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Venlafaxine reverses chronic fatigue-induced behavioral, biochemical and neurochemical alterations in mice

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Abstract

A state of chronic fatigue was produced in mice by subjecting them to forced swim inside a rectangular jar of specific dimensions everyday for a 6 min session for 15 days. Immobility period was recorded on alternate days. The effect of venlafaxine, a dual reuptake inhibitor of serotonin and norepinephrine was evaluated in this murine model of chronic fatigue. Venlafaxine was administered daily and on the days of testing, it was injected 30 min before forced swim session. On the 16th day i.e. 24 h after the last dose of venlafaxine, various behavioral, biochemical and neurotransmitter estimations in the brain were carried out. There was a significant increase in immobility period in vehicle treated mice on successive days, the maximum immobility score reaching on the 7th day and sustained till 15th day. Behavioral parameters revealed hyperlocomotion, anxiety response, muscle incoordination, hyperalgesia and memory deficit. Biochemical analysis showed a significant increase in lipid peroxidation, nitrite and myeloperoxidase levels and a decrease in the reduced glutathione (GSH) levels in brain homogenates. Further, there was a decrease in adrenal ascorbic acid following chronic forced swim. The neurotransmitter estimations in the brain samples revealed a decrease in norepinephrine, serotonin and dopamine levels on chronic exposure to forced swim for 15 days. Daily treatment with venlafaxine (8 and 16 mg/kg, i.p.) for 15 days produced a significant reduction in immobility period and reversed various behavioral, biochemical and neurotransmitter alterations induced by chronic fatigue. Venlafaxine could be of therapeutic potential in the treatment of chronic fatigue.

Keywords: Venlafaxine; Chronic fatigue; Behavioral alterations; Neurotransmitters; Oxidative stress

1. Introduction

Exposure to a stressful stimulus or chronic fatigue is perceived as a threat to the organism's homeostasis and elicits a variety of symptoms encompassing behavioral, biochemical and neurochemical aspects. Syndromes of medically unexplained chronic fatigue may include chronic fatigue syndrome (CFS) or idiopathic chronic fatigue. It is debated that the psychiatric disorders are common in both chronic fatigue (CF) and chronic fatigue syndrome (CFS) (Schmaling et al., 2003). No physical signs are specific to either CFS or CF, and there are no diagnostic tests to identify these syndromes. These syndromes are based on symptom complaints, and may be characterized as heterogeneous with multiple etiologies possibly involved (Schmaling et al., 2003). In fact, chronic fatigue includes fewer symptoms than chronic fatigue syndrome (Buchwald et al., 1997).

Chronic fatigue is an illness characterized by profound disabling fatigue accompanied by numerous neuro- and psychosomatic complaints (Roy-Byrne et al., 2002; Wessely et al., 1996). Various neuroendocrine abnormalities contribute to the impaired energy and mood in this illness (Singh et al., 2002). It is debated that too much of exhaustion, mental stress or depression can lead to the chronic fatigue. Recent studies have demonstrated the involvement of oxidative stress in the pathology and clinical symptoms of chronic fatigue (Singh et al., 2002). Because of the unclear etiology and the resultant heterogeneity of the syndrome population, there are no firmly established treatment recommendations for this condition.

Major depression is the most significant factor in the differential diagnosis of chronic fatigue (Roy-Byrne et al., 2002). In

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other words, this illness may represent a sub-syndromal form of major depression or anxiety disorder (Roy-Byrne et al., 2002). This is consistent with the previous speculations that chronic fatigue more closely resembled "atypical depression" with its prominent anxiety and somatic symptoms with poor response to conventional antidepressant therapy (Gold et al., 1995; Terman et al., 1998). On the contrary, even though patients suffering from chronic fatigue syndrome responded to the antidepressant therapy particularly to selective serotonin reuptake inhibitors, some failed to show improvement with fluoxetine in a dose of 20 mg daily (Vercoulen et al., 1996).

Venlafaxine, a novel antidepressant inhibits selectively re-uptake of both serotonin as well as norepinephrine (Redrobe et al., 1998). It exhibits six- to seven-fold selectivity for inhibition of serotonin reuptake as compared to norepinephrine reuptake in rat brain synaptosomal preparations and a 15- to 30-fold higher affinity for serotonin transporter (SERT) binding sites as compared to those of norepinephrine transporter (NET) (Gould et al., 2006). Because of its dual reuptake inhibiting properties, venlafaxine may produce a better and complete antidepressant action as compared to selective serotonin reuptake inhibitors like fluoxetine (Hellerstein et al., 1999). In the various clinical studies conducted with venlafaxine, it has shown superior efficacy to SSRIs in severe major depressive episodes, treatment-resistant depression, depressive symptom remission and obsessive compulsive disorder (Gutierrez et al., 2003, Goodnick, 1996). In one of the studies, venlafaxine in a dose range of 37.5 mg thrice a day to 75 mg thrice a day for total of 6 weeks was found to improve the symptoms of chronic fatigue syndrome (Goodnick, 1996).

The present study was carried out to assess the effect of venlafaxine in murine model of chronic fatigue. Behavioral, biochemical and neurochemical correlates of chronic fatigue and the possible mechanism of anti-fatigue action of venlafaxine were evaluated.

2. Materials and methods

2.1. Animals

Male albino mice (Laca strain) weighing between 22 and 30 g bred in Central Animal House (CAH) facility of the Panjab University, Chandigarh, India were used. The animals were housed under standard laboratory conditions and maintained on natural light and dark cycle and had free access to food and water. Animals were acclimatized to laboratory conditions before the experiment. Each animal was used only once. All the experiments were carried out between 0900 and 1500 h. The experimental protocols were approved by Institutional Animal Ethics Committee (IAEC) and conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals.

