Antidepressant Effect of *Tetragonia tetragonoides* (Pall.) Kuntze Extract on Serotonin Turnover

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1. Introduction

Depression is a psychiatric disease as well as a chronic, recurring, and potentially life-threatening illness. The main symptoms of depression are characterized by a mood imbalance, loss of interest, and unhappiness [1]. The symptoms of depression during menopause are similar to general depression, although menopausal depression is highly affected by hormone fluctuation [2, 3]. Hormonal fluctuations such as elevation of follicle-stimulating hormone and reduction of ovarian hormone levels, i.e., estrogen and progesterone, are a common phenomenon during menopause [4].

*Tetragonia tetragonoides* (Pall.) Kuntze (TTK), known in Korea as Beonhaengcho, has similar textures and flavor properties as spinach. TTK therapy has been shown to alleviate menopausal symptoms and treat hepatic cell metabolism [5–7]. In a recent report, various herbal extracts improved menopausal symptoms such as hot flashes, weight gain, and involutional depression [8–10]. *Hypericum perforatum*, *Rhodiola rosea*, and *Crocus sativus* have also been used as alternative therapeutics for the treatment of general depressive symptoms, inhibition of monoamine reuptake, and sensitization of neurotransmitter receptors [11–13].

Serotonin or 5-hydroxytryptamine (5-HT), a monoamine neurotransmitter, plays a critical role in the pathophysiology of mood disorders, i.e., anxiety disorder and depression [14, 15]. Serotonin controls central nervous system (CNS) function, including sleep, endocrine secretion, motor function, and cognition [16, 17]. Many antidepressant drugs target the release of serotonin and its transport system, including
serotonin transporter (SERT) and inhibition of serotonin reuptake activity [18]. Selective serotonin reuptake inhibitor (SSRI) is used for treatment of depression, bone loss, and peripheral errors in postmenopausal women [19, 20]. The serotonergic systems and reproductive endocrinology have been linked in various reports, which are markedly related in mood change and behavior patterns [21, 22]. Fluoxetine is the only SSRI registered for the treatment of depression in postmenopausal women in the Unites States [23]. In addition, molecular imaging studies have observed reduction of brain SERT binding in major depressive disorders. Translational level of SERT might affect SSRI efficacy either directly or through adaptive changes in serotonergic function [24, 25].

Tryptophan hydroxylase (TPH) belongs to the enzyme superfamily of aromatic amino acid hydroxylases and is the regulator of serotonin synthesis and serotonin activity in the brain [26, 27]. There are two isoforms of TPH (TPH-1 and TPH-2), which mediate the synthesis of most peripheral serotonin and are predominantly expressed in the gut, thymus, spleen, and pineal gland [28, 29]. Especially, TPH-2 is a neuronal-specific enzyme that is predominantly expressed in the neurons of raphe nuclei in the brain stem, and it is the rate-limiting enzyme in serotonin synthesis as well as a key factor for serotonin transmission in the CNS [30, 31]. Recent studies have assessed the recurrence of patients suffering from depression, and targeting of TPH-2 was shown to improve the effectiveness of antidepressant medications [32, 33].

As mentioned before, we hypothesized that TTK might be effective in improving menopausal depression, which is known to have various pharmacological effects on improving the symptoms of menopausal symptoms, antiobesity, and hepatocyte apoptosis induction. In the present study, we examined the therapeutic effect of TTK on serotonergic system through the expression of TPHs, SERT and 5-HT reuptake activity in in vitro model. We also used ovariectomized (OVX) rats to investigate whether or not TTK can elevate serum levels of serotonin and improve immobility time in an in vivo model.

2. Materials and Methods

2.1. Preparation of TTK Extract. TTK was purchased from a commercial vender at Kwangmyung-Dang (Ulsan, South Korea). All raw materials and extracts were deposited at the Korea Institute of Oriental Medicine (KIOM130081-3). Dried Korea). All raw materials and extracts were deposited at the a commercial vender at Kwangmyung-Dang (Ulsan, South

2.2. Cell Lines and Cell Culture. The rat basophilic leukemia cell line RBL-2H3 was purchased from the ATCC (American Type Culture Collection). RBL-2H3 cells were maintained in Minimum Essential Medium (MEM) α containing 10% fetal bovine serum, 100 U/mL of penicillin, and 100 mg/mL of streptomycin in a humidified atmosphere containing 5% CO₂ at 37°C.

