Antidepressant-like Effects of *Polygonatum humile* Fisch via Serotonergic System in Mouse Model: Involvement of the Serotonin Subunit 6 Receptor

Dool-Ri Oh¹, Yujin Kim¹, Kyo-Nyeo Oh¹, Yonguk Kim¹ and Donghyuck Bae^{1*}

¹Jeonnam Bioindustry Foundation, Jeonnam Institute of Natural Resources Research (JINR), Jeollanamdo 59338, Republic of Korea.

Authors' contributions

This work was carried out in collaboration among all authors. Authors DRO and YK designed the experiment, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author DB managed the analyses of the study and wrote the review & editing of the manuscript. Authors KNO and YK was involved with experimentation. All authors read and approved the final manuscript.

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ABSTRACT

Polygonatum humile Fisch is commonly called Sookjuk in Korea. The roots of *P. humile* are typically used in traditional medicine as an anti-rheumatic, demulcent, and sedative agent. The aim of this study was to evaluate the potential of the *P. humile* extracts to treat depression. Mice were administered *P. humile* water extract (PH) and were subjected to the forced swim test (FST), tail suspension test (TST), and open-field test (OFT). The levels of brain monoamines including serotonin (5-HT), norepinephrine, and dopamine were evaluated. In addition, the PH antidepressant-like effects were studied based on the regulation of 5-HT-mediated Ca^{2+} and extracellular signal-regulated kinase 1/2 (ERK1/2) in human 1321N1 cells stably expressing the 5-HT subunit 6 receptor (5-HT₆). In FST and TST, PH (300 mg/kg) significantly reduced the immobility time without changing the locomotor activity in mice. In addition, PH enhanced the level of 5-HT in the mice brain. The results further indicated an inhibitory activity of PH on both the 5-HT₆ receptor expression level and the 5-HT₆ receptor dependent downstream signal

*Corresponding author: E-mail: bdhyuch@naver.com;

pathway ERK1/2. These findings indicated antidepressant-like effects of PH as evidenced by decreased immobility time in mice through a 5-HT₆ receptor antagonistic mechanism.

Keywords: *Polygonatum humile*; *fisch*; antidepressant; serotonin; serotonin subunit 6 (5-HT₆).

1. INTRODUCTION

Serotonin (5-hydroxytryptamine, 5-HT) is an important monoamine neurotransmitter found in both the central nervous system and peripheral nervous system [1]. Previous reports have demonstrated that 5-HT plays a role in anxiety, emotion, cognition, and depression. The 5-HT receptors are divided into seven families, namely, the 5-HT₁₋₇ receptors, with a total of 14 distinct subtypes. All the 5-HT receptors are known as G-protein-coupled receptors, except for the 5-HT₃ receptor, which is a member of ligand-gated ion channels. In particular, 5-HT₆ receptor is coupled to the G_{αs} protein, which results in the activation of adenylyl cyclase, thereby increasing the intracellular cyclic AMP (cAMP)-protein kinase A signaling [2]. In addition, 5-HT₆ plays an important role in cognition, mood, psychosis, and depression [3,4]. Findings from recent pharmacological studies indicated that both the blockade and stimulation of 5-HT₆ may evoke antidepressant-like effects [5]. In addition, preclinical studies have shown that SB399885, a 5-HT₆ antagonist, exerts an antidepressant-like effect in the forced swimming test (FST) and tail suspension test (TST) in rats and mice [6,7]. Moreover, SB258585, which is a selective 5-HT₆ antagonist, is currently being developed for the treatment of depression and anxiety [8]. Thus, both antagonists and agonists of the 5-HT₆ receptor may be useful in treating depression.

Polygonatum humile Fisch, commonly called Sookjuk in Korea, belongs to the family Convallariaceae. The roots and rhizomes of *P. humile* have been used in traditional medicine, particularly for their anti-rheumatism, demulcent, and sedative effects [9]. In modern phytochemical studies, several components of *P. humile* have been identified, including polyphenols and flavonoids [10]. However, antidepressant-like effects of *P. humile* have not yet been reported.

The aim of the present study was to evaluate the effect of the blockade of the 5-HT₆ receptor on the antidepressant-like effects of *P. humile* water extract (PH). We examined whether PH

produced antidepressant-like effects in FST, TST, and open-field test (OFT) in mice. Additionally, the effects of PH on the regulation of the brain monoamines levels were examined. In addition, we used human 1321N1 cells stably expressing the 5-HT₆ receptor to evaluate the effects of PH on the regulation of 5-HT-mediated Ca²⁺ and the extracellular signal-regulated kinase 1/2 (ERK1/2), which is a key signal transduction mediator of the 5-HT₆ receptor.

