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Anxiolytic-like effects of acute and chronic treatment with *Achillea millefolium* L. extract

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ABSTRACT

Ethnopharmacological relevance: Achillea millefolium L. (Asteraceae), known as yarrow ("mil folhas"), has been used as folk medicine for gastrointestinal disorders, inflammation, anxiety, and insomnia.
Aim: To evaluate the potential anxiolytic-like effect of hydroalcoholic extract of *Achillea millefolium* L. in animal models.
Methods: The present study evaluated the effects of the hydroalcoholic extract from the aerial parts of *Achillea millefolium* L. in mice subjected to the elevated plus-maze, marble-burying, and open-field tests. Additionally, the $GABA_A$/benzodiazepine (BDZ) mediation of the effects of *Achillea millefolium* was evaluated by pretreatment with the noncompetitive $GABA_A$ receptor antagonist picrotoxin and the BDZ antagonist flumazenil and by [^{3}H]-flunitrazepam binding to the BDZ site on the $GABA_A$ receptor.
Results: Achillea millefolium exerted anxiolytic-like effects in the elevated plus-maze and marble-burying test after acute and chronic (25 days) administration at doses that did not alter locomotor activity. This behavioral profile was similar to diazepam. The effects of *Achillea millefolium* in the elevated plus-maze were not altered by picrotoxin pretreatment but were partially blocked by flumazenil. Furthermore, *Achillea millefolium* did not induce any changes in [^{3}H]-flunitrazepam binding.
Conclusion: The results indicate that the orally administered hydroalcoholic extract of *Achillea millefolium* L. exerted anxiolytic-like effects that likely were not mediated by $GABA_A$/BDZ neurotransmission and did not present tolerance after short-term, repeated administration.

1. Introduction

Numerous traditionally used plants exhibit pharmacological properties with great potential for therapeutic applications in the treatment of central nervous system disorders, such as anxiety disorders (Carlini, 2003; Faustino et al., 2010). *Achillea millefolium* L. (Asteraceae), known as yarrow ("mil folhas"), is a perennial herb that has been used for hundreds of years in folk medicine in several countries (Chandler et al., 1982; Wichtl and Bisset, 1989). In Germany and Italy, it is used for the treatment of gastrointestinal disturbances. In Britain and North America, it is used to treat bleeding (Bruneton, 1999; Willuhn, 2002; Applequist and Moerman, 2011). In Brazil, its main indication is for the treatment of pain, wounds, inflammation, and gastrointestinal complaints (Dalsenter et al., 2004; Cavalcanti et al., 2006; Pires et al., 2009), although plant infusion or the decoction of the aerial parts of the plant is indicated for "calmness" (Manfrini et al., 2009). This latter indication is also seen in Mexico (Molina-Hernandez et al., 2004).

Previous preclinical studies have corroborated its safety and antinociceptive and antiulcer effects (Dalsenter et al., 2004; Cavalcanti et al., 2006; Pires et al., 2009). Although *Achillea millefolium* L. has been proposed as a folk remedy in the treatment of central nervous system disorders, very few data have been published supporting this claimed ethomedical action. Molina-Hernandez et al. (2004) used a conflict operant procedure and showed that the anticonflict-like actions of an aqueous extract of

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the flowers of *Achillea millefolium* L. administered intraperitoneally vary according to the estrous cycle phase of female rats.

The search for new anxiolytic drugs is supported by the adverse effects associated with the current drug treatments for generalized anxiety disorder. Benzodiazepines (BDZs) can lead to disturbing effects, such as amnesia, dependence liability, and sedation. Buspirone is associated with low patient satisfaction. Moreover, antidepressants can lead to sexual dysfunction, insomnia, and gastrointestinal disturbances (Andreatini et al., 2001; Carlini, 2003; Mitte et al., 2005). Medicinal plants continue to have anxiolytic potential (Carlini, 2003; Faustino et al., 2010).

The elevated plus maze is likely the most frequently used animal model for studying anxiety and anxiolytic-like drugs (Carobrez and Bertoglio, 2005). It is based on the conflict between the fear of open areas on the open arms and neophobia (fear of new environments), which elicit avoidance behavior, and the drive to explore new environments, which elicits approach behavior (Rodgers and Cole, 1994; Rodgers and Dalvi, 1997). Thus, it is related to unconditioned behavior, although some learned behavior occurs during the experimental session (Rodgers and Cole, 1994; Carobrez and Bertoglio, 2005). Most anxiolytic-like drugs increase open arm exploration, reflected by an increase in the percentage of entries into and time spent on the open arms, at doses that do not affect locomotor activity (Lister, 1987; Borsini et al., 2002). However, debatable is whether the elevated plus maze can detect the anxiolytic-like effects of antidepressants, such as selective serotonin reuptake inhibitors (Rodgers and Cole, 1994; Borsini et al., 2002). Marble-burying behavior in mice and rats is based on the behavior of burying harmless objects (Nicolas et al., 2006; Thomas et al., 2009). This model is also related to an unconditioned behavior. Anxiolytic-like drugs decrease the number of marbles buried during a session at doses that do not alter locomotor activity (Njung'e and Handley, 1991; Borsini et al., 2002; Nicolas et al., 2006). These models have some differences. The elevated plus maze is affected by an animal's previous maze experience, whereas marble-burying behavior is stable across repeated tests (Rodgers and Cole, 1994; Carobrez and Bertoglio, 2005; Thomas et al., 2009; Gomes et al., 2011). There are some criticisms of the marble-burying model as a model of anxiety or defensive behavior (Londei et al., 1998; Thomas et al., 2009). Marble-burying behavior has been considered a perseverative behavior related to obsessive compulsive disorder (Londei et al., 1998; Costa et al., 2011; Gomes et al., 2011). However, it is sensitive to benzodiazepines and antidepressants (Njung'e and Handley, 1991; Borsini et al., 2002; Nicolas et al., 2006), which may make it suitable for screening anxiolytic-like drugs. Considering the possibility of false-positive and false-negative results with these animal models (Borsini et al., 2002; Nicolas et al., 2006), in the present study these two models were used in a complementary manner.

