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Research report

Repeated arctigenin treatment produces antidepressant- and anxiolytic-like effects in mice

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ABSTRACT

Depression is the root of various diseases. It is one of the most debilitating conditions globally. Antidepressant drugs are usually the first-line of depression treatment. Arctigenin (ARC), one of active ingredient of *Arctium lappa L*, has been found to exert neuroprotective, anti-decrepitude, and anti-inflammatory activities. Thus, the aim of this study was to investigate the potential antidepressant- and anxiolytic-like effects of ARC using acute and chronic mild stress (CMS) mice model. ICR mice model received acute stress or chronic mild stress assessed by open field test (OFT), novelty suppressed feeding (NSF), sucrose preference test (SPT), forced-swimming test (FST), and tail suspension test (TST). After the final test, blood was collected to detect the serum levels of angiogenin (ANG), thrombopoietin (TPO), and vascular endothelial growth factor (VEGF) by enzyme-linked immunosorbent assay (ELISA). The behavioral results showed that repeated ARC (10, 30 mg/kg) administration significantly relieved the antidepressant- and anxiolytic-like effects. And repeated ARC administration at the dose of 10 and 30 mg/kg could significantly block depressive- and anxiety-like behaviors caused by CMS. Finally, ELISA results showed that ARC administration increased the serum levels of angiogenin (ANG), thrombopoietin (TPO), and vascular endothelial growth factor (VEGF). Results showed that chronic ARC administration produces antidepressant- and anxiolytic-like effects, which provides direct evidence for the first time that ARC may be a novel strategy for the treatment of depression and even stress-related disorders. The present data supports further exploration for developing ARC administration as a novel therapeutic strategy for depression and even stress-related disorders.

1. Introduction

Depression is one of the most severe and devitalizing psychiatric illnesses with symptoms including: feeling sad most of the time, feeling restless or jittery, worthlessness, hopelessness, or helplessness, loss of pleasure or interest in activities one used to enjoy, frequent thoughts of death or suicide, physical or slow mental responses, feeling a lot of guilt for no reason, thinking the same thoughts things over and over again. However, the symptoms of depression are not the same for every patient. Clinical studies have shown that stressful life event is one of the

Abbreviations: AD, Alzheimer's disease; ANG, angiogenin; ANOVA, analysis of variance; ARC, arctigenin; CMS, chronic mild stress; ELISA, enzyme-linked immunosorbent assay; FLU, fluoxetine hydrochloride; FST, forced-swimming test; MAOI, monoamine oxidase inhibitor; NSF, novelty suppressed feeding; OFT, open field test; SNRI, selective noradrenalin reuptake inhibitor; SPT, sucrose preference test; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressants; TPO, thrombopoietin; TST, tail suspension test; VEGF, vascular endothelial growth factor

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main pathogenic factor in psychiatric disorders such as depression, in addition a stressful life event plays a key role in the onset and progression of depressive disease in human (Hill et al., 2012). The World Health Organization calculates that more than 350 million people of all ages may suffer from depression. Incredibly, over 3000 suicides occur each day due to depression.

Despite treatment of depression, side effects and other adverse reactions to chemical antidepressants still exist; mainly including hypotension, sexual dysfunction, agitation, emaciation, thirst, constipation, and obesity. However more natural antidepressants are being found which contain fewer side effects. Cipadesin A, a limonoid component extracted from *Xylocarpus granatum*, has antidepressant action in acute stressed mice model of depression, which likely occurs by inhibiting the Hypothalamus-Pituitary-adrenal(HPA) axis activity response to stress (Gao et al., 2016). Sal B, extracted from *Salvia miltiorrhiza*, significantly reduced the immobility time in both the FST and TST tests, without affecting locomotion in spontaneous motor activity (Feng et al., 2012). Ginsenoside Rg1 displayed antidepressant activity through the regulation of the HPA and the HPG axis (Mou et al., 2017). Berberine markedly protects rats from various symptoms of chronic stress and depression (Zhu et al., 2017). Our research focused on is the chemical arctigenin (ARC). ARC (chemical structure shown in Fig.1.) is a lignans derived from *Arctium lappa L.* and has been widely used in traditional Chinese medicine for treating inflammation. It has also been widely used in North America and Europe for hundreds of years (Chan et al., 2011; Cho et al., 2004). The bioactivity and pharmacological functions reported for ARC include anti-cancer, anti-inflammatory, anti-diabetic, antimicrobial, anti-heat shock, and antiviral activities (Chan et al., 2011; Fang et al., 2015; Jurado-Ramos et al., 2009; Shi et al., 2015; Zhao et al., 2013). Recent studies have demonstrated the neuroprotective effects of ARC in the brain. ARC protects cortical neurons from glutamate-induced toxicity by binding to kainate receptor (Jang et al., 2002). ARC showed protective effect in ischemic stroke through inhibition of neuroinflammation, and ameliorated memory impairment in Alzheimer's disease mouse models (Fan et al., 2012). In addition, ARC is an effective endoplasmic reticulum stress alleviator, which protects HepG2 cells against endoplasmic reticulum stress through activating adenosine monophosphate activated protein kinase (AMPK), finally attenuating protein translation and reducing endoplasmic reticulum load (Gu et al., 2012). ARC decreased H_2O_2-induced reactive oxygen species production and enhanced antioxidant capacity of the skeletal muscles (Wu et al., 2014). But the potential effects of ARC on depressive- and anxiety-like behaviors are still unknown. The present study is aimed to investigate the effects of ARC on stress-induced depressive- and anxiety-like behaviors and the possible underlying mechanism.

