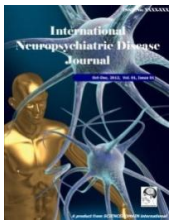

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Chronic Administration of *Bacopa monniera* Alleviates Depressive Like Behavior and Increases the Expression of ERK1/2 in Hippocampus and Pre-Frontal Cortex of Chronic Unpredictable Stress Induced Rats

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Authors' contributions:

This work was carried out in collaboration between all authors. Authors ACM and GS designed the experiments and wrote the first draft of the main manuscript. Authors SH and SK preformed all the scientific experiments and typed the manuscript, made the graphical figures and statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Aim of the present study is to investigate the effect of *Bacopa monniera* in chronic unpredictable stress (CUS) induced depression in Sprague Dawley rats and its effect on ERK protein level.
Study Design: Rats were subjected to CUS procedure and daily administration of BM intragastrically for consecutive 28 days.
Place and Duration of Study: Department of Physiology, Raja Peary Mohan College, Uttarpara, Hooghly, West Bengal, India from February 2012-to July 2014.
Methods: Various types of unpredictable stressors were applied for consecutive 28 days during

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CUS procedure including foot shock (1 mA, 1s duration, average 1 shock/min) for 60 min, 5 min cold water swim (at 4°C), 1 min tail pinch (1 cm from the end of the tail), 48 hour food deprivation, 24 hour water deprivation, and overnight illumination. The effect of BM treatment in CUS-induced depression was examined applying behavioral tests on rats including the sucrose consumption test that indicates anhedonia like behavioral change, open field test points out decreased locomotor activity which is indicative of a behavioral change that may reflect certain aspects of refractory depression and elevated plus maze test indicates decreased anxiety. We examined the possible mechanism of BM by measuring corticosterone level (ELISA), ERK1/2 and P-ERK1/2 level in rat hippocampus and prefrontal cortex (Western blot).

Results: Four weeks of CUS exposure induced depression-like behavior in rats which is evidenced by significant decreases in sucrose consumption, decreased locomotor activity and short time spent in open arms (P=.05).In addition, it was found that corticosterone levels, ERK1/2, Phospho-ERK1/2 was significantly lower (*P*=.05) in CUS-treated rats. Daily administration of 80 mg/kg body weight of BM during the CUS interval significantly suppressed behavioral changes and reduced corticosterone levels, ERK1/2 and Phospho-ERK1/2 protein levels in the hippocampus and prefrontal cortex.

Conclusion: The present study advocates that BM can attenuate depression and also confirms that 80 mg/kg doses of BM extract have significantly higher antidepressant-like activity.

Keywords: Antidepressant; Bacopa monniera; chronic unpredictable stress; extracellular signal regulated kinase; open field test; sucrose preference test; elevated plus maze.

1. INTRODUCTION

Depression is nowadays a commonly occurring psychiatric disorder characterized by a pervasive low mood, loss of interest, sleep and psychomotor disturbances, decreased food-intake, body-weight and suicidal tendencies [1]. It has been suggested that neuronal atrophy in the hippocampus and cortex is involved in the pathogenesis of depression [1,2]. Neurotrophins modulate neuronal plasticity, inhibit cell death cascades and increase cell survival proteins that are responsible for proliferation, differentiation and maintenance of central nervous system (CNS) neurons [2,3]. It may be the important factor involved in the development and treatment of depression. Recently, the role for the extracellular signal regulated kinase (ERK) pathway in the molecular mechanism of depression is increasingly becoming the focus of much research interest, and a growing body of evidence indicates that the ERK pathway may participate in the neuronal modulation of depression [4,5,6,7]. Several studies demonstrated that the ERK signal pathway participated in stress response and correlated with the stress-induced depressive-like behaviors, which suggests that the ERK signal pathway may participate in the molecular mechanism of depression [4,5,6,7]. Our recent studies have shown that chronic antidepressant drug treatment up-regulates the neurotrophins Brain Derived Neurotrophic Factor (BDNF) [1,8]. BDNF that binds with the cognate receptor Trkβ that leads to the activation of ERKs in the downstream signaling pathway which activates cAMP response element binding protein (CREB) [9,10]. Hence, ERKs could also represent a major target of antidepressant activity. Therefore, we decided to focus on the mitogen-activated protein kinase (MAPK) pathway and specifically the ERK pathway based on the evidence that these proteins represent multifunctional signaling integrators involved in the regulation of gene transcription [11].

Rodents exposed to different types of unpredictable stress for long time are considered as well established model to study the effect of herbal treatment on chronic stress induced depression [1,12,8,2]. Our recent studies demonstrated that chronic unpredictable stress (CUS) induced depression is a model to determine whether chronic BM treatment can alleviate stress-induced behavioral abnormalities [1,8]. In order to further confirm the role of the ERK 1/2 signal system in the molecular mechanism of depression and to explore the potential target of antidepressant, the present study was designed to investigate the effect of BM on the ERK 1/2 signal system and the depressive like behaviors in CUS rats. In the present experiment, a series of behavioral tests, including open field test, elevated plus maze test and sucrose preference test are done with good face validity and the highest degree of pharmacological predictive validity [1,2]. It was applied to assess emotion, locomotor activity,

anhedonia/ of depression like behavior, as well as anxiety.

