Anxiolytic and anti-depressant effects of hydroalcoholic extract from *Erythrina variegata* and its possible mechanism of action

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Abstract

Background: *Erythrina variegata* has been widely used as a traditional medicine.

Objective: The study was designed to evaluate the anxiolytic and anti-depressant effects of an extract from *Erythrina variegata*.

Methods: The extract was evaluated for anxiolytic and anti-depressant action using the elevated plus maze, light/dark box, open field, forced swimming and tail suspension tests in mice. The mechanism of action was further elucidated using high-performance liquid chromatography with fluorescence detection methods to assay the levels of five neurotransmitters in brain.

Results: The extract exhibited significant increase in the percentage of the open arms entries and the time spent in the open arms in the elevated plus maze test. The results of the light/dark box test revealed a significant increase in the amount of time spent in the light chamber. Extract- treated mice also produced significant increase in the number of crossings and rearings in the open field test.In the forced swimming and tail suspension tests, the extract was able to promote significant decrease in the immobility time. In addition, the extract significantly altered the levels of five neurotransmitters in the brain tissue.

Conclusion: These findings suggest that *Erythrina variegata* presents potential anxiolytic and anti-depressant activity, and the mechanism may be related to the alteration of neurotransmitter levels.

Keywords: Anxiolytic; anti-depressant; *Erythrina variegata*; elevated plus maze; forced swimming; neurotransmitters.

DOI: https://dx.doi.org/10.4314/ahs.v19i3.28

Cite as: *Chu H-B, Tan Y-D, Li Y-J, Cheng B-B, Rao B-Q, Zhou L-S. Anxiolytic and anti-depressant effects of hydroalcoholic extract from Erythrina variegata and its possible mechanism of action. Afri Health Sci. 2019;19(3): 2526 -2536. https://dx.doi.org/10.4314/ahs.v19i3.28*

Introduction

According to the World Health Organization report[1], approximately 450 million people suffer from a mental or behavioral disorder. Depression and anxiety are two of the most common mental disorders, exacting a pervasive toll on the individual and impairing numerous aspects of quality-of-life by inducing physical, social, emotional, and occupational dysfunction[2-5]. The complexities of the central nervous system make diagnoses, treatment, and amelioration of these illnesses exceptionally difficult. In the etiology and pathophysiology of depressive disorders, chronic stress is one of the most important contributing factors[6]. This explains the strong comorbidity between depression and anxiety[7] and the similar efficacy of pharmacological therapies for both disorders[8]. However, because of the limited efficacy of current drugs, the need for newer, better-tolerated and more efficacious treatments remains high.

As a result, there has been increased interest in the use of complementary and alternative medicines (CAM) as a natural method for treating numerous types of anxiety and depression[9]. In recent years, many traditional Chinese medicinal plants have been successfully used to prevent or treat anxiety and depression[10].

The genus *Erythrina* (*Fabaceae*) consists of 110-200 tropical trees and shrubs, which are widely distributed in tropical and sub-tropical regions. The plants under the *Erythrina* genus are collectively known as "coral tree". It is typically found on sandy soil in littoral forest, and sometimes in coastal forest up to 250m in elevation[11]. Many species of the *Erythrina* genus are used in folk medicines to treat central nervous system diseases, including *E. velutina*, *E. mulungu* and *E. mysorensis*, which present the sedative, neu-

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romuscular blocking[12], anti-nociceptive, anxiolytic and depressant activities[13-19].

E. variegata is a medium sized deciduous small tree with prickly stems and branches, leaves with triangular leaflets and large coral red flowers and grows all over the tropics. In traditional medicine, different parts of *E. variegata* have been used to produce nervine sedation, febrifuge, anti-asthmatic, and anti-epileptic effects, and its leaves have been used for the treatment of patients with various conditions, such as liver ascites, convulsions, and arthritis, among other disorders. It has also been shown to have potential for treating patients with conditions such as fever, inflammation, bacterial infection, insomnia, helminthiasis, coughing, cuts, and wounds[20-23].

In view of all observations cited above, this influenced us to design and conduct the present study to evaluate the impact of alcoholic extract of *E. variegata* bark in different behavioral models in mice and to elucidate its mechanism of action. The anxiolytic- and antidepressant-like effects were assessed in the elevated plus maze, light/dark box, open field, forced swimming and tail suspension tests, respectively.