2. MATERIALS AND METHODS

2.1 Reagents

Dulbecco's Modified Eagle's Medium (DMEM) was purchased from Hyclone Laboratories Inc. (Logan, UT, USA). Fetal bovine serum (FBS), 100 U/mL penicillin, 100 µg/mL streptomycin, sodium pyruvate, Lipofectamine™ 2000, pluronic F-127, and Fura-2/acetoxymethyl ester (AM) were purchased from Invitrogen Inc. (Grand Island, NY, USA). Geneticin (G-418) was purchased from Calbiochem (San Diego, CA, USA). Serotonin (5-HT), norepinephrine (NE), and dopamine (DA) enzyme-linked immunosorbent assay kits were purchased from Abnova Corp. (Taipei City, Taiwan). The primary antibodies against p44/42 MAPK (ERK1/2) and phosphorylated-p44/42 MAPK (p-ERK1/2), and the HRP-conjugated anti-rabbit IgG were purchased from Cell signaling Technology (Beverly, MA, USA).

2.2 Preparation of the PH Extract

The *P. humile* Fisch (specimen voucher number: JINR-PH-D-124) whole plant used in this study was collected (May 2013) in the Gangjin County (Jeollanamdo, Republic of Korea). *P. humile* (0.5 kg) was extracted using 20 volumes of water at 100 °C for 4 h. The extracted solution was then filtered, concentrated with an evaporator under a vacuum, and freeze-dried. The dried PH was stored at 4 °C to avoid compound degradation before further use in the experiments.

2.3 Animals

Male 6-week-old ICR mice weighing 25–29 g were purchased from the Central Lab Animal Inc.

(Seoul, Republic of Korea). The animals were maintained at a constant room temperature of 22 ± 2 °C with a humidity of 50 ± 5 %, and had free access to water and food under a 12:12-h light:dark cycle (lights on at 8:00 am). The animals were acclimatized to the laboratory environment for 4 days before the beginning of the experiments.

2.4 Experimental Groups and Drug Administration

The mice were randomly assigned to three groups (n = 5) based on the treatment as follows: Group I, control (vehicle); Group II, PH extract at 100 mg/kg/day (PH 100); and Group III, PH extract at 300 mg/kg/day (PH 300). Oral administration of PH (100 and 300 mg/kg/day) was continued for 14 days. After 30 min of the last PH administration, the animals were subjected to the behavioral tests.

2.5 Forced Swim Test (FST)

During the FST, mice were individually placed in a Plexiglas cylinder (diameter 15 cm) filled with 20 cm of water at 22–25 °C, and the duration of immobility, climbing, and swimming behaviors was scored during the last 5 min of the 6-min test. Immobility was scored when a mouse stopped struggling and remained floating motionless in the water, making only those movements necessary to keep its head above the water surface.

2.6 Tail Suspension Test (TST)

The immobility induced by tail suspension was carried out according to the method described by Steru et al. [11], with modification. Mice were suspended on the edge of a table 50 cm above the floor by an adhesive tape placed approximately 1 cm from the tip of the tail. The behavior of the mice was recorded at 6 min, and the immobility time was determined by an observer. Immobility was defined as the absence of any limb or body movements. Mice were considered immobile only when they hung passively and completely motionless.

2.7 Open-Field Test (OFT)

The general locomotor activity was evaluated using the OFT, based on a method described previously with slight modifications [12]. The OFT apparatus consisted of a 60 × 60 × 20 cm

wooden box, divided into 25 equal squares. Each mouse was gently placed in a corner of the apparatus and observed for 5 min. A count was considered when the mouse completed a crossing from one square to the next. After each trial, the apparatus was cleaned with 70% ethanol.

2.8 Tissue Sampling

The mice were sacrificed immediately after the OFT. Blood was collected during decapitation. The serum was separated using refrigerated centrifugation at 3,000 rpm and 4 °C for 20 min, and stored at –80 °C until further analysis. The brains were quickly removed, and the brain tissues immediately frozen in liquid nitrogen and stored at –80 °C until further analysis.

