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Bacopa monniera Treatment Reverses Chronic Unpredictable Stress Induced Depressive like Behavior by Increasing Expression of Neurotrophins in Rat Brain

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ABSTRACT

Role of *Bacopa monniera* in retaining memory & learning is well known. Some recent observations have also indicated its possible role as antidepressant. However, conclusive evidences are still lacking. Therefore, in the present investigation we aim to investigate the therapeutic efficacy of *Bacopa monniera* in alleviating the symptoms of stress related disorders in rats exposed to chronic unpredictable stress. Graded doses of *Bacopa monniera* were applied on CUS exposed rats. Sucrose preference test and open field exploratory behavioral test were used to assess the stress related behaviors. Furthermore, the physiological response of stress effects assessed by measuring plasma corticosterone level by RIA and ELISA was carried on to estimate endogenous BDNF and NGF protein levels in hippocampus and pre frontal cortex. Following exposure of 4 weeks chronic unpredictable stress and treatment with different doses of *Bacopa monniera* showed 120 mg/kg body weight to be significantly effective in ameliorating the behavioral and biochemical responses of chronic stress in rats. Therefore, the present study indicates the antidepressant property of *Bacopa monniera* and it also shows translational value of *Bacopa monniera* treatment modality as therapeutic approach to combat stress induced depression.

Keywords: *Bacopa monniera*, BDNF, Corticosterone, Depression, NGF.

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INTRODUCTION

Depression is a common and potentially life threatening condition that affect individuals over the course of their lifetime and causes various mental and physical problems that sometimes lead to suicidal attempts with high rate of morbidity and high risk of mortality [1-2]. According to World Health Organization, depression is now the fourth most prevalent cause of loss of manpower and it will become the second by the year 2020 [3-4]. At present, several antidepressant drugs are clinically used such as MAOIs, SSRIs, SNRIs and NRIs but most of these drugs have unwanted side effects [5-6] and also these drugs only produce remission in 30% of patients because multiple pathogenic factors are involved in depression. Therefore, instead of using these drugs, seeking safe and effective antidepressant drugs from traditional herbs having properties to combat both anxiety and depression with lesser side effects might be useful for such clinical conditions [6-7].

It has been suggested that neuronal atrophy in the hippocampus and cortex is involved in the pathogenesis of depression [8]. 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 neurons [9]. It may be the important factor involved in the development and treatment of depression. Brain-derived neurotrophic factor (BDNF) and Nerve growth factor (NGF) are the most abundant neurotrophins in the central nervous system [10-11]. Previous studies revealed that neurotrophins in the hippocampus and cortex have been involved in the pathophysiology of stress-related behavior and depression. Several studies have reported that antidepressant treatments might exert beneficial action by regulating synthesis and/or release of BDNF or NGF in the hippocampus and cortex [8],[10]. BDNF and NGF in the hippocampus and cortex can serve as growth factors for monitoring the development and therapeutic intervention of stress induced depression and neuropsychiatric disorders.

Bacopa monniera (BM) is a perennial creeping annual plant found throughout the India in wet, damp and marshy areas. Commonly known as Brahmi, the plant has been used to increase intellect and memory for almost 3000 years by ayurvedic medical practitioners. Triterpenoid, saponins, bacosides, bacopasides present in BM are considered to be responsible for enhancing cognitive functions which helps to enhance memory [12-13]. Bacopasaponins constituents have been shown to facilitate mental retention in avoidance response in rats, and to reverse amnesic effects of neurotoxin, scopolamine, phenytoin, electroshock, and immobilization stress [14-15]. Previous studies reported that BM has potent neuropsycopharmacological activities in stress induced depression in rats [7],[16]. According to pharmacological profile of BM, it is reasonable to assume that the extract may also have some neuroprotective activities [17-18].

Chronic unpredictable stress (CUS) induced depression of animal model can be used for evaluating the efficacy of antidepressant candidates through behavioral tests including widely validated the open field test and sucrose preference tests [4],[19]. Exposure to CUS produces deficits in locomotors activity and sucrose consumption. Normal animals show increased

locomotors and exploration activity while chronic stressed animals show decreased activity in a novel open field. Anhedonia is reflected by reduced consumption or preference to sweetened solutions [20]. 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 [8]. Therefore, the present study was designed to validate the effects of BM extract (40, 80, 120 mg/kg b.w.) p.o. on stress induced model of behavioral depression in rats.

