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Antidepressant-like Effect of Kaempferol and Quercitirin, Isolated from *Opuntia ficus-indica* var. *saboten*

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ABSTRACT

Opuntia ficus-indica var. saboten. is widely cultivated in Jeju Island (South Korea) for use in manufacture of health foods. This study described antidepressant effect of two flavonoids (kaempferol and quercitrin) isolated from the *Opuntia ficus-indica var. saboten*. The expression of the hypothalamic POMC mRNA or plasma β-endorphin levels were increased by extract of Opintia ficus-indica var. saboten or its flavoniods administered orally. In addition, antidepressant activity was studied using tail suspension test (TST), forced swimming test (FST) and rota-rod test in chronically restraint immobilization stress group in mice. After restraint stress (2 hrs/day for 14 days), animals were kept in cage for 14 days without any further stress, bet with drugs. Mice were fed with a diet supplemented for 14 days and during the behavioral test period with kaempferol or quercitrin (30 mg/kg/day). POMC mRNA or plasma β-endorphin level was increased by extract of *Opintia ficus-indica var. saboten* and its flavoniods. In addition, immobility time in TST and FST was significantly reduced by kaempferol or quercitrin. In rota-rod test, the time of permanence was maintained to the semblance of control group in turning at 15 rpm. Our results suggest that two flavonoids (kaempferol and quercitrin) isolated from the *Opuntia ficus-indica var. saboten*. show a potent antidepressant effect.

Key words: kaempferol, quercitirin, antidepressant, *Opuntia ficus-indica var. saboten*

INTRODUCTION

Chronic stress is thought to be a risk factor for psychosomatic psychiatric illnesses, such as anxiety and depression disorders (Ader and Cohen, 1993; McEwen and Stellar, 1993). Stress alters the homeostasis of many regions of the body, including neural, endocrine, immune, and digestive systems. Depression is a disorder characterized by a broad range of symptoms, including altered mood and cognitive functions, and recurrent thoughts of death or suicide. In contrast with the normal experiences of sadness, clinical depression is a chronic disease

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that can interfere significantly in the individual's life quality. Depression constitutes the second most common chronic condition in clinical practice (Whooley and Simon, 2000) and would become the second leading cause of premature death or disability worldwide by the year 2020 (WHO, 1999). Approximately two-thirds of the anxious or depressed patients respond to the currently available treatments but the magnitude of improvement is still disappointing (Mora et al., 2006). Although there are many effective antidepressants available today, the current armentarium of therapy is often inadequate with unsatisfactory results in about one third of all subjects treated. This necessitates the development of new and more effective antidepressants from traditional medicinal plants whose psychotherapeutic potential has been assessed in a variety of animal models (Zhang, 2004).

Opuntia ficus-indica var. saboten is widely cultivated in Jeju Island (South Korea) for use in manufacture of health foods such as tea, jam and juice. Its fruits and stems have been traditionally used in oriental folk medicines to treat diabetes, hypertension, asthma, burns, edema and indigestion (Lopez, 1995; Ahn, 1998). It has also been reported that the extracts of fruits or stems exhibit hypoglycemic (Ibanez-Camacho and Roman-Ramos, 1979; Trejo-Gonzalez et al., 1996), anti-ulcer (Galati et al., 2001), and anti-allergic activities (Lee et al., 2000). In addition, Park et al. (1998) reported analgesic and anti-inflammatory activity of the fruits and the stem extracts and isolated β-sitosterol as an active anti-inflammatory principle from the stem extract (Park et al., 2001). However, antidepressant-related effect of *Opuntia ficus-indica var. saboten*. has not been well characterized. Thus, the medicinal importance of this plant motivated us to investigate its possible antidepressant effect.

In the present study, the possible antidepressant effect of two flavonoids (kaempferol and quercitrin) isolated from the *Opuntia ficus-indica var. saboten* were examined. Here we demonstrate that both kaempferol and quercitrin shows anti-depressant effect in chronic stress mice model, consisting of restraint for 2 hr daily for 14 days.

MATERIALS AND METHODS

These experiments were approved by the University of Hallym Animal Care and Use Committee (registration number: Hallym 2009-05). All procedures were conducted in accordance with the 'Guide for Care and Use of Laboratory Animals' published by the National Institutes of Health.

Experimental animals

Male ICR mice (MJ Co., Seoul, Korea) weighing 20～25 g were used for all the experiments. Animals were housed 5 per cage in a room maintained at 22±0.5°C with an alternating 12 hr light-dark cycle. Food and water were available ad libitum. The animals were allowed to adapt to the laboratory for at least 2 hr before testing and were only used once. Experiments were performed during the light phase of the cycle (10:00～17:00).

