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Antidepressant-like effects of neferine in the forced swimming test involve the serotonin$_{1A}$ (5-HT$_{1A}$) receptor in mice

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ABSTRACT

The effects of neferine, an alkaloid of *Nelumbo nucifera* Gaertner embryos, on immobility in the forced swimming test, which is used to evaluate antidepressants, were investigated in mice. The administration of neferine from 25 to 100 mg/kg i.p. elicited anti-immobility effects in mice. The molecular dose effects of neferine in the forced swimming test were almost equal to those of the typical antidepressants maprotiline and imipramine. The involvement of the 5-HT receptor subtypes was also studied using 5-HT receptor antagonists. Anti-immobility effects of neferine are antagonized by the serotonin$_{1A}$ (5-HT$_{1A}$) receptor antagonist, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide (WAY 100635). However, the 5-HT$_{1B}$ receptor antagonist, 3-[3-(dimethylamino)propyl]-4-hydroxy-N-[4-(4-pyridinyl)phenyl] benzamide dihydrochloride (GR 55562), the 5-HT$_2$ receptor antagonist, 6-methyl-1-(methylethyl)-ergoline-8β-carboxylic acid 2-hydroxy-1-methylpropyl ester (LY 53857), the 5-HT$_3$ receptor antagonist, ondansetron and the 5-HT$_4$ receptor antagonist, 4-amino-5-chloro-2-methoxy-benzoic acid 2-(diethylamino)ethyl ester (SDZ 205,557) did not affect the anti-immobility effects of neferine. The anti-immobility effect of the selective 5-HT$_{1A}$ receptor agonist, 8-hydroxy-2-(di-*n*-propylamino)tetaralin (8-OH-DPAT) was also antagonized by WAY 100635. Furthermore, co-administration of subactive doses of neferine (10 mg/kg) and 8-OH-DPAT (0.1 mg/kg) produced synergistic antidepressant-like effects. These results suggest that neferine shows antidepressant-like effects in mice similar to typical antidepressants and that these effects are mediated by the 5-HT$_{1A}$ receptor. Therefore, the central effects of neferine are likely to be linked to serotonergic neurotransmission.

1. Introduction

Embryos of the seeds of *Nelumbo nucifera* Gaertner are used in Chinese traditional medicine and have sedative, antipyretic and hemostat effects in humans (Chiang, 1978). Benzylisoquinoline alkaloids, including neferine, liensinine, and isoliensinine, are present in this herbal medicine (Furukawa, 1966). However, detailed studies on the effects of this medicine or its alkaloids on the central nervous system have not been performed. We recently reported that extracts of embryos of the seeds of *N. nucifera* Gaertner and its main alkaloid neferine (Fig. 1) inhibited locomotor activity in mice (Sugimoto et al., 2008). Furthermore, we found that neferine elicited hypothermic effects and potentiated thiopental-induced sleeping in mice (Sugimoto et al., 2008). These results are the first findings of the central effects of neferine. Moreover, we reported that neferine has anti-anxiety effects similar to diazepam in the elevated plus maze test, although neferine did not affect motor coordination, different from diazepam (Sugimoto et al., 2008).

It is well known that benzodiazepine derivatives show anti-anxiety effects through the benzodiazepine receptor coupled with the GABAergic system. Recently it was recognized that serotonergic neurotransmission is involved in anxiety. Serotonin (5-HT) receptors are involved in anxiety, and serotonergic agents, such as tandospirone and buspirone, are clinically used as anxiolytics (Deakin, 1998). In addition, selective serotonin reuptake inhibitors (SSRIs) are effective in anxiety disorders (Figgitt and McClellan, 2000). SSRIs are known to have strong antidepressant effects, similar to tricyclic antidepressants such as imipramine (Borsini, 1995). Since we found that neferine possesses anti-anxiety effects, it is presumed that the pharmacological effects of neferine affect serotonergic neurons, and neferine may have antidepressant effects. However, this has not yet been confirmed. The forced swimming test in mice is used to evaluate antidepressants and

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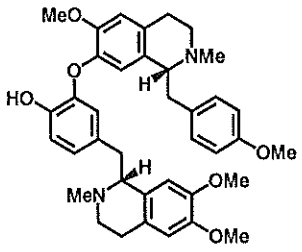


Fig. 1. Chemical structure of neferine.

many antidepressants can shorten immobility time in this test (Porsolt et al., 1977, 1979). In this paper, to assess its antidepressant effects, we studied the effects of neferine on the immobility time in mice in the forced swimming test and the involvement of 5-HT receptors in anti-immobility effects of neferine.

