



Antidepressant-like effect of low molecular proanthocyanidin in mice: Involvement of monoaminergic system

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ABSTRACT

Proanthocyanidin is a phenolic product present in plants which has antioxidant, antinociceptive and neuroprotective properties, without inducing significant toxicological effects. The present study tested the hypothesis that low molecular proanthocyanidin from grapes that has optimized bioavailability, would exert antidepressant-like activities in behavioral despair tests. The results suggested that oral administration proanthocyanidin at doses of 25 and 50 mg/kg for 7 days significantly reduced the duration of immobility in both the tail suspension and forced swimming tests. The doses that affected the immobile response did not affect locomotor activity. In addition, the neurochemical and neuropharmacological assays showed that proanthocyanidin produced a marked increase of 5-HT levels at 25 and 50 mg/kg in three brain regions, the frontal cortex, hippocampus and hypothalamus. Noradrenaline and dopamine levels were also increased when higher dose of proanthocyanidin (50 mg/kg) administration both in the frontal cortex and hippocampus. These effects were similar to those observed for the classical antidepressant imipramine (10 mg/kg, i.p.). Moreover, Our study suggested that proanthocyanidin (12.5, 25 and 50 mg/kg) dose dependently inhibited monoamine oxidase-A (MAO-A) activity, while MAO-B inhibitory activity was also found at higher doses (25 and 50 mg/kg) after 7 days administration. MAO-A selective inhibitor, moclobemide (20 mg/kg, i.g.) produced MAO-A inhibition of 70.5% in the mouse brain. These findings suggest that the antidepressant-like effects of proanthocyanidin may involve the central monoaminergic neurotransmitter systems.

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1. Introduction

Depression is a major psychiatric disorder affecting nearly 21% of the world population and imposes a substantial health burden on society (Nemeroff, 2007; Yu et al., 2002). There are three main kinds of classical antidepressants in clinical practice, including tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs) and monoamine oxidase inhibitors (MAOIs). Most of these drugs, however, have undesirable side effects and their mechanisms of action have not been satisfactorily resolved. A growing number of herbal medicines are being introduced into psychiatric practice, many of which have comparable efficacy to prescription medications with

lower side effects. This makes herbal therapies as desirable alternative treatment for severe depression (Thachil et al., 2007).

Grape seed extract is one of the most widely consumed fruits worldwide and consists of ~90% proanthocyanidins and 7% other polyphenols (flavonoids). Proanthocyanidin is a kind of phenolic product (oligonols are catechin-type monomers, dimers and trimers, as well as oligomeric proanthocyanidins) present in plant, has received attention recently (Peng et al., 2005; Dalbo et al., 2006). Experimental and clinical studies have shown that proanthocyanidin has an antioxidant effect, antinociceptive and cardioprotective properties, without inducing significant toxicological effects (Preuss et al., 2000; Uchida et al., 2008; Sato et al., 2001). Recently, the grape seed proanthocyanidins extract has been indicated to be efficacious in reducing A β -induced oxidative insult and dysfunction in PC12 cells (Aruoma et al., 2006). However, information regarding the antidepressant activity of proanthocyanidin is lacking. Some dietary-derived food constituents, including proanthocyanidins, attenuate the activity of the monoamine oxidase (MAO) in C6 glial cells (Mazzio et al., 1998; Xu et al., 2005a). MAO causes oxidative deamination of biogenic amines and xenobiotics, and regulates the intracellular concentration

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of catecholamines and serotonin in the brain (Yu et al., 1992). Therefore, the abnormal function of this enzyme is thought to be involved in several psychiatric and age-related neurological disorders, including depression and Parkinson's disease.

With the view of the above observations, we wondered if high percentage of low molecular proanthocyanidin (catechin-type monomers and oligomeric proanthocyanidins, the easily absorbed forms) from grape seed extract (with optimized bioavailability), would exert antidepressant-like activities in behavioral despair tests. Evidence suggests that drugs that are effective antidepressants increase the availability of monoamines such as serotonin, noradrenaline and dopamine. This effect is due to either by preventing enzymatic breakdown, as in the case of monoamine oxidase inhibitors, or by preventing monoamine reuptake, as in the case of the tricyclic antidepressants. The present study was designed to investigate the potential antidepressant-like effects of proanthocyanidin through behavioral, biochemical and neurochemical approaches.