Oral administration

Oral administration was performed with gage in a volume of 500 μl/ 25 g bodyweight.

The making of crude extract from Opintia ficus-indica var. saboten

Opuntia ficus-indica var. saboten (300 g) was dissolved in 80% ethanol (1,500 ml) and extracted as refluxing for 3 hours, and then the extract was filtered for obtaining A. This process was repeated again once to obtain B from residue. A and B were mixed. This mixture was decompressed and dried for using as extract of *Opuntia ficus-indica var. saboten*.

Isolation of total RNA

Three animals of each group were dissected for Northern blot analysis. Every result was confirmed by three times independent experiments. Total cellular RNA was extracted from dissected pituitary gland and hypothalamus tissues using a rapid guanidine thiocyanate-watersaturated phenol/chloroform extraction procedure and subsequent precipitation with acidic sodium acetate (Chomczynski and Sacchi, 1987). Total cellular RNA in the aqueous phase was precipitated with ice-cold isopropyl alcohol. Isolated RNA samples were subjected to spectrophotometric analysis at 260 nm and 280 nm.

Preparation of digoxigenin (DIG)-labeled cRNA probes

The cRNA probe for POMC (Civelli et al., 1982) was synthesized *in vitro* from linearized expression vectors which contained SP6 or T7 viral promoter. One microgram of linearized plasmid was mixed with RNA labeling mixture that contained ATP, CTP, GTP and DIG-labeled-UTP, transcription buffer, and SP6 or T7 RNA polymerase. After incubation at 37°C for 2 h, the mixture was co-incubated with DNase I (RNase free) at 37°C for 15 min, precipitated in ethanol containing lithium chloride at 70°C for 30 min, and washed with 70% chilled ethanol.

Non-isotopic northern blot analysis

Isolated RNA samples were dissolved in 40～50 ml water and a 400X diluted RNA solution was subjected to spectrophotometric analysis at 260 and 280 nm. Ten micrograms of total RNA and an equal volume of RNA loading buffer (50% glycerol, 1 mM EDTA) were denatured in 65°C for 10 min and subsequently cooled on ice. The denatured RNA samples were electrophoresed on 1% agarose-formaldehyde gels under 6070 V and transferred to nylon hybond-N hybridization membrane sheets (Amersham, Buckinghamshire, UK). After UV cross-linking, the membranes were pre-hybridized at 68°C for at least 1 h in a prehybridization buffer (5X SSC, 50% formamide, 0.02% SDS, 0.1% sodium N-lauroyl sarcosine, and 2% blocking reagent). The DIG-labeled POMC probe was added to the prehybridization buffer containing 50% formamide. The membranes were incubated overnight at 68°C in a shaking waterbath, and washed twice for 10 min per wash in 2X SSC and 0.1% SDS at room temperature. Then the membranes were washed twice for 15 min per wash 0.1X SSC and 0.1% SDS. After equilibrating the membranes in Buffer I (100 mM maleic acid [pH 7.5] and 150 mM NaCl) for 1 min, the membranes were gently agitated in Buffer II (1% blocking reagent in Buffer I) for 30～60 min. The membranes were hybridized with the diluted anti-DIG-alkaline phosphatase (1 : 10,000 [75 mU/ml]) in Buffer II for 30 min. After washing the membranes twice for 15 min per wash in 0.3% Tween 20 (in Buffer I), the membranes were equilibrated in Buffer III (100 mM Tris-HCl [pH 9.5], 100 mM NaCl, and 50 mM $MgCl_2$) for 2 min. Diluted disodium 3-[4-methoxyspiro (1,2-dioxietane-3,2'-[5'-chloro] tricycle [3.3.1.1.3,7decan]-4-yl) phenyl phosphate (CSPD) (Boehringer Mannheim) (1 : 100 dilution in Buffer III) was spread over the surface of the membranes. After incubation of the membranes at 37°C for 15～20 min, the membranes were exposed to Hyperfilm-ECL (Amersham) for detection of the chemiluminescent signal. For rehybridization, blots were washed for 20 min at room temperature in sterilized (Millipore) water. The membranes were washed overnight at 65°C in 50 mM Tris-HCl (pH 8.0), 50% dimethylformamide and 1% SDS to remove the hybridized probe and rehybridized to the DIG-labeled rat cyclophilin cRNA probe, a gene encoding peptidyl-prolyl cis-trans isomerase which is constitutively constitutively expressed in most mammalian tissues with the exception of skeletal muscles (Danielson et al., 1988; Takahashi et al., 1989).