2.2. Experimental procedure

2.2.1. Measurement of immobility time

The test procedure has been earlier validated in many of our previous studies as an animal model for testing antidepressant activity (Dhir and Kulkarni, 2007a,b,c,d; Parale and Kulkarni, 1986; Kulkarni and Mehta, 1985). In brief, mice were individually forced to swim inside a rectangular glass jar ($25 \times 12 \times 25$ cm^3) containing 15 cm of water maintained at 23–25 °C; the total duration of immobility during a 6 min test was recorded with the help of stop-watch. The animal was judged to be immobile when it ceased struggling and remained floating motionless in water, making only those movements necessary to keep its head above water. The animals were forced to swim 6 min everyday for total of 15 days, but the recording of immobility period was done on alternate days.

2.3. Behavioral assessment

Various behavioral parameters were assessed in mice 24 h after the last chronic forced swim test.

2.4. Measurement of locomotor activity

Locomotor activity (ambulations) was measured by using computerized actophotometer (IMCORP) for a period of 5 min. Mice were individually placed in a transparent plastic cage ($30 \times 23 \times 22$ cm^3) and were allowed to acclimatize to the observation chamber for a period of 2 min. The locomotion was expressed in terms of total counts per 5 min per animal (Dhir et al., 2005; Akula et al., 2007).

2.5. Rota-rod test

Mice were subjected to motor function evaluation by placing them individually on rota rod, which was adjusted to the speed of 25 rpm. The fall-off time was recorded for each mouse and the longest period any animal was kept on the rod was 300 s (Singh and Kulkarni, 2002).

2.6. Measurement of anxiety in the mirror chamber

The anxiety behavior was measured using the mirror chamber. During the 5 min test session the following parameters was noted (i) latency to enter the mirror chamber (ii) the total time spent in mirror chamber, and (iii) the number of entries animal made into the mirror chamber. Animals were placed individually at the distal corner of the mirror chamber at the beginning of the test. An anxiogenic response was defined as decreased number of entries and time spent in the mirror chamber (Kulkarni and Reddy, 1996).

2.7. Measurement of cognitive behavior using plus-maze test

Cognitive behavior was noted by using elevated plus-maze learning task (Reddy and Kulkarni, 1998). Transfer latency (TL) that is the time taken by the animal to move from the open arm to enclosed arm, was considered as an index of learned task (memory process). The elevated plus maze consisted of two open arms (50×10 cm) and two closed arms ($50 \times 10 \times 40$ cm) with an open roof. The maze was elevated to a height of 25 cm from the floor. The animal was placed individually at the end of either of the open arms and the initial transfer latency was noted

on the first day. If the animal did not enter an enclosed arm within 90 s, it was gently pushed in to the enclosed arm and the transfer latency was assigned as 90 s. To become acquainted with the maze, the animals were allowed to explore the plus maze for 20 s after reaching the closed arm and then returned to its home cage. Retention of the learned task was assessed 24 h after the 1st day trial and % retention of the memory was calculated from the initial transfer latency.

2.8. Measurement of hyperalgesia

Tail-immersion test was used to assess hyperalgesic effect in mice. Each mouse was placed individually in restrainer leaving the tail hanging out freely. The terminal 1 cm part of the tail was immersed in a water bath maintained at 47 °C. The withdrawal latency was defined as the time for the animal to withdraw its tail from water. A cut-off time of 15 s was used to prevent damage to the tail (Dhir et al., 2005). Hyperalgesic response was defined when the average latency of tail flick response was significantly decreased compared to that of unstressed animals measured on day 16.

2.9. Biochemical parameters

All the animals were sacrificed by decapitation on day 16 following behavioral assessment. The brains removed, rinsed in isotonic saline and weighed. A 10% (w/v) tissue homogenate was prepared with 0.1 M phosphate buffer (pH 7.4). The post nuclear fraction was obtained by centrifugation of the homogenate at 12,000 ×*g* for 20 min at 4 °C.

2.10. Lipid peroxidation assay

The quantitative measurement of lipid peroxidation in the whole brain was measured according to the method of Wills (Wills, 1966). The amount of malondialdehyde (MDA) formed was measured by the reaction with thiobarbituric acid at 532 nm using Perkin Elmer lambda 20 spectrophotometer. The results were expressed as nmol of MDA/mg protein using the molar extinction coefficient of chromophore ($1.56 \times 10\ M^{-1}\ cm^{-1}$).

2.11. Estimation of reduced glutathione (GSH)

Reduced glutathione in the brain was estimated according to the method of Ellman (Ellman, 1959). A 0.75 ml of homogenate was precipitated with 0.75 ml of 4% sulfosalicylic acid by keeping the mixture at 4 °C for 1 h and the samples were immediately centrifuged at 1200 ×g for 15 min at 4 °C. The assay mixture contains 0.5 ml of supernatant and 4.5 ml of 0.01 M dithiobisnitrobenzoic acid (DTNB). The yellow color developed was read immediately at 412 nm using Perkin Elmer lambda 20 spectrophotometer. The results were expressed as nmol GSH per mg protein.

