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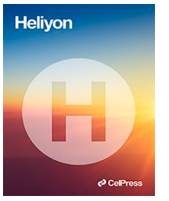

Research article

A soy protein enzymatic digest mitigates Nrf2-related oxidative stress and attenuates depression-like behavior in a mouse model of sub-chronic restraint stress

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ABSTRACT

Continuous oxidative stress conditions have been identified as a major cause of various neuropsychiatric disorders, including depression. The present study investigated the potential antidepressant-like effects of a soy protein enzymatic digest (SPD) containing soy-deprestatin, which is a soy-derived peptide with reported antidepressant-like effects, as well as its ability to mitigate oxidative stress in the brain caused by sub-chronic restraint stress. Mice were divided into two groups: a control group and restraint stress group. The restraint stress group was further divided into two groups administered water or SPD. After repeated short-time restraints over five days, we evaluated immobility times in the tail suspension test, and antioxidant enzyme activities, glutathione levels, oxidative stress maker levels, and the gene expression levels of Nrf2 and antioxidant enzymes in the brain. The results obtained showed that the oral administration of SPD reduced immobility times in mice exposed to restraint stress. In comparisons with the water-treated restraint group, the administration of SPD restored superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase activities and glutathione levels and prevented restraint stress-induced increases in malondialdehyde, carbonyl protein, and 8-OHdG levels in the restraint stress group. In addition, high expression levels of Nrf2, HO-1, NQO-1 and GCLC were observed in the SPD-treated restraint group. These results suggest that SPD attenuated repeated restraint stress-induced depression-like behaviors by mitigating oxidative stress through the activation of the Nrf2 signaling pathway.

1. Introduction

According to the World Health Organization (WHO), stress is defined as any type of change that causes physical, emotional, or psychological strain. Moderate stress is good for individuals to perform and protect themselves. However, the living environment in modern society is rapidly changing, causing excessive stress. The effects of excessive stress in daily life manifest in all areas of the body, including the cardiovascular, respiratory, digestive, nervous, and urinary systems, and may be risk factors for depression, schizophrenia, anxiety disorders, and sleep disorders [1–4].

Oxidative stress is defined as an imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms. The production of ROS is a normal process as long as it is in moderate amounts, and these ROS have many physiological roles,

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including intracellular signaling and defense against pathogens [5]. On the other hand, stressful lifestyle habits including psychological stress, poor dietary choices, excessive drinking, smoking, and limited sleep may increase the generation of ROS, leading to oxidative stress, a condition in which the production of ROS exceeds antioxidant defense mechanisms. Continuous oxidative stress conditions damage cellular proteins, lipids, carbohydrates, and nucleic acids, and may ultimately result in a number of diseases, such as cardiovascular, pulmonary, renal, and oncological diseases [6–8].

In addition, oxidative stress has been implicated in the progression of multiple neuropsychiatric disorders, including major depressive disorder, bipolar disorder, schizophrenia, and anxiety disorders [9–12]. A recent study suggested that the toxic function of kynurenine pathway metabolites from SARS-CoV-2-induced hyperinflammation is another mechanism that involves oxidative stress and triggers or exacerbates major depressive disorder [13].

The gut and brain are closely related via the autonomic nervous system and humoral factors, such as hormones and cytokines. This gut-brain communication is also involved in emotional regulation and mood modulation, and changes in the intestinal flora by lactobacillus were found to attenuate anxiety and depressive behavior in mice via the vagus nerve [14].

Soy-deprestatin, a soy-derived peptide that is produced by the enzymatic digestion of soybean β-conglycinin, was shown to exert antidepressant-like effects in mice following its oral administration through gut-brain communication. The antidepressant-like effects of orally administered soy-deprestatin were abolished by blockade of the vagus nerve, indicating that its effects are achieved by signals transmitted from the gut to the brain through the vagus nerve. Therefore, the effects of soy-deprestatin were abolished by treatments with antagonists of serotonin 5-HT_{1A}, dopamine D_1, and $GABA_A$ receptors, suggesting that these pathways are mediated in this order [15]. However, the mechanisms underlying the antidepressant-like effects of soy-deprestatin in the brain currently remain unknown.

In the present study, we used a mouse model with repetitive short periods of time restraints. This sub-chronic restraint stress induced oxidative stress in the brain, as previously reported [16,17]. We herein demonstrated that a soy protein enzymatic digest (SPD) containing soy-deprestatin affected oxidative stress and depression-like behavior in mice, in which oxidative stress was induced in the brain, regardless of its weak antioxidant activity.

2. Materials and Methods

2.1. Preparation of a SPD product containing soy-deprestatin

SPD (soy protein enzymatic digest) was prepared as follows; soy protein isolate (SPI, SUPRO 661, Koyo Mercantile) was digested with thermolysin (THERMOASE PC10F, Amano Enzyme) at 60 °C, and then dried to powder. SPD was also available for purchase from UHA Mikakuto as SOYLAX®.

