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RESEARCH ARTICLE

THE EFFECT OF CURCUMIN (*CURCUMA DOMESTICA* VAL. EXTRACT) ADMINISTRATION ON APOPTOSIS THROUGH *PRO BRAIN-DERIVED NEUTROPHIC FACTOR* EXPRESSION AFTERTRAUMATIC BRAIN INJURY IN THE RATS *SPRAGUE-DAWLEY*

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ABSTRACT

Background: Traumatic brain injury is one of the leading causes of death and disability in those of reproductive age, with approximately 0.5-1 million cases occurring per year. During the period of the brain injury, a cascade reaction occurs that causes apoptosis. Curcumin enhances the expression of brain-derived neurotrophic factor(BDNF) via BDNF coding gene transcription. The different kind of injury creates neurotropic factors and cytokine which protect the existence of excitotoxicity, such as BDNF. The aim of this study was to evaluate the effects of curcumin on pro-BDNF(pBDNF) expression and apoptosis on the traumatic brain cellof *Sprague-Dawley*rats. **Methods:** This study was a laboratory test on experimental animals with post-test only control group design. The sample size was 33 rats divided into 3 groups: the negative control group A (normal rats without curcumin treatment), the positive control group B (trauma model without curcumin treatment), and the trauma model group C (given oral curcumin extract treatment 200 mg per weight (kg) daily for 6 days). **Results:** There were significant differences in pBDNF expression between the three groups (p=0.000) with ANOVA methods. The positive control group B showed significant differences to the negative control group A and intervention group (p=0.000). Apoptosis showed significant differences between the three groups, which were as high as those for pBDNF expression. However, there were was no difference between the negative control group A and the intervention group. **Conclusion:** This study proves that curcumin stimulates the decreased of BDNF expression and decreases apoptosis in rat brain cells in traumatic models.

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INTRODUCTION

Traumatic brain injury is a direct or indirect mechanical trauma to the head which can result in impaired neurological, physical, cognitive and psychosocial function; this may be temporary or permanent. Traumatic brain injury is one of the leading causes of death and disability in the reproductive age group, with approximately 0.5-1 million cases occurring per year. The incidence of traumatic brain injury in Indonesia is estimated to reach 500,000 cases each year (Indharty, 2013). Based on the Indonesian Health Profile produced by the Ministry of Health of the Republic of Indonesia (MoHRI), head injuries are included in the top ten diseases treated in the hospital. The incidence of head injury in Indonesia is between 6 and 12% of all cases of head injury with a mortality rate of up to 23-37%. Data on cases of head injury in RSUP H. Adam Malik Medan in 2010 were available for 1627 cases, 274 of which required surgery (Indharty, 2013). Ten percent of the patients died before arriving at the hospital. Of those arriving at the hospital, 80% were classified as mild traumatic brain injury, 10% were included as moderate traumatic brain injury and 10% were included as severe traumatic brain injury (Ministry of Health Republic of Indonesia, 2012). During head injury, an inflammatory cascade reaction occurs that will trigger apoptosis. Several different types of injuries cause the release of neurotrophic and cytokine factors, which can protect neurons from excitotoxicity, one of which is brain-derived neurotrophic factor (BDNF). BDNF, a member of the neurotropin family, is known to be a major factor contributing to the survival of nerve cells, as well as being involved in proliferation and differentiation, and the regulation of synapse function in the central nervous system. BDNF expression in the cerebellum is particularly high in granular cells (Juananda,