2.12. Nitrite estimation

Nitrite (NO^{2-}) is the stable end product of nitric oxide (NO) in living system. Accumulation of nitrite was measured in cell-free supernatants from brain homogenate by spectrophotometer assay based on Greiss reaction (Green et al., 1982). Briefly, the supernatant of brain homogenate was mixed with equal volume of Greiss reagent (1% sulphanilamide/0.1% naphthylethylene-diamine dihydrochloride/2.5% phosphoric acid) and incubated at room temperature for 10 min to yield a chromophore. Absorbance was read at 543 nm spectrophotometrically. The nitrite concentration was calculated from a standard curve using sodium nitrite as standard and expressed as micromolar nitrite per milliliter homogenate.

2.13. Adrenal ascorbic acid

The adrenal glands removed, rinsed in isotonic saline and weighed. A 1% (w/v) tissue homogenate was prepared with 0.1 M phosphate buffer (pH 7.4) and centrifuged at 12,000 ×g for 10 min, at 4 °C. The adrenal ascorbic acid levels were determined by 2, 4-dinitrophenyl hydrazine method described by Roe and Kuether (Roe and Kuether, 1943) and expressed as microgram of ascorbic acid per mg of adrenal tissue.

2.14. Protein estimation

The protein content was measured according to the method of Lowry (Lowry et al., 1951) using bovine serum albumin as standard.

2.15. Measurement of biogenic amines

Biogenic amines (norepinephrine, serotonin and dopamine) were estimated by High Performance Liquid Chromatography (HPLC) with Electrochemical Detector (ECD) by the method of Beyer et al. (2002). Waters® standard system consisting of a high pressure isocratic pump, a 20 µl sample injector valve, C18 reverse phase column and electrochemical detector. Data was recorded and analyzed with the help of Empower software. Mobile phase consists of 0.15 M sodium dihydrogen phosphate, 0.25 mM ethylenediaminetetraacetic acid, 1.75 mM 1-octane sulfonic acid, 2% isopropanol and 4% methanol (pH 4.8). Electrochemical conditions for the experiment were +0.800 V, sensitivity ranges from 1–100 nA. Separation was carried out at a flow rate of 1 ml/min. Samples (20 µl) were injected manually. Whole brain was homogenized in homogenizing solution containing 0.1 M perchloric acid. After that samples were centrifuged at 24,000 g for 15 min. The supernatant was further filtered through 0.25 µm nylon filters before injecting in the High Performance Liquid Chromatography injection pump. Data was recorded and analyzed with the help of Empower® software provided by Waters®.

2.16. Drugs and treatment

Venlafaxine (Panacea Biotec, Punjab, India) was dissolved in distilled water and it was administered intraperitoneally at two doses, 8 and 16 mg/kg was administered intraperitoneally daily 30 min before the animals were subjected to forced swim for a total of 15 days. The selection of doses was according to the

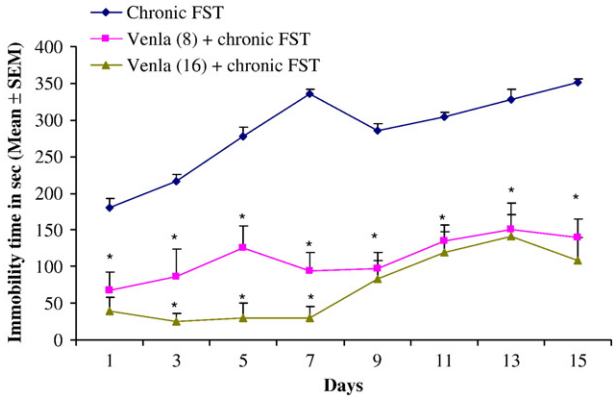

Fig. 1. Effect of chronic administration of venlafaxine (8 and 16 mg/kg i.p.) on mean immobility period on alternate days 1 through 15 in mice subjected to chronic swimming. *$P<0.05$ as compared to chronic forced swim test (FST) control group (ANOVA followed by Dunnett's test) ($n=6–8$).

previous studies reported from our laboratory (Dhir and Kulkarni, 2007b,c; Kulkarni and Dhir, 2007).

2.17. Statistical analysis

Results are expressed as mean±S.E.M. The significance of the difference in the responses of treatment groups in comparison to the control was determined by One Way Analysis of Variance (ANOVA) followed by Dunnett's test. $P<0.05$ was considered statistically significant.

3. Results

3.1. Reversal by venlafaxine (8 and 16 mg/kg., i.p.) of chronic forced swim-induced immobility period in mice

Chronic exposure to forced swimming produced a significant increase in immobility period in control mice, the maximum response attained on day 7 and maintained till the 15th day. Daily administration of venlafaxine (8 and 16 mg/kg., i.p.) for 15 days 30 min prior to forced swim test (FST) reversed the mean immobility period as assessed on alternate days, i.e., 1, 3, 5, 7, 9, 11, 13 and 15th day of the study, respectively (Fig. 1).

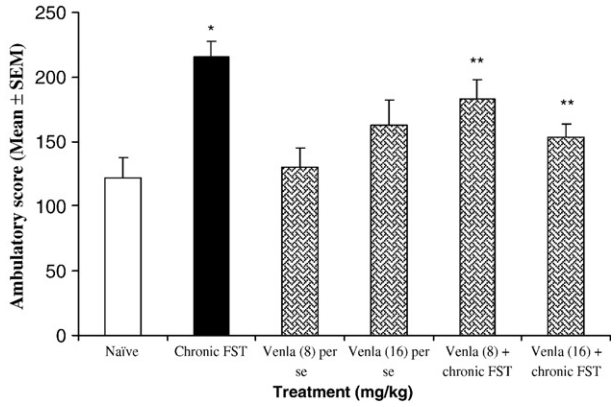

Fig. 2. Effect of chronic administration of venlafaxine (8 and 16 mg/kg i.p.) on chronic swimming-induced locomotor activity. Ambulatory activity was assessed on the 16th day of the study. *$P<0.05$ as compared to naive group. **$P<0.05$ as compared to chronic forced swim test (FST) control group (ANOVA followed by Dunnett's test) ($n=6–8$).

