ORIGINAL ARTICLE / ÖZGÜN MAKALE

THE EFFECTS OF MELATONIN, FLUOXETINE AND THEIR COMBINATIONS ON STRESS-INDUCED BEHAVIORAL AND COGNITIVE IMPAIRMENTS IN MICE

FARELERDE STRESİN NEDEN OLDUĞU DAVRANIŞSAL VE KOGNİTİF BOZUKLUKLARA MELATONİN, FLUOKSETİN VE KOMBİNASYONLARININ ETKİSİ

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ABSTRACT

Objective: *Melatonin (Mlt) is a hormone with antidepressant and antioxidant features. It is well known that Mlt protects brain cells from reactive oxygen species and that the brain's high oxygen consumption and lipid content make it particularly vulnerable to oxidative stress caused by prolonged stress. This study aims to investigate the effects of melatonin, fluoxetine (Flx) and their combinations on emotional memory, depression, and anxiety-like behavioral changes induced by immobilization (Imb) stress.*

Material and Method: *48 male Balb/c mice were divided into eight groups: Cnt (control), Imb, Imb+Mlt, Imb+Flx, Imb+Mlt+Flx, Mlt, Flx and Mlt+Flx. For seven days in a row, the mice underwent daily immobilization stress for 6 hours. Mice were treated with Mlt (10 mg/kg) and Flx (20 mg/kg) or saline 30 min prior to the immobilization stress procedure. All animals were subjected to behavioral tests; forced swimming test (FST), open field test (OFT), elevated plus maze (EPM), passive avoidance test (PAT), and hot plate (HP) test. After all behavioral tests, brain tissues were obtained for malondialdehyde (MDA) level analysis.*

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Result and Discussion: *OFT test data showed the time spent in the central zone and the number of entrances to the central area were lower in the Imb group compared to the Cnt group; these were higher in the Imb+Flx, Imb+Mlt, Imb+Mlt+Flx groups compared to the Imb group. Also, according to the data obtained from FST, immobile time was significantly higher in the Imb group compared to the Cnt group, and it was lower in the Imb+Flx, Imb+Mlt, Imb+Mlt+Flx groups compared to the Imb group. Besides, it was demonstrated that the emotional memory index was statistically higher in the Imb group compared to the Cnt group, and the increasing of memory index returned to normal in the Imb+Mlt and Imb+Mlt+Flx groups with PAT. Furthermore, lipid peroxidation level, which increased in the Imb group, decreased significantly in the Imb+Flx, Imb+Mlt, and Imb+Mlt+Flx groups. As a result, it was observed that melatonin has antidepressant, anxiolytic, antioxidant effects and normalized emotional memory. Also, melatonin, fluoxetine, and their combinations exert similar effects in the present study.*

Keywords: *Anxiety, depression, emotional memory, melatonin, stress*

ÖZ

Amaç: *Melatonin (Mlt) antidepresan ve antioksidan özellikleri olduğu bilinen, yüksek lipit içeriği ve oksijen tüketimi nedeniyle oksidatif strese oldukça duyarlı olan beyin dokularını uzun süreli stresin neden olduğu reaktif oksijen türlerine karşı koruyan bir hormondur. Bu çalışmada melatonin, fluoksetin (Flx) ve kombinasyonlarının immobilizasyon stres ile meydana gelen duygusal öğrenme, depresyon ve anksiyete benzeri davranışsal değişiklikler üzerine etkilerinin araştırılması amaçlanmıştır.*

Gereç ve Yöntem: *48 erkek Balb/c fare 8 gruba ayrılmıştır: Cnt (kontrol), Imb (immobilizasyon), Imb+Mlt, Imb+Flx, Imb+Mlt+Flx, Mlt, Flx ve Mlt+Flx. Farelere immobilizasyon stress prosedüründen 30 dk önce Mlt (10 mg/kg), Flx (20 mg/kg) veya salin enjekte edildi. İmmobilizasyon stress prosedürü 7 ardışık gün, günde 6 saat uygulandı. Ardından tüm denekler davranış testlerine tabi tutuldu: zorunlu yüzme (FST), açık alan (OFT), yükseltilmiş artı labirenti (EPM), pasif sakınma (PAT) ve hot plate (HP) testi. Davranış testlerinden sonra malondialdehit (MDA) düzeyi analizi için beyin dokuları izole edildi.*

Sonuç ve Tartışma: : *Açık alan testi verilerine göre, Imb grubunda Cnt grubuna göre azalan santral alana giriş sayısı ve santral alanda geçirilen süre Imb+Flx, Imb+Mlt, Imb+Mlt+Flx gruplarında artmıştır. Ayrıca, zorunlu yüzme testinde Imb grubunda Cnt grubuna göre artan immobil zaman Imb+Flx, Imb+Mlt, Imb+Mlt+Flx gruplarında azalmıştır. Bunun yanı sıra pasif sakınma testine göre Imb grubunda Cnt grubuna göre artan duygusal öğrenme indeksinin Imb+Mlt ve Imb+Mlt+Flx gruplarında normale döndüğü gösterilmiştir. Bununla birlikte Imb grubunda artan MDA düzeyinin Imb+Flx, Imb+Mlt ve Imb+Mlt+Flx gruplarında anlamlı olarak azaldığı görülmüştür. Sonuç olarak melatoninin antidepresan, anksiyolitik ve antioksidan etkilerinin olduğu ve duygusal öğrenmeyi normalleştirdiği gösterilmiştir. Ayrıca bu çalışma ile, melatonin, fluoksetin ve kombinasyonlarının benzer etkili olduğu gösterilmiştir.*

Anahtar Kelimeler: : *Anksiyete, depresyon, duygusal öğrenme, melatonin, stres*

INTRODUCTION

Stress is defined as physical and pathological changes in the body that disrupt the organism's homeostasis and are assumed to be the major cause of many diseases [1]. We are exposed to stress in all aspects of everyday life, which seriously influences cognitive and emotional functions [2]. Chronic stress has been associated with a higher risk of a variety of physical and mental conditions, such as depression, anxiety, and cognitive impairment [3]. In addition, neurochemical and hormonal changes during stress have been related to oxidative stress [4]. Chronic stress has been reported to increase lipid peroxidation, nitrite levels, catalase activity, and glutathione levels in the brain, all related to oxidative stress [1].

