Ameliorating Effect of the Edible Mushroom *Hericium erinaceus* on Depressive-Like Behavior in Ovariectomized Rats

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Estrogen deficiency during menopause causes a variety of neurological symptoms, including depression. The edible Lion’s Mane mushroom, *Hericium erinaceus* (Bull.: Fr.) Pers. (HE), is a medicinal mushroom that has the potential for a neuroprotective effect and ameliorating neurological diseases, such as depression, anxiety, and neurodegenerative diseases. HE contains phytoestrogens, including daidzein and genistein. However, the ameliorating effect of HE on menopausal symptoms is not well understood. Here we investigated the impact of methanol extract of the HE fruiting body on depressive-like behavior in postmenopausal model rats. The activation of estrogen receptor alpha (ERα) causes body weight loss and uterine weight gain. Body weight gain and uterine weight loss by estrogen deficiency in ovariectomized (OVX) rats were reversed with 17β-estradiol (E2) but not with HE. Thus, the phytoestrogens in HE may hardly activate ERα. Estrogen receptor beta (ERβ) is expressed in the brain, and activation of ERβ ameliorates menopausal depressive symptoms. Notably, depressive-like behavior in OVX rats evaluated in forced swim test was reduced by administration of not only E2 but also HE for 92 d. Long-term activation of ERα increases the risk of breast and uterine cancers. HE, therefore, may be effective in treating menopausal depression without the risk of carcinogenesis caused by ERα activation.

**Key words** *Hericium erinaceus*; depression; menopause; phytoestrogen; ovariectomy; rat

INTRODUCTION

During menopause, women experience a decrease in estrogen levels and various symptoms that negatively impact a woman’s QOL. Menopausal symptoms include depression as well as hot flashes and osteoporosis. The risk of developing depression increases by approximately 2.5 times with a woman’s QOL. Menopausal symptoms include depression and various symptoms that negatively impact a woman’s QOL. Menopausal symptoms include depression as well as hot flashes and osteoporosis. The risk of developing depression increases by approximately 2.5 times with a woman’s QOL. Hormone replacement therapy (HRT) is used to treat menopausal symptoms, particularly effectively treating autonomic imbalances such as hot flashes, sweating, and tachycardia. For menopausal depression, antidepressants are generally the first choice for treatment. HRT may also be used to treat menopausal depression, although some reports indicate that its effectiveness is limited. In cases where selective serotonin reuptake inhibitors (SSRIs) are used for depression in women over 50 years of age, the therapeutic effect of SSRIs can be significantly enhanced when used in combination with HRT. In addition to psychiatric symptoms, HRT may also improve cognitive function. Regarding adverse events of HRT, HRT with 17β-estradiol (E2) increases the risk of cancers such as breast and endometrial cancer, albeit very slightly.

In recent years, extensive research on medicinal mushrooms has been conducted to assess their potential for treating various conditions as safer alternatives to drug-dependent therapies. We previously showed a beneficial effect of *Pleurotus eryngii* for treating menopausal memory impairment and depression by using ovariectomized (OVX) rats. *Hericium erinaceus* (HE) is known as Lion’s Mane mushroom or *yamabushitake* in Japan. HE is a medicinal mushroom that has the potential for treating various conditions, including depression, anxiety, neurodegenerative diseases, dementia, cognitive impairment, diabetes, cancer, and spinal cord injury. It has also been shown to promote neurite outgrowth and nerve regrowth and to have a neuroprotective effect.

HE contains phytoestrogens. Two isoflavones, daidzein and genistein, have been isolated from HE mycelium. Poly-saccharides and secondary metabolites such as erinacines, hericins, hericenones, resorcinols, steroids, mono- and di-terpenes, and volatile aromatic compounds have also been isolated from HE. Hericenone, a bioactive compound, shows an antidepressant-like effect. Hericenones C and D can induce the synthesis of nerve growth factors and stimulate neurogenesis in the hippocampus. HE, therefore, has the potential to improve the neurological symptoms of menopause. However, the ameliorating effect of HE on menopausal depression re-
mains unclear. This study investigated the ameliorative effect of HE fruiting body on menopausal depression using menopausal model rats.

