Antidepressant Like Effect of Sodium Orthovanadate in A Mouse Model of Chronic Unpredictable Mild Stress.

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Research Article

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Abstract

Depression is a psychiatric disorder characterized by low esteem, anhedonia, social deficit, and lack of interest. Decreased BDNF and impaired TrKB signaling be associated with depression. In our study, depressive-like behavior was induced in mice by chronic unpredictable mild stress (CUMS) model. Various behavioral tests like tail suspension test (TST), open field test (OFT), and sucrose preference test (SPT); biochemical analyses for corticosterone, reduced glutathione (GSH), lipid peroxidation (LPO), superoxide dismutase (SOD), nitric oxide (NO) and ELISA for BDNF were performed. Body weight was measured every week. Depressive-like behavior was associated with increased oxidative stress in the brain and subsequent reduction of BDNF. Further, sodium orthovanadate (SOV), a protein tyrosine phosphatase inhibitor was used as a test drug as it is reported to stimulate BDNF levels. Sodium orthovanadate (SOV-5 mg/kg, 10 mg/kg) and fluoxetine (10 mg/kg) was given to mice orally for 21 days before 30 minutes of stress induction. The behavioral tests reflected depressive-like behavior in CUMS, which was attenuated by both SOV and fluoxetine. SOV at 10 mg/kg has demonstrated significant results in our study by decreasing malondialdehyde levels (MDA/LPO), NO levels, and increasing GSH and SOD in both the cortex and hippocampus. Besides, ELISA revealed the elevation of BDNF levels in the treatment groups (SOV-5 mg/kg, 10 mg/kg, and FLX-10 mg/kg) as compared with the disease group (CUMS). Therefore, the treatment with SOV appeared to reverse both oxidative and nitrosative stress. Decreased serum corticosterone levels (SOV-5 mg/kg, 10 mg/kg); FLX (10 mg/kg) + SOV (5 mg/kg); FLX-10 mg/kg and *per-se*) and elevated BDNF level (SOV-5 mg/kg, 10 mg/kg and FLX-10 mg/kg) were associated with attenuation of depressive-like behavior. The findings of this preliminary study indicate that SOV has the potential to restore antidepressant-like effect or prevention of stress-induced anhedonia and so further molecular mechanisms will be warranted for clinical translation.

1. Introduction

Major Depressive Disorder (MDD) is one of the most prevalent, recurrent, and debilitating psychopathology forms. Epidemiological surveys indicate that the lifetime prevalence of MDD is 16.6%, with estimates as high as 21.3% in women [1].

The monoamine hypothesis formulated in the 1990s suggested deficiency or imbalances in the monoamine neurotransmitters, such as serotonin (5-HT), dopamine (DA), and norepinephrine (NE), as the cause of depression. The antidepressants are prescribed to treat mild to severe depression. However, despite the increased synaptic content of monoamine neurotransmitters, tricyclic antidepressants and selective serotonin reuptake inhibitors produce their effect after a lag period. Moreover, they are useful in only 50% of patients. Approximately 30–50% of patients don't even respond to their initial antidepressant trial, and the remission rates are as low as 37.5% [2]. This phenomenon probably results from the complex and multifactorial MDD etiology, which comprises psychosocial, biological, environmental, and genetic factors, explaining why most patients fail to respond to the standard monoaminergic antidepressants [3]. This limitation led to a paradigm shift towards the neurotrophin hypothesis, as

depression is associated with neuronal atrophy and neuronal loss in specific brain regions in several clinical and preclinical studies [4, 5].

Considering neurotrophins, the brain-derived neurotrophic factor (BDNF) is the major neurotrophin present in the central nervous system, which regulates neurogenesis [6, 7] as it has a prominent role in the growth, differentiation, maturation, and survival of neurons. It also promotes the formation of dendritic spines and thus improves the transmission efficiency of synapses by increasing their number. Hence it is vital for synaptic plasticity and augmentation of neurotransmission [7]. BDNF triggers the intracellular downstream signaling via multiple pathways i.e., phosphatidylinositol 3-kinase (PI3K), phospholipase Cyclase (PLC-), and mitogen-activated protein kinase (MAPK) [8, 9]. This results in the activation of cAMP response element-binding protein (CREB), modulating the expression of BDNF levels [10], thus improving synaptic plasticity and cell survival.

Several clinical and preclinical studies have implicated a close association between BDNF and depressive-like behavior. It has been suggested that in depression, the BDNF-TrkB pathway gets impaired, resulting in the reduced secretion of BDNF [11, 12], and the studies reflect that the treatment with antidepressant consequently increases the BDNF levels [13, 14]. Moreover, many clinically used antidepressants were found to increase BDNF levels and their effects are ascribed to this effect also [15–17].

Stressful life events trigger HPA axis hyperactivation in about 70% of depressive patients [18]. Increased corticosterone levels associated with chronic stress [19] impairs hippocampal BDNF function, which supports the hippocampal atrophy reported in major depression [20]. Stress also promotes the production of proinflammatory cytokines in the brain microglia, resulting in reduced hippocampal neurogenesis [21]. The inflammatory cytokines affect the phosphorylation of the BDNF receptor (TrkB), thereby interfering with BDNF signaling [22, 23].

Notably, sodium orthovanadate (SOV) is an inorganic compound belonging to the vanadium family, having a role as protein tyrosine phosphatase inhibitor (PTP) [24]. Protein tyrosine phosphatase inhibition by SOV has been reported to result in the activation of the PI3K/AKT, PLC-, and MAPK pathway, which further stimulates BDNF levels [25], improves cell survival, synaptic plasticity, and delay neuronal damage [26]. Additionally, a study done on subarachnoid hemorrhage in rats has revealed that the administration of SOV resulted in the elevation of the BDNF levels, and SOV protected cortical and hippocampal neurons after experimental subarachnoid hemorrhage by increasing BDNF levels [27]. Also, sodium orthovanadate elicits an antioxidant property by regulating levels of SOD, GPx, catalase, LPO, and glutathione in the diabetic rat [28].

With the above background, we hypothesized that SOV could have a beneficial effect in a rodent model of major depression. Further, the aim was to compare it with the clinically used fluoxetine in CUMS induced rodent model of depression to see whether the antidepressant-like effect by SOV is better than the fluoxetine or not.

2. Material And Methods

2.1 Experimental animals

Male Balb/c male mice with a weight ranging between 29–34 g were procured from Central Research Institute Kasauli and Central Animal House of Panjab University Chandigarh, India. All the mice were housed in a room at a temperature of 25 ± 2°C with proper light-dark periods (12h light/12h dark) with water and food provided *ad libitum*. This experiment was conducted between the period from 09:00 to 17:00 according to the guidelines provided by the Committee for Control and Supervision of Experiments on Animals (CPCSEA). The study protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Panjab University, Chandigarh, with an approval number of PU/45/99/CPCSEA/IAEC/2019/303.

2.2 Drugs and treatments

Sodium orthovanadate (SOV) (catalog no.: S6508-10G) and fluoxetine (FLX) were procured from Sigma-Aldrich (St. Louis, MO, United States). Distilled water was used to dissolve SOV and administered orally with the help of an oral gavage. Normal saline, i.e., 0.9% NaCl, was used to dissolve the fluoxetine that was administered orally in a dose of 10 mg/kg [29]. Doses were decided in accordance with the previously reported studies [27, 30] which have shown attenuation of neuronal death and oxidative damage along with alleviation of mitochondrial dysfunction. Mouse ELISA kit for BDNF estimation was procured from Elabscience (catalog no.: E-EL-M0203), USA. The whole treatment was given daily starting from day 8 to 28 days.

2.3 Experimental design

Animals (n = 42) were randomly divided into seven groups, containing an equal number of animals (n = 6) as depicted in Table 1.

Initially, for 1st week, normal saline water (0.9% NaCl) was given to the control group (that was not subjected to CUMS) and the CUMS group. From day 8th, treatment was given to all the groups before 30 minutes of stress induction [CUMS group and *per-se* group (CUMS + SOV 10 mg/kg)] till the 28 days. On 29th and 30th day sucrose preference test (SPT) was performed, whereas a tail suspension test (TST) was performed on the 31st day. To check the effect on locomotor activity, an open field test (OFT) was performed on the 32nd day. The duration of the protocol lasted for 33 days from the induction of CUMS model until the sacrifice of animals and body weight was analyzed weekly. On the 33rd day, blood was collected from the animals under anesthesia, and then animals were euthanized for isolation of cortex and hippocampus to perform biochemical analysis and ELISA (Fig. 1).

Table 1
Allocation of animals in the groups

Sr. No.	Groups	No. of Animals	Duration of Treatment
1.	Normal Control	6	1st – 28th
2.	CUMS	6	1st – 28th
3.	CUMS + SOV (5 mg/kg p.o.)	6	8th – 28th
4.	CUMS + SOV (10 mg/kg p.o.)	6	8th – 28th
5.	CUMS + FLX (10 mg/kg p.o.)	6	8th – 28th
6.	CUMS+ [SOV (5 mg/kg) + FLX (10 mg/kg)]	6	8th – 28th
7.	SOV (10 mg/kg p.o.)	6	8th – 28th

2.4 Procedure for CUMS

Chronic unpredictable mild stress (CUMS) has been widely used in animals to mimic depressive-like behavior in humans [31]. Animals were exposed to CUMS for 28 days except for the control and *per-se* group. The different types of stressors were given to the animal both on a regular and repetitive basis till the sacrifice of animals as depicted in Table 2 [32].