MATERIALS AND METHODS

Animals:

Male Sprague-Dawley rats were used in the current experiment. At the start of the experiment, rats were of the same age (approximately 2 months) weighing 224±1.5 gm. All rats were individually housed in temperature controlled (22–24°C) room for at least 1 week prior to the experimentation, with *ad libitum* access to food and water. Rats were maintained on a 12 h light/dark cycle (lights on at 6 a.m. off at 6 p.m.). All experimental protocols were designed to minimize the number of animals and sufferings were approved by the Institutional Animal Ethics Committee (IAEC) of the Raja Peary Mohan College, Uttarpara, Hooghly, West Bengal, India. Socially housed male rats were randomly assigned to 6 experimental groups prior to the experiment.

Drugs:

BM Extract (≥40% w/w) was purchased from Natural Remedies Pvt. Ltd., Bangalore, India and prepared the solution by dissolving 450 mg of dried powder in 80 ml saline water (0.9%) and used for the study. Imipramine hydrochloride (IMI), a tricyclic antidepressant, was purchased from Sigma–Aldrich (MO, USA), prepared the solution by dissolving in saline water (0.9%) and used as positive control for antidepressant action. All other reagents and solvents were of analytical grade.

CUS procedures:

Rats were randomly selected to 6 groups, each group having 8 individuals. The groups were: vehicle control, vehicle plus CUS, CUS plus BM 40, CUS plus BM 80, CUS plus BM 120 and CUS plus IMI. The CUS group rats were exposed to various types of unpredictable stressors for consecutive 28 days (vide Table 1). One of these stressors selected randomly and was given every day between 9.00 a.m. to 12 a.m. to CUS exposed rats for consecutive 28 days. We have administered graded doses of BM using 40, 80 and 120 mg/ kg body weight and CUS plus IMI group was given 20 mg/ kg (IMI) (p.o.). BM and IMI were administered intra-gastrically with the help of a specially designed feeding needle, 1 hour before each stressor applied once daily at the same time (8 a.m.–9 a.m.) for 28 days.

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Table 1: Chronic unpredictable stressors (CUS) experimental schedule

No. of days	Stressors
Day 1	30 inescapable foot shocks (0.8 mA intensity and 15 s duration with interval of 45 s) for 30 minutes.
Day 2	48 hours food deprivation
Day 3	24 hour water deprivation
Day 4	5 minutes cold water swim (4ºC)
Day 5	60 min restraint
Day 6	overnight illumination
Day 7	24 hr social defeat
Day 8	24 hour water deprivation
Day 9	30 inescapable foot shocks (0.8 mA intensity and 15 s duration with interval of 45 s) for 30 minutes.
Day 10	48 hours food deprivation
Day 11	24 hr social defeat
Day 12	60 min restraint
Day 13	30 inescapable foot shocks (0.8 mA intensity and 15 s duration with interval of 45 s) for 30 minutes.
Day 14	overnight illumination
Day 15	5 minutes cold water swim (4ºC)
Day 16	24 hr social defeat
Day 17	48 hours food deprivation
Day 18	5 minutes cold water swim (4ºC)
Day 19	30 inescapable foot shocks (0.8 mA intensity and 15 s duration with interval of 45 s) for 30 minutes.
Day 20	24 hour water deprivation
Day 21	overnight illumination
Day 22	24 hr social defeat
Day 23	30 inescapable foot shocks (0.8 mA intensity and 15 s duration with interval of 45 s) for 30 minutes.
Day 24	5 minutes cold water swim (4ºC)
Day 25	48 hours food deprivation
Day 26	60 min restraint
Day 27	overnight illumination
Day 28	60 min restraint

Open Field Test:

The open field test was conducted at the beginning and the end of the CUS procedure. The open-field apparatus consists of a square wooden arena 100 cm ×100 cm, with a 50-cm-high side wall, the floor marked with a grid dividing it into 25 equal size squares. Rats were placed in the central square for exploration and observed for 5 min. Number of square crossing (with at least three paws) and No. of rearing (standing upright on hind legs) was recorded. The apparatus was cleaned with alcohol and dried before and after each experiment.