At present, several antidepressant drugs are clinically used such as monoamine oxidase inhibitors (MAOIs), tri-cyclic antidepressants (ADTs) such as selective serotonin re-uptake inhibitors (SSRIs), serotonin- norepinephrine re-uptake inhibitors (SNRIs) and norepinephrine re-uptake inhibitors (NRIs) but most of these drugs have unwanted side effects [2,8]. Bacopa monniera (*BM*) (Brahmi, family: Scrophulariaceae) [1], is mentioned as a rasayana and has been used in the Ayurvedic system of medicine for centuries and is advised to be useful in anxiety disorders, clinical mental disorders, obsessive compulsive disorders, hysteria, epilepsy and insomnia according to Ayurvedic materia medica. From many centuries people used herbs and herbal preparations as medicine and some of the herbal medicines may be effective alternatives in the treatment of neuropsychiatric diseases such as depression. Traditionally, it was used as a brain tonic to enhance memory development and learning. It grows naturally in wet soil, shallow water, and marshes in India [13,14,15,16]. Bacopa includes many active constituents like the alkaloids Brahmine and herpestine, saponins d-mannitol and hersaponin, acid A, monnierin, betulic acid, stigmastarol, beta-sitosterol, as well as numerous bacosides and bacopasaponins those are responsible for pharmacological effects. Bacosides A and B are the constituents responsible for Bacopa's cognitive effects. Several clinical studies have confirmed the beneficial actions of BM and its pharmacological actions are mainly attributed to the bacosides present in it [17,18,19]. Our recent studies confirmed that BM can alleviate stress-induced behavioral abnormalities and cause alterations of neurotrophins such as BDNF in hippocampus and PFC [1,8].

The ERK pathway is activated by neurotrophins and other neuroactive chemicals and is involved in survival, differentiation, structural and functional plasticity of neurons. A common cellular response to monoamines and neurotrophins is the activation of extracellular signal-regulated kinase (ERK), which belongs to the MAPK family [11,14]. ERK pathway is the major convergence point in all signal transduction pathways regulating cellular growth and differentiation and neuronal plasticity. The p44 ERK (ERK1) and p42 ERK (ERK2) are activated by phosphorylation in response to many neurotransmitters including 5-HT, norepinephrine, dopamine, growth factors and neurotrophins [20,21]. The phosphorylation of ERK1 and ERK2 is mediated by the MAPK/ERK kinase (MEK). Alterations of brain monoamines and neurotrophins in depression and after antidepressant treatment suggest that the dysfunction of ERK or inadequate phosphorylation of ERK might be involved in the pathology of depression [5,6]. ERK1 and ERK2 are prominently found in hippocampus and prefrontal cortex (PFC), which are key limbic regions in brain most likely to be implicated in stress response and depression [1,8]. Recent research indicates that BDNF can ameliorate depression via activating the ERK pathway [4,5,6], and the chronic antidepressant treatment can induce ERK phosphorylation (phospho-ERK1/2) [6,22]. These data suggest that the ERK pathway may be the potential target of antidepressant treatment and participate in the molecular mechanism of depression. However, there is very little direct evidence for the role of BM in the ERK pathway in depression.

2. MATERIALS AND METHODS

2.1 Preparation of BM Extract

Dried extract of BM was purchased (≥40% total bacosides) from Natural Remedies Pvt. Ltd., Bangalore, India. The extract contains total bacosides 40.0–50.0%, along with a number of chemical constituents, namely bacoside A3 (>2.7%), bacopaside II (>3.6%), Jujubogenin isomer of bacopa saponin C (>4.5%), bacopasaponin C (>3.0%), bacopaside I (>4.5%). This was prepared as mentioned in our previous published [1,8] paper and administered intragastrically at the dose of 80 mg/kg body weight (b.w.) once daily.

2.2 Drug treatments

Imipramine hydrochloride (≥99%, crystalline) (IMI), a tricyclic antidepressant, was purchased from Sigma-Aldrich (St. Louis, MO) and used as positive control for antidepressant action. IMI was applied intragastrically at the dose of 20mg/kg b.w. once daily [1].

2.3 Experimental Animals

Male Sprague-Dawley rats weighing 180–220 g were obtained from the animal care and maintenance division of Chittaranjan National Cancer Institute (CNCI), Kolkata, India. The animals were maintained on a 12 h light/dark

cycle (lights on at 6:00 a.m., lights off at 6:00 p.m.) under controlled temperature conditions (22±2°C), and given standard food and water ad libitum. They were allowed to acclimatize for 7 days before use. The rats were randomly assigned to four group, Vehicle control, CUS plus vehicle, CUS plus BM (80 mg/kg b.w.) and CUS plus IMI (20 mg/kg b.w.). BM and IMI were each given intragastrically 60 min before each stressor applied once every day for 4 weeks. BM and IMI were dissolved in normal physiological saline (0.9% NaCl) and the animals in the control and CUS groups were treated with equal volume of physiological saline as vehicle [1]. All experiments conformed to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA, New Delhi Reg. No.: 1148/PO/ac/07/CPCSEA dated 13.02.2012). The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Raja Peary Mohan College, Uttarpara, Hooghly (W.B.). Effort was made to minimize the number and suffering of the animals.

