Harmaline Attenuates Stress-Induced Depression-Like Behaviour and Biochemical Alterations in Mice

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Abstract

Depression is a debilitating psychiatric disorder affecting millions worldwide. The Chronic Unpredictable Mild Stress (CUMS) model is widely used to simulate depression-related behavioural and neurobiological changes in rodents. This study evaluated the antidepressant-like effects of harmaline, a monoamine oxidase (MAO) inhibitor derived from *Peganum harmala*, in comparison to imipramine, a standard antidepressant. Mice exposed to CUMS received daily oral doses of harmaline (15 mg/kg) or imipramine (30 mg/kg) for 21 days. Harmaline significantly improved behavioural performance in the open field test, forced swim test, and sucrose preference test, indicating a reversal of depression-like symptoms. Biochemical analyses showed that harmaline, consistent with its MAO-inhibitory activity, reduced serum corticosterone levels and increased brain concentrations of monoamines, namely, serotonin, norepinephrine, and dopamine, involved in mood regulation. Additionally, harmaline attenuated oxidative stress by lowering malondialdehyde (MDA) levels and enhancing antioxidant defences such as catalase and glutathione. It also reduced pro-inflammatory cytokines, including tumour necrosis factor-alpha and interleukin-6, suggesting an anti-neuroinflammatory effect. These outcomes were comparable to those seen with imipramine. Overall, the findings support harmaline's potential as an agent for mitigating stress-induced depressive disorders through the modulation of monoamines, oxidative stress, and neuroinflammation.

Introduction

Depression is a pervasive mental health disorder affecting individuals across all demographic groups and remains one of the leading healthcare challenges globally. With millions of individuals impacted by this condition, depression poses substantial burdens not only to the patients but also to healthcare systems and clinicians who strive to manage its complex symptomatology and outcomes [1–4]. Despite advancements in treatment modalities, many patients experience either incomplete remission or relapse, highlighting the need for more effective therapeutic interventions. Among the various biochemical pathways implicated in depression, the monoamine hypothesis has remained a cornerstone in understanding its pathophysiology. Monoamine oxidase (MAO) enzymes, which are responsible for the degradation of key neurotransmitters such as norepinephrine (NE), serotonin (5-HT), and dopamine, are of particular interest in this regard. These neurotransmitters play vital roles in mood regulation, and their depletion has long been associated with the development of depressive symptoms. Consequently, MAO inhibitors (MAOIs) have emerged as a pivotal class of drugs in the treatment of depression, particularly in cases resistant to conventional antidepressants [4–8].

MAO inhibitors function by preventing the breakdown of these neurotransmitters, thereby increasing their availability in the synaptic cleft and enhancing synaptic transmission, which can lead to a reduction in depressive symptoms. Though effective, the use of MAOIs has been somewhat limited due to their side-effect profile and dietary restrictions. Nonetheless, they remain a valuable tool in treating treatment-resistant depression, and ongoing research continues to explore their therapeutic potential [9–10].

In recent years, the neurobiological understanding of depression has expanded beyond the monoamine hypothesis. Emerging evidence points to the dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis as a critical factor in the development and persistence of depression. Hyperactivity of the HPA axis leads to elevated serum cortisol levels, which have been consistently observed in depressed patients [11–13]. Additionally, alterations in neurotrophic factors such as brain-derived neurotrophic factor (BDNF) have been implicated in the pathophysiology of depression, with lower levels of BDNF contributing to neuronal atrophy and impaired neuroplasticity in the brain [14–15]. Furthermore, a growing body of research underscores the role of neuroinflammation in depression, with elevated levels of pro-inflammatory cytokines, such as tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), being linked to depressive symptoms [16–19].

Among the various natural compounds being investigated for their potential antidepressant effects, *Peganum harmala* L., commonly known as Syrian rue, has garnered significant attention. This medicinal herb contains β-carboline alkaloids, including harmaline and harmine, which have been reported to possess potent MAO inhibitory activity [22–24]. This class of compounds offer a promising alternative to synthetic MAOIs, with the potential for fewer side effects and additional therapeutic benefits. In this context, harmaline has emerged as a particularly intriguing candidate for further study.

This study aims to evaluate the pharmacological effects of harmaline in comparison with imipramine, a well-established tricyclic antidepressant, on various behavioural, biochemical, and neurobiological parameters in a chronic unpredictable mild stress (CUMS) model of depression. Specifically, the study will assess CUMS-induced behavioural changes, serum cortisol levels, brain levels of key neurotransmitters (NE, dopamine, and serotonin), neuroinflammatory cytokines (TNF-α, IL-6), and markers of oxidative stress, including malondialdehyde (MDA), catalase activity, and glutathione levels. By investigating these parameters, the study seeks to provide a comprehensive understanding of the potential antidepressant effects of harmaline, with a particular focus on its ability to modulate both monoaminergic and non-monoaminergic pathways involved in the pathophysiology of depression.