2.3. Reagents and Antibodies. ASP (4-Di-1-ASP (4-(4-(Dimethylamino)styryl)-N-Methylpyridinium Iodide)) was obtained from Invitrogen (Carlsbad, CA, USA). LPS (Lipopolysaccharide) and Fluoxetine were purchased from Sigma-Aldrich (St Louis, MO, USA). Stock solutions were prepared in dimethyl sulfoxide (DMSO) and stored at -20°C. ASP and LPS were diluted in fresh medium before each experiment, and the final concentration of DMSO was <0.1%.

2.4. Reverse Transcription and Real-Time PCR. Total RNA was extracted using RNeasy Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. cDNA was synthesized using 1 μg of total RNA with a Thermo Scientific RevertAid First Strand cDNA Synthesis Kit (Thermo, Massachusetts, USA) and amplified by RT-PCR using AmpliTaq Gold DNA polymerase and Quantitative real-time PCR. cDNA was amplified using Premix ExTaq (TaKaRa Bio Inc., Shiga, Japan) with SYBR Premix EX Taq (TaKaRa Bio Inc.) using the BI PRISM 7500HT Sequence Detection System (Applied Biosystems, USA). Primer was synthesized by Macrogen Inc. (Seoul, South Korea). Actin expression was used as a control, and the primers used for RT-PCR are listed in Table 1.

2.5. Cell Growth Inhibition Assay. Cell viability was assessed using MTT assays. A total of 1x10⁵ cells/mL were seeded in 96-well plates, incubated for 16 h, and treated for 72 h with TTK at 37°C. After treatment, medium was replaced with an equal volume of fresh medium containing 2 mg/mL of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich, St Louis, MO, USA) diluted in PBS and incubated for 4 h at 37°C, after which the medium was discarded. After confirming formation of formazan, medium was completely removed and analyzed by adding 150 μL of DMSO to melt the formazan. Cell viability was determined by measuring absorbance at 540 nm using an ELISA microplate reader (Synergy HTX Multi-Mode Reader; BioTek, Winooski, VT, USA).

2.6. 5-HT Uptake Assay. 5-HT transport activity into RBL-2H3 cells was assessed by ASP assay. Briefly, RBL-2H3 cells were harvested at approximately 60-80% confluence and seeded in 24-well plates at a density of 5x10⁵ cells/well in 500 μL of nucleoside-free MEM α containing 10% fetal bovine serum. Cells were untreated or treated with TTK at different concentrations (50 or 100 μg/mL). After 16 h, medium was replaced with serum-free medium, followed by incubation for 4 h at 37°C. Cells were then washed with PBS buffer three times, incubated with medium containing 1 mM ASP, and dissolved in DMSO at 37°C for 1 h in the dark. Next, ASP-containing medium was removed, and cells were washed three times with PBS. Fluorescence density was measured using a multifluorescent microplate reader (SpectraMax Paradigm Multi-Mode Microplate Reader; Molecular devices, Sunnyvale, California, USA) at λex=475 nm and λem=605 nm.

2.7. Experimental Animals and Treatments. Female Sprague-Dawley rats (6-weeks-old, weight 130-150 g, total n=32, n=8
were considered to be statistically significant. PRISM software (v6.0; GraphPad, CA, USA).

or ANOVA with Tukey for multiple comparison tests using Paired Student’s t-tests were used to compare each group/two.fitted./one.fitted/zero.fitted. Statistical Analysis.

other than that necessary to keep the rat’s head above the experiment. Immobility was defined as no additional activity.

The rats were exposed to a pretest for 10 min prior to the experiment. The swimming test was a polycarbonate cylinder (diameter: 300 mm, depth: 400 mm) filled with room temperature water. Passive and immobile after a period of vigorous activity. The swimming test, and this method involves the rats becoming/stimulating SERT, which plays a critical role in depression/stimulating SERT, which plays a critical role in depression.

SERT expression. On the contrary, TPH-1 and SERT expression 100

TPH-1 is not significantly expressed in the brain [34]. We demonstrated that treatment of RBL-2H3 cells with 50 and 100 μg/mL of TTK significantly increased TPH-2 mRNA expression. On the contrary, TPH-1 and SERT expression decreased upon TTK treatment (Figure 2).

2.8. Serum Serotonin Analysis. Blood samples were collected directly from the inferior vena cava using a 1-mL syringe at the end of the experiment. Serum was obtained by centrifugation at 4,000 x g for 10 min and stored at -70°C until use. Serum serotonin levels were measured using a Serotonin ELISA kit (Abnova; Taipei, Taiwan) according to the manufacturer’s instructions.

