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Artigo

Effect of *Artemisia annua* L. leaves essential oil and ethanol extract on behavioral assays

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RESUMO: "Efeito do extrato etanólico bruto e óleo essencial das folhas de *Artemisia annua* em modelos comportamentais". *Artemisia annua* tem sido utilizada tradicionalmente para o tratamento de malária e febre na China devido à presença do princípio ativo, artemisinina. O presente trabalho avaliou a atividade central de do óleo essencial obtido por hidrodestilação e do extrato etanólico bruto de folhas frescas de *A. annua* em modelo *in vivo* como parte de um *screening* farmacológico dessa espécie. Sono induzido por pentobarbital, nado forçado e o ensaio de campo aberto são modelos de estudo conhecidos para o estudo de fármacos sobre depressão induzida. A administração do óleo essencial ou extrato bruto etanólico de *A. annua* aumentaram o tempo de imobilidade no teste do nado forçado. Por outro lado, diminuíram outros parâmetros no campo aberto, como ambulação, exploração, o ato de lamber as patas ou se lamber. Ambos produtos aumentaram o tempo de sono induzido por pentobarbital, com o óleo essencial apresentando um efeito superior ao do extrato. Pela análise dos resultados, é possível sugerir que tanto o extrato bem como o óleo essencial podem atuar como depressores do Sistema Nervoso Central (SNC).

Unitermos: *Artemisia annua*, Asteraceae, SNC, *in vivo*.

ABSTRACT: *Artemisia annua* has been used as a traditional plant for the treatment of malaria and fever in China because of the presence of its active compound, artemisinin. The present study evaluated the central activity of the essential oil and the crude ethanol extract of *A. annua* L. in animals as a part of a psychopharmacological screening of this plant. The extract was prepared in ethanol (AEE) and the essential oil (AEO) obtained by hydrodistillation, both with fresh leaves. Induced immobility, the forced swimming test (FST) and the open-field test (OFT) are well-known animal models to study drug-induced depression. The administration of *A. annua* essential oil or crude ethanol extract increased the immobility time in the FST and decreased other activities (ambulation, exploration, rearing and grooming) in the OFT in animals. Both AEO and AEE prolonged pentobarbital-induced sleep as well, but the essential oil had a marked effect. Observing these results, it is possible to suggest that *A. annua* crude ethanol extract and essential oil could act as depressors on the Central Nervous System (CNS).

Keywords: *Artemisia annua*, Asteraceae, CNS, *in vivo*.

INTRODUCTION

Artemisia annua L. has been used in traditional Chinese medicine for treatment of malaria and fever because its contents of artemisinin, a sesquiterpen found in this specie and in *A. apieceae* in amounts enough to be used as a therapeutic agent (Klayman, 1985; Simon et al., 1990). The chemical composition of *A. annua* has been studied extensively (Perazzo et al., 2003; Foglio, 1996; Carnat et al., 1985; Marques et al., 2006) and it is comprised of a complex mixture, including linalool, cineol, *p*-cymene, thujone and camphor. These compounds have been studied to evaluate its effect on the Central Nervous System (CNS), presenting a facility to cross biological membranes because of its elevated liposolubility and might affect the CNS (Robbers et al., 1996). In the present study, behavioral assays have been conducted with the essential oil (AEO) and the crude ethanolic extract (AEE) of this plant in rodents to evaluate its central effects.

MATERIAL AND METHODS

Collection of the vegetal material

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A. annua L. leaves (Hybrid CPQBA 2/39 x PL5) were collected in April 2001 from the experimental field of medicinal plants of Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas (CPQBA) - UNICAMP, located in Paulínia, SP, Brazil. A voucher specimen is deposited at CPQBA/UNICAMP, under registration number CPQBA- 12.46.

