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Ameliorating effects of essential oil from Acori graminei rhizoma on learning and memory in aged rats and mice

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Abstract

Although there are normal cognitive changes that take place as a person becomes older, ageing in humans is generally associated with a deterioration of cognitive performance, in particular of learning and memory. There are a number of herbal medicines that are reported to improve brain function and intelligence. In the present study, the ameliorating effects of an essential oil extracted from Acori graminei rhizoma on learning and memory in aged, dysmnesia rats and mice were determined using the step-down passive avoidance test and Y maze. Oral administration of the essential oil (0.02, 0.04 and 0.08 g kg^{-1}) to rats for 30 days and to mice for 15 days improved the latency and number of errors in aged, dysmnesia rats and mice. The cerebral neurotransmitters in aged rats given the essential oil (0.02, 0.04, 0.08 g kg^{-1}) for 30 days were also investigated, and increased levels of norepinephrine, dopamine and serotonin, and decreased levels of acetylcholinesterase activity were found. The results suggest that the essential oil improves cognitive function in aged animals possibly by increasing norepinephrine, dopamine and serotonin relative levels, and by decreasing the activity of acetylcholinesterase in the cerebra.

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Introduction

As life expectancy increases there is concern about the incidence of senile dementia, which results from degeneration of the brain in the absence of cerebrovascular disease and which has very high prevalence in aged people. The prevalence is estimated at 1.53% between the ages of 65 and 69 years; between 60 and 80 years, the relative incidence of senile dementia increases exponentially with age, doubling every 5 years (Preston 1986; Brayne et al 1995). Between the ages of 90 and 94 years, the prevalence is 31.48% of the population and this increases to as high as 44.48% between the ages of 95 and 99 years (Ritchie & Kildea 1995). Thus, senile dementia is an aged-related disorder.

There are normal cognitive changes that take place as a person becomes older. Ageing in humans is associated with deterioration of cognitive performance, in particular of learning and memory (Erickson & Barnes 2003). Cognitive deficits are a debilitating consequence of ageing (Forster et al 1994). Older humans (Uttl & Graf 1993; Wilkniss et al 1997; Newman & Kaszniak 2000), monkeys (Lai et al 1995; Rapp et al 1997), rats (Barnes 1979; Markowska et al 1989; Gallagher & Rapp 1997) and mice (Bach et al 1999) all show impairments in learning and memory compared with their younger counterparts. For example, Uttl & Graf (1993) studied the ability of groups of people aged from 15 to 74 years to navigate through and remember spatial location information in a museum exhibit. The subjects showed an age-related decline in memory for the location of target exhibits that began to appear during the sixth decade. Although changes in memory with age can be variable between individuals, and all types of memory are not affected equally, alterations in memory can be observed objectively at least by the fifth decade in humans (Albert et al 1987), and many in this age group notice subtle changes in memory. Impairment and decline in cognitive function can also be detected in non-demented older people who eventually progress to clinically

recognizable dementia (Rubin et al 1998; Albert et al 2001; Chen et al 2001; Morris et al 2001; Howieson et al 2003; Amieva et al 2005; Tierney et al 2005).

For more than a millennium, herbal remedies have been used, apparently safely and effectively, in Asian countries, especially in China, Japan and Korea, to alleviate various symptoms of cognitive deficits and to facilitate learning and memory. There are a number of herbal medicines that have been characterized for their effects on brain function, such as *Panax ginseng* (Jin et al 1999), *Polygala tenuifolia* (Chen et al 2004), *Ginkgo biloba* (Rai et al 1991; Topic et al 2002; Olga et al 2006) and *Acorus gramineus* (Bombi et al 2003; Yoshifumi & Wing 2003; Oh et al 2004).

Acori graminei rhizoma (AGR) has been used as a treatment for various diseases of the brain and for its effects of tranquilizing the mind and promoting intelligence; in combination with other herbal drugs, it is one of the most prescribed herbal remedies for the treatment of various neurological disorders such as epilepsy, cerebrovascular diseases and senile dementia, including Alzheimer's disease (Liao et al 1998; Hsieh et al 2000). Anecdotal clinical experiences support the notion that AGR is safe and effective in treating and/or alleviating the symptoms of these diseases. Among 75 of the most famous Chinese complex prescriptions characterized as promoting intelligence in past dynasties in China, more than half contain AGR (Liu & Liu 2005).

