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Original Article

The Antidepressant Effect of Fluoxetine and Mozart K448 Combination Therapy on Hippocampal Serotonin and BDNF Levels

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ABSTRACT

Despite the use of fluoxetine as a first-line therapy, some patients do not show a good therapeutic effect. Effective antidepressant therapy will reverse the low serotonin and BDNF levels found in depression. Mozart K. 448 was reported to yield a good therapeutic effect for depression. Based on findings, the combination of Mozart K. 448 and fluoxetine as a therapy for depression is very rare. Therefore, this study aimed to determine the effect of the combined therapy of fluoxetine and Mozart K. 448 on hippocampal serotonin and BDNF levels in an animal model of depression under CUMS conditions. In this study, the animal model of depression was administered three different treatments, i.e. fluoxetine, Mozart, or fluoxetine-Mozart combined therapy, respectively. Hippocampal serotonin and BDNF levels were assessed after 21 days of treatment. Statistical analysis was then carried out using T-test or Mann-Whitney test and ANOVA or Kruskal-Wallis. The fluoxetine-Mozart group has higher BDNF levels, but lower serotonin levels compared to other groups with values of 1,694±0.215 and 44,533±3,275, respectively.

Key words: Fluoxetine, Mozart, Serotonin, BDNF, Hippocampus.

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INTRODUCTION

Fluoxetine is a first-line antidepressant.[1,2] Fluoxetine can increase the low serotonin levels as well as low BDNF levels found in depression.[3,4] Despite its usefulness, some patients did not respond well to this treatment.[5,6] Music is one of the alternative and complementary therapies available for those patients.[7,8] Slow rhythm music can increase BDNF levels in the brain.[9] Mozart K448 is one of the music that can increase serotonin in certain brain areas.[10] Mozart's music is one of depression's alternative therapy, and it acts differently on several areas of the brain to improve mood.[9-13]

Depression can show widely varied manifestations.[14,15] The animal model used to evaluate the therapeutic effects of certain treatments should be able to mimic various pathogenesis and symptoms of depression in humans.[16-18] An animal model of depression shows decreased serotonin and BDNF levels compared to the normal control.[19-22] These changes can be reversed using effective management.[19,23]

A combination of music and antidepressant therapy can be designed to obtain a better outcome in depressive patients.[24] Therefore, this study aims to determine the combined effects of music and fluoxetine therapy on hippocampal serotonin and BDNF levels.

METHODS

This study was carried out from January to March 2022 in the experimental animal laboratory (LPHC), Faculty of Medicine, Brawijaya University, Malang, Indonesia. The protocol was reviewed and approved by the Animal Care and Use Committee, Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia, with reference number 2.KE.120.10.2021. Minimal numbers of animals were used and all efforts were done to minimize suffering. This study was carried out to fulfill the requirements for a doctoral program.

Animals and study design

Male Wistar rats obtained from PT. Indoanilab (Bogor, Indonesia) undergoes one week of acclimatization. Later, they were randomly divided into two groups, namely control (no CUMS given) and CUMS (CUMS given). The CUMS group received psychological dominant CUMS protocols (cold swimming, foot shock, forced swimming, tail pierced, immobilization, no bedding, bright light, tail tied, isolation in a narrow dark space, predator exposure, wet bedding, and continuous light) by randomly giving 1 or 2 unexpected treatments daily for 21 days.[25] During the process, the same stressor was not given for two consecutive days.[26-28] A sucrose preference test was carried out before and after the use of CUMS to determine the successful creation of an animal model of depression. The CUMS procedure was carried out continuously in the treatment period for another 21 days.

All animals were group-housed with two rats per cage, and they were separated by a wire mesh. The conditions in the room included a temperature of 23 $\pm 2^0$C and humidity of 40 to 70% with 12 hours/12 hours light/dark cycle (lights on at 6:00 AM). The samples were fed ad libitum with food, water, and sucrose 1.5%. The food given was weighed every morning before feeding, and the weight of the leftover was obtained the next day. Water and sucrose solutions were freshly prepared daily in separate bottles.

The inclusion criteria included male rats aged fourteen weeks old, which were exhibiting depressive-like behavior in the treatment group, as assessed by SPT. Meanwhile, the exclusion criteria were physical illness and disability, which were

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assessed by a veterinarian. The animals in the treatment group were randomly assigned to four groups, namely CUMS (CUMS + water), fluoxetine (CUMS + fluoxetine), Mozart (CUMS + Mozart K. 448 + water), and fluoxetine + Mozart (CUMS + Mozart K. 448 + fluoxetine) group. The control was given standard care and water using gavage feeding. Fluoxetine was dissolved in water at 2 ml/kg and given in a dose of 10 mg/kg body weight through gavage feeding. Dose selection for fluoxetine was carried out based on a previous study.[29] Mozart K. 448 was administered at a dosage of 60 – 80 dB from 06.00 PM – 06.00 AM. Water was also given in a dose of 2 ml/kg body weight through gavage feeding. A separate room was provided for groups without exposure to music, and all treatment lasted for 21 days. The design of the study is summarized in Figure 1.