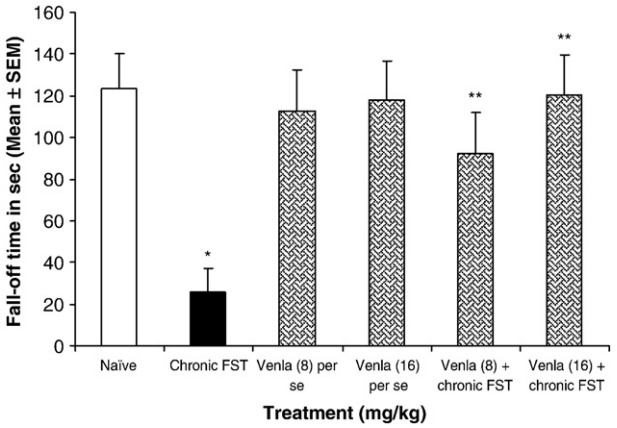

Fig. 3. Effect of chronic administration of venlafaxine (8 and 16 mg/kg i.p.) on chronic swimming-induced mean fall-off time in rota-rod test. Mean fall-off time was assessed on the 16th day of the study. *$P<0.05$ as compared to naive group. **$P<0.05$ as compared to chronic forced swim test (FST) control group (ANOVA followed by Dunnett's test) ($n=6–8$).

3.2. Behavioral assessment

The animals chronically exposed to forced swimming, showed an increase in the locomotor activity compared to unstressed mice. While venlafaxine *per se* groups did not show any significant change in the locomotor activity but its chronic treatment (8 and 16 mg/kg, i.p. for 15 days) decreased the ambulatory scores in chronic forced swim test (Fig. 2).

Similarly, animals chronically stressed to FST showed a significant decrease in the fall-off time as compared to vehicle treated group, thus displaying muscle incoordination. Daily

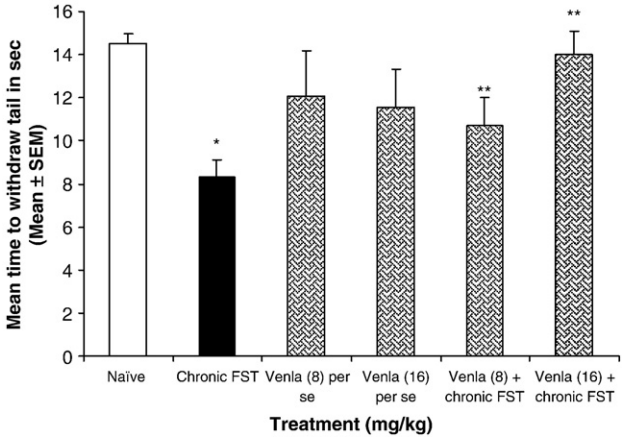

Fig. 4. Effect of chronic administration of venlafaxine (8 and 16 mg/kg i.p.) on chronic swimming-induced hyperalgesia assessed by tail-immersion test. The tail withdrawal latencies were assessed on the 16th day of the study. *$P<0.05$ as compared to naive group. **$P<0.05$ as compared to chronic forced swim test (FST) control group (ANOVA followed by Dunnett's test) ($n=6–8$).

treatment with venlafaxine (8 and 16 mg/kg, i.p. for 15 days) before the exposure increased the mean fall-off time as compared to chronic FST group, but the effect was not dose-dependent (Fig. 3).

Animals chronically subjected to FST showed a significant decrease in tail withdrawal latency indicating hyperalgesia as compared to unstressed mice. Chronic treatment with venlafaxine (8 and 16 mg/kg, i.p. for 15 days) significantly attenuated the development of the hyperalgesia in chronically stressed animals (Fig. 4).

Chronic fatigue produced anxiety response in mice as the latency to enter the mirror chamber was significantly increased (Fig. 5A), decreased the number of entries (Fig. 5B), and also decreased the mean time spent in the mirror chamber (Fig. 5A) as compared to unstressed mice. Daily treatment with venlafaxine (8 and 16 mg/kg, i.p. for 15 days) reversed these responses in chronically exposed mice.

Similarly, chronic fatigue significantly decreased percent retention of memory in mice as compared to unstressed mice which was reversed by chronic administration of venlafaxine (8 and 16 mg/kg, i.p. for 15 days) (Fig. 6).

Daily treatment with venlafaxine (8 and 16 mg/kg, i.p. for 15 days) *per se* did not affect all the behavioral parameters.

