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Psychopharmacological Assessment of *Pfaffia glomerata* Roots (Extract BNT-08) in Rodents

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A pharmacological assessment of the standardized extract (BNT-08) of *Pfaffia glomerata* roots was performed in young mice submitted to acute treatment with several doses (i.p.), in young and old mice submitted to chronic oral treatment for 150 days or with water (control groups) and in old mice at a dose of 100 mg/kg of extract. Acute tests involved an initial screening, spontaneous movements, rota-rod, barbiturate sleeping time and passive avoidance were carried out. The chronic test involved mortality assessment, body weight and learning and memory in a T-maze left/right discrimination test and in the passive avoidance model. Of the acute tests only the sleeping time test showed relevant differences between the groups. With the chronic treatment, a relevant decrease of the number of sessions necessary for learning in the group of old mice treated with the extract was evident. A partial reversal of the memory deficit induced by age in the old mice treated with the extract was found in the passive avoidance test. The results suggest that the standardized extract from *Pfaffia glomerata* roots promoted an increase in both learning and memory of old mice treated in the chronic test.

Keywords: Pfaffia glomerata; extract BNT-08; brazilian ginseng; learning and memory; aged rats.

INTRODUCTION

The increase in the population's longevity has made the search for medications that lessen the general processes of aging a continuing one during the past years (WHO, 1989). From this effort, several results have been obtained, as in the case of the standardized extract of *Ginkgo biloba* L. leaves, presenting evidence from extensive studies in animals and humans that it is beneficial for memory problems, labyrinthitis and dizziness (Schulz *et al.*, 2001). Korean ginseng (*Panax ginseng* C. A. Meyer) root was also the object of positive investigations, emphasizing several results in learning, memory and physical capacity, both in animals and humans (Petkov and Mosharrof, 1987; Ni *et al.*, 1993).

In Brazil, some native species are used to improve memory, attention and reasoning, such as guaraná (*Paullinia cupana* Kunth.) and catuaba (*Anemopaegma mirandum* (Cham.) DC) (Carlini, 1995). Recently, the plant popularly referred to as 'nó-de-cachorro' (*Heteropteris aphrodisiaca* O.Mach.) was assessed in models aimed at the activity and memory of rodents; chronic treatment with extracts of these species restored the age deficits detected in experimental models with the passive avoidance and the T-maze discrimination test (Galvão, 1997; Galvão *et al.*, 2002).

In addition to these plants, the local population developed the habit of making use of Amaranthaceae species for the same purposes as Korean ginseng, that is, as general tonics chronically taken to increase mental and physical moods, including in the sexual area. In this context, the following are mentioned as 'Brazilian ginsengs': the species *Pfaffia glomerata* (Spreng.) Pedersen and *Hebanthe paniculata* Martius (Marques, 1998; Borsch and Pedersen, 1997). These species have tuberous roots having humanoid forms similar to those of the Korean ginseng (Oliveira *et al.*, 1980; Akisue *et al.*, 1992). Chemically, both contain triterpenic saponins, with the presence of pfaffosides and alantoine in *H. paniculata* and of ecdysteroids in *P. glomerata* (Nakai *et al*., 1984; Nishimoto *et al*., 1990; Nishimoto, 1992).

Pfaffia glomerata is popularly known as 'fáfia', 'paratudo', 'corango sempre-viva' and 'acônito', among other names (Carriconde *et al.*, 1996). It has a wide geographical distribution over Brazil, occurring abundantly between the States of Paraná and Mato Grosso do Sul, at the margins and islands of River Paraná (Carriconde, 1994; Van den Berg, 1982; Vazzoler *et al.*, 1997). With regard to the pharmaceutical market, this is the species that provides monthly about 80 tons of roots supplied to the national and international market under the name 'suma' and erroneously

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identified as *Pfaffia paniculata* (Marques, 1998; Vigo *et al.*, 2002).