AGR, the rhizome of *Acorus gramineus* Schott. (Araceae), contains up to 4.86% essential oil (EO) (Li et al 2005), which is mainly composed of β-asarone (63.2–81.2%) and α-asarone (8.8–13.7%) (Chang & Pui-Hay But 1986). AGR has been shown to affect the central dopaminergic and GABA systems (Liao et al 1998), and its major component, asarone, has a neuroprotective effect against excitotoxic neural death (Cho et al 2000, 2002). Hsieh et al (2000) reported cognitive enhancing effects of AGR on scopolamine-induced amnesia in rats. In addition, AGR has protective effects against ischaemia-induced neuronal loss and learning and memory impairment in rats (Bombi et al 2003). Until now, as far as we are aware, there have been no reports on the facilitating effects of EO from AGR on learning and memory in aged animals.

In the present study, we investigated the effects of EO from AGR on cognitive function in young and aged animals, and in chemically induced dysmnesia subjects. Learning and memory parameters were evaluated using the step-down passive avoidance task and Y maze as previous studies have shown that aged animals and chemically induced dysmnesia subjects perform poorly compared with their young counterparts in these types of tests (Luo et al 2003; da Silva et al 2004). The step-down passive avoidance task examines the passive avoidance response, while spatial memory can be determined by using the Y maze. Although some investigators have argued against the usefulness of passive avoidance testing to evaluate learning and memory in animals (LeDoux 1993), it is generally accepted as an indicator of long-term memory. In addition, this study also examined the effects of EO on levels of the monoaminergic neurotransmitters norepinephrine (NE), dopamine (DA) and serotonin (5-HT), and on the activity of acetylcholinesterase (AChE) in the cerebra of aged rats, all of which are known to be important in the mediation of learning and memory processes.

Materials and Methods

Preparation of EO from AGR

AGR was purchased from Chengdu Tong-ren-tang Pharmaceutical Group and identified as the rhizoma of *Acorus tatarinowii* Schott. by Professor D.-G. Wan (The Pharmacy Faculty of Chengdu University of Traditional Chinese Medicine, Chengdu, China). The EO was extracted by hydrodistillation. The dried rhizomes were broken into small segments, 10 kg of which were immersed in 50 L distilled water and boiled in a distillation apparatus for 10 h. The yield of EO was 3.04% (v/w) and it was stored at 4°C until utilized.

Gas chromatography–mass spectrometry (GC-MS) analysis

GC-MS was performed with an Agilent 6890 gas chromatography instrument coupled to an Agilent 5973 mass spectrometer and Agilent ChemStation software (Agilent Technologies, Palo Alto, CA, USA). Compounds were separated on a 30 m × 0.32 mm i.d. capillary column coated with 0.25-μm film of 5% phenyl methyl siloxane. The temperature program began at 60°C and was held for 1 min, then increased at a rate of 3°C min^{-1} to 220°C and held for 5 min. Split injection (2 μL) was conducted with a split ratio of 1:30, and helium was used as the carrier gas at a flow rate of 1.3 mL min^{-1}. The spectrometers were operated in electron-impact mode and the ionization energy was 70 eV. The inlet and ionization source temperatures were 250°C and 280°C, respectively. The GC-MS analysis showed that the main components of the EO were β-asaricin and α-asaricin, having a relative content 63.37% and 4.12% (w/w), respectively.

Administration of EO from AGR

EO was dissolved in 3% Tween-80 and distilled water before administration. Three groups of animals (n = 10) were orally administered 0.02, 0.04 and 0.08 g kg^{-1} EO per day by intubation; the mice were treated for 15 days while the rats were treated for 30 days. Two further groups of animals (normal and control) were orally administered the vehicle (3% Tween-80), and they were run concurrently with EO-treated groups. EO and vehicle were given in a volume of 10 mL kg^{-1} irrespective of dose.

