

RESEARCH ARTICLE

Psychopharmacological effects of *Artemisia copa* aqueous extract in mice

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Abstract

Objective: To evaluate the aqueous extract from aerial parts of *Artemisia copa* Phil. (Asteraceae) administered orally for its psychopharmacological activities in several experimental models

Methods: The extract was administered p.o. in Swiss albino mice and tested on pentobarbital-induced hypnosis, locomotor activity, exploration in the hole-board, anxiolytic like profile evaluated in the marble-burying test and anticonvulsant activity on convulsions induced by pentylenetetrazol.

Results: *Artemisia copa* at doses up to 1.5 g/kg produced a dose-dependent sleep induction and potentiation of sub-hypnotic and hypnotic doses of pentobarbital. The extract also produced a dose-dependent increase and decrease in the spontaneous motor activity (0.5–1.5 g/kg, respectively), no disruption or a decrease on exploratory (hole-board) behavioral profiles (0.5–1.5 g/kg respectively) and a dose-related anxiolytic-like activity as indicated by increases in the percentage of marbles they left uncovered in the marble-burying test at doses (0.5 g/kg) that do not disrupt the motor activity. In addition, the extract (1.5 g/kg) produced a significant increase in the latency time and a decrease in the duration of seizures and mortality induced by PTZ 75 mg/kg in mice.

Conclusion: These results suggest that the aqueous extract of *Artemisia copa* may contain sedative principles with potential anxiolytic and anticonvulsant activities.

Keywords: Anticonvulsant activity; anxiolytic activity; aqueous extract; *Artemisia copa*; sedative activity

Introduction

Artemisia copa Phil. (Asteraceae), commonly known as “copa-copa”, is a small and very branched bush with a height of 30–60 cm that grows over 3000 m in the north-west of Argentina and in the north of Chile. The plant is regularly sold in local markets and herb health stores, and infusions of the aerial parts are used in popular medicine as antitussive, digestive, for lowering fever, for pulmonary diseases and hypertension (Giberti, 1983). The leaves, macerated in alcohol, are also used locally to rub off rheumatic pains (Ratera & Ratera, 1980).

In a previous phytochemical study, four flavonoids (jaceidin, jaceidin-7-methyl-ether, luteolin and kaempferol-6-methyl-ether 3-rhamnoglucoside) were isolated from *Artemisia copa* (Quarenghi et al., 1991). Luteolin and its derivatives have been described to exert several interesting pharmacological activities, namely antitumoral, antioxidant, anti-inflammatory, analgesic (Block et al., 1998; Kotanidou et al., 2002) and anxiolytic (Coleta et al., 2008). Pharmacological studies of the plant revealed that the aqueous extract of aerial parts of *A. copa* possess analgesic and anti-inflammatory activity (Acevedo et al., 2005).

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In this paper we studied the anticonvulsant activity of *A. copa*, considering that several genera of the Compositae family, including the genus *Artemisia*, have been used in traditional medicine to treat epilepsy in Brazil and other countries (de Lima et al., 1992).

Taking into account all that information, this study was to carry out a psychopharmacological screening of *Artemisia copa* in different experimental models in mice.

Materials and methods

Plant collection

The aerial parts of *Artemisia copa* Phil. (Asteraceae), were collected and identified by Gustavo Giberti in Antofagasta de la Sierra, Catamarca Province, Argentina, in November 2008. A voucher specimen has been deposited at the Museo de Farmacobotánica Juan A. Domínguez, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina, under BAF 10313.

Preparation of *A. copa* aqueous extract

The plant material was dried under airflow in an oven at $45 \pm 1^\circ\text{C}$ and powdered mechanically. The aqueous extract was prepared by macerating 50 g of powdered plant material for 20 min using 500 mL of boiling water. The extract was filtered and freeze-dried. The yield of the infusion was 12.2% w/v. The lyophilized aqueous extract of *A. copa* was dissolved in physiological saline on the day of experiment.

Drugs

The following drugs were used: Diazepam (DZ) (Hoffmann-La Roche), pentobarbital sodium (PB), pentylenetetrazole (PTZ) (Sigma, Saint Louis, MO).

Experimental animals and treatments

Female albino Swiss mice (25–30 g) were used. They were housed in groups of 10/cage for a minimum of 3 days prior to pharmacological studies. They were kept in a room maintained at $25 \pm 1^\circ\text{C}$ with free access to standard laboratory food and tap water under a 12 h light/dark cycle (light from 8.00 a.m. to 8.00 p.m.) and used taking into account international guiding principles and local regulations concerning the care and use of laboratory animals for biomedical research (ANMAT, 1996). All animals were fasted 8 h with tap water *ad libitum* prior to experiment. A minimum of 10 animals per group was used in all tests.