Material and Methods

Animals

Male Swiss albino mice weighing 23–29 g (8–10 weeks of age) were obtained from the Serum Institute of India, Pune, India and 6 mice were housed in each polycarbonate cage with corncob as bedding material. All mice were maintained in a temperature-controlled room (22 ± 3°C) in a 12-hour light-dark cycle and were supplied chow diet (Amrut Lab, Pune, India) and RO water, ad libitum. All experimental procedures to be carried out on the mice were approved by the institutional animal ethics committee SIPS/IAEC/2014-15/01.

Chemicals and reagents

Imipramine (CAS: 50-49-7) and Harmaline (CAS: 304-21-2) were purchased from Yucca Enterprises, Mumbai, India. Serum corticosterone, oxidative stress marker (catalase, glutathione and MDA conversion) assay kits and BDNF, TNF-α, IL-6, serotonin, dopamine and epinephrine enzyme-linked immunosorbent assay (ELISA) kits were purchased from Krishgen Biosystems. Protease inhibitor cocktail, BCA protein estimation kit and other reagents and chemicals were purchased from Sigma Aldrich, India.

Experimental design

18 mice of 36 were exposed to CUMS protocol, while the remaining 18 mice were maintained under normal housing conditions (non-CUMS). Both CUMS and non-CUMS exposed mice were divided into 3 treatment groups each (n = 6) in separate cages and one-week post CUMS and non-CUMS exposure the mice in respective treatment groups were given a daily dose of either vehicle (10 ml/kg), imipramine (30 mg/kg) or harmaline (15 mg/kg) by oral gavage for a period of 24 days, imipramine and harmaline were formulated in a carboxymethylcellulose (1%, w/v) suspension that served as vehicle.

Chronic unpredictable mild stress (CUMS) protocol

CUMS exposure in mice was conducted with some modifications to the procedure as described elsewhere [25, 26]. Briefly, mice in CUMS arm of the study were exposed to different stressor paradigms for 4 weeks, as shown in Table 1 (first week without treatment and next 3 weeks treated with drugs). The non-stressed mice were undisturbed except for routine cage cleaning. The detailed schematic protocol is shown in Table 1.

Table 1- CUMS protocol with particulars of mild stressors in mice

Behavioural studies: Safety observations such as excitement, convulsions, itching and tremors: Both non-CUMS and CUMS exposed mice in all treatment groups were observed daily for any abnormalities in behavioural signs such as excitation, convulsion, itching and tremors. Behavioural assessment was carried out through various tests as described below on the days indicated in the study design (Fig. 1).

Open-field test (OFT)

The ambulatory behaviour in mice from all treatment groups was assessed in an open-field test as described previously [27]. The apparatus consisted of a wooden box measuring 40 cm × 60 cm × 50 cm. The floor of the arena was divided into 12 equal squares. The number of squares crossed with all paws (crossings) and rearing frequency were counted in a 6-minute session. The apparatus was cleaned with 10% ethanol between tests to hide prior animal cues.

Sucrose preference test (SPT)

Chronic dosing with test compounds, imipramine, harmaline or vehicle controls for 21 days, one week post CUMS or non-CUMS exposure, mice in all treatment groups were subjected to sucrose preference test (29th day of the study) as per the method described elsewhere [28]. Briefly, 72 hours prior to testing, mice in all treatment groups were acclimatized to 1% sucrose solution (w/v) by placing two bottles of 1% sucrose solution in each cage for 24 hours. The volumes of sucrose solution and water consumed in one hour were recorded, and sucrose preference (%) was determined in harmaline, imipramine or vehicle-treated groups.

Forced swim test (FST)

The immobility time during the forced swim test was recorded as per the procedure described elsewhere [29]. Mice in all treatment groups were placed in a cylindrical tank (10 cm diameter, 25 cm height) containing temperature-controlled water (25°C ± 2) filled to 19 cm height level and the immobility time (seconds) was recorded.

Biochemical evaluation

Upon completion of all behavioural tests, blood samples were collected from all mice by cardiac puncture, serum fractions were separated and stored at -20 ºC until analysis. Mice in all treatment groups were killed by decapitation, and brain samples were collected, washed with ice-cold phosphate buffer saline (PBS). The prefrontal cortex and hippocampal regions were collected on ice from all mice brains, wet tissue weights were recorded, snap frozen in liquid nitrogen and stored at -80 ºC until processing for biochemical analysis. All tissue samples were homogenized in (1:10 w/v; tissue weight to 50 mM phosphate buffer (pH 7.4), 1 mM EDTA, 0.1% Triton X-100 and protease inhibitor cocktail (Sigma-Aldrich, as per manufacturer's instructions), centrifuged at 16000 x g at 4 ºC for 10 minutes and supernatants were collected, and protein estimation was carried out by using bicinchoninic acid assay method, using manufacturer's protocol. Equal amounts of protein homogenate (1 mg/ml) from each sample were used for BDNF, TNF-α, IL-6 ELISA assays and for lipid peroxidation, glutathione reduction and catalase activity.