2.9. Forced Swimming Test. Rats were subjected to a forced swimming test, and this method involves the rats becoming passive and immobile after a period of vigorous activity. The test apparatus was a polycarbonate cylinder (diameter: 300 mm, depth: 400 mm) filled with room temperature water. The rats were exposed to a pretest for 10 min prior to the experiment. Immobility was defined as no additional activity other than that necessary to keep the rat’s head above the water.

2.10. Statistical Analysis. Data is presented as means ± SD. Paired Student’s t-tests were used to compare each group or ANOVA with Tukey for multiple comparison tests using PRISM software (v6.0; Graph Pad, CA, USA). P values < 0.05 were considered to be statistically significant.

3. Results

3.1. Cytotoxicity Effect of TTK on RBL-2H3. RBL-2H3 cells were treated with various concentrations of TTK for 72 h. Upon treatment with 10-1000 μg/mL of TTK, viability rate of cells treated with 500 μg/mL of TTK was approximately 80% compared to that of nontreatment. Based on the data, a subsequent experiment was conducted using a concentration of TTK below 500 μg/mL (Figure 1).

3.2. Effects of TTK on TPH-1, TPH-2, and SERT mRNA Expression. The effects of TTK on mRNA expression levels of TPH-1, TPH-2, and SERT, which are associated with serotonin synthesis and uptake, were assessed. Especially, TPH-2 is expressed in peripheral tissues of the brain, which is important in the regulation of mood disorders, whereas TPH-1 is not significantly expressed in the brain [34]. We demonstrated that treatment of RBL-2H3 cells with 50 and 100 μg/mL of TTK significantly increased TPH-2 mRNA expression. On the contrary, TPH-1 and SERT expression decreased upon TTK treatment (Figure 2).

3.3. Effects of TTK on 5-HT Uptake by RBL-2H3 Cells. To further explore the effects of TTK on 5-HT uptake by RBL-2H3 cells, 5-HT uptake was analyzed by measuring SERT activities based on ASP fluorescent intensities in RBL-2H3 cells. LPS is known to enhance 5-HT uptake by stimulating SERT, which plays a critical role in depression [34], whereas Fluoxetine is known to reduce 5-HT uptake by acting as an SSRI. The results showed that 5-HT uptake significantly decreased upon TTK treatment in a dose-dependent manner similar to the effect of 10 μM Fluoxetine (Figure 3).
3.4. Serum Serotonin Level and Immobility Time. Dietary supplementation with TTK extract affected serum levels of serotonin in rats. As shown in Figure 4, serum serotonin levels were significantly elevated by supplementation with 1% or 2% TTK extract compared to the vehicle group. However, serum levels of serotonin were not altered in the 0.5% TTK-treated group. The effect of TTK on immobility time in rats is shown in Figure 5. The doses of TTK extract (1% and 2%) significantly reduced the duration of immobility in comparison with vehicle control, but its effect was not shown in 0.5% TTK-treated group. The maximal antidepressant effects of TTK extract were obtained with 1% TTK diet, and there were no significant differences between 1% and 2% TTK. These results indicate that TTK extract improved immobility in the forced swimming test, suggesting this beneficial compound ameliorated depression in rats.
TTK extract in RBL-2H3 cells. that SERT transcription was significantly down-regulated by influence synaptic 5-HT activity [40, 41]. We also observed transcription induces TPH activity and 5-HT release, which interface for neurotransmitters [39]. Up-regulation of TPH-2 and peripherals might be linked, acting as a regulatory in the brain [33, 38]. Interestingly, the serotonins of brain gut, whereas TPH-2 is the predominant gene transcribed mainly in peripherals such as enterochromaffin cells of the isoforms, TPH-1 and TPH-2 [33, 38]. TPH-1 is utilized is involved in the serotonin synthesis and has two different concentrations of TTK extract. TPH is an isoenzyme, and it TPH-1 was significant reduced following treatment with all (50 and 100 μg/mL of TTK. Quantitative real-time RT-PCR analysis revealed that mRNA expression of TPH-2 was dose-dependently up-regulated by TTK extract (50 and 100 μg/mL). In addition, mRNA expression of TPH-1 was significant reduced following treatment with all concentrations of TTK extract. TPH is an isoenzyme, and it is involved in the serotonin synthesis and has two different isoforms, TPH-1 and TPH-2 [33, 38]. TPH-1 is utilized mainly in peripherals such as enterochromaffin cells of the gut, whereas TPH-2 is the predominant gene transcribed in the brain [33, 38]. Interestingly, the serotonin of brain and peripherals might be linked, acting as a regulatory interface for neurotransmitters [39]. Up-regulation of TPH-2 transcription induces TPH activity and 5-HT release, which influence synaptic 5-HT activity [40, 41]. We also observed that SERT transcription was significantly down-regulated by TTK extract in RBL-2H3 cells.

