

## Original Research Article

# Anti-depressant-like effect of curculigoside isolated from *Curculigo orchoides* Gaertn root

Jing Wang<sup>1\*</sup>, Xiao-Li Zhao<sup>2</sup> and Lan Gao<sup>3</sup>

<sup>1</sup>Neurology Department, Shanxi Provincial People's Hospital, Taiyuan 030012, <sup>2</sup>Neurology Department, The First Hospital of Xi'an City, Xi'an 710002, Shanxi, <sup>3</sup>Beijing Huilongguan Hospital, Beijing 100096, China

\*For correspondence: Email: [doctorwangjing@126.com](mailto:doctorwangjing@126.com); Tel/Fax: +86-0351-4960171

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### Abstract

**Purpose:** To investigate the anti-depressant-like activity of curculigoside from *Curculigo orchoides* Gaertn and its underlying mechanism(s).

**Methods:** Antidepressant-like activity was determined in mice through forced swimming test (FST), tail suspending test (TST), and open field test (OFT). Mechanism of action was investigated by measuring levels of dopamine (DA), norepinephrine (NE), and 5-hydroxytryptamine (5-HT) in chronic mild stress (CMS) rats using high-performance liquid chromatography-electron capture detector (HPLC-ECD). Western blotting was used to investigate the effect of curculigoside on the expression of brain-derived neurotrophic factor (BDNF) protein in rats.

**Results:** In FST and TST, treatment of mice with curculigoside (10, 20, 40 mg/kg, p.o.) significantly reduced immobility time, which was, however, unaffected by locomotor activity when assessed in the OFT. The treatment led to significant increases in DA, NE and 5-HT, and up-regulation of BDNF protein expression in the hippocampus of the CMS rats.

**Conclusion:** These results demonstrate that curculigoside exerts significant anti-depressant-like activity on mice, the mechanism of which involves increase in the levels of DA, NE, 5-HT, and up-regulation of BDNF expression. Thus, curculigoside can be considered a potential drug candidate for the treatment of depressive disorders.

**Keywords:** *Curculigo orchoides* Gaertn, Curculigoside, Antidepressant-like activity, Behavioral studies

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## INTRODUCTION

Depression, a common mental disorder, is a leading cause of disability all over the world [1]. It may be long-lasting or recurrent, and it substantially affects the thoughts, behavior, feelings and sense of well-being of affected individuals. In recent years, there has been an increase in depression-associated morbidity and mortality in the world. According to a World Health Organization (WHO) report, there are about 350 million people with depression [2]. By 2030, researchers predict that depression will be

one of the three leading causes of illness and a major contributor to the overall global burden of disease [2]. Mild depression may not require any professional treatment, but prolonged or severe depression often requires medication and professional management. Although there have been some improvement in the pharmacotherapy of depression, approximately 30–40 percent of depressive patients are still unable to achieve full remission [3]. Therefore, there is an urgent need for development of effective and alternative anti-depressant drugs with low side-effects.

Curculigoside, a phenolic glycoside, is extracted from the roots of *Curculigo orchioides* Gaertn, and it is the major bioactive compound in this herbal plant [4]. *Curculigo orchioides* Gaertn belongs to the family Amaryllidaceae and is often used in traditional Chinese medicine for treating impotence, limb limpness, diarrhea and arthritis [5]. In India, *C. orchioides* is used as a potent immuno-modulator and aphrodisiac in Ayurvedic medical system [5].

Curculigoside has been reported to exhibit various pharmacological properties. These include anti-oxidant, anti-osteoporotic and neuro-protective activities [6-9]. In addition, a study has shown that curculigoside improves cognitive function (learning and memory) in aged rats by inhibition of cerebral acetyl cholinesterase (AChE) activity and down-regulation of expression of  $\beta$ -site APP-cleaving enzyme 1 (BACE1) in the hippocampus [4].

To the best of our knowledge, only a few publications have reported the antidepressant properties of curculigoside isolated from *C. orchioides*. Moreover, not much is known about the mechanism by which it exerts its pharmacological action. The present study was carried out to investigate the antidepressant-like activity of curculigoside, and its mechanism of action.