Plasma β-endorphin ELISA analysis

The blood sample was collected three times (30, 90 and 180 min) or 90 min after oral administration of the diluted-extract (1,000 and 2,000 mg/kg) or kaempferol (10, 25 and 50 mg/kg) or quercitrin (10, 25 and 50 mg/kg), and then the serum was obtained by centrifuge (4°C, 1,600×g, 15 min). We observed the serum β-endorphin level with ELISA kit (Phoenix pharmaceutical, INC). The immunoplaste in this kit is pre-coated with secondary antibody and nonspecific binding sites are blocked. The secondary antibody can bind to the Fc fragment of the primary antibody (peptide antibody) whose Fab fragment will be competitively bound by both biotinylated peptide and peptide standard or targeted peptide in sample. The biotinylated peptide is able to interact with streptavidin-horseradish peroxidase (SA-HRP) which catalyzes the substrate solution composed of 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide to produce a blue colored solution. The enzyme-substrate reaction is stopped by hydrogen chloride (HCL) and the solution turns to yellow.

Restraint stress and drug treatment

Stress treatment procedure has been described previously (Kim and Han, 2006). To deliver rest-

raint, 8-week-old mice weighing 22～23 g were individually placed head-first into a well-ventilated 50-ml conical tube and plugged with a 3 cm long middle tube and finally the cap of the 50 ml tube. Mice were not able to move forward or backward in this device. This restraint stress was delivered to animals at set times daily from 10 AM. Control mice remained in their original cages and were left undisturbed in this home environment. After restraint stress administration, restrained animals were returned to their normal home environments by housing them in pairs. This procedure was repeated for 14 days unless otherwise indicated. After restraint, animals were kept in their normal home cages for additional fourteen days without any further stress, but with drugs. Mice were fed with a diet supplemented for 14 days and during the behavioral test period with kaempferol or quercitrin (Sigma) (30 mg/kg/day). Behavioral tests were performed in the sequence of TST, FST, and rota-rod tests.

Tail suspension test (TST)

Mice were suspended individually by their tail from a metal rod. The rod was fixed 50 cm above the surface of a table covered with soft cloth in a sound-isolated room. The tip of the tail was fixed using adhesive Scotch tape; the duration of the test was 6 min. The immobility time was determined by an observer, using a stopwatch, who was unaware of the strain (Steru et al., 1985; Bilkei-Gorzo et al., 2002).

Forced swim test (FST)

Mice were placed in a Plexiglas cylinder (height: 27 cm, diameter: 15 cm) containing water at 24°C and a depth of 14 cm so that they could neither escape nor touch the bottom. Mice were subjected to a 15-min pre swim. After a 15-min test, the animals were dried quickly with a towel and returned to home cages. One day later, mice were forced to swim for 6 min. The animals were habituated for the first 1 min and behavior was noted over the next 5 min. Immobility time was defined as the summed time during which animals remained floating with all limbs motionless. This scoring method has been described previously (Porsolt et al., 1977; Armario et al., 1988; 1991; Kim and Han, 2006).

Rota-rod test

Mice were evaluated on their motor activity using Rota-rod tested at 10 or 15 rpm. The time mice remained on the Rota Rod was recorded. Maximum time was adjusted to 5 min.

Drugs

Kaempferol and quercitrin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Extract of *Opintia ficus-indica var. saboten* and quercitirin were dissolved in saline. Kaempferol was prepared following steps: (A) 1 g of kaempferol was dissolved in 0.5 ml of ethanol plus 0.5 ml of polyethylene glycol 400. (B) Separately, 100 mg of sodium carboxymethylcellulose was dissolved in 9 ml of distilled water. (C) Finally, Solution (A) and Solution (B) were vigorously mixed. This solution excluding kaempferol was used as vehicle control. All drugs were prepared just before use.

The Fig. 1 is structures of kaempferol and quercitrin.

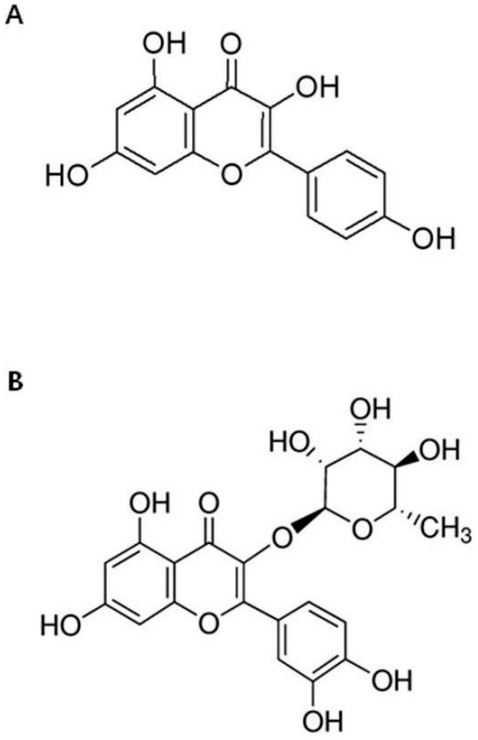

Fig. 1. Structures of kaempferol and quercitirin.