Sucrose Preference Test:

Rats were deprived of water for 24 hours. After that each rat was placed in a separate cage having two bottles; one containing 100 ml water and another containing 100 ml 1% sucrose solution. After 1 hr both the amount of water and sucrose solution was measured and recorded. The ratio of amount of sucrose solution to that of total solution consumed within 1 hr represented the parameter of hedonic behavior. Sucrose preference test was carried at the start and end of the 28 days CUS exposure and performed during 9 a.m. - 12 a.m.

Determination of Plasma Corticosterone levels:

Plasma corticosterone levels were measured in all six 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 kit (ICN Biomedical, Costa Mesa, CA, USA).

Measurement of BDNF & NGF protein levels in the hippocampus and frontal cortex:

Endogenous BDNF & NGF levels were measured in hippocampus and frontal cortex using enzyme linked immunosorbent assay (ELISA) kit according to the manufacturer instructions (Chemicon, USA). Hippocampus and frontal cortex were immediately isolated after anesthesia was over. Briefly, hippocampus and frontal cortex were homogenized in phosphate buffer solution (PBS) with 1 mM phenylmethylsulfonyl fluoride (PMSF) and 1 mM EGTA. Microtiter plates (96-well flat bottom) were coated for 24 h with the sample diluted 1:2 in sample diluent. The plates were then washed four times with sample diluent, and a monoclonal anti-BDNF and anti-NGF rabbit antibody diluted to 1:1000 sample diluent was added to each well and incubated for 3 h at room temperature. After washing, a peroxidase conjugated anti-rabbit antibody (diluted 1:1000) was added to each well and incubated at room temperature for 2 h. After addition of streptavidin-enzyme, substrate was added followed by stop solution. The amounts of BDNF & NGF were determined by absorbance in 450 nm (Tecan Infinite M200). A standard curve was produced and it ranged from 7.8 to 500 pg/mL of BDNF and NGF. This curve was obtained from a direct relationship between optical density and BDNF concentration. Total protein concentration was measured by Lowry's method using bovine serum albumin (BSA) as a standard.

Statistical analysis:

The Statistical Package for the Social Science (SPSS) 15.0 was utilized for statistical analyses. All data were expressed as mean ± SEM.

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RESULTS

The Effects of BM on rats in open field exploratory behavior test:

Figure 1 and Figure 2 show the effect of BM treatment on CUS exposed rats in open field test. Open field test was carried on to assess the ambulation and rearing activity. CUS rats exhibited depressed behavior as evidenced by decreased no. of crossing (Figure 1) and rearing (Figure 2) activity compared to vehicle control group rats. Treatment with BM at 120 mg/kg significantly ($p<0.05$) reverses the CUS induced behavioral changes as observed by increased no. of crossing and rearing activity in comparison to CUS exposed rats. IMI treatment (20 mg/kg) as positive control also significantly ($p<0.05$) alters the number of crossing and rearing in CUS exposed rats.

Figure 1: Effect of BM treatment on the number of crossings in the open field test of CUS exposed rats. Values given are the mean ±SEM (n = 8).*p<0.05 as compared with the CUS group.

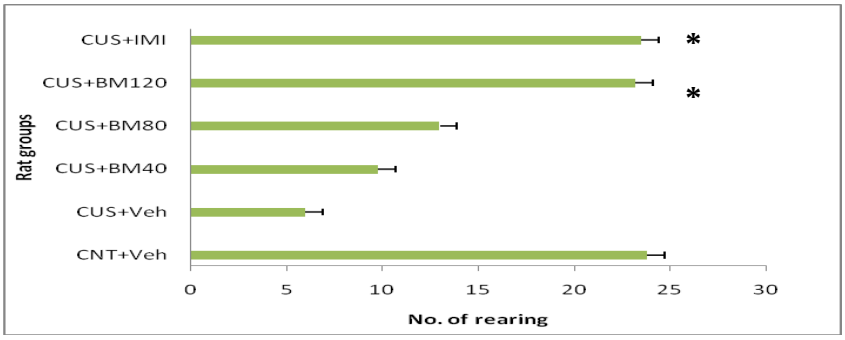

Figure 2: Effect of BM treatment on the numbers of rearing in the open field test of CUS exposed rats. Values given are the mean ±SEM (n = 8).*p<0.05 as compared with the CUS group.

