

Effect of Soursop Leaf And Royal Jelly on Brain Neuronal Edema And Apoptosis in Rat Cerebral Contusion Model Mediated by Bdnf/Il-1 β Ratio

Arif Kusumo Rahardjo*, Moch Istiadjid Eddy Santoso**

*) Department Of General Surgery, Faculty Of Medicine, Brawijaya University Malang, Dr. Saiful Anwar Malang General Hospital

***) Professor Of Neurosurgery Department, Faculty Of Medicine, Brawijaya University Malang, Dr. Saiful Anwar Malang General Hospital

Abstract: Brain injury causes disability and death. Soursop leaf extract has been widely studied as anti-inflammatory and anti oxidant in non-trauma cases. The anti-inflammatory and neurotrophic effects of Royal Jelly has also been studied. The role of soursop leaf extract and Royal Jelly on cerebral contusion need to be studied.

The aim of this study is to analyze the effect of Soursop leaf extract and Royal Jelly in cerebral contusion rat model to cerebral edema and apoptosis mediated by BDNF/IL-1 β ratio.

Rat is grouped into 5 groups, with 5 rats as the member. Cerebral contusion model made by applying 1,6 Joule of energy. Control group (K) didn't receive Soursop leaf neither Royal Jelly. P1 Group is given 100 mg/kg Soursop leaf extract, P2 Group is given 200 mg/kg Soursop leaf extract, P3 Group is given 400 mg/kg Soursop leaf extract, P4 Group is given 100 mg/kg Royal Jelly for 7 days. Cells underwent edema dan apoptosis were counted (TUNEL), BDNF and IL-1 β were measured (ELISA).

Soursop leaf extract decreased apoptosis (p-value<0,05), decreased IL-1 β and increased BDNF (p-value<0,05). Royal Jelly decreased cerebral edema and apoptosis (p-value<0,05), decreased IL-1 β and increased BDNF (p-value<0,05). The increased BDNF/IL-1 β ratio caused decreased cells underwent cerebral edema and apoptosis (p-value<0,05).

Both administration of Soursop leaf extract and Royal Jelly significantly decrease cerebral neuronal edema and apoptosis, decrease IL-1 β and increase BDNF. The decrease of edema and apoptosis are mediated by increment of BDNF/IL-1 β ratio.

Keywords: cerebral contusion, soursop leaf extract, royal jelly.

I. Introduction

Brain injury resulted in nearly half of the deaths due to trauma. Cerebral edema that occurs after brain injury is the leading cause of disability and death of brain injury patients. Cerebral edema treatment strategies that exist today as diverse as osmotic fluids, hyperventilation, sedation, neuromuscular relaxant, hypothermic, and surgical intervention [1].

Brain injury will cause the body's response is to recover himself. Recovery depends on the conditions of neurotransmitter / ionic and presence of neurotrophic (or what is known as a growth factor). One neurotrophic well known is the brain-derived neurotrophic factor (BDNF), which is secreted when there is brain injury. Some research indicates BDNF fix the motor neurobehavioral and cognitive function [2]. BDNF is a growth factor expression was more available in the brain compared to the peripheral nerves.

The changes in brain injury involves various mediators of the inflammatory response. Today a key mediator recognized is Interleukin-1 β (IL-1 β). Decrease the amount of IL-1 β is shown to improve the condition of the brain at the cellular and behavioral conditions brain injury [3].

Herbal therapy is now widely used in modern medicine. However the effectiveness of herbal therapies have been noted by a number of studies [4]. Botanical Garden of the World recorded more than 2200 species of plants around the world have the potential to be used as drugs. The growth of herbal based industries reach 7-15% per year. Analysis of the World Health Organization, 80% of world population uses a variety of plant-based products [5]. One type of herbal medicinal plants are considered useful is *Annona muricata* (soursop), this plant contains flavonoids that are believed as a source of antioxidants and anti-inflammatory that could be expected to reduce the edema in the brain cells [6,7]. There are various kinds of research that has been conducted on the effects of antioxidants soursop leaves on the condition diabetes mellitus [8]. However, research on the effects of soursop leaf brain injury still has not been done.

Royal Jelly is secreted by glands hypopharynx belongs to the worker bees, to feed the young larvae and adult queen bee. Royal Jelly has antioxidant effects, neurotrophic, anti-inflammatory and immunomodulatory

[4]. Researchers questioned whether *Annona muricata* Soursop Leaf Extract and Royal Jelly can serve as an effective anti-inflammatory to reduce cell death and apoptosis in brain injury, through the mediation of IL-1 β and BDNF?

II. Aim And Objectives

2.1 Aim

2.1.1 To analyze the effect of *Annona muricata* Soursop Leaf Extract and Royal Jelly to cerebral edema and apoptosis of brain injury, through the mediation of IL-1 β and BDNF.

2.2 Objectives

2.2.1 Analyze and explain the effect of Soursop Leaf Extract and Royal Jelly in rats brain injury to the reduced levels of Interleukin-1 β .

2.2.2 Analyze and explain the effect of Soursop Leaf Extract and Royal Jelly in rats brain injury to the increase in BDNF.

2.2.3 Analyze and explain the effect of Soursop Leaf Extract and Royal Jelly in rats brain injury to the decline of cerebral edema.

2.2.4 Analyze and explain the effect of Soursop Leaf Extract and Royal Jelly in rats brain injury to a decrease in the number of apoptotic neurons.

2.2.5 Analyze and explain the effect of Soursop Leaf Extract and Royal Jelly in rats brain injury to the reduction of cerebral edema and apoptosis of neurons through the agency Ratio BDNF / IL-1 β .

