

## **Title**

**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 when a person becomes older, aging in humans is generally associated with deterioration of cognitive performance and, in particular, of learning and memory. These cognitive deficits can cause debilitating consequences due to aging. There are a number of herbal medicines which are reported to improve brain function including intelligence. In the present study, ameliorating effects of essential oil (EO), extracted from *Acori graminei* rhizoma (AGR), on learning and memory in aged, dysmnnesia rats and mice were determined by the use of step-down type passive avoidance test or Y type maze trial. Oral administration of EO (0.02, 0.04, 0.08 g/kg, in rats given for 30d and in mice for 15d) evidently improved the latency and number of errors in aged, dysmnnesia rats and mice. The cerebral neurotransmitters in aged rats given EO (0.02, 0.04, 0.08 g/kg for 30d) were also investigated and, increased levels of norepinephrine (NE), dopamine (DA), serotonin (5-HT) and decreased levels of acetylcholine esterase (AChE) activity were found. These results suggest that EO improves cognitive function of aged animals and may do so by relatively increasing NE, DA, 5-HT levels and by decreasing the activity of AChE in the cerebra.

## **Introduction**

Life expectancy is increasing and 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. Its prevalence is estimated at 1.53% between 65 and 69 years of age and from the age of 60-80 years relative

incidence of senile dementia increases exponentially with age, doubling every five years (Preston, 1986; Brayne et al, 1995). However, between the ages of 90 and 94, the prevalence is 31.48% of the population (Karen et al, 1995) and these increases as high as 44.48% in the age-range 95-99 years (Karen et al, 1995) thus, senile dementia is an aged-related disorder.

There are normal cognitive changes that take place when a person becomes older. Aging in humans is associated with deterioration of cognitive performance and, in particular, of learning and memory (Erickson et al., 2003) and cognitive deficits are the debilitating consequences of aging (Forster et al., 1994). Old, normal aged humans (Uttl et al., 1993; Wilkniss et al., 1997; Newman et al., 2000), old monkeys (Lai et al., 1995; Rapp et al., 1997), old rats (Barnes, 1979; Markowska et al., 1989; Gallagher et al, 1997) and old mice (Bach et al., 1999) all show impairments in learning and memory compared with their younger counterparts. For example, Uttl and Graf (1993) studied the ability of groups of people from 15 to 74 years of age 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, which 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 recognisable 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, as a treatment for alleviating various symptoms of cognitive deficits and facilitating learning and memory. There are a number of herbal medicines which 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), *Acorus gramineus* (Bombi et al., 2003; Yoshifumi et al., 2003; Oh MH et al., 2004), and so on.

*Acori graminei rhizoma* (AGR) has been used as a treatment for illnesses of the brain and for its actions of regaining consciousness, tranquilizing the mind, and promoting intelligence, which, in combination with other herbal drugs, is one of the most prescribed herbal remedies for treating various kinds of neurological disorders such as epilepsy, cerebrovascular diseases, and senile dementia including AD (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 symptoms of these diseases. Among 75 of the most famous Chinese complex prescriptions characterized by promoting intelligence in past dynasties in China, more than half of these prescriptions contain AGR (Liu et al., 2005).

AGR, the rhizome of *Acorus gramineus* Schott. (Araceae), contains up to 4.86% of essential oil (EO) (Li et al., 2005), which is mainly composed of  $\beta$ -asarone (63.2-81.2%) and  $\alpha$ -asarone (8.8-13.7%) (Chang et al., 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 ischemia-induced neuronal loss and learning and memory damage in rats (Bombi et al., 2003). As far as we are aware, no reports have been issued on the facilitating effects of EO from AGR on learning and memory in aged animals until the present time.

In the current experiments, we investigated the effects of EO from AGR on cognitive functions of young, aged animals and chemical-induced dysmnnesia subjects. Learning and memory parameters in these subjects were evaluated using step-down type passive avoidance task and Y type maze trial. Previous studies have shown that aged animals and chemical-induced dysmnnesia subjects perform poorly compared with young in the animal models used (Luo et al., 2003; Adriana et al., 2004). Step-down trial can examine ability of passive avoidance response, while ability of spatial memory in animals can also be determined by the use of Y-maze task. 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 monoaminergic neurotransmitters (NE, DA and 5-HT), together with AChE activity in cerebra of aged rats, all of which are known to be important to 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, a pharmacognosist, from The Pharmacy Faculty of Chengdu University of Traditional Chinese Medicine (Chengdu, China). The EO was extracted by the use of hydrodistillation. The dried rhizomes were broken into small segments, 10kg of which were immersed in 50L distilled water, and boiled in a distillation apparatus for 10h. The yield of EO was 3.04% (v/w) and it was stored at +4°C until utilized.