## EXPERIMENTAL

### Plant material

Roots of *C. orchioides* were purchased from Tong-ren-tang Pharmaceutical Group (Taiyuan, China) in 2014 and identified by a taxonomist at the Department of Traditional Chinese Medicine in Shanxi Provincial People's Hospital (No. 29 Taiyuan Twin Towers Temple Street, Taiyuan, China). A voucher specimen of *C. orchioides* (201401wj) was kept in our laboratory for future reference.

### Chemicals

Methanol (MeOH) (analytical reagent, AR), ethyl acetate (AR), n-butanol (AR) and petroleum ether (AR) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China); silica gel for column chromatography (100 – 200 and 200 – 300 mesh) was obtained from Qingdao Haiyang Chemical Co., Ltd. (Qingdao, China); Sephadex LH-20 was supplied by GE Healthcare Co. (Skokie, IL, USA); imipramine was purchased from Aladdin Reagent Co. Ltd. (Shanghai, China), BCA protein assay reagent

was purchased from Beyotime (Haimen, China); goat-anti-rabbit/rat horseradish-peroxidase-conjugated (HRP) secondary antibody was a product of Beyotime Biotechnology (Haimen, China). Others include brain-derived neurotrophic factor (BDNF) antibody (Santa Cruz Biotechnology CA, USA) and  $\beta$ -actin antibody (Abcam Biotechnology, Cambridge, MA, USA). All other reagents used in the present investigation were of AR grade.

### Preparation of pure curculigoside

The dried roots of *C. orchioides* were powdered and extracted by refluxing three times with 75 % aqueous ethanol, each for 90 min. The filtrates were concentrated by rotary evaporation at 60 °C in vacuum. Subsequently, the extract was re-extracted with ethyl acetate, and then the ethyl acetate extract (ACE) was fractionated by silica-gel column chromatography. Elution was carried out with graded volume ratios of petroleum ether and acetone (20 : 1, 15 : 1, 10 : 1, 5 : 1, 1 : 1 and 1 : 2) to obtain a series sub-fractions (I-IV) based on thin-layer chromatography (TLC) analysis. Then, fraction III was subjected to Sephadex LH-20 chromatography and eluted with MeOH to obtain curculigoside [4].

### Animals

The animals were purchased from the Laboratory Animal Center of Shanxi Institute of Chinese Medicine (Shanxi, China). Experimental groups consisted of Institute of Cancer Research (ICR) mice (male, 20 ± 2 g) or Sprague-Dawley (SD) rats (220 ± 20 g). Animals were housed at 25 °C under a 12 h light/12 h dark cycle, with free access to standard laboratory chow and water. Each animal was used only once in the experiment. The experimental protocols were approved by the Animal Care and Use Committee of Shanxi Provincial People's Hospital (approval no. Animal-2015-1017#). The animals were handled according to standard protocols for the use of laboratory animals [10].

### Experimental protocol

The anti-depressive effect of curculigoside was studied in mice using forced swimming test (FST), tail suspending test (TST), and open field test (OFT). The levels of dopamine (DA), norepinephrine (NE), and 5-hydroxytryptamine (5-HT) in the brain of chronic mild stress (CMS) rats were measured by HPLC-ECD. Furthermore, the effects of curculigoside on the expression of brain-derived neurotrophic factor (BDNF) protein in the hippocampus of the CMS rats were determined by western blotting.

In these experiments, 50 mice or rats were randomly divided into 5 groups ( $n = 10$ ), including one control, one positive control and three curculigoside testing groups. Based on previous investigations [4], the doses of the curculigoside were 10, 20 and 40 mg/kg. Imipramine (for positive control group) was administered by intraperitoneal (i.p.) injection, while curculigoside was administered orally. The control group was treated with an equivalent volume of the vehicle (DMSO) in which curculigoside was suspended.

### Forced swimming test (FST)

The forced swimming test (FST) was carried out on mice as reported previously [11]. Mice were pre-treated with curculigoside (10, 20, 40 mg/kg, p.o.) 1 h before the FST, and imipramine (15 mg/kg, i.p.) was administered 30 min before the test. The mice were dropped individually into glass cylinders ( $14 \times 14 \times 25$  cm) containing water 20 cm deep at  $25 \pm 2$  °C. Then mice were allowed to swim for 6 min in the water and their immobility durations were recorded during the last 4 min.

