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The Mediation Effects of Interleukin-6 and Cortisol in Hippocampus In Relationship beween Interleukin-6 and Cortisol in Plasma to Effect of Psychological Stressor on Serotonin Transporter (SERT) Distribution and Apoptotic Index of Hippocampus

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Abstract : The aim of this research is to investigate the Mediation Effects of Interleukin-6 and Cortisol in Hippocampus In Relationship beween Interleukin-6 and Cortisol in Plasma to Effect of Psychological Stressor on Serotonin Transporter (SERT) Distribution and Apoptotic Index of Hippocampus. This study uses data Suparno, using a sample of 24 female rats Rattus Wistar novergicus 10-12 weeks of age 150-185 gram weight, which is exposed to predators in the form of two cats who were selected empirically the level of aggressiveness of the rat before and after treatment, then taken blood plasma as well as neuronal networks (pyramidal neurons) hippocampal CA3 area, with a thick slice preparations 2-4 lm. The variables of this research are: independent variables (Interleukin-6 or IL-6 and Cortisol in Plasma), mediation variables (Interleukin-6 and Cortisol in Hippocampus), and dependent variables (SERT and Apoptotic in Hippocampus). The result shows that (1) there is mediation Effects of Interleukin-6 in Hippocampus In Relationship beween Interleukin-6 and Cortisol in Plasma to Effect of Psychological Stressor on Serotonin Transporter (SERT) Distribution and Apoptotic Index of Hippocampus, (2) there is mediation Effects of Cortisol in Hippocampus In Relationship beween Interleukin-6 and Cortisol in Plasma to Effect of Psychological Stressor on Serotonin Transporter (SERT) Distribution and Apoptotic Index of Hippocampus.
Key words: Interleukin-6, cortisol, SERT, Apoptotic Index, Mediation Effect.

Introduction

In the sphere of life in urban areas, it is impossible to avoid exposure to various stressors, including psychological stressors. The impact of exposure to psychological stressors can be the eustres conditions (standard mild / moderate) and distress (weight class). Eustres conditions can still trigger effects that are positive, that motivate individuals even harder to achieve the target of his life, while the condition of distress causing physical and psychological suffering.

On the condition of distress occurs functional changes such as hormonal changes and neurotransmission, including increased activity of noradrenergic and cortisol levels, if chronically will lead to structural changes, such as atrophy of pyramidal cells and decrease the volume of the hippocampus, as well as increasing the activity of the axis of the hypothalamo-pituitariadrenal (HPA axis) that lead to changes in levels of interleukin-6 (IL-6). IL-6 has a positive correlation with the neurotransmitter norepinephrine[1,2,3]

Distress conditions associated with blood cortisol concentrations and the concentration of IL-6 plasma or distribution in the central nervous system. Exposure to psychological stressors (exposure to predators) stimulates the hypothalamus, pituitary and adrenal glands, which form the HPA axis and related to the effects of stress, such as increased levels of cortisol and catecholamines are closely linked to levels of serotonin and dopamine in the brain. Increased cortisol levels were positively correlated with increased catecholamine levels, namely adrenaline, norepinephrine, and dopamine[4,5,6].

Interleukin-6 (IL-6) which is one of the proinflammatory cytokines (key immune mediators), and its receptor, are located in different areas of the brain including the hypothalamus and the hippocampus, which is centrally involved in mediating emotions and behavior. The cytokines IL-6 participates also in the immune system and the neuroendocrine networks. IL-6 plays a role similar to the role it endokrinik autokrinik and parakriniknya, is one of the main cytokines that stimulate the HPA axis during "stress inflammation"[6]. IL-6 is a potential stimulator of the CRH (hypothalamus) with the impact of the activation of the HPA axis and the release of glucocorticoids[7]. Serotonergic malfunctions most commonly associated with mental disorders, making it useful as a marker for mental disorders[8,9].