Outcome measure

Weekly body weight measurement, sucrose preference test, and forced swimming test were done and published elsewhere. All rats were euthanized after the treatment period. The hippocampus was retrieved to measure serotonin levels and BDNF levels using ELISA (E0866Ra and E0476Ra respectively). Hippocampal tissue was taken, rinsed in cold PBS (pH 7.4), and weighed before homogenization. The sample was homogenized in PBS (tissue mass (g): PBS volume (mL) = 1:9) using a glass homogenizer on ice, followed by sonication. Centrifugation was carried out for 15 minutes at 12,000 RPM (4°C), and the supernatant was collected to measure the hippocampal serotonin levels and BDNF levels based on the manufacturer's instructions.

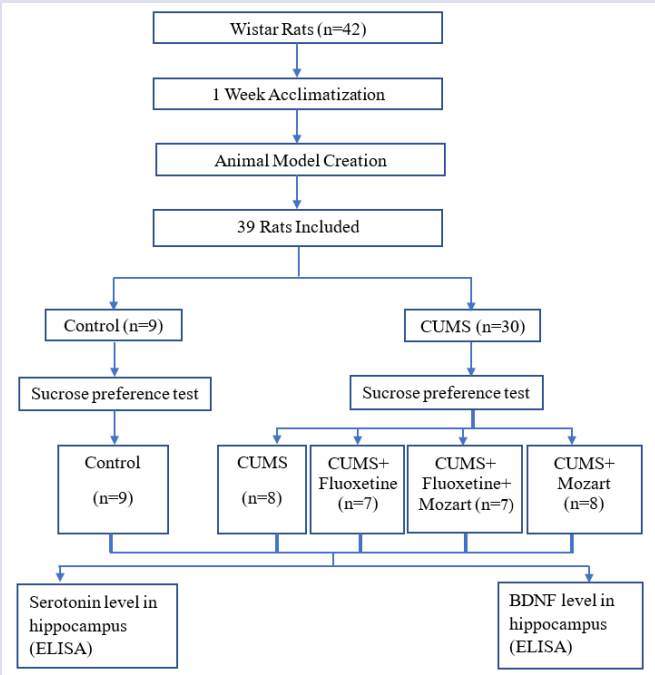

Figure 1: Study's design

Statistical analysis

Data were presented as mean ± SD, and the difference between groups was analyzed using ANOVA or Kruskal-Wallis test, depending on the homogeneity. Furthermore, differences between the CUMS and fluoxetine-Mozart groups were analyzed with a t-test or Mann-Whitney test, depending on whether the data distribution was normally distributed or not (Kolmogorov-Smirnov test). A p-value of <0.05 was considered statistically significant in this study. Data analysis was carried out using IBM SPSS Statistics software version 23.0 (IBM Corp., Armonk, NY, USA).

RESULTS

A total of 39 rats with body weights of 92-159 g were included in this study (33 and nine rats in CUMS and control groups, respectively). Serotonin levels and BDNF levels between groups showed a significant difference, as shown in Table 1. Based on the results, the fluoxetine + Mozart group had the lowest serotonin level and the highest BDNF levels compared to the fluoxetine group and the CUMS group. Both the Mozart group and the fluoxetine + Mozart group show no significant difference compared to the control group, as shown in Table 2.

DISCUSSION

Despite the high efficacy and relatively well-tolerated antidepressant drug,[30,31] some patients show a low response to the drug.[30] Additional treatment with other modalities needs to be considered to optimize the treatment's result.[31] Mozart K. 448 is one of the music that can be used as a complementary therapy to improve depressive management.[32-34] This study revealed that the combination of fluoxetine and Mozart can be used as an option for depression management, as it yields a similar response to control in terms of hippocampal BDNF levels (p>0.05).

The CUMS is a method commonly used to obtain a valid animal model of depression.[35] The modification in this study was done to mimic a human stressor that is psychologically dominant.[25] The CUMS protocol consists of chronic and unpredictable mild stressor exposure for 21

Table 1: Serotonin and BDNF between groups.

	ELISA level (ng/mL), mean ± SD					p value
	CUMS (n=8)	CUMS+F (n=7)	CUMS+F+M (n=7)	CUMS+M (n=8)	Control (n=9)	
Serotonin	50,242 ± 4,411	45,079 ± 4,432	44,533 ± 3,275	46,579 ± 4,047	50,672 ± 3,458	0.007**[a]
BDNF	1,757 ± 0.125	1,510 ± 0.229	1,694 ± 0.215	1,646 ± 0.090	1,759 ± 0.130	0.031*[a]

[a]ANOVA*significant, p value <0.05 **significant, p value <0.01.

Table 2: Significance comparison between groups.

