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Cognitive enhancement in aged rats after chronic administration of *Equisetum arvense* L. with demonstrated antioxidant properties in vitro

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Abstract

The aim of this work was to verify if chronic administration of the hydroalcoholic extract of stems from *Equisetum arvense* (HAE) reverses the cognitive impairment in aged rats, as well as, evaluates it in vitro antioxidant properties. Chronic administration of HAE at dose of 50 mg/kg, i.p., improved both short- and long-term retention of inhibitory avoidance task and ameliorated the cognitive performance in reference and working memory version of the Morris Water Maze. No differences were found between all three groups of young controls, aged controls and EHA-treated animals with regard to the open field and elevated plus maze tests. Indeed, no toxicity manifestations were observed during treatment. In vitro assays revealed that HAE diminished the thiobarbituric acid reactive substances as well as nitrite formation, but did not alter catalase activity. Thus, the cognitive enhancement effects of the HAE may be attributed, at least in part, to it antioxidant action.

Keywords: Equisetum arvense; Learning; Memory; Aging; Oxidative stress

1. Introduction

In both humans and animals, aging is associated with a slow deterioration of cognitive performance and, in particular, of learning and memory (Ingram et al., 1994; Grady and Craik, 2000). Oxidative damage has long been proposed to be critically involved in several pathological manifestations of aging, including dementia (Floyd, 1991; Veerendra Kumar and Gupta, 2002), a cognitive disorder characterized by loss of intellectual ability sufficiently severe as to interfere with one's occupational or social activities, could be of several types and invariably involves impairment of memory (Dhingra et al., 2004).

Numerous studies have reported increases in protein oxidation and lipid peroxidation in various regions of aged mammalian brains (Oliver et al., 1987; Smith et al., 1991; Leutner et al., 2001; Liu et al., 2002a,b). These findings led to the notion that antioxidant defense mechanisms in the brain are not sufficient to prevent age-related increase in oxidative damage and that drug with antioxidant effects might be beneficial for preserving brain function. In fact, previous studies have indicated that oxidative damage can be attenuated relatively rapidly in aged animals after

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administration of certain antioxidant compounds (Carney et al., 1991; Dubey et al., 1995). The decrease in oxidative damage produced under these conditions has been associated with improvement in cognitive and/or psychomotor functions (Carney et al., 1991; Forster and Lal, 1999).

Thus, efforts have been directed to find therapeutic agents, both synthetic and natural, that could reduce the oxidative damage and promote a functional recovery in aged subjects. *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 is being grown in several regions of Europe in addition to North, Central and South America (Joly, 1979). In folk medicine it is used as an anti-inflammatory agent (Hoffman, 1990; Ody and Kindersley, 1993). Recent work in our laboratory showed an analgesic and anti-inflammatory effects of hydroalcoholic extract of stems from *E. arvense* (HAE) (Do Monte et al., 2004). In this work, phytochemical analysis revealed the presence of flavonoids, sterols, tannins and saponins. Several findings in the literature related antioxidant properties of numerous substances that contain flavonoids (Lim et al., 2002; Singh et al., 2003; Vitor et al., 2004; Han et al., 2004).

In view of these findings, it is possible that the HAE presents an antioxidant effect, and thus, can improve the cognitive deficits in aged rats. To address this question, the present work verifies if chronic treatment with HAE enhances the cognitive functions in aged rats, as well as, verifies its possible in vitro antioxidant effects.

2. Material and methods

2.1. Plant material and preparation of hydroalcoholic extract

The plant was collected in Santa Catarina State, South of Brazil, during the summer of 2002. Botanical material was classified by Dra. Claudete Schrage Nuernberg (Department of Agricultural Botanic, State University of Santa Catarina, Lages, Brazil). A voucher sample has been deposited at the Herbarium of the Medicinal Plants of the State University of Santa Catarina. The dried stem of *E. arvense* were minced and extracted with 50% ethanol–water, being stirred and macerated at room temperature (21 ± 3 °C) for 15 days. The ethanol was evaporated and the extract was stored in the concentration of 5% at −20 °C, until use. The extract was suspended in 0.9% NaCl solution to the desired concentration just before use.

