Journal of Ethnopharmacology 125 (2009) 36–40

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Experimental evidence of the anaphrodisiac activity of *Humulus lupulus* L. in naïve male rats

P. Zanoli[a,*], M. Zavatti[b], M. Rivasi[a], A. Benelli[b], R. Avallone[a], M. Baraldi[c]

[a] Department of Biomedical Sciences, Section of Pharmacology, University of Modena and Reggio Emilia, 41100 Modena, Italy
[b] Department of Anatomy and Histology, University of Modena and Reggio Emilia, 41100 Modena, Italy
[c] National InterUniversity Consortium for the Study of Natural Active Principles (CINSPAN), University of Modena and Reggio Emilia, 41100 Modena, Italy

ARTICLE INFO

Article history:
Received 6 March 2009
Received in revised form 15 June 2009
Accepted 18 June 2009
Available online 26 June 2009

Keywords:
Humulus lupulus
8-Prenylnaringenin
Male rat
Sexual behavior

ABSTRACT

Ethnopharmacological relevance: In the folk medicine *Humulus lupulus* L. (hops) is mainly recommended as a mild sedative with antispasmodic and digestive properties. It is also reputed to exert an anaphrodisiac effect but it is still lacking the experimental evidence of this activity.
Aim of the study: To evaluate the influence of *Humulus lupulus* extract on sexual behavior of both naïve and sexually potent male rats; thereafter to investigate the role of 8-prenylnarigenin (8-PN) in the effect displayed by the hop extract.
Materials and methods: Sprague–Dawley male rats both naïve and sexually potent were acutely administered with the hop extract dosed at 5, 10, 25 and 50 mg/kg. In addition the extract was administered daily for 10 consecutive days at the dose of 0.25 mg/kg/day in sexually potent animals. The pure compound 8-PN was acutely administered in naïve rats at the dosages of 5, 12.5 and 25 μg/kg. All the animals were screened for their sexual behavior manifestation during the mating test.
Results: In naïve rats the acute administration of *Humulus lupulus* extract at the doses of 25 and 50 mg/kg significantly reduced the percentage of mounting and ejaculating animals, in comparison to vehicle controls. The other parameters recorded during the mating test were not affected by the hop extract. In sexually potent rats nor the acute neither the repeated administration of the extract modified their copulatory behavior.
The pure compound 8-PN failed to influence male sexual behavior of naïve rats.
Conclusion: Humulus lupulus extract exerted an anaphrodisiac effect only in naïve rats by inhibiting their mounting and ejaculating behavior. The presence of 8-PN in the extract could be only partially involved in the observed anaphrodisiac effect.

1. Introduction

The hop plant (*Humulus lupulus* L.) is a climbing vine belonging to the hemp family of Cannabinaceae. It is widely cultivated throughout the temperate regions in the world being an essential raw material in the brewing industry. It was firstly used for its preservative activity, but it also adds bitterness and aroma to beer. Hops or hop cones are the female inflorescences made up of membranaceous bracts covered with tiny glands holding the stick yellow resin named lupulin. The hop cones contain essential oil, bitter acids, flavonoid glycosides, catechins and prenylated chalcones (Stevens et al., 1997; Malizia et al., 1999; De Keukeleire et al., 2003). The pharmacognostic, phytochemical and pharmacological aspects of *Humulus lupulus* were summarized in a recent review (Zanoli and Zavatti, 2008).

Humulus lupulus has a long history as medicinal plant useful to treat sleep disturbances, restlessness and excitability besides to promote healthy digestion (Blumenthal, 1998). Moreover it was used as a folk remedy to treat a wide range of complaints, including spasms, cough, fever, inflammation, earache and toothache (Grieve, 1971). The plant has also gained reputation of male anaphrodisiac, but this claim is largely based on folk opinion rather than on scientific demonstration (Duke, 1985; Weiss, 1988). Nevertheless, an herbal veterinary product (named Sedovet), containing *Humulus lupulus* and *Escholtia californica*, is commercially available with the claim to treat restlessness, aggressiveness and hypersexuality in cats and dogs. Hence the primary aim of the present study was to assess the effect of *Humulus lupulus* extract on sexual behavior of male rats. Subsequently we tempted to elucidate the role of a phytoestrogenic component of hops, 8-prenylnaringenin (8-PN), in the

* Corresponding author at: Dipartimento di Scienze Biomediche, Sezione di Farmacologia, Via Campi 287, I-41100 Modena, Italy. Tel.: +39 0592055165; fax: +39 0592055376.
E-mail address: zanoli.paola@unimore.it (P. Zanoli).

0378-8741/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2009.06.018

observed effect. We focused our attention on 8-PN because it was recognized to display *in vitro* the major activity among different compounds (xanthohumol, isoxanthohumol, 8-prenylnaringenin, and 6-prenylnaringenin) identified in a hop polyphenolic fraction showing estrogenic activity (Milligan et al., 1999, 2000). The estrogenic property of 8-PN was confirmed in different *in vivo* experiments (Milligan et al., 2002; Diel et al., 2004), including the ovariectomized rat model (Rimoldi et al., 2006; Overk et al., 2008). At our knowledge the effect of 8-PN in male rats was never investigated; only one study performed *in vitro* (a yeast-based androgen receptor assay) showed an anti-androgenic activity of 8-PN (Zierau et al., 2003).