The serotonergic system is one of the most important determinants of psychological state, emotions, sleep, and hunger-satiety [5]. Fluoxetine inhibits serotonin reuptake, increases serotonin levels in the synaptic gap, and produces antidepressant effects [6]. Fluoxetine is a potent antidepressant and anxiolytic agent that increases serotonin levels and protects brain cells from oxidative damage [7]. Melatonin is a circadian rhythm-related hormone synthesized from serotonin and released from the

pineal gland in the dark [6]. The antagonist effect of agomelatine on 5-HT2C serotonin receptor and agonist effect on MT1/MT2 melatonin receptors [8] has directed scientists to investigate the antidepressant effect profile of melatonin [6]. It has been suggested in studies that melatonin can prevent oxidative damage by various mechanisms [9, 10]. The use of drug combinations in treating cognitive and behavioral disorders stimulates various pharmacological pathways and allows the drugs to have additive or synergistic effects [11].

This study aimed to investigate the therapeutic effects of melatonin, fluoxetine, and their combinations on stress-induced pathways in mice. For this purpose, an OFT was used to determine locomotor activity and anxiety level. The effects of drugs on depression were investigated using an FST. Anxiety assessment was performed using an EPM test; emotional memory functions were measured using a PAT. MDA, a lipid peroxidation biomarker, was used to investigate brain oxidative damage and medications' effects on the damage.

MATERIAL AND METHOD

Experimental Animals

In the study, 48 male Balb/c mice weighing an average of 35-40 grams were obtained from Dicle University Health Sciences Application and Research Center (DÜSAM) with the approval of ethical committee number 16 dated 13.04.2016. The animals were maintained at a fixed room temperature (23±2°C) under a 12h light/ dark cycle. Tap water and food were provided ad libitum. Each mouse was tested individually and only once per day. The principles regarding "Protection of Animal Rights" were carefully followed during the study.

Experimental Design

As in previous studies, the mice in the Imb groups underwent immobilization stress in cages too small to move around for 6 hours a day for 7 consecutive days [12]. Stressed mice were also restricted from food and water for 6 hours. Injections were made 30 minutes before the immobilization. Mlt (10 mg/kg, Sigma Chemical Co, St. Louis, MO, USA) [13] was dissolved in 5% ethanol and diluted with saline, while Flx (20 mg/kg, Sigma Chemical Co, St. Louis, MO, USA) [14] was dissolved in saline containing 5% ethanol (Table 1). All drugs and saline solution were administered ip with a total injection volume of 0.1 ml. Behavioral tests; OFT, FST, PAT, EPM, and HP tests were started on the eighth day. The mice were subjected to one test every day. After the behavioral tests, the mice were sacrificed with cervical decapitation under ether anesthesia, and their brain tissues were removed. MDA levels, an end product of lipid peroxidation, were measured in brain tissues.

Table 1. Experimental Design

Groups(n=6)	Immobilization (Imb)	Melatonin (10mg/kg)	Fluoxetine (20mg/kg)
1. Control (Cnt)			
2. Immobilization (Imb)	*		
3.Immobilization+Melatonin (Imb+Mlt)	*	*	
4.Immobilization+Fluoxetine (Imb+Flx)	*		*
5.Immobilization+Melatonin+Fluoxetine (Imb+Mlt+Flx)	*	*	*
6. Melatonin (Mlt)		*	
7. Fluoxetine (Flx)			*
8.Melatonin+Fluoxetine (Mlt+Flx)		*	*

Open field test (OFT)

It is a test used to evaluate the spontaneous locomotor activities of subjects, such as exploring and walking. In addition, with the OFT, the anxiety caused by the conflict between the subjects' desire to explore and their inner fears due to staying in the open area can also be measured [15].

OFT; it is a 40x40x20cm dimension, illuminated by a light source, all surfaces in black color. The behavior of the subjects is recorded with the camera placed on the device and evaluated with the Ethovision XT 11.0 (Noldus Inf. Tech. Netherlands). Mice are left in the middle of the field, and their movements are recorded for 5 minutes. The duration of their stay in the central area and the number of entrances to this area, their total distance, and speeds in the open field were calculated.

Forced swimming test (FST)

The FST is often used in depression research to study the effectiveness of antidepressants. This test was executed in a transparent cylinder tank filled with 30 cm water (diameter:20 cm, height: 50 cm). The escape efforts of the subjects who were left in the tank filled with water were monitored, and they were expected to remain immobile within a certain period (6 minutes) [16]. The fact that subjects gave up their efforts to get rid of the situation in a short time was interpreted in direct proportion to high depression levels. During the test, the temperature of the water in the tank was kept constant (24-26 ° C) to prevent additional stress on the subjects. And all the movements of the subjects were recorded with the help of a camera. After the experiment, the time spent mobile and immobile was analyzed using the Ethovision XT 11 (Noldus Inf. Tech. Netherlands) program.

Passive Avoidance test (PAT)

The PAT is based on the subjects avoiding painful stimuli (mild foot shock). It is generally used in evaluating emotional memory in rodents [17].

The PAT apparatus consists of two sections (one bright, one dark, width-length-height: 11x12x20 cm) connected by an automatic sliding door. The bright section is illuminated with white light, while in the dark section, there is a steel grid with wires parallel to each other and connected to the shock device.

The PAT was performed in two consecutive days. On the first day (acquisition trial), the subjects were exposed to 2000 lux of light, and after waiting for 60 seconds to get used to the environment, the door between the two compartments was opened. The subjects waited to enter the dark section with their instincts, and the step-through latencies (time to escape from the light) were recorded. After the entrance, the automatic door was closed, and the subjects were given a 0.5 mA electric shock for 1 second with the help of the steel grid on the floor. After waiting for 3 seconds, the experiment was terminated. This test was repeated 24 hours later in order to evaluate the gain and recall in the memory of fear.

On the second day (retention trial), after the subjects were exposed to 2000 lux light for 30 seconds, the intermediate compartment was opened, and the step-through latencies (avoidance from darkness) were recorded. The difference between both step-through latency was accepted as the memory index. The mice that did not enter the dark section for five minutes were considered well remembered, and the experiment was terminated.

Elevated Plus Maze (EPM) test

This test can evaluate the subjects' anxiety levels. Typically, subjects prefer to stay in closed arms and avoid open arms. Subjects with no anxiety demonstrate high environmental recognition behavior and spend more time in open arms [18].

The EPM has a 5x5 cm central area with two open and two closed arms, each 35 cm long. Its height from the ground is approximately 50 cm, and it is an entirely black-painted apparatus in the form of + (plus).

In our study, the subject's movements placed in the middle of the apparatus were recorded with a video camera for 5 minutes (300 seconds). The time spent in central, open, and closed arms and the number of entrances to central, open, and closed arms were calculated using the Ethovision XT 11 (Noldus Inf. Tech. Netherlands) program based on the video recordings.