The literature was poor with regard to data about *P. glomerata*, there having been only a single study in the area of microbiology (Alcântara and Andrade, 1994), one study in the area of adaptogenous effects (Nicolodi *et al.*, 2002) and several agronomic studies (Mattos *et al.*, 1997; Montanari Jr. *et al.*, 2002; Ming *et al.*, 2002; Figueiredo *et al.*, 2002). In the area of the nervous central system, de-Paris and collaborators (2000) reported a preliminary psychopharmacological assessment of *P. glomerata* roots in rodents. By employing several experimental models (open-field, sleeping time, pentylenotetrazol-induced convulsions, elevated pluz-maze, step-down inhibitory avoidance task and forced swimming) and doses acutely administered, the results showed interference of habituation in the open-field, a decrease in the latency and an increase in the barbiturate sleep time, a partial protection in the convulsions induced by pentylenotetrazol and a decrease in the memory retention (i.p. doses) and practically no relevance in the doses administered orally. These data present a diazepan-like profile and conflict with the popular use of the plant (Carriconde, 1994).

Since the plants considered as a 'tonic' by the population that makes use of them are used chronically, their activities are similar to the concepts attributed to the plants referred to as adaptogenus (Galvão *et al.*, 2002). This concept has been developed during the past 40 years, with emphasis given to the works of Brekman and Dardymov (1969), who reported phytotherapic preparations having a low toxicity and that did not produce acute effects, which required an extended administration in order to exercise their effects, mainly those that help the organism to maintain the homeostatic equilibrium (Wagner *et al.*, 1994).

Considering this context, the purpose of this study was to assess the general psychopharmacological effects of *Pfaffia glomerata* roots, by employing a chemically standardized extract (BNT-08 – patent pending), as well as to accomplish both acute and chronic treatments in young and old rodents. Complementarily, the acute toxicology in mice was assessed (DL_{50}).

MATERIALS AND METHODS

Animals. For the acute pharmacological tests, albino male mice were used, having a Swiss lineage, aged 3–4 months and weighing 30–40 g. For the chronic pharmacological tests, male Wistar rats were used aged 3–4 months (young) weighing 200–300 g and another group aged 20–24 months (old) and weighing 300–400 g. For the DL_{50} test, male young Wistar rats (5 months old) were used. The animals came from the central *biotery* of the Psychobiology Department of the School of Medicine of São Paulo, being kept in *bioteries* separated per sex, with a controlled temperature (22° ± 2 °C) and a light–dark cycle of 12 h. The protocol was submitted to the Animal Ethics Committee of the Federal University of Sao Paulo, having been approved without restrictions.

Chemicals. The drugs scopolamine, sodium pentobarbital (Sigma Chemical Company, St Louis, USA) and β-ecdisone (Merck, Germany) were used in the pharmacological tests and for standardization of the extract.

Plant material. *Pfaffia glomerata* was collected in the municipality of Porto Rico (PR), assembled in exsicates and submitted to the specialist in Amaranthaceae, Professor Josafá Siqueira, of the Catholic Pontific University (Rio de Janeiro, Brazil); samples of the collection identified are deposited in the collection of Herbarium Friburgense (FCAB) under number 5426. The roots were collected, washed, dried in a greenhouse having circulating air and were assessed regarding their quality (Farmacopéia, 1988).

Lyophilized extract preparation. Hydroalcohol extracts were obtained from the roots (10%) by means of the process of turbolysis for 30 min, with temperature control (Voigt, 1993), which were filtered, concentrated, lyophilized and stored in a vacuum dessicator in a freezer. The extracts were solubilized in water before being administered.

Standardization. The crude drug and lyophilized extracts were standardized in terms of *β-ecdysone* in a high efficient liquid chromatography system coupled with a Hewlett Packard integrator, model 1050. One gram of the dry and pulverized roots was extracted with methanol in a soxhlet for 4 h; the extract was concentrated, distilled water added and the solution was centrifuged for 10 min at 3000 rpm. It was filtered and the filtrate was analysed. Solutions of *β-ecdisone* were prepared (contents of 99.9%) in methanol at concentrations of 40, 120 and 200 ppm and were injected in the chromatographer in ascending order. The conditions employed were as follows: Lichrospher 100 (RP 18) 5 μm (Merck) column, 250 × 4 mm; temperature of 27 °C; run time of 18 min; flow of 1 mL/min; methanol–water mobile phase (40:60 – v/v). UV detector was employed.

Acute toxicological test (DL_{50}). One group of 10 young male rats was submitted to a 12 h fast and then a dose of 3 g/kg of lyophilized extract was given oraly. The animals were then kept under constant observation for 2 h and at 4, 8 and 12 h after that. The follow-up of the group's behaviour and the mortality was continued up to 14 days after administration (Brito, 1997).