Table 2
Schedule of CUMS

Sr. No.	Days	Stressors	Duration
1	Day 1	Food + Water Deprivation	24 h
2	Day 2	Empty bottle + Foreign Object	1 h + 23 h
3	Day 3	Forced Swim* + Overnight illumination	6 min + 12 h
4	Day 4	Restraint stress + cage tilt**	2 h + 7 h
5	Day 5	Predator Sound + Food deprivation	10 min + 24 h
6	Day 6	Water deprivation + Overnight illumination	24 h + 12 h
7	Day 7	Cage tilt** + Empty bottle	7 h + 1 h
* Forced swim was done at a water temperature of about 10–12º C for 8–10 min.			
** Cage was tilted or inclined at 45º.			

2.5 Behavioral studies

2.5.1 Open field test (OFT)

To find out any effect of the drug on locomotor activity, OFT was conducted. The number of lines crossed was evaluated in the open field paradigm under normal daylight. The whole procedure was performed as per the previously described study [33]. Before the commencement of OFT, mice were acclimatized to the environment for 2–3 min. The test apparatus consisted of an arena with a measurement of 50 cm x 30 cm and painted with black color. The floor of the test apparatus consisted of 25 virtually produced grids made with the help of Ethovision. Each mouse was placed in the middle of the arena and then allowed to explore freely. The number of lines crossed by the mice with all their paws within each grid in 6 min was evaluated. After each testing, the apparatus was cleaned with 70% ethanol to remove any odor and clues of the previous mouse made by its urine and fecal content. Results were expressed as the number of lines crossed.

2.5.2 Sucrose preference test (SPT)

A state of anhedonia characterizes depression, and lowered sucrose consumption in rodents is a clear-cut indication of this state. The procedure was performed as described previously [34]. Briefly, 30 h before the test, mice were deprived of water and food, then 2 bottles were placed in the cage containing 1% sucrose solution (w/v) and regular tap water, respectively. Animals were freely allowed to access both the bottles for 24 h. At the end of 24 h, the sucrose preference (%) was calculated as below. Results were expressed as the percentage of sucrose consumed.

Sucrose consumption

Sucrose consumption + Water consumption

2.5.3 Tail suspension test (TST)

This test was performed as per the previously described procedure [35]. Before the conduction of TST, all mice were acclimatized to surroundings for 2–3 min. Mice were isolated from any external sound and visuals and placed 50–55 cm above the ground by fixing the tail on the frontal lever (approx. 1 cm from tip) with adhesive tape. During the total 6 min of the test, acclimatization was done for an initial 2 min, and the remaining time was utilized for recording the immobility of mice on the kymograph. Results were expressed as immobility time (sec).

2.5.4 Bodyweight measurement

Animal body weight was measured every week of the experimental protocol to observe for the changes. Results were expressed as grams.

2.6 Biochemical estimations

2.6.1 Tissue homogenate preparation

After completing all the behavioral evaluations, the animals were euthanized by cervical dislocation. Before cervical dislocation, anesthesia [ketamine (70 mg/kg) and xylazine (10 mg/kg)] was injected intraperitoneally. During the anesthetic state, the blood was collected through the retro-orbital plexus and

stored in the Eppendorf containing EDTA. Afterward, the animals were sacrificed; brains were isolated and perfused with PBS. The entire cortex and hippocampus were isolated later from the whole brain. The isolated tissues were stored and homogenized in 10% (w/v) homogenization buffer (comprising of 10 mM Tris-HCl, 150 mM $MgCl_2$, 1mM EDTA, 1% Triton X 100, pH equivalent to 7.4) and centrifuged at 10,000 rpm and 4º C for 20 min. After the centrifugation, the supernatant was isolated by pipette and stored at − 80º C for various antioxidant assays and ELISA. Further, the plasma was separated by the process of centrifugation at 10,000 rpm for 10 min and stored at -80ºC for further estimations.

2.6.2 Estimation of Protein

The biuret method was used for the quantification of protein [36]. The standard curve of bovine serum albumin was used to determine protein concentration expressed in mg/ml. Thus, the values obtained were used in the calculations of other biochemical results.

2.6.3 Reduced glutathione (GSH) assay

Reduced glutathione was estimated based on a previous study [37]. In the method, 100 µl of the supernatant of tissue homogenate was added to 1 ml of 4% w/v sulfosalicylic acid. The precipitate was formed, and the reaction mixture was kept at temperature 2–8°C in the refrigerator. After one hour, samples were centrifuged in a cold centrifuge, i.e., 4°C, at a rotation of 1200 g for 15 min. Pellet was discarded to obtain the supernatant. Furthermore, 100 µl of this supernatant, 2.7 ml of 0.1 M phosphate buffer (pH 8), and 200 µl of 0.1 M 5,5-dithiobis-2-nitrobenzoic acid (DTNB-Ellman's reagent) were mixed to produce a pale-yellow color. The color produced was read at 412 nm with a UV–visible spectrophotometer (Perkin Elmer, USA). The calculation was done by applying the molar extinction coefficient of 1.36×10^4 M^{-1} $cm^{-1,}$ and the results were expressed as µM GSH per mg protein.

2.6.4 Estimation of lipid peroxidation (LPO)

LPO was conducted by evaluating malondialdehyde levels [38]. Concisely, 0.5 ml of tissue homogenate was added to 0.5 ml of Tris-HCl followed by 2 h of incubation at 37 ºC. To the above mixture, 1.0 ml of 10% trichloroacetic acid (TCA), was added and then centrifuged at 1000g for 10 min. Then, 1.0 ml of supernatant from the above solution was pipetted out and mixed with 1.0 ml of thiobarbituric acid (0.67% w/v). The tubes containing the mixture were put in boiling water, for 10 min, followed by cooling and the addition of 1.0 ml of double-distilled water. Reading for absorbance was noted at 532 nm (UV-VIS Spectrophotometer, Perkin Elmer, Lambda 20). Levels of MDA were measured and expressed as the amount of MDA (nmoles/mg protein).

2.6.5 Estimation of superoxide dismutase (SOD)

SOD was estimated as per described study [39]. 0.1 mM EDTA at 10.8 pH, 96 mM nitro blue tetrazolium (NBT), and 50 mM sodium carbonate mixture were prepared. The supernatant of tissue homogenate (50 µl) was added to the above mixture followed by hydroxylamine hydrochloride (0.5 ml) results in the oxidation of hydroxylamine hydrochloride. Finally, absorbance was measured at 560 nm wavelength for 2 min and SOD values were calculated as SOD units/mg protein.

2.6.6 Estimation of plasma corticosterone

The plasma corticosterone estimation was done as previously described [40]. For the assessment of corticosterone levels in the blood (plasma), the reagents respectively reagent A (0.10% p-nitroso-N, N-dimethylaniline in ethanol), reagent B (0.10 % phenol in ethanol), and reagent C (1% aqueous solution of potassium ferricyanide) were prepared freshly. An equal volume of ethanol and sample (1 ml) was added to reagent A, and then the final solution was stored in ice water for 5 min only. Further, 0.5 ml of 0.10 M NaOH was added and incubated for the duration of 5 h at 0ºC. Upon completing the above procedure, 2 ml of buffer (0.20 M boric acid, 0.20 M KCl, and 0.20 M KOH) was added along with the addition of reagent B and reagent C in the resulted solution. Then, the final solution prepared was kept for 10 min at 20 ± 2□C. Lastly, the reading was noted down at 650 nm (UV-VIS Spectrophotometer, Perkin Elmer, Lambda 20).

2.6.7 Estimation of nitrite (NO)

For nitrite assay, equal volumes, i.e., 100 µl of tissue homogenate sample, was mixed with 100 µl of Griess reagent (0.1% naphthyl ethylenediamine dihydrochloride + 1% sulphanilamide in 5% phosphoric acid). This mixture was incubated at 25–30°C for 10 min in a dark place. Absorbance readings were taken in a UV–visible spectrophotometer at 540 nm. Nitrite concentrations were interpreted from a standard curve of sodium nitrite solution, and results were expressed as µg of nitrite per mg protein [41].

2.7 Molecular estimations

2.7.1 Enzyme-linked immunosorbent assay (ELISA)

ELISA was performed in the hippocampal and cortical tissue of the brain as they are the major brain regions associated with mood disorders.

2.7.1.1 BDNF protein

BDNF has a significant role in neurogenesis and synaptic plasticity. Levels of BDNF were analyzed using a mouse BDNF ELISA kit (Elabscience). 100 µl of standard/tissue homogenate was added to each well of the pre-coated plates and incubated for 90 min at 37^{O}C. The liquid was removed, and 100 µl of biotinylated detection antibody was added to each of the wells and incubated for 60 min at 37^{O}C. The plate was washed 3 times using wash buffer. Then 100 µl of HRP conjugate was added to each of the wells and kept at 37^{O}C for 30 min. The plate was then again washed 5 times, and 90 µl of substrate reagent was added to it and incubated for 15–30 min at 37^{O}C for blue color development. Finally, 50 µl of stop solution was added to each well, and a color change to yellow was observed. The optical density of the reaction was measured at 450 nm. Results were expressed as pg/ml of BDNF.

2.8 Statistical Analysis

Analysis of data was done using a one-way ANOVA or two-way ANOVA followed by a Tukey's post hoc test or Bonferroni's post hoc test respectively for multiple comparisons. Statistically significant effects were defined as those with levels of P-values < 0.05. The standard error of the mean was represented by error bars. Prism Graphpad 5.0 (GraphPad Software Inc., Ca, USA) was used to analyze the data.