Hot Plate (HP) Test

HP test measured the pain (analgesic effect) reaction latencies. The test was carried out on a system with a metal floor at 52 °C and surrounded by a plexiglass cover (H26 cm, D19 cm). The experiment was terminated if the subjects licked their hind paws or jumped (max 30 seconds) [19].

Biochemical Analysis of Lipid Peroxidation Levels

MDA is one of the lipid peroxidation products. Low pH and high-temperature MDA react with nucleophilic addition reaction with 2-thiobarbituric acid (TBA) and form a product that can measure the peak absorbance at 532 nm in the form of red fluorescent, 1: 2, MDA: TBA.

3 ml phosphoric acid (1%) and 1 ml TBA (0.6%) were added to 0.5 ml homogenate in the centrifuge tube, and the mixture was heated in a hot water bath for 45 minutes. After the mixture had cooled, 4 ml of n-butanol was added to the mixture and mixed on vortex for 1 min. The resulting mixture was centrifuged at 20,000 rpm for 20 minutes. The supernatant obtained after centrifugation was transferred to a new tube, and its absorbance at 532 nm was measured. 1,1,3,3-tetramethoxypropane was used as standard [20].

Statistical Analysis:

Data are expressed as mean ± standard error. SPSS statistics program (Chicago, Ill. USA) was used to analyze of data obtained. Kruskal-Wallis test was used to compare multiple groups, and the Mann-Whitney U test was used to compare binary groups. The Wilcoxon test was used to compare weights between the first and final days. $p < 0.05$ was accepted for statistical significance.

RESULT AND DISCUSSION

Weights of the subjects were measured before starting the experiment and after the behavioral tests ended. When the initial and final weights of the subjects were compared, the weights of the subjects in the Cnt group insignificantly increased. The subjects' weights in all other groups except Imb+Flx and Flx groups statistically decreased (Figure 1, $p < 0.05$).
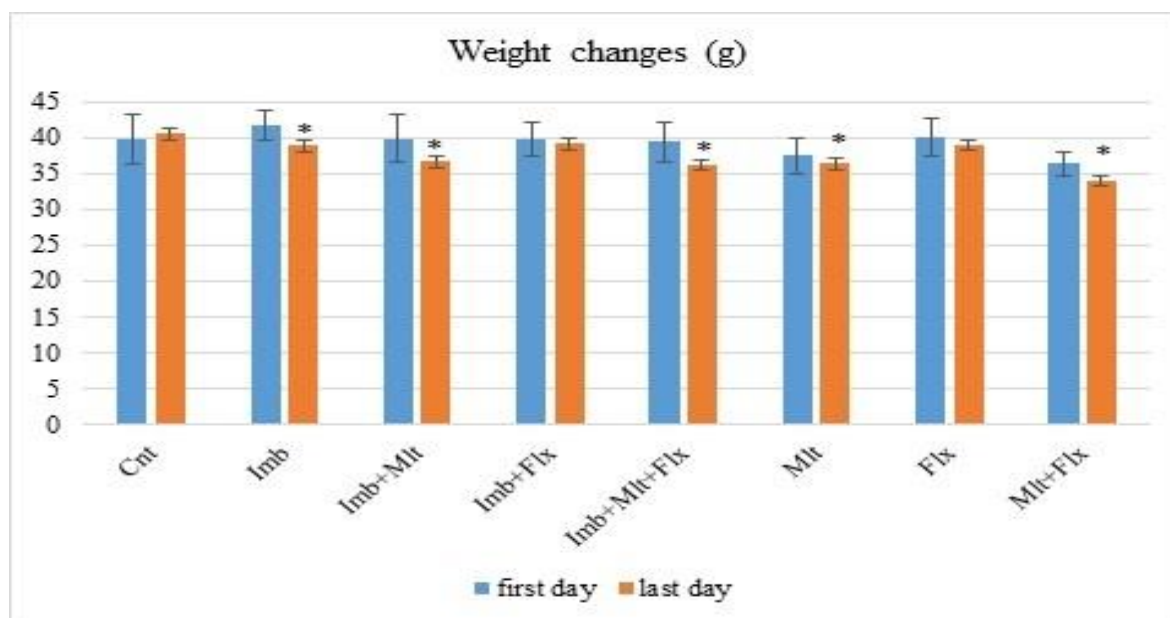

Figure 1. Weight changes compared with the initial and final weights of the subjects [*]$p < 0.05$

The time spent in the central area was lower in the Imb group compared to the Cnt group ($p < 0.01$, Figure 2). Compared to the Imb group, the time spent in the central zone and the number of entrances

to the central area were significantly higher in all drug-applied groups (p<0.01, Figure 2). Moreover, the number of entrances to the central area was higher than in the Imb+Mlt+Flx group compared to Imb+Mlt and Imb+Flx groups (p<0.05, Figure 2). The Imb group moved significantly less distance than the Cnt group (p<0.01, Figure 3). Total distance and mean velocity in all drug-applied groups were significantly higher compared to the Imb group (p<0.01, Figure 3). In addition, total distance and mean velocity in the Imb+Flx and Imb+Mlt+Flx groups were significantly higher compared to the Imb+Mlt group (p<0.05, Figure 3). And also, total distance and mean velocity in the Mlt+Flx group were significantly higher compared to the Mlt group (p<0.05, Figure 3).
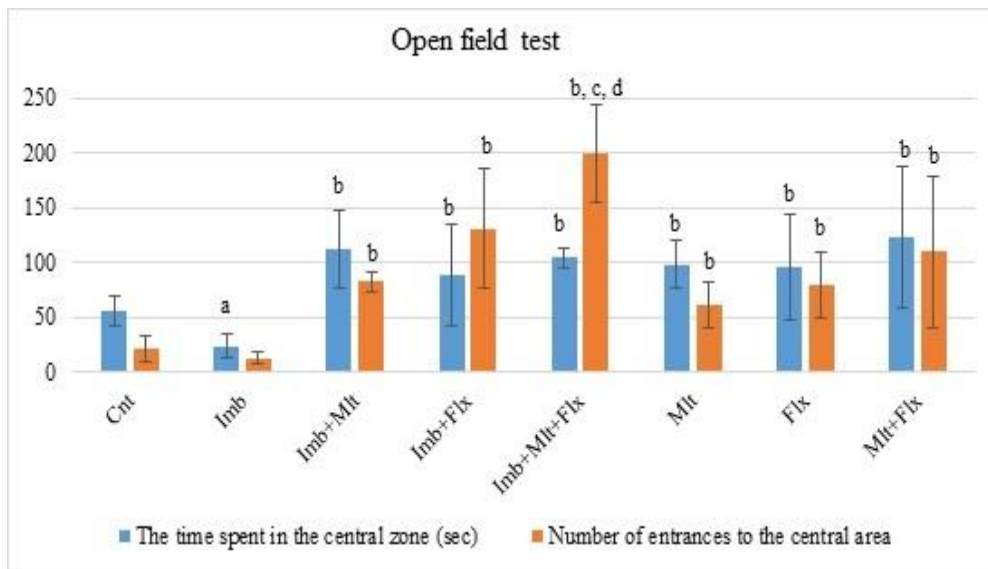
Figure 2. Open field test compared with Cnt [a]p<0.01, compared with Imb [b]p<0.01, compared with Imb+Mlt [c]p<0.05, compared with Imb+Flx [d]p<0.05.