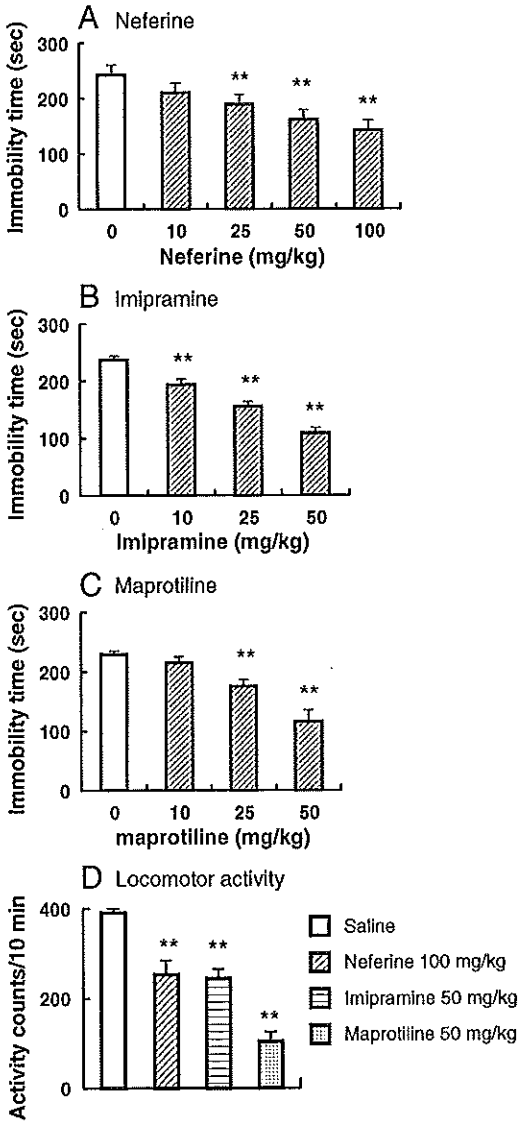


Fig. 2. Effects of neferine, imipramine and maprotiline on immobility in forced swimming test and locomotor activity in mice. Results are shown as mean ± S.E.M. ($N = 5–10$). Drugs were given i.p. 30 min before test. ** $P<0.01$ vs. saline of respective group. (A) Neferine $F(4,31) = 11.15$, $P<0.05$. (B) Imipramine $F(3,28) = 22.09$, $P<0.0001$. (C) Maprotiline $F(3,23) = 16.61$, $P<0.01$. (D) Locomotor activity $F(3,16) = 60.82$, $P<0.0001$.

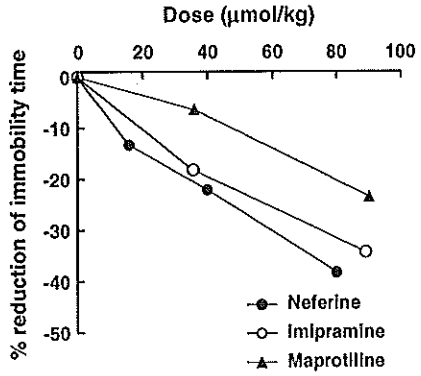


Fig. 3. Comparison of the potency of the anti-immobility effects of neferine, imipramine and maprotiline in mice.

2. Materials and methods

2.1. Extraction and isolation from plant materials

Embryos of the seeds of *N. nucifera* Gaertner were purchased from Longhua Hospital (Shanghai University of Traditional Chinese Medicine, Shanghai, China). A voucher specimen was deposited in the laboratory of Kobe Pharmaceutical University.

Neferine was isolated and identified from embryos of the seeds of *N. nucifera* according to the method described in our previous report (Sugimoto et al., 2008). Isolated neferine was prepared as the hydrochloride salt.

2.2. Animals

Male ICR mice weighing 25–30 g (5 weeks old) were purchased from SLC Japan (Japan). Mice had free access to food and water and were maintained on a 12 h dark/light cycle in a room with controlled temperature (23 ± 1 °C) and humidity (55 ± 5%). Experiments were performed in accordance with the Guiding Principles for the Care and

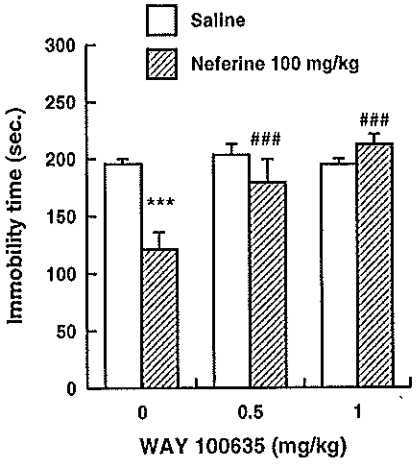
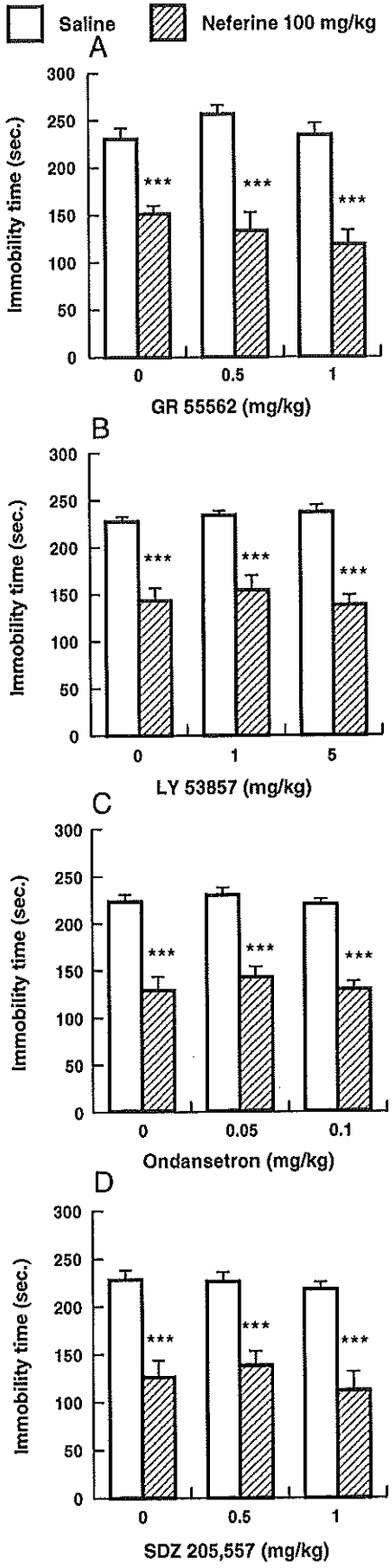


