Hypnotic effect of ecdysterone isolated from *Pfaffia glomerata* (Spreng.) Pedersen

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**ABSTRACT:** In this study the depressant effect of fractions from *P. glomerata* was initially evaluated using the mice barbiturate sleeping time test as reference. The fractions tested were the CHCl₃, the EtOAc, the n-BuOH and the aqueous fraction obtained from *P. glomerata* subterraneous parts. Only the pretreatment with the lipophilic fraction (CHCl₃: EtOAc, 1:1, w/w) increased the barbiturate sleeping time (i.p 500 mg/kg; v.o. 1000 mg/kg). Ecdysterone, the main substance isolated from this lipophilic fraction, was identified by spectroscopic methods and its content in the ethanol extract was determined as 1.4% (w/w) by HPLC. In order to investigate the hypothesis of ecdysterone displaying a depressant effect on nervous central system, an evaluation toward the hypnotic-sedative and anxiolytic effects of this drug was carried out. Ecdysterone 100 mg/kg, i.p, increased the barbiturate sleeping time without provoking hypothermia; when administered by oral route its minimal effective dose was 400 mg/kg. On the other hand, ecdysterone (100 mg/kg, i.p; 400 mg/kg, p.o) did not impair motor coordination and was ineffective on pentylenetetrazole-induced convulsion, elevated plus-maze and step-down inhibitory avoidance tests, indicating that at these doses the drug does not present an anxiolytic profile and does not cause manifest neurotoxic effects as well. In conclusion, the lipophilic fraction from *P. glomerata* presents a hypnotic effect being ecdysterone one of the compounds responsible for this CNS activity.

**Keywords:** *Pfaffia glomerata*, Amaranthaceae, ecdysterone, central depressant effect, pentobarbital-induced sleeping time.

**INTRODUCTION**

The genus *Pfaffia* (Amaranthaceae) comprises about ninety species distributed through Central and South America, twenty-seven of them being described in Brazil (Taniguchi et al., 1997). *Pfaffia paniculata*, popularly known as “Brazilian ginseng” (Oliveira, 1986), is the most employed and commercialized species in Brazil as a surrogate for *Panax* spp. (ginseng - Araliaceae). Furthermore, the substitution of *P. paniculata* by *Pfaffia glomerata* is also common due to falsification or botanical misidentification (De-Paris et al., 2000). Recently some quality parameters to differentiate between *P. paniculata* and *P. glomerata* roots have been described considering their botanical and chemical characteristics (Gosmann et al., 2003). Ecdysterone (Figure 1) was only found in *P. glomerata*, as already described (Shiobara et al., 1993), so it seems that
this compound could be a good marker for differentiation of both species. Allantoin, ecdysteroids, pfafic acid and their glycosides (nortriterpene saponins), stigmasterol and sitosterol have been isolated from subterraneous parts of Pfaffia species (Nakai et al., 1984, Nishimoto et al., 1984, Takemoto et al., 1982).

Pharmacological studies with *P. glomerata* roots evidenced a gastroprotective effect probably mediated by histaminergic pathway and an enhanced production of nitric oxide in the stomach (Freitas et al., 2003, 2004). An ethanol extract of this species did not show antiviral, antiproliferative, antifungal or MAO inhibitory activities in vitro (Gossmann et al., 2003). A crude hydroalcoholic extract of *P. glomerata* roots presented analgesic and anti-inflammatory activities (Neto et al., 2005).

Regarding to the central nervous system action, the administration of a crude ethanol extract of *P. glomerata* by intraperitoneal route produces a depressor effect in the barbiturate sleeping time test and an amnesic effect in adult rodents (De-Paris et al., 2000; Vigo et al., 2003). On the other hand, Marques et al. (2004) reported a barbiturate sleeping time decrease and an improvement in learning and memory in old mice chronically treated.

The aim of this work was to evaluate the central nervous activity of *P. glomerata* fractions and its main isolated compound toward the hypnotic-sedative, anxiolytic and memory effects.

