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Original Research Article

Curcumin evokes antidepressant-like effects in mice by regulating miR-124/brain derived neurotrophic factor

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Abstract

Purpose: *To investigate the effect and mechanism of curcumin on depression in mice*
Methods: *Mice were subjected to chronic unpredictable mild stress (CUMS), and behavioural changes were evaluated by sucrose preference test (SPT) and forced swimming test (FST). CUMS-treated mice received curcumin at a concentration of 50, 100, or 200 mg/kg. The level of MiR-124 was measured by real-time polymerase chain reaction (RT-PCR). Brain-derived neurotrophic factor (BDNF) levels were evaluated by western blotting.*
Results: *CUMS induced depressive behaviour in mice, with increase in miR-124 and decrease in BDNF. Curcumin inhibited miR-124 expression and promoted BDNF in a dose-dependent manner in CUMS-treated mice. Brain-derived neurotrophic factor was the direct target of miR-124, decreasing the transcription of BDNF, but this was reversed by curcumin in vitro. MicroRNA-124 overexpression aggravated CUMS-induced depressive symptoms including loss of appetite, less sucrose consumption, shorter swimming time, and longer immobility time ($p < 0.001$). The effects were attenuated by curcumin.*
Conclusion: *Curcumin alleviates CUMS-induced depressive behaviour by regulating miR-124/BDNF, suggesting that curcumin may a viable treatment option for depression.*

Keywords: *Curcumin, MiR-124, Brain-derived neurotrophic factor, Depression, Chronic unpredictable mild stress (CUMS)*

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INTRODUCTION

Depression is a common psychiatric disorder characterised by emotional or physical problems such as low mood, loss of memory, hopelessness, and insomnia [1]. Stressful life incidents may increase morbidity or the possibility of depression. Evidence from rodent stress models indicates that chronic unpredictable mild stress (CUMS) is a useful method for induction of depression and anxiety behaviours [2]. There are many hypotheses about the pathogenesis of depression. The neurotrophic hypothesis holds that neurotrophic factors, especially brain-derived neurotrophic factor (BDNF), are critical for neuronal survival and synaptic plasticity and are involved in the development of depression [3].

MiRNAs are 20 to 25-nucleotide long non-coding regulatory RNAs that affect diverse biological processes by regulating mRNA translation or gene expression [4]. miRNAs are abundant in the brain and play vital roles in many physiological processes[5]. Many studies have reported that miRNA dysregulation affects depression course in patients with major depressive disorder (MDD) patients, as well as in animal models [6,7]. Furthermore, miR-124 is abundant in the mouse brain [8]. In a clinical study, miR-124 levels in peripheral blood mononuclear cells (PBMCs) in MDD patients were significantly higher than in healthy people. After treatment, the level of miR-124 in patients decreased markedly [9], which suggesting that miR-124 plays a vital function in the process of MDD.

Conventional antidepressants such as those that affect 5-hydroxytryptamine [5-HT] signalling may not fully resolve the psychiatric condition. Clinical and experimental research indicate that natural products have good therapeutic effects on stress-induced depression [10]. Curcumin (CUR), the main component isolated from *Curcuma longa* L, influences several biomarkers in patients with depression [11] and exerts antidepressant-like effects in an animal model of depression associated with BDNF [12]. One study reported that BDNF was a target gene of miR-124, and hippocampal miR-124 disorder may induce psychiatric illnesses through its target gene, *BDNF* [13]. The aim of this study was to investigate whether curcumin exerts an antidepressant effect by regulating miR-124/BDNF.

EXPERIMENTAL

Animals and treatment

Male C57BL/6 mice, purchased from the Beijing Vital River Laboratory Animal Company, were acclimated for 1 week (light/dark cycle, 22 °C, humidity controlled). All experiments were in accordance with the Guide for the Care and Use of Laboratory Animals [14] and were approved by the Animal Ethics Committee of First Affiliated Hospital of WenZhou Medical University (approval no. 2017-211). Curcumin was purchased from Sigma-Aldrich (St. Louis, MO, USA). The mice were randomly divided into five groups: control, stress (CUMS), CUMS + curcumin (50 mg/kg), CUMS + curcumin (100 mg/kg), and CUMS + curcumin (200 mg/kg). Curcumin was administered daily for 5weeks before exposure to CUMS. The CUMS protocol was performed as previously described [15] with a few modifications. Each day for 5 weeks, mice were exposed to different stressors such as food or water deprivation, tail pinching, continuous lighting overnight, cage tilting (30°), hot (45 °C) or cold (5 °C) water exposure, or soiled cage bedding.