3. Results

3.1 Effect of SOV, fluoxetine and their combination on locomotor activity in open field test

A significant effect in the locomotion was observed across the groups after one-way ANOVA followed by Tukey's post hoc test [$F_{(6,29)} = 8.21$ ($p < 0.001$)]. CUMS for 28 consecutive days significantly ($p < 0.001$) decreased the ambulatory score as compared to the control group. Treatment with SOV (10 mg/kg) and fluoxetine significantly increased the ambulatory score when compared to CUMS group ($p < 0.05$). Treatment with a combination group showed no significant difference in the ambulatory score than the CUMS and control group. The *per-se* group did not show any difference compared with the control group (Fig. 2).

Values are expressed as mean ± SEM. For statistical significance, $^{*}p < 0.05$ compared to the control group; #p < 0.05 compared to CUMS group (One-way ANOVA followed by Tukey's post hoc test). CUMS: Chronic Unpredictable Mild Stress, SOV: Sodium orthovanadate.

Effect of SOV, fluoxetine and their combination on the consumption of sucrose in a Sucrose preference test

The sucrose preference test was calculated by one-way ANOVA followed by Tukey's post hoc test [$F_{(6,37)} = 0.77$ ($p < 0.001$)]. Sucrose preference was significantly reduced in CUMS group compared to the control group ($p < 0.001$). Treatment with SOV (5 mg/kg and 10 mg/kg), fluoxetine, and combination groups significantly increased percentage sucrose preference as compared to CUMS group ($P < 0.001$). Fluoxetine was not showing any significant difference among treatment groups. *Per-se* group demonstrated a significant decrease in the sucrose consumption as compared to the control group ($p < 0.001$) (Fig. 3)

Values are expressed as mean ± SEM. For statistical significance, $^{*}p < 0.05$ compared to the control group; #p < 0.05 compared to CUMS group (One-way ANOVA followed by Tukey's post hoc test). CUMS: Chronic Unpredictable Mild Stress, SOV: Sodium orthovanadate.

3.3 Effect of SOV, fluoxetine and their combination on an immobility duration in tail suspension test

The one-way ANOVA followed by Tukey's post hoc test has suggested a significant effect across groups ($F_{(6,17)} = 5.14$ ($p < 0.001$)]. Induction of depressive-like behavior by CUMS was evident by a significant

increase in the immobility time ($p < 0.001$) as compared to the control group. Comparable effects were seen in the treatment with sodium orthovanadate [5 mg/kg ($p < 0.001$) and 10 mg/kg ($p < 0.05$)] and fluoxetine [10 mg/kg ($p < 0.05$)] that significantly decreased the immobility time when compared to CUMS group. Treatment with the combination drug also significantly reduced the immobility time when compared with CUMS group ($P < 0.001$). *Per-se* group did not show any significant effect compared to the control group (Fig. 4).

Values are expressed as mean ± SEM. Statistical significance was $^{*}p < 0.05$ compared to the control group; #$p < 0.05$ compared to CUMS group (One-way ANOVA followed by Tukey's post hoc test). CUMS: Chronic Unpredictable Mild Stress, SOV: Sodium orthovanadate.

3.4 Effect of SOV, fluoxetine and their combination on body weight

Bodyweight was observed to be significantly low in CUMS group from day 8 to day 33 as compared to the control group ($p < 0.001$). Treatment with SOV (5 mg/kg and 10 mg/kg) produced no significant body weight change when compared with the CUMS group. However, fluoxetine treatment significantly increased body weight compared to CUMS group on day 15 ($p < 0.05$), on day 28, and day 33 ($p < 0.01$). Similarly, combination treatment also significantly increased the bodyweight of CUMS rats as compared to CUMS group from day 22 to 33 ($p < 0.001$). *The per-se* group showed a significant decrease in body weight as compared to the control group from day 15 to 33 ($p < 0.001$) (Table 3).

Table 3
Effect of sodium orthovanadate, fluoxetine, and their combination on the body weight.

GROUPS							
Control	Disease (CUMS + Vehicle)	CUMS + SOV (5mg/kg)	CUMS + SOV (10mg/kg)	CUMS + [(SOV (5mg/kg) +FLX (10mg/kg)]	CUMS + FLX (10mg/kg)	SOV (10mg/kg)	
1	33.50 ± 0.8	30.86 ± 0.7	29.94 ± 1.5	29.43 ± 0.4	31.02 ± 0.9	29.28 ± 0.7	29.14 ± 0.8
8	34.50 ± 0.6	29.5 ± 0.3*	30.23 ± 1.6	29.43 ± 0.3	33.12 ± .17	32.71 ± 1.5	31.42 ± 0.4
15	37.50 ± 1.2	27.5 ± 0.4*	27.08 ± 1.5@	26.51 ± 0.8@	31.76 ± 2.3	32.85 ± 1.6#	29.85 ± 1.2*
22	37.34 ± 1.2	28.5 ± 1.3*	28.08 ± 1.4	26.57 ± 1.6@	36.87 ± 1.6#	32.85 ± 1.6	27.71 ± 1.3*
28	35.34 ± 0.8	25.8 ± 1.7*	26.75 ± 1.1@	26.85 ± 1.5@	41.56 ± 1.7#	37.14 ± 1.5#	26.57 ± 1.7*
33	39.35 ± 1	24.5 ± 1.6*	30.75 ± 1.6#,@	29.14 ± 1.3@	34.68 ± 2.0#	38.66 ± 1.8#	29.34 ± 1.1*

Values are expressed as mean ± SEM. For statistical significance, *$p < 0.05$ as compared to the control group; #$p < 0.05$ as compared to CUMS group; @$p < 0.05$ as compared to fluoxetine group (Two-Way ANOVA followed by Bonferroni's post hoc test). CUMS: Chronic Unpredictable Mild Stress, SOV: Sodium Orthovanadate.

Effect of SOV, fluoxetine and their combination on the level of GSH, LPO, and activity of SOD

A significant effect was observed across the groups on antioxidant enzyme activity of GSH, LPO and SOD in cortex after one-way ANNOVA followed by Tuckey's post hoc test [$F_{(6,14)} = 8.15$ ($p < 0.001$)], [$F_{(6,14)} = 8.43$ ($p < 0.001$)] and [$F_{(6,14)} = 20.32$ ($p < 0.001$)] respectively, and in hippocampus [$F_{(6,14)} = 10.95$ ($p < 0.001$)], [$F_{(6,14)} = 6.22$ ($p < 0.001$)] and [$F_{(6,14)} = 29.22$ ($p < 0.001$)] respectively. In both the hippocampus and cortex areas in CUMS group, the levels of GSH were decreased ($p < 0.001$) significantly compared to the control group. Treatment with sodium orthovanadate (5 mg/kg and 10 mg/kg) significantly increased the levels of GSH in both cortex ($p < 0.05$ and $p < 0.01$) and hippocampus ($p < 0.05$). Fluoxetine showed a significant increase in the levels of GSH in both cortex and hippocampus compared to the CUMS group ($p < 0.05$). In addition to above, combination of SOV (5 mg/kg) and FLX (10 mg/kg) significantly increased the levels of GSH on comparing with CUMS ($p < 0.05$). *Per-se* group did not show any significant result in the GSH levels compared to the control group, both in the hippocampus and cortex (Fig. 5).

Values are expressed as mean ± SEM. For statistical significance, $^{*}p < 0.05$ compared to the control group; #p < 0.05 compared to CUMS group (One-way ANOVA followed by Tukey's post hoc test). CUMS: Chronic Unpredictable Mild Stress, SOV: Sodium Orthovanadate.

The level of LPO in CUMS group was found to be significantly increased in both cortex ($p < 0.001$) and hippocampus ($p < 0.05$) as compared to the control group. Treatment with sodium orthovanadate (10 mg/kg), fluoxetine, and combination group significantly decreased the levels of LPO as compared to CUMS group in both cortex ($p < 0.01$) and hippocampus ($p < 0.05$). In addition to the above, sodium orthovanadate (5 mg/kg) significantly decreased the levels of LPO in the cortex region ($p < 0.001$). In both cortex and hippocampus, the lipid peroxidation did not increase in the *per-se* group as compared to the control group (Fig. 6).

Values are expressed as mean ± SEM. For statistical significance, $^{*}p < 0.05$ compared to the control group; #p < 0.05 compared to CUMS group (One-way ANOVA followed by Tukey's post hoc test). CUMS: Chronic Unpredictable Mild Stress, SOV: Sodium Orthovanadate.

The antioxidant enzyme SOD was significantly decreased in both the hippocampal and cortical regions of the brain in CUMS group compared to control mice ($p < 0.001$). Treatment with sodium orthovanadate (10 mg/kg) showed a significant increase in the level of SOD in both hippocampus ($p < 0.01$) and cortex ($p < 0.05$) as compared with CUMS group. Fluoxetine showed a significant increase in the SOD levels in the hippocampus as compared to CUMS group ($p < 0.001$), and also SOD levels in the combination group were found to be increased significantly in the hippocampus as compared to CUMS group ($p < 0.01$). *Per-se* group significantly decreased the SOD levels in both hippocampus and cortex as compared to the control group ($p < 0.05$) (Fig. 7).

Values are expressed as mean ± SEM. For statistical significance, $^{*}p < 0.05$ as compared to the control group; $^{\#}p < 0.05$ as compared to CUMS group, $^{@}p < 0.05$ as compared to fluoxetine (One-way ANOVA followed by Tukey's post hoc test). CUMS: Chronic Unpredictable Mild Stress, SOV: Sodium Orthovanadate.

