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Nelumbinis Semen Reverses a Decrease in 5-HT_{1A} Receptor Binding Induced by Chronic Mild Stress, a Depression-like Symptom

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Depression is associated with a dysfunctional serotonin (5-hydroxytryptamine; 5-HT) system. More recently, several lines of evidence suggest that an important factor in the development of depression may be a deficit in the function and expression of 5-HT_{1A} receptors. The present study assessed if *Nelumbinis Semen* (*N. s.*) had an anti-depression effect through reversing a decrease in 5-HT_{1A} receptor binding in rats with depression-like symptoms induced by chronic mild stress. Using a 5-HT_{1A} receptor binding assay, with a specific 5-HT_{1A} receptor agonist, 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino) tetralin), the mechanism of the anti-depression effect of *N. s.* on rats was investigated, and the effects compared with two well-known anti-depressants, *Hyperium Perforatum* (St. Johns Wort) and fluoxetine (Prozac). Animals were divided into five groups: the normal (N) group without chronic mild stress (CMS), the control (C) group under CMS for 8 weeks, the *Nelumbinis Semen* (*N. s.*) treatment group under CMS for 8 weeks, the *Hyperium Perforatum* (*H. p.*) treatment group under CMS for 8 weeks and finally, the fluoxetine (F) treatment group under CMS for 8 weeks. Each treatment was administered to rats during the last 4 weeks of the 8-week CMS. A sucrose intake test was performed to test the anti-depression effect of *N. s.* The *N. s.* treatment significantly reversed the decreased sucrose intake under CMS ($P<0.05$ compared to control group under CMS). In the CA2 and CA3 regions of the hippocampus, both *N. s.* and *H. p.* reversed the CMS-induced decrease in 5-HT_{1A} receptor binding. In the I to II regions of the frontal cortex, *N. s.* and *H. p.* also reversed the CMS-induced decrease in 5-HT_{1A} receptor binding, and even showed a significant increase in 5-HT_{1A} receptor binding compared to the F treatment group (*N. s. vs.* P, $p<0.05$, *H. p. vs.* P, $p<0.05$). However, in the hypothalamus, all treatments reversed the CMS-induced decrease in 5-HT_{1A} receptor binding. This reversal effect of *N. s.* on the decrease in 5-HT_{1A} receptor binding in the frontal cortex, hippocampus and hypothalamus of rat brains was very similar to that of *H. p*, but different from that of F. It is concluded that *N. s.* presents an anti-depression effect through enhancing 5-HT_{1A} receptor binding.

Key words: Dysfunctional 5-HT system, 5-HT_{1A} receptor, *Nelumbinis Semen*, Anti-depression, Chronic mild stress

INTRODUCTION

Depression is a recurrent and life threatening mental illness, with high morbidity and mortality. The World Health Organization estimates that depression is now the fourth most important worldwide cause of loss in human disability adjusted life years, and predicts it will have become the second most by the year 2020 (Nowak *et al.*, 2003). It has been established that depressed patients have a dysfunctional serotonin (5-hydroxytryptamine; 5-HT) system. More recently, several lines of evidence have emerged that specifically implicate a dysfunction in the 5-

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HT_{1A} receptor in depression (Leitch *et al.*, 2003). A decrease in the 5-HT_{1A} binding potential, as determined by positron emission tomography, has been demonstrated in multiple forebrain areas, including the frontal cortex and hippocampus, of depressed patients (Drevets *et al.*, 1999 and 2000; Sargent *et al.*, 2000). It has also been reported that chronic treatment with corticosterone induces a depression-like symptom in rodents and attenuates the 5-HT_{1A} receptor density in the hippocampus and hypothalamus (Leitch *et al.*, 2003). Most anti-depressant medications function by normalizing the 5-HT_{1A} receptor density, thus relieving depression (Haddjeri *et al.*, 1998; Leonard, 1992). *Nelumbinis Semen* has been widely used in Korean traditional medicine as a remedy for insomnia, anxiety and womens post-menstrual-pause depression. It was recently found that this herbal medicine presented an anti-depressive effect in rats under a forced swim-induced depression-like symptom (data not shown). However, there has been no molecular indication of an anti-depression effect through normalization of 5-HT_{1A} receptor binding by treatments with *Nelumbinis Semen*. Therefore, the present study assessed the molecular aspects of the anti-depression effect of *Nelumbinis Semen* on rats under depression-like symptoms induced by chronic mild stress (CMS), using a 5-HT_{1A} receptor binding assay with a specific 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT), and compared the results with two well-known anti-depressants, *Hyperium Perforatum* (St. John's wart) and fluoxetine (Prozac).

