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Sedative and anticonvulsant effects of hydroalcoholic extract of *Equisetum arvense*

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Abstract

The hydroalcoholic extract of *Equisetum arvense* (HAE) tested at the doses of 200 and 400 mg/kg showed a significant activity on the open-field, enhanced the number of falls in the rota-rod reducing the time of permanence in the bar and increased the sleeping time (46% and 74%) in the barbiturate-induced sleeping time. In the pentylenetetrazole-seizure, it increased the first convulsion latency, diminished the severity of convulsions, reduced the percentage of animals which developed convulsion (50% and 25%) and protected animals from death. On the contrary, in the elevated plus maze, the doses 50, 100 and 150 mg/kg did not affect the evaluated parameters. Thus, HAE presented anticonvulsant and sedative effects. Phytochemical analysis detected the presence of tannins, saponins, sterols and flavonoids.

Keywords: Hydroalcoholic extract; *Equisetum arvense*; CNS

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1. Introduction

Equisetum arvense L. (Equisetaceae, traditional name:"horsetail") is a plant showing aerial stems, branched with regular verticilies 2–23 mm in diameter, terminal strobile in the branches and in the main stem (10-mm long and 4 mm in diameter). It glowed in several regions of Europe and North, Central and South America [1]. Several studies showed a hypoglycemic [2,3] and diuretic activity [4–8] of some species of horsetail. In contrast, less is know about the role of *E. arvense* in central nervous system.

The few available reports indicate that equine and bovine intoxication with *E. arvense* are characterized by a short period of excitation (irritability, tremor and ataxia), followed by a period of sedation [9,10]. The aim of this work was to investigate the CNS activity of *E. arvense*, studying the effect of its hydroalcoholic extract (HAE) in rats.

2. Experimental

2.1. Plant material

E. arvense, collected in Santa Catarina State, Brazil, during the summer of 2002, was identified by Dra. Claudete Schrage Nuernberg, Department of Agricultural Botanic, State University of Santa Catarina, Lages, Brazil. A voucher sample has been deposited in the Herbarium of the Medicinal Plants of the State University of Santa Catarina.

2.2. Preparation of the hydroalcoholic extract

E. arvense dried and minced stems were extracted with 50% EtOH–water at 21 ± 3 °C for 15 days. Ethanol was evaporated and the extract obtained was stored at -20 °C.

2.3. Animals

Male Wistar rats (200–250 g) obtained from the Animal House of the State University of Santa Catarina were used. All animals were maintained under controlled temperature (23 ± 1 °C) and a 12 h dark/light cycle (light on at 07:00 am). They were fed with standard rodent diet and water available ad libitum.

2.4. Behavioral tests

2.4.1. Open field test

Open field test was used to evaluate the exploratory activity and emotional response of the animals. The apparatus consists of an arena of white wood (150 cm diameter) enclosed by stainless steel walls and divided in 19 squares by black lines. The open field was placed inside a light- and sound-attenuated room. The animals received intraperitoneally saline or HAE at doses of 50, 100, 200 or 400 mg/kg. After 30 min, each animal was placed in the center of the arena, and during the following 5 min were observed; the number of squares crossed (with four paws), rearing and latency for the first crossing (as measures of

exploratory activity), numbers of grooming and fecal bolus (as measures of emotionality) [11].

2.4.2. Rota rod

Animal was placed with the four paws on a 7 cm diameter bar, 24 cm above the floor, which was turning at 12 rev./min. All animals were trained to remain in the bar for three consecutive trials of 1 min each. In the next day, the animals received intraperitoneally saline or HAE 50, 100, 200 or 400 mg/kg. After 30 min, each animal was placed individually in the bar, for three consecutive trials of 1 min. The total number of falls and the time remained in the bar was calculated at the end of the three trials [12].

2.4.3. Barbiturate-induced sleeping time

According to Ferrini et al. [13], rat sleep was induced by the intraperitoneal administration of 40 mg/kg of pentobarbital. Thirty minutes before pentobarbital administration, the animals received saline or HAE 50, 100, 200 or 400 mg/kg i.p. Latency time of sleep (time to loose the righting reflex) and sleeping time (duration of loss of the righting reflex) were registered.

