan, and tyrosine (Sigma–Aldrich). Amino acids were visualized by spraying with 0.3% ninhydrin in n-butanol containing 3% acetic acid and heated for 10 min. Amino acids, including GABA were prominent in the total extract and amino acid extract, and virtually absent in the amino acid reduced extract. Using NIH ImageJ for simple densitometry, GABA content was semi-quantified as compared to a 1 mg/ml (10 μl) GABA standard.

Passiflora extracts for in vivo testing in CF-1 mice

Passiflora herb source: Fresh Passiflora herb (flower, fruit, leaf, and stem) was collected from the wild in Salisbury, NC, USA by Botanical Supply, Inc. and obtained from OWH (Lot# PAS035FWBO). A voucher specimen was deposited with the herbarium at Portland State University and confirmed to be *Passiflora incarnata* L. A portion of the batch was air-dried in a drying room at OWH. Moisture content of the fresh and dried herb was determined as 75% and 5% respectively, using an A&D MF-50 moisture analyzer.

Extraction methods: Five different extracts were prepared from fresh or dried Passiflora herb as described in Table 1. Extract PAS 1 was prepared at OWH, whereas extracts PAS 4, 5, 7 and 8 were prepared at OHSU. Ethanol was removed from extracts using a rotary evaporator, and residual water by freeze drying. The weight of the freeze-dried residue was calculated as percent of the weight of fresh or dried herb extracted, and corrected for moisture content to enable comparison of the extracts (Table 1).

HPLC: A chemical fingerprint of the freeze-dried *Passiflora* extracts was obtained using an Agilent HPLC–DAD apparatus with an Econosil 5 micron C18 column. A stepwise, binary gradient of acetonitrile and water, each containing 0.1% acetic acid, was applied. The percentages of acetonitrile were 5, 25, 50 and 70 at 0, 20, 35 and 40 min respectively, with a return to starting conditions from 45 to 48 min. UV absorbance was monitored at 205 nm, 254 nm, 290 nm, 330 nm, and 350 nm. For LC–MS, full scan electrospray ionization mass spectra of the eluent were obtained using a ThermoElectron LCQ Advantage 3D Ion trap tandem mass spectrometer (San Jose, CA). The ionization source was operated in the negative mode with spray voltage 4500 V, sheath gas 35, auxiliary gas 15, capillary temperature 275 °C and tube lens 40 V. Total and individual flavonoids were estimated against vitexin as a standard, using peak areas obtained at 330 nm.

Hippocampal slice physiology: Male Sprague–Dawley albino rats were obtained from and housed in the OHSU NSI vivarium. Care and use of the animals was approved by the OHSU Animal Research Committee and performed according to its policies and guidelines. Hippocampal slices were prepared from young (2–3 weeks old) rats and kept viable by perfusion with O_2-saturated artificial CSF as described (Rossi et al. 2000). Electrical currents induced in the cell membrane by *Passiflora* total extract and amino acid reduced extract were measured under whole-cell voltage clamp conditions (Rossi et al. 2000). All current traces and the mean values are from CA1 pyramidal cells voltage-clamped at −30 mV with E_{Cl^-} set to −60 mV, so $GABA_A$ mediated chloride currents were outward currents. Blocking a $GABA_A$ mediated current resulted in an inward current. All experiments were performed in the presence of the glutamate receptor antagonist kynurenic acid (1 mM).

In vivo studies in CF-1 mice: Male CF-1 mice were obtained from and housed in the OHSU vivarium. Care and use of the animals was approved by the OHSU Animal Research Committee and performed according to its policies and guidelines. Each of the 5 different passionflower extracts was tested on a group of CF-1 mice after continuous administration in the drinking water (1000 mg freeze-dried extract/kg/day; equivalent to between 4.2 and 13 g of dry herb/kg/day depending on preparation method) for 1 week and

Table 1
Extraction methods of the five extracts from *P. incarnata*.

Extract	Condition of herb	Extraction temperature	Solvent	Duration of extraction	Extract weight as % w/w of plant material extracted	Extract weight as % w/w of dry plant material[a]
PAS 1	Fresh	25 °C	65% ethanol	14 days	2.12	8.5
PAS 4	Fresh	100 °C then 4 °C	Water	75 min	6.00	24.0
			Water	21 h		
PAS 5	Dried	4 °C	65% ethanol	14 days	7.30	7.7
PAS 7	Dried	100 °C then 4 °C	65% ethanol	65 min	9.34	9.8
			65% ethanol	19 h		
PAS 8	Dried	100 °C then 4 °C	Water	60 min	8.40	8.8
			Water	20 h		

Extraction methods used for the five different extracts from *Passiflora incarnata* whole herb, and the relative yields of each extraction method are shown.

[a] Corrected for moisture content, i.e.75% of fresh herb and 5% of dry herb.