Antidepressant-like effects of young green barley leaf (*Hordeum vulgare* L.) in the mouse forced swimming test

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ABSTRACT

Background: Young green barley leaf is one of the richest sources of antioxidants and has been widely consumed for health management in Japan. In this study, we examined whether oral administration of young green barley leaf has an antidepressant effect on the forced swimming test in mice. **Materials and Methods:** Mice were individually forced to swim in an open cylindrical container, one hour after oral administration of young green barley leaf (400 or 1000 mg / kg) or imipramine (100 mg / kg). Expression of mRNA for nerve growth factor (NGF), brain-derived neurotrophic factor, and glucocorticoid receptor in the brain was analyzed using real-time quantitative polymerase chain reaction (PCR). **Results:** There was a significant antidepressant-like effect in the forced swimming test; both 400 and 1000 mg / kg young green barley leaves, as well as the positive control imipramine (100 mg / kg), reduced the immobility duration compared to the vehicle group. The expression of mRNA for NGF detected in the hippocampus immediately after the last swimming test was higher than that in the non-swimming group (Nil). Oral administration of imipramine suppressed this increase to the level of the Nil group. Young green barley leaf (400 and 1000 mg / kg) also showed a moderate decrease in the expression of mRNA for NGF, in a dose-dependent manner. **Conclusion:** Oral administration of young green barley leaf is able to produce an antidepressant-like effect in the forced swimming test. Consequently it is possible that the antidepressant-like effects of the young green barley leaf are, at least in part, mediated by an inhibition of the increase in the hippocampus levels of NGF.

Key words: Antidepressant-like effect, corticosterone, forced swimming test, hippocampus, nerve growth factor, young green barley leaf

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INTRODUCTION

Depression is characterized by a wide range of debilitating emotional and physical symptoms, and is among the top three leading causes of disease burden worldwide.[1] Furthermore, depression is one of the important risk factors for suicide.[2] Numerous neural pathways are involved in the pathophysiology of depression. Stressful life events that involve threat, loss, humiliation or defeat, influence the onset and course of depression.[3-6]

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There is growing interest in the use of natural products to maintain health, particularly from a preventive point of view. One factor driving this interest is the increase in stressful life events in developed countries. Furthermore, it has become accepted that too much stress may be linked to various diseases and poor health. As dietary supplements and natural products marketed to maintain health or alleviate adverse effects on lifestyle are more widely used, consumers and healthcare providers continue to require more information based on scientific evidence.

Young green barley leaves are a good natural source of vitamins and minerals and its juice is widely consumed for health reasons in Japan. Young green barley leaves are one of the richest sources of antioxidants and contains the flavones C-glycoside, saponarin, and lutonarin.[7,8] Furthermore, it has been shown that barley and young green barley leaf exhibit a physiological activity that

includes hypolipidemic[9,10] and anti-ulcer effects,[11] via its antioxidative action. There have been a number of unpublished, but reputable concerns on the antidepressive effects of the young green barley leaf in consumers in Japan. Results of recent studies have indicated that antioxidants such as, α-tocopherol,[12] ascorbic acid[13] and *Hypericum perforatum* extract[14] have an antidepressant-like effect. Therefore, it is possible that young green barley leaf, which contains antioxidative flavonoids, may also exhibit an antidepressant effect. However, the putative antidepressant effect of the young green barley leaf has not been investigated experimentally, and therefore, its efficacy remains unclear. The forced swimming test is widely used to assess the antidepressant properties of new products. It is known that effective antidepressant treatments decrease immobility duration in the test.[15] Therefore, in the present study, we aim to examine whether oral administration of young green barley leaf has an antidepressant effect on mice in the forced swimming test. In addition, we have investigated the expression of mRNA for nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and glucocorticoid receptor (GR), which have been implicated in hypothalamic–pituitary–adrenal (HPA) axis activity and may have a role in the stress response of the hippocampus.

MATERIALS AND METHODS

Animals

All experiments and procedures were approved by the Chiba University Institutional Animal Care and Use Committee. Male Imprinting Control Region (ICR) mice, six weeks of age, were obtained from Japan SLC Inc. (Hamamatsu, Japan), and housed under controlled light (0700 – 1900 hours) and temperature (24°C) conditions, with food and water available *ad libitum*.

Materials

Young green barley leaves (*Hordeum vulgare* L. var. *nudum* Hook), 20 – 35 cm in height, were supplied by JPD Co. Ltd. (Hyogo, Japan), who collected the specimens in the Ohita prefecture, Japan, during November, and subsequently extracted juice from the leaves to produce a dried powder, in accordance with the company's guide to Good Manufacturing Practice. Imipramine hydrochloride and dexamethasone were obtained from Sigma Chemical (St. Louis, MO), and corticosterone was obtained from Wako Pure Chemical Institute (Osaka, Japan). Young green barley leaf and imipramine were dissolved in distilled water.