Dopamine, serotonin and norepinephrine estimation in brain samples

Brain levels of neurotransmitters, namely, dopamine, serotonin and norepinephrine, were determined in cortical and hippocampal brain tissue homogenate samples by the ELISA method as per the manufacturer's instructions using commercially available kits.

Estimation of serum corticosterone and brain BDNF, TNF-α and IL-6

Serum samples from all mice were used for the estimation of corticosterone levels. A commercial ELISA kit was used to determine the serum corticosterone levels as per the manufacturer's instructions. The

corticosterone concentration values were expressed in µg/ml of serum. BDNF, IL-6, and TNF-α were estimated in cortical brain tissue homogenates as per the manufacturer's instructions using commercial ELISA kits.

Brain oxidative stress marker profiles

Lipid peroxidation

Estimation of malondialdehyde (MDA) formation in the brain, as a marker of lipid peroxidation, was measured in brain homogenate samples of mice in all treatment groups as described elsewhere [30, 31]. Briefly equal volumes (2 ml) of the tissue homogenate and trichloroacetic acid (10% w/v) were mixed. The mixture was then cooled for 15 min and centrifuged to obtain supernatants. To 0.5 ml supernatant, 3 ml of thiobarbituric acid (0.67%) was added, the reaction mixture was then kept in boiling water for 10 minutes, cooled, and absorbance was measured at 535 nm using a UV spectrophotometer (Shimadzu 1700). The amount of MDA produced from each sample was expressed as nM of MDA/mg of wet tissue weight.

Estimation of catalase (CAT) activity

Catalase activity on brain tissue homogenates was carried out using a procedure described elsewhere [32]. Briefly, brain homogenate samples from mice in all treatment groups were diluted 20 times using phosphate buffer. 2 ml of this diluted homogenate was taken to which 1 ml of hydrogen peroxide (8.5 µ-l of H_2O_2 in 2.5 ml phosphate buffer) was added. The change in absorbance was read at 240 nm against a blank on Shimadzu 1700 UV Spectrophotometer. The CAT activity was expressed as µM of H_2O_2 formed/min/mg of wet tissue.

Estimation of reduced glutathione (GSH)

Reduced GSH levels were determined in the brain tissue homogenates using a procedure described elsewhere [32]. Equal volumes of tissue homogenate supernatants from all mouse brains (cerebral cortex) were mixed with 20% trichloroacetic acid (TCA). Aliquot 250 µl of supernatant was transferred to a test tube, and to this 2 ml of DTNB [5, 5-dithiobis (2-nitrobenzoic acid), 0.6 mM] was added. The final volume was made up to 3 ml with phosphate buffer (0.2 M, pH 8.0), and the absorbance of the resultant solution was measured by spectrophotometric analysis at 412 nm (Shimadzu 1700 UV).

Statistical Analyses

All data are expressed as mean ± SEM. The statistical analysis was carried out using GraphPad Prism software and two-way analysis of variance (ANOVA) followed by the Tukey post hoc test. A p-value of < 0.05 was considered to be statistically significant.

Results

Body weights

A steady trend of body weight gain was observed in all treatment groups, regardless of the treatment, over the 4-week study period. However, no significant changes in body weight were noted in mice in all treatment groups due to treatment with harmaline, imipramine or vehicle with or without stress exposure during the entire course of the study (Fig. 2).

Behavioural testing

As a measure of safety, any abnormalities in behavioural signs such as excitation, convulsion, itching and tremors due to drug treatment were observed daily during the study period in both non- CUMS and CUMS exposed groups. No such abnormalities in these behavioural signs were observed in mice due to drug treatment. However, exposure to stress, as seen in mice in CUMS arm of the study, behavioural abnormalities were evident on subjecting the animals to the open field test, sucrose preference and forced swim test as described below.

Open field test

In the open field test CUMS exposure in mice led to a significant reduction in the number of crossings and rearing frequency ($p < 0.0001$). Treatment with harmaline as well as imipramine significantly increased the number of crossings scores as compared to vehicle-treated controls ($p < 0.0001$). Exposure to CUMS led to a significant reduction in rearing frequency as compared to vehicle-treated controls in the non-CUMS expose mice ($p < 0.0001$). Treatment with both harmaline and imipramine showed a trend towards increasing the rearing frequency, but this effect was not statistically significant (Fig. 3a and 3b).

Forced Swim Test

Exposure to CUMS led to a significant increase in immobility time as compared to non-CUMS vehicle control mice ($P < 0.001$). In the non-CUMS group, treatment with imipramine ($p < 0.001$) led to a significant reduction in immobility time as compared with the vehicle group mice, while harmaline failed to bring about a significant reduction in immobility time. Exposure to CUMS led to increased immobility times that were significantly reduced by harmaline ($p < 0.05$) as compared to the vehicle control group (Fig. 3c).