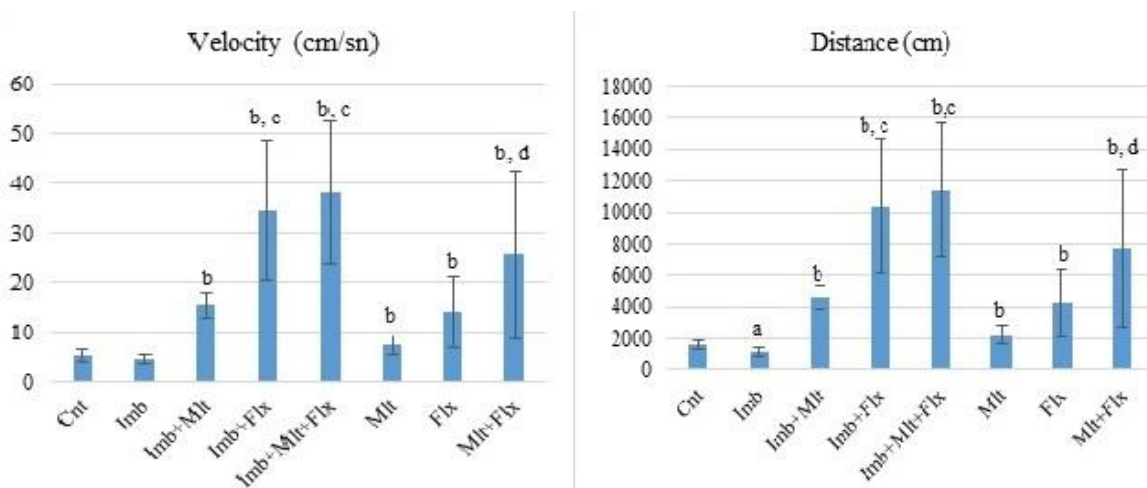**Figure 3.** Open field test compared with Cnt [a]p<0.01, compared with Imb [b]p<0.01, compared with Imb+Mlt [c]p<0.05, compared with Mlt [d]p<0.05

No statistically significant differences were obtained with the elevated plus maze test. (Figure 4, Figure 5)

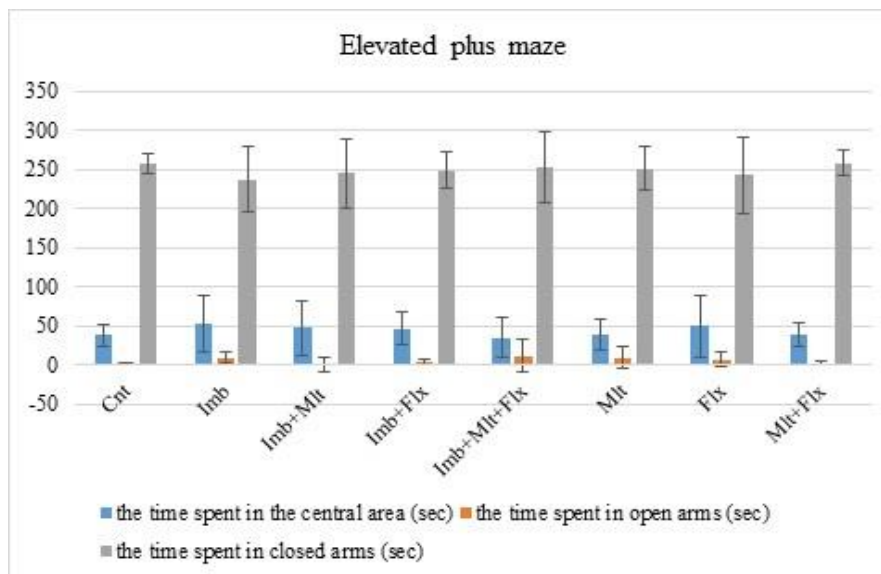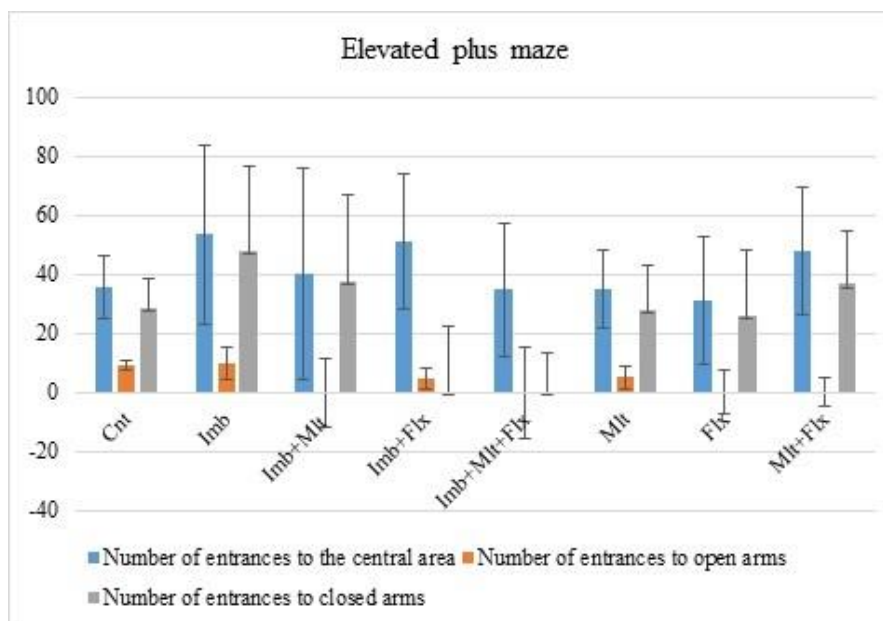**Figure 4.** Elevated plus maze

Figure 5. Elevated plus maze

The duration of immobilization was significantly higher in the Imb group compared to the Cnt group (p <0.05, Figure 6). In the Imb+drug or only drug groups, the duration of immobilization was statistically lower than the Imb group (p <0.01, Figure 6). On the other hand, while the mobilization duration in the Imb group was lower than the Cnt group (p <0.05, Figure 6), the mobilization duration in the Imb+drug and only drug groups was higher than the Imb group (p <0.01, Figure 6).