### Tail suspension test (TST)

Total duration of immobility induced by tail suspension was determined in mice as described previously [11]. Curculigoside (10, 20, 40 mg/kg, p.o.) was administered 1 h before TST, while imipramine (15 mg/kg, i.p.) was given 30 min before the test. Mice were individually suspended 50 cm above the floor in clear black Plexiglass boxes ( $30 \times 30 \times 45$  cm) by adhesive tape placed approximately 1 cm from the tip of the tail. When the mice hung passively and completely motionless, they were considered immobile. Immobility time was recorded during a 6-min test.

### Evaluation of locomotor activity

This open field test (OFT) was performed to eliminate possible influence of drug treatments on locomotor activity. The test was carried out in clear black Plexiglas boxes ( $41 \times 41 \times 41$  cm) equipped with the video-based Ethovision System (Noldus, Wageningen, The Netherlands). Illumination was identical to that used for FST and TST. Mice were pre-treated with curculigoside (10, 20, 40 mg/kg, p.o.) 1 h prior to the OFT, or imipramine (15 mg/kg, i.p.) 30 min before the test. In the experiment, mice were individually and gently placed in the center of the open-field apparatus and locomotor activity was measured by video for 5 min. This measurement was taken from 9 o'clock in the morning. The

total ambulatory distance and the frequency of rearing were recorded as the horizontal locomotor activity.

### Preparation of chronic mild stress (CMS) model rats

Chronic mild stress (CMS) model was established in rats in line with previous methods [4,12] but with some modifications. The rats were exposed to the following ten stressors for 28 consecutive days: (1) overnight illumination; (2) deprivation of food or water (24 h); (3) restricted access to food (24 h); (4) clip on the tail for 1 min; (5) forced swimming (30 min, water temperature 25 °C); (6) forced swimming (5 min, water temperature 10 °C); (7) soiled cage (200 ml water spilled onto 100 g sawdust bedding); (8) wet bedding for 24 h; (9) white noise (approx. 110 dB); and (10) cage tilt (45°, 24 h). The rats were stimulated with one stressor every day. Each stressor was used two or three times, but the same stressor was not repeated in four days. Rats in the control group were housed without any stressors in a separate room, with free access to food and water. The rats were treated with curculigoside (10, 20, 40 mg/kg, p.o.) or imipramine (15 mg/kg, i.p.) once a day. The control group was treated with an equivalent volume of DMSO once a day at the same time.

### Determination of DA, NE, and 5-HT in the brain of CMS rats

After 4 weeks of stress procedure, all rats were sacrificed by cervical dislocation. The brain of each rat was removed and the hippocampus tissue was isolated, and weighed. DA, NE, and 5-HT were measured by high performance liquid chromatography-electron capture detector (HPLC-ECD).

### Assessment of brain-derived neurotrophic factor (BDNF) expression by western blotting

The hippocampus samples were homogenized, and total proteins were extracted by western blot and IP cell lysis buffer kit. Protein content was quantified with BCA Protein Assay Reagent. Then equal amounts of protein (40 µg) were separated by sodium dodecyl sulfate-polyacrylamide electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF). Subsequently, the membrane was probed with anti-BDNF rabbit polyclonal IgG (1:200) and anti-β-actin rabbit polyclonal IgG (1: 200) at 4 °C for 12 h, followed by incubation with horseradish-peroxidase-conjugated goat anti-rabbit IgG. Finally, the immune-reactive bands were visualized with ECL-detecting reagent.

## Data analysis

All data are presented as mean  $\pm$  standard deviation (SD). Statistical analysis was performed via one-way analysis of variance, followed by Dunnett's test using SPSS software version 19.0, and differences were considered significant at  $p < 0.05$ .

## RESULTS

### Identification of curculigoside

The structure of the isolated chemical compound was identified by  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  as curculigoside in line with previous reports [4,13]. The spectral data and chemical structure of curculigoside are shown in Table 1 and Figure 1 respectively.

