Improvement of Depression-Like Behavior and Memory Impairment with the Ethanol Extract of *Pleurotus eryngii* in Ovariectomized Rats

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Received August 19, 2013; accepted September 17, 2013

Ethanol extract of *Pleurotus eryngii* (DC.) Quél. has estrogen-like activities that protect against bone loss caused by estrogen deficiency. In the present study, we investigated the effect of *P. eryngii* on depression-like behavior and memory impairment in ovariectomized (OVX) rats. Immobility time during a forced swimming test was significantly longer for OVX rats than for sham-operated rats. The depression-like behavior in OVX rats was improved by long-term administration of the ethanol extract of *P. eryngii* (500 mg/kg body weight (b.w.)/d). Spatial memory impairment in OVX rats assessed by the Morris water maze test was also improved by *P. eryngii* extract without any effect on motility. These results suggested that *P. eryngii* extract has estrogen-like improvement activity against depression-like behavior and memory impairment in OVX rats. Additionally, increase in the amount of synaptosomal zinc after ovariectomy was inhibited by *P. eryngii* extract. Since zinc in synaptic vesicles is important for memory function and is linked to the pathophysiology of depression, normalization of zinc signaling would be involved in the beneficial effect of *P. eryngii* extract on neurological disorders after ovariectomy.

**Key words** *Pleurotus eryngii*; synaptosomal zinc; depression; memory impairment; ovariectomized rat; post-menopausal syndrome

Women who are in a state of estrogen deficiency are at risk for neurological symptoms such as depression and memory impairment. The risk for depression is twice higher in women than men and increases with menopause. Estrogen can be used as an adjunct therapy for patients with depression who do not respond to conventional antidepressant treatment.

Also, episodic memory after surgical menopause is improved by prompt estrogen administration, although natural menopause is not associated with substantial cognitive change.

Estrogen or estrogen-progestin replacement therapy, however, increase the risk of breast and endometrial cancers with long-term usage. A clinical trial, HABITS: hormonal replacement therapy after breast cancer—is it safe?, was terminated because the risk of breast cancer in women who had breast cancer previously reached an unacceptably high level with hormone replacement therapy (HRT). In the 2002 Women’s Health Initiative, health risks exceeded benefits from HRT in healthy postmenopausal women, resulting in termination of the trial. Considering the negative aspect of HRT, it would be helpful to find alternative and naturally occurring substances that minimize postmenopausal syndrome.

Recently, mushrooms are gaining importance as functional food and therapeutics in maintaining brain health. Among the mushrooms, *Pleurotus giganteus* was reported to have neurite outgrowth effects.

Also known as king oyster mushroom, is gaining popularity as a culinary mushroom and is commercially grown in several countries including Japan. *P. eryngii* produced several biological active compounds such as β-1,3-glucans, lovastatin, pleu- reryn, eryngin, ribonuclease, 17β-estradiol, eryngeolysin, ergothionine and protein xb68AB. These compounds from *P. eryngii* have been reported to show various medicinal effects including immune modulation, anti-tumor, anti-infection and anti-osteoporosis effects. Shimizu et al. reported that the ethanol extract of *P. eryngii* contains estrogen-like compounds that protect against bone loss caused by estrogen deficiency.

In the present study, we investigated the effect of estrogen-like compounds contained in *P. eryngii* on depression-like behavior and memory impairment in ovariectomized (OVX) rats.

Blood zinc level is affected by ovarian steroid deprivation and menopausal hormone therapy. In the brain, approximately 20% of total zinc exists in presynaptic vesicles. Synaptic zinc is an important modulator of neurotransmission, synaptic plasticity and recognition memory and is involved in the pathogenesis of depression, Alzheimer’s disease and excitotoxic neuron injury. Synaptosomal zinc level is affected by both endogenous and exogenous estradiol. Therefore, we also investigated the effect of *P. eryngii* extract on the change in synaptosomal zinc level after ovariectomy.

**MATERIALS AND METHODS**

Ethanol Extractions of *P. eryngii* Freeze-dried of *P. eryngii* (1.4 kg, Hokuto PLE No. 2, Hokuto, Nagano, Japan) was milled and extracted with ethanol (8 L, Wako Pure Chem-

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ical Industries, Ltd., Osaka, Japan) at 40°C for 24 h. After filtration through a filter paper (No. 2, Advantec, Tokyo, Japan), the residue was extracted with ethanol again in the same manner. The extracts were concentrated with an evaporator and then freeze-dried. The freeze-dried extract was dissolved in water (50 mg/mL) and stored at −20°C.

