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Original Article

Behavioral and neuronal biochemical possible effects in experimental induced chronic mild stress in male albino rats under the effect of oral barley administration in comparison to venlafaxine

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Abstract: Venlafaxine is an antidepressant of choice, whose effectiveness could be modified by a commonly used medicinal plant and nutrient. The current study had evaluated the barley extract (1 g/kg) when compared to or combined to venlafaxine (32 mg/kg) in a rat stress model. The present study was conducted on 40 male Wister albino rats; divided to five groups. Four groups were subjected to social chronic mild stress. Drugs or saline were orally daily administered one week before stress induction and extended up to ten weeks. Behavioral, brain biochemical tests and serum magnesium were assessed at the end. The study revealed significant change in the combined group on behavioral tests; forced swim test, elevated plus maze and saccharin preference test when compared to barley extract group. Furthermore, there was significant reduction in brain malondialdehyde level, no significant change in brain nitric oxide level, while significant increase in serum magnesium level was noticed. Whereas, the barley extract group recorded a lowest significant improvement in behavioral, brain and serum biochemical tests. It could be concluded that barley and venlafaxine together had muffled the oxidant stress and increased brain serotonin, serum magnesium level that might had a crucial role in experimental induced chronic mild stress in rats.

Keywords: Barley extract, chronic mild stress, venlafaxine

Introduction

Chronic stressful events in life are risk factors for many diseases, in spite of acquisition of physiological and behavioral adaptations. The stress response consists of neural and endocrine mechanisms whose function is to regain homeostasis through cortisol and catecholamines surge for example. Cessation of stress terminates these responses. However, very intense or long lasting or chronic stresses may result in new biological equilibrium [1, 2]. The exact mechanism of depression is unknown; however, most of theories concentrate on the role of neurotransmitter serotonin. Most of the serotonergic, noradrenergic and dopaminergic neurons are located in midbrain and brainstem nuclei and project to large areas of the entire brain. Almost every compound that inhibits monoamine reuptake or its breakdown, leading to an increased concentration of monoamines in the synaptic cleft, has been proven to be a clinically effective antidepressant [3]. That was the base for monoamine theory for the nowadays clinically used antidepressants. The selective serotonin reuptake inhibitors (SSRIs) are among several classes of antidepressants that rather include the tricyclic and related cyclic antidepressants, the monoamine oxidase inhibitors [4-6].

SSRIs allosterically inhibit the serotonin transporter by binding the receptor at a site other than active binding site for serotonin. At therapeutic doses, about 80% of the activity of the transporter is inhibited. SSRIs have modest effects on beta adrenoceptor and norepinephrine transporter. Binding to the serotonin transporter is associated with tonic inhibition of the dopamine system; SSRIs do not bind aggres-

sively to histamine and muscarinic receptors [7]. Venlafaxine; is one member of SSNRIs used in long term continuation therapy of depressive obsessive patients. In spite of its safe pharmacokinetic data proved as a sole therapy in several clinical trials, the fear from serotonin syndrome and extrapyramidal manifestations is still hazardous specially if given within a regimen combating resistant depression [8].

Moreover, the role nitric oxide (NO) plays in memory, learning, oxidative and social stress has been identified since decades. Its molecular, biochemical, physiological or hemodynamic basis or pharmacological interventions had a role in neurodegenerative phases, ischemic reperfusion injury and finally in depressive, stressful clinical or experimental problem [9-11].

The experimental studies using medicinal plants led to approval of newer modalities for depression or stress management [12-15]. Furthermore, many depression models and behavioral tests with high validity and suitability had been modified over the last decades [16-18]. Within the medicinal plants that could be valuable in this era, there is barley. Barley, the tryptophan, lignan, selenium, copper and magnesium source; is a member of the gluten grain. As tryptophan is a precursor for serotonin, thus barley extract acts as a wrestler in this era of psychiatric illness and may be useful in treatment of depression [19-22].

For the above mentioned reasons, the current research was designed to compare the therapeutic effects of venlafaxine and barely in chronic mild stress (CMS) in rats on brain serotonin and nitric oxide levels as well as plasma magnesium levels in CMS.