Forced swimming test

The forced swimming test was conducted between 10:30 and 15:00 hours. One hour after the oral administration of young green barley leaf (400 or 1000 mg / kg), imipramine (100 mg / kg), or distilled water (vehicle), individual mice were forced to swim in an open cylindrical container (diameter 20 cm, height 40 cm), containing 30 cm water at 25 ± 1°C. The total duration of immobility was recorded in a six-minute period. The mice were judged to be immobile when they ceased struggling and remained floating motionless in the water, making only those movements necessary to keep their heads above water. A decrease in the duration of immobility was indicative of an antidepressant-like effect.[15]

Experimental schedule

Mice, with the exception of non-swimming (Nil) mice, were subjected to the forced swimming test once a day for three days. After the first measurement of immobility duration on day zero, the mice were divided into four groups, with equal immobility duration in each group. One hour before the second (day 1) and the third forced swimming (day 2) tests, the mice were administered oral young green barley leaf, imipramine or vehicle; the Nil group received vehicle without undergoing the swimming test.

Measurement of serum corticosterone

Blood was collected by retro-orbital bleeding immediately after the final forced swimming test, and allowed to clot at room temperature for one hour, and centrifuged at 1,000 *g* for 20 minutes. Serum was collected and stored at -30°C prior to the analysis. Serum levels of corticosterone were determined by high-performance liquid chromatography (HPLC). Briefly, 20 µl aliquots of standards or samples were transferred to 1.5 ml Eppendorf centrifuge tubes. A 25 µl aliquot of internal standard solution (dexamethasone, 1 µg / ml final concentration) was added to the serum followed by 200 µl ethyl acetate, and briefly mixed on a vortex mixer. The mixture was centrifuged at 5,000 *g* for five minutes at 4°C to remove the precipitated proteins. An 80 µl aliquot of 0.05 M sodium hydroxide was added to the supernatant and mixed on a vortex mixer. The mixture was centrifuged at 5,000 *g* for five minutes at 4°C. The supernatant was then transferred to a 1.5 ml Eppendorf centrifuge tube and evaporated to dryness using a centrifugal concentrator (DNA-mini, Heto, Denmark). Subsequently, a 25 µl HPLC mobile phase (30% acetonitrile / 70% water / 0.1% formic acid) was added and transferred to a 250 µl injection vial. A 5 µl aliquot of the sample or standard solution in the injection vial was subjected to HPLC analysis. A Shiseido Nanospace SI-2 (Tokyo, Japan) HPLC system was used to analyze corticosterone. A Unison UK-C-18 column (1.5 x 250 mm, 3 µm, Imtakt Corp., Kyoto, Japan) was used at 40°C, with a flow rate of 100 µl / min. The mobile phase was 30% acetonitrile / 70% water / 0.1% formic acid, and corticosterone was detected at a wavelength of 240 nm.

Expression of mRNA for NGF, BDNF, and GR in the brain

Immediately after the final forced swimming test, the mice were killed by decapitation and their cerebral cortex and hippocampus were removed according to the method of Hagihara *et al.*[16] The cerebral cortex and hippocampus were homogenized in a lysis buffer (Buffer RLT, Qiagen, Hilden, Germany) containing 2-mercaptoethanol (Wako, Osaka, Japan), and after centrifugation (36,670 *g*) for ten or three minutes for cerebral cortex or hippocampus, respectively, the supernatant was collected and the total RNA was extracted using an RNeasy Mini Kit (Qiagen). cDNA was prepared from the RNA by reverse transcription using a PrimeScript RT reagent kit (Takara Bio INC., Shiga, Japan). Real-time quantitative PCR was performed on a Step One TM Real Time PCR System (Applied Biosystems Inc., Carlsbad, CA) using SYBR Premix Ex Taq for mouse β-actin, NGF, BDNF, and GR, in accordance with the manufacturer's instructions (Takara Bio INC.). Results were expressed as the relative mRNA level, corrected with reference to β-actin mRNA as an internal control.

Statistical analysis

All data are presented as mean ± SEM. The statistical significance was analyzed using the Dunnett's method for multiple comparisons. Statistical differences in the two groups were analyzed using the Student's *t*-test or Welch's test after an *F*-test. Differences at $P < 0.05$ were considered statistically significant. All statistical analyses were conducted using StatLight software (Yukms Co., Ltd. Tokyo, Japan).

