Molecular mechanism underlying antidepressant activity of curcumin

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Abstract

Curcumin, a yellow pigment extracted from rhizomes of the plant *Curcuma longa*, has been widely used as food additive and also as herbal medicine throughout Asia. It has been used to effectively manage mental stress and depression-related disorder. Unpredictable chronic mild stress (UCMS) has long been used as a model of depression. Antidepressant agents can reverse most effects of UCMS. We hypothesized that curcumin may alleviate stress induced depression. Thus in present study we assessed whether curcumin treatment affect behavior in UCMS treated ICR mice. Mice were exposed to UCMS for 5 weeks and anhedonia was evaluated by weekly monitoring of sucrose consumption. curcumin (10 and 20 mg/kg/day, i.p.) or imipramine (20 mg/kg/day, i.p.) or vehicle were continuously administered the last two weeks of UCMS. Behavioral tests were performed over the last week of UCMS. We found that subjecting animals to the UCMS protocol resulted in decreasing sucrose intake and increasing immobility time in tail suspension test (TST), the tests have been used to detect helpless behavior. The results showed that cucrucmin 20 mg/kg and imipramine 20 mg/kg significantly decrease the immobility time in both test. Only imipramine exhibited the decreasing in locomotor activity in open field test. In addition, the anxiety test was performed in this study to determine the effect of curcumin in anxiolytic activity. In light/dark preferences test, the percentage of time reduction in dark zone and induction time in light zone of mice were treated with curcumin and imipramine compared to the stressed-control mice. Curcumin and imipramine treatment increased the spending time in open arm and decreased the spending time in close arm in elevated plus maze test. Moreover, we also found that UCMS procedure decreased the BDNF mRNA expression in the frontal cortex when compare with non stress mice and curcumin and imipramine treatment significantly reverse the effect of UCMS by increasing the BDNF mRNA expression. There are many studies reported that antidepressant treatment can block the UCMS-induced down regulate BDNF mRNA expression. These results provide compelling evidence that the behavioral effects of curcumin in chronically stressed animals may be related to their modulating effects on other organ. In addition some evidence has hypothesis that, curcumin has an property of MAO inhibitor that was effect on Catecholamine neurotransmitter(6) such as Serotonin, Epinephrine, Dopamine etc. these are regulate

the function of mood. Furthermore, curcumin treatment exhibited the antidepressant activity via increasing the BDNF mRNA expression in frontal cortex similar to imipramine, a reference antidepressant. Although BDNF gene we have selected in this study but many gene are relevant on the depression not only BDNF gene and also should be study further gene expression on the other organs.

Keywords; Curcumin, unpredictable chronic mild stress, antidepressant, BDNF

Introduction

A Major Depression disorder (MDD) manifests with the symptoms at the psychological, behavioral and physiological levels. An episoderequires the presence for at least 2 weeks of one or two core symptoms: dysphoricmood and anhedonia (a loss of interest or pleasure in activities that usually would beenjoyed). In addition, four of the following symptoms must be present (three if bothcore symptoms are present): disturbances of sleep, feelings of worthlessness or guilt,inability to concentrate or think, increased or decreased psychomotor activity,decreased sexual drive, appetite disturbance or weight change and suicidal thoughts.

A Major Depression disorder preference is increasing. The World Health Organization estimated that in year 2020 this disorder will become the second largest health problem in human inferior to heart disease. There are three groups of antidepressant that are tricyclic antidepressants (TCAs), Monoamine oxidase inhibitors (MAOIs), both of TCA and MAOI are the first of antidepressant shown their ability facilitated to noradrenergic and/or serotonergic neurotransmission, which correlated with behavioral excitation. But in the mid-1970s, the third group of antidepressant was found by related to serotonin, serotonin reuptake inhibitors (SSRIs). Despite the last group is commonly prescribed in the present, there are many undesirable adverse effects of the antidepressants. Some of the various side effect from the different antidepressants are dry mouth, urinary retention, blurred vision, constipation, sedation (can interfere with driving or operating machinery), sleep disruption, weight gain, headache, nausea, gastrointestinal disturbance/diarrhea, abdominal pain, inability to achieve an erection, inability to achieve an orgasm (men and women), loss of libido, agitation, anxiety etc [Woelk, H. (2000)]. Thus the herbal medicine is the major sources of drug, which may relate to depression therapy.

Figure 1: The yellow pigments of curcumin that extracted from *Curcuma longa*

Curcumin, a yellow pigment extracted from rhizomes of the plant *Curcuma longa*, has been widely used as food additive and also as herbal medicine throughout Asia. In China, curcumin is a major constituent of Xiaoyao-san, the traditional Chinese medicine. This regimen has been used to effectively manage mental stress and

depression-related disorder.