2. Materials and methods

2.1. Animals

Male ICR mice (7 weeks old) were housed at a constant temperature (21 ± 2°C) and humidity (55% ± 5%) with 12 h/12 h light/dark cycles (lights on at 20:00 pm and off at 08:00 am) and free access to food and water. All drug administration and behavioral tests were performed in a dark phase. All animal procedures were confirmed by the Local Animal Use Committee of Hebei Medical University and performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals of China.

2.2. Drugs

Arctigenin (CAS: 7770-78-7,HPLC ≥ 98%,) was obtained from Shanghai Yuanye Bio-Technology Co., Ltd. Fluoxetine hydrochloride (CAS:56296-78-7) was bought from SIGMA-RICH company. ARC and FLU were dissolved in normal saline.

2.3. Forced-swimming test (FST)

The FST was performed as previous studies (Gu et al., 2014a; Shi et al., 2012)· Mice were put into a 10 cm diameter × 35 cm height plastic cylinder filled with 23–25 °C water as high as 20 cm. The floating time and latency to floating time were measured. The definition of floating is the absence of movement except motions required to retaining the animal's head above the water. Results were showed as floating time of mice in the last 4 min of the 6 min session and the latency to floating time from moving to floating in the first 2 min.

2.4. Open field test (OFT)

Learning from previous study (Wu et al., 2016), the equipment consists of a square arena (40 cm × 40 cm × 35 cm). Placing the mice in the center of the cage by itself, then the crossing activity was counted as horizontal locomotor activity for 5 min. This test was videotaped and the time in the central zone (20 cm × 20 cm) was recorded to reflect the anxiety-like behaviors of mice (Szakacs et al., 2015a).

2.5. Tail suspension test (TST)

The TST was implemented according to previous reports (Gu et al., 2014b; Zhu et al., 2012). Briefly speaking, mice were suspended 40 cm above the floor by adhesive plaster placed roughly 1 cm from the tip of the tail for 6 min. Immobility was defined as the motionlessness of limb or body movements, without those caused by breath when the mice hung passively and were perfectly motionless. During the test, mice were separated from each other to obstruct possible visual and acoustical influences. The results were showed the time (in seconds) that mice spent immobile in the last 4 min of the 6 min session and the latency to immobility tome in the first 2 min.

2.6. Sucrose preference test (SPT)

Just like the previous studies (Miura et al., 2008; Szakacs et al., 2015b), the mice were trained to accommodate to a 1% sucrose solution (w/v) for 48 h prior to the experiment; two bottles of a 1% sucrose solution were laid in each cage. After adaptation, the mice were deprived of food and water for 24 h, then following the SPT, during which mice were housed individually in cages for 24 h with exposed two identical bottles: one was filled with a 1% sucrose solution, another was filled with water. Sucrose and water consumption (in g) were weighted. Sucrose preference (%) = consumption × 100/(sucrose consumption + water consumption).

2.7. Novelty-suppressed feeding (NSF)

The NSF was imitated the previous studies (Liu et al., 2015; Shi et al., 2012). The mice were deprived of food and water for 24 h before the test in their home cages. On the test day, mice were separately placed in an open field arena (40 cm × 40 cm × 35 cm) with a morsel of food placed in the center. First put each mouse in a corner of the cage. The latency time to approach the food and begin eating was recorded (in seconds) as the major test parameter (maximum time, 5 min). Next each mouse was taken back to its home cage, the consumption of food during the first 10 min was quantified immediately to eliminate the possibility that stress affected normal appetite. The more anxiety the longer time it takes to begin eating in a novel environment.

2.8. Chronic mild stress (CMS)

The CMS was adapted from previous reports, with minor changes (Shi et al., 2012; Tian et al., 2014). In brief, mice were exposed to a variable sequence environment, with a series of unpredictable stressors for 28 days. A total of 9 different stressors were used; two stressors were