The Effects of BM on rats in sucrose preference test:

Figure 3 show that rats exhibit no significant variation in preference to sucrose solution before the CUS procedure. Following 4 weeks of CUS showed decreased percentage of sucrose consumption in CUS exposed rats compare to vehicle control group rats. Treatment with BM at 80 and 120 mg/kg body weight significantly ($p<0.05$) increases the percentage of sucrose consumption compare to CUS group rats. IMI treatment (20 mg/kg) as positive control also

significantly ($p<0.05$) increases the percentage of sucrose consumption in CUS exposed rats. However, treatment with BM 120 mg/kg dose did not completely reverse the sucrose preference to the baseline.

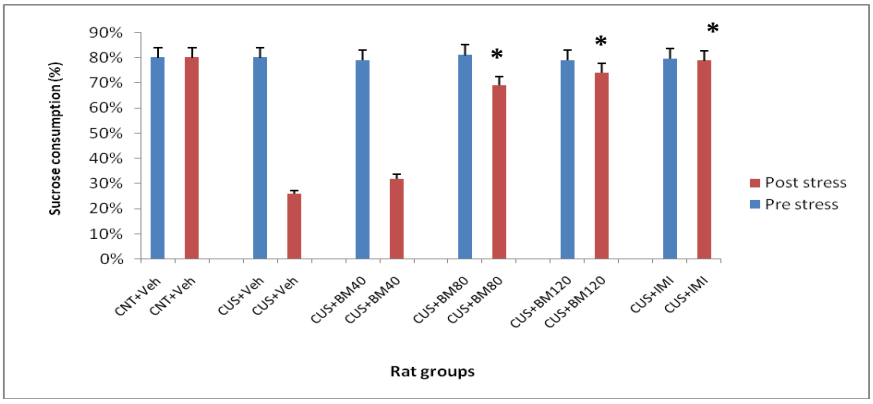

Figure 3: Effect of BM treatment on the level of sucrose consumption of CUS exposed rats. Values given are the mean ±SEM (n = 8).*p<0.05 as compared with the CUS plus Vehicle group.

The Effects of BM on serum corticosterone levels:

CUS group rats showed the elevation in serum corticosterone level due to 4 weeks of CUS in comparison to vehicle control groups (Figure 4). The stress induced increase of serum corticosterone levels were significantly ($p<0.05$) reduced in rats those were treated with BM at 120 mg/kg dose or IMI (20 mg/kg) compare to CUS group rats. The reductions were robust, but not same as of the non-stressed control rats.

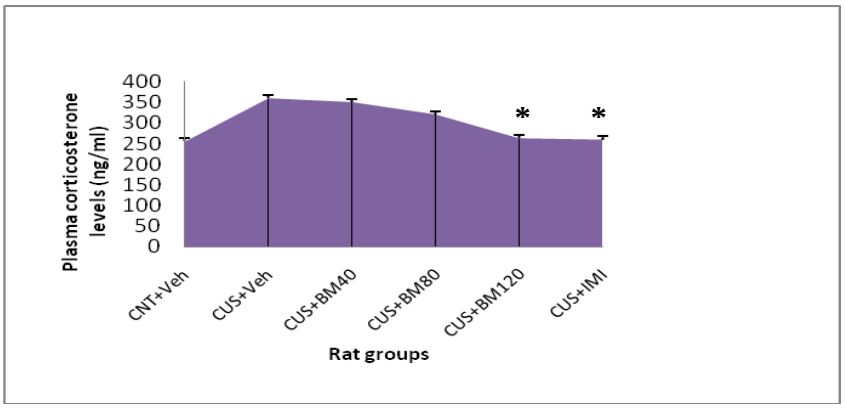

Figure 4: Effect of BM treatment on plasma corticosterone levels of CUS exposed rats. Values given are the mean ±SEM (n = 6).*p < 0.05 as compared with the CUS group.