Forced swimming test (FST)

The protocol was performed as previously reported [16]. Each mouse was placed in a water-filled cylinder (diameter, 20 cm and height, 50 cm). The mice were not able to touch the bottom of the container during the test. The test sessions were video-taped and analysed by two independent observers. The mouse was considered depressive when it stopped the active behaviour (swimming) and floated instead. The amounts of time spent swimming and immobile were recorded.

Sucrose preference test (SPT)

The protocol was performed as previously described [17]. Two bottles (1% sucrose [w/v]or water) were placed on each cage. Water and sucrose solution intakes were measured by weighing the bottles. The positions of the bottles were exchanged every 24 h to avoid side bias.

Cell culture

The 293T cell line was purchased from American Type Culture Collection (Manassas, VA, USA) and cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% (v/v) foetal bovine serum (FBS).

Cell transfection

Antisense miR-124 and non-specific miRNA were transfected into 293T cells with Lipofectamine 2000 transfection reagent (Invitrogen, Carlsbad, CA, USA). Cells were collected 36 h after transfection.

Luciferase reporter assay

The 3′-UTR of *BDNF* containing miR-124 binding sites was cloned downstream of the luciferase gene in the pGL-3 vector (Promega, Madison, WI, USA). Cells were transfected with different miRNAs and analysed using Dual-Luciferase reporter assay system (Promega) after 48 h.

Western blotting

The tissues or cells were lysed in RIPA buffer and proteins were quantified using the bicinchoninic acid method. Equal amounts of protein were separated on 10% SDS-PAGE and transferred onto PVDF membrane (Millipore,

Burlington, MA, USA). After blocking, the membranes were incubated with BDNF antibodies (#3897, Cell Signaling Technology, Danvers, MA, USA) at a dilution of 1:1000. The protein bands were detected using an imaging machine and enhanced chemiluminescence (ECL) system according to the manufacturer's instructions and quantified with ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Real-time polymerase chain reaction (RT-PCR)

Total RNA was extracted with TRIzol Reagent and reverse-transcribed to cDNA using the GoScript™ Reverse Transcription System according to the manufacturer's instructions. Quantitative RT-PCR was performed using SYBR Green PCR Master Mix reagent kits (Promega).

Statistical analysis

Data were analysed by one-way analysis of variance (ANOVA) with Tukey's tests using SPSS statistical software (SPSS, Inc., Chicago, IL, USA) and are presented as mean ±standard deviation (SD).

RESULTS

Confirmation of CUMS-induced depression

The FST, SPT, and food latency tests were performed to measure depressive behaviours and the results are shown in Figure 1 A. CUMS-treated mice exhibited latency to food, lower preference for sucrose consumption, marked decrease in swimming time, and increased immobility time ($p < 0.001$) when compared with control animals. Collectively, the behavioural results suggest that the CUMS protocol successfully induced depression in the mice.

Hippocampal BDNF and miR-124 levels in CUMS-treated mice

To investigate the mechanisms of CUMS-induced depression, level of miR-124 (Figure 1 B) were measured by qRT-PCR and BDNF (Figure 1 C) was measured by western blotting. Over time, the miR-124 level in the CUMS group increased, while that of BDNF decreased, which suggest that CUMS-induced depression was partly regulated by miR-124/BDNF.

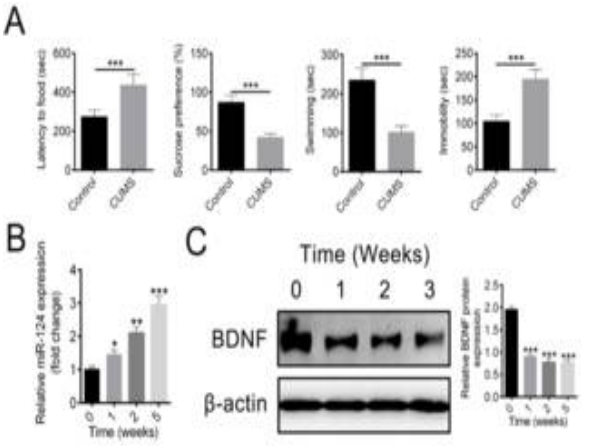

Figure 1: miR-124/BDNF levels in CUMS-induced depression. (A) Behaviour tests in animals with CUMS-induced depression. (B) Hippocampal miR-124 levels measured by qRT-PCR. (C) Hippocampal BDNF expression measured by western blotting;*p< 0.05, ** p< 0.01, *** p< 0.001

Effect of curcumin on miR-124 and BDNF levels

CUMS-treated mice were treated with different concentrations of curcumin (0, 50, 100, 200 mg/kg). As shown in Figure 2, curcumin significantly decreased hippocampal miR-124 and increased BDNF expression.