Pharmacological screening. Groups of mice received the lyophilized product intraperitoneally (doses of 1, 10, 100, 200 and 1000 mg/kg) or orally (dose of 100 mg/kg), and observed at 5, 15 and 30 min, 1, 2, 4 and 24 h noting any signs of pharmacological and/or toxic effects (Carlini, 1972).

Spontaneous movement. Wooden boxes were used, and movements were registered for 60 min using photoelectrical cells and an electromechanical counter (Oliveira *et al.*, 1991). An experiment was performed with three groups of mice treated acutely by the intraperitoneal route with saline or 10 and 100 mg/kg of the lyophilized extract BNT-08.

Motor coordination ('rota-rod'). The 'rota-rod' corresponds to a rotatory bar having a rough surface, 3 cm in diameter and 60 cm long, elevated to 40 cm and

rotating at a speed of 12 rpm). Three groups of mice treated acutely by the intraperitoneal route with saline or 10 or 100 mg/kg of the extract were placed on the device at 30, 60 and 120 min after the administration, noting the time spent on the rod.

Potentiation of sodium pentobarbital sleeping time. Three groups of mice were treated intraperitoneally with saline or 10 or 100 mg/kg of the extract. After 60 min, they received a dose of 50 mg/kg of sodium pentobarbital, as a measure of the latency for sleeping and the time between the loss and the recovery of the animal's righting reflex (Carlini *et al*., 1986).

Passive avoidance test. The device consisted of two interconnected boxes, a light and a dark one, the floor of dark part allowing the application of shocks. The animal was placed in the light box, the passage between the chambers was opened and the time that the animal took to pass through it noted. The passage was then closed, and a shock was applied (0.6 mA per 1 s duration), the animal was removed from it and left in the cage for 24 h; it was then placed in the equipment again, and the new time – retention latency noted (Galvão, 1997). In the acute test four groups of mice were used, which received different treatments intraperitoneally. The first group received only saline, the second received the lyophilized extract (100 mg/kg), the third received 2 mg/kg of scopolamine and the fourth group received the 100 mg/kg extract followed by 2 mg/kg scopolamine. The second test involved chronic oral treatment (150 days) of young and old rats treated with water (young and old controls) and old mice treated with 100 mg/kg of the BNT-08 extract.

Right-left discrimination test ('T' labyrinth). Three groups of male rats were used, one of them being young and receiving only water (control young group) and two groups of old mice were pooled into one group that also received only water (control old group). Another group of old mice was treated with 100 mg/kg of the BNT-08 lyophilized extract (Pfaffia old group). The treatment was performed by the oral route (gavage) for 150 days. In this period, the animals were trained and tested in a cross-like labyrinth made of wood, in which one of the arms was blocked, rendering it into a T labyrinth, where pieces of candies were put as reinforcement. A test was carried out to see which of the animal's sides or which animal would be reinforced, and kept constant for the entire duration of the equipment. Each of the sessions involved six attempts, in which the rat was placed at the end of the starting arm, thus making its passage free for choosing one of the sides; in case they hit it, they received a piece of candy. This phase was developed until the animal reached twelve hits in two consecutive days (Aggleton *et al.*, 1989).

Mortality and body weight. From the beginning of the chronic administration, the behaviour and occurrence of mortality were noted daily, and each week, the animals were weighed on electronic scales up to the end of the experiments.

Statistical analysis. The variance analysis of a one-way (ANOVA) and two-way ANOVA were employed, followed by comparisons, when necessary, by the Duncan test; and also the Kruskal–Wallis test followed by the Mann–Whitney (one-tailed) *U*-test and chi-square (χ^2) test. A significance level of 0.05 or less was considered statistically different.

RESULTS

Quality control

The plant roots were axial, elongated, having different sizes and forms; their external surfaces having a coloration that varied from light yellow to brownish shades; when the root was fresh, its surface was smooth with spaced circular marks; when dried, longitudinal wrinkles arose overall; the form of some pieces resembling humanoid aspects. The HPLC data demonstrated the following contents of β-ecdysone: 0.76% for the dried powdered roots and 1.07% for the lyophilized extract (retention time of 12.305 and 11.796, respectively).

Acute tests

The acute toxicology test used male rats ($n = 10$) that received a dose of 3 g/kg of the lyophilized product by the oral route (gavage). The animals were observed for up to 14 days and no behavioural change was noted, and there was no mortality in this period. Hence, the DL_{50} (oral) of the lyophilized extract BNT-08 was above 3 g/kg.