2.4 CUS Procedures

CUS procedure was done as described in our previous publication according to Banerjee et al. [1] and Hazra et al. [8]. Briefly, CUS consisted of a variety of unpredictable stressors, including foot shock (1 mA, 1s duration, average 1 shock/min) for 60 min, 5 min cold water swim (at 4°C), 1 min tail pinch (1 cm from the end of the tail), 48 h food deprivation, 24 h water deprivation, and overnight illumination. One of these stressors (in random order) was given every day for 28 days.

2.5 Sucrose Preference Test

The test was performed as described in our previous publication [1,8]. Briefly, before the test, the rats were trained to adapt to two bottles of sucrose solution (1%, w/v). After the adaptation procedure, the rats were deprived of water and food for 24 h. The rats were given free access to two bottles containing 100 mL of sucrose solution (1%, w/v) and 100 mL of water, respectively. After 60 min, the volume of both the consumed sucrose solution and water was recorded.

2.6 Open Field Test

The open field test was carried out according to our previous published paper [1,8]. Briefly, the open field apparatus consisted of a square wooden arena (100×100×50 cm) with a black surface covering the inside walls. The floor of the wooden arena was divided equally into 25 squares marked by black lines. In each test, a single rat was placed in the center of the arena and allowed to explore freely. The number of crossings (squares crossed with all paws) and rearing (raise of the front paws) were recorded during a test period of 5 min [1]. This wooden apparatus was cleaned with a detergent and dried after occupancy by each rat.

2.7 Elevated Plus Maze

The maze was elevated to a height of 70 cm with two open (50 x 10 cm) and two enclosed arms (50 x 10 x 50 cm), arranged so that the arms of the same type were opposite each other, and connected by an open central area (10 x 10 cm) [12]. At the beginning of the experiment, rats were placed in the center of the maze, facing one of the enclosed arms, and observed for 4 min. Time spent in open arms and number of open and closed-arm entries (defined as entry of all four limbs into an arm of the maze) were recorded during a test period of 5 min [6]. The wooden apparatus was cleaned with a detergent and dried after occupancy by each rat.

2.8 Determination of Plasma Corticosterone Levels

Plasma corticosterone levels were measured in the plasma of all four rat groups. Blood samples were collected after sacrificing the animals and centrifuge immediately at 2000g at 4ºC for 15 min. Corticisterone levels were measured using commercially available Radioimmunoassay (RIA) kit (ICN Biomedical, Costa Mesa, CA, USA) [8].

2.9 Tissue Sample Collection

The rats were sacrificed by decapitation after twenty four hours of the last behavioral test to assess any biochemical, neurochemical changes that may have occurred in the hippocampus and frontal cortex of rat brain. The whole brain of each rat was rapidly removed and chilled in an ice cold saline solution. Various brain areas, including the hippocampus and prefrontal cortex (PFC), were dissected on a cold plate and immediately frozen in liquid nitrogen. The tissue samples were stored at -80ºC until assay [1].

2.10 Quantitation of ERK1/2 and Phospho ERK1/2 in Hippocampus and PFC by Western Blot analysis.

Immunolabeling of ERK1/2, P-ERK1/2 was determined by the procedure described before [1]. Tissues were homogenized in homogenizing buffer containing 5 mM Tris–HCl (pH 7.4), 0.1% EDTA, 2 mM leupeptin, 0.5 mM phenylmethylsulfonyl fluoride, 1.45 mM pepstatin, 0.2 unit/ml aprotinin, and 2 mM DTT. The homogenate was centrifuged at 980g for 10 min at 4 ºC. Protein concentration was measured by Lowry's method. Equal volumes of protein samples (20 µl containing 50 µg proteins) were loaded onto 7.5% (w/v) polyacrylamide gel and subsequently transferred to a nitrocellulose membrane. Blots were incubated in blocking buffer for 2 h at room temperature. The blots were incubated overnight with primary antibodies ERK1/2, P-ERK1/2 (Pierce Biotechnology, Rockford, IL, USA.) at a dilution of 1:1000 and 1: 500 and after washing, it was incubated with horseradish-peroxidase-linked secondary antibodies (anti-mouse IgG; 1:3000) for 4 h at room temperature. The membranes were exposed to chemiluminescence (ECL) film and were probed with anti-β-actin monoclonal antibody (1:10,000 dilutions in 3% BSA, Sigma, St. Louis, MO, USA). The OD value of each protein band was analyzed (SmartView Pro Imager System, USA) and determined by using the OD of the corresponding β-actin band.

2.11 Statistical Analysis

The Statistical Package for the Social Science (SPSS) 15.0 was utilized for statistical analyses. All data were expressed as mean± SEM and have been statistically analyzed with one way ANOVA followed by unpaired Student's t-test. *P* values less than .05 were considered statistically significant. SEM means standard error of the mean.