2.2. Animals

10 young (8-weeks-old, 200–250 g) and 22 aged (80-weeks-old, 510–650 g) male Wistar rats were used. The animals were housed in standard environmental conditions (22 ± 1 °C, humidity 60 ± 5% and a 12 h/12 h dark/light cycle—light on at 7:00 a.m.), with food and water available ad libitum in accordance to the Guide for the Care and Use of Laboratory Animals, US Department of Health and Human Services, 1985. The protocol of experiment was approved by the Ethical Committee of Research of the University in which the experiment was done (Federal University of São Paulo, Brazil).

2.3. Drug treatment

Eight weeks prior to testing, the aged rats were randomly assigned to groups that received once daily (at 18:00 h) saline (aged control group) or HAE at dose of 50 mg/kg, i.p. (HAE group). In a pilot study, this dose (but not the doses of 200 and 400 mg/kg) produced neither motor impairments nor sedative effects when administered acutely. The treatment was performed until the last day of the behavioral procedures. The young control group was composed of 10 naïve young rats that received neither saline nor HAE injections.

2.4. Behavioral procedures

2.4.1. Elevated plus maze

The apparatus was made of wood and consisted of two opposite open arms, 50 × 10 cm and two enclosed arms, 50 × 10 × 40 cm, elevated to a height of 50 cm above the floor. Each animal was placed on the center platform of the maze facing the enclosed arm and the number of entries and the time of permanence in the open and closed arms were recorded during a 5 min session (Pellow et al., 1985).

2.4.2. Open Field

Besides measurement of exploratory activity, the open field test was performed as an indicator of drug toxicity, as well as, to rule out any confounding effect on the cognitive tests. 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. Each animal was placed in the center of the arena, and was observed for the number of both fecal boli produced and squares crossed (with four paws) in addition to rearing and grooming behaviors during 5 min (Archer, 1973).

2.4.3. Step-through inhibitory avoidance

A two-compartment apparatus from Ugo Basile, Italy was used. Each rat was placed individually inside the light compartment of the apparatus. When the animal entered the black compartment with all four paws, a foot shock of 1 mA was delivered for 1 s (training session). The latency for the animal to enter the black compartment was recorded (baseline) up to a cut-off time of 298 s. The test sessions were carried out 1.5 (short-term memory) and 24 h (long-term memory) after training (Izquierdo et al., 1999). Each animal was placed again in the light compartment, and the

time taken by the animal to cross to the black compartment was recorded (retention latencies). If the animal did not cross within 298 s, it was removed from the apparatus and a latency of 298 s was attributed.

2.4.4. Morris Water Maze (MWM)

The apparatus consisted of a circular pool containing a movable platform. During the trial, the rat was monitored in the platform with a chromotrack video tracking system (Ethovision, Amsterdam, The Netherlands). The software package (Ethovision) was used to acquire the following parameters in each trial: time to find the platform and swimming velocity.

2.4.4.1. Reference memory version. Each animal was given 16 trials: four trials per day on 4 consecutive days (acquisition phase). A trial was initiated by lowering the rat into the water by its tail while facing the wall along the side of the pool at a predetermined position. The trial was finished when the rat found the platform or when 120 s had elapsed. If the rat succeeded in finding the platform within 120 s, the latency was recorded and it was allowed to remain on the platform for 30 s. If the rat failed to find the platform within 120 s, it was manually guided to the platform and there maintained for 30 s. At the end of each trial, animals were dried and kept warm in a heated box for 8 min before the next trial. The position of the platform was the same in all trials of acquisition phase and it was submersed 2 cm under the water surface. Probe trials (retention phase) was performed in three distinct periods: just prior to the first day (probe 1) and second day (probe 2) of acquisition, as well as, in the next day after the acquisition phase (probe 3). The platform was removed from the tank and the time spent in the quadrant of the platform was registered for 60 s. This protocol was adapted from the one proposed by Morris et al. (1982).