Composition multimalfungsi central neurotransmission, can be estimated from the large degree of malfunctions serotonergic neurotransmission, which reflected transporterserotonin (SERT) platelets. SERT affinity and density measurement is more accurate than the measurements of the levels of serotonin blood or urine, because it describes the real conditions of serotonergic neurotransmission at synapses slit[10].

Serotonin transporter (SERT) is a high-affinity transporter protein located in the plasma membrane of the pre-synaptic nerve endings[11]. SERT mengkatalisir movement of serotonin (5HT) pass through cell membranes. In the brain, serotonin SERT cleaning of extra cellular space, modulate the strength and duration of serotonergic signaling[12].

The location of neurotransmitter receptors and transporters are in the cell membrane, so that the structural changes in the hippocampus will have an impact on changes in the receptor and transporter changes (affinity and density) of neurotransmitters. These conditions will result in a multi malfunctions of some of the processes of neurotransmission in the brain (central) and peripherally with different compositions individually. Multi malfunctions of neurotransmission processes in the brain (central) is allegedly closely associated with mental disorders type of distress. Composition of multi malfunctions neurotransmission central varying individually, can be detected or predicted from the function of SERT hippocampus (represented density and affinity SERT hippocampus, and reflected also in the transporter-serotonin that are in platelets), as well as changes in the structure of the hippocampus, which include volume reduction and atrophy of hippocampal CA3 pyramidal neurons of the hippocampus area[13].

From various studies above, mental disorders are estimated to have changes in the type of distress function and structure of the hippocampus, and also related to the concentration of interleukin - 6 plasma and blood cortisol levels as well as an affinity or SERT density in platelets and the hippocampus (the brain). So far it has not been done exhaustive research on the relationship between changes in the concentration of Interleukin-6 and plasma cortisol concentrations with SERT distribution along with the apoptotic index hippocampus in the brain in the context of mental disorders type of distress. Therefore, this study wanted to investigate the Mediation Effects of Interleukin-6 and Cortisol in Hippocampus In Relationship beween Interleukin-6 and Cortisol in Plasma to Effect of Psychological Stressor on Serotonin Transporter (SERT) Distribution and Apoptotic Index of Hippocampus.

Theoretical Review: Mediation Effect

Research in the field of psychiatry as described above, often investigates a system. Where, the system is a relationship among variables, and is called model. They are use structural equation modeling (SEM) to analyze a model. The model is usually has many variables and relationships and a mediating variable is often in the model. A variable may be called a mediator "to the extent that it accounts for the relation between the predictor and the criterion"[14]. Mediation is the name given to models in which the effect of an antecedent or independent variable (X) on the dependent variable (Y) is transmitted through a third intervening or mediating variables (M). In other words, X affects M, which in turn affects Y[15]. A researcher can determine if mediation exist in several ways, i.e. investigate the mediating variable use a structural equation modeling (SEM) analysis. They calculated this analysis use AMOS software. Figure 1 shows a diagram of a single mediator model.

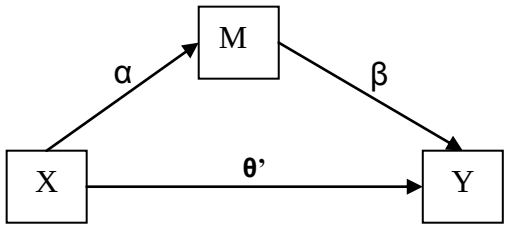

Figure 1. Illustration of a mediation design, X affects Y indirectly through M.

Variable M considered a mediator if (1) X significantly affects M ($\boldsymbol{\alpha} \neq 0$) and M significantly affects Y controlling for X ($\boldsymbol{\beta} \neq 0$). This is called a conventional method.