Animals

Mice and rats were obtained from the Experimental Animal Center of Chengdu University of Traditional Chinese Medicine (Chengdu, China). They were housed in a regulated environment (20 ± 1°C), with a 12-h light/dark cycle (lights on: 08:00–20:00 hours) and were grouped as follows. Young and aged male Kunming mice (30 days of age, 18–22 g and 12–13 months of age, 40–50 g, respectively), Grade II, Certificate No 2000-7.

Young and aged male Sprague–Dawley rats, (90 days of age, 180–220 g and 24–25 months of age, 550–650 g, respectively), Grade II, Certificate No 2000-8. Food and water were given ad libitum, except for the duration of the experimental period. On the day of the experiment, the animals were allowed to acclimatize to the experimental environmental conditions for approximately 60 min before the start of the experiment. All animal treatments were strictly in accordance with international ethical guidelines and the National Institutes of Health Guide concerning the Care and Use of Laboratory Animals, and the experiments were carried out with the approval of the Committee of Experimental Animal Administration of the University.

In the experiment on the effects of EO on learning performances in aged mice, 40 aged mice were randomly divided into four groups (n = 10): three EO treatment groups (0.02, 0.04, 0.08 g kg^{-1} per day) and one control group. Another 10 young mice served as the normal group. In the experiment on the effects of EO on learning performances in aged rats, 40 aged rats were randomly divided into four groups (n = 10): three EO treatment groups (0.02, 0.04, 0.08 g kg^{-1} per day) and one control group. Another 10 young rats served as the normal group. In the experiments on the effects of EO on dysmnesia, animals were induced by scopolamine (mice), sodium nitrite (rats) or ethanol (mice); 40 young animals were randomly divided into four groups (n = 10): three EO treatment groups (0.02, 0.04, 0.08 g kg^{-1} per day) and one control group. Another ten young subjects served as the normal group. In the experiment on the effects of EO on cerebral 5-HT, NE and DA levels in aged rats, 40 aged rats were randomly divided into four groups (n = 10): three EO treatment groups (0.02, 0.04, 0.08 g kg^{-1} per day) and one control group. Another ten young rats served as the normal group. In the experiment on the effects of EO on cerebral AChE activity in aged rats, the grouping was the same as outlined above.

Chemicals and modelling

Scopolamine (Mingxing Pharmaceutical Factory, Guangzhou, China) and sodium nitrite (Chengdu Chemical Reagent Factory, China) were dissolved in sterile 0.9% saline, respectively. Ethanol was diluted to a concentration of 30% (v/v) with distilled water. All chemicals were administered intraperitoneally in a volume of 5 mL kg^{-1} irrespective of dose. Control and normal animals received respective solvent injections, and they were run concurrently with the drug-treated groups.

In the experiments on the effects of EO on dysmnesia, scopolamine (1 mg kg^{-1}, i.p.) was administered 30 min before the training trial and induced memory acquisition impairment in mice; sodium nitrite (120 mg kg^{-1}, i.p.) was injected immediately after the training trial and induced memory consolidation impairment in rats; and, finally, 30% alcohol (1.5 g kg^{-1}, i.p.) was injected 30 min before the testing trial and induced memory retrieval impairment in mice.

Behavioural testing

Step-down test

The method described by Xu et al (2002) served as the reference. The apparatus consisted of an acrylic box (20 × 20 × 20 cm) with a stainless-steel grid floor and a wooden platform (4 × 4 × 4 cm) fixed at the centre of the box. Electric shocks (36 V) were delivered to the grid floor for 6 s with an isolated pulse stimulator. At the beginning of the training trial, mice were placed in the box to adapt for 3 min. After 3 min, electric shocks were delivered and the mice jumped onto the platform to avoid noxious stimulation. The shocks were maintained for 5 min. After a 24-h interval, mice were again placed on the platform and their latency to step down onto the grid with all four paws for the first time and the number of errors subjected to shocks within 5 min were measured as learning performances.