### Anti-depression-like effects of curculigoside in FST

The effects of curculigoside on total duration of immobility induced by FST in mice are shown in Fig. 2. Curculigoside (at doses of 10, 20 and 40 mg/kg) significantly decreased the duration of

immobility ( $p < 0.01$ ) in a dose-dependent manner, when compared with the control group. The imipramine at 15 mg/kg, also significantly reduced immobility duration relative to control.

### Effect of curculigoside on depressive-like mice

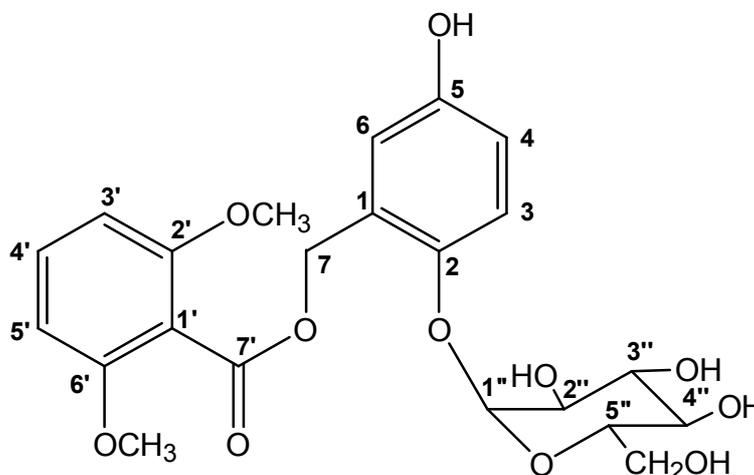
The effects of curculigoside on immobility time were also determined in TST in mice. As shown in Fig. 3, imipramine (15 mg/kg), significantly decreased duration of immobility ( $p < 0.01$ ) when compared to the vehicle-treated control group. Interestingly, curculigoside (10, 20 and 40 mg/kg) also produced significant reduction in immobility time ( $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.01$ , respectively), in a dose-dependent manner.

### Effect of curculigoside on locomotor activity

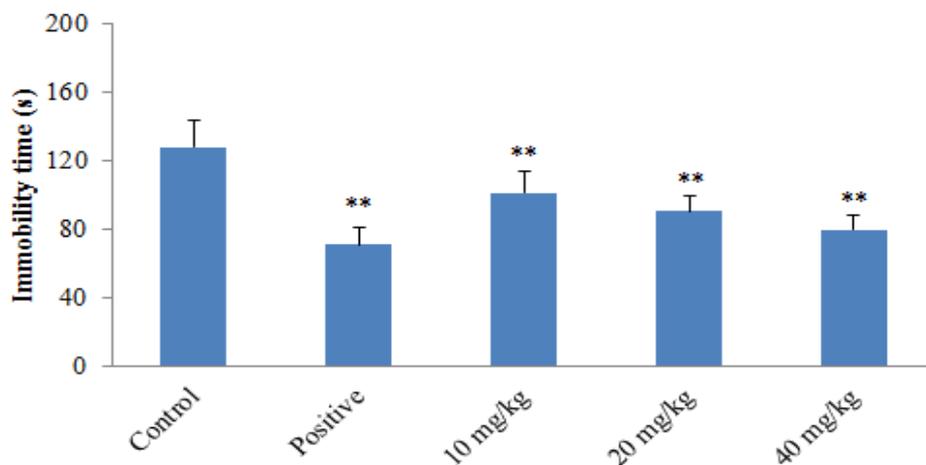
As can be seen from Fig. 4, imipramine (15 mg/kg) significantly increased locomotor activity when compared with the vehicle-treated control group ( $p < 0.01$ ). However, treatment with curculigoside at all the tested doses did not affect mobility performance.