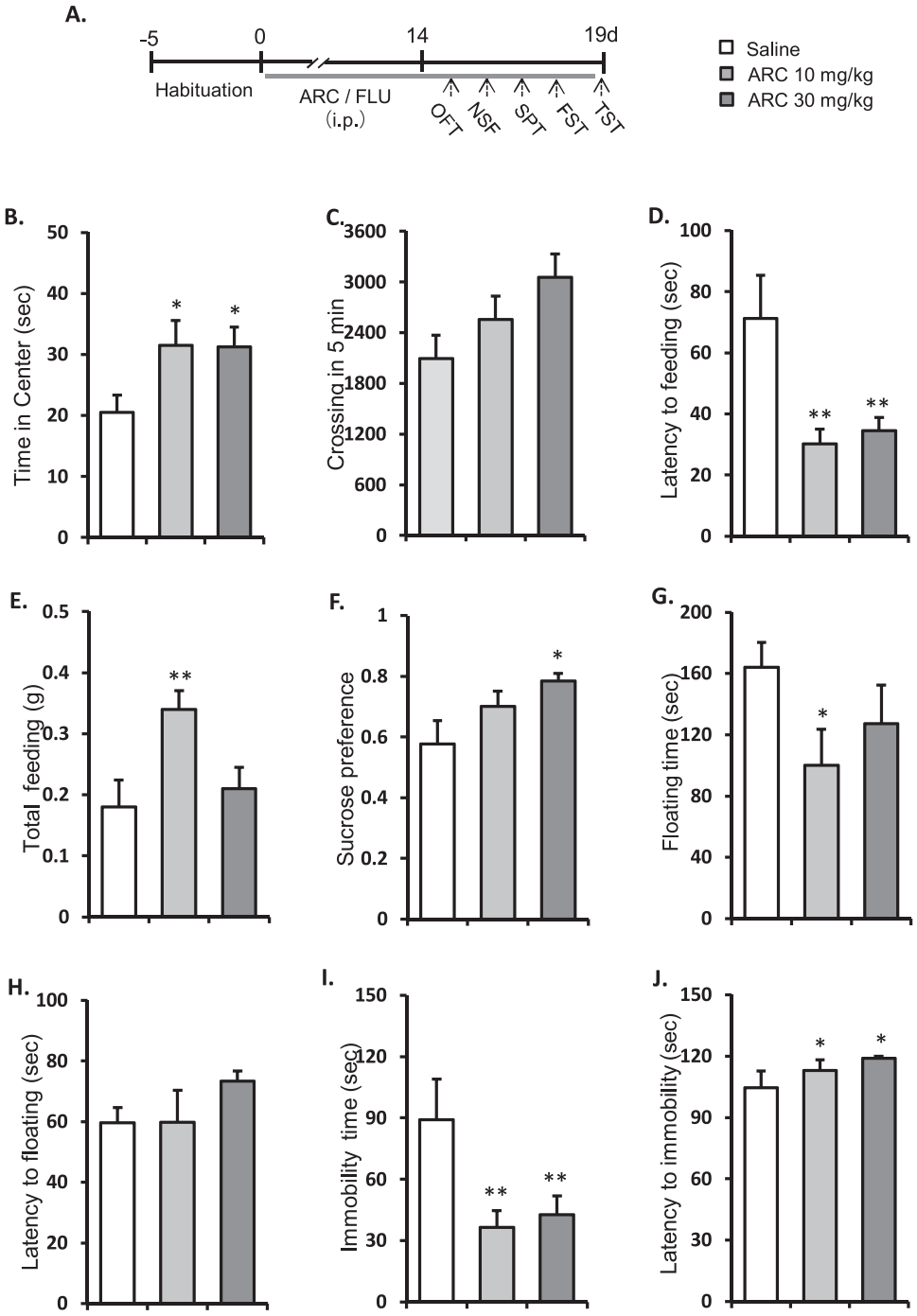

Fig. 1. Chemical structure of arctigenin (ARC).

used per day. The stressors included forced cold swim for 5 min, tilted cages for 24 h, tail clamp for 1 min, restraint for 2 h, light/dark cycle reversal for 24 h, dampened bedding for 24 h, divested of food for 24 h, divested water for 24 h and empty cage for 24 h. Control mice were handled daily without any stress in the housing room.

2.9. Enzyme-linked immunosorbent assay

We detected the serum levels of ANG, TPO, VEGF by ELISA according to our previous study. Briefly, 1 ml of blood was collected from the eyeball. Blood samples were kept at room temperature for 1 h, then centrifuged at 4000 rpm for 10 min. The serum (supernatant fraction) was transferred into a new tube and stored at -80°C for subsequent assays. Serum ANG, TPO and VEGF levels were measured with commercially available ELISA kits (ANG, ml650713; TPO, ml651370; VEGF, ml002076, mlbio, China) according to the manufacturer's instructions. To exclude the potential impact of diurnal rhythm on mouse hormone levels, blood samples were collected in the same time window of 2:00–4:00 pm.

2.10. Experimental design

2.10.1. Experiment 1: effects of repeated ARC administration on depressive- and anxiety-like behaviors in acutely stressed mice

As shown in Fig. 2A, experiment 1 was aimed to determine the effects of ARC on depressive- and anxiety-like behaviors in mice