2.4.4.2. Working memory version. Two days after the reference memory test, the animals were submitted to working memory version. The procedure utilized was the same as that of the reference memory version. However, cued pattern taped in the room during the reference memory were changed. Indeed, in each subsequent training day the platform position was moved to distinct localization, in a pseudorandom way. The position utilized in reference memory version was not available in the working memory version. Indeed, the inter-trial interval is the minimum possible, that is, the subsequent trial was performed immediately after the current trial, to ensure that the animal maintained the information on line during the execution of the test. No probe trial was performed in this version. This protocol was based in the study performed by Miyoshi et al. (2002).

2.4.4.3. Cued version. Two days after the working memory version, the animal were submitted to cued version, similar to the reference memory procedures, except that the position of the escape platform was cued by a 7-cm diameter white ball attached to the top of the platform and protruding above the water. Furthermore, in this version the position of the platform was always changed in each trial of the day. The protocol of this memory task was adapted from the study of Packard and McGaugh (1992).

2.5. Toxicological study

Daily, during the treatment period, just after injections, the aged animals (control and that received HAE) were weighted and for 30 min its behaviors were observed (Mattei et al., 1998). The symptoms evaluated include: piloerection, alteration in motor activity, tremors, convulsion, alteration in posture, abdominal constriction, eyelid ptosis, loss of reflexes, lacrimation and salivation. There were no obvious changes in body weights or clinical symptoms in the animals that received HAE compared to animals that received saline.

2.6. Assessment of antioxidant capacity in vitro

Antioxidant activity was evaluated by measuring the production of thiobarbituric acid (TBARS), as indicator of lipid peroxidation (Agar et al., 1999), and the catalase activity (Chance and Maehly, 1995). Naïve Wistar rats (150–200g) were decapitated, and its brain was removed rapidly under standard conditions at 4 °C and stored for 24 h at −20 °C (Mattei et al., 1998, 2001; Auddy et al., 2003). The whole brain besides the cerebellum was homogenized in 50 mM potassium phosphate buffer (pH 7.4) and the concentration adjusted to 1g wet weight of brain per 60 ml. Then 250 μl of the homogenate in the absence (control) or presence of lyophilized HAE (10 and 20 μg/ml) was obtained for the studies that followed.

2.6.1. Measurement of TBARS

The homogenates were incubated in a water bath for 1 h at 37 °C. After incubation, 400 μl of 35% perchloric acid was added to stop spontaneous lipoperoxidation. We obtained 600 μl of supernatant by centrifugation at 1400 g for 10 min, and added it to 200 μl of 1.2% sodium 2-thiobarbiturate solution. The mixture was then placed in a water bath and heated for 30 min at 95–100 °C. After the solution cooled, the absorbance was measured at a wavelength of 532 nm. The protein concentration was determined by using Lowry assay (Lowry et al., 1951).

2.6.2. Nitrite determination

For the assessment of nitrite, derived from nitric oxide (NO), 100 μl of Griess reagent (1% sulfanilamide in 1% H_3PO_4/0.1% *N*-(1-naphthyl)-ethylenediamine dihydrochloride/1% H_3PO_4/distilled water, 1:1:1:1) were added to 100 μl of brain homogenates or to 100 μl of $NaNO_2$ at concentrations ranging from 0.75 to 100 μM (standard curve). For the blanks, 100 μl of the Griess reagent were

added to 100 µl of the cell culture medium. The absorbance was measured with a reader plate at 560 nm. The standard curve was used for the determination of nitrite concentrations in samples (Green et al., 1981).