2.9 Serum Biochemical Parameters

Serum glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), blood urea nitrogen (BUN), and creatinine levels were measured using the appropriate kits (DRICHEM 4000i, FUGI-FILM, Tokyo, Japan).

2.10 Measurement of Brain Monoamines

Quantification of monoamines was performed according to the method described previously [13], with some modifications. Briefly, the brain tissues were homogenized on ice in a 10-fold volume of an extraction buffer (PRO-PREP™ protein extraction solution, iNtRON Biotechnology, Sungnam, Korea), and incubated for 2 h at 4 °C with shaking. Subsequently, the lysates were centrifuged separately at 13,000 rpm for 20 min at 4 °C. Protein contents were determined using the bicinchoninic acid protein assay reagent (Thermo Scientific, Rockford, IL) with bovine serum albumin as standard. The monoamines, 5-HT, NE, and DA were determined in the homogenates of the brain tissues as per the method described by the manufacturer (Abnova).

2.11 Cell Culture and Transfection

Human astrocytoma 1321N1 cells, stably expressing the human 5-HT₆ receptor gene (cat. ES-316-CV, clone C1, PerkinElmer, Boston, MA, USA), were grown in DMEM supplemented with 10% FBS, 1 mM sodium pyruvate, and 0.4 mg/mL G-418 for receptor expression selection, and incubated at 37°C in a 5% CO₂ incubator.

For transient transfection with the G-protein chimeric gene (G_{qs5}), 1×10^5 cells/mL were placed on a 96-well black wall/clear bottom (BD Falcon, Franklin Lakes, NJ) and transfected with G_{qs5} using Lipofectamine™ 2000 for 48h following the manufacturer's instructions.

2.12 Assay of 5-HT₆ Receptor Activity using the High-Throughput Screening (HTS) FDSS6000 System

The concentration of intracellular calcium ($[Ca^{2+}]_i$) was measured using Fura-2/AM and monitored by exciting wavelengths of 340/380 nm. Briefly, 1321N1 cells were washed one time with N-(2-hydroxyethyl)piperazine-N'-2-ethanesulfonic acid (HEPES)-buffered solution (150 mMNaCl, 5 mM KCl, 1 mM $MgCl_2$, 10 mM HEPES, 10 mM glucose, 2 mM $CaCl_2$, pH 7.4) and loaded with 5 μ M Fura-2/AM and 0.001% pluronic F-127 at 37 °C in a 5% CO₂ incubator. After 60-min incubation, the cells were washed three times with the HEPES-buffered solution and maintained with a volume of 80 μ L/well in 96-well plates. Cells were monitored by exciting wavelengths of 340/380 nm. For the antagonist experiments, cells were pre-incubated with the PH extract for 1 min before the addition of a 5-HT agonist. All data were collected and analyzed using the BD pathway 855 system and the associated AttoVision imaging software (BD Biosciences, San Jose, CA).

2.13 Western Blot Analysis

The 5-HT₆ receptor stably expressing 1321N1 cells were placed in 6-well plates at a density of 1×10^6 cells/mL. After 24 h, the cells were incubated with serum-free DMEM for 2 h, and with PH extract alone for 15 min. The antagonistic effects were measured after exposure to the PH extract for 15 min, and subsequent treatment with 100 μ M 5-HT for 10 min. Subsequently, the cells were washed with cold phosphate-buffered saline and lysed using the PRO-PREP protein extraction kit (iNtRON Biotechnology). Protein contents were determined using the bicinchoninic acid protein assay reagent (Thermo Scientific) with bovine serum albumin as standard. The total proteins were incubated in boiling water for 5 min. Samples were run at 100 μ g/mL of protein on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels, using the Power Pac Basic electrophoresis apparatus (Bio-Rad, Hercules, CA). Subsequently, the protein samples were transferred to polyvinylidene

difluoride (PVDF) membranes (0.45 mm pore size, Pall, USA). After blocking for 1 h at room temperature in blocking solution (1× Tris-buffered saline [TBS] containing 0.2% Tween 20 and 5% skim milk), the membranes were washed three times with washing solution (1× TBS containing 0.2% Tween 20). The membranes were incubated with the primary antibodies anti-p-ERK1/2 (1:1000) and anti-ERK1/2 (1:1000) overnight at 4 °C. After washing five times in washing solution, the membranes were incubated in diluted HRP-conjugated anti-rabbit IgG secondary antibody (1:5000) for 1 h at room temperature. The detection was performed according to the manufacturer's instructions using a Chemiluminescence Detection Kit (Merck Millipore, Darmstadt, Germany).