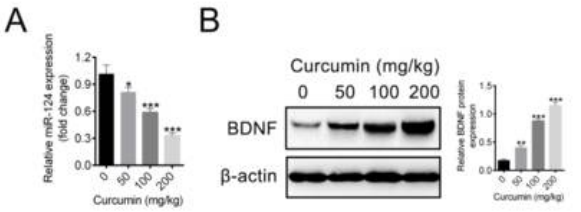

Figure 2: Effect of curcumin on miR-124 level by RT-PCR (A) and BDNF level by western blotting (B);*p< 0.05, ** p< 0.01, *** p< 0.001

Curcumin regulates BDNF expression through miR-124

The target genes of miR-124 were predicted using the bioinformatic databases (TargetScan). As shown in Figure 3 A, the complementary sequence of miR-124 was found in the 3′-UTR of the BDNF mRNA sequence. To determine if miR-124 regulates BDNF expression, 293T cells were transfected with an miR-124 plasmid, and 3′-UTR luciferase activity of BDNF was measured. The transfection efficiency of miR-124 mimics were analysed by RT-PCR, and the results indicated that the miR-124 level was increased in transfected 293T cells when compared with control. Importantly, miR-124 overexpression

reduced BDNF transcription when compared with control (Figure 3 C).

Curcumin alone increased BDNF expression when compared with mimic negative control (NC-mimic) (Figure 3 D). The miR-124 decreased the transcription of *BDNF*, but was inhibited by curcumin, which demonstrated that curcumin could increase *BDNF* transcription by regulating miR-124. Western blotting confirmed that protein levels of BDNF (Figure 3 E) were in accordance with mRNA levels measured by RT-PCR.

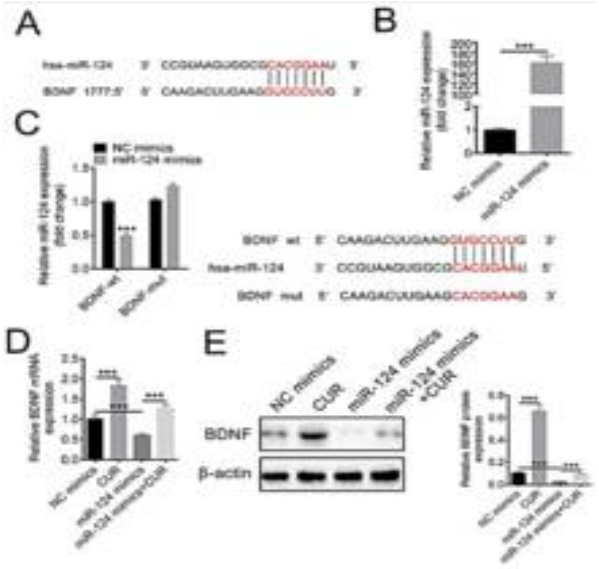

Figure 3: Curcumin regulated expression of BDNF through miR-124.A. Complementary sequence of miR-124 in the *BDNF* 3′-UTR regions. B. luciferase activities of miR-124-transfected 293T cells. All experiments were performed in triplicate. C. *BDNF* expression of miR-124 mimic-transfected 293T cells analysed by RT-PCR. D. *BDNF* transcription of normal or miR-124-overexpressing 293T cells and the effects of curcumin measured by RT-PCR. E. BDNF protein levels in normal or miR-124-overexpressing 293T cells and the effects of curcumin measured by western blotting; *** $p< 0.001$

Up-regulation of miR-124 aggravates CUMS-induced depressive behaviours

To confirm the effects of miR-124 on CUMS-induced depression *in vivo*, adenoviruses (with miR-124 overexpression or negative control) were transfected into normal or CUMS-treated mice. The transfection efficiencies were analysed by RT-PCR, and the results revealed significantly increased miR-124 levels in adenovirus-treated mice (Ad-miR-124) in both the control and CUMS groups (Ad-miR) as shown in Figure 4 A. Detailed behavioural changes are shown in Figure 4 B. For wild-type mice (control) with miR-124 overexpression, the latency to food increased and swimming time decreased. There were no significant differences in sucrose preference or immobility time. In CUMS-treated mice with miR-124 overexpression, there were changes in all four measures latency to food, sucrose preference, swimming time and immobility time changes. Animals treated with plus miR-124 overexpression displayed latency to food, reduced sucrose consumption, decreased swimming time, and increased immobility time ($p < 0.001$) when compared with CUMS treatment alone. The results demonstrate that up-regulation of miR-124 aggravates CUMS-induced depression.