III. Materials And Method

3.1 Subject of research

This research uses experimental animals as research subjects. Kind of animal was *Rattus norvegicus* Wistar strain, aged 12-14 weeks with a mean weight of 250-300 grams. Male sex, healthy condition of the experimental animals without any defects, active movement. Obtained from the Laboratory of Physiology of the Faculty of Medicine, University of Brawijaya. From the point of raising placed in a cage to the site of the study. Male sex been so pathophysiological process after a brain injury can be observed more clearly without the influence of the hormone progesterone.

3.2 The inclusion and exclusion criteria

3.2.1 Criteria for inclusion

- Healthy (active movement)
- Weight 250-300 g
- Males
- Age 12-14 weeks

3.2.2 Exclusion criteria

- Mice that were sick, disabled
- Mice that died during the study

3.3 Materials

1. Soursop Leaf Extract, taken from Materia Medica Government Office for Herbs Research.
2. Royal Jelly, acquired from Faculty of Animal Husbandry Brawijaya University Malang.
3. Material for examination of apoptosis: the rat brain tissue, Apoptech (Enzyme-Proteinase K, Apoptag, DAB liquid), apoptosis kit manufactured by Immunochemistry Inc.
5. Material examination IL-1 β : rat brain tissue, Complete ELISA Kit, pink-ONE, Rat IL-1 β (Komabiotech Inc.)

3.4 Study design

The research design was used to test hypotheses: experimental studies, randomized controlled trials (randomized controlled trial), posttest only design, single blinded. Researchers do not know where the rats given Soursop Leaf Extract and Royal Jelly.

3.5 Treatment of subjects

3.5.1 Treatment

3.5.1.1 Preparation of brain injury models

Each animal anesthetized with ketamine 100 mg / kg intramuscularly [9]. Then made observations on the success of anesthesia. This success has seen the loss of reflex reaction to painful stimuli to the skin of the

belly, beard hair, and between the toes. After anesthesia successful, dropped a load of 0.2 kg pass through a cylindrical tube from a height of 0.8 m (1.6 Joules of energy) on the head of the experimental animals are put on fixation devices. Conflicts aimed at point 9 mm anterior to the coronary suture. This action will result in diffuse brain injury in rats [10].

3.5.1.2 Administration of Soursop Leaf Extract

After the treatment of brain injury action, the patient returned to the cage. Four hours later awarded Leaf Extract oral administration using a sonde in accordance with the division of the group, each within 5 minutes. Animals are maintained. Award Leaf Extract Soursop then repeated every 24 hours for 7 days in a row. Inside the cage, the animals try to get food, water and air freely.

- a. P1 group. Animals in this group is given Soursop Leaf extract at a dose of 100 mg / kg.
- b. P2 group. Animals in this group Soursop Leaf Extract is given at a dose of 200 mg / kg.
- c. P3 group. Animals in this group Soursop Leaf Extract is given at a dose of 400 mg / kg.

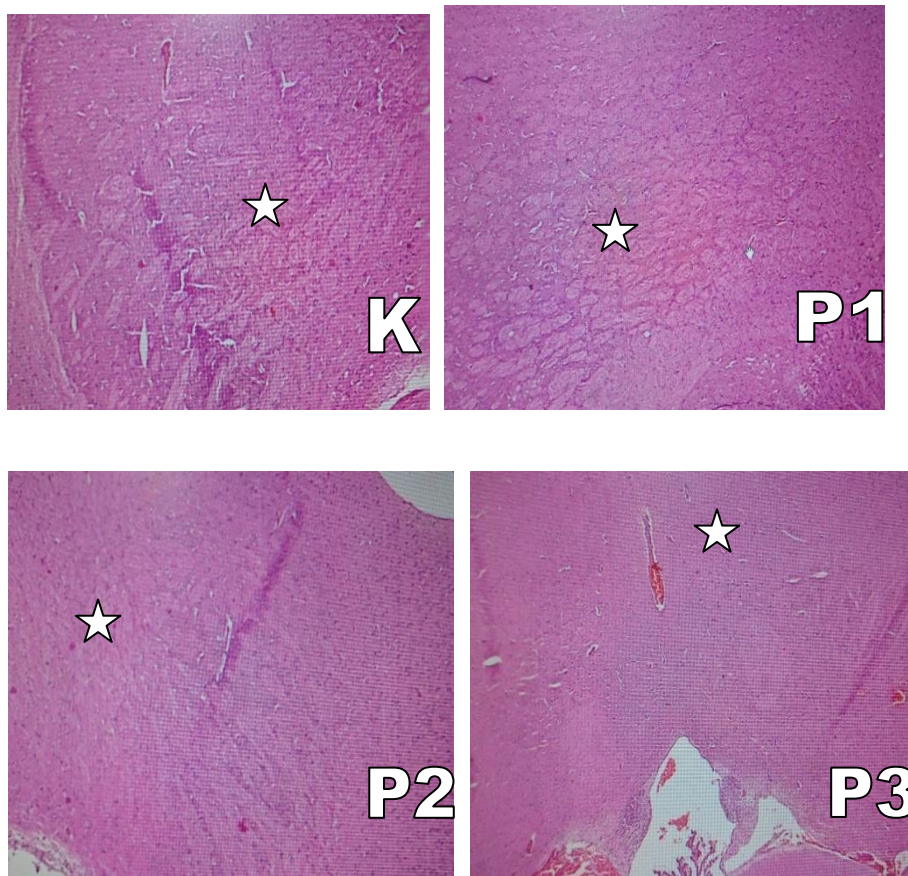
3.5.1.3 Administration of Royal Jelly

After the treatment of brain injury action, the patient returned to the cage. Four hours later awarded the RJ administration orally at a dose of 100 mg / kg, respectively within 5 minutes. Animals are maintained. Award RJ then repeated every 24 hours for 7 days in a row. Inside the cage, the animals try to get food, water and air freely.