RESULTS

Effects on forced swimming test

The immobility duration in the forced swimming test in the vehicle group increased from day 0 to day 2. There was a significant treatment effect in the forced swimming test, with oral administration of 400 and 1000 mg / kg young green barley leaf, reducing the immobility duration compared to the vehicle group on day 2, indicating that the plant was effective for producing an antidepressant-like effect in this behavioral model [Figure 1]. The positive control, imipramine (100 mg / kg), further decreased the immobility duration in the forced swimming test, also indicating a significant antidepressant-like effect.

Effects on the concentration of corticosterone in mouse serum

We measured the serum glucocorticoid level to investigate the effect of young green barley leaf on the HPA axis. The serum concentration of corticosterone in the vehicle group increased significantly compared to the Nil group after the final forced swimming test. Oral administration of imipramine (100 mg/kg) significantly reduced the concentration of corticosterone, which had been elevated by the forced swimming tests. By contrast, oral administration of young green barley leaf (400 and 1000 mg/kg) failed to significantly change the serum corticosterone level [Figure 2].

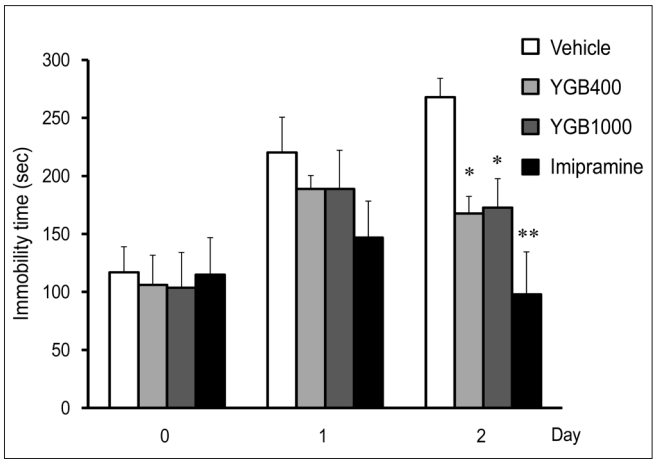

Figure 1: Effects of young green barley leaf extract on immobility duration in mice subjected to forced swimming
Values represent the mean ± SEM for eight mice. **P* < 0.05 and ***P* < 0.01 versus the vehicle group (Dunnett's multiple comparisons). YGB 400 and YGB 1000: young green barley leaf extract at doses of 400 and 1000 mg / kg. Imipramine dose was 100 mg / kg.

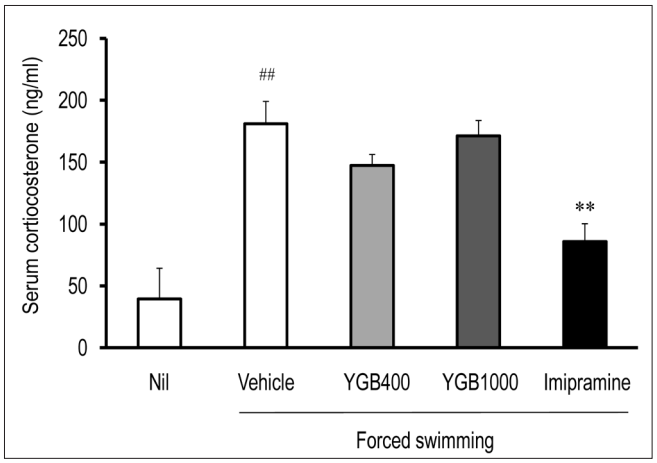

Figure 2: Effects of young green barley leaf extract on the serum corticosterone concentration in mice subjected to forced swimming
Values represent the mean ± SEM for eight mice. ***P* < 0.01 versus the vehicle group (Dunnett's multiple comparisons). ##*P* < 0.01 versus Nil (Student's *t* test). Nil: non-swimming group; YGB400 and YGB1000: young green barley leaf extract at doses of 400 and 1000 mg / kg. Imipramine dose was 100 mg / kg.

Effects on the expression of mRNA for NGF, BDNF, and GR in mouse brain

Using quantitative PCR, we analyzed the effect of the forced swimming test, performed once a day for three

days, in the presence and absence of young green barley leaf, on the expression of mRNA for the neurotrophic factors NGF, BDNF, and GR. The expression of mRNA for NGF detected in the hippocampus immediately after the last swimming test was 31% higher than in the non-swimming Nil group. Oral administration of imipramine, the positive control, completely suppressed this increase ($P = 0.057$), whereas, young green barley leaf (400 and 1000 mg / kg) produced a moderate decrease in NGF mRNA in a dose-dependent manner [Table 1]. Although only a modest increase in mRNA for NGF was observed in the cerebral cortex, a similar decrease was observed following the administration of young green barley leaf or imipramine. By contrast, the levels of mRNA for BDNF and GR were not obviously different in the vehicle group and the Nil group in either the hippocampus or the cerebral cortex [Table 1]. There was no obvious change in body weight in any group during the experimental period (data not shown).