Unpredictable chronic mild stress (UCMS) has long been used as a model of depression. In the UCMS model, rats or mice are exposed sequentially, over a period of weeks, to a variety of mild stressors, and the measure most commonly used to track the effects is a decrease in consumption of a palatable sweet solution. The model has good predictive validity (behavioral changes are reversed by chronic treatment with a wide variety of antidepressants), face validity (almost all demonstrable symptoms of depression have been demonstrated), and construct validity (UCMS causes a generalized decrease in responsiveness to rewards, comparable to anhedonia, the core symptom of the melancholic subtype of major depressive disorder)[(Willner,1997)]. Animal models are indispensable in clarifying the pathophysiology that underlies depression, depression–cognition interactions, and in searching for new antidepressants. Several animal models have been established, such as forced swimming test (FST), tail suspension test (TST), learned helplessness (LH) and chronic mild stress (CMS). These models have been used as reliable research tools to screen effective antidepressants and to further research into pathophysiology of depression. However, the value of these animal models in defining the impact of depression producing stressors on spatial learning and memory remains uncertain.[(L. Song et al,2006)]. The UCMS animal model has been also used to evaluate anxiety-like behaviors which is an important element in the development of depression by some behavioral assay such as the elevated plus maze and ligh/dark paradigm as the evaluation of anxiety-like effects.[(Yann S. M.,2006)]. And Genetic level may involve with depression, from our review literature shown that Brain-derived neurotrophic factor (BDNF) is a neurotrophin important for neuronal development and synaptic plasticity. However, it has also been recently implicated in the etiology and treatment of psychiatric disorders, including depression [(F Angelucci et al, 2005)]. And a number of findings suggest that BDNF action could be impaired in depression and stress-related affective disorders, and that BDNF is involved in the etiology of these illnesses. Chronic administration of several antidepressants, including selective serotonin reuptake inhibitors, increases BDNF expression in the hippocampus. Another study demonstrated that centrally administered BDNF produces antidepressant-like activity in learned helplessness paradigms and the forced swim test. Therefore, up regulation of BDNF in response to antidepressant treatment could have similar behavioral effects. This is further supported by an animal study demonstrating that environmental stressors, such as immobilization, decreased central BDNF mRNA. Indeed, chronic stress down regulates neurotrophin synthesis causing atrophy.

Materials and Methods

Animals

Male ICR mice, weight about 20 – 25 g, were obtained at the age of 3 weeks. They were housed in groups of 6 – 7 per cage. Housing conditions were thermostatically maintained at ambient temperature (22 ± 1℃) and 12 hours light/dark cycle. They were fed with standard diet and water *ad libitum* and were allowed to acclimate 7 daysbefore they were used. In case of sucrose preference, animals were fasted for 18 hours before they were test. They were randomly di-

vided into 5 groups, which are non-stressed control group and the other 4 groups of stressed mice with unpredictable chronic mild stress.

Drugs and drug administration

After 21 days of continuous to the UCMS sequence of the below-described, mildly stressful situation, when sucrose consumption was reduced significantly in stressed animals to levels not significantly different among them, the four groups of stressed animals were assigned randomly to one of the following treatment: (1) vehicle control, carboxymethlycellulose solution, (2) curcumin(MP Biomedicals), 10 mg/kg body wt.; (3) curcumin, 20 mg/kg body wt.; (4) Imipramine (Nacalaitesque), 20 mg/kg body wt. All drugs were administered intraperitoneally (i.p.) once daily for last two weeks of UCMS.

Unpredictable chronic mild stress

At the start of the experiment, the animal were first trained to consume a 2% sucrose solution for a 48-h period in their cages with no food or water following food and water deprivation for 18 h. They were given sucrose for 1 h per day on the five consecutive days at the end of training in order to group the mice. The mice in the experimental groups were then subjected to UCMS for 5 weeks. The UCMS procedure consisted of a variety of unpredictable mild stressors including two periods of tilted cage 45° (12h), two periods of 1 h restricted access to food (5 micropellets), two periods of exposure to empty bottle (3h), one period of 21 h wet cage (200 ml water in 100 g

Protocol I: Chronic Mild Stress Activity (CMS) schedule

Activity/Days	Thu	Fri	Sat	Sun	Mon	Tue	Wed
- Food and water deprivation (18h)	Thu; 15.00 – Fri; 9.00						
- Sucrose preference (1h)		9.00 – 10.00					
- Tiled cage 45° (12h)	8.00 – 20.00		Sat; 22.00 – Sun; 10.00				
- Restricted access to food 5 micro pellets (1h)		20.00 – 21.00			16.00 – 17.00		
- Exposure to empty bottle (3h)			9.00 – 12.00		19.00 – 22.00		
- Wet cage 200 ml water in 100 g sawdust bedding (21h)						Tue;13.00 – Wed;10.00	
- Light exposure (36h)	Thu; 18.00			Sun; 6.00 – Mon; 18.00			Wed; 6.00 –
- Intermittent sound (3h, 5h)			12.00 – 17.00			16.00 – 21.00	
- Paired caging (2h)	20.00 – 22.00	14.00 – 16.00					

sawdust bedding), two periods of light exposure (36h), two periods of intermittent sound (3h, 5h), two periods of paired caging (2h) and food and water deprivation for 18 h before 2% sucrose solution consumption. These stressors were randomly scheduled over a one-week period and repeated throughout the 5-week experiment.

Sucrose consumption

Sucrose intake were measured once weekly after the end of UCMS, 18 h of food and water deprivation. Consumption was measure by weighing the pre-weighed bottle at the end of the test. Baseline was measured at the week 0 before started of UCMS.