The Effects of BM on BDNF protein levels in Hippocampus and frontal cortex:

Effect of BM treatment on BDNF protein levels in hippocampus and frontal cortex of vehicle control and CUS treated rats are shown in Figure 5 and 6 respectively. 28 days CUS

exposure decreases BDNF protein levels in both hippocampus and PFC in comparison to vehicle control group rats. CUS exposed rats, treated with BM 40 and BM 80 dose (40 and 80 mg/kg of b.w.) do not significantly restore BDNF protein levels in hippocampus and frontal cortex but BM 120 dose (120 mg/kg of b.w.) shows significant ($p<0.05$) elevation of BDNF protein levels in both hippocampus and PFC in comparison to CUS treated group rats. IMI (20 mg/kg), treated as positive control show the same alterations.

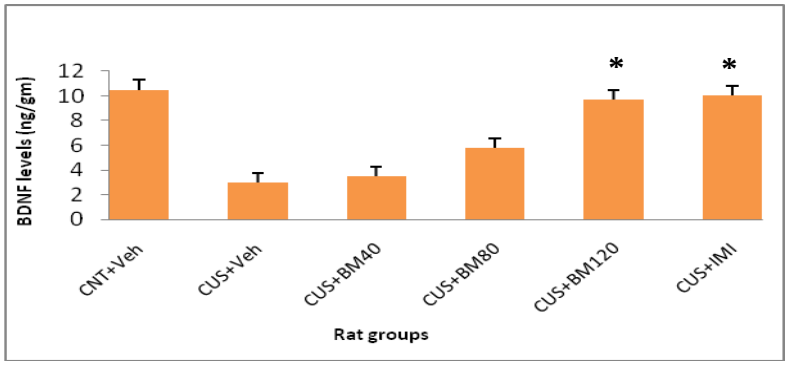

Figure 5: Effect of BM treatment on brain derived neurotrophin factor (BDNF) protein levels in the hippocampus of CUS exposed rats. Values given are the mean ±SEM (n = 6).*p < 0.05 as compared with the CUS group.

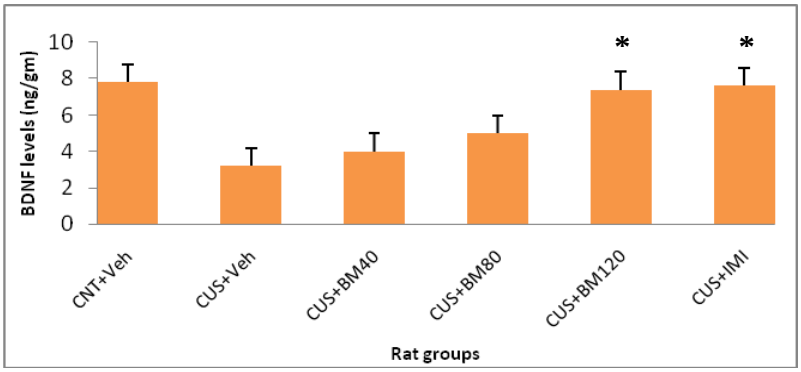

Figure 6: Effect of BM treatment on brain derived neurotrophin factor (BDNF) protein levels in the pre frontal cortex of CUS exposed rats. Values given are the mean ±SEM (n = 6).*p < 0.05 as compared with the CUS group.

Effect of BM on NGF protein levels in the frontal cortex and hippocampus:

Effect of BM treatment on NGF protein levels in the frontal cortex and hippocampus of vehicle control and CUS-treated rats are shown in Figure 7 and 8 respectively. There were no significant changes in the NGF protein levels in the hippocampus among all rats. On the other hand, the exposure to CUS caused a decrease in NGF protein levels in the frontal cortex of rats, as compared to the vehicle control group of rats. BM 40 and BM 80 did not significantly restore NGF protein levels in frontal cortex. BM treatment at the dose of 120 mg/kg b.w. significantly ($p<0.05$) increased the NGF protein levels in the frontal cortex of rats exposed to CUS in comparison to the only CUS-treated group. Treatment with IMI (20 mg/kg) also significantly ($p<0.05$) increased the NGF protein levels in the frontal cortex in CUS-treated rats.