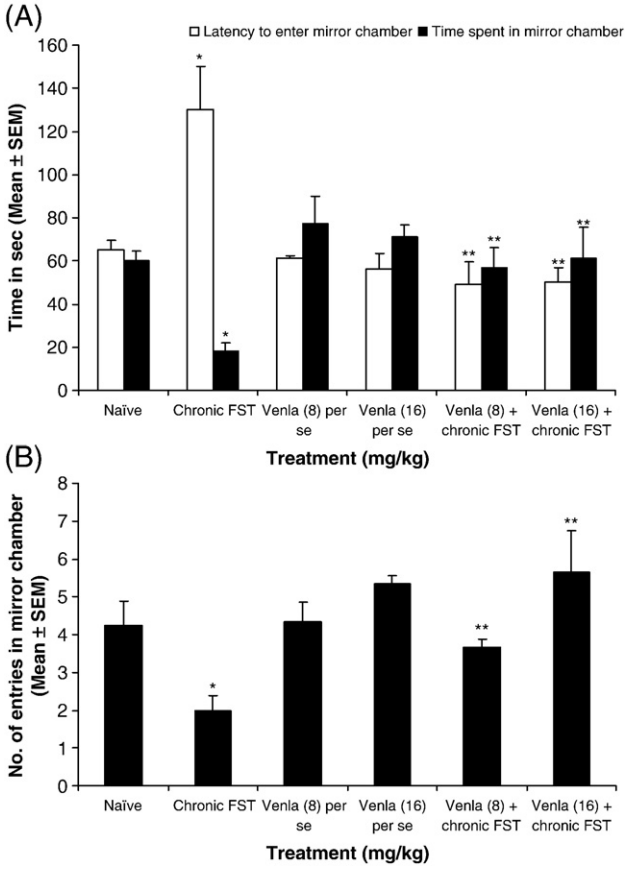

Fig. 5. Effect of chronic administration of venlafaxine (8 and 16 mg/kg i.p.) on chronic swimming-induced anxiety in the mirror chamber test. (A) The latency and time spent in mirror chamber (Sec) and (B) the number of entries in the mirror chamber was assessed on the 16th day of the study. *$P<0.05$ as compared to naive group. **$P<0.05$ as compared to chronic forced swim test (FST) control group (ANOVA followed by Dunnett's test) ($n=6–8$).

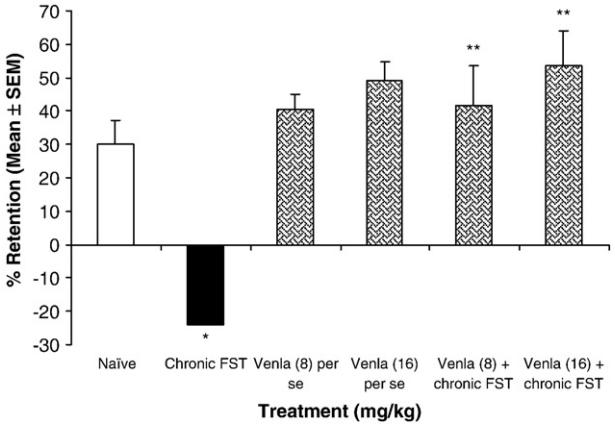

Fig. 6. Effect of chronic administration of venlafaxine (8 and 16 mg/kg i.p.) on chronic swimming-induced-memory deficit in the plus-maze paradigm. The retention of memory was assessed on the 16th day of the study. *$P<0.05$ as compared to naive group. **$P<0.05$ as compared to chronic forced swim test (FST) control group (ANOVA followed by Dunnett's test) ($n=6–8$).

3.3. Biochemical parameters

In animals chronically exposed to forced swimming, there was a significant increase in the whole brain MDA levels compared to unstressed mice. Chronic treatment of venlafaxine (8 and 16 mg/kg, i.p. for 15 days) decreased the MDA levels in chronic FST (Fig. 7A).

Similarly, animals chronically subjected to FST showed a significant decrease in the whole brain reduced glutathione levels as compared to vehicle treated group. Daily treatment with venlafaxine (8 and 16 mg/kg, i.p. for 15 days) before the exposure significantly improved the depleted reduced GSH levels in (Fig. 7B).

In addition, animals chronically subjected to FST showed an increase in the whole brain nitrite levels as compared to vehicle treated group. Daily treatment with venlafaxine (8 and 16 mg/kg, i.p. for 15 days) before exposure significantly attenuated the increased nitrite levels (Fig. 7C).

The myeloperoxidase (MPO) activity was significantly higher in chronically stressed mice. Daily treatment with venlafaxine (8 and 16 mg/kg, i.p. for 15 days) before exposure significantly attenuated the increased myeloperoxidase levels (Fig. 7D).

Also, animals chronically subjected to FST showed a significant decrease in the adrenal ascorbic acid levels compared to vehicle treated group. Daily treatment with venlafaxine (8 and 16 mg/kg, i.p. for 15 days) before exposure significantly improved the depleted adrenal ascorbic acid levels (Fig. 8).

Daily treatment with venlafaxine (8 and 16 mg/kg, i.p. for 15 days) *per se* did not affect all the biochemical parameters.

3.4. Neurochemical parameters

Animals chronically stressed to FST showed a significant decrease in neurotransmitters *viz.* norepinephrine, dopamine and serotonin levels in the mouse brain. Daily treatment with