The amount of soy-deprestatin in SPD was measured as follows. SPD powder dissolved in 0.1% polypeptone was analyzed by injecting 10 μL into LC/MS/MS. Analyses were performed using Acquity Arc (Waters) for liquid chromatography (LC), 3200 Q Trap (ABsciex) for mass spectrometry (MS), and CAPCELL PAK C18 UG80 (φ2.0 × 150 mm, 5 μm; Osaka soda) for columns. Separation was performed using 0.1% formic acid - 0.1% formic acid/acetonitrile at a flow rate of 0.2 mL/min, under 0–30% (0–15 min), 30% (15–20 min), and 30–100% (20–25 min) gradient conditions. MS was performed by using the positive ion mode of electrospray ionization. The monitored precursor/fragment ions were *m/z* 1113.4/101.2 for soy-deprestatin. MS conditions were as follows: curtain gas, 20 psi; collision gas, 3; ion spray voltage, 5000 V, temperature, 600 °C; ion source gas 1, 30 psi; ion source gas 2, 30 psi. Quantitative measurements of soy-deprestatin were performed by creating a calibration curve using synthetic peptides (LSSTQAQQSY; Genscript).

2.2. Animals

Five-week-old male ddY (Slc:ddY) mice were purchased from Japan SLC (Shizuoka, Japan) and maintained at 21–23 °C with free access to food and tap water under a 12-h light:dark cycle (lights on at 07:00 a.m.). All animal experiments were performed at the Katagiri VMD Office (Kobe, Japan) in strict accordance with the Guidelines for the Proper Conduct of Animal Experiments of the Science Council of Japan. Experimental protocols and procedures were approved by the Animal Experiment Committee of the Katagiri VMD Office (MKT22-04).

2.3. Experimental design

After habituation for at least one week, mice were randomly divided into three groups with 13 mice each to receive different administrations: control with vehicle administration (Control), sub-chronic restraint stress with vehicle administration (Restraint-Control), and sub-chronic restraint stress with SPD administration (Restraint-SPD). Sub-chronic restraint stress was induced as previously described [16–19], with slight modifications. Briefly, mice subjected to sub-chronic restraint stress were gently placed into a well-ventilated 50-mL centrifuge tube without access to food and water for 150 min every day and for 5 consecutive days. Mice in the control group were housed in their original cages without food and water, similar to restraint stress mice. SPD was dissolved in purified water and was administered orally to mice by gavage at a dose of 100 mg/kg body weight 60 min prior to restraint stress exposure.

2.4. Tail suspension test

The tail suspension test is a classic behavioral test and one of the most frequently used tasks to evaluate depression-like behavior in mice. We performed the tail suspension test 120 min after release from the last restraint stress according to a previously reported

method [15,20] with minor modifications. In brief, mice were suspended 30 cm above the floor with adhesive tape placed ~2 cm from the extremity. Results are shown as the immobility time within 6 min.

2.5. Brain tissue preparation

After the tail suspension test, mice were sacrificed by deep anesthesia and the brains were collected. Brains were longitudinally cut into hemispheric sections. One half of the brain was used for a biochemical analysis and was stored at −80 °C until processed. The other half of the brain was used for a quantitative real-time PCR (qPCR) analysis and was immediately placed in RNAlater (Sigma), immersed overnight at 4 °C, and stored at −80 °C until processed.

In the biochemical analysis, brain tissue was homogenized by a disperser (IKA, T10). The homogenate was centrifuged and the supernatant was collected and stored at −80 °C for further biochemical analyses. Protein concentrations were assessed by the BCA protein assay kit (Thermo Scientific Pierce) with bovine serum albumin as the standard.

2.6. Evaluation of antioxidant enzyme activities

We examined the activities of the following antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione reductase (GR). SOD, CAT, GPX, and GR activities in the brain were measured using a commercially available bioassay kit from the Cayman Chemical for SOD, CAT, and GPX and the BioAssay System for GR, according to the manufacturer's instructions.

2.7. Evaluation of glutathione levels

Reduced and oxidized forms of glutathione (GSH and GSSG, respectively) were measured using a GSSG/GSH Quantification Kit (Dojindo) according to the manufacturer's instructions. Total glutathione and GSSG levels were measured with this kit and the quantity of GSH was assessed by subtracting the amount of GSSG from that of total glutathione.

2.8. Evaluation of oxidative stress markers

We measured the levels of malondialdehyde (MDA), carbonyl protein, and 8-hydroxy-2′-deoxyguanosine (8-OHdG) as oxidative stress markers. The content of MDA, a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid reactive substances (TBARS) using a TBARS (TCA Method) Assay Kit (Cayman Chemical). Carbonyl protein, a measure of protein peroxidation, was measured using a Carbonyl Protein ELISA kit (Nikken SEIL) after dinitrophenylhydrazine derivatization (DNP) according to the manufacturer's instructions. Quantitative measurements of DNA damage were performed as 8-OHdG and assessed using a highly sensitive ELISA Kit for 8-OHdG (Nikken SEIL).