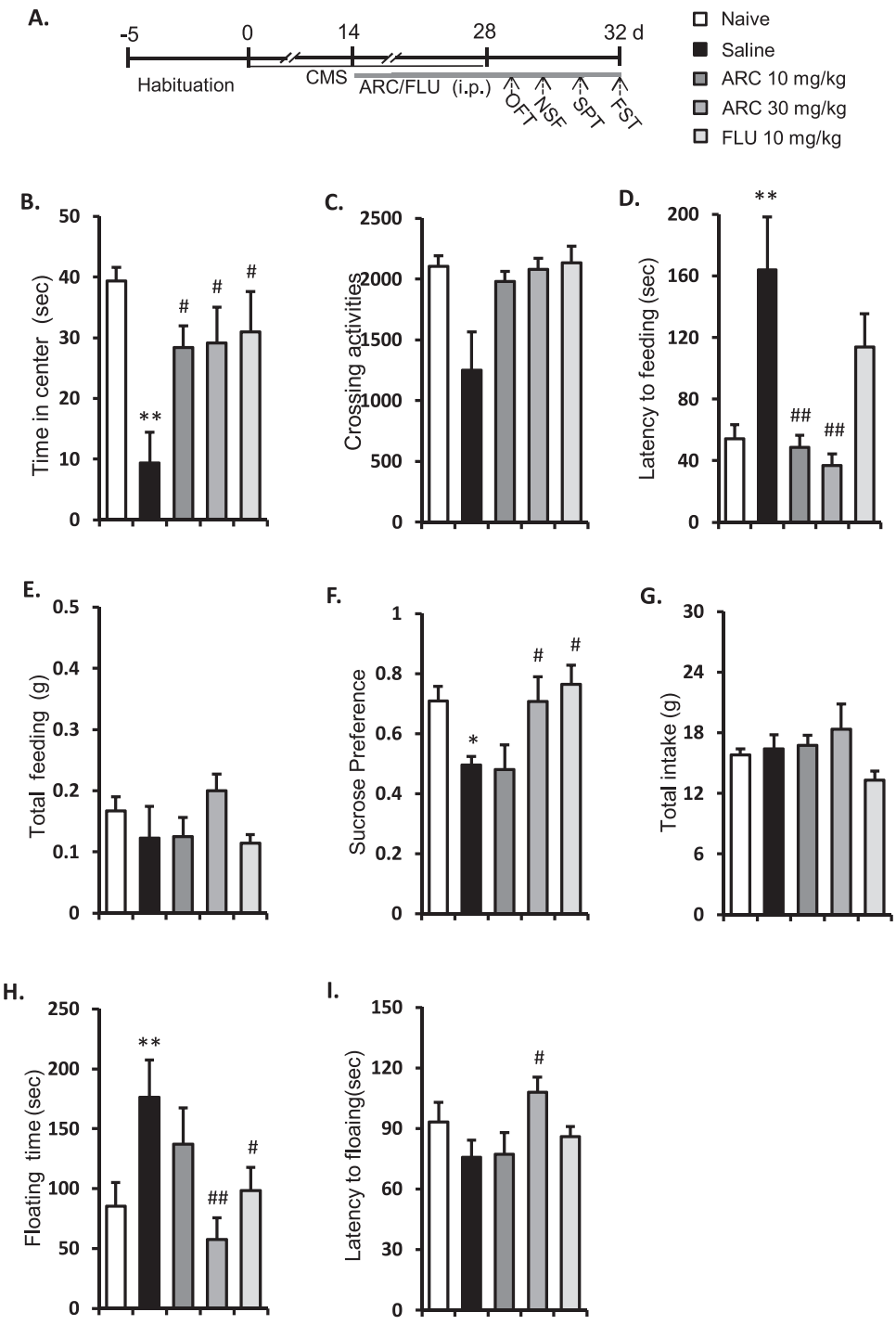

Fig. 2. ARC produced antidepressant- and anxiolytic-like behaviors in mice. (A) Experimental procedure. After a 5-day adaptation period, the mice were given daily administration of saline, ARC(10 or 30 mg/kg, i.p.) for 14 days. Beginning on day 15, behavioral tests were conducted to assess the depressive- and anxiety-like behaviors. ARC administration significantly decreased immobility time in TST (B) and the floating time in the FST (D). ARC increased the sucrose preference in the SPT(F). ARC significantly decreased the latency to feeding (G),nevertheless, there is a difference in the total feeding in homecage during the NSF test(H).ARC significantly increased the time spent in the central zone (I) without affecting the crossing activities (*J*) in the OFT. * $p < 0.05$, ** $p < 0.01$ versus the saline-treated group. n = 6–10 per group.

responded to acute stress After a 5-day habituation period, the mice were randomly divided into 3 groups (n = 6–10 per group) and were injected (i.p.) with saline, ARC (10, or 30 mg/kg) daily for 14 consecutive days. Behavioral tests, including SPT, NSF, OPT, TST and FST, were conducted 24 h after the last ARC treatment.

2.10.2. Experiment 2: effects of repeated ARC administration on depressive- and anxiety-like behaviors in chronically stressed mice

To further assess the effects of ARC on depressive- and anxiety-like behaviors in mice after chronic stress. CMS procedure was used in this experiment. Mice were divided into 5 groups (n = 6–10 per group): Control + saline, CMS + saline, CMS + ARC (3 mg/kg), CMS + ARC (10 mg/kg) and CMS + FlU (10 mg/kg). After a 5-day habituation, mice in CMS groups were treated with a consecutive 28-day chronic stress procedure. Since the 14th day during CMS procedure, CMS-treated mice were randomly divided into 4 subgroups and were injected with saline (10 ml/kg, i.p.), ARC (10 mg/kg, i.p.), ARC (30 mg/kg, i.p.) or FLU (10 mg/kg, i.p.) daily for 14 days. Mice in control group were left in their home cages only with saline injections daily for 14 days. Behavioral tests, including SPT, NSF, OPT and FST, were conducted 24 h after the last drug treatment (see Fig. 3A).

