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The Effect of Mirtazapine on Reducing Chronic Stress in Male Rats

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Abstract

Background: Mirtazapine (Mtz) has attracted attention for its anti-anxiety properties. We aimed to explore the impacts of chronic unpredictable stress (CUS) using Mtz treatment as a post-traumatic stress disorder (PTSD) drug and to determine body weight and plasma corticosterone levels at various times in rats.

Methods: Adult male Wistar rats were administered nine days of CUS in this research and then received Mtz after CUS or when the CUS began. At the end of the CUS protocol, the rats were subjected to the elevated plus maze (EPM) test and open-field test (OFT) (for anxiety-like behavior) followed by the forced swimming test (FST) and tail suspension test (TST) (for depression behavior). The CUS protocol included body weight measurement, a sucrose preference test (SPT), and plasma corticosterone (CORT) levels.

Results: Significantly decreased body weight and increased plasma CORT levels were seen in the CUS group. Mtz at 10 mg/kg significantly increased body weight in rats after being exposed to CUS, demonstrating anti-anxiety activity. The process was discovered to be linked with a decline in plasma CORT and no significant difference was seen with respect to body weight compared with the control group, as we found in one of the treated groups (Mtz after CUS).

Conclusion: A decrease in CORT levels in serum plasma and modulated body weight might be a key mechanism by which Mtz exerts its therapeutic potential as an antidepressant, and it would be safe to take orally after stressful conditions.

Keywords: Animal models; Mirtazapine; Open-field test; Sucrose preference test; Corticosterone.

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Introduction

Stress is a common occurrence throughout all phases of life.[1] Restoring homeostasis (allostasis) is facilitated by stress.[2] Stressors are defined as factors that significantly deviate from homeostasis.[3] Allostasis mediators such as cortisol and adrenaline are advantageous when produced in response to stress or lifestyle variables such as nutrition, sleep, and exercise.[4] Most studies attempting to establish a relationship between stress exposure and life histories have concentrated on environmental stressors such as weather, predators, and food shortages.[3] The brain and body are entirely integrated.[2] Stress may come from an external source, such as the environment or an internal perception of the individual. This latter form can cause anxiety and/or other negative emotions and feelings such as pain, sadness, pressure, and others, leading to significant psychological disorders such as post-traumatic stress disorder (PTSD).[1] Various studies have shown that 70%-90% of diseases are related to stress.[5] The stress response is regulated by the sympathetic-adreno-medullar (SAM) axis, the hypothalamus-pituitary-adrenal (HPA) axis, and the immune function.[6] When a period of stress is sustained, it develops into chronic stress. Chronic stress can lead to various illnesses, including depression.[7] The stress hormone cortisol is released to counteract the stress and preserve homeostasis. On the other hand, prolonged cortisol production causes immunosuppression.[8] Chronic stress is thought to inhibit the immune system by promoting the activity of inflammatory molecules such as IL-6.[9] Stress has acted as a valuable heuristic for academics, allowing them to combine patterns that reveal various phases of the process relating stressful life experiences to disease.[10] Recently, pharmaceutical techniques have been widely used in the treatment of PTSD. Hence, mirtazapine (Mtz), along with serotonin-norepinephrine reuptake inhibitors and selective serotonin reuptake inhibitors, are common first-line therapies.[11] A stress reaction is elicited by any physical or psychological stimulus that disrupts homeostasis.[6] Chronic and acute stress affect the human body differently. Acute stress, as a stress response, forces the body for a "physiological stress" event and therefore is advantageous to health, but chronic stress can have the

opposite effect and be harmful.[12] Chronic unpredictable stress (CUS) raises an organism's allostatic burden and disrupts homeostasis. Depression can be triggered by stressful events in a person's life, resulting in poorer function of specific organs such as the immune and cardiovascular systems.[4] According to the description of a possibly frequent depression subtype, called "stress-induced depression," it is highlighted that the relationship between stress and depression might be more profound than previously thought.[13] A worldwide prevalence of 4.4% and an annual occurrence of 3.0% make depression one of the most frequent illnesses.[14] Sequeira-Cordero et al established a CUS animal paradigm that links chronic stress with depression and a dependable method for creating a rat model of depression.[15]