Investigation of the nature and functioning of mediation variables must proceed along two distinct dimension: (a) the ontological content of the variable and (b) the grounds upon which the scientist is able to justify his introduction of M into the estimation of Y from X[16]. The formal heuristic analysis often used to detect simple mediation effects is straightforward and follows directly from the definition of a mediator provide by Baron and Kenny. Variable M is considered a mediator if (1) X significantly predicts Y (i.e., $\boldsymbol{\theta} \neq 0$ in figure 2), (2) X significantly predicts M (i.e., $\boldsymbol{\alpha} \neq 0$ in figure 1), and (3) M significantly predicts Y controlling for X (i.e., $\boldsymbol{\beta} \neq 0$ in figure 1).

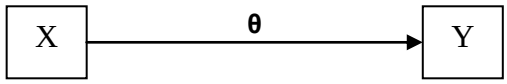

Figure 2. Illustration of direct effect, X affects Y

Baron and Kenny discuss several analyses that should be performed and the results assessed with respect to the criteria just described. These criteria are assessed by estimating the following equations:

$Y = \delta_1 + \boldsymbol{\theta} X$
$M = \delta_2 + \boldsymbol{\alpha} X$
$Y = \delta_3 + \boldsymbol{\theta}'X + \boldsymbol{\beta} M$

where δ_i is an intercept coefficient. When the effect of X on Y decrease to zero with the inclusion of M, perfect mediation (complete mediation) is said to have occurred. When the effect of X on Y decreases by a nontrivial amount, but not to zero, partial mediation is said to have occurred[14,17].

Test of the intervening variable effect are useful because they examine processes by which variables are related. There are three types test of significance of intervening variables effect, i.e.: causal steps, differences in coefficients, and product of coefficients. Causal steps is consist Judd and Kenny, Baron and Kenny, and Joint Significant of **α** and **β** method**.** Differences in coefficients is consist Freedman and Schatzkin, McGuigan and Langholtz, Clogg *et al.*, and Olkin and Finn method. Product of coefficients consist Sobel first-order solution, Aroian second-order exact solution, and Goodman unbiased solution, MacKinnon *et al.*, and Babko and Riek method[18].

Materials and Methods

This study uses data Suparno[19], using a sample of 24 female rats Rattus Wistar novergicus 10-12 weeks of age 150-185 gram weight, which is exposed to predators in the form of two cats who were selected empirically the level of aggressiveness of the rat before and after treatment, then taken blood plasma as well as neuronal networks (pyramidal neurons) hippocampal CA3 area, with a thick slice preparations 2-4 lm.

The variables of this research are: independent variables (Interleukin-6 or IL-6 and Cortisol in Plasma), mediation variables (Interleukin-6 and Cortisol in Hippocampus), and dependent variables (SERT and Apoptotic in Hippocampus). The measurement of each variables are follow: (1) Measurement of concentrations of IL - 6 rat plasma by enzyme immunoassay using mouse Quantikine IL - 6 immunoassay (R & D). Read on

microplate reader (BioRad-505) at 450 / 490nm, (2) Measurement of plasma cortisol concentrations rats by enzyme immunoassay using Cortisol ELISA. Normal cortisol levels afternoons (pk 16.00) 30-150 ng / ml (83-414 nmol / l). Calculate the results: the standard concentration semilogarithmic graph paper (abscissa, logarithmic) are plotted against the optical levels listed (ordinate, linear), (3) Distribution measurement of cortisol, IL - 6, and SERT as well as the index of apoptosis of hippocampus by measuring mouse brain trying to do a series of cuts in the area CA3 hippocampus, then staining with immunohistochemical detection kit (Dako), and successively performed: Making paraffin blocks network, process deparafinisasi, the coloring process hematoxilin-eosin (mayer), labeling of DNA fragmentation (examination of apoptosis by the method of tunnels), staining methods immunositokimia cortisol, IL6, and SERT, the counting process IL - 6, cortisol and SERT as well as apoptosis modeling distress with exposure to predators.