2. Materials and methods

2.1. Quantification of 8-PN in Humulus lupulus CO_2 extract by HPLC analysis

2.1.1. Chemicals

(±)-8-Prenylnaringenin (8-PN) was purchased from Sigma–Aldrich (Milan, Italy). HPLC-grade methanol and acetonitrile, and phosphoric acid of analytical grade were purchased from Carlo Erba (Milan, Italy). Distilled water deionized by Milli-Q Water Purification System (Millipore, Bedford, MA, USA) was used for the preparation of all samples and solutions.

2.1.2. Standard and sample preparation

The main stock solution of 8-PN was prepared by dissolving weighted quantities in the initial mobile phase used for the chromatographic separation (H_2O:phosphoric acid 0.5%:acetonitrile:methanol 68:22:4:6) and Tween 80 (5%). 8-PN was prepared at the concentration of 1 mg/ml. The standard mixtures for calibration were prepared by diluting the stock solution in the same mobile phase with concentrations ranging from 0.25 to 500 μg/ml. Stock solution and standard mixtures were stored in darkness at −20 °C.

Prior to HPLC analysis the extract was properly diluted in the initial mobile phase used for the chromatographic separation.

2.1.3. Apparatus and chromatographic conditions

The HPLC system consisted of a LaChrom L-7100 quaternary pump, a L-7612 Vacuum Solvent Degasser for in line degassing of the mobile phase, a rheodyne manual injector with a 50 μl loop and a LaChrom L-7455 Diode Array Detector. Data analysis was performed using a D-7000 computer interface and a D-7000 HPLC System Manager software (Merck - Hitachi, Darmstadt, Germany). The chromatographic separation was performed on an analytical Microsorb-MV^{TM} RP-C_{18} 100 Å column (250 mm × 4.6 mm), placed in a water bath set at 40 °C. The solvents used for gradient elution were H_2O (A), phosphoric acid 0.5% (B), acetonitrile (C) and methanol (D); the flow rate was set at 1 ml/min, and the gradient elution profile is presented in Table 1. After every injection, the column was re-equilibrated with initial conditions for 15 min. UV–vis absorbance spectra (λ = 198–708 nm) were collected continuously during the course of each chromatogram and used for the peak purity determination. Peak of 8-PN in the samples was identified by comparing its retention time and UV–vis spectrum with that of the reference standard, after subtraction of the corresponding base spectrum. Peak areas obtained at 220 nm were used for the construction of calibration curves.

2.2. Behavioral experiments

2.2.1. Animals

Male and female Sprague–Dawley rats, weighing from 180 g (females) to 220 g (males), were purchased from Harlan Laboratories (Udine, Italy). They were housed two per cage, males and females separately, and maintained in standard conditions at 22 ± 1 °C and 55–60% relative humidity with a reversed 12 h light/dark cycle. Food in pellet (Global Diet, 2018, Mucedola s.r.l., Milan, Italy) and water were freely available. After one week-adaptation period, the females were bilaterally ovariectomized under ketamine hydrochloride (Ketavet 100®, Farmaceutici Gellini Spa, Italy) plus xylazine hydrochloride (Rompun®, Bayer, Germany) anesthesia and allowed to recover from the surgery for three weeks. They were brought into estrous by sequential subcutaneous injections of 500 μg/rat estradiol benzoate (Estradiolo AMSA®, AMSA) and 500 μg/rat progesterone (Prontogest®, AMSA), 48 and 4 h before mating tests, respectively. They were screened with non-experimental sexually experienced males and only those exhibiting good sexual receptivity (solicitation behavior and lordosis in response to mounting) and no rejection behavior, were used.

Animal care, maintenance and surgery were conducted in accordance with the Italian law (D.L. n. 116/1992) and European legislation (EEC n. 86/609). The experimental design and procedures received the approval of the Bioethical Committee of the Italian Institute of Health.

2.2.2. Treatments

Humulus lupulus CO_2 extract, supplied by Indena Spa (Milan, Italy), was dissolved in Tween 80 (10%) and deionized water. It was administered by oral gavage (p. os) in a volume of 5 ml/kg both acutely, at doses of 5, 10, 25 and 50 mg/kg b.w., and subchronically at a daily dose of 0.25 mg/kg b.w. for 10 consecutive days. Control animals received an equal amount of vehicle solution. Mating tests were performed 45 min after the acute administration or 24 h after the last administration of subchronic treatment. The pure compound 8-PN, purchased by Sigma–Aldrich (Milan, Italy), was dissolved in Tween 80 (5%) and water; then it was acutely administered p. os at different dosages (5–12.5–25 μg/kg) 45 min before the mating test. The 8-PN dosages were based on the amount of 8-PN held in *Humulus lupulus* extract administered in rats.

2.2.3. Mating test

Male sexual behavior was monitored by trained observers, unware of the experimental design, in a separate room under a dim red illumination, as described by Ågmo (1997). Single male rats were placed in rectangular glass observation cages (40 cm × 50 cm × 40 cm) for 5 min before the introduction of a sexually receptive female. The following parameters of sexual behavior were recorded in accordance to Ågmo (1997):

(1) *mount latency (ML)*: time from the introduction of the female until the first mount with pelvic thrusting;

Table 1
Gradient elution system.

Time (min)	% A, H_2O	% B, 0.5% phosphoric acid	% C, acetonitrile	% D, methanol
0	68	22	4	6
18	58	22	8	12
23	54	22	12	12
40	36	22	30	12