Though they are sometimes used for anxiety, atypical antidepressants, such as Mtz, commonly treat severe depression.[16,17] Several studies suggest that venlafaxine and Mtz provide better results than fluoxetine.[18,19] Mtz, as suggested by Ribeiro et al, is an appealing alternative for treating panic disorder because of its features.[20] Mtz has a unique and complicated mechanism of action based on its antagonistic effects on the α2-adrenergic receptors, pre and post-synaptic, with no selectivity between the two, as well as effects on the serotonergic 5-HT3, 5-HT2A, and 5-HT2C receptors.[21] For this reason, Mtz has been used to treat moderate to severe depression.[22] A previous study showed that at a 10 mg/kg dose, Mtz had positive results in reducing stress on neurobehavioral development in adult rats.[23] Consequently, Mtz appears as a treatment for depression that does not cause anxiogenic effects and does not induce sexual dysfunction in clinical practice.[24] However, other researchers found that Mtz is not sedative in rats when given chronically.[25] Several studies mentioned a positive correlation between Mtz and body weight gain.[26,27] However, there is no study to determine the suitable time to take Mtz (for example, when stress begins or following a stressful condition). On the other hand, fluoxetine, as another antidepressant, showed a reduction in body weight[28] which is not selected for the target of our study. Therefore, in the current experiment, we tried this drug orally for a short time to study other factors such as body weight, corticosterone (CORT) levels, and its therapeutic effect on anxiety, depression and stress at the right administration time.

The open field test (OFT) has been employed across several experimental models to assess neuromuscular impacts, including stroke, drug psychotic consequences, stress/anxiety, age, sex, circadian cycles, neuromuscular illnesses of genetic origin, and environmental factors.[29] Activities in the open field center can suggest a decrease in anxiety and/or an increase in exploration.[30] Similarly, the elevated plus maze (EPM) model assumes that presenting an elevated and open-arm maze creates a conflict. In contrast, the frequency of open-arm entrances and spending time in the exposed arms offer are indicators of anxiety-induced suppression of normal exploratory behavior.[31] Based on their responses, rodents are subjected to the EPM test to investigate whether drugs are anxiolytic or anxiogenic. An observation of a decline in the period spent on closed arms, an enhance in spending time on exposed arms, as well as a reduction in the frequency of passes in closed arms may indicate reduced anxiety levels in the EPM test.[32] It is dependent on the animal's typical behavioral approach to investigate new areas and its dread of open, highly lighted environments; in other words, it assesses approaching and aversion.[33] The primary benefit of the EPM technique is that it makes use of rats' innate preference for dark, enclosed areas and their unconditioned dread of heights and aversion to open spaces.[32] Anxiolytic medicines enhance the quantity of time spending on open arms; as a result, animals that are afraid of open areas are more likely to be classified as nervous.[33] We aimed to evaluate anxiety-like behavior of Mtz in rats at a dosage of 10 mg/kg/d employing some behavioral assays, sucrose preference test (SPT) and analysis of the CORT level in the blood plasma. Additionally, the present study sought to determine at which level of stress does Mtz show a beneficial effect on physiological parameters such as body weight and CORT levels.

Materials and Methods

Animals and Treatments

In the present research, 6-7-week-old Sprague Dawley Wistar male rats weighing 180-250 g were bought from the Razi Vaccine and Serum Research Institute (RVSRI). They were kept in a 12-hour cycle of light and darkness and fed commercial rat chow. Rats were allowed at least one week to become used to the animal groups. All tests were performed following guidelines established by the Malek-Ashtar University Medical Ethics Committee, the Animals (Scientific Procedures) Act of 1986 and related procedures, as well as a recommendation from National Institutes of Health (NIH) for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). A daily body weight measurement was taken before each treatment. We have made every attempt to alleviate the pain. Mtz (Remeron) 30 mg was purchased from Hexal Co., Denmark. Mtz was administered orally every day.

Experimental Protocols

The animals were randomly allocated into the following five categories.

1. Animals were administrated by saline and non-stressed conditions (1 mL/kg, $n=8$, Ctrl).
2. Untreated rats underwent chronic unpredictable stress for nine consecutive days ($n=8$, CUS).
3. For nine days, rats were under CUS and then treated with Mtz until day 14 (10 mg/kg, o.p., $n=8$,

Mtz after CUS).

4. Rats were treated with Mtz simultaneously as chronic stress started (10 mg/kg, o.p., $n=8$, CUS+Mtz).
5. Rats only received Mtz for 14 days without stress (10 mg/kg, o.p., $n=8$, Mtz).

The group was set up separately with time intervals to prevent time interference in capturing behavioral stimuli and increase work accuracy in conducting data experiments (Figure 1).

Chronic Unpredictable Stress Procedure

The CUS (sub-chronic) procedure was applied to five groups for nine consecutive days and treated with Mtz (only in one dose, 10 mg/kg) differently. Rats were subjected to either CUS groups or a non-stressed group (control). The control (Ctrl) group rats were fed and watered daily without any stress conditions. The cages were changed every three days. As part of the CUS procedure, various mild stressors were introduced. A total of nine stressors were listed in the protocol: swim stress for 10 minutes, movement restriction for 3 hours, lack of water consumption for 24 hours, movement restrictions in 4°C for 90 minutes, isolation for 24 hours, deficiency of meals for 24 hours, water restriction for 24 hours, movement restrictions in 4°C for 2 hours, and food deprivation for 24 hours. Table 1 shows the CUS procedure described by Li et al.[34]