The memory index of the Imb group was significantly higher than the Cnt group (p<0.01, Figure 7). The Imb+Mlt and Imb+Mlt+Flx groups' memory indexes were significantly lower than the Imb

group (p<0.05, Figure 7). In addition, the Imb+Flx group memory index was significantly higher than the Imb+Mlt group (p<0.05, Figure 7).
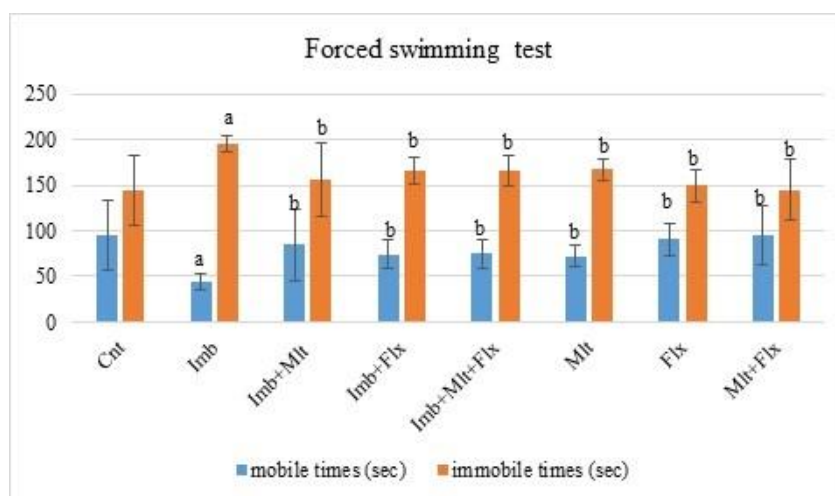

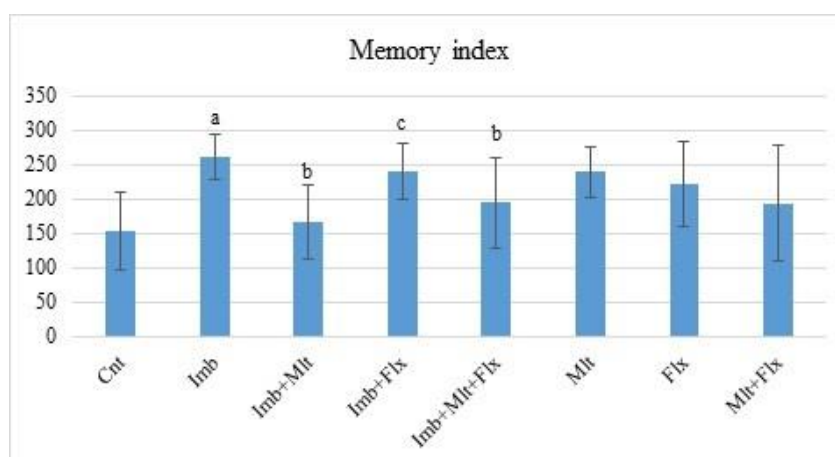**Figure 6.** Forced swimming test result compared with Cnt, [a]p<0.05, compared with Imb [b]p<0.01

Figure 7. Memory index of the subjects in the passive avoidance test compared with Cnt [a]p<0.01, compared with Imb [b]p<0.05, compared with Imb+Mlt [c]p<0.05.

No statistically significant differences were obtained between the groups' pain reaction latencies (p>0.05, Figure 8).

The MDA levels of the Imb group were significantly higher than the Cnt group (p<0.01, Figure 9). Mlt, Flx, and Mlt+Flx treated groups' MDA levels were significantly lower than in the Imb group (p<0.05, Figure 9).

The immobilization stress model strongly mimics physical and psychological stress and is widely used to induce experimental stress [21]. This model has been demonstrated to cause anxiety, depression-like behavior, and changes in pain reaction, body weight, and locomotor activity. Furthermore, due to this procedure increased nitrite and MDA levels and decreased glutathione and adrenal ascorbic acid levels were reported [1, 3, 22].

In this study, the Imb group's weight gain was significantly lower than that of the Cnt group. While in a study, weight gain diminishes with the decrease in food intake in the immobilization stress groups [23], others did not exert a difference between the Imb and Cnt groups [6, 24].

The emotional memory index obtained from the PA tests revealed the Imb group had a significantly higher index than the Cnt group. While some researchers have reported that stress impairs learning and memory [25, 26], others have found that stress improves memory [27, 28]. According to Das et al. (2000), cognitive functions improved due to the suppression of brain acetylcholinesterase activity in the acute immobilization stress group; acetylcholinesterase activity was normalized with the development of adaptive processes in the cholinergic system in the case of continuous stress exposure [29].
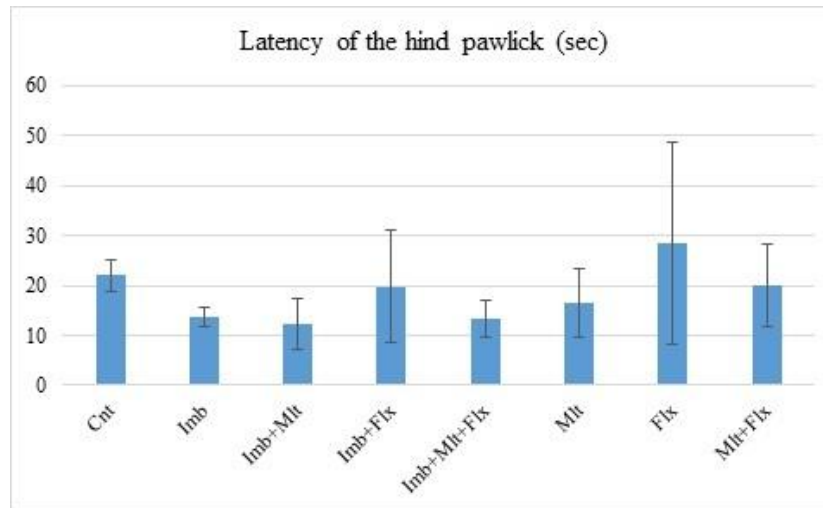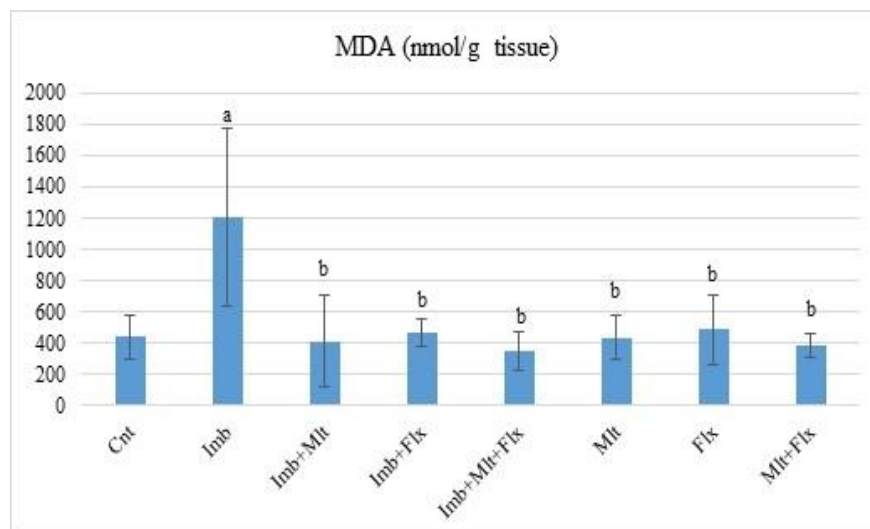