2.14 Statistical Analysis

Data were presented as the mean $\pm$ standard error of the mean (SEM). The data were statistically evaluated using the Student's t-test or one-way analysis of variance (ANOVA). Differences among groups were assessed using Duncan's multiple range tests. All statistical analyses were performed using GraphPad Prism (GraphPad Inc., San Diego, California, USA), and statistical significance was considered at $p < 0.05$.

3. RESULTS AND DISCUSSION

According to the World Health report, approximately 264 million people suffer from a mental or behavioral disorder [14]. Depression is commonly known as the mental disorder associated with feeling sad, anxious, hopeless, worthless, and guilty. Depression is also associated with several other psychiatric disorders, poor health, and increased risk of suicide [15]. Therefore, recent studies have reported that many of the available antidepressant compounds act via different mechanisms including the serotonergic, noradrenergic, and/or dopaminergic systems.

The TST and FST behavioral tests are widely used for the screening of antidepressant drugs [16]. In FST, mice are forced to swim in a restricted space from which they cannot escape, and are forced to exhibit a characteristic behavior of immobility. Immobility behavior, reflecting a state of despair, is reduced by several agents such as amphetamine and atropine, which are therapeutically effective in human depression. The TST also induces a state of despair in

animals. This immobility is claimed to reproduce a condition similar to human depression [11, 17]. In the present study, we confirmed the antidepressant-like effect of PH in both the FST and TST. Oral administration of PH (300 mg/kg/day) significantly decreased the immobility time in FST (Fig. 1). On the other hand, administration of PH (300 mg/kg/day) was associated with increased duration of swimming ($p < 0.05$) and climbing behaviors ($p > 0.05$). In addition, PH (300 mg/kg/day) also reduced the

immobility time in the TST ($p < 0.05$, Fig. 2). The results also indicated that the administration of PH at 100 mg/kg did not induce any changes in the immobility time in both the FST and TST ($p > 0.05$). Based on these results, we concluded that PH at a dose of 300 mg/kg might exert antidepressant effects. Fig. 3 depicts the effects of PH on the OFT results. The results indicated no alterations in the locomotor activity of mice treated with PH regardless of the dose used compared with the control groups.

Fig. 1. Evidence of the antidepressant effect of PH in mice in the FST. Duration of immobility, swimming, and climbing behaviors recorded in the 5-min test session of FST. PH 100, *P. humile* extract 100 mg/kg; PH 300, *P. humile* extract 300 mg/kg. Values are expressed as the mean $\pm$ standard error of the mean (n = 5). * $p < 0.05$ compared with the respective control group

Fig. 2. Evidence of the antidepressant effect of PH in mice in the TST. Duration of immobility behaviors recorded in the 6-min test. ns, not significant; PH 100, *P. humile* extract 100 mg/kg; PH 300, *P. humile* extract 300 mg/kg. Values are expressed as the mean $\pm$ standard error of the mean (n = 5). * $p < 0.05$ compared with the control group

Fig. 3. Effects of PH on the mice locomotor activity (number of crossings) in the OFT. Locomotor activity was scored during 5-min test. PH 100, *P.humile* extract 100 mg/kg; PH 300, *P.humile* extract 300 mg/kg. Values are expressed as the mean $\pm$ standard error of the mean (n = 5)

We investigated the acute oral toxicity of PH (100 and 300 mg/kg/day) after administration in mice for 14 days. The body weight, food intake, organ weight, and serum parameters are summarized in Table 1. The body weight, food intake, and organ weight were not significantly different between the control group and the PH groups. Furthermore, to evaluate the potential toxic effects of ingesting PH, serum toxicological markers indicating liver and kidney injuries were measured at the end of the experiment. The levels of GOT, GPT, creatinine, and BUN were not significantly changed in PH-treated mice compared with the levels in the control group. These results indicated that exposure to PH did not cause obvious toxic effects.