Sucrose preference test

No significant difference in sucrose preference was shown by mice in the non-CUMS group by treatment with harmaline or imipramine as compared to vehicle control. A significant reduction in sucrose preference was observed due to the CUMS exposure in vehicle-treated mice. However, treatment of CUMS exposed mice with harmaline ($p < 0.05$) brought about significant improvement in their sucrose preference as compared to the vehicle-treated controls (Fig. 3d).

Effect of drug treatment on biochemical parameters

Brain neurotransmitter levels (serotonin, norepinephrine and dopamine)

Brain serotonin levels were unchanged in the non-CUMS mice with or without drug treatment (Fig. 4a). CUMS exposure also did not affect the mice's brain levels of serotonin as compared to non-CUMS controls. However, treatment with harmaline ($p < 0.0001$) brought about a highly significant increase in brain serotonin levels to that of vehicle treated vehicle-treated CUMS control mice.

No statistically significant changes were seen in the brain dopamine levels of mice from all treatment groups with or without CUMS exposure (Fig. 4b). No change in the brain norepinephrine levels was noted in the non-CUMS group mice treated with harmaline, as compared to vehicle controls. However, a small but significant ($p < 0.05$) increase in brain norepinephrine was observed in the harmaline-treated group as compared to the vehicle control group in CUMS exposed mice (Fig. 4c).

Serum corticosterone and brain BDNF, IL-6 and TNF-α levels

Serum corticosterone

CUMS exposure led to a significant increase in serum corticosterone levels as compared to the vehicle treatment group in non-CUMS mice. Treatment with harmaline as well as imipramine normalized serum corticosterone levels as compared to vehicle-treated controls in CUMS exposed mice ($p < 0.0001$; Fig. 5a).

Brain BDNF levels

CUMS exposure led to a significant reduction in brain BDNF levels as compared to the vehicle control group in non-CUMS mice ($p < 0.0001$). Treatment with harmaline as well as imipramine brought about a highly significant increase in the brain BDNF levels as compared to CUMS vehicle group ($p < 0.0001$; Fig. 5b).

Brain IL-6 and TNF-α levels

No difference in the brain levels of IL-6 in the different drug treatment groups was seen in the non-CUMS exposed mice. However, there was a significant increase in the brain IL-6 levels in the mice exposed to CUMS, and treatment with imipramine ($p < 0.05$) as well as harmaline ($p < 0.001$) led to a significant reduction in IL-6 levels as compared to the vehicle-treated controls (Fig. 5c). CUMS exposure led to a significant increase in the brain TNF-α levels as compared to non-CUMS vehicle controls ($p < 0.001$). Treatment with harmaline as well as imipramine showed a trend towards a slight reduction in brain TNF-α levels; however, no statistically significant difference was seen as compared to CUMS vehicle controls (Fig. 5d).

Brain oxidative stress markers (MDA levels, catalase activity and GSH levels)

Brain malonaldehyde levels were unchanged in the different treatment groups in the non- CUMS arm of the study. Elevation in the malonaldehyde brain levels was seen in the vehicle group of the CUMS arm, while treatment with harmaline as well as imipramine led to a significant reduction in brain malonaldehyde levels ($P < 0.001$) (Fig. 6a).

Brain catalase activity was comparable in the different treatment groups in the non- CUMS arm of the study. However, there was a reduction in brain catalase activity on exposure to CUMS, which was unaffected by the drug treatment ($P < 0.05$) (Fig. 6b). Treatment with imipramine in CUMS cohort significantly reduced glutathione levels as compared to the vehicle CUMS cohort, while harmaline brought about a small reduction, which was not statistically significant (Fig. 6b).

Discussion

The present study highlights the antidepressant potential of harmaline, a β-carboline alkaloid obtained from *Peganum harmala* (commonly known as Syrian rue), in a mouse model of depression induced by Chronic Unpredictable Mild Stress (CUMS) [22–24]. In previous in vitro studies, harmaline has demonstrated monoamine oxidase (MAO) inhibitory activity with IC_{50} values ranging from 0.07 μM to 0.15 μM [37, 38]. This variability in IC_{50} may be attributed to differences in experimental assay conditions and protocols.

In the current study, harmaline was administered orally at a dose of 15 mg/kg once daily, with imipramine (30 mg/kg) used as the reference standard. Both treatments were compared to vehicle controls in mice exposed to stress and non-stress conditions. Results from behavioural paradigms, including the open field test, forced swim test, and sucrose preference test, showed that harmaline significantly ameliorated stress-induced behavioural deficits. Like imipramine, harmaline improved locomotor activity, reduced immobility, and increased sucrose preference, collectively indicating antidepressant-like activity in the CUMS model.