Behavioral Tests

Elevated Plus Maze Test

This experiment examined rats' anxious behavior because of their innate distaste for open and elevated places. This apparatus, which Handley and Mithani[35] have described, is plus-shaped and made up of four elevated arms. The tool comprised two closed arms and two open arms, constructed from acrylic material and positioned 50 cm off the ground. The EPM was made of the main base (10 cm × 10 cm) consisting of two opposing open arms (50 cm × 10 cm × 1 cm height) and two closed arms (50 cm × 10 cm × 40 cm height).[36,37] At the start of every experiment, rats were put in the middle, facing open arms, and given five minutes to wander freely. The motion path of the animals was captured using a digital camera and evaluated by software (Mobile Datum Information Technology Co.,

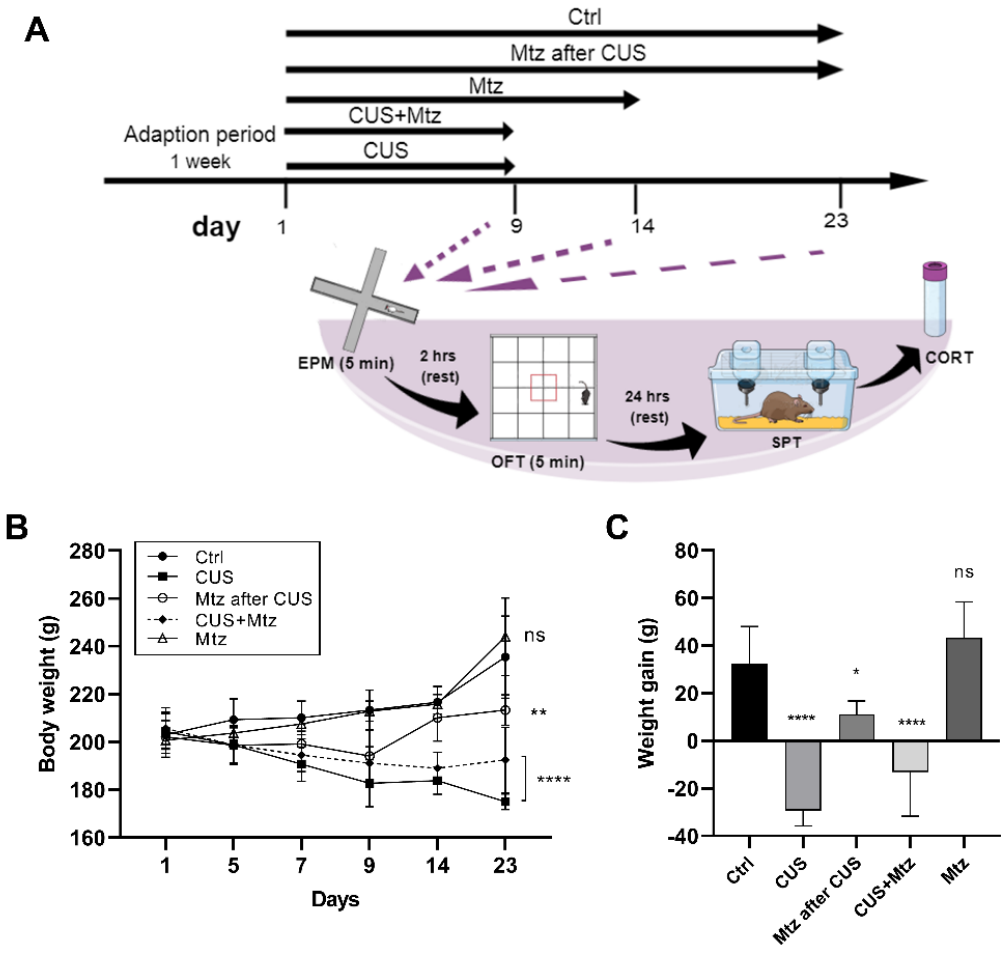

Figure 1. A Simplified Experimental Design Procedure for the Chronic Unpredictable Stress (CUS) **(A)**, body weight of rats subjected to CUS **(B)**, and weight gain during various experiments in the CUS protocol **(C)**. The data are expressed as mean (g) ± SD ($n=8$/group). One-way ANOVA was used to comparison between groups using Dunnett's multiple comparison test. P value were used to compare the treated groups and the Ctrl group, * $P<0.05$, ** $P<0.01$, **** $P<0.0001$; ns = no significant.

Table 1. Chronic Unpredictable Stress Technique

Day	Stressor	Duration
1	Swim stress in glass tank (44 × 33 × 30)	10 min
2	Movement restrictions	3 h
3	Water deprivation	24 h
4	Movement restrictions in 4°C	90 min
5	Isolation	24 h
6	Food deprivation	24 h
7	Water deprivation	24 h
8	Movement restrictions in 4°C	2 h
9	Food deprivation	24 h

Ltd., Shanghai, China). This study examined total arms entries (open and closed), open-arms, and closed-arms entry percentages.