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2015). Both granular cells and Purkinje cells express BDNF receptors i.e. tropomyosin-receptor kinase B (TrkB). Deletion of the BDNF gene in mice increases granular cell death, inhibits the growth of Purkinje cell dendrites, and affects the cerebellum foliation pattern. Several previous studies have suggested that BDNF may protect nerve cells from injuries induced by hypoglycaemia, ischaemia, hypoxia and other neurotoxicity mechanisms. *Curcuma xanthorhiza* Roxb is a member of the Zingiberaceae family plant which is widely grown and used as a raw material for traditional medicine in Indonesia. Empirically, *temulawak* rhizome is known to have many benefits, one of which is as an antioxidant. The active components responsible for antioxidants in the ginger rhizome are curcumin, desmethoxycurcumin and bisdemethoxycurcumin. Some research results show that the ginger rhizome has an antioxidant effect. Rao's research suggests that curcumin is more active than vitamin E and beta carotene. This is because the role of curcumin as an antioxidant that prevents free radicals cannot be separated from the structure of curcumin compounds. In addition to being antioxidants, curcumin compounds also have neuroprotective properties. This is due to the high lipophilic nature that causes the increased disposition of curcumin in the brain. *In vitro* and *in vivo* studies suggest that curcumin may prevent neurodegenerative processes due to oxidative stress. *In vitro,* curcumin can inhibit lipid peroxidation in the brain. In addition, curcumin may prevent the increase of malondialdehyde (lipid peroxidation product) and improve the reduction of glutathione in oxidative stress-induced rats with sour acid. Although there have been many studies explaining the effect of giving curcumin extracts in terms of neuronal cell protection, there has been no study linking the use of curcumin to pro-BDNF expression. Based on the assumption that curcumin is neuroprotective, it is suspected that there may be an effect of curcumin on pro-BDNF expression. Therefore, the objective of this study was to identify the effect of curcumin on pro-BDNF expression after traumatic brain injury.

MATERIALS AND METHODS

This research is a laboratory test in experimental animal model with a post-test only control group design. This design allows researchers to measure the effect of treatment (intervention) on the experimental group by comparing the experimental group with the control group. However, it does not allow the researcher to determine the extent to which or how much the change occurred, because the tests were performed at the end of the treatment (rather than to determine initial data).(4)This research was approved by the Health Research Ethical Committee, Medical Faculty of University of Sumatera Utara/ H.Adam Malik General Hospital.

Selection and acclimatisation of rats: The experimental animals used in this study were healthy Sprague-Dawley rats, aged 2.5-3 months old, weighing 280-320 grams, which were obtained from the Faculty of Veterinary Medicine, Bogor Agricultural University. The selection of rats as experimental animals is based on the consideration that Sprague-Dawley rats are genetically like humans and have adaptability to the laboratory environment.(5)The sample allocation (grouping) uses simple random sampling by first numbering each mouse. Therefore, experimental animals, experimental sites, and other research materials can be said to be homogeneous. The sample size was 33, consisting of 11 of each group. There are three groups of the sample.

Group A includes healthy rats with eye shining conditions, fur that is not dull, with a good level of activity and a good appetite. Group B contains rats which were given traumatic brain injury with modification of Feeney TBI Weight drop model without the administration of curcumin extract, while group C includes the rats which were given traumatic brain injury and administered with curcuminextract.All rats were then adapted to laboratory conditions for 7 days in order to adapt to the environment and were fed BR I during adaptation.

Treatment: Group A (negative control group) was sacrificed by cervical dislocation in day 7, after which the brains were taken for the manufacture of immunohistochemical preparations. Group B (positive control group) was given a brain injury treatment on day 1, and was then observed and treated for 6 days. The rats were then sacrificed on the 7th day by cervical dislocation after anaesthesia with ether, and the brains were taken for the manufacture of immunohistochemical preparations. Group C (intervention group) animals were given a brain injury on day 1 and treated with curcumin extract (200 mg orally per day for 6 days); then, rats were sacrificed on day 7 by cervical dislocation after anaesthesia with ether, before the brains were taken for the preparation of immunohistochemical samples. The administration of curcumin extract at doses of 200 mg was given orally each day for 6 days. Observations were performed after 6 days after treatment, due to the expression TUNEL assay system on the injured frontal cortex reaching a peak after 24 hours (Federer, 1983). The Sprague-Dawley male rat underwent craniotomy 4 mm into the right fronto-parietal to obtain exposure to the dura, with the opening centred at 1.5 mm posterior of the bregma and 2.5 mm laterally from the midline; a 40 gr load was dropped on the intact bone from a height of 100 cm, with a load diameter of 2.5 mm. Immediately after TBI, the scalp was covered with stitches (Silk 3.0 cutting) and the animal was returned to the cage. For the negative control group, the surgical craniotomy was performed, without the load injury (Majid Motaghinejada, 2017).