MATERIAL AND METHODS

Animals

The 6 week old male Wister rats, weighing 180 to 200 grams, were supplied by Jung-Ang Experimental Animal Center (Seoul, Korea). The rats were housed under restricted and pathogen free conditions (room temperature: 21±2°C, relative humidity: 70-80%, light cycle: 07:00 19:00) and allowed free access to their diets and tap water, except during the scheduled CMS. The rats were adapted to this environment for 1 week prior to the experiments.

Preparation of anti-depressants

The sprayed-dry extracts of *Nelumbinis Semen* used were purchased from the Sun-Ten Pharmaceutical Company, Taiwan. *Hyperium Perforatum* and fluoxetine were used as the positive controls. The *Hyperatum Perforatum* was purchased from HBC Protocols Company (Los Angeles, CA, USA). The Hypericin, the standard material in *Hyperatum Perforatum*, was 0.3%, which is a widely used index for its use in animal studies (Khalifa, 2001). Fluoxetine (Prozac), a selective serotonin reuptake inhibitor (SSRI), was purchased from Sigma Company (St. Louis, MO, USA).

Chronic mild stress

In order to induce CMS and depression-like behavior, rats were consistently exposed to various mild stressors, such as overnight illumination, food and/or water deprivation, cage tilt and change of cage mate for an 8 week period, according to the Willners method, as described previously (Willner *et al.*, 1987). These stressors were changed every few hours over the 8 week period. The weekly schedule of the CMS procedures is shown in Table I.

Drug administration

Nelumbinis Semen (4 g/kg), *Hyperium Perforatum* (2.68 g/kg) or fluoxetine (10 mg/kg) was administered orally (*Nelumbinis Semen* and *Hyperium Perforatum*) or intraperitoneally (fluoxetine) for the last 4 weeks during the 8 week-CMS.

Sucrose intake

For the sucrose intake test, subjects were accustomed to consume 1% sucrose solution prior to the start of the experiment. They were exposed to 1% sucrose solution for 48 h in their home cages, but without any food or water available. The sucrose solution was then provided to the rats for 1 h each day for four consecutive days. The sucrose intake test was administered weekly, between 14:00 and 15:00 oclock on every Monday. Prior to each test, the animals were deprived of food and water for 20 hrs. The sucrose solution consumption was recorded by weighing the bottles before and after the test.

Receptor autoradiography

Brains of rats were removed immediately after decapitation, and frozen on dry ice. Coronal sections of 20 μm were prepared at -20°C using a microtome cryostat (Leica, Germany). The sections were thaw-mounted on gelatin-coated microscope slides (Superfrost®/Plus, Fisher Scientific, USA) and stored at -80°C until used. 5-HT_{1A} receptor autoradiograms were generated, according to the method described previously (Ase *et al.*, 1999), with some modifications. Briefly, for 5-HT_{1A} receptors, the sections were pre-incubated for 15 min in 50 mM Tris-HCl buffer (pH 7.4), and then incubated for 60 min in the same pre-incubation buffer, with the addition of 2 nM [^{3}H]8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino) tetralin) (170 Ci/mmol, NEN Life Science, Boston, MA, USA), at room temperature. Non-specific binding was assessed in the presence of 10 μM serotonin-HCl (Radja *et al.*, 1993). After incubation, both sets of slides were rinsed three times for one minute in the buffer (Tris-HCl, 50 mM at

