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BEHAVIOURAL AND ANTIOXIDANT PROFILE OF VENLAFAXINE TREATED ANIMAL MODEL OF DEPRESSION

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ABSTRACT

Venlafaxine is the first Serotonin Norepinephrine reuptake inhibitor (SNRI) and is one of the First-line antidepressants currently used owing to its rapid onset and decreased propensity to cause anticholinergic effects and cardiotoxicity. The mechanisms of psychopathology of depression are multifaceted and include evidence that oxidative stress may play an important role. In the present study the effect of Venlafaxine treatment on the antioxidant profile of Chronic Unpredictable Mild Stress induced male Wistar rats was determined and correlated with behavioural assessment following administration of Venlafaxine for 21 days. The results showed a significant decrease in sucrose consumption on stress induction, which increased significantly during Venlafaxine treatment. The period of immobility during the forced swim test was also found to be decreased with treatment, whereas the activities of enzymic and non-enzymic antioxidants were found to be significantly increased.

KEYWORDS: Depression, Venlafaxine, Oxidative stress, Antioxidants, Behavioural assessment, Anhedonia

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INTRODUCTION

Major depression is a serious, recurrent disorder linked to diminished role functioning, quality of life, medical morbidity and mortality[1]. Depression is related to the normal emotions of sadness and bereavement, but it does not remit when the external cause of these emotions dissipate, and is disproportionate to their cause. Classic severe states of depression often have no external precipitating cause[2]. Depression has been reported to be one of the most common psychiatric disorders in outpatient clinic populations and also a common disorder observed in community based studies[3]. Recent reports state that Depression affects 121 million people worldwide and is responsible for 850,000 deaths every year[4]. New research showed that 15% of the population from high-income countries (compared to 11% for low/middle-income countries) were likely to get depression over their lifetime with 5.5% having had depression in the last year[5]. A study determined the prevalence of depression in a major city in South India to be 15.1%. The study also reported that age, gender and socio-economic status were also contributing factors for depression among the urban population[6]. Another recent study conducted among the elderly population in a slum of Medinipur town, West Bengal found that 59.8% of the sample population were suffering from depression and that the prevalence increased with age of the responder[7]. Stress is described as a state of threatened homeostasis provoked by psychological, physiological or environmental stressors[8]. Stressor can either be an internal or external stimulus, which activates the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system resulting in an altered physiological state[9]. Stressful conditions are hypothesised to precipitate anxiety and depression, leading to an excessive production of free radicals which in turn results in oxidative stress[10]. Evidences show reduced antioxidant defences in depression, as indicated by lowered levels of key antioxidants and antioxidant enzymes. Several studies show reduced levels of serum Vitamin E[11,12,13], glutathione, glutathione peroxidase and a lowered total antioxidant status[14,15]. Venlafaxine is a Serotonin Norepinephrine reuptake inhibitor (SNRI) as it blocks the synaptosomal uptake of noradrenaline and serotonin and, to a lesser extent dopamine uptake[16]. It is one of the First-line antidepressants currently used owing to its rapid onset and decreased propensity to cause anticholinergic effects and cardiotoxicity. A previous study demonstrated that Venlafaxine treatment decreased the lipid peroxidation in the cerebral cortex of depression-induced animals[17].

MATERIALS AND METHODS

A. PROCUREMENT OF ANIMALS

The Ethical clearance for the study was obtained from the Institutional Animal Ethics Committee, PSG IMS & R, Coimbatore (Proposal No. 195/2013/IAEC). Wistar Male albino rats of 120 ± 20 g procured from the Animal Facility, PSG IMS & R were used for the study. The animals were acclimatized under standard laboratory conditions with controlled temperature, humidity and diet with a 12 hour day and night cycle for the first week.

B. EXPERIMENTAL SET UP

The experimental animals were divided into 4 groups of 6 animals each.