2.9. qPCR analysis

Brain tissue samples stored in RNAlater were homogenized by the bead beating grinder and lysis system, FastPrep-24 (MP-bio). Total RNA was extracted from the homogenate using an RNeasy Plus Universal Mini Kit (Qiagen), and reverse transcription to cDNA was performed using an iScript Advanced cDNA Synthesis Kit (Bio-Rad). qPCR was performed on a Bio-Rad CFX Connect real-time PCR detection system (Bio-Rad) using a SsoAdvanced Universal SYBR Green Supermix (Bio-Rad) and PrimePCR primers, nuclear factor (erythroid-derived 2)-like 2 (Nrf2; qMmuCID0021433), hemeoxygenase-1 (HO-1; qMmuCID0040051), NADPH quinone oxidoreductase 1 (Nqo-1; qMmuCID0017701), and glutamyl cysteine ligase catalytic subunit (GCLC; qMmuCID0019217). The relative expression of genes was measured with the ΔΔCt method using β-actin (qMmuCED0027505) as an internal standard.

2.10. Evaluation of antioxidant activity in vitro

Hydrophilic or lipophilic oxygen radical absorbance capacity (H-ORAC or L-ORAC) assays were performed using an improved method that was modified and validated by the National Agriculture and Food Research Organization (NARO) from the original method for ORAC measurements for use in evaluations of the antioxidant capacity of agricultural products and food extracts [21,22] The blank-corrected area under the fluorescence decay curve for each sample was plotted against the concentration of the standard antioxidant, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and ORAC values were expressed as Trolox equivalents (μmol Trolox equivalent/L).

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was measured using a DPPH Antioxidant Assay Kit (Dojindo) according to the manufacturer's instructions. DPPH radical scavenging activity is expressed in terms of the Trolox equivalent antioxidant capacity (TEAC), which is defined as the concentration at which 50% of the DPPH radical is scavenged, calculated based on the DPPH radical scavenging rate for Trolox standards and samples.

SOD-like activity in relation to the elimination of ROS was measured using a SOD Assay Kit-WST (Dojindo) according to the technical manual provided by the manufacturer. SOD-like activity was evaluated by the inhibition rate by SOD-like materials in the reaction in which 2-(4-iodophenyl)-3-(4-nitrophenyl-5-(2,4-disulfo-phenyl)-2H-tetrazolium, monosodium salt (WST-1) is reduced by superoxide anions to produce a water-soluble formazan dye. SOD-like activity was calculated as follows: one unit of SOD is defined as

the amount of the enzyme in a sample solution (20 μL) that inhibits the reduction reaction of WST-1 with superoxide anions by 50%.

2.11. Statistical analysis

Data are expressed as the mean ± standard deviation (SD). Statistical analyses were performed using a one-way ANOVA followed by Tukey's multiple comparison test. $p < 0.05$ was considered to be significant.

3. Results

3.1. Soy-deprestatin is present in SPD

SPD (soy protein enzymatic digest) is a food ingredient that contains the soy protein-derived antidepressant peptide soy-deprestatin. Soy-deprestatin is a peptide consisting of 10 residues of LSSTQAQQSY, produced by the enzymatic digestion of soy protein. The calibration curve prepared using synthetic soy-deprestatin as a standard (Fig. 1A), and the results of LC/MS/MS measurement of SPD (Fig. 1B) showed that the soy-deprestatin contained in SPD was 0.091%. Therefore, the dose of 100 mg/kg body weight of SPD used in this study corresponded to 0.091 mg/kg body weight of soy-deprestatin.

3.2. SPD ingestion attenuates depression-like behavior in a sub-chronic restraint stress model

After exposure to repeated restraint stress for 5 days, immobility times in the tail suspension test were longer in the restraint group than in the control group (Fig. 2). The oral administration of SPD at 100 mg/kg for 5 days completely reversed this increase in immobility times in the restraint control group, indicating that SPD exerted antidepressant-like effects in restrained mice.

3.3. Orally administered SPD activates antioxidant enzymes in a model of sub-chronic restraint stress

Previous studies indicated that repeated restraint stress increased oxidative stress and decreased antioxidant enzyme activities [16,23,24]. The present results showed that SOD, CAT, GPX, and GR activities in the brain were markedly lower in the restraint group than in the control group. The decreases induced in SOD, CAT, GPX, and GR activities by restraint stress were restored by the administration of SPD for five days, but remained unchanged in the untreated restraint group (Fig. 3A–D), indicating that the oral intake of SPD affected the activities of antioxidant enzymes in the brain.