A researcher can determine the mediating variable, and whether it is complete or partial, if mediation exists in several ways. Hair[20] rule is used in this research. If path labeled **θ**' is expected to be zero due to be mediation (representing complete mediation), a SEM model can represent mediation by including only the path **α** and **β** in the model. But, if **θ**' is does not to be zero, we require to investigate the mediating variable. A series steps can be followed to evaluate mediation, these steps apply using SEM or any other general linear model approach.

1. Establish that the necessary individual relationships have statistically significant relationships:

1. X is related to Y: Here we are establishing that the direct relationship does exist.
2. X is related to M: Here we establish that the mediator is related the "input" construct
3. M is related to Y: Here we establish that the mediator does have relationship with the outcome construct.

2. Estimate an initial model with only the direct effect (**θ**) between X and Y. Then estimate second model adding in the mediating variable (M) and the two path estimates (**α** and **β).** Then asses the extent of mediation as follows:

1. If the relationship between X and Y (**θ**') remain significant and unchanged once M is include in the model as an additional predictor (X and M now predict Y), then mediation not supported.
2. If **θ**' is reduced but remains significant when M is include as an additional predictor, then partial mediation is supported
3. If **θ**' is reduced to a point where it is not statistically significantly after M is included as a mediating construct, full (complete) mediation is supported [1].

SEM was used for data analysis and Hair[20]. rule was used to investigate. AMOS software is used for data analysis[21].

Result and Discussion

Figure 1 and 2 reports the result of investigation the mediating variable. The table shows the Sobel Test method result that all of model is a mediating variable.

The result shows that there is mediation Effects of Interleukin-6 in Hippocampus In Relationship beween Interleukin-6 and Cortisol in Plasma to Effect of Psychological Stressor on Serotonin Transporter (SERT) Distribution and Apoptotic Index of Hippocampus ($P < 0.05$). The positive mediation effect ($B>0$) show that (1) the higher the value of Interleukin-6 in Plasma, will lead to higher value of Psychological Stressor on Serotonin Transporter (SERT) Distribution and Apoptotic Index of Hippocampus, (2) the higher the value of Cortisol in Plasma, will lead to higher value of Psychological Stressor on Serotonin Transporter (SERT) Distribution and Apoptotic Index of Hippocampus.