Materials and methods

Plant material and extract preparation

The bark of *E. variegata L.* (*Leguminosae*) was collected from the regions of Zhejiang Province of China in the month of September 2016, and identified from Department of Pharmacy of Jinggangshan University by Prof. Zhaochang Liang. The dried and powdered bark (100 g) of *E. variegata* was extracted three times with 95% alcohol (300 mL) under reflux for 3 hours. The resulting solvent was eliminated under reduced pressure to obtain a dried extract, with a yield of 18.5 g/100 g of the starting crude material. The residue was dissolved in water for final suitable concentrations.

Animals

Male Kunming mice (18-22 g) were purchased from Hunan SJA Laboratory Animal Co., Ltd, and were fed a commercial diet and water ad libitum. The animals were housed in cages with food and water ad libitum and maintained on a natural 12 h of light and dark cycle. Ethical clearance for performing the experiments on animals was obtained from Institutional Animal Ethics Committee, School of Medicine, Jinggangshan University.

Experimental design

The anxiolytic activities were examined by using the elevated plus maze (EPM), light/dark box and open field tests. The forced swimming and tail suspension tests were performed to evaluate antidepressant activities. Animals were treated orally with the extract of *E. variegata*. Controls received vehicle (water) at the same volume (10 mL/kg) administered by the same route as the treated groups. Diazepam and fluoxetine, used as standards, were administered orally after dissolution in distilled water. The treatments of all groups were continued for 7 days. The mice were allowed to rest for 30 min after the last feeding. To each test, a total of 60 male mice were randomly divided into five groups of 12 mice each. The different groups were treated with: water (vehicle), extract (50, 100 and 200 mg/kg), diazepam (DZP, 2 mg/kg) or fluoxetine (FLU, 10 mg/kg).

Elevated plus maze test

The elevated plus maze test for mice consisted of two perpendicular open arms (30×5 cm) and two closed arms (30×5×25 cm) also in perpendicular position[24]. The open and closed arms were connected by a central platform (5×5 cm). The entire apparatus was elevated 45 cm and four lights were placed above the maze. The mice were taken from their home cages and transported to the apparatus for 5 min. The animal was placed at the center of the plus maze with its nose in the direction of one of the open arms. Entry into an arm was defined as the animal placing all four paws onto the arm. The percentage of the open arms entries (OE%) and the percentage of the time spent in the open arms (OT%) were recorded as the anxiety index. All tests sessions were taped by using a video camera. After each test, the maze was carefully cleaned up with a wet tissue paper (10 % ethanol solution).

Light/dark box test

The apparatus consisted of a cage having dimensions 21 cm × 42 cm × 25 cm. A partition with door was placed to divide it into two sections of equal size. The first section was white and second was black colored. The light illumination in the first section was kept bright and second was dim. Each mouse was placed separately in the center place of white box while facing door present in the partition. The observation period of each animal was 5 min. All tests sessions were taped using a video camera. After each test, the maze was carefully cleaned up with a wet tissue paper (10 % ethanol solution). Anxiolytic ativ-

ity was evaluated in terms of percentage of time spent in light and dark area[25].

Open field test

The open field test was used to evaluate the exploratory activity of the animal for 5 min. The apparatus consisted of a wooden box (50×50×50 cm). The arena of the open field was divided into 25 squares (10×10 cm) of equal area. The experimental room was dark and sound attenuated. The open field arena was illuminated with a lamp, focusing on the field from a height of about 75-100 cm. The mouse was placed in the central square and then the number of squares the mouse crossed was measured (crossing) and the number of times the mouse stood on hind limbs (rearing) was observed and recorded[26].

Forced swimming test

The forced swimming test is the most widely used and recognized pharmacological model for assessing anti-depressant activity[27]. Briefly, mice were forced to swim individually in a glass cylinder (diameter 10 cm, height 25 cm), containing fresh water up to a height of 10cm at 25cm. All animals were forced to swim for a 6 min period and the total duration of immobility was recorded during the last 4 min with a video camera. Mice were considered immobile when they floated in the water without struggling and making only those movements necessary to keep their heads above the water.