3.6 Effect of SOV, fluoxetine and their combination on the levels of plasma corticosterone and NO

Significant effects were observed on plasma corticosterone levels across the groups after analysis by one-way ANOVA followed by Tukey's post hoc test [$F_{(6,14)} = 37.06$ ($p < 0.001$]. The plasma corticosterone levels were significantly increased in CUMS group as compared to the control group ($P < 0.001$). Treatment with sodium orthovanadate (5mg/kg and 10 mg/kg), fluoxetine, and combination group significantly decreased the levels of plasma corticosterone as compared to CUMS group ($p < 0.001$). The *per-se* group produced a significant decrease in plasma corticosterone levels as compared to the control group ($p < 0.001$) (Fig. 8).

Values are expressed as mean ± SEM. For statistical significance, $^{*}p < 0.05$ compared to the control group; #p < 0.05 compared to CUMS group (One-way ANOVA followed by Tukey's post hoc test). CUMS: Chronic Unpredictable Mild Stress, SOV: Sodium Orthovanadate.

One-way ANOVA followed by Tukey's post hoc test has demonstrated a significant effects on nitrite levels in both cortex [$F_{(6,14)} = 24.36$ ($p < 0.001$)] and hippocampus [$F_{(6,14)} = 27.67$ ($p < 0.001$)] across groups. CUMS significantly increased nitrite levels in both cortex and hippocampus ($p < 0.001$) compared to the control group. Treatment with SOV (10 mg/kg) significantly decreased the nitric oxide levels in both hippocampus ($p < 0.001$) and cortex ($p < 0.05$) as compared to CUMS group. Fluoxetine also showed a significant decrease in nitrite levels when compared to CUMS group in both cortex ($p < 0.05$) and hippocampus ($p < 0.001$) regions. However, SOV (10 mg/kg) in both cortex ($p < 0.05$) and hippocampus ($p < 0.001$) area significantly decreased NO levels as compared to CUMS group. No significant difference has been observed between combination and CUMS group in both cortex and hippocampus. *Per-se* group significantly increased the levels of nitrite as compared to the control group ($p < 0.001$) (Fig. 9).

Values are expressed as mean ± SEM. For statistical significance, $^{*}p < 0.05$ as compared to the control group; $^{\#}p < 0.05$ as compared to CUMS group, $^{@}p < 0.05$ as compared to fluoxetine (One-way ANOVA followed by Tukey's post hoc test). CUMS: Chronic Unpredictable Mild Stress, SOV: Sodium Orthovanadate.

3.7 Effect of SOV and fluoxetine on the levels of brain-derived neurotrophic factor

BDNF levels were observed to be decreased significantly across the groups in both cortex [($F_{(4,5)} = 11.43$ ($p < 0.001$)] and hippocampus [$F_{(4.5)} = 19.57$ ($p < 0.001$)]. BDNF levels were decreased in CUMS group as compared to the control group ($p < 0.05$). Treatment with SOV (5 mg/kg and 10 mg/kg) and fluoxetine significantly increased the BDNF levels in both hippocampus and cortex as compared to CUMS group ($p < 0.05$) (Fig. 10). No significant difference has been observed between FLX (10 mg/kg) and SOV (5 mg/kg, 10 mg/kg) groups in both cortex and hippocampus.

Values are expressed as mean ± SEM. For statistical significance, $^{*}p < 0.05$ compared to the control group; #p < 0.05 compared to CUMS group (One-way ANOVA followed by Tukey's post hoc test). CUMS: Chronic Unpredictable Mild Stress, SOV: Sodium Orthovanadate.

4 Discussion

In our present study, we evaluated SOV for the antidepressant-like effects by analyzing various behavioral tests, anti-oxidative enzyme activity, and BDNF levels associated with depressive-like behavior. Corticosterone, a stress hormone, is an indicator of anxiety and depressive-like behavior in an individual. Stress elevates corticosterone levels by activating the HPA axis, resulting in neuronal atrophy arising due to decreased brain levels of neurotrophins like BDNF. Corticosterone decreases BDNF mRNA expression

gradually, resulting in diminished levels of BDNF protein translation [42]. BDNF is one of the growth factors that trigger neuronal survival after BDNF–TrkB signaling. Impairment in the neurotrophins, mainly BDNF, leads to depressive-like behavior, increased hippocampal dendritic atrophy, cell death, and reduced LTP.

Chronic stress causes HPA axis dysregulation, and many studies have reported a decrease in the proliferation and survival of hippocampal neurons when the HPA axis is dysregulated [43]. Moreover, chronic stress-induced HPA axis dysfunctioning results in the production of proinflammatory cytokines [21]. Neuroinflammation leads to oxidative stress and both together generate a vicious cycle resulting in reduced hippocampal neurogenesis.

CUMS paradigm, which is a well-validated model of depression produced by the set of stressors in rodents [44]. CUMS significantly increased the levels of corticosterone depicting a state of stress. However, the treatment with SOV significantly decreased plasma corticosterone levels. The effect produced by SOV *per-se* on corticosterone levels is consistent with previous findings where vanadium compound attenuated corticosterone levels in rats [45]. According to a prior study, the chronic FLX treatment in CUMS exposed rats also normalized the corticosterone levels [46] as reported in our study.

CUMS also downregulates the levels of BDNF and CREB, resulting in a depressive-like behavior [47] and so, we measured the levels of BDNF in both hippocampus and cortex. The results have demonstrated that both the doses of SOV and FLX significantly elevated the BDNF levels in CUMS rats. The effect of SOV be through It was observed that fluoxetine significantly increased the levels of BDNF in both hippocampus and cortex. A published report suggested that PTP1B down-regulates the neuronal BDNF-TrkB pathway through the dephosphorylation of TrkB, whereas PTP1B inhibition boosts BDNF signaling [48]. It is well postulated that SOV, directly acting at tyrosine residues of TrkB, preserves its signaling and also recovers tyrosine kinase activity of TrkB by upregulating m-BDNF [30]. Moreover, antidepressants like fluoxetine may be increasing BDNF levels via triggering transcription regulators, i.e., CREB [49].

CUMS has also shown significant effects on body weight where body weight indicates the pathogenesis of the disease, and it was found that CUMS rats demonstrated a significant decrease in weight compared to the control group, thus depicting one of the core signs of a depressive-like behavior [50]. In our study, treatment with SOV produced no significant gain in body weight compared to CUMS group. However, SOV *per-se* has made a significant decrease in weight gain as compared to the control group. The above peculiar effect of SOV concerning bodyweight has supported our results with the previous finding in which vanadium-fed dams had lower food intakes and weight gains than controls during pregnancy [51]. Vanadium compound-induced weight loss could also be attributed to the reduction in neuropeptide Y synthesis (NPY), which is responsible for the stimulation of appetite [52]. At the same time, significant weight gain was observed in the fluoxetine and control groups. In the former case, the antidepressant tends to increase body weight [53], appetite, and a study conducted on humans also support our results regarding the elevation of body weight by fluoxetine as serotonin is responsible for appetite [54].

Increased immobility in the tail suspension test (TST), regarded as a condition of 'failure to adapt to stress' [55] was produced after CUMS induction. Significant associations were observed between decreased immobility and the potency of antidepressants in tail suspension tests [56].

CUMS model shows a declined sensitivity to reward, termed as the anhedonic state [57] and, is one of the core symptoms of depressive-like behavior. A study confirmed that mice exposed to CUMS consume less sucrose fluid [58], supporting our findings. An open field test was conducted to analyze the locomotor activity and explore the novel environment [38]. Many studies depict the decline in the number of line crossing activity (OFT) in CUMS group, reflecting depressive-like behavior [58]. Our study also showed a similar finding reflecting the effect of CUMS in the induction of depressive-like behavior. However, both the SOV doses in the present study improved the core features of depressive-like symptoms in rodents like anhedonia, despair behavior, and hypo-locomotion. SOV *per-se* resulted in decreased sucrose consumption that could result from its roles in improving leptin and insulin signaling, which play an essential role in regulating energy balance through food-associated reward control [48].

Hippocampal oxidative stress is induced due to low BDNF after chronic unpredictable mild stress. Numerous studies have highlighted that stress significantly decreases BDNF mRNA expression in the frontal cortex and hippocampus [59], thus indicating a link between BDNF and oxidative stress. A study found that BDNF downregulates ethanol-induced cellular oxidative stress and apoptosis in developing hypothalamic neuronal cells [23] thus affirming antioxidant-like activities of BDNF [5]. In the present study, we analyzed MDA levels (an indicator of lipid peroxidation), NO, GSH, and SOD in the hippocampal and cortical tissues of mice exposed to CUMS. CUMS exposure resulted in the generation of oxidative stress and nitrosative stress in both the cortex and hippocampus. However, chronic SOV treatment has ameliorated oxidative and nitrosative stress in the brain, emphasizing the role of BDNF in mediating antioxidant effects [60]. SOV significantly increased SOD levels after CUMS exposure and the findings are consistent with a previous study indicating the neuroprotective effect of SOV [59]. In our present study, FLX (10 mg/kg) in combination with SOV (5 mg/kg) has demonstrated a significant enhancement in the GSH and SOD levels.

MDD is associated with lipid peroxidation and decreased antioxidative enzyme activities resulting in reactive oxygen species (ROS) generation [59, 61]. Neuronal degeneration is a significant consequence of ROS generation. *In vitro* studies suggested that ROS act in a neurotoxic as well as in a neuroprotective manner, which is enhanced by TrkB [62]. However, ROS has a significant role in psychiatric disease due to the vulnerability of the central nervous system to oxidative stress, and CUMS results in induction of ROS expression by Akt pathway modulation [63]. In addition to the above finding, a study conducted on cisplatin-induced ROS suggested that BDNF attenuates ROS generation, resulting in a decline in ROS levels [64]. Indeed, a specific pathway has not been evolved fully to support the link between ROS and TrkB.