2.6.3. Catalase activity

It was measured by the methods that employ hydrogen peroxide to generate H_2O and O_2 (Chance and Maehly, 1995). The substrate mixture contained 0.3 ml of hydrogen peroxide in 50 ml of 0.05 M phosphate buffer, pH 7.0. The sample aliquot (20 µl) diluted with phosphate buffer was added to 980 µl of the substrate mixture. Absorbances were read after 1 and 6 min at 230 nm. A standard curve was established using purified catalase (Sigma MO, USA) and results were expressed as mM/min/µg protein.

2.7. Statistical analysis

The data obtained from elevated plus maze, open field and antioxidant assays were analyzed by a parametric two-way ANOVA, followed by Tukey test. In all versions of the MWM, a parametric two-way ANOVA for repeated measures, followed by Tukey test was performed. Non-parametric Kruskall–Wallis, followed by Mann–Whitney test was utilized for data obtained in the inhibitory avoidance test.

3. Results

3.1. Behavioral tests

The results obtained in the inhibitory avoidance test, showed that only the aged control animals presented an impairment of both short-term retention ($U=10$; $Z=3.02$; $p<0.01$) and long-term retention ($U=0$; $Z=3.78$; $p<0.01$) when compared to young control group. In addition, it differed from aged animals that received HAE, in the long-term retention ($U=26$; $Z=2.24$; $p<0.05$). No difference was observed among all experimental groups in the training latency ($U=46$; $Z=1.06$; $p=0.81$) (see Fig. 1).

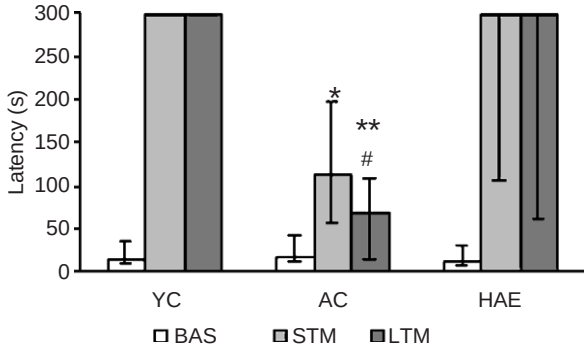

Fig. 1. Training and retention latencies in the inhibitory avoidance paradigm of young control, aged control and aged animals that received hydroalcoholic extract of *Equisetum arvense* at dose of 50 mg/kg. The data were expressed as median and interquartil interval. YC=young control group ($n=10$). AC=aged control group ($n=10$). HAE=aged animals which received *E. arvense* ($n=12$). BAS=baseline latency; STM=short-term retention latency; LTM=long-term retention latency. Kruskall–Wallis followed by Mann–Whitney test. *$p<0.05$, **$p<0.01$ in relation to young control group, $^{\#}p<0.05$ in relation to aged animals that received HAE.