Different doses of the lyophilized aqueous extract of *A. copa* between 0.2 and 1.5 g/kg were administered p.o. in a volume of 0.1 mL/10 g body weight 60 min before tests. Control mice (saline 10 mL/kg) were tested in parallel with those animals receiving drug treatment.

Evaluation of pharmacological activities

Psychopharmacological assays

Hypnogenic activity. Vehicle or *A. copa* aqueous extract (0.2, 0.4, 0.8 and 1.5 g/kg) were administered p.o. to mice 60 min before i.p. injection of sub-hypnotic or hypnotic doses of PB (30 and 40 mg/kg, respectively). In both experiments and for each mouse, the time spent between loss and recovering of the righting reflex on each treatment was recorded.

Studies on spontaneous motor activity. Spontaneous locomotor activity (SMA) was recorded in a 5 min period with a photocell activity meter apparatus (Animex, LKB, Farad, Sweden). DZ was used as a reference drug and administered at different doses of 0.3, 0.5, 1 and 3 mg/kg, i.p., 30 min before the test. Two doses of *A. copa* aqueous extract (0.5 and 1.5 g/kg) were tested.

Studies on exploratory behavior. The hole-board test (Perez et al., 1998) was used for this study. The animals were singly placed in a hole-board apparatus (Ugo Basile, Italy) and the number of times mice dipped their heads into the holes during a 5-min trial was recorded for control and treated groups (*A. copa* 0.3, 0.5 and 1.5 g/kg). A decrease in the head-dips, compared to controls, reveals a sedative behavior.

Marble-burying test. The marble-burying test (MBT) consisted of a Plexiglas cage $23 \times 17 \times 14$ cm with a smooth lid punctured by small ventilation holes. The floor was covered with a 5 cm layer of sawdust and 24 glass marbles 1.5 cm diameter, evenly spaced against the walls ($4 \times 8 \times 4 \times 8$) of the cage (Young et al., 2006). The mouse was placed in the cage for 30 min after which it was removed and the burying response quantified by counting the number of marbles that were more than two thirds covered with sawdust. An anxiolytic-like effect is assumed from drug-induced decreases in marbles buried (or the reciprocal measure of increases in marbles left uncovered). Drug treatments and doses were as follows: DZ 0.3, 0.5, 1 and 3 mg/kg, i.p. administered 30 min before the test, and *A. copa* aqueous extract 0.5 and 1.5 g/kg, p.o., 60 min before.

Pentylenetetrazol-induced seizures. The mice were treated with pentylenetetrazol (75 mg/kg, i.p.) 60 min before *A. copa* aqueous extract (1.5 g/kg, p.o.), and the latencies (s) to onset of the first convulsion, duration (s) and lethality were recorded. Animals devoid of seizures were considered protected.

Statistical analysis

All data were expressed as mean \pm SEM (standard error of mean). The statistical tests used were one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test and the non parametric χ^2 test, when correspond. A value of $P < 0.05$ was considered significant.

Results

The *A. copa* aqueous extract at doses of 0.2, 0.4 and 0.8 g/kg does not induce or potentiate sleeping time produced by sub-hypnotic (30 mg/kg) or hypnotic doses of PB (40 mg/kg), but given at the dose of 1.5 g/kg produced a significant increment of the sleep in both treatments, ($P < 0.01$) (Figure 1).

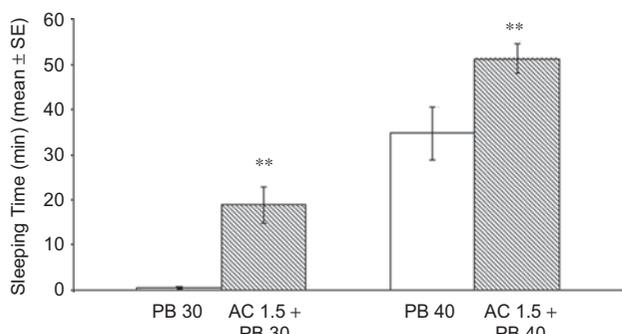


Figure 1. Effect of AC on the sleeping time in Swiss mice of subhypnotic and hypnotic doses of pentobarbital. Mice were given *A. copa* aqueous extract at doses of 0.2, 0.4, 0.8 and 1.5 g/kg, p.o., 60 min before PB. Doses up to 0.8 g/kg do not induce or potentiate sleeping time produced by sub-hypnotic (30 mg/kg) or hypnotic doses of PB (40 mg/kg). Each bar represents the mean \pm SEM of the sleeping time (min) of ten mice. ** $P < 0.01$ versus control (ANOVA followed by Dunnett's test).

