



Neuronal Cell Death Induces Depressive Disorder in Rats Depression-Like Behaviors Caused by Chronic Stress

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ABSTRACT

Introduction: Depression is believed to be a disorder in which an increase in serotonin activity in the brain. This has implications for the development of various antidepressant drugs that work to increase serotonin levels, by inhibiting serotonin reuptake. However, management with antidepressants is still believed to be not optimal, there are still various problems that have not been able to be solved only by increasing serotonin levels in the brain. Therefore, it is necessary to do further exploration to find out other possible pathophysiology of depressive disorders. This study intended to explore the role of apoptosis of neuronal cells in the prefrontal cortex to answer the hypothesis that depression was not only caused by increased serotonin levels but also there was a role of dead neuronal cells in the prefrontal cortex which will trigger the body's homeostatic efforts to compensate by increasing serotonin levels. **Methods:** A total of 30 male Wistar rats (200 ± 20 g) were obtained from Eureka Research Laboratory (Palembang, Indonesia). Experimental animals were placed in cages under controlled conditions (12 hours of light / dark cycles with temperatures of 22 ± 1 °C and humidity of 40-60%), fed and drank ad libitum. Experimental animals with depression model were induced using Chronic Mild Stress (CMS). CMS procedures were performed with mild stressors such as repeated cold stress (4 °C), space reduction in the homecage, changed cages and social interaction with other animals of the CMS group. To assess whether animal were being depression or not, the animal were tested using Forced Swimming Test (FST). After induction, rats were randomly divided into two groups which each contained 15 animals: the normal control group (not induced CMS) and the CMS group (negative control). Furthermore, the animal model was performed perfusion to maintain organ when evacuation was done, cell damage did not occur. To evaluate cell organ, immunohistochemistry examination and ELISA examination was performed. All data are presented as mean ± standard deviation and all statistical analyzes are performed with the SPSS 25 (IBM) program. **Result:** This research showed that CMS animal model has a greater duration of immobility than the normal group and serotonin level in CMS animal models decreased almost threefold compared to the normal group. In addition, there were increased expression of caspase-3 indicates that more neuronal cells suffered from apoptosis. So, in this research, it was clearly stated that in depressive disorder, there were elevation of neuronal cell apoptosis in the prefrontal cortex. **Conclusion:** Neuronal cell apoptosis in the prefrontal cortex plays a role in the pathophysiology of depression through activation of negative feedback on serotonin production.

1. Introduction

Depression is a clinical condition characterized by a decrease in mood, in the form of feelings of sadness and decreased activity. This disorder is sometimes underestimated by the public, even by doctors. People

are more afraid if they experience cardiovascular disorders than if they experience depressive disorders. This causes a decrease in public awareness and the academic community related to this disorder. Theories

related to the pathophysiology of depression still need to be explored further, as the pathophysiology of depression is not as clear as other medical disorders such as cardiovascular disorders.

It is believed that depression is caused by neurotransmitter dysfunction in the brain, especially the neurotransmitter serotonin. Neurotransmitters are being the most guilty parties to depression. Depression is believed to be a disorder in which an increase in serotonin activity in the brain. This has implications for the development of various antidepressant drugs that work to increase serotonin levels, by inhibiting serotonin reuptake.¹⁻³ However, management with antidepressants is still believed to be not optimal, there are still various problems that have not been able to be solved only by increasing serotonin levels in the brain. Therefore, it is necessary to do further exploration to find out other possible pathophysiology of depressive disorders.

Serotonin is produced by raphe nucleus in the brain stem, whose serotonin produced in will act in neuron cells located in prefrontal cortex. If something happens to the neuron cells in the prefrontal cortex, for example the occurrence of apoptosis, it will cause as much as serotonin levels to be increased, it will not be able to provide an optimal effect. The prefrontal cortex is the center of emotional regulation, decision making, social cognition and psychopathology.⁴

This study was aimed to explore the role of neuronal cells in the prefrontal cortex in depressed conditions. This study intended to explore the role of apoptosis of neuronal cells in the prefrontal cortex to answer the hypothesis that depression was not only caused by increased serotonin levels but also there was a role of dead neuronal cells in the prefrontal

cortex which will trigger the body's homeostatic efforts to compensate by increasing serotonin levels.

2. Methods

Research Subject

A total of 30 male Wistar rats (200 ± 20 g) were obtained from Eureka Research Laboratory (Palembang, Indonesia). Experimental animals were placed in cages under controlled conditions (12 hours of light / dark cycles with temperatures of $22 \pm 1^\circ\text{C}$ and humidity of 40-60%), fed and drank ad libitum. All animal treatments and experimental procedures were approved by the Ethics and Humanities Commission of the Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia (No.267 / kptfkunsri-rsmh / 2019).