2.4.4. Elevated plus maze

The elevated plus maze apparatus [14] was made of wood and consisted of two opposite open arms, 50×10 cm (surrounded by 1 cm high Plexiglas), and two enclosed arms, $50 \times 10 \times 40$ cm, elevated to a height of 50 cm above the floor. The junction area of the four arms (central platform) measured 10×10 cm. The maze was placed inside a light- and sound-attenuated room. The animals received saline or HAE 50, 100 or 150 mg/kg i.p. After 30 min, each animal was placed on the center platform of the maze facing the enclosed arm. The percentage of time spent in the open arms in relation to both open and enclosed arms, the percentage of entries into the open arms in relation to both open and enclosed arms and the number of total entries in both arms were recorded [15].

2.4.5. Pentylenetetrazole seizures [16]

Animals were treated with saline or HAE 50, 100, 200 and 400 mg/kg i.p. Thirty minutes later, seizures were induced by the intraperitoneal administration of 60 mg/kg of pentylenetetrazole. The following parameters were observed during the first 30 min: severity of convulsions, percentage of death per group, percentage of animals which developed seizures per group, latency for death and latency to the first convulsion. Seizures were scored as follows [17]: stage 0, no response; stage 1, ear and facial twitching; stage 2, convulsive twitching axially through the body; stage 3, myoclonic jerks and rearing; stage 4, rolling over onto a side position, wild running and wild jumping; and stage 5, generalized clonic–tonic convulsion. Latency to death and latency to the first convulsion were measured to a cut-off time of 1800 s.

2.4.6. Preliminary acute toxicity determination

Preliminary acute toxicity of HAE was determined for groups of eight animals at doses of 1.0, 2.0 and 5.0 g/kg i.p. Animals receiving saline served as controls. The groups were

Table 1
Effect of hydroalcoholic extract from *E. arvense* (HAE) on open field test in rats

Groups	Squares crossed	Rearing	First crossing latency (s)	Grooming	Fecal bolus
Saline	59.2 ± 4.4	6.2 ± 0.8	6.6 ± 1.2	4.0 ± 0.7	5.0 ± 0.8
HAE (50 mg/kg)	49.8 ± 6.3	3.1 ± 0.7*	4.9 ± 1.8	1.5 ± 0.4*	1.8 ± 0.6*
HAE (100 mg/kg)	43.2 ± 3.4	0.5 ± 0.3**	2.8 ± 0.5	0.9 ± 0.2**	1.4 ± 0.4**
HAE (200 mg/kg)	27.4 ± 4.8**	0.2 ± 0.2**	8.5 ± 1.4	1.1 ± 0.4**	1.5 ± 0.6*
HAE (400 mg/kg)	13.1 ± 3.2**	0.1 ± 0.1**	79.8 ± 48.1	0.4 ± 0.2**	0.5 ± 0.3**

$N=8$.
Squares crossed, rearing, first crossing latency, grooming and fecal bolus are expressed as mean ± S.E.M.
*$P<0.05$ and **$P<0.01$ (ANOVA test followed by the Bonferroni/Dunn post-hoc).

observed at 0, 30, 60, 120, 180 and 240 min after treatment. In the next day, the number of survivors was recorded.

2.5. Statistical analysis

The percentage of deaths and percentage of animals developing seizures per group were analyzed by the Chi-square test. The severity of convulsion, latency of death and latency for the first convulsion were analyzed by the non-parametric Kruskal–Wallis test followed by the Mann–Whitney test when necessary. The other parameters were analyzed by the parametric ANOVA test followed by the Bonferroni/Dunn test when necessary.

3. Results and discussion

Table 1 shows the effects of HAE in the open-field test. HAE at all doses tested decreased the number of rearing, grooming and the number of fecal bolus. After treatment with 200 and 400 mg/kg i.p., only a decrease in the number of crossing (54 and 78%, respectively) was observed. Neither doses of the HAE showed a statistical difference when compared with control group in the latency for the first crossing.