Fig. 4. Effects of WAY 100635 on neferine-induced anti-immobility in mice. Results are shown as mean ± S.E.M. ($N = 5–8$). Neferine at 100 mg/kg was given i.p. WAY 100635 was injected i.p. 30 min before neferine. *** $P<0.001$ vs. saline of respective group. ### $P<0.001$ vs. saline + neferine-treated group. Pretreatment: $F(2,32) = 7.33$, $P<0.01$. Treatment: $F(2,32) = 7.71$, $P<0.01$. Pretreatment × treatment interaction: $F(2,32) = 7.33$, $P<0.01$.



Use of Laboratory Animals approved by The Japanese Pharmacological Society.

2.3. Forced swimming test

The forced swimming test was performed according to the methods described by Porsolt et al. (1977) and our previous reports (Sugimoto et al., 2002). Each mouse was placed in a 25-cm glass cylinder (10 cm diameter) containing 10 cm water at 23±1 °C. Immobility was recorded during a 6-min swimming test. A mouse was judged to be immobile when it floated and its hindlimbs were immobile, and only small movement of the forepaws was made to keep its head above water.

2.4. Drug treatment

Imipramine HCl, maprotiline HCl, 8-hydroxy-2-di(*n*-propylamino) tetralin HBr (8-OH-DPAT), *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide maleate (WAY 100635), 6-methyl-1-(methylethyl)-ergoline-8β-carboxylic acid 2-hydroxy-1-methylpropyl ester maleate (LY 53857), ondansetron hydrochloride and 4-amino-5-chloro-2-methoxy-benzoic acid 2-(diethylamino)ethyl ester HCl (SDZ 205,557) were obtained from Sigma (U.S.A.). 3-[3-(Dimethylamino)propyl]-4-hydroxy-N-[4-(4-pyridinyl)phenyl]benzamide dihydrochloride (GR 55562) was purchased from Tocris (U.K.). All drugs were dissolved in saline and administered i.p. except for 8-OH-DPAT, which was given subcutaneously (s.c.). Volume of administration was 0.1 ml/10 g body weight. Mice in the control group received saline. 5-HT receptor antagonists were given 30 min before neferine or 8-OH-DPAT. The forced swimming test was performed 30 min after treatment with neferine, imipramine, maprotiline and 8-OH-DPAT.

2.5. Measurement of locomotor activity

The locomotor activity of mice was measured using a digital counter with an infrared sensor (DAS system, Neuroscience Inc., Japan) following the method described in previous reports (Yamada et al., 2004). An infrared sensor was set over an open-top clear polycarbonate cage (22.5×33.8×14.0 cm) into which each mouse was placed. Locomotor activity was determined over a period of 10 min. The apparatus detects and records a digital count of the horizontal movements of animals.

2.6. Statistics

Differences between groups of animals treated with saline or drugs alone were analyzed by one-way analysis of variance (ANOVA) followed by the Dunnett's multiple comparison post-hoc test. Other data was analyzed by two-way ANOVA with pretreatment or co-administration, and drugs treatment showing anti-immobility effects as the main factors. Pairwise follow-up comparisons of individual treatment groups were analyzed by Tukey's multiple comparison post-hoc test.