Meanwhile, a study conducted on cell lines revealed that vanadate compound *per-se* could generate ROS resulting in the decreased levels of SOD via MAPK pathway activation [65]. And our study has also

demonstrated that SOV *per-se* decreased SOD and increased nitrite levels per-se while it does not affect MDA levels. Whereas it produced a significant antioxidant effect in CUMS exposed rats supported by a previously conducted study [59].

Our study resulted that CUMS exposure increased nitrite levels, whereas SOV (*per-se)* also increased nitrite levels that may be via the Akt pathway activation [66] while SOV at a dose of 10 mg/kg significantly decreased its levels. However, the combination of SOV 5 mg/kg and FLX 10 mg/kg have not shown a significant effect in NO levels with CUMS group. This might be due to the activation of the Nrf2 pathway by FLX [67] and Akt pathway by SOV (Aid, Kazantseva et al. 2007), which has demonstrated a synergistic effect on NO upregulation. CUMS also activates microglia, which further regulate the production of inflammatory cytokines. These inflammatory markers are the leading cause for the production of nitrites in the brain whereas protein tyrosine phosphatase 1B (PTP1B), a member of the protein tyrosine phosphatases (PTPs) family, positively regulates neuroinflammation by causing dephosphorylation of proteins at tyrosine residues. SOV (10 mg/kg), a PTP inhibitor reduces this feature induced by CUMS, resulting in a decline in nitrite levels than the CUMS group [68].

From the above findings, we observed that the SOV has depicted an antidepressant-like effect which could be attributed to its antioxidant and BDNF increasing activity. However, the effect was found to be comparable with the standard drug fluoxetine and neither the combination of SOV with fluoxetine produced any synergistic effect.

5 Conclusion

BDNF has many divergent roles in the neuroscience area, and its regulatory effect on the TrkB receptor opens avenues for further research on psychiatric and neurological disorders. PTP inhibitors positively modulate BDNF-TrKB signaling. In our present study, SOV has demonstrated the enhanced BDNF levels at a dose of 10 mg/kg and prominently reversed the oxidative stress markers, reduced corticosterone levels, and ameliorated depressive-like behavior in the animals. However detailed molecular mechanisms were not deciphered in the present study and so future studies, are warranted to affirm the role of SOV in major depression.

Declarations

Declaration of interest

None.

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Conflict of interest

The authors declare no conflict of interest.

Availability of data and material

Not Applicable

Code availability

Not Applicable

Authors' contributions

Angel Joshi has conducted the entire study and has also written a whole manuscript. Ansab Akhtar has performed the editing of the manuscript and supports the conduction of the study. Priyanka Saroj has supported the conduction of various biochemical assays. Anurag Kuhad and Sangeeta Pilkhwal Sah had reviewed, edited, and finalized the manuscript. In addition to the above, both Anurag Kuhad and Sangeeta Pilkhwal Sah had provided technical support in the whole conducted study. The authors declare that all data were generated in-house and that no paper mill was used.

Ethics approval

The study protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Panjab University, Chandigarh, with an approval number of PU/45/99/CPCSEA/IAEC/2019/303.

Consent to participate

Not Applicable

Consent for publication

Not Applicable

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Figures

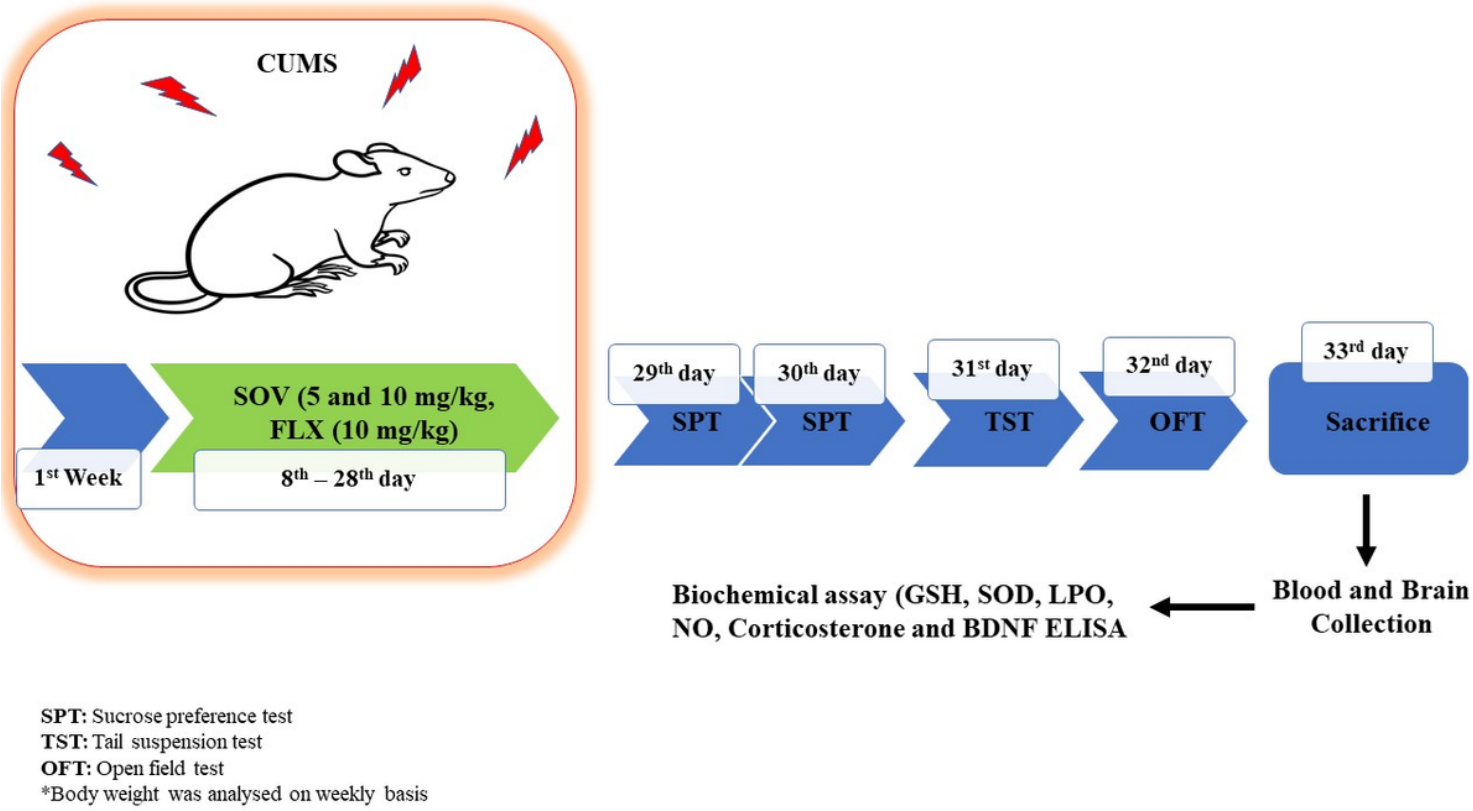

Figure 1

Experimental design.

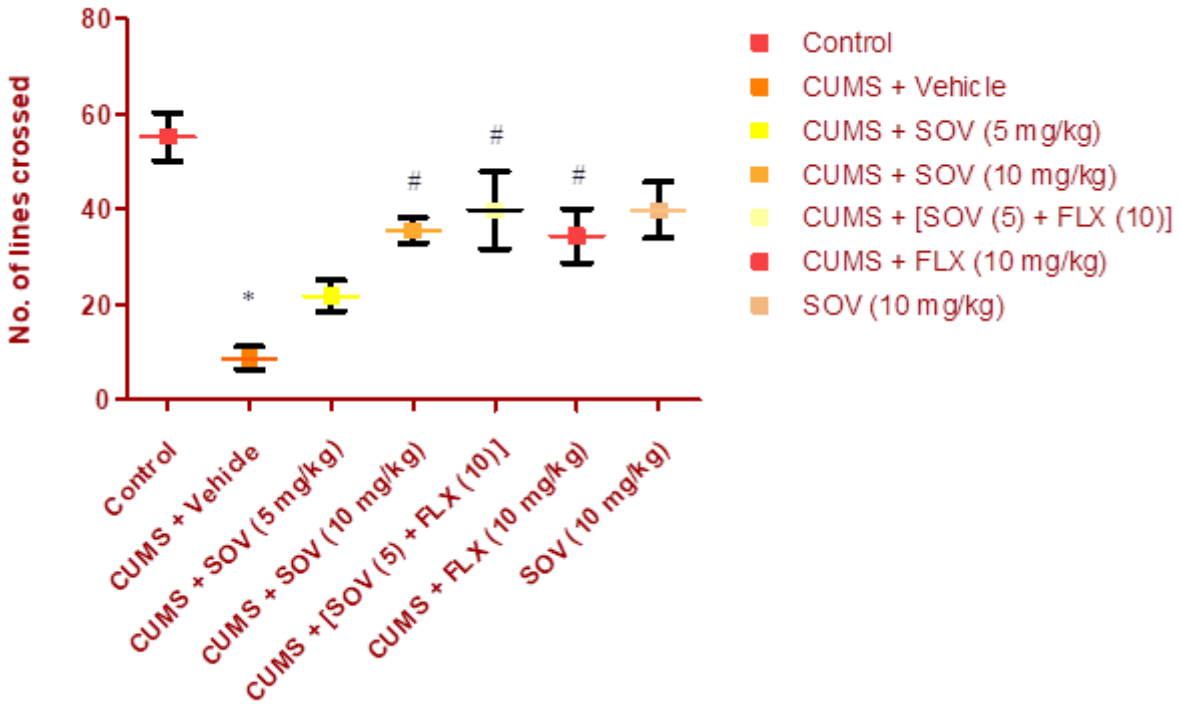

Figure 2

Effect of SOV, fluoxetine, and their combination on locomotor activity in the open field test.