### **GC–MS analysis**

GC–MS was performed with an Agilent 6890 gas chromatography instrument coupled to an Agilent 5973 mass spectrometer and an Agilent ChemStation software (Agilent Technologies, Palo Alto, CA, USA). Compounds were separated on a 30m×0.32mm i.d. capillary column coated with 0.25 µm film 5% phenyl methyl siloxane. The column temperature program began at 60°C and was held for 1 min, then increased at a rate of 3°C min<sup>-1</sup> to 220 °C and held for 5 min. Split injection (2µl) was conducted with a split ratio of 1:30 and helium was used as carrier gas at a flow rate of 1.3 ml min<sup>-1</sup>. The spectrometers were operated in electron-impact (EI) mode and the ionization energy was 70 eV. The inlet, ionization source temperatures were 250°C and 280°C, respectively. The GC-MS analysis showed that the main components of 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 prior to administration. Three groups of animals (n=10) were orally administered 0.02, 0.04, 0.08g/kg.d<sup>-1</sup> of EO by intubation respectively, mice were administered for 15 days whilst the rats were

administered for 30 days. Two more groups of animals (normal and control groups) were orally administered 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 body weight irrespective of dose.

### **Animals and grouping**

Animals, obtained from Experimental Animal Center of Chengdu University of Traditional Chinese Medicine (Chengdu, China), were housed in a regulated environment ( $20\pm1^{\circ}\text{C}$ ), with a 12-h light and 12-h dark cycle (08:00-20:00, light) and were grouped as follows: Young and aged male Kunming mice, (30 days of age, 18-22g; 12-13 months of age, 40-50g, respectively), Grade II, Certificate No 2000-7. Young and aged male Sprague–Dawley rats, (90 days of age, 180-220g; 24-25 months of age, 550-650g, respectively), Grade II, Certificate No 2000-8. Food and water were given ad libitum, except for the duration of the experimental session. On the day of the experiment, animals were brought to the experimental room and allowed to habituate to the environmental conditions for a period of approximately 60 min before the beginning of the experiment. All animal treatments were strictly in accordance with international ethical guidelines and National Institutes of Health Guide concerning the Care and Use of Laboratory Animals and the experiments were carried out under 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, forty aged mice were divided into four groups ( $n=10$ ) ad libitum, namely, three EO-treated groups (0.02, 0.04, 0.08g/kg.d<sup>-1</sup>) and one control group. Another ten young mice served as normal group. In the experiment on the effects of EO on learning performances in

aged rats, forty aged rats were divided into four groups (n=10) ad libitum, viz. three EO-treated groups (0.02, 0.04, 0.08g/kg.d<sup>-1</sup>) and one control group and another ten 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 subjects were divided into four groups (n=10) ad libitum, viz. three EO-treated groups (0.02, 0.04, 0.08g/kg.d<sup>-1</sup>) and one control group. Another ten young subjects served as normal group. In the experiment on the effects of EO on cerebral 5-HT, NE, and DA levels in aged rats, forty aged rats were divided into four groups (n=10) ad libitum, viz. three EO-treated groups (0.02, 0.04, 0.08g/kg.d<sup>-1</sup>) and one control group. Another ten young rats served as normal group. In the experiment on the effects of EO on cerebral AChE activity in aged rats, grouping was the same as outlined above.

### **Chemicals and modeling**

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 body weight irrespective of dose. Control and normal animals received respective solvent injections, and they were run concurrently with drug-treated groups.

In experiments on the effects of EO on dysmnesia animal models, Scopolamine (1 mg/kg, i.p.) was administered 30 min before the training trial and induced memory acquisition impairment of mouse; sodium nitrite (120mg/kg, i.p.) was injected immediately after the training trial and induced memory consolidation impairment of rat;



and finally 30% of alcohol (1.5g/kg, i.p.) was injected thirty minutes before testing trial and induced memory retrieval impairment of mice.