3.4. Orally administered SPD increases antioxidants in a sub-chronic restraint stress model

Glutathione is one of the antioxidants that protect cells from ROS, such as free radicals and peroxides. It normally exists in the body in its reduced form (GSH); however, when exposed to oxidative stress, it is converted from GSH to its oxidized form (GSSG), and the ratio of GSH to GSSG is used as an indicator of oxidative stress. Previous studies reported that repeated restraint stress or chronic variable unpredictable mild stress decreased GSH levels in mice and rats [16,25–27]. We measured the levels of GSH and GSSG and the ratio of GSH/GSSG in the brain with and without the administration of SPD. As shown in Fig. 4, GSH levels were significantly lower in the restraint group than in the control group, and the oral administration of SPD restored GSH levels in stressed mice to the same levels as those in the control group (Fig. 4A). GSSG levels were higher in the restraint and SPD-treated restraint groups than in the control group (Fig. 4B). In contrast, the GSH/GSSG ratio was significantly lower in the restraint group than in the control group, and was restored in the SPD-treated restraint group to the same levels as those in the control group (Fig. 4C). These results indicate that the intake of SPD increased antioxidants in the brain and reduced oxidative stress to the same level as that in the control group.

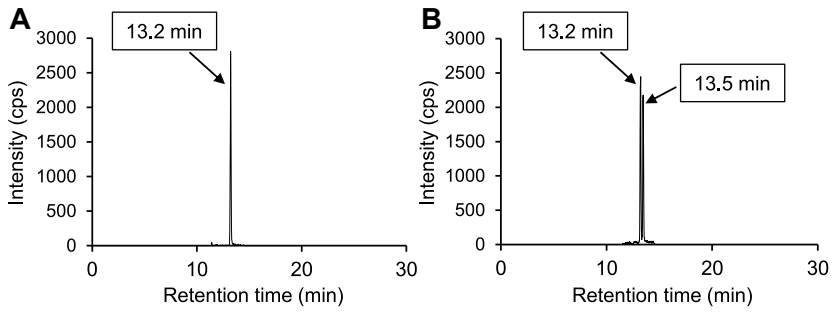

Fig. 1. Analysis of soy-deprestatin using LC/MS/MS. Synthetic soy-deprestatin (1023 ng/ml) (A), and SPD solution (1.0 mg/ml) (B) were analyzed by LC/MS/MS as described in Materials and Methods section. The peak of 13.2 min is that of soy-deprestatin.

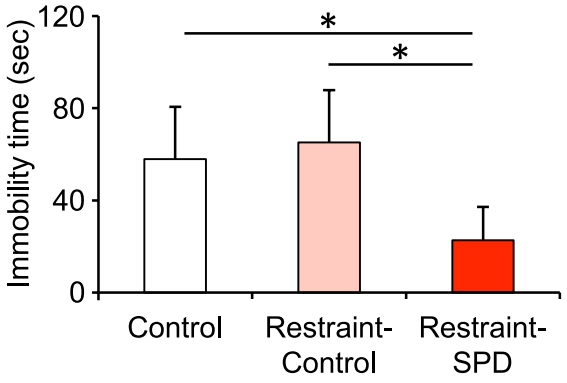

Fig. 2. Antidepressant-like effects of SPD in the tail suspension test after its oral administration to sub-chronic restraint stress mice.Mice in the sub-chronic restraint stress group administered purified water (Restraint-Control) or the sub-chronic restraint stress group administered 100 mg/kg of SPD 60 min prior to restraint stress (Restraint-SPD) were exposed to restraint stress for 5 days. Mice in the non-stress group (Control) received purified water. Mice were subjected to the tail suspension test 120 min from their release from the last restraint stress exposure. Immobility times during the 6-min test were measured. Results are expressed as the mean ± SD of 13 mice in each group. $^*P < 0.05$.