3. RESULTS

3.1 The Effect of Chronic BM Treatment on Sucrose Consumption in CUS exposed rats

Fig. 1 shows the effect of BM treatment on the sucrose consumption in CUS exposed rats. There are significant variations found in one way anova ($F_{3,\ 31}$=108.41; *P=.05*) due to four week period of CUS exposure along with BM treatment. Sucrose consumption level in the animals were significantly reduced (t_{CUS}=22.33, df =35, *P=.05*) compared to the vehicle controls. Chronic BM treatment of 80 mg/kg daily doses significantly increases the sucrose consumption rate in CUS exposed rats (t_{BM80}=12.45, df= 35, *P=.05*) compared to vehicle treated CUS exposed rats. IMI (20 mg/kg) applied as positive control also significantly increased the level of sucrose consumption in CUS exposed rats (t_{IMI20}=18.26, df=35, *P=.05*) (Results are based on unpaired Student's t-test). There was no significant difference in the percentage of sucrose consumption among all rats (data not shown) before CUS treatment.

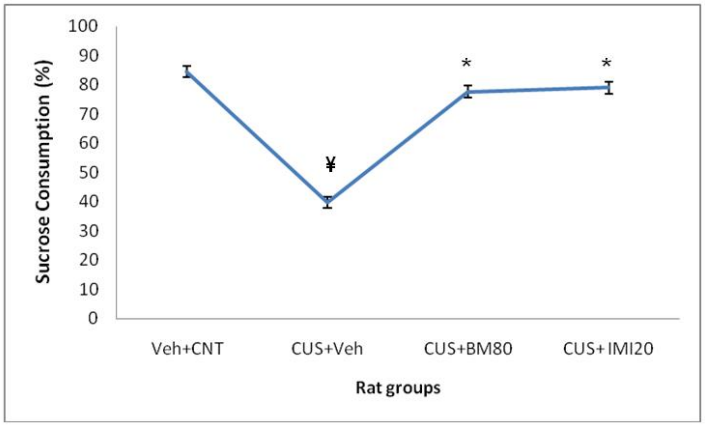

Fig. 1. The effect of BM on the level of sucrose consumption in CUS exposed rats. Values given are the mean±SEM (n=8). Results shown above are based on statistical comparison, using unpaired Student's t test

**P=.05 as compared with the CUS group, ¥ P=.05 as compared with the Vehicle control group*

3.2 The Effect of Chronic BM Treatment on Open Field Test in CUS exposed Rats

Figs. 2 and 3 show how BM treatment alters locomotor activity in open field test in CUS exposed rats. Significant variation was observed by one way anova in locomotor activity by the number of crossings ($^{Crossingxc}F_{3,\ 47}$=231.40; *P=.05*) (Fig. 2) and rearings ($^{Rearing}F_{3,\ 47}$= 89.12; *P=.05*) (Fig. 3) in the open field test. Significant reduction was found in the number of crossings (t_{CUS}= 24.26, df=24, *P=.05*) (Fig. 2) and rearings (t_{CUS}=30.71, df=35, *P=.05*) (Fig. 3) among rats at interval of four week period of CUS exposure as compared to the vehicle controls. Significant increase in the number of crossings (t_{BM80}=7.64, df=35, *P=.05*) and rearings (t_{BM80}=5.61, df=35, *P=.05*) (Fig. 3) were observed with chronic BM 80 (80 mg/kg b.w.) treatment, as compared to the CUS plus vehicle grouped rats. IMI (20 mg/kg b.w.) shows similar effects by significant increase in the number of crossings (t_{IMI20}=12.53, df=35, *P=.05*) (Fig. 2) and rearings (t_{IMI20}=13.41, df=35, *P=.05*) (Fig. 3) in CUS exposed rats (Results are based on unpaired Student's t-test). There were no significant differences found before CUS in the number of crossings and rearings among all rats (data not shown).

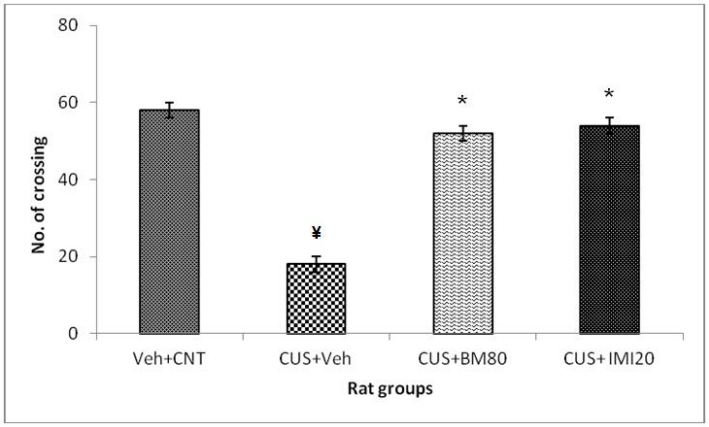

Fig. 2. The effect of BM on the number of crossings in the open field test in CUS exposed rats. Values given are the mean±SEM (n=8). Results shown above are based on statistical comparison, using unpaired Student's t test.