Giving treatment at four hours post-trauma based on the pathology of cerebral edema and brain circulation disorders ranging from the first hour and last until the 7th day [11]. Neuroendocrine changes ranging from 30 minutes post-trauma [12]. Peak increase of neurotrophin BDNF post-traumatic happened to the third hour [13] and last up to 72 hours post-injury. So that in the fourth hour of physiological body has to secrete BDNF fullest.

IV. Results

4.1 Cerebral edema



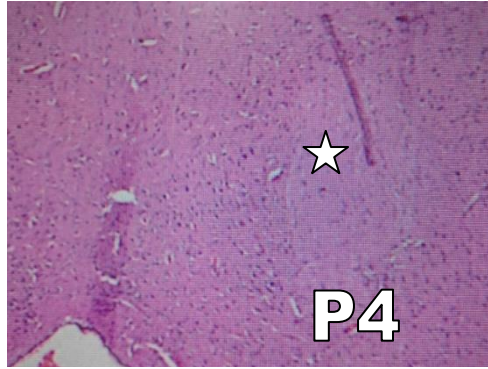


Figure 1 Cerebral edema after brain injury manufacture and delivery of treatment.

Description: Histological examination with Hematoxylin-eosin staining. The area of edema was measured using a measuring device on the computer after scanning slides. Area edema appear as pale colored areas and interstitial inflammation (star).

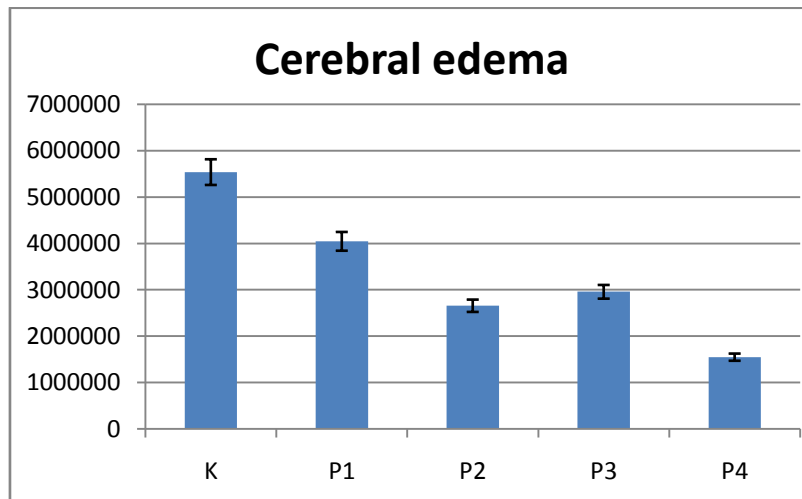
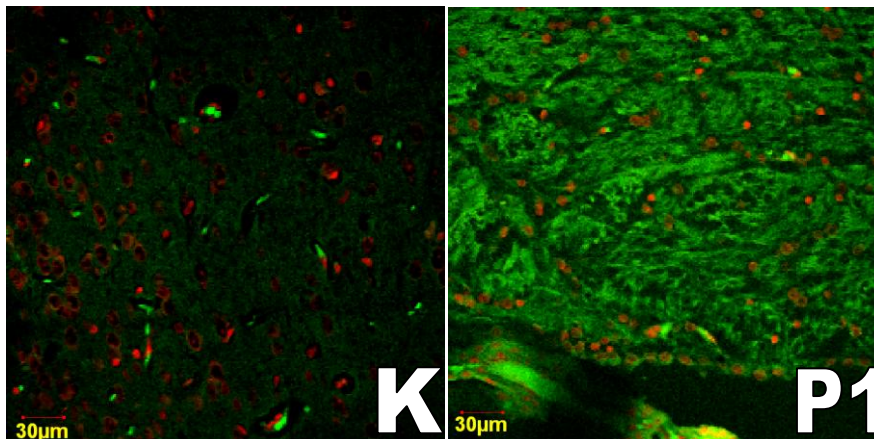


Figure 2 Cerebral edema

Description: The graph of the average area of cerebral edema after each treatment group

The highest mean of cerebral edema obtained with the control group (5,538,324.0360), and the lowest obtained in treatment P4 (1,549,128.3080; $p < 0.005$).