Statistical analysis

ELISA data was presented as the mean±SEM. The statistical significance of differences between groups was assessed with one-way ANOVA with Bonferroni's post-hoc test using GraphPad Prism version 4.0 for Windows XP (GraphPad Software, San Diego, CA, USA); $p<0.05$ was considered significant. Behavior test data two-sample comparisons were carried out using the Student's t-test, and multiple comparisons were made using one-way ANOVA, followed by the Newman-Keuls multiple range test. All data are presented as mean ±SEM, and statistical significance was accepted at the 5% level unless otherwise indicated.

RESULTS

The expression of the hypothalamic POMC mRNA and plasma β-endorphin level elicited by extract of Opintia ficus-indica var. saboten administered orally

Extract of *Ofuntia ficus-indica var. saboten* (1 or 2 g/kg) was administered orally in mice, and hypothalamus was dissected or blood sample was collected at 30, 90 and 180 min after administration of extract of *Ofuntia ficus-indica var. saboten*. The control group was administered 0.9% normal saline orally instead of the extract. In the northern-blot assay, we found that POMC mRNA was increased in extract-treated group compared to control group at all times (Fig. 2A). In ELISA method, we found that plasma β-endorphin level was increased by extract of *Opintia ficus-indica var. saboten* (Fig. 2B). In 30 min after oral administration, plasma β-endorphin level was increased in extreat-treated group of 2,000 mg/kg compared to control group. In 90 min after oral administration, plasma β-endorphin level was increased slightly in both extract-treated group, but not significantly. In 180 min after oral administration, plasma β-endorphin level was increased significantly in dose dependent manner in extract-treated group (1 and 2 g/kg) compared to control group.

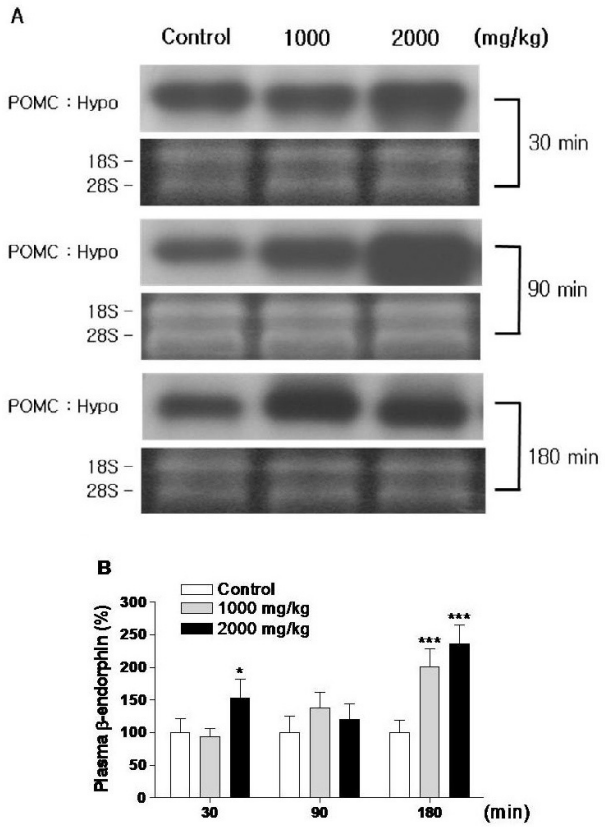

Fig. 2. The expression of the hypothalamic POMC mRNA and plasma β-endorphin level elicited by extract of *Opintia ficus-indica var. saboten* administered orally. (A) For Northern blot analysis, the whole mouse hypothalamus was dissected. Northern blot analysis was performed at time points (30, 90 and 180 min) after saline or extract of *Opintia ficus-indica var. saboten* (1,000 and 2,000 mg/kg) administered orally. Total constitutively expressed ribosomal RNA (18S and 28S) was used as an internal loading control. The number of animal used for each group was 3. (B) For ELISA, the whole mouse blood sample was collected. ELISA was performed at time points (30, 90 and 180 min) after saline or extract of *Opintia ficus-indica var. saboten* (1,000 and 2,000 mg/kg) administered orally. The vertical bars indicate the standard error of the mean. The number of animal used for each group was 8～10 (*$p<0.05$, ***$p<0.001$, compared with control group).