Tail suspension test

The tail suspension test allows fast evaluation of drugs psychotropic effects. The animals were subjected to the short-term, inescapable stress of being suspended by their tail and develop an immobile posture. Animals were suspended 50 cm above the floor by means of an adhesive tape, placed approximately 1 cm from the top of the tail. The time during which mice remained immobile was measured during a test period of 6 min. Mice were considered immobile only when they hung passively and completely motionless. Immobility time is defined as the activity of stressed mice.

Forced swimming test

The mice were individual to swum for 15 min in glass cylinder (height: 27 cm, diameter: 20 cm) containing 10 cm of water at 25℃ for pretest. A 24-h after pretest, mice were placed in glass cylinder again for 6 min test and was recorded the last 4 min of testing period. A mouse was judged to be immobile when it discontinued struggling and remained floating motionless in the water, making only a small movement necessary to keep its head above water.

Open field analysis

In the last week of UCMS and drug exposure, the open field analysis was performed. The open field consist of a black walls and base divided into 16 (4 × 4) identical sectors by white stripes. The animals were placed in the central sector and measured the movement of mice for 5 min manually. The open field arena was thoroughly cleaned between each test. Motility was scored when animals crossed a sector border with both its hind limbs or rearing and grooming. This test can determine the effect of drug on motor function.

Light/Dark preference

The light dark (LD) test is used to evaluate the relative anxiety status of mice. The light dark paradigm in mice is based on a conflict between the innate aversion to brightly illuminated areas and the spontaneous exploratory activity. If given a choice between a large brightly compartment versus a small dark compartment, mice spontaneously prefer the dark. Anxiolytic com-

pounds have been found to increase the total duration of time spent there. Anxiogenic compounds are observed to work in the opposite way.

Elevated plus-maze

The elevated plus-maze was constructed from black acrylic plateand elevated to a height of 50 cm. It consisted of two open arms (50 x 10 cm) and two enclosed arms (30 x 5 x 15 cm). Each mouse was placed in the central square facing an open arm, and allowed to explore the maze for 5 min. The maze was cleaned thoroughly before each test. The percentage of time spent on the open arms (time on open arms/(time on open arms + time on closed arms) x 100), the percentage open arm entries (open arm entries/total entries x 100), and total number of entries were determined. An entry was defined as three of the four paws being on the arm.

Semiquantitative revers transcription-polymerase chain reaction (RT-PCR)

Total RNA extraction

Homogenize tissue samples in 1 ml of TRIZOL reagent. Add 0.2 ml of chloroform per 1 ml of TRIZOL Reagent. Cap sample tubes securely. Vortex samples vigorously for 15 seconds and incubate them at room temperature for 2 to 3 minutes. Centrifuge the samples at no more than 12,000 x g for 15 minutes at 4 ºC. Following centrifugation, the mixture separates into lower red, phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase. Transfer upper aqueous phase carefully without disturbing the interphase into fresh tube. Measure the volume of the aqueous phase (The volume of the aqueous phase is about 60% of the volume of TRIZOL Reagent used for homogenization). Precipitate the RNA from the aqueous phase by mixing with isopropyl alcohol. Use 0.5 ml of isopropyl alcohol per 1 ml of TRIZOL Reagent used for the initial homogenization. Incubate samples at 15 to 30 ºC for 10 minutes and centrifuge at not more than 12,000 x g for 10 minutes at 4 ºC. The RNA precipitate, often invisible before centrifugation, forms a gel-like pellet on the side and bottom of the tube. Remove the supernatant completely. Wash the RNA pellet once with 75% ethanol, adding at least 1 ml of 75% ethanol per 1 ml of TRIZOL Reagent used for the initial homogenization. Mix the samples by vortexing and centrifuge at no more than 7,500 x g for 5 minutes at 4 ºC. Repeat above washing procedure once. Remove all leftover ethanol. Air-dry or vacuum dry RNA pellet for 5-10 minutes. Do not dry the RNA pellet by centrifuge under vacuum. It is important not to let the RNA pellet dry completely as this will greatly decrease its solubility. Dissolve RNA in 40 μl RNase free water by passing solution a few times through a pipette tip, keep in -20 ºC refrigerator as stock RNA.

Reverse transcription

Pipette 2 μl of 0.5 μg/μl RNA sample to nuclease-free microcentrifuge tube. Add 10 μl master mix1 [1 μl oligo (dT) 12-18, 4 μl 10 mM dNTP Mix (10 mM each dATP, dGTP, dCTP and dTTP at neutral pH) and RNase free water adjust to 10 μl] Heat mixture to 65 ºC for 5 min and quick chill

on ice. Add 7 μl master mix2 [4 μl 5X First-Strand Buffer, 2 μl 0.1 M DTT, 0.2 μl RNase Inhibitor, 0.8 μl RNase free water] Mix contents of the tube gently and incubate at 37 ºC for 2 min. Add 1 μl (200 units) of M-MLV RT, and mix by pipetting gently up and down. Incubate 50 min at 37 ºC and inactivate the reaction by heating at 70 ºC for 15 min.