**P=.05 as compared with the CUS group, ¥ P=.05 as compared with the Vehicle control group*

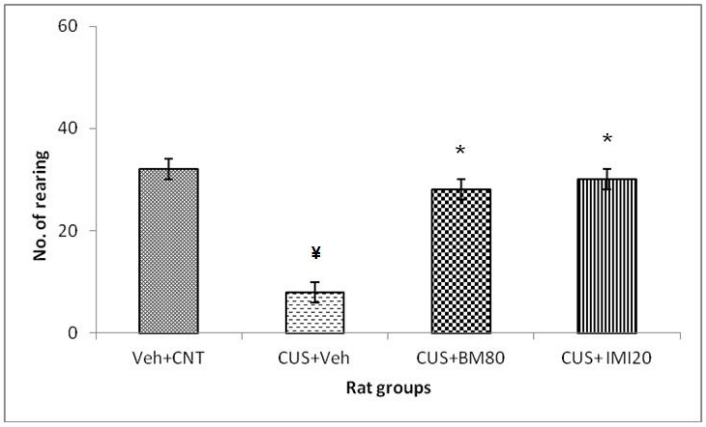

Fig. 3. The effect of BM on the number of rearings in the open field test in CUS exposed rats. Values given are the mean±SEM (n=8). Results shown above are based on statistical comparison, using unpaired Student's t test.

**P=.05 as compared with the CUS group, ¥ P=.05 as compared with the Vehicle control group*

3.3 The Effect of Chronic BM Treatment on Elevated Plus Maze in CUS Exposed Rats

Fig. 4 shows the effect of BM on time spent in arms in CUS rats. Significant variation was assessed by the percentage of time spent in open arms and in closed arm in one way anova ($F_{3, 47}$=158.96, *P=.05*). Before CUS there was no such significant differences (data not shown) but after four weeks period of CUS exposure, significant reduction of time spent in open arm and increased time spent in closed arm (t_{CUS}= 51.45, df=35, *P=.05*) was observed. BM 80 mg/kg of b.w. significantly increased the percentage of time spent into open arms and significantly reduced time spent in closed arm (t_{BM80}=16.80, df=35, *P=.05*) in CUS exposed rats. IMI 20 mg/kg b.w. treatment also significantly increased the percentage of time spent into open arms and significantly reduced time spent in closed arm (t_{Imi}=17.72, df=35, *P=.05*) in CUS exposed rats (Results are based on unpaired Student's t-test).

3.4 The Effect of BM Treatment on Serum Corticosterone Levels in CUS Exposed Rats

Fig. 5 shows that CUS group rats showed the variation/ increase in serum corticosterone level due to 4 weeks of CUS in comparison to vehicle control groups in one way anova ($F_{3, 47=}$ 102.69, *P=.05*). The CUS induced significant increase of serum corticosterone (t_{CUS}= 49.17, df=35, *P=.05*) levels in CUS group rats compared to vehicle control group rats. Corticosterone levels significantly reduced (t_{BM80}= 29.45, df =35, *P=.05*) in rats treated with BM at 80 mg/kg dose or IMI 20 (as positive control) compare to CUS group rats (t_{IMI20}= 25.17, df=35, *P=.05*) (Results are based on unpaired Student's t-test).

3.5 The Effect of Chronic BM Treatment on Expression of ERK1/2 and Phospho ERK1/2 in the Hippocampus and PFC in CUS exposed rats

Figs. 6 and 7 shows that one-way ANOVA indicated that the ERK1 and ERK2 level significantly differed both in the hippocampus ($^{ERK1}F_{3, 47}$= 11.57, *P=.05* and $^{ERK2}F_{3, 47}$= 9.32, *P=.05*) and in the prefrontal cortex, ($^{ERK1}F_{3, 47}$ = 3.71 and $^{ERK2}F_{3, 47}$ = 5.71, *P=.05*) among all rat groups. Four week period of CUS significantly reduced the expression of both ERK1 and ERK2 in hippocampus ($^{ERK1}t_{CUS}$= 17.54, df=41, *P=.05*; $^{ERK2}t_{CUS}$= 20.33, df=41, *P=.05*) and PFC ($^{ERK1}t_{CUS}$= 21.11, df=41, *P=.05*; $^{ERK2}t_{CUS}$= 24.53, df=41, *P=.05*) in CUS group of rats compared to vehicle control group. Chronic administration of BM 80 had significant reversing effect and significantly increased ERK1 and ERK2 level both in the hippocampus ($^{ERK1}t_{BM80}$= 18.78, df=41, *P=.05*; $^{ERK2}t_{BM80}$= 23.33, df=41, *P=.05*) and in the prefrontal cortex ($^{ERK1}t_{BM80}$= 14.16, df=41, *P=.05*; $^{ERK2}t_{BM80}$= 19.57, df=41, *P=.05* respectively) in comparison to CUS group of rats. Positive control IMI 20 also shows similar effect of reversing ERK1 and ERK2 significantly in hippocampus ($^{ERK1}t_{IMI20}$= 12.81, df=41, *P=.05*; $^{ERK2}t_{IMI20}$= 13.37, df=41, *P=.05*) and in PFC ($^{ERK1}t_{IMI20}$= 9.64, df=41, *P=.05*; $^{ERK2}t_{IMI20}$= 11.45, df=41, *P=.05* respectively) when compared to CUS induced rats.

