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The Effct of Extrac Propolis on DNF (Brain Derived Neurothropic Factors) and Monitoring GCS on Severe Head Injury with Diffuse Axonal Injury

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I. Introduction

More than 80% of patients who came to the emergency room were accompanied by head injuries. For each death, there were two cases with permanent disability, commonly secondary head injury(Narayan, 1991).

Brain-derived neurotrophic factor (BDNF)is one of the many neurotrophinsthat have properties as increasingly popular growth factor investigated as a neurotropic factor (Barde et al., 1982; Leibrock et al., 1989). BDNF is a growth factor playing a role in repair, defense, neuronal cell growth, and synapse plasticity. These molecules function to trigger the growth and differentiation of new neurons and synapses and playing an important role in the development of normal nerves (Leibl *et al.*, 2000).

Propolis containing 15% flavonoids is a potent antioxidant and neuroprotectant. The administration of propoliscan reduce the oxidation of neuron cell walls, especially in trauma border zones. Further study showed that propolis was able to increase the role of BDNF as anti apoptosis by inhibiting caspase-3 activation. Propolis has the potential as an alternative therapy in increasing BDNF expression and decreasing apoptosis that occurs in TBI so that it is expected to prevent secondary injury in TBI complications(Aliyazicioglu, 2011).

This study was conducted to assess the effect of administration of propolis extract on cerebrospinal fluid of BDNF levels and its relation to clinical improvement of severe head injury patients with diffuse axonal injury.

II. Research Method

This study was a clinical experimental study with a randomized double-blinded pre and post-test controlled trial design approach covering 18 subjects. The subjects in this study were all patients with severe head injuries (GCS 6-8) treated at Saiful Anwar Hospital Malang for a 12-week period.

Subjects who met the inclusion criteria of severe head injuries (GCS 6-8)with diffuse axonal injury, ICP monitor installed within 24 hours post trauma and aged 16-40 years were taken. The subject's exclusion criteria were no nuclear family (father/mother/ biological child) tosign a consenttoparticipate in the study, abnormalities that can cause a second-hit head injury, including a history of previous strokes, intracranial infections, previousdegenerative brain disorder, brain tumors, extracranial trauma accompanying (pneumothorax, flail chest, multiple rib fractures, vascular rupture, abdominal bleeding), factors of comorbidchronic diseases such as diabetes mellitus and chronic hypertension.

Patients with severe head injuries were divided into 2 groups, EP and K groups. Each group consisted of 10 severe head injuries patients with diffuse axonal injury. Both groups were given oxygen therapy, intravenous fluids, analgesics, antibiotics, and proton pump inhibitors (PPI)/anti H2 receptors (anti H2R) and fasted for 1 x 24 hours. In both groups, ICP was installed and cerebrospinal fluid samples were taken. The EP group was given additional feeding of 1500 mg/day propolis extract, while group K was given additional aquabides feeding as a placebo. Treatment was continued until 21 days of treatment. On day 5 after ventricular catheter placement, cerebrospinal fluid samples were collected through the ventricular catheter in both sample groups. BDNF levels were examined in both sample groups and Glasgow Coma Scale was assessed in both sample groups, then the data was analyzed.

Severe head injuries were head injuries patients with Glasgow Coma Scale of 6–8. Diffuse axonal injury treatment is established clinically and CT scan shows no bleeding abnormalities.

DAI is diffuse axon damage in the cerebral hemisphere, corpus callosum, brain stem and cerebellum (pedunculus). DAI treatment and CT Scan examination were clinically established, including: 1. Clinical Treatment: It was obtained a decrease in consciousness and neurological deficits in patients. 2. CT Scan: There was a presence of small hemorrhagic lesions in the corpus callosum, upper brain stem, corticomedullary

junction, parasagittal region, and basal ganglia. Brain computed tomographic (CT) was found to be innaccurate in predicting patient outcomes and not related to GCS scores and patient's neurological status.

Propolis extract is a propolis preparation on the market with BPOM certification. The preparation was then dissolved in 15 cc sterile aquabides before being given to the patient through a nasogastric tube. The dosage was 1500 mg per day divided into 3 doses given through a nasogastric tube.