Table 2: Summary of the primer pair for house keeping gene (β-actin), stress-related genes (BDNF, CREB) and product length.

Gene	**Primer Sequence** **Top line : forward primer** **Bottom line : reverse primer**	**Product length**
β-actin	5′-AAC GGT CTC ACG TCA GTG TA-3′ 5′-GTG ACA GCA TTG CTT CTG TG-3′	220 bp
BDNF	5′-GAC AAG GCA ACT TGG CCT AC-3′ 5′-CCT GTC ACA CAC GCT CAG CTC-3′	334 bp

Semi-quantitative RT-PCR

The PCR reaction mixture; 1 μl of cDNA, 4 μl of 5x PCR buffer, 2 μl of deoxyneucleoside triphosphate mixture, 2 μl of magnesium chloride, 2 μl of primer pair (Table 2), 0.4 μl of Taq polymerase and 8.6 μl of distilled water to give final volume 20 μl. In general, PCR was performed with a preheating cycle at 95 ℃ for 2 min, denaturation, annealing, elongation and reaction cycles were carried out follow Table 3. Aliquots of PCR products were analyzed by gel electrophoresis with 10% polyacrylamide gel stained in ethidium bromide, photographed under UV light and analyzed by GeneSnap and Gene ToolsMatch Software

Statistic Analysis

Data are expressed as the mean ± S.E.M. and analyzed by t-test between non-stress group and UCMS group and one-way analysis of variance (ANOVA) followed by the Student–Newman –Keuls test for multiple comparisons among different groups. Differences with $p < 0.05$ were considered significant.

Results

Sucrose consumption

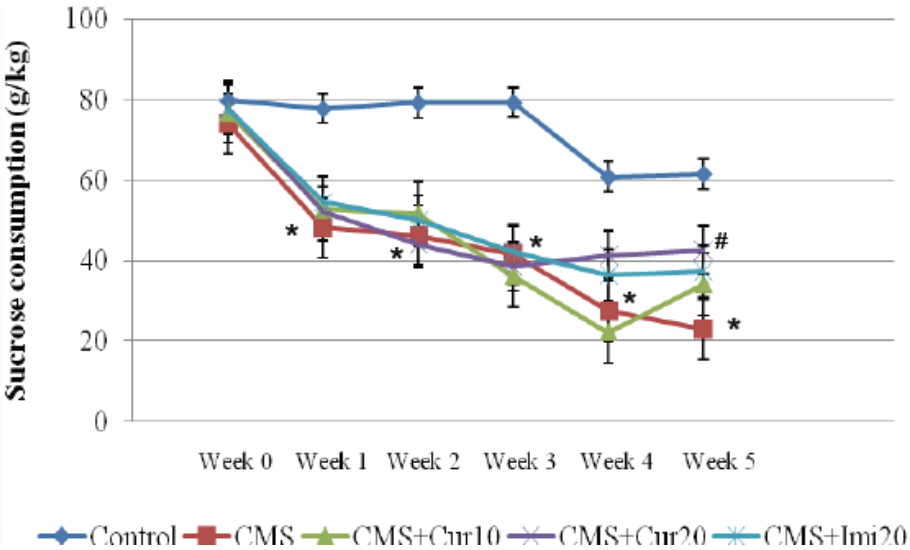

Figure 2: Effects of Curcumin on sucrose consumption of mice exposed to unpredictable chronic mild stress (UCMS) (mean ± S.E.M., n = 10 – 13). Chronic treatment of Curcumin (10, 20 mg/kg, i.p.) was given the last 2 weeks of the 5-week unpredictable chronic mild stress procedure. $^{\#}P< 0.05$ compared with non-stressed control group; $^{*}P< 0.05$ compared with UCMS + vehicle group.

In the sucrose solution-training phase (base line phase, week 0), sucrose consumption did not differ significantly among the group. The UCMS significantly decreased the consumption of the 2%sucrose solution from 74.14 mg/kg in the base line week to 22.91 mg/kg in the last week of UCMS procedure. Treatment with curcumin and imipramine caused a gradual recovery of the sucrose intake. The decreasing of sucrose preference in all groups of in the last 2 weeks, which is drug treatment period, may cause from the injection of drug. Despite the injection of drug was made them like exposed to stress condition, the mice showed recovery of sucrose intake in the last week of UCMS procedure. At last week of experiment, the amount of sucrose preference taken by the stressed mice receiving curcumin 20 mg/kg and imipramine 20 mg/kg were significantly increase when compare with the vehicle-treated stressed mice.