In the pharmacological screening, abdominal contortions ('writhes') were apparent after the doses administered intraperitoneally, but not after the oral route; a decrease of motor activity and stereotypia in some doses, scaling behaviour and grooming at a dose of 10 mg/kg (i.p.), and ruffled hairs at doses of 200 and 1000 mg/kg (i.p.) and 100 mg/kg (oral) were observed. In the other acute tests, differences occurred only between the control and treated group in the sleeping time, with a shorter sleep time recorded in mice treated with 100 mg/kg (i.p.) (Table 1).

The data from the passive avoidance acute test are shown in Fig. 1, where the damage promoted by scopolamine may be determined both in the acquisition and retention of the animals' memory. On the other hand, it also shows that the BNT-08 extract at a dose of 100 mg/kg (i.p.), although not reversing the deficit promoted by the scopolamine (Pfaf+scop group), also did not cause damage in the acquisition or retention of the mice's normal memory used in the experiment when separately administered (Pfaffia group).

Chronic tests

Right-left discrimination test ('T' labyrinth). By counting the number of sessions necessary to achieve the learning criteria set forth for the T labyrinth (12 consecutive hits), statistically relevant differences between the old group and the young one were found, as expected; however, comparing the experimental *Pfaffia* 100 mg/kg group with the old control group, and there were no differences between the results of the young

Table 1. Results (average ± SE/SD) of the acutely treated mice tests with 10 and 100 mg/kg (i.p.) of *Pfaffia glomerata* (BNT-08) lyophilized extract

Test	Stage	Control	*Pfaffia* (10 mg/kg)	*Pfaffia* (100 mg/kg)
Spontaneous movement (n = 10)	10 min	218 ± 32	218 ± 29	200 ± 26
	30 min	453 ± 85	402 ± 61	344 ± 35
	60 min	683 ± 129	579 ± 101	579 ± 68
Motor coordination (n = 10)	30 min	57.8 ± 4.7	57.2 ± 8.8	55.4 ± 10.2
	60 min	57.0 ± 6.5	57.5 ± 5.4	54.3 ± 12.0
	120 min	58.1 ± 6.0	59.5 ± 1.6	58.0 ± 6.3
Sleeping time (n = 10)	Latency	3.2 ± 0.4	3.0 ± 0.0	3.0 ± 0.0
	Total time	136.0 ± 12.8	132.9 ± 12.5	93.1 ± 11.4[a]

[a] Statistically different from the control – ANOVA ($p \leq 0.05$ – Duncan's test).

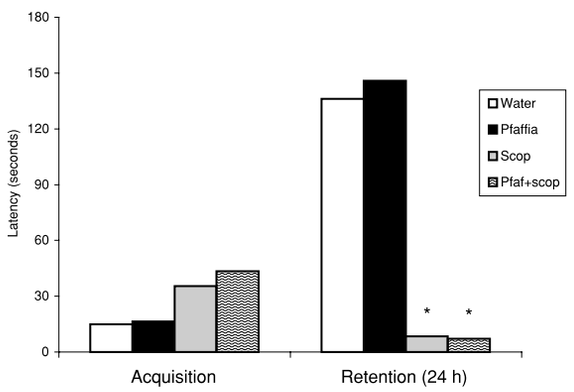

Figure 1. Latency for acquisition and retention (24 h later) phases of the passive avoidance test by young mice acutely treated (i.p.) with water (n = 9), 100 mg/kg *Pfaffia glomerata* extract BNT-08 (n = 9), scopolamine 2 mg/kg (n = 10) and 100 mg/kg *Pfaffia glomerata* extract + scopolamine 2 mg/kg (n = 10). The values are expressed as medians. * Indicates statistical difference from the water and *Pfaffia* groups in the retention phase.

control group and the *Pfaffia* 100 mg/kg group (Fig. 2). This means that the old group that received the extract BNT-08 chronically obtained a performance equivalent to that of the young rats group. In terms of the learning curve, it was found that the performance of the old rats treated with the lyophilized extract BNT-08 was very different from that of the old control rats, which needed a longer period of time (days) in order to achieve the criteria necessary (Fig. 3).