Table I. Weekly schedule of chronic mild stress procedures

Day Time	Monday	Tuesday	Wednesday (overday lighting)		Thursday		Friday (overday lighting)	Saturday	Sunday
am 00:00	water and food deprivation (20 hours)	soiled cage (15 hours)	water deprivation (16 hours)		grouped housing (17 h)	water and food depri-vation (20 h)		45°cage tilt (17 hours)	stro-bo-scope lighting (9 hours)
01:00									
02:00									
03:00									
04:00									
05:00									
06:00									
07:00									
08:00							White noise (5 hours)		
09:00		stroboscope lighting (9 hours)							
10:00			empty wate bottle						
11:00			cage tilt (7 hours)						
pm 12:00									
13:00									
14:00	sucrose test				stro-bo-scope lighting (9 h)	restricted access to food(2 h)			
15:00	White noise (3 hours)								
16:00									
17:00							[Behavior Test]		
18:00	soiled cage (15 hours)	water deprivation (16 hours)	grouped housing (17 h)	water and food deprivation (20 h)					water and food deprivation (20 hours)
19:00							45°cage tilt (17 hours)	stro-bo-scope lighting (9 hours)	
20:00									
21:00									
22:00									
23:00									

4°C), followed by one brief wash in cold distilled water, and then immediately dried in a stream of cool air.

Analysis of autoradiograms

Dried tissue sections were placed next to Kodak BioMax MR film (Eastman Kodak Co., Rochester, NY, USA), and following a 5-week exposure period, developed in room temperature developer for 3 min and then fixed for 3 min. Autoradiograms were analyzed by a digital scanning densitometer (Personal Densitometer, Molecular Dynamics, Sunnyvale, CA, USA), using the image acquisition and analysis program, ImageQuant 3.3 (Molecular Dynamics, Sunnyvale, CA, USA). A standard [^{3}H] Microscales™ (Amersham Biosciences, Piscataway, NJ, USA) curve was used to convert the density levels into nCi per milligram or gram of protein, with the mean density measured from at least two sections per region per rat.

Statistical analysis

The results are presented as the mean value ± SEM. Statistical significance was compared between each treatment group and the control by a One-Way-ANOVA and its post-hoc test, the Student-Newman-Keuls test. Results with $p<0.05$ were considered statistically significant.

RESULTS

The reversal effect of decreased sucrose intake induced under CMS by *N. s.* treatment

The decrease in sucrose intake has been demonstrated

as a core symptom of depression in the CMS animal model (Katz, 1982; Willner *et al.*, 1987). Thus, whether *N. s.* treatment could reverse the decreased sucrose intake upon CMS exposure was investigated. There was no significant difference in the sucrose intakes between the groups before induction of CMS (see column "Before" in Table II). CMS treatment for 4 weeks induced significant decrease in the sucrose intakes in all groups compared with the normal group ($P<0.05$ compared to N, see column "After 4 weeks" in Table II). *N. s.* treatment significantly reversed the decrease in sucrose intake, similarly to that with F treatment [$P<0.05$ compared to C, *N. s.*: 12.467 ± 1.069 mL (74% increase compared to C, n=6), F: 10.283 ± 0.709 mL (43% increase compared to C, n=6), see column "Treatment for last 4 weeks" in Table II]. This result suggests that *N. s.* has a distinct anti-depressant effect, as presented by the reversal in the decreased sucrose intake.

Table II. The recovery of decreased sucrose intake after anti-depressant treatment during the last 4 weeks of the 8-week CMS

Treatment	Before	After 4 weeks	Treatment for last 4 weeks
N (n=6)	10.750±1.510	13.650±0.655	16.333±1.021#
C (n=6)	10.650±0.673	9.067±0.603*	7.167±0.724
N. s. (n=6)	9.467±1.555	9.917±0.637*	12.467±1.069#
F (n=6)	12 ±2.231	9.283±0.513*	10.283±0.709#
H. p. (n=6)	11.617±0.922	9.2 ±0.795*	17.4 ±1.192#

Each number presents the mean value ± SEM of the sucrose intake volume (mL) from six rats per group, with the four groups denoted by treatment: normal group (N), the control group (C), *Nelumbinis Semen* treatment group (*N. s.*), *Hyperium Perforatum* treatment group (*H. p.*) and fluoxetine treatment group (F). *P < 0.05 compared to N, #P < 0.05 compared to C.