DISCUSSION

In this study, we used the forced swimming test to evaluate the antidepressant-like effect of young green barley leaf in mice. The forced swimming test is an animal model that is used as an experimental paradigm for the assessment of despair / depression-like behavior. It is also commonly used as a screening test for the antidepressant properties of drugs. Our results show that young green barley leaf administered orally significantly reduces immobility duration in the mouse forced swimming test. Thus, to our knowledge, this study is the first to suggest that the young green barley leaf has a favorable antidepressant-like profile.

The hippocampus is a region in the brain that plays a central role in emotional processing, and is implicated in the regulation and control of anxious response and conditioned fear.[17] Hippocampal function is also influenced by the presence of the neurotrophins NGF and BDNF, which are proteins involved in the growth, survival, and function of neurons in the central nervous system. In the present study, the expression of mRNA for NGF in the hippocampus was increased by forced swimming compared to that of non-swimming (Nil) mice. It was previously shown that acute exposure to forced swimming tended to increase the level of NGF in the hippocampus.[18] Moreover, increased levels of circulating NGF have been observed during stressful conditions in both humans and laboratory animals.[19] These findings are consistent with our results. It has also been previously reported that repeated exposure of mice to minimal electroconvulsive shock stress exerted a neuroprotective action via the upregulation of NGF expression in the hippocampus,[20] thus suggesting that increased expression of NGF in the vehicle group in our study might have a neuroprotective action against the stress of forced swimming. It was recently suggested that an anti-stress effect of exposure to music in mice is associated with reduction in NGF levels in the hypothalamus.[21] These results support the hypothesis that the reduction in NGF levels by administration of young green barley leaf may contribute to its antidepressant-like effect on forced swimming in mice.

Stress potently modulates anxiety- and depression-related behaviors. In response to stressors, the HPA axis is activated, resulting in the release of glucocorticoids from the adrenal cortex.[22] Glucocorticoids bind to GRs to mediate the peripheral stress response and also inhibit further activation of the HPA axis by negative feedback to the central nervous system.[23] In the present study, administration of the young green barley leaf did not significantly affect the serum gloucocorticoid levels or the expression of mRNA for GR. This suggests that the antidepressant-like effect of the young green barley leaf is not mediated by its effect on the HPA axis.

Table 1: Effects of young green barley leaf extract on the expression of mRNA for NGF, BDNF, and GR in the hippocampus and cerebral cortex of mice subjected to forced swimming

	Nil	Vehicle	YGB 400	YGB 1000	Imipramine
NGF / β-actin					
Hippocampus	1.00±0.12	1.31±0.12	1.22±0.14	1.11±0.10	0.97±0.13
Cerebral codex	1.00±0.07	1.14±0.06	1.09±0.04	1.06±0.09	0.96±0.07
BDNF / β-actin					
Hippocampus	1.00±0.05	1.11±0.04	1.08±0.06	1.02±0.05	1.04±0.05
Cerebral codex	1.00±0.08	0.92±0.05	0.82±0.05	0.93±0.06	0.95±0.05
GR / β-actin					
Hippocampus	1.00±0.06	1.14±0.06	1.11±0.05	0.94±0.07	1.03±0.09
Cerebral cortex	1.00±0.05	1.04±0.07	0.93±0.08	0.92±0.05	0.86±0.06

The total RNA was extracted from each brain immediately after the last forced swimming test. Real-time quantitative PCR was conducted using specific primers for NGF, BDNF, and GR. The mRNA levels in each group were normalized to the corresponding β-actin mRNA levels, and the mean value of the Nil group was set at 1.0. Values represented the mean ± SEM for seven to eight mice. Nil: non-swimming group; YGB 400 and YGB 1000: young green barley leaf extract at doses of 400 and 1000 mg / kg. Imipramine dose was 100 mg / kg.

The forced swimming test did not affect the expression of mRNA for BDNF. In contrast to our findings, a recent study indicated that short acute stress induces a significant increase in BDNF mRNA in the rat hippocampus.[24] This discrepancy might be attributable to the different animal models used, but further study will be needed to clarify this possibility.

Taken together, our results demonstrate that oral administration of young green barley leaf is able to produce an antidepressant-like effect in the forced swimming test. Moreover, it is assumed that the antidepressant-like effect of the young green barley leaf on mouse forced swimming might be mediated, at least in part, by its inhibitory effect on the increase in expression of mRNA for NGF in the hippocampus.

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