Biochemical assessments further revealed the neuroprotective effects of harmaline. Daily oral administration of harmaline significantly reduced serum corticosterone levels, a hallmark of hypothalamic-pituitary-adrenal (HPA) axis dysregulation commonly observed in depressive disorders [11–13]. Additionally, harmaline treatment restored levels of key brain monoamines, serotonin and norepinephrine, both of which play essential roles in regulating mood and emotional behaviour [34]. The increased availability of these neurotransmitters supports the hypothesis that harmaline exerts its antidepressant effects at least in part via MAO inhibition, akin to the mechanism of action of conventional MAO inhibitor antidepressants [9, 10].

Importantly, harmaline also mitigated oxidative stress, as evidenced by a reduction in malondialdehyde (MDA) levels and increased activity of endogenous antioxidant enzymes such as catalase and glutathione [35]. Since oxidative damage has been implicated in the pathophysiology of depression, these findings suggest that harmaline confers neuroprotection through its antioxidant activity [36, 37]. The antioxidant and MAO-inhibitory properties of *Peganum harmala* and its alkaloids, as well as related

β-carboline-containing plants such as *Banisteriopsis caapi*, may serve as potential agents in pharmacological treatment of depression [22–24, 33].

Moreover, harmaline exhibited anti-inflammatory activity, as shown by reduced levels of pro-inflammatory cytokines including tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6). Given that chronic stress is often accompanied by neuroinflammation, which contributes to neuronal dysfunction and depressive symptomatology, this anti-inflammatory effect may represent another important mechanism underlying harmaline's antidepressant action [11].

Taken together, the findings of this study demonstrate that harmaline exhibits comparable efficacy to imipramine in reversing behavioural and biochemical impairments induced by CUMS. These results suggest that harmaline, through its multi-target actions including MAO inhibition, antioxidative effects, and anti-inflammatory activity, may serve as a promising candidate for the development of novel antidepressant therapies, especially for cases involving complex neurobiological disruptions.

Conclusion

The present study demonstrates that harmaline, a β-carboline alkaloid isolated from *Peganum harmala*, exerts marked antidepressant-like effects in mice subjected to Chronic Unpredictable Mild Stress (CUMS). Harmaline treatment led to notable improvements in depressive behaviour, reduced oxidative stress, and favourable modulation of key biochemical parameters, including serum corticosterone, central monoaminergic neurotransmitters, and pro-inflammatory cytokines. These outcomes suggest that harmaline and structurally related molecules possess therapeutic potential for managing stress-associated depressive disorders, showing efficacy comparable to that of the established antidepressant, imipramine. Therefore, harmaline or similar bioactive compounds derived from *Peganum harmala* warrant further investigation as a multimodal candidate for antidepressant drug development. Future studies should aim to explore its pharmacokinetics, safety profile, and long-term efficacy to support its progression toward clinical evaluation in human depression.

Ethics statement

All procedures for animal experiments were carried out in accordance with the CCSEA, Government of India, for animal care. All experimental procedures carried out on mice were approved by the Institutional Animal Ethics Committee SIPS/IAEC/2014-15/01.

Declarations

Declaration of Competing Interest

The authors claim no conflict of interest. All authors have approved the final article.

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Author Contribution

Conceptualization: KJ; Methodology: KJ, FR, NS; Investigation: FR, NS, AR, SP; Data Curation: KJ, AR; Formal Analysis: KJ, NS; Writing – Original Draft: KJ; Writing – Review & Editing: KJ, FR, NS, AR, SP; Supervision/Guidance: PP; Project Administration: PP; Funding Acquisition: PP

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Tables

Table 1- CUMS protocol with particulars of mild stressors in mice

Day	Duration	Stressor paradigms
Monday	24 h	Food deprivation
	24 h	Water deprivation
Tuesday	1 h	Empty bottle
	24 h	Foreign object
Wednesday	06 min	Forced swimming
	12 h	Overnight illumination
Thursday	2 h	Restraint
	7 h	Cage tilt (45°)
Friday	12 h	Food deprivation
	24 h	Soiled cage
Saturday	24 h	Water deprivation
	12 h	Overnight illumination
Sunday	1 h	Empty bottle
	7 h	Cage tilt (45°)