## **Behavioral testing**

### **Step-down test**

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

### **Y-maze test**

The Y-maze was used for behavioral 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 (60l×16w×32h cm) made of dark opaque Plexiglas and these three arms were symmetrically disposed at 120° to each other. Arms I and III were non-safety zone (shocks were administered via these) and arm II was a safety zone (on the top of which there was an insulated grid floor of 16×25cm). Rats were placed on the top of arm I and a fixed resistance shock source was connected to an automatically operated switch and electric shocks (50V) were

applied. After shocking, rats escaped from foot shocks by accidentally entering the top of arm II and this was counted as one practice and the rats were repeatedly trained for this procedure a further 10 times. After a 24h interval the rats were successively tested for 10 times and their latency to enter safety zone (i.e. insulated grid floor) from 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, 5-HT levels**

For determination of cerebral levels of 5-HT, NE, and DA, three groups of aged rats (n=10) received 0.02, 0.04 or 0.08 g/kg.d<sup>-1</sup> po of EO for 30d before decapitation. Two more groups of animals (n=10), serving as normal group (young rats) and control group (aged rats), were given orally vehicle by intubation respectively, and run concurrently with EO-treated groups. Animals were decapitated and skulls were split on ice and salt mixture and the cerebra were isolated, weighed, and homogenized in ice-cold *n*-butanol solution (5 ml/g tissue) according to Miller et al. (1970) and Biochemistry group of acupuncture and Meridian Research Institute of TCM academy (1975). Homogenization was performed using ice-cold homogenizer for 1 min and a 20% homogenate was made which was then centrifuged at 3000 rpm for 5 min. Supernatant (2.5ml) 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 rpm for 5 min. The aqueous phases were used for the estimation of 5-HT, NE, and DA levels employing the fluorospectrophotometry (850 type, HIITACHI Corp. Japan) reported by Ciarlone (1978).

### **Assay of AChE activity**

For the determination of cerebral AChE activity, three groups of aged rats (n=10) received 0.02, 0.04 or 0.08 g/kg.d<sup>-1</sup> po of EO for 30d before decapitation. Two more groups of animals (n=10), serving as normal group (young rats) and control group (aged rats), were given orally vehicle by intubation respectively, and run concurrently with EO-treated groups. Animals were decapitated and skulls were split on ice and salt mixture and 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 Zhengxin Ni (1990).

### **Statistical Analysis**

The data were analyzed using a statistical package (SPSS10.0). The data for multiple comparisons were performed by one-way ANOVA followed by Dunnett's *t*-test. *P*<0.05 was considered statistically significant and all results are presented as the mean±SD.

## **Results**

### **Effects of EO on learning performances in aged mice (Table 1)**

In the control group containing aged mice, the latencies significantly shortened and the number of errors markedly increased compared to the normal group by the step-down test. In contrast, in aged mice treated by EO (0.04, 0.08g/kg.d<sup>-1</sup>) for 15 days, learning performances were manifestly improved, except at the lower dose group of EO (0.02g/kg.d<sup>-1</sup>), which was similar to that observed in the control group.

### **Effects of EO on learning performances in aged rats (Table 2)**

In aged rats of control group, the latencies were significantly prolonged and the number

of errors markedly increased in contrast to young rats by the Y-maze test. However, in EO-treated groups (0.04, 0.08g/kg.d<sup>-1</sup> still for 30 days), learning performances were manifestly improved, except for the number of errors at the lower dose group of EO (0.02g/kg.d<sup>-1</sup>), which had no statistical significance compared with control group.

#### **Effects of EO on dysmnesia mice induced by scopolamine (Table 3)**

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

#### **Effects of EO on dysmnesia rats induced by sodium nitrite (Table 4)**

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 improvement in performances of rats at doses of 0.02, 0.04, 0.08g/kg, that is to say, the latencies evidently shortened and the number of errors markedly decreased compared to the control group.

#### **Effects of EO on dysmnesia mice induced by ethanol (Table 5)**

Thirty minutes before testing trial, mice were intraperitoneally injected with 30% alcohol, which evidently impaired the step-down type passive avoidance test performances as indicated in Table 5. EO (0.02, 0.04, 0.08g/kg) increased step-down latencies and decreased the number of errors in dose-dependent manner, the EO-treated groups differed significantly from the control group.