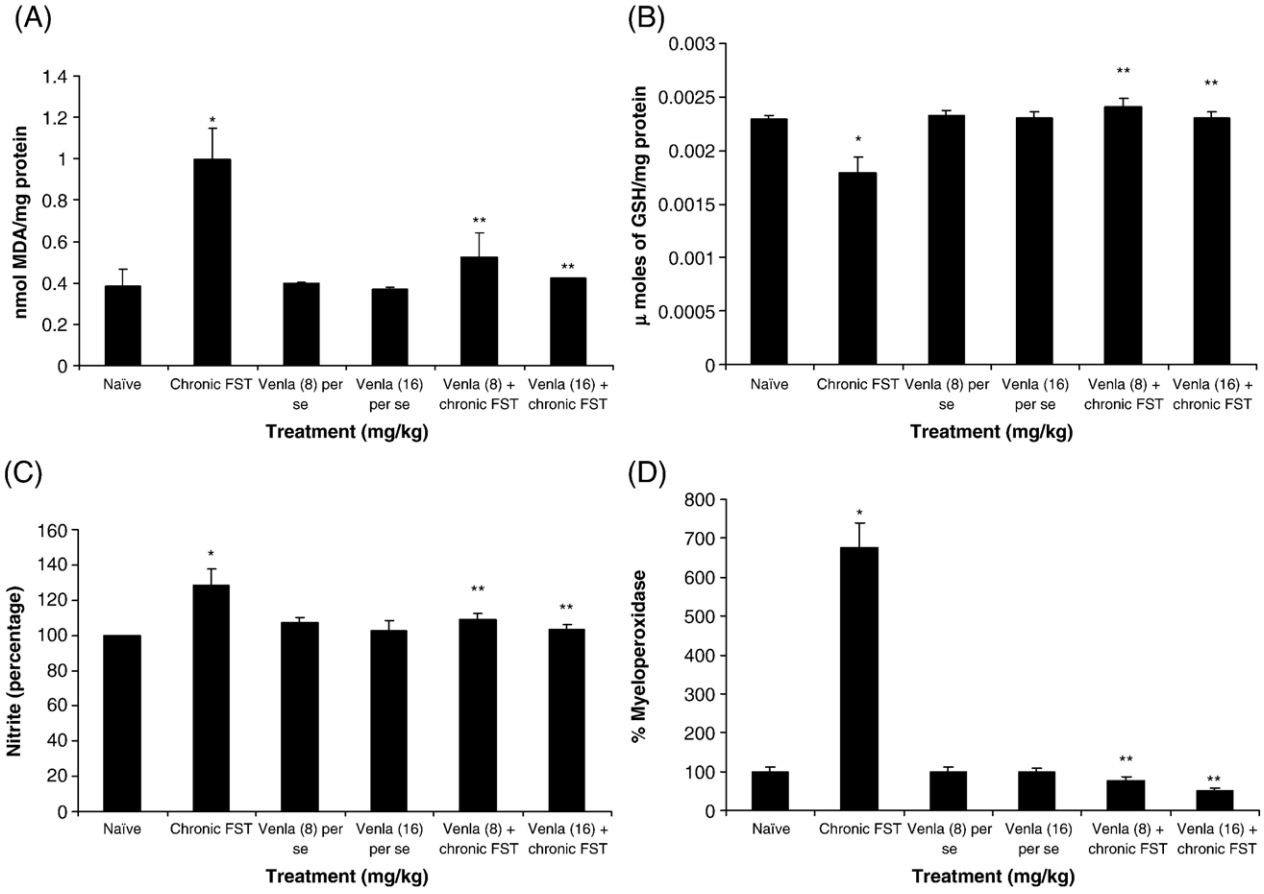

Fig. 7. Chronic effect of venlafaxine (8 and 16 mg/kg i.p.) on chronic swimming-induced alterations in (A) lipid peroxidation (B) reduced glutathione (C) nitrite levels (D) myeloperoxidase levels in mice. Various parameters were assessed on the 16th day of the study. *$P<0.05$ as compared to naive group. **$P<0.05$ as compared to chronic forced swim test (FST) control group (ANOVA followed by Dunnett's test) ($n=6–8$).

venlafaxine (8 and 16 mg/kg i.p. for 15 days) before exposure restored the decreased brain biogenic amines (Fig. 9).

Animals treated with venlafaxine at 8 mg/kg i.p. *per se* displayed increase levels of norepinephrine while at a dose of

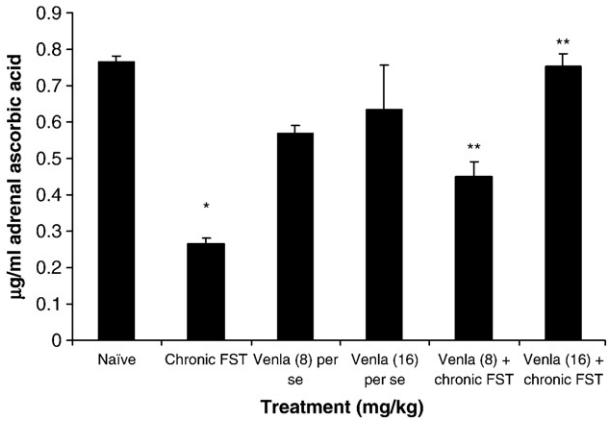

Fig. 8. Chronic effect of venlafaxine (8 and 16 mg/kg i.p.) on chronic swimming-induced alterations in adrenal ascorbic acid in mice. Adrenal ascorbic acid was assessed on the 16th day of the study. *$P<0.05$ as compared to naive group. **$P<0.05$ as compared to chronic forced swim test (FST) control group (ANOVA followed by Dunnett's test) ($n=6–8$).