2. Materials and methods

2.1. Animals

Male ICR mice (20–25 g) were obtained from the Animal Center of Shanghai Branch, Chinese Academy of Sciences. On arrival, the animals were housed eight per cage and acclimatized to a colony room with controlled ambient temperature (22 ± 1 °C), humidity ($50 \pm 10\%$) and a 12 h natural light/dark cycle. They were fed with standard diet and water *ad libitum* and were allowed to acclimate 7 days before they were used. The experiments were performed between 10:00 h and 14:00 h. All experiments were conducted in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985), and approved by the Wenzhou Medical College Committee on Animal Care and Use.

2.2. Drugs and drug administration

Proanthocyanidin (95%) was purchased from Tianjin Jianfeng Natural Product R&D Co., Ltd (Tianjin, China), which contained catechin-type monomers and oligomeric proanthocyanidin (60–80% oligomers), the easily absorbed forms. Imipramine hydrochloride, kynuramine dihydrobromide, 4-hydroxyquinoline, clorgyline, deprenyl, 5-hydroxytryptamine (5-HT), noradrenaline, dopamine and 5-hydroxyindoleacetic acid (5-HIAA) were purchased from Sigma Chemical Co., (USA). Moclobemide hydrochloride and sodium carboxymethyl cellulose were provided by the Beijing Institute of Pharmacology and Toxicology (China). For oral administration (via gavage, *i.g.*), proanthocyanidin was dissolved in 0.5% sodium carboxymethyl cellulose and diluted to the desired concentration on the day of testing and moclobemide was dissolved in redistilled water. For intraperitoneal injection, imipramine was dissolved in redistilled water.

The plasma concentration of proanthocyanidin peaks 1 h after a single dose is orally administered to rats (Koga et al., 1999). All drugs were administered acutely (1 day) or chronically (7 days) between 10:00 and 14:00 h. The behavioral tests were conducted 1 h after the acute (1 day) and the final proanthocyanidin treatment (7 days). The neurochemical and biochemical assays were started in mice 1 h after the last administration of 7 days. The effects of positive antidepressants, moclobemide (20 mg/kg, *i.g.*) and imipramine (10 mg/kg, *i.p.*), were tested 1 h or 30 min respectively, after the last drugs treatment as previously described (Xu et al., 2005b).

2.3. Tail suspension test

The tail suspension test was based on the method of Steru (Steru et al., 1985) as our previous work (Xu et al., 2005b). Animals were suspended 50 cm above the floor by means of an adhesive tape, placed

approximately 1 cm from the tip of the tail. The time during which mice remained immobile was quantified during a test period of 6 min. Mice were considered immobile only when they hung passively and completely motionless.

2.4. Forced swimming test

The forced swimming test employed was similar to that described elsewhere (Porsolt et al., 1977; Porsolt et al., 1978) with minor modification (Xu et al., 2005a). Briefly, mice had a swimming-stress session for 15 min (pre-test), 24 h before being individually placed in glass cylinders (height: 25 cm; diameter: 10 cm; containing 10 cm of water at 24 ± 1 °C) for 6 min (test). A mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only small movements necessary to keep its head above water. The duration of immobility was recorded during the last 4 min of the 6-min testing period.

2.5. Locomotor activity

The assessment of locomotor activity was carried out on mice using a slightly modified method (Xu et al., 2005b). Briefly, the locomotor activity of the mice was measured by an ambulator with five activity chambers (JZZ98, Institute of Materia Medica, Chinese Academy of Medical Sciences, China). Mice were placed in the chambers and their paws contacted or disconnected the active bars producing random configurations that were converted into pulses. The pulses, which were proportional to the locomotor activity of the mice, were automatically recorded as the cumulative total counts of motor activity. Mice were placed in test chambers, 5 min prior to the evaluation for acclimatization and then locomotion counts were recorded for a period of 10 min.

2.6. Determination of monoamines and metabolites

Mice were decapitated and their brains were rapidly removed and frozen on dry ice. Various brain areas, including the frontal cortex, hippocampus and hypothalamus, were dissected on a cold plate (-16 °C) according to Franklin and Paxinos (1997). The tissue samples were weighed and stored at -80 °C until homogenization.