MATERIALS AND METHODS

Experimental Animals Female Wistar rats (10 weeks old) were purchased from Japan SLC (Hamamatsu, Japan) and used for the experiment after two weeks of acclimatization. Rats had ad libitum access to a regular chow (MF®; Oriental Yeast, Tokyo, Japan) and tap water until the start of the experiment. Rats were kept in a temperature- and humidity-controlled room (23 ± 1 °C, 55 ± 5% humidity) with a 12-h light/dark cycle. The animals were cared for under the guidelines established by the University of Shizuoka. All animal experiments were pre-approved by the animal ethics committee of the University of Shizuoka (Approval No. 156168).

Methanol Extraction Freeze-dried fruiting body powder of 2 kg HE was extracted with 8 L methanol (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) at 50 °C for 24 h. After filtration through filter paper (No. 2, Advantec, Tokyo, Japan), the residue was extracted with 8 L methanol again in the same manner. After removing the solvent with an evaporator, 560 g of HE extract was obtained. A voucher specimen of the mushroom HE (KLU-M 1232) was deposited in the Herbarium of the University of Malaya, Malaysia. Chemical profiles of compounds in methanol extract of HE was shown in Supplementary Figs. S1–4.35)

Ovariectomy and Administration Bilateral oварiectomies were carried out under anesthesia with a mixture of butorphanol tartrate (Meiji Seika Pharma, Tokyo, Japan, 2.5 mg/kg body weight), medetomidine hydrochloride (FUJIFILM Wako Pure Chemical Corporation, 0.375 mg/kg body weight), and midazolam (FUJIFILM Wako Pure Chemical Corporation, 2 mg/kg body weight). Similar operations were carried out in the sham-operated rat group except for the ovariectomy. Two days later, the administration of HE or E2 (FUJIFILM Wako Pure Chemical Corporation) was started. HE was administered by feeding with a chow containing 1% HE extract prepared by a contractor (Oriental Yeast, Tokyo, Japan). E2 was given by drinking water containing 1 mg/100 mL E2 first prepared as 100 mg/mL in dimethyl sulfoxide and then diluted to 1 mg/100 mL with tap water. Rats had ad libitum access to the chows and drinking water. Rats were dissected on day 98 after ovariectomy, and uterine weight was measured.

Morris Water Maze Test The Morris water maze test was performed 87 d after the ovariectomy. A pool 130 cm in diameter was filled with water (22 ± 2 °C), and a transparent escape platform was submerged in the pool. In the training session, rats were released into the water and trained to find the platform for up to 40 s. In the case of failure, rats were guided to the platform by hand. Rats were kept on the platform for 10 s and then picked up. Trials were repeated four times a day for five days. The release position of the rats was randomly changed every time. Rats with clearly different behavior during the training process were not used. In the probe test session, rats were released into the pool after platform removal. The pool was virtually divided into four sections: east, west, south, north, and south. The time the rat stayed in the quadrant where the platform had been placed was measured for 60 s using a video tracking system (ANY-maze Ver. 5.3; Muromachi Kikai, Tokyo, Japan).

Forced Swim Test The forced swim test was performed 94 d after the ovariectomy. Each rat was placed for 15 min in an acrylic cylinder (20 cm in diameter, 50 cm in height) filled with water (22 ± 2 °C) to 22 cm. After 24 h, the rat was placed for 5 min in the cylinder again. Behavior was recorded with a video camera. The time the rats were passively floating in the water was measured as immobility time.

Statistical Analysis The following multiple comparisons were used: one-way ANOVA followed by Dunnett’s multiple comparison test or two-way repeated-measures ANOVA followed by Bonferroni’s multiple comparison test. Statistical analysis was performed by GraphPad Prism (GraphPad Software, La Jolla, CA, U.S.A.). Outliers were excluded based on the Smirnov–Grubbs test. Data are shown as means with a standard error of the mean.