Clinical condition is the general condition of the patient assessed using Glasgow Coma Scale. In this study, the difference in the Glasgow Coma Scale scores was assessed in the initial and final assessment. Glasgow Coma Scale is an assessment of quantitative level of consciousness based on three components, namely eye response, verbal response andmotor response. It has a score between 3 and 15.

BDNF is the 2nd member of neurotrophic family of growth factor obtained in the nerve tissue most commonly found in cortex and hippocampus, but it can also be detected in cerebrospinal fluid, and examined by the ELISA method. The unit is ng/mL. The scale is continuous data. BDNF day-0 was the BDNF level taken from the ventricular catheter when installing the ICP monitor. BDNF day-5 was a BDNF level taken from the ventricular catheter just before it was removed. BDNF was examined using ELISA according to Ray Biotech, Inc. The wells with antibodies were dripped with a standard and sample therapies in which the sample and standard therapies have been prepared of 100ul. It was incubated for 150 minutes, stirred at room temperature, then washed with buffer. The washed wells were dripped with a 100ul biotin labeled antibody solution, then incubated for 60 minutes, and stirred at room temperature. Then, 100% streptavidin was added, incubated for 45 minutes and stirred at room temperature, then washed. 100ul subtract solution was added to the washed wells, then incubated for 30 minutes. After that, 50ul stopolution was given. Wells were read using Elisa reader with a wavelength of 450nm.

Data Analysis

The calculation process used SPSS version 17.0 computer software. The difference test between treatments (EP and K groups) was carried out by paired t-test (parametric test) with an alternative of Mann-Whitney test (non-parametric test). Correlation test or relationship between administration of propolis extract and clinical improvement of patients was performed by Pearson correlation test (to determine the correlation significance and relationship strength) and regression test (to determine the effect of administration of propolis extract on clinical improvement).

III. Result

A total of 18 patients who met the inclusion criteria were then divided into two groups

Basic Characteristics of Research Subject

The basic characteristics of the subject including gender, age, address, education level, onset of head injury, and comorbidity can be seen in Table 1.

Table 1 Basic Characteristics of Research Subject

Characteristic		Control Group (n=9)	Propolis Group (n=9)
Age	15-20 years	2	3
	20-30 years	6	5
	>30 years	1	1
Gender	Male	7	8
	Female	2	1
Address	Malang	9	9
	Outside Malang	0	0
Education Level	Primary-Secondary S.	0	0
	HS/Vocational HS	3	4
	Higher Education	6	5
Onset of Injury	<2 hours	5	6
	2-4 hours	4	3
	>4 hours	0	0
Comorbidity	DM	0	0
	HT	0	0
	Dyslipidemia	0	0
Outcome	Died	1	0

Difference in Levels of *Brain-derived Neurotrophic Factor* (BDNF)

The results showed that BDNF level on day 0 in the control group (4.49±0.38 pg/mL) was not different from the group with propolis treatment (4.85±0.52 pg/mL) (independent T-test, p = 0.580). Further analysis showed that BDNF level on day 5 in the group with propolistreatment(7.32±0.44 pg/mL) was significantly higher than the control group (6.08±0.36 pg/mL) (T-test, p = 0.046). Increased BDNF levels from days 0 to 5 in

the treatment group (2.47±0.12 pg/mL) were significantly higher than the control group (1.59±0.08 pg/mL) (T-test, p = 0.000). Figures 1 and 2 show differences in BDNF levels and increased BDNF in the control and treatment groups.

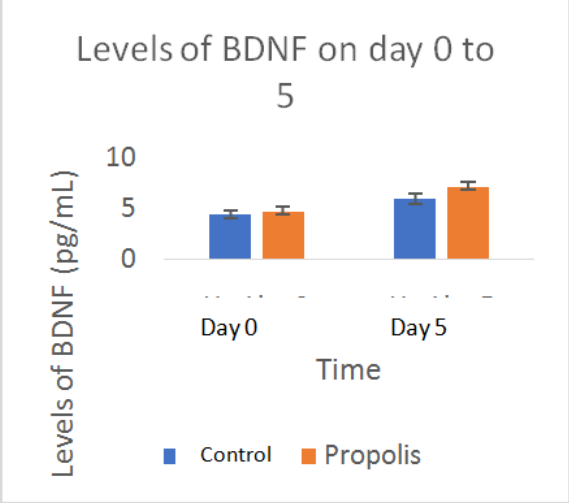

Figure 1 Comparison of BDNF levels in the control group and propolis group on day 0 and day 5