Figure 8. Hot plate test result

Figure 9. MDA levels compared with Cnt [a]p<0.01, compared with Imb [b]p<0.05

In studies conducted with the FST used to assess depression levels, the Imb group's immobilization duration was significantly higher than the Cnt group [6, 21]. Increased duration of immobilization in the FST is interpreted in favor of depression. In our study, compared to the Cnt group, the duration of immobilization in the FST was significantly higher in the Imb group.

According to OFT data, the Imb group spent less time in the center than the Cnt group, which was evaluated as an anxiety-like behavior in the Imb group. This study has demonstrated consistent results with other studies in which stress causes anxiety [30, 31]. No significant difference was observed between the Imb and the Cnt group regarding the number of entries to the central area and velocity.

However, in accordance with the literature, in the present study, the distance covered by the Imb group was significantly lower compared to the Cnt [22, 30]. This demonstrated that the Imb group's locomotor activity increased. On the other hand, in the previous study, according to the OFT data, no difference was reported between the Cnt group and the Imb group in terms of the time spent in the central area, the number of the entrance to the central area, and the distance they moved [6].

In the immobilization stress group, stress-related weight loss, anxiety, depression, emotional learning-memory enhancement, decreased locomotion, and increased lipid peroxidation levels were all successfully observed.

Fluoxetine was utilized to prevent stress-induced depression, anxiety-like behavior, and oxidative stress, which has been defined as an antidepressant, anxiolytic, and antioxidant in the literature [9,32].

Melatonin, released by the pineal gland, is reported to have significant functions in regulating circadian rhythm, immune system response, weight regulation, reproduction, tumor development suppression, jet-lag, and oxidation prevention [33]. It was reported that melatonin also has antidepressant and anxiolytic effects [10,34]

In the weight control we performed at the beginning and end of this study, the weight gain of the Imb, Imb+Mlt, Imb+Mlt+Flx, Mlt, and Mlt+Flx groups was considerably lowered. In other words, the Mlt treatment reduced weight gain in all groups, and the weight gain reduction was higher in the Mlt group than in the Imb group, while the difference was not statistically significant. A previous study reported that there is no relationship between melatonin and weight gain [6], while some other studies reported that melatonin causes weight gain [35] or weight loss [22].

In our study, the memory index data in the Imb+Mlt and Imb+Mlt+Flx groups were considerably lower than in the Imb group with the PAT. These results show that emotional memory, which increases with stress, decreases with Mlt. A recent study reported that melatonin enhanced impaired spatial learning and working memory by reducing oxidative stress [36]. Another research concluded that the level of melatonin decreases in the early stages of Alzheimer's disease and that melatonin treatment can improve cognitive abilities in these patients [37]. On the other hand, genetic deletion of melatonin receptors has been reported to improve cognitive and motor performance [38].

In this study, the duration of immobility on the FST was significantly lower in the Imb+Mlt, Imb+Flx, and Imb+Mlt+Flx groups than in the Imb group. The incidence of depression-like behavior was lower in all the drug-applied groups than in the Cnt group. It was noted that getting fluoxetine and melatonin together had no additional benefit. Numerous studies have demonstrated that melatonin has an antidepressant effect [39, 40]. In the previous study, similar to this study, in the FST, the duration of mobility in the Imb group was lower compared to the Cnt group, and the duration of mobility in the Imb+Mlt, Imb+Flx, Imb+Mlt+Flx groups was higher compared to the Imb group. However, Mlt+Flx was found to be more effective than the application of these drugs alone in immobilization stress [6].

The OF and the EPM tests were used to assess anxiety. While the EPM test yielded no significant results, in comparison to the Imb group, the time spent in the center area and also the number of entrances to the central area was higher in the Imb+Mlt, Imb+Flx, and Imb+Mlt+Flx groups with the OFT. These results indicate that increased anxiety levels with stress application can be ameliorated with the mentioned drugs. However, giving the drugs alone or in combination provided no benefit. A study reported that melatonin showed anxiolytic effects by increasing the number of open arm entrances and duration in the EPM test [41].

MDA is a marker of oxidative stress-mediated lipid peroxidation in the central nervous system and other organs. It was revealed that lipid peroxidation, which increases with stress, affects depression, anxiety, and learning-memory pathology [42]. In a previous study, it was also reported that melatonin pretreatment reduced lipid peroxidation [43]. Our study displayed that MDA levels, which increased with immobilization stress, decreased significantly with Mlt, Flx, and Mlt+Flx treatments. However, the combined drugs did not provide more benefits than using them alone.

The immobilization stress model was successfully carried out in this study. It was observed that the Mlt, Flx, and Mlt+Flx significantly reduced depression, anxiety, and MDA levels in immobilization stress group. In addition, it was observed that the Mlt, Flx, and Mlt+Flx significantly reduced depression, anxiety, and MDA levels in the immobilization stress group. In

addition, the improved emotional learning memory in the Imb group returned to baseline with the Mlt and Mlt+Flx applications. According to these results, no significant difference was observed between the administration of melatonin, fluoxetine and their combinations.

In conclusion, our study demonstrated that melatonin, fluoxetine, and their combinations have beneficial effects on stress-induced alterations in behavior and oxidative parameters.

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AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

All experiment procedures and protocols used in the study were approved by the Dicle University Local Committee on Animal Research Ethics (No: 16, dated 13.04.2016).