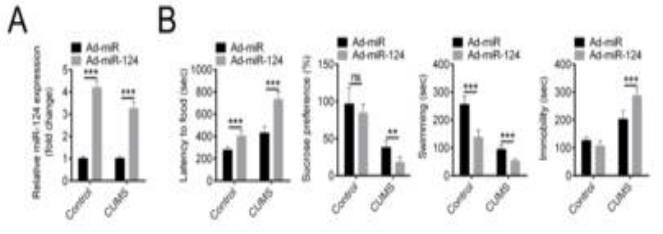

Figure 4: miR-124 overexpression aggravates CUMS-induced depression. A. miR-124 expression in normal or CUMS-treated mice transfected with adenovirus (overexpressed miR-124). B. Behaviour changes (latency to food, sucrose preference, Swimming time and immobility time) of different groups;**$p< 0.01$, ***$p< 0.001$

Curcumin exerts anti-depressive effects by regulating miR-124/BDNF

To confirm the effects of curcumin on CUMS-induced depression and miR-124/BDNF, the CUMS-treated mice was transfected with miR-124 adenovirus. As shown in Figure 5 A, miR-124 adenovirus transfection induced miR-124 overexpression in CUMS-treated mice (miR-124 mimics vs. CUMS) and was inhibited by curcumin (miR-124 mimics + CUR vs. miR-124 mimics). Furthermore, curcumin alone inhibited miR-124 expression in CUMS mice (CUR vs. CUMS). The pattern of BDNF expression was opposite that of miR-124 (Figure 5 B). The behaviour changes in the four groups are shown in Figure 5 C. MiR-124 overexpression enhanced CUMS-induced depressive behaviours (miR-124 mimics vs. CUMS), including latency to food, less sucrose consumption, shorter swimming time, and longer immobility time. Curcumin treatment significantly alleviated depressive behaviours (miR-124 mimics + CUR vs. miR-124 mimics). Furthermore, curcumin alone alleviates depressive symptoms in CUMS mice (CUR vs. CUMS). The results indicate that curcumin exerts anti-depressive effects by regulating miR-124 /BDNF.

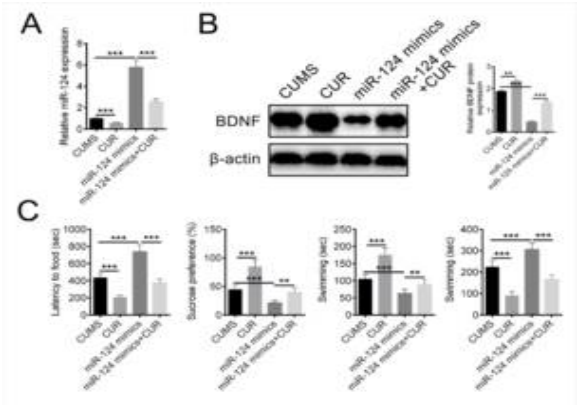

Figure 5: Curcumin exerts anti-depressive effects by regulating miR-124 /BDNF. (A)RT-PCR results of miR-124 expression in normal, CUMS-treated, and curcumin-treated mice transfected with adenovirus to overexpress miR-124. (B)Western blots of BDNF expression in normal, CUMS-treated, or curcumin-treated mice transfected with adenovirus to overexpress miR-124. (C) Behaviour changes (latency to food, sucrose preference, swimming time, and immobility time) in each group; **$p< 0.01$, ***$p< 0.001$

DISCUSSION

Depression is a common mental disorder characterised by symptoms including sadness, loss of interest and pleasure, low self-esteem, and sleep disturbances. Many factors such as genetics, environmental factors, monoamine deficiency, immunologic factors, and neurogenesis may contribute to the occurrence of depression. No single hypothesis could describe the complex pathophysiologic mechanisms of depression [18]. The outcomes of standard clinical treatment with pharmacotherapy or psychotherapy are highly variable, can be expensive, and may have adverse effects [19]. Herbal medicine or natural product extracts given as complementary or alternative medicine, such as resveratrol[20] and curcumin [11], have been effective treatments for depression.