Effects of kaempferol and quercitrin on the plasma β-endorphin level in blood

Kaempferol or quercitrin (10, 25 and 50 mg/kg) was administered orally in mice, and blood sample was collected after 90 min. In ELISA method, we found that plasma β-endorphin level was increased by kaempferol and quercitrin (Fig. 3). Plasma β-endorphin level was increased significantly in dose dependent manner in kaempferol or quercitrin treated group compared to control group.

Antidepressant-like effects of kaempferol and quercitrin

After restraint stress (2 hrs/day for 14 days), animals were kept in cage for another 14 days

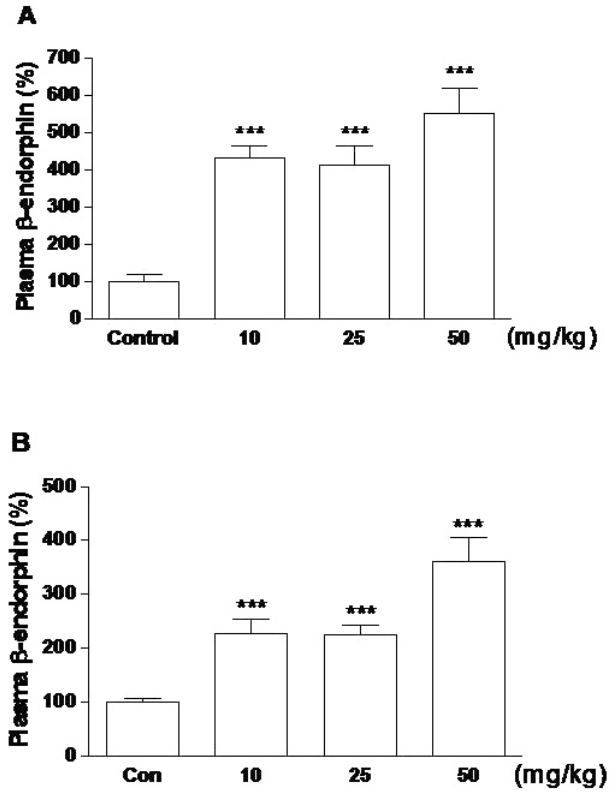

Fig. 3. The plasma β-endorphin level elicited by kaempferol and quercitirin administered orally. For ELISA, the whole mouse blood sample was collected. ELISA was performed at 90 min after vehicle control or kaempferol (10, 25 and 50 mg/kg) (A) and saline or quercitirin (10, 25 and 50 mg/kg) (B) administered orally. The vertical bars indicate the standard error of the mean. The number of animal used for each group was 8~10 (***p $<$0.001, compared with control group).

without any further stress, bet with drugs. Mice were fed with a diet supplemented for 14 days and during the behavioral test period with kaempferol or quercitrin (30 mg/kg/day). Behavioral tests were performed in the sequence of TST, FST, and rota-rod tests tests as described below (Fig. 4A). In TST test, the immobility time was increased in chronically restraint stress group compared to control group. Both kaempferol and quercitrin attenuated the total immobilization time to the control level. (Fig. 4B). In FST test, the immobility time was increased in chronically restraint stress group compared to control group, similar to the result shown in TST test. Both kaempferol and quercitrin attenuated the total immobilization time to the control level (Fig. 4C). In rota-rod test, the time of permanence in chronically restraint stress group was decreased significantly compared to control group during at the speed of 15 rpm. Both kaempferol and quercitrin reversed the time of permanence to the control level. However, during at the speed of 10 rpm, no different response was observed in all groups (Fig. 4D).

DISCUSSION

The opioid system has been implicated in the mechanism of action of some antidepressants. In this regard, it has been proposed that involvement of the opioid system in the antidepressants' mechanism of action may be necessary in order to prove effective in the treatment of severe depression (Schreiber et al., 2002). Excessive stress is related to hyperpiesia, diabetes, a gastric ulcer and depression. Reaction against stressful stimuli is necessary to maintain human homeostasis. The endocrine, metabolism, autoimmune and psychological disease arises when this homeostasis is destroyed. CRH (corticotropin-releasing hormone), arginine vasopressin, peptide, glucocorticoid, catecholamine are related to stressful system. Especially, POMC is a precursor of β-endorphin composed of active peptides such as ACTH (adrenocorticotropin), melanotropin, α, β, and α-MSH in other brain regions as well as pituitary gland. These peptides were produced by complicate translation of POMC (Krieger et al., 1977). As mentioned above, stressful system reactivation destroys stress circuit homeostasis as releasing sustained ACTH when chronic stressful stimuli were applied (Sapolsky, 2003). In addition, β-endorphin positive cell body in arcuate nucleus project to PVN (Paraventricular nucleus) (Akil et al., 1984), suggesting that CRH in paraventricular nucleus (PVN) of hypothalamus correlate with in β-endorphin positive neuron in arcuate nucleus. These suggest that β-endorphin may perform an important physiological role in stressful response.