Y-maze test

The Y-maze was used for behavioural testing of spatial recognition memory and the method described by Xu et al (2002) served as the reference. The apparatus with a conductive grid floor consisted of three identical arms (60 cm long × 16 cm wide × 32 cm high) made of dark opaque Plexiglas and these three arms were symmetrically disposed at 120° to each other. Arms 1 and 3 were non-safety zones (shocks were administered via these); arm 2 was a safety zone (on the top of which there was an insulated grid floor 16 × 25 cm). Rats were placed on the top of arm 1; a fixed resistance shock source was connected to an automatically operated switch and electric shocks (50 V) were applied. After shocking, the rats escaped from foot shocks by entering the top of arm 2; this was counted as one practice and the rats were repeatedly trained for this procedure a further 10 times. After a 24-h interval, the rats were successively tested 10 times and their latency to enter the safety zone (i.e. the insulated grid floor) from the non-safety zone for the first time and number of errors displayed by entering the non-safety zone within 10 times were recorded as learning performances.

Assays of NE, DA and 5-HT levels

To determine cerebral levels of 5-HT, NE and DA, three groups of aged rats (n = 10) received EO (0.02, 0.04 or 0.08 g kg^{-1} per day) orally for 30 days before decapitation. Two more groups of animals (n = 10), the normal group (young rats) and control group (aged rats), were given the vehicle orally by intubation and run concurrently with EO-treated groups. Animals were decapitated and skulls were split on an ice and salt mixture. The cerebra were isolated, weighed and homogenized in ice-cold *n*-butanol solution (5 mL (g tissue)$^{-1}$) according to Miller et al (1970) and the Biochemistry Group of Acupuncture and Meridian Research Institute of TCM Academy (1975). Homogenization was performed using an ice-cold homogenizer for 1 min and a 20% homogenate was made and then centrifuged at 3000 rev min^{-1} for 5 min. Supernatant (2.5 mL) was then transferred to a tube containing 1.6 mL of 0.2 N acetic acid and 5 mL *n*-heptane. After mixing on a vortex mixer for 30 s, the tubes were centrifuged at 3000 rev min^{-1} for 5 min. The aqueous phases were used for the estimation of 5-HT, NE and DA levels by fluorospectrophotometry (850 type; HIITACHI Corp. Japan) as reported by Ciarlone (1978).

Assay of AChE activity

For the determination of cerebral AChE activity, three groups of aged rats (n = 10) received EO (0.02, 0.04 or 0.08 g kg^{-1} per day) orally for 30 days before decapitation. Two more groups of animals (n = 10), the normal group (young rats) and control group (aged rats), were given the vehicle orally by intubation and run concurrently with the EO-treated groups. Animals were decapitated and skulls were split on an ice and salt mixture. The cerebra were isolated, weighed and homogenized in phosphate buffer, pH 8.0 (1 mL/40 mg tissue) for 1 min and a 4% homogenate was made. Estimation of the cerebral AChE activity was performed with some modifications to the assay described by Ellman et al (1961) and Ni (1990).

Statistical analysis

The data were analysed using a the SPSS 10.0 statistical package. The data for multiple comparisons were performed by one-way analysis of variance followed by Dunnett's *t*-test. $P < 0.05$ was considered statistically significant and all results are presented as the mean ± s.d.

Results

Effects of EO on learning performances in aged mice in the step-down test

In the control group of aged mice, the latencies were significantly shortened and the number of errors markedly increased compared with the normal group in the step-down test (Table 1). In contrast, in aged mice treated by EO (0.04 and 0.08 g kg^{-1} per day) for 15 days, learning performances were manifestly improved, except in the lower dose group (EO 0.02 g kg^{-1} per day) which was similar to that observed in the control group.

Effects of EO on learning performances in aged rats in the Y-maze test

In the control group of aged rats, the latencies were significantly prolonged and the number of errors markedly increased in contrast with young rats in the Y-maze test (Table 2). However, in EO-treated groups (0.04 and 0.08 g kg^{-1} per day for 30 days), learning performances were manifestly improved, except for the number of errors in the lower dose group of EO (0.02 g kg^{-1} per day) which was not significantly different compared with the control group.