The contents of 5-HT, noradrenaline, dopamine and 5-HIAA were measured as described previously (Nitta et al., 1992; Xu et al., 2005a) using high-performance liquid chromatography (HPLC) with electrochemical detection with minor modifications. Each frozen tissue sample was homogenized by ultrasonication in 200 μ l of 0.4 M perchloric acid (solution A). The homogenate was kept on ice for 1 h and then centrifuged at $12,000 \times g$ (4 °C) for 20 min. The pellet was discarded. An aliquot of 160 μ l of supernatant was added to 80 μ l of solution B (containing 0.2 M potassium citrate, 0.3 M dipotassium hydrogen phosphate and 0.2 M EDTA). The mixture was kept on ice for 1 h and then centrifuged at $12,000 \times g$ (4 °C) for 20 min again. Twenty μ l of the resultant supernatant was directly injected into an ESA liquid chromatography system equipped with a reversed-phase C₁₈ column (150 \times 4.6 mm I.D., 5 μ m) and an electrochemical detector (ESA CoulArray, Chelmsford, MA, USA.). The detector potential was set at 50, 100, 200, 300, 400, and 500 mV, respectively. The mobile phase consisted of 125 mM citric acid-sodium citrate (pH 4.3), 0.1 mM EDTA, 1.2 mM sodium octanesulfonate and 16% methanol. The flow rate was 1.0 ml/min. The tissue levels of monoamine were expressed in terms of nanograms per gram of tissue.

2.7. Measurements of monoamine oxidase activity

Mice were sacrificed and the brain tissues were rapidly frozen (-80 °C) until analyzed. Mouse brain monoamine oxidase activity was measured following the procedure described previously

(Chakrabarti et al., 1998; Xu et al., 2005b) with a slight modification. Briefly, the brain tissues were homogenized with 4 ml of phosphate buffer (pH 7.4, 0.05 M). The activities of monoamine oxidase-A and -B in brain tissues were measured in the presence of either 1 μ M deprenyl (type B inhibitor) or clorgyline (type A inhibitor). For lysis of the membranes, the tissue homogenate was treated with 0.4 ml of 20% Triton X-100, 2.5 ml of phosphate buffer (pH 7.4) was then mixed with 0.2 ml of the tissue homogenate. The mixture was preincubated at 37 °C for 15 min. Then 30 μ l of 2.19 mM kynuramine dihydrobromide was added to the reaction mixture (final concentration 22 μ M) as substrate. Samples were then incubated at 37 °C for 30 min again. After incubation, the reaction was terminated by adding 0.2 ml of 5 M perchloric acid. After cooling and centrifugation at 1500 \times g for 10 min, an aliquot of 0.5 ml of the supernatant was added to 2.5 ml of 1 M NaOH. The fluorescence intensity was detected with excitation at 315 nm and emission at 380 nm using a fluorescence spectrometer. The concentration of 4-hydroxyquinoline was estimated from a corresponding standard fluorescence curve of 4-hydroxyquinoline. Monoamine oxidase activity was expressed as nmol of 4-hydroxyquinoline formed/30 min/mg protein. Protein concentrations were determined by the method of Bradford (Bradford, 1976).

After tail suspension or forced swimming test, the hippocampus, frontal cortex and hypothalamus brain regions were tested for monoamine levels. In preliminary studies, no differences were found in monoamine levels regardless of whether mice were subjected to the tail suspension or forced swimming test. Therefore, all control data mice were vehicle treated and subjected to the forced swimming test in neurochemical and biochemical assays.

2.8. Data analysis

Results were expressed as the mean \pm standard error of the mean (S.E.M.). All data were analyzed statistically using one-way analysis of variance (ANOVA), followed by a post hoc Dunnett's test. Differences with $P < 0.05$ were considered statistically significant.

3. Results

3.1. The effects of proanthocyanidin on the duration of immobility in the tail suspension and forced swimming test

As shown in Fig. 1, the effects of proanthocyanidin were evaluated in mouse tail suspension test. Proanthocyanidin showed a tendency to reduce the duration of immobility after 1-day treatment. However, proanthocyanidin at the doses of 12.5, 25 and 50 mg/kg reduced, in a dose-dependent manner, the duration of immobility after 7 days treatment, resulting in a 17.2%, 30.7% and 40.8% immobility reduction

compared with the control group, respectively [$F(4,35) = 12.43$, $P < 0.001$ vs. control group] (Fig. 1).

In the forced swimming test, these same doses of proanthocyanidin also significantly inhibited immobility with a respective percent reduction of 21.0%, 32.5% and 38.6% after 7 days administration [$F(4,35) = 7.22$, $P < 0.001$ vs. control group], though there was not obvious change on 1 day treatment (Fig. 2). In both models of depression, the effects of proanthocyanidin were similar to those observed for the classical antidepressant imipramine (10 mg/kg, i.p.) after 7 days treatment. The percentages of inhibition for imipramine were 65.3% and 52.1% in the tail suspension and forced swimming tests after 7 days treatment, respectively ($P < 0.001$; $P < 0.001$).