**Experimental Animals** Female Wistar rats were purchased from Japan SLC (Hamamatsu, Japan). Rats had ad libitum access to standard chow and tap water and were kept under a controlled 12-h light/dark cycle in a temperature- and humidity-controlled room (23 ± 1°C, humidity of 55 ± 5%). All experiments were performed in accordance with the Japanese Pharmacological Society guide for the care and use of laboratory animals, and the protocols were pre-approved by the Animal Ethical Committee of the University of Shizuoka.

**Ovariectomy and Administration** Bilateral ovariectomies were carried out under chloral hydrate anesthesia (400 mg/kg body weight (b.w.), intraperitoneally (i.p.)) in 10-week-old rats. Similar operations were carried out for the sham-operated rats except for removal of ovaries. After 2 d, OVX rats were administered *P. eryngii* extract (500 mg/kg b.w./d, per os (p.o.)) or tap water (10 mL/kg b.w./d, p.o., OVX), and sham-operated rats were administered tap water (10 mL/kg/d, p.o., Sham) every day for 82 d. Body weights were measured every week.

**Forced Swim Test** The forced swim test was performed in an acrylic cylinder (20 cm in diameter, 50 cm in height) filled to a height of 22 cm with water maintained at (22 ± 2°C). Rats were individually placed in the cylinder for 6 min. Behavior during the test was recorded with a video camera to measure immobility time accurately. A rat was judged to be immobile when it remained floating passively in the water.

**Morris Water Maze Test** A pool of 130 cm in diameter was filled with water (22 ± 2°C), and a transparent platform was submerged in the pool. Rats were released into the water and trained to find the platform within a maximum time of 40 s. The trials were repeated 8 times in one day for 3 d. Releasing location was changed randomly in each trial. The last 8 trials were analyzed as the test session.

**Motility Measurement** The pool was divided into four quadrants virtually, and total entry times into each quadrant zone were measured during the first 4 trials of the Morris water maze test. Motility was assessed as entry times/latency time (s).

**Synaptosome Preparation** The right hemisphere in 5 mL of 0.32 M sucrose was homogenized with 12 strokes of a Potter–Elvehjem tissue grinder at 800 rpm. After centrifugation at 1000 g for 10 min, the supernatant was centrifuged again. The supernatant was layered onto 4 mL of 1.2 M sucrose and centrifuged at 160000 g for 15 min (himac CP65 β, Hitachi Koki, Tokyo, Japan). The synaptosome-containing band at the interface between the 1.2 M and 0.32 M sucrose layers was collected, diluted with 4 mL of 0.32 M sucrose, and then layered onto 4 mL of 0.8 M sucrose. After centrifugation at 160000 g for 15 min, the pellet was collected as synaptosomes.

**Zinc Measurement** The synaptosomes were diluted with 100 μL of 1% nitric acid and then centrifuged at 160000 g for 3 min. Zinc concentration in the supernatant was measured using the colorimetric method with 5-Br-PAPS (zinc assay kit LS-MPR, Metallogenics, Chiba, Japan) and a microplate reader (Infinite M200, Tecan, Männedorf, Switzerland) with absorbance at 560 nm.

**Statistical Analysis** Student’s *t*-test was used for comparison of the means of unpaired data. For multiple comparisons, ANOVA followed by Newman–Keuls multiple comparison test was performed.

**RESULTS** At 16 d after ovariectomies, the body weight of OVX rats (187.1 ± 3.0 g) was significantly greater than that of sham-operated rats (177.0 ± 1.6 g, *p* < 0.01, Student’s *t*-test). At 79 d after ovariectomies, OVX rats continued to show significantly greater body weight than that of sham-operated rats. On the other hand, long-term administration of the ethanol extract of *P. eryngii* did not affect the body weight of OVX rats (Fig. 1).

The effect of *P. eryngii* extract on depression-like behavior in OVX rats was investigated by the forced swim test. Immobility time was significantly longer for OVX rats than for sham-operated rats at 79 d after surgery. When the OVX rats were administrated *P. eryngii* extract for 77 d, immobility time of the OVX rats was significantly shortened to the levels of sham-operated rats (Fig. 2).

We also investigated the effect of *P. eryngii* extract on memory impairment in OVX rats by the Morris water maze test. Latency time until arriving at the hidden platform was significantly increased for OVX rats compared to that for sham-operated rats at 65–67 d after surgery. This memory impairment in OVX rats was improved by the administration of *P. eryngii* extract (Fig. 3A). Motility during the training session did not show a significant difference within each group (Fig. 3B).