Effects of EO on dysmnesia mice induced by scopolamine

A dose of 1 mg kg^{-1} scopolamine was injected 30 min before the training trial in the induced memory acquisition impairment model, and this impaired the step-down type passive avoidance test performance of mice (Table 3). Mice in the control group displayed poor performances, and the latencies were shortened and the number of errors increased as determined by the step-down test. In contrast, EO (0.02, 0.04 and 0.08 g kg^{-1}) given for 15 days significantly improved the performances of dysmnesia mice.

Effects of EO on dysmnesia rats induced by sodium nitrite

The results presented in Table 4 indicate that sodium nitrite impaired the Y-maze type test performances of rats. EO produced an overall statistically significant and dose-dependent

Table 1 Effects of essential oil from Acori graminei rhizoma on learning performances in aged mice

Group	n	Dose (g kg^{-1} per day)	Latency (s)	Number of errors (time/5 min)
Normal	10	Vehicle	135.30 ± 21.88	2.10 ± 1.73
Control	10	Vehicle	75.10 ± 20.63†††	7.70 ± 4.22†††
Essential oil	10	0.08	112.60 ± 31.52**	3.80 ± 2.35*
Essential oil	10	0.04	104.40 ± 28.22*	4.70 ± 2.11*
Essential oil	10	0.02	96.60 ± 30.11	6.30 ± 3.65

*$P < 0.05$, **$P < 0.01$ significantly different compared with control aged mice. $^{\dagger\dagger\dagger}P < 0.001$ significantly different compared with normal mice. Data are presented as mean ± s.d.

Table 2 Effects of essential oil from Acori graminei rhizoma on learning performances in aged rats

Group	n	Dose (g kg^{-1} per day)	Latency (s)	Number of errors (time/5 min)
Normal	10	Vehicle	13.40 ± 6.02	2.60 ± 2.41
Control	10	Vehicle	40.50 ± 22.09††	6.50 ± 2.27††
Essential oil	10	0.08	17.90 ± 9.15**	3.00 ± 2.45**
Essential oil	10	0.04	17.60 ± 9.57**	3.00 ± 2.11**
Essential oil	10	0.02	21.50 ± 16.29*	5.00 ± 2.00

*$P < 0.05$, **$P < 0.01$ significantly different compared with control aged rats. $^{\dagger\dagger}P < 0.01$ significantly different compared with normal rats. Data are presented as mean ± s.d.

Table 3 Effects of essential oil from Acori graminei rhizoma on dysmnesia in mice induced by scopolamine

Group	n	Dose (g kg^{-1} per day)	Latency (s)	Number of errors (time/5 min)
Normal	10	Vehicle	116.70 ± 59.05	2.20 ± 1.87
Control	10	Vehicle	40.30 ± 27.31††	6.70 ± 3.59††
Essential oil	10	0.08	83.00 ± 47.66*	2.60 ± 1.84**
Essential oil	10	0.04	88.40 ± 57.30*	2.10 ± 2.51**
Essential oil	10	0.02	88.50 ± 48.67*	2.80 ± 1.93**

*$P < 0.05$, **$P < 0.01$ significantly different compared with control mice. $^{\dagger\dagger}P < 0.01$ significantly different compared with normal mice. Data are presented as mean ± s.d.

Table 4 Effects of essential oil from Acori graminei rhizoma on dysmnesia in rats induced by sodium nitrite

Group	n	Dose (g kg^{-1} per day)	Latency (s)	Number of errors (time/5 min)
Normal	10	Vehicle	16.10 ± 7.81	2.40 ± 2.06
Control	10	Vehicle	35.50 ± 19.72††	5.20 ± 1.32††
Essential oil	10	0.08	17.10 ± 10.56*	2.70 ± 1.94**
Essential oil	10	0.04	18.70 ± 10.61*	2.90 ± 2.38*
Essential oil	10	0.02	19.20 ± 8.15*	3.60 ± 1.26*

*$P < 0.05$, **$P < 0.01$ significantly different compared with control rats. $^{\dagger\dagger}P < 0.01$ significantly different compared with normal rats. Data are presented as mean ± s.d.

improvement in the performances of rats at doses of 0.02, 0.04 and 0.08 g kg^{-1}, that is to say, the latencies were shortened and the number of errors markedly decreased compared with the control group.