3.2. The effects of proanthocyanidin on mouse locomotor activity

To exclude the false positive effect in behavioral despair tests, the effects of proanthocyanidin on locomotor activity in mice were tested (Fig. 3). Neither proanthocyanidin (12.5 to 50 mg/kg) nor imipramine (10 mg/kg) after 7 days administration affected locomotor activity at doses that significantly reduced immobility response in the mouse tail suspension and forced swimming tests [$F(4,35) = 0.57$, $P = 0.69$ vs. control group].

3.3. The effects of proanthocyanidin involvement of monoamine neurotransmitter levels in different brain regions of mice

The effects of proanthocyanidin and imipramine after 7 consecutive day treatments on mouse frontal cortex monoamine neurotransmitters 5-HT (and its metabolites 5-HIAA), noradrenaline and dopamine levels were shown in Table 1. The monoamine levels of control animals in present study are in agreement with the previous studies (Xu et al., 2005b; Kulkarni et al., 2008). 5-HT levels in the frontal cortex were significantly increased after administration with 25 and 50 mg/kg proanthocyanidin or 10 mg/kg imipramine [$F(4, 35) = 4.45$, $P < 0.01$ vs. control group]. Noradrenaline and dopamine levels were also increased after higher dose of proanthocyanidin (50 mg/kg) administration [$P < 0.05$; $P < 0.05$ vs. control group]. Both proanthocyanidin and imipramine (10 mg/kg) exhibited a decreased 5-HT turnover (a ratio of 5-HIAA/5-HT) in this region [$F(4, 35) = 2.90$, $P < 0.05$ vs. control group]. A tendency for decreased DOPAC levels was seen when 50 mg/kg proanthocyanidin was administered.

As shown in Table 2, an increase in 5-HT levels in the hippocampus was also observed following proanthocyanidin (25 and 50 mg/kg) or imipramine (10 mg/kg) [$F(4, 35) = 3.92$, $P < 0.01$ vs. control group] administration. The noradrenaline and dopamine levels were increased only after 50 mg/kg proanthocyanidin administration in

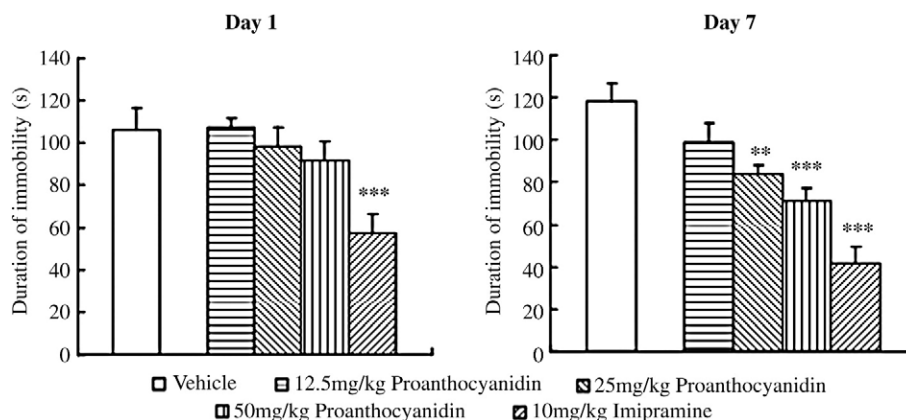


Fig. 1. The effects of proanthocyanidin on the duration of immobility in the tail suspension test. Mice were administered vehicle, proanthocyanidin (12.5, 25 and 50 mg/kg) or imipramine (10 mg/kg) for 1 day (Day 1) and 7 days (Day 7). Values were the mean \pm S.E.M. with 8 mice in each group. ** $P < 0.01$, *** $P < 0.001$ vs. the vehicle control group.