Sample collection: On the 7th day, mice in groups A, B and C were killed by cervical dislocation after anaesthesia with ether and rat brain surgery was performed. In group A, the rats were killed on the seventh day of the study, by cervical dislocation after anaesthesia with ether, and rodent abdominal surgery wsa performed to remove the brain. Brain tissue was fixed in 10% formalin for 15-24 hours. After that, dehydration was achieved using stratified alcohols (30%, 50%, 70%, 80%, 96% and absolute) for 60 min each. Every 60 minutes, this was cleared using xylol 2 times. Then, infiltration with soft paraffin for 60 minutes was performed at a temperature of 48°C. Then, the block was placed in hard paraffin on the mould and left for a day. The next day, the blocks were attached to the holder and cutting was performed using a rotary microtome to a thickness of 5-7 µm. After that, they were mounted on the object glass coated with poly-L-lysine (Anderson, 2002). The paraffin blocks were soaked in xylol 2 times each for 5 minutes. After that, rehydration was performed using serial alcohol (absolute, 96%, 80%, 70%, 50% and 30%) for 5 minutes each. Finally, samples were rinsed in dH_2O for 5 minutes (Federer, 1983)

Immunohistochemical methods Apoptosis (Thermo Scientific): The slide was washed using PBS pH 7.4 once for 5 minutes. Endogenous peroxide blocking was performed using 3% H_2O_2 for 15 minutes. Samples were washed using PBS pH

7.4 three times, each for 3 minutes. Unspecified proteins were blocked using 5% FBS containing 0.25% Triton X-100 and washed using PBS pH 7.4 three times, for 3 minutes each. The incubation process was performed using the primary antibody Tunel assay (apoptosis cat number sigma #11684817910) for 60 minutes and washed using PBS pH 7.4 three times, each for 3 minutes. Samples were incubated with a conjugated anti-biotin mouse antibody for one hour at room temperature and washed using PBS pH 7.4 three times, each for 3 minutes. Incubation with SA-HRP (Strep-Avidin Horseradish Peroxidase) was performed for 10 minutes, and then washed using PBS pH 7.4 three times, for 3 minutes each. Samples were the titrated with DAB (DiaminoBenzidine) and incubated for 5-15 minutes, before being washed using PBS pH 7.4 three times, for 5 minutes each. Counterstaining was performed with Mayer Haematoxylin for 1 minute and samples were washed using tap water. Finally, samples were rinsed with dH_2O and air-dried, before being mounted using entrain and covered with a cover slip. Slides were observed on a light microscope.

Cell Counting (tunnel assay) on the contusions of the cerebral cortex and the penumbra region: Cells (tunnel assay) on the contusions of the cerebral cortex and the penumbra region were counted. Positive molecule expression with primary antibodies will look brown under a 1000X light microscope. Cerebral cortical contusion cells were calculated to lie in the cerebral cortex region, and positive cells were counted in 20 fields of view (HPF) in each sample.