Passive avoidance (chronic). By using the animals submitted to the chronic treatment with water or BNT-08 for 150 days (young control group, old control group and old group of *Pfaffia* 100 mg/kg), it was noted that there were no differences in the behaviour of the groups in the acquisition phase (averages of 21, 9 and 20, respectively). In the retention phase carried out 24 h later, the young control group showed a statistically different behaviour to that of the old control group, as expected in the model in which the young animals increased the latency for entry in the device (Md = 300) and the old ones decreased the latency as a result of the damage caused by age (Md = 235). The old *Pfaffia* group showed an intermediate behaviour between the control groups (Md = 276), without a statistical difference in relation to the young control group indicating a partial reversal of the memory deficit associated with age and detected in the device (Fig. 4). It must be emphasized that the old control group did not present a standard behaviour, with average response times much higher than normally expected for old mice in this model.

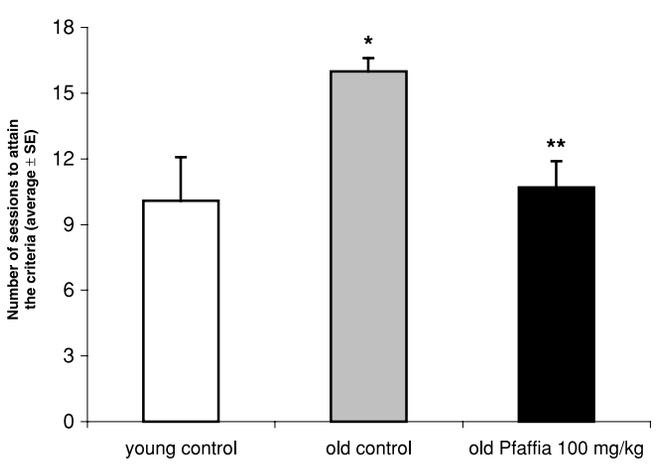

Figure 2. Number of sessions required to attain the learning criteria in the T maze by young control rats (n = 12), aged control rats (n = 12) and aged rats chronically treated with 100 mg/kg *Pfaffia glomerata* extract BNT-08 (n = 10) for 150 days. The values are expressed as mean ± S.E. ***** Indicates statistical difference from the young control group. ****** Indicates statistical difference from the aged control group.

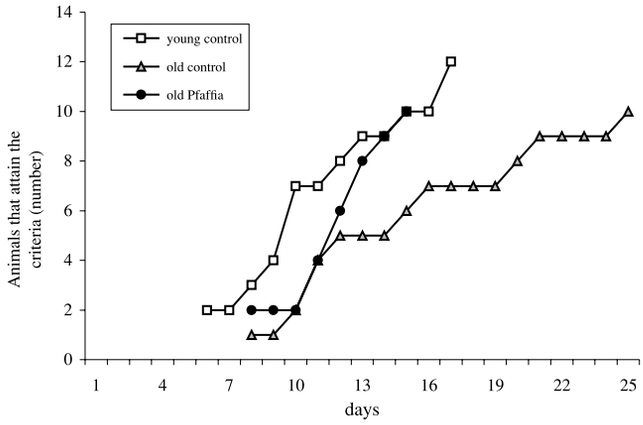

Figure 3. Curve of learning in the left/right discrimination test of young control rats (n = 12), old control rats (n = 12) and old rats chronically treated with 100 mg/kg *Pfaffia glomerata* extract BNT-08 (n = 10).

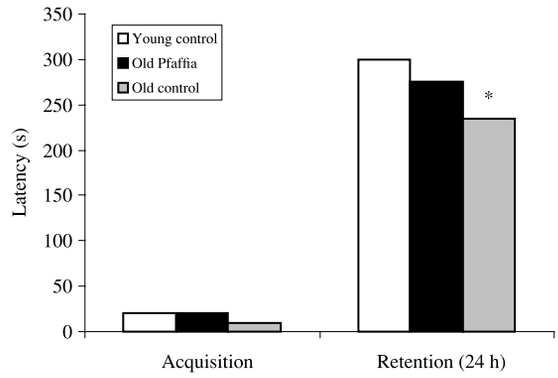

Figure 4. Latency for the acquisition and retention (24 h later) phases of the passive avoidance test by young control rats (n = 14), old control rats (n = 12) and old rats chronically treated with 100 mg/kg *Pfaffia glomerata* extract BNT-08 (n = 13). The values were expressed as medians. * Indicates statistical difference from the young control group.