Figures

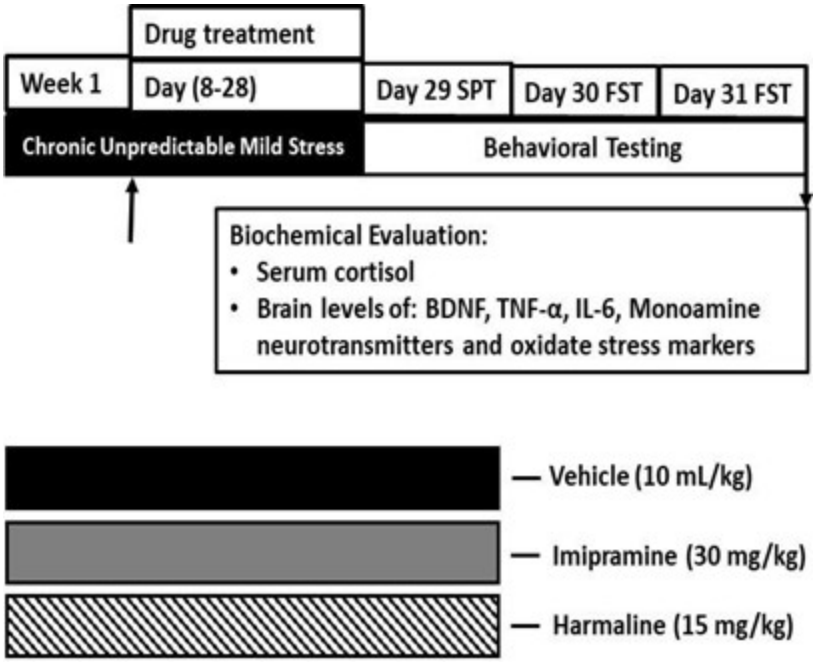

Figure 1

Diagrammatic representation of the study design.

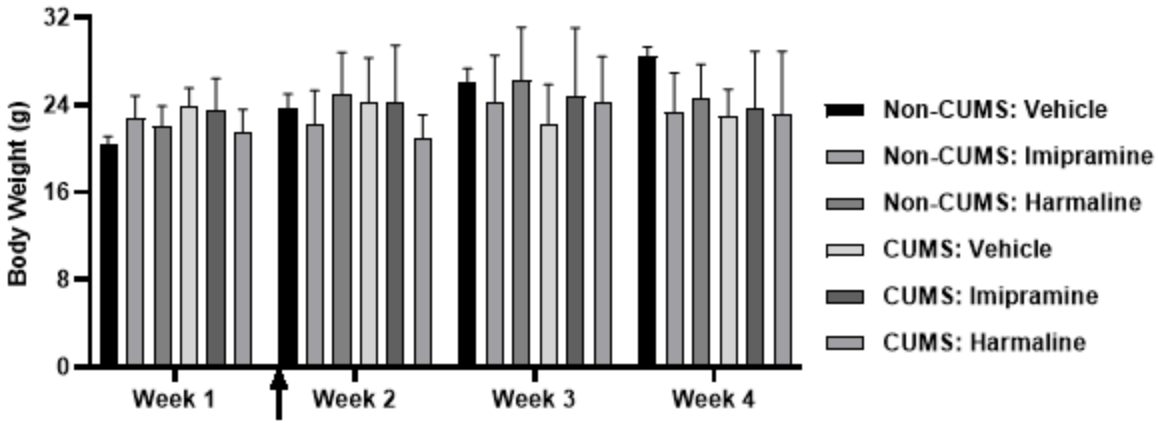

Figure 2

Weekly body weights recorded in mice from all treatment groups.