Tail suspension test

Mice were assessed in the tail suspension test, which was performed with a computerized device, allowing four animals to be tested at one time. In a chamber that was both acoustically and visually isolated, an individual mouse was suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during the last 4 min of a total of 6 min session. Immobility was scored as a failure to make any struggling movements, and attempts to catch the adhesive tape or body torsions or jerks.

Measurement of neurotransmitter levels

Detections of the levels of dopamine, noradrenaline, serotonin, glutamate and gamma-aminobutyric acid (GABA) in brain were estimated by using high-performance liquid chromatography with fluorescence detection (HPLC-FD). After the end of behavioral experiments in EPM or forced swimming test, the mice were killed by decapitation and the brain was dissected immediately. After recording weight of the brain, it was frozen at -80°c until analysis. Samples were homogenized in 2 ml of ice cold 0.6 M perchloric acid and the resulting mixture was centrifuged at 10,000 ×g for 15 min at 4 °C. The supernatant was centrifuged again at 12,000 ×g for 10 min at 4 °C and divided into A and B portions. Portion A was used for detections of the levels of dopamine, noradrenaline and serotonin and portion B for glutamate and GABA. The instrument parameters used for HPLC-FD were as follows: Agilent chromatographic system, Diamonsil ODS column (4.6mm × 250mm), mobile phase for portion A sample: methanol-buffer (buffer: 0.1mol NaAc, 0.1mmol EDTA-2Na, pH 5.1) and for portion B sample: methanol-buffer (buffer: 50 mmol NaAc, 1/100 (v/v) THF); flow rate: 1.0 mL/min; injection volume: 10 L; column temperature: 25°c. Excitation and emission of the fluorescence detector were set to 290, 330 nm for portion A, and 338, 425 nm for portion B, respectively. The precolumn derivative reagent (phthalic aldehyde) was added into portion B sample before injection. Peaks were identified by comparing the retention time of sample and standard. The concentration of each neurotransmitter in the sample was analyzed according to their area under the curve using their straight-line equation. The levels of neurotransmitter were expressed as ng/g (for dopamine, noradrenaline and serotonin) and μg/g (for glutamate and GABA).

Statistical analysis

Expression of data was done as mean ± standard error of mean (per group n=12). The normally distributed data were subjected to two-way ANOVA followed by Dunnett's test. $p<0.05$ was considered statistically significant.

Results

Elevated plus maze test

In EPM test, the dose-effects of extract at 50, 100 and 200 mg/kg are shown in Figure 1. Compared with the vehicle group, the group of animals treated with diazepam (DZP) and the extract at 50 and 100 mg/kg showed significant increase ($p<0.01$or $p<0.05$) in the OE% as well as OT%. The 100 mg/kg group showed higher levels of OE% ($p<0.05$) than those of the 200 and 50 mg/kg groups, indicating that 100 mg/kg dose is significantly more effective than those of other doses of the extract. No significant differences in the OE% as well as OT% were found between the 200 mg/kg group and vehicle

group. However, extract at doses of 50–200 mg/kg produced a lower level of OE% as well as OT%, when compared with the DZP group.