### **Effects of EO on cerebral 5-HT, NE, and DA levels in aged rats (Table 6)**

As can be seen in Table 6 in the control group consisting of aged rats the levels of cerebral NE, DA, and 5-HT were significantly decreased compared to those observed in the rats in the normal group. When the aged rats were given EO at a dosage of 0.08 g/kg the levels of NE, DA and 5HT were similar to those observed in the normal group, this difference being statistically significant. At an EO dosage of 0.04g/kg only NE and DA levels were significantly increased when compared to the values observed in the control group. However, at an EO dosage of 0.02g/kg only NE levels were statistically significant when compared to the values observed in the control group. At this dosage although there was some improvement in the levels of DA and 5HT, this change was not statistically significant.

### **Effects of EO on AChE activity in cerebra of aged rats (Figure 1)**

As can be seen in Fig.1, in the control group consisting of aged rats there was a significant increase in the activity of cerebral AChE when compared to the values observed in the cerebra of the rats in the normal group. This increase in the activity of AChE was significantly reversed when the rats were given EO at dosages of 0.02, 0.04, 0.08 g/kg 30 days when compared to the control group.

## **Discussion**

Ethologic investigation of learning and memory is at present one of the most reliable targets reflecting levels of animal intelligence. Many nootropic studies investigate animal's behavior changes using step-down, step-through, maze test, etc., which are often applied for the determination of capabilities of passive avoidance and spatial

memory in animals (Jair 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 in 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 test. The results demonstrated improvements in learning performances in aged mice receiving EO by an increased latency and a decreased number of errors in the step-down test, and in aged rats receiving EO by a decreased latency and a decreased number of errors in the Y-maze test.

Generally, memory as measured by changes in an animal's behavior some time after learning, is considered to be a process that has several stages, including acquisition, consolidation and retrieval (Abel and 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 and Lattal, 2001).

Some chemical agents, such as scopolamine, sodium nitrite and ethanol, impair memory in animals trained on a step-down type passive avoidance and a radial maze type task (Lu, 2001; Jill et al., 2003; Reinaldo et al., 2005), which are used to measure the three stages of memory process depending on drug-treated period. As revealed by many studies on cognitive function, a single intraperitoneal injection of scopolamine

hydrobromide (0.5-1mg/kg) 10-30 min prior to training significantly impairs the ability for animal's memory acquisition (Anagnostaras et al., 1995; Gibbs et al., 1998; Jill et al., 2003). Sodium nitrite when injected immediately after training trial demolishes memory consolidation, and 10-30% ethanol injected 30 min before testing trial disturbs memory retrieval (Vikas et al., 2000; Luo et al., 2003; Kim et al., 2005). In the present study, mice given scopolamine or 30% ethanol, displayed poor performances, whose latency shortened and the number of errors were 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 behavior in dysmnesia animals to a great degree. EO (0.02, 0.04, and 0.08 g/kg) showed a dose-dependent effect on acquired learning of mice with scopolamine-induced dysmnesia. The observed results of EO at the same doses on other memory stages also revealed a dose-dependent effect, such as consolidation and retrieval. Taking all these observations into account including latency and error numbers, we proposed that EO at the dose of 0.02 g/kg 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 acetylcholine (ACh), dopamine (DA), norepinephrine (NE), and, serotonin (5-HT) play an important role in the learning and memory processes (Arjan, 1996; Trond, 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 and 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 and 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<sub>1</sub> receptor such as SCH2330, worsens performance in the delayed response paradigm (Didriksen, 1995). This is consistent with anatomical data indicating that the D<sub>1</sub> 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 dopamine in the brain. Central serotonin (5-HT) has been linked to emotional processes (Hashimoto et al., 1999) and plays a particular role in emotionally related tasks and despite the lack of functional specialisation, the serotonergic system plays a significant role in learning and memory (Buhot et al., 2000). Administration of 5-HT<sub>2A/2C</sub> or 5-HT<sub>4</sub> receptor agonists or 5-HT<sub>1A</sub> or 5-HT<sub>3</sub> and 5-HT<sub>1B</sub> receptor antagonists prevents memory impairment (Buhot et al., 2000) and has utility in 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) whilst 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 neurotransmitter. The administration of diethyldithiocarbamate, an inhibitor of the enzyme dopamine-beta-hydroxylase, to rats, depletes norepinephrine stores in the brain, and produces complete retention failure of passive avoidance learning (Stein et al., 1971; Hamburg et al., 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).