Animal Model Depression

Experimental animals with depression model were induced using Chronic Mild Stress (CMS). CMS is a form of stress induction performed on experimental animals in a chronic manner for 4 weeks. CMS procedures were performed with mild stressors such as repeated cold stress (4°C), space reduction in the homecage, changed cages within the CMS group, cage tilt, empty cage, intermittent air puff, wet bedding, white noise, overnight illumination and social interaction with other animals of the CMS group. The particular context (stressor applied during preceding dark phase) of the restriction water intervals involved overnight illumination (Sunday-Monday), wet cage and cage tilt (Tuesday-Wednesday and Thursday-Friday) and changed cages (Wednesday- Thursday), respectively.⁵⁻⁶ As the following schedule:

Table 1. CMS Procedure Schedule Light Phase Dark Phase

	First half	Second half	First 2 hours	Remaining 10 hrs
Monday	Cold Stress (2x30 min)	Cold stress (30 min)	Restriction water	Space reduction
Tuesday	Changed room	Air Puff (3x3 intermitent)	Wet Cage	Wet cage
Wednesday	Wet Cage	Social Interaction	Foreign cage	Foreign cage
Thursday	Foreign Cage	Social Interaction	Restriction water	Cage Tilt
Friday	Empty Cage	Changed room	White noise	White Noise
Saturday	White Noise	Changed room	Changed room	Changed room
Sunday	Changed room	Changed room	Overnight illumination with 700 lx	Overnight illumination with 700 lx

Clinical Test: Forced Swimming Test (FST)

Forced Swimming Test (FST) is a test conducted to assess mobility in animal model. First the FST vehicle was prepared, in the form of a cylindrical tube with a height of 60 cm and a diameter of 30 cm, then filled with water as high as 40 cm. Water was replaced every test per rats. Tests were conducted between 9:00 and 15:00. Each animal was acclimatized for 10 minutes first, then the mobility duration was measured in 5 minutes. Then, the mobility of rats was measured in seconds.⁷

Treatment of Animal Models

After induction for 4 weeks, rats were randomly divided into two groups which each contained 15 animals: the normal control group (not induced CMS) and the CMS group (negative control). Furthermore, the animal model was performed perfusion to maintain organ when evacuation was done, cell damage did not occur. Before perfusion was performed, the animal was anesthetized with ketamine (5 mg / kgBW) and xylazine (0.2 mg / kgBW) intraperitoneally. Perfusion was done by inserting perfusor fluid, Paraformaldehyde 4%, through the left ventricle of the heart, followed by a tear in the right atrium of the heart so that blood will come out of the right atrium replaced by perfusor fluid. 8-9 Perfusion continued until the fluid coming out of the right atrium becomes clear. Evacuation of the animal's brain was done by

performing a craniotomy, through sulcus coronarius from calvaria, so that the animal's brain organ will be obtained and a coronal division is performed to obtain the prefrontal cortex. Prefrontal cortex was then added to the fixation fluid, Neutral Buffer Formalin 10% (NBF).

Immunohistochemistry (IHC) Examination

Prefrontal cortex which has been immersed to the fixation fluid, was dehydrated using alcohol and xylene, then paraffinized and then cut as thick as 5 um using a rotary microtome (Leica). Later, tissue was placed on coated object glass. Then, the tissue was rehydrated using xylene and alcohol with a concentration of 96%, 90%, 80% and 70% and rinsed with tap water. The next stage, retrieval antigen was carried out with the HIER (Heat Induced Epitop Retrieval) method, where the slides were put into a citrate buffer solution, then heated at a temperature of 95oC for 60 minutes. Then, 1: 700 (Cloud Clone) Caspase-3 antibody was painted, followed by overnight incubation at 4oC. The next stage is to paint with a secondary antibody, Biotinylated- HRP (Horseradish Peroxidase), incubation for 1 hour, at room temperature. Next, giving chromogen. Next, dehydration process was carried out again using concentration and xylene alcohol. 10-13 The next step was mounting and evaluating Caspase-3 expressions

using Imagej Software, so that the percentage of Caspase-3 expressions will be obtained.