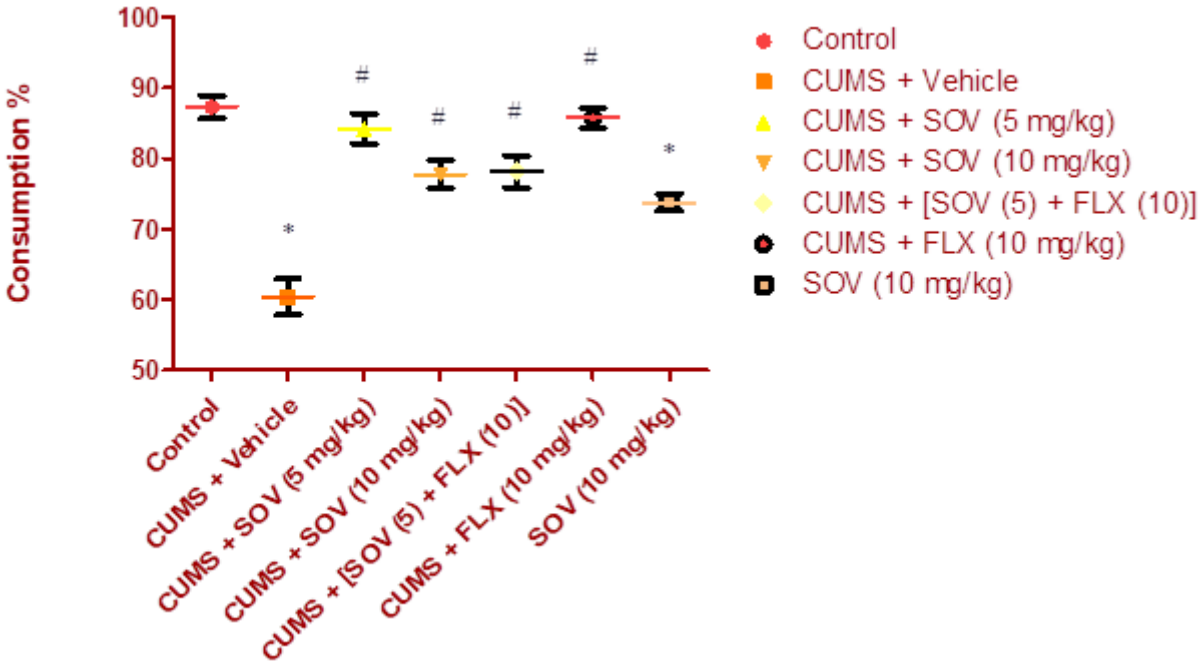

Figure 3

Effect of SOV, fluoxetine and their combination on the consumption of sucrose in a sucrose preference test

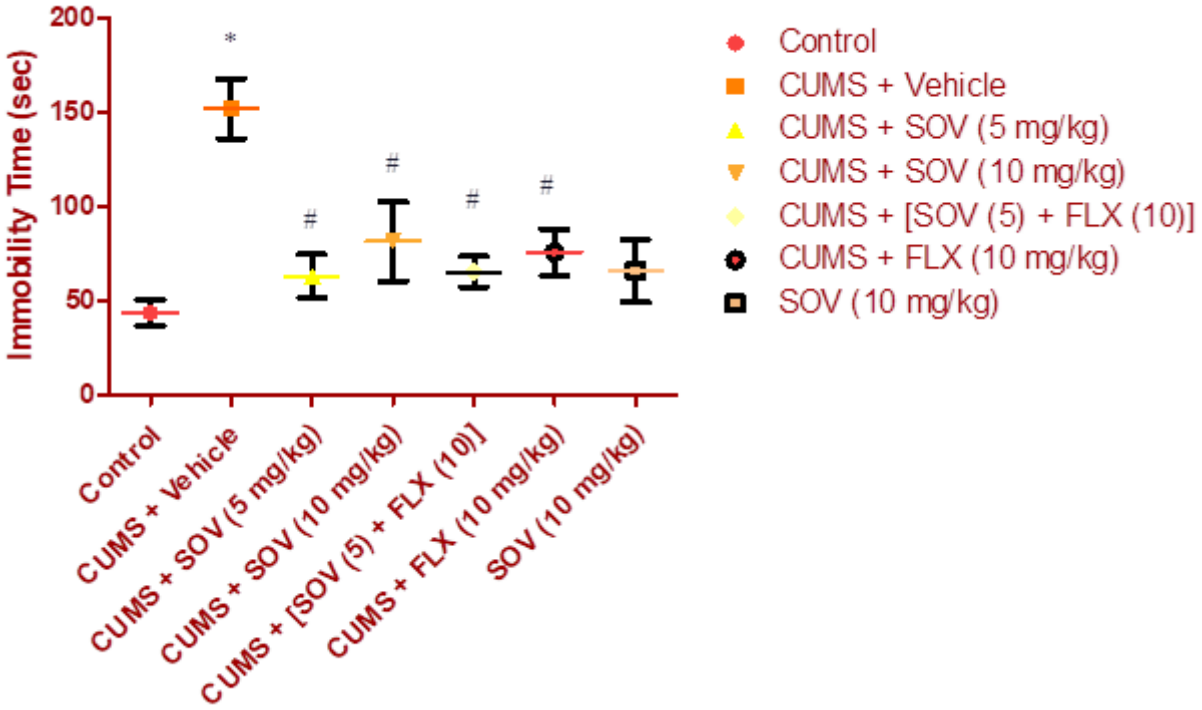

Figure 4

Effect of SOV, fluoxetine and their combination on an immobility duration in tail suspension test

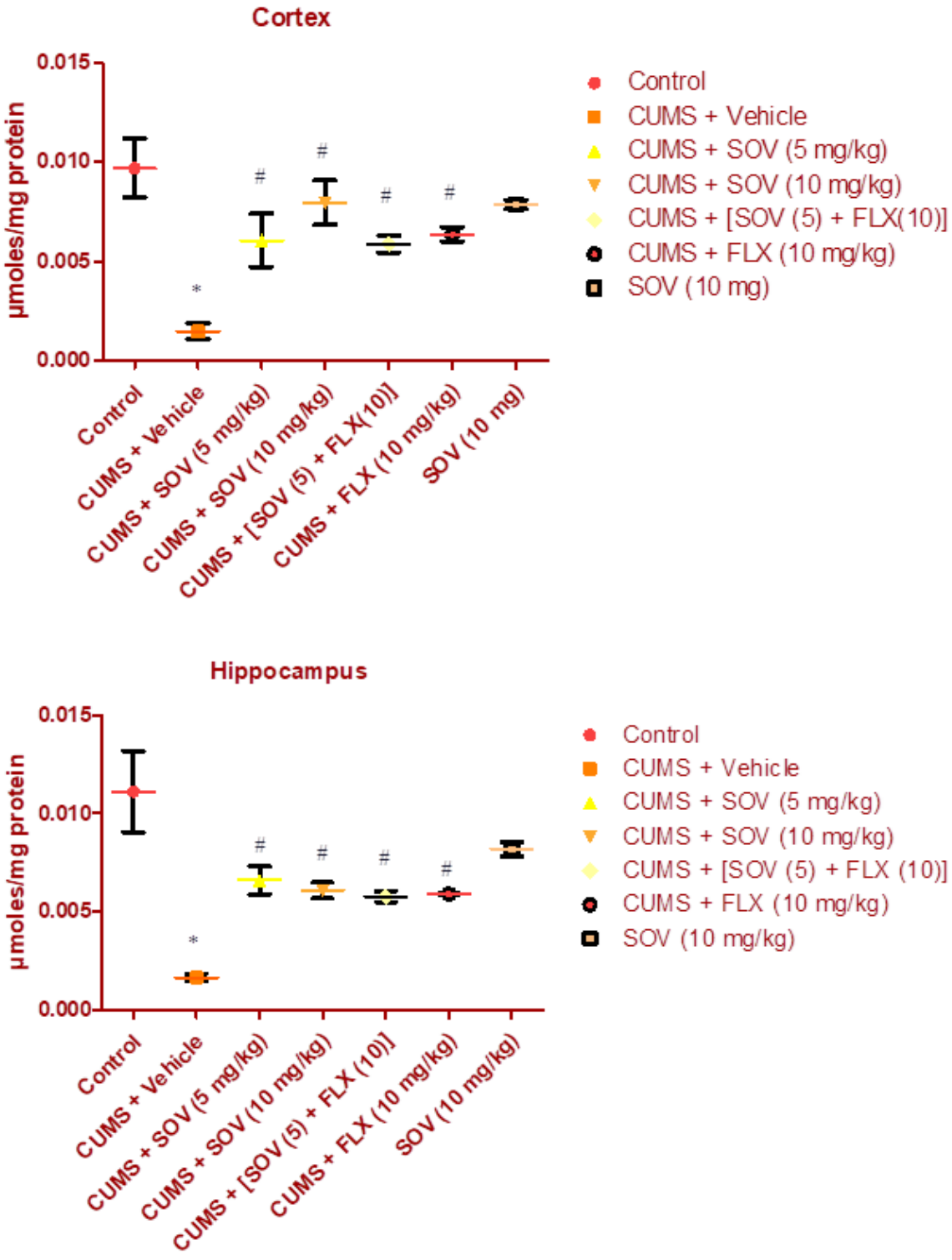

Figure 5

Effect of SOV, fluoxetine and their combination on GSH levels in cortex and hippocampus of CUMS induced depressive mice.