In the present study, we show that POMC mRNA or plasma β-endorphin level was increased by extract of *Opintia ficus-indica var. saboten*. and its flavoniods such as kaempferol and quercitrin. These results suggest that the *Opuntia ficus-indica var. saboten*. and its two flavonoids increase β-endorphin, which may perform as an important physiological regulator in response to depression.

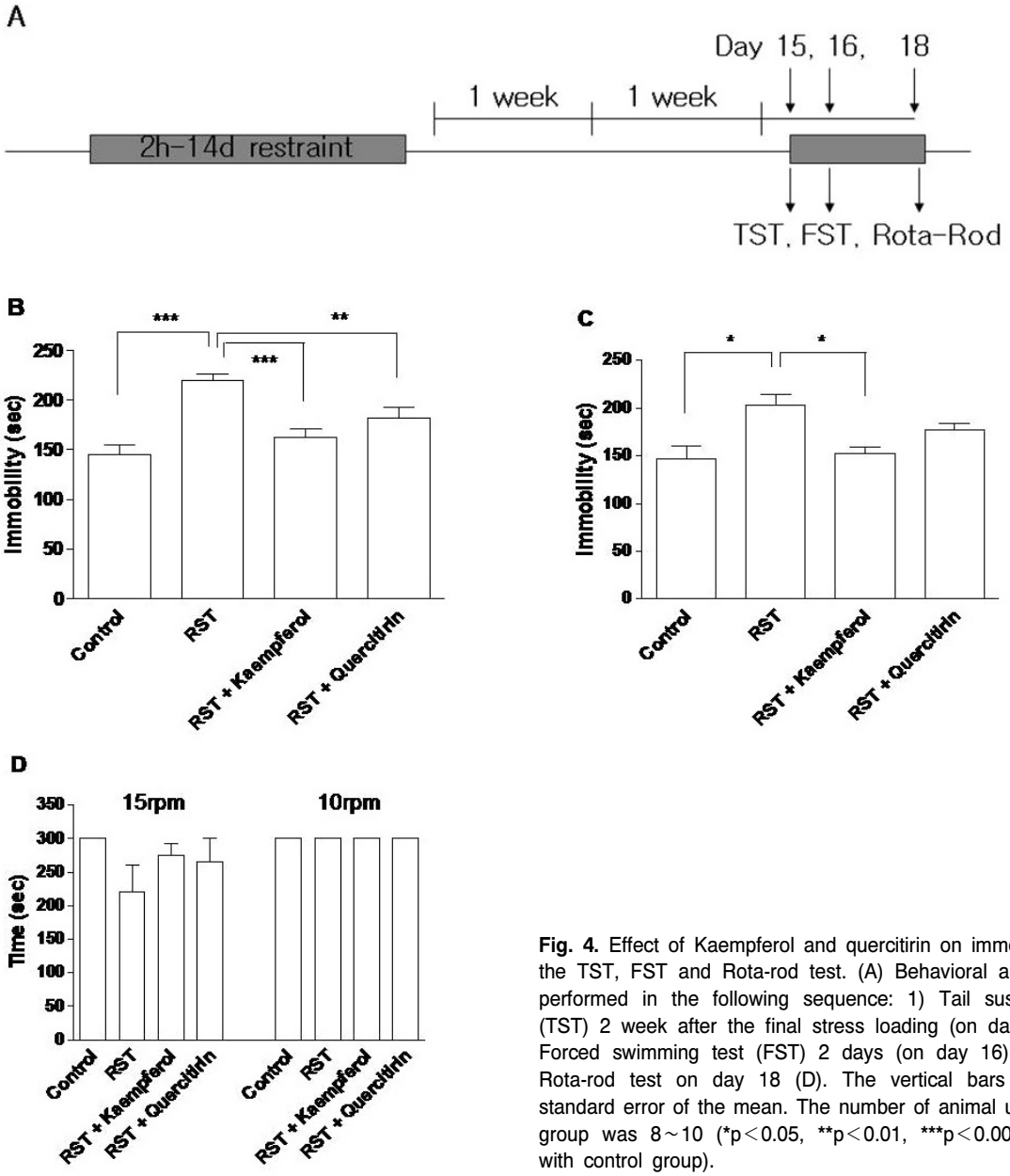

Fig. 4. Effect of Kaempferol and quercitirin on immobility time in the TST, FST and Rota-rod test. (A) Behavioral analyses were performed in the following sequence: 1) Tail suspension test (TST) 2 week after the final stress loading (on day 15) (B), 2) Forced swimming test (FST) 2 days (on day 16) (C), and 3) Rota-rod test on day 18 (D). The vertical bars indicate the standard error of the mean. The number of animal used for each group was 8~10 (*$p<0.05$, **$p<0.01$, ***$p<0.001$, compared with control group).