Furthermore, the proportion of open arm crossings [open entrances/(open + closed entries) 100] and the proportion of spending time in open arms [(open arms/300) 100] was calculated. The maze was wiped using an alcohol solution before each experiment.[36] Based on methods by Mazor et al[38] and Rao et al,[33] which take into account the length of the period in exposed arms relative to the overall quantity of spending time investigating an apparatus, an anxiety index was calculated. The following formula was used to calculate the anxiety index:

$$\text{Anxiety index} = 1 - \left(\frac{\text{Open} - \text{arm time}}{\text{Total time}}\right) + \left(\frac{\text{Open} - \text{arm entries}}{\text{Total entries}}\right) / 2$$

Sucrose Preference Test

The SPT was used following earlier published methods with slight modification.[12] Each animal was housed in an individual cage. In addition, two containers of 1% w/v sucrose solution were administered 72 hours before the test. The 1% sugar solution container was swapped with a water supply for the following 24 hours to allow the animals to adapt to the sucrose solution. The rats were deprived of nourishment for 24 hours following the adaption phase. In each cage, two pre-weighed glass flasks, one holding normal water and another carrying sucrose solution (1%), were located side by side as part of the SPT. Each animal had the option of drinking a different bottle. For one hour, the animals were supplied with drinking water. Each bottle's initial and final weights were recorded in one set. The formula below was used to calculate the proportion of sucrose preference[39]:

$$\%\text{Sucrose preference index} = \frac{\text{Sucrose consumption}}{\text{Sucrose} + \text{Water consumption}} \times 100$$

Open-Field Test

Plexiglas open field containers (90 × 90 cm^2 with a 42 cm height) were measured to quantify body movements. Black lines were painted on the cardboard on the box's floor, splitting the floor into 18 cm × 18 cm squares. Gridlines, consisting of four 11 cm distances from each wall, divide open fields into centers and surroundings. Based on dependent measures, spending time in the middle, distance moved in the middle, and area covered in the middle divided by the whole distance crossed were calculated. The number of squares crossed and the number of animals reared were recorded after reviewing each video by an observer who was not informed of the treatment regimen. Each rat was back in its cage as soon as the test was completed.

Tail Suspension Test

The total time of immobility generated by the tail suspension test (TST) was assessed according to the procedure reported by Modarresi Chahardehi et al.[40] Adhesive tape was attached roughly 1 cm from the end of the tail to suspend the rat 70 cm above the floor. The immobility period was manually assessed using a timer during a 6-minute test session. The absence of movement in their limbs and bodies, other than when they breathed or hung passively, was considered immobility. The number of seconds spent motionless was acquired as a parameter.

Forced Swimming Test

Hartmann et al conducted a forced swimming test (FST) following the OFT to examine the choice of coping strategies during stressful and inescapable circumstances.[41] It was found that rats could not contact the bottom of the beaker with their rear paws or tails or climb out of a 2-L glass beaker (13 × 24 cm) filled with water supply (21 ±1°C, height = 15 cm). The animals were toweled off following the trial to avoid getting cold, which lasted for 6 minutes.

Measurement of Corticosterone

Chloroform was used to anesthetize rats, and blood samples were obtained directly via their hearts. Following the manufacturer's instructions using the Corticosterone ELISA Kit (Cat # KA0468), the CORT level in plasma was determined by centrifugation at 1600 g for 10 minutes. An ELISA reader calibrated to 450 nm was used to measure the absorbance of the samples.

Statistical Analysis

Data are expressed as mean and standard deviation (SD). Our findings were analyzed using GraphPad Prism® 8.1 software for Windows (GraphPad Software, San Diego, CA, USA). One-way analysis of variance (ANOVA) with Dunnett's test was used to compare the groups and time exposure (short-term and long-term). The tracking paths were assessed utilizing a computerized video-tracking system by Kinovea software (version 0.9.5, http://www.kinovea.org). *P* values under 0.05, 0.01, 0.001, or 0.0001 were considered statistically significant.

Results

Body Weight

The CUS and CUS+Mtz groups showed a significant reduction ($F=1.503$; df = 3, 3; $P<0.05$) and ($F=5.477$; df = 3, 3; $P<0.05$) in body weight at their endpoints, respectively, vs. the Ctrl group (Figure 1). After day-14 of the CUS protocol, animals' bodyweight in the Ctrl increased; however, the Mtz group reached its endpoint at day 14 with no significant difference from the Ctrl group ($P>0.05$). Another group, Mtz after CUS, which received Mtz at 10 mg/kg after stress, showed a reduction in body weight with no statistically significant change from the Ctrl group. However, after the seventh day, it entered stable conditions and had an increasing trend. Although weight gain was not affected in the Mtz after CUS group did not affect weight gain compared with the Ctrl and CUS groups (Figure 1B). Based on Figure 1C, only the Mtz after CUS and Mtz groups showed weight gain in rats. However, for weight gain, the Mtz after CUS group was significantly lower than Mtz and Ctrl groups ($P=0.0107$).