Based on previous studies, the monoamines hypothesis of depression has been established whereby one of the mechanisms underlying depression includes the significant depletion of monoamines, such as 5-HT, DA, and NE [18]. In the present study, we demonstrated that daily administration of PH for 14 days significantly enhanced the 5-HT levels, indicating that the PH exerted antidepressant-like effects through the serotonergic system. As shown in Table 2, when mice were exposed to PH (300 mg/kg/day, oral administration) for 14 days, the 5-HT levels in the brain tissues were increased compared with the control groups ($p < 0.05$). However, the DA and NE levels did not significantly increase compared with the control groups ($p > 0.05$). These results suggested that PH is dependent on the

serotonergic system and the elevation of 5-HT levels in the mice brain.

The 5-HT₆ receptor plays an important role in the treatment of depression, cognition, and emotion. Thus, 5-HT₆ receptor agonists may be useful in treating depression. For example, LY-586713, a 5-HT₆ receptor agonist, increased the hippocampal BDNF expression, which is a cellular index of antidepressant actions [19]. In addition, the 5-HT₆ receptor agonist EMD 386088 produced antidepressant-like and anxiolytic effects [20-21]. The blockade of the 5-HT₆ receptor also elicits anxiolytic and antidepressant-like effects. Other preclinical studies have demonstrated that 5-HT₆ receptor antagonists such as SB399885 and SB258585 exert antidepressant-like effects in rodents [6-8]. In this study, we screened natural products to identify novel ligands of the 5-HT₆ receptor using the HTS FDSS6000 system (data not shown). Among those, the PH showed the strongest inhibitory effect on the activity of the 5-HT₆ receptor. The PH at concentrations of 1, 3, 10, 30, and 100 μ M inhibited the 5-HT-mediated Ca^{2+} increase in 1321N1 cells stably expressing the 5-HT₆ receptor up to 36.69 ± 1.99 %, 60.50 ± 0.93 %, 61.36 ± 0.34 %, 73.20 ± 0.20 %, and 78.11 ± 0.49 %, respectively. In addition, the half maximal inhibitory concentration (IC_{50}) value was 2.12 ± 5.39 μ M (Fig. 4A). We also examined the effect of SB399885, which is a selective antagonist of the 5-HT₆ receptor, and obtained an IC_{50} value of 3.46 ± 2.27 μ M (Fig. 4B). As

shown in Figs. 4C and 4D, the application of PH (100 µg/mL) and SB399885 (10 µM) with 1-min pretreatment rapidly and significantly inhibited the increase of 5-HT-mediated Ca^{2+} . These results indicated a PH potent antagonistic activity against the human 5-HT₆ receptor.

The 5-HT₆ receptor binds to adenylate cyclase and consequently triggers a cAMP-dependent signaling pathway. A key point signal transduction mediator in this pathway is the ERK1/2. In the present study, we examined whether the PH extract itself produced any effect on the ERK1/2 phosphorylation. Based on our

results, PH did not affect the ERK1/2 phosphorylation (Fig. 5A). We also assessed whether PH antagonizes the 5-HT-mediated ERK1/2 phosphorylation. As a standard, we used the selective 5-HT₆ receptor antagonist SB399885 (30 µM). The administration of PH (100 µg/mL) and SB399885 (3 µM) resulted in the inhibition of the 5-HT-mediated ERK1/2 phosphorylation (Figs. 5B and 5C). These results indicated that the antidepressant-like effects of PH can be elicited by decreased behavioral immobility through the 5-HT₆ receptor antagonistic mechanism.

Fig. 4. Effects of PH and SB399885 (5-HT₆ receptor antagonist, positive control) on 5-HT-induced increase of the intracellular Ca^{2+} concentration $[\text{Ca}^{2+}]_i$ in human astrocytoma 1321N1 cells stably expressing the 5-HT₆ receptor. These 1321N1 cells were transfected with G-protein chimeric gene (G_{qs5}) and loaded with Fura-2/AM. The percentage inhibition was calculated as $100 \times [(\text{Ratio}_{5\text{-HT}} - \text{Ratio}_{\text{sample}}) / \text{Ratio}_{5\text{-HT}}]$. (A) Inhibition effect of the various concentrations of PH on 5-HT-induced $[\text{Ca}^{2+}]_i$ increase. (B) Inhibition effect of the various concentrations of SB399885 on 5-HT-induced $[\text{Ca}^{2+}]_i$ increase. (C) Representative tracing of the $[\text{Ca}^{2+}]_i$ response evoked by 5-HT (100 µM) alone or 5-HT plus PH (100 µg/mL). (D) Representative tracing of the $[\text{Ca}^{2+}]_i$ response evoked by 5-HT (100 µM) alone or 5-HT plus SB399885 (10 µM). PH, *P. humile* extract; 5-HT, serotonin 100 µM. Values are expressed as the mean $\pm$ standard error of the mean (n = 5). ** $p < 0.01$ and * $p < 0.001$ compared with 5-HT**