Fig. 5. Effects of several 5-HT receptor antagonists on neferine-induced anti-immobility in mice. Results are shown as mean±S.E.M. (N=5–8). Neferine at 100 mg/kg was given i.p. 5-HT receptor antagonists were injected i.p. 30 min before neferine. *** $P<0.001$ vs. saline of respective group. (A) GR 55562. Pretreatment: $F(2,26)=1.07$, $P>0.05$. Treatment: $F(1,26)=94.64$, $P<0.0001$. Pretreatment×treatment interaction: $F(2,26)=1.52$, $P>0.05$. (B) LY 53857. Pretreatment: $F(2,26)=0.42$. Treatment: $F(1,26)=111.23$, $P<0.0001$. Pretreatment×treatment interaction: $F(2,26)=0.45$, $P>0.05$. (C) Ondansetron pretreatment: $F(2,29)=0.73$, $P>0.05$. Treatment: $F(1,29)=113.62$, $P<0.0001$. Pretreatment×treatment interaction: $F(2,29)=0.06$, $P>0.05$. (D) SDZ 205,557 Pretreatment: $F(2,29)=0.95$, $P>0.05$. Treatment: $F(1,29)=86.27$, $P<0.001$. Pretreatment×treatment interaction: $F(2,29)=0.27$, $P>0.05$.

3. Results

3.1. Effects of neferine, imipramine and maprotiline on the immobility time of mice

Fig. 2A shows the effects of neferine on the immobility time of mice. Anti-immobility effects of neferine at doses ranging from 10 to 100 mg/kg were examined 30 min after its administration based on previous studies on its sedative and anxiolytic effects (Sugimoto et al., 2008). Neferine elicited apparent anti-immobility effects dose-dependently (Fig. 2A). Fig. 2B and C shows the significant anti-immobility effects of both imipramine and maprotiline. Fig. 2D shows effects of neferine, imipramine and maprotiline on locomotor activity in mice 30 min after the injection. Neferine, imipramine and maprotiline decreased locomotor activity. Fig. 3 shows a comparison of the anti-immobility effects of neferine, imipramine and maprotiline. In a comparison of the molecular doses per body weight, neferine showed the most potent anti-immobility effects.

3.2. Effects of 5-HT receptor antagonists on neferine-induced anti-immobility effects in mice

Figs. 4 and 5 illustrate the effects of 5-HT receptor antagonists on the anti-immobility effects elicited by neferine. The 5-HT_{1A} receptor antagonist WAY 100635 (0.5 and 1 mg/kg) significantly blocked anti-immobility effects (Fig. 4). However, the 5-HT_{1B} receptor antagonist, GR 55562 (0.5 and 1 mg/kg), the 5-HT_2 receptor antagonist, LY 53857 (1 and 5 mg/kg), the 5-HT_3 receptor antagonist, ondansetron (0.05 and 0.1 mg/kg) and the 5-HT_4 receptor antagonist, SDZ 205,557 (0.5 and 1 mg/kg) did not affect neferine-induced anti-immobility effects (Fig. 5). The doses and administration time of 5-HT receptor antagonists were chosen based on information about how they block their respective receptors (Nair and Gudelsky, 2005; Jeong et al., 2004; Le et al., 2006; Chojnacka-Wojcik et al., 1991; Yamada and Sugimoto, 2002).

The locomotor activities after neferine and 5-HT receptor antagonist treatment are shown in Table 1. Neferine at a dose of 100 mg/kg reduced locomotor activity as shown in our previous study (Sugimoto et al., 2008). 5-HT receptor antagonists did not increase baseline locomotor activity and neferine-induced hypolocomotion.

3.3. Effects of WAY 100635 on 8-OH-DPAT-induced anti-immobility effects and co-administration with subactive doses of neferine and 8-OH-DPAT on immobility time in mice

Fig. 6A shows that 8-OH-DPAT dose-dependently reduced the immobility time in mice. Pretreatment with WAY 100635 reduced the anti-immobility effects induced by 8-OH-DPAT (Fig. 6B).

Fig. 7A shows the immobility time after co-administration of subactive doses of neferine (10 mg/kg) and 8-OH-DPAT (0.1 mg/kg). Co-administration with neferine at 10 mg/kg and 8-OH-DPAT at 0.1 mg/kg elicited a significant anti-immobility effect. Changes in locomotor activity after administration of 8-OH-DPAT with WAY 100635 or neferine are shown in Figs.6C and 7B, respectively. No changes in locomotor activity after treatment with these drugs were observed.

4. Discussion

Embryos of the seeds of *N. nucifera* are known to have suppressive effects on the central nervous system, such as sedative activity, or to reduce fever in humans (Chiang, 1978). Our previous findings demonstrated for the first time that extracts and the major alkaloid neferine from embryos of the seeds of *N. nucifera* have apparent sedative

Table 1
Locomotor activity after the injection of 5-HT receptor blockers and neferine.