Behavioral Assays

Elevated Plus Maze Test

The results of this experiment are depicted in Figures 2A-E, representing the frequency of entrances in open and closed arms and the proportion of time spent in open and closed arms. Male rats in the Ctrl, CUS, and treatment groups had their time in the open and closed arms recorded in seconds as time spent in the middle of EPM. On the other hand, Mtz after CUS group, spent a considerably greater amount of time in the open arms ($P=0.0002$), followed by CUS+Mtz ($P=0.0036$) and Mtz groups ($P=0.0451$) (Figure 2A). Compared to the Ctrl group, open arms entries in the CUS+Mtz and Mtz groups increased by 16.9% and 22.6%, respectively. In contrast, only the CUS+Mtz and Mtz groups, with $P=0.0381$ and $P=0.0062$, showed a statistically significant improvement over the CUS group (Figure 2B). It became clear that there was a noticeable trend in terms of frequency of entries and the proportion of spending time in the open arms. Compared to spending time in the open arm, the percentage of time spent in closed and open arms was shown to have a distinct pattern of significant changes.

As shown in both figures (Figure 2C and D), rats spend significantly more time in closed arms than open arms, indicating a rise in anxiety mood in rats exposed to CUS events ($P<0.01$ and $P<0.001$). A total amount of entrances, including open and closed arms, and cumulative time spent on this test can be used to calculate an anxiety index score that ranges from 0 to 1 if open-arm period and open-arm entrances are taken into consideration. In this study, the anxiety index shows a range from 0.6 to nearly 0.9, with Mtz after CUS and CUS+Mtz revealing a significant difference in anxiety levels of 0.011 and 0.001, respectively (Figure 2F).

Sucrose Preference Test

A significant decline was observed in all animals that underwent the CUS protocol in week 2 in their sucrose

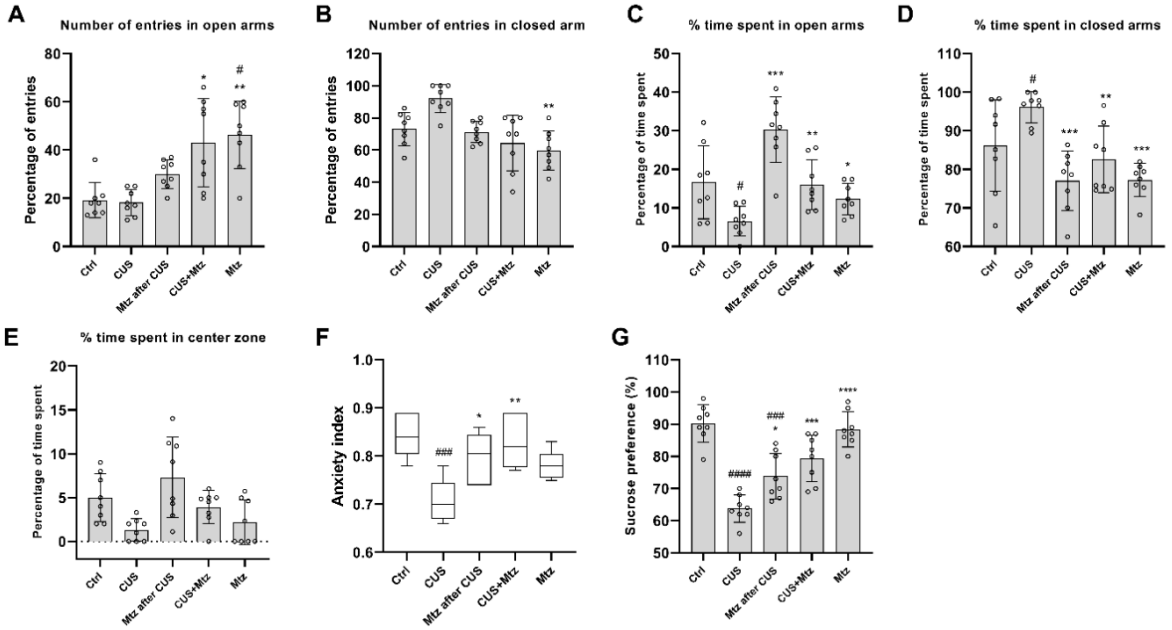

Figure 2. Effects of Various Models of Anxiety-Like Behaviors Mixed or Without Mirtazapine in the Elevated Plus Maze Test in Open and Closed Arms and Sucrose Preference Test. **(A)** the frequency of entries into the open arms; **(B)** the frequency of entries into the closed arms; **(C)** proportion of time spent in the open arms; **(D)** proportion of time spent in the closed arms; **(E)** percentage of time spent in middle zone, **(F)** anxiety index, a correlation exists between the anxiety index and open arm times, and **(G)** sucrose preference test. The whiskers of the box plot indicate minimum to maximum. Data represents the mean ± SEM ($n=8$/group). ### $P<0.001$, against the control group (Ctrl); * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$ against the CUS group; one-way ANOVA followed by Dunnett's multiple comparison test. The groups and the control group showed no statistically significant differences.

preference compared to the Ctrl group (Figure 2G). A one-way ANOVA found a statistically significant difference between the CUS group and Mtz after CUS group. The CUS group showed a substantial ($P<0.0001$) decrease in the sucrose preference assay. Treatment with Mtz showed a slightly increasing trend. Compared with the CUS group, the Mtz group showed the highest level of sucrose ($P<0.0001$), whereas the Ctrl group did not exhibit any significant difference ($P>0.05$).