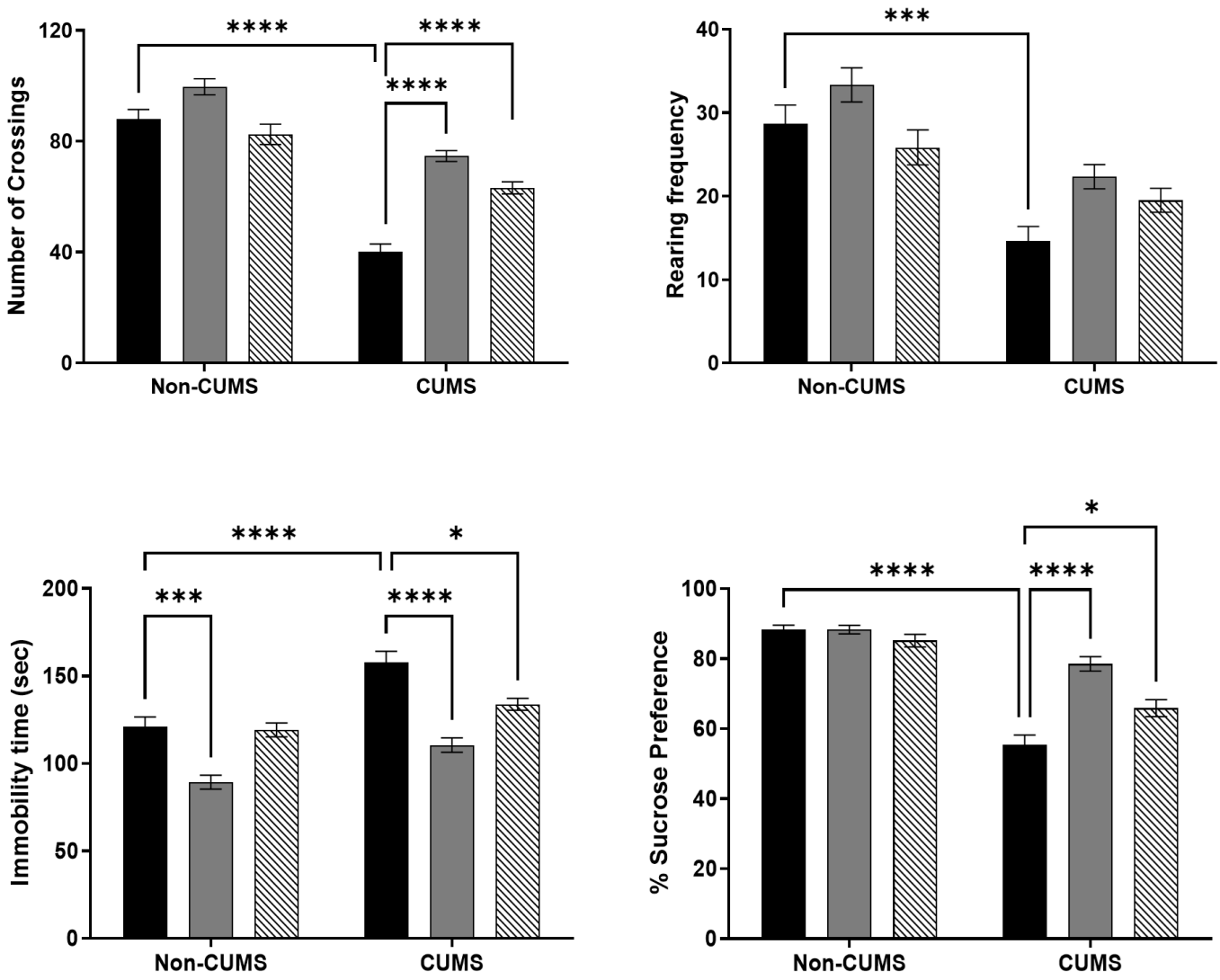

Figure 3

(a, b) open field test, forced swim test (c) and Sucrose preference test (d) results in mice from all treatment groups.

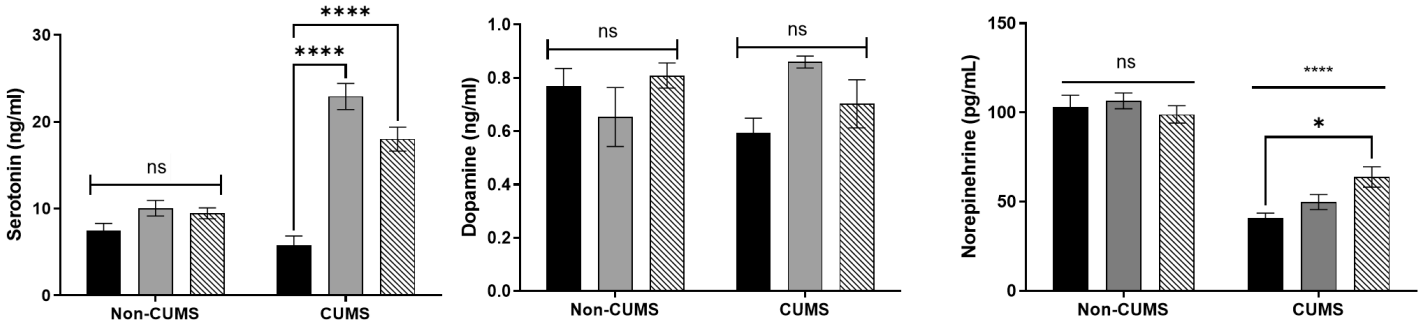

Figure 4

Monoamine, a. dopamine, b. serotonin and c. norepinephrine, levels in brain homogenate samples from mice from all treatment groups