4.2 Apoptosis



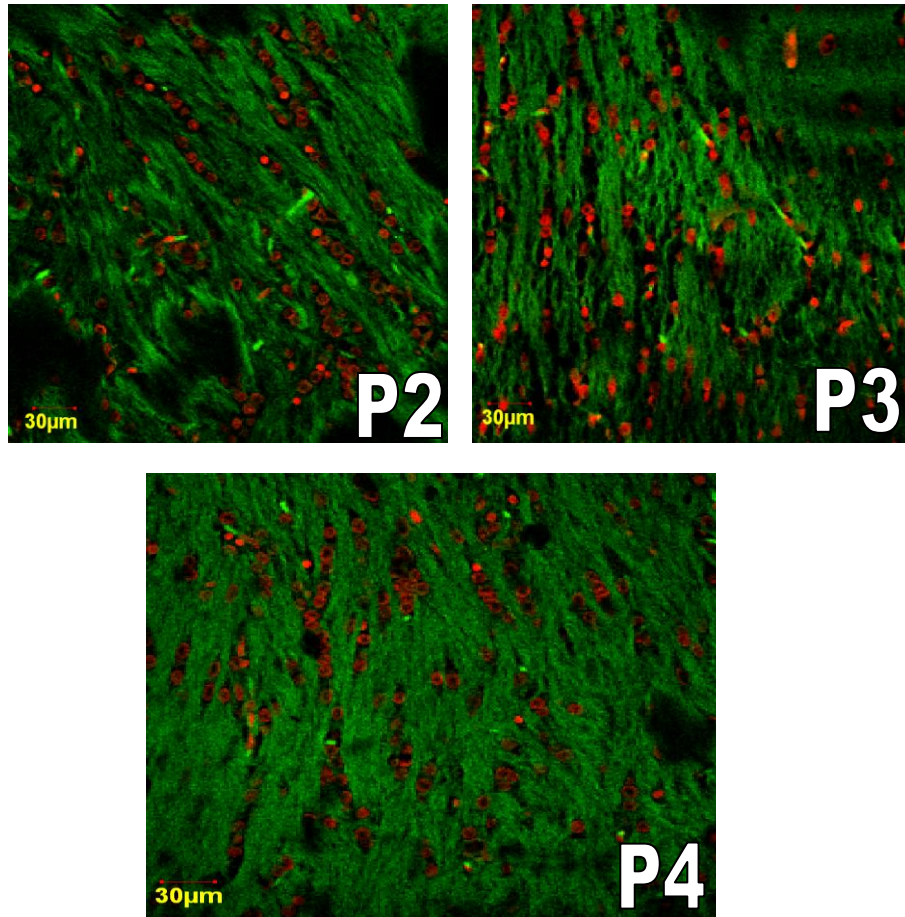


Figure 3. Microscopic neuronal apoptosis 400x magnification.

Description: Cells undergoing apoptosis has a characteristic morphological shrinkage. Nuclei appear round, condensed, fragmented.

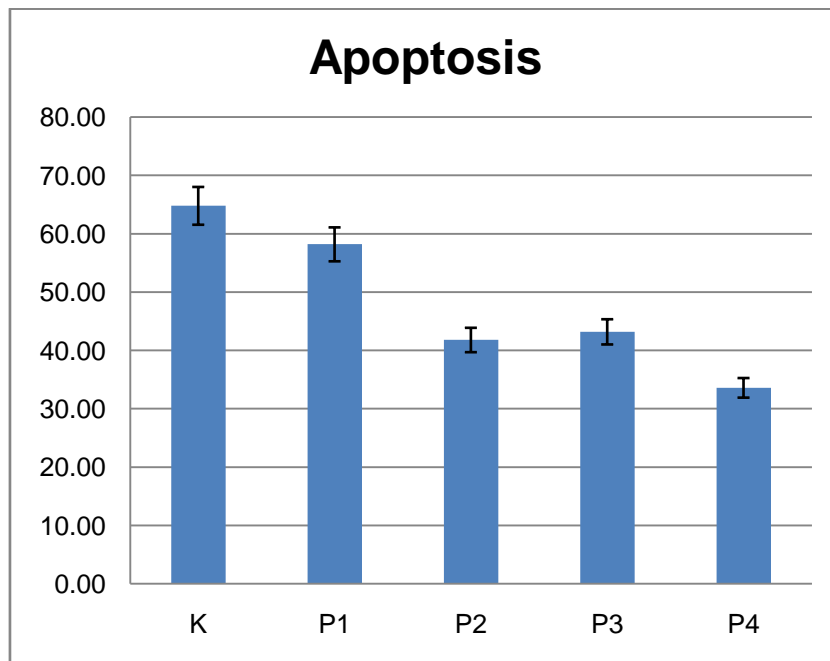


Figure 4. Apoptosis

Description: Mean of the number of apoptotic neurons per treatment group

The highest apoptosis obtained in the control group (64.800), and the lowest obtained at treatment P4 (33.600; $p < 0.005$).

4.3 Level of IL-1 β

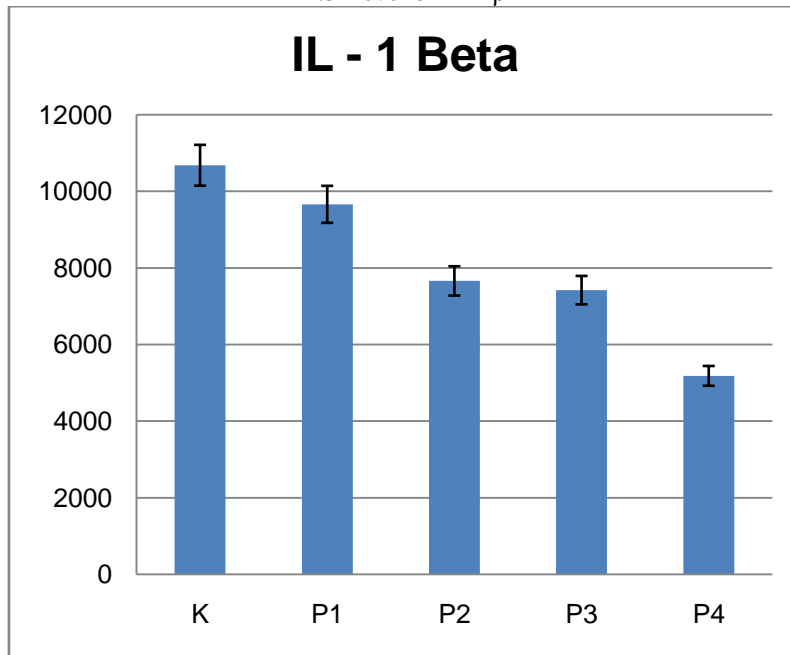


Figure 5. The level of IL-1 β

Description: The mean of the levels of IL-1 β each treatment group

The highest level of IL-1 β were obtained in control group (10680), and the lowest obtained in group P4 (5180; $p < 0.005$)