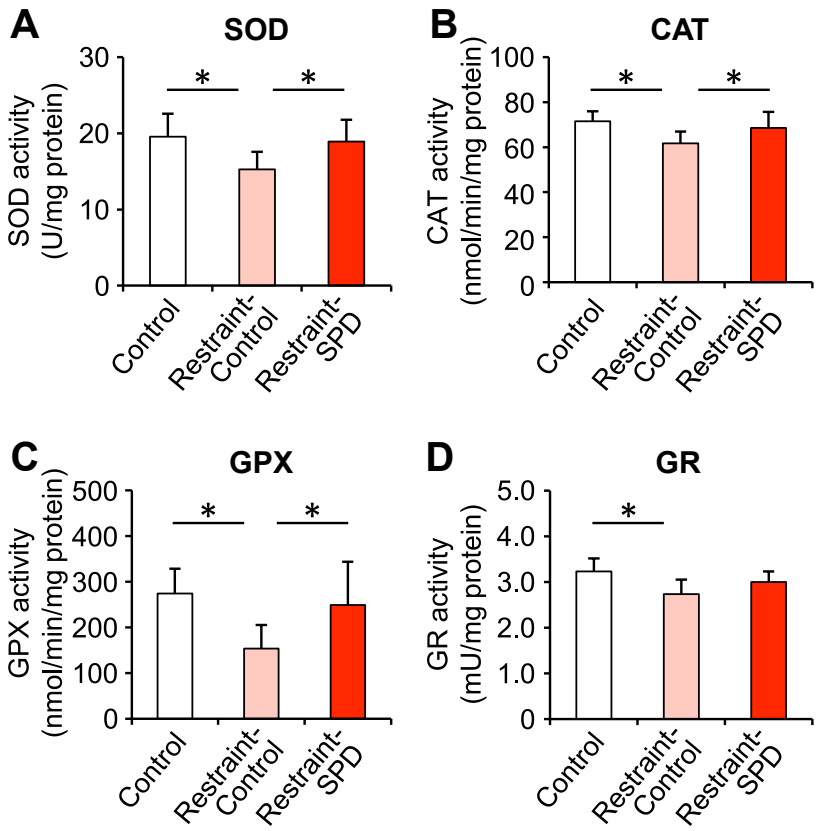

Fig. 3. Effects of SPD on the activity of antioxidant enzymes in sub-chronic restraint stress mice.The activities of SOD (A), CAT (B), GPX (C), and GR (D) in the brains of mice in the non-stress group administered water (Control), the sub-chronic restraint stress group administered water (Restraint-Control), or the sub-chronic restraint stress group administered SPD (Restraint-SPD) were evaluated. After the completion of the tail suspension test, brain tissue was removed, homogenized, and the homogenate was used for measurements. Results are expressed as the mean ± SD of 13 mice in each group. $^*P < 0.05$.

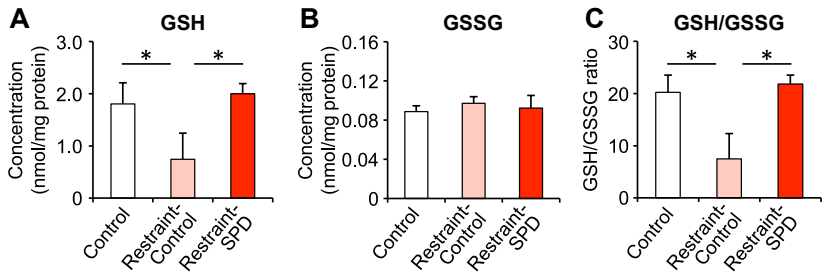

Fig. 4. Effects of SPD on changes in glutathione in sub-chronic restraint stress mice.The levels of GSH (A) and GSSG (B) and the ratio of GSH/GSSG (C) in the brains of mice in the non-stress group administered water (Control), the sub-chronic restraint stress group administered water (Restraint-Control), or the sub-chronic restraint stress group administered SPD (Restraint-SPD) were evaluated. After the completion of the tail suspension test, brain tissue was removed, homogenized, and the homogenate was used for measurements. Results are expressed as the mean ± SD of 7 mice in each group. $^*P < 0.05$.

3.5. Oral SPD administration decreases oxidative stress markers that are increased in sub-chronic restraint stress

To measure changes in oxidative stress in the brain due to restraint stress and the effects of SPD administration, we measured oxidative stress markers. Quantitative measurements of lipid and protein peroxidation and DNA damage induced by ROS in the brain were evaluated as the production of MDA, carbonyl protein, and 8-OHdG. The present results showed that MDA, carbonyl protein, and 8-OHdG levels in the brain were markedly higher in the restraint group than in the control group. On the other hand, the oral administration of SPD for five days prevented restraint stress-induced increases in MDA, carbonyl protein and 8-OHdG (Fig. 5A–C). These results indicate that the intake of SPD reduced oxidative stress in the brain.

3.6. SPD intake increases Nrf2 expression in a mouse model of sub-chronic restraint stress

We measured the expression levels of the Nrf2, HO-1, NQO-1, and GCLC genes. Nrf2 is a transcription factor that functions as a master regulator of the antioxidant response, while HO-1, NQO-1, and GCLC are its downstream antioxidant enzymes. As shown in Fig. 6A - D, the mRNA expression levels of Nrf2, HO-1, NQO-1, and GCLC were significantly lower in the restraint group than in the control group, and the oral administration of SPD prevented the decreases observed in Nrf2, HO-1, NQO-1, and GCLC in the restraint group.

3.7. Antioxidant activities of soy-deprestatin and SPD in vitro

Since soy-deprestatin was completely dissolved in water at a concentration of 1.0 mg/mL, whereas SPD was not, we presumed that SPD contained hydrophilic and lipophilic components and measured ORAC using two different methods, H-ORAC and L-ORAC. Soy-deprestatin and SPD showed the lowest H-ORAC and L-ORAC values, approximately one-tenth of vitamin C (VitC) and vitamin E (VitE), and one two-hundredth of *trans*-resveratrol (*t*-Res) (Fig. 7A and B). In addition to the ORAC assay, which is a hydrogen atom transfer (HAT)-based method, antioxidant capacity was measured by the DPPH radical scavenging assay, which is a single electron transfer (SET)-based method, and the SOD-like activity assay, which evaluates the efficiency of the reactivity of superoxide anions suppressed by antioxidants. Fig. 7C shows that the DPPH radical scavenging rates of soy-deprestatin and SPD were undetectable at the concentrations tested. The TEAC of VitC and *t*-Res were calculated as 1063.28 and 902.52 μg Trolox equivalent/μg, respectively, whereas those of soy-deprestatin and SPD were not evaluated because the DPPH radical scavenging rate did not reach 50% at any