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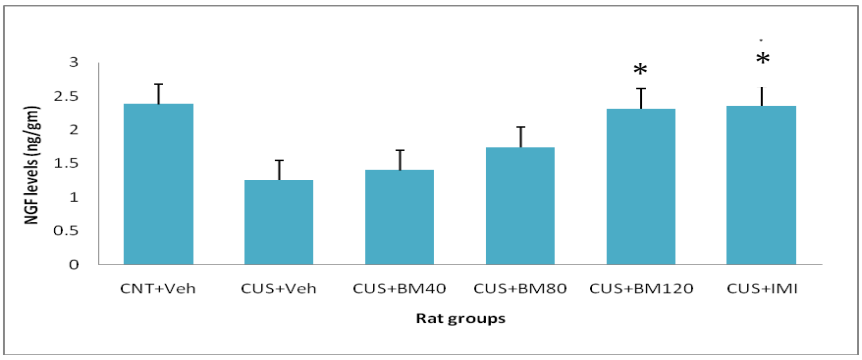

Figure 7: Effect of BM treatment on nerve growth factor (NGF) protein levels in the frontal cortex of CUS exposed rats. Values given are the mean ±SEM (n = 6).*p < 0.05 as compared with the CUS group.

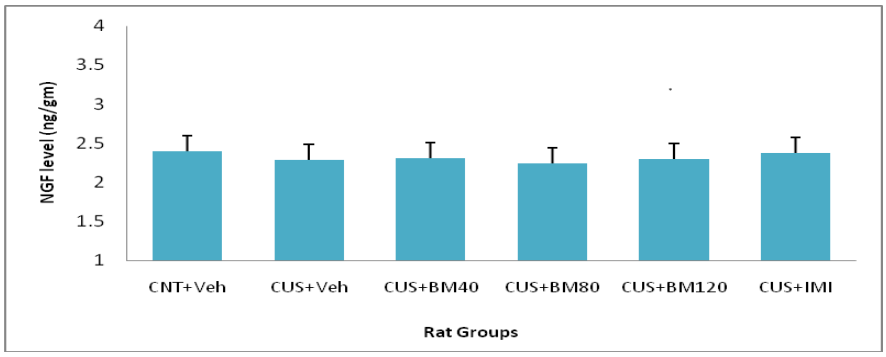

Figure 8: Effect of BM treatment on nerve growth factor (NGF) protein levels in the hippocampus of CUS exposed rats. Values given are the mean ±SEM (n = 6).

DISCUSSION

Chronic administration of various unpredictable stresses, a procedure known as chronic unpredictable stress (CUS), is an appropriate model for the experimental investigation of depression [8],[21]. In this regard, an animal model of CUS-induced depression has been developed to simulate the pathogenesis of depression. Several studies suggest that CUS can induce behavioral and physiological changes resembling symptoms of clinical depression and that CUS-induced depression model can be used for evaluating the efficacy of antidepressant drug through behavioral tests like sucrose preference, and open-field tests [22]. In the present study, we investigated the effects of BM on CUS exposed rats, using two widely validated models like sucrose preference and open field tests to assess their behavior and measuring BDNF, NGF protein levels in the hippocampus and pre frontal cortex [4]. The changes in sucrose consumption, locomotors and rearing activity, corticosterone levels and BDNF levels in both hippocampus and pre frontal cortex and NGF protein level in frontal cortex are predominant in 28 days CUS rat group. However, the stress induced changes could be reversed by chronic administration of BM in a dose dependent manner [7] showing the most significant antidepressant effect as manifested from biochemical and behavioral changes following

administration of BM 120 mg/kg treatment schedule. Effect of BM seems to be similar to IMI which is used as positive control showing its efficacy of BM as antidepressant.