ELISA (Enzyme-Linked Immunosorbant Assay) Examination

Serotonin levels in the prefrontal cortex were examined by Rat ELISA Serotonin ELISA kit (Cloud Clone), based on the protocol found in the manufacturer's protocols. Briefly, 50 µl standard diluent or serum samples were added to wells that have been coated with anti-serotonin and incubated at 37 ° C for 30 minutes. After the plates were washed, 100 µl of a biotinylated antibody solution was added and incubated for 30 minutes at 37 ° C. After washing three times, 50 ul avidin-peroxidase complex solution was added and incubated for 15 minutes at 37 ° C. After washing, 50 µl of the tetramethylbenzidine color solution was added and incubated in the dark for 15 minutes at 37 ° C. Finally, 50 ul stop solutions were

added to stop the reaction and optical density (OD) values were measured using an ELISA reader (Biorad), wavelength 450 nm.¹⁴⁻¹⁵

Data Analysis

All data are presented as mean ± standard deviation and all statistical analyzes are performed with the SPSS 25 (IBM) program. One way ANOVA followed by post hoc analysis was conducted to assess differences in the mean expression and levels of each protein as well as clinical data. P <0.05 was determined as an indication that there were significant differences in the mean levels.

3. Results

Duration of immobility shows a clinical picture of depression in CMS animal models. Figure 1 shows that the CMS animal model showed a greater duration of immobility than the normal group.

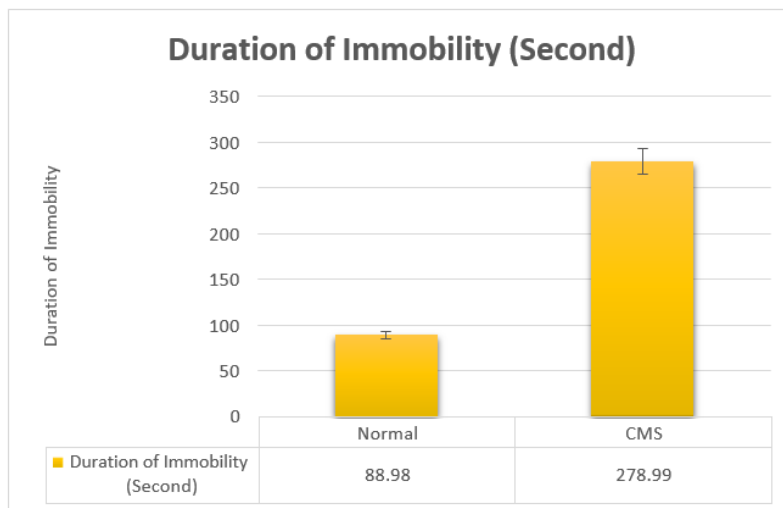


Figure 1. Duration of immobility in rats for forced swimming test; * VS Normal, p<0,05

Serotonin is the main neurotransmitter that is believed to play a role in the initiation of depressive disorders. Table 1 shows that the level of serotonin in

CMS animal models decreased almost threefold compared to the normal group.

Table 1. Serotonin Level in Cortex Prefrontal Rats

No.	Group	Serotonin Level(pg/mL) ± SD	P Value*
1.	Normal	178.86 ± 15,76	-
2.	CMS	54,54 ± 3,12	0,00

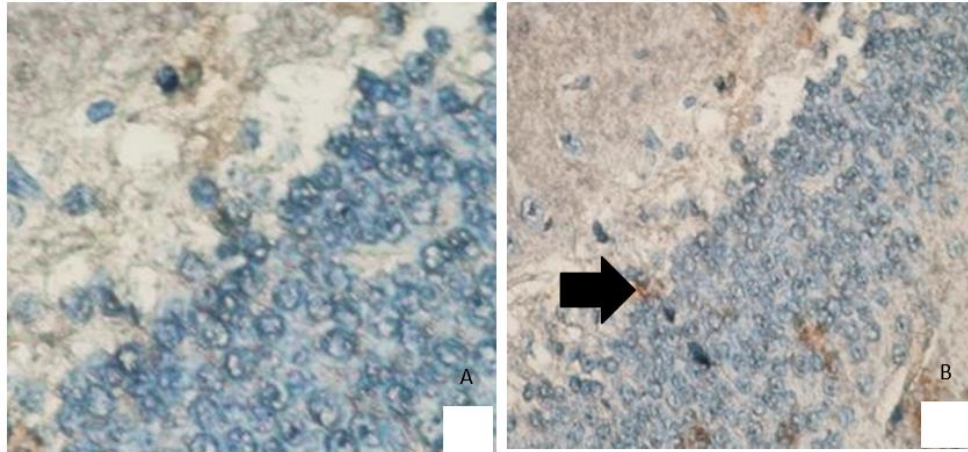


Figure 2. Caspase 3 Expression in Cortex Prefrontal Rats. Black Arrows : Caspase-3 Expression. Magnificant 400x. A: Normal, B: CMS

Caspase-3 is the main protein that plays a role in the process of apoptosis or programmed cell death. Increased expression of caspase-3 indicates that more neuronal cells suffered from apoptosis. As can be seen

in Table 2 which shows that depression increases neuronal cell apoptosis in the prefrontal cortex.