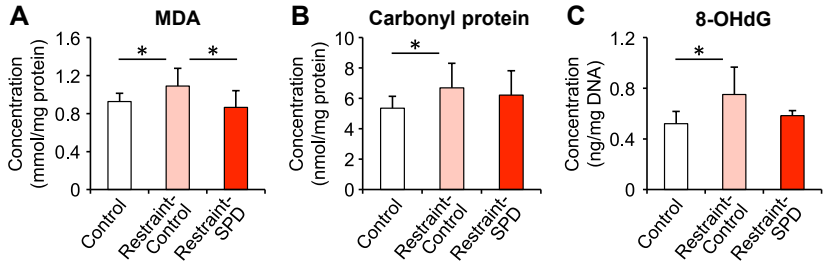

Fig. 5. Effects of SPD on oxidative stress markers in sub-chronic restraint stress mice.The levels of MDA (A), carbonyl protein (B), and 8-OHdG (C) in the brains of mice in the non-stress group administered water (Control), the sub-chronic restraint stress group administered water (Restraint-Control), or the sub-chronic restraint stress group administered SPD (Restraint-SPD) were evaluated. The brain tissue homogenate was used for measurements of MDA and carbonyl protein levels. The level of 8-OHdG was measured after DNA was extracted from brain tissue and hydrolyzed. Results are expressed as the mean ± SD of 13 mice per group for MDA and carbonyl protein and 7 mice per group for 8-OHdG. $^*P < 0.05$.

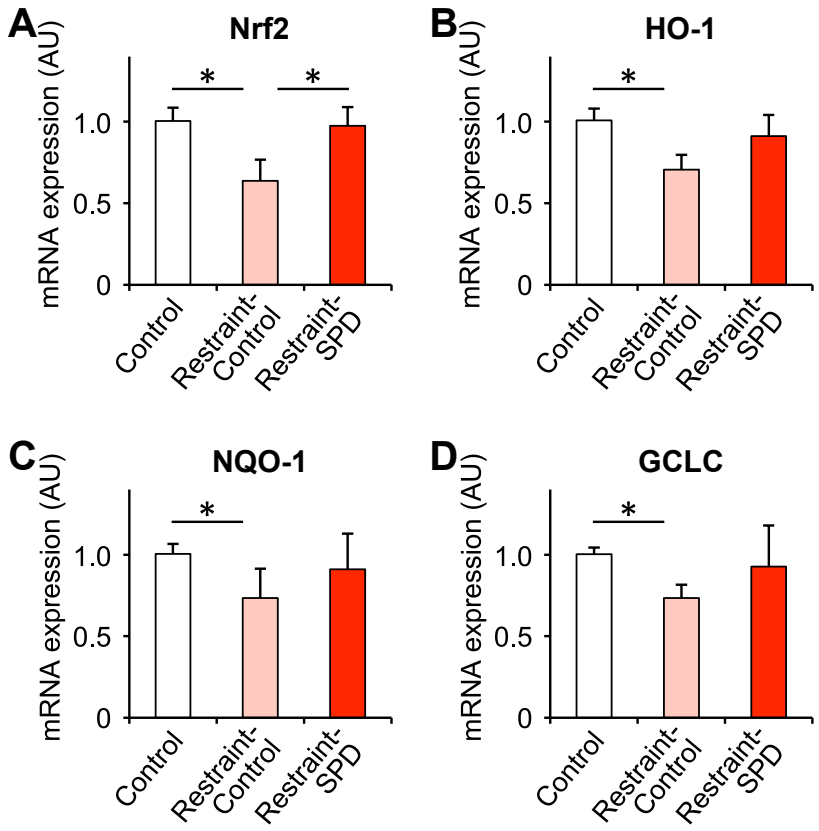

Fig. 6. Effects of SPD on mRNA expression for Nrf2 and antioxidant enzymes in sub-chronic restraint stress mice.The mRNA expression levels of Nrf2 (A), HO-1 (B), NQO-1 (C), and GCLC (D) in the brains of mice in the non-stress group administered water (Control), the sub-chronic restraint stress group administered water (Restraint-Control), or the sub-chronic restraint stress group administered SPD (Restraint-SPD) were measured by real-time PCR. Results are expressed as the mean ± SD of 6 mice in each group. $^*P < 0.05$.

concentration. Similarly, inhibition rates in the SOD-like activity assay for soy-deprestatin and SPD were undetectable and also did not reach 50% at the concentrations tested (Fig. 7D); therefore, it was not possible to evaluate SOD-like activity. On the other hand, the SOD-like activities of VitC and (−)-epigallocatechin-3-gallate (EGCg) were 315.10 and 8739.42 U/mg.

4. Discussion

The World Mental Health Report by WHO in 2022 estimated that already common conditions, such as depression and anxiety, have increased by more than 25% since the COVID-19 pandemic began, and also that 246 million (3153 cases per 100,000 population) have major depressive disorder and 374 million (4802 per 100,000 population) have anxiety disorders [28]. Mental health issues, such as depression and anxiety disorders, are pressing issues that need to be resolved.