Mortality and body weight of young and old rats. During the 150 treatment days, there were 11 deaths in the old control group and 14 deaths in the *Pfaffia* old control group, with no statistical difference between the groups. The body weight during the chronic treatment showed that after day 54 of administration, the old rats from the *Pfaffia* group started to lose weight, becoming different to the other old control group. This difference disappeared on day 75, but soon reappeared on day 104, remaining unaltered and increasing until the end of treatment, also reaching significance in relation to the young control group (Fig. 5). These data suggest some harmful effects of the treatment with the BNT-08 extract on the body weight of the old rats.

DISCUSSION AND CONCLUSION

The species *Pfaffia glomerata* is referred to as one of the 'Brazilian ginsengs', presenting, both in popular and commercial uses basically the same therapeutic indications as Korean ginseng, which is noted for its use as a 'tonic', both cerebral and general for the entire organism (Brasmédica, s.d.). The object of this research was to concentrate efforts on the species that is effectively being traded and used by the population, contributing to a clarification of the commercial confusion that occurs between *Pfaffia glomerata* and *Hebanthe paniculata* (Vigo *et al.*, 2002).

From properly identified raw materials an attempt was made to use a standardized extract of the roots, so that the concentration of the active ingredients and reproducibility was possible in studies in the pharmacopoeic control group and in dosing of the marker. In another aspect, the extracts were prepared according to the technique of turbolysis, which is a method that is validated for its efficiency, rapidity and simplicity of execution when compared with the classic methods of both maceration and percolation (Mello, 1989; Franco, 1990; Cardoso, 1990), and also by avoiding the exposure of the substances present in the crude drug to high temperatures such as occurs in methods involving infusion, decoction or reflux in a soxhlet.

The standardization of the BNT-08 extract was made based on β-ecdysone, considered to be the main component of *P. glomerata* roots (Oliveira, 1986). The contents obtained showed values, in the dry roots (0.76%), similar to those found by other authors (0.67%–0.71% and 0.64% – Magalhães, 1998; Carriconde *et al.*, 1996). The extraction and lyophilization processes yielded a small concentration of saponine expressed in *β-ecdisone* (1.07% in the lyophilized extract).

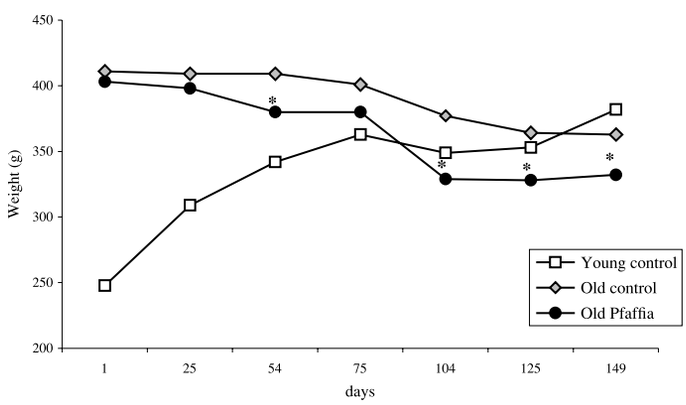

Figure 5. Body weight of young control rats (n = 14), old control rats (n = 12) and old rats chronically treated with 100 mg/kg *Pfaffia glomerata* extract BNT-08. The values were expressed as mean ± SD. * Indicates statistical difference from the old control group.

With regard to the pharmacological results, the data obtained in the acute tests showed statistical significance only in the sleeping time test at a dose of 100 mg/kg of lyophilized extract promoting a decrease in the total sleeping time compared with the control (93.1 ± 11.4 versus 136.0 ± 12.8 min; $p = 0.026$). This result is interesting, because it is more common to find no specific depressive effects with plants (Carlini, 1972), and the obtained data appear to be of a more stimulating character.

This result conflicts with the data of De-Paris and collaborators (2000), that reported a depressive effect with *P. glomerata* 500 mg/kg (i.p.) extract, with a reduction in latency and potentiation of the sleep time induced by pentobarbital. In the passive avoidance test with mice, by employing 2 mg/kg scopolamine as on inducing agent for memory deficits, the lyophilized extract did not change the acquisition or retention latencies in this model, that is, it was not capable of antagonizing the anticholinergic effects of scopolamine, but neither caused any deficit when administered alone. Also, in this case, the acute results were different to those obtained by De-Paris and collaborators (2000), who observed damage in learning and memory of the mice in the retention phase of the 'step-down passive avoidance' experimental model. Since the work of these authors employed different extraction methods (extraction through hot reflux in a soxhlet for 6 h), a different dose of the extract and used only acute treatment, the results are not comparable.