Pretreatment	Treatment	Activity counts/10 min	Two way ANOVA
Saline	Saline	375 ± 15.6	Pretreatment $F(2,24)=0.06$, $P>0.05$.
Saline	Neferine	207 ± 15.5[a]	Treatment $F(1,24)=66.26$, $P<0.0001$.
WAY 100635 0.5 mg/kg	Saline	362 ± 17.3	Pretreatment × treatment interaction $F(2,24)=0.08$, $P>0.05$.
WAY 100635 0.5 mg/kg	Neferine	210 ± 22.2[b]	
WAY 100635 1 mg/kg	Saline	379 ± 21.3	
WAY 100635 1 mg/kg	Neferine	210 ± 34.7[a]	
Saline	Saline	388 ± 13.0	Pretreatment $F(2,24)=0.26$, $P>0.05$.
Saline	Neferine	221 ± 14.8[a]	Treatment $F(1,24)=54.18$, $P<0.0001$.
GR 55562 0.5 mg/kg	Saline	378 ± 14.5	Pretreatment × treatment interaction $F(2,24)=0.50$, $P>0.05$.
GR 55562 0.5 mg/kg	Neferine	259 ± 47.7[b]	
GR 55562 1 mg/kg	Saline	376 ± 7.2	
GR 55562 1 mg/kg	Neferine	230 ± 22.8[b]	
Saline	Saline	393 ± 9.0	Pretreatment $F(2,24)=9.37$, $P<0.001$.
Saline	Neferine	246 ± 37.6[b]	Treatment $F(1,24)=147.61$, $P<0.0001$.
LY 53857 1 mg/kg	Saline	344 ± 12.8	Pretreatment × treatment interaction $(2,24)=5.09$, $P<0.05$.
LY 53857 1 mg/kg	Neferine	122 ± 28.7[a,c]	
LY 53857 5 mg/kg	Saline	384 ± 16.8	
LY 53857 5 mg/kg	Neferine	95.4 ± 13.1[a,c]	
Saline	Saline	376 ± 20.0	Pretreatment $F(2,24)=0.40$, $P>0.05$.
Saline	Neferine	250 ± 21.5[b]	Treatment $F(1,24)=38.70$, $P<0.0001$.
Ondansetron 0.05 mg/kg	Saline	351 ± 29.6	Pretreatment × treatment interaction $F(2,24)=0.19$, $P>0.05$.
Ondansetron 0.05 mg/kg	Neferine	233 ± 21.7[b]	
Ondansetron 0.1 mg/kg	Saline	368 ± 27.6	
Ondansetron 0.1 mg/kg	Neferine	220 ± 32.1[b]	
Saline	Saline	403 ± 12.3	Pretreatment $F(2,24)=0.48$, $P>0.05$.
Saline	Neferine	237 ± 8.9[a]	Treatment $F(1,24)=54.26$, $P<0.0001$.
SDZ 205,557 0.5 mg/kg	Saline	371 ± 15.3	Pretreatment × treatment interaction $F(2,24)=0.28$, $P>0.05$.
SDZ 205,557 0.5 mg/kg	Neferine	241 ± 30.0[a]	
SDZ 205,557 1 mg/kg	Saline	404 ± 17.8	
SDZ 205,557 1 mg/kg	Neferine	257 ± 43.9[a]	

Results are shown as mean ± S.E.M. ($N=5$).
[a] $P<0.001$ vs. saline of respective group.
[b] $P<0.01$ vs. saline of respective group.
[c] $P<0.001$ vs. saline + neferine.