4.4 Level of BDNF

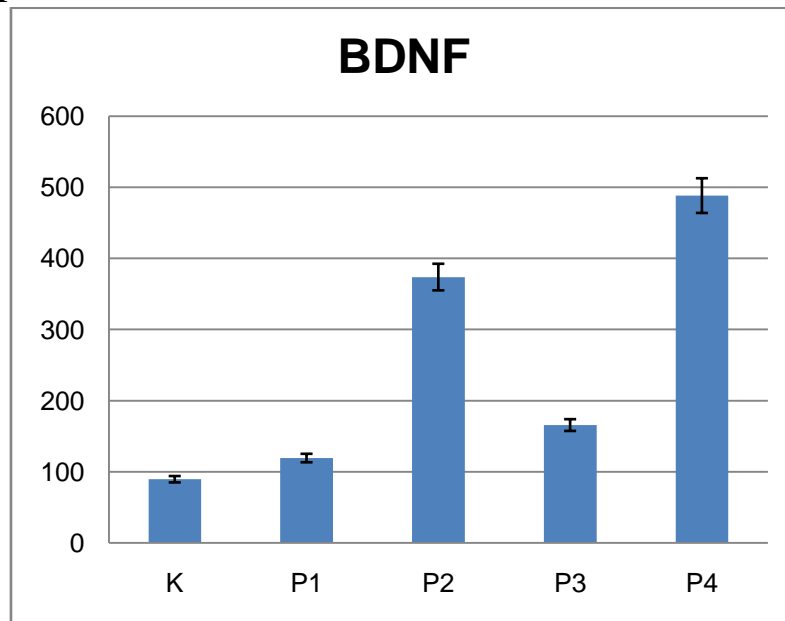


Figure 6. Level of BDNF

Description: Mean levels of BDNF of each treatment group

The highest mean obtained at treatment P4 (488,400; $p < 0.005$), and the lowest obtained in the control (89,400).

4.5 Effect of Ratio (BDNF/IL-1 beta) against Apoptosis

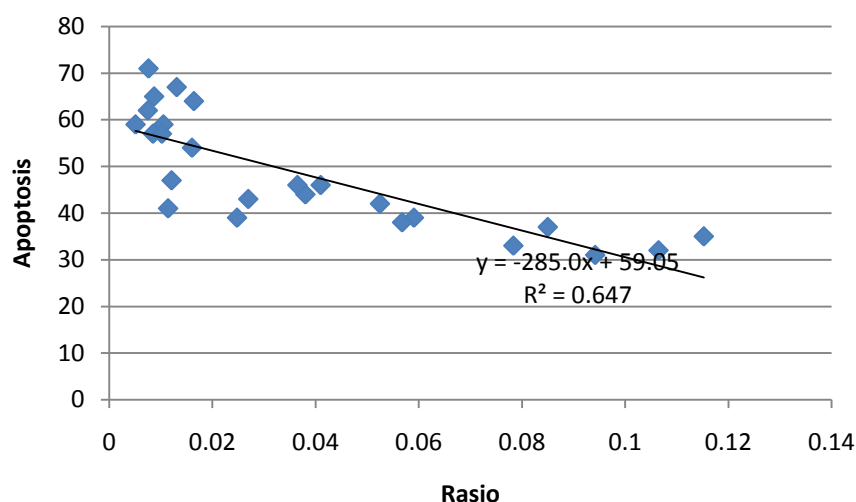


Figure 7. Curve relationship between the ratio of apoptosis

Description: Increasing the ratio will be followed by a decrease in apoptosis Ratio (BDNF / IL-1 beta) significantly affects the number variable apoptosis ($p < 0.005$).

4.6 Effect of Ratio (BDNF / IL-1 beta) against Cerebral edema

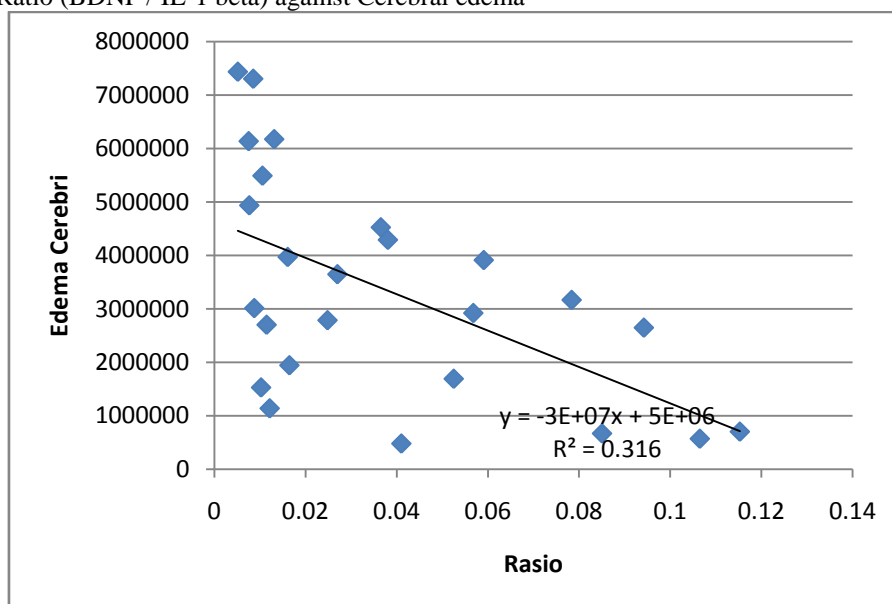


Figure 8. Curve relationship between the ratio with cerebral edema

Description: Increasing the ratio will be followed by a decrease in cerebral edema Ratio (BDNF / IL-1 beta) significantly affects the number variable cerebral edema ($p < 0.005$).