RESULTS

The data from three groups were tested for normality. Based on the Shapiro-Wilk test, all data were distributed normally. Table 1 shows the mean levels of apoptosis and pBDNF in the three groups. Group B, or the positive control, showed the highest score for apoptosis cells, while group C, with curcumin administration, had the lowest score. The positive control group also showed the highest level of BDNF. In contrast, the negative control group had a BDNF score similar to that of the intervention group. Data were analysed using the ANOVA test to compare the mean of three groups. There was a significant difference in apoptosis between the three groups (p=0.000). In addition, the difference in pro-BDNF for the three groups is shown in Table 2. The analysis was continued with the post hoc test to investigate the difference in apoptosis and pro-BDNF in the two groups. The results show a significant difference in apoptosis and pro-BDNF expression between the positive and negative control groups as well as between the positive control and intervention groups: p=0.000 and p=0.000, respectively. However, there was no difference in apoptosis expression between the negative control and intervention groups (p=0.224) as well as in pro-BDNF expression (p=0.979), as shown in Table 3. The statistical results of apoptosis cell expression between groups were consistent with the immunohistochemical results, as shown in Figure 1. A is an overview of the negative control group (Group A); B is a description of the group given TBI (Group B; and C is a description of the group given TBI and curcumin (Group C). In addition, Figure 2 illustrates the immunohistochemical comparison of the BDNF expression between groups.

DISCUSSION

The results showed that there was a significant difference in the expression of pBDNF and apoptosis between positive and negative control groups. The results also showed that there was a significant difference between the positive control group and the intervention group with regard to the expression of BDNF and apoptosis. This indicated that curcumin has the ability to increase the expression of pBDNF when brain injury occurs. The role of curcumin in increasing pBDNF expression is related to the transcription of the pBDNF coding gene. Curcumin proved to increase the levels of cAMP and ERK (extracellular signal regulated kinases) and p38 kinase. The cAMP compound will activate Protein Kinase A (PKA), an enzyme needed to increase the cAMP-response element binding (CREB) protein activity. This activated CREB will then occupy the promoter in the gene encoding BDNF and begin the process of gene transcription, which causes the production of pBDNF protein to a greater extent (Bathina, 2015). Curcumin is also capable of enhancing mitochondrial biogenesis as well as being able to suppress the release of various pro-inflammatory cytokines such as interleukin 6 and tumour necrosis factor-alpha (TNFα). All of these pathways describe the role of curcumin as an antioxidant, anti-inflammatory, anti-apoptotic and neuroprotective factor (Caldeira *et al*., 2007). Exogenous BDNF results in an increase in the dendritic length and complexity of pyramidal neurons in the developing visual cortex layer by layer. This suggests that BDNF not only promotes neuronal growth but also modulates specific patterns in dendritic growth. Furthermore, the inhibition of spontaneous electrical activity, synaptic transmission, or L-type calcium channels prevents an increase in dendritic growth induced by exogenous BDNF, suggesting that neurons must be active enough to respond to the increased action of BDNF growth.

In the central nervous system trauma model, using adult mouse ganglion retina cells (RGC), it was observed that *in vivo* injections of BDNF improved neural viability by activating the TrkB, MAPK and PI3K-PKB pathways and inhibiting apoptosis-induced caspase-3. Neurogenesis in the hypothalamus is enhanced by the long-term continuous administration of BDNF for 12 days (Ray, 2002). BDNF not only increases NMDA levels and intracellular calcium concentrations, but also relieves NMDA Mg^{2+} receptors and promotes long-term changes in synaptic activity. A reduction of TrkB and BDNF reduces LTP induction. Thus, BDNF is involved in NMDA receptor traffic by increasing calcium influx, leading to the release of post-synaptic BDNF which, in turn, increases the pre-synaptic vesicles, and increases LTP and synaptic plasticity (Risling *et al*., 2011). TBI develops over a period of hours or days after the initial impact to the head. Secondary injury is associated with the synthesis and release of various neurochemicals that affect brain metabolism, and alter cerebral blood flow, homeostasis, and other neuronal injury that occur at TBI (Dharmajaya *et al*., 2018; Galvin, 2003) The disruption of cerebral blood flow, excitotoxicity, reactive oxygen species (ROS), inflammation, and apoptosis were pathogenesis for secondary brain injury. Caspase-dependent pathways consist of an extrinsic pathway initiated by death receptors and intrinsic pathways that are initiated by mitochondria. The activation of initiator caspase from both pathways will activate caspase 3 and execute the process of apoptosis (Radecki *et al*., 2005). This study shows linear results in the expression of pBDNF and apoptosis in both the positive control group and the intervention group with curcumin extract. In the positive control group the mean apoptotic expression (11.55) and pBDNF mean (16.27) increased when compared with the negative control group.