Effects of EO on dysmnesia mice induced by ethanol

At 30 min before testing trial, mice were injected with 30% alcohol, which evidently impaired the step-down type passive avoidance test performances (Table 5). EO (0.02, 0.04 and 0.08 g kg^{-1}) increased step-down latencies and decreased the number of errors in a dose-dependent manner. The EO-treated groups were significantly different compared with the control group.

Effects of EO on cerebral 5-HT, NE and DA levels in aged rats

In the control group of aged rats, the levels of cerebral NE, DA and 5-HT were significantly decreased compared with those observed in rats in the normal group (Table 6). When

Table 5 Effects of essential oil from Acori graminei rhizoma on dysmnesia in mice induced by ethanol

Group	n	Dose (g kg^{-1} per day)	Latency (s)	Number of errors (time/5 min)
Normal	10	Vehicle	95.40 ± 35.52	3.70 ± 0.82
Control	10	Vehicle	22.40 ± 10.57††	7.80 ± 4.10††
Essential oil	10	0.08	91.40 ± 50.12**	3.60 ± 1.58**
Essential oil	10	0.04	88.80 ± 49.58**	3.80 ± 1.62**
Essential oil	10	0.02	77.70 ± 45.85*	4.30 ± 2.91*

*$P < 0.05$, **$P < 0.01$ significantly different compared with control mice. $^{\dagger\dagger}P < 0.01$ significantly different compared with normal mice. Data are presented as mean ± s.d.

Table 6 Effects of essential oil from Acori graminei rhizoma on cerebral serotonin (5-HT), norepinephrine (NE) and dopamine (DA) levels in aged rats

Group	n	Dose (g kg^{-1} per day^{1})	5-HT (ng g^{-1})	NE (ng g^{-1})	DA (ng g^{-1})
Normal	10	Vehicle	552.80 ± 134.31	476.20 ± 73.87	3655.50 ± 1086.66
Control	10	Vehicle	399.50 ± 151.11†	381.70 ± 34.61††	2430.40 ± 692.58†
Essential oil	10	0.08	658.20 ± 220.43**	441.70 ± 64.79*	4269.90 ± 1586.87**
Essential oil	10	0.04	540.30 ± 214.74	569.90 ± 86.47***	3482.80 ± 926.59*
Essential oil	10	0.02	425.20 ± 153.98	430.10 ± 43.57*	2817.70 ± 1145.74

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ significantly different compared with control aged rats. $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ significantly different compared with normal rats. Data are presented as mean ± s.d.

the aged rats were given EO at a dose of 0.08 g kg^{-1} the levels of NE, DA and 5-HT were similar to those observed in the normal group. At an EO dose of 0.04 g kg^{-1} only NE and DA levels were significantly increased compared with the values observed in the control group. However, at an EO dose of 0.02 g kg^{-1} only NE levels were statistically significant compared with the values observed in the control group. At this dose, although there was some improvement in the levels of DA and 5-HT, the change was not statistically significant.

Effects of EO on AChE activity in cerebra of aged rats

As can be seen in Figure 1, in the control group of aged rats there was a significant increase in the activity of cerebral AChE compared with the values observed in the cerebra of rats in the normal group. This increase in the activity of AChE was significantly reversed when the rats were given EO at doses of 0.02, 0.04 and 0.08 g kg^{-1} for 30 days compared with the control group.

Discussion

Ethology, the study of animal behaviour, including learning and memory, is at present one of the most reliable determinants of animal intelligence. Many nootropic studies have investigated changes in animal behaviour using the step-down, step-through and maze tests, which are often applied for the determination of capabilities of passive avoidance and spatial memory (Guilherme dos Santos et al 2005; Farr et al 2006; Türkmen et al 2006; Um et al 2006). Cognitive deficits are often observed in aged humans, as well as a result of various neurological conditions. The present study assessed the effects of EO from AGR on learning and memory in aged mice and rats using step-down and Y-maze tests. The results demonstrated improvements in learning performances in aged mice receiving EO as evidenced by an increased latency and a decreased number of errors in the step-down test, and in aged rats receiving EO as evidenced by a decreased latency and a decreased number of errors in the Y-maze test.