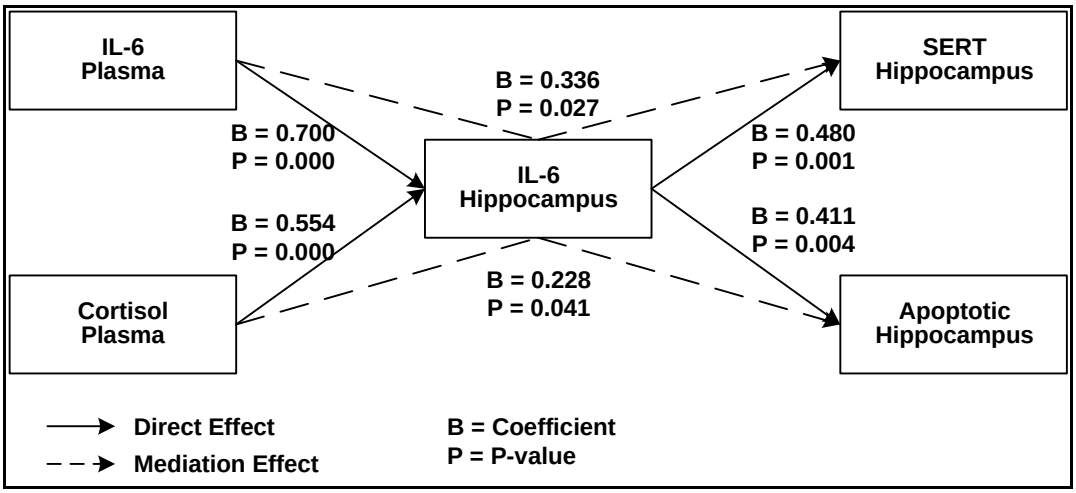

Figure 1. Mediation Effects of Interleukin-6 in Hippocampus

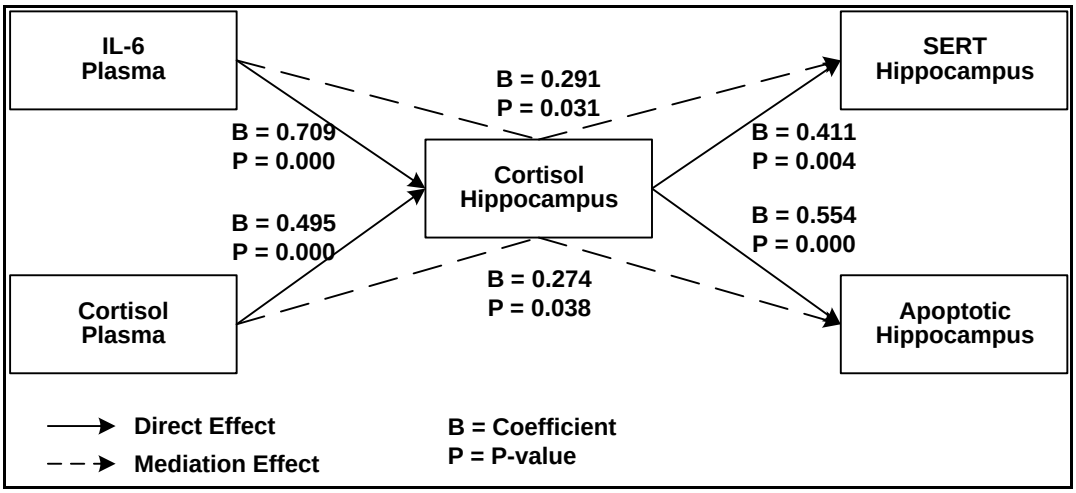

Figure 2. Mediation Effects of Cortisol in Hippocampus

The result shows that there is mediation Effects of Cortisol in Hippocampus In Relationship beween Interleukin-6 and Cortisol in Plasma to Effect of Psychological Stressor on Serotonin Transporter (SERT) Distribution and Apoptotic Index of Hippocampus ($P < 0.05$). The positive mediation effect ($B>0$) show that (1) the higher the value of Interleukin-6 in Plasma, will lead to higher value of Psychological Stressor on Serotonin Transporter (SERT) Distribution and Apoptotic Index of Hippocampus, (2) the higher the value of Cortisol in Plasma, will lead to higher value of Psychological Stressor on Serotonin Transporter (SERT) Distribution and Apoptotic Index of Hippocampus.

The findings indicate that the distribution Increased IL - 6 and cortisol hippocampus, also shown to increase the distribution of SERT and the apoptotic index hippocampus; mengningat strong correlation and positive between the distribution of IL-6 hippocampus with the distribution of SERT hippocampus, between the distribution of IL-6 hippocampus with apoptotic index hippocampus, between the distribution of cortisol hippocampus with the distribution of SERT hippocampus, between cortisol distribution hippocampus hippocampus with apoptosis index. Increased distribution of IL-6 hippocampus hippocampus increases SERT distribution (87.73%) and hippocampus apoptosis index (67.49%). Increased cortisol distribution hippocampus hippocampus increases SERT distribution of 85.68% and 114.00% apoptotic index hippocampus. These three findings to prove that, cortisol can directly lead to changes in the structure of the hippocampus. The findings of the third study are consistent with previous studies that prove that prolonged stress in experimental animals (rats) damage in the hippocampus which is plastic with a marker of biological form of reduction of pyramidal neurons in area CA3 hippocampus, and this effect is mediated by glucocorticoids increases the flow of calcium ions in in the hippocampus. IL-6 may affect neurogenesis through a number of mechanisms which different

which is a variation of the physiological condition. IL-6 is also centrally acting stimulate the HPA axis, then increase the circulation of glucocorticoids. Previously, the researchers find any evidence of the existence of multidireksional communication between various immune system, autonomic, hormonal and central nervous system.