Since persistent stress that feels unmanageable may lead to depression, we introduced a restraint stress model in which mice were immobilized for 150 min per day for 5 consecutive days. The results obtained revealed that repeated restraint stress on mice increased immobility times in the tail suspension test and also that the oral administration of SPD (soy protein enzymatic digest) reversed this increase. The present results indicate that SPD attenuated depression-like behavior induced by sub-chronic restraint stress (Fig. 2).

Since oxidative stress has emerged as a major cause of depression [29], we evaluated oxidative parameters. In the present study, we showed that the enzyme activities of SOD, CAT, GPX, and GR and the level of GSH were higher in the SPD-treated restraint group than in the water-treated restraint group (Figs. 3 and 4). We also found that the levels of MDA, carbonyl protein, and 8-OHdG were lower in the SPD-treated restraint group than in the water-treated restraint group (Fig. 5). These results suggest that the antidepressant-like effects of SPD observed in the present study were supported by its antioxidant properties.

Oxidative stress is involved in the development and progression of many diseases, the transcription factor Nrf2 regulates oxidative stress by modulating the expression of antioxidant and detoxification metabolic enzymes [30]. Oxidative stress and Nrf2 signaling pathway have recently become a subject of great interest as therapeutic targets for various diseases including diabetes, diabetic retinopathy, and rheumatoid arthritis [31–33]. Similarly, a large body of literature suggests that the Nrf2 signaling pathway plays an important role in mood disorders such as depression, and natural compounds such as sulforaphane, curcumin, quercetin, and resveratrol act as Nrf2 activators and have shown significant antidepressant effects in preclinical experiments [34–37]. In our study,

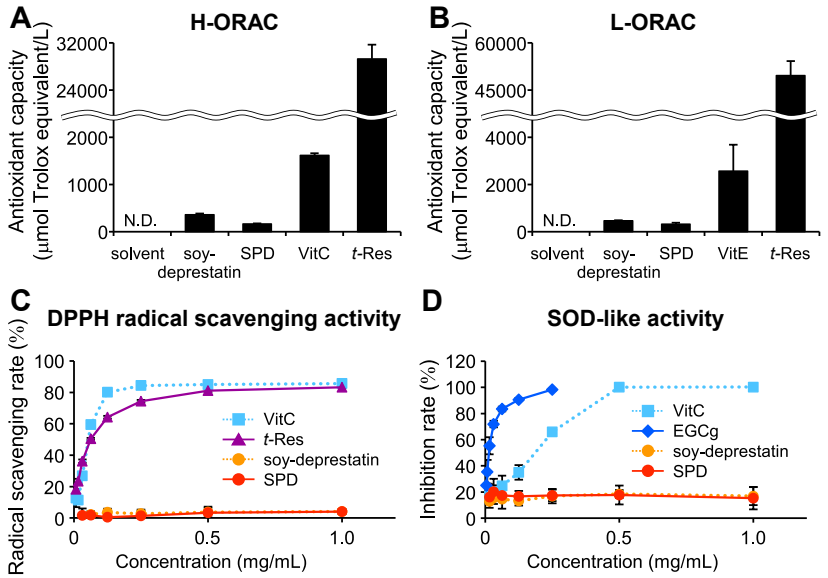

Fig. 7. Antioxidant activities of soy-deprestatin and SPD. Hydrophilic (A) or lipophilic (B) oxygen radical absorbance capacity (H-ORAC or L-ORAC), 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (C), and SOD-like activity (superoxide anion radical scavenging effect) (D) were measured to evaluate the antioxidant activities of soy-deprestatin and SPD by an *in vitro* assay. Antioxidant activities were expressed as the antioxidant capacity equivalent of Trolox (A, B), DPPH radical scavenging rate (C), and the inhibition rate of the reaction that produces formazan dye (D). Vitamin C (VitC), vitamin E (VitE), *trans*-resveratrol (*t*-Res), and (−)-epigallocatechin-3-gallate (EGCg) were used as a positive control. N.D. in panels A and B means not detected. Results are expressed as the mean ± SD of triplicate experiments.

the mRNA expression levels of Nrf2 gene and its downstream antioxidant enzyme genes, HO-1, NQO-1, and GCLC were higher in the SPD-treated restraint group than in the water-treated restraint group (Fig. 6). Although our study cannot clearly demonstrate a causal relationship due to the limitation that we have not conducted experiments that block the Nrf2 pathway and observe the expression of relevant factors, it is possible that SPD mitigates oxidative stress through activation of the Nrf2 signaling pathway. In the future, SPD, as well as other Nrf2 activators, may have great potential for clinical application by expanding our knowledge of the effects of SPD on other mood disorders such as anxiety and cognitive functions such as learning and memory, in which oxidative stress and the Nrf2 pathway have been implicated.

The soybean (*Glycine max*) is a leguminous plant that is believed to have originated in East Asia and has been widely cultivated as an agricultural crop since ancient times. Soy foods are widely consumed throughout the world. Soybeans have long been recognized as sources of high-quality protein and healthy fat and are a rich source of a number of vitamins and minerals [38].