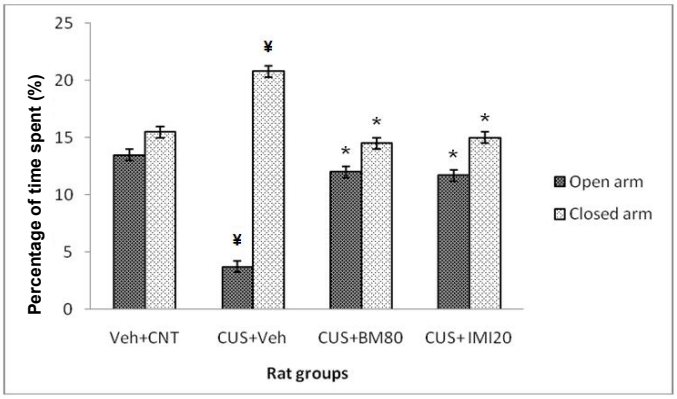

Fig. 4. The effect of BM on the percentage of time spent in open and closed arms in elevated plus maze test in CUS exposed rats. Values given are the mean±SEM (n=8). Results shown above are based on statistical comparison, using unpaired Student's t test.

**P=.05 as compared with the CUS group, ¥ P=.05 as compared with the Vehicle control group*

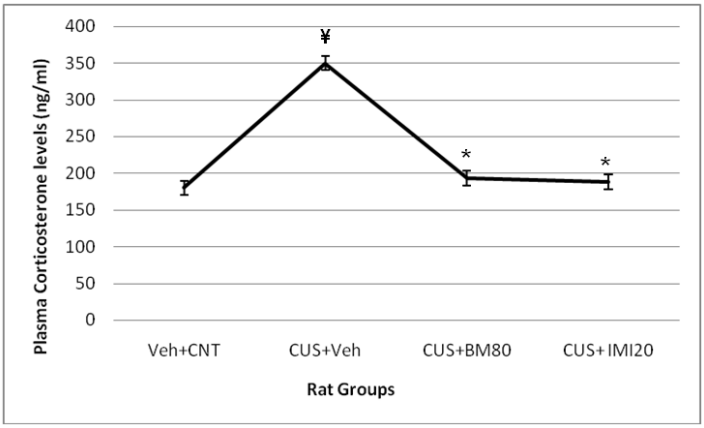

Fig. 5. The effect of BM on plasma corticosterone levels of CUS exposed rats. Values given are the mean ±SEM (n = 8). Results shown above are based on statistical comparison, using unpaired Student's t test.

**P=.05 as compared with the CUS group, ¥ P=.05 as compared with the Vehicle control group*

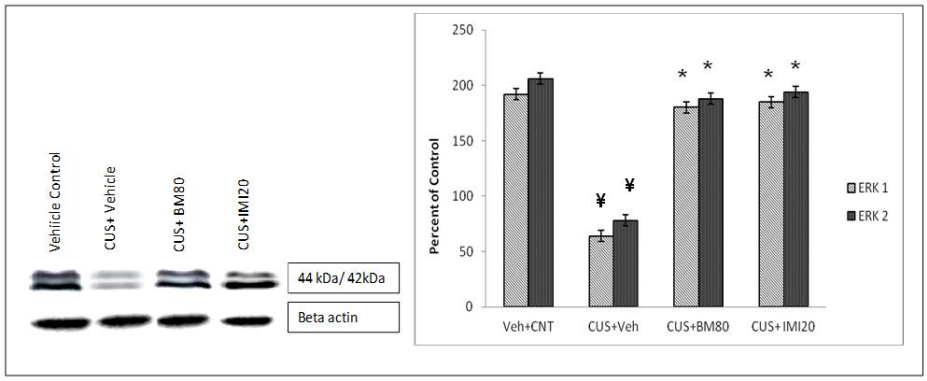

Fig. 6. The effect of BM on ERK 1 and ERK 2 protein levels in the hippocampus of CUS exposed rats. Values given are the mean±SEM (n=8). Results shown above are based on statistical comparison, using unpaired Student's t test.

**P=.05 as compared with the CUS group, ¥ P=.05 as compared with the Vehicle control group*

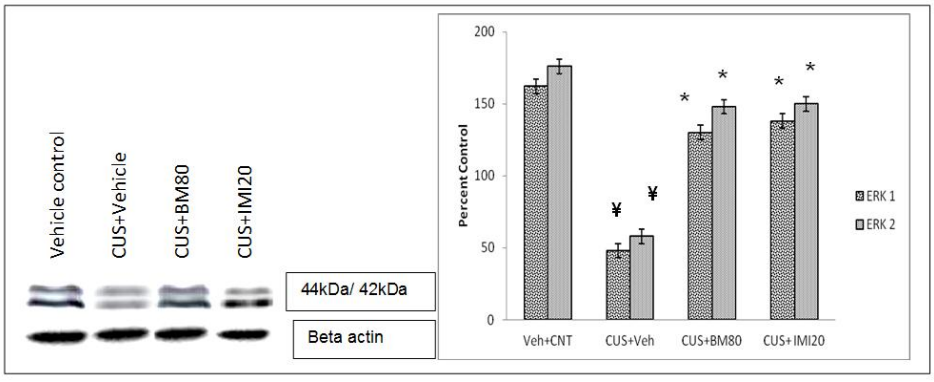

Fig. 7. The effect of BM on ERK 1 and ERK 2 protein levels in the PFC of CUS exposed rats. Values given are the mean±SEM (n=8). Results shown above are based on statistical comparison, using unpaired Student's t test.