Open-Field Test, Tail Suspension Test and Forced Swimming Test

In OFT, Mtz and Ctrl groups differed significantly from each other, as shown by a one-way ANOVA ($F=0.8725$; df = 3, 19; $P=0.0256$), as well as for the Mtz group versus CUS group ($F=0.9762$; df = 3, 19; $P=0.0196$). However, in the Mtz after CUS group compared to the rest of the groups, these effects were higher (except for the Mtz group) and were statistically insignificant (Figure 3B).

We used the TST and FST tests (Figures 3D and 3E, respectively) to investigate how CUS might lead to depressive-like symptoms. CUS+Mtz ($P=0.0035$) and Mtz ($P=0.0086$) groups showed a significant decrease in immobility over the Ctrl group in Figure 3D. There were significant differences in immobility between the Mtz and Ctrl groups in the FST test (Figure 3E, $P=0.0396$) employing one-way ANOVA using Dunnett's test.

Biochemical Analysis

Plasma Corticosterone Levels

According to the one-way ANOVA, there was a significant difference between the two groups with respect to this parameter ($F=3.064$; df = 4, 25; $P<0.0001$). A considerable difference ($P<0.0001$) in plasma CORT levels could be found between the CUS and Ctrl groups using Dunnett's test (Figure 4). In the Mtz group, CORT

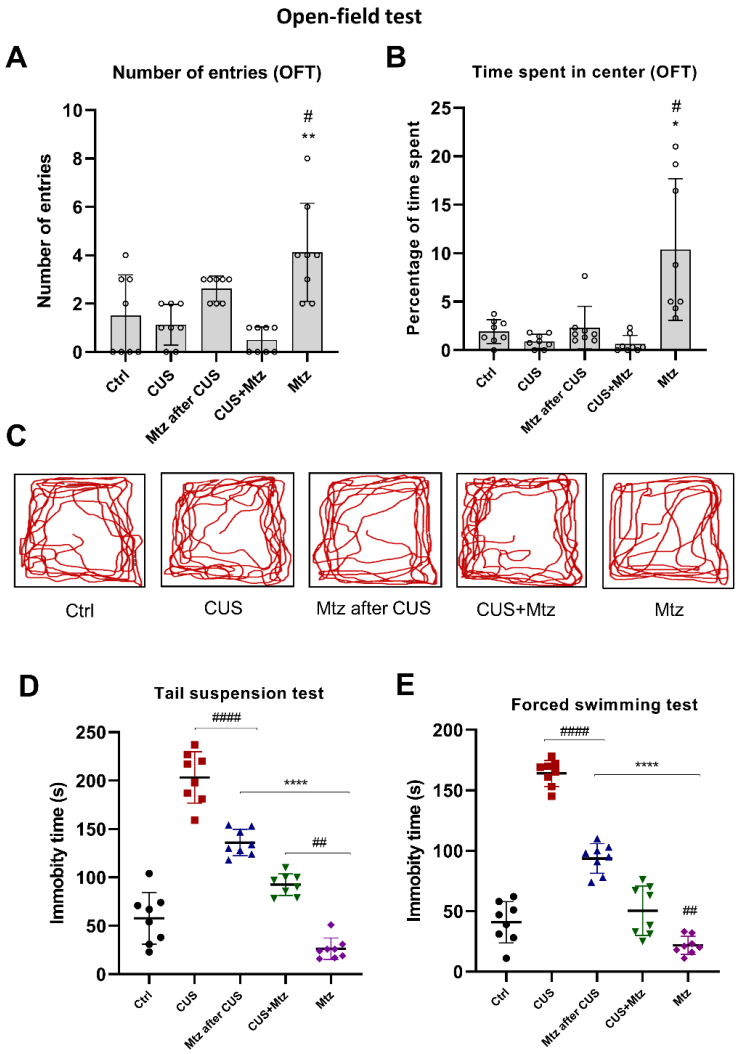

Figure 3. Comparison of Three Behavioral Tests on the CUS Tested and Control Groups. **(A)** the percentage of time spent in the center of the OFT, **(B)** the number of entries to the center of the OFT, **(C)** representative images from the open field test tracking the pace of rats, **(D)** tail suspension test, and **(E)** forced swimming test. Data represent the mean ± S.D. ($n=8$/group). $^{\#}P<0.05$, vs. the control group (Ctrl); $^{**}P<0.01$, $^{*}P<0.05$ vs. the CUS group; compared to the control group (Ctrl) and CUS group, one-way ANOVA using Dunnett's multiple comparison test were used to examine differences among groups. The groups and the control group showed no statistically significant differences.