SERT is a member of neurotransmitter-sodium symporter, and it plays an important role in the released and extinguished serotonin through transport across the presynaptic membrane [41], and numerous studies have reported the antidepressant effects of reduction of SERT mRNA expression [42, 43]. In the current study, we investigated whether or not reduction of SERT transcription can regulate 5-HT reuptake activity in TTK-treated RBL-2H3 cells. Our results show that 5-HT reuptake activity was dose-dependently reduced by TTK treatment in LPS-induced RBL-2H3 cells, and this pattern was also observed in the SSRI (Fluoxetine; 10 μM)-treated group. The serotonergic system has long been related in the pathogenesis of depression, and the potential evidence involves the inhibition of depression by SSRIs [44]. Other studies have indicated that herbal extracts and their ingredients may exert SSRI-like effects in in vitro and in vivo models [44, 45]. These data suggest that TTK extract may be critical for expression of TPH-1, TPH-2, and SERT as well as inhibition of 5-HT reuptake activity in RBL-2H3 cells via a mechanism similar to those of SSRIs.

In the present study, we determined that rats fed TTK extract (0.5%, 1%, and 2%) for 8 weeks showed up-regulation of serotonin levels in serum or the reduction of immobility in behavioral test upon 1% and 2% TTK treatment, and the data have already been collected by patent office in South Korea [46]. Peripheral serotonin has long been reported to be the suitable marker for diagnosis of depression, as blood serotonin shares a similar serotonin uptake and release mechanism as serotonergic neurons [47–49]. Furthermore, the forced swimming test is widely used to screen potential antidepressive effects in vivo, and antidepressants reduce immobility time in this test [50, 51]. Several important clinical implications have emerged based on the finding that 5-HT is associated with the behavioral effects of SSRIs in the forced swimming test [50, 51].

The present study assessed the expression of TPH-1, TPH-2, and SERT as well as 5-HT reuptake activity in RBL-2H3 cells and described serum levels of serotonin and immobility time in OVX female rats. Based on the findings, it appears that TTK may be a potent therapeutic agent for the treatment of depression based on its regulation of serotonin-mediated genes and peripheral serotonin level(s) in our in vitro and in vivo model. However, TTK crude extract contains variety of compounds, and further studies are needed on fractions or partial purification of the extract to identify the active pharmaceutical ingredient among the TTK-derivative compounds.

4. Discussion

Herbal extracts have recently been reported as complementary therapies for antidepressant purposes, and this type of treatment is expected to prevent onset of mood disorders and regulate serotonin reuptake activity [35, 36]. However, these alternative remedies are limited in the treatment of severe depression [9, 37]. In the present study, we examined TPH-1 and TPH-2 mRNA expression levels in RBL-2H3 cells after treatment with 0, 50, and 100 μg/mL of TTK. Quantitative real-time RT-PCR analysis revealed that mRNA expression of TPH-2 was dose-dependently up-regulated by TTK extract (50 and 100 μg/mL). In addition, mRNA expression of TPH-1 was significant reduced following treatment with all concentrations of TTK extract. TPH is an isoenzyme, and it is involved in the serotonin synthesis and has two different isoforms, TPH-1 and TPH-2 [33, 38]. TPH-1 is utilized mainly in peripherals such as enterochromaffin cells of the gut, whereas TPH-2 is the predominant gene transcribed in the brain [33, 38]. Interestingly, the serotonin of brain and peripherals might be linked, acting as a regulatory interface for neurotransmitters [39]. Up-regulation of TPH-2 transcription induces TPH activity and 5-HT release, which influence synaptic 5-HT activity [40, 41]. We also observed that SERT transcription was significantly down-regulated by TTK extract in RBL-2H3 cells.

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Data Availability

All data used to support the findings of this study are included within the article.

Conflicts of Interest

All authors declared that there are no conflicts of interest.

Authors’ Contributions

Hyun Yang, Hye Jin Kim, Eui-Ju Hong, Bo-Jeong Pyun, Byung-Seob Ko, and Hye Won Lee performed the research, analyzed the data, and wrote the manuscript; Eui-Ju Hong and Hyun Yang performed in vivo experiments and data analysis; Hye Jin Kim performed in vitro experiments and data analysis. All authors read and approved the final manuscript. Hyun Yang and Hye Jin Kim contributed equally.

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