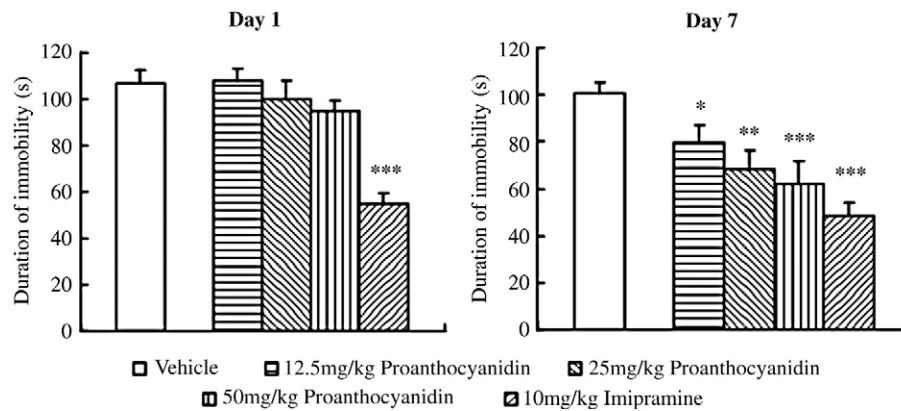


Fig. 2. The effects of proanthocyanidin on the duration of immobility in the forced swimming test. Mice were administered vehicle, proanthocyanidin (12.5, 25 and 50 mg/kg) or imipramine (10 mg/kg) for 1 day (Day 1) and 7 days (Day 7). Values were the mean \pm S.E.M. with 8 mice in each group. * P <0.05, ** P <0.01 and *** P <0.001 vs. the vehicle control group.

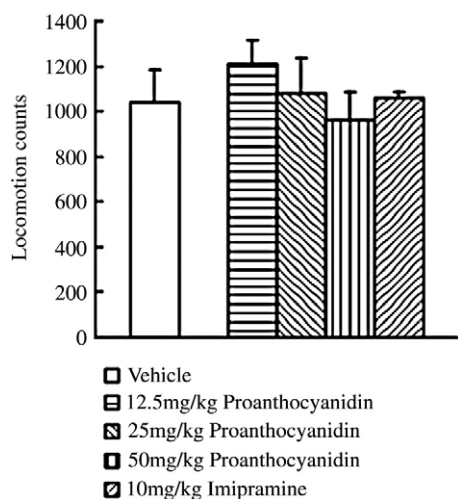


Fig. 3. The effects of proanthocyanidin on locomotor activity in mice. Mice were administered vehicle, proanthocyanidin (12.5, 25 and 50 mg/kg) or imipramine (10 mg/kg) for 7 days. The locomotion counts were recorded for 10 min. Values were the mean \pm S.E.M. with 8 mice in each group.

this region [P <0.05; P <0.05 vs. control group]. Furthermore, post hoc test showed that 25 and 50 mg/kg proanthocyanidin significantly decreased the 5-HT turnover [P <0.01; P <0.01 vs. control group]. We observed a tendency for decreased DOPAC levels after proanthocyanidin treatment (50 mg/kg) in this region, although the changes were not significant.

In the hypothalamus, we found a significantly increased 5-HT levels at doses of 25 and 50 mg/kg proanthocyanidin and 10 mg/kg imipramine administration [$F(4, 35) = 5.52$, P <0.05 vs. control group]. No significant change in noradrenaline, dopamine, DOPAC

and 5-HT turnover after proanthocyanidin treatment were observed in this region (Table 3).

3.4. The effects of proanthocyanidin on monoamine oxidase activity in mouse brain

The inhibition of type A and B monoamine oxidase activities by proanthocyanidin in mouse brain was shown in Table 4. Proanthocyanidin at doses of 12.5 to 50 mg/kg, the mouse brain monoamine oxidase-A (deprenyl-treated) activity was inhibited by 8.3%, 10.8% and 15.1%, respectively [$F(4,35) = 38.51$, P <0.001 vs. control group]. We also found 2.5%, 8.7% and 10.3% inhibition of monoamine oxidase-B (clorgyline-treated) activity at doses of 12.5, 25 and 50 mg/kg [$F(4,35) = 3.99$, P <0.01 vs. control group]. Moclobemide (20 mg/kg) produced monoamine oxidase-A inhibition of 70.5% [P <0.001 vs. control group], but it did not affect monoamine oxidase-B activity after 7 days administration. Imipramine did not show any significant effects on both monoamine oxidase A or B.

4. Discussion

In the present study, the antidepressant-like effects of proanthocyanidin were evaluated in two behavioral models, the tail suspension and forced swimming tasks. The immobility behavior displayed in mice when subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioral despair, which in turn may reflect depressive disorders in humans. Besides behavioral abnormality, neurobiological evidence in both animals and depressive patients indicate the role of the monoaminergic system in the pathophysiology of mental depression (Elhwuegi, 2004). The present study provides pharmacological and neurochemical evidence for the involvement of monoamines particularly serotonin and MAO-A enzyme system in the antidepressant-like activities of proanthocyanidin.

Table 1

The effects of proanthocyanidin on the concentrations of monoamines and their metabolites in the frontal cortex of mice.