RESULTS

HE Extract Did Not Affect Daily Food Intake and Body Weight after Ovariectomy Bilateral ovariectomies were carried out in 12-week-old rats. Two days later, HE extract and E2 were administered for 94 d (Fig. 1A). The daily food intake was recorded at 21, 49, and 77 d after the ovariectomy. The daily food intake was not significantly different in each treatment group (two-way repeated-measures ANOVA: \( F_{1,18} = 15.3, p = 0.0013 \) for treatment; \( F_{2,18} = 15.5, p = 0.0001 \) for days). HE extract did not affect this increased food intake after ovariectomy. The daily intake of HE extract was approximately 140–155 mg throughout the entire period.

Estrogen deficiency in OVX rats resulted in significantly higher body weight gain than the sham-operated rats after 30 d from the ovariectomy (Fig. 1B). On day 72 after ovariectomy, the increased body weight of OVX rats was not significantly affected by HE extract but was reduced to the level of the sham-operated rats by E2 (one-way ANOVA, \( F_{3,36} = 36.7, p < 0.0001 \)) (Fig. 1C).

HE Extract Did not Affect Uterine Weight after Ovariectomy On day 96 after ovariectomy, uterine weight was lower than in the sham-operated rats (Fig. 2). The decreased uterine weight by the ovariectomy was not significantly affected by HE extract administration but was increased by E2 administration (one-way ANOVA, \( F_{3,35} = 68.1, p < 0.0001 \)).

HE Extract Did Not Affect Memory Impairment after Ovariectomy Hippocampus-dependent spatial memory was assessed by the Morris water maze on day 87 after ovariectomy. In the training session, latency time to platform arrival was decreased over the course of the training but was not significantly different in each treatment group (two-way repeated-measures ANOVA: \( F_{4,120} = 1.16, p = 0.342 \) for treatment; \( F_{4,120} = 32.3, p < 0.0001 \) for days, data not shown). In the probe test session, time spent in the target quadrant was significantly reduced by ovariectomy (Fig. 3). Memory impairment in OVX rats was not significantly improved by the administration of HE extract but tended to be improved by E2 (one-way ANOVA, \( F_{3,38} = 3.34, p = 0.033 \)).

HE Extract Ameliorated Depressive-Like Behavior after Ovariectomy Depression was assessed as immobility time in the forced swimming test on day 94 after ovariectomy. The OVX rats showed the longest immobility among the four

B) Changes in body weight for 72 d after ovariectomy. C) Body weight at day 72 after ovariectomy. Sham, n = 11; OVX, n = 11; OVX + HE, n = 10; and OVX + E$_2$, n = 8. ***p < 0.001 vs. sham; †††p < 0.001 vs. OVX (one-way ANOVA followed by Dunnett’s multiple comparison test).

Table 1. Effect of HE Extract on Daily Food Intake in OVX Rats

<table>
<thead>
<tr>
<th>Days after OVX</th>
<th>Sham (g/d)</th>
<th>OVX (g/d)</th>
<th>OVX + HE (g/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>14.0 ± 0.3</td>
<td>15.3 ± 0.3*</td>
<td>15.0 ± 0.3</td>
</tr>
<tr>
<td>49</td>
<td>13.8 ± 0.4</td>
<td>14.9 ± 0.3</td>
<td>14.6 ± 0.2</td>
</tr>
<tr>
<td>77</td>
<td>12.1 ± 0.7</td>
<td>13.9 ± 0.4**</td>
<td>14.0 ± 0.3**</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01 vs. sham (two-way repeated-measures ANOVA followed by Bonferroni’s multiple comparison test).

DISCUSSION

In the present study, we investigated the ameliorating effect of HE on menopausal depression using a menopausal animal model. OVX rats exhibit depressive-like behavior due to estrogen deficiency. We confirmed that depressive-like behavior in OVX rats, as assessed by the forced swimming test, was ameliorated by administration of HE as well as E$_2$.

Phytoestrogens such as daidzein and genistein have been isolated from HE. Phytoestrogens can cross the blood-brain barrier, and both daidzein and genistein bind more strongly to estrogen receptor beta (ER$\beta$) than to ER$\alpha$, respectively. ER$\beta$ is expressed at relatively high levels in the prostate, ovary, lung, bladder, brain, uterus, and testis. In the brain, ER$\beta$ expressed in the dorsal raphe, hippocampus, and medial amygdala is associated with depression. Activation of ER$\beta$ reduces depressive-like behavior during the forced swim test. The improvement of depressive symptoms by ER$\beta$ activation is due to promoting serotonin synthesis by induction of tryptophan hydroxylase and brain-derived neurotrophic factors synthesis. Thus, it is presumed that activation of ER$\beta$ is partially responsible for the effectiveness of HE in ameliorating depression-like behavior. However, since HE contains many bioactive substances, we cannot rule out the possibility that there are other causes.
than activation of ERβ for the ameliorative effects of HE.