**Table 1:**  $^1\text{H NMR}$  (600 Hz) and  $^{13}\text{C NMR}$  (150 Hz) data of curculigoside in DMSO- $d_6$  ( $\delta$ , ppm)

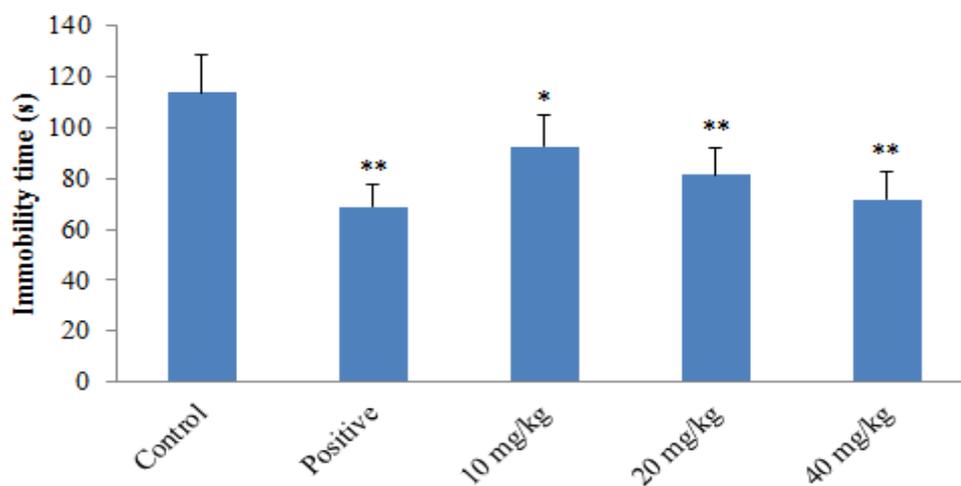
No.	$\delta_{\text{H}}(\text{J})$	$\delta_{\text{C}}$	No.	$\delta_{\text{H}}(\text{J})$	$\delta_{\text{C}}$
1		128.1	5'	6.77 (1H, d, $J = 8.1$ ),	112.9
2		148.2	6'		157.1
3	6.95 (1H, d, $J = 8.7$ )	118.0	7'		167.3
4	6.55 (1H, dd, $J = 3.2, 8.9$ )	115.9	1''	4.68 (1H, d, $J = 7.7$ )	104.1
5		151.9	2''		74.2
6	6.80 (1H, d, $J = 3.2$ )	115.3	3''		78.1
7	5.35 (2H, s)	62.4	4''		69.7
1'		105.3	5''		76.1
2'		157.2	6''		61.3
3'	6.79 (1H, d, $J = 8.1$ ),	115.0	5-OH	9.12 (1H, s)	
4'	7.29 (1H, t, $J = 8.1$ )	136.1	-OCH <sub>3</sub>	3.93 (6H)	57.4



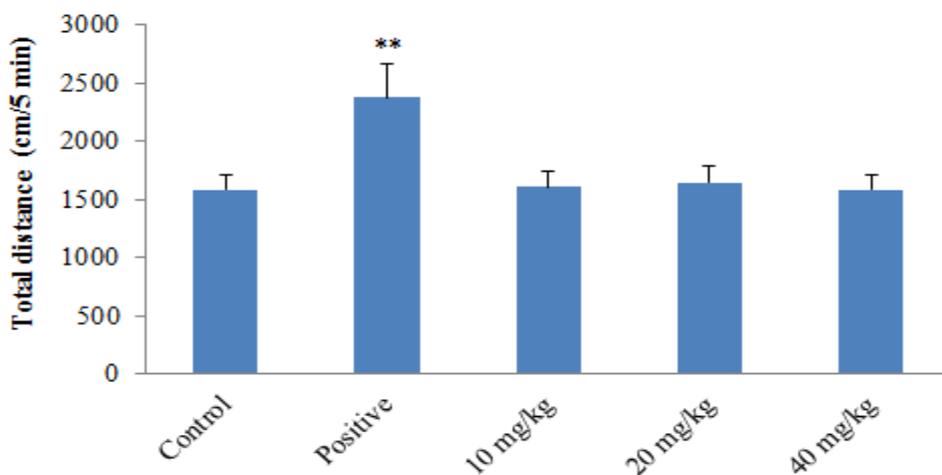
**Figure 1:** Structure of curculigoside



**Figure 2:** Effect of the curculigoside on immobility time in FST experiment. Imipramine (15 mg/kg) was used as reference drug. Data are presented as mean  $\pm$  SD (n = 10). The asterisks indicate significant differences from the control, \* $p$  < 0.05, \*\* $p$  < 0.01



**Figure 3:** Effect of the curculigoside on immobility time in TST. Imipramine (15 mg/kg) was used as standard drug. Data are presented as mean  $\pm$  SD (n = 10). The asterisks indicate significant differences from the control, \* $p$  < 0.05, \*\* $p$  < 0.01



**Figure 4:** Effect of the curculigoside on spontaneous behavior in the OFT experiment. Imipramine (15 mg/kg) was used as reference drug. Data are presented as mean  $\pm$  SD (n = 10). The asterisks indicate significant differences from the control, \*\* $p$  < 0.01.