The results demonstrate that curcumin alleviated depressive behaviours induced by CUMS via regulating miR-124/BDNF. Accumulating evidence suggests that curcumin affects many physiopathologic processes by regulating numerous molecular targets including transcription factors, cytokines, and enzymes [21]. Previous studies reported that curcumin exerts antidepressant effects in both patients with major depression [11] and experimental animal models [22]. Thus, these results agree with those of others.

MiRNAs are small non-coding regulatory RNAs that are abundant in the brain; they affect diverse brain biological processes and could be therapeutic targets for depression [23]. MiR-124-3p expression is highly dysregulated in stressed rats and MDD pathophysiology [24]. Selective inhibition ofmiR-124 in the hippocampus has anti-depressive effects in rats [25]. In the present study, CUMS induced depressive behaviours and reduced miR-124 levels. Curcumin attenuated CUMS-induced depressive behaviours and increased miR-124 levels, indicating that curcumin could improve depression partly via targeting miR-124. Researchers also reported that miR-124 directly targets BDNF in hippocampal areas, which contributes to depressive behaviours in the development of autism spectrum disorders [13].

Brain derived neurotrophic factor (BDNF), a neurotrophin highly expressed in the hippocampus, regulates neuronal proliferation, differentiation, and survival [26]. According to 'neurotrophic hypothesis', it is involved in the pathophysiology of depression [27]. Clinical research indicates that antidepressants increase hippocampal BDNF expression [28]. The concentrations of BDNF and pro-BDNF (a precursor protein) in human blood serves as diagnostic biomarkers for MDD [29]. Hurley *et al* found that chronic curcumin administration reduced immobility time and increased hippocampal BDNF expression in Wistar Kyoto rats (a model of depression) [12]. These findings confirm previous research that CUMS led to depressive behaviours and reduced BDNF, while curcumin attenuated depressive symptoms by regulating miR-124/BDNF *in vitro* and *in vivo*.

CONCLUSION

Curcumin alleviates CUMS-induced depressive behaviour by regulating miR-124/BDNF. These results indicate that miR-124 is a potential target for treating neurological disorders, while curcumin is a viable treatment option for depression.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We 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 the authors. Yanghong Yi and Jing Li designed all the experiments and revised the paper. Weian Chen performed the

experiments, Yanhong Yi wrote the paper and Jing Li final approval.