Aging is often accompanied by some alterations in the neurotransmitter systems of humans and other mammals (Arranz et al., 1996; Magnone et al., 2000; Monica et al., 2003). Most of the studies on brain physiology in aging 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 published work appears to agree with respect to several aspects such as the reductions of the levels of neurotransmitters, including ACh, DA, NE, 5-HT which have been demonstrated in the aging brain (Habib et al., 2001; Monica et al., 2003). Our findings demonstrate that reductions in the levels of DA, NE, 5-HT significantly decreased and AchE activity markedly increased in aging brain which is consisted with earlier reports (Habib et al., 2001; Monica 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, 5-HT whilst significantly decreasing the activity of AChE in the cerebra of aged rats.

## **Conclusion**

In summary, 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, 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 and Watanabe, 1996; Trond, 2003), further studies should be directed towards investigating the effect of EO on glutamatergic neurotransmitter in brain areas implicated in the control of learning and memory processes. In addition, Beta-asarone and alpha-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 et al., 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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## Tables

Table 1 Effects of EO on learning performances in aged mice

Group	N	Dose (g/kg.d <sup>-1</sup> )	Latency (second)	Number of errors (time/5min)
Normal	10	vehicle	135.30±21.88	2.10±1.73
Control	10	vehicle	75.10±20.63 <sup>△△</sup>	7.70±4.22 <sup>△△</sup>
Essential oil	10	0.08	112.60±31.52 <sup>**</sup>	3.80±2.35 <sup>*</sup>
Essential oil	10	0.04	104.40±28.22 <sup>*</sup>	4.70±2.11 <sup>*</sup>
Essential oil	10	0.02	96.60±30.11	6.30±3.65

<sup>△△△</sup>*P* < 0.001 compared with normal mice; <sup>\*</sup>*P* < 0.05, <sup>\*\*</sup>*P* < 0.01 compared with control aged mice. Data are presented as mean ± SD.

Table 2 Effects of EO on learning performances in aged rats

Group	N	Dose (g/kg.d <sup>-1</sup> )	Latency (second)	Number of errors (time/10 times)
Normal	10	vehicle	13.40±6.02	2.60±2.41
Control	10	vehicle	40.50±22.09 <sup>△△</sup>	6.50±2.27 <sup>△△</sup>
Essential oil	10	0.08	17.90±9.15 <sup>**</sup>	3.00±2.45 <sup>**</sup>
Essential oil	10	0.04	17.60±9.57 <sup>**</sup>	3.00±2.11 <sup>**</sup>
Essential oil	10	0.02	21.50±16.29 <sup>*</sup>	5.00±2.00

<sup>△△</sup>*P* < 0.01 compared with normal rats; <sup>\*</sup>*P* < 0.05, <sup>\*\*</sup>*P* < 0.01 compared with control aged rats. Data are presented as mean ± SD.

Table 3 Effects of EO on dysmnesia mice induced by scopolamine

Group	N	Dose (g/kg.d <sup>-1</sup> )	Latency (second)	Number of errors (time/5min)
Normal	10	vehicle	116.70±59.05	2.20±1.87
Control	10	vehicle	40.30±27.31 <sup>△△</sup>	6.70±3.59 <sup>△△</sup>
Essential oil	10	0.08	83.00±47.66 <sup>*</sup>	2.60±1.84 <sup>**</sup>
Essential oil	10	0.04	88.40±57.30 <sup>*</sup>	2.10±2.51 <sup>**</sup>
Essential oil	10	0.02	88.50±48.67 <sup>*</sup>	2.80±1.93 <sup>**</sup>

<sup>△△</sup>*P* < 0.01 compared with normal mice; <sup>\*</sup>*P* < 0.05, <sup>\*\*</sup>*P* < 0.01 compared with control mice. Data are presented as mean ± SD.