5-HT_{1A} receptor binding in CA2 region of hippocampus

Fig. 1 shows a representative autoradiogram of the 5-HT_{1A} receptor in a rats brain. The CA2 region of the hippocampus is known as the major component of the memory circuit, and the hippocampus has been reported to be shrunken and damaged in depressed patients. Most depressed patients present a memory deficiency due to such damage (MacQueen *et al.*, 2003). Also, several avenues of evidence have shown that antidepressants, such as tricyclic antidepressants (TCA), recovered neuronal damage and even induced neurogenesis in this region (Banasr *et al.*, 2004). The 5-HT_{1A} receptor agonist, 8-OH-DPAT, binding in the CA2 region of the hippocampus was examined, and the binding capacities of other commonly used anti-depressants, *H. p.* and fluoxetine, were compared with the newly proposed anti-depressant, *N. s*, on rats under CMS (see Table III). *Nelumbinis Semen* reversed to normal the decrease of the 5-HT_{1A} receptor binding induced by CMS [14% increase compared to C]. Such a reversal by *N. s.* was very similar to that with *H. p.*

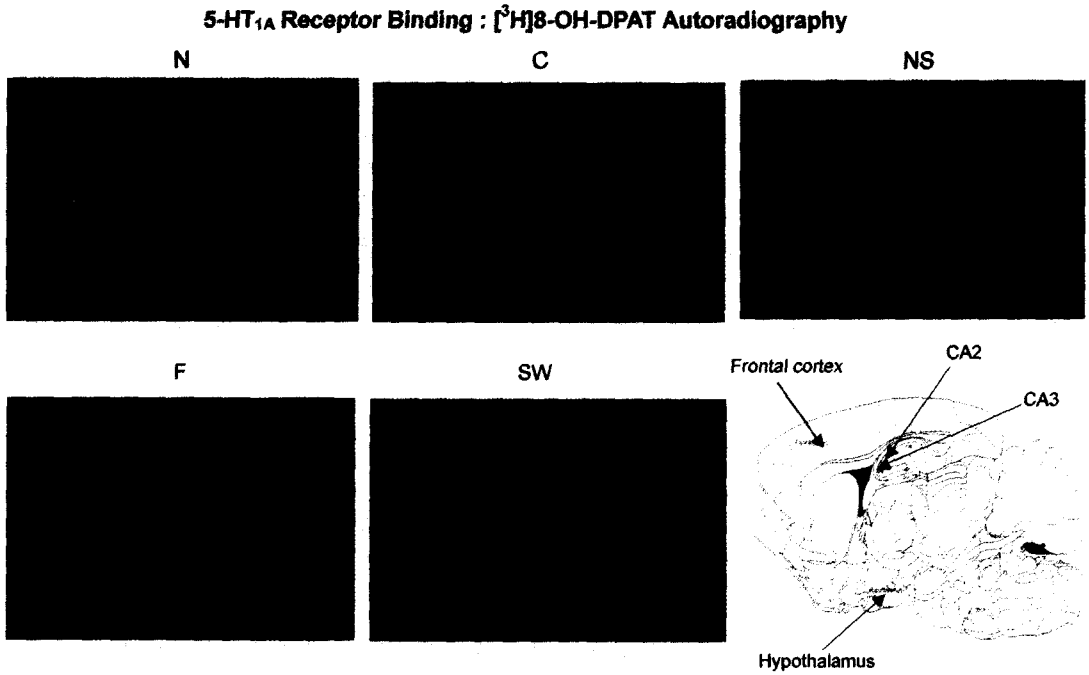

Fig. 1. Representative autoradiogram of 5-HT_{1A} receptor binding after the anti-depressant treatments during CMS. Normal group (N), the control group (C), *Nelumbinis Semen* treatment group (*N. s.*), *Hyperium Perforatum* treatment group (*H. p.*) and fluoxetine treatment group (F) in each brain region.

treatment [14% increase compared to C], but this effect was further decreased by F treatment compared to the control [12% decrease compared to C].