In the right–left discrimination test, with the ongoing course of the sessions, the young rats quickly learned the defined behaviour (10.1 ± 3.8 sessions). The old control group, as expected, found difficulty in learning, needing a greater number of sessions to achieve the criterion necessary (16.0 ± 6.8 sessions). On the other hand, the group of old rats that received the lyophilized extract reached the criterion in a number of sessions equivalent to that of the young group (10.7 ± 2.3) demonstrating that the extract promoted benefits in the acquisition and retention of the determined behaviour, leading the animals to performance levels equivalent to those of the young ones, and statistically different from the old group that did not receive the plant extract.

The passive avoidance test performed with these same groups of rats chronically treated provided positive results that are added to those of the labyrinth. The group of young control rats showed a high latency in the retention phase (Md = 319 s), but the group of control old rats, for which a low entrance latency would be expected in the dark side of the equipment, demonstrating a low level of memory retention, behaving in an uncommon manner in the face of what was theoretically expected and had a high latency value (Md = 189 s), even though statistically different from the young control group. The old pfaffia group presented intermediate values between the two controls (Md = 272 s), thus showing an increase in latency compared with the old control group and showing a partial reversal of the memory deficit associated with the animals' age in this model.

The learning and performance deficits of old mice in labyrinth models are well described in the literature (Aggleton *et al.*, 1989), and include results with several plants that lessen these deficits and improve the performance of these animals (Nishizawa *et al.*, 1991; Ghosal *et al.*, 1993; Yabe *et al.*, 1996; Galvão *et al.*, 2002). In particular, the species *Panax ginseng* has already demonstrated positive effects in the damage induced by scopolamine measured in the radial labyrinth (Yamaguchi *et al.*, 1996) or in the T labyrinth, as used in this case (Ni *et al.*, 1993). This last author refers to the behaviour assessed in this model ('working memory') as a type of memory analogous to recent memory (immediate) in humans, which is damaged in cases of insanity rather than remote memory.

The cases mentioned in the literature used scopolamine as an amnesic agent and the benefits generally found are associated with the positive effects in the cholinergic system. In the case of *Pfaffia glomerata*, despite the positive effects in the T labyrinth and passive avoidance tests, there is no way of knowing how to associate these benefits with one or other of the neurotransmitter systems. The lack of relevant effects in the acute passive avoidance with scopolamine diminishes the evidence that cholinergic effects are involved.

The positive data found in the passive avoidance tests and T labyrinth, however, were obtained only after chronic treatment in a manner similar to that suggested by the popular use of this plant. This suggests that its action mechanism may be similar to that of the plants referred to as adaptogens, that is, that increase the nonspecific resistance of organisms submitted to several stressful factors, causing a state of adaptation to exceptional situations (Carlini, 1995). The action mechanisms of the adapting elements are not yet entirely elucidated, but it is known that they modulate the hypophyseal – adrenal axis with several repercussions in the organism, mainly lengthening the physiological adaptation of the organism to several stressful situations, thus protecting the energy sources of depletion and accelerating the biosynthesis and nucleic acids (Wagner *et al.*, 1994; Panossian *et al.*, 1999).

The results showed a body weight gain in the young control group and a progressive fall in weight of the old groups, resulting from a decrease of proteins in the muscular mass. However, the old pfaffia group started to lose weight more rapidly than the old control group; this difference appeared on day 54, disappeared on day 75, but soon reappeared on day 104 of treatment, remaining and increasing until the end of the treatment. Since activity in the labyrinth involved periods in which the rats were left fasting for about 15 h (from day 83 of administration until the end of the treatment), an exceptional situation, in addition to the daily load of the ingestion of the lyophilized extract, must have contributed to the promotion of a higher loss of weight in the old pfaffia group.

Finally, the assessment of mortality between the groups at the end of the treatment period showed that the deaths occurring in the old control group (11 deaths among 23 animals) were not statistically different from those of the pfaffia old group (13 deaths among 27 animals), increasing evidence that, even during long-term administration, the lyophilized extract did not show evidence of toxicity.

The data presented continue research with the purpose of gaining more knowledge of *Pfaffia glomerata* with regard to learning mechanisms and memory.

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