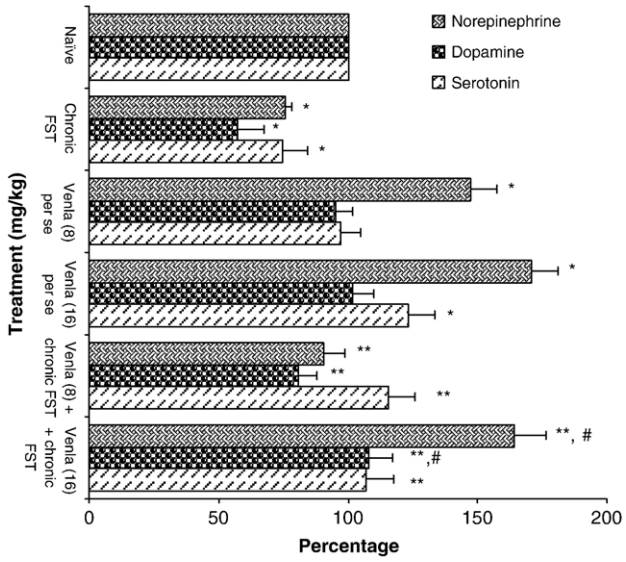

Fig. 9. Chronic effect of venlafaxine (8 and 16 mg/kg i.p.) on chronic swimming-induced alterations in biogenic amines in the brain. Biogenic amines were assessed on the 16th day of the study *$P<0.05$ as compared to naive group. **$P<0.05$ as compared to chronic forced swim test (FST) control group. $^{\#}P$ as compared to venlafaxine (8 mg/kg., i.p.)+chronic forced swim test (FST) group (ANOVA followed by Dunnett's test) ($n=6–8$).

16 mg/kg, both norepinephrine and serotonin was increased (Fig. 9).

4. Discussion

Chronic fatigue is relatively a common disorder. In addition to the characteristic persistent fatigue, the patients often complain of a number of symptoms including headache, joint pain, gastrointestinal disturbances, cognitive dysfunction, visual disturbances, and paraesthesia. It has been suggested that chronic fatigue syndrome is associated with a decrease of central synaptic transmission of neurotransmitters like serotonin and glutamate (Miwa and Takikawa, 2007). Attenuation of serotonin neurotransmission can be due to increased expression of serotonin transporter (Miwa and Takikawa, 2007). It is well established that there is a high lifetime prevalence of affective symptoms, such as depression, dysthymia and anxiety in the chronic fatigue population and there are many overlapping symptoms between chronic fatigue and major depression (Roy-Byrne et al., 2002; Białyszewski, 1993). Therefore, various antidepressant drugs have found clinical utility in this syndrome. However, patients suffering from chronic fatigue demonstrated poor response to conventional antidepressant therapy (Gold et al., 1995; Terman et al., 1998).

In the present study, we have employed the forced swimming activity to induce a state of chronic fatigue in animals (Singh et al., 2002). Animals were made to swim daily for 6 min over a total period of 15 days. It was found that the immobility (fatigue response) period increased to maximum on day 7 and persisted up to day 15. These chronically fatigued animals showed an early fall from the rota rod, increased anxiety response in mirror chamber and increased pain sensation. Similarly, the biochemical evidences indicated oxidative stress (increased lipid peroxidation, nitrite, myeloperoxidase and decrease in reduced glutathione levels) and depletion of various brain neurotransmitter levels. The core finding of the present study is that venlafaxine attenuates various behavioral, biochemical and neurochemical alterations due to chronic fatigue caused by daily exposures of mice to forced swim for 15 days. The two doses of venlafaxine used in the present study had shown to be effective in both forced swim and the tail-suspension tests and without having effect *per se* on the locomotor activity (Dhir and Kulkarni, 2007b,c).

Although the involvement of serotonin neurotransmission and the effect of antidepressants in the pharmacotherapy of chronic fatigue syndrome are known, however, none of the studies have elucidated the anti-fatigue action of venlafaxine.

Venlafaxine is a representative of a new class of antidepressants which inhibit selectively the reuptake of serotonin and noradrenaline. ED50 of venlafaxine in decreasing the immobility period in mouse forced swim test and tail-suspension test was found to be 8.5 (2.90–24.88) mg/kg., i.p. and 12 (6.04–23.85) mg/kg., i.p. respectively (Kulkarni and Dhir, 2007). In our previous studies, we have shown the involvement of L-arginine-nitric oxide-cyclic guanosine monophosphate pathway in the antidepressant activity of venlafaxine (Dhir and Kulkarni, 2007b). Similarly, sigma receptor modulation is involved in its antidepressant activity (Dhir and Kulkarni, 2007c).

Chronically stressed mice showed enhanced sensitivity to amphetamine-induced hyperlocomotion (Cabib et al., 1995; Strekalova et al., 2005). Usually, the patients with fatigue syndrome suffer from the comorbid mood or anxiety syndrome (Roy-Byrne et al., 2002; Katon and Walker, 1993). In the present study, the locomotor activity in mice was increased 24 h after the last episode of forced swim test, which may be due to the reason that animals are more anxious 24 h after the last forced swim session. Daily administration with venlafaxine before stressing to FST prevented the hyperlocomotion and anxiogenic effect.

Cognitive problems are another most disruptive and disabling symptoms of chronic fatigue. Patients with chronic fatigue often have difficulties with concentration and memory (McDonald et al., 1993). It is debated that the cognitive impairment in fatigued patients is strongly related to psychiatric disorder, especially depressed mood, but not fatigue, anxiety, or objective performance (McDonald et al., 1993). Transfer latency in the elevated plus-maze task has been taken as an index of memory impairment and is carried out according to the previously standardized procedure in our laboratory (Reddy and Kulkarni., 1998, 1999). The plus-maze model is used to measure the effect of drugs particularly on spatial long-term memory (Reddy and Kulkarni., 1998, 1999). In the present study, chronically fatigued mice demonstrated memory impairment and the daily treatment with venlafaxine prevented the memory dysfunction in stressed mice. It is debated that the patients with significant complaints of mental fatigue exhibit significant impairment in the spatial working memory and sustained attention (rapid visual information processing) tasks when compared to patients with low complaints of mental fatigue and nonfatigued subjects (Capuron et al., 2006).