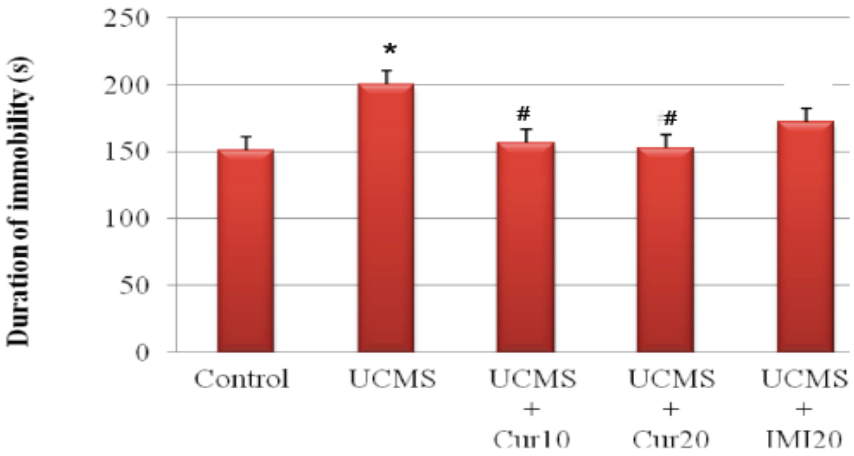

Figure 3: Effect of Curcumin on the forced swimming test of UCMS stressed mice (mean ± S.E.M., n = 8 – 12). The mice were administered vehicle, Curcumin (10, 20 mg/kg, i.p.) or Imipramine (20 mg/kg, i.p.). The mean immobility time of stressed-control mice was 200.57 ± 11.02 s. The respective percent reduction in immobility time was 21.81%, 23.88% and 14.15% for Curcumin 10 mg/kg, 20 mg/kg and Imipramine 20 mg/kg. #$P < 0.05$ vs. the stressed-control mice and showed the
significantly different between non-stressed control and stressed-control mice (*$P = 0.006$, *t-test*).

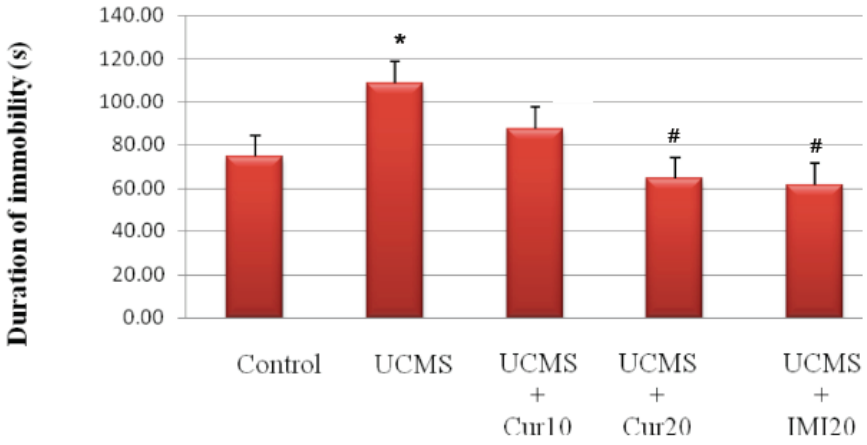

Figure 4: Effect of Curcumin on the tail suspension test of UCMS stressed mice (mean ± S.E.M., n = 8 – 12). The mice were administered vehicle, Curcumin (10, 20 mg/kg, i.p.) or Imipramine (20 mg/kg, i.p.). The mean immobility time of stressed-control mice was 108.76 ± 8.08 s. The respective percent reduction in immobility time was 19.75%, 40.90% and 43.35% for Curcumin 10 mg/kg, 20 mg/kg and Imipramine 20 mg/kg. $^{\#}P < 0.05$ vs. the stressed-control mice.

Forced swimming test and tail suspension test

The effect of administration with curcumin in forced swimming test and tail suspension test at the dose of curcumin with 10 and 20 mg/kg and imipramine with 20 mg/kg. The duration of immobility time in forced swimming test, resulting in 21.81%, 23.88% and 14.15% immobility

reduction of dose with curcumin 10, 20 mg/kg and imipramine 20 mg/kg, respectively compared to the UCMS stressed-control mice. In the tail suspension test, these same doses of curcumin and imipramine also significantly inhibited immobility with a respective percent reduction of 19.75%, 40.90% and 43.35%. In both models of depression, the effects of curcumin were similar to those observed for the classical antidepressant Imipramine (20mg/kg).