5-HT_{1A} receptor binding in CA3 region of hippocampus

The CA3 region of the hippocampus is also known as a major component of the memory circuit, and the hippocampus has been reported to be shrunken and damaged in depressed patients (MacQueen *et al.*, 2003). As shown in Table III, *Nelumbinis Semen* reversed to normal the decrease of 5-HT_{1A} receptor binding induced by CMS (14% increase compared to C). Such a reversal by *N. s.* was very similar to that with *H. p.* treatment (4% increase compared to C), but F treatment decreased the 5-HT_{1A} receptor binding even more dramatically than in the control (2% decrease compared to C). Taken together with the results from the CA2 and CA3 regions, these data suggest that *N. s.* might alleviate depression and recover the memory deficiency induced by depression, through recovery of the 5-HT_{1A} receptor density in the hippocampus, and such an effect might be similar to that with *H. p,* but not to that with F.

5-HT_{1A} receptor binding in I II region of frontal cortex

The I II region of the frontal cortex (pre-frontal region) is known to play a major role in cognitive function, and this pre-frontal region has be reported to be damaged in depressed patients (Bench *et al.*, 1992; Soares and Mann, 1997). Most depressed patients present with cognitive function deficiency, as measured by the WAIS test (Devanand *et al.*, 2003). In the molecular mechanism of depression, the dysfunction of 5-HT_{1A} receptors in the pre-frontal cortex has been reported, with such a dysfunction likely to cause deficiency in cognitive function (Buhot *et al.*, 2000; Keck and Lakoski, 1996). The results of agonist binding in the fontal cortex region have demonstrated that *Nelumbinis Semen* reversed the decrease in the 5-HT_{1A} receptor density induced by CMS compared to that in the normal control (11% increase compared to C). Such a reversal caused by *N. s.* was very similar to that with *H. p.* treatment (54% increase compared to C), but the F treatment decreased the 5-HT_{1A} receptor binding even more dramatically (46% decrease compared to C). The 5-HT_{1A} receptor bindings of N, *N. s.* and *H. p.* were significantly different from that with F [N: 6.553 ± 0.537 nCi/mg protein (61% difference compared to F, $p<0.05$, n=4), *N. s.*: 6.314 ± 0.451 nCi/mg protein (57% difference compared to F, $p<0.05$, n=4), *H. p.*: 8.773 ± 0.908 nCi/mg protein (100% increase compared to F, $p<0.05$, n=4)].

These results suggest that *N. s.* might cure depression and recover the cognitive function deficiency induced by depression, through normalization of 5-HT_{1A} receptor functions in frontal cortex area, and such an effect might be similar to that with SW, but not with F.

5-HT_{1A} receptor binding in hypothalamus

The hypothalamus has been reported to play a major role in the induction of depression, by releasing too much cortisol when a stressful environment was present. It was also reported that a tremendous increase of cortisol causes dysfunction in the 5-HT_{1A} receptor. Most depressed patients present a significantly increased cortisol level (Leitch *et al.*, 2003). *Nelumbinis Semen* reversed this decrease of the 5-HT_{1A} receptor binding induced by CMS by 42% compared to the C. Such a reversal by *N. s.* is very similar to those by *H. p* (44% increase compared to C) and F treatments (71% increase compared to C). These results suggest that *N. s.* might cure depression and recover the 5-HT_{1A} receptor function deficiency of the hypothalamus through normalization of the 5-HT_{1A} receptor function.