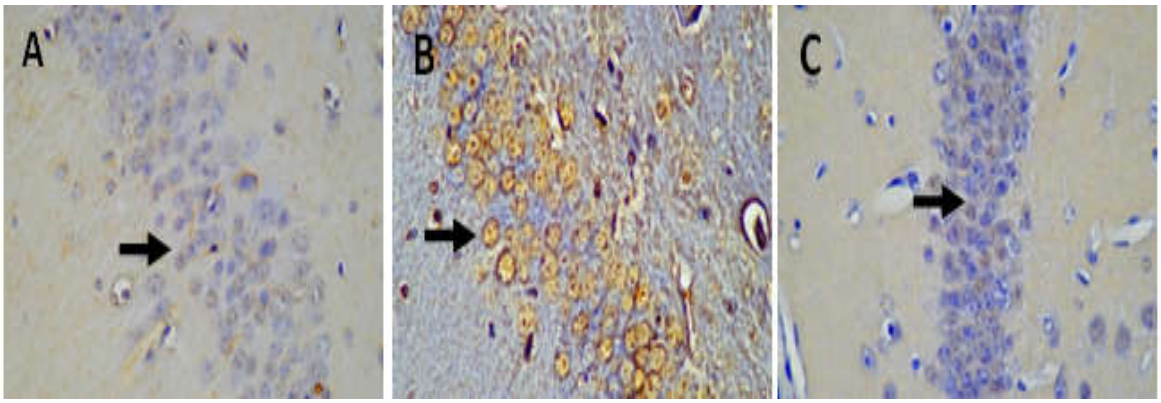

Figure 1. Comparison of apoptosis cells expression on Subgranular Zone (SGZ) from Hippocampus Dentate Gyrus (DG). (Immunohistochemical, Olympus BX 50 magnification 1000x)

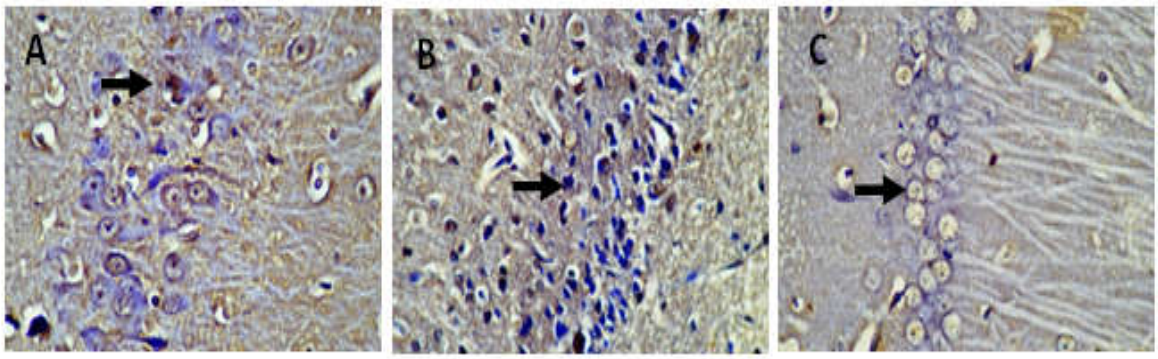

Figure 2. Comparison of pBDNF expression on Subgranular Zone (SGZ) from Hippocampus Dentate Gyrus (DG) (Immunohistochemical, Olympus BX 50 magnification 1000x)

Table 1. Mean of apoptosis and pBDNF by three groups

	Groups	N	Mean	Std. deviation	95% Confidence interval	
					Lower bound	Upper bound
Apoptosis	Negative control	11	4.82	2.040	3.45	6.19
	Positive control	11	11.55	1.508	10.53	12.56
	Intervention	11	3.55	1.695	2.41	4.68
pBDNF	Negative control	11	8.09	1.375	7.17	9.01
	Positive control	11	16.27	2.796	14.39	18.15
	Intervention	11	8.27	2.149	6.83	9.72