The main finding of the present investigation suggests the antidepressant activities of two flavonoids (kaempferol and quercitrin) isolated from the *Opuntia ficus-indica var. saboten*. as manifested in forced swimming test, tail suspension test and rota-rod test models of depression. Both kaempferol and quercitrin significantly reduced the immobility period in both TST and FST. In addition, the time of permanence was maintained to the semblance of control group in rota-rod test.

The TST and FST are widely used for screening potential antidepressants. Antidepressants reduce the immobility time in both TST and FST. The immobility behavior displayed in rodents when subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioral despair which in turn may reflect depressive disorders in humans. There is, indeed, a significant correlation between clinical potency and effectiveness of antidepressants in both models (Porsolt et al., 1977; Steru et al., 1985; Cryan et al., 2002). Rota-rod test is classical models for screening central nervous system actions providing information about psychomotor performance, anxiety and depression.

To establish a practical animal model for chronic stress studies, in the present study we examined the usefulness of the rationale that the psychiatric traits anxiety and depression-related behavior can be used as stress assessment parameters. After restraint stress (2 hrs.day for 14 days), animals were kept in cage for another 14 days of period without any further stress, bet with drugs. Mice were fed with a diet supplemented for 14 days and during the behavioral test period with kaempferol or quercitrin (30 mg/kg/day).

Pretreatment with kaempferol or quercitrin exhibited significant of immobility time in TST and FST. So, the escape-directed behaviours with minimal immobile posture showed by kaempferol or quercitrin treated rats may be due to its attenuating effect in endogenous depression. The underlying principle measuring the lack of active coping behavior is identical in the TST and FST, but their variability in response to certain antidepressants indicates potentially different substrates and neurochemical pathways mediating performance in these tests. These issues may underlie the observed behavioral differences (Bai et al., 2001). Furthermore, one of the most important differences between these two models is the response to drugs in both tests and the apparent increased sensitivity of the TST. The mouse FST has not traditionally been viewed as a consistently sensitive model for detecting selective serotonin reuptake inhibitor activity, whereas these antidepressants are generally reported as active in the TST (Cryan et al., 2005). Moreover, the TST was proposed to have a greater pharmacological sensitivity as compared with the FST (Thierry et al., 1986; Cryan et al., 2005). Remarkably, TST detects the anti-immobility effects of a wide array of antidepressants, including tricyclic antidepressants (TCA), selective serotonin reuptake inhibitors (SSRI), monoamine oxidase inhibitors (MAOI), electro-convulsive shock (ECS), and even atypical antidepressants. Thus, the activity of kaempferol or quercitrin could involve one of the mechanisms of the established agents as described above.

In summary, our results suggest that *Opuntia ficus-indica var. saboten*. and two flavonoids (kaempferol and quercitrin) isolated from the *Opuntia ficus-indica var. saboten*. exert antidepressant effect in experimental depression animal models. Thus, *Opuntia ficus-indica var. saboten*., kaempferol, and quercitrin can be developed for the usefulness remedy for depression treatment.

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REFERENCES

Ader R and Cohen N (1993) Psychomeuroimmunology: conditioning and stress. *Annual Review of Psychology* 44: 53-85.

Ahn DK (1998) *Illustrated Book of Korean Medicinal Herbs*. Kyohak Publishing Co, Korea seoul. pp 497.

Akil H, Watson SJ, Young E, Lewis ME, Khachaturian H and Walker JM (1984) Endogenous opioids: biology and function. *Annual Review of Neuroscience* 7:223-255.

Armario A, Gavalda A and Marti O (1988) Forced swimming test in rats: effect of desipramine administration and the period of exposure to the test on struggling behavior, swimming, immobility and defecation rate. *Eur J Pharmacol* 158:207-212.

Armario A, Gill M, Marti J, Pol O and Balasch J (1991) Influence of various acte stressors on the activity of adult male rats in a holeboard and in the forced swim test. *Pharmacol Biochem Behav* 39:373-377.

Bai F, Li X, Clay M, Lindstrom T and Skolnick P (2001) Intra and interstrain differences in models of "behavioral despair". *Pharmacol Biochem Behav* 70:187-192.

Bilkei-Gorzo A, Racz I, Michel K and Zimmer A (2002) Diminished anxiety- and depression-related behaviors in mice with selective deletion of the Tac 1 gene. *J Neurosci* 22:10046-10052.

Chomczynski P and Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Analytical Biochemistry* 162:156-159.