2.10.3. Experiment 3: effects of repeated arctigenin administration on serum levels of ANG, TPT, and VEGF, in acutely stressed mice

As shown in (Fig.4A), experiment 3 was aimed to investigate whether the antidepressant-like effects of ARC are associated with

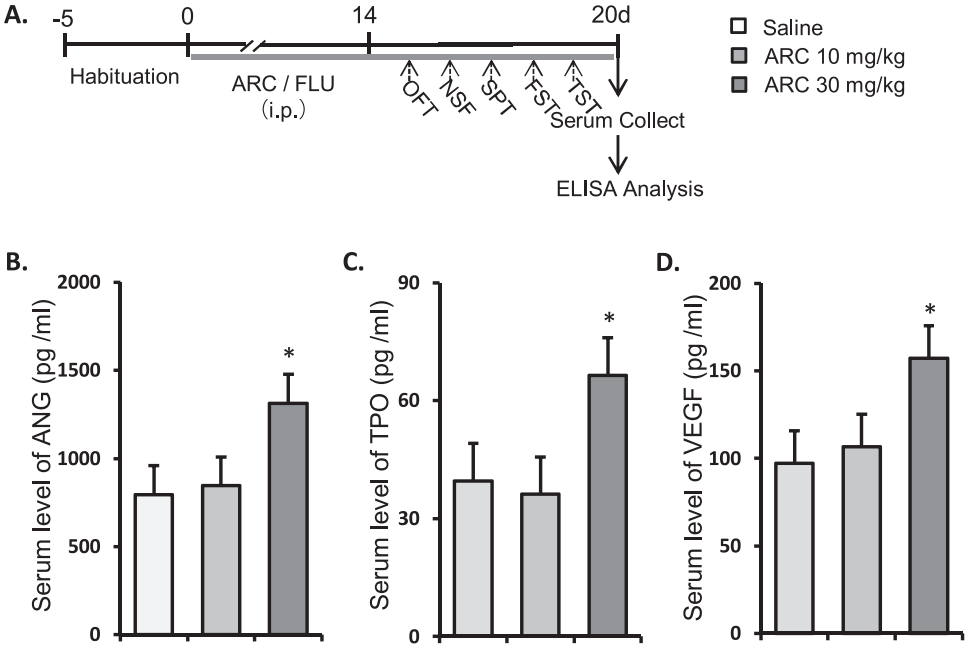

Fig. 3. ARC reversed the depressive- and anxiety-like behaviors in chronically stressed mice. (A) Experimental procedure. After a 5-day adaptation period, mice were treated by chronic stress for 28 days. On day 14, mice were injected with saline, ARC(10 or 30 mg/kg, i.p.) or Flu daily 0.5 h before stress for 14 days. During day 28–30, behavioral tests were conducted to assess the depressive- and anxiety-like behaviors. ARC significantly increased the sucrose preference (B) without affecting the total intake (C) compared with saline-treated CMS group. ARC significantly increased the floating time in the FST (D) and increased the time spent in the central zone (F) but exist some differences in the crossing activities (G) of chronically stressed mice during the OFT. ARC significantly decreased the latency to feeding (H) without affecting the total feeding in homecage (I) of chronically stressed mice during the NSF test. * $p < 0.05$ and ** $p < 0.01$ versus the naive group. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ versus the saline-treated CMS group; n = 6–10 per group.

vasculogenesis. Fifteen mice were used and were divided into three groups (n = 5 per groups) with CMS and drug treatments similar to those in the experiment 1. Mice were decapitated 24 h after the last drug treatment and the blood was collected to detect the serum concentration of ANG, TPO, and VEGF by ELISA analysis.

2.10.4. Experiment 4: effects of repeated arctigenin administration on serum levels of ANG and TPO in chronically stressed mice

Experiment 5 was aimed at investigating whether the antidepressant-like effects of ARC are associated with vasculogenesis. And twenty-four mice were used and were divided into five groups (n = 4–6 per groups) with CMS and drug treatment similar to those in the experiment 2. Mice were decapitated 24 h after the last drug treatment, and the blood was collected to detect the serum concentration of ANG and TPO by ELISA analysis. (see Fig. 5A)

2.11. Data analysis

Data are expressed as the mean ± SEM. Statistical analysis of the data from acute and chronically stressed mice was performed by one-way analysis of variance (ANOVA), respectively, which was followed by a post hoc Dunnett's test. (For details, see Section 3). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Repeated ARC administration inhibited the depressive- and anxiety-like behaviors in acutely stressed mice

One-way ANOVA of the TST date revealed a significant effect of ARC dose (Fig. 2B,C). Post hoc analyzes showed that repeated ARC treatment for 14 days at dose of 10, 30 mg/kg significantly reduced