V. Discussion

In severe traumatic brain injury occurs to any event that is very complex. Damage caused by a primary brain injury will cause interference at the level of atoms and molecules that contribute to the function and metabolism of cells. At the level of atoms and molecules will be a series of disruption of homeostasis some ions such as Ca^{2+} ions, the formation of excessive free radicals, the formation of molecules that are toxic so that at the cellular level will be interference with cellular integrity, cell swelling, necrosis and even apoptosis. If this process continues, the brain as an organ would be disrupted so that the nervous system will be malfunctioning. With the onset of nerve dysfunction patient can fall into various degrees of impaired consciousness [14].

Neuron membrane fatty acids are susceptible to oxidative stress, because the brain receives 20% of cardiac output. Therefore, to maintain the integrity of neurons from oxidative stress should be reduced such as

by anti-oxidants. Neuronal damage will increase the expression of BDNF synthesis, so that BDNF increases. The increase in BDNF will help the growth of neurons, defense, repair of neurons and synaptic plasticity and anti-apoptosis [15,16].

Soursop leaves have chemical constituents saponins, flavonoids, tannins, acids kafeat (Soemarji, 2002). Phytosterols, Ca oxalate, alkaloids mirisine also reportedly contained in the leaves of the soursop (Iswari, 2002). Obtained from the leaves contain flavonoids-like [4,6,17]. Flavonoids in various studies showed a potent antioxidant to inhibit the oxidation of arachidonic acid [18].

Adewole et al [8] say that Soursop Leaf Extract doses of 25-100 mg / kg intraperitoneally is safe in rats. After all relatively high doses (> 200 mg / kg) intraperitoneally cause toxic or lethal in mice. In the study also said that a single dose of *Annona muricata* extract significantly reduced blood glucose levels and increase serum insulin in rat models of diabetes. In mice given streptozocin (STZ) which induces oxidative stress in the liver tissue indicate that extracts of the leaves of *Annona muricata* have antioxidant effects that inhibit oxidative damage caused STZ known from decreasing levels of malondialdehyde (MDA) [8]. Research conducted by de Sousa et al. [19] indicates that Graviola Leaf Extract has antinociceptive and anti-inflammatory effects.

Royal Jelly is a material that has the effect of anti-lipid peroxidation. There are studies that have reported the neuroprotective effects of Royal Jelly [20].

This study showed that administration of Soursop Leaf Extract can reduce inflammation in the brains of mice, consistent with research Adewole & Ojowole [8] but STZ-induced inflammation in mice. Royal Jelly has anti-inflammatory effects, according to research Kohno [21] which showed anti-inflammatory activity in experimental animals.

Increased levels of BDNF expected because of the effects of Royal Jelly on the synthesis of BDNF, and thus lower the apoptosis, according to research Aslan [20], which shows that Royal Jelly has antioxidant and neuroprotective effects against rabbit model treated with spinal cord injury.

In this study, a significant difference, the average number of apoptosis and cerebral edema, brain tissue taken from mice with brain injury after administration Soursop Leaf Extract and Royal Jelly. The treatment group Royal Jelly, showed a decrease edema (72.03%), decreased apoptosis (46.23%), a decrease in IL-1 β (51.5%), the increase in BDNF (446.31%).

Compared with the control, reduction of cerebral edema and apoptosis, obtained significant group Royal Jelly dose administration of 100 mg / kg and Soursop Leaf Extract dose of 200 mg / kg (apoptosis); Royal Jelly dose of 100 mg / kg, and Soursop Leaf Extract dose of 200 mg / kg and 400 mg / kg (cerebral edema). This happens because the expected anti-inflammatory and antioxidant effects of Soursop Leaf Extract [8]. While Royal Jelly has antioxidant effects and inhibit lipid peroxidation [20].

IL-1 β is one of the few materials proinflammatory role in brain injury. IL-1 β is secreted when there are triggers inflammatory pathways and pathway free radicals in brain injury. Decreased levels of IL-1 β loaded significantly in the treatment group Royal Jelly dose administration of 100 mg / kg and Soursop Leaf Extract dose of 200 mg / kg and 400 mg / kg. It is estimated that as a result of anti-inflammatory and antioxidant effects of Soursop Leaf Extract [8], as well as the antioxidant effects of Royal Jelly [20].

BDNF is a compound that has the effect of trophic (plays in the lives and development of neurons). Various kinds of stimulus will increase BDNF gene expression. Examples are light stimulation, osmotic, whisker stimulus, and physical activity increase BDNF mRNA expression in various areas in the brain [15].

Increased levels of BDNF loaded significantly in the treatment group Royal Jelly dose administration of 100 mg / kg and Soursop Leaf Extract dose of 200 mg / kg. It is estimated that, when giving treatment Soursop Leaf Extract and Royal Jelly, appeared stimulus in the brain that ultimately increases brain levels of BDNF. The effect of increased BDNF in the delivery of Soursop Leaf Extract 200 mg / kg higher than the dose of 400 mg / kg body weight, may be due to the effects of toxicity [8].

To complete understanding of the relationship between the decrease in the number of cerebral edema and apoptosis with decreased levels of IL-1 β , and increased levels of BDNF, used regression analysis. From this analysis, it was found the relationship between the decrease in the number of cerebral edema and apoptosis to decreased levels of IL-1 β . There is also a correlation between the decrease in the number of cerebral edema and apoptosis to increased levels of BDNF.