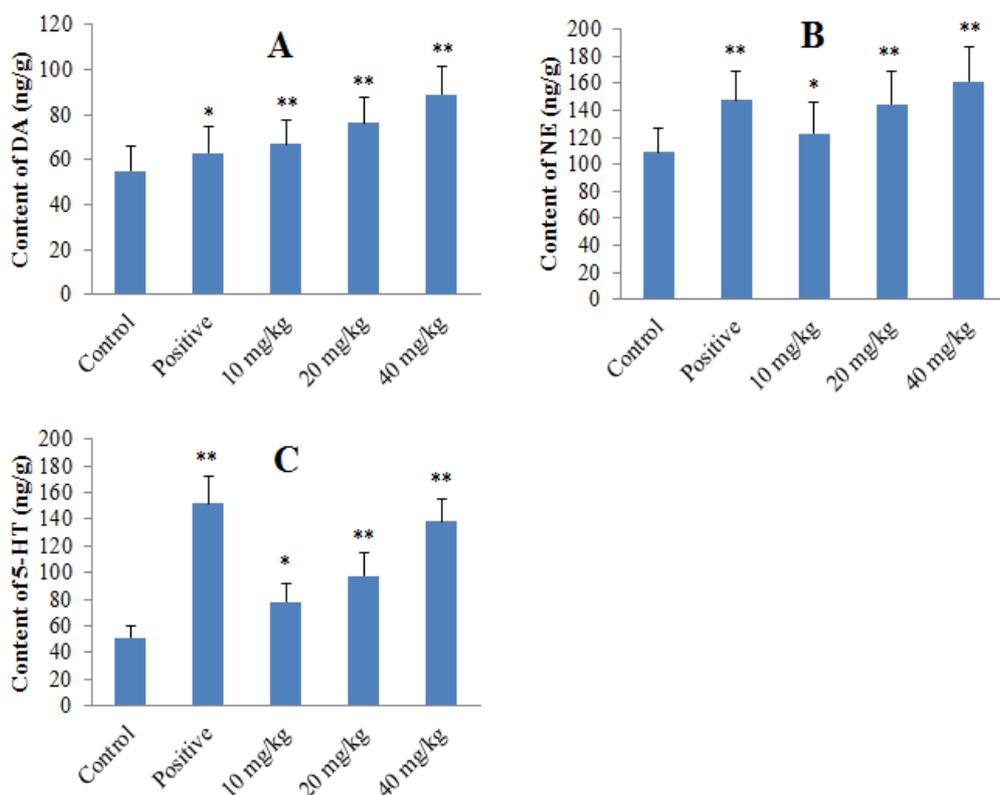
**Effect of curculigoside on levels of DA, NE, and 5-HT in CMS rats**

The effect of curculigoside on the levels of DA, NE, and 5-HT in CMS rats are shown in Fig. 5. The reference drug imipramine (15 mg/kg) significantly increased levels of DA, NE, and 5-HT in CMS rats ( $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.01$ , respectively), when compared with the vehicle-treated control group. Similarly, curculigoside, at doses of 10, 20, 40 mg/kg, also resulted in increased levels of DA in the hippocampus of CMS rats ( $p < 0.01$ ). After treatment with different concentrations of curculigoside, the