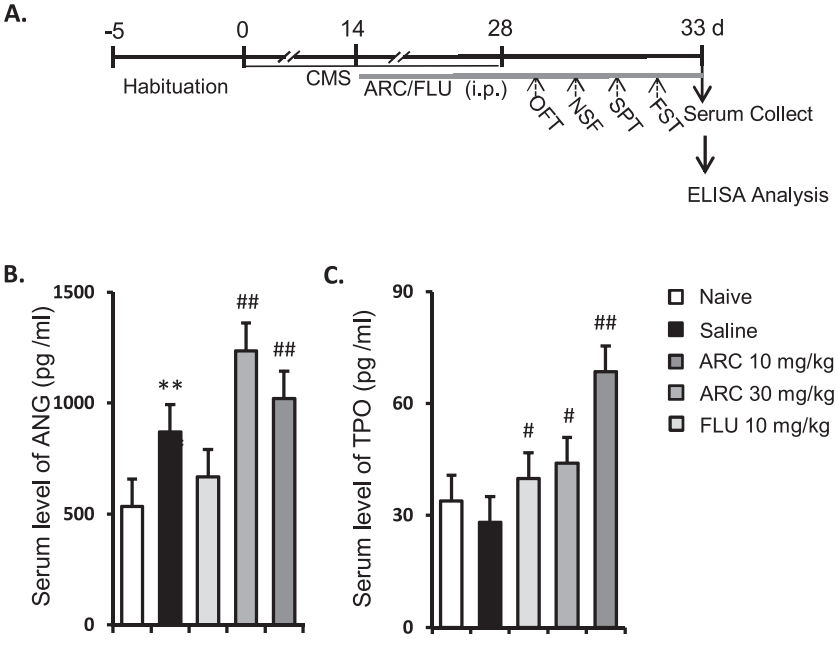

Fig. 4. ARC reversed the acute stress-induced increase of serum levels of ANG, TPO and VEGF. (A) Experimental procedure. After a 5-day adaptation period, the mice were given daily administration of saline, ARC (10 or 30 mg/kg, i.p.) for 14 days. On day 18, 1 ml of blood was collected from eyeball bleeding for ELISA analysis. ARC (30 mg/kg, i.p.) significantly increased the ANG (B) and TPO (C) levels in chronically stressed mice. * $p < 0.05$ and ** $p < 0.01$ versus the saline-treated group. n = 5 per group.

immobility time ($F_{2,21} = 6.038$, $P < 0.01$ both) and increased the latency of immobility time ($F_{2,21} = 4.543$, $P < 0.05$ both) compared with saline-treated mice.

One-way ANOVA of the FST date showed a significant effect of ARC dose (Fig. 2D). Post hoc analyzes showed that repeated ARC treatment for 14 days at dose of 10 mg/kg ($F_{2,20} = 4.151$, $P < 0.01$) significantly reduced floating time compared with saline-treated mice.

One-way ANOVA of the SPT date indicated a significant effect of ARC dose (Fig. 2F). Post-hoc analyzes showed that repeated ARC treatment for 14 days at dose of 30 mg/kg ($F_{2,28} = 2.842$, $P < 0.05$) significantly increased sucrose preference compared with saline-treated mice. There was no difference in total intake. The results elicited that ARC was able to produce antidepressant-like effects in the FST, TST, and SPT.

Next, the potential anxiolytic effects of ARC were also evaluated. One-way ANOVA analysis of the data revealed a significant effect of ARC dose in the OFT and in the NSF. Post-hoc analyzes showed that repeated ARC treatment at the doses of 10, 30 mg/kg ($F_{2,22} = 7.935$, $P < 0.01$ both) significantly decreased the latency to feeding time compared with that of saline-treated control mice in the NSF (Fig.2G). Repeated ARC treatment at all doses extended the time in the center ($F_{2,23} = 3.874$, $P < 0.05$ both) compared with vehicle group in the OFT (Fig. 2I). The results elicited that ARC was able to produce anxiolytic-like effects in mice responded to acute stress.

These data indicated that repeated ARC administration exerted significant antidepressant- and anxiolytic-like activities in mice exposed to acute stress.

3.2. Repeated ARC administration blocked the depressive- and anxiety-like behaviors in CMS-treated mice

The potential antidepressant-like effects of ARC were firstly evaluated by SPT. One-way ANOVA revealed that ARC treatment significantly increased the sucrose preference in the SPT (Fig. 3B). Post-hoc analyses vehicle group obviously reduced sucrose preference ($t_{10.707} = 3.795$, $P < 0.01$). The same as the classic antidepressant agent FLU, ARC treatment at 30mg/kg/d increased sucrose preference compared with vehicle group ($F_{3,25} = 4.249$, $P < 0.05$, $P < 0.05$ respectively). There is no difference in total intake (Fig. 3C). Then in FST, mice subjected CMS extended floating time compared with naive group ($t_{5.912} = -3.33$, $P < 0.05$), while the treatment at ARC (30mg/kg/d) and FLU shortened it compared with vehicle group ($F_{3,25} = 3.531$, $P < 0.01$, $P < 0.05$ respectively) (Fig. 3D). And extended the latency of floating time at ARC treatment (30 mg/kg) ($F_{3,25} = 2.83$, $P < 0.05$). (Fig. 3E)