VI. Conclusions

From the results of this study concluded that administration of Soursop Leaf Extract and Royal Jelly in mice with brain injuries for 7 days:

1. Giving Soursop Leaf Extract and Royal Jelly in rats who suffered brain injuries reduce levels of IL-1 β .
2. Giving Soursop Leaf Extract and Royal Jelly in rats brain injury increases levels of BDNF.
3. Royal Jelly in rats brain injury decreases the amount of cerebral edema.
4. Soursop Leaf Extract and Royal Jelly in rats brain injury decreases the amount of apoptosis.
5. Giving Soursop Leaf Extract and Royal Jelly in rats brain injury decrease cerebral edema and apoptosis of

neurons through increasing the ratio of BDNF / IL-1 β .

VII. Tables

Table 1. Mean body weight of experimental animals

Sample	K	P1	P2	P3	P4
1	262	264	270	265	263
2	258	275	273	270	271
3	264	272	271	261	265
4	260	262	259	258	269
5	272	265	261	263	261
Mean	263.2	267.6	266.8	263.4	265.8

Table 2. Analysis of animal body weight

	N	Minimum	Maximum	Mean	Std. Deviation	Variance	F	Sig.
K	5	258.0	272.0	263.200	5.4037	29.200	.714	.592
P1	5	262.0	275.0	267.600	5.5946	31.300		
P2	5	259.0	273.0	266.800	6.3403	40.200		
P3	5	258.0	270.0	263.400	4.5056	20.300		
P4	5	261.0	271.0	265.800	4.1473	17.200		

Table 3. Animal treatment

Group	Cerebral Contusion	Soursop Leaf Extract	Royal Jelly
K (Control)	+	-	-
P1	+	100 mg/kg	-
P2	+	200 mg/kg	-
P3	+	400 mg/kg	-
P4	+	-	100 mg/kg

Table 4. Area of cerebral edema

Group	Average cerebral edema (micron ²)
K	5538324,04
P1	4046254,92
P2	2657322,59
P3	2958208,06
P4	1549128,31

Table 5. Significance test of mean of cerebral edema

Group	Mean	St. Dev.	F hit	Sig.
K (control)	5538324,0360	1666276,02261	4.000*	0.015
P1 (soursop leaf extract 100 mg)	4046254,9240	2422744,45058		
P2 (soursop leaf extract 200 mg)	2657322,5880	1580567,43873		
P3 (soursop leaf extract 400 mg)	2958208,0580	1258905,74440		
P4 (Royal Jelly)	1549128,3080	1252735,77986		
Total	3349847,5828	2071071,33394		

Table 6. Apoptosis

Sample	K	P1	P2	P3	P4
1	64	64	48	47	37
2	59	57	44	43	32
3	65	59	44	41	35
4	68	54	45	46	34
5	62	55	42	41	33
Mean	63,6	57,8	44,6	43,6	34,2

Table 7. Significance test of mean of apoptosis

Group	Mean	St. Dev.	F hit	Sig.
K (control)	64.800	4.6044	64.838*	0.000
P1 (soursop leaf extract100 mg)	58.200	3.7014		
P2 (soursop leaf extract200 mg)	41.800	3.3466		
P3 (soursop leaf extract400 mg)	43.200	3.3466		
P4 (Royal Jelly)	33.600	2.4083		
Total	48.320	12.1200		

Table 8. Level of IL-1 β (pg/mL)

Sample	K	P1	P2	P3	P4
1	10400	9200	8200	7200	5400
2	10200	9200	7300	7200	4800
3	10000	9900	7400	7100	4200
4	10900	10100	8600	6800	5500
5	11900	9900	6800	8800	6000
Mean	10680	9660	7660	7420	5180

Table 9. Significance test of level of IL-1 β

Group	Mean	St. Dev.	F hit	Sig.
K (control)	10680.000	759.6052	47.738*	0.000
P1 (soursop leaf extract100 mg)	9660.000	427.7850		
P2 (soursop leaf extract200 mg)	7660.000	726.6361		
P3 (soursop leaf extract400 mg)	7420.000	788.6698		
P4 (ekstrak Royal Jelly)	5180.000	694.2622		
Total	8120.000	2050.6097		

Table 10. Level of BDNF (pg/mL)

Sample	K	P1	P2	P3	P4
1	136	151	336	87	459
2	52	78	383	194	511
3	87	104	437	81	484
4	83	162	327	248	518
5	89	101	386	218	470
Mean	89.4	119.2	373.8	165.6	488.4

Table 11. Significance test of level of BDNF

Perlakuan	Mean	St. Dev.	F hit	Sig.
K (control)	89.400	30.0716	71.260*	0.000
P1 (soursop leaf extract100 mg)	119.200	35.7169		
P2 (soursop leaf extract200 mg)	373.800	44.2911		
P3 (soursop leaf extract400 mg)	165.600	76.9370		
P4 (ekstrak Royal Jelly)	488.400	25.5402		
Total	247.280	165.0246		

Table 12. Test of Mean of Ratio BDNF/IL-1 β

Group	Rata-rata	St. Dev.	F hit	Sig.
K (control)	0.008	0.0029	72.538*	0.000
P1 (soursop leaf extract100 mg)	0.012	0.0036		
P2 (soursop leaf extract200 mg)	0.049	0.0095		
P3 (soursop leaf extract400 mg)	0.022	0.0106		
P4 (ekstrak Royal Jelly)	0.096	0.0151		
Total	0.038	0.0342		