Table 2. The difference of apoptosis and pBDNF between three groups (Anova test)

	Groups	Mean square	p value
Apoptosis	Between groups	203.273	0.000
	Within groups	3.103	
pBDNF	Between groups	240.121	0.000
	Within groups	4.776	

Table 3. The difference of apoptosis and pBDNF between two groups (Post hoc test)

	Group		Mean Difference	p value
Apoptosis	Negative control	Positive control	-6.727	0.000
		Intervention	1.273	0.224
	Positive control	Negative control	6.727	0.000
		Intervention	8.000	0.000
	Intervention	Negative control	-1.273	0.224
		Positive control	-8.000	0.000
pBDNF	Negative control	Positive control	-8.182	0.000
		Intervention	-0.182	0.979
	Positive control	Negative control	8.182	0.000
		Intervention	8.000	0.000
	Intervention	Negative control	0.182	0.979
		Positive control	-8.000	0.000

Likewise with the intervention group, the mean of apoptotic expression (3.55) and the average pBDNF (8.27) decreased and tended to re-approach the expression in the negative control group. A number of recent studies have demonstrated that pBDNF can effectively activate cellular events that depend on p75NTR, including apoptosis and chemotaxis, and that pBDNF and proNGF are thought to have a causal role in neuronal or oligodendroglial death in the brain or injured spinal cord (Kim, 2010). The TNF receptor class, p75NTR is one of the most important, it is widely known to be a group consisting of "bait" receptors that regulate the biological activity of transmembrane On the other hand, recent research states that proNGF, a precursor form of NGF, is more active than mature NGF in inducing apoptosis after binding to p75NTR Although pro-domain BDNF and NGF only produce areas of similarity, previous studies support the hypothesis of p75NTR. that local BDNF can increase neuronal apoptosis in vivo (Blaha, 2000). Sortilin receptors exhibit high affinity for pBDNF, the strength of this affinity can be disturbed by the sortilin antagonist, neurotensin. Based on an analysis of the structure and function of proNGF and analysis of neurotensin bonds with cysteine-rich areas in ectodomainsortilin, it is possible that pBDNF interacts with the same area of sortilin. In fact, the affinity between proGNF and pBDNF with sortilin shows that pBDNF is a ligand that has a stronger bond. Although the findings show that pBDNF has better binding with sortilin, it is still unclear whether pBDNF is a ligand that has a better affinity than proNGF in inducing cellular apoptosis: a comparison of these two proneurotropins using SCG nerve culture does not produce a significant difference (Conte *et al*., 2008). However, this in vitro experiment cannot rule out the possibility of decaying an important part of ectodomainsortilin, and that the solution form of sortilin binds to pBDNF to interfere with proapoptotic activity. Of course, the previously formed pBDNF and sortilin complexes are less active than the proBDNF in inhibiting cell death. This observation shows that the bioactivity of pBDNF can be determined by the local concentration of dissolved sortilin. Finally, our findings that mature NGF can withstand apoptotic activity from pBDNF further support the idea that neuronal death / survival in vivo is regulated by a number of extrinsic signals (Kaplan, 2010).

Conclusion

Curcumin is able to decrease the expression of pro-BDNF through inhibit the apoptosis process in traumatic brain injury.

What is already know on this topic

- The curcumin extract administration increases the expression of BDNF and inhibits the process of apotosis on rats brain tissue after traumatic brain injury
- BDNF improves nerve survival by activating the TrkB, MAPK and PI3K-PKB pathways and inhibiting caspase-3-induced apoptosis.
- Curcumin and pBDNF are knwon as inhibitor of apoptosis process that can be used to the traumatic brain injury.

Competing interests: This study had no competing interest.