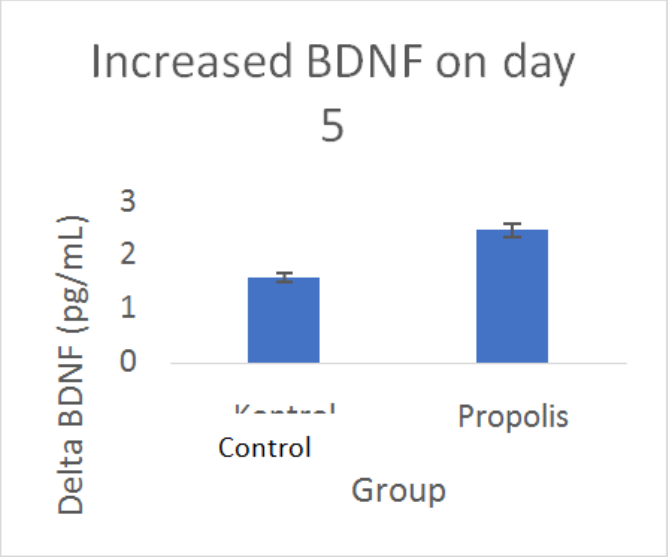

Figure 2 Comparison of increased BDNF levels in the control group and propolis group on day 5.

Difference in*Glasgow Coma Scale* (GCS) Scores

The results showed that GCS score on day 0 in the control group (6.44±0.24) was not different from the group with propolis treatment (6.67±0.24) (Mann-Whitney test, p = 0.489). Further analysis also showed that GCS score on day 5 in the group with propolistreatment (7.44 ± 0.29) was insignificantly higher than the control group (7.11±0.2) (Mann-Whitney test, p = 0.436). Increased consciousness on day 5 in the propolis group was higher (0.78±0.22) than in the control group (0.67±0.17) but not significant (Mann-Whitney test, p = 0.796). After the follow-up on day 5, 1 patient in the control group died on the treatment day 12.

On day 21, GCS scores in the propolis group (9.89±0.2) showed a significant clinical improvement compared to the control group (8.88±0.29) (Mann-Whitney test, p = 0.027). The increase in GCS scores on day 21 also showed a significant difference compared to day 0 between the propolis group (3.22±0.15) and the control group (2.25±0.25) (Mann-Whitney test, p = 0.011). Figures 3 and 4 show differences in GCS scores and increased GCS scores in the control and treatment groups.

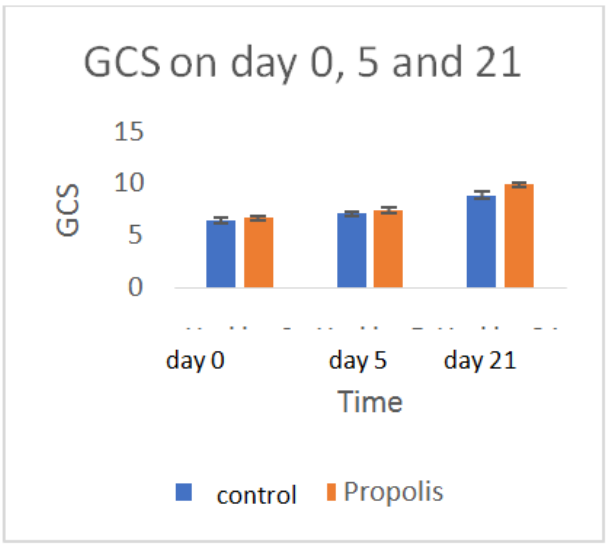

Figure 3 Comparison of GCS scores between thecontrol group and thepropolis group on day 0, day 5, and day 21.