Table 2. Caspase 3 Expression in Cortex Prefrontal Rats

No.	Group	Caspase 3 Expression(%) ± SD	P Value*
1.	Normal	2.86 ± 0,76	-
2.	CMS	53,87 ± 3,62	0,00

4. Discussion

Increased expression of the caspase-3 protein in neuronal cells in the prefrontal cortex indicates an increase in apoptotic activity in neuronal cells. The results of this study indicate that there are other pathophysiological mechanisms that play a role in depressive conditions, namely the occurrence of neuronal cell apoptosis in the prefrontal cortex. Prefrontal cortex is part of brain that plays a role in the regulation of emotions, decision making, social cognition and psychopathology.

Stress conditions will trigger Hypothalamic-Pytuitary-Adrenal Glands (HPA) axis activation. Activation of HPA Axis will evoke cortisol production from supraadrenal gland. Increased cortisol levels further trigger the activation of glutamate neurotransmitter in the brain, which will make the individual alert and alert. A continuous increase of the

glutamate neurotransmitter will cause glutamate intoxication. Furthermore, this will lead to cell stress, which will trigger the activation of apoptosis from neuronal cells (prefrontal neuronal cortex cells).¹¹

Apoptosis in prefrontal neuronal cortex cells will cause cell population decline, and definitely it will be followed by a decrease in receptors, especially serotonin receptors.^{1,2} The decrease in these receptors send a negative feedback mechanism related to serotonin production in the raphe nucleus. This will cause a decrease in serotonin production.

5. Conclusion

Neuronal cell apoptosis in the prefrontal cortex plays a role in the pathophysiology of depression through activation of negative feedback on serotonin production.

6. References

1. Wulandari, Patricia. Biomolecular Aspects of Schizophrenia. *Bioscientia Medicina : Journal of Biomedicine and Translational Research*, [S.l.], v. 3, n. 2, p. 38-43, may 2019. ISSN 2598-0580, doi: <https://doi.org/10.32539/bsm.v3i2.88>.
2. R. Hidayat, U. Sriwijaya, M. I. Saleh, U. Sriwijaya, N. Parisa, and U. Sriwijaya, "Effect of supplementation kayu manis (*Cinnamomum burmannii*) extract in neuronal cell death protection in wistar rats lir-psychotic on haloperidol therapy In Neuronal Cell Death Protection In Wistar Rats Lir-Psychotic On," no. February, pp. 14–17, 2018.
3. G. Amodeo, M. A. Trusso, and A. Fagiolini, "Depression and Inflammation : Disentangling a Clear Yet Complex and Multifaceted Link Review," vol. 7, pp. 448–457, 2017.
4. J. P. Kongsman, "Inflammation and Depression : A Nervous Plea for Psychiatry to Not Become Immune to Interpretation," 2019.
5. O. Köhler, J. Krogh, O. Mors, and M. Eriksen, "Inflammation in Depression and the Potential for Anti-Inflammatory Treatment," 2016.
6. M. C. Schweizer, M. S. H. Henniger, and I. Sillaber, "Chronic Mild Stress (CMS) in Mice : Of Anhedonia , ' Anomalous Anxiolysis ' and Activity," vol. 4, no. 1, 2009.
7. C. Yang, Y. Hu, Z. Zhou, G. Zhang, and J. Yang, "Acute administration of ketamine in rats increases hippocampal BDNF and mTOR levels during forced swimming test," no. August 2012, pp. 3–8, 2013.
8. H. M. Abelaira and G. Z. Re, "Animal models as tools to study the pathophysiology of depression," pp. 112–120, 2017.
9. Y. Zhao, R. Ma, J. Shen, H. Su, D. Xing, and L. Du, "A mouse model of depression induced by repeated corticosterone injections," no. March 2008, 2017.
10. M. Stepanichev, N. N. Dygalo, G. Grigoryan, G. T. Shishkina, and N. Gulyaeva, "Rodent Models of Depression: Neurotrophic and Neuroinflammatory Biomarkers," vol. 2017, 2017.
11. V. Zeldetz et al., "A New Method for Inducing a Depression-Like Behavior in Rats A New Method for Inducing a Depression-Like Behavior in Rats," no. February, 2018.
12. A. H. Miller and C. L. Raison, "imperative to modern treatment target," vol. 16, no. 1, pp. 22–34, 2017.
13. K. Belovicova, E. Bogi, K. Csatlosova, and M. Dubovicky, "Animal tests for anxiety-like and depression-like behavior in rats," vol. 10, no. 1, pp. 40–43, 2017.
14. M. Berk et al., "So depression is an inflammatory disease , but where does the inflammation come from?," *BMC Med.*, vol. 11, no. 1, p. 1, 2013.
15. A. V Kalueff and P. Tuohimaa, "Experimental modeling of anxiety and depression," pp. 439–448, 2014.