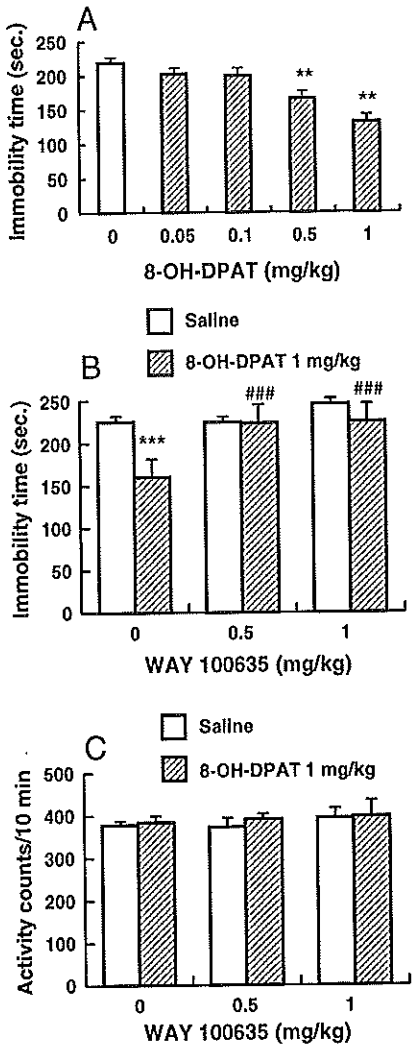


Fig.6. Effects of 8-OH-DPAT on immobility and effects of co-administration of WAY 100635 and 8-OH-DPAT on immobility and locomotor activity. Results are shown as mean ± S.E.M. (N = 5–10). 8-OH-DPAT was given s.c. 30 min before test. (A) Effects of 8-OH-DPAT on immobility in mice. ** P<0.01 vs. saline. $F(4,25)$ = 12.11, P<0.05. (B) Effects of WAY 100635 on 8-OH-DPAT-induced anti-immobility effects in mice. 8-OH-DPAT at 1 mg/kg was given s.c. WAY 100635 was injected i.p. 30 min before 8-O-DPHA T. 30 min after the injection of 8-OH-DPAT, activity counts were measured during 10 min. *** P<0.001 vs. saline of respective group. ### P<0.001 vs. saline + neferine-treated group. Pretreatment: $F(2,29)$ = 5.62, P<0.01. Treatment: $F(1,29)$ = 4.48, P<0.05. Pretreatment × treatment interaction: $F(2,29)$ = 5.90, P<0.01. (C) Locomotor activity after treatment with WAY 100635 and 8-OH-DPAT in mice. 8-OH-DPAT at 1 mg/kg was given s.c. WAY 100635 was injected i.p. 30 min before 8-O-DPHA T. Pretreatment: $F(2,24)$ = 0.26, P>0.05. Treatment: $F(1,24)$ = 0.24, P>0.05. Pretreatment × treatment interaction: $F(1,24)$ = 0.06, P>0.05.

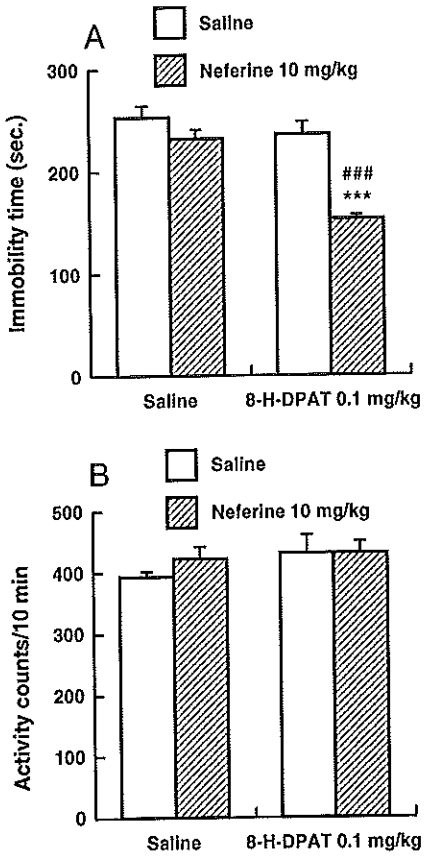


Fig. 7. Effects of co-administration of 8-OH-DPAT and neferine at subactive doses on the immobility and locomotor activity in mice. Results are shown as mean ± S.E.M. (N = 5–8). 8-OH-DPAT at 0.1 mg/kg was given s.c. Neferine at 10 mg/kg was injected i.p. 8-OH-DPAT and neferine were injected simultaneously. *** P<0.001 vs. saline of respective group. ### P<0.001 vs. saline + neferine-treated group. (A) Effects of 8-OH-DPAT at 0.1 mg/kg on immobility time treated with neferine at 10 mg/kg on immobility time in mice. Pretreatment: $F(2,29)$ = 5.62, P<0.01. Treatment: $F(1,29)$ = 4.48, P<0.05. Pretreatment × treatment interaction: $F(2,29)$ = 5.90, P<0.01. (B) Locomotor activity after injection of neferine and 8-OH-DPAT in mice. 8-OH-DPAT treatment: $F(1,16)$ = 1.22, P>005. Neferine treatment: $F(1,16)$ = 0.50, P>0.05. 8-OH-DPAT × neferine interaction: $F(1,16)$ = 0.4915, P>0.05.

effects on mice (Sugimoto et al., 2008). This indicates that the main alkaloid neferine may play a major role in the central depressive effects of embryos of the seeds of *N. nucifera*. Further pharmacological analysis of neferine in mice revealed that neferine has anxiolytic effects in mice following the elevated plus maze test (Sugimoto et al., 2008). These results suggest that embryos of the seeds of *N. nucifera* and neferine may be useful for treating psychiatric disorders.

Depression is a common, serious illness that debilitates motivation. Depression is observed in more than 9% of the population in the Western world (Schloss and Henn, 2004). Depression is related to monoamines in the brain, especially to serotonin (5-HT) and noradrenaline. Elevation of 5-HT or noradrenaline in the synapses improves depression and many antidepressants act on these neurotransmitter systems (Briley and Moret, 1993). At present, depression is treated with antidepressants; however, some depressed patients are resistant to commonly used antidepressants (reviewed in Richelson, 2003), and therefore, novel antidepressants are required as a substitute. Herbal medicine or agents from traditional plants may be effective against depression and St. John's Wort is often used to treat depressed patients in Europe (Linde et al., 2005; Sarris, 2007). We previously found that neferine from embryos of the seeds of *N. nucifera* had sedative and anxiolytic effects (Sugimoto et al., 2008). Recently, some anxiolytics have shown antidepressant effects. Serotonergic anxiolytic drugs, including azapirone derivatives, such as gepirone or tandospirone, may also be effective against depression (Chojnacka-Wojcik et al., 1991; Yamada et al., 2003). We therefore examined the effects of neferine on immobility time in the forced swimming test.