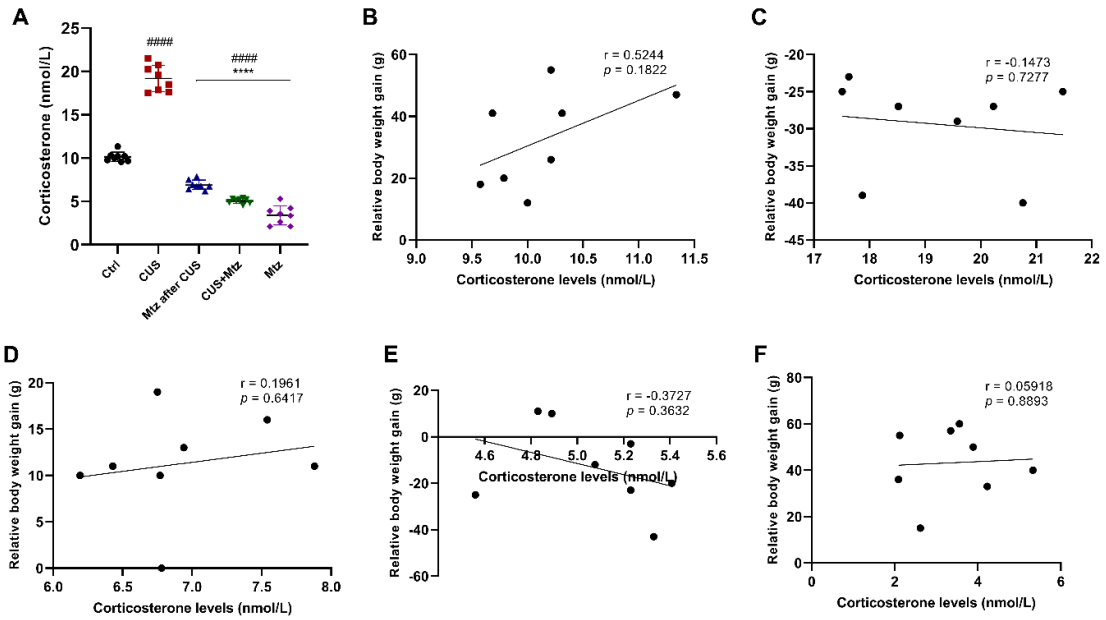

Figure 4. The Correlations of Physiological Factors Such as Body Weight and Corticosterone Levels Together. **(A)** Plasma corticosterone (nmol/L) measured in rats in response to behavioral tests. **(B)** corticosterone levels in control (Ctrl) group, which positively correlated with body weight gain after 23 days of experiment. Relative body weight in CUS group was **(C)** negatively correlated with corticosterone levels, **(D)** positively correlated with corticosterone levels in Mtz after CUS group following 23 days. **(E)** corticosterone levels in CUS+Mtz group revealed negatively correlated with body weight, while **(F)** in Mtz group positively correlated with body weight after 14 days. The data is expressed as the mean ± SD. r = Pearson's correlation, *P* = significant value.

levels significantly ($P<0.0001$) compared to the CUS group. The plasma CORT levels in the Mtz after CUS and CUS+Mtz groups both dropped significantly ($P<0.0001$) compared the CUS group.

A Pearson's correlation showed interesting differences between body weight groups (Figure 4B-E). Based on the results in Figure 4, CORT levels were only positively correlated with body weight gain following 23 and 14 days of experiment in Mtz after CUS and Mtz groups (r = 01961, 95% CI: -0.5901-0.7914, $P=0.6417$ (Figure 4D); r = 0.05918, 95% CI: -0.6736-0.7333, $P=0.8893$ [Figure 4F]).