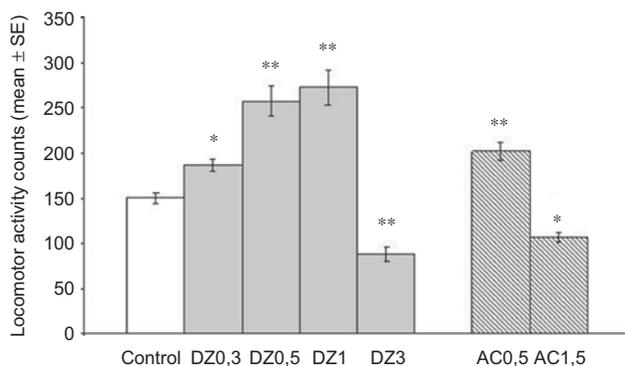


Figure 2. Effect of *A. copa* on the spontaneous motor activity during a 5-min test session. AC (0.5 and 1.5 g/kg, p.o.) was administered 60 min before test and DZ at doses between 0.3 and 3 mg/kg, i.p., 30 min before. Each bar represents the mean \pm SEM of the SMA of ten mice. * $P < 0.05$; ** $P < 0.01$ versus control (ANOVA followed by Dunnett's test).

The overall effects of the p.o. administration of the plant extract on SMA are shown in Figure 2. *A. copa* produced a dose-dependent and significant increase and decrease in the SMA at doses of 0.5 and 1.5 g/kg ($P < 0.01$ and $P < 0.05$, respectively). DZ also showed a significant increase on the SMA (0.3, 0.5 and 1 mg/kg) and a decrease at 3 mg/kg.

In the hole-board test, the *A. copa* aqueous extract (1.5 g/kg) showed a significant decrease in the number of nose-pokes ($P < 0.01$) without effects at doses of 0.3 and 0.5 g/kg, compared to control. This decline reveals a sedative effect at high doses (Figure 3).

Figure 4 indicates that low doses of DZ between 0.3 and 1 mg/kg and *A. copa* aqueous extract at the dose of 0.5 g/kg, displayed a significant anxiolytic-like activity indicated by increases in the percentage of marbles they left uncovered on top of their bedding material. Animals

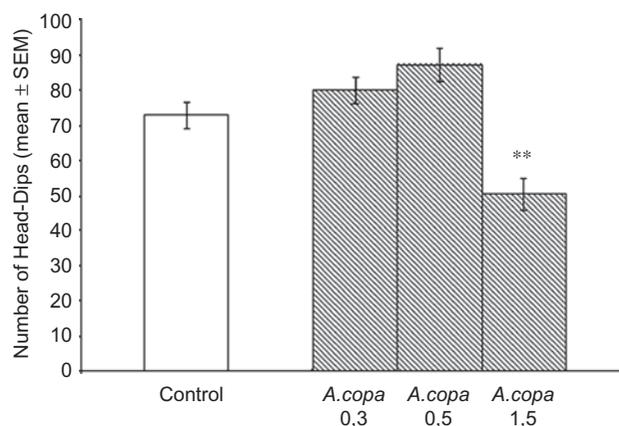


Figure 3. Exploratory behavior in the hole-board. Effects of *A. copa* aqueous extract administered 60 min before the test during 5 min in mice. *A. copa* (0.3, 0.5 and 1.5 g/kg, p.o.) was administered 60 min before the test. Each bar represents the mean \pm SEM of the nose-pokes of ten mice. * $P < 0.01$ (ANOVA followed by Dunnett's test).

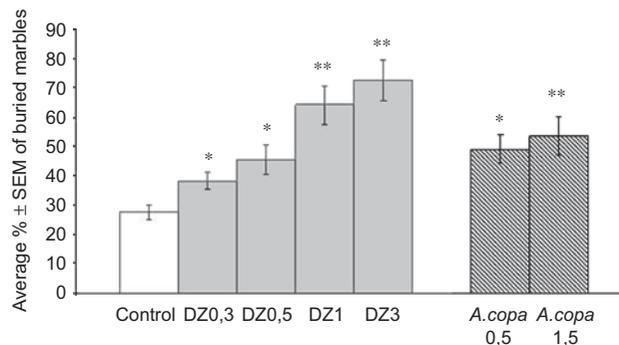


Figure 4. Effects of *A. copa* on the behavior of mice in the marble burying test. Mice were given *A. copa* aqueous extract at doses of 0.5 and 1.5 g/kg, p.o., 60 min before the test and DZ at several doses (0.3, 0.5, 1 and 3 mg/kg, i.p.) 30 min before. Each bar represents the average % (mean \pm SEM) of marbles that remained uncovered for ten mice. * $P < 0.05$; ** $P < 0.01$ versus control (ANOVA followed by Dunnett's test).