Open field analysis

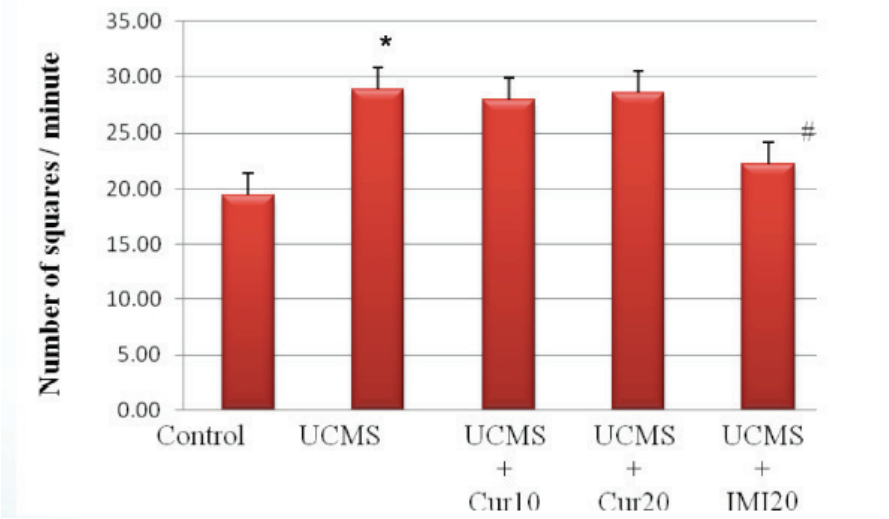

Figure 5: Effect of Curcumin on the open field analysis activity (mean ± S.E.M., n = 8 –12). Chronic treatment of vehicle 20 mg/kg, Curcumin with 10, 20 mg/kg and Imipramine 20 mg/kg was given during last 2 weeks of UCMS procedure and resulting in significant difference between imipramine and stressed-control mice group (#$P <$ 0.05) and also differently significant between the non-stressed control group and the stressed-control group (*P = 0.021, *t-test*).

The UCMS stressed-control mice showed show significant difference in total activity compared with non-stressed control mice in 5-min open field analysis. After 2 weeks of treatment, they showed that have significantly differences of mice were administered imipramine 20 mg/kg between the stressed-control mice group.

Light/Dark preference

Mice receiving UCMS procedure show the significant decrease in the latency to dark zone. After treatment with curcumin and imipramine the latency time were significantly increase. No significant difference in spending time in dark zone and light zone between UCMS group and treatment group. The respect percentage of dark zone timereduction were 21.75%, 31.73% and 31.28% in dose of curcumin and imipramine compared to stressed-control mice. The mean of time preference of dark zone of UCMS group was 214.34 ± 1.38. The percentage time induction of light zone were 54.15%, 79.01% and 77.90% in dose of curcumin and imipramine compared to stressed-control mice. The mean of light time preference in the light zone of UCMS group was 85.96 ± 9.87.

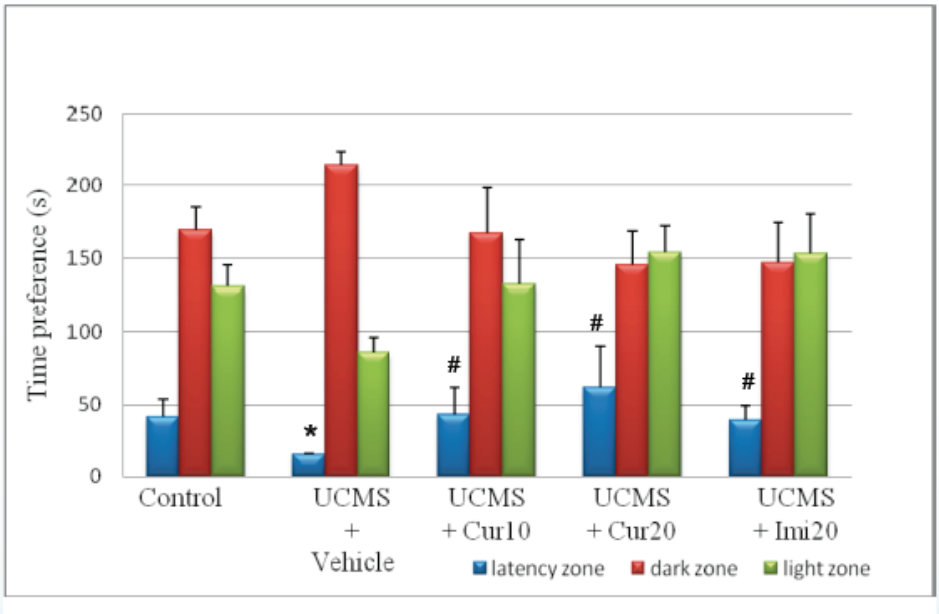

Figure 6: Effect of Curcumin on light-dark preference with the UCMS stressed mice and non-stressed control (mean ± S.E.M., n = 8 – 12), #$P < 0.05$, compared between curcumin 20 mg/kg and imipramine20 mg/kg with the stressed-control mice, *$P = 0.006$, *t-test* vs. non-stressed control group.

Elevated plus maze

UCMS mice show significantly decrease in the spending time in open arm and close arm when compare with the non stress mice. Only curcumin 20 mg/kg significantly reverse the effect of UCMS stress in both spending time in open arm and close arm. Imipramine show significantly increase only spending time in close arm.