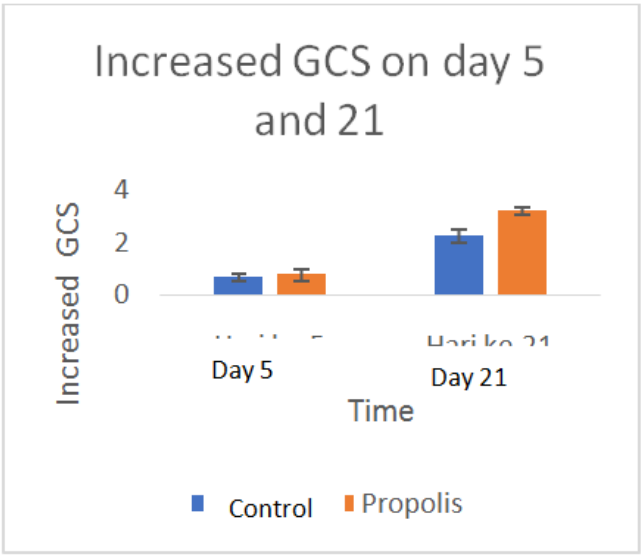

Figure 4 Comparison of the increased GCS scores on day 5 and day 21 for both groups.

Correlation between Increased *Brain-derived Neurotrophic Factor* (BDNF) and *Glasgow Coma Scale* (GCS) Score

BDNF levels did not show a significant correlation with increased GCS score on day 5 ($p = 0.477$; $r = 0.179$). Interestingly, increased BDNF showed a strong correlation with clinical improvement as indicated by increased GCS score on day 21 ($p = 0.22$; $r = 0.551$). These results indicate that the initial increase in BDNF was related to improvements in central nervous system function, or in this case, consciousness on day 21.

The result of the linear regression test showed that increased BDNF had an effect on the clinical improvement of the patient with a R2 value of 0.307, meaning that 30.7% of clinical improvement of patients on day 21 was affected by increased BDNF.

IV. Discussion

This studywas an experimental study with a post-test only controlled group design aimed to investigate the effect of propolis adjuvant therapy on clinical improvement (GCS) and BDNF levels. Based on the basiccharacteristics of the subject, there was no difference in the distribution of gender and age of the patients.

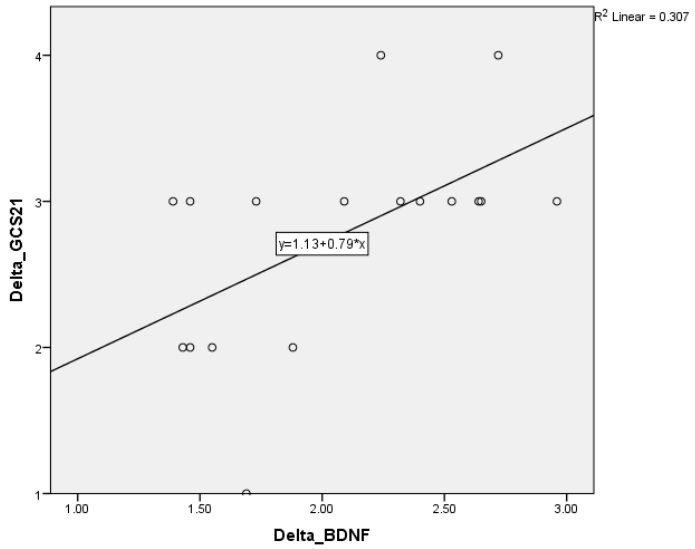

Figure 5 Graph of correlation between increased BDNF levels and increased GCS scores on day 21.

In addition, to minimize the bias, the study subjects were excluded for diseases other than the causes of head injury (diffuse axonal injury) such as diabetes, hypertension, and dyslipidemia.

Difference in the Levels of *Brain-derived Neurotrophic Factor* (BDNF)

BDNF has been widely investigated as a substance that serves to repair neurons, especially in terms of regulation of neuronal plasticity, neuronal cell growth, neuronal cell proliferation, neuronal cell survival and memory (Cohen-Cory et al, 2009; Khalin et al. 2016). The administration of BDNF molecules using poly nanoparticles (lactide-co-glycolide) has been shown to reduce the score of neurological severity in mice with traumatic brain injury (TBI) model (Khalin et al, 2016; Wurzelmann et al, 2017). In addition, BDNF has been investigated to be used as a predictor for the diagnostic and prognosis of patients with traumatic brain injury (Failla et al, 2016; Korley et al, 2016).