Research findings show that the role of IL - 6 as a mediator of stress is more dominant compared to cortisol, considering IL - 6 plasma can improve the distribution of IL - 6 and cortisol hippocampus higher (63.79% and 77.86%) compared to plasma cortisol (38.19% and 41.98%). Recently it was believed that, the holder of the central role of a response to stress is cortisol[4], given previous studies proved that prolonged stress in experimental animals (mice) will cause damage in the hippocampus which is plastic with a marker of biological form of reduction of pyramidal neurons in area CA3 hippocampal , and this effect is mediated by glucocorticoids that can improve the flow of calcium ions in the hippocampus which has a key role in the destruction of CA3 pyramidal neurons in the hippocampus area.

Research findings that cortisol (through mediation in the hippocampus) can lead to structural changes in the hippocampus. These findings are consistent with prior research that proves that prolonged stress in experimental animals (mice) will cause damage in the hippocampus which is plastic with a marker of biological form of reduction of pyramidal neurons in area CA3 hippocampus, and this effect is mediated by glucocorticoids that can improve the flow of calcium ions in the hippocampus which has a key role in the destruction area CA3 pyramidal neurons in the hippocampus. IL-6 may affect neurogenesis through a number of different mechanisms that are variations of physiological conditions. IL-6 may also works centrally to stimulate the HPA axis, then increase the circulation of glucocorticoids. In some studies the experts to get evidence multidireksional communication between various immune system, autonomic, hormonal and central nervous system[7].

Weaknesses were observed and found in this study include (1) Procedure laboratory in this research is the method of semiquantitative, while the molecular diagnosis (diagnosis genotypic) requires quantitative methods (measurement transporter using means radioligan), (2) Not researching the "transporter" of the system neurotransmission others (GABA, dopamine, noradrenaline).

Conclusion and Reccomendation

Based on the analysis above, the conclusion of this research are folow: (1) there is mediation Effects of Interleukin-6 in Hippocampus In Relationship beween Interleukin-6 and Cortisol in Plasma to Effect of Psychological Stressor on Serotonin Transporter (SERT) Distribution and Apoptotic Index of Hippocampus, (2) there is mediation Effects of Cortisol in Hippocampus In Relationship beween Interleukin-6 and Cortisol in Plasma to Effect of Psychological Stressor on Serotonin Transporter (SERT) Distribution and Apoptotic Index of Hippocampus. The conventional method to investigate the mediating variable is simple and easy but not sufficient. Investigation by Baron and Kenny methods or Hair[20] rule to determine the mediating variable is still necessary. The rule to investigate the mediating variables is should completed, namely (1) if **θ** is not statistically significant, (2) **α** is significant, **β** is significant, and (3) **θ**' is significant and increase when M is include as an additional predictor, then partial mediation is supported. In this research, can be shown to be that the serotonin transporter (SERT), IL - 6, cortisol and IL-1β can be used to establish the molecular diagnosis (diagnosis genotypic) from a psychiatric disorder, as well as to complete the consideration of treatment of chronic diseases that are resistant therapy (eg, chronic gastritis).

However, these benefits can still be further enhanced quality with advanced research; therefore it is recommended to do (1) Further studies examining the correlation between affinity and density transporter-serotonin platelet with changes in the function and structure of the hippocampus (malfunctioning SERT & Apoptosis region CA3 hippocampal; in vivo, quantitative in nature). (2) Study further on the role of glutamate, and other neurotransmission transporter, in the pathophysiology of mental disorders in experimental animals; expanded research into other brain areas such as the dorsal raphe, locus coeruleus and prefrontal areas (in vivo).