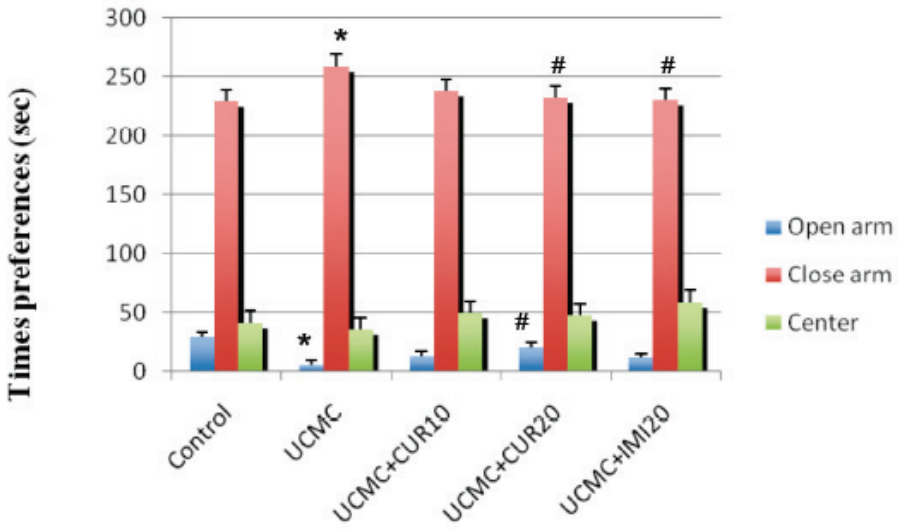

Figure 7: Effects of curcumin on the elevated plus maze of UCMS stressed mice (mean ±S.E.M., n = 8). The result showed significantly differences among non-stressed control andstressed control groups (*$P \leq 0.001$, t-test) and #$P < 0.05$ vs. stressed control group.

Semi quantitative RT-PCR analysis

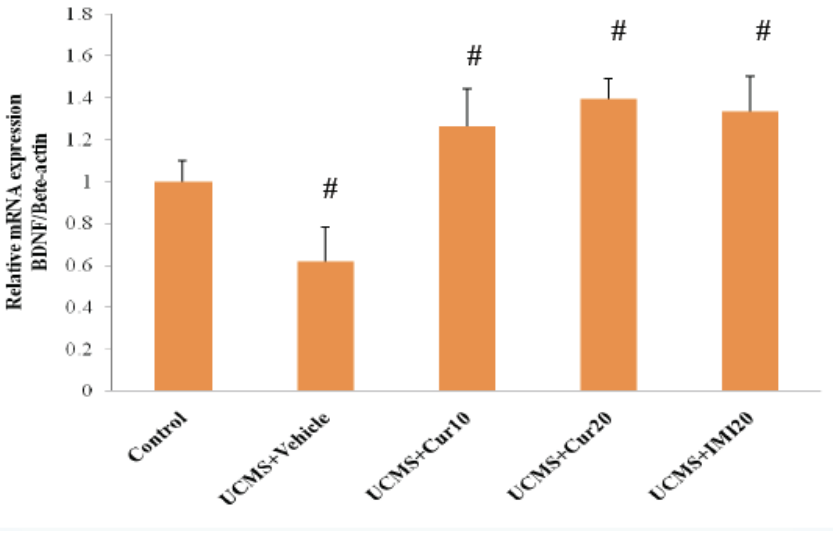

Figure 8: Effect of Curcumin on relative mRNA expression with the UCMS stressed mice on chronic curcumin treatment 10 mg/kg and 20 mg/kg i.p.on UCMS were changed by increased, (mean ± S.E.M, n = 4-6), (#$P < 0.05$) , whereas UCMS+vehicle was decreased (#$P < 0.05$). And UCMS+IMI were increase attenuated BDNF levels. (*P <0.05), whereas each treatments compare with control group.

UCMS procedure significantly reduced the relative BDNF mRNA expression when compare with non stress treatment. Curcumin and imipramine treatment group significantly increased the BDNF mRNA expression when compare with UCMS+vehicle mice.

Discussion

The UCMS model of depression involves in the presentation of a series of varied and unpredictable environment stressors, such as two periods of tilted cage 45° (12h), two periods of 1 h restricted access to food (5 micropellets), two periods of exposure to empty bottle (3h), one period of 21 h wet cage (200 ml water in 100 g sawdust bedding), two periods of light exposure (36h), two periods of intermittent sound (3h, 5h), two periods of paired caging (2h) and food and water deprivation for 18 h. Following such exposure, mice have been reported with anhedonia effect, measured by 2% sucrose consumption. To assured these results are from UCMS procedure and sucrose preferences; we performed the behavioral activities to test the effect of curcumin substances and used the classical antidepressant, imipramine, as standard of treatment. The forced swimming test and tail suspension test were performed for determining the antidepressant effect. Curcumin 20 mg/kg and imipramine 20 mg/kg showed the antidepressant activity in both test. Only imipramine exhibited the decreasing in locomotor activity in open field test. In addition, the anxiety test was performed in this study to determine the effect of curcumin in anxiolytic activity. Light/dark preferences and elevated plus maze were performed to evaluate the anxiolytic activity of curcumin. The results showed that curcumin had the anxiolytic activity

by decreasing the latency to dark zone in light/dark preference and decreased spending time in close arm and increased the spending time in open arm. UCMS induced behavioral changed were reverse by chronic antidepressant medication and long-term curcumin consumption. In addition curcumin and imipramine reversed the effect of UCMS on BNDF expression. Interestingly, we found that curcumin increased BDNF levels quite similar to the imipramine ($p > 0.05$) In fact; chronic antidepressants could produce long-term adaptation in cellular signaling mechanisms in mice. The ability of curcumin to up-regulate BDNF mRNA expression is also considered to prove its potential mechanism as antidepressant agent. Some mechanism have an involve with curcumin to explain, why it is involve to depression increased cell proliferation and neuronal populations may be a mechanism by which curcumin treatment overcomes the stress-induced behavioral abnormalities and neuronal damage. Moreover, curcumin treatment, via up-regulation of 5-HT1A receptors and BDNF, may reverse or protect neurons from further damage in response to chronic stress, which may underlie the therapeutic actions of curcumin (8). And the topic of dose of curcumin 10 mg/kg and 20 mg/kg are not significantly different ($p > 0.05$) on relative gene expression, they are seem to the dose of curcumin 10 mg/kg, 20 mg/kg gives the efficiency to promote BDNF mRNA expression closely for each other.