The tricyclic antidepressant imipramine and selective noradrenaline reuptake inhibitor maprotiline elicited apparent anti-immobility effects in mice, consistent with previous reports (Porsolt et al., 1979; Yamada and Sugimoto, 2001, 2002). Neferine dose-dependently shortened the immobility time in mice, indicating its antidepressant-like effects. The anti-immobility effect of neferine was not caused by ambulation, because neferine did not increase locomotor activity. The doses of neferine that induced anti-immobility effects were almost in the same range as those causing sedative or anxiolytic effects in mice (Sugimoto et al., 2008). In comparison with the efficacy of imipramine and maprotiline at molecular doses, neferine displayed strong anti-immobility

effects; therefore, these results suggest that neferine has antidepressant-like effects in addition to anxiolytic effects.

As mentioned above, it is suggested that depression is strongly related to the serotonergic system. Specifically, it has been reported that anti-immobility effects are related to 5-HT receptors and thus, we studied the involvement of 5-HT receptors in the anti-immobility effects of neferine. It is well known that 5-HT receptors are divided into many subtypes. It was previously reported that 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_3 and 5-HT_4 receptors were related to the anti-immobility effects of antidepressants in rodents (Redrobe and Bourin, 1997; Redrobe et al., 1996; Lucas et al., 2007). We therefore examined the effects of antagonists of these 5-HT receptor subtypes on the anti-immobility effects of neferine.

The 5-HT_{1A} receptor antagonist, WAY 100635 significantly reduced anti-immobility effects. However, the 5-HT_{1B} receptor antagonist, GR 55562 did not influence the anti-immobility effects of neferine. The non-selective 5-HT_2 receptor antagonist, LY 53857 also did not modify the anti-immobility effects of neferine. In addition, neither of the 5-HT_3 and 5-HT_4 receptor antagonists, ondansetron nor SDZ 205,557 showed any effects. These results suggest that the 5-HT_{1A} receptor is strongly related to the anti-immobility effects of neferine and that neferine may influence the serotonergic system. Chojnacka-Wojcik et al. (1991) reported that the 5-HT_{1A} receptor participated in the anti-immobility effects of gepirone in rats. This previous result, showing the involvement of the 5-HT_{1A} receptor in anti-immobility effects, is consistent with our present data on neferine. As demonstrated herein, the selective 5-HT_{1A} receptor agonist 8-OH-DPAT reduced the immobility time in mice and was antagonized by pretreatment with WAY 100635, which can block the effects of neferine. In addition, co-administration of subactive doses of neferine and 8-OH-DPAT produced significant anti-immobility effects. Although neferine decreased locomotor activity, 5-HT receptor antagonists and 8-OH-DPAT did not increase locomotor activity. Therefore, locomotion is not related to the anti-immobility effects of neferine. Taken together, these results strongly support the idea that the 5-HT_{1A} receptor is connected to the anti-immobility effects of neferine in mice.

The present results indicate that neferine elicits anti-immobility effects in mice, indicating that it possesses antidepressant-like effects. Since neferine is a major alkaloid of embryos of the seeds of *N. nucifera* (Chiang, 1978), this herbal medicine may be effective against depression in humans. The antidepressant-like effects of neferine demonstrated in this study may suggest that embryos of the seeds of *N. nucifera* may be useful to treat psychiatric diseases, including anxiety and depression. The forced swimming test is widely used for assessing the activity of antidepressants (Porsolt et al., 1979). Although this test is useful for detection of antidepressants, clinical efficacy in humans should be evaluated in detail (Cryan and Slattery, 2007; Kalueff et al., 2007).

We demonstrated that anti-immobility effects of neferine are mediated by the 5-HT_{1A} receptor. It is assumed that neferine acts as a direct 5-HT_{1A} receptor agonist, similar to 8-OH-DPAT or azapirone derivatives, and/or may enhance the activity of serotonergic neurons as a result of inhibiting 5-HT reuptake or the activation of 5-HT metabolism. Other mechanisms of the serotonergic systems may be implicated in the anti-immobility effects of neferine. Further detailed studies are required and are now under investigation in our laboratory.

References

Borsini, F., 1995. Role of the serotonergic system in the forced swimming test. Neurosci. Biobehav. Rev. 19, 377–395.

Briley, M., Moret, C., 1993. Neurobiological mechanisms involved in antidepressant therapies. J. Clin. Neuropharmacol. 16, 24–35.

Chiang, Su New Medical College (Ed.), 1978. Zhong-yao-dai-ci-dian (Dictionary of Chinese Crude Drugs). Shanghai Scientific Technologic Publisher, Shanghai, p.1806.

Chojnacka-Wojcik, E., Tatarczynska, E., Golembiowska, K., Przegalinski, E., 1991. Involvement of 5-HT_{1A} receptors in the antidepressant-like activity of gepirone in the forced swimming test in rats. Neuropharmacology 30, 711–717.

Cryan, J.F., Slattery, D.A., 2007. Animal models of mood disorders: recent development. Curr. Opin. Psychiatry 20, 1–7.