References

1. Bleich A, Brown SL, Kahn R, van Praag HM. The role of serotonin in schizophrenia. Schizophr Bull.1988; 14: 297-315

2. Charney DS, Nestler EJ. Neurobiology of Mental Illness. 2nd Oxford University Press, Inc. New York. 2004
3. Chrousos, G. P. (1998). Stressors, Stress, and Neuroendocrine Integration of the Adaptive Response: The 1997 Hans Selye Memorial Lecture. Annals NYAS Online.1998; 851: 311-335
4. Dubovsky SL, Thomas M. Serotonergic Mechanisms and Current and Future Psychiatric Practice.J Clin Psychiatry. 1995; 56 (suppl 2): 38-48
5. Figueiredo.HF, Bodie.,BL Tauchi. M, C. Mark Dolgas. Herman. JP. Stress Integration after Acute and Chronic Predator Stress: Differential Activation of Central Stress Circuitry and Sensitization of the Hypothalamo-Pituitary-Adrenocortical Axis. Endocrinology ; 2003;144 (12): 5249-5258
6. Fuchs E, Czeh B, Michaelis T, de Biurrun G, Watanabe T, Frahm J. Synaptic plasticity and tianeptine: structural regulation. Eur Psychiatry. 2002; 17 suppl 3: 311-317.
7. Ganong, WF. Review of Medical Physiology . 17 ed., Appleton & Lange, Norwalk, Connecticut. 1995; 251-261, 368-369. 8. Jie-Guang Chen and Gary Rudnick. Permeation and gating residues in serotonin transporter Proc Natl Acad Sci U S A. February 1. 2000; 97 (3): 1044–1049
8. McEwen, BS. Adrenal steroids, the hippocampus, and the amygdala: relationship to anxiety and depression . Medicographia. 1994; 16: 60-63
9. McKittrick CR, Magarinos AM, Blanchard DC, Blanchard RJ, McEwen BS, Sakai RR. Chronic social stress reduces dendritic arbors in CA3 of hippocampus and decreases binding to serotonin tranporter sites. Synapse. 2000; 36:85-94.
10. Montanez S, Daws LC, Gould GG, Frazer A. Serotonin (5-HT) transporter (SERT) function after graded destruction of serotonergic neurons. J Neurochem. 2003 ; 87(4):861-7.
11. Nutt DJ. The neuropharmacology of serotonin and noradrenaline in depression. International Clinical Psychopharmacology. 2002; 17 [suppl]: S1-S12
12. Papanicolaou DA, Wilder RL, Manolagas SC, Chrousos GP. The pathophysiologic roles of interleukin-6 in human disease. Ann Intern Med. 1998;128:127–137
13. Preacher, K.J. and A.F. Hayes (2004) SPSS and SAS Procedures for Estimating Indirect Effects in Simple Mediation Models. *Behavior Research Methods, Instrument, and Computers.* 36 (4). 717-731
14. Fritz, M.S. and D.P. MacKinnon. (2008). A Graphical Representation on the Mediated Effect. *Behavior Research Methods*. 40 (1). 55-60
15. Rozeboom, W.W. (1956) Mediation Variables in Scientific Theory. *Psychological Review*. **63** (4). 249-264
16. Preacher, K.J. and G.J. Leonerdelli (2007) *Calculation for the Sobel Test an Interactive Calculation Tool for Mediation Test*.
17. MacKinnon , D.P., C.M. Lockwood, J.M. Hoffman, S.G. West, and V. Sheets (2002) A Comparison of Methods to Test Mediation and Other Intervening Variable Effects. *Psychol Methods*. **7** (1), 80-114
18. Suparno (2007). *The Effect of Psychological Stressor on Serotonin Transporter (SERT) Distribution and Apoptotic Index of Hippocampus*. Jurnal Kedokteran Brawijaya, Vol XXIII(3), December 2007, 107-115.
19. Hair Jr., J.F., W.C. Black, B.J. Babin, and R.E Anderson (2010) *Multivariate Data Analysis* 7th Ed. Pearson Prectice Hall (e-books)
20. Arbuckle, J.L. and W. Worthke (1999) *AMOS 4.0 User's Guide*. USA: SmallWaters Corporation.

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