DISCUSSION

Depression is a mental disease that results in losses of interests, pleasure and responses to daily living and pleasurable activities. The symptoms of depression include sadness, anxiety, apathy, sleeping problems, loss of

Table III. The recovery of the decrease in 5-HT_{1A} receptor binding after the anti-depressant treatments during the last 4 weeks of the 8-week CMS, i.e. the molecular anti-depression effect of *N. s.*

	CA2	CA3	Frontal cortex (I-II)	Hypothalamus
N	8.893±0.892 (n=4)	10.351±0.806 (n=4)	6.553±0.537 (n=4)*	11.793±1.782 (n=4)
C	8.4 ±0.188 (n=3)	9.425±0.81 (n=3)	5.711±0.865 (n=3)*	7.428±2.596 (n=4)
N. s.	9.567±0.891 (n=2)	10.708±0.813 (n=4)	6.314±0.451 (n=4)*	10.526±3.367 (n=2)
F	7.378±0.297 (n=3)	9.216±0.426 (n=3)	3.09 ±0.323 (n=3)	12.701±2.021 (n=2)
H. p.	9.64 ±0.817 (n=4)	9.795±0.747 (n=4)	8.773±0.908 (n=4)*	10.721±0.904 (n=4)

Each number represents the mean value ± SEM of the 5-$HT1_A$ receptor binding intensity (nCi/mg protein) in two to four rats per group, with the four groups denoted by treatment: normal group (N), the control group (C), *Nelumbinis Semen* treatment group (*N. s.*), *Hyperium Perforatum* treatment group (*H. p.*) and fluoxetine treatment group (F) in each brain region. *$P < 0.05$ compared to F.

appetite and sexual desire, feelings of worthlessness, and most importantly, suicidal thoughts. Because the risk of suicide is dramatically increased for patients with depression, a quick and effective treatment of the illness is often required (Willner, 1991; Willner, 1997a; Willner, 1984; Willner, 1997b; Willner *et al.*, 1996; Willner *et al.*, 1987). In order to develop effective anti-depressants, various animal models of depression have been formulated to induce depression-like symptoms (Katz, 1981). Stressors invented by Katz and his colleagues during the initial stage of developing depression models included such severe methods as intense foot-shock, cold-water immersion, and food and water deprivation (Willner, 1984). Chronic stress induced by these stressors often led to impairments of the behavior maintained by brain stimulation reward, and could not be recovered with the use of most anti-depressants (Katz, 1982; Zacharko *et al.*, 1984; Zacharko *et al.*, 1983). Such severe stressors are not appropriate stimulators for depression-like conditions, since commonly used anti-depressants can reverse the effect called "anhedonia", the loss of responsiveness to pleasant events, which is a core symptom of depression (Katz, 1982; Zacharko *et al.*, 1984; Zacharko *et al.*, 1983). Studies strongly suggest that repeated, weak stressors are effective methods for inducing depression-like symptoms in an animal model, as they reflect human depression symptoms attributed to the weak, consistent and chronic stress in modern society. The model of chronic mild stress (CMS) developed by Willner and colleagues is one of the most widely accepted animal models of depression, with a high degree of validity and reliability (Katz, 1981; Papp *et al.*, 1991). In a typical experiment involving this model, rats (Willner *et al.*, 1987) or mice (Monleon *et al.*, 1995) are consistently exposed to various mild stressors, such as overnight illumination, food and/or water deprivation, cage tilt and change of cage mate. These stressors would change every few hours, over periods of weeks or months and induce anhedonia. Furthermore, it also decreases the consumption and preference for palatable weak (1-2%) sucrose solution, which is concrete behavior of anhedonia when present in animals under CMS. It is known that this decrease in the consumption of sucrose solution continues for several weeks, even after discontinuation of CMS. Treatment with most anti-depressants will cause the consumption of sucrose solution to return to normal (Muscat *et al.*, 1992). Thus, the CMS model was chosen in the present study for its ease of inducing a depression-like symptom. *N. s.* treatment significantly reversed the decreased sucrose intake under CMS ($P<0.05$ compared to control group under CMS). Some potential therapeutic effects, such as hepato-protective, anti-oxidative (Sohn *et al.*, 2003) and anti-arrhythmic actions (Li *et al.*, 1989; Li *et al.*, 1990) have been reported for *Nelumbinis Semen* extracts. It is known that various alkaloids are contained within *Nelumbinis Semen* (Wang *et al.