REFERENCES

Anderson G, Bancroft J. 2002. Theory and practice of histological techniques. London: Churchill Livingstone.

Bathina S., Das UN. 2015. Brain-derived neurotrophic factor and its clinical implications. *Arch Med Sci.,* 11:1164–78.

Blaha GR, Raghupathi R, Saatman KE, McIntosh TK. Brain-derived neurotrophic factor administration after traumatic brain injury in the rat does not protect against behavioral or histological deficits. *Neuroscience*. 2000.99:483–493.

Caldeira MV., Melo CV., Pereira DB., Carvalho RF., Carvalho AL., Duarte CB. 2007. BDNF regulates the expression and traffic of NMDA receptors in cultured hippocampal neurons. Mol Cell Neurosci. 35:208–19.

Conte V, Raghupathi R, Watson DJ, Fujimoto S, Royo NC, Marklund N, *et al*. TrkB gene transfer does not alter hippocampal neuronal loss and cognitive deficits following traumatic brain injury in mice. Restor Neurol Neurosci 2008. 26:45–56.

Dharmajaya R, Sari DK, Ganie RA. Determinants of Glasgow outcome scale in patients with severe traumatic brain injury for better quality of life. Earth Environ. Sci. 2018; 125 :1-6

Federer WT. 1983. Principles of statistical design with special reference to experiment and treatment design. (69)345:1-27.

Galvin KA, Oorschot DE. Continuous low-dose treatment with brain-derived neurotrophic factor or neurotrophin-3 protects striatal medium spiny neurons from mild neonatal hypoxia/ischemia: a stereological study. *Neuroscience* 2003;118:1023–1032.

Indharty RS. 2013. The increase of serum Bcl-2 concentration in moderate head injury outcome: The role of ACTH4-10Pro8-Gly9-Pro10. *Asian J Neurosurg*., 2013; 8(2):83–89.

Juananda D. 2015. Pengaruh Stres Kronikterhadap Otak: Kajian Biomolekular Hormon Glukokortikoiddan Regulasi Brain Derived Neurotrophic Factor (BDNF_ Pasca Stress di Cerebelum. *JIK*. 2015;9(2):65 - 70

Kaplan GB, Vasterling JJ, Vedak PC. Brain-derived neurotrophic factor in traumatic brain injury, post-traumatic stress disorder, and their comorbid conditions: role in pathogenesis and treatment. *Behav Pharm.* 2010. 21:427–437

Kim HJ, Lee JH, Kim SH 2010. Therapeutic effects of human mesenchymal stem cells on traumatic brain injury in rats: secretion of neurotrophic factors and inhibition of apoptosis. *J Neurotrauma.* 27:131–844.

Majid Motaghinejada, Manijeh Motevaliana, Sulail Fatimab, Hajar Hashemia, Mina Gholamic Curcumin confers neuroprotection against alcohol-induced hippocampal neurodegeneration via CREB-BDNF pathway in rats. BiomedPharm. 2017;87:721–740.

Ministry of Health Republic of Indonesia. 2012. *Profilkesehatan Indonesia tahun 2011.* Jakarta, Indonesia: Ministry of Health Republic of Indonesia.

Prins M, Greco T, Giza CC. 2013. The pathophysiology of traumatic brain injury at a glance. *Dis Model Mech.,* 6(6):1307-1315.

Radecki DT, Brown LM, Martinez J, Teyler TJ 2005. BDNF protects against stress-induced impairments in spatial learning and memory and LTP. *Hippocampus.* 2005. 15:246–253.

Ray SK., Dixon CE., Banik NL. 2002. Molecular mechanisms in the pathogenesis of traumatic brain injury. *Histol Histopathol*. 2002;17:1137–1152.

Risling M, Plantman S, Angeria M, Rostami E, Bellander B-M, Kirkegaard M, *et al*. Mechanisms of blast induced brain injuries, experimental studiesin rats. *Neuroimage*. 2011; 54: 89-97.

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