The data from rota rod test (Table 2) show that HAE at doses of 200 and 400 mg/kg increased significantly the number of falls and reduced the time permanence in the bar

Table 2
Effect of hydroalcoholic extract from *E. arvense* (HAE) on Rota rod test in rats

Groups	Number of falls	Time permanence (s)
Saline	0 (1–0)	169.2 ± 5.4
HAE (50 mg/kg)	1 (2–0)	161.3 ± 7.7
HAE (100 mg/kg)	1 (3–0)	137.2 ± 15.7
HAE (200 mg/kg)	3 (3–3)*	51.5 ± 14.3*
HAE (400 mg/kg)	3 (3–3)*	21 ± 7.2*

$N=8$.
Number of falls was expressed as median with range in parentheses and time of permanence in the bar as mean ± S.E.M.
* $P<0.01$ (ANOVA test followed by Bonferroni/Dunn post-hoc).

Table 3
Effect of hydroalcoholic extract from *E. arvense* (HAE) on barbiturate-induced sleeping time test in rats

Groups	Latency of sleeping time (s)	Sleeping time (min)
Saline	133.2 ± 5.5	139 ± 9.3
HAE (50 mg/kg)	108.2 ± 3.7	156.2 ± 14.6
HAE (100 mg/kg)	131.8 ± 20.3	177.6 ± 8.5
HAE (200 mg/kg)	121.4 ± 27.9	203.2 ± 10.2*
HAE (400 mg/kg)	78 ± 15.5	242.2 ± 17.7**[a]

$N=8$.
Latency of sleeping time and sleeping time are expressed as mean ± S.E.M.
*$P<0.05$ and **$P<0.01$ (ANOVA test followed by Bonferroni/Dunn post-hoc).
[a] Two animals died during experiment and were not considered in the statistical analysis of sleeping time parameter.

(70% and 88%, respectively). These doses also increased the sleeping time (46% and 74%, respectively) in the pentobarbital-induced sleeping time test (Table 3).

No effect was observed on elevated plus maze test (data not shown).

In the pentylenetetrazole-seizures test (Table 4), all doses of HAE (50, 100, 200 and 400 mg/kg i.p.) increased the latency to the first convulsion and diminished the severity of convulsions. Only at doses of 200 and 400 mg/kg i.p., HAE decreased the percentage of animals developing convulsions in each group. All the doses of HAE protected from deaths. The preliminary acute toxicity study showed that HAE induced mortality at doses of 2 (12.5%) and 5 g/kg i.p. (37.5%), respectively. All the treated animals showed a transitory respiratory depression and an elevated sedation, which persisted at 240 min of observation. These effects were dose dependent.

Broudiscou and Lassalas reveled which flavonoids portion of HAE from *E. arvense* contains isoquercitrin [18]. Kang et al. [19] demonstrated that isoquercitrin increased the sleeping time in the barbiturate- and diazepam-induced sleeping time, a good indication of sedative effect [19]. Thus, the currently observed effects might be explained, at least in part, by the action of isoquercitrin. Obviously, other as yet still unknown compounds might have a role in the central effect of the extract.

Table 4
Effect of hydroalcoholic extract from *E. arvense* (HAE) on pentylenetetrazole seizure test in rats

Groups	Convulsion severity	Death (%)	Development of convulsion (%)	Death latency (s)	First convulsion latency (s)
Saline	3.5 (4–3)	12	100	1651 ± 149	87 ± 11
HAE (50 mg/kg)	2 (3–0)*	0	88	1800 ± 0	349 ± 208*
HAE (100 mg/kg)	2 (2–0)**	0	88	1800 ± 0	378 ± 205*
HAE (200 mg/kg)	1 (2–0)**	0	50[#]	1800 ± 0	1105 ± 263**
HAE (400 mg/kg)	0 (2–0)**	0	25[##]	1800 ± 0	1446 ± 231**

$N=8$.
Latencies to death and to first convulsion are expressed as mean ± S.E.M. and convulsion severity as median with range in parentheses.
*$P<0.05$ and **$P<0.01$ (Kruskal–Wallis test followed by the Mann–Whitney post-hoc).
[#]$P<0.05$ and [##]$P<0.01$ (Chi-square test).

To summarize, the hydroalcoholic stem extract of *E. arvense* showed sedative and anticonvulsant effects.

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