REFERENCES

1. Zhao J, Qi XR, Gao SF, Lu J, van Wamelen DJ, Kamphuis W, Bao AM, Swaab DF. Different stress-related gene expression in depression and suicide. J Psychiatr Res 2015; 68: 176-185.
2. Ayuob NN, Firgany AEL, El-Mansy AA, Ali S. Can Ocimum basilicum relieve chronic unpredictable mild stress-induced depression in mice? Exper Molecular Pathol 2017; 103(2): 153-161.
3. Angelucci F, Brene S, Mathe AA. BDNF in schizophrenia, depression and corresponding animal models. Mol Psychiatry. 2005; 10(4): 345-352.
4. Flynt AS, Lai EC. Biological principles of microRNA-mediated regulation: shared themes amid diversity. Nat Rev Genet 2008; 9(11): 831-842.
5. Kosik KS. The neuronal microRNA system. Nat Rev Neurosci 2006; 7(12): 911-920.
6. Cao MQ, Chen DH, Zhang CH, Wu ZZ. Screening of specific microRNA in hippocampus of depression model rats and intervention effect of Chaihu Shugan San. Zhongguo Zhong Yao Za Zhi 2013; 38(10): 1585-1589.
7. Fan HM, Sun XY, Guo W, Zhong AF, Niu W, Zhao L, Dai YH, Guo ZM, Zhang LY, Lu J. Differential expression of microRNA in peripheral blood mononuclear cells as specific biomarker for major depressive disorder patients. J Psychia Res 2014; 59: 45-52.
8. Lagos-Quintana M, Rauhut R, Yalcin A, Meyer J, Lendeckel W, Tuschl T. Identification of tissue-specific microRNAs from mouse. Curr Biol 2002; 12(9): 735-739.
9. He S, Liu X, Jiang K, Peng D, Hong W, Fang Y, Qian Y, Yu S, Li H. Alterations of microRNA-124 expression in peripheral blood mononuclear cells in pre- and post-treatment patients with major depressive disorder. J Psychiatr Res 2016; 78: 65-71.
10. Xu Y, Zhang C, Wu F, Xu X, Wang G, Lin M, Yu Y, An Y, Pan J. Piperine potentiates the effects of trans-resveratrol on stress-induced depressive-like behavior: involvement of monoaminergic system and cAMP-dependent pathway. Metab Brain Dis 2016; 31(4): 837-848.
11. Lopresti AL, Maes M, Meddens MJ, Maker GL, Arnoldussen E, Drummond PD. Curcumin and major depression: a randomised, double-blind, placebo-controlled trial investigating the potential of peripheral biomarkers to predict treatment response and antidepressant mechanisms of change. Eur Neuropsychopharmacol 2015; 25(1): 38-50.
12. Hurley LL, Akinfiresoye L, Nwulia E, Kamiya A, Kulkarni AA, Tizabi Y. Antidepressant-like effects of curcumin in WKY rat model of depression is associated with an increase in hippocampal BDNF. Behav Brain Res 2013; 239: 27-30.
13. Bahi A. Hippocampal BDNF overexpression or microR124a silencing reduces anxiety- and autism-like behaviors in rats. Behav Brain Res 2017; 326: 281-290.
14. Council NR: Guide for the Care and Use of Laboratory Animals: National Academies Press; 2010.
15. Bhatt S, Shukla P, Raval J, Goswami S. Role of Aspirin and Dexamethasone against Experimentally Induced Depression in Rats. Basic Clin Pharmacol Toxicol 2016; 119(1): 10-18.
16. Porsolt RD, Bertin A, Jalfre M. Behavioral despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn Ther 1977; 229(2): 327-336.
17. Willner P, Towell A, Sampson D, Sophokleous S, Muscat R. Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. Psychopharmacol (Berl) 1987; 93(3): 358-364.
18. Thompson SM, Kallarackal AJ, Kvarta MD, Van Dyke AM, LeGates TA, Cai X. An excitatory synapse hypothesis of depression. Trends Neurosci 2015; 38(5): 279-294.
19. Gartlehner G, Hansen RA, Morgan LC, Thaler K, Lux L, Van Noord M, Mager U, Thieda P, Gaynes BN, Wilkins T et al. Comparative benefits and harms of second-generation antidepressants for treating major depressive disorder: an updated meta-analysis. Ann Intern Med 2011; 155(11): 772-785.
20. Shen J, Xu L, Qu C, Sun H, Zhang J. Resveratrol prevents cognitive deficits induced by chronic unpredictable mild stress: Sirt1/miR-134 signalling pathway regulates CREB/BDNF expression in hippocampus in vivo and in vitro. Behav Brain Res 2018; 349: 1-7.
21. Goel A, Kunnumakkara AB, Aggarwal BB. Curcumin as "Curecumin": From kitchen to clinic. Biochem Pharmacol 2008; 75(4): 787-809.
22. Li YC, Wang FM, Pan Y, Qiang LQ, Cheng G, Zhang WY, Kong LD. Antidepressant-like effects of curcumin on serotonergic receptor-coupled AC-cAMP pathway in chronic unpredictable mild stress of rats. Prog Neuropsychopharmacol Biol Psychia 2009; 33(3): 435-449.
23. Brites D, Fernandes A. Neuroinflammation and Depression: Microglia Activation, Extracellular Microvesicles and microRNA Dysregulation. Front Cell Neurosci 2015; 9(476).
24. B R, M D, C SR. Identification of microRNA-124-3p as a Putative Epigenetic Signature of Major Depressive Disorder. Neuropsychopharmacol 2017; 42(4): 864-875.
25. Bahi A, Chandrasekar V, Dreyer JL. Selective lentiviral-mediated suppression of microRNA124a in the hippocampus evokes antidepressants-like effects in rats. Psychoneuroendocrinol 2014; 46: 78-87.
26. C H, M H, A BY. BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. Nature 1991; 350(6315): 230-232.

27. Duman RS. Role of neurotrophic factors in the etiology and treatment of mood disorders. Neuromolecul Med 2004; 5(1): 11-25.
28. Lam P, Cheng CY, Hong CJ, Tsai SJ. Association study of a brain-derived neurotrophic factor (Val66Met) genetic polymorphism and panic disorder. Neuropsychobiol 2004; 49(4): 178-181.
29. K H. Brain-derived neurotrophic factor (BDNF) and its precursor proBDNF as diagnostic biomarkers for major depressive disorder and bipolar disorder. European Archiv Psychiatr Clin Neurosci 2015, 265(1):83-84. 2015; 265(1): 83-84.