Extraction procedure

Fresh leaves of *A. annua* (450 g) were submitted to dynamic maceration with ethanol (99.0%) for four hours. The macerate was filtered and this procedure was repeated. Concentration of the extracts under reduced pressure provided 51.07 g (yield 11.35%) of crude ethanol extract (AEE). The residue was suspended in 3% Tween 80 in saline solution (100 mg AEE/mL).

Essential oil extraction procedure

The essential oil was obtained using the Method I of the Brazilian Pharmacopoeia (2nd edition, 1959). Fresh leaves of *A. annua* (500 g) were distilled for three hours using a Clevenger apparatus, yielding 1.28%. The essential oil obtained (AEO) was added to a 5% Tween 80 in 0.9% saline solution (100 mg AEO/mL) just before administration to the animals.

Animals

Male rats (*Rattus norvegicus* - albinus, Wistar), specific pathogen free, weighing 150 - 200 g, acquired from the Animal Experimental Center of Universidade Estadual de Campinas were used. The animals were kept in five animal groups in polyethylene boxes, in a controlled environment (23 ± 2 °C), for 12 hour shifts with dark/light control, with food and water *ad libitum*, for 7 days before the experiments. This study was conducted according to internationally accepted principles of laboratory animal use.

Treatment and dose

The animals were treated by intraperitoneal via (i.p.), and the used doses were 470 mg/kg for the essential oil and 450 mg/kg for crude ethanol extract (Perazzo et al., 2003).

Pentobarbital-induced sleeping-time

Three groups of rats (n = 8) were first injected (i.p.) with the control solution (3% Tween 80 0.9% in saline solution), AEO (470 mg/kg) or AEE (450 mg/kg). Thirty minutes later, 40 mg/kg of sodium pentobarbital (Cristália Ind. Farmacêutica Co.) was given i.p. and the latency time of lost reflexes and the time elapsed between the loss and recovery of the righting reflex was recorded as the sleeping time (Lima et al., 1993).

Forced swimming test

The method consists in placing a rat in cylinder (40 cm height, 18 cm diameter) containing 15 cm water maintained at 25 °C. After 15 minutes (pre-test session), the rat is dried for 15 min in a heated enclosure (32 °C). Twenty-four hours later, the animals receive the treatment (AEO 470 mg/kg, AEE 450 mg/kg) and are exposed again to the conditions outlined above, and the total swimming time and immobility during a five-minute period was registered in the test session (Porsolt et al., 1977). This experiment was recorded for further analysis.

Open-field test

The exploratory activity of the rats was also observed in an open-field. The AEO (470 mg/kg), AEE (450 mg/kg) or control solution were given 30 minutes before the rat has been placed into an unknown open-field (100 x 100 x 50 cm). The ambulation, exploration, rearing and grooming of the rat in the open-field were recorded for 5 minutes. This test can be used for evaluation of the effect of the drugs on the locomotor activity of the rats (Borsini et al., 1986). This experiment was also recorded for further analysis.

Statistical analysis

The statistical analyses were done using Analysis of Variance (ANOVA) followed by the Tukey-Kramer multiple comparison test (Sokal and Rohlf, 1995). Results with $p < 0.05$ were considered to be significant. Data are expressed as mean ± S.D.

RESULTS

Pharmacological assays

In the sleeping time induced by pentobarbital, it is possible verify that the administration of both AEO (0.8 ± 0.37 min.) and AEE (4.6 ± 0.54 min.) presented the latency time for the induction of the depressive action to be shorter than that of the control group (5.6 ± 0.42 min.). Regarding the total sleeping time, all the groups were significantly different (Figure 1). The group treated with AEO (219.0 ± 7.34 min.) presented the higher sleeping time, significantly different from the group treated with AEE (137.5 ± 6.39 min.) and from the control (78.6 ± 3.31 min.). The group treated with AEE was also different from the control group ($p < 0.05$). These results are shown in the Figure 2.