Vehicle treated non-stressed control group rats show increased ambulation (Numbers of lines crossed in a novel open field) and rearing (Number of times animal stood on their hind limbs) activity indicating their instinct for exploration in a new environment. Rats prior to CUS for 28 days show decreased ambulation and rearing activity in a novel open field [4],[8]. However, chronic administration of BM at 120 mg/kg body weight significantly ($p<0.05$) restores their numbers of lines crossing and rearing activity that expressed their interest for exploration indicating again antidepressant property of BM. Taken together, results obtained from behavioral studies indicated that long term BM treatment produced an antidepressant-like action in CUS-induced depression model in rats.

Sucrose preference test is an indicator of anhedonia-like behavioral change. Anhedonia [20], a core symptom of major depression, was modeled by inducing a decrease in responsiveness to rewards reflected by a reduced consumption and/or preference of sweetened solutions. In this study, vehicle treated CUS exposed rats show less preference to sucrose solution than vehicle treated non stressed control group rats, a behavior known as anhedonia [22]. Chronic treatment of BM alters this behavioral activity among CUS exposed rats. The dose of BM 80 and 120 significantly ($p<0.05$) restores the rate of sucrose consumption which indicates the antidepressant property of BM.

It is well established that following stress exposure the HPA axis is being activated leading to increased plasma corticosterone level after its release from the adrenal cortex [23]. This increased plasma corticosterone level has thus been considered as a well known peripheral biomarker to assess the degree of physiological response of stress [8]. Therefore, in the present study, significantly ($p<0.05$) increased plasma corticosterone level following CUS established that present rodent model indicates reliability of the model to study the antidepressant effect of BM. In the present investigation, the 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. Among the three graded doses tested, administration of BM 120 showed itself most significant in down regulation of plasma corticosterone compared to untreated CUS group rats.

We found that the antidepressant-like effect of BM on CUS induced depression was associated with the expressed content of BDNF and NGF in selective brain tissues [4],[11]. Recent reports suggest that hippocampus and pre frontal cortex in brain regions are not only affected by stress response but also involved in regulation of stress, mood, anxiety and memory [20],[22]. BDNF plays an important role in pathophysiology of mood disorders, the mechanism of action of psychotropic agents, and the course of complex cognitive process such as memory [9]. BDNF seems to be one of the key molecular mediators of synaptic plasticity, and it increases the cell survival proteins which help in proliferation and maintenance of central nervous system neurons [24-25]. The result agreed with the present finding that BDNF seems to be an important factor in the development and treatment of depression [9-10].

Present study shows that rats exposed to CUS have decreased BDNF expression in both hippocampus and pre frontal cortex area [4] [11]. It also shows that BDNF protein level in the hippocampus and PFC after administration of CUS is well correlated with decreased ambulation, exploration and low preference to sweet consumption [22]. Present study shows CUS exposure was found to decrease BDNF protein and mRNA levels in the hippocampus and frontal cortex of rats. Interestingly following long term BM at 120 mg/kg body weight treatment, BDNF level in hippocampus and PFC which showed normalisation ($p<0.05$) can also be correlated with normalisation of ambulation, exploration and low preference to sweet consumption indicating the antidepressant effect of BM.

In addition, it has been well demonstrated that NGF in the frontal cortex is also involved in the pathogenesis of depression. Other researchers have reported in their study that post-mortem of a suicide victims has shown decreased NGF protein levels in the frontal cortex [4][8][11]. Present study also revealed that NGF protein level was found to be decreased in the frontal cortex of rats exposed to CUS, while long term treatment of BM at 120 mg/kg b.w. significantly ($p<0.05$) increased NGF protein levels in the frontal cortex of CUS-treated rats. On the other hand, in the present study, we found that CUS and long-term BM treatment did not alter NGF protein levels in the hippocampus of CUS-treated rats. The unchanged levels of NGF in the hippocampal area do not suggest a major contribution of NGF in the resistance to develop stress.

CONCLUSION

These preliminary results though show antidepressant property of BM, its molecular mechanism of action is yet to be ascertained and is the subject of our ongoing research in our laboratory. Since BM is already in clinical use for restoration and normalisation of memory and learning; however, the present study shows translational value of BM treatment modality as therapeutic approach to combat stress related disorders and depression.

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