Fig. 5. Effects of PH and SB399885 (5-HT₆ receptor antagonist, positive control) on ERK1/2 phosphorylation in human astrocytoma 1321N1 cells stably expressing the 5-HT₆ receptor. The 1321N1 cells were treated with the indicated drugs for 15 min (A). For the evaluation of the antagonistic effects, cells were pretreated with 5-HT (100 µM) and incubated for 10 min after the pretreatment with the indicated drugs for 15 min (B). (C) Quantitative analysis of the relative ERK1/2 phosphorylation. The SB399885 was used at 3 µM, and PH was used at 30 and 100 µg/mL. 5-HT, serotonin 100 µM; ns, not significant; PH 30, *P.humile* extract 30 µg/mL; PH 100, *P.humile* extract 100 µg/mL. Values are expressed as the mean $\pm$ standard error of the mean (n = 5). ** $p < 0.01$ compared with 5-HT

Table 1. Effects of PH on the body weight, organ weight, and blood parameters in mice. Values are expressed as the mean $\pm$ standard error of the mean (n = 5)

	Control	PH 100 mg/kg	PH 300 mg/kg
Body weight			
Initial Body weight (g)	31.01 $\pm$ 0.88	31.15 $\pm$ 0.74	31.05 $\pm$ 0.44
Final Body weight (g)	32.52 $\pm$ 0.83	34.91 $\pm$ 0.73	34.28 $\pm$ 0.42
Body weight gain (g)	1.51 $\pm$ 1.46	3.76 $\pm$ 0.98	3.23 $\pm$ 0.75
Organ weight			
Kidney (g)	0.59 $\pm$ 0.03	0.62 $\pm$ 0.01	0.60 $\pm$ 0.01
Spleen (g)	0.13 $\pm$ 0.01	0.11 $\pm$ 0.00	0.16 $\pm$ 0.01
Liver (g)	1.66 $\pm$ 0.08	1.98 $\pm$ 0.05	2.04 $\pm$ 0.03
Blood parameter			
GOT (U/L)	71.33 $\pm$ 5.72	53.00 $\pm$ 3.51	67.00 $\pm$ 5.06
GPT (U/L)	22.33 $\pm$ 1.35	20.25 $\pm$ 1.30	22.33 $\pm$ 1.51
Creatinine (mg/dL)	0.13 $\pm$ 0.01	0.13 $\pm$ 0.01	0.17 $\pm$ 0.02
BUN (mg/dL)	23.97 $\pm$ 0.57	23.40 $\pm$ 0.98	26.83 $\pm$ 1.80

Table 2. Effects of PH on the monoamine neurotransmitter levels in the mouse brain. Values are expressed as the mean $\pm$ standard error of the mean (n = 5). 5-HT, serotonin; DA, dopamine; NE, norepinephrine. * $p < 0.05$ compared with the control group

Monoamines (ng/mg protein)	Control	PH 100 mg/kg	PH 300 mg/kg
5-HT	2,530.16 $\pm$ 3.25	2,713.48 $\pm$ 36.21	3,064.28 $\pm$ 30.84*
DA	260.85 $\pm$ 4.03	276.01 $\pm$ 9.97	298.46 $\pm$ 8.54
NE	65.66 $\pm$ 1.05	68.15 $\pm$ 2.25	74.65 $\pm$ 1.67

Polyphenols and flavonoids have been identified in PH [10]; in particular, naringenin, curcumin, ellagic acid, rutin, quercetin, apigenin, and resveratrol showed possible antidepressant and anxiolytic actions [22]. However, the underlying mechanisms of these PH antidepressant effects remain unknown. Further studies will be required in order to define the functional compounds that regulate the antidepressant-like effects of PH through the 5-HT₆ receptor blockade.

4. CONCLUSION

The present findings indicated that PH, which is a new herbal medication, can regulate depression through the action of the 5-HT₆ receptor antagonistic mechanism.

ETHICAL APPROVAL

All experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at Jeonnam Institute of Natural Resources Research (approval no. JINR-1906-2019). All animal experiments were conducted in accordance with the IACUC guidelines.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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