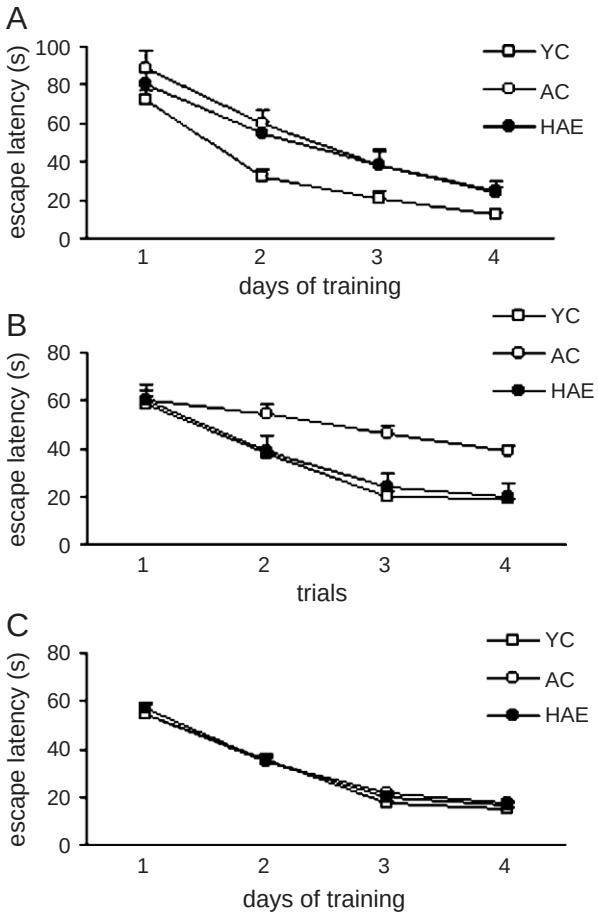

Fig. 2. Latency to found the platform in three distinct versions of the MWM in the young and aged control group, as well as in aged animals that received hydroalcoholic extract of *Equisetum arvense* at dose of 50 mg/kg. The data were expressed as mean±S.E.M. YC=young control group ($n=10$). AC=aged control group ($n=10$). HAE=aged animals which received *E. arvense* ($n=12$). (A) Reference memory version. There was a significant main effect of HAE treatment ($F_{(2,29)}=3.57$, $p<0.05$) and days of training ($F_{(3,87)}=137.40$, $p<0.001$), but no significant interaction between these factors was observed ($F_{(6,87)}=1.11$, $p=0.36$). (B) Working memory version. There was a significant main effect of HAE treatment ($F_{(2,29)}=5.54$, $p<0.01$) and trials effect ($F_{(3,87)}=67.12$, $p<0.001$). Indeed, an interaction was found between HAE treatment and trials factors ($F_{(6,87)}=0.36$, $p<0.01$). (C) Cued version. There was no significant main effect of HAE treatment ($F_{(2,29)}=0.74$, $p=0.48$) and no significant interaction between treatment and day of training ($F_{(6,87)}=0.36$, $p=0.90$).

In the reference memory version of the MWM there was a significant main effect of HAE treatment ($F_{(2,29)}=3.57$, $p<0,05$) and days of training ($F_{(3,87)}=137.40$, $p<0.001$), but no significant interaction between these factors was shown ($F_{(6,87)}=1.11$, $p=0.36$). The HAE treatment diminished the escape latency in the aged animals, despite the fact that all experimental groups were able to learn the spatial reference information (Fig. 2A). In regard to the probe trials,

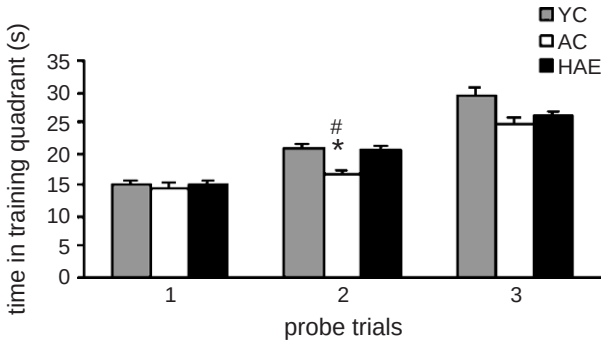

Fig. 3. Time spent in the platform quadrant during probe trials in young and aged controls, as well as in aged animals that received hydroalcoholic extract of *Equisetum arvense* at dose of 50 mg/kg. Probe trial 1 (before the first reference memory day), probe trial 2 (24 h after the second reference memory day) and probe trial 3 (24 h after the fourth reference memory day). The data were expressed as mean±S.E.M. YC=young control group ($n=10$). AC=aged control group ($n=10$). HAE=aged animals that received *E. arvense* ($n=12$). There was a significant main effect of HAE administration ($F_{(2,29)}=10.33$, $p<0.01$) and probe trial ($F_{(2,58)}=173.08$, $p<0.01$). Indeed, a significant interaction between these factors was found ($F_{(4,58)}=2.88$, $p<0.05$). *$p<0.01$ in relation to young control group; $^{\#}p<0.01$ in relation to aged animals that received HAE treatment.