Table 13. Test Results Summary Simple Linear Regression Ratio Relations BDNF / IL-1 β with Apoptosis

	Coefficients	t hitung	Sig.	F	Sig.
Constant	59.058			42.270	0.000
Rasio (BDNF/IL-1 beta)	-285.050	-6.502	0.000		
R ² = 0.648					
F table = 4.279					

Table 14. Test Results Summary Simple Linear Regression Ratio Relations BDNF / IL-1 β with Cerebral edema

	Coefficients	t hitung	Sig.	F	Sig.
Constant	4631741,845			10.629	0.003
Rasio (BDNF/IL-1 beta)	-34028455,349	-3,260	0.003		
R ² = 0.316					
F table = 4.279					

References

- [1]. Alejandro, A.R. Treatment of Cerebral Edema, The Neurologist, 12(2), 2006, 59-73.
- [2]. Gennarelli TA, Graham DI. Neuropathology, in Silver JM, McAllister TW, Yudofsky SC (eds.) Textbook of Traumatic Brain Injury. (American Psychiatric Publishing : Washington DC, 2005) 43-44.
- [3]. Clausen F, Hånell A, Israelsson C, Hedin J, Ebendal T, Mir AK, Gram H, Marklund N. Neutralization of interleukin-1 β reduces cerebral edema and tissue loss and improves late cognitive outcome following traumatic brain injury in mice. European Journal of

- Neuroscience, 34(1), 2006, 110–123.
- [4]. Bent S. Herbal medicine in the united states: review of efficacy, safety, and regulation. *Journal of General Internal Medicine*, 23(6), 2008, 854-9.
- [5]. El-Tahir KED & Bakeet D. The Black Seed *Nigella Sativa* Linn – Amine for Multicures: A Plan for Urgent Clinical Evaluation of Its Volatile Oil. *J.T.U Med Sc*, 2006.
- [6]. Khar et al. Differential Cytotoxic Effects of *Annona Squamosa* seed Extract on Human Tumor Cell Lines: Role of Reactive Oxygen Species and Oxidative Stress in Hepatocytes of Streptozotocin treated diabetic rats. *Complementary and Alternative Medicines*. Indian Academy of Sciences, 2005.
- [7]. Wene K. A Flavonol Triglicoside and Investigation of The Antioxidant and Cell Stimulating Activities Of *Annona Muricata* Linn. *Arch Pharm Res* 35, 2012.
- [8]. Adewole SO & Ojewole AO. Protective Effects of *Annona Muricata* Linn. (Annonaceae) Leaf Aqueous Extract on Serum Lipid Profiles and Oxidative Stress in Hepatocytes of Streptozotocin treated diabetic rats. *Complementary and Alternative Medicines*. African Journal Traditional, 2008.
- [9]. Istiadjid MES. Analgesia dan anestesia yang umum dipakai pada hewan laboratorium. In Istiadjid MES (ed.) *Buku Ajar Etik Penelitian Kesehatan* (Malang: Universitas Brawijaya Press, 2011) 281-283.
- [10]. Cernak, I. Animal Models of Head Trauma. *The Journal of the American Society for Experimental NeuroTherapeutic* 2, 2005, 410–422.
- [11]. Bareyre F, Wahl F, McIntosh TK, Stutzmann JM. Time Course of Cerebral Edema after Traumatic Brain Injury in Rats: Effects of Riluzole and Mannitol. *Journal of Neurotrauma*, 14(11) 2009, 839-849.
- [12]. Lucas SM, Rothwell NJ, Gibson RM. The role of inflammation in CNS injury and disease. *British Journal of Pharmacology*, 147(S1), 2009, 232-241.
- [13]. Hicks RR, Numan S, Dhillon HS, Prasad MR, Seroogy KB. Alterations in BDNF and NT-3 mRNAs in rat hippocampus after experimental brain trauma. *Molecular Brain Research* (48), 1997, 401–406.
- [14]. Kasan U. Cedera otak patofisiologi penanganan dan biomolekuler. (Surabaya, Faculty of Medicine Airlangga University, 2006).
- [15]. Binder DK, Scharfman HE. Brain-derived neurotrophic factor. *Growth Factors*. 22(3), 2004, 123-131.
- [16]. Slemmer JE, Weber JT, DeZeeuw CI. Cell death, glial protein alterations and elevated S-100B release in cerebellar cell cultures following mechanically induced trauma. *Brain research*, 10(21), 2004, 159-166.
- [17]. Zakirof SK. Alkaloids from *Annona Muricata* leaves. *Chemistry of Natural Compounds*, 47(2), 2011.
- [18]. Miller LK. Baculovirus interaction with host apoptotic pathways. *Journal of cellular physiology*, 173(2), 2007, 178-82.
- [19]. De Sousa OV, Vieira GD, de Pinho JRG, Yamamoto CH & Alves MS. Antinociceptive and Anti-Inflammatory Activities of the Ethanol Extract on *Annona Muricata* L. Leaves in Animal Models. *International Journal of Molecular Sciences*, 11. 2010, 2067-2078
- [20]. Aslan A, Cemek M, Buyukokuroglu ME, Altunbas K, Bas O, Yusuf Yurumez. Royal jelly can diminish secondary neuronal damage after experimental spinal cord injury in rabbits. *Food and Chemical Toxicology*, 50, 2012, 2554–2559.
- [21]. Kohno K, Okamoto I, Sano O, Arai N, Iwaki K, Ikeda M, Kurimoto M. Royal Jelly Inhibits the Production of Proinflammatory Cytokines by Activated Macrophages. *Biosciences Biotechnology Biochemical*, 68(1), 2004, 138-145.