**MATERIAL AND METHODS**

**Plant material**

*Pfaffia glomerata* (Spreng.) Pedersen subterraneous parts were obtained from the cultivated area of the Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas (CPQBA/UNICAMP, Campinas, SP, Brazil) and a voucher specimen is kept in the herbarium-UNICAMP (CPQBA 0238). Roots and rhizomes (subterraneous parts) from *Pfaffia* species were obtained from the cultivated subterraneous parts (1000 g) were obtained using soxhlet and thence triturated to powder.

**Ethanol extract and fractions from Pfaffia glomerata**

The ethanol extract from *Pfaffia glomerata* subterraneous parts was prepared as already described (De-Paris et al., 2000). The fractions from *Pfaffia glomerata* subterraneous parts (1000 g) were obtained using soxhlet during 12 h and, successively, solvents of increasing polarity to obtain the CHCl₃ (2 g, 0.2% w/w), the EtOAc (7 g, 0.7% w/w) and the n-BuOH (16 g, 1.6% w/w) fractions, which were evaporated, separately, to dryness. The remainder vegetal residue was submitted to decoction under stirring during 1 h, and then the resulting aqueous fraction (600 g, 60% w/w) was lyophilized. CHCl₃, and EtOAc fractions were pooled due to similar TLC profile. The fractions used in pharmacological experiments were named CAE (CHCl₃ and EtOAc, 1:1, w/w), BUT (n-BuOH) and AQU (aqueous).

**Isolation of ecdysterone**

The main constituent in the organic fraction was isolated from *Pfaffia glomerata* roots (1500 g) through soxhlet using EtOAc. EtOAc fraction was concentrated until half volume and cooled resulting in a precipitate with a major compound which was purified using CHCl₃ until obtaining a white powder (5 g, 0.3% yield, w/w, relating to the dried plant). The isolated product was identified as ecdysterone (ECD) (Figure 1) by spectroscopic and HPLC analysis. FAB-MS spectrum was performed on a MS50 spectrometer. ¹H and ¹³C NMR spectra were recorded on Bruker AMX 500 spectrometer.

**HPLC quantification of ecdysterone**

The quantification of ecdysterone (ECD) present in the ethanol extract of *P. glomerata* was carried out in a liquid chromatograph Shimadzu LC-10A as already described using an HPLC methodology previously validated (Zimmer et al., 2005).

**Animals**

Adult male Wistar rats (weight 200-300 g) and adult male CF1 mice (weight 25-30 g) from Fundação Estadual de Produção e Pesquisa em Saúde (FEPPS, Porto Alegre, RS, Brazil) breeding colony were used. The animals were housed in plastic cages, five by cage, under a 12 h light/dark cycle (lights on at 7:00 a.m.) at constant temperature of 23 ± 1 °C with free access to standard certified rodent diet and tap water. All experiments were performed between 10:00 and 16:00 h.

All experiments were approved by the Research Ethical Committee of Universidade Federal do Rio Grande do Sul (# 2003236).

**Drugs and treatments**

Pentobarbital sodium salt (PTB, Abbot®, São Paulo, SP, Brazil), pentylentetrazole (PTZ, Sigma®, St. Louis, MO) and diazepam (DZP, Valium®, Roche®) were used.
The aqueous fraction, pentobarbital, pentylenetetrazole were dissolved in physiological saline (NaCl 0.9%). Other fractions, ecdysterone and diazepam were suspended in saline with the addition of polysorbate 80 at 1% v/v. All administrations were made in a volume of 1 ml/100 g body weight (mice), except for the inhibitory avoidance task, where the rats were treated with a 2 ml/kg volume. When oral route was used, all the animals were fasted for 6 h before testing.

**Barbiturate sleeping time test**

Different groups of mice were treated with different *P. glomerata* fractions or ecdysterone, saline (SAL), saline + polysorbate 80 1% (TWE) and diazepam by intraperitoneal and oral routes. Thirty minutes after intraperitoneal injection and 60 min after gavage, all groups received pentobarbital (40 mg/kg, i.p.) and the time elapsed between the loss and voluntary recovery of the righting reflex was recorded as sleeping time. A ceiling of 240 min was imposed in this measure, i.e., animals whose sleeping time was over 240 min were counted as 240 min. Sleep latency was also recorded. The room temperature was kept at 23 ± 1 ℃.