**P=.05 as compared with the CUS group, ¥ P=.05 as compared with the Vehicle control group*

Figs. 8 and 9 shows that for the levels of pERK1 and pERK2, one-way ANOVA indicated significant differences among groups both in hippocampus ($^{ERK1}F_{3,47}$=14.92, *P=.05*, and $^{ERK2}F_{3,47}$= 9.33, *P=.05*) and in PFC ($^{ERK1}F_{3,47}$=4.65, *P=.05*, and $^{ERK2}F_{3,47}$= 6.21, *P=.05*) among all rat groups. After four weeks of CUS, pERK1 and pERK2 was significantly decreased in hippocampus ($^{pERK1}t_{CUS}$= 7.33, df=41, *P=.05*; $^{pERK2}t_{CUS}$= 12.19, df=41, *P=.05*) and in PFC ($^{ERK1}t_{CUS}$= 10.24, df=41, *P=.05*; $^{ERK2}t_{CUS}$= 8.43, df=41, *P=.05*). Chronic administration of BM 80 significantly increased pERK1 and pERK2 level both in the hippocampus ($^{pERK1}t_{BM80}$= 16.17, df=41, *P=.05*; $^{pERK2}t_{BM80}$= 18.65, df=41, *P=.05*) and in the prefrontal cortex ($^{Hippo}t_{BM80}$= 14.16, df=41, *P=.05*; $^{PFC}t_{BM80}$= 19.57, df=41, *P=.05* respectively) in comparison to CUS group of rats. Positive control IMI 20 also shows similar effect of reversing ERK1 and ERK2 significantly in hippocampus ($^{pERK1}t_{IMI20}$= 9.81, df=41, *P=.05*; $^{pERK2}t_{IMI20}$= 11.72, df=41, *P=.05*) and in PFC ($^{pERK1}t_{IMI20}$= 7.25, df=41, *P=.05*; $^{pERK2}t_{IMI20}$= 8.54, df=41, *P=.05* respectively) when compared to CUS induced rats.

4. DISCUSSION

As advocated CUS induced depression in rodents resemble the symptoms of clinical depression, so it can be employed to assess the efficacy of antidepressant through behavioral tests including the sucrose preference, open field and elevated plus maze [1,2,22,23,24,25,26].

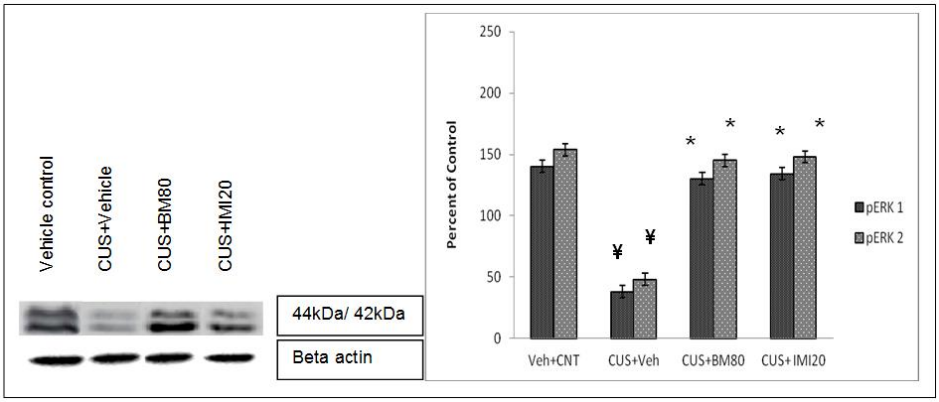

Fig. 8. The effect of BM on P-ERK 1 and P-ERK 2 protein levels in the hippocampus of CUS exposed rats. Values given are the mean±SEM (n=8). Results shown above are based on statistical comparison, using unpaired Student's t test.