Next by OFT and NSF indicated ARC work up the anxiolytic. One-way of ANOVA of the OFT data revealed a significant effect of ARC dose. Post-hoc analyses indicated that normal saline treatment strikingly reduced the time in the center ($t_{15} = 2.227$, $P < 0.01$). With the same effect of FLU treatment, ARC treatment at the dose of 10, 30mg/kg/d increased the time in center compared with vehicle group ($F_{3,26} = 3.383$, $P < 0.01$, $P < 0.05$, $P < 0.05$ respectively) (Fig. 3F). Nevertheless, CMS reduced the crossing activities ($t_{15} = 2.227$, $P < 0.05$), then ARC and FLU treatment showed increased crossing activities ($F_{3,27} = 5.016$, $P < 0.05$, both) (Fig. 3G). One-way ANOVA of the NSF data also revealed a significant effect of ARC. Compared with naive group the mice subjected to CMS exposed significant anxiety-like behaviors, reflected by an extended latency of feeding ($t_{15} = -3.257$, $P < 0.01$). The repeated ARC (10, 30 mg/kg/d) treatment reversed the anxiety-like behaviors of mice induced by CMS, decreased the latency of feeding ($F_{3,27} = 8.224$, $P < 0.01$,$P < 0.01$) (Fig. 3H), without any significant effects on the total feeding in home cages (Fig. 3I).

All behavioral results indicated that repeated ARC administration significantly reversed depressive- and anxiety-like behaviors in CMS mice.

3.3. Effects of ARC on peripheral ANG, TPO and VEGF levels in mice exposed to acute stress

The effects of ARC on vasculogenesis were evaluated using ELISA analysis. The results showed that two weeks of ARC (30 mg/kg) administration significantly increased the serum ANG ($F_{2,12} = 6.042$, $P < 0.05$), TPO ($F_{2,11} = 8.15$, $P < 0.01$), and VEGF ($F_{2,11} = 8.15$, $P < 0.01$) level compared with vehicle group (Fig.4B, C, and D).

3.4. Effects of ARC on peripheral ANG, TPO levels in mice exposed to Chronic stress

The results showed that four weeks of CMS significantly increased the serum ANG ($t_{5.184} = -5.292$, $P < 0.01$) levels compared with the control group (Fig.5B), and the same as FLU, ARC (30mg/kg) administration further significantly increased the serum ANG level ($F_{3,16} = 14.138$, $p < 0.01$) compared with vehicle group. Similar to the effects of FLU administration, ARC (10, 30 mg/kg) administration significantly increased the serum TPO ($F_{3,16} = 32.976$, $P < 0.01$ both) levels (Fig. 5C).

4. Discussion

In the present study, using mice in acute and chronic stress model, we explored whether repeated ARC treatment has antidepressant- and anxiolytic-like effects in adult mice. The behavioral results showed that repeated ARC administration indeed has antidepressant- and anxiolytic-like effects in the mice exposed to either acute or chronic stress. It is noteworthy that the antidepressant-like efficiency of ARC equals to FLU, which is generally used as antidepressant in the clinic. Considering the comparable efficiency to prescription medication with widely neuroprotective effects and no significant side effects (Zhang et al., 2013), ARC should be well researched as a potentially new antidepressant to be used clinically.

In this study, a depression model was established based on a chronic mild stress regimen. In rodents, the depressive behaviors and neurobiological changes caused by chronic mild stress are similar with those depressive patients (Henn and Vollmayr, 2005; Murray et al., 2008). Therefore, the animal model of CMS has been commonly employed in preclinical antidepressant assessment for probing the nosogenesis of depressive disorder and more beneficial therapeutic drug (Cryan and Holmes, 2005). The scheme of CMS was performed as previously described. Mice were habituated to 1% sucrose solution for 2 days then subjected to a CMS protocol. Briefly, this plan consists of the sequential application of a variety of mild stressors, including forced cold swim for 5 min, tilted cages (45∘) for 24 h, tail clamp for 1 min, restraint for 2 h, light/dark cycle reversal for 24 h, dampened bedding for 24 h, divested of food for 24 h, divested water for 24 h and empty cage for 24 h. All stressors were randomly interspersed throughout the stress period. Mice were exposed to CMS for 4 consecutive weeks to induce depression-like behavior. Compared with the control group, the general condition of stressed mice did not change significantly, but there were some behavioral effects such as hopelessness, anorexia, lack of interest, and reduced learning and memory ability. Next, we used behavioral tests to evaluate the state of depression. SPT was a most widely used behavioral testing methods to evaluate the anhedonia of depressed animals, the lower sucrose preference means the higher level of depression in the animal (Murray et al., 2008). FST and TST are two reliable experimental models for assessing depressive like behaviors and screening antidepressant drugs (Shi et al., 2012; Tian et al., 2014), the longer the immobility time, the higher level of depression of animals. NSF and OFT are two classic behavioral tests to evaluate anxiety. The shorter time in the center or the longer time to eating means that the more anxious. Our results showed that ARC significantly relieved CMS-induced depression and anxiety-like behaviors in mice, as characterized by increased sucrose consumption in the SPT, reduced the time to

feeding without affecting the total feeding in the NSF and reduced immobility time in the FST and TST, indicating that ARC can be considered as an antidepressant drug to cure depression in clinical. In acute stress, ARC treatment significantly increased sucrose consumption in the SPT and the time in center in the OFT, reduced time to feeding in the NSF and the immobility time in the FST, highlighting that ARC can increase the anti-pressure ability.