Civelli O, Birnberg N and Herbert E (1982) Detection and quantitation of pro-opiomelanocortin mRNA in pituitary and brain tissues from different species. *J Biol Chem* 257: 6783-6787.

Cryan JF, Markou A and Lucki I (2002) Assessing antidepressant activity in rodents: recent developments and future needs. *Trends in Pharmacological Sciences* 23: 238-245.

Cryan JF, Mombereau C and Vassout A (2005) The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic syudies in mice. *Neuroscience and Biobehavioral Reviews* 29:571-

625.

Danielson PE, Forss-Petter S, Brow MA, Calavetta L, Douglass J, Milner RJ and Sutcliffe JG (1988) p1B15: A cDNA clone of the rat mRNA encoding cyclophilin. *DNA* 7:261-267.

Galati EM, Monforte MT, Tripodo MM, d' Aquino A and Mondello MR (2001) Antiulcer activity of Opuntia ficus-indica (L.) Mill. (Cactaeceae): ultrastructural study. *J Ethnopharmacol* 76:1-9.

Ibanez-camacho R and Roman-Ramos R (1979) Hypoglycemia effect of *Opuntia cactus*. *Archivos de Investigación Médica* 10:223-230.

Kim KS and Han PL (2006) Optimization of chronic stress paradigms using anxiety- and depression-like behavioral parameters. *J Neurosci Res* 83:497-507.

Krieger DT, Liotta A and Brownstein MJ (1977) Presence of corticotropin in limbic system of normal and hypophysectomized rats. *Brain Research* 123:575-579.

Lee NH, Yoon JS, Lee BH, Choi BW and Park KH (2000) Screening of the radical scavenging effects, tyrosinase inhibition, and anti-allergic activities using *Opuntia ficus-indica*. *Korean Journal of Pharmacognosy* 31:412-415.

Lopez D (1995) A review: use of the fruits and stem of prickly pear cactus (*Opuntia* spp.) into human food. *Food Sci Tech Int* 1:65-74.

McEwen BS and Stellar E (1993) Stress and the individual: mechanisms leading to disease. *Archives of Internal Medicine* 153:2093-2101.

Mora S, Millian R, Lungenstrass H, Diiaz-Vieliz G, Morian JA, Herrera-Ruiz M and Tortoriello J (2006) The hydroalcoholic extract of *Salvia elegans* induces anxiolytic- and antidepressant-like effects in rats. *J Ethnopharmacol* 106: 76-81.

Park EH, Kahng JH, Lee SH and Shin KH (2001) An anti-inflammatory principle from cactus. *Fitoterapia* 72:288-290.

Park EH, Kahng JH and Paek EA (1998) Studies on the pharmacological actions of cactus: identification of its anti-inflammatory effect. *Archives of Pharmacal Research* 21:30-34.

Porsolt RD, Bertin A and Jalfre M (1977) Behavioral despair in mice: a primary screening test for antidepressants. *Archives internationales de pharmacodynamie et de thérapie* 229:327-336.

Porsolt RD, Le Pichon M and Jalfre M (1977) Depression: a new animal model sensitive to antidepressant treatments. *Nature* 226:730-732.

Sapolsky RM (2003) Stress and plasticity in the limbic system. *Neurochemical Research* 28:1735-1742.

Schreiber S, Bleich A and Pick CG (2002) Venlafaxine and mirtazapine: different mechanisms of antidepressant action, common opioid-mediated antinociceptive effect-a possible opioid involvement in severe depression? *J Mol Neurosci* 18:143-149.

Steru L, Chermat R, Thierry B and Simon P (1985) The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology* 85:367-370.

Takahashi N, Hayano T and Suzuki M (1989) Peptidyl-prolyl cis-trans isomerase is the cyclosporin A-binding protein cyclophilin. *Nature* 337:473-475.

Thierry B, Steru L, Simon P and Porsolt RD (1986) The tail suspension test: ethical considerations. *Psychopharmacology* 90:284-285.

Trejo-Gonzalez A, Gabriel-Ortiz G, Puebla-Perez AM, Huizar-Contreras MD, Munguia-Mazarigos MR, Mejia-Arreguin S and Calva E (1996) A purified extract from prickly pear cactus (*Opuntia fulginosa*) controls experimentally indyced diabetes in rats. *J Ethnopharmacol* 55:27-33.

WHO (1999) WHO Director-General unveils new global strategies for mental health. Press Release WHO/99-67.

Whooley MA and Simon GE (2000) Managing depression in medical outpatients. *N Engl J Med* 343:1942-1950.

Zhang ZJ (2004) Therapeutic effects of herbal extracts and constituents in animal models of psychiatric disorders. *Life Sciences* 75:1659-1699.