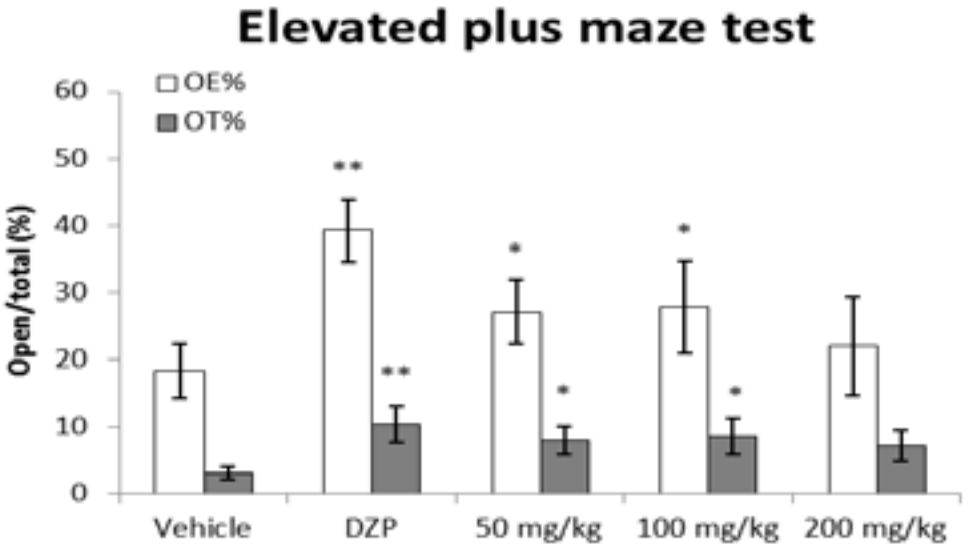

Figure 1: Anxiolytic effect of extract on the OE% and OT% of EPM mice.Mean (±s) percentage of open arm entries (OE%) or of time spent in the open arms (OT%) in the mice placed at the center of a plus-maze and given a 5-min test. $^{*}p<0.05$ and $^{**}p<0.01$ comparedwith the vehicle group.

Open field test

In the open field test, diazepam-treated mice showed significant increase ($p < 0.01$) in the number of squares crossed during 5-min interval of test as compared to vehicle-treated groups (Figure 3). Hydroalcoholic extract-treated mice at two doses (50 and 200 mg/kg) produced significant increase in the number of crossings ($p < 0.05$). Extract-treated mice (50 and/or 100 mg/kg) also produced significant increase in the number of rearings ($p < 0.01$ and/or $p < 0.05$). No significant differences in the crossing times were found between the 100 mg/kg group and vehicle group. The significant differences in the rearing times were not found between the DZP, 200 mg/kg groups and vehicle group. Extract at doses of 50–200 mg/kg produced a lower level of the crossing times, when compared with the DZP group. However, extract at doses of 50 and 100 mg/kg produced a higher level of the rearing times, when compared with the DZP group.

Forced swimming test

To examine the possible anti-depressant-like effect of the extract, we subjected the mice to forced swimming test. As shown in Figure 4, the animal treated with fluoxetine (FLU, 10 mg/kg) showed significant decrease ($p<0.05$) in the immobility time when compared to the vehicle group treated with water. Also, the mice treated with extract at 50, 100 and 200 mg/kg showed lower levels of immobility time. The 200 mg/kg group has lower level of immobility time ($p<0.01$) than those of the FLU, 50 and 100 mg/kg groups, indicating that 200 mg/kg dose is significantly more effective than those of other dose and fluoxetine (10 mg/kg).

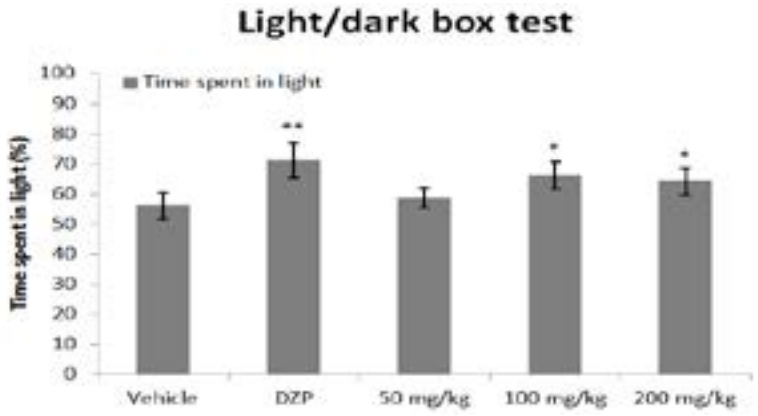

Figure 2: Anxiolytic effect of extract on performance in the light/dark box test. Mean (±s)percentage of time spent in the light area in the mice placed at the light/dark box and given a 5-min test. $^{*}p<0.05$ and $^{**}p<0.01$ comparedwith the vehicle group.