Table 4 Effects of EO on dysmnesia rats induced by sodium nitrite

Group	N	Dose (g/kg.d <sup>-1</sup> )	Latency (second)	Number of errors (time/10 times)
Normal	10	vehicle	16.10±7.81	2.40±2.06
Control	10	vehicle	35.50±19.72 <sup>△△</sup>	5.20±1.32 <sup>△△</sup>
Essential oil	10	0.08	17.10±10.56 <sup>*</sup>	2.70±1.94 <sup>**</sup>
Essential oil	10	0.04	18.70±10.61 <sup>*</sup>	2.90±2.38 <sup>*</sup>
Essential oil	10	0.02	19.20±8.15 <sup>*</sup>	3.60±1.26 <sup>*</sup>

<sup>△△</sup>*P* < 0.01 compared with normal rats; <sup>\*</sup>*P* < 0.05, <sup>\*\*</sup>*P* < 0.01 compared with control rats. Data are presented as mean ± SD.

Table 5 Effects of EO on dysmnnesia mice induced by ethanol

Group	N	Dose (g/kg.d <sup>-1</sup> )	Latency (second)	Number of errors (time/5min)
Normal	10	vehicle	95.40±35.52	3.70±0.82
Control	10	vehicle	22.40±10.57 <sup>△△</sup>	7.80±4.10 <sup>△△</sup>
Essential oil	10	0.08	91.40±50.12 <sup>**</sup>	3.60±1.58 <sup>**</sup>
Essential oil	10	0.04	88.80±49.58 <sup>**</sup>	3.80±1.62 <sup>**</sup>
Essential oil	10	0.02	77.70±45.85 <sup>*</sup>	4.30±2.91 <sup>*</sup>

<sup>△△</sup> $P < 0.01$  compared with normal mice; <sup>\*</sup> $P < 0.05$ , <sup>\*\*</sup> $P < 0.01$  compared with control mice. Data are presented as mean ± SD.

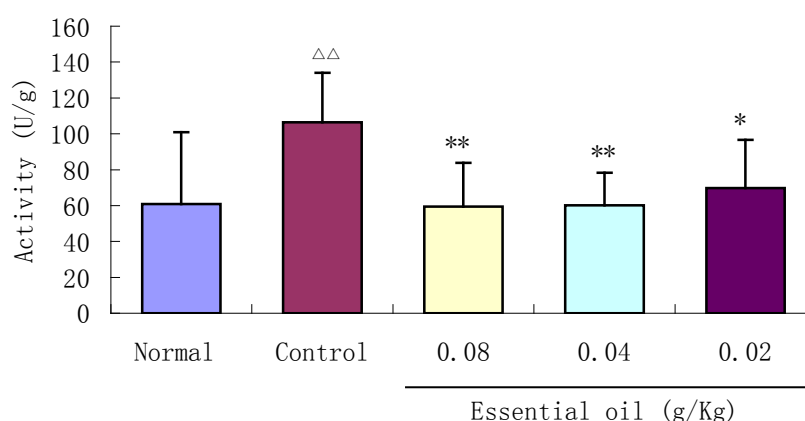
Table 6 Effects of EO on cerebral 5-HT, NE, and DA levels in aged rats

Group	N	Dose (g/kg.d <sup>-1</sup> )	5-HT (ng/g)	NE (ng/g)	DA (ng/g)
Normal	10	vehicle	552.80±134.31	476.20±73.87	3655.50±1086.66
Control	10	vehicle	399.50±151.11 <sup>△</sup>	381.70±34.61 <sup>△△</sup>	2430.40±692.58 <sup>△</sup>
Essential oil	10	0.08	658.20±220.43 <sup>**</sup>	441.70±64.79 <sup>*</sup>	4269.90±1586.87 <sup>**</sup>
Essential oil	10	0.04	540.30±214.74	569.90±86.47 <sup>***</sup>	3482.80±926.59 <sup>*</sup>
Essential oil	10	0.02	425.20±153.98	430.10±43.57 <sup>*</sup>	2817.70±1145.74

<sup>△</sup> $P < 0.05$ , <sup>△△</sup> $P < 0.01$  compared with normal rats; <sup>\*</sup> $P < 0.05$ , <sup>\*\*</sup> $P < 0.01$ , <sup>\*\*\*</sup> $P < 0.001$  compared with control aged rats. Data are presented as mean ± SD.

## Figure

Fig. 1 Effects of EO on cerebral AChE activity in aged rats



<sup>△△</sup> $P < 0.01$  compared with normal rats; <sup>\*</sup> $P < 0.05$ , <sup>\*\*</sup> $P < 0.01$  compared with control aged rats. Ten animals were used per group. The values represent the mean ± SD.