Group	Dose (mg/kg)	Frontal cortex (ng/g)					
		5-HT	5-HIAA	5-HIAA/5-HT	Noradrenaline	Dopamine	DOPAC
Control		515.3 \pm 28.2	125.5 \pm 13.5	0.24 \pm 0.02	273.9 \pm 15.5	17.4 \pm 3.3	24.1 \pm 2.7
Proanthocyanidin	12.5	520.5 \pm 28.1	123.2 \pm 9.4	0.24 \pm 0.02	274.3 \pm 17.2	19.0 \pm 2.7	23.5 \pm 3.5
	25	605.6 \pm 22.2*	125.1 \pm 14.2	0.21 \pm 0.02	299.0 \pm 18.9	23.3 \pm 2.5	21.2 \pm 1.9
	50	628.8 \pm 24.0**	113.8 \pm 6.3	0.18 \pm 0.01*	334.9 \pm 24.4*	29.3 \pm 4.6*	19.7 \pm 2.1
Imipramine	10	609.9 \pm 24.4*	112.4 \pm 6.4	0.18 \pm 0.01*	338.5 \pm 21.8*	19.6 \pm 2.1	19.2 \pm 1.8

Values were the mean \pm S.E.M. expressed as nanograms per gram of tissue of 8 mice in each group. Data analysis was performed using Dunnett's *t*-test. * P <0.05, ** P <0.01, compared with control group.

Table 2

The effects of proanthocyanidin on the concentrations of monoamines and their metabolites in the hippocampus of mice.

Group	Dose (mg/kg)	Hippocampus (ng/g)					
		5-HT	5-HIAA	5-HIAA/5-HT	Noradrenaline	Dopamine	DOPAC
Control		615.9 ± 22.1	195.1 ± 10	0.32 ± 0.02	283.7 ± 12.4	23.6 ± 4.1	14.7 ± 2.8
Proanthocyanidin	12.5	635.5 ± 14.7	184.7 ± 9	0.29 ± 0.01	271.8 ± 18.5	22.6 ± 2.4	14.3 ± 1.4
	25	692.0 ± 24.3*	174.8 ± 9	0.25 ± 0.01**	281.5 ± 19.7	30.5 ± 3.6	13.5 ± 2.2
	50	708.5 ± 19.9**	179.9 ± 13.6	0.25 ± 0.01**	342.0 ± 15.9*	36.1 ± 3.3*	12.2 ± 2.3
Imipramine	10	702.4 ± 20.8**	180.2 ± 11.7	0.26 ± 0.02**	349.6 ± 27.8*	34.1 ± 2.4*	14.2 ± 1.1

Values were the mean ± S.E.M. expressed as nanograms per gram of tissue of 8 mice in each group. Data analysis was performed using Dunnett's *t*-test. **P* < 0.05, ***P* < 0.01, compared with control group.

Table 3

The effects of proanthocyanidin on the concentrations of monoamines and their metabolites in the hypothalamus of mice.

Group	Dose (mg/kg)	Hypothalamus (ng/g)					
		5-HT	5-HIAA	5-HIAA/5-HT	Noradrenaline	Dopamine	DOPAC
Control		424.4 ± 23.3	94.9 ± 7.4	0.22 ± 0.01	299.4 ± 19.3	16.1 ± 1.8	12.3 ± 1.4
Proanthocyanidin	12.5	426.3 ± 26.7	92.6 ± 11.7	0.22 ± 0.02	302.0 ± 21.2	18.4 ± 2.0	13.1 ± 2.1
	25	493.8 ± 13.7*	103.5 ± 8.6	0.21 ± 0.02	317.1 ± 16.6	21.1 ± 2.0	13.5 ± 2.2
	50	549.5 ± 20.5**	96.8 ± 6.3	0.18 ± 0.03	345.4 ± 12.2	20.9 ± 1.6	12.6 ± 1.7
Imipramine	10	510.0 ± 28.7*	89.0 ± 13.5	0.17 ± 0.02*	368.0 ± 21.8*	17.5 ± 2.7	12.4 ± 1.9

Values were the mean ± S.E.M. expressed as nanograms per gram of tissue of 8 mice in each group. Data analysis was performed using Dunnett's *t*-test. **P* < 0.05, ***P* < 0.01, compared with control group.