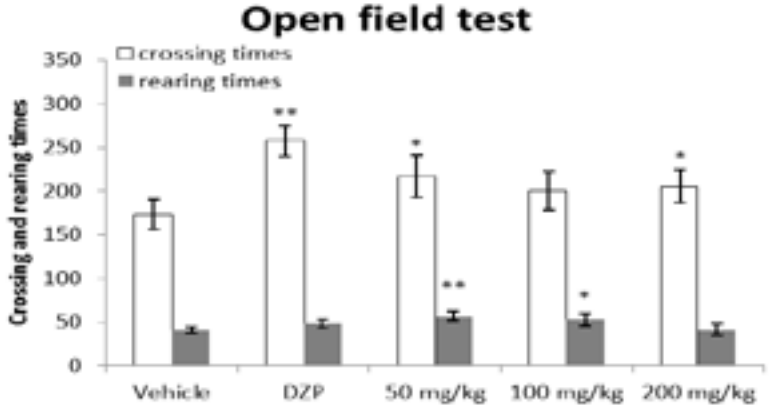

Figure 3: Anxiolytic effect of extract on performance in the open field test.Mean (±s)number of squares the mouse crossed (crossing) and number of times the mouse stood on hind limbs (rearing) in the mice placed at the open field and given a 5-min test. $^{*}p<0.05$ and $^{**}p<0.01$ compared with the vehicle group.

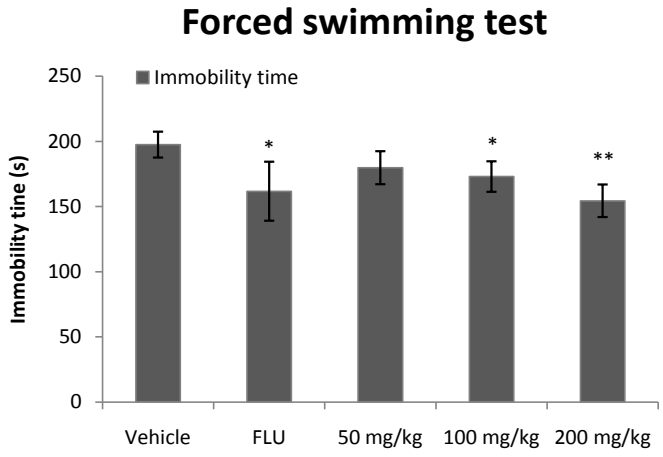

Figure 4: Antidepressanteffect of extract on performance in the forced swimming test. Mean (±s) immobility time in the mice placed at the forced swimming test and given a final 4 min of the 6-min test.$^{*}p<0.05$ and $^{**}p<0.01$ compared with the vehicle group.

Tail suspension test

Comparison between the effects of extract at various doses with respect to control vehicle and the standard drug fluoxetine has been presented in Figure 5. The extract (50 and 200 mg/kg) significantly ($p<0.05$) decreased the immobility time as compared to control group. However, the extract (100 mg/kg) did not show significant decline in immobility time. Fluoxetine treated mice showed greater decline in immobility time and showed significant ($p<0.01$) reduction in immobility time compared to control group. However, the extracts at doses of 50–200 mg/kg produced a lower level of the immobility time, when compared with the FLU group.

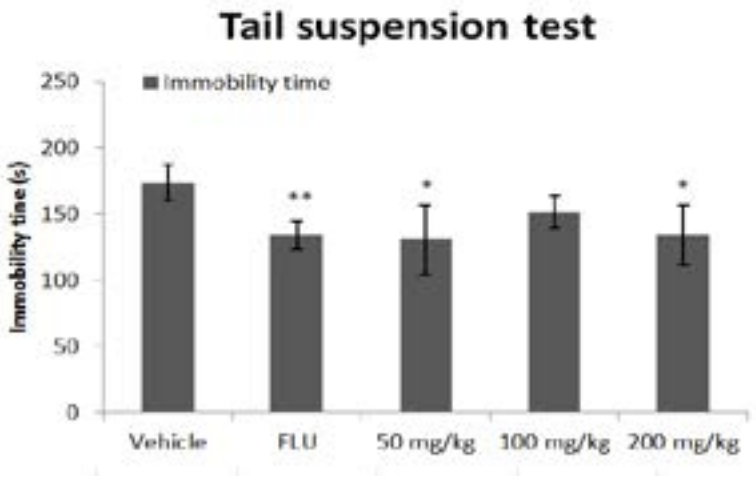

Figure 5: Antidepressanteffect of extract on performance in the tail suspension test.Mean (±s) immobility time in the mice placed at the tail suspension test and given a final 4 min of the 6-min test. $^{*}p<0.05$ and $^{**}p<0.01$ compared with the vehicle group.