**P=.05 as compared with the CUS group, ¥ P=.05 as compared with the Vehicle control group*

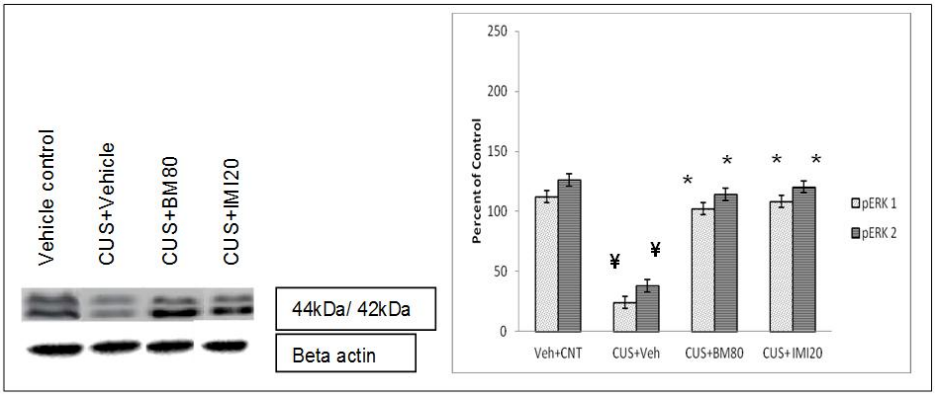

Fig. 9. The Effect of BM on P-ERK 1 and P-ERK 2 protein levels in the PFC of CUS exposed rats. Values given are the mean±SEM (n=8). Results shown above are based on statistical comparison, using unpaired Student's t test.

**P=.05 as compared with the CUS group, ¥ P=.05 as compared with the Vehicle control group*

The present results demonstrated that CUS induced depressive like behavior in rats, and chronically administered *Bacopa monniera* (80 mg/ kg b.w.) reversed almost all the behavioral alterations observed in the model. Imipramine was used as a positive control which showed similar effects like BM. ERK1, ERK2 and pERK1, pERK2 levels in hippocampus and PFC were restored by both BM and IMI doses.

Anhedonia like behavioral change was assessed by sucrose preference test [1,8,23,25]. Rats consumed less sucrose solution compared to vehicle controlled rats due to 28 days period of CUS. Behavioral alterations were suppressed by administering BM that indicates antidepressant like action of the herbal product. In general control animals show increased locomotor activity in a novel open field [1,2,8]. Behavioral changes were observed in rats due to CUS. Locomotor activities were decreased in a novel open field that points out certain aspects of refractory depression, or loss of interest [23]. This behavior also significantly altered by chronic administration of BM and indicates antidepressant like effect of it. The elevated plus maze test is used to study anxiety in a novel open space. Vehicle control animals show reduced entries in open arms, shorter time spent in open arms than the closed arms that indicates anxiety. It is further affected when rats are subjected for CUS [5,6,7,27]. After administration of BM and IMI, the behavior was reversed and animals spent more time in open arm than the CUS group of rats, which certainly identifies BM have anti anxiolytic like effect of it.

HPA axis is being activated leading to increased plasma corticosterone level after its release from the adrenal cortex [28]. This increased plasma corticosterone level has thus been considered as a well known peripheral biomarker to assess the degree of physiological response of stress [2,8]. In the present study, significant increase of plasma corticosterone level following CUS established that present rat model indicates reliability of the model to study the antidepressant effect of BM. Plasma corticosterone levels (ng/ml) were measured and it shows that CUS caused an elevation of plasma corticosterone level compare to vehicle control group rats. Administration of BM 80 showed itself most significant in decreasing of plasma corticosterone compared to CUS group rats.

It was reported from our laboratory and published that hippocampus and PFC are key limbic regions of brain that are affected by stress responses and critically involved in the regulation of depression [1,6,29,30]. Recently, several studies investigated the direct role for the ERK pathway in the pathophysiology of depressive-like behavior [31,32] and our results corroborated with it. Bcl-2, BAD, CREB, and BDNF are targets downstream of ERK/RSK and play key roles in neuronal development, neuronal survival, and long-term neuronal plasticity [33,34,35]. *BM* increased the ERK1 and ERK2 in the hippocampus and prefrontal cortex and reversed the stress-induced disruption of the P-ERK1 and P-ERK2, which is indicated by the increased level of the P-ERK1 and P-ERK2 in the hippocampus and prefrontal cortex in CUS plus BM group compared to CUS group. Recent publications indicate that lithium and valproate largely used for the treatment of bipolar disorder illness, stimulate the ERK pathway [32] but the effect exerted by *BM* on the ERK signal system in brain has been poorly documented. To our knowledge there are very few studies to date to investigate the effect of *BM* on the CUS-induced alterations in ERK pathway, and the present study demonstrates that *BM* reverses the stress induced decrease of ERK1, ERK2 and also P-ERK1, P-ERK2 in the hippocampus and prefrontal cortex. Thus ERK pathway, as a potential molecular mechanism underlying functional regulations, its disruption in the hippocampus and prefrontal cortex may produce anxiety-like behavior and locomotor impairment, respectively.

5. CONCLUSION

In conclusion, our study demonstrates the disrupted activities of the ERK and P-ERK in hippocampus and PFC in rats that displayed depressive-like behaviors after receiving CUS. The reduction in the activities of the ERK in both the regions and the depressive-like behaviors exhibited in CUS induced rats were reversed by chronic BM treatment. Herein, the data advocate that somehow ERK signal system may implicate in mediating stress response, and the neuronal mechanism of depression. Moreover, studies are needed to examine the relation between neurotrophins and ERK in depression and whether may be involved in the mechanism by which BM is effective as an anti- depressive therapeutic agent.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "All experiments conformed to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA, New Delhi Reg. No.: 1148/PO/ac/07/CPCSEA dated 13.02.2012). The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Raja Peary Mohan College, Uttarpara, Hooghly (W.B.), India."

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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