**Rota-rod motor coordination test**

The rota-rod consisted of a cylinder of 3 cm of diameter at a height of 21 cm from the base. One day before testing, mice were placed on the cylinder for training during 5 min. On the test day, the animals were placed on the bar and selected based on their ability to remain at least 90 s continuously on the rotating bar at the speed of 5 rpm. Immediately after, the selected mice were treated with the test substances and replaced on the cylinder 60 min later. In both sessions, the parameters registered were number of falls and the maximum time of permanence on the bar through 5 min. The following groups were tested: TWE (saline + polysorbate 80 1%, n = 9), ecdysterone 400 mg/kg (n = 9), ecdysterone 800 mg/kg (n = 8) and diazepam (5 mg/kg, n = 10). All groups were treated orally.

**Effect on pentylenetetrazole-induced convulsions**

Groups of mice were treated with ecdysterone 100 mg/kg (n = 13), TWE (saline + polysorbate 80 1%, n = 14) and diazepam (1 mg/kg, n = 10) by intraperitoneal route. pentylenetetrazole (80 mg/kg i.p.) was given 30 min after the intraperitoneal administration. The latency and duration of the first convolution and number of death were taken into account.

**Elevated plus-maze**

The elevated plus-maze consists of two open arms (30 x 10 cm) and two enclosed arms (30 x 10 x 15 cm), arranged in such a way that the two arms of each type were opposite one another. The maze is 50 cm
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![Graph A](image)

![Graph B](image)

**Figure 3.** Effects of pretreatment with ECD (100 mg/kg i.p.; n = 12) and diazepam (DZP 1 mg/kg i.p.; n = 16) on latency (A) and sleeping time (B) induced by pentobarbital (40 mg/kg i.p.) in mice. Control group: TWE - saline + polysorbate 80 1% i.p. (n = 13).

A) The data are reported as median and interquartile intervals (*different from TWE group. Kruskal-Wallis; H = 17.16; p < 0.001).

B) The data are reported as mean ± standard error (*different from TWE group. ANOVA; F2,40 = 33.63; p < 0.001).

**Figure 4.** Effects of pretreatment with ECD (100, 200, 400 e 800 mg/kg p.o.; n = 12) and diazepam (DZP 2 mg/kg p.o.; n = 13) on sleeping time induced by pentobarbital (40 mg/kg i.p.) in mice. Control group: TWE - saline + polysorbate 80 1% p.o. (n = 13). The data are reported as mean ± standard error (*different from TWE group. ANOVA; F5,69 = 8.29; p < 0.001).

**Table 1.** Performance of mice treated with ecdysterone 400 mg/kg and 800 mg/kg p.o. (ECD), diazepam 5 mg/kg p.o. (BZD) and saline + polysorbate 80 1% p.o (TWE) in the rotarod test.

<table>
<thead>
<tr>
<th></th>
<th>Number of falls</th>
<th>Maximum time of permanence (s)</th>
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<tbody>
<tr>
<td></td>
<td>T0</td>
<td>T60</td>
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<tr>
<td>TWE</td>
<td>2.2 ± 0.9</td>
<td>1.2 ± 0.6</td>
</tr>
<tr>
<td>BZD</td>
<td>2.6 ± 0.9</td>
<td>9.0 ± 2.0*</td>
</tr>
<tr>
<td>ECD 400</td>
<td>0.7 ± 0.4</td>
<td>3.1 ± 1.3</td>
</tr>
<tr>
<td>ECD 800</td>
<td>1.7 ± 0.5</td>
<td>1.6 ± 0.7</td>
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</tbody>
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T0: immediately before treating; T60: sixty minutes after treating. The data are reported as mean ± standard error (*different from T0. Two way repeated measures ANOVA; F1,71 = 5.09; p < 0.05).
All statistical analyses were done using the Statistical analysis
mg/kg, i.p., n = 9).
ecdysterone 100 mg/kg, i.p. (ECD) and apomorphine (1
glomerata
following treatments were used: saline (i.p. n = 8),
done 15, 30, 60 and 90 min after the drug injection. The
determination of basal temperature as described
from Training session. Two way repeated measures ANOVA;
neuractive steroid (NAS).
It is plausible that ecdysterone represents a type of