	Comparison between groups									
	CUMS Vs control	CUMS+F Vs control	CUMS+F+M Vs control	CUMS +M Vs control	CUMS Vs CUMS+F	CUMS Vs CUMS +F+M	CUMS Vs CUMS +M	CUMS+F Vs CUMS+F+M	CUMS+F Vs CUMS+M	CUMS+F+M Vs CUMS+M
Serotonin	0.825[a]	0.013*[a]	0.003*[a]	0.040*[a]	0.042*[a]	0.015*[a]	0.105[a]	0.798[a]	0.505[a]	0.306[a]
BDNF	0.984[a]	0.030*[a]	0.471[a]	0.059[a]	0.031*[a]	0.493[a]	0.060[a]	0.146[a]	0.180[a]	0.569[a]

[a]T-test *Significant, p value <0.05 ** Significant, p value <0.01.

days. The sucrose preference test was used to evaluate the successful creation of an animal model of depression, as it represents anhedonia.[36] This study used psychologically dominant CUMS modification for 21 days to create an animal model of depression. The CUMS protocols were still given during the treatment periods.

Lower serotonin levels and BDNF levels were found in the depression compared to the normal control.[3,4] A decrease in brain serotonin levels alone is not sufficient to cause symptoms of depression.[37] A combination of serotonin pathway disruption and neurobiological vulnerability plays a role in the pathophysiology of depression.[38] Improvements in serotonin pathways contribute to depression improvements.[37] Lower BDNF levels that can be reversed in a well-tolerated treatment is found in an animal model of depression,[23] as well as in human.[39] This study found that the CUMS group indeed showed lower serotonin levels compared to the control group. While it did not achieve a significant difference statistically ($p>0.05$), it did show a trend that fits with the depression paradigm. This study also found that the CUMS group had lower serotonin levels than the control group, although the difference was not significant statistically ($p>0.05$). Marzban *et al*. found that Mozart K. 448 can indeed increase hippocampal BDNF levels.[11] Moraes *et al*. found that music exposure can increase serotonin.[10] Among the treatments group, the fluoxetine + Mozart group had the highest BDNF levels (1,6940.215), while the Mozart group had the highest serotonin levels (46,579±4,047) in this study

Serotonin and BDNF affect each other.[40] Decreased BDNF levels will affect serotonin sensitivity.[40] Subsequently, this will reduce resilience to the environmental stressor and cause phenotype alteration that will last for life.[37,40] Although fluoxetine increases serotonin levels, the response will not be optimal if the BDNF level is decreased.[40,41] This study found that the fluoxetine + Mozart group had the highest BDNF levels with the lowest serotonin levels, although it did not reach statistical significance.

This study provided additional data related to the impact of fluoxetine and Mozart K. 448 combined therapy on serotonin levels and BDNF levels. This study has several limitations. First, hippocampal serotonin levels and hippocampal BDNF levels measurement were done after the treatment was given only. Second, the number of cells as well as neuron complexity in the hippocampus was not evaluated. Third, the serotonin and BDNF receptors were not assessed in this study.

CONCLUSION

The group treated with the combined therapy of fluoxetine and Mozart for 21 days had higher BDNF levels, but lower serotonin levels compared to other groups. This study found that therapy in addition to standard therapy did not necessarily provide a better outcome in all aspects. The addition of Mozart to fluoxetine treatment resulted in higher BDNF levels compared to either Mozart or fluoxetine alone.

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CONFLICTS OF INTEREST

There is no competing interest.

AUTHOR'S CONTRIBUTION

LP, II, and MMM: designed the research, methodology, validation, formal analysis and review, and editing. LP: data collection, and writing original draft preparation. All authors have read, reviewed, and approved the final manuscript.

RECOMMENDATIONS

The addition of Mozart to fluoxetine treatment can be used as it resulted in higher BDNF levels compared to either Mozart or fluoxetine alone. Further research needs to be done to unveil the pathways of depression improvement in Mozart and fluoxetine combined therapy.

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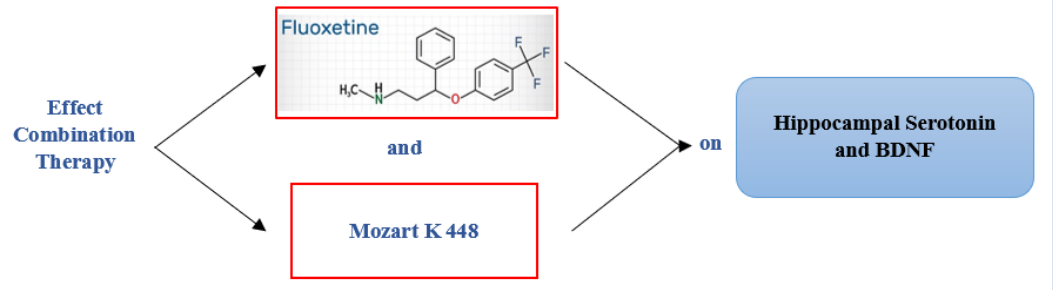
GRAPHICAL ABSTRACT



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