*, 1991; Zelenski, 1977). Among those alkaloids, neferine is known for its anti-arrhythmic action (Li *et al.*, 1989; Li *et al.*, 1990), nulciferine for its tranquilization action (Nieto-Sampedro *et al.*, 1980) and isoquecertin for its spasmolytic action (Lozoya *et al.*, 1994). Most of all, the anonaine, asimilobine, isoquercitrin, hyperoside, lirinidine and nornuciferine components of the *Nelumbinis Semen* are known to have anti-depression effects, as evaluated by neurotransmitter reuptake inhibition and the Forced Swim Test (Butterweck *et al.*, 2000; Hasrat *et al.*, 1997; Protais *et al.*, 1995; Shoji *et al.*, 1987). It has been established that depressed patients have a dysfunctional 5-HT system (Leitch *et al.*, 2003); more specifically, a decrease in the 5-HT_{1A} binding potential, as determined by positron emission tomography, has been demonstrated in multiple forebrain areas, including the frontal cortex and hippocampus, of depressed patients (Drevets *et al.*, 2000; Drevets *et al.*, 1999; Sargent *et al.*, 2000). Also, it has been reported that chronic corticosterone treatment induces a depression-like symptom in rodents; it attenuates the 5-HT_{1A} receptor density in hypothalamus (Leitch *et al.*, 2003). Thus, most anti-depressants are believed to normalize the 5-HT_{1A} receptor density. Our results strongly suggest that *Nelumbinis Semen* presents an anti-depression effect through enhancement of 5-HT_{1A} receptor binding. These effects are likely to be mediated by the ingredients in *Nelumbinis Semen,* such as the known serotonin receptor agonists, anonaine and asimilobine. Patterns of 5-HT_{1A} receptor binding in the hippocampus, pre-frontal cortex and hypothalamus are similar to those of SW, but different from those of F. The receptor binding induced by F was decreased in all regions examined, with the exception of the hypothalamus. Fluoxetine greatly influences the 5-HT receptor binding through tremendous increases in the serotonin concentration. Among several 5-HT receptors, the 5-HT_{1A} receptor has been reported to be down-regulated and dysfunctional in the presence of too much serotonin induced by fluoxetine (Dremencov *et al.*, 2003). Therefore, it is thought that fluoxetine could increase the concentration of serotonin in the hippocampus and pre-frontal cortex, resulting in down-regulation and dysfunction of the 5-HT_{1A} receptor, except in the hypothalamus, a region having only few 5-HT_{1A} receptors. In the hippocampus, postsynaptic 5-HT_{1A} receptor activation increases local cholinergic and dopaminergic neurotransmission (Dremencov *et al.*, 2003). Hippocampal application of a 5-HT_{1A} receptor agonist was found to attenuate the cognitive deficits caused by brain injury (Kline *et al.*, 2001). It is also proposed that postsynaptic 5-HT_{1A} receptor activation increases local cholinergic and dopaminergic or serotonergic neurotransmission through activation of cAMP

formation in the cortex where the 5-HT_{1A} receptors are mostly postsynaptic (Celada *et al.*, 2004; Elena Castro *et al.*, 2003). Therefore, it is possible that *N. s.* and *H. p.* could increase local cholinergic and dopaminergic or norephinergic neurotransmission through activation of cAMP formation in the hippocampus and pre-frontal cortex. It is well known that *H. p.* shows an anti-depression effect through the enhancement of dopamine or norephinephrine instead of serotonin, and those neurotransmitters are as important as serotonin in depression (Butterweck *et al.*, 1997; Muller *et al.*, 1997). Therefore, these results suggest that F might not recover the cognitive function deficiency in the pre-frontal cortex or the memory deficiency in the hippocampus induced by depression, because of its down-regulation and dysfunction of the 5-HT_{1A} receptor. However, it is thought that *Nelumbinis Semen* might be a more effective and safe drug as it establishes recovery of the 5-HT_{1A} receptor function. These results support the basis for development of a novel anti-depressant based on the pharmacological action of *Nelumbinis Semen*. However, the molecular mechanism underlying such an anti-depression effect of *Nelumbinis Semen* requires further study before firm conclusion are drawn and therapies designed.

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