Material and methods

Drugs and chemicals

Venlafaxine: EFFEXOR 75 Wyeth Ireland. Barely: Barley was obtained from the Egyptian Market, washed in cold water, after been dried well; the aqueous extract of barley was daily prepared by diluting one volume of well grinded plant to 10 volume of water at 80 °C in stopper flask. After shaking well, it is allowed to stand for 10 minutes then cooled and filtered to be used within 12 hours. Saccharin 1% powder was supplied by El Gomhoreya Company, Egypt. Other chemicals used in the study were purchased from E-Merck, Germany; all were of the analytic grade.

Animals

40 male Wistar albino rats weighing 180-250 gms were acclimatized in standard cages (4 rats per cage) for two weeks before study in Mowassah Animal House, Faculty of Medicine Alexandria University. Housing was maintained under a 12 h light/12 h dark cycle, water and food were allowed *ad libitum* throughout the study with the exception of periods of chronic mild stress (CMS) according to the designed research protocol. Animal care procedures followed and were accepted by the Alexandria Faculty of Medicine Ethical Committee, in compliance with national and international guidelines in experimental researches. The rats were divided randomly to five groups; each contained 8 rats. Group A: (Control group): rats were subjected to standard conditions and oral daily 1 ml saline administration for 9 weeks. Group B (non-treated stress group): rats were subjected to stress regimen and oral daily 1 ml saline administration for 9 weeks. Group C (Barley treated stress group): rats were treated with aqueous extract of Barley 1 g/kg orally as a single daily dose one week before stress induction and continued for another 9 weeks [21]. Group D (Venlafaxine treated stress Group): rats were treated with venlafaxine, 32 mg/kg orally once daily: one week before stress induction and continued for another 9 weeks [15]. Group E (Combined Barley and venlafaxine treated stress group): rats were treated with barely and venlafaxine in previous doses one week before stress induction and continued for another 9 weeks.

Chronic mild stress (CMS) induction

Within two weeks, rats were subjected to two periods of stress regimen with the following stressors: food deprivation for 24 h, day-night reversal, soiled bedding (150 ml water per cage) for 22 h, cage tilting (45 degree inclined) for 22 h, crowded housing (8 animals per cage) for 12 h, exposure to a novel odor (household air freshener) for 12 h, restraint stress for 20 min, cold stress 4-8 °C and heat stress 38-39 °C for 20 min and intermittent noise (80 dB) for 5 h for 3 periods [13].

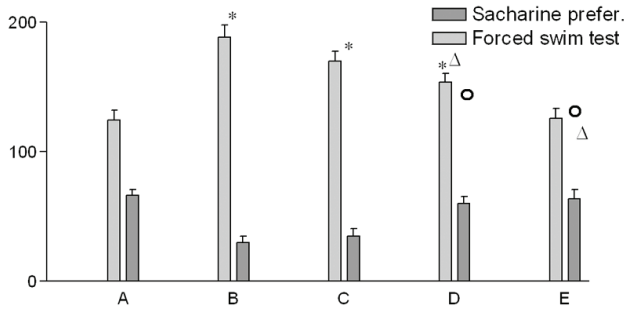

Figure 1. Mean of total immobility time (seconds) in forced swim test (FST), and percentage of saccharine preference for normal saline treated control group A, non treated stress group B, Barely extract (1 g/kg) treated group C, venlafaxine (32 mg/kg) treated group D and their combination treated group E; orally daily for one week before and continued nine weeks after induction of chronic mild stress in rats. Values are expressed as Mean S.E.M (n=8). $^{\Delta}$p is significant compared to Group B; *p is significant compared to Group A; ^{o}p is Significant compared to Group C.

Behavioral tests

All rats were evaluated in two sets, basal behavioral tests before drug or vehicle administration and after 10 weeks at the end of study. Each set was performed over three days. Both forced swim test and elevated plus maize, behavioral tests were video-taped using hand held Sony Cyber-shot digital camera, model number DSC-H70. They included:

Forced swim test (FST)

All studied group of animals were individually forced to swim in an open cylindrical container and the total amount of time each animal remained immobile during a 5minute session was recorded as immobility time. The rat was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. A decrease in the duration of immobility time in seconds was an indicative of an antidepressant-like effect [23].

Elevated plus maze

In the modified Elevated plus maze, the assessment of anxiety behavior of rodents was done by comparing time spent on the open arms to the time spent on the closed arms. It relies upon rodents' tendency toward dark, enclosed spaces (approach) and an unconditioned fear of heights/open spaces (avoidance) [24].