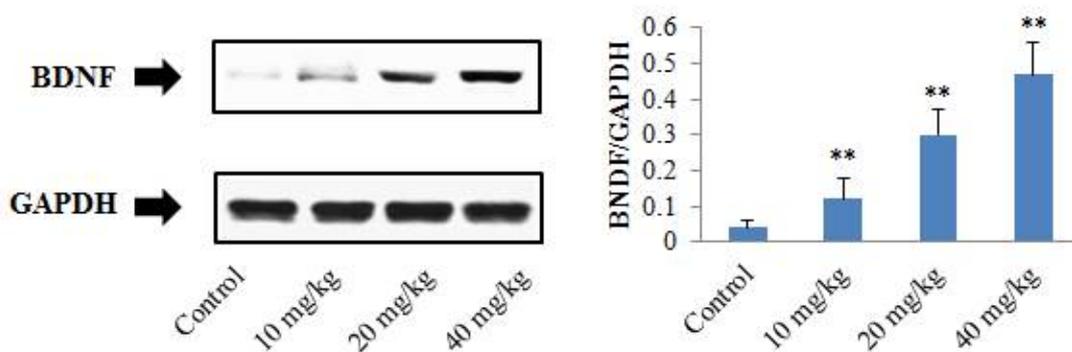
levels of NE and 5-HT were also significantly increased in a dose-dependent manner ( $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.01$ , respectively).

**Effect of curculigoside on the expression of BDNF in CMS rats**

In Fig. 6, it can be observed that curculigoside, at doses of 10, 20, 40mg/kg, significantly up-regulated protein expression levels of BDNF in the hippocampus of CMS rats ( $p < 0.01$ ), in a dose-dependent manner, when compared with the control group.



**Figure 5:** Effect of curculigoside on DA (A), NE (B), and 5-HT (C) levels in the hippocampus of CMS rats. Imipramine (15 mg/kg) was used as reference drug. Data are represented as mean  $\pm$  SD (n = 10). The asterisks indicate significant differences from the control, \* $p < 0.05$ , \*\* $p < 0.01$



**Figure 6:** Effect of the curculigoside on BDNF expression in brain. Data are represented as mean  $\pm$  SD (n = 4). The asterisks indicate significant differences from the control, \*\* $p < 0.01$

## DISCUSSION

In this study, an animal behaviour model for FST, TST and OFT was used to investigate the antidepressant-like activity of curculigoside. FST and TST are preliminary and simple "behavior despair" models which are widely used to screen for anti-depressant drugs [14,15]. The behavior despair test is centered on a rodent's response to the threat of drowning, and its result has been used to determine susceptibility to negative mood.

The results of the present study show that in the FST and TST on mice, treatment with curculigoside significantly decreased immobility time in a dose-dependent manner, which indicates that curculigoside has antidepressant activity in the "behavior despair" test. OFT is used to assess general locomotor activity levels and anxiety in rodents. Curculigoside did not significantly affect locomotor activity of mice in the open-field test, which shows that the treatments given had no effect on mobility performance, and further demonstrates that curculigoside has antidepressant activity in mice. Elevations in the brain levels of DA, NE, and 5-HT in the brain have been reported to be effective in the treatment of depression [16]. DA, NE, and 5-HT are neurotransmitters and neuro-modulators. Many anti-depressant drugs elevate these neurotransmitters. In the present investigation, treatment with curculigoside significantly enhanced the levels of DA, NE, and 5-HT in the hippocampus of CMS rats, which indicates that the antidepressant activity of curculigoside relates to increases in levels of neurotransmitters.

BDNF is a secretory protein of the neurotrophin family which has been reported to be implicated in depression [17]. A previous study has reported that knock-out of BDNF in the dentate gyrus produced a depression-like response [18]. In addition, it has been reported that depressed patients often manifest decreased serum BDNF levels [19]. In the present study, treatment with curculigoside up-regulated expression levels of BDNF in the hippocampus of CMS rats, which suggests that the antidepressant-like effect of curculigoside is associated with boosting BDNF levels.

## CONCLUSION

This study demonstrates that curculigoside possesses significant antidepressant-like activity, which mechanisms most likely involve increases in the levels of DA, NE, 5-HT as well as enhanced expression of BDNF. Thus,

curculigoside has a potential as a new drug candidate for the treatment of depressive disorders.

## DECLARATIONS

### Conflict of Interest

No conflict of interest associated with this work.

### Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

## REFERENCES

- Grosso G, Micek A, Pajak A, Castellano S, Galvano F. Coffee, tea, caffeine and risk of depression: a systematic review and dose-response meta-analysis of observational studies. *Mol Nutr Food Res* 2016; 60: 223-234.
- Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *Plos med* 2006; 3(11): 442.
- Wei YB, Melas PA, Wegener G, Mathé AA, Lavebratt C. Antidepressant-like effect of sodium butyrate is associated with an increase in TET1 and in 5-hydroxymethylation levels in the Bdnf gene. *Int J Neuropsychoph* 2015; 18(2): 1-10.
- Wu X-Y, Li J-Z, Guo J-Z, Hou B-Y. Ameliorative effects of curculigoside from *Curculigo orchioides* Gaertn on learning and memory in aged rats. *Molecules* 2012; 17(9): 10108-10118.
- Nie Y, Dong X, He Y, Yuan T, Han T, Rahman K, Qin L, Zhang Q. Medicinal plants of genus *Curculigo*: traditional uses and a phytochemical and ethnopharmacological review. *J Ethnopharmacol* 2013; 147(3): 547-563.
- Wang YK, Hong YJ, Wei M, Wu Y, Huang ZQ, Chen RZ, Chen HZ. Curculigoside attenuates human umbilical vein endothelial cell injury induced by H<sub>2</sub>O<sub>2</sub>. *J Ethnopharmacol* 2010; 132(1): 233-239.
- Lakshmi V, Pandey K, Puri A, Saxena R, Saxena K. Immunostimulant principles from *Curculigo orchioides*. *J Ethnopharmacol* 2003; 89(2): 181-184.
- Wang Y, Zhao L, Wang Y, Xu J, Nie Y, Guo Y, Tong Y, Qin L, Zhang Q. Curculigoside isolated from *Curculigo orchioides* prevents hydrogen peroxide-induced dysfunction and oxidative damage in calvarial osteoblasts. *ACTABioch Bioph Sin* 2012: 014.
- Tian Z, Yu W, Liu H-b, Zhang N, Li X-b, Zhao M-g, Liu S-b. Neuroprotective effects of curculigoside against NMDA-induced neuronal excitotoxicity in vitro. *Food Chem Toxicol* 2012; 50(11): 4010-4015.

10. National Institute of Health, USA. Public health service policy on humane care and use of laboratory animals; 2002.
11. Duan D, Chen L, Yang X, Tu Y, Jiao S. Antidepressant-like effect of essential oil isolated from *Toona ciliata* Roem. var. *yunnanensis*. *J Nat Med* 2015; 69(2): 191-197.
12. An L, Zhang Y-Z, Yu N-J, Liu X-M, Zhao N, Yuan L, Li Y-F. Role for serotonin in the antidepressant-like effect of a flavonoid extract of *Xiaobuxin-Tang*. *Pharmacol Biochem Be* 2008; 89(4): 572-580.
13. Fu, D.X.; Lei, G.Q.; Cheng, X.W.; Chen, J.K.; Zhou, T.S. Curculigoside C, a new phenolicglucoside from Rhizomes of *Curculigoorchioides*. *Acta Bot Sin* 2004; 4: 621–624.
14. Lucki I. The forced swimming test as a model for core and component behavioral effects of antidepressant drugs. *Behav Pharmacol* 1997; 8(6-7): 523-532.
15. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology* 1985; 85(3): 367-370.
16. Koch S, Perry KW, Nelson DL, Conway RG, Threlkeld PG, Bymaster FP. R-fluoxetine increases extracellular DA, NE, as well as 5-HT in rat prefrontal cortex and hypothalamus: an *in vivo* microdialysis and receptor binding study. *Neuropsychopharmacology* 2002; 27(6): 949-959.
17. Martinowich K, Manji H, Lu B. New insights into BDNF function in depression and anxiety. *Nat Neurosci* 2007; 10(9): 1089-1093.
18. Taliaz D, Stall N, Dar D, Zangen A. Knockdown of brain-derived neurotrophic factor in specific brain sites precipitates behaviors associated with depression and reduces neurogenesis. *Mol Psychiatr* 2010; 15(1): 80-92.
19. Castrén E, Rantamäki T. The role of BDNF and its receptors in depression and antidepressant drug action: reactivation of developmental plasticity. *Dev Neurobiol* 2010; 70(5): 289-297.