Isoflavones are one of the key components responsible for the health benefits of soybeans. Previous studies demonstrated the antioxidant and antidepressant-like effects of isoflavones [39–41]. In the present study, antioxidant and antidepressant-like effects were observed following the administration of SPD, but were unlikely to be induced by isoflavones because SPD is a digest of a soy protein isolate and, thus, does not contain isoflavones.

Soy protein is mainly composed of the storage proteins β-conglycinin and glycinin, which account for 80–90% of the total protein in soybeans [42]. In recent years, soy-derived peptides produced from soy proteins by proteolytic enzymes or other means have also been found to have functions and are attracting increasing attention [42,43]. Studies on the physiological functions of soy-derived peptides revealed lipid-lowering, antidiabetic, antihypertensive, anticancer, antioxidant, and anti-inflammatory effects [42,43]. Furthermore, some soy-derived peptides have been implicated in mental health. For example, a chymotrypsin degradation product (βCGα323-333; FLSSTEAQQSY) consisting of the undecapeptide of the β-conglycinin α-subunit was found to exert anxiolytic-like effects in the elevated plus-maze and open field tests in mice [44]. Similarly, a decapeptide (LSSTQAQQSY) produced by the thermolysin digestion of β-conglycinin is a soy-deprestatin that exerted antidepressant-like effects in the tail suspension test and forced swimming test in mice [15].

Since SPD is an enzymatic digest of soy protein, it contains soy-deprestatin. Although further studies are needed to identify all of the constituents responsible for the antidepressant-like effects of SPD, the majority of the effects observed in the present study appeared to be attributed to soy-deprestatin.

Soy-deprestatin has been shown to exert antidepressant-like effects when administered orally, but not intraperitoneally [15]. The results shown in Fig. 7 suggest that soy-deprestatin by itself exhibited no direct antioxidant activity, and even if soy-deprestatin crossed the blood-brain barrier and reached the brain, it was not expected to act against oxidative stress, which is consistent with these findings. Similar to soy-deprestatin, the direct antioxidant activity of SPD was negligible (Fig. 7); however, mice in the SPD-treated restraint group showed the attenuation of oxidative stress and depression-like behavior. Therefore, SPD appears to exert its antioxidant and antidepressant-like effects in the brain through gut-brain communication; however, the underlying mechanisms remain

unknown. Soy-deprestatin and SPD, when taken orally, can be considered to exert their antidepressant-like effects through a novel mechanism in which they exhibit antioxidant activities in the brain through gut-brain communication without acting in the brain themselves.

The amelioration of oxidative stress was noted in the SPD-treated restraint group. Previous studies reported that food-derived ingredients, such as polyphenols, carotenoids, and polyunsaturated fatty acids, exerted antioxidant effects [45–47]; however, many enter the bloodstream after ingestion, pass through the blood-brain barrier, and then enter the brain, where they act directly. In addition, difficulties are associated with controlling the amounts of some components, such as resveratrol, curcumin, and astaxanthin, reaching the brain due to their low bioavailability [48–50]. On the other hand, the effects of soy-deprestatin were found to be mediated through gut-brain communication, without entering the bloodstream, and experiments using mice showed that antidepressant-like effects were detected at least 30 min after the oral administration of soy-deprestatin or a thermolysin digest of β-conglycinin [15].

Soy-deprestatin may exhibit its effects through the pathway mediated by the serotonin 5-HT_{1A}, dopamine D_1, or $GABA_A$ receptor systems; however, its relationship with antioxidant activity remains unknown. Although further studies are warranted, soy-deprestatin may be a food material that exerts antioxidant effects early after its ingestion without being absorbed in the body.

This is the first study to show that SPD containing soy-deprestatin exerted antioxidant and antidepressant-like effects. Soy-deprestatin may exert its antidepressant-like effects through a different mechanism than already-known food ingredients or drugs. Since over a third of patients with major depressive disorder do not have an adequate response to first-line antidepressant treatments [51,52], SPD and soy-deprestatin have potential as candidates for novel antidepressants.

5. Conclusion

The present study confirmed that SPD improved the antioxidant status by activating the Nrf2 signaling pathway in a mouse model of sub-chronic restraint stress, and provided evidence to support the antidepressant-like effects of SPD. These effects may be mediated through gut-brain communication. The results obtained herein indicate the potential of SPD in the treatment and management of stress conditions.

Ethics statement

All animal experiments were performed in strict accordance with the Guidelines for the Proper Conduct of Animal Experiments of the Science Council of Japan. Experimental protocols and procedures were approved by the Animal Experiment Committee of the Katagiri VMD Office (MKT22-04).

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Takuwa Yasuda: Writing – original draft, Investigation, Conceptualization. **Yasuhiro Kashima:** Investigation.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Takuwa Yasuda reports a relationship with UHA Mikakuto Co., Ltd. that includes: employment. Yasuhiro Kashima reports a relationship with UHA Mikakuto Co., Ltd. that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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