Measurement of neurotransmitter levels

After the end of EPM behavioral experiments, the neurotransmitter levels of dopamine, noradrenaline, serotonin, glutamate and GABA, were quantified by HPLC-FD in the brain of mice (Table 1). The diazepam (DZP) and 50 mg/kg dosage groups showed a decrease in levels of dopamine, and 100 and 200mg/kg dosage groups had an increase in levels of dopamine when compared to the group treated with water. Moreover, in this test the effects of extract at dose of 50, 100, and 200mg/kg indicated the effects that increased after 7 days of treatment. The 50 mg/kg dosage groups showed similar decrease to levels of noradrenaline and serotonin. The effects of extract at dose of 50, 100, and 200mg/kg presented a trend to increase the levels of noradrenaline and serotonin after 7 consecutive days of treatment. In addition, statistical analysis indicated a significant decrease ($p<0.01$) in glutamate level in mice treated with extract at 50 and 100 mg/kg dose. At the same time, the GABA levels in the above groups had obvious decrease ($p<0.05$) when compared with vehicle group. The glutamate/GABA ratio showed significant decrease ($p<0.01$) in the mice treated with diazepam and extracts (50 and 100 mg/kg).

Table 1: The levels of dopamine, noradrenaline, serotonin, glutamate and GABA in brain tissue of mice in EPM

Group	dopamine (ng/g)	noradrenaline (ng/g)	serotonin (ng/g)	glutamate (μg/g)	GABA (μg/g)	glutamate/GABA
Vehicle	735.7±85.0	771.7±88.9	537.4±50.0	1499.6±209.5	3396.2±277.8	0.44±0.05
DZP	713.1±68.5	780.9±92.1	541.1±86.7	928.1±112.0**	3041.6±232.2**	0.31±0.03**
50 mg/kg	679.6±84.4	689.3±101.4	509.6±66.8	1104.7±218.0**	3154.6±233.7*	0.35±0.06**
100 mg/kg	764.6±123.4	780.3±149.8	527.1±54.3	1104.7±284.4**	3138.7±300.4	0.35±0.07**
200 mg/kg	823.4±113.7	822.5±157.4	584.1±91.9	1218.4±295.1*	2974.4±454.0*	0.43±0.14

Data are presented as mean ± SE (n=12). * $p<0.05$, ** $p<0.01$ vs. vehicle group

Similarly, the above five neurotransmitter levels were quantified by HPLC-FD in the brain of mice after end of forced swimming test behavioral experiments (Table 2). The animal treated with diazepam (2 mg/kg) and extracts (50, 100 and 200 mg/kg) showed decrease in levels of dopamine and noradrenaline when compared to the mice treated with water; while the diazepam (DZP) group showed significant decrease ($p<0.05$) in level of dopamine. The levels of serotonin showed significant decrease in diazepam treated and 100 mg/kg ($p<0.01$) and 200 mg/kg ($p<0.05$) extract treated groups compared to the vehicle group. On the other hand, the data indicated a significant decrease ($p<0.01$) in glutamate level in mice treated with extract at 50 and 100 mg/kg dose. At the same time, the GABA levels in the above groups had obvious decrease when compared with vehicle group. The glutamate/GABA ratio showed significant decrease ($p<0.01$) in the mice treated with diazepam and extracts (50 and 100 mg/kg).

Table 2: The levels of dopamine, noradrenaline, serotonin, glutamate and GABA in brain tissue of mice in forced swimming test

Group	dopamine (ng/g)	noradrenaline (ng/g)	serotonin (ng/g)	glutamate (μg/g)	GABA (μg/g)	glutamate/GABA
Vehicle	791.1±63.6	949.8±147.0	546.9±49.2	1688.0±156.7	2720.2±197.8	0.62±0.05
FLU	733.9±46.6*	842.7±91.4	502.5±58.6*	1695.9±189.3	2617.2±136.0	0.65±0.05
50 mg/kg	732.5±162.5	854.1±166.7	549.7±92.8	1329.4±272.6**	2626.2±453.1	0.51±0.10**
100 mg/kg	788.0±52.3	909.2±71.4	469.7±52.2**	1318.1±241.7**	2447.1±418.8	0.54±0.05**
200 mg/kg	788.0±78.4	864.5±76.6	506.4±53.7*	1495.8±288.4	2663.3±630.0	0.55±0.13