Previous studies have demonstrated that various stress conditions induce hyperalgesia to thermal, chemical and mechanical stimuli (Quintero et al., 2000). The dysfunction of the hypothalamo-pituitary-adrenocortical axis (HPA axis) and multiple neurotransmitter systems in the central nervous system (CNS), including endogenous opioid, serotonergic and noradrenergic systems, has been reported (Imbe et al., 2006). Antidepressants belonging to various categories have been known to induce a dose-dependent antinociceptive effect in the formalin test in rats (Yokogawa et al., 2002). In a recent study, it was suggested that venlafaxine displayed antidepressant and analgesic activity by inhibiting the activity of locus coeruleus neurons which is further modulated by alpha (2)-adrenergic and 5-HT (1A) receptors (Berrocoso and Mica, 2007). In the present study, there is hyperalgesic response in chronically fatigued mice which was attenuated by daily treatment with venlafaxine.

It has been suggested that chronic fatigue illness can stimulate numerous pathways leading to increased production of free radicals. The pro-inflammatory cytokines are reported to be increased in major depression (Anisman et al., 2002) and sickness behavior (Rönnbäck and Hansson, 2004). This is interesting as there are overlaps between mental fatigue and these disorders (Rönnbäck and Hansson, 2004). Various studies have indicated the up-regulation of inducible nitric oxide synthase enzyme in patients suffering from stress and related disorders (Harvey et al., 2004). Increased inducible nitric oxide

synthase (iNOS) expression can lead to increase production of nitric oxide, which can initiate inflammatory process in the body. Moreover, nitric oxide together with increased expression of cyclooxygenase-2 (COX-2) can further deteriorate the illness (Maes et al., 2007). In one of the clinical studies, it has been revealed that levels of isoprostanes positively correlated with the symptoms of chronic fatigue (Kennedy et al., 2005) and there was an increased oxidation of lipids (Kennedy et al., 2005). Similarly, the studies conducted in our laboratory have shown that oxidative stress is involved in the murine model of chronic fatigue. There was an enhancement of malionaldehyde levels (an indicator of lipid peroxidation), nitrite and myeloperoxidase levels and there was decrease in reduced glutathione levels in the whole brain. Myeloperoxidase activity is an indication of the polymorphonuclear leukocytes recruitment in the brain.

There are reports that show that the endogenous neurotransmitter serotonin may work as an innate antioxidant defense mechanism in the CNS (Kang et al., 2001). Serotonin is known to exert a protective effect in the hippocampus and attenuate the behavioral consequences of stress by activating 5-HT_{1A} serotonin receptors. These effects may mediate the therapeutic actions of several antidepressants (Joca et al., 2007) including venlafaxine. In fact, curcumin a natural antioxidant and active in mouse model of depression is known to protect hippocampal neurons from damage induced by chronic stress *via* the up-regulation of 5-HT_{1A} serotonin receptors and brain derived neurotrophic factor, which may underlie the therapeutic actions of curcumin (Xu et al., 2007). In one of the study, chronic fluoxetine administration to stressed animals for 21 days prevented restraint stress-induced oxidative damage with an efficacy similar to that of turmeric (Zafir and Banu, 2007). Noradrenaline, another neurotransmitter is also known to protect cortical neurons against microglial-induced cell death (Jose et al., 2005). In a recent study, it has been shown that noradrenaline can protect neurons from Aβ-induced damage, and suggest that its actions may involve activation of PPARδ and increases in glutathione production (Jose et al., 2007). Similarly, intracellular reactive oxygen species (ROS) are known to be drastically reduced by treatment with noradrenaline (Troadec et al., 2001). These studies indicate that the neurotransmitter itself acted as an antioxidant. Increased oxidative stress is also known to damage dopaminergic neurons in the brain (Testa et al., 2005). Patients with dopaminergic deficiencies show symptomatic mental fatigue resulting from a failure to maintain adequate levels of dopaminergic transmission resulting in impaired cognitive control (Lorist et al., 2005). With this background, the study was extended to measurement of neurotransmitter alterations in the brain. Animals with 15 days chronic FST displayed decrease in norepinephrine, dopamine as well as serotonin levels in the brain which was reversed by treatment with venlafaxine. Therefore, from the present study, it is emphasized that the drugs which increase the serotonin, noradrenaline or dopamine levels in the brain could be used as drug therapy in the patients suffering from chronic fatigue. It can be hypothetized that the increase in neurotransmitter levels *viz.* serotonin, noradrenaline and dopamine by the administration of venlafaxine may be responsible for its antioxidant action.

It is well reported that oxidative stress stimulated the hypothalamic–pituitary–adrenal resulting in increased production of corticosterone (Kipp and Revers, 1987). In humans and animals, adrenal cortex contains a higher concentration of ascorbic acid than other tissues and the acute administration of adrenocorticotrophic hormone (ACTH) caused decrease in ascorbic acid levels (Kipp and Revers, 1987; Laney et al., 1990). The present data demonstrates that chronic exposure to FST caused significant depletion of ascorbic acid suggesting that ACTH was greatly stimulated. In the present study, there was decrease in adrenal ascorbic acid in stressed animals which was reversed with venlafaxine.

In summary, the present study revealed that venlafaxine, a dual reuptake inhibitor was effective in reversing chronic fatigue-induced various behavioral, biochemical and neurochemical alterations in mice.

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