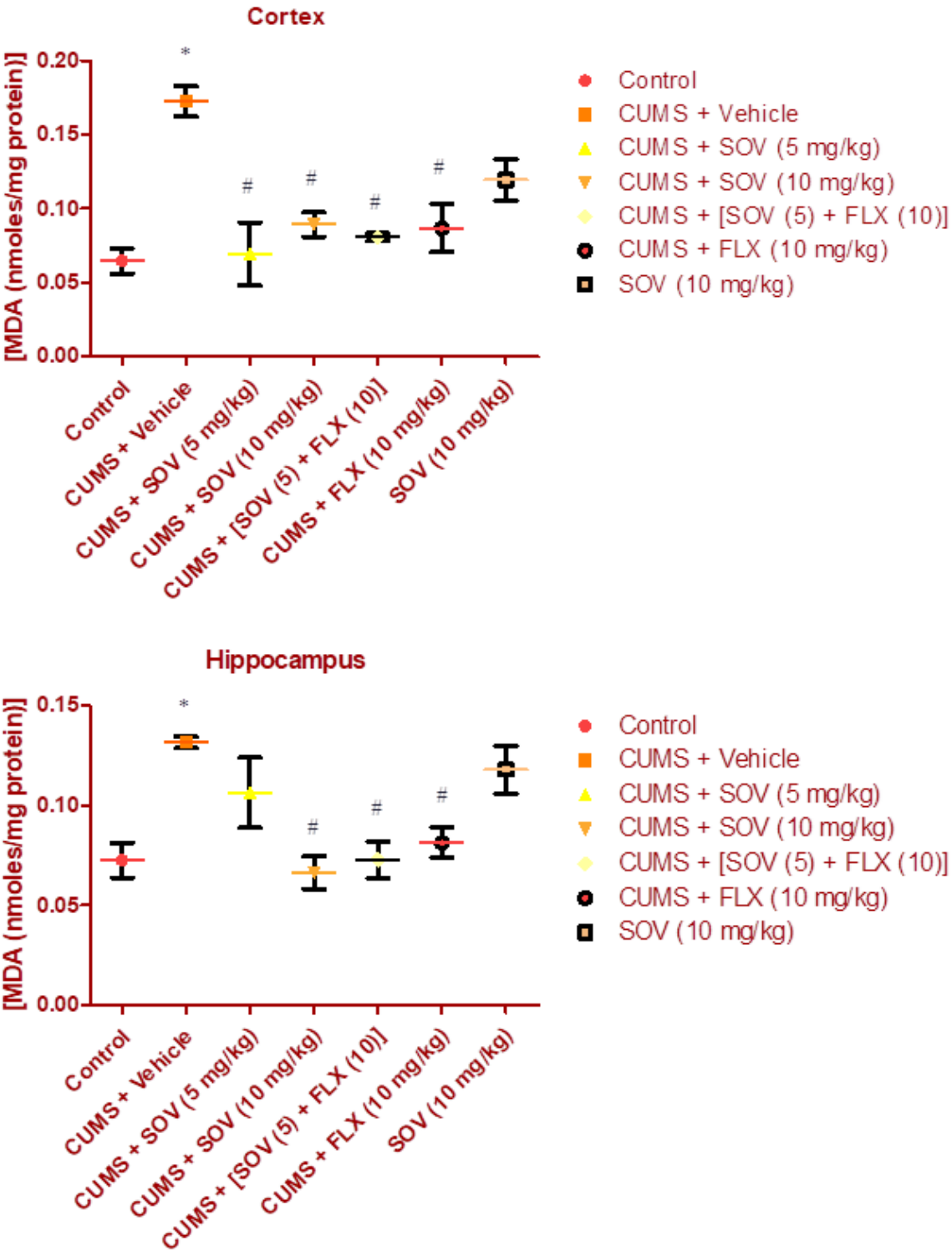

Figure 6

Effect of SOV, fluoxetine and their combination on LPO levels in cortex and hippocampus of CUMS induced depressive mice.

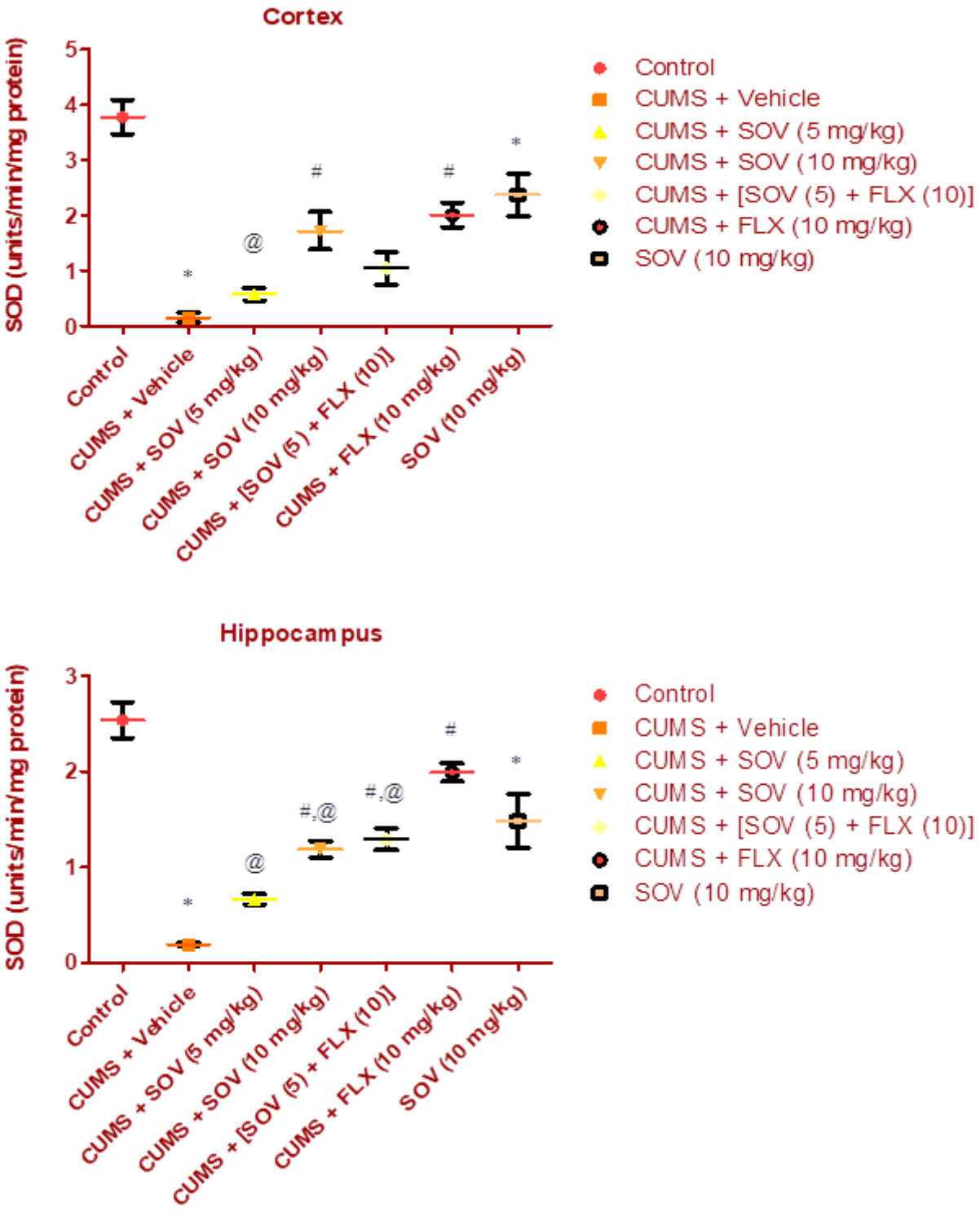

Figure 7

Effect of SOV, fluoxetine and their combination on the activity of SOD in cortex and hippocampus of CUMS induced depressive mice.