REFERENCES

1. Sahin, E., Gumuslu, S. (2007). Immobilization stress in rat tissues: alterations in protein oxidation, lipid peroxidation and antioxidant defense system. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 144(4), 342-347. [CrossRef]

2. Kang, Y.J., Sim, Y.B., Park, S.H., Sharma, N., Suh, H.W. (2015). Involvement of alpha(2)-adrenergic receptor in the regulation of the blood glucose level induced by immobilization stress. Archives of Pharmacal Research, 38(5), 921-929. [CrossRef]

3. Chiba, S., Numakawa, T., Ninomiya, M., Richards, M.C., Wakabayashi, C., Kunugi, H. (2012). Chronic restraint stress causes anxiety- and depression-like behaviors, downregulates glucocorticoid receptor expression, and attenuates glutamate release induced by brain-derived neurotrophic factor in the prefrontal cortex. Progress in Neuropsychopharmacology and Biological Psychiatry, 39(1), 112-119. [CrossRef]

4. Samarghandian, S., Azimi-Nezhad, M., Farkhondeh, T., Samini, F. (2017). Anti-oxidative effects of curcumin on immobilization-induced oxidative stress in rat brain, liver and kidney. Biomedicine & Pharmacotherapy, 87, 223-229. [CrossRef]

5. Azouzi, S., Santuz, H., Morandat, S., Pereira, C., Cote, F., Hermine, O., El Kirat, K., Colin, Y., Le Van Kim, C., Etchebest, C., Amireault, P. (2017). Antioxidant and membrane binding properties of serotonin protect lipids from oxidation. Biophysical Journal, 112(9), 1863-1873. [CrossRef]

6. Li, K., Shen, S., Ji, Y.T., Li, X.Y., Zhang, L.S., Wang, X.D. (2018). Melatonin augments the effects of fluoxetine on depression-like behavior and hippocampal BDNF-TrkB signaling. Neuroscience Bulletin, 34(2), 303-311. [CrossRef]

7. Avitsur, R., Grinshpahet, R., Goren, N., Weinstein, I., Kirshenboim, O., Chlebowski, N. (2016). Prenatal SSRI alters the hormonal and behavioral responses to stress in female mice: Possible role for glucocorticoid resistance. Hormones and Behavior, 84, 41-49. [CrossRef]

8. Tchekalarova, J., Stoynova, T., Ilieva, K., Mitreva, R., Atanasova, M. (2018). Agomelatine treatment corrects symptoms of depression and anxiety by restoring the disrupted melatonin circadian rhythms of rats exposed to chronic constant light. Pharmacology Biochemistry and Behavior, 171, 1-9. [CrossRef]

9. Rebai, R., Jasmin, L., Boudah, A. (2017). The antidepressant effect of melatonin and fluoxetine in diabetic rats is associated with a reduction of the oxidative stress in the prefrontal and hippocampal cortices. Brain Research Bulletin, 134, 142-150. [CrossRef]

10. Ergenc, M., Ozacmak, H.S., Turan, I., Ozacmak, V.H. (2022). Melatonin reverses depressive and anxiety like-behaviours induced by diabetes: involvement of oxidative stress, age, rage and S100B levels in the hippocampus and prefrontal cortex of rats. Archives of Physiology and Biochemistry, 128, 402-410. [CrossRef]

11. Roni, M.A., Rahman, S. (2015) Effects of lobeline and reboxetine, fluoxetine, or bupropion combination on depression-like behaviors in mice. Pharmacology Biochemistry and Behavior, 139(Pt A), 1-6. [CrossRef]

12. Dhir, A., Padi, S.S., Naidu, P.S., Kulkarni, S.K. (2006). Protective effect of naproxen (non-selective COX-inhibitor) or rofecoxib (selective COX-2 inhibitor) on immobilization stress-induced behavioral and biochemical alterations in mice. European Journal of Pharmacology, 535(1-3), 192-198. [CrossRef]

13. Taniguti, E.H., Ferreira, Y.S., Stupp, I., Fraga-Junior, E.B., Mendonça, C.B., Rossi, F.L., Ynoue, H.N., Doneda, D.L., Lopes, L., Lima, E., Buss, Z.S., Vandresen-Filho, S. (2018). Neuroprotective effect of melatonin against lipopolysaccharide-induced depressive-like behavior in mice. Physiology & Behavior, 188, 270-275. [CrossRef]

14. Kudryashov, N.V., Kalinina, T.S., Shimshirt, A.A., Korolev, A.O., Volkova, A.V., Voronina, T.A. (2018). Antidepressant-like effect of fluoxetine may depend on translocator protein activity and pretest session duration in forced swimming test in mice. Behavioural Pharmacology, 29(4), 375-378. [CrossRef]

15. Kumar, N., Singh, N., Jaggi, A.S. (2012). Anti-stress effects of cilnidipine and nimodipine in immobilization subjected mice. Physiology and Behavior, 105(5), 1148-1155. [CrossRef]

16. Castagne, V., Moser, P., Roux, S., Porsolt, R.D. (2011). Rodent models of depression: forced swim and tail suspension behavioral despair tests in rats and mice. Current Protocols in Neuroscience, Chapter 8, Unit 8 10A. [CrossRef]

17. McGaugh, J.L., Cahill, L., Roozendaal, B. (1996). Involvement of the amygdala in memory storage: interaction with other brain systems. Proceedings of the National Academy of Sciences of the United States of America, 93(24), 13508-13514. [CrossRef]

18. Walf, A.A., Frye, C.A. (2007). The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nature Protocols, 2(2), 322-328. [CrossRef]

19. Bannon, A.W., Malmberg, A.B. (2007). Models of nociception: hot-plate, tail-flick, and formalin tests in rodents. Current Protocols in Neuroscience, 41, 8.9.1-8.9.16. [CrossRef]

20. Pompella, A., Maellaro, E., Casini, A.F., Ferrali, M., Ciccoli, L., Comporti, M. (1987). Measurement of lipid peroxidation in vivo: a comparison of different procedures. Lipids, 22(3), 206-211. [CrossRef]

21. Samad, N., Saleem, A., Yasmin, F., Shehzad, M.A. (2018). Quercetin protects against stress-induced anxiety- and depression-like behavior and improves memory in male mice. Physiological Research, 67(5), 795-808. [CrossRef]

22. Kumar, A., Kaur, G., Rinwa, P. (2014). Buspirone along with melatonin attenuates oxidative damage and anxiety-like behavior in a mouse model of immobilization stress. Chinese Journal of Natural Medicines, 12(8), 582-589. [CrossRef]