This study showed that administration of propolis can significantly increase BDNF levels on day 5 compared to group with standard therapy. This can be caused by a neuroprotective effect of propolis. Propolis is rich in flavonoids and polyphenols (Ghasem et al., 2007) and caffeoyliquinic acid (Nakajima et al, 2007) and has been investigated to have neuroprotective effects on several models of neurological disorders such as cerebral ischemia, neuronal apoptosis, and encephalomyelitis (Navarro and Boveris, 2009). In addition, propolis also contains caffeic acid phenethyl ester (CAPE) which has a selective bond affinity in human estrogen receptor β (hERβ), activates the nuclear pathway and can subsequently increase BDNF levels (Aliyazicioglu et al, 2011).

The administration of propolis in Parkinson's disease mice model showed that propolis water extract can increase BDNF levels and decrease oxidative stress parameters in dopaminergic neurons (Safari et al, 2015). In vitro study on SH-SY5Y human neuronal cell culture in the model of Alzheimer's disease showed that the administration of propolis can increase BDNF expression through activation of the transcriptional pathway (Ni et al, 2017). Other studies in vivo also showed that administration of propolis extract can increase BDNF levels in mice with traumatic brain injury (Annis et al, 2015). In mice model with spinal injury, propolis was able to maintain BDNF levels in the lesion site compared to the control group (Kasai et al, 2011).

However, other studies in vitro showed different results. The treatment of using propolis in general can increase levels of neurotrophic growth factor (NGF) but not BDNF in the culture of dental pulp cells (DPCs) and these findings have implications for further use of propolis in neurodegenerative diseases (Kudo et al, 2015). Although it showed different results on BDNF levels, it can be seen that propolis generally has a neuroprotective effect.

Difference in *Glasgow Coma Scale* (GCS) Scores

This study showed that propolis can provide an improvement in GCS scores on day 21, but not on day 5. This was probably due to the clinical improvement of head injury patients requiring sufficient duration, especially on activation of the neuron plasticity pathway. The administration of propolis has been shown to increase BDNF levels on day 5 and this was the initial improvement in central nervous system function in patients with head injuries.

Previous studies have been conducted to determine the clinical effects of propolis on various models such as Parkinson's disease, brain trauma, and ischemia. However, the studies on the effects of the administration of propolis to patients with head injuries were limited. In animal model with Parkinson's disease, propolis has been shown to improve contralateral turns behavior in animal model with Parkinson's disease with rotational behaviour assessment method induced by apomorphine (Safari et al, 2015). Clinical improvements tested with locomotor function in animals with spinal injury animal model have also been reported with high doses of propolis (1 or 5 mg/kg) intraperitoneally (Kasai et al, 2011). Although studies directly determining the effects of propolis on consciousness (GCS) of patients with head trauma have not yet been published, previous studies have reported that increased BDNF levels can clinically improve neurodegenerative disorder and trauma. Thus, it can be concluded that increased BDNF in patients on day 5 may affect the clinical improvement of patients on day 21. This wasin accordance with the result of this study that increased BDNF levels in patients was strongly correlated with increased GCS scores on day 21.

Research Limitation

This study only analyzed the levels of BDNF as a factor in improving brain tissue and clinical improvement of patients as indicated by consciousness. The mechanism for improving patient consciousness, especially on day 21 and influential factors need to be further investigated. In addition, markers of oxidative stress and inflammation, which also play a role in clinical improvement, need to be examined in this study. However, this study can only be used as a preliminary study on the neuroprotective effect of propolis on the setting of head trauma cases especially diffuse axonal injury.

V. Conclusion

The administration of propolis adjuvant therapy in severe head injury patients with a diagnosis of diffuse axonal injury can increase BDNF levels more significantly on day 5 and improve GCS scores on day 21 marked by an increase in GCS scores.

Further research is needed to find out the mechanism of action of propolis in increasing BDNF levels and its correlation with clinical improvement both in vitro and in vivo and to assess the effects of propolis administration with a larger number of subjects with the parameters of inflammatory marker, apoptosis, and oxidative stress in the patient's cerebrospinal fluid with a head injury.

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