In the FST, the administration of AEO significantly decreased the total swimming time (0.47

Table 1. Effect of i.p. administration of AEO (470 mg/kg) and AEE (450 mg/kg) on the total swimming time and immobility in the forced swimming test in rats.

	Control	AEO (470 mg/kg)	AEE (450 mg/kg)
Swimming time (min)	4.3 ± 0.15^a	0.47 ± 0.86^b	3.0 ± 0.47^c
Immobility (min)	0.2 ± 0.15^a	4.30 ± 1.10^b	1.2 ± 0.21^a

Different letters present statistically significant results ($p < 0.05$ - ANOVA followed by the Tukey - Kramer multiple comparison test). Data are expressed as mean ± S.D.

Table 2. Effect of i.p. administration of AEO (470 mg/kg) and AEE (450 mg/kg) on the open-field test in rats.

Parameter (unit)	Control	AEO (470 mg/kg)	AEE (450 mg/kg)
Deambulation	87.0 ± 31.5^a	4.3 ± 3.6^b	76.2 ± 7.69^a
Exploration	20.8 ± 5.60^a	0.60 ± 0.89^b	12.6 ± 3.43^a
Rearing	20.4 ± 4.39^a	0.0 ± 0.0^b	14.2 ± 1.48^c
Grooming	20.2 ± 9.20^a	0.0 ± 0.00^b	5.2 ± 0.83^b

Different letters present statistically significant results ($p< 0.05$ - ANOVA followed by the Tukey - Kramer multiple comparison test. Data are expressed as mean ± S.D.

± 0.86 min.) when compared to the control time (4.3 ± 0.15 min.). The control group was different from the group treated with AEE (3.0 ± 0.47 min.) as well (Table 1).

The immobility in the group treated with AEO (4.3 ± 1.10 min.) was greater and statistically different from the AEE group (1.2 ± 0.21 min.). In the open-field test, the AEO inhibited the locomotor activity (4.3 ± 3.60), while both AEE (76.2 ± 7.69) and the control (87.0 ± 31.5) did not show this effect. In the exploratory activity, all groups were statistically different, but the one treated with AEO (0.60 ± 0.89) presented a weak exploratory activity when compared to the control (20.8 ± 5.60) and the AEE group (12.6 ± 3.43). Results are presented in Table 2.

The number of rearing in the group treated with AEO was significantly different from the group treated with AEE (14.2 ± 1.48), and both groups were different of the control (20.4 ± 4.39). The number of grooming was greater in the control group (20.2 ± 9.20), and it was significant to the groups treated with AEO and AEE (5.2 ± 0.83). These results are in Table 1.

DISCUSSION

As a part of the pharmacological screening of this plant and its effect on the CNS, our group first described the chromatographic analysis of the AEO (Perazzo et al., 2003). Its composition includes major terpenes compounds as 1,8-cineol (20.42%), camphor (22.68%) and linalool (3.82%). The major identified monoterpene compound was p-cymene (12.21%), as

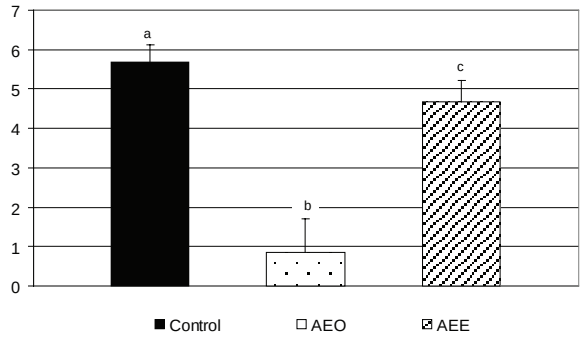

Figure 1. Effect of i.p. administration of AEO (470 mg/kg) and AEE (450 mg/kg) on pentobarbital-induced sleeping time (minutes to the lost of the righting reflex) in rats. Different letters present statistically significant results ($p < 0.05$ - ANOVA followed by the Tukey - Kramer multiple comparison test). Data are expressed as mean ± S.D.