23. Sakhri, F.Z., Adachi, N., Zerizer, S., Ohashi, Y., Ikemoto, H., Tsukada, M., Kabouche, Z., Hisamitsu, T., Sunagawa, M. (2021). Behavioral and neurological improvement by Cydonia oblonga fruit extract in chronic immobilization stress rats. Phytotherapy Research, 35(4), 2074-2084. [CrossRef]

24. Macedo, I.C., Rozisky, J.R., Oliveira, C., Oliveira, C.M., Laste, G., Nonose, Y., Santos, V.S., Marquez, P.R., Ribeiro, M.F.M., Caumo, W., Torres, I.L.S. (2015). Chronic stress associated with hypercaloric diet changes the hippocampal BDNF levels in male Wistar rats. Neuropeptides, 51, 75-81. [CrossRef]

25. Faucher, P., Huguet, C., Mons, N., Micheau, J. (2022). Acute pre-learning stress selectively impairs hippocampus-dependent fear memory consolidation: Behavioral and molecular evidence. Neurobiology of Learning and Memory, 188, 107585. [CrossRef]

26. Woo, H., Hong, C.J., Jung, S., Choe, S., Yu, S.W. (2018). Chronic restraint stress induces hippocampal memory deficits by impairing insulin signaling. Molecular Brain, 11(1), 37. [CrossRef]

27. Kumar, R.S., Narayanan, S.N., Kumar, N., Nayak, S. (2018). Exposure to enriched environment restores altered passive avoidance learning and ameliorates hippocampal injury in male albino wistar rats subjected to chronic restraint stress. International Journal of Applied and Basic Medical Research, 8(4), 231-236. [CrossRef]

28. Rajbhandari, A.K., Gonzalez, S.T., Fanselow, M.S. (2018). Stress-enhanced fear learning, a robust rodent model of post-traumatic stress disorder. Journal of Visiulized Experiments, 140, 58306. [CrossRef]

29. Das, A., Kapoor, K., Sayeepriyadarshini, A.T., Dikshit, M., Palit, G., Nath, C. (2000). Immobilization stress-induced changes in brain acetylcholinesterase activity and cognitive function in mice. Pharmacological Research, 42(3), 213-217. [CrossRef]

30. Gomaa, A.M., Galal, H.M., Abou-Elgait, A.T. (2017). Neuroprotective effects of melatonin administration against chronic immobilization stress in rats. International Journal of Physiology, Pathophysiology and Pharmacology, 9(2), 16-27.

31. Banagozar Mohammadi, A., Torbati, M., Farajdokht, F., Sadigh-Eteghad, S., Fazljou, S.M.B., Vatandoust, S.M., Golzari, S.E.J., Mahmoudi, J. (2019). Sericin alleviates restraint stress induced depressive- and anxiety-like behaviors via modulation of oxidative stress, neuroinflammation and apoptosis in the prefrontal cortex and hippocampus. Brain Research, 1715, 47-56. [CrossRef]

32. Mendez-David, I., Tritschler, L., Ali, Z.E., Damiens, M.H., Pallardy, M., David, D.J., Kerdine-Römer, S., Gardier, A.M. (2015). Nrf2-signaling and BDNF: A new target for the antidepressant-like activity of chronic fluoxetine treatment in a mouse model of anxiety/depression. Neuroscience Letters, 597, 121-126. [CrossRef]

33. Samanta, S. (2022). Physiological and pharmacological perspectives of melatonin. Archives of Physiology and Biochemistry, 128, 1346-1367. [CrossRef]

34. Nie, L., Wei, G., Peng, S., Qu, Z., Yang, Y., Yang, Q., Huang, X., Liu, J., Zhuang, Z., Yang, X. (2017). Melatonin ameliorates anxiety and depression-like behaviors and modulates proteomic changes in triple transgenic mice of Alzheimer's disease. Biofactors, 43(4), 593-611. [CrossRef]

35. Tamura, I., Tamura, H., Kawamoto-Jozaki, M., Doi-Tanaka, Y., Takagi, H., Shirafuta, Y., Mihara, Y., Maekawa, R., Taketani, T., Sato, S., Sugino, N. (2021). Long-term melatonin treatment attenuates body weight gain with aging in female mice. Journal of Endocrinology, 251(1), 15-25. [CrossRef]

36. Lamtai, M., Azirar, S., Zghari, O., Ouakki, S., El Hessni, A., Mesfioui, A., Ouichou, A. (2021). Melatonin ameliorates cadmium-induced affective and cognitive impairments and hippocampal oxidative stress in rat. Biological Trace Element Research, 199(4), 1445-1455. [CrossRef]

37. Lin, C. H., Chiu, C.C., Lane, H.Y. (2021). Trough melatonin levels differ between early and late phases of Alzheimer Disease. Clinical Psychopharmacology and Neuroscience, 19(1), 135-144. [CrossRef]

38. O'Neal-Moffitt, G., Pilli, J., Kumar, S.S., Olcese, J. (2014). Genetic deletion of MT(1)/MT(2) melatonin receptors enhances murine cognitive and motor performance. Neuroscience, 277, 506-521. [CrossRef]

39. Stefanovic, B., Spasojevic, N., Jovanovic, P., Jasnic, N., Djordjevic, J., Dronjak, S. (2016). Melatonin mediated antidepressant-like effect in the hippocampus of chronic stress-induced depression rats: Regulating vesicular monoamine transporter 2 and monoamine oxidase A levels. European Neuropsychopharmacology, 26(10), 1629-1637. [CrossRef]

40. Sun, X., Wang, M., Wang, Y., Lian, B., Sun, H., Wang, G., Li, Q., Sun, L. (2017). Melatonin produces a rapid onset and prolonged efficacy in reducing depression-like behaviors in adult rats exposed to chronic unpredictable mild stress. Neuroscience Letters, 642, 129-135. [CrossRef]

41. Spasojevic, N., Stefanovic, B., Jovanovic, P., Dronjak, S. (2016). Anxiety and hyperlocomotion induced by chronic unpredictable mild stress can be moderated with melatonin treatment. Folia Biologica (Praha), 62(6), 250-257.

42. Karimi, S.A., Salehi, I., Shykhi, T., Zare, S., Komaki, A. (2019). Effects of exposure to extremely low-frequency electromagnetic fields on spatial and passive avoidance learning and memory, anxiety-like behavior and oxidative stress in male rats. Behavioral Brain Research, 359, 630-638. [CrossRef]

43. Pal, R., Gulati, K., Banerjee, B.D., Ray, A. (2016). Pharmacological and biochemical studies on the protective effects of melatonin during stress-induced behavioral and immunological changes in relation to oxidative stress in rats. Canadian Journal of Physiolology and Pharmacology, 94(3), 296-301. [CrossRef]