there was a significant main effect of HAE administration ($F_{(2,29)}=10.33$, $p<0.001$) and probe trial ($F_{(2,58)}=173.08$, $p<0.001$). Indeed, a significant interaction between these factors was found ($F_{(4,58)}=2.88$, $p<0.05$). The HAE was able to increase the time spent in the platform quadrant during the second probe trial. As expected, no difference between groups was found in the first and third probe trials, because the first probe was performed prior to acquisition phase, and the third probe was carried out after the acquisition phase, when all groups acquired the information (see Fig. 3). In the working memory version of the MWM, there was a significant main effect of HAE treatment ($F_{(2,29)}=5.54$, $p<0.01$) and trials effect ($F_{(3,87)}=67.12$, $p<0.001$). Indeed, an interaction was observed between HAE treatment and trials factors ($F_{(6,87)}=0.36$, $p<0.01$). The administration of HAE diminished the escape latency of the aged groups within each day, a good indicative of cognitive improvement (see Fig. 2B). In the cued version of the MWM, as shown in the Fig. 2C, there was no significant main effect of HAE treatment ($F_{(2,29)}=0.74$, $p=0.48$) and no significant interaction between treatment and day

Table 1
Number of squares crossed, rearing, fecal bolus and time of grooming during the open field paradigm, in the young and aged controls, as well as in aged animals that received hydroalcoholic extract of *Equisetum arvense* at dose of 50 mg/kg, i.p.

Groups	Squares crossed	Rearing	Time of grooming (s)	Fecal bolus
YC ($n=10$)	89.3±7.4	8.4±2.3	16.6±4.4	4.1±0.8
AC ($n=10$)	81.2±6.2	7.2±1.8	15.6±2.6	4.8±0.6
HAE ($n=12$)	81.2±5.7	5.4±1.2	17±2	5.5±0.7

The data were expressed as mean±S.E.M. YC=young control group. AC=aged control group. HAE=aged animals with received *E. arvense*.

Table 2
Percentage of entries and percentage of time spent in open arms related to both open and closed arms during the elevated plus maze test, in the young and aged controls, as well as in aged animals that received hydroalcoholic extract of *Equisetum arvense* at dose of 50 mg/kg, i.p.

Groups	Entries in open arms (%)	Time spent in open arms (%)
YC ($n=10$)	29.8±4.1	22.1±4.3
AC ($n=10$)	32±2.7	18.5±3.1
HAE ($n=12$)	28.8±5.7	12.8±3.0

The data were expressed as mean±S.E.M. YC=young control group. AC=aged control group. HAE=aged animals with received *E. arvense*.

of training ($F_{(6,87)}=0.36$, $p=0.90$). However, a significant main effect of days of training was observed ($F_{(3,87)}=217.46$, $p<0.001$). These findings indicated that both experimental groups presented similar learning habit.

In relation to open field test, no difference was found between groups in regard to number of squares crossed ($F_{(2,29)}=0.52$), rearing ($F_{(2,29)}=0.76$), time in grooming ($F_{(2,29)}=0.06$) and number of fecal bolus ($F_{(2,29)}=0.98$) (see Table 1). Indeed, in the elevated plus maze, all experimental groups explored equally the open arms of the apparatus ($F_{(2,29)}=0.56$, for percentage of entries; $F_{(2,29)}=1.21$, for percentage of time in permanence) (Table 2).

3.2. In vitro antioxidant assays

Concerning to the in vitro antioxidant assays, the brains submitted to oxidative stress (stress group) showed an increase in the lipid peroxidation (thiobarbituric-acid reacting substances — TBARS) (29%), nitrite contents (342%) and catalase activity (440%) when compared to brains not submitted to oxidative stress (control group). Otherwise, the HAE at the doses of 10 and 20 mg/kg diminished both TBARS and nitrite contents, when compared to control ($p<0.05$ and $p<0.01$, respectively) and stress ($p<0.01$) groups. (See Table 3).