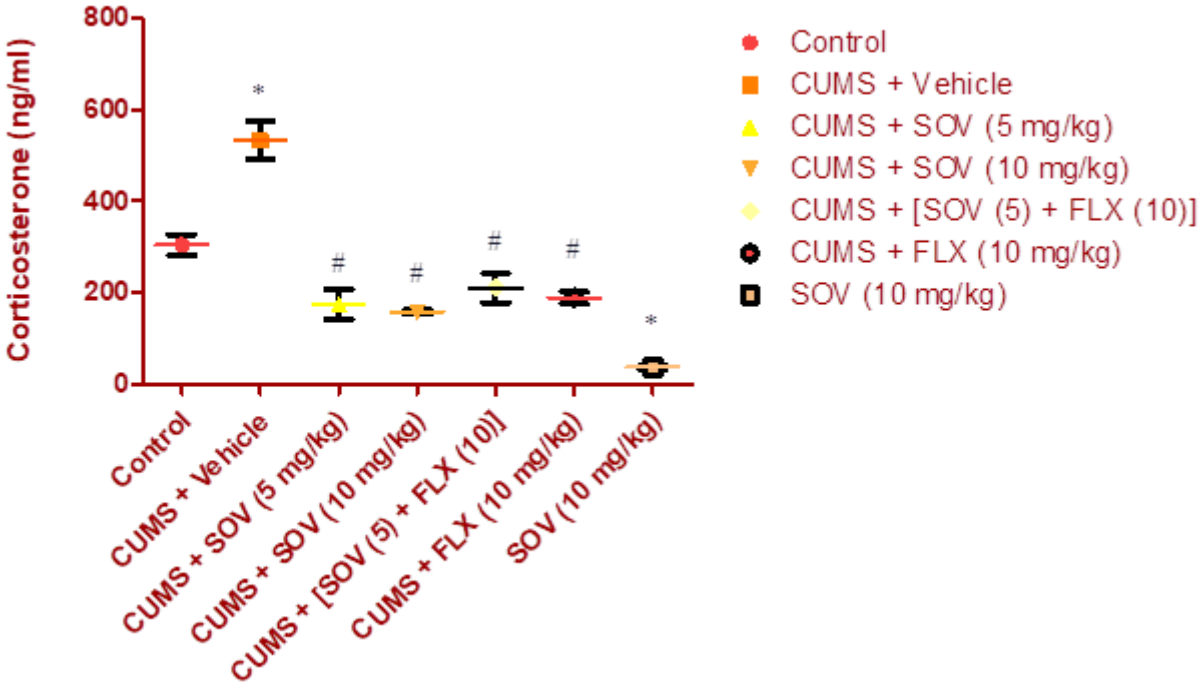

Figure 8

Effect of SOV, fluoxetine and their combination on corticosterone levels in the plasma of CUMS induced depressive mice.

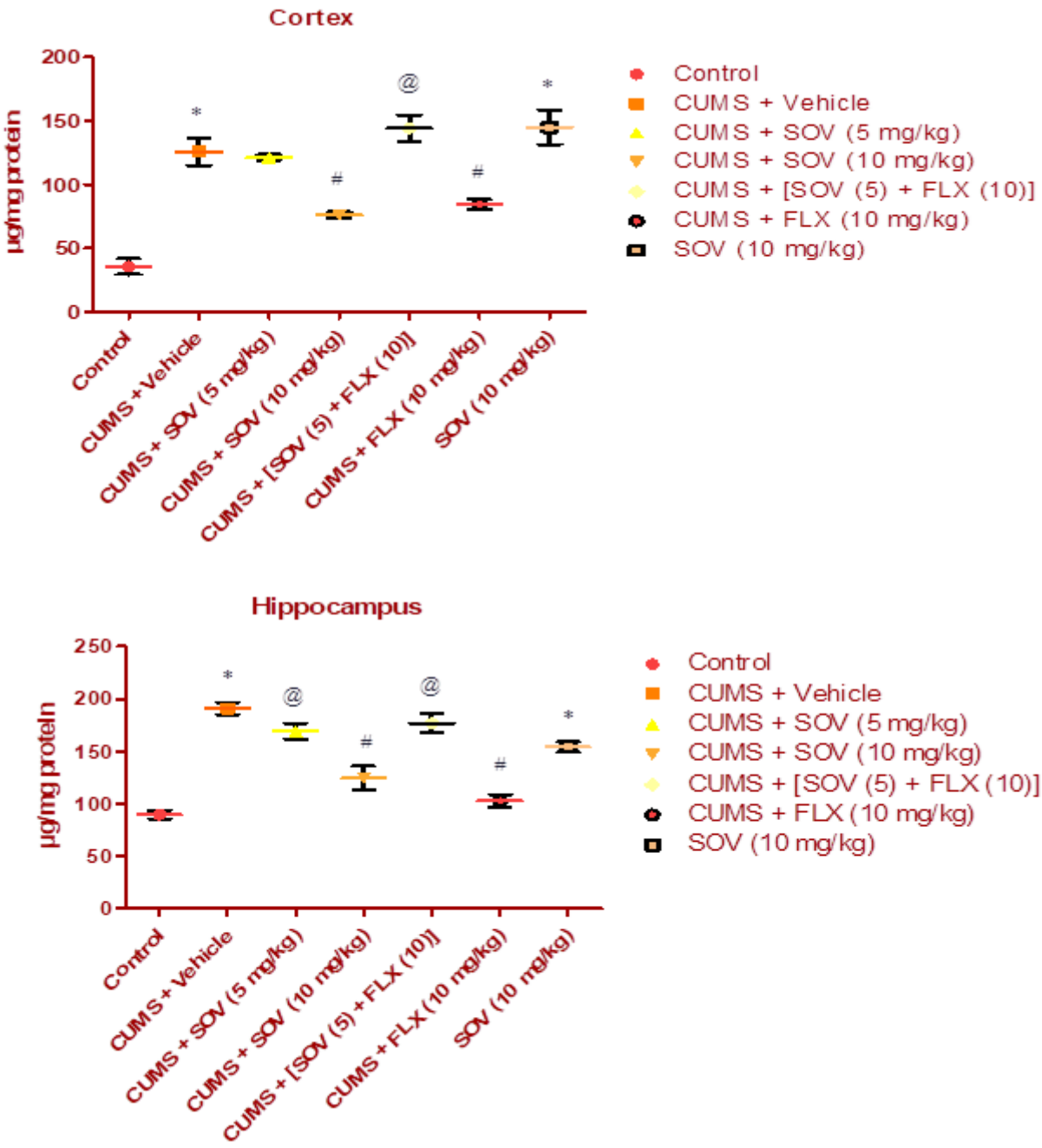

Figure 9

Effect of SOV, fluoxetine and their combination on the levels of NO in cortex and hippocampus of CUMS induced depressive mice.

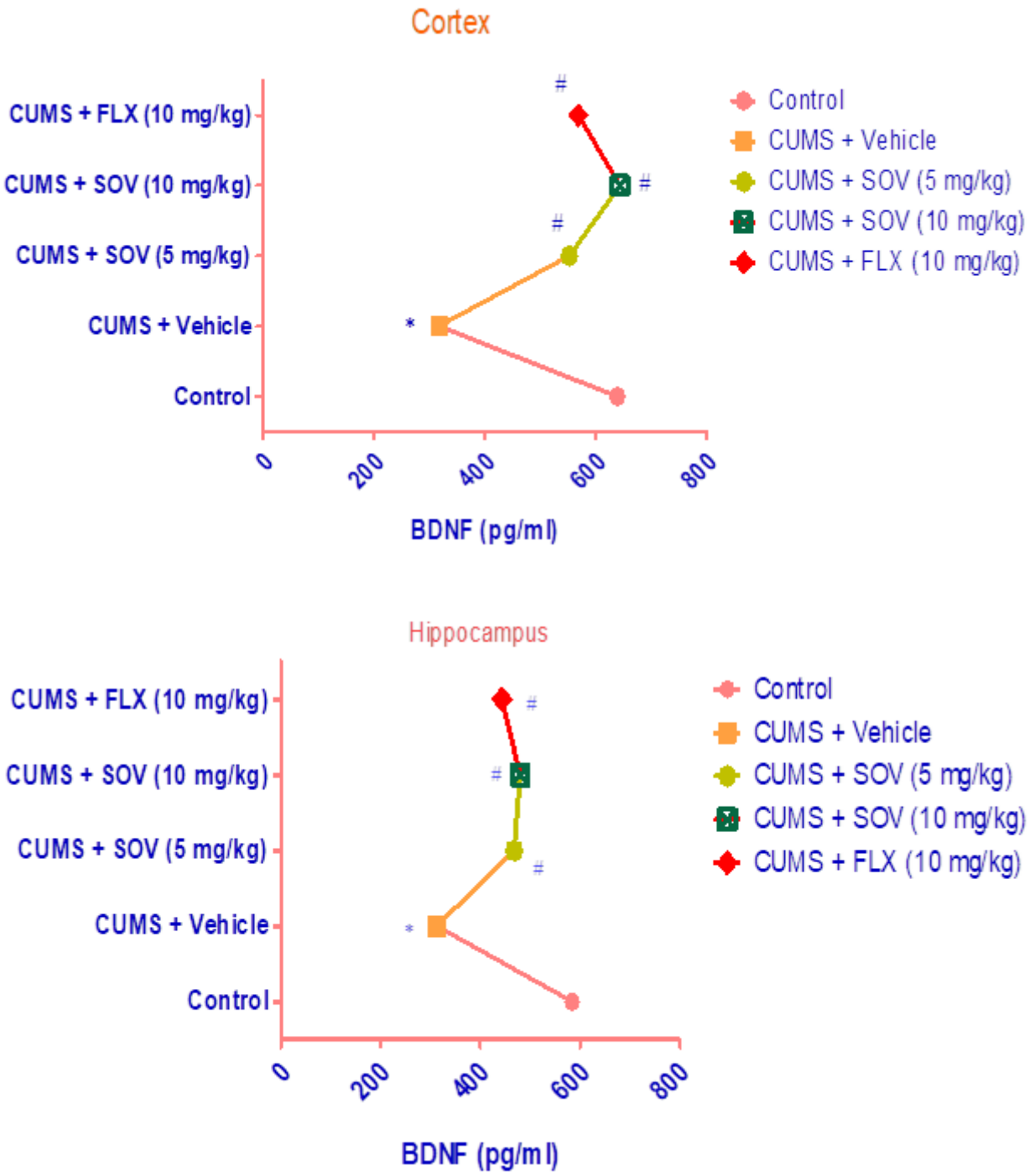

Figure 10

Effect of SOV and fluoxetine on the levels of BDNF in cortex and hippocampus.