Depression is the outcome of an eventual inability to cope with a stream of dissimilar unpleasant stimuli imposed by the environment. Animal models are widely used in pre-clinical antidepressant evaluation and to provide insights into the neuropathology of depression (Garcia, 2002). The tail suspension and forced swimming tests are the most widely used tools for inducing behavioral deficits which can subsequently be reversed by antidepressant treatments. There is a significant correlation between clinical potency and effectiveness of antidepressants in both models (Machado et al., 2009). The present study demonstrated that proanthocyanidin at oral doses of 12.5, 25 and 50 mg/kg exerted a tendency to reduce the immobility time after acute administration (1 day) both in the tail suspension and forced swimming tests in mice. Acute treatment with the classical antidepressant imipramine (10 mg/kg, i.p.) reduced the immobility time significantly. However, a dose-response reduction in the duration of immobility were observed after administration of proanthocyanidin (12.5, 25 and 50 mg/kg) for 7 days. These effects were largely comparable to those found with imipramine (10 mg/kg for 7 days, i.p.). Furthermore, we did not observe any evidence indicating a change in locomotor activity in mice at the doses that significantly improved antidepressant performance. Thus, the antidepressant-like effect of proanthocyanidin is unlikely to be due to an increase in locomotor activity. These results clearly demonstrated for the first time, the antidepressant-like efficacy of proanthocyanidin in the mouse models of depression.

Monoamine neurotransmitters play important roles in depression and in mediating behavioral effects of antidepressant drugs (Xia et al., 2007). Most of the antidepressants currently used exert their primary neurochemical effects by regulating synaptic concentrations of serotonin, noradrenaline and dopamine. In addition to having a role as a conventional neurotransmitter, 5-HT is involved in the regulation of mood, sleep, memory, learning and sexual behavior as a crucial 'fine tuner' of normal and pathological processes, all of which are deranged to varying extents in patients with severe depression (Naughton et al., 2000). Furthermore, Blier and colleagues (1994) have reported that enhancement of 5-HT neurotransmission might underlie the therapeutic response to different types of antidepressants.

The present study focused on three brain regions, the frontal cortex, hippocampus and hypothalamus, which involved in emotional, motivational, and mnemonic processes may be related to the

expression of depression (Butterweck et al., 2002). Our results showed that both proanthocyanidin and imipramine mainly affected concentrations of 5-HT in the frontal cortex, hippocampus and hypothalamus after 7 days treatment. Furthermore, we used the ratio of 5-HIAA/5-HT (the metabolite to neurotransmitter ratios), a usual indicator of serotonergic activity, as a way to reflect neurotransmitter utilization to compare proanthocyanidin and tricyclic antidepressant imipramine which is well documented to decrease monoamine turnover. The significant decrease in 5-HT turnover was due to both increase in 5-HT levels and decrease in 5-HIAA concentrations. Proanthocyanidin at doses of 25 or 50 mg/kg significantly decreased 5-HT turnover in the frontal cortex and hippocampus. Imipramine also reduced the 5-HT turnover in the three brain regions after 7 days treatment at 10 mg/kg. These results suggest that the enhanced serotonin level and the reduced 5-HT turnover produced by proanthocyanidin may be related, at least in part, to an effect on monoamine metabolism.

Generally, the most widely accepted hypotheses of the biological basis of depression implicate noradrenaline or 5-HT system dysfunction. Consistent with this view, most anti-depressant drugs exert their action by elevating synaptic monoamine concentrations. Measurement of noradrenaline after proanthocyanidin administration (50 mg/kg) showed an increase in central noradrenaline concentrations both

Table 4

The inhibitory effects of proanthocyanidin on type A and type B monoamine oxidase activities in mouse brain.

Group	Dose (mg/kg)	Monoamine oxidase-A activity (nmol/30 min/mg protein)	Monoamine oxidase-B activity (nmol/30 min / mg protein)
Control		55.6 ± 1.3	191.0 ± 3.0
Proanthocyanidin	12.5	51.0 ± 2.0*	186.2 ± 5.6
	25	49.6 ± 1.5**	174.4 ± 4.8*
	50	47.2 ± 1.1**	171.4 ± 7.1*
Imipramine	10	52.11 ± 1.0	184.1 ± 5.2
Moclobemide	20	15.3 ± 0.6***	194.4 ± 4.0

Monoamine oxidase-A or monoamine oxidase-B activity was determined fluorimetrically using kynuramine as a substrate in the presence of 1 μM deprenyl or clorgyline, respectively. Values were the mean ± S.E.M. with 8 mice in each group. Data analysis was performed using Dunnett's *t*-test. **P* < 0.05, ***P* < 0.01 and ****P* < 0.001, compared with control group.

in the frontal cortex and hippocampus. These proanthocyanidin effects were similar to imipramine, and also agreed with our preliminary data that suggests proanthocyanidin (50 mg/kg for 7 days, i.g.) antagonized the syndromes induced by reserpine, such as ptosis and hypothermia, indicating its action on the metabolism and/or transmission of noradrenaline. Thus, the influence on the process of noradrenaline in the nerve terminals might underlie the antidepressant-like response to proanthocyanidin.

The results of different studies recently also support a role of the dopaminergic system in depression. In particular, psychomotor retarded depressive patients exhibited lower levels of metabolite of dopamine, while the selective dopamine and noradrenaline reuptake inhibitor (DNRI) has proven antidepressant efficacy in the treatment of depression (Clausius et al., 2009). In our study, a significantly increased concentration of dopamine in the frontal cortex and hippocampus were found when 50 mg/kg proanthocyanidin was administered for 7 days. In addition, trends towards decreased DOPAC levels both in the frontal cortex and the hippocampus were observed when mice treated with 50 mg/kg proanthocyanidin. This suggests that the drug regulates dopaminergic function by influencing the synthesis or metabolism of dopamine and this might also be involved as a mechanism of antidepressant therapy. However, the extent to which the effect of proanthocyanidin in increasing dopamine levels contributes to its antidepressant-like activity needs to be investigated further.

Monoamine oxidase is a mitochondrial bound isoenzyme which catalyze the oxidative deamination of monoamine neurotransmitters, such as 5-HT, noradrenaline and dopamine. MAO is classified into two types, A and B, according to their sensitivity towards specificity substrates and acetylinic inhibitors. MAO-A shows a higher affinity for 5-HT and noradrenaline, whereas MAO-B prefers phenylethylamine and is inactivated by deprenyl as a selective inhibitor. Most antidepressant drugs today, increase the central monoamine levels and reverse the depressive-like behavior either by inhibiting MAO enzyme (moclobemide) or by influencing reuptake mechanism (imipramine) (Bhutani et al., 2009). However, monoamine oxidase inhibitors (MAOI) are often more effective than tricyclic antidepressants (imipramine) for atypical or refractory depression (Heydendael and Jacobson, 2008; Lee and Chen, 2007). In order to verify whether the increase of monoamines resulted from the inhibition of MAO activity, we evaluated mouse brain monoamine oxidase activity after proanthocyanidin administration for 7 days. Indeed, we tested the acute action of proanthocyanidin on MAO activities in our preliminary study, but the significant effects were not found (data not shown). These results were consistent with the data from the behavioral tests. However, unlike the MAO-A inhibitor moclobemide, proanthocyanidin's oral administration to mice for 7 days dose-dependently inhibited MAO-A activity (12.5, 25 and 50 mg/kg), the MAO-B inhibitory activity was also observed at higher doses (25 and 50 mg/kg). This finding is in agreement with previous *in vitro* study which indicates that chronic treatment with some polyphenolic products, including proanthocyanidin, exhibit MAO inhibiting activity (Mazzio et al., 1998). Recently, MAO-B inhibitors are found to have neuroprotective properties and have significant antidepressant effects in mice, suggesting that MAO-B also plays a role in pathophysiology of depression (Bortolato et al., 2008). Furthermore, the novel chemical categories of drugs suggest that the novel brain-selective MAO-AB inhibitors, such as TV-3326, are found to be devoid of the "cheese reaction" (e.g., acute hypertension and headache, also known as the tyramine-provoked events) (Youdim and Weinstock, 2004). Thus, the discovery of a new generation of MAO inhibitors allows for better control of the degree of drug effects and may lead to an improvement of treatment for the various types of depression. Low molecular proanthocyanidin is such a phenolic product with potential beneficial effects as an antioxidant nutraceutical, although its effect remains to be further clarified. The significant MAO inhibitory effect in brain presently is highly indicative of the ability of proanthocyanidin to pass through the blood-

brain barrier, a function that is critical for neuroprotection. Moreover, low molecular proanthocyanidins, especially oligomers, are responsible for the endothelium-dependent relaxation of blood vessels by increasing nitric oxide production (Fitzpatrick et al., 2000). These properties indicate proanthocyanidin may reduce the risk of "cheese reaction", which makes it a viable therapeutic treatment for depression in future clinical practice. However, the efficacy and safety of proanthocyanidin at relevant doses need to be further studied.

Taken together, proanthocyanidin possesses antidepressant-like effects in behavioral despair tests through the central monoaminergic system, which was mainly related to the serotonergic activation by MAO-A inhibition. The convergence of this finding suggests that proanthocyanidin may be useful as a natural antidepressant agent.

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