with doses greater than 3 mg/kg for DZ and 1.5 g/kg for *A. copa* also increased the percentage of marbles they left uncovered ($P < 0.01$) but in this case as a result of sedative activity.

A. copa aqueous extract produces a significant increase in the latency ($P < 0.05$) and a decrease in the duration of seizures and lethality ($P < 0.01$ and 0.05 , respectively) induced by PTZ (Table 1) at doses which also affected locomotor activity (1.5 g/kg).

Discussion

The results of the present study demonstrate that the p.o. administration of increasing doses of an aqueous extract of *A. copa*, causes several effects including a dose related increase and decrease of SMA, an induction and potentiation of sub-hypnotic and hypnotic doses of PB, a dose related anxiolytic-like activity in the marble burying assay and a protective action against convulsions induced by PTZ.

Animals, such as mice and rats, bury objects in the bedding material of their cage. One example of this type of behavior occurs when mice are placed in an environment which contains glass marbles as unfamiliar objects. An anxiolytic-like effect is assumed from pretreatment with drugs that induced reduction in number of marbles buried (or the reciprocal measure of increases in marbles left uncovered) without concomitant impairments in tests that are typically used as potential indicators of side-effect liability such as locomotor and exploratory screen assays (Malick, 1987).

The administration of diazepam inhibits marble-burying behavior at doses that not disrupt the motor activity (Young et al., 2006). In a similar way, the *A. copa* aqueous extract-like low doses of DZ present an anxiolytic effect at doses (0.5 g/kg) that do not produce a decrease in the activities evaluated in the SMA and hole-board tests. The increase in locomotor activity is a consequence of the anxiolytic effect produced by low doses of *A. copa* and diazepam (Hiller & Zetler, 1996).

It is known that the efficacy of the γ -aminobutyric acid (GABA) system plays an inhibitory role on activity of the central nervous system (CNS). In this study, *A. copa* was effective against PTZ-induced seizures, a receptor antagonist that reduces the activity of GABA_A

(Kasture et al., 2000). This may suggest that the anticonvulsant action of *A. copa* is mediated by a modulatory action on the channel of GABA_A/benzodiazepine receptor complex, increasing the concentration of GABA in brain.

Since the discovery that certain flavonoids (namely flavones) specifically recognise the central BDZ receptors, several efforts have been made to identify naturally occurring GABA(A) receptor benzodiazepine binding site ligands. Flavonoid derivatives with a flavone-like structure such as apigenin and chrysin have been reported for their anxiolytic-like activity in different animal models of anxiety. One of the constituents present in the aqueous extract of *A. copa*, luteolin (3',4',5,7-tetrahydroxyflavone) (Quarenghi et al., 1991), is a widespread flavonoid aglycon and has CNS activity with anxiolytic-like effects despite the low affinity for the shown *in vitro*. Our findings suggest a possible interaction with other neurotransmitter systems but we cannot rule out the possibility that luteolin's metabolites might show a higher affinity for the BDZ-R *in vivo*, thus eliciting the evident anxiolytic-like effects through a GABAergic mechanism (Coleta et al., 2008).

These results suggest that the aqueous extract of *Artemisia copa*, contains sedative principles with potential anxiolytic and anticonvulsant activities. However, further studies including toxicological evaluations and the use of all-purified fractions are necessary to confirm and extend the present findings.

Declaration of interest

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Table 1. Influence of acute pretreatment of mice with AC aqueous extract p.o. on latency and duration to convulsions induced by PTZ (75 mg/kg, i.p.). Data are expressed as means \pm SEM. * $P < 0.05$; ** $P < 0.01$ (ANOVA followed by Dunnett's test) and *** $P < 0.05$ (χ^2).

Treatment	Latency (s)	Duration (s)	Lethality (%)
Vehicle (n = 15)	80.27 \pm 7.55	293.13 \pm 62.36	33.33
<i>A. copa</i>	174.18 \pm 42.69*	116.82 \pm 19.63**	9***
1.5 g/kg (n = 17)			

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