Data are presented as mean ± SE (n=12). * $p<0.05$, ** $p<0.01$ vs. vehicle group

Discussion

The present work evaluated the anxiolytic and antidepressant activity of various doses of the extract of *E. variegata* in mice employing behavioral animal models, including the elevated plus maze, light/darbox, open field, forced swimming and tail suspension tests. These tests are classic and standard models for screening central nervous system actions providing information about anxiety and depressant performance. Animal models of anxiety and depression are typically based on exposure of animals to a stressful condition and a specific test for measuring behavioral and physiological responses[28].

Both the EPM and light/dark box test have been widely used to evaluate neurobehavioral profiles of rodents under conditions of anxiety. In the EPM test, exposure animals upon to an open arm causes an approach conflict that is significantly robust than the response elicited by exposure to an enclosed arm of the maze. Thus, open/enclosed arm entries and time spent in respective arms provide a measure of fear provoked by suppression of exploratory action. Generally, classical anxiolytic antagonizes these behavioral changes, and benzodiazepines are used to validate the anxiolytic activity. Similarly, in light/dark box test, anxiety is generated by the conflict between the tendency to explore and the initial tendency to avoid the unfamiliar environment and can be evaluated according to the number of transitions in to and the time spent in the light chamber where an increase in these parameters is considered to reflect anxiolytic-like properties[29]. In the present work, a clear anxiolytic-like activity of a hydroalcoholic extract of *E. variegata* has been demonstrated. This extract was able to increase significantly the time spent and number of entries mice into the aversive arm of the plus-maze and light/dark box tests. These results indicate anxiolytic activity comparable with that produced by DZP, the standard anxiolytic drug.

The open field test is a standard neophobic test of anxiety. In this test, rodents naturally tend to avoid open spaces[30]. Thus, animals removed from their acclimatized cage and placed in environment express anxiety and fear, by showing alteration in all or some parameters. Anxiolytic treatments reduce such fearful behavior of animals in open field. Statistical analysis of the data obtained from these experiments supported anxiolytic-like activity of extracts at both the doses (50 and 100 mg/kg) as its effect shows significant increase in the number of squares crossed and the number of rearings, as compared to the vehicle-treated group, which indicates its anxiolytic-like effect.

The forced swimming and the tail suspension tests are behavioral despair tests useful for probing the pathological mechanism of depression and for the evaluation of

antidepressant drugs[31]. Characteristic behavior scored in both tests is termed immobility, reflecting a behavioral state of despair, as seen in human depression[32]. The effects of extract of *E. variegata* on the duration of immobility in the above mouse depressive models have showed that treating animals with the extract orally for 7 days reduced significantly the duration of immobility both in forced swimming and the tail suspension tests at the doses of 50, 100 and 200 mg/kg.

The dose-response relationship of extracts in the EPM, light/dark box, open field and forced swimming, tail suspension models appears to be rough bell-shaped. This explains why the 100 mg/kg dose appears to be superior to the 50 and 200 mg/kg. This phenomenon remains poorly understood although many of the most important biological events show receptor desensitization.

To date, the biological explanations for many types of anxiety and depressant disorders remain inadequate. Postulations have implicated a dysregulation of specific neurotransmitters such as serotonin, dopamine and GABA as potential causes for both depression and anxiety disorders[33].

Alterations in the brain monoamines dopamine (DA) and serotonin (5-HT) have been implicated in the etiology and/or pharmacotherapy of multiple mental disorders including schizophrenia and depression. Serotonin is a monoamine neurotransmitter and is found predominantly in the gut, however is also synthesized in the central nervous system where it plays a role in regulating mood, memory and various other functions[34]. Noradrenergic hyperactivity has been established as a critical component of the stress response and abnormal noradrenergic signal is consistently implicated in anxiety related behavior[35]. The diazepam (DZP) and 50 mg/kg dosage groups showed a decrease in levels of dopamine, and 100 and 200mg/kg dosage groups had an increase in levels of dopamine when compared to the group treated with water. Moreover, in this test the effects of extract at dose of 50, 100, and 200mg/kg presented a trend to increase after 7 consecutive days of treatment. The 50 mg/kg dosage groups showed similar decrease to levels of noradrenaline and serotonin. The effects of extract at dose of 50, 100, and 200mg/kg presented a trend to increase the levels of noradrenaline and serotonin after 7 consecutive days of treatment. Results indicated that extract decreased the levels of dopamine, noradrenaline and serotonin in mice, suggesting that the extract has a regulating effect on monoamine neurotransmitters after central nervous system disorder and it might be one of the anxiolytic or anti-depressant functional mechanism.