Depression is a disease with specific symptoms that can be diagnosed and cured. Fluoxetine and venlafaxine are the classical antidepressant used in clinic (Tian et al., 2014; Zhang et al., 2013). However, it is reported that up to 45%–87% of patients with depressive disorder have significant anxiety symptoms (Braam et al., 2014; Gili et al., 2013; Johansson et al., 2013; Lin et al., 2014). Anxious depression as a formal term has been used in 1966 to describe depression associating with significant anxiety symptoms (Overall et al., 1966). Depressive disorders comorbid with anxiety (anxiety disorder and/ or anxiety symptoms) is a common clinical phenomenon, the latter is an important factor leading to antidepressant therapy impedance. Thus, it is necessary to explore antidepressant and anxiolytic-like effects drugs to cure anxious depression. In our study, we found that ARC is both an antidepressant and an anxiolytic.

Even if the patient's symptoms are alleviated in clinical trials, they may still not know all the causes of the disease. Nearly a half century the development of antidepressants went through from tricyclic antidepressants (TCA), monoamine oxidase inhibitor (MAOI) to selective serotonin reuptake inhibitor (SSRI), selective noradrenalin reuptake inhibitor (SNRI). A series of studies have shown that depression is associated with inflammation such as the NLRP3 inflammasome mediates stress-induced depression via immune activation (Zhang et al., 2015). And hypothalamic-pituitary-adrenal (HPA)-axis dysregulation implicated in the development of depression (Zeni et al., 2017). During the mid-part of the twentieth century, people put forward the view of "monoamine metabolic abnormal hypothesis", considering that the causes of depression was a poor synaptic membrane within the monoamine neurotransmitters, and increased synaptic interstitial neurotransmitters is a common way to treat all aspects of depression. Nowadays, with modern development, researchers have found some new pathogenesis of depression. A report suggests that the DAPK1 interaction with the NMDAR GluN2B subunit acts as a critical component in the pathophysiology of depression and is a potential target for new antidepressant treatments (Li et al., 2018). The results of Liyi Zhang et al suggest that altered the expression of hsa_circRNA_103636 in peripheral blood mononuclear cells is a potential novel biomarker for the diagnosis and treatment of major depressive disorder (Cui et al., 2016). More and more comprehensive pathogenesis of depression is being discovered.

Angiogenesis is closely related to the development and prognosis of the disease, and the vascular system provides nutrition and oxygen for the tissue. Innutrition is in great relationship with depression, which was ignored in the past treatment. Studies have shown that the lack of neurotransmitters can easily to lead to depression. At the same time aerobic exercise can reduces the hormones, glucose and fat that was released by the depressed body, and then enhances the ability of the adrenal medulla to secrete catecholamines to alleviates depression. VEGF is potent factor acting on endothelial cells to absorb more nutrients and oxygen by improving the permeability of blood vessels. Studies have shown that owing to the fast growth of tumors and central area blood supply insufficiency leads to tissue hypoxia and necrosis, stimulating the production of VEGF, inducing endothelial cell proliferation. Most studies reported that high peripheral VEGF level in major depressive disorder patients compared with healthy control (Buttenschon et al., 2015; Fang et al., 2014; Sharma et al., 2016). An article recorded serum VEGF level in AD patients with depression were significantly higher than AD patients without depression or the healthy control (Jung et al., 2015). VEGF is also neurotrophic and its expression in brain has been reported to be up-regulated by antidepressants treatment (Fournier and Duman, 2012; Huang et al., 2012; Menard et al., 2016; Suzuki et al., 2014). In this study, ARC treatment induced the level of VEGF, effectively controls the occurrence of depression. Therefore, hypoxia is a stimulus factor to produce VEGF, elevated VEGF can help to absorb more oxygen to protect the body. But whether hypoxia can stimulate an increase in ANG levels is somewhat of a of controversy. Angiopoietin plays a key role in maintaining vascular stability, promoting angiogenesis, maintaining vascular integrity and enhancing the protective effect of ANG on brain microvascular endothelial cells (Chen et al., 2016). Hypoxia can increase ANG-2 levels in culturing glial and endothelial cell. In this study, the CMS-treated stimulated the ANG levels increased, with the treatment of ARC, the ANG level was further increased to maintain vascular stability and protect the brain's microvascular endothelial cells. Besides the effect of promoting hemostasis and accelerate the function of coagulation, platelet also can support capillary endothelial cells and offer nutrition for it, so that reduces the brittleness of capillary to maintains the integrity of the capillary wall. In this study, mice exposed to CMS showed a decreasing trend of TPO, ARC treatment increased its level, which promoting the platelet levels increased, supporting vascular endothelial cells and intaking more oxygen and nutrition, finally contributing to the treatment of depression. And in acute stress model, ARC treatment increased the serum level both of ANR and TPO.

5. Conclusion

In sum, results from these studies suggest that Arctigenin (ARC), one of an active ingredient in Great Burdock Achene, exerts significant antidepressant- and anxiolytic-like effects, which are likely to be associated with its regulation on angiogenesis and functions of platelets. Our present data support further exploration for developing ARC as a novel therapeutic strategy for depression and even other stress-related disorders.

Conflict of interest

All the authors hereby declare that they have no competing financial interests.

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