Group I **:** Normal Control
Group II **:** Depression induced
Group III **:** Treatment with Venlafaxine low dose (16 mg/kg body weight)
Group IV **:** Treatment with Venlafaxine high dose (32 mg/kg body weight)

C. INDUCTION OF STRESS BY CHRONIC UNPREDICTABLE MILD STRESS (CUMS) PROTOCOL[18]

The following stressors were presented to the experimental animals for a period of 24 days in a random fashion such that the stressors were repeated in an unpredictable manner. This prevented the animals from adapting to the stressors.

1. Food deprivation for 24 hrs
2. Day – night reversal (0600 hrs – 1800 hrs)

3. Soiled bedding (~ 150 mL water per cage of38 x 23 x 15) for 22 hrs
4. Cage tilting (~ 45° inclined) for 22 hrs
5. Crowded housing (10 animals per cage of 38 x 23 x 15) for 12 hrs
6. Exposure to a novel odour (household air freshner) for 12 hrs
7. Restraint stress for 20 minutes
8. Cold stress (4° - 8°C) for 20 minutes
9. Heat stress (38° - 39°C) for 20 minutes

D. BEHAVIOURAL ASSESSMENT

1. ***Behavioural despair test***[19] ***(Forced Swim test):*** The rats were allowed to swim individually in a chamber (45 x 12 x 45 cm) containing fresh water (25 ± 2°C), of height 35 cm, such that the rat could not touch the bottom of the cylinder with its limb or tail or climb over the edge of the chamber. Two swim sessions were conducted, an initial 15 minute pre-test followed by a 6 minute test, 24 hrs later. The period of immobility after an initial 2 – 3 minute period of vigorous activity was recorded. A rat was considered immobile when it remained floating in the water without struggling, making only minimum movements to keep its head above water. The total duration of immobility was recorded during the next 4 minutes of the total 6 minute long test. The rats were then allowed to dry in a pre-warmed enclosure (~32°C) before being returned to their cages.
2. ***Sucrose consumption test:*** During this test, mice were given, for 24 h, a free choice between two bottles, one with 1% sucrose solution and another with tap water. To prevent possible effects of side preference in drinking behaviour, the positions of the bottles were switched after 12 h. No previous food or water deprivation was applied before the test. The consumption of water and sucrose solution was estimated simultaneously in control and experimental groups. The preference for sucrose was calculated as a percentage of consumed sucrose solution of the total amount of liquid drunk[20,21,22].

E. COLLECTION OF SERUM AND BRAIN TISSUES

At the end of the treatment period (21 days), the animals were anesthetized under mild chloroform. Blood was collected by cardiac puncture and the serum was separated by centrifugation at 2500 rpm for 15 minutes. The whole brain was excised immediately and thoroughly washed in ice cold saline before use. A 10% homogenate of the whole brain was prepared using 0.1M cold Tris-HCl buffer (pH 7.4) in potter homogenizer fitted with a Teflon plunger running at 600 revolutions per minute for 4 minutes. The homogenate was used for the assay of protein, various enzymic and non-enzymic antioxidants.

F. BIOCHEMICAL ANALYSIS

The serum and tissue homogenate were used for the assay of Protein by the method of Lowry *et al*. *(*1957)[23], Superoxide Dismutase (SOD) by the method of Marklund and Marklund (1974)[24], Catalase (CAT) by the method of Sinha (1972)[25], Glutathione Peroxidase (GPx) by the method of Ellman (1959)[26], Vitamin C by the method of Omaye *et al*. (1979)[27] and Lipid peroxidation by the method of Niehius and Samuelsson (1968)[28].

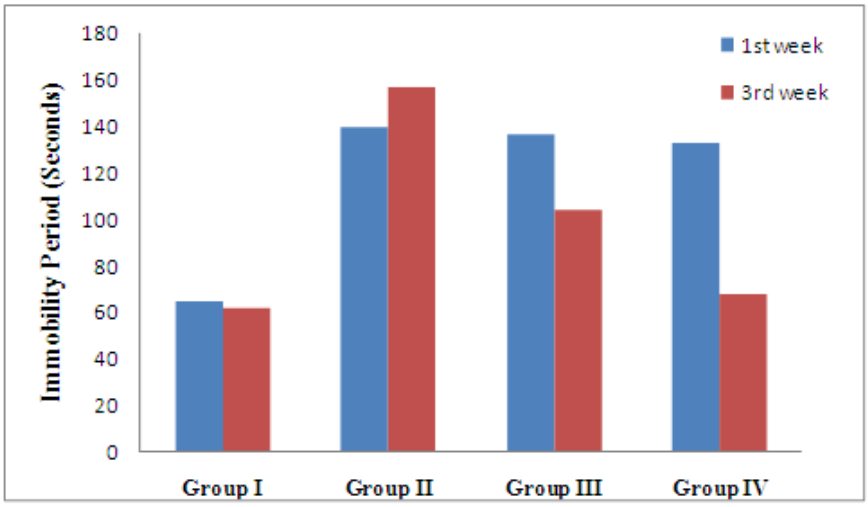
RESULTS

After two days of stress induction, the animals were orally administered with the Venlafaxine solution. At the end of the first week (7th day) of Venlafaxine treatment, the Forced Swim Test (FST) was administered to all the experimental animals to assess the severity of stress induced. The results (Table I) showed a significant increase in the immobility time in Groups II, III and IV as compared to those of Group I. At the end of the Venlafaxine treatment (21st day), FST was performed once again to assess the effect of treatment with low and high doses of Venlafaxine as against that of the untreated animals. The results show that the immobility time has decreased significantly in Group IV as compared to those of the Group II and III and was found to be similar to that of Group I (Figure 1).

TABLEI
RESULTS OF FORCED SWIM TEST

S. No	Group	Dosage	Immobility (seconds)	
			1st week	3rd week
1.	I	-	64.73 ± 47.13	61.41 ± 31.81
2.	II	-	139.67 ± 29.41	157 ± 46.19
3.	III	16 mg/kg body weight	136.33 ± 36.09	103.83 ± 31.83
4.	IV	32 mg/kg body weight	133.17 ± 51.14	67.67 ± 52.61

FIGURE 1
FORCED SWIM TEST (FST)

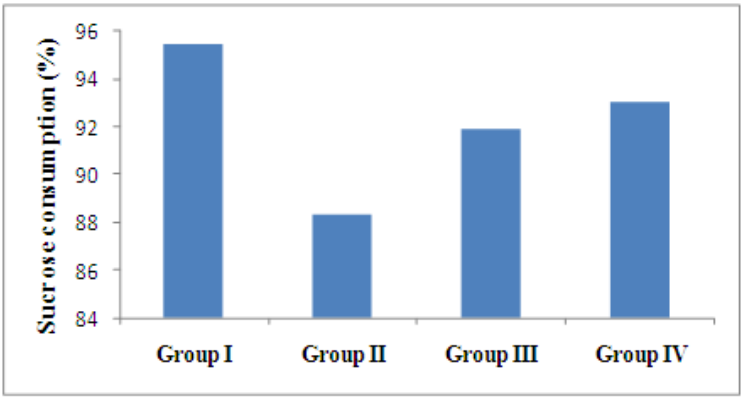


The animals were separated and kept in individual cages after FST on the 21st day. They were then provided with 100 ml of both 1% Sucrose solution and drinking water in separate containers overnight. The following day (after 24 hours) the volume of 1% Sucrose solution and drinking water consumed was measured and percentage of sucrose preference (Table II). The results show that Group IV had an increased sucrose preference when compared to that of Group II and III, which was found to be similar to that of Group I (Figure 2).

TABLE II
RESULTS OF THE SUCROSE CONSUMPTION TEST

S. No	Group	1% Sucrose solution consumed (ml)	Water consumed (ml)	Total volume of fluids consumed (ml)	Sucrose preference (%)
1.	I	65.08 ± 2.13	4.03 ± 0.41	66.74 ± 5.07	95.46 ± 1.04
2.	II	42.93 ± 9.18	5.22 ± 2.76	37.72 ± 6.89	88.31 ± 4.06
3.	III	54.55 ± 7.03	4.30 ± 0.86	60.17 ± 5.16	91.93 ± 2.40
4.	IV	64.63 ± 4.67	4.47 ± 0.49	50.25 ± 7.40	93.02 ± 1.23

FIGURE 2
SUCROSE CONSUMPTION TEST

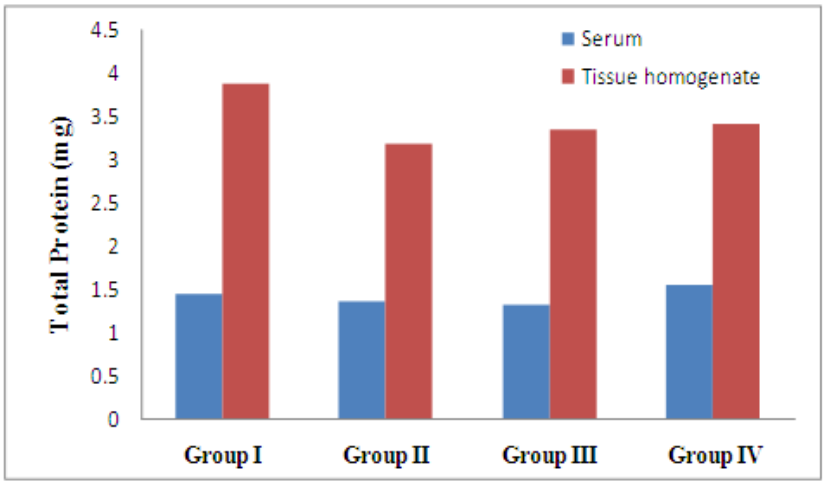


At the end of the Venlafaxine treatment period (21 days), the study animals were anesthetized under mild chloroform. Blood was collected by cardiac puncture from which serum was separated for the biochemical studies. The whole brain was excised immediately and thoroughly washed in ice cold saline to remove any traces of blood before use. The whole brain was used to prepare the tissue homogenate for the Biochemical studies. The amount of total proteins present in the serum and brain tissue homogenate was estimated with BSA as standard using the method of Lowry *et al* (Table III). The results show a significant increase in the serum total proteins in the high dose treated group as compared to the control, induced and low dose treated groups. A significant increase in the total protein was observed in the tissue homogenate of Group IV, which was found to be near normal levels when compared with that of Groups II and III where it was significantly decreased (Figure 3).

TABLEIII
ESTIMATION OF TOTAL PROTEIN

S. No	Group	Dosage	Total protein in Serum (mg/ml)	Total protein in Tissue homogenate (mg/g tissue)
1.	I	-	1.44 ± 0.12	3.87 ±0.92
2.	II	-	1.36 ± 0.07	3.17 ± 0.15
3.	III	16 mg/kg body weight	1.30 ± 0.19	3.33 ± 0.46
4.	IV	32 mg/kg body weight	1.55 ± 0.07	3.40 ± 0.83

FIGURE 3
ESTIMATION OF TOTAL PROTEINS



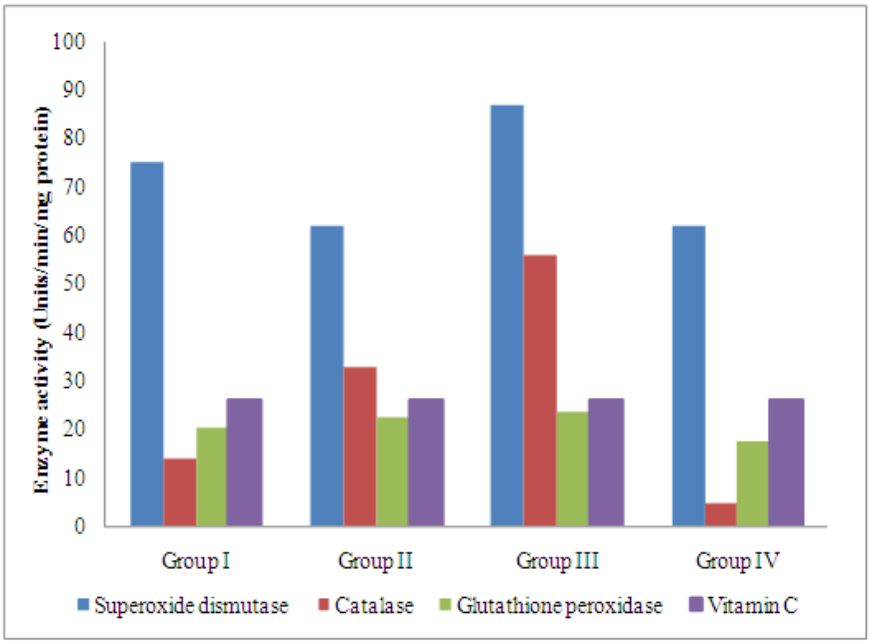
The results of the biochemical assays performed using serum are tabulated in Table IV and those using the tissue homogenate are tabulated in Table V.

TABLE IV
ANTIOXIDANTACTIVITY IN THE SERUM

Group	SOD (Units/min/ mg serum protein)	CAT (µmoles of H_2O_2 consumed/min/mg serum protein)	GPx (µmoles of GSH oxidized/min/mg serum protein)	Vitamin C (µg/mg serum protein)	LPO (µmoles TBARS/mg serum protein)
Control	0.75±0.21	13.92±7.86	20.38±1.05	261.31±28.37	17.86±11.85
Induced	0.62±0.21	32.63±37.43	22.40±2.59	258.04±19.37	21.83±14.23
Lowdose	0.87±0.42	55.95±82.05	23.53±4.83	260.36±26.92	16.80±04.76
Highdose	0.62±0.24	04.46±5.21	17.40±1.31	261.21±14.23	10.29±3.52

The values are expressed as mean ± SD (n=6)

FIGURE 4
ASSAY OF ENZYMIC ANTIOXIDANTS IN SERUM



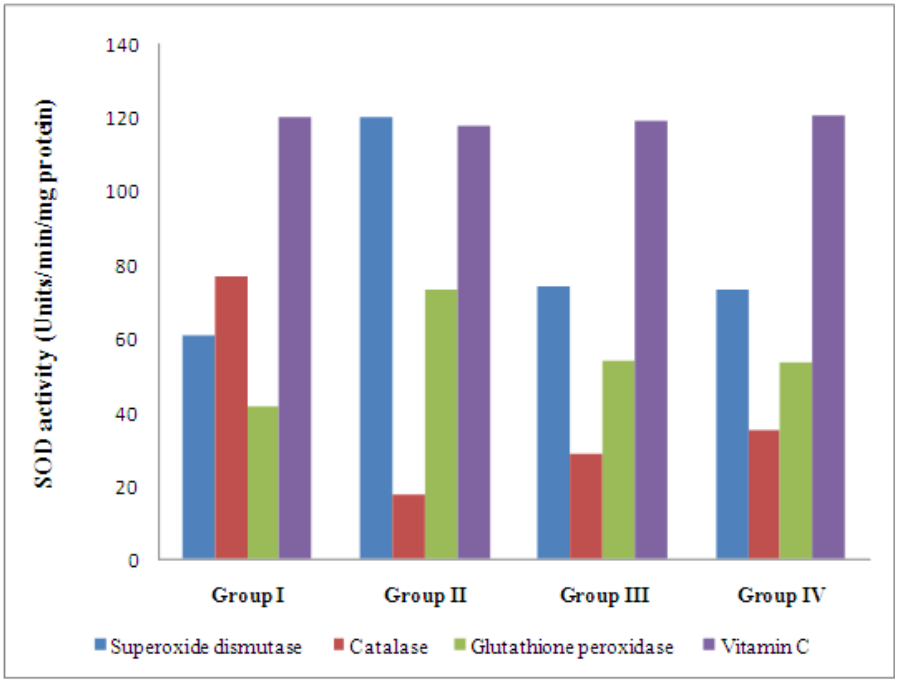
The enzymic activity of Superoxide Dismutase in serum was found to be significantly increased after treatment in Group III to near normal levels when compared with that of Group II and IV. On the other hand, the activity in the tissue homogenate was found to be significantly increased in Group II when compared to Groups III and IV which were found to have similar values. The activity of Catalase in serum was found to be significantly increased in Group III when compared to that of Groups II and IV, which was similar to that of the SOD activity in serum, whereas the activity in tissue homogenate was found to slightly increase in the Group IV when compared with that of Groups II and III. The activity of Glutathione peroxidase in serum and tissue homogenate was assayed using the reaction of glutathione with DTNB. The serum Glutathione peroxidase activity was found to be slightly increased in Group III when compared with that of Groups II and III, whereas the activity in tissue homogenate was found to be significantly increased in Group II when compared to that of Groups III and IV.

TABLE V
ANTIOXIDANT ACTIVITY IN THE BRAIN TISSUE HOMOGENATE

Group	SOD (Units/min/mg protein)	CAT (µmoles of H_2O_2 consumed/min/mg protein)	GPx (µmoles of GSH oxidized/min/mg protein)	Vitamin C (µg/mg protein)	LPO (µmoles TBARS/mg protein)
Control	0.61±0.02	76.85±32.29	412.94±69.34	1204.21±421.24	237.29±46.66
Induced	1.20±0.23	17.41±8.59	730.61±46.86	1179.97±91.90	288.04±23.40
LowDose	0.74±0.21	28.40±17.07	539.58±80.44	1193.70±161.04	315.91±69.50
HighDose	0.73±0.18	34.82±11.97	532.45±113.01	1204.76±312.88	256.83±54.67

The values are expressed as mean ± SD (n=6)

FIGURE 5
ASSAY OF ENZYMIC ANTIOXIDANTS IN TISSUE HOMOGENATE



The amount of Ascorbic acid in both serum and tissue homogenate was estimated by the method of Omaye *et al*., and was found to be significantly increased in Group IV to near normal levels when compared to that of Groups II and III.Lipid Peroxidation in the serum and tissue homogenate was estimated colorimetrically using TBARSand was found to be significantly decreased to near normal levels in Group IV when compared to that of Groups II and III.

DISCUSSION

A study reported that Major depression is accompanied by lower serum Vitamin E concentrations, suggesting lower antioxidant defenses against lipid peroxidation[11].Another study suggested that the lower plasma levels of a-tocopherol in depression are not related to their dietary intake[12]. Yet another study found that blood levels of Vitamins A, C and E were decreased in patients of Generalised anxiety disorder and depression. On supplementation with these vitamins their anxiety and depression scores were significantly decreased indicating a possible role of these antioxidants[13]. Another study

found statistically significant correlations between SOD and MDA, and SOD and NO in Affective disorders[14].A study using herbal medicine found that Chronic Mild Stress produced anxiogenic and depressive behaviour in experimental rats with metabolic disturbance, where significant decrease in SOD, CAT levels and increase in lipid peroxidation levels was observed. The study also found that the herbal drug treatment significantly increased SOD, CAT, and ascorbic acid level and controlled the lipid peroxidation in different tissues[17]. A recent study reported increased SOD and CAT activity and decreased lipid and protein damage in the male rat prefrontal cortex and hippocampus after both acute and chronic treatment with the antidepressant drug imipramine[18]. The results of the present study confirm the previous findings that the levels of several enzymic and non-enzymic antioxidants are decreased in the serum and brain tissue of experimental animal models[11,12,13,14,17,18]. The study also showed that a significant decrease in sucrose consumption was observed on stress induction, which increased significantly during high dose of Venlafaxine treatment. The period of immobility during the forced swim test was significantly increased on stress induction, which was then found to be decreased on treatment with high dose of Venlafaxine. The levels of enzymic and non-enzymic antioxidants were found to be significantly increased with the low dose of Venlafaxine and total antioxidant capacity in terms of Lipid peroxidation was also found to be significantly decreased following administration of low dose of Venlafaxine.

CONCLUSION

The study reaffirms the findings of the previous studies that antioxidants are significantly decreased and that play an important role in the pathophysiology of Major depression. The study also suggests that the anti-depressive activity of Venlafaxine and other major antidepressants could be due a possible increase in the total antioxidant activity. Further studies on the biochemical pathways might reveal their possible mechanism of action. The findings also suggest that chronic treatment with Venlafaxine has an anti-stress and neuro-protective effect.

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