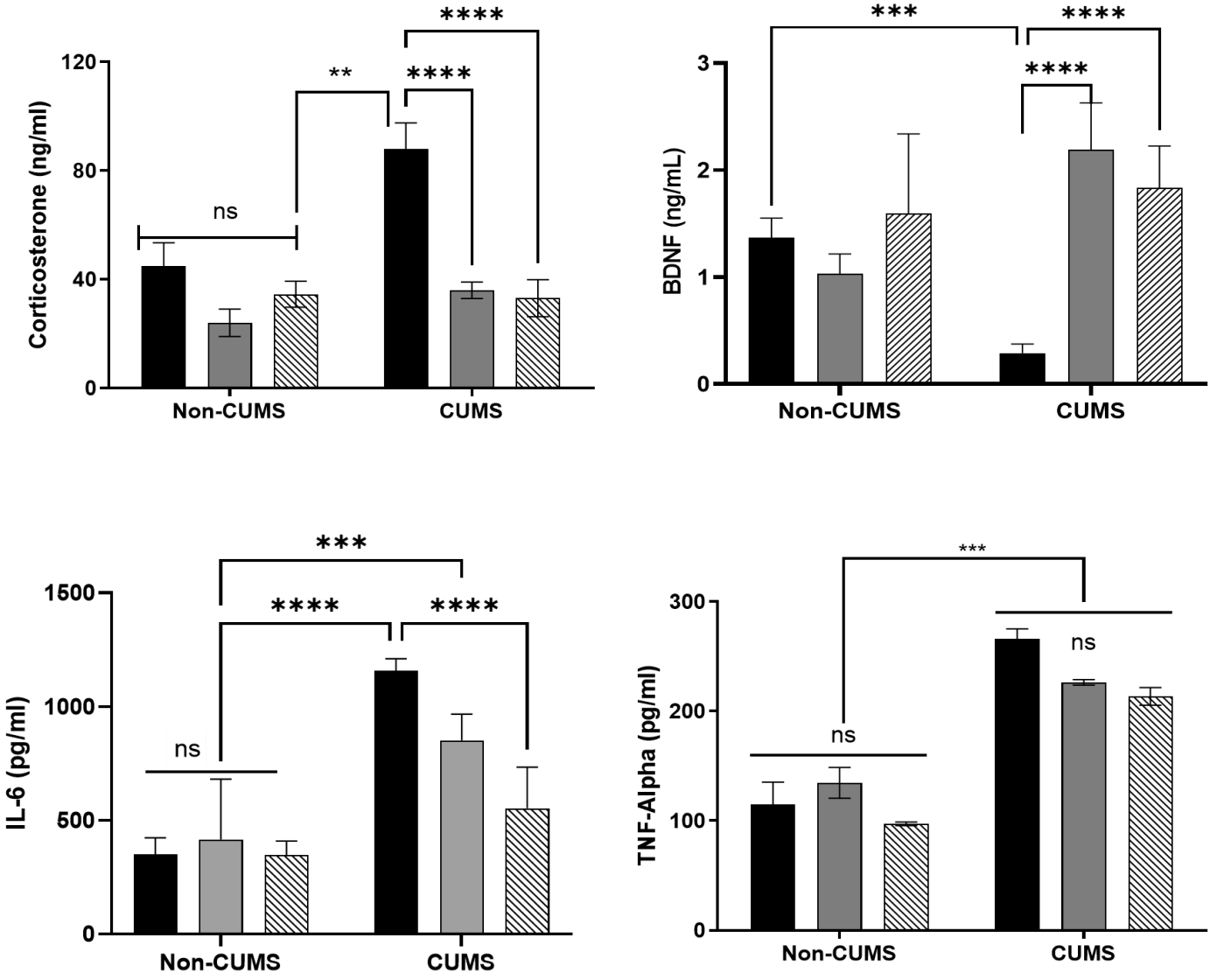

Figure 5

(a, b, c, d) Serum corticosterone, BDNF, TNF-α and IL-6 levels in brain homogenate samples from mice from all treatment groups

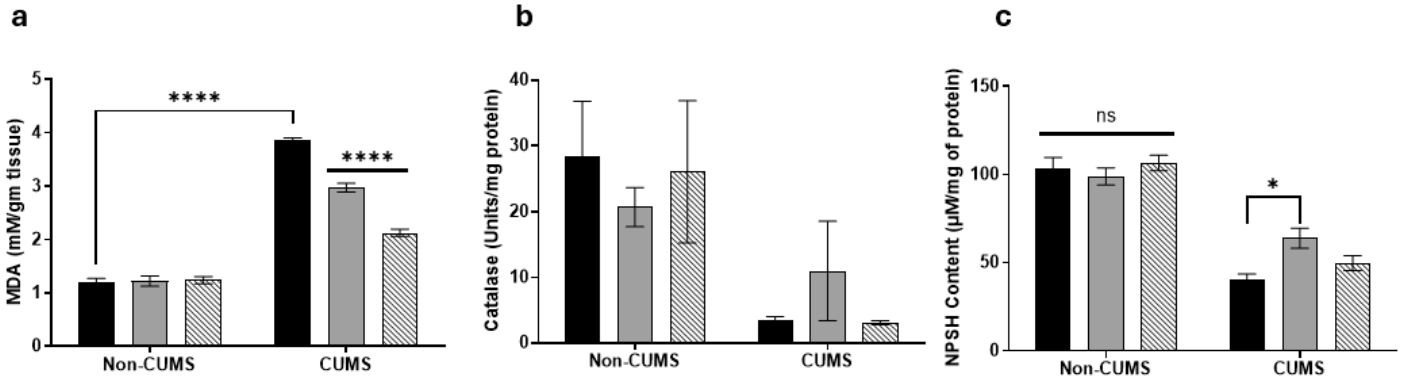

Figure 6

(a, b, c) Effect of drug treatment on oxidative stress parameters in brain homogenate samples from mice from all groups