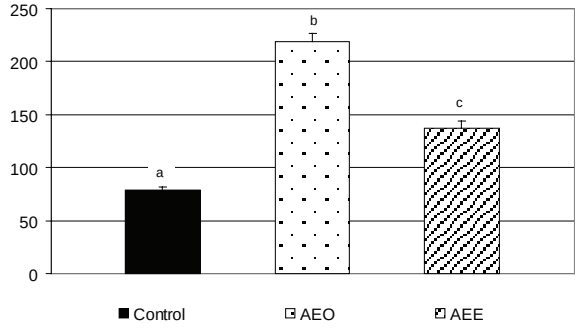

Figure 2. Effect of i.p. administration of AEO (470 mg/kg) and AEE (450 mg/kg) on pentobarbital-induced total sleeping time (minutes) in rats. Different letters present statistically significant results ($p < 0.05$ - ANOVA followed by the Tukey - Kramer multiple comparison test). Data are expressed as mean ± S.D.

found by other authors (Carnat et al., 1985; Foglio, 1996). The major sesquiterpene compounds identified were germacrene D (3.54%) and *trans*-caryophyllene (2.08%). The AEE was analyzed to verify the common compounds. The AEE analysis showed that it was possible to identify camphor, β-cubebene and *trans*-caryophyllene (Perazzo et al., 2003).

Regarding the pharmacological tests, the sleeping time assay evaluates the depressant activity of a drug by increasing the sleeping time induced by barbiturics. The latency time for the loss of the righting reflex was decreased in both treated groups. Total sleeping time was increased in the AEO group by 278% and the AEE also increase this time in 175%, both compared to the control. The difference of the results obtained suggests that the main constituents of the essential oil, which present cineol and p-cymene, monoterpenes with depressant potential found in amounts enough to produce this action might be responsible for these results (Robbers et al., 1996). The

AEO and AEE at the doses tested produced sedation and decreased spontaneous motor activity.

In the FST, the AEO induced a significant reduction in the immobility time compared to the AEE group, an effect that was also observed in the open-field test. This suggests that the sedation and decreased motor activity were involved in the action seen in the FST and that the effect of immobility in the FST could be central rather than peripheral origin, considering the chemical composition and polarity of each drug. This result agrees with findings of other authors (Ali et al., 1995; Ali et al., 1998).

The mechanisms by which AEO and AEE act out remain unclear. However, the present results obtained with AEO and AEE suggest that both may contain several active compounds acting on the CNS by different paths.

Cholinergic drugs may cause an increase in the immobility time in FST and decrease ambulation on an open-field (Bartholini et al., 1985; Herman et al., 1981), whereas cholinomimetics increase it (Herman et al., 1981). The administration of AEO increased the immobility time, and that may be explained by an increase in cholinergic activity, as described in our previous study (Perazzo et al., 2003).

Antidepressant drugs, such as fluoxetine (serotoninergic agents), increase the total swimming time, decreasing immobility time (Kirby & Lucki, 1997), just as dopaminergics (Duncan et al., 1985; Kitada et al., 1986), knockout mice for dopamine receptor (Dulawa et al., 1999) or noradrenergic drugs (Porsolt et al., 1977).

In the OFT, decreases in ambulation, exploration, rearing and cleaning are signs of the depressant activity of the drugs (Borsini et al., 1986). When the animals were submitted to this test, it was noted that the treatment with AEO inhibited the motor activity when compared to the control group. On the other hand, treatment with AEE did not show a significant difference in this activity. The AEO decreased all of these parameters when compared to the control, probably because of its chemical composition.

The results found in this study show that the AEE presented different activities when compared to AEO, especially in the ambulation and exploration. This difference could be attributed to the different compounds present in AEO and AEE. According to the results, it is possible to suggest that the AEO has a marked depressant potential, and the AEE does not.

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