On the other hand, activation of ERα induces a loss of body weight and a gain in uterine weight. ERα is abundantly expressed in the uterus, testis, pituitary, ovary, kidney, epididymis, and adrenal gland. In this study, the HE extract did not significantly affect body weight gain and uterine weight loss by estrogen deficiency in OVX rats. Thus, the phytoestrogens in HE may hardly activate ERα. Long-term activation of ERα induces breast cancer, uterine cancer, and cardiovascular disease. In addition, activation of ERβ attenuates the growth of cancer cells. Thus, HE is considered to have a low risk of cancer due to ERα activation.

In the present study, the HE methanolic extract did not ameliorate the hippocampal-dependent spatial memory impairment in the OVX rats. Previously, it was reported that dietary supplementation of HE improved the recognition memory in the novel object recognition test and increased neurotransmission at the hippocampal mossy fiber-CA3 synapse in wild-type mice. However, HE did not affect the hippocampal-dependent spatial memory evaluated using a Y maze and an object location task, which is consistent with our findings.

There are limitations to this study. First, there is no direct evidence that the improvement of depressive-like behavior in OVX rats is caused by ERβ activation with HE. In order to prove the direct involvement of ERβ activation in the ameliorative effect of HE, animal experiments using ERβ knockout mice, ERβ agonists (e.g., diarylpropionitrile), and ERβ antagonists (e.g., PHTPP) will be required. Second, HE is not entirely free of activation to ERα. Since genistein does not affect uterine weight, the activating effect of genistein on ERα is not strong. However, as previously reported, phytoestrogens can bind not only to ERβ but also to ERα. Third, we cannot conclude from the change in body weight that there is no activating effect of HE on ERα. Body weight is more strongly affected by ERα activation than ERβ, but ERβ activation has been reported to affect adiposity and body weight. Since ERβ agonists do not affect uterine weight, uterine weight is considered more appropriate for evaluating activity against ERα. Fourth, since we do not have a group of sham-operated rats administered with HE, we cannot properly evaluate whether the improvement effect of HE is limited to the depression caused by estrogen deficiency. As ERβ is also expressed in the male brains, the activation of ERβ by HE may positively affect the neurological function in males as well.

Fig. 2. Effect of HE Extract on Uterine Weight in OVX Rats
Sham, n = 11; OVX, n = 10; OVX + HE, n = 10; and OVX + E2, n = 8. ***p < 0.001 vs. sham; †††p < 0.001 vs. OVX (one-way ANOVA followed by Dunnett’s multiple comparison test).

Fig. 3. Effect of HE Extract on Memory Impairment in OVX Rats
Hippocampus-dependent spatial memory was assessed by the Morris water maze. Time spent in the target quadrant was measured for 60-s in the probe test session. Dashed lines represent the chance level. Sham, n = 10; OVX, n = 10; OVX + HE, n = 8; and OVX + E2, n = 5. *p < 0.001 vs. sham (one-way ANOVA followed by Dunnnett’s multiple comparison test).

Fig. 4. Ameliorating Effect of HE on Depressive-Like Behavior Exhibited by OVX Rats
The change in immobility time (A) and the total immobility time (B) were measured for 5 min in the forced swimming test. Sham, n = 11; OVX, n = 11; OVX + HE, n = 10; and OVX + E2, n = 8. *p < 0.05 vs. sham; †p < 0.05, ††p < 0.01 vs. OVX (one-way ANOVA followed by Dunnett’s multiple comparison test).
In summary, depressive-like behavior in OVX rats evaluated in the forced swim test was reduced by HE administration for 92 d. Since HE hardly activates ERα, HE may be effective in treating menopausal depression with a low risk of carcinogenesis caused by ERα activation.

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Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials This article contains supplementary materials.

REFERENCES