Table 3
Lipid peroxidation, nitrite contents and catalase activity in the brain submitted to oxidative stress and incubated with or without hydroalcoholic extract of *Equisetum arvense* at dose of 10 and 20 mg/kg

	TBARS (%)	Nitrite (mM)	Catalase (mM/min/μg protein)
Control ($n=04-05$)	1.38±0.04	55.91±3.07	1624.41±826.29
OE ($n=05-06$)	1.78±0.06 **	135.61±4.83**	8771.52±2288.64*
HAE10 ($n=06$)	1.03±0.10 *,#	43.39±1.36*,#	8016.43±1540.05
HAE20 ($n=05-06$)	0.47±0.11**,#	28.18±2.54**,#	6568.86±1189.51

The data were expressed as mean±S.E.M. Two-way ANOVA followed by Tukey post hoc, *$P<0.05$, **$P<0.01$ in relation to control group, $^{\#}P<0.01$ in relation to OE group. The TBARS and nitrite formation, as well as catalase activity were measured in the absence (OE) or presence of HAE (HAE10 and HAE20, for 10 and 20 μg/ml, respectively) after 1 h of incubation of brain homogenate at 37 °C.

4. Discussion

The results of present work revealed that chronic treatment with hydroalcoholic extract of stems from *E. arvense* prevents cognitive deficits in aged rats. Indeed, the in vitro assays showed a clear antioxidant effect.

A preliminary acute toxicity study conducted in our lab showed that HAE induced mortality at dose of 2 and 5 g/kg (12.5% and 37.5%, respectively). All treated animals were sedated while demonstrating transitory respiratory depression. These effects persisted for 240 min and were dose-dependent. According to Hodgson (1997), substances with LD50s higher than 5 g/kg can be considered non-toxic. In addition, chronic administration of the dose utilized in the present study did not result in toxicity which may support the intense, chronic and popular use of the plant among humans as an herbal medicine (Hoffman, 1990; Ody and Kindersley, 1993).

In the inhibitory avoidance test, the short- and long-term retentions in aged control rats were impaired. The administration of HAE improved these cognitive deficits, because the animals that received the HAE, presented similar short- and long-term retentions when compared to young control animals. Several works in the literature show that aged rats performed more poorly in the inhibitory avoidance and that antioxidant drugs prevent the cognitive impairment in this paradigm (Raghavendra and Kulkarni, 2001; Yasui et al., 2002; Parle and Dhingra, 2003; Singh et al., 2003). Singh et al. showed that 30 days of quercetin treatment improve the short- and long-term retention of aged mice, but not young mice, in the inhibitory avoidance (Singh et al., 2003). Cognitive enhancement in aged mice in the same paradigm has also occurred following 30 days of treatment with acetyl-L-carnitine (Yasui et al., 2002) and melatonin (Raghavendra and Kulkarni, 2001). Indeed, Parle and Dhingra verified that only 1 week treatment with ascorbic acid was sufficient to improve retention latencies in aged mice, during inhibitory avoidance test (Parle and Dhingra, 2003). However, in the work of Socci et al., 4–5 months of treatment with several antioxidant drugs did not change the retention parameters of aged rats in the inhibitory avoidance (Socci et al., 1995).