Conclusion

The effect of Curcumin can reversed the decrease of Anhedonicbehavior, whichinvolves with depressionby monitor the sucrose consumption. These changes were reversed by chronic curcumin administration (10 or 20 mg/kg, p.o.). According to 2% sucrose consumption, at the last 2 weeks of UCMS procedure, resulting in significantly differences of sucrose consumption among their groups. In the group of Curcumin and Imipramine treated, the mice showed the induction of sucrose preferences compared to the stressed-control mice.The effect of administration with Curcumin in forced swimming test and tail suspension test at the dose of Curcumin with 10 and 20 mg/kg and Imipramine with 20 mg/kg. The duration of immobility time in forced swimming test, resulting in 21.81%, 23.88% and 14.15% immobility reduction of dose with Curcumin 10, 20 mg/kg and Imipramine 20 mg/kg, respectively compared to the UCMS stressed-control mice. In the tail suspension test, these same doses of Curcumin and Imipramine also significantly inhibited immobility with a respective percent reduction of 19.75%, 40.90% and 43.35%. In both models of depression, the effects of Curcumin were similar to those observed for the classical antidepressant Imipramine (20mg/kg). In case of open field test, they showed no differences among their groups but there is significantly differences between stressed-control and Imipramine mice and showed as the same result as light/dark preference. The light/dark preferences showed the percentage of time reduction in dark zone and induction time in light zone of mice were treated with Curcumin and Imipramine compared to the stressed-control mice. In addition, we also found that the unpredictable chronic stress procedure induced a down-regulation of brain-derived neurotrophic factor (BDNF) protein levels (1), in the frontal cortex of UCMS mice. Furthermore,

these stress-induced decreases in BDNF were also blocked by chronic curcumin administration (10 or 20 mg/kg, p.o.). These results provide compelling evidence that the behavioral effects of curcumin in chronically stressed animals may be related to their modulating effects on other organ. In addition some evidence has hypothesis that, curcumin has an property of MAO inhibitor that was effect on Catecholamine neurotransmitter(6) such as Serotonin, Epinephrine, Dopamine etc. these are regulate the function of mood. Although BDNF gene we have selected in this study but many gene are relevant on the depression not only BDNF gene and also should be study further gene expression on the other organs.

Reference

Angelucci F, Brene S, Mathe AA. BDNF in schizophrenia, depression and corresponding animal models. Mol Psychiatry. 2005 Jan 18;10(4):345-352.

Chen Y, Wang H, Xia X, Kung H, Pan Y, Kong L., 2007. Behavioral and biochemical studies of total furocoumarins from seed of *Psoralea corylifolia* in the chronic mild stress model of depression in mice. Phytomedicine. 14, 523-529.

Fumagalli F, Racagni G, Colombo E, Riva MA. BDNF gene expression is reduced in the frontal cortex of dopamine transporter knockout mice. Mol Psychiatry. 0 ;8(11):898-899.

Holmes A, Murphy DL, Crawley JN. Reduced aggression in mice lacking the serotonin transporter. Psychopharmacology (Berl). 2002 May ;161(2):160-7.

Li Y, Wang F, Pan Y, Qiang L, Cheng G, Zhang W, et al. Antidepressant-like effects of curcumin on serotonergic receptor-coupled AC-cAMP pathway in chronic unpredictable mild stress of rats. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2009 Apr 30;33(3):435-449.

Li S, Wang C, Wang M, Li W, Matsumoto K, Tang Y., 2007. Antidepressant like effects of piperine in chronic mild stress treated mice and its possible mechanisms. Life Sci. 80, 1373-1381.

Wang R, Xu Y, Wu H, Li Y, Li Y, Guo J, Li X., 2008. The antidepressant effects of curcumin in the forced swimming test involve 5-HT1 and 5-HT2 receptors. Eur. J. Pharmacol. 578, 43-50.

Xu Y, Ku B, Cui L, Li X, Barish PA, Foster TC, et al. Curcumin reverses impaired hippocampal neurogenesis and increases serotonin receptor 1A mRNA and brain-derived neurotrophic factor expression in chronically stressed rats. Brain Research. 2007 Aug 8;11629-18.

Xu Y, Ku B, Tie L, Yao H, Jiang W, Ma X, Li X., 2006. Curcumin reverse the effect of chronic stress on behevior, the HPA axis, BDNF expression and phosphorylation of CREB. Brain Res. 1122, 56-64.

Xu Y, Ku B, Yao H, Lin Y, Ma X, Zhang Y, Li X., 2005. The effect of curcumin on depressive-like behaviors in mice. Eur. J. Pharmcol. 518, 40-46.