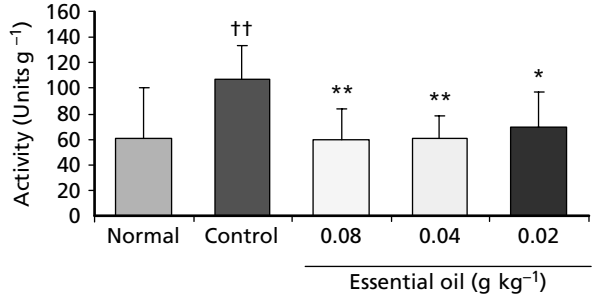

Figure 1 Effects of essential oil from Acori graminei rhizoma on cerebral acetylcholinesterase activity in aged rats. *$P < 0.05$, **$P < 0.01$ significantly different compared with control aged rats. $^{††}P < 0.01$ significantly different compared with normal rats. Values represent the mean ± s.d. (n = 10 animals per group).

Generally, memory as measured by changes in an animal's behaviour some time after learning is considered to be a process that has several stages, including acquisition, consolidation and retrieval (Abel & Lattal 2001). The use of pharmacological, genetic and lesion approaches has helped to define the brain systems and molecular processes important for these different stages of memory. For example, in the experiment containing contextual fear conditioning, memory acquisition occurs as the animal learns an association between a context and a shock. During consolidation, which can last from minutes to days, this memory is moved from a labile to a more fixed state, and during retrieval, the animal is returned to the conditioning context, where memory for the context/shock association is assessed (Abel & Lattal 2001).

Some chemical agents, such as scopolamine, sodium nitrite and ethanol, impair memory in animals trained in step-down type passive avoidance and radial maze type tasks (Lu 2001; Daniel et al 2003; Takahashi et al 2005), which are used to measure the three stages of the memory process depending on drug-treated period. As revealed by many studies on cognitive function, a single intraperitoneal injection of scopolamine hydrobromide (0.5–1 mg kg^{-1}) 10–30 min before training significantly impairs the animal's memory acquisition (Anagnostaras et al 1995; Gibbs et al 1998; Daniel et al 2003). Sodium nitrite when injected immediately after the training trial demolishes memory consolidation, and 10–30% ethanol injected 30 min before the testing trial disturbs memory retrieval (Vikas et al 2000; Luo et al 2003; Hellemans et al 2005). In the present study, mice given scopolamine or 30% ethanol displayed poor performances, the latency of which was shortened and the number of errors increased as determined by the step-down test; administration of sodium nitrite in rats evidently increased the latency and the number of errors. The administration of EO from AGR improved cognitive behaviour in dysmnesia animals to a large degree. EO (0.02, 0.04, and 0.08 g kg^{-1}) showed a dose-dependent effect on acquired learning by mice with scopolamine-induced dysmnesia. EO at the same doses on other memory stages, such as consolidation and retrieval, also revealed a dose-dependent effect. Taking all these observations into account, including latency and error numbers, we propose that EO at a dose of 0.02 g kg^{-1} can overcome amnesia at the three stages of the memory process.

Learning and memory is one of the most important functions of the brain, which is associated with complex neurophysiologic and neurochemical changes. Many neurotransmitters, including acetyl choline (ACh), DA, NE and 5-HT play an important role in learning and memory processes (Blokland 1996; Myhrer 2003). ACh has been related to attentional processes (Himmelheber et al 2000) and plays an important role in cognitive processing. It has been reported that administration of scopolamine or atropine induces memory dysfunction in rats, primates and humans (Blozovski et al 1977; Drachman 1977; Aigner & Mishkin 1986). This drug-induced impairment is subsequently reversed after displacement of the blocking agent (Dawson et al 1992), and by the use of AChE inhibitors. These drugs act by preventing the breakdown of ACh in the synaptic cleft. The administration of physostigmine to both young and aged monkeys produces an overall improvement of mnemonic processes in both groups (Bartus & Uehara