differences between the activities of the ethanol extract
ecdysterone when administered orally could be a
of modifying neural activities. NAS are involved in
several psychiatric disorders, including depression
syndromes, stress responses, anxiety disorders and
memory processes and pre-menstrual syndrome (Amin
was determined as 1.4% (w/w) in relation to the dried
extract.
The fractions CAE, BUT and AQU were administered
by intraperitoneal route at 500 mg/kg and
tested on the barbiturate sleeping test. Only the
pretreatment with CAE increased the sleeping time
(Figure 2A) without any effect on the latency to sleep
(data not shown). This lipophilic fraction kept its effect
on the sleeping time when orally administered (1000
mg/kg, Figure 2B). Following, ecdysterone, the main
substance isolated from CAE, was tested. Ecdysterone
100 mg/kg i.p. caused a decrease in the latency and an
increase on the sleeping time (Figures 3A and 3B). The
same treatment did not alter the core temperature of mice
(data not shown). These results indicate a depressant
effect of ecdysterone. Thus, the effect of ecdysterone per
os (100, 200, 400 and 800 mg/kg) on the pentobarbital-
sleeping time was evaluated. Ecdysterone 400 mg/kg
and 800 mg/kg, p.o., increased the sleeping time (Figure
4) but it did not change the latency to sleep in any tested
dose (data not shown).
These results suggest that ecdysterone is
responsible for the depressant effect of the ethanol
extract on the barbiturate sleeping time test. The
differences between the activities of the ethanol extract
and ecdysterone when administered orally could be a
consequence of the low systemic levels obtained for
ecdysterone after the ethanol extract administration due
to the drug poor bioavailability by this route in addition
to its low content in this extract (1.4%).
Ecdysterone is a steroid molecule which is
extensively studied as an insect’s hormone. More
recently some biological activities in mammals have
been reported on normal and tumor cellular metabolism
(Wu & Wang., 2003, Konovalova et al., 2002).
Ecdysterone also improved the learning and memory in
the Morris Water Maze and increased the expression of
c-fos into the hippocampus of rats (Yang et al., 2004).
Thus, it is plausible that ecdysterone represents a type of
neuractive steroid (NAS).
The term neuroactive steroid (NAS) refers to
steroids which, independent of their origin, are capable
of modifying neural activities. NAS are involved in
several psychiatric disorders, including depression
syndromes, stress responses, anxiety disorders and
memory processes and pre-menstrual syndrome (Amin
et al., 2006). The neurosteroid dehydroepiandrosterone
cause an increase in the sleep time induced by ethanol
or pentobarbital (Melchior & Ritzman, 1992). Some
NAS have been shown to exert hypnotic, sedative and
anticonvulsive effects, mainly through GABA_\text{A}
receptor modulation (for review see Dubrovsky, 2005).
Thus the hypothesis of ecdysterone presenting
effects on GABA system was investigated by testing
the drug on animal models of anxiety, convulsions and
memory which are recognized as useful tools to detect
benzodiazepine-like or GABAergic drugs (Izquierdo &

In addition, since substances with hypnotic-sedative action have a great potential to interfere with motor activity parameters the effect of ecdysterone (400 and 800 mg/kg v.o.) was evaluated in the rotarod test. It did not alter any parameter evaluated (Table 1), demonstrating that it does not impair the motor coordination of the animals and does not manifest neurotoxic effects as well.

Non of the parameters evaluated in the plus-maze test were modified by the pre-administration of ecdysterone (400 mg/kg v.o.) (Table 2). Ecdysterone (100 mg/kg i.p.) still did not protect the mice from the PTZ-induced convulsions (Table 3). Additionally, ecdysterone (400 mg/kg v.o.) did not incite any change in the step-down inhibitory avoidance rat’s performance (Table 4).

This test is a classical model to take measurements of memory with a strong aversive component. Thus, this result point out that probably ecdysterone is not the substance responsible for the amnesic effect previously reported to the ethanol extract (De-Paris et al., 2000).

With the results obtained so far it can be assumed that the lipophilic fraction from P. glomerata when acutely administered to adult rodents present a hypnotic effect that could be attributed to ecdysterone. This effect seems not to be mediated by the GABAergic system since ecdysterone was ineffective in animals models considered predictive of benzodiazepine-like or GABAergic effects.

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REFERENCES