Since estrogen influences zinc transporter expression and zinc level in synaptic vesicles, we studied the effect of *P. eryngii* extract on synaptosomal zinc level of OVX rats. Zinc level in synaptosomal fractions prepared from OVX rats was significantly higher than that in fractions from sham-operated rats. The increased synaptosomal zinc level in OVX rats was significantly decreased by administration of *P. eryngii* extract (Fig. 4).

**DISCUSSION**

Estrogen deficiency increases the risk for metabolic syndrome and osteoporosis as well as depression and memory impairment.3,19,20) The importance of estrogen levels for leading to these symptoms is supported by the OVX rodent, which is in a state of estrogen deprivation. OVX rodents show body weight gain, excessive feeding21–24) and osteoporosis25) following ovariectomy, which are attenuated by administration of estradiol. OVX rats also show depression-like behavior as assessed by the forced swim test21,26,27) or the tail suspension test.28) The depression-like behavior of OVX rats is improved by administration of estradiol29–32) or phytoestrogen,33) the effect of which is probably mediated by monoaminergic systems.34–38) Additionally, OVX rats display impairment of spatial memory and decrease of spine density in the hippocampus and prefrontal cortex.39–41) Administration of estradiol improves spatial memory deficits in OVX rats by increase in spine density and alteration of monoaminergic activity.42,43)

Our data indicate that body weight of OVX rats was signifi-
Significantly increased following bilateral ovariectomy. Additionally, immobility time during the forced swim test and latency time to find the hidden platform during the Morris water maze were significantly increased in OVX rats compared to those in sham-operated rats. These results indicate that OVX rats at 9–12 weeks after ovariectomy are useful as a model for post-menopausal depression and memory impairment.

Two different types of estrogen receptor (ER), ERα and ERβ, have been identified. The beneficial effect of estradiol on excessive feeding and body weight gain in OVX mice is not shown in ERα knockout mice. ERα is necessary for normal food intake and body weight, while ERβ is not. On the other hand, The depression-like behavior of OVX rats was improved by the ERβ-selective agonist diarylpropionitrile, but it was not improved by estradiol in ERβ knockout mice and it was aggravated by infusion of antisense oligodeoxynucleotides for ERβ in OVX rats. Recognition memory assessed by object recognition and placement memory tasks is enhanced by administration of estradiol or an ERβ-selective agonist, but not by administration of an ERα-selective agonist, in OVX rats. Therefore, the beneficial effect of estradiol on depression-like behavior and spatial memory deficits after ovariectomy is mediated by activation of ERβ rather than by activation of ERα.

We examined the effect of the ethanol extract of *P. eryngii* on postmenopausal depression and memory impairment. The depression-like behavior and memory impairment in OVX rats were improved by long-term administration of *P. eryngii* extract.

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Fig. 1. Effect of *P. eryngii* Extract on Body Weight of OVX Rats

A, Time table for the experiment. Ovariectomies were carried out at day 0 in 10-week-old rats. *P. eryngii* extract was administered from day 2 for 82d. B, Changes in body weights of sham-operated (Sham), OVX and *P. eryngii* extract-administered OVX (OVX+*P. ery.*) rats are shown. C, Body weights at day 79 are shown. Each value is the mean±S.E.M. (Sham, n=10; OVX, n=10; OVX+*P. ery.*, n=8). Asterisks indicate significant differences (*p<0.05, ANOVA followed by Newman–Keuls multiple comparison test).

Fig. 2. Improvement of Depression-Like Behavior in OVX Rats by Long-Term Administration of *P. eryngii* Extract

A, Accumulated immobility time during the forced-swim test was measured in sham-operated, OVX and *P. eryngii* extract-administered OVX rats at day 79 after ovariectomy. B, Immobility time for 6min is shown. Each value is the mean±S.E.M. (Sham, n=10; OVX, n=10; OVX+*P. ery.*, n=8). Asterisks indicate significant differences (*p<0.05, ANOVA followed by Newman–Keuls multiple comparison test).
The zinc level in a mammalian body is influenced by estrogen. After ovariectomy, the high concentration of synaptosomal zinc in the OVX mouse is lowered by administration of estrogen. We examined the effect of *P. eryngii* extract on the synaptosomal zinc level in OVX rats, and we found that the increment of synaptosomal zinc level in OVX rats was decreased to the level in sham-operated rats by administration of *P. eryngii* extract. This result suggested that *P. eryngii* extract enables the synaptic vesicular zinc level in OVX rats to recover.

In conclusion, the ethanol extract of *P. eryngii* has an estrogen-like beneficial effect on depression and memory impairment in OVX rats. Normalization of zinc signaling is thought to be involved in this effect of *P. eryngii* extract on neurological disorders after ovariectomy.

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