Deakin, J.F., 1998. The role of serotonin in panic, anxiety and depression. J. Int.Clin. Psychopharmacol. 13 (Suppl 4), S1–S5.

Figgitt, D., McClellan, K., 2000. Fluvoxamine. An updated review of its use in the management of adults with anxiety disorders. Drugs 60, 925–954.

Furukawa, H., 1966. Studies on the alkaloids of *Nelumbo nucifera* Gaertn. NMR spectra of liensinine type alkaloids. Yakugaku Zasshi 86, 883–886.

Jeong, C.Y., Choi, J.I., Yoon, M.H., 2004. Roles of serotonin receptor subtypes for the antinociception of 5-HT in the spinal cord of rats. Eur. J. Pharmacol. 502, 205–211.

Kalueff, A.V., Wheaton, M., Murphy, D.L., 2007. What's wrong with my mouse model? Advances and strategies in animal modeling of anxiety and depression. Behav. Brain Res. 179, 1–18.

Le, A.D., Funk, D., Harding, S., Juzytsch, W., Fletcher, P.J., Shaham, Y., 2006. Effects of dexfenfluramine and 5-HT3 receptor antagonist on stress-induced reinstatement of alcohol seeking in rats. Psychopharmacology 186, 82–92.

Linde, K., Berner, M., Egger, M., Mulrow, C., 2005. St John's Wort for depression: meta analysis of randomised controlled trials. Br. J. Psychiatry 186, 99–107.

Lucas, G., Rymar, V., Mnie-Filali, O., Bosgaard, C., Manta, S., Lambas-Senas, L., Wilborg, O., Haddjeri, N., Pineyro, G., Sadikoto, A.F., Debonnel, G., 2007. Serotonin(4)(5-HT4) receptor agonists are putative antidepressants with a rapid onset of action. Neuron 55, 712–725.

Nair, S.G., Gudelsky, G.A., 2005. 3, 4-Methylenedioxymethamphetamine(MDMA) enhances the release of acetylcholine by 5-HT4 and D1 receptor mechanisms in the rat prefrontal cortex. Synapse 58, 229–235.

Porsolt, R.D., Bertin, A., Jalfre, M., 1977. Behavioural despair in mice: a primary screening test for antidepressants. Arch. Int. Pharmacodyn. Ther. 229, 327–336.

Porsolt, R.D., Bertin, A., Blavert, N., Denie, M., Jalfre, M., 1979. Immobility induced by forced swimming in rats: effects of agents which modify central catecholamine and serotonin activity. Eur. J. Pharmacol. 57, 201–210.

Redrobe, J.P., Bourin, M., 1997. Partial role of 5-HT_2 and 5-HT_3 receptors in the activity of antidepressants in the mouse forced swimming test. Eur. J. Pharmacol. 325, 129–135.

Redrobe, J.P., MacSweeney, C.P., Bourin, M., 1996. The role of 5-HT_{1A} and 5-HT_{1B} receptors in antidepressant drug actions in the mouse forced swimming test Eur. J. Pharmacol. 318, 213–220.

Richelson, E., 2003. Interactions of antidepressants with neurotransmitter transporters and receptors and their clinical relevance. J. Clin. Psychiatry 64 (Suppl 13), 5–12.

Sarris, J., 2007. Herbal medicines in the treatment of psychiatric disorders: a systematic review. Phytother. Res. 21, 703–716.

Schloss, P., Henn, F.A., 2004. New insights into the mechanisms of antidepressant. Pharmacol. Ther. 102, 47–60.

Sugimoto, Y., Yamada, S., Yamada, J., 2002. The 5-HT2 receptor antagonist reduces immobility of mice treated with the atypical antidepressant mianserin in the forced swimming test. Biol. Pharm. Bull. 25, 1479–1481.

Sugimoto, Y., Furutani, S., Itoh, A., Tanahashi, T., Nakajima, H., Oshiro, H., Sun, S., Yamada, J., 2008. Effects of extracts and neferine from the embryo of *Nelumbo nucifera* seeds on the central nervous system. Phytomedicine 15, 1117–1124.

Yamada, J., Sugimoto, Y., 2001. Effects of 5-HT_2 receptor antagonists on the anti-immobility effects of imipramine in the forced swimming test with mice. Eur. J. Pharmacol. 427, 221–225.

Yamada, J., Sugimoto, Y., 2002. Differential effects of the 5-HT_2 receptor antagonist on the anti-immobility effects of noradrenaline and serotonin reuptake inhibitors in the forced swimming test. Brain Res. 958, 161–165.

Yamada, K., Yagi, G., Kanba, S., 2003. Clinical efficacy of tandospirone augmentation in patients with major depressive disorder: a randomized controlled trial. Psychiatry Clin. Neurosci. 57, 183–187.

Yamada, J., Sugimoto, Y., Yamada, S., 2004. Involvement of dopamine receptors in the anti-immobility effects of dopamine re-uptake inhibitors in the forced swimming test. Eur. J. Pharmacol. 504, 207–211.