Discussion

It is estimated that up to 15% of all adults worldwide suffer from depression at some point in their lifetime.[42] We aimed to evaluate the effect of Mtz at various times of administration during the CUS protocol or before or after this situation, where prior studies only focused on the administration of this drug for the depression mode, and not evaluating when the individual must consider to taking it. Also, we tried to find a correlation between weight gain and CORT levels to determine the best time to use Mtz without making it harmful to the body. Hence, we performed three treatment groups in the animal behavior study under the CUS condition: sub-chronic (~9 days), chronic (~23 days), and mixed Mtz treatments. Mtz has been shown to have no sedative effect at a dosage of 30 mg/kg (in 30 days or longer), significantly reduce depressive and anxiety symptoms throughout continuous drug withdrawal and permanently reduce the behavioral symptoms (using single or combined) in animals and individuals.[43] Hence, in our study, we evaluated Mtz for two weeks of treatment. After 14 days of oral Mtz at a dosage of 10 mg/kg, there was a rise in the amount of spending time in the open arms following the induction of CUS (Mtz after CUS, $P<0.001$) but not the frequency of entrances into the open arms ($P>0.05$), according to this study. More spending time and entries were found to be spent on the center arm, according to EPM results (data not shown). However, for the OFT, several studies have demonstrated that antidepressant drugs show no effect in this test.[44] Their results were consistent with our results in the OFT. Hence, we performed another test (EPM) to evaluate depression and anxiety-like behavior. The results indicated the effect of Mtz on the recovery process of the CUS condition. The CUS procedure reduced animal body weight, consistent with previously published results.[12,45] Monoamine deficiency is also caused by this procedure, which reduces the amounts of dopamine in the hippocampus and prefrontal cortex, as well as 5-HT and norepinephrine in the brain.[46] A current study demonstrated that CUS conditions might induce depression-like symptoms, such as despair behavior and anhedonia, as evidenced by decreased sucrose intake in the SPT, especially in the Mtz after CUS group. All two Mtz treatments decreased weight gain; however, Mtz after CUS did not demonstrate a significant difference from the control group. According to Salazar-Juarez et al, chronic stress conditions treated with Mtz at a concentration of 15 mg/kg led to increased body mass, whereas levels higher than 30 mg/kg had no effect.[25] Jia et al mentioned that

Mtz, compared to fluoxetine, enhanced food intake from 2-weeks to two months.[28] On the other hand, Mtz is a particular serotonergic and adrenergic antidepressant.[16] In healthy people, Mtz has been demonstrated to suppress cortisol secretion dose-dependent acutely. The antioxidant properties of Mtz may account for its antioxidative effects and chemoprotective actions in the rat brain following cisplatin-induced oxidative stress and DNA damage,[47] protecting rats against cisplatin-induced testicular injury.[48]

In addition, CUS exposure resulted in a substantial rise in blood CORT levels in rats, indicating HPA axis hyperfunction, consistent with earlier research. A substantial drop in CORT levels was found in Mtz after CUS, Mtz+CUS, and the Mtz groups. These treatment groups considerably lowered these increased CORT levels, indicating that they may exert their antidepressant properties by decreasing HPA axis hyperactivity. Plasma CORT levels rose after the CUS procedure, whereas dopamine, serotonin, and noradrenaline levels in the hippocampus and prefrontal cortex declined.[12] Nevertheless, the animals' ability to maintain a high level of CORT after re-exposure to the plus-maze was the most obvious indication that they are under the impact of stressful situations.[49] Based on the prior study by Abdul Shukkoor et al, CUS increased NF-κB levels in the prefrontal cortex and the hippocampus. Therefore, the CUS protocol caused inflammation in rats' hippocampus and prefrontal cortex.[12] Zhu et al discovered that after repeated Mtz administration, elevated NF-κB levels in the brain declined.[50] In addition, Mtz inhibits cerebral proinflammatory cytokines and NF-κB activity in the CNS, which are factors that influence neuropathic pain.[50] As a result, our findings are consistent with previous researchers[50,51] and point to the anti-inflammatory and antidepressant activity of Mtz. The alpha-2 presynaptic adrenoceptors are also blocked by Mtz, which increases norepinephrine and serotonin neurotransmission and causes a dramatic increase in serotonin levels at nerve terminals.[52] As mentioned previously, this medication is a noradrenergic, serotonergic antidepressant and an α-2 antagonist.[53]

Our study showed that the Mtz after CUS group decreased CORT levels and reduced CUS-induced depression-like symptoms along with weight gain in rats. These findings suggest that administrating Mtz following CUS conditions may have antidepressant action in treating chronic stress like depression. However, further research is needed to determine the significance of Mtz's acute inhibitory effects on cortisol release for its antidepressant effects.

Conclusion

Using behavioral tests and the SPT, this study found that Mtz had antidepressant-like effects in male rats under a chronic unpredictable mild stressful model of depression. A decline in plasma CORT levels and an increase in body weight were also associated with Mtz's effect after CUS condition via oral administration.

Authors' contribution

SMM and MANK designed the study; SBM collected behavioral and CORT data; AMC analyzed the data, wrote and edited the manuscript.

Conflict of Interest Disclosures

The authors declare no conflicts of interest.

Data Availability

The data can be obtained upon request.

Ethical Statement

All tests were performed following guidelines established by the Iran Medical University and Shahid Beheshti Medical Ethics Committee (approval ethics no. IR.SBMU.RETECH.REC.1395.671), the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, and the National Institutes of Health guide for the care and use of Laboratory Animals (NIH Publications No. 8023, revised 1978). This study was conducted with permission under ethic code IR.SBMU.RETECH.REC.1395.671.

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