The mean performance level of our untreated aged controls confirms previous findings, showing that aged Wistar rats exhibit deficits in reference and working memory, but not in cued-version of the MWM (Socci et al., 1995; Liu et al., 2002a,b; Kiray et al., 2004). In the reference memory version, these animals needed more time to find the platform at the beginning of the acquisition (first two days) and spent less time in the platform quadrant during the retention phase (second retention test, performed 24 h after the second acquisition day). Chronic administration of HAE seems to alleviate the performance deficits of the aged rats, because the old animals treated with HAE found the platform equally with young controls throughout the acquisition phase and spent time in the platform quadrant similarly to young controls during retention phase (in all sessions). In the working memory version, the aged controls do not acquire the information throughout the four trials; however, in the old animals that received HAE, the acquisition performance was similar to young controls. As verified in the present work, antioxidant compounds improved the performance of aged animals in the MWM (Socci et al., 1995; Stackman et al., 2003). Socci et al. showed that aged rats that received 4–5 months treatment with several antioxidants performed better than aged rats which received saline, in the MWM test (Socci et al., 1995). Stackman et al. verified that 6 months of treatment with Ginkgo biloba extract enhanced the performance of transgenic animal that over expresses a mutant form of human Abeta precursor protein. These animals exhibit age-related cognitive deficits in the MWM (Stackman et al., 2003).

Despite the fact that animals of both aged groups swim more slowly throughout the experiments in all version of the MWM, when compared to young controls, with no difference from each other (data not showed), no differences were observed between groups in relation to ambulatory activity in the open field and in the latency to enter in the dark compartment of the inhibitory avoidance apparatus during the training. Therefore, the beneficial effect of HAE on the MWM and inhibitory avoidance paradigms are unlikely to be a result of differences in motor abilities. Indeed, because no difference was found in grooming time and number of fecal bolus in the open field, as well as in the percentage of entries and time spent in the open arms of the elevated plus maze, alterations in the motivational and/or emotional state of the animal, which could have affected performance in both inhibitory avoidance and water maze learning, can be ruled out.

Reactive oxygen species (ROS) can be highly damaging to cells due to the oxidation of essential cellular constituents such as lipids, proteins and DNA. The brain is particularly susceptible to oxidation by ROS because of its dependency on aerobic metabolism, large contents of polyunsaturated lipid in the mitochondrial and plasma membranes of brain cells and its low antioxidant defenses, such the antioxidant enzyme catalase (Reiter, 1995). The ROS-production-mediated protein oxidation can be measured by tyrosine nitration (Rong et al., 1999), as well as, lipid peroxidation as indicated by malondialdehyde (Bruce and Baudry, 1995). Thus, the increment of TBARS and nitrite contents found in the stress group indicates clear oxidative processes, whereas the highest levels of catalase represent a compensatory neuroprotector mechanism against oxidative stress. Because the HAE diminished the ROS production but not altered the catalase activity, it could be proposed that the HAE presents an antioxidant effect that is not changed to the increase of catalase activity.

In the literature, several natural products with antioxidant proprieties have been reverting the cognitive impairment in aged rats (Veerendra Kumar and Gupta, 2002; Singh et al., 2003; Stackman et al., 2003; El-Sherbiny et al., 2003; Dhingra et al., 2004). Phytochemical analysis of the extract

utilized in the present work revealed the presence of tannins, saponins, flavonoids and sterols. Broudiscou and Lassalas showed that the flavonoids portion of hydroalcoholic extract of *E. arvense* basically contain isoquercitrin (Broudiscou and Lassalas, 2000). According to several papers, this compound presented significant antioxidant properties. The isoquercitrin has been isolated from *Spartium junceum* (Yesilada et al., 2000), *Juniperus chinensis* (Lim et al., 2002), *Aceriphyllum rossii* (Han et al., 2004), *Pterospartum tridentatum* (Vitor et al., 2004) and others. In these works, both isoquercitrin and flavonoid portion of the extract from these plants presented a clear antioxidant effects. Thus, the antioxidant proprieties of HAE can be attributed, at least in part, to isoquercitrin.

To summarize, chronic administration of HAE improved the cognitive deficits in aged rats, and this effect can be due, at least in part, to its antioxidant action. It is possible that some compound of the flavonoid fraction of the extract, namely isoquercitrin, plays a crucial role in this action.

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