Multiple lines of evidence strongly implicate glutamate in anxiety and depressant disorders. There are abnormal levels of glutamate and various glutamate receptor classes in the brains of patients with anxiety disorders, and glutamate levels are altered in rodents by stressors[36]. Glutamate is the most abundant excitatory neurotransmitter in the brain, and acts pre- and postsynaptically by activating diverse receptors characterized by their structural properties. Glutamate ionotropic and metabotropic receptors regulate neurotransmission across excitatory synapses, and modulate several physiological brain functions such as synaptic plasticity, learning, and memory[37]. The present results showed that, compared to the vehicle group, the levels of glutamate in 50 and 100 mg/kg dose groups had different decrease ($p<0.01$), which suggest that the anxiolytic and anti-depressant effect of *E. variegata* was possibly involved in the decrease of glutamate level (Table 1 and 2). In addition, GABA is known as an inhibitory neurotransmitter present almost exclusively in the central nervous system, and GABAergic dysfunction causes mood disorders or neurological disorders such as seizures, anxiety, and depression[38]. Our present study showed that the levels of GABA in standard and extract dose groups had obvious decrease, when compared to the vehicle group treated with water. It might be speculated that the net effect of this reduction in GABA concentrations would be to reduce the overall inhibitory influence of GABA on neural circuits involved in responding to stress or threat[39].

GABA is synthesized from glutamate by removal of an α-carboxyl group by glutamic acid decarboxylase. The synthetic relationship between GABA and glutamate suggests that concentrations of each may be influenced by the other[40]. As shown in Table 1 and 2, the glutamate/GABA ratio showed significant decrease in the mice treated with standard and extract, when compared to the vehicle group. The experimental data implied further the anxiolytic and depression effect of *E. variegata* was possibly involved in the alteration of glutamate and GABA levels. However, the precise mechanism underlying this change still requires further investigations.

Some studies showed that phytochemicals like alkaloids,

flavonoids, phenolic acids, lignans, cinnamates, terpenes and saponins possess anxiolytic effects in a wide range of animal models of anxiety[41]. The inhibitory effects of flavonoids on monoamine oxidases have attracted great interest, since alterations in monoaminergic transmission are reported to be related to neurodegenerative diseases such as Parkinson's and ADs and psychiatric disorders such as depression and anxiety, thus monoamine oxidases may be considered as targets for the treatment of these multi-factorial diseases[42].

Erythrina plants are known to produce alkaloids, flavonoids, and terpenes[43-45]. Three isolated alkaloids from *E. mulungu* plants have shown anxiolytic effects in different animal models[46]. The natural flavonoids compound luteolin has been reported to have anti-depressant, anti-nociceptive and anxiolytic-like effects, which possibly involve the mechanisms of modulating GABA signaling[47].Preliminary phytochemical studies on *E. variegata* exposed the presence of carbohydrates, protein, alkaloids, steroids, phytosterols, terpenoids, saponin, glycosides, tannins and phenols, isoflavonesand flavonoids[48-51]. The current display of anxiolytic and anti-depressant activity may be related to *E. variegata* ingredients, but the exact relationship needs further investigation.

Conclusion

The hydroalcoholic extract of the bark of *E. variegata* possesses anxiolytic and anti-depressant properties in mice. The alteration of neurotransmitters level was observed in present study, which is possibly involved in mediating anxiolytic and anti-depressant action. Further investigations are warranted for elucidating the exact mechanism and bioactive compounds responsible for the observed effects.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (31260084) and Natural Science Foundation of Jiangxi Province (20142BAB205024).

Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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