1979). DA has been associated with motivational processes (Wilson et al 1995) and has a special role in appetitively motivated tasks. The use of dopaminergic antagonists, especially those more selective for the D_1 receptor such as SCH2330, worsens performance in the delayed response paradigm (Didriksen 1995). This is consistent with anatomical data indicating that the D_1 receptor is the most abundant dopaminergic receptor in the mammalian brain (Cortes et al 1989; Goldman-Rakic et al 1990), and therefore is the most likely to be involved in the cognitive processes mediated by DA in the brain. Central 5-HT has been linked to emotional processes (Hashimoto et al 1999) and plays a particular role in emotionally related tasks; despite the lack of functional specialization, the serotonergic system plays a significant role in learning and memory (Buhot et al 2000). Administration of $5\text{-HT}_{2A/2C}$ or 5-HT_4 receptor agonists or 5-HT_{1A} or 5-HT_3 and 5-HT_{1B} receptor antagonists prevents memory impairment (Buhot et al 2000) and is used in the treatment of Alzheimer's disease and amnesia (Menses 1998). L-Tryptophan, the precursor of serotonin, is reported to enhance memory functions in schizophrenic and depressed patients (Riedel et al 1999; Levkovitz et al 2003), while NE has been relevant to learning and memory consolidation, possibly by acting as a regulation of signal (Crow 1968; Kety 1970). Cognitive deficits induced by various lesions to the locus ceruleus are reversible by the administration of drugs that enhance noradrenergic neurotransmitters. The administration of diethyldithiocarbamate, an inhibitor of the enzyme dopamine-β-hydroxylase, to rats depletes norepinephrine stores in the brain, and produces complete retention failure of passive avoidance learning (Stein & Wise 1971; Hamburg & Cohen 1973). Subsequently, normal learning of the passive avoidance task is restored in diethyldithiocarbamate-treated rats with a single intraventricular dose of NE (Stein et al 1975).

Ageing is often accompanied by some alterations in the neurotransmitter systems of humans and other mammals (Arranz et al 1996; Magnone et al 2000; De la Fuente et al 2003). Most of the studies on brain physiology in ageing have been performed in rodents and the results do not always show consistent changes in the neurochemical parameters. Some of the discrepancies observed may be due to species or strain differences. Nevertheless, the current work appears to agree with respect to several aspects such as the reductions of the levels of neurotransmitters, including ACh, DA, NE and 5-HT, which have been demonstrated in the ageing brain (Rehman & Masson 2001; De la Fuente et al 2003). Our findings demonstrate that reductions in the levels of DA, NE and 5-HT significantly decreased and AChE activity markedly increased in the ageing brain, which is consistent with earlier reports (Rehman & Masson 2001; De la Fuente et al 2003). However, the most significant result of our study is that the administration of EO caused significant increases in the levels of DA, NE and 5-HT, while significantly decreasing the activity of AChE in the cerebra of aged rats.

Conclusion

The administration of EO significantly enhances learning performances in aged rats and mice. It also ameliorates memory deficits in amnesic rats and mice induced by chemical agents, which are associated with increases of NE, DA and 5-HT levels, and a decrease in the activity of AChE in the cerebra. Since a desirable cognitive effect has been reported for glutamate (Ohno & Watanabe 1996; Myhrer 2003), further studies should be directed towards investigating the effect of EO on glutamatergic neurotransmitters in brain areas implicated in the control of learning and memory processes. In addition, β-asarone and α-asarone are two major components of EO extracted from Acori graminei rhizome, which are able to ameliorate learning and memory; however, their facilitating effects on cognitive function are inferior to EO (Hu et al 1999; Wu & Fang 2004). Since EO is an effective part of Acori graminei rhizoma and contains a mixture of active components, it may be exerting its effects through multiple components. Although this is an animal-based study, it is proposed that EO may be effective in the improvement of brain function in elderly humans.

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