Thus, the objective of the present study was to evaluate the effects of acute and chronic oral treatment with the hydroalcoholic extract of the aerial parts of *Achillea millefolium* L. in mice subjected to the elevated plus maze, marble-burying, and open-field tests. Additionally, the $GABA_A$/benzodiazepine (BDZ) mediation of the effects of *Achillea millefolium* was evaluated by pretreatment with the noncompetitive $GABA_A$ receptor antagonist picrotoxin and the BDZ antagonist flumazenil and by [^{3}H]-flunitrazepam binding to the BDZ site on the $GABA_A$ receptor. Considering that apigenin is an important constituent of *Achillea millefolium* extracts (Pires et al., 2009; Tuberoso et al., 2009) and that previous studies have suggested an anxiolytic-like effect of this compound (Viola et al., 1995; Paladini et al., 1999), the effect of apigenin was also investigated, at a dose range similar to that which would be found in *Achillea millefolium* extract (0.1–1.1%; Pires et al., 2009; Tuberoso et al., 2009), in the elevated plus-maze and marble-burying test.

2. Materials and methods

2.1. Plant material

The *Achillea millefolium* used in our experiments was collected in July 2007 from the botanical garden of the Universidade Paranaense campus, Umuarama, Brazil, at an altitude of 430 m above sea level (S23°47′55–W53°18′48). The plant was identified by Dr. Mariza Barion Romagnolo (Department of Botany, Universidade Paranaense, PR, Brazil). Voucher specimens were deposited at the herbarium of the university (specimen no. 1896).

2.2. Preparation of hydroalcoholic extract

The aerial parts (leaves, stalks, and stems) of *Achillea millefolium* were air-dried in an oven at 40 °C for 4 days, and then the dry plant was cut and pulverized. The dried, powdered plant material was macerated for 7 days using 90% ethanol as a solvent. The solvent was then eliminated using a rotary vacuum evaporator under reduced pressure and lyophilized, yielding an extract of 17.4% of the dry material (Potrich et al., 2010). The hydroalcoholic extract was dissolved in a 5% Tween-80 aqueous solution and administered at doses ranging from 30 to 600 mg/kg.

2.3. Animals

Animals were adult albino Swiss male mice (30–45 g) from our breeding stock. They were housed in groups in polypropylene cages with wood shavings as bedding, under a controlled 12 h/12 h light/dark cycle (lights on at 7:00 a.m.) and controlled temperature (22 °C). The animals had free access to water and food, with the exception of 1 h before and during the experiments. The animals were not specifically handled prior to the experiments, with the exception of handling during necessary animal care (e.g., cleaning the cages) and drug administration (weighing, tail marking, and drug administration).

2.4. Drugs and treatments

The hydroalcoholic extract of *Achillea millefolium* (30–300 mg/kg), diazepam (0.75 mg/kg; Sigma, USA), and *Achillea millefolium* vehicle were administered by gavage (per os, p.o.) 1 h before the tests. Flumazenil (1.0 mg/kg, Sigma; Lolli et al., 2007; Kamei et al., 2009) and picrotoxin (1.0 mg/kg, Sigma; Stankevicius et al., 2008) were administered intraperitoneally (i.p.) 30 min before the administration of the hydroalcoholic extract of *Achillea millefolium* (300 mg/kg). All treatments were administered at a constant volume of 10 mL/kg body weight. The *Achillea millefolium* dose was based on a preliminary pharmacological screening protocol (Pires et al., 2009) with different extract doses (100, 300, and 600 mg/kg) administered to mice. At all doses used, no signs of acute toxicity (e.g., seizures, abdominal pain, and death) were observed.

The apigenin doses (0.3, 1.0, and 3.0 mg/kg; Sigma) were calculated based on apigenin content in *Achillea millefolium* extract: 0.1% (Tuberoso et al., 2009)–1.1% (Pires et al., 2009).

2.5. Behavioral tests

In the acute experiments, each animal was tested in only one behavioral test, whereas in the chronic experiment, the same animal was tested in the open-field test 5 min before testing in the elevated plus maze. The mice were habituated to the experimental room 1 h before the tests. The procedures used in the present study were in accordance with the guidelines for animal research care