Saccharine preference test

The rats had free access of their standard rodent diet. On the first day (habituation); each cage was supplied with two identical graduated water bottles, each containing 250 ml of water. On the next day (test), regular water in one of the bottles was replaced with 0.1% saccharin diluted in tap water. The test was performed starting from 5:00 PM up to 24 hours. Taste preference was expressed as percent of the volume of saccharin solution of a total volume of fluid (saccharin plus regular water) consumed over 24 hours [25].

Biochemical measures

After the second behavioral tests had been accomplished, animals were fasted then subjected to light ether anesthesia. Blood samples were collected from main aortic trunk in EDTA coated tubes and centrifuged by Rotofix 32A centrifuge of Hettitch 3000 G rpm immediately for collection of plasma. It was stored at -20 °C for measuring magnesium level.

Then, rats were decapitated and brain was removed on ice, weighed and homogenized by Glascole homogenizer. The supernatant was separated by Rotofix 32A centrifuge of Hettitch at 4 °C and 16000 G rpm and stored at -20 °C, the sample was divided into 3 aliquots to measure serotonin [26], NO [27] and malondialdehyde (MDA) [28].

Brain serotonin level was measured competitive inhibition enzyme immunoassay technique using ELISA kit (CSB-E14951r) supplied by IBL Immuno-Biological Laboratories [26].

Determination of serum NO was done through nitrite assays. It depends on the addition of Griess reagents which convert nitrite into a deep purple azo compound, photometric measurement of the absorbance due to this azo chromophore accurately determines nitrite concentration, by (nitric oxide assay kit-Biodiagnostic) [27].

Total magnesium concentration in blood serum was determined by calmagite method. The method is based on the specific binding of cal-

magite, a metallochromic indicator, and magnesium at alkaline pH with the resulting shift in the absorption wavelength of the complex. The intensity of the chromophore formed is proportional to the concentration of magnesium in the sample [29].

Statistical analysis

The changes in behavioral tests and biochemical results were expressed as the mean of individual response±standard deviation. Differences among group were compared using one-way ANOVA through the Statistical Package of (Minitab 16.0) soft ware. Fisher test was used as a post hoc test for comparison among groups. Statistical significance was set at *P* values lower than 0.05 [30]. Figures were done through the Graph Pad prism version 5 soft ware.

Results

Behavioral tests

Time of immobility in forced swim test (FST): The mean time, in seconds±MSE in normal control group A was 124.50±7.35 seconds while in control non treated stress group B was significantly increased to 188.25±9.39. Treatment of rats with barely or venlafaxine or both together showed a significant decrease of immobility time as compared to non treated group (169.75±7.81, 153.75±6.54, 125.88±7.35 respectively). In comparing the pretreated groups, the present study revealed that the immobility time in group E was significantly lower than group C and D, whereas no significant difference was detected between group C and D. The F value for all groups was 12.79, where *P* value < 0.0001 (**Figure 1**).

Percentage of saccharin preference: The mean value for saccharine consumption in normal control group was 66.25±4.60. In stress control group B, it was significantly lowered; 30.00±4.63. Other treated stress groups showed significant increase in comparison to control non treated stress group B. The values were 35.00±5.67, 60.00±5.35 and 63.75±7.06 for groups C, D and E respectively. Moreover, group E and D showed a significant higher value as compared to group C, whereas no significant difference was detected between group E and D. The F value was 9.58, *p* value was < 0.0001 (**Figure 1**).

Time spent in closed and open arms in EPM test: The mean values for time spent in closed arm and that for open arm (seconds) were 173.13±2.43 and 125.63±3.99 respectively for control normal group A. While in stress non treated control group B, a significant increase was detected in time spent in closed arm and a significant decrease in time spent in open arm as compared to group A (193.63±4.68, 107.25±2.41 respectively). All treated groups showed a significant lower time spent in closed arm as compared to group B (147.63±2.58, 141.38±3.15, 143.88±7.31 for group C, D & E respectively) and a significant longer time spent in open arm (160.00±1.84 for group C, 152.50±1.45 for group D & 170.13±0.515 for group E). Fisher post hoc test showed the highest significant value was for group E in comparison to other treated groups; The F value for all groups was 122.64, *p* value was 0.000 (**Figure 2**).

Biochemical tests

MDA level (nmole/g tissue): Mean MDA brain tissue level in non treated control group A was 34.64±2.41, while it was significantly higher in non treated stress control group B; 83.21±6.70. The other three treated groups; group C, D and E showed significant lowered MDA brain tissue level as compared to stress control group as presented in **Table 1**. No statistical significant difference between groups D and E. Both groups D and E have significant lower MDA level compared to group C. F value was 42.30, p was 0.000.

Nitric oxide (NO) brain level (μmol/gm tissue): This analyzed parameter showed no significant change among treated groups in comparison to stress non treated control group. F value was 1.72; *p* value was 0.17. Post hoc fisher test showed significant higher NO level in group B compared to group A (**Table 1**).

Serotonin brain level: The mean value for serotonin brain level in control normal group A was 17.750±0.750. It was significantly lowered in non treated stress group B to 8.375±0.596. Group E showed the highest statistical significant increase in serotonin brain level among all treated groups. No statistical difference

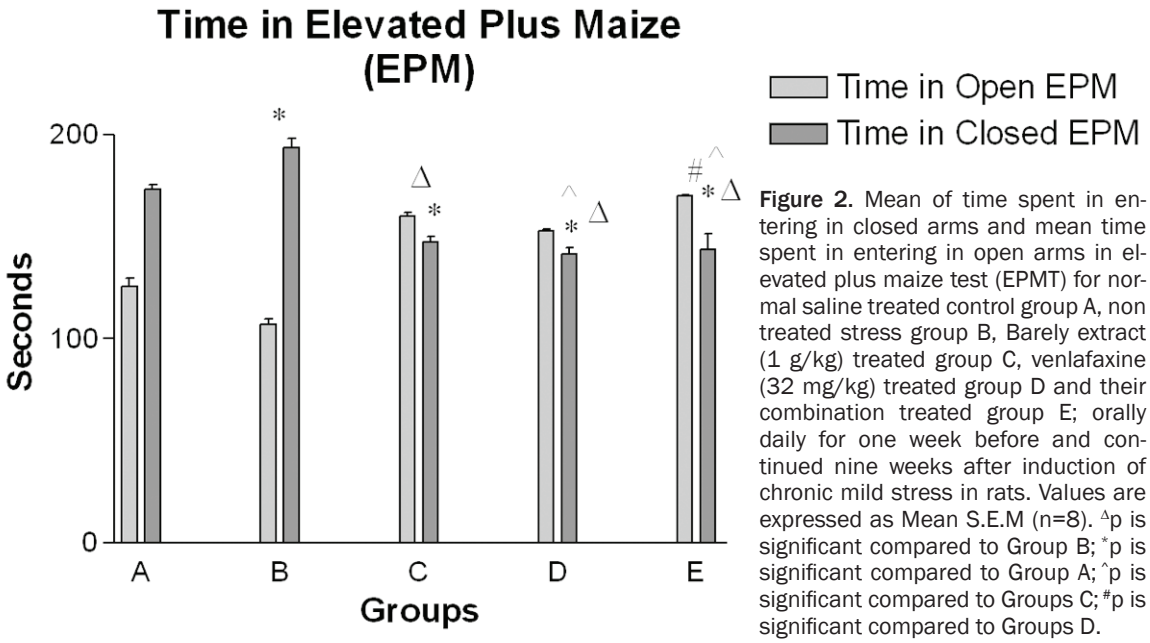

Figure 2. Mean of time spent in entering in closed arms and mean time spent in entering in open arms in elevated plus maize test (EPMT) for normal saline treated control group A, non treated stress group B, Barely extract (1 g/kg) treated group C, venlafaxine (32 mg/kg) treated group D and their combination treated group E; orally daily for one week before and continued nine weeks after induction of chronic mild stress in rats. Values are expressed as Mean S.E.M (n=8). $^{\Delta}p$ is significant compared to Group B; $^{*}p$ is significant compared to Group A; $^{\wedge}p$ is significant compared to Groups C; $^{\#}p$ is significant compared to Groups D.

between groups C and D. The F value was 19.18, *p* value was 0.0001 (**Table 1**).

Serum magnesium (mg/dl): The mean serum magnesium level for the control group A was 2.932±0.147. It showed a non significant increase in non treated stress control group B; 3.456±0.169. The barley treated stress group showed significant rise compared to normal control group A but no significant change was detected as compared to stress group B. The other groups D and E showed significant reduction compared to both groups B and C. Group B showed statistical significant higher serum magnesium level compared to normal control group A, but no significant difference when compared to stress control group B. The mean value for the combined treated stress group E was significantly lowered compared to all other groups (1.948±0.166). The F value was 12.38, *p* value was < 0.001 (**Table 1**).

Discussion

Due to the clinical and etiological heterogeneity of major depressive disorder, it has been difficult to elucidate its pathophysiology. The monoamine-deficiency theory posits that the underlying path physiological basis of depression is a depletion of the neurotransmitters serotonin, norepinephrine or dopamine in the central nervous system. Serotonin is the most extensively studied neurotransmitter in depression. The current study revealed that treatment of rats with venlafaxine produced a significant improvement in immobility time, recorded in forced swim test, indicating improvement of CMS. The results are consistent with Dhir A et al, where they proved effectiveness of venlafaxine in increasing doses from 2 mg/kg up to 16 mg/kg in treatment of depression as it produced significant dose dependent shortening of immobility time in mice. Moreover, their results are enforced with addition of the alpha 2-adrenoceptor agonist; yohimbine. They concluded that alpha 2-adrenoceptors strongly affect monoaminergic neurotransmission by enhancing not only noradrenergic but also serotonergic neurotransmission [31]. On the other hand, Cryan JF et al, tried to explore the role of serotonin receptors in behavioral tests in general including the FST. They concluded that SSRIs as antidepressants had improved the immobility time in FST because of the increase in serotonergic transmission, while the increased climbing behavior, they had noticed, was attributed to adrenergic transmission and corticotrophin releasing factor [32]. In another study, Craft et al, had another view after studying sertraline (serotonin reuptake inhibitor) 10 mg/kg or desipramine (norepinephrine reuptake inhibitor) 10 mg/kg in rats pre-exposed to the FST in mid-pregnancy, neither subchronic nor chronic treatment with desipramine or ser-

Table 1. Mean of MDA brain levels (nmole/g tissue), Nitric oxide brain levels (μmol/gm tissue), Serotonin level and serum magnesium levels (mg/dl) for normal saline treated control group A, non treated stress group B, Barely extract (1 g/kg) treated group C, venlafaxine (32 mg/kg) treated group D and their combination treated group E; orally daily for one week before and continued nine weeks after induction of chronic mild stress in rats. Values are expressed as Mean S.E.M (n=8)

Groups	MDA level (nmole/g tissue)	NO level (μmol/gm tissue)	Serotonin level	Serum Magnesium (mg/dl)
A	34.64±2.41	25.99±2.17	17.75±0.75	2.93±0.15
B	83.21±6.70*	33.93±2.95*	8.375±0.596*	3.456±0.169
C	55.99±1.82$^{*\Delta}$	30.41±2.37	14.00±1.15$^{*\Delta}$	3.601±0.31*
D	30.58±2.28$^{\Delta\wedge}$	26.40±2.73	14.00±0.71$^{*\Delta}$	2.143±0.24$^{\Delta\wedge}$
E	32.14±0.97$^{\Delta\wedge}$	27.30±2.45	16.00±0.71$^{\Delta}$	1.948±0.17$^{*\Delta\wedge}$
F value	42.30	1.72	19.18	12.38
p value	0.000	0.167	0.0001	<0.001

$^{\Delta}$p is significant compared to Group B; *p is significant compared to Group A; $^{\wedge}$p is significant compared to Groups C; $^{\#}$p is significant compared to Groups D.

traline decreased immobility on postpartum day 2. On the contrary, chronic desipramine significantly decreased immobility in virgin controls. They stated that a reduction in some "active coping behaviors" but no significant change in immobility under the use of those antidepressant drugs [33]. The present study also revealed improvement in behavioral tests in chronic mild stress in rats with the use of barley extract, indicating improvement of the depressive state. However, the effect of barely is less significant than venlafaxine. Barley extract (1 g/kg) to rats had the highest improvement magnitude when combined to venlafaxine. The results agreed with Yamaura et al, who examined young green barley leaf in mice in two different doses 400 and 1000 mg/kg orally compared to imipramine as a tricyclic antidepressant. Barley green leaf showed dose dependent improvement in immobility time in FST. They attributed the barley antidepressant effect possibly to be mediated by inhibitory effect on hippocampal expression of mRNA for nerve growth factor [34]. Unfortunately, the single study in literature, used barley in stress or depression, done by Yamaura et al, focused only on FST, didn't assess other behavioral tests as saccharin preference or elevated plus maize test. They were evidenced in our study to be parallel to results of FST where the highest significance consumption or saccharine preference was for group E; combined barley and venlafaxine treatment followed by venlafaxine group, then by barley extract group. Kumar et al, had elucidated the venlafaxine modulatory role on nitric oxide and oxidative stress in two different doses of venlafaxine (5 and 10 mg/kg) for one week in an experimental model of chronic behavior despair in mice. They studied immobility periods on every alternate days in addition to plus maze tests when compared to naïve animals. In their study, seven days venlafaxine (5 and 10 mg/kg) treatment significantly caused anti-anxiety-like effect, improved locomotor activity and attenuated oxidative damage, reduced lipid peroxidation, nitrite concentration and caused restoration of reduce glutathione and catalase activity as compared to control. They suggested that NO modulation might be involved in the protective effects of venlafaxine [35]. In our study, we used higher doses of venlafaxine 32 mg/kg for longer duration that produced improvement in behavioral tests and oxidative stress parameter MDA, but there was no significant fall in brain NO levels either in venlafaxine treated group or combined with barley extract. On contrary to our results, regarding NO levels under the effect of venlafaxine, Krass et al, proved its role in suppression of synthesis of NO through the use of NO precursor; L-arginine that pledge the antidepressant effect of both imipramine and venlafaxine in mice and modify FST locomotion values [36].

Matching to our results at least in part, regarding the oxidative stress, Abdel-Wahab and Salama, who examined the antidepressant effect of long-term treatment (21 days) of venlafaxine in doses in three doses 5, 10 and 20 mg/kg/day, i.p. was tested using forced swimming test (FST) and tail suspension test (TST) in mice. They proved the improvement of oxidative stress parameters as glutathione transfer-

ase activity, reduced glutathione and the lipid peroxidation product MDA. They correlated the antidepressant effect of venlafaxine to antagonizing the oxidative stress and enhancing the antioxidant defense mechanisms. In relation to NO, their results were significant at the mentioned doses in mice for 21 days [37]. Our results using venlafaxine for more than 21 days in rats either as a sole therapy in group D or combined to barley extract in group E were not significant.

About 1% of total body magnesium is located extracellular mainly in serum and RBCs. Regarding the CNS, There is balance between cerebrospinal fluid (CSF) magnesium concentration and plasma magnesium concentration which is regulated by the active transport between these two compartments [38]. Being a cofactor for hundreds of enzymes involved in numerous metabolically important reactions, including those for oxidative stress, this might interpret our finding in the current study that there was increase in serum magnesium level associated with reduction of brain MDA levels in treated groups with highest significant level in group E, the combined treated group by both venlafaxine and barely extract.

Furthermore, activation of the NMDA receptor ion channel is blocked by magnesium in a voltage-dependent manner, and this blockade occurs when the concentration of magnesium is less than 1 mM, which is within the range of the magnesium level found in CSF and plasma. Lowering extracellular magnesium concentration was found to increase central hyperexcitability due to the disinhibition of the NMDA receptor channel [38, 39]. Many experimental evidences supported the role of magnesium in depression and other psychiatric illness as mania [38-42]. The current study support the magnesium impact to experimental depression in rats; where significant increase in its serum level has been proved in all treated stressed groups. The role of magnesium as a constituent in barley extract couldn't be pledged in our study, neither is denied. The presence of other constituents as beta glucan might have a role in combating the oxidative stress and hence the anti depressant effect in the current study. This opinion was proved in another experimental model by Delaney B et al, who proved the anti-oxidant anti-atherogenic effect of barley [43]. On the other hand venlafaxine anti depressant role in our study could be also interrelated by its, at least in part, anti-oxidant effect. Eren I et al, study on venlafaxine in depressed rats supported our results [44], proved the anti oxidant modulating effect to depression in their model. However, no study in literature enforced the relationship between serotonin magnesium and nitric oxide in stress or depression model in experimental work.

Conclusion

It can be complemented in our study that barley extract orally administered had reinforced the venlafaxine antidepressant and antioxidant effect when combined together in experimental induced chronic mild stress model in rats. Their compliment effect had explored further on brain serotonin and magnesium serum level. No significant effect on brain nitric oxide level had been proved in the current study by any of the treated regimen.

Conflict of interest

The authors declare that there is no conflict of interest.

Abbreviations

SSNRIs, The selective serotonin nor adrenaline reuptake inhibitors; NO, Nitric oxide; CMS, Chronic mild stress; FST, Forced swim test; EPM, Elevated Plus Maize test.

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