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Antidepressant-like effects of flavonoids extracted from *Apocynum venetum* leaves in mice: the involvement of monoaminergic system in mice

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The present study investigated a possible antidepressant-like activity of flavonoids extracted from *Apocynum venetum* leaves using two predictive tests for antidepressant effect on rodents: the forced swimming test (FST) and the tail suspension test (TST). Additionally, the monoaminergic mechanisms involved in the antidepressant-like effect of AV-extract in the mouse forced swimming test (FST) were evaluated. The extract (25, 50 and 100 mg/kg, i.g.) produced antidepressant-like effects in the FST and TST, without accompanying changes in ambulation distances (open-field test). The antidepressant-like effects of AV-extract (50 mg/kg, i.g.) was prevented by the pretreatment of mice with ketanserin (5 mg/kg, s.c., a serotonin 5-HT_{2A} receptor antagonist), cyproheptadine (3 mg/kg, i.g., a serotonin 5-HT_2 receptor antagonist), prazosin (1 mg/kg, i.g., an α_1-adrenoceptor antagonist), yohimbine (1 mg/kg, i.g., an α_2-adrenoceptor antagonist) or propranolol (2 mg/kg, i.g., aβ-adrenoceptor antagonist), SCH23390 (0.05 mg/kg, i.g., a dopamine D_1 receptor antagonist) and sulpiride (50 mg/kg, i.g., a dopamine D_2 receptor antagonist). By contrast, pretreatment of mice with WAY 100635 (0.1 mg/kg, s.c., a serotonin 5-HT_{1A} receptor antagonist) did not counteract the antidepressant-like effect of AV-extract in the TST. It can be concluded that the AV-extract produces an antidepressant-like effects in the FST and in the TST through interaction with the serotonergic (5-HT_{2A} and 5-HT_2 receptors), noradrenergic (α_1-adrenoceptor, α_2-adrenoceptor, a β-adrenoceptor) and dopaminergic (D_1 and D_2 receptors) systems. Taken together, our results suggest that AV-extract deserves further investigation as a putative alternative therapeutic tool that could help the conventional pharmacotherapy of depression.

Key words: Antidepressant, AV-extract, serotonergic, noradrenergic, dopaminergic, tail suspension test, forced swimming test.

INTRODUCTION

Depression is a major psychiatric disorder affecting nearly 17% of the world population and imposes a substantial health burden on society (Nemeroff, 2007; Yu et al., 2002). According to the monoamine theory, which is a widely accepted explanation for depression, the major neurochemical process in depression is the

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impairment of monoaminergic functions and the decrease of serotonin, noradrenaline and dopamine levels (Delgado, 2000). Antidepressants increase the availability of these monoamines at the synapse, which may induce longer-term adaptive changes by modulating the monoaminergic functions and promoting neurogenesis (Elhwuegi, 2004; Dailly et al., 2004). Serotonergic, noradrenergic and dopaminergic systems represent the major targets of current therapeutic treatments and drug development. Yet, there are a few major issues with conventional antidepression drugs. (1) Low remission rate: Although they are mostly effective, only about 50% of individuals with depression showed full remission (Berton and Nestler, 2006). (2) Low side effects: There are antidepressants that are not suitable for many patients because of the side effects. (3) Slow action: For many of these drugs, it takes several weeks to achieve their clinical efficacy. Hence, there is a growing interest in complementary and alternative medicine (CAM) among depression patients, with the general belief that "Natural is better" (Pilkington et al., 2006; Sarris and Kavanagh, 2009). Examples of plants with confirmed anti-depression effects include *Akebiae quinata* (Zhou et al., 2010), *Albizzia julibrissin* (Kim et al., 2007) and *Bupleurum falcatum* (Kwon et al., 2010).

Discovering new sources of natural products for depression treatment will continue to be an important field of research. *Apocynum venetum* L. (Apocynaceae) has shown a great promise for its antidepressant-like effects. A flavonoid extract from its leaves was found to markedly shorten the immobility time in a forced swimming test (FST), indicating antidepressant activities (Butterweck et al., 2001). The main flavonoids in *A. venetum* leaves are hyperoside and isoquercitrin (Butterweck et al., 2001). There is evidence that the *A. venetum* extract affects monoamine levels (Butterweck et al., 2003), we are interested in investigating the underlying mechanisms. Recently, we demonstrated that flavonoids from *A. venetum* leaves could generate a neuroprotective effect on corticosterone-induced neurotoxicity in PC12 cells (Zheng et al., 2011).

The purpose of the present study was to gain further insight into a possible involvement of noradrenergic, dopaminergic and serotonergic systems using laboratory mice.

MATERIALS AND METHODS

Plant material and preparation of the flavonoids extracted from Apocynum venetum leaves

Dry Leaves of *A. venetum* leaves was purchased from Tong Ren Tang Co., Beijing. A sample of 10 g of leaves was extracted three times in a refluxed condenser for 1 h each with 200 ml 70% ethanol. The combined extract was evaporated until dryness, dissolved in 20 ml hot water, adjusted to pH 3.0 with sulfuric acid, and then filtered. The filtrate was chromatographed on a macroporous resin D101 column (10 × 80 cm, Naikai Chemical Co.China) and eluted sequentially with 100 ml water and 70% 100 ml ethanol. The aqueous ethanol fraction was evaporated to dryness to obtain *A. venetum* leaves extract. The extract contained 3.5% hyperoside and 3.7% isoquercitrin, respectively. High performance liquid chromatography (HPLC) analytical conditions were as follows: Column: SHISEIDO CAPCELL PAKC18 (UG) 4.6 mm i.d. 3150 mm, detector at 330 nm, mobile phase: 0.1% TFA in water/0.1% TFA in acetonitrile 85: 15, flow rate: 1.0 ml/min. Equipment: Waters 2695 with a Waters 2998 DAD detector. Hyperoside appeared at 33.8 min and isoquercitrin at 39.7 min.

Animals

Male ICR mice weighing 18 to 22 g were purchased from the Experimental Animal Center, Changchun Institute of Biological Products, Jilin, China. Animals were under a normal 12 h/12 h light/dark schedule (with the lights on at 07:00 a.m). Ambient temperature and relative humidity were maintained at 22 ± 1°C and at 55 ± 5%, respectively. All mice had free access to tap water and food pellets, and were given a standard chow and water *ad libitum* for the duration of the study. All procedures were performed in accordance with the published guidelines of the China Council on Animal Care (Regulations for the Administration of Affairs Concerning Experimental Animals, approved by the State Council on October 31, 1988 and promulgated by Decree No. 2 of the State Science and Technology Commission on November 14, 1988). All efforts were made to minimize animals suffering and to reduce the number of animals used in the experiments.

Chemicals

The following drugs were used: hyperoside, isoquercitrin, WAY-100635 (a serotonin5-HT1A receptor antagonist), ketanserin (a serotonin 5-HT_{2A} receptor antagonist), cyproheptadine (a serotonin 5-HT2 receptor antagonist), prazosin (an α_1-adrenoceptor antagonist), propranolol (a β-adrenoceptor antagonist), yohimbine (an α_2-adrenoceptor antagonist), sulpiride (a dopamine D2 receptor antagonist), SCH23390 (a dopamine D1 receptor antagonist) were purchased from Sigma-Aldrich (St.Louis, MO, USA). Fluoxetine hydrochloride was purchased from Shanghai Zhongxi Pharmaceutical Co., Ltd. (Shanghai, China). All other chemicals were of high-purity analytical grade obtained from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China).

Drug treatments

Different groups of mice, 10 animals per group, were used for drug treatment and for each test. All drugs were administered by intragastric (i.g.) in a constant volume of 10 ml/kg body weight, except fluoxetine that were administered by oral (p.o.) gavage, and WAY 100635 and SCH23390 which were administered by subcutaneous route (s.c.). Appropriate vehicle treated groups were also assessed simultaneously. The animals were used only once in the test.

Experiment 1: To text the antidepressant-like effect of AV-extract

Animals were divided into five experimental groups: one 0.9% saline control group, one fluoxetine group (5 mg/kg) and three AV-extract treatment groups (25, 50, and 100 mg/kg). The administration volume was 20 ml/kg-body weight. To investigate its possible antidepressant-like effect, AV-extract was administrated by oral route 60 min before the forced swimming test (FST), tail suspension test (TST) or OFT.

Experiment 2: **To assess the involvement of the serotonergic system in the antidepressant-like effect of AV-extract**

To assess the involvement of the serotonergic system in the antidepressant-like effect of AV-extract in the TST, mice were pretreated with WAY 100635 (0.1 mg/kg, s.c., a serotonin5-HT_{1A} receptor antagonist), cyproheptadine (3 mg/kg, i.g., a serotonin 5-HT_2 receptor antagonist), ketanserin (5 mg/kg, i.g., a serotonin 5-HT_{2A} receptor antagonist) or vehicle. After 60 min, they received AV-extract (50 mg/kg, i.g.) or vehicle, and were tested in the TST 60 min later.

Experiment 3: T**o investigate the possible involvement of the noradrenergic system in the antidepressant-like effect of AV-extract**

To investigate the possible involvement of the noradrenergic system in the antidepressant-like effect of AV-extract in the TST, animals were pretreated with prazosin (1 mg/kg, i.g., an α_1-adrenoceptor antagonist), yohimbine (1 mg/kg, i.g., an α_2-adrenoceptor antagonist), propranolol (2 mg/kg, i.g., a β-adrenoceptor antagonist) or vehicle. After 60 min they received AV-extract (50 mg/kg, i.g.) or vehicle which were tested in the TST 60 min later.

Experiment 4: **To test the possible involvement of the dopaminergic system in the antidepressant like effect of AV-extract**

To test the possible involvement of the dopaminergic system in the antidepressant like effect of AV-extract in the TST, animals were pretreated with SCH23390 (0.05 mg/kg, s.c., a dopamine D_1 receptor antagonist), sulpiride (50 mg/kg, i.g., a dopamine D_2 receptor antagonist) or vehicle. After 60 min they received AV-extract (50 mg/kg, i.g.) or vehicle and were tested in the TST 60 min later. The doses of the drugs used above were determined according to literature data, which were reported not to increase the locomotor activity (Gay et al., 2010).

Forced swimming test

Mice were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), containing 19 cm of water (depth) at 25 ± 1°C; the total amount of time each animal remained immobile during a 6-min session was recorded (in seconds) as immobility time, as described previously (Machado et al., 2009). Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. A decrease in the duration of immobility is indicative of an antidepressant-like effect (Porsolt et al., 1977).

Tail suspension test

The tail suspension test (TST) was conducted as previously described (Steru et al., 1985), with some modifications. Briefly, mice were individually suspended by the tail with a clamp (10 mm from the tail tip in a box (250 × 250 × 300 mm) with the head 50 mm from the bottom. Testing was carried out in a darkened room with minimal background noise. Each mouse was suspended for a total of 6 min, and the duration of immobility was recorded during the final 4 min interval of the test. Mice were considered immobile only when they hung passively and completely motionless. The test sessions were recorded by a video camera and scored by an observer blind to treatment.

Open-field test

To assess the effects of the extract from AV-extract on locomotor activity, the mice were individually housed in a rectangular container made of dark polyethylene (40 × 40 × 25 cm) in a dimly lit room equipped with a video camera above the center of the floor, as described previously (Kim et al., 2007) with slight modification, and locomotor activity was measured. The locomotor activity was monitored by a computerized video-tracking system using the S-MART program (Pan Lab, Barcelona, Spain). The animals were allowed to adapt for 1 h in the container, and the distance they traveled was recorded during the last 10 min of a total 20 min test. The locomotor activity was measured in centimeters. The floor surface of each chamber was thoroughly cleaned with 70% ethanol between tests.

Statistical analyses

All data were expressed as mean ± standard error of mean (SEM). To compare experimental and control groups, we used one- or two-way ANOVA, followed by post-hoc Dunnett's test using the SPSS software (SPSS Inc., Chicago, USA). A value of $P < 0.05$ was considered statistically significant for analysis. The figures were obtained by the Statistical Analysis System (Graph Pad Prism 4, Graph Pad Software, Inc., San Diego, CA).

RESULTS

Effects of AV-extract on the immobility time in the FST and TST and on the locomotor activity in the OFT

AV-extract or the conventional antidepressant fluoxetine given by oral route significantly decreased the immobility time in the FST [$F(4, 45) = 16.25$, $P < 0.001$] and TST [$F(4, 45) = 22.37$, $P < 0.001$]. Administration of AV-extract (20, 50,100 mg/kg) and fluoxetine (5 mg/kg) resulted in a significant decrease in the immobility time. As shown by the OFT, AV-extract (25, 50 and 100 mg/kg) did not lead to significant changes in ambulation distance (2212.6 ± 4 8.6, 2253 ± 89.9, and 2275.2 ± 98.9 mm, respectively) relative to the vehicle group (2297.3 ± 100 mm). This indicates a lack of apparent association of immobility in the tests with changes in locomotor activity.

Involvement of the serotonergic system

The result presented in Figure 2A showed that the pretreatment of animals with ketanserin (5 mg/kg, i.g., a 5-HT2A receptor antagonist) was able to prevent the anti-immobility effect of AV-extract (50 mg/kg, i.g.) in the TST. A two-way ANOVA revealed significant differences of AV-extract treatment [$F(1, 36) = 14.46$, $P < 0.01$] and ketanserin pretreatment × AV-extract treatment interaction [$F(1, 36) = 13.68$, $P < 0.01$], but not of ketanserin pretreatment [$F(1, 36) = 2.47$, $P > 0.05$]. Figure 2B showed that the pretreatment of mice with cyproheptadine

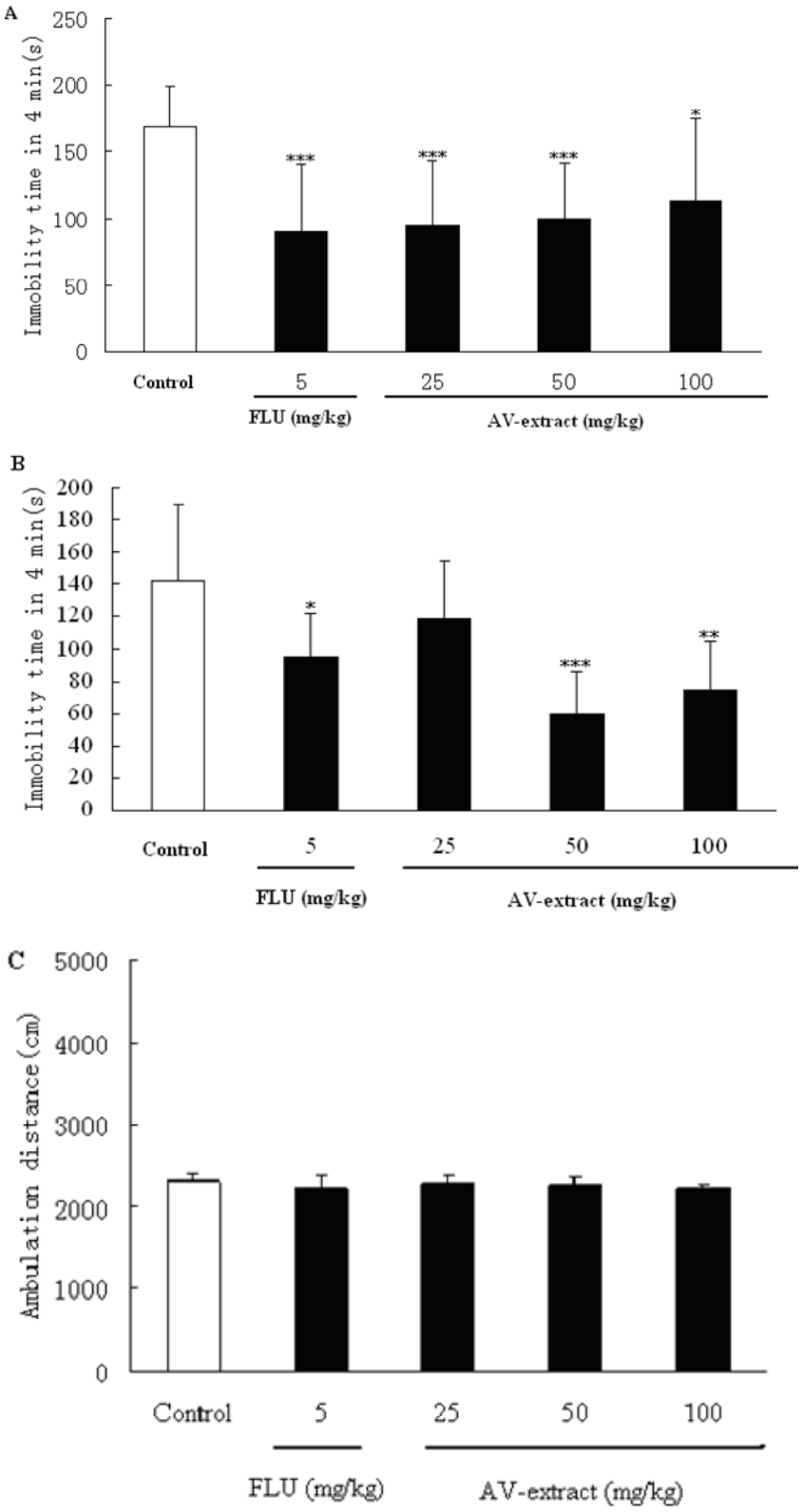

Figure 1. Effects of AV-extract and fluoxetine on immobility time in the mouse FST (A), TST (B), and OFT (C).
The immobility time was measured in mice receiving 0.9% saline (vehicle), 25, 50 and 100 mg/kg AV-extract, 5 mg/kg fluoxetine for 10 days. The number of mice in each group was 10. Data were expressed as mean ± S.E.M. *P < 0.05 and **P < 0.01 and ***P < 0.001 vs FST or TST or OFT with vehicle group.

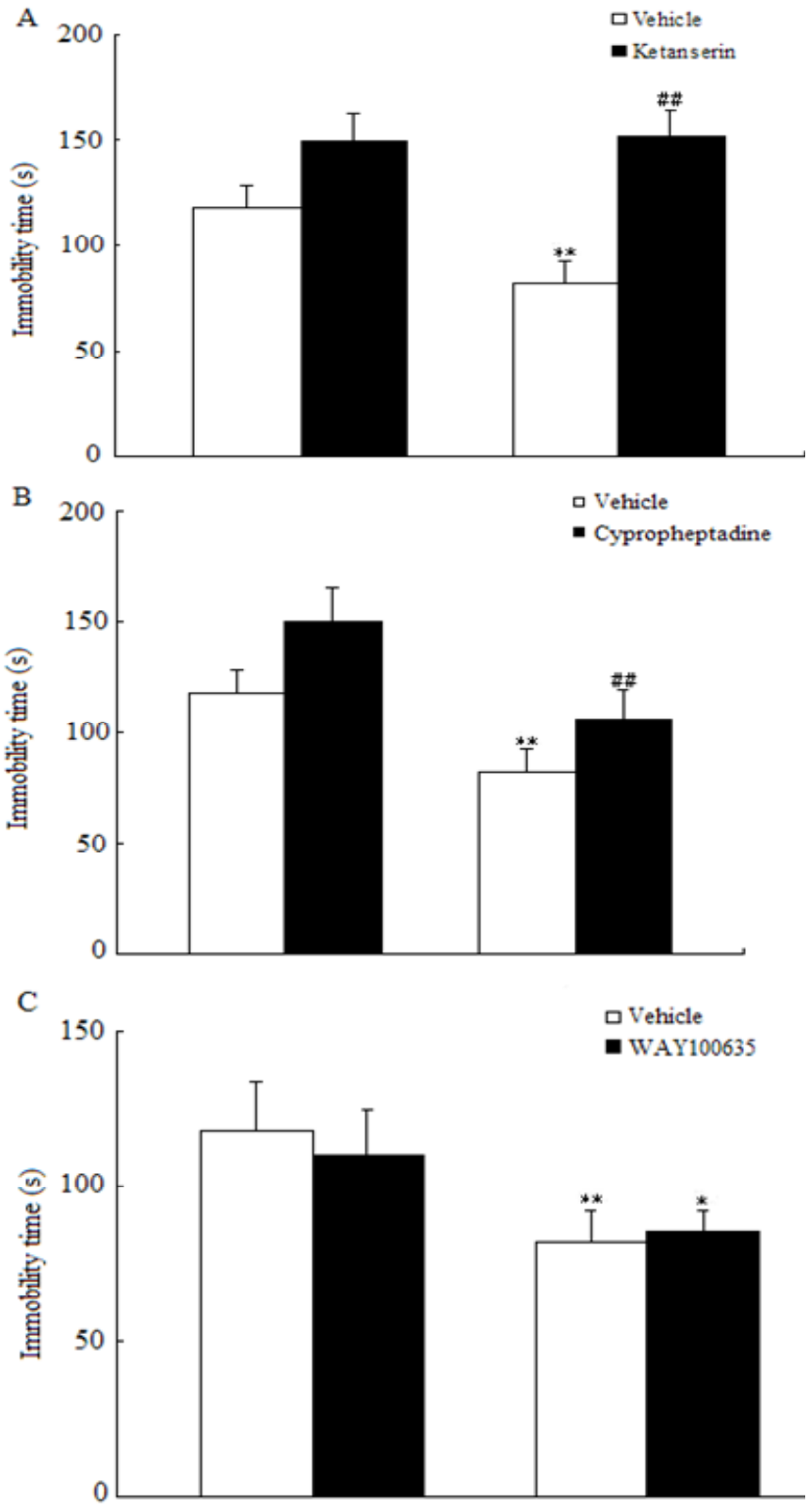

Figure 2. Effects of pretreatment with ketanserin (5mg/kg, i.p., A), cyproheptadine (3 mg/kg, i.p., B) and WAY 100635 (0.1 mg/kg, s.c., C) on the AV-extract (50 mg/kg, i.g.)-induced reduction in immobility time in the mouse TST.
The number of animals in each group was 10. Data were expressed as mean ± S.E.M. ***P*<0.01 vs. TST with vehicle group, ##*P*<0.01 vs. AV-extract group pretreated with vehicle.

(3 mg/kg, i.g., a serotonin 5-HT2 receptor antagonist) was able to block the antidepressant-like effect of AV-extract (50 mg/kg, i.g.) in the TST. A two-way ANOVA revealed significant differences of AV-extract treatment [F (1, 36) = 14.46 $P < 0.01$], cyproheptadine pretreatment [F (1, 36) = 15.63, $P < 0.01$] and cyproheptadine pre-treatment × AV-extract treatment interaction [F (1, 36) = 29.42, $P < 0.01$]. Figure 2C showed that the pretreatment of mice with WAY 100635 (0.1 mg/kg, s.c., a serotonin 5-HT_{1A} receptor antagonist) did not block the antidepressant-like effect of AV-extract in the TST. A two-way ANOVA revealed significant differences of AV-extract treatment [F (1, 36) = 10.2, $P < 0.01$], but not of WAY 100635 pretreatment [F (1, 36) = 3.31, $P > 0.05$], and WAY 100635 pretreatment × AV-extract treatment interaction [F (1, 36) = 0.48, $P > 0.05$]. Post hoc analyses indicated that the pre-treatment of mice with ketanserin and cyproheptadine but not WAY 100635 prevented the decrease in immobility time in the TST produced by the administration of the AV-extract.

Involvement of the noradrenergic system

The result presented in Figure 3A showed that the pretreatment of animals with prazosin (1 mg/kg, i.p., an α1-adrenoceptor antagonist) was able to inhibit the anti-immobility effect of AV-extract (50 mg/kg, i.g.) in the TST. A two-way ANOVA revealed significant differences of AV-extract treatment [F (1, 36) = 14.42, $P < 0.01$], prazosin pretreatment [F (1, 36) = 4.25, $P < 0.05$] and prazosin pretreatment × AV-extract treatment interaction [F (1, 36) = 6.47, $P < 0.01$]. Figure 3B showed that the pretreatment of mice with yohimbine (1 mg/kg, i.p., an α2-adrenoceptor antagonist) was able to prevent the antidepressant-like effect of AV-extract (50 mg/kg, i.g.) in the TST. A two-way ANOVA revealed significant differences of AV-extract treatment [F (1, 36) = 7.21, $P < 0.01$], and Yohimbine pretreatment × AV-extract treatment interaction [F (1, 36) = 19.24, $P < 0.01$], but not of yohimbine pretreatment [F (1, 36) = 0.55, $P > 0.05$]. Figure 3C showed that the pretreatment of mice with propranolol (2 mg/kg, i.p., aβ-adrenoceptor antagonist) was able to prevent the antidepressant-like effect of AV-extract (50 mg/kg, i.g.) in the TST. A two-way ANOVA revealed significant differences of AV-extract treatment [F (1, 36) = 7.28, $P < 0.01$] and propranolol pretreatment × AV-extract treatment interaction [F (1, 36) = 27.18, $P < 0.01$], but not of propranolol pretreatment [F (1, 36) = 0.73, $P > 0.05$]. Post hoc analyses indicated that the anti-immobility effect of the AV-extract was completely prevented by pre-treatment of animals with prazosin, yohimbine or propranolol.

Involvement of the dopaminergic system

The result presented in Figure 4A showed that the pretreatment of animals with SCH23390 (0.05 mg/kg, s.c., a dopamine D1 receptor antagonist) was able to prevent the anti-immobility effect of AV-extract (50 mg/kg, i.g.) in the TST. A two-way ANOVA revealed significant differences of AV-extract treatment [F (1, 36) = 5.71, $P < 0.05$] and SCH23390 pretreatment × AV-extract treatment interaction [F (1, 36) = 22.85, $P < 0.01$], but not of SCH23390 pretreatment [F (1, 36) = 0.26, $P > 0.05$]. Figure 4B showed that the pretreatment of mice with sulpiride (50 mg/kg, i.p., a dopamine D2 receptor antagonist) was able to block the antidepressant-like effect of nobiletin (50 mg/kg, i.g.) in the TST. A two-way ANOVA revealed significant differences of AV-extract treatment [F (1, 36) = 4.42, $P < 0.05$], sulpiride pretreatment [F (1, 36) = 5.19, $P < 0.05$] and sulpiride pretreatment × AV-extract treatment interaction [F (1, 36) = 8.35, $P < 0.01$]. Post hoc analyses indicated that the pre-treatment of mice with SCH23390 and sulpiride prevented the decrease in immobility time in the TST produced by the administration of the AV-extract.

DISCUSSION

Behavioral study is an important approach in evaluating anti-depressant drugs, and forced swimming test (FST) and tail suspension test (TST) are popular tools. The characteristic behavior scored in these tests, termed “immobility”, is an indicator of the degrees of severity of behavioral despair; the lower are the scores, the less severe is the despair. Antidepressant drugs are able to reduce the immobility time in rodents (Porsolt et al., 1977). The antidepressant-like effect of *A. venetum* was first demonstrated by Butterweck et al. (2001) using a forced swimming test (FST) in rats, and the present study has provided more detailed behavioral data using mice. Our results showed that administration at 100, 50 and 25 mg/kg (i.g.) for 10 days significantly reduced immobility time in FST ($P < 0.001$, $p < 0.05$ and $P < 0.01$, respectively; Figure 1A). Administration at 100, 50 mg/kg (i.g.) also significantly reduced immobility time in TST ($P < 0.01$ and $P < 0.001$, respectively; Figure 1B). In line with these observations, treatments with AV-extract at 100, 50 and 25 mg/kg did not cause significant changes in amulation distance (2212.6 ± 48.6, 2253 ± 89.9 and 2275.2 ± 98.9 mm, respectively) compared to the vehicle group (2297.3 ± 100 mm; Figure 1C). These effects were comparable to those found with fluoexetine treatment. Taken together, AV-extract possesses a clear antidepressant-like effect in all animal models used.

It is generally accepted that improving brain mono-aminergic functions is effective in treating depression, and the serotonergic, noradrenergic or dopaminergic systems have become the targets for development of antidepressants (Lambert et al., 2000; Esposito, 2006). The serotonergic system has long been implicated in the pathogenesis of anxiety and depression (Heninger et al., 1996). Some of the most compelling evidence involves the

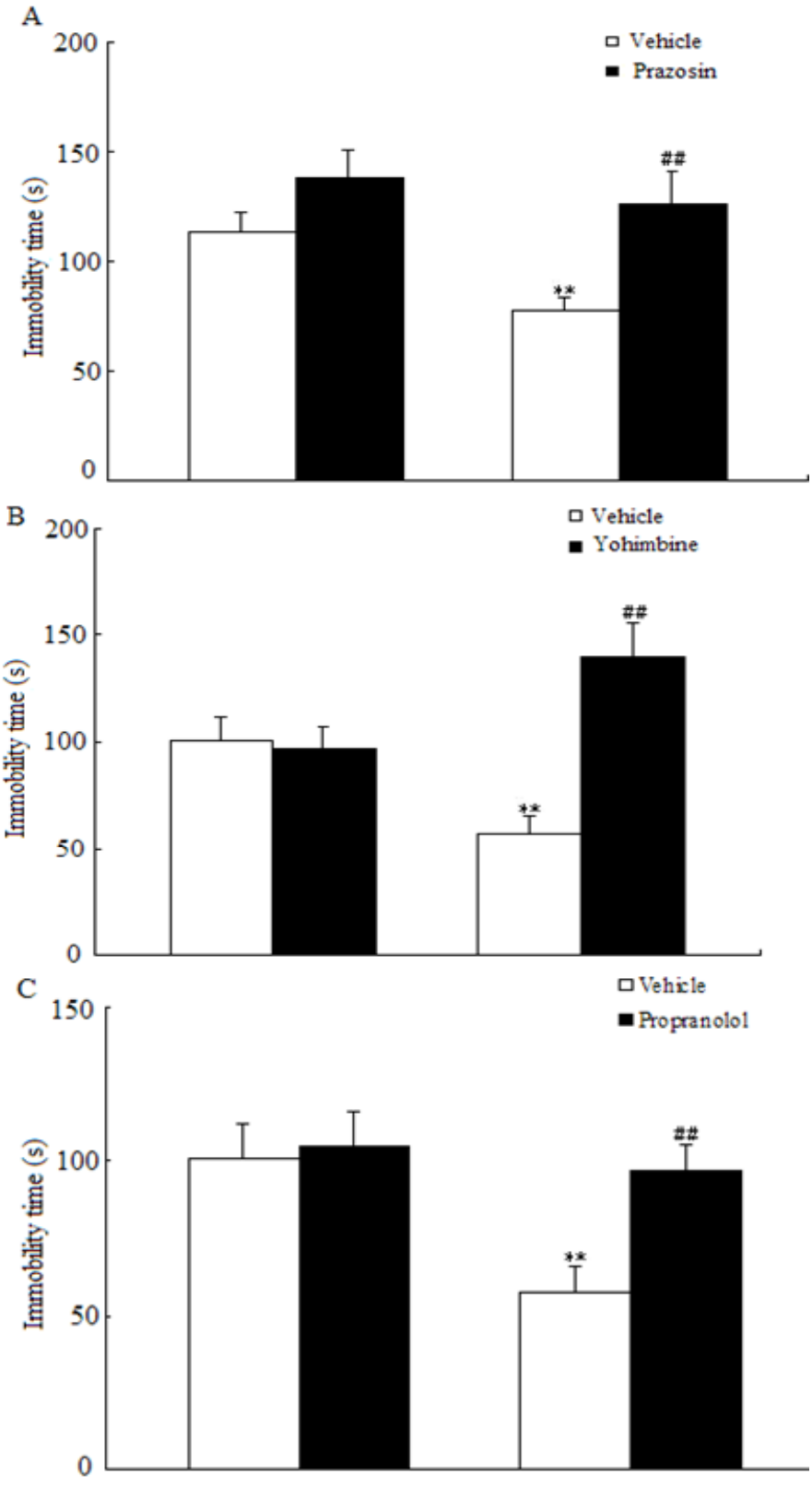

Figure 3. Effects of pretreatment with prazosin (1 mg/kg, i.p., A), yohimbine (1mg/kg, i.p., B) and propranolol (2 mg/kg, i.p., C) on the AV-extract (50 mg/kg, i.g.)-induced reduction in immobility time in the mouse TST. The number of animals in each group was 10. Data were expressed as mean ± S.E.M. $^{**}P<0.01$ vs. TST with vehicle group, $^{\#\#}P<0.01$ vs. AV-extract group pretreated with vehicle.

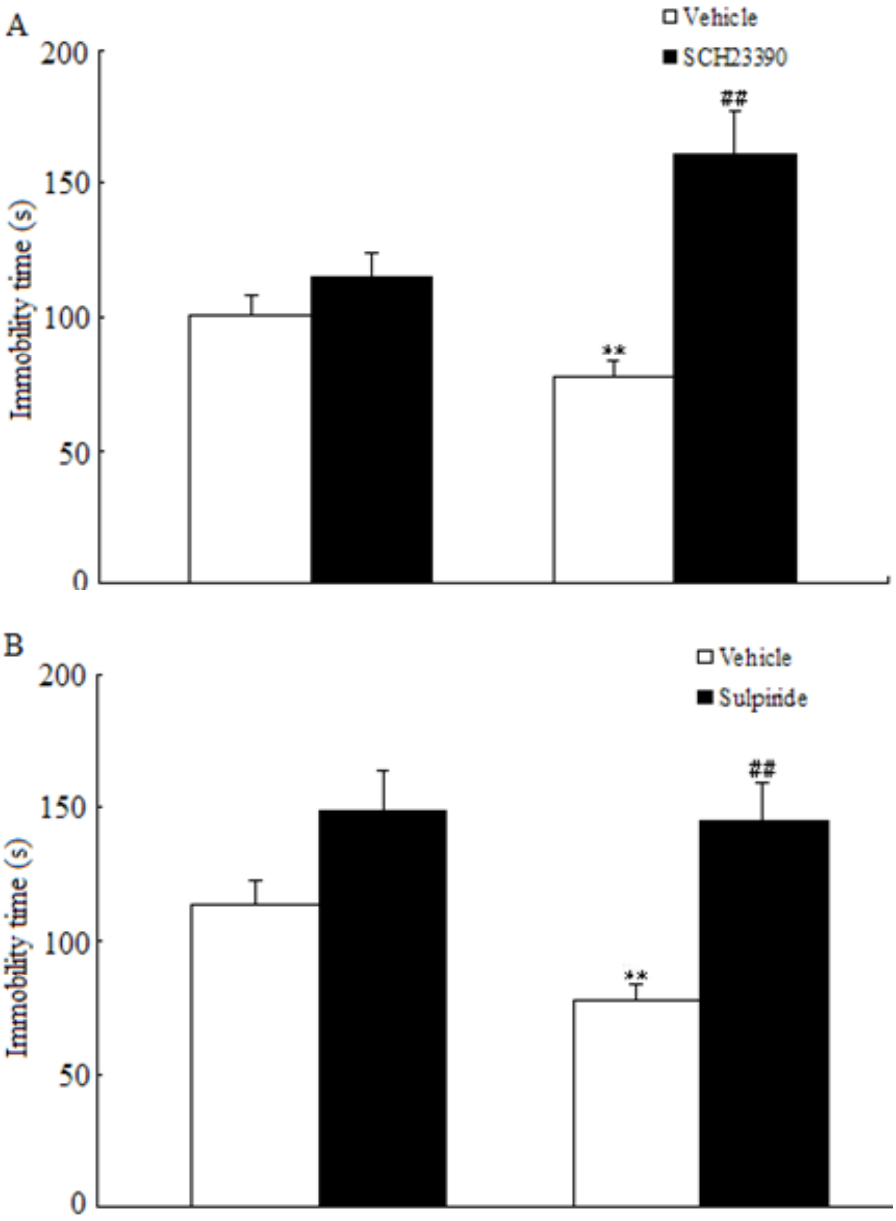

Figure 4. Effects of pretreatment with SCH23390 (0.05 mg/kg, s.c., A) and sulpiride (50mg/kg, i.g., B) on the AV-extract (50 mg/kg, i.g.)-induced reduction in immobility time in the mouse TST.
The number of animals in each group was 10. Data were expressed as mean ± S.E.M. $^{**}P<0.01$ vs. TST with vehicle group, $^{\#\#}P<0.01$ vs. AV-extract group pretreated with vehicle.

alleviation of depression caused by serotonin selective reuptake inhibitors (SSRIs), which increase the availability of serotonin at the synapse (Malagie et al., 2002). The serotonergic system is a neuromodulatory system interacting with other neurotransmissions and participating in the elaboration of an adapted response of the central nervous system to external stimuli (Grimaldi et al., 1999). Depressions are often associated with a down regulation of 5-HT_{1A} receptors in the hippocampus and in the temporal lobe (Gross et al., 2000). 5-HT_2 receptors, especially 5-HT_{2A} and 5-HT_{2C} subtypes, have been shown to be involved in neurochemical changes mediated by antidepressants (Cryan and Lucki, 2000; Elhwuegi, 2004). Preclinical reports showed that the preferential 5-HT_{2A} receptor agonist DOI enhances the antidepressant-like effect of some compounds (Zomkowski et al., 2004) in the mouse FST. Accordingly, antidepressant desipramine treatment decreased $5HT_2$ receptors densities in the rat brain (Goodnough and Bake, 1992). In the present study, the pretreatment with pretreatment with

5-HT_2 receptor antagonist cyproheptadine and 5-HT_{2A} receptor antagonist ketanserin prevent the anti-immobility effect induced by AV-extract in the TST, whereas WAY 100635 was ineffective in reversing the immobility time in the TST; these results are indicative of participation of 5-HT_2 and 5-HT_{2A} receptors, but not 5-HT_{1A} receptor, in the antidepressant-like effect of AV-extract in the mouse TST (Figure 2).

Noradrenergic system is another target for antidepressants (Maj et al., 2000). Increased levels of α_1-adrenoceptor were found in the prefrontal cortex of depressed individuals (García-Sevilla et al., 1999). A down regulation of a_2-adrenergic receptors was documented in depression (Brunello et al., 2003). There is an up-regulation of β-adrenoceptor in depressed patients and a down-regulation after chronic antidepressant treatment in mice (Leonard et al., 1997). In our study, pretreatment of mice with prazosin and yohimbine or propranolol was able to reverse the antidepressant-like effect of the AV-extract, suggesting participation of α_1-, α_2- and αβ-adrenoceptors in the antidepressant-like effect of the extract in mouse (Figure 3).

The relationship between dopaminergic system and depression was confirmed by the fact that antidepressants act on the dopaminergic system (Klimek et al., 2002). Common symptoms of depression such as anhedonia, dysphoria and avolition are likely caused by a functional deficit of dopaminergic transmission (Heinz et al., 1994). Many antidepressant drugs such as SSRIs, imipramine (a tricyclic antidepressant) and bupropion (an atypical antidepressant that inhibits the reuptake of dopamine) act activating dopamine D_1 and D_2 receptors (Renard et al., 2001; Yamada et al., 2004). In our experiments, the pretreatment with SCH23390 or sulpiride inhibited the anti-immobility effect of AV-extract. Thus, our results suggested a participation of both dopamine D_1 and D_2 receptors in the antidepressant-like effects of AV-extract.

In summary, our results provided pharmacological and biochemical evidence for the previously reported antidepressant-like effects of AV-extract in the FST (Butterweck et al, 2001). We have further shown that these effects are apparently related to noradrenergic (α_1-adrenoceptor, α_2-adrenoceptor, aβ-adrenoceptor), dopaminergic (D_1 and D_2 receptors) and serotonergic (5-HT_{2A} and 5-HT_2 receptors).

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REFERENCES

Berton O, Nestler EJ (2006). New approaches to antidepressant drug discovery: beyond monoamines. Nat. Rev. Neurosci. 7:137-151.

Brunello N, Blier P, Judd LL, Mendlewicz J, Nelson CJ, Souery D, Zohar J, Racagni G (2003). Noradrenaline in mood and anxiety disorders: basic and clinical studies. Int. Clin. Psychopharmacol. 18:191-202.

Butterweck V, Nishibe S, Sasaki T, Uchida M (2001). Antidepressant effects of *Apocynum venetum* leaves in the forced swimming test. Biol. Pharm. Bull. 24:848–851.

Butterweck V, Simbrey K, Seo S, Sasaki T, Nishibe S (2003). Long-term effects of an Apocynum venetum extract on brain monoamine levels and beta-AR density in rats. Pharmacol. Biochem. Behav. 75:557-564.

Cryan JF, Lucki I (2000). Antidepressant-like behavioral effects mediated by 5-Hydroxytryp-tamine (2C) receptors. J. Pharmacol. Exp. Ther. 295:120–126.

Dailly E, Chenu F, Renard CE, Bourin M (2004). Dopamine, depression and antidepressants. Fundam. Clin. Pharmacol. 18:601-607.

Delgado PL (2000). Depression: the case for a monoamine deficiency. J Clin Psychiatry., 61:7-11.

Elhwuegi AS (2004). Central monoamines and their role in major depression. Prog. Neuropsychopharmacol. Biol. Psychiatry 28:435–451.

Esposito E (2006). Serotonin-dopamine interaction as a focus of novel antidepressant drugs. Curr. Drug Targets 7:177-185.

García-Sevilla JA, Escribá PV, Ozaita A, La Harpe R, Walzer C, Eytan A, Guimón J (1999). Up-regulation of immunolabeled alpha2A-adrenoceptors, Gi coupling proteins, and regulatory receptor kinases in the prefrontal cortex of depressed suicides. J. Neurochem. 72:282-291.

Gay BM, Prigol M, Stein AL, Nogueira CW (2010). Antidepressant-like pharmacological profile of 3-(4-fluorophenylselenyl)-2, 5-diphenylselenophene: Involvement of serotonergic system. Neuropharmacology 59:172-179.

Goodnough DB, Baker GB (1994). 5-Hydroxytryptamine and beta-adrenergic receptor regulation in rat brain following chronic treatment with desipramine and fluoxetine alone and in combination. J. Neurochem. 62:2262-2268.

Grimaldi B, Bonnin A, Fillion MP, Prudhomme N, Fillion G (1999). 5-Hydroxytryptamine-moduline: a novel endogenous peptide involved in the control of anxiety. Neuroscience 93:1223-1225.

Gross C, Santarelli L, Brunner D, Zhuang X, Hen R (2000). Altered fear circuits in 5-HT_{1A} receptor KO mice. Biol. Psychiatry 48:1157-1163

Heinz A, Schmidt LG, Reischies FM (1994). Anhedonia in schizophrenic, depressed, or alcohol-dependent patients--neurobiological correlates. Pharmacopsychiatry 27:7-10.

Heninger GR, Delgado PL, Charney DS (1996). The revised monoamine theory of depression:a modulatory role for monoamines, based on new findings from monoamine depletion experiments in humans. Pharmacopsychiatry 29:2-11.

Kim JH, Kim SY, Lee SY, Jang CG (2007). Antidepressant-like effects of *Albizzia julibrissin* in mice: involvement of the 5-HT_{1A} receptor system. Pharmacol. Biochem. Behav. 87:41–47.

Klimek V, Schenck JE, Han H, Stockmeier CA, Ordway GA (2002). Dopaminergic abnormalities in amygdaloid nuclei in major depression: a postmortem study. Biol. Psychiatry 52:740-748.

Kwon S, Lee B, Kim M, Lee H, Park HJ, Hahm DH (2010). Antidepressant-like effect of the methanolic extract from *Bupleurum falcatum* in the tail suspension test. Prog. Neuropsychopharmacol. Biol. Psychiatry 34:265–270.

Lambert G, Johansson M, Agren H, Friberg P (2000). Reduced brain norepinephrine and dopamine release in treatment-refractory depressive illness: evidence in support of the catecholamine hypothesis of mood disorders. Arch. Gen. Psychiatry 57:787-793.

Leonard BE, Johansson M, Agren H, Friberg P (1997). Noradrenaline in basic models of depression. Eur. Neuropsychopharmacol. 7:11-6

Machado DG, Bettio, LE, Cunha MP, Capra JC, Dalmarco JB, Pizzolatti MG, Rodrigues AL (2009). Antidepressant-like effect of the extract of *Rosmarinus officinalis* in mice: involvement of the monoaminergic system. Prog. Neuropsychopharmacol. Biol. Psychiatry 33:642-650.

Maj J, Rogóz Z, Dlaboga D, Dziedzicka-Wasylewska M (2000). Pharmacological effects of milnacipran, a new antidepressant, given repeatedly on the alpha1-adrenergic and serotonergic 5-HT_{2A} systems. J. Neural Transm. 107:1345-1359.

Malagie I, David DJ, Jolliet P, Hen R, Bourin M, Gardier AM (2002). Improved efficacy of fluoxetine in increasing hippocampal 5-HT outflow in 5-HT_{1B} receptor knockout mice. Eur. J. Pharmacol. 443:99-104.

Nemeroff CB (2007). The burden of severe depression: a review of diagnostic challenges and treatment alternatives. J. Psychiatr. Res. 41:189-206.

Pilkington K, Rampes H, Richardson J (2006). Complementary medicine for depression. Expert Rev. Neurother. 6:1741-51.

Porsolt RD, Bertin A, Jalfre M (1977). Behavioural despair in mice: a primary screening test for antidepressants. Arch. Int. Pharmacodynamie Ther. 229:327-336.

Renard CE, Fiocco AJ, Clenet F, Hascoet M, Bourin M (2001). Is dopamine implicated in the antidepressant-like effects of selective serotonin reuptake inhibitors in the mouse forced swimming test? Psychopharmacology 159:42-50.

Sarris J, Kavanagh DJ (2009). Kava and St. John's Wort: current evidence for use in mood and anxiety disorders. J. Altern. .Complement. Med. 15:827-836.

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

Yamada J, Sugimoto Y, Yamada S (2004). Involvement of dopamine receptors in the anti-immobility effects of dopamine reuptake inhibitors in the forced swimming test. Eur. J. Pharmacol. 504:207-211.

Yu ZF, Kong LD, Chen Y (2002). Antidepressant activity of aqueous extracts of *Curcuma longa* in mice. J. Ethnopharmacol. 83:161-165.

Zomkowski ADE, Rosa AO, Lin J, Santos ARS, Calixto JB, Rodrigues ALS (2004). Evidence for serotonin receptor subtypes involvement in agmatine antidepressant-like effect in the mouse forced swimming test. Brain Res. 1023: 253-263.

Zheng MZ, Liu CM, Pan FG, Shi DF, Ma FS, Zhang YC, Zhang YJ (2011). Protective effects of flavonoids extract from *Apocynum venetum* leaves against corticosterone-induced neurotoxicity in PC12 cells. Cell Mol. Neurobiol. 31:421-428.

Zhou D, Jin H, Lin HB, Yang XM, Cheng YF, Deng FJ, Xu JP (2010). Antidepressant effect of the extracts